OLYMPUS

User Manual

OLYMPUS Stream [Ver.2-5]

IMAGING ANALYSIS SOFTWARE

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Contents

1. Before you start

1.1. Which documentation comes along with your software?

The documentation for your software consists of several parts: the installation manual, the online help, and PDF manuals which were installed together with your software.

Where do you find which information?

A quick setup guide describing the software activation is delivered with your software.

On the setup-DVD, several PDF manuals are provided.

- In the installation manual, you can find the system requirements. Additionally, you can find out how to install and configure your software.
- In the user manual, you will find both an introduction to the product and an explanation of the user interface. By using the extensive step-by-step instructions you can quickly learn the most important procedures for using this software.
- The database is explained in its own user manual.

In the online help, you can find detailed help for all elements of your software. An individual help topic is available for every command, every toolbar, every tool window and every dialog box.

New users are advised to use the manual to introduce themselves to the product and to use the online help for more detailed questions at a later date.

Writing convention used in the documentation

In this documentation, the term "your software" will be used for OLYMPUS Stream.

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Example images

The DVD that comes with your software contains, among a lot of other data, also images that show different examples of use for your software. You can load these so-called example images from the DVD. However, in many cases, installing the example images on your local hard disk or on a network drive is more helpful. Then the example images will always be available, no matter where the DVD with the software currently is.

Note: Your software's user documentation often refers to these example images. You can directly follow some step-by-step instructions when you load the corresponding example image.

You can open and view the example images with your software. Additionally, you can use the example images to test some of your software's functions, for example, the automatic image analysis, the image processing or the report creation.

Due to the fact that the example images also contain multi-dimensional images like Z-stacks or time stacks, making use of them enables you to quickly load images that require more complex acquisition settings.

Installing example images

You can install the example images after you've installed the software, or at any later point in time.

To do so, insert the DVD that contains the software into the DVD drive. If the installation wizard starts, browse to the directory that contains the example images and install them.

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1.2. Online help for your software

In the online help, you can find detailed help for all elements of your software. An individual help topic is available for every command, every toolbar, every tool window and every dialog box.

When you use the help mode, you'll have access to most online topics. As soon as you use the context help, you will find yourself in the help mode. In the help mode, a question mark will be attached to the mouse pointer. Then you will be able to call for help on almost all of your software's functions.

Switching to the help mode

There are various ways of switching to the help mode.

- Click the *Context Help* button. You can find this button on the *Standard* toolbar.
- Use the *Help > Context Help* menu command.
- Use the [Shift + F1] shortcut.

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1.3. About your software

Note: Not every software package contains all of the features!

To support the different requirements on the software optimally, a variety of packages are available for your software. The larger software packages contain more features than the smaller packages. For example, the smaller packages contain only restricted database functionality.
Some of the functions described are, therefore, of no relevance to users of smaller packages.

Acquiring images

You can use your system to acquire high resolution images of a sample in a few steps. Your system is comprised of your software and the hardware, e.g., microscope and camera. During image acquisition, the data from the camera which is mounted on your microscope will be read out and displayed on your computer's monitor.

You can first examine the live-image and adjust it optimally. The live-image will be constantly updated, i.e., when you, for example, move the stage to a different position, the live-image will be changed accordingly. You can switch the live-image on and off and acquire a photo of the parts of the sample that interest you. When you do this, you will create a digital image that you can save and process or analyze with a variety of your software's functions.

Acquiring and viewing multi-dimensional images

A multi-dimensional image is always made up of several frames. These have, for example, been acquired at different times, or in different focus positions. With your software you can, e.g., acquire a time stack or a Z-stack. For optimum viewing of multi-dimensional images, use the separate navigation bar that is shown directly in the image window when a multi-dimensional image is loaded.

Acquiring an EFI image

With your software, you can acquire images which have a practically unlimited depth of focus. These images are called EFI images. EFI is the abbreviation for "Extended Focal Imaging". For the creation of an EFI image, the software determines which of the pixels from the differently focused frames in a Z-stack are the sharpest, and calculates an image that is sharply focused in all areas from them.

Acquiring stitched images

When your system is equipped with a motorized XY-stage: Use the *XY-Positions/MIA* acquisition process to acquire a stitched image of a larger part of the sample. MIA stands for Multiple Image Alignment. During the acquisition, this acquisition process directly combines all of the images that are acquired, into a stitched image, just like a puzzle.

When your system is not equipped with a motorized XY-stage: Use the *Manual MIA* acquisition process and manually move the stage to have the different, adjoining parts of the sample put on display one after the other. By using this acquisition process directly during the acquisition, you combine all of the images that are acquired into a stitched image, just like a puzzle.

Saving documents in a database

You can insert not only images, but also documents which have another file format into a database. That enables you to store all manner of data that belongs together in one location. Search and filter functions make it quick and easy to locate documents.

Images will, by default, be saved in the TIF or VSI file format. When you save an acquired image in TIF format, a lot of important image information will be automatically saved with it, for example, data concerning the camera used, the exposure time, the resolution, the time of creation, and so on. You can later view this data again whenever you want, simply by opening the image with your software. You do not need to collect this data separately.

A PDF manual for your database is installed together with your software.

Measuring images

You can make various measurements on images, and, e.g., measure the length of a line, the perimeter of an ellipse or an angle in degrees. The measurement objects will be displayed in the image's drawing layer, and can be faded in and out. The measurement results will be shown in a sheet and can be differently sorted by a click of your mouse. You can export measurement results, for example, to the XLS format (for further editing in the MS-Excel application program).

You can measure an image, or several images at the same time, according to different material science analysis processes.
The *Materials Solutions* tool window works similarly to a software wizard. As soon as you've started an analysis process you'll be guided step by step through the measurement.
The following material science analysis processes are available:

- Chart Comparison
- Grains Intercept

- Grains Planimetric
- Layer Thickness
- Cast Iron
- Inclusions Worst Field
- Inclusion Content
- Throwing Power
- Porosity
- Phase Analysis
- Particle Distribution
- Automatic Measurement
- Coating Thickness
- Dendrite Arm Spacing

Processing images

You can process the acquired images and retroactively optimize the image quality according to your requirements. Numerous filters and functions are available for this purpose, e.g., various smoothing or sharpness filters, and functions to optimize the contrast. As well as this, you can mirror the images and also rotate them through an arbitrary number of degrees.

Analyzing images automatically

With an automatic image analysis, your software searches for areas in an image that have the same intensity, or color. All of the areas that have the same intensity, or color will be assigned to a phase, and evaluated. This makes it possible to automate typical measurement tasks. You can, for example, determine the area ratios of the different phases in an image.

Creating reports

You can document the results of your work in a report. To do this, select the required page templates and images in the *Report Composer* tool window, for example, and generate an MS-Word report.

Alternatively, you can create a report in the MS-Excel format straight from your software, containing the last image to be measured and all of the measurement results, for example. MS-Excel reports are particularly useful for users who want to analyze the data and measurement results gained in the image analysis program further in MS-Excel.

In case you want to insert images, workbooks or charts from your software into new or existing MS-Word, MS-Excel, or MS-PowerPoint documents, use a special Olympus add-in for this. With the help of this add-in, you can access all documents and data that you created with your image analysis program from MS-Word, MS-Excel, or MS-PowerPoint. You can apply different settings to all the report's images, detail zooms, for example. It's sufficient for your image analysis program to be open in the background.

Controlling the microscope

You can control your microscope's motorized parts via the software. For example, you can change an objective, load an ND filter, or open and close a shutter, with your

software. To make this communication function, the components must not only be motorized, but also have been configured in the software.

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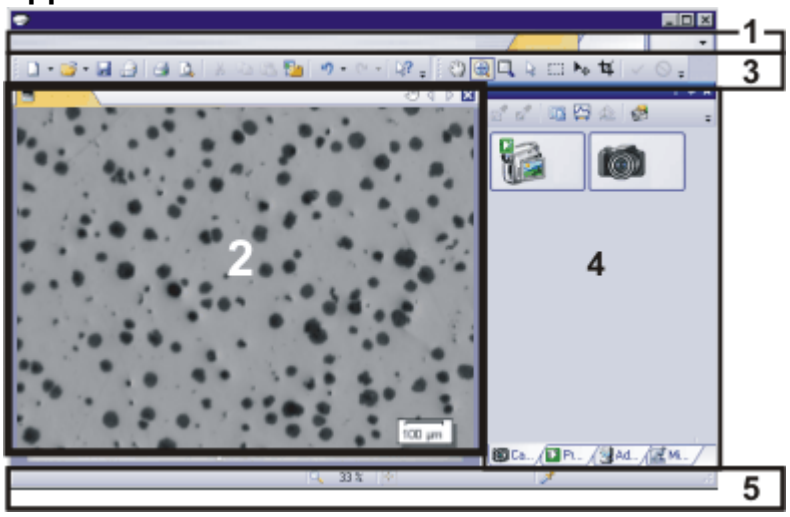
2. User interface

The graphical user interface determines your software's appearance. It specifies which menus there are, how the individual functions can be called up, how and where data, e.g. images, is displayed, and much more. Here, the basic elements of the user interface are described.

Note: Your software's user interface can be adapted to suit the requirements of individual users and tasks. You can, e.g., configure the toolbars, create new layouts, or modify the document group in such a way that several images can be displayed at the same time.

Appearance of the user interface



The illustration shows the schematic user interface with its basic elements.

(**1**) Menu bar
(**2**) Document group
(**3**) Toolbars
(**4**) Tool windows
(**5**) Status bar

(1) Menu bar

You can call up many commands by using the corresponding menu. Your software's menu bar can be configured to suit your requirements. Use the *Tools > Customization > Start Customize Mode...* command to add menus, modify, or delete them.

(2) Document group

The document group contains all loaded documents. These can be of all supported document types.
When you start your software, the document group is empty. While you use your software it gets filled - e.g., when you load or acquire images, or perform various image processing operations to change the source image and create a new one.

(3) Toolbars

Commands you use frequently are linked to a button providing you with quick and easy access to these functions. Please note, that there are many functions which are only accessible via a toolbar, e.g., the drawing functions required for annotating an image. Use the *Tools > Customization > Start Customize Mode...* command to modify a toolbar's appearance to suit your requirements.

(4) Tool windows

Tool windows combine functions into groups. These may be very different functions. For example, in the *Properties* tool window, you can find all the information available on the active document.

In contrast to dialog boxes, tool windows remain visible on the user interface as long as they are switched on. That gives you access to the settings in the tool windows at all times.

(5) Status bar

The status bar shows, e.g., a brief description of each function. Simply move the mouse pointer over the command or button for this information.
You can also find additional information in the status bar.

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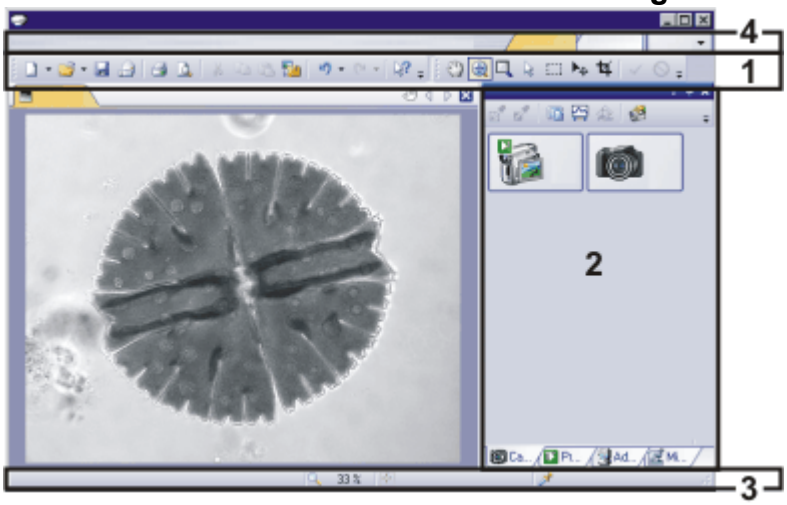
2.1. Overview - Layouts

What is a layout?
Your software's user interface is to a great extent configurable, so that it can easily be adapted to meet the requirements of individual users or of different tasks. You can define a so-called "layout" that is suitable for the task on hand. A "layout" is an arrangement of the control elements on your monitor that is optimal for the task on hand. In any layout, only the software functions that are important in respect to this layout will be available.

Example: The *Camera Control* tool window is only of importance when you acquire images. When instead of that, you want to measure images, you don't need that tool window.
That's why the “Acquisition” layout contains the *Camera Control* tool window, whereas in the “Processing” layout it's hidden.

Which elements of the user interface belong to the layout?



The illustration shows you the elements of the user interface that belong to the layout. The layout saves the element's size and position, regardless of whether they have been shown or hidden. When, for example, you have brought the *Windows* toolbar into a layout, it will only be available for this one layout.

(**1**) Toolbars
(**2**) Tool windows
(**3**) Status bar
(**4**) Menu bar

Switching to a layout

To switch backwards and forwards between different layouts, click on the right-hand side in the menu bar on the name of the layout you want, or use the *View > Layout* command.

Which predefined layouts are there?

For important tasks several layouts have already been defined. The following layouts are available:

Working with a database ("Database" layout)
Acquiring images ("Acquisition" layout)
Viewing and processing images ("Processing" layout)
Generating a report ("Reporting" layout)
Analyzing images using deep learning (layout "Deep Learning")

In contrast to your own layouts, predefined layouts can't be deleted. Therefore, you can always restore a predefined layout back to its originally defined form. To do this, select the predefined layout, and use the *View > Layout > Reset Current Layout* command.

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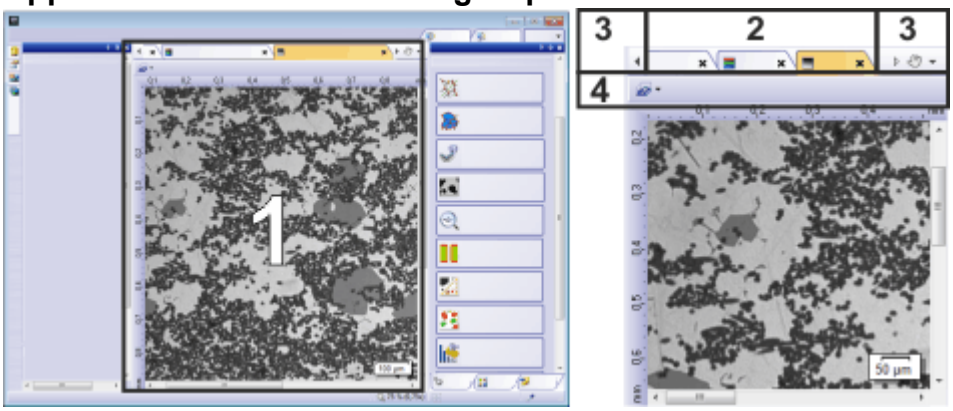
2.2. Document group

The document group contains all loaded documents. As a rule, images will be loaded. You can also find other types of documents in the document group, charts for example.

Appearance of the document group



(**1**) Document group in the user interface
(**2**) Document bar in the document group
(**3**) Buttons in the document bar
(**4**) Toolbar in the image window

(1) Document group in the user interface

You will find the document group in the middle of the user interface. In it you will find all of the documents that have been loaded, and of course also all of the images that have been acquired. The live-image and the images resulting from any image processing function are also displayed there.

Note: At the same time, up to 150 documents can be loaded in the document group.

(2) Document bar in the document group

The document bar is the document group's header.

For every loaded document, an individual tab showing the document name will be set up in the document group. Click the name of a document in the document bar to have this document displayed in the document group. The name of the active document will be shown in color. Each type of document is identified by its own icon.

At the top right of each tab, a small [**x**] button is located. Click the button with the cross to close the document. If it has not yet been saved, the *Unsaved Documents* dialog box will open. You can then decide whether or not you still need the data.

(3) Buttons in the document bar

The document bar contains several buttons, on the left and on the right.

Hand button

Click the button with a hand on it to extract the document group from the user interface. In this way you will create a document window that you can freely position or change in size.

If you would like to merge two document groups, click the button with the hand in one of the two document groups. With the left mouse button depressed, drag the document group with all the files loaded in it, onto an existing one.

Prerequisite: You can only position document groups as you wish when you are in the expert mode. In standard mode the button with the hand is not available.

Arrow buttons

You can find two arrow buttons at the top left and the top right of the document group.

When your software starts, the arrow buttons are inactive. The arrow buttons will only become active when you have loaded so many documents that all of their names can no longer be displayed in the document group.

If you have loaded so many images that all of their names can no longer be displayed in the document group, click one of the two arrows. This scrolls the fields with the document names to the left or to the right. That will enable you to see the documents that were previously not shown.

List of loaded documents

Click the small arrow on the right to open a list of all of the loaded documents. If you are using more than one document group, the loaded documents are sorted by document group. A horizontal line divides the document groups from each other.

Left click the document that you want to have displayed on your monitor.

Alternatively, you can use the *Documents* tool window or the *Gallery* tool window to get an overview of the documents that have been loaded.

(4) Toolbar in the image window

Navigation bar in the image window

Multi-dimensional images, time stacks for example, have their own navigation bar directly in the image window. Use this navigation bar to set or to change how a multi-dimensional image is to be displayed on your monitor.

There are some other document types with their own navigation bar directly in the image window. One example is a report instruction.

Selecting image window views

There can be more than one view for the same image. For example, with an image series you can display in the image window either an individual image or an overview of all of the individual images. There is a menu with all of the image window view options for the active image on the image window’s toolbar.

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2.3. Tool Windows

What is a tool window?

Tool windows combine functions into groups. These may be very different functions. For example, in the *Properties* tool window, you can find all the information available on the active document.

In contrast to dialog boxes, tool windows keep visible on the user interface as long as they are switched on. That gives you access to the settings in the tool windows at all times.

Showing and hiding tool windows

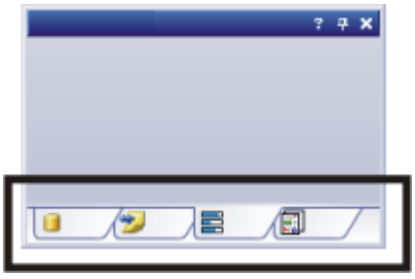
Which tool windows are shown by default depends on the layout you have chosen. You can, at any time, make specific tool windows appear and disappear manually. To do so, use the *View > Tool Windows* command.

Position of the tool windows

The user interface is to a large degree configurable. For this reason, tool windows can be docked, freely positioned, or integrated in document groups.

Docked tool windows

Tool windows can be docked to the left or right of the document window, or below it. To save space, several tool windows may lie on top of each other. They are then arranged as tabs. In this case, activate the required tool window by clicking the title of the corresponding tab below the window.



Freely positioned tool windows

You can only position tool windows as you wish when you are in the expert mode.

You can at any time float a tool window. The tool window then behaves exactly the way a dialog box does. To release a tool window from its docked position, click on its header with your left mouse button. Then, while pressing the left mouse button, drag the tool window to wherever you want it.

Saving the tool window's position

Tool windows and their positions are saved together with the layout and are available at the same position the next time you start your software. A return to the original layout using the *View > Layout > Reset Current Layout* command will have the result that only the tool windows that are defined by default for this layout will be displayed.

Buttons in the header

In the header of every tool window, you will find the three buttons *Help*, *Enable Auto Hide*, and *Close*.

Click the *Help* button to open the online help for the tool window.
Click the *Enable Auto Hide* button to minimize the tool window.
Click the *Close* button to hide the tool window. You can make it reappear at any time, for example, with the *View > Tool Windows* command.

Context menu of the header

To open a context menu, right click a tool window's header. The context menu can contain the *Auto Hide* and *Transparency* commands.

Additionally, the context menu contains a list of all of the tool windows that are available. Every tool window is identified by its own icon. The icons of the currently displayed tool windows will appear clicked. You can recognize this status by the icon's background color.
Use this list to make tool windows appear.

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2.4. Working with documents

You can choose from a number of possibilities when you want to open, save, or close documents. As a rule, these documents will be images. In addition, your software supports other document types as well.

Saving documents

You should always save important documents immediately following their acquisition. You can recognize documents that have not been saved by the star icon after the document's name.

There are a number of ways in which you can save documents.

1. To save a single document, activate the document in the document group. Then use the *File > Save As...* command or press [Ctrl + S] on your keyboard.
2. Use the *Documents* tool window.

   Select the desired document and use the *Save* command in the context menu. For the selection of documents, the standard MS-Windows conventions for multiple selection are valid.
3. Use the *Gallery* tool window.

   Select the desired document and use the *Save* command in the context menu. For the selection of documents, the standard MS-Windows conventions for multiple selection are valid.
4. Save your documents in a database. That enables you to store all manner of data that belongs together in one location. Search and filter functions make it quick and easy to locate saved documents.

Automatic save

1. When you exit your software, all data that has not yet been saved will be listed in the *Unsaved Documents* dialog box. This gives you the chance to decide which document you still want to save.
2. You can also configure your software in such a way that all images are saved automatically after image acquisition. To do so, use the *Acquisition Settings > Saving* dialog box.

   Here, you can also configure your software in such a way that all images are automatically saved in a database after the image acquisition.

Closing documents

There are a number of ways in which you can close documents.

1. Use the *Documents* tool window.

   Select the document you want and use the *Close* command in the context menu. For the selection of documents, the standard MS-Windows conventions for multiple selection are valid.

2. To close a single document, activate the document in the document group and use the *File > Close* command. Alternatively, you can click the button with the cross [**x**]. You can find this button at the top right of the document tab next to the document name.
3. Use the *Gallery* tool window.

   Select the document you want and use the *Close* command in the context menu. For the selection of documents, the standard MS-Windows conventions for multiple selection are valid.

Closing all documents

To close all loaded documents use the *Close All* command or press [Ctrl + Alt + W] on your keyboard. You will find this command in the *File* menu, and in both the *Documents* and the *Gallery* tool windows' context menu.

Closing a document immediately

To close a document immediately without a query, close it with the [Shift] key depressed. Data you have not saved will be lost.

Opening documents

There are a number of ways in which you can open or load documents.

1. Use the *File > Open...* command.
2. Use the *File Explorer* tool window.
   To load a single image, double click on the image file in the *File Explorer* tool window.
   To load several images simultaneously, select the images and with the left mouse button depressed, drag them into the document group. For the selection of images the standard MS-Windows conventions for multiple selection are valid.
3. Drag the document you want, directly out of the MS-Windows Explorer, onto your software's document group.
4. To load documents from a database into the document group, use the *Database > Load Documents...* command.

Note: At the same time, up to 150 documents can be loaded in the document group.

Generating a test image

If you want to get used to your software, then sometimes any image suffices to try out a function.
Press [Ctrl + Shift + Alt + T] to generate a color test image.
With the [Ctrl + Alt + T] shortcut, you can generate a test image that is made up of 256 gray values.

Activating documents in the document group

There are several ways to activate one of the documents that has been loaded into the document group and thus display it on your monitor.

1. Use the *Documents* tool window. Click the desired document there.
2. Use the *Gallery* tool window. Click the desired document there.

3. Click the title of the desired document in the document group.
4. Click the small arrow at the top right of the document group to open a list of all of the loaded documents. Left click the document that you want to have displayed on your monitor.
5. In the *Window* menu, you will find a list of all of the documents that have been loaded. Select the document you want from this list.

Document group and database

Please note that in the *Database* layout the document group will not be shown. Select one of the other layouts, e.g., the *Processing* layout, to have the document group displayed.

Attaching a document to an e-mail

1. Load the documents you want to attach to your e-mail.
2. Use the *File > Send E-mail...* command.
3. Check whether all documents you want to attach are selected.
4. Click the *Send* button to generate an e-mail with the selected documents included as attachments.
   - You will receive a warning message if the sum of file sizes of all documents exceeds the maximum permitted size.
   - A new e-mail form will be opened by your e-mail program. Your e-mail program does not have to be already running for this to happen. The e-mail contains all of the selected image and document files as attachments.
     As long as the e-mail form remains open, you cannot use your software or your e-mail program. The e-mail form cannot be minimized, no can other e-mails be generated, nor can you read any incoming e-mails. You can't close the *Send E-mail* dialog box nor continue working.
5. Enter the recipient's address and your message and then send off your e-mail.

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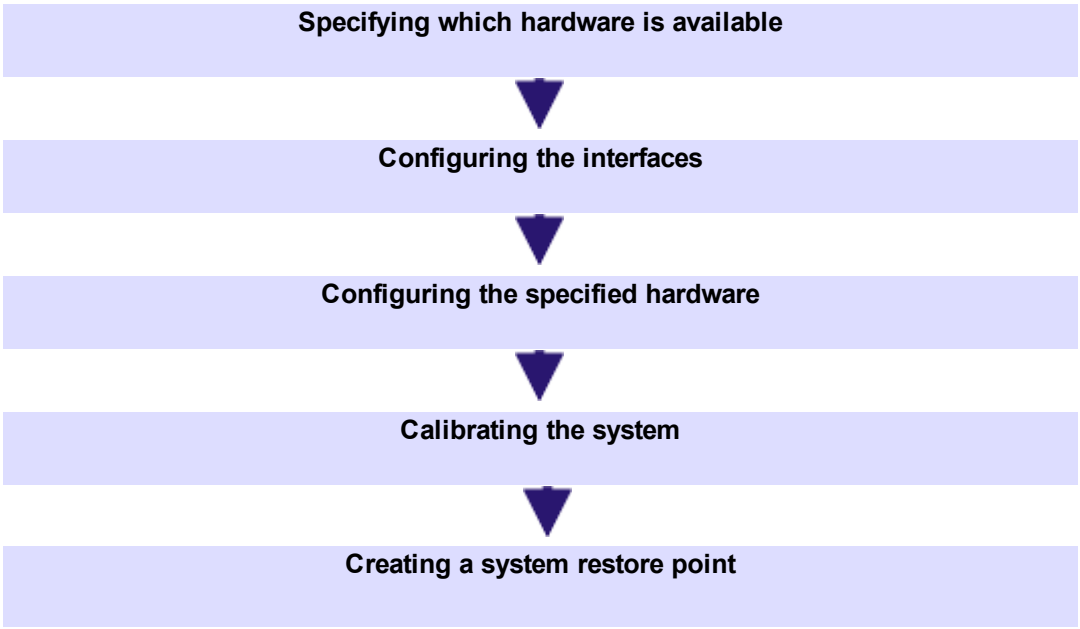
3. System configuration

3.1. Overview - System Configuration

Why do you have to configure the system?
After successfully installing your software you will need to first configure your image analysis system, then calibrate it. Only when you have done this will you have made the preparations that are necessary to ensure that you will be able to acquire high quality images that are correctly calibrated. When you work with a motorized microscope, you will also need to configure the existing hardware, to enable the program to control the motorized parts of your microscope.

3.1.1. Process flow of the configuration

To set up your system, the following steps are necessary:



Specifying which hardware is available
Your software has to know which hardware components your microscope is equipped with. Only these hardware components can be configured and subsequently controlled by the software. In the *Acquire > Devices > Device List* dialog box, you select the hardware components that are available on your microscope.

Configuring the interfaces
Use the *Acquire > Devices > Interfaces* command to configure the interfaces between your microscope or other motorized components, and the PC on which your software runs. Normally, the interfaces will automatically be configured properly.

Configuring the specified hardware
Usually, various different devices, such as a camera, a microscope and/or a stage will belong to your system. Use the *Acquire > Devices > Device Settings...* dialog box to

configure the connected devices so that they can be correctly actuated by your software.

Additionally, you can find all camera settings in the *Device Settings* dialog box.

Calibrating the system

When all of the hardware components have been registered with your software and have been configured, the functioning of the system is already ensured. However, it's only really easy to work with the system and to acquire top quality images, when you have calibrated your software. The detailed information that helps you to make optimal acquisitions will then be available.

Your software offers a wizard that will help you while you go through the individual calibration processes. Use the *Acquire > Calibrations...* command to start the software wizard.

Creating a system restore point

Use the *Create System Restore Point...* function to create a restore point for your system. Configurations that were applied to the image analysis system are saved in a system restore point. You can find the command in the *Acquire > Devices > Create System Restore Point...* menu.

3.1.2. About the system configuration

When do you have to configure the system?

You will only need to completely configure and calibrate your system anew when you have installed the software on your PC for the first time, and then start it. When you later change the way your microscope is equipped, you will only need to change the configuration of certain hardware components, and possibly also recalibrate them.

Necessary user rights for the system configuration

To be able to configure the system, you have to be logged in to your software with administrator or power user rights. If you have installed the software yourself you will automatically have been assigned Administrator rights.

In contrast, other users that also want to work with the software are given the *User* role. With this user role, the system configuration cannot be changed or viewed, i.e., the *Device List* and *Device Settings* dialog boxes cannot be opened.

For this reason, the software administrator has to assign the necessary user rights to those users who did not themselves install the software, but who are to be allowed to view or change the system configuration. Start the software as administrator and select the *Tools > User Rights...* command to open the *User Rights* dialog box. In it, select the required user, then click the *Properties...* button.

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3.2. Configuring the system

In order to acquire correctly calibrated images, the software requires information about your camera, the objectives and the microscope camera adaptor's

magnification. Set up your system with this in mind.

Preconditions

Your software is installed and the camera is connected to your PC. The camera driver is installed in MS-Windows.

Specifying which hardware is available

1. Start your software.

Setting up a new hardware configuration

2. Use the *Acquire > Devices > Device List...* command.
3.  Click the *Create New Device Configuration* button.
   - The *Create New Device Configuration* dialog box opens.
4. Enter a name for the new hardware configuration in the *Name* field. It is a good idea to choose a name combining the microscope and camera names, for example BX51_DP26.
   - Under this name, you can later reload this hardware configuration in the *Device Settings* dialog box.
5. Select the *Copy current device configuration* option if you have previously chosen your camera and microscope. Otherwise, choose the *Empty device configuration* option.
6. Close the *Create New Device Configuration* dialog box with *OK* to return to the *Device List* dialog box.
   - You will then find the new hardware configuration entered in the *Configuration* field.
   - Once you have completely set up a new hardware configuration, all entries from the *Device List* will be empty. You can now enter a completely new definition for the hardware configuration.

Defining the hardware configuration

Define the new hardware configuration in the *Device List* dialog box. Begin with the specifications for the camera and the microscope.

7. Select your camera (e.g. DP26) from the *Camera 1* list.
8. Select your microscope (e.g. BX51) from the *Frame* list. If your microscope isn't listed, select the *Manual Microscope* entry.
   - Once you have chosen a microscope, the options in the *Device List* dialog box change. For some microscopes there are default settings.

Examples of default settings:

   - For the manual microscope BX51, the *Manual Nosepiece* entry from the *Nosepiece* list is preset.
   - For the manual stereo microscope SZX10, the *Manual Nosepiece* and *Manual Zoom / Magnification Changer* entries are preset.
9. For some microscopes, you need to choose the port on which your camera is mounted (e.g. *Side (left)*). You find the list to the right of the camera list.

10. All other settings, such as nosepiece, observation filter wheel, shutter and condensor are appropriately preset, independent of your microscope. Check your settings and, if necessary, adjust them to suit your microscope equipment.

Initializing your devices

11. Close the *Device List* dialog box with *OK*.
    - Your hardware configuration will be automatically saved.
    - You can return to the default configuration whenever you want to. To do so, use the *Acquire > Devices > Device Settings...* command. Select the *Default* entry in the *Configuration* list.
    - As soon as you close the *Device List* dialog box, your software will try to set up the connection to the specified devices. You can see whether the devices can be successfully controlled in the *Acquire > Devices > Device Settings* device box.

Configuring the specified hardware

1. Use the *Acquire > Devices > Device Settings...* command.
   - In the tree view on the left side, you can find all hardware components that you have chosen in the device list.
2. Select the *Lightpath* entry in the *Sort by* list.

Configuring your camera

3. In the tree view on the left-hand side, expand the *Camera > <camera name>* entry (e.g. DP26).
4. Select the *Camera Adapter* entry.
5. Select your camera adapter's magnification on the right-hand side of the *Magnification* list. The magnification is imprinted on your camera adapter. Common values are 1.00 or 0.63.

Configuring the objective nosepiece

6. In the tree view, select the *General > Manual Nosepiece* entry if you have a manual microscope.
   In the tree view, select the *General > <Name of nosepiece>* entry if you have a motorized microscope.
   - On the right hand side of the dialog box, the current configuration of the nosepiece will be displayed. When you configure the software for the first time, the fields for the details referring to your objectives will be empty.
7. Choose the objectives which are currently fitted to the nosepiece from the right-hand side of the *Magnification* lists. Start with the smallest magnification and increase up through the higher magnifications. You can read the magnification off of the objective.
8. Choose each corresponding objective from the *Objective Type* list. The type is written on the objective.
   - In the *Description* field, a description of the objective will be suggested. You may change the description of the objective in the *Description* field, if you wish.

9. If the objectives don't use air as their refraction medium, select the immersion medium from the *Refraction Index* list. In this case, you find an appropriate label on the objective.

Configuring the mirror turret

10. Select the *General > <Name of the mirror turret>* entry in the tree view.
11. Make a selection for every position, whether it is occupied or not. For occupied positions, either select a filter or fluorescence cube being used from the *Filter* list, or enter the name of your filter module.
12. Select the *Free* entry for positions that have been purposely left free to keep the light path free of optical elements.
    For example, where the mirror turret is concerned, it is especially important that one position is kept free, in order not to impede the light path for the transmitted light microscopy.

Finishing system configuration

13. Close the *Device Settings* dialog box with *OK*.
    - In certain cases, a message appears telling you to check the calibrations. You can perform calibration now or later.
14. To have this toolbar displayed, use the *View > Toolbars > Microscope Control* command.
    - The *Microscope Control* toolbar contains buttons with all of your objectives with correct color codes.
    - For stereo microscopes or inverted microscopes, you find the zoom factors in the list to the right of the objectives.

Note: Use the *Create System Restore Point...* function to create a restore point for your system. Configurations that were applied to the image analysis system are saved in a system restore point. You can find the command in the *Acquire > Devices > Create System Restore Point...* menu.

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4. Image Acquisition

4.1. Overview - Acquisition processes

Your software offers a wide range of different acquisition processes.

4.1.1. Basic acquisition processes

Use the *Camera Control* tool window to acquire images and movies.

Acquisition process - Snapshot

You can use your software to acquire high resolution images in a very short period of time.

Acquisition process - Movie

You can use your software to record a movie. When you do this, your camera will acquire as many images as it can within an arbitrary period of time. The movie can be saved as a file in the AVI or VSI format. You can use your software to play it back.

4.1.2. Complex acquisition processes

Use the *Process Manager* tool window to handle complex acquisition processes.

Acquisition process - Time Lapse

With the automatic acquisition process *Time Lapse* you acquire a series of frames one after the other. This series of individual images makes up a time stack. A time stack shows you how an area of a sample changes with time. You can play back a time stack just as you do a movie.

If your microscope stage is equipped with a motorized Z-drive, when you acquire a time stack you can use the autofocus. You can find a description of the individual settings along with the description of the acquisition process.

Acquisition process - Z-stack

Use the automatic acquisition process *Z-Stack* to acquire a Z-stack. A Z-stack contains frames acquired at different focus positions. That is to say, the microscope stage was located in a different Z-position for the acquisition of each frame.

Alternatively, you can also acquire an EFI image with the *Z-Stack* acquisition process. Then a resulting image (EFI image) with a practically unlimited depth of focus is automatically calculated from the Z-stack that has been acquired. Such an image is

focused throughout all of its segments. EFI is the abbreviation for Extended Focal Imaging.

Acquisition process - XY-Positions/MIA

You can only use this acquisition process when your microscope is equipped with a motorized XY-stage. With this acquisition process you can carry out one or more automatic acquisition processes at different positions on the sample or acquire a stitched image of a larger sample position.

If your microscope stage is equipped with a motorized Z-drive, you can use the autofocus for this acquisition process. You can find a description of the individual settings along with the description of the acquisition process.

Acquisition process - Instant EFI

Use the manual acquisition process *Instant EFI* to acquire an EFI image at the camera's current position that is sharply focused all over.

Acquisition process - Manual MIA

When you use the *Manual MIA* acquisition process, you move the stage manually in such a way that different, adjoining sample areas are shown. Every time you click one of the buttons with an arrow, an image is acquired. With this acquisition process, you combine all of the images that are acquired, directly during the acquisition, just like a puzzle, into a stitched image. The stitched image will display a large sample segment in a higher X/Y-resolution than would be possible with a single acquisition.

Acquisition process - Instant MIA

For the *Instant MIA* acquisition process, you slowly move the stage manually over all of the positions on the sample that you want to acquire for the MIA image. Your software acquires images continuously and automatically assembles them. You just have to start the acquisition process, the acquisition of the individual images takes place automatically as you move the stage.

Acquisition process - MIX Light Source

With the *MIX Light Source* automatic acquisition process, you acquire a time stack where every frame shows the sample with different LEDs of the MIX light source switched on. Therefore, the light hits the sample from different angles and the sample can be illuminated from a 360° angle.

The MIX light source is a hardware component that is only available for certain microscopes (BX53M family, GX53, MX63, MX63L). This is why the *MIX Light Source* acquisition process is deactivated if you are using a different microscope or if the MIX light source wasn't selected in the microscope's device configuration.

Acquisition process - VisiLED MC 1500

With the *VisiLED MC 1500* automatic acquisition process, you acquire a time stack where every frame shows the sample with different LEDs of the VisiLED MC 1500 ring illumination. Therefore, the light hits the sample from different angles and the sample can be illuminated from a 360° angle.

The VisiLED MC 1500 is an optional hardware component for stereo microscopes (for example, SZX 7). This is why the *VisiLED MC 1500* acquisition process is deactivated if you are using a different microscope or if the *VisiLED MC 1500* light source wasn't selected in the microscope's device configuration.

4.1.3. Combination of several acquisition processes

You can combine several automatic acquisition processes. To do so, click the corresponding button for each acquisition process you require.

Note: Which automatic acquisition processes you can combine with each other, depends on your software.

Note: In the smaller software packages, the automated acquisition processes are only available when the *Automation* software solution is active.

When you combine the two acquisition processes *Z-Stack* and *XY-Positions/MIA* to acquire a Z-stack at several positions on your sample, to begin with, the complete Z-stack at the first position will be acquired. When that has been done, your system will move to the next position, and will acquire the next Z-stack etc...

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4.2. Acquiring a snapshot

You can use your software to acquire high resolution images in a very short period of time. For your first acquisition you should carry out these instructions step for step. Then, when you later make other acquisitions, you will notice that for similar types of sample many of the settings you made for the first acquisition can be adopted without change.

1. Switch to the *Acquisition* layout. To do this, use, e.g., the *View > Layout > Acquisition* command.
   - You can find the *Microscope Control* (**1**) toolbar at the upper edge of the user interface, right below the menu bar.
     To the right of the document group, you can find the *Camera Control* (**2**) tool window.

Selecting an objective

2. On the *Microscope Control* toolbar, click the button with the objective that you use for the image acquisition.

Switching on the live-image

3. In the *Camera Control* tool window, click the *Live* button.
   - The live-image (**3**) will now be shown in the document group. A new image document will automatically be created for the live-image.
4. Go to the required position on the sample.

Setting the image quality

5. Bring the sample into focus. The *Focus Indicator* toolbar is there for you to use when you are focusing on your sample.

Note: For some cameras, the *Focus Peaking* function is available to help you to focus on your sample.

6. Check the color reproduction. If necessary, carry out a white balance.
7. Check the exposure time. You can either use the automatic exposure time function, or enter the exposure time manually.
8. Select the resolution you want.

Acquiring and saving an image

9. In the *Camera Control* tool window, click the *Snapshot* button.
   - The acquired image will be shown in the document group.
10. Use the *File > Save As...* command to save the image. Use the recommended TIF or VSI file format.

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4.3. Acquiring HDR images

4.3.1. Overview - HDR images

What are HDR images?

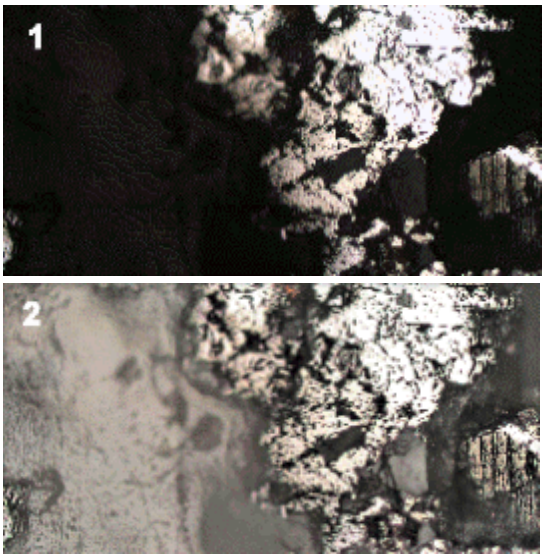
Under the microscope, certain samples (with very reflective metal surfaces, for example) can have such strong differences in brightness that it is impossible to find an exposure time that is suitable for all areas of the sample.

For such samples, an HDR image acquisition is recommended. **HDR** stands for **H**igh **D**ynamic **R**ange. Dynamic range relates to the capacity of cameras, or image processing software, to display both the bright and the dark segments of an image well.

Before acquiring an HDR image, the necessary exposure range needs to be determined for the current sample. The exposure range is made up of a minimum and maximum exposure time as well as several exposure times between them. Several individual images are then taken of the sample with differing exposure times, so that no image segment is left over or underexposed.

Your software then detects the best exposed pixels in each acquired individual image and merges them into one new image. Under correctly defined acquisition conditions, the HDR image will no longer contain any under or overexposed image segments.

Just like with images acquired with Extended Focal Imaging (EFI image), an HDR image is a rendered image containing information from several images.



Here, you can see an image acquired of a very reflective metal surface. Example 1 shows an image which was not acquired using HDR. The reflective segments of the surface are correctly exposed, whereas other segments are completely underexposed. Example 2 shows an image which was acquired using HDR. Without overexposing the reflective segments of the surface, now the structures in the dark image segments, which were not recognizable before, are visible.

Determining the exposure range

A recently determined exposure range will continue to be used for all HDR images until you let your software determine the exposure range anew. It is irrelevant whether the exposure range had been determined automatically or manually.

If you are acquiring several images of the same or similar parts of a sample, you don't need to determine the exposure range each time. If you change the sample or adjust settings on the microscope, it is recommended to determine the exposure range anew (either automatically or manually).

HDR images and acquisition processes

You can also insert an HDR image acquisition into an acquisition process, such as during the acquisition of a time stack or a Z-stack. The *Process Manager* tool window informs you about the status of the HDR image acquisition. If the *Enable HDR* check box is selected in the *Camera control* tool window, the *Process Manager* tool window shows the *Active* entry in the *HDR* field. If the check box is deselected, the *Process Manager* tool window shows *Off* in the *HDR* field.

HDR images and movie recording

It is not possible to record movies with HDR. Because of this, the *Enable HDR* check box is ignored while the *Movie recording* check box is selected.

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4.3.2. Acquiring an HDR image with an automatically set exposure range

With this procedure, your software automatically determines the exposure range. To do so, your camera automatically acquires a set of images with various exposure times and measures the amount of over and underexposed pixels. The exposure time continues to change until the amount of over and underexposed pixels is within defined limits. At this point, the exposure range has been defined. How much the exposure time is adjusted by is determined by your software with regards to the minimum and maximum exposure time.

Preparations

1. Switch to the *Acquisition* layout. To do this, use the *View > Layout > Acquisition* command, for example.
2. On the *Microscope Control* toolbar, click the button with the objective that you want to use for the acquisition of the HDR image.
3. Switch to the live mode, and select the optimal settings for your acquisition in the *Camera Control* tool window. Carry out a white balance. Then select an exposure time meaning that no part of the sample is overexposed.
   - The automatic exposure time detection uses this value as a basis and raises the exposure time so as to correctly light even the dark parts of the sample.
4. Search for the part of the sample which you want to acquire an HDR image of. This should be a position which has such significant differences in brightness that not all segments can be shown with optimal lighting.
5. Finish the live mode.

Acquiring HDR image

6. In the *Camera Control* tool window, select the *Enable HDR* check box.
   - In the upper part of the tool window, the *Snapshot* button changes to the *HDR* button.
     
   - If the *Enable Halation Removal* button in the *Halation Removal* group was switched on, this switches it off automatically. This is because the *HDR* and *Halation Removal* acquisition modes can't be used simultaneously.
7. In the *Determine exposure range* group, click the *Automatic* button to have the exposure range determined automatically.
   - The necessary exposure range will now be determined. To do so, the camera automatically acquires several images which only differ in exposure time. This acquisition occurs in the background, which means that the images are not shown in the document group. The exposure range determined in this way will continue to be used for all HDR images until you let your software determine the exposure range anew.
   - Determining the exposure range automatically takes about 30 seconds. Pay attention to the progress bar located in the status bar. When all elements in the

tool window are active again, the process has finished. In the *Total time* field, you can now see how long is needed for the HDR image acquisition.

- If, in the *Acquisition Settings > Acquisition > HDR* dialog box, the *Automatic HDR preview* check box is selected, the HDR image will be acquired and shown automatically, once the exposure range has been set.

8. If the HDR image has not been acquired automatically, click the *HDR* button in the *Camera Control* tool window to start the image acquisition.
   - The image acquisition will begin. Pay attention to the progress bar located in the status bar.

     

     It shows how long the acquisition has taken so far and the total acquisition time. The progress bar contains the *Cancel* button, which you can use to stop the current image acquisition.
   - After the acquisition has been completed the HDR image will be shown in the document group.
9. Check the image. If you want to change the settings (to use a different algorithm for the output rendering, for example), open the *Acquisition Settings* dialog box. Select the *Acquisition > HDR* entry in the tree view.
10. If you don't want to change any settings, use the *File > Save As...* command to save the image. Use the recommended TIF or VSI file format.
    - These are the only formats which also save all the image information including the HDR entries together with the image. This means that you can always see whether or not an image was acquired using HDR. Open the *Properties* tool window, and look at the data in the *Camera* group.

4.3.3. Acquiring more HDR images without setting the exposure range anew

If you have just acquired HDR images of the same or a similar sample, as a rule, it is not necessary to determine the dynamic range anew. In this case, you have already completed the preparations for acquisition (such as carrying out a white balance) and set the HDR image acquisition settings correctly (such as choosing the optimal algorithm used for output rendering) anyway.

In such circumstances, acquiring an HDR image is especially easy. Do the following:

1. In the *Camera Control* tool window, select the *Enable HDR* check box.
2. Click the *HDR* button in the *Camera Control* tool window to start the image acquisition.
   - The image acquisition will begin. After the acquisition has been completed the HDR image will be shown in the document group.
3. Check the image before saving it.
   - This step can be left out if your software is configured to import images into a database directly after acquisition.

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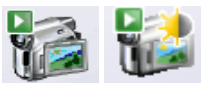
4.3.4. LiveHDR

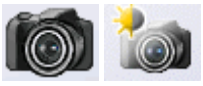
If you are using a DP74 camera, the *LiveHDR* function is available in addition to the *HDR* function. This function displays the live-image as an HDR image. You can acquire HDR images and HDR movies in this mode.

1. In the *DP74* group, select the *Use LiveHDR* check box.
   - This displays the *LiveHDR* group in the *Camera Control* tool window instead of the *HDR* group.
   - Different buttons appear in the *LiveHDR* group depending on what hardware your PC is equipped with.
2. Activate liveHDR mode in the *LiveHDR* group.

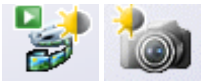
   If your PC has an NVIDIA graphics card that supports CUDA 2.1 or higher, click the *Fast LiveHDR* button or the *Fine LiveHDR* button.

   Click the *LiveHDR* button if your PC isn't equipped with the recommended graphics card.
   - The *Live* button changes to the *LiveHDR* button.

     
   - The *Snapshot* button changes to the *HDR* button.

     
   - If you select the *Movie recording* check box, the *HDR* button is replaced with

the *HDR Movie* button.



3. Specify whether to adjust the LiveHDR image automatically or manually.
   - The *Automatic adjustment* option automatically adjusts the settings for the LiveHDR image. This option gives you two settings for optimizing the LiveHDR mode.

     Select the *Remove halation* option to reduce interference from bright reflected light, thus increasing the image quality.

     Select the *Enhance texture* option to increase the texture in the sample, making edges and structures more pronounced.
   - The *Manual adjustment* option enables you to use the slide controls to adjust the LiveHDR image manually.
4. Click the *LiveHDR* button at the top of the tool window to activate the LiveHDR acquisition.
   - The live-image is displayed as a LiveHDR image.
5. Use the *HDR* button or the *HDR Movie* button to acquire an HDR image or an HDR movie.

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4.4. Acquiring an image without halation

Prerequisite: You can only acquire images without halation if your microscope is equipped with a rotational light source.

An image without halation is a computed image from which overexposure or reflections, that can be caused when light hits the sample from different angles, have been removed.

For images without halation, your software acquires a time stack in which every frame shows the sample with different LEDs of the rotational light source switched on. This makes the light hit the sample from different angles. Your software then takes certain pixels of the frames of the time stack and combines them into a new image. The acquisition of the frames takes place in the background. They are not shown in the document group and are not saved.

Which pixels in the frames are used depends on the projection that has been selected for the image without halation. For example, if the minimum intensity projection is selected, the darkest pixels in each of the frames will be used.

Rotational light source

Your software supports two different rotational light sources:
The MIX light source is an optional hardware component that is only available for certain microscopes (BX53M family, GX53, MX63, MX63L).
The *VisiLED MC 1500* light source is an optional hardware component for stereo microscopes.

Acquiring an image without halation

Prerequisite: The following step-by-step instructions describe how to acquire an image using a MIX light source. Acquiring an image using a *VisiLED MC 1500* light source, works in the same way.

Preparations

1. Switch to the *Acquisition* layout. To do this, use the *View > Layout > Acquisition* command, for example.
2. On the *Microscope Control* toolbar, click the button with the objective that you want to use for the acquisition of the image without halation.
3. Switch to live mode and select optimal settings for your acquisition, in the *Camera Control* tool window. Focus on the sample and, if necessary, perform a white balance. Set the exposure time automatically or select a suitable exposure time manually.

Note: The algorithm that is used to compute an image without halation sometimes delivers the best results when the image is slightly overexposed.
If this is the case for your samples, set the exposure time manually to achieve a slight overexposure. Alternatively, you can have the exposure time be determined automatically and select a positive value in the *Exposure compensation* list.

4. Finish the live mode.

Acquiring an image without halation

1. In the *Halation Removal* group, select the *Enable Halation Removal* check box.
   - The MIX light source is switched on as an additional light source. The setting for the existing illumination components (the reflected light LED for example) is not changed.
   - If the *Enable HDR* button in the *HDR* group was switched on, this switches it off automatically. This is because the *HDR* and *Halation Removal* acquisition modes can't be used simultaneously.

   - The appearance of the *Acquire a snapshot without halation* button at the top of the tool window changes.
2. Select whether to switch on 1 or 2 segments in the MIX light source at the same time (corresponding to 4 or 8 LEDs) for the acquisition of each frame. To do this, click one of these buttons.

3. Set the brightness of the LEDs of the MIX light source.
   - You can adjust the intensity of the MIX light source's LEDs continuously from 0 (no light) to 100% (full light strength). Usually a light intensity of 100% works well.
4. Select the step size. The step size determines how many new LEDs are used for the acquisition of the next frame. You can select between the *22.5°* and *45°* entries. If you select the *45°* entry for example, the LEDs being used move on by 2 positions each time a frame is acquired.

- The selection that you make affects the number of frames that are acquired and the time it takes to acquire them. You can't, however, see the number of acquired images because they aren't shown in the document group.

5. Select the projection that is to be used for computing the image without halation.
   - For example, if the minimum intensity projection is selected, the darkest pixels in each of the frames will be used.

6. Click the *Acquire a snapshot without halation* button.
   - The acquisition begins. Pay attention to the progress bar that is displayed in the status bar at the bottom left.
   - Your camera now automatically acquires several frames, using different LEDs of the MIX light source for each one. The acquisition of the frames takes place in the background. They are not shown in the document group and are not saved.
   - Your microscope's handswitch (the BX3M-HS for example) and the graphical preview of the MIX light source in the *Microscope Control* tool window indicate which LEDs are currently being used.
   - After the acquisition has been completed, the image without halation will be shown in the document group.
7. Use the *File > Save As...* command to save the image. Use the recommended TIF or VSI file format.
   - These are the only formats which also save all the image information concerning halation removal together with the image. This allows you to check at any time whether the *Enable Halation Removal* check box was selected when the image was acquired. Open the *Properties* tool window, and look at the data in the *Camera* group.
   - If you are using TIF or VSI file format you can also read out the saved acquisition parameters from the currently selected image and reapply them to your system. You can use the *Acquire > Restore Device Status* command to do this.

8. If you want to copy your settings for the selected segment and for the brightness of the MIX light source to the *Microscope Control* tool window: Click the *Apply Settings* button.

Note: The step size settings aren't transferred because an image without halation can only be acquired with two step sizes, (*22.5°* and *45°*) and four step sizes are available in the *Microscope Control* tool window.

- In the *Microscope Control* tool window, the same settings for the selected segment and the brightness of the MIX light source are now selected.

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4.5. Acquiring movies and time stacks

With your software you can acquire movies and time stacks.

4.5.1. Acquiring a movie

You can use your software to record a movie. When you do this, your camera will acquire as many images as it can within an arbitrary period of time.

1. Switch to the *Acquisition* layout. To do this, use the *View > Layout > Acquisition* command, for example.

Setting the magnification

2. On the *Microscope Control* toolbar, click the button with the objective that you want to use for the movie acquisition.

Selecting the storage location

3. In the *Camera Control* tool window's toolbar, click the *Acquisition Settings* button.
   - The *Acquisition Settings* dialog box opens.
4. Select the *Saving > Movie* entry in the tree structure.
5. You have to decide how a movie is to be saved after the acquisition. Select the *File system* entry in the *Automatic save > Destination* list to automatically save the movies you have acquired.
   - The *Path* field located in the *Directory* group shows the directory that will currently be used when your movies are automatically saved.
6. Click the [...] button next to the *Path* field to alter the directory.
7. In the *File type* list, select the file format in which you want to save the movie. You can save the movie either as a VSI image or as an AVI video. You can select the *AVI Video File (*.avi)* entry.

Selecting the compression method

8. Click the *Options...* button when you want to compress the AVI file in order to reduce the movie's file size.
9. Select, for example, the *Motion JPEG* entry from the *Encoder* list.

   Select the *Medium* entry in the *Quality* list.

   Close the *Movie Options* dialog box with *OK*.

Note: Compressing the movie is only possible if the selected compression method (codec) has already been installed on your PC. If the compression method has not been installed the AVI file will be saved uncompressed.
The selected compression method must also be available on the PC that is used for playing back the AVI. Otherwise the quality of the AVI may be considerably worse when the AVI is played back.

10. Close the *Acquisition Settings* dialog box with *OK*.

Setting the image quality

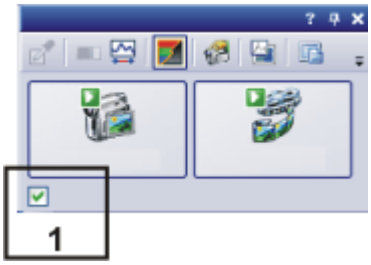
11. Switch to the live-mode and select the optimal settings for movie acquisition in the *Camera Control* tool window. Pay special attention to setting the correct exposure time.
    - This exposure time will not be changed during the movie recording. Even if you

have set the exposure time to automatic, the exposure time won't be adjusted while the movie is being recorded.

12. Find the segment of the sample that interests you and focus on it.

Switching to movie recording mode

13. Select the *Movie recording* check box (**1**). The check box can be found below the *Live* button in the *Camera Control* tool window.



- The *Snapshot* button will be replaced by the *Movie* button.

Starting movie recording

14. Click the *Movie* button to start the movie recording.
    - The live-image will be shown and the recording of the movie will start immediately.
    - In the status bar a progress indicator is displayed. At the left of the slash the number of already acquired images will be indicated. At the right of the slash an estimation of the maximum possible number of images will be shown. This number depends on your camera's image size and cannot exceed 2 GB.

    - This icon on the *Movie* button will indicate that a movie is being recorded at the moment.

Stopping movie recording

15. Click the *Movie* button again to end the movie recording.
    - The first image of the movie will be displayed.
    - The navigation bar for time stacks will be shown in the document group. Use this navigation bar to play the movie.
    - The software will remain in the movie recording mode until you clear the *Movie recording* check box.

4.5.2. Acquiring a time stack

In a time stack all frames have been acquired at different points of time. With a time stack you can document the way the position on the sample changes with time. To begin with, for the acquisition of a time stack make the same settings in the *Camera Control* tool window as you do for the acquisition of a snapshot. Additionally, in the *Process Manager* tool window, you have to define the time sequence in which the images are to be acquired.

Example: You want to acquire a time stack over a period of 10 seconds. One image is to be acquired every second.

1. Switch to the *Acquisition* layout. To do this, use the *View > Layout > Acquisition* command, for example.

Setting the magnification

2. On the *Microscope Control* toolbar, click the button with the objective that you want to use for the movie acquisition.
   If you are using a magnification changer, you will also have to select the magnification value used.

Setting the image quality

3. Switch to the live mode, and select the optimal settings for your acquisition, in the *Camera Control* tool window. Pay special attention to setting the correct exposure time. This exposure time will be used for all of the frames in the time stack.
4. Choose the resolution you want for the time stack's frames, from the *Resolution > Snapshot/Process* list.
5. Find the segment of the sample that interests you and focus on it.

Selecting the acquisition process

6. Activate the *Process Manager* tool window.
7. Select the *Automatic Processes* option.

8. Click the *Time Lapse* button.
   - The button will appear clicked. You can recognize this status by the button's colored background.
   - The [*t*] group will be automatically displayed in the tool window.
9. Should another acquisition process be active, e.g., *Z-Stack*, click the button to switch off the acquisition process.
   - The group with the various acquisition processes could, for example, now look like this:

Setting the acquisition parameters

10. Clear the check boxes *Start delay* and *As fast as possible*.
11. Specify the time that the complete acquisition is to take, e.g., 10 seconds. Enter the value 00000:00:10,000 in the *Recording time* field, to set the recording time to 10 seconds. You can directly edit every number in the field. To do so, simply click in front of the number you want to edit.
12. Select the radio button on the right-hand side of the *Recording time* field to specify that the acquisition time is no longer to be changed.
    - The  lock icon will automatically appear beside the selected radio button.
13. Specify how many frames are to be acquired.
    Enter e.g., 10 in the *Cycles* field.

- The *Interval* field will be updated. It shows you the time that will elapse between two consecutive frames.

Acquiring a time stack

14. Click the *Start* button.
    - The acquisition of the time stack will start immediately.

    - The *Start* button changes into the *Pause* button. A click on this button will interrupt the acquisition process.

    - The *Stop* button will become active. A click on this button will stop the acquisition process. The images of the time stack acquired until this moment will be preserved.
    - At the bottom left, in the status bar, the progress bar will appear. It indicates the number of images that are still to be acquired.

    - The acquisition has been completed when you can see the *Start* button in the *Process Manager* tool window again, and the progress bar is no longer displayed.
    - You will see the time stack you've acquired in the image window. Use the navigation bar located in the image window to view the time stack.
    - By default, the time stack that has been acquired will be saved automatically. The storage directory is shown in the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.

Note: When other programs are running on your PC, for instance a virus scanning program, it can interfere with the performance when a time stack is being acquired.

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4.5.3. Acquiring a time stack using the MIX light source acquisition process

Use the *MIX Light Source* acquisition process to acquire a time stack where every frame shows the sample with different LEDs of the MIX light source switched on. The light hits the sample from different angles. This makes the details of the sample more distinct than they would be if the sample was evenly illuminated.

Note: The MIX light source is an optional hardware component that is only available for certain microscopes (BX53M family, GX53, MX63, MX63L). This is why the *MIX Light Source* acquisition process is deactivated if you are using a different microscope or if the MIX light source wasn't selected in the microscope's device configuration.

Prerequisite: In the smaller software packages, the *MIX Light Source* acquisition process is only available when the *Automation* solution is active.

Before you start the acquisition process

1. Switch to the *Acquisition* layout. To do this, use the *View > Layout > Acquisition* command, for example.

2. Find the segment of the sample that interests you and focus on it.
3. In the *Options > Images > View* dialog box, specify whether to display the angle at which the light from the MIX light source hits the sample under each frame instead of displaying the acquisition time there. If you want to display the angle, select the *Display angle instead of time if available* check box.

Selecting the acquisition process

4. Activate the *Process Manager* tool window.
5. Select the *Automatic Processes* option.

6. Click the *MIX Light Source* button.
   - The button will appear clicked. You can recognize this status by the button's colored background.
   - The *MIX Light Source* group will be automatically displayed in the tool window.

Setting the acquisition parameters

Note: The settings that you make here are only valid for the duration of the *MIX Light Source* acquisition process. If other settings were made for the MIX light source in the *Microscope Control* tool window, these will not be changed. The settings made in the *Halation Removal* group in the *Camera Control* tool window are also not adopted by the *MIX Light Source* acquisition process.

7. Select whether to switch on 1 or 2 segments in the MIX light source at the same time (corresponding to 4 or 8 LEDs) for the acquisition of each frame. To do this, click one of these buttons.

8. Set the brightness of the LEDs of the MIX light source.
   - You can adjust the intensity of the MIX light source's LEDs continuously from 0 (no light) to 100% (full light strength).
9. Select the step size. The step size determines how many new LEDs are used for the acquisition of the next frame. You can select between the *22.5°*, *45°*, *90°* and *180°* entries. If you select the *45°* entry for example, the LEDs being used move on by 2 positions each time a frame is acquired.
   - The selection that you make affects the number of frames that are acquired and the time it takes to acquire them:
     at *22.5°*, 16 frames are acquired
     at *45°*, 8 frames are acquired
     at *90°*, 4 frames are acquired
     at *180°*, 2 frames are acquired
10. Decide whether you want to keep the current settings for the microscope's reflected light LED for the acquisition process. You can specify here, for example, for the microscope's reflected light LED to be used at a particular intensity for the acquisition process.
    - The settings that you make here are displayed in the *Microscope Control* tool window as well.

11. Click the *Start* button.
    - The acquisition of the time stack will start immediately.
    - The acquisition has been completed when you can see the *Start* button in the *Process Manager* tool window again, and the progress bar is no longer displayed.
    - You will see the time stack you've acquired in the image window.
    - By default, the time stack that has been acquired will be saved automatically. The storage directory is shown in the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.

Viewing the time stack

12. Use the navigation bar located in the image window to view the time stack. You can switch to tile view to look at the frames that have been acquired. Click the *Play* button to start the animation with the current settings.
    - You can calculate minimum intensity, maximum intensity, and mean intensity projection images from the multi-dimensional image retroactively.

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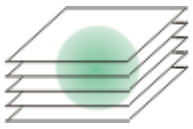
4.6. Acquiring Z-stacks

A Z-stack contains frames acquired at different focus positions. That is to say, the microscope stage was located in a different Z-position for the acquisition of each frame.

Note: You can only use the *Z-Stack* acquisition process when your stage is equipped with a motorized Z-drive.

Example: You want to acquire a Z-stack. The sample is approximately 50 µm thick. The Z-distance between two frames is to be 2 µm.



1. Switch to the *Acquisition* layout. To do this, use, e.g., the *View > Layout > Acquisition* command.

Selecting an objective

2. On the *Microscope Control* toolbar, click the button with the objective that you want to use for the image acquisition.

Setting the image quality

3. Switch to the live mode, and select the optimal settings for your acquisition, in the *Camera Control* tool window. Pay special attention to setting the correct exposure time. This exposure time will be used for all of the frames in the Z-stack.
4. Search out the required position in the sample.

Selecting the acquisition process

5. Activate the *Process Manager* tool window.
6. Select the *Automatic Processes* option.
7.  Click the *Z-Stack* button.
   - The button will appear clicked. You can recognize this status by the button's colored background.
   - The [*Z*] group will be automatically displayed in the tool window.

Setting the acquisition parameters

8. Select the *Range* entry in the *Define* list.
9. Enter the Z-range you want, in the *Range* field. In this example, enter a little more than the sample's thickness (= 50 µm), e.g., the value 60.
10. In the *Step Size* field, enter the required Z-distance, e.g., the value 2, for a Z-distance of 2 µm. The value should roughly correspond to your objective's depth of focus.
    - In the *Z-Slices* field you will then be shown how many frames are to be acquired. In this example 31 frames will be acquired.
11. Find the segment of the sample that interests you and focus on it. To do this, use the arrow buttons in the [*Z*] group. The buttons with a double arrow move the stage in larger steps.

Acquiring an image

12.  Click the *Start* button.
    - Your software now moves the Z-drive of the microscope stage to the start position. The starting positions lies half of the Z-range deeper than the stage's current Z-position.
    - The acquisition of the Z-stack will begin as soon as the starting position has been reached. The microscope stage moves upwards step by step and acquires an image at each new Z-position.
    -  The acquisition has been completed when you can see the *Start* button in the *Process Manager* tool window again, and the progress bar is no longer displayed.
    - You can see the acquired Z-stack in the image window. Use the navigation bar located in the image window to view the Z-stack.
    - The Z-stack that has been acquired will be automatically saved. You can set the storage directory in the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.

Note: When other programs are running in the background on your PC, for instance a virus scanning program, it can interfere with the performance when a Z-stack is being acquired.

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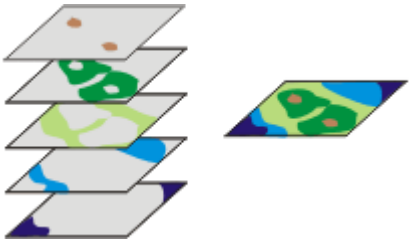
4.7. Acquiring an EFI image

What is EFI?

EFI is the abbreviation for Extended Focal Imaging. By using the "EFI" acquisition process you can acquire images with your microscope which have practically unlimited depth of focus. To do this, EFI uses a series of differently focused individual images (focus series) to calculate a resulting image (EFI image) that is focused in all of its parts.



At the left hand-side, the illustration shows a number of frames that were acquired at different Z-positions. In each of these frames there are only a few image segments that are displayed sharply focused. These segments are shown in color. These sharply focused image segments will be assembled into the EFI image (right).

Creating an EFI image

Your software offers you several ways of creating an EFI image.

Acquiring an EFI image without a motorized Z-drive
Acquiring an EFI image with a motorized Z-drive

4.7.1. Acquiring an EFI image without a motorized Z-drive

Example: You have a thick section in the transmitted light mode, or a sample with a very rough surface in the reflected light mode, e.g., with holes, grooves, bumps peaks or slanting planes. In the image it's only possible to bring one layer of the section or only part of the surface sharply into focus, higher-lying or deeper-lying areas are outside the depth of focus range. Acquire a Z-stack of the complete thickness or height of the sample, and have the EFI image calculated for you.

In this case, you can use the manual *Instant EFI* acquisition process to acquire a sharply focused image of all of the sample.

Note: You can use the *Instant EFI* acquisition process with every microscope. a motorized Z-drive or a Z-encoder aren't required.

Note: If your stage has a Z-drive or a Z-encoder, you can also acquire a height map with the *Instant EFI* acquisition process.

Selecting the acquisition process

1. Use the *View > Tool Windows > Process Manager* command to make the *Process Manager* tool window appear.
2. Select the *Manual Processes* option.

3. Click the *Instant EFI* button.
   - The button will appear clicked. You can recognize this status by the button's colored background.
   - The *Instant EFI* group will be automatically displayed in the tool window.

Setting the acquisition parameters

4. From the *Algorithm* list, select the *Reflected light* entry, when you use your light, or stereo microscope in the reflected light mode.
5. If you work with a stereo microscope, select the *Automatic frame alignment* check box.
   If you don't work with a stereo microscope, clear the *Automatic frame alignment* check box.

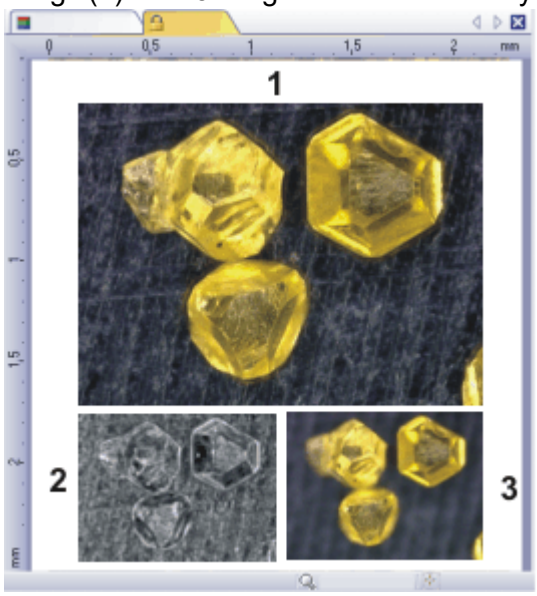
Preparing EFI acquisition

6. Use the *View > Tool Windows > Camera Control* command to make the *Camera Control* tool window appear.

7. In the *Camera Control* tool window, click the *Live* button.
8. In the live-image, move the microscope focus to the Z-position where either the lowest or the highest position on the sample is only just out of focus.
9. Check the exposure time, and correct it if necessary. When the *Instant EFI* acquisition process has been started, the exposure time will be kept constant during the whole of the acquisition.

Acquiring an EFI image

10. In the *Process Manager* tool window, click the *Start* button.
    - The live-image in the document group will divide itself into 3 images. On the bottom right, you'll still see the live-image (**3**). On the bottom left, you'll see the sharpness map (**2**). The large image above them is the composite resulting image (**1**). The 3 images will be continually updated.



11. Use your microscope's Z-drive to move your stage slowly through the height range of the sample's surface.
    - Your software will acquire images at the various focal planes, then it will set them together. While this is being done, the camera will acquire the images as quickly as possible. The sharpness value of individual pixels will be calculated for every image. If the sharpness values are higher than in the previous images, the pixels in the composite EFI image will be adopted. The EFI image contains the pixels with the highest sharpness values from all of the images acquired up till then.
    - The sharpness map at the bottom left will show you which image areas will be sharply reproduced in the EFI image. The brighter a pixel is in the sharpness map, the higher is its sharpness value in the EFI image.
    - Once the acquisition process has been started, the sharpness map should only be bright at the deepest or highest parts of the sample, the rest of the map is dark.
12. Focus on the sample slowly once through all the focal planes.
    After each change of the focus position, wait until you see that further areas become brighter in the sharpness map.
    - As the process continues, more and more areas in the sharpness map should become brighter. At the same time the EFI image will also get better and better.
13. Check the EFI image and the sharpness map. Are all areas of the image now sharp? Are there any areas in the sharpness map that are still dark?
    Focus on these areas and have additional images calculated into the EFI image.
    Continue acquiring additional images until the whole sample has been sharply reproduced.
14.  In the *Process Manager* tool window, click the *Stop* button.
    - The resulting image is not a Z-stack, but a standard image.
    - The EFI image will be automatically saved. You can set the storage directory in the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.
15.  In the *Camera Control* tool window, click the *Live* button again to release it.

4.7.2. Acquiring an EFI image with a motorized Z-drive

Example: You have a thick section in the transmitted light mode, or a sample with a very rough surface in the reflected light mode, e.g., with holes, grooves, bumps peaks or slanting planes. In the image it's only possible to bring one layer of the section or only part of the surface sharply into focus, higher-lying or deeper-lying areas are outside the depth of focus range. Acquire a Z-stack of the complete thickness or height of the sample, and have the EFI image calculated for you.

You can use the automatic *Z-stack* acquisition process, to acquire a sharply focused image of all of the sample.

Prerequisite: You can only use the *Z-Stack* acquisition process when your stage is equipped with a motorized Z-drive.

Setting the EFI parameters

1. Activate the *Process Manager* tool window.
2.  To open the *Acquisition Settings* dialog box, click the *Acquisition Settings* button in the tool window's toolbar.
3. Select the *Acquisition > Automatic EFI* entry in the tree view.
4. In the *Algorithm* list, select the *Transmitted light (exponential)* entry, if you're working in transmitted light mode, and the *Reflected light*, entry if you're working in reflected light mode.
5. Select the *Automatic frame alignment* check box when you're working with a stereo microscope and acquiring the sample at a viewing angle.
   Otherwise, clear this check box.
6. Close the *Acquisition Settings* dialog box with *OK*.

Preparing Z-stack acquisition

7. Carry out all the microscope settings.
8. In the *Microscope Control* toolbar, click the button corresponding to the objective you've set.
9. Activate the *Camera Control* tool window.
10. Switch to the live mode.
11. Optimize the exposure time. The exposure time will be kept constant during the acquisition of the Z-stack.
12.  Click the *Autofocus* button in the *Camera Control* tool window's toolbar to focus.

Setting the Z-stack parameters

13. Activate the *Process Manager* tool window.
14.  Select the *Z-Stack* acquisition process.
15. Select the *Top and bottom* entry in the *Define* list.
16. Use the arrow buttons in the [*Z*] group to move your stage to the Z-position at which the lowest-lying position on the sample is sharply focused.

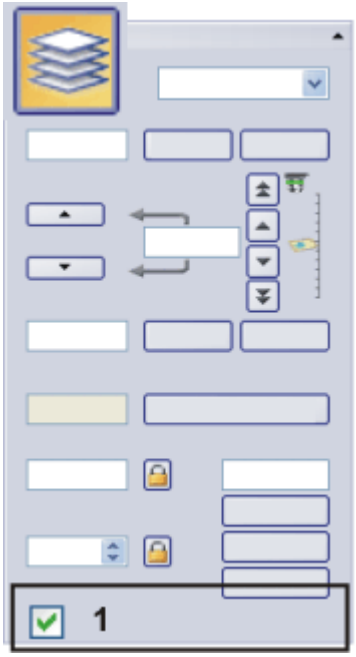
The arrow buttons move the stage either by steps of 2 µm or of 20 µm.

- The stage's current position will be shown to you in the *Position* field.

17. Click the top *Set* button to define the starting position for the Z-stack acquisition.
    - The current Z-position will be adopted in the *Start* field.
18. Use the arrow buttons in the [*Z*] group to move your stage to the Z-position at which the highest-lying position on the sample is sharply focused.
19. Click the bottom *Set* button to define the position at which the Z-stack acquisition is to end.
    - The current Z-position will be adopted in the *End* field.
20. In the *Step Size* field, enter the distance between two frames in the Z-stack. This Z-distance should be small enough to ensure that no positions on the sample between two images remain blurred. The higher your objective's Numerical Aperture is, the smaller the Z-spacing should be.
21. Use the [Enter] key to confirm the Z-distance that you've set.
    - The number of images in the stack will be automatically calculated on the basis of the Start and End values, and the Z-distance.

Starting EFI acquisition

22. Select the *Extended Focal Imaging* (**1**) check box. You find the check box at the bottom of the [*Z*] group located in the *Process Manager* tool window.



23. Finish the live mode.
24. Click the *Start* button.
    - The EFI acquisition begins immediately.
    - The acquisition will begin. After the acquisition has been completed the EFI image will be shown in the document group. This image was calculated from the variously focused separate images.

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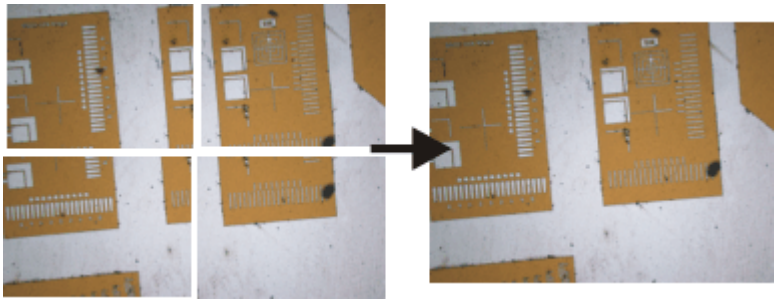
4.8. Creating stitched images

What is a stitched image?

If you acquire a stitched image, move the stage in a way that different, adjoining parts of the sample are shown. All of the images that are acquired are combined, just like a puzzle, into a stitched image. The stitched image will display a large part of the sample in a higher X/Y-resolution than would be possible with a simple snapshot.



The illustration shows left, four separate images. On the right you see the stitched image made up from the four images.

Creating a stitched image

Your software offers you several ways of creating a stitched image.

Acquiring a stitched image by moving the stage (Instant MIA)
Acquiring a stitched image without a motorized XY-stage (Manual MIA)
Acquiring a stitched image with a motorized XY-stage (XY-Positions/MIA)
Acquiring a stitched image with extended depth of focus
Automatically acquiring several stitched images
Combining individual images into a stitched image

Note: If image defects on the edge of an image decrease the quality of the stitched image or hinder the assembly of the individual images, you can clip these images during acquisition with *Subarray* mode in the *Camera Control* tool window.

Materials science analysis processes on stitched images

The *Materials Solutions* tool window offers several materials science analysis processes. Most of these processes can also be applied to stitched images, provided that you are working with a motorized XY-microscope stage. The acquisition of these stitched images are defined in the *Stage path settings* step within the materials science analysis process. In this case, you won't need the acquisition processes described in this topic.

4.8.1. Acquiring a stitched image by moving the stage (Instant MIA)

Preconditions

For the acquisition of stitched images, it's very important that your system has been correctly set up. If, for example, the shading correction wasn't performed correctly, the

individual images create a tiled effect in the stitched image. It's also very important that the camera is aligned parallel to the stage's XY-axes. When the camera is askew in relation to the stage, the individual images in the stitched image will also be askew in relation to one another. The angle between camera and stage should be smaller than 1°.

Making settings for the acquisition of an image

1. Switch to the *Acquisition* layout. To do this, use the *View > Layout > Acquisition* command, for example. The *Camera Control* tool window and the *Process Manager* tool window are displayed automatically.
2. Use the default acquisition settings for the *Instant MIA* process. To do so, open the *Acquisition Settings > Acquisition > Instant MIA* dialog box. Click the *Default* button and close the dialog box.

   - You can open this dialog box, for example, via the *Process Manager* tool window. In the tool window's toolbar, click the *Acquisition Settings* button. Select the *Acquisition > Instant MIA* entry in the tree view.
3. Select the microscope settings you want. In particular, select the required magnification. If you have defined observation methods, select the required observation method.
   - In this case, the background color of the stitched image depends on the observation method that has been selected. The background is automatically black for all fluorescence observation methods and all darkfield observation methods. The background is white for all other observation methods.

Selecting, configuring and starting the acquisition process

4. Activate the *Process Manager* tool window.

5. Select the *Manual Processes* option, and click the *Instant MIA* button.
6. Check the configuration of this acquisition process.

7. Click the *Start* button.
   - The *Adjust Acquisition Conditions* dialog box opens.
   - Your software will automatically switch to the live mode.
   - The camera resolution is set to the value that is specified in the acquisition settings.
   - You can't use HDR with the *Instant MIA* acquisition process. If HDR is activated when you start this acquisition process, you receive an error message to this effect. Deactivate HDR in the *Camera Control* tool window and restart the acquisition process.
   - Your software checks how much storage capacity is available. If too little storage capacity is available, an error message appears.
8. Select the optimal settings for your acquisition in the *Camera Control* tool window. You can still adjust the camera resolution as well.
   - The settings are applied to all of the individual images that make up the stitched image (exposure time, resolution, subarray, the white balance).

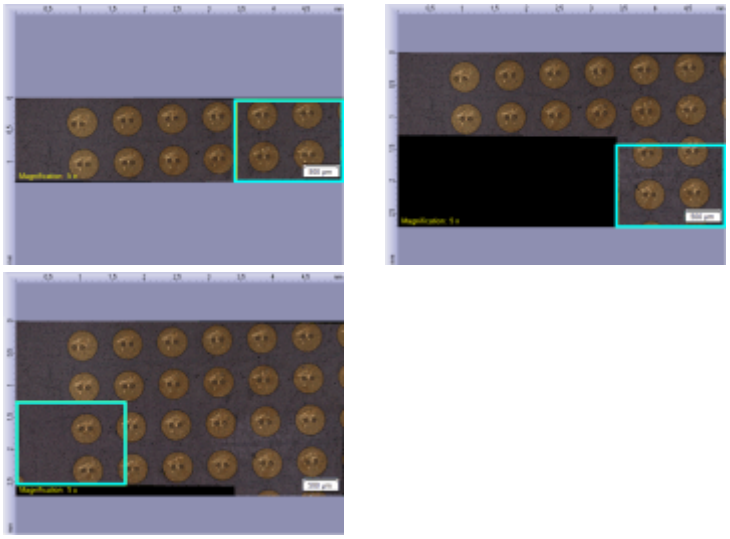
- The focus setting that is now made is, by default, also used for all of the individual images that make up the stitched image. The autofocus function is deactivated during the *Instant MIA* acquisition process. You can, however, still adjust the focus manually while the acquisition process is running. This is only possible in a special focus view.

Note: It's especially important that the sample is well exposed and that the current exposure time is as short as possible. If the exposure time is too long, you receive an error message.

9. Find the position on the sample at which you want to start acquiring the stitched image.
10. In the *Adjust Acquisition Conditions* dialog box, click the *Start* button.
    - The first image of the stitched image is displayed in the image window.
    - Most of the software's functions are now not available. Camera control is also locked.
    - The software switches to a special MIA image view. This view uses the MIA cursor. It consists of a square frame that can have different colors (see the table below).

Acquiring a stitched image

11. Slowly move the stage to the next position on the sample.
    - The camera acquires images continuously as long as you move the stage. The individual images are immediately assembled. You can watch how the stitched image grows, in the image window.
    - If required, use the mouse wheel to zoom in to or out of the stitched image. Alternatively, you can also use the *Zoom* toolbar for this.



Display of the stitched image during the *Instant MIA* acquisition process. The MIA cursor indicates the status of the image acquisition.

12. Pay attention to the MIA cursor. The color of the frame indicates the status of the image acquisition.

	A light blue frame means that there are no problems with assembling the stitched image.
	A yellow frame means that it's still possible to assemble the images. The settings, however, aren't optimal. It could be that the stage was moved too quickly, for example.
	An orange frame means that the stitching position was temporarily lost. It could be that the stage was moved too quickly, for example, or that the sample has too little image information at the current stage position for the images to be assembled. However, your software can often find the stitching position again in this state by its own means.
	A red frame indicates that the stitching position was definitely lost. Your software can't find the stitching position again in this state by its own means. However, in certain cases, you can manually bring your software into a state where the stitching position is found again. Alternatively you can now finish the *Instant MIA* acquisition process. The stitched image contains all information that had been acquired until the stitching position was lost.

Adjusting the focus on the sample

13. If you need to adjust the focus on the sample (for example, if you navigate to a slightly thicker position on the sample), click the *Focus View* button. You'll find the button in the *Instant MIA* group, located in the *Process Manager* tool window.
    - The *Focus View* button now becomes the *MIA Image View* button.
14. Adjust the focus on the image. Either use the focus knob on the microscope for this, or if your microscope has a motorized Z-drive, use the slide control in the *Microscope Control* tool window. The autofocus function can't be used while the *Instant MIA* acquisition process is activated.
    - In focus view, the live-image is displayed in a new tab. The MIA image view remains on its own tab in the image window. The stitched image, however, is not refreshed as long as you stay in focus view.
15. When you've adjusted the focus on the sample, click the *MIA Image View* button.
    - Switch back to the MIA image view and you can continue with the image acquisition.

Note: The *Instant MIA* acquisition process can’t run indefinitely. The acquisition process ends automatically after about 30 minutes.

Stopping image acquisition

16. Click the *Stop* button when you want to end the acquisition of the stitched image.
    - You see the completed stitched image, in the image window. It is usually not rectangular, but instead contains empty areas on its borders. In the stitched image, these areas are by default colored in white, or in black with dark field images.

You can also select any background color you want. To do so, select the *Select background color* check box in the acquisition settings.

- The stitched image will, by default, be automatically saved in a database. Alternatively, you can select a storage location, or switch off the automatic saving. To do so, use the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.
- The images composing the stitched image will not be saved separately.

4.8.2. Acquiring a stitched image without a motorized XY-stage (Manual MIA)

Example: You want to acquire an image of a large sample area. Use the *Manual MIA* acquisition process to acquire several individual images of adjoining positions on the sample, and to have them combined into a stitched image. MIA stands for Multiple Image Alignment.

Prerequisite

The camera is aligned parallel to the XY-stage. The angle between camera and stage should be smaller than 1°.

1. Switch to the *Acquisition* layout. To do this, use the *View > Layout > Acquisition* command, for example.

Selecting microscope settings

2. Select the microscope settings you want. In particular, select the required magnification. To do this, on the *Microscope Control* toolbar, click the button with the objective that you want to use for the acquisition of the stitched image. If you are using a magnification changer, you will also have to select the magnification value used.

   If you have defined observation methods, select the required observation method instead.

   - In this case, the background color of the stitched image depends on the observation method that has been selected. The background is automatically black for all fluorescence observation methods and all darkfield observation methods. The background is white for all other observation methods.

Setting the image quality

3. Switch to the live mode, and select the optimal settings for your acquisition, in the *Camera Control* tool window. Pay special attention to setting the correct exposure time. This exposure time will be used for all of the stitched image's individual images.

   - If you are using a DP74 camera, the live-image can be displayed as a LiveHDR image.

4. Find the position on the sample at which you want to start acquiring the stitched image.
5. Finish the live mode.

Selecting the acquisition process

6. Activate the *Process Manager* tool window.
7. Select the *Manual Processes* option.

8. Click the *Manual MIA* button.
   - The button will appear clicked. You can recognize this status by the button's colored background.
   - The *Manual MIA* group will be automatically displayed in the tool window.

   - Should the *Instant EFI* acquisition process have been active, it will be automatically switched off. You can, however, use images with extended depth of focus for the stitched image. To do this, before you acquire each of the individual images, click the *Instant EFI* button located in the *Manual MIA* group.

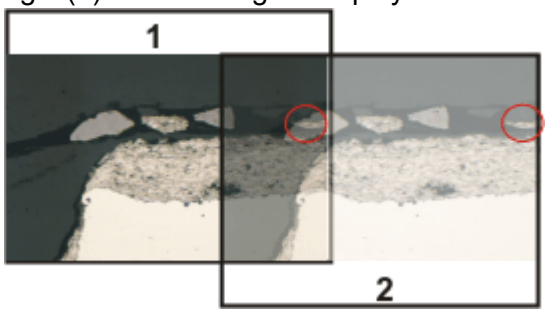
Setting the acquisition parameters

9. Make quite certain that the *Auto Align* button appears clicked. It should then look like this.
   - Then your software will search for the same image structures in neighboring individual images. The stitched image will be put together in such a way that image areas that are the same will be superimposed.

Acquiring a stitched image

10. Click the *Start* button.
    - Your software switches into the live mode.
11. Bring the sample into focus.

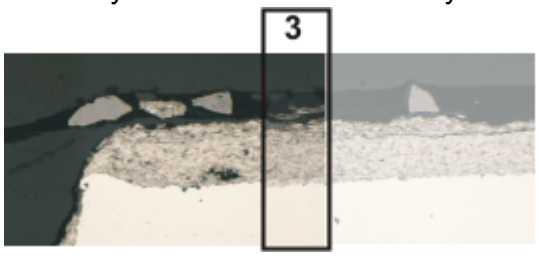
12. Click on one of the arrow buttons to set the side of the current image at which the next image is to be arranged. For example, click this button if the next image is to be laid to the right of the current image.
    - Your system now acquires an image at the current position on the sample. In the image window you now see on the left (**1**) the acquired image, and on the right (**2**) the live-image is displayed.



      Because you haven't moved the sample, the live-image is still displaying the current position on the sample. This means that you are now looking at two displays of the current image.
      The two images overlap. Because the live-image is transparent, both images are displayed in the overlap area.
13. Make a note of a significant structure on the live-image's right border. You will find the same sample structure in the overlap area. On the illustration, a significant

structure has been indicated by a circle.

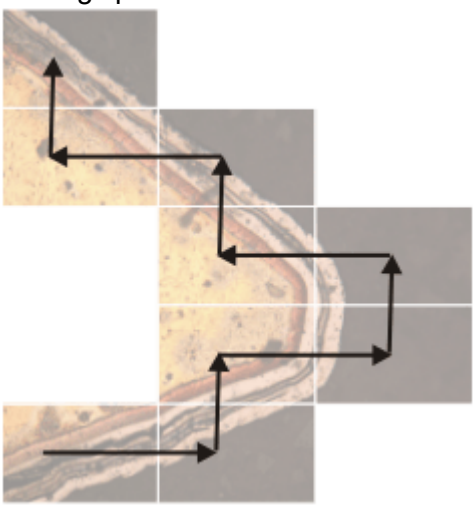
14. Now move the stage very slowly to make the structure on the live-image move to the left. Keep moving the stage until the image structures in the overlap area lie as exactly over each other as possible. The image structures need not lie precisely over each other, since your software will match the individual images with each other.
    - In the overlap area (**3**), the same image segments are shown now. This enables your software to seamlessly combine the two images.



    - You can reverse the direction in which your stage moves, in the *Device Settings > Stage* dialog box. Depending on how you can best orient yourself, the live-image will then move to the left or to the right, when you move your stage to the right.

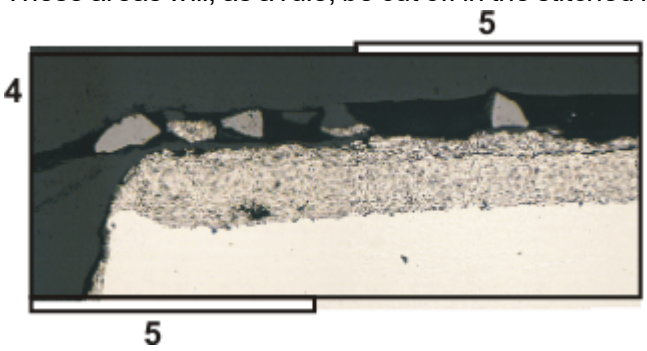
15. Check whether both images have been correctly combined. Otherwise, you can undo the last step by using the *Undo last frame* button. You can then move the stage again, and match the structures better.
    - During the acquisition, you can change the current stitched image's zoom factor, e.g., to see certain parts in the overlap area better.

16. Define your way through the sample, with the arrow buttons, and follow that with the stage.
    In this manner, you can display a sample in any form you like in the stitched image. The illustration shows a stitched image that is made up of 9 individual images, and the stage path.



17. Click the *Stop* button when you want to end the acquisition of the stitched image.
    - You see the completed stitched image (**4**) in the image window.
      Since the individual images can lie a little askew of each other, the stitched image isn't as a rule, rectangular, but contains empty areas on its borders (**5**).

These areas will, as a rule, be cut off in the stitched image.

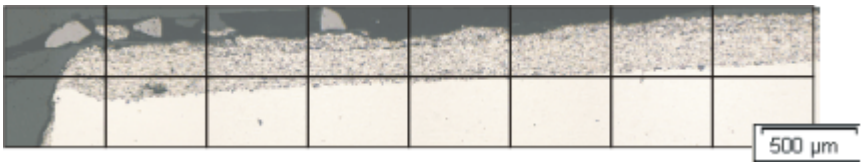


- The stitched image will, by default, be automatically saved in a database. Alternatively, you can select a storage location, or switch off the automatic saving. To do so, use the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.

Properties of the stitched image

- By default, in the overlap area, the intensity values of two adjoining individual images will be matched with each other to make the image's overall impression homogeneous.
- Stitched images are calibrated. This means that you can measure distances and objects on a stitched image.

4.8.3. Acquiring a stitched image with a motorized XY-stage (XY-Positions/MIA)



Example: You want to acquire an image of a large sample area. Use the automatic *XY-Positions/MIA* acquisition process to scan an area of the sample and to have adjoining images combined into one stitched image. MIA stands for Multiple Image Alignment.

Prerequisite: You can only use the *XY-Positions/MIA* acquisition process if your microscope is equipped with a motorized XY-stage.

Preconditions

- The stage has been set up and initialized, i.e. its stage limits have been defined.
- The camera is aligned parallel to the XY-stage. The angle between camera and stage should be smaller than 1°.
- The shading correction has been set up.

1. Switch to the *Acquisition* layout. To do this, use the *View > Layout > Acquisition* command, for example.

Selecting microscope settings

2. Select the microscope settings you want. In particular, select the required magnification. To do this, on the *Microscope Control* toolbar, click the button with the objective that you want to use for the acquisition of the stitched image. If you are using a magnification changer, you will also have to select the magnification value used.

   If you have defined observation methods, select the required observation method instead.

   - In this case, the background color of the stitched image depends on the observation method that has been selected. The background is automatically black for all fluorescence observation methods and all darkfield observation methods. By default, the background is white for all other observation methods.

Selecting the acquisition process

3. Activate the *Process Manager* tool window.
4. Select the *Automatic Processes* option.

5. Click the *XY-Positions/MIA* button.
   - The button will appear clicked. You can recognize this status by the button's colored background.
   - The *XY* group will be automatically displayed in the tool window.

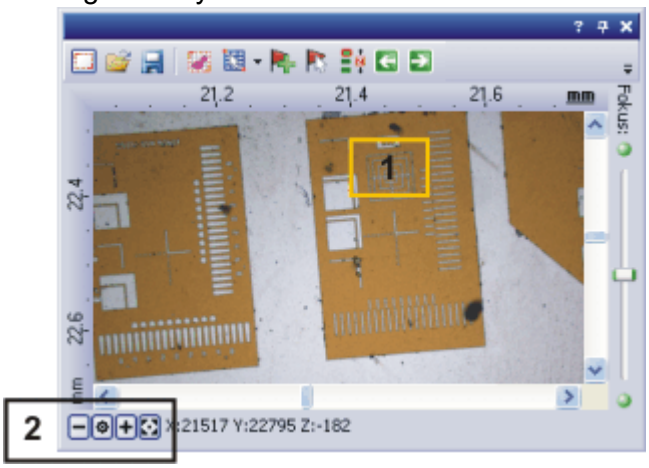
Using the software autofocus

6. If your microscope is equipped with a motorized Z-drive, you can switch on a software autofocus.
   In the *Process Manager* tool window, click the *Autofocus* button.
   - The *Autofocus* group will be automatically displayed in the tool window.
7. In the *Autofocus* group, select the *Multiposition / MIA autofocus* check box.
   If the sample surface is not plane or if it is inclined to the objective, choose the *Every MIA frame* option. Now, the software autofocus will be performed before every image acquisition.

Putting the stage navigator on display

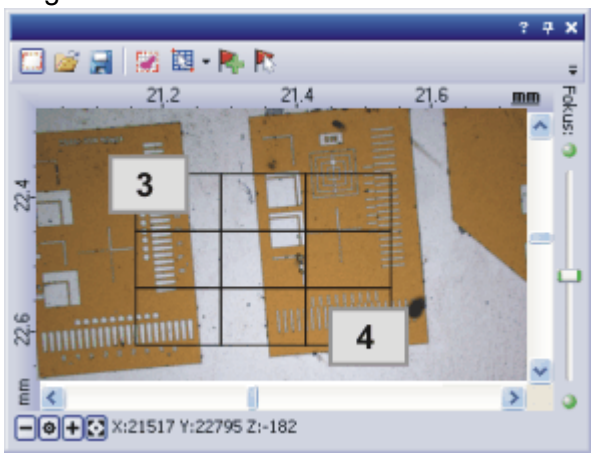
8. In the *Process Manager* tool window, click this button .
   - The *Stage Navigator* tool window will be shown. When you have acquired an overview image of your sample, you will see this area of the image in the stage navigator's image segment.
9. Set the magnification for the image segment in the *Stage Navigator* tool window.
   To do this, use the zoom buttons at the bottom left of the tool window (**2**).
   The current stage position will be shown by a yellow rectangle in the image segment (**1**). You should choose a magnification that enables you to see this

rectangle clearly.



Defining the MIA scan area

10. In the *Process Manager* tool window, click this button .
    - The system will automatically switch into the live mode.
    - The *Define MIA Scanning Area* dialog box opens.
11. Move the XY-stage to the top left-hand corner of the MIA scan area you want (**3**).
12. Focus, then select the optimal settings for your acquisition in the *Camera Control* tool window. Pay special attention to setting the correct exposure time. This exposure time will be used for all of the stitched image's individual images.
13. Confirm the starting position in the *Define MIA Scanning Area* dialog box, with *OK*.
14. Move the XY-stage to the bottom right-hand corner of the MIA scan area (**4**). Confirm this position in the *Define MIA Scanning Area* dialog box, with *OK*.
    - In the *Stage Navigator* tool window, the MIA scan areas that have been defined are displayed. Here, you can immediately see how many individual images are required for the acquisition of the stitched image, when the current magnification is used.



Acquiring a stitched image

15. Click the *Start* button.
    - The acquisition begins immediately. The individual images are acquired, then immediately assembled. You can watch how the stitched image grows, in the image window.
    - In the status bar at the bottom left of the user interface, you can find a progress bar, the number of images already acquired, and the total number of frames (e.g., 3/9).

    - The acquisition has been completed when you can see the *Start* button in the *Process Manager* tool window again, and the progress bar is no longer displayed.
    - You see the completed stitched image, in the image window. The individual images won't be saved separately.
    - The stitched image will, by default, be automatically saved in a database. Alternatively, you can select a storage location, or switch off the automatic saving. To do so, use the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.

4.8.4. Acquiring a stitched image with extended depth of focus

When you acquire a stitched image of a thick section or an uneven surface, in some cases not all of the areas of the sample will be sharply displayed. In this case, you can combine the acquisition of a stitched image with the *EFI* (Extended Focal Imaging) acquisition process. By doing this, you'll make sure that the stitched image is sharply focused everywhere.

Note: The acquisition of a stitched image with extended depth of focus, is both with and without, a motorized XY-stage, possible.

Without a motorized XY-stage

1. Start the *Manual MIA* acquisition process.
2. Click the *Instant EFI* button, in the *Manual MIA* group.

    - The *Instant EFI* acquisition process will start at once. Instead of the live-image, you now see the EFI image.
3. Now move your microscope's Z-drive slowly and change the focusing of the image. Observe how the EFI image builds itself up.
    - For each image that is acquired, the sharpest image segment is adopted in the EFI image.
4. When all of the image structures are sharply displayed, click one of the direction arrows in the *Manual MIA* group to continue with the acquisition of the stitched image.

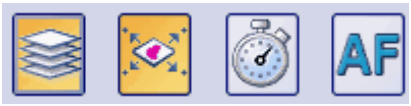
Note: You now see the live-image with the last focus settings. That means that normally, the live-image won't be in focus.

5. Bring the image into focus.
6. Repeat the last steps for each of the stitched image's individual images for which you want to use the *Instant EFI* acquisition process.
7. Click the *Stop* button when you want to end the acquisition of the stitched image.
   - You see the completed stitched image, in the image window.

With a motorized XY-stage

Prerequisite: You can only use the *EFI* acquisition process when your stage is equipped with a motorized Z-drive.

1. Select the *XY-Positions/MIA* acquisition process.
2. Define an MIA scan area.
   You can find a step-by-step instruction for doing this further above.
3. Additionally, select the *Z-Stack* acquisition process.
   - In the group with the different acquisition processes, two of them are now active:



4. Define all of the parameters for the Z-stack's acquisition.
5. In the [*Z*] group, select the *Extended Focal Imaging* check box.
6. Click the *Start* button to begin the acquisition of the stitched image.
   - At each of the MIA scan area's stage positions, a Z-stack will first be acquired, then the EFI image calculated from it. The EFI images will be combined into a stitched image.
   - When the acquisition process has been completed, you'll see the finished stitched image in the image window.

4.8.5. Automatically acquiring several stitched images

You can define several MIA scan areas on the sample. When the acquisition has started, all of the MIA scan areas will be moved to, one after the other, and a stitched image will be acquired at every position.

1. Select the *XY-Positions/MIA* acquisition process.
2. Define several MIA scan areas. You can find a step-by-step instruction on how to define an MIA scan area further above.

   Begin with the area of the sample that is to be scanned first.

Putting the stage navigator on display

3. In the *Process Manager* tool window, click this button .
   - The *Stage Navigator* tool window will be shown. When you have acquired an overview image of your sample, you will see this area of the image in the stage navigator's image segment.

- In the *Stage Navigator* tool window, the MIA scan areas that have been defined are displayed. They are numbered serially in the order in which they were defined.

Acquiring stitched images

4. Click the *Start* button to begin the acquisition of the stitched image.
   - Each of the MIA scan areas will now be scanned, and the stitched image created. The scan areas will be scanned in the order that is predefined by the numbering.
   - All of the stitched images will be acquired with the current camera, and current acquisition settings.
   - When the acquisition process has been completed, you'll find a stitched image for each of the MIA scan areas, in the document group.

4.8.6. Combining individual images into a stitched image

Use the *Process > Multiple Image Alignment* menu command to have several separate images combined, as with a puzzle, into a stitched image. The individual images will be combined in their full X/Y-resolution. The stitched image will thus display a large sample segment in a higher X/Y-resolution than would be possible with a single acquisition.

Acquiring images

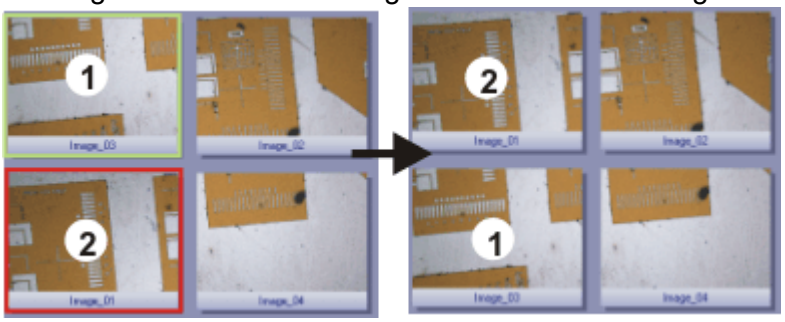
1. Load the images you want to combine or acquire a suitable set of images.
   - All of the images you want to combine must be of the same image type. You can't, e.g., have a gray-value image combined with a true-color image.
   - When you acquire the images, number their names sequentially, e.g., "Image001", "Image002" and so on. In many cases, the images will then already be arranged in the right order in the *Multiple Image Alignment* dialog box.

Selecting images

2. Open the *Gallery* tool window. To do this, you can use the *View > Tool Windows > Gallery* command.
3. Select all of the images you want to combine, in the *Gallery* tool window.

Assembling images

4. Use the *Process > Multiple Image Alignment* command. This command is only active when more than one image of the same image type has been selected.
   - The dialog box's stitching area will display a preview of the individual images.
5. If necessary, while keeping your left mouse button depressed, drag on the bottom left-hand corner of the dialog window to enlarge it. Alternatively, double click the header of the dialog box to enlarge the dialog box to full-screen size.
6. Check whether the images' positions are correct. You can change the arrangement of the individual images, e.g., by exchanging two images in the stitching area using Drag&Drop.
   - The illustration shows the stitching area with four individual images. On the left, the images 1 and 2 are not in the correct position. Image 1 (green frame) will therefore be dragged onto image 2 (red frame). On the right, you see the stitching area after the two images have been interchanged.

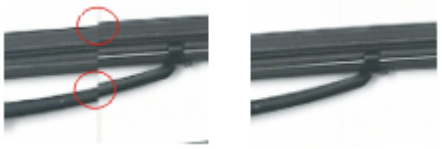


7. When the individual images overlap, select the *Correlation* entry in the *Output > Alignment* list.
   Then your software will search for the same image structures in neighboring individual images. The stitched image will be put together in such a way that image areas that are the same will be superimposed.
8. Click the *OK* button to carry out the automatic image alignment.
   - The *Multiple Image Alignment - Manual Align* dialog box opens.
   - The stitched image will be displayed.

Checking a stitched image

9. Check the stitched image on display.
   Use the zoom buttons in the dialog box to zoom in the stitched image in the dialog box.

10. Should individual images have been incorrectly assembled, you can manually shift one or more of them, in respect to one another.
    To do this, click in the image you want to shift, then drag it with your left mouse button depressed, in the required direction.
    - The currently selected image will be displayed semi-transparently to make it easier for you to find the point of contact with the neighboring image.



    - Two images were not correctly aligned with each other. There is a misalignment. When the manual alignment has been made, the two images fit together seamlessly.
11. Select the *Cut Edges* check box to clip the image in such a way that there are no longer any empty areas visible on its borders.
    - In the preview, the image edges that are to be clipped will be displayed semi-transparently.
12. Select the *Equalize* check box if the images aren't homogeneously illuminated.
    Then the intensity values of the individual images will be matched with one another, which will make the background appear more homogeneous.
13. Click the *OK* button.
    - A new image with the name *Image_<consecutive No.>* will be created.

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5. Image processing

5.1. Commenting on images

There are several different ways of adding notes to an image.

Using drawing objects

The *Drawing* toolbar makes a variety of drawing functions (line, rectangle, ellipse, text) available to you, as well as options for color selection and line styles.

Using annotations

You can use the *Annotations* tool window to mark interesting positions in an image, to name them and to save them. You can give each position a text or audio annotation. In this way you will be able to jump to the position in the image that you want with one mouse click, and this will be immediately shown in the magnification you want.

Use this possibility especially when you are commenting on very large images.

Entering an image comment

The *Properties* tool window will show you all of the available information from the document group on the active image.

You can also supplement this information with a text annotation of your own on the image. Enter your comment in the *Note* field.

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5.2. Processing images

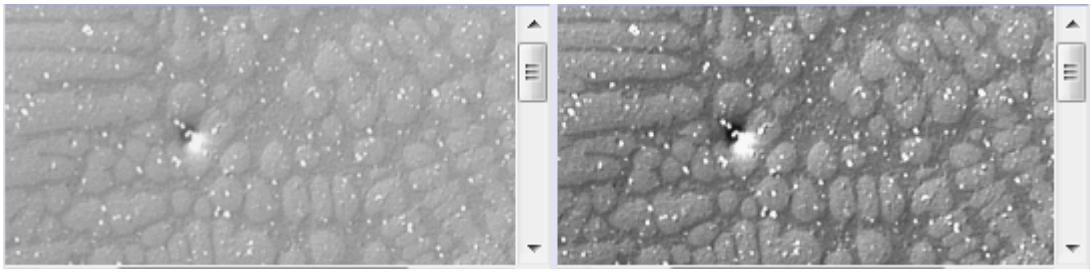
The *Process* menu offers numerous image processing functions with which you can change an acquired image (for example increase the image contrast or the image sharpness).

1. Load the image you want to process, or activate the image in the document group.
   - Please note that the *Process* menu will only be visible when an image is loaded and active in the document group.

2. Use one of the commands in the *Process* menu, e.g., *Process > Enhancements > Adjust Intensity* .
   - The image processing dialog box opens. The image processing operation that is active will be shown in the dialog boxes header.

3. Click the small arrow next to the *Preview* button to open a list of all of the preview functions. Select the *Original and Preview* entry.
   - This preview function displays the same image segment twice in the dialog box. The first one shown is the source image. The second is the image that results when the current parameters are used.
   - Most of the image processing operations need one or two of the parameters that are shown in the *Settings* group.

4. Select the *Create new document as output* check box to create a new image. When you do this, the source image remains unchanged.
   Clear the check box if you want the image processing function to change the source image. Now no new image document will be created. As long as you have not yet saved the image, you can use the *Edit > Undo* command to restore the source image.
5. Change the image processing operation's parameters. You can decrease the gamma value and increase the brightness, for example.
   - Every time you change a parameter, the new resulting image will be displayed in the preview window.
6. Click the *Default* button, to readopt the preset parameters in the *Settings* group, when the current parameter doesn't make sense to you.
7. When you have found the optimal parameters, click the *OK* button to have the active image processing operation applied to the image with the active parameters.
   - The image processing dialog box closes.
   - By default, the image processing function doesn't change the source image. A new image document is created instead.
   - The new image document will not be saved automatically. This is indicated by the asterisk after the image name in the document group.



The source image (**left**) has low contrast. Adjust the intensity to get significantly better contrast in the resulting image (**right**).

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6. Analyzing images using deep learning

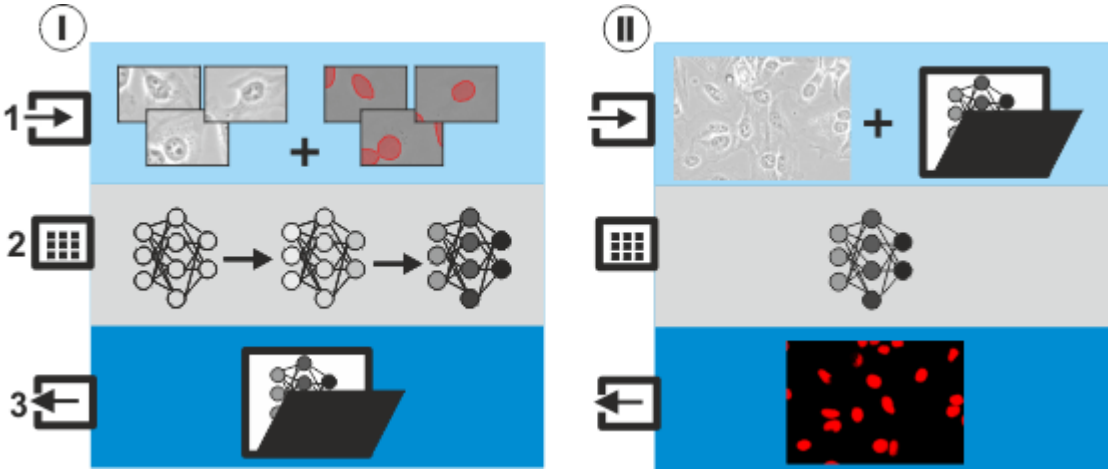
What is Deep Learning?

Many tasks require your software to detect objects in an image. One example of this is dyeing cells with fluorescing markers in order to observe and to analyze biological processes. For this to work, the cells must be automatically detected in the microscopic image acquisitions.

Deep learning can solve many of these detection tasks. Deep learning is one of the methods that machine learning uses. Deep learning uses artificial neural networks that belong to a class of algorithm that is more or less inspired by the human brain. The successful detection of images of cats in an image database containing millions of animal images is the most well known example of a detection task solved with deep learning.

To be able to use deep learning, two phases are required.
In the **training phase** the neural network is trained using images of objects. These are the same objects that you later want the neural network to detect in other images, the inference images. The interesting thing is that no parameters whatsoever that characterize the objects must be specified, their size or a description of their appearance for example. All that is required is for an expert to label the objects clearly. In the example with cats mentioned above, the neural network was trained using images of animals but a person was required to specify for each of the training images whether it contained a cat or not.

The second phase is the **inference phase**. The inference phase analyzes an unknown image, the inference image, using a neural network. In the example with the images of cats, a system can use a neural network to decide whether any image of an animal contains a cat or not.



The illustration shows the training phase (**I**) and the inference phase (**II**). The input data (**1**), the computation (**2**) and the output data (**3**) are shown.

The input data (**1**) in **training phase** (**I**) is microscopic images and the ground truth. In this case, the ground truth consists of the labels that indicate the objects you want to identify.In this example, the objects you want to identify are cell nuclei. However, you

can also search for something else.
During the training process (**2**), your software computes appropriate parameters for the neural network. The computation runs in the background and may take a long time depending on the task and the computer being used.

After a successful training process, the objects you want to identify will be correctly detected. The training process results in a neural network with particular parameters. You save the neural network as a parameter set in a file (**3**). So the output data of the training phase is a parameter set that you can now use in your software.

In the **inference phase** (**II**) you use the saved neural network to perform an analysis on an inference image (**1**). The inference image is a microscopic image with similar objects to those in the training images. The result of the analysis using the neural network (**2**) is a probability map that informs about the likelihood for each pixel in the inference image that it belongs to one of the objects you want to identify.

Deep learning in your software

You can use the *Deep Learning* software solution to create training images, and to train neural networks. You use your own images for the training process. As a rule, you will need to train and save an individual neural network for each task that you want to solve with the help of a neural network. For the training process you require a powerful computer and an expert who can clearly label the objects you want to identify in the image.

You don't require the *Deep Learning* software solution to use a neural network in your software. You can import a neural network that was created at a different workstation.

Note: Both the training phase and the inference phase take place exclusively with your own data and on your own computer.

The difference between an analysis using a neural network, and an object analysis based on thresholds.

In the *Count and Measure* tool window, your software offers an alternative method to detect and to measure objects in images. This method of object analysis uses thresholds that are set on the distribution of color or gray intensity values in the image. This object analysis based on thresholds has a number of limitations.

It requires that the objects you want to identify are distinct from the background either in color or in intensity. Objects that touch or overlap can also not be easily detected with this method. A neural network enables you to find objects that could not be found using a standard object analysis based on thresholds.

General process flow of an analysis using a neural network

The following steps are required to train and apply a neural network using your software.

Perform the training phase

Step 1: Acquiring training images

Acquire the training images. Use similar acquisition conditions (exposure, magnification) for the training images to those you will later use to acquire the inference images that you want to analyze with the neural network.

Step 2: Creating training labels

On the training images, define the objects that you want the neural network to detect. You can define the objects either automatically or manually. If you want to define the objects automatically, use the *Count and Measure* tool window to perform an automatic object analysis using thresholds. If you want to define objects manually, use the *Training Labels* tool window.

Step 3: Training and saving a neural network

Select the training configuration for the neural network and a training duration. Start the training process.

Follow the progress of the training process. During the training process, you can check the results on validation images at different points in time.

When your training process has been successful, save a new neural network as a parameter set.

Use the *Deep Learning* layout to train and save a neural network.

Performing the inference phase

In your software, there are several ways of performing an analysis using a saved neural network.

Option 1: Performing an object analysis on a probability map

This option first performs an analysis using a neural network. Then an object analysis is performed on the probability map.

To perform the analysis using a neural network, use the *Process > Deep Learning > Neural Network Processing* command. The neural network will identifies object in the inference image. The result is a probability map. For each pixel of the inference image, the probability map indicates the likelihood that the pixel belongs to an object.

Then use the *Probability Layer Segmentation* software function in the *Count and Measure* tool window to perform an object analysis on the probability map. This will detect and measure the objects in the probability map.

Option 2: Performing an object analysis using a neural network

This option combines the analysis using a neural network, and the object analysis into one step.

Use the *Neural network Segmentation* software function in the *Count and Measure* tool window to perform an analysis using a neural network on the inference image. The neural network will identify objects in the inference image. Then the objects will then be detected and measured immediately. You can view the measurement results in the *Count and Measure Results* tool window.

Software and hardware requirements

Software requirements

You must purchase the *Deep Learning* software solution to train a neural network.

Which graphics cards are supported?

A large amount of data must be processed to train a neural network. This demands a lot of the PC's hardware equipment and requires a fast graphics card. To be suitable, a graphics card must support CUDA technology.

The two graphics cards listed below were successfully tested. However, due to technical progress, this list can change frequently. Contact your Olympus sales representative if you have any questions about suitable graphics cards.

NVIDIA Quadro P4000
NVIDIA Quadro RTX 4000

What are training images?

The neural networks that you train with your software should be able to identify very specific types of objects in an image. The software requires training images to perform a training. The number of training images required to train the neural network very much depends on the task.

Requirements for the training images

The training images must meet the following requirements:

Images with training labels	All training images must contain training labels. The training labels form the ground truth for the training of the neural network. You can automatically create the training labels using an object analysis. However, this will only be successful if the objects to be found have a color or intensity that is distinct from the background. Use the *Count and Measure* tool window to perform an automatic object analysis. If the training images aren't suitable for an automatic object analysis, you can create the required training labels manually. Use the *Training Labels* tool window to manually draw the training labels. The training labels are displayed on their own image layer. This image layer is called *User Labels*.
Training	With your software, you can create neural networks that search for different classes of

label classes	objects simultaneously in images. The training images for these types of neural networks will then contain different training label classes. A separate class is defined for each object type.
Image size	The minimum size for training images is 1024x1024 pixels. The training images don't have to be of the same size. As long as a training image has the required size, you may use it. If you are using a very large image with very many objects as a training image, a single image may suffice to train the neural network. Please note that this requirement only applies for the training images. You can later also apply the neural network to images that have a different image size.
XY-calibration	All of the training images must have approximately the same calibration. If you acquired all the training images with the same objective magnification, this requirement will be fulfilled.
Image format	Training images must be in one of the following image file formats: VSI, TIF, TIFF, BTF.

The probability map

A deep learning analysis produces a probability map. For each pixel of the inference image, the probability map indicates the likelihood that the pixel belongs to an object. You can define the color that the probability map will use in the *Training Labels* tool window. The probability map isn't a binary image. It has varying intensity values. The intensity corresponds to the probability that an object that was predicted is actually present in the inference image. If an object on the probability map is only very lightly colored, there is a small probability that an object actually exists at that position. If the object is heavily colored, there is a high probability that the prediction is accurate.

The probability map is a separate image layer that is superimposed on the inference image. Use the *Layers* tool window to show or hide the probability map. You can also extract the probability layer.

When the neural network was trained using several training label classes, a separate probability map is calculated for all defined training label classes. In this case, use the *Dimension Selector* tool window to show or hide the probability maps for the individual training label classes.

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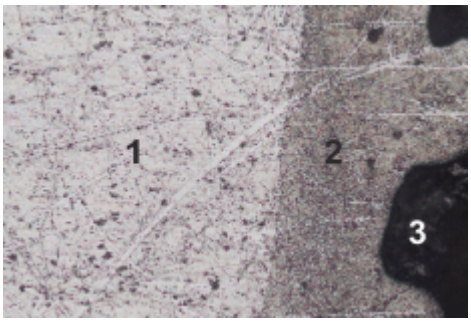
6.1. Working with deep learning

Example: Let's say you want to examine samples that have been exposed to heat, due to welding for example, and this has created a second phase. You want to measure the area covered by each phase. Scratches and tears make it difficult to automatically identify the phases on the basis of color or intensity.
Use the *Deep Learning* software solution to train a neural network that can detect the phases in the images. You can then perform a phase analysis on the images.



The illustration show the image of a sample with two phases, (**1**) and (**2**). The dark areas (**3**) don't belong to either phase. You are going to measure the area covered by phases (**1**) and (**2**).

The following step-by-step instructions take you through the whole process of a deep learning analysis using this example.

Step 1: Creating training labels manually

Use the *Training Labels* tool window to manually define training labels on the training images.

▼

Step 2: Training and saving a neural network

▼

Step 3: Performing a phase analysis using the neural network

Preparations

To train a neural network you need suitable training images.

1. Acquire images of typical areas on the sample. Use acquisition conditions that are as similar as possible to those for the images that you will later analyze. For example, select the same objective magnification and similar exposure conditions.

   The number of training images required to train the neural network very much depends on your use case. If you want to check the results during the training process, acquire enough images so that one of the images can be reserved as a validation image. This will give you a visual way of checking the results of the training while it is in progress.
2. Load the images that you want to use as training images in your software.
   - During the installation of your software some sample images have been installed, too. You can follow these step-by-step instructions using the example images.

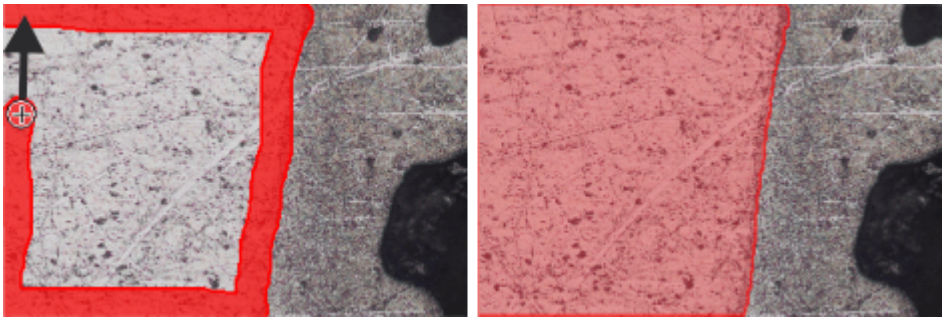
Step 1: Creating training labels

All of the images that you want to use to train a neural network must have at least two image layers. One image layer contains the image that was acquired of the objects you want to identify. The other image layer contains the training labels that clearly define

for the software the objects that you want to identify. You can manually draw the training labels on the image.

1. Activate the first image on which you want to define the training labels.
2. If the *Training Labels* tool window isn't displayed, use the *View > Tool Windows > Training Labels* command to show it.
3.  In the *Trainings Labels* tool window, click the *New Training Label Class* button.
   - The last used training label classes will be overwritten.
   - A new training label class is created. The training label class is called *Class1* and the color of the first training label class is red.
   - When you create a training label class, the *User Labels* image layer is added to the active image. All training labels will be defined on this image layer.
4. Enter a name for the training label class. To do this, double click the *Name* name cell in the table in the *Training Label Classes* group. For this example, name the training label class *BrightPhase*.
5. If necessary, select the *BrightPhase* training label class in the *Training label Classes* group. Click one of the buttons in the *Training Labels* group to switch to the corresponding edit mode.

    For example, click the *Create Training Labels - Fill* button to automatically fill the training label that you draw.
   - The button becomes active, indicating which edit mode is active.
   - The edit mode remains active until you explicitly end it.
6. While pressing the left mouse button, outline all of the segments in the image that belong to the bright phase. You don't have to be completely exact when you do this. If the bright areas are not touching each other, you will need to define several training labels.
   - In this drawing mode, the training labels are automatically filled as soon as the ends of the line meet.
   - The training label is drawn in the *User Labels* image layer.
   - The color of the training label corresponds to the color of the class to which it belongs.
   - The training labels are transparent when they are drawn. This ensures that the objects under the training label remain visible.



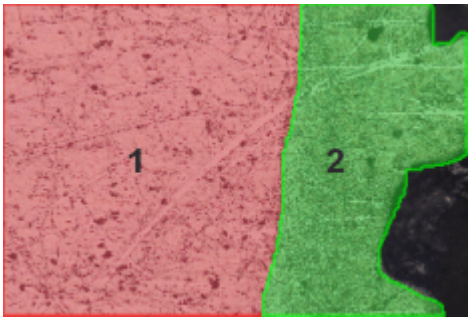
The bright phase is indicated with a red training label.

7. You can at any time correct the training labels that have been drawn. You can delete whole training labels or just parts of them. You can expand training labels and you can draw new training labels. To do this, select the training label class and use the buttons in the *Training Labels* group.
8. Now define the second training label class, *DarkPhase*.

To do this, in the *Trainings Labels* tool window click the *New Training Label Class* button again.

9. Outline the second phase in the image. It's ok for the training labels to overlap a little.

   If the training labels that have already been drawn are in the way, click the eye icon in the row for the *BrightPhase* training label class. This will hide the training labels that define the bright phase. Click in the *Visible* cell to show the training labels again.



The required training labels have been defined on this training image. The training labels belong to two different training label classes, one for the bright phase and one for the dark phase. The image background is not allocated to either of the training label classes.

10. Save the training label classes that have been defined. To do this, click this button in the *Training Label Classes* group. Save the parameter set using the name *Phase Analysis*.
11. Save the training image with the training labels that have been defined. To do so, use the *File > Save As* command.
12. Load the next training image.

13. Click this button in the *Training Label Classes* group and load the *Phase Analysis* parameter set.
14. Select one of the training label classes and draw the appropriate training labels in the image.

    When you train a neural network, the same training label classes must be defined in all of the training images. You don't have to define a training label for every training label class. The number of training labels in this training label class will then be 0. This enables you to include in the training images images that only have one of the phases you want to identify.
15. Load some more images that you want to use as training images. For each image, load the *Phase Analysis* parameter set again and draw the appropriate training labels on the image.

16. Release the *Create Training Labels - Fill* button in the *Training Labels* tool window to leave edit mode.
17. Save the training images.
    - You can now use the training images to train a neural network.

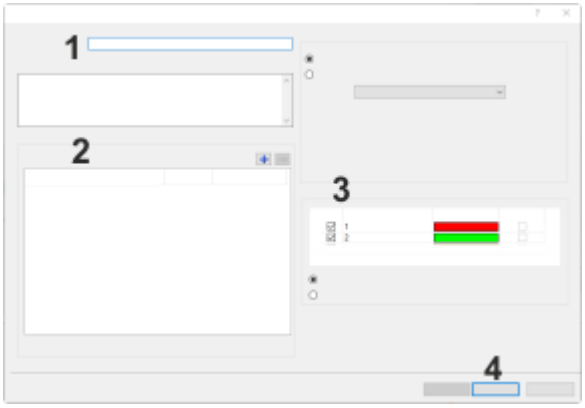
Step 2: Training and saving a neural network

Use the training images from step 1 to train a neural network. You want the neural network to detect bright and dark phases in an image.

1. Switch to the *Deep Learning* layout. To do this, click the *Deep Learning* tab at the top right of the user interface.
    - The *Deep Learning* layout has been designed especially for training neural networks. The *Deep Learning* layout always has the same structure and fills the whole user interface. You are not able to display any additional tool windows or toolbars. You are also not able to hide any of the functions that are displayed in the layout.
2. Click the *New Training* button. You can find the button at the top left of the *Deep Learning* layout.
    - The *New Training: Input and Output* dialog box opens.

Specifying the input and output for the training

Make the necessary settings in the *New Training: Input and Output* dialog box.



1. In the *Name* field (**1**), enter a descriptive name for the neural network that you want to create. For this example, you can name the training *Phase Analysis*.

   In the *Description* field, enter a good description of the new neural network.
    - Your software continually checks the settings in the *New Training* dialog box. If a setting has not yet been made or if a settings is incorrect, a message appears at the bottom right of the dialog box.

      Before you have selected the images, a message appears stating that no input images have been specified yet. This message disappears as soon as you add the training images.
2. Click the [+] button in the *Images* group (**3**).

   Navigate to the directory in which your training images are saved and select the

training images.

Click the *Open* button to load the training images.

- The imported training images are displayed in the *Images* group.
- The *Training label classes* group lists the training label classes that have been defined for the training images.

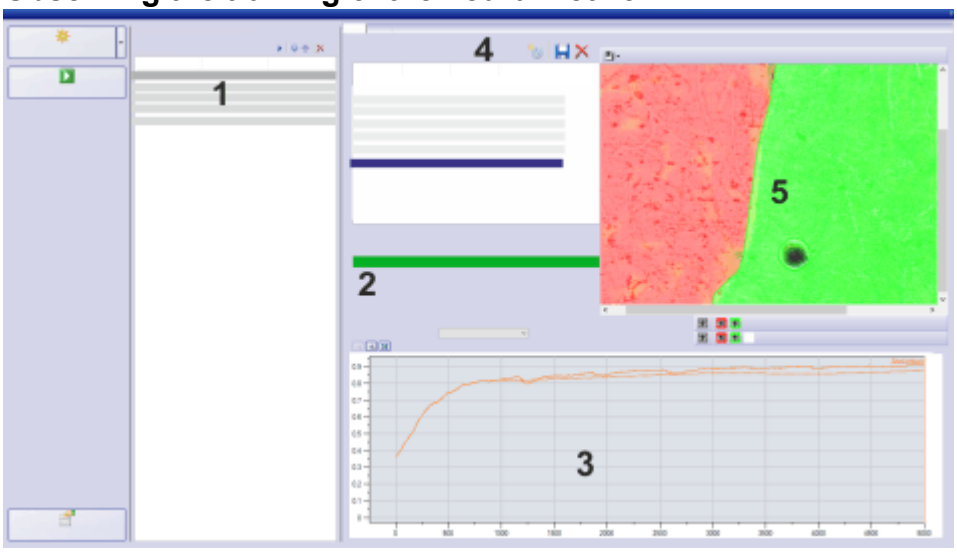
4. Make sure that the check boxes next to classes *1* and *2* are marked in the *Training Label Classes* (**3**) group. This way both classes will be taken into account by the training process.
5. With phase analyses, a pixel can't belong to two different phases at the same time. For this reason, select the *multiclass classification* option in the *Training label classes* group. The neural network will now allocate each pixel to the phase for which its probability is the highest.
6. Keep the default settings in the *Input channels* group.
7. Click the *Next* button (**4**) to select the model for the neural network.
   - The *New Trainings: Parameters* dialog box opens.
8. The *Deep Learning* software solution uses pre-configured models for the neural network. From the *Available training configurations* list, select the model that you want to use for the training process. To the right of the selected model, you can find a detailed technical description.

   Which parameter set delivers the best results depends strongly on the application. The *Standard Network* parameter set is suggested for most standard tasks.

   For this example, select the *Standard Network* parameter set.
9. The phases are easily recognizable in the training images. In this example, you can reduce the duration of the training process. In the *Training duration* list, select the *Iteration limit* entry.

   Enter the required number of iterations in the field to the right of the *Training duration* list. For this particular example, select 5000 iterations.
10. Click the *Start* button to start the training process.
    - The *New Training* dialog box closes.
    - You can follow the progress of the training in the *Deep Learning* layout.

Observing the training of the neural network



The *Deep Learning* layout will look similar to this while the training is in progress.

1. You can follow the progress of the training in the *Deep Learning* layout.
   - The trainings that your have defined or already performed are listed in the training list. When a new training begins, it is inserted into the top position in the training list (**1**). The training that is in progress has the *Running* status.
   - The progress bar (**2**) shows when the training is expected to finish. The progress bar tells you the total number of iterations that have already been performed, and how many are yet to come. The time remaining in the training process is displayed next to it.
   - Your software provides several quality indicators. These enable you to check the quality of the neural network. The *Similarity* quality indicator is displayed by default in the diagram (**3**). The diagram is refreshed constantly while the training is in progress.

     The *Similarity* value is between 0 and 1. The closer the value is to 1, the better the prediction of the neural network is. In this example, the curve climbs and approaches the value of 1. This curve shows that the neural network that is being trained is finding the phases increasingly well.
   - The neural network consists of a parameter set. This parameter set is varied during the training and adapted to the training images. Your software saves the current parameter set at regular intervals and uses it to create checkpoints. You can use these to check the quality of the neural network. The checkpoints are listed in the *Available checkpoints* (**4**) list.

     In this example, checkpoint 1 is created after 1000 iterations. This checkpoint reflects the development of the neural network's parameter set at 1000 iterations.
   - The validation image (**5**) shows the result that belongs to the selected checkpoint. This means that, at checkpoint 1, the neural network analyzes the validation image with the parameters that were calculated after 1000 iterations.

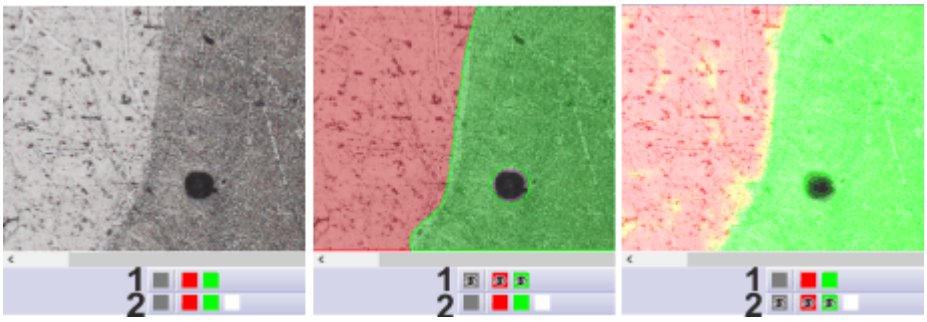
     For the first checkpoint in the list, the calculation has not yet started. No neural network has yet been calculated. The validation image shows one of the

training images without a probability map. For each pixel, the probability map indicates the degree of probability that the pixel belongs to a class.

2. Take a look at the probability map for one of the checkpoints that has already been computed. You can select checkpoint 3 in the *Available checkpoints* list for example.
   - The preview window in the *Deep Learning* layout displays all of the image layers in the validation image superimposed on top of each other. Use the buttons over and under the validation image to show and hide the different image layers.

     The *Training label classes* (**1**) buttons under the validation image correspond to the training label classes that have been defined. In this example, two training label classes are active for the training job. The red training label class contains the training labels for the bright phase. The green training label class contains the training labels for the dark phase.

     When you analyze an image using a neural network, the result is a probability map. The *Probability* (**2**) buttons correspond to the probability maps for the individual training label classes. A separate probability map is created for each training label class. Additionally, a probability map is always created for the background.
3. Click on one of the buttons to show or hide the corresponding image layer. You can repeatedly click on the gray *Training label classes* button under the validation image.
   - The training labels appear and disappear. This allows you to judge whether the neural network belonging to the selected checkpoint is finding the phases as wanted.



The illustrations show a validation image in the *Deep Learning* layout. In the image on the left, the training labels and the probability map are not displayed. In the middle, the training labels (**1**) are shown. On the right, the probability map (**2**) is shown. The probability map largely corresponds with the training labels. Because the probability maps for the two phases overlap, some areas in the probability map are yellow (overlap of red and green).

Saving a neural network

1. Wait until the neural network training is finished.

Note: You can continue to use your software while a training process is running. You can also define additional training processes. The trainings will then automatically be performed one after the other.

- After the training is finished, its status changes from *Running* to *Done*.
- The progress bar shows you that the training is finished.

2. Select the checkpoint at which the similarity is the highest. As a rule, it will be the last checkpoint.
3. Click the *Save Neural Network* button. You can find the button above the *Available checkpoints* list.
   - The *Save Neural Network As* dialog box opens.
4. Enter a descriptive name for the neural network in the *Name* field. For this example, use the name *Phase Analysis*.

   Use the *Description* field to describe the task and the training images being used.

   If you want other users of your software to be able to use the neural network, select the *Public* option. Select the *Private* option if you want the neural network to be available only to yourself.

   Click the *Save* button.
5. You can now use the *Phase Analysis* neural network to detect and measure bright and dark phases in images.

Step 3: Performing a phase analysis using the neural network

1. Acquire the images that you want to analyze. Select acquisition conditions that are as similar as possible to those used to acquire the training images. For example, select the same objective magnification and similar exposure conditions.

Note: You can of course apply the neural network to existing images. In this case, load the image that you want to analyze.

2. Open the *Options* dialog box by clicking the *Count and Measure Options* button, located in the *Count and Measure* tool window.
3. Click the *Count and Measure > Classification* entry in the tree view. Select the *Phase* classification scheme. Now, all image segments that belong to one phase also belong to one object class.
4. Click the *Count and Measure > Detection* entry in the tree view. Select the *Borders - frame > Truncate* option.
5. Select the measurement parameters for the phase analysis.

   In the tree view, click the *Count and Measure > Measurements* entry.

   Click the *Select Object Measurements* button to select suitable measurement parameters for the object measurement. Select the *Area* measurement parameter and close the dialog box.

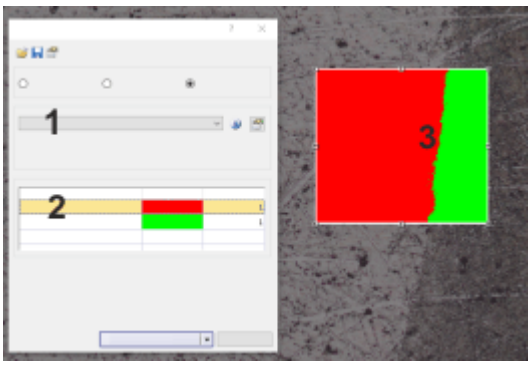
   Click the *Select Class Measurements* button to select suitable measurement parameters for the class measurement. Select the *Object Class*, *Sum (Area)* and *Area Fraction Objects* measurement parameters. Close the dialog box.

   Close the *Options* dialog box.
6. In the *Count and Measure* tool window, click the small arrow next to the threshold setting button. The button displays the number 1. Select the *Neural Network Segmentation*

command in the menu to open the *Neural Network Segmentation* dialog box.

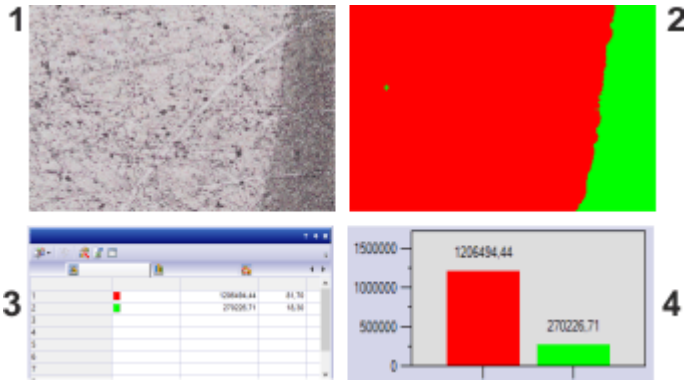
7. In the *Neural Network* list (**1**) select the *Phase Analysis* neural network.
   - In the *Neural Network Segmentation* dialog box, the training label classes are listed in the *Phases* (**2**) group. In this example there are two training label classes, *BrightPhase* and *DarkPhase*.
   - Your software starts the analysis as soon as a suitable neural network has been selected. This can take several minutes. Pay attention to the progress bar located in the status bar.
   - In the preview area (**3**) you can see which parts of the sample are defined as first phase and which parts of the sample are defined as second phase. For the display, the colors that are currently selected in the *Color* field are used.

Note: You can accelerate the computation of the preview image by reducing the size of the preview area in the image window. To do this, drag one of the preview area's handles towards the center.



The illustration shows the *Neural Network Segmentation* dialog box and the preview window.

8. Click the *Count and Measure* button to get the results.
   - The results are displayed in the *Count and Measure Results* tool window in the *Class Measurements* results view. For each phase, you see the area that this phase takes up in the image.



The result of a phase analysis: The image that was analyzed (**1**) now has the *Detected Objects* image layer (**2**) which contains the phases that were detected. The area fraction of each phase is displayed in the results table (**3**). The class histogram (**4**) shows the area distribution in a bar chart.

Note: The phase analysis is also available in the analysis processes in the *Materials Solutions* tool window. The *Deep Learning* solution can't be used together with phase analysis in the *Materials Solutions* tool window.

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7. Interactive measurements

7.1. Overview

Your software offers a wide range of measurement functions. They enable you to quickly count objects and measure segments and areas. All the results will be saved together with the image and can also be output as a sheet.

Prerequisite

For making measurements, correctly calibrated images are an essential prerequisite.

Images that you have acquired with your software will have been automatically correctly calibrated when you have specified the objective you used. If your system has a motorized nosepiece or an encoder for the nosepiece, the correct magnification is automatically read-out before the image acquisition.

Should the image not yet have been calibrated, use the *Image > Calibrate Image...* command to carry out a calibration.

Additional measurement functions in your software

In addition to the interactive measurement functions, your software offers you a further range of measurement functions.

Line Profile	Use the *Line Profile* tool window to measure the intensity profile along a line on an image.
3D Profile	Use the *3D Profile* tool window to make measurements on a height map.
Automatic image analysis	You can detect and analyze objects in images with your software.
Materials Solutions	Use the *Materials Solutions* tool window to measure an image, or several images at the same time, according to different material science analysis processes.

Selecting the measurement environment

Measuring with help of the tool window

Switch to the *Processing* layout when you want to measure images. You can find the *Measurement and ROI* tool window in the bottom section of this layout. In this tool window you have fast access to all measurement functions and settings which effect measurements. This tool window is at the same time the measurement display and contains all of the values that have been measured on the active image.

Note: Should, right at the bottom of the user interface, several tool windows lie one over the other, activate the *Measurement and ROI* tool window, by clicking on the header of the *Measurement and ROI* tab. The tabs can be found under the tool windows.

Starting a measurement

Begin a measurement by selecting the measurement function you want. You can find the measurement function in the *Measurement and ROI* tool window, on the *Measurement and ROI* toolbar, or in the *Measure* menu.

Working in the measurement mode

As soon as you have clicked a measurement function, your software will automatically switch to a measurement mode. In the measurement mode, your mouse pointer will take on the shape of a cross on the image. A small icon indicating the selected measurement function attaches itself to the bottom right of the mouse pointer.

You can make as many measurements on the active image as you like using the measurement function that has been selected. The continuous measurement mode is valid for all loaded images. You can, therefore, easily measure numerous images one after the other.

The selected measurement function's button will keep its clicked appearance and in this way show you the current measurement function. You can recognize this status by the button's background color.

Finishing the measurement mode

You can explicitly switch off the measurement mode. To do this, click on the active measurement function's button again.

You automatically turn off the measurement mode when you switch to a different mouse pointer mode. For example, click the *Select Measurement Objects* button to switch to the selection mode. You can find the button either in the *Measurement and ROI* tool window or on the toolbar. You can select and edit measurement objects in this mouse pointer mode.

Changing the default measurement mode

The continuous measurement mode described above is preset by default. You can change this default setting. To do this, use the *Tools > Options...* command. Select the *Measurement and ROI > General* entry in the tree view. Select the *Switch to 'Select Measurement Objects' mode after creating a measurement object* check box. Then, when you have completed a measurement, you will automatically leave the measurement mode again. This means you have to select the measurement function again before you start each interactive measurement.

Displaying and saving measurement results

The measurement results will be displayed directly on the image and in the *Measurement and ROI* tool window. Should this tool window not be visible, use the *View > Tool Windows > Measurement and ROI* command to display the tool window.

Saving the measurement results

The measurements will be saved along with the image, if you save the image in the TIF or VSI file format. You can, however, also export the measurement results in a results sheet, and save this as a file.

Showing and hiding measurement results in an image

The measurement results will be shown on the image in a special data layer, the measurement layer. On your monitor, image and measurement layer are shown together. The data of each, however, is individually stored if you use the TIF or VSI image file format. Try and picture the measurement layer as a transparency which is placed over the image. When you measure an image, the image data will not be changed by having the measurement results displayed on it.

You can, at any time, hide or show the measurement layers.
To do so, use the *Layers* tool window. There you have access to all of an image's layers. The eye icon identifies all of the layers that are currently on display on your monitor.
Click the eye icon in front of the *Measurement and ROI* layer to hide the measurements. Click an empty cell without an eye icon to make the corresponding layer reappear.

Creating an Excel report that displays the measurement results

You can create an Excel report that contains the image that was measured as well as its the measurement results. To do, click the *Create Excel Report* button located on the *Measurement and ROI* tool window's toolbar. The *Create Excel Report* dialog box opens. In this dialog box you select the Excel template and the data that you want the Excel report to use. When you confirm your selection, the MS-Excel application program opens and the report is displayed.

Editing measurements

You can edit existing measurement objects at any time. The measurement values in the *Measurement and ROI* tool window will be correspondingly updated.

Note: When you load an image file with measurement objects, it is only possible to edit the measurement objects if the image file has been saved in the TIF or VSI image file format.

Selecting measurement objects

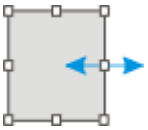
Before you can edit measurement objects, you have to select them. To do so, click the *Select Measurement Objects* button, and then select the measurement object(s). You can find the button either in the *Measurement and ROI* tool window or on the toolbar.

If the image is very large and many measurement objects have been defined, it can be difficult to find a particular measurement object in the image. In this case, select the measurement object that you are searching for in the *Measurement and ROI* tool window. Click your right mouse button and select the *Navigate to Measurement Object* command in the context menu. The measurement object you are looking for is then displayed in the image window.

Changing the position and size of measurement objects

You can move a whole measurement object while keeping the left mouse button pressed.

You can also change the size of a measurement object. Move the pointer onto a marker. By dragging the marker with the mouse button depressed, you can adjust the frame's size as wished.



Change the measurement object by moving the handles.

Deleting measurement objects

Click the [Del] key on your keyboard in order to delete the selected measurement object. You can select measurement objects that you want to delete in the image and also in the sheet in the *Measurement and ROI* tool window.

Changing the color, font, and line thickness of individual measurement objects

You can, at any time, change the color, font, and line thickness, of individual measurement objects. Select one or more measurement objects in an image and click your right mouse button to open a context menu. In the context menu you'll find the following commands. You can use them to change the appearance of the selected measurement objects.

Change Color
Helper Lines
Change Line Thickness
Change Font

Measuring in the live mode

All of the measurement functions are also available in the live-image. You can therefore, e.g., quickly measure a segment in the live-image.

When you finish the live mode with the *Acquire > Snapshot* command, the measurements that you carried out in the live-image are applied to the image that was acquired.

Measuring on different image and document types

Measuring on image series

You can combine a series of individual images into one image. What results is a time stack in which all of the frames have been acquired at different times, for example.

You can make measurements on every frame. Display the required frame on your monitor. To do this, use the navigation bar in the image window. Then carry out the measurement on this frame. The measurement will be permanently linked to this frame, i.e., the measurement will only be displayed on your monitor when the frame on which you made this measurement is also on display.
The measurement results will be shown in the *Measurement and ROI* tool window. You can give every measurement the number of the frame on which it was made. For time stacks, for example, you can do this using the *Index (t)* measurement parameter.

Measuring on multi-layer images

With some functions, e.g., with the *Image > Combine Color Images...* function, a multi-layer image will be created. This multi-layer image is made up of several layers.

Measurements always apply to one image layer. For this purpose, show the image layer on your monitor, on which you want to make measurements. To do so, use the *Layers* tool window. Then carry out the measurement on this image layer. The measurement will be permanently linked to this image layer, i.e., the measurement will only be displayed on your monitor when the image layer on which you made this measurement is also on display.
The measurement results will be shown in the *Measurement and ROI* tool window. You can give every measurement the name of the image layer on which it was made. To do this, use the *Layer* measurement parameter.

Measuring on charts

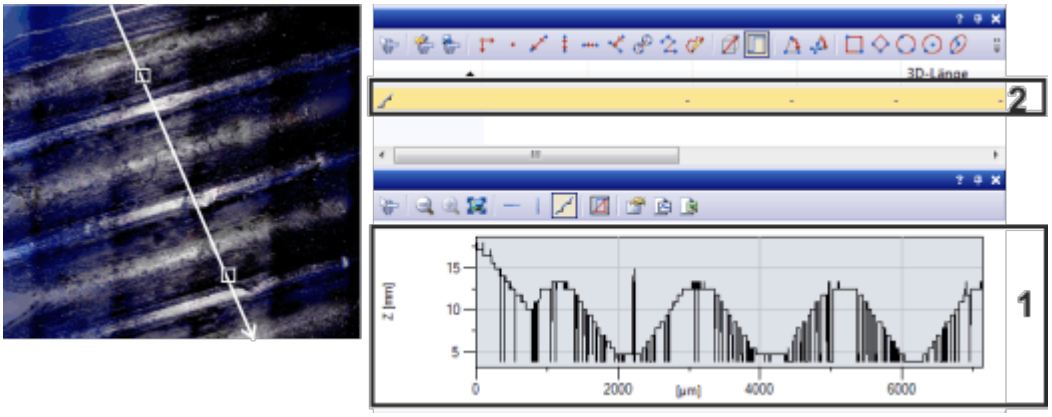
Your software has its own chart document. A chart can be saved, edited and also measured.
Use the *Line Profile* tool window to measure the intensity profile along a line on an image, for example. In the tool window, click the *Export to Chart* button to export the line profile to a chart.

As soon as a chart has become active in the document group, the *Measurement and ROI* tool window changes its appearance. From then on, only the measurement functions that you can use for charts are available.

	Name of the button	Description
	Horizontal Line	In a chart, measure the horizontal distance between two interactively determined points.
	Multiple Horizontal Lines	In a chart, measure the horizontal distance between a reference line and an interactively determined point.

Measuring on images that contain height information

Your software supports images that contain height information. Use the *3D Profile* tool window to define a height profile on one of these height maps. You can measure the height difference between two points on the height profile, for example. To do this, define the measurement object in the*3D Profile* tool window. The measurement results will be shown in the *Measurement and ROI* tool window. You can use all of the measurement parameters that are of the *3D Line* object type.



The 3D profile line is shown in white in the image on the left. The *3D Profile* tool window displays the corresponding 3D profile (**1**). In the *Measurement and ROI* tool window, a measurement object (**2**) has been created for the 3D line.

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7.2. Measuring images

Your software offers a wide range of measurement functions. They enable you to quickly count objects, and measure distances and areas on an image.

The following step-by-step instructions present the measurement functions to you by way of several examples.

Measuring image objects interactively
Outputting various measurement parameters
Measuring several images

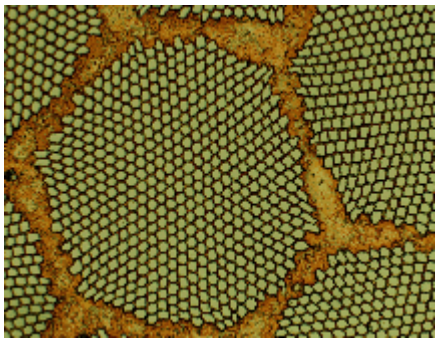
Measuring image objects interactively

Example: You want to measure the filaments in a supraconductor.
To do this, load a suitable image, or acquire one. Measure the diameter of several of the hexagonal filaments, in each case between the opposing vertices.
Subsequently edit the measurement. Delete some of the measurements you've made.
Enter the results in a MS-Excel sheet.

1. Use the *View > Tool Windows > Measurement and ROI* command to display the *Measurement and ROI* tool window.
   - You'll find the tool window at the lower edge of the user interface. It's possible that it may be covered by the *Count and Measure Results* tool window. Click the *Measurement and ROI* tab at the bottom of the user interface to bring the tool window into the foreground.

Loading an image

2. Acquire an image or load one.



- During the installation of your software some sample images have been installed, too. You can follow these step-by-step instructions for measuring images if you use the SupraConductor.tif example image.

Setting the labeling color

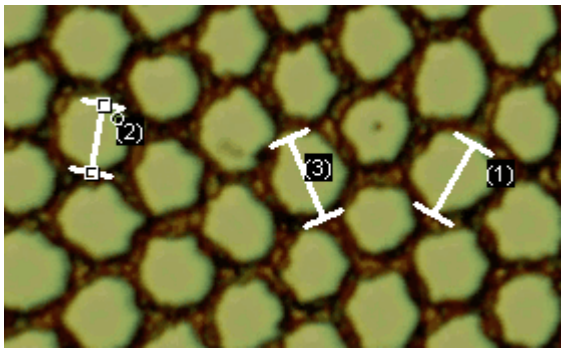
The measurement results will, in accord with the default settings, be written in red in the image, without a background. This can be hard to read on some images. Change the labeling settings.

3. Use the *Tools > Options...* command.
4. Click the *Measurement and ROI > Measurement Display* entry in the tree view.
5. Click in the *Background Color* field and choose a color, black for example.
6. Select the *Text color > Fixed colors* option and select a suitable color from the palette. Select the color white to display the measurements in white and the labels in white on a black background.
7. Close the dialog box with *OK*.

Measuring lengths

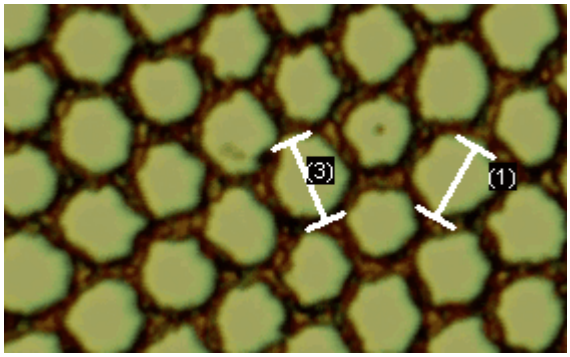
8. Click the *Arbitrary Line* button, located on the toolbar at the top of the tool window.
9. Click with your left mouse button at the starting point and end point of the reference distance.
10. If you have measured a reference distance, you can immediately proceed with the next measurement.

11. Click the *Arbitrary Line* button again to end the length measurement.
12. Take a look at the results in the tool window and in the image.
    - The illustration shows the image with three executed measurements. Measurement 2 has been selected.



Deleting measurements

13. Click one of the measurement results in the *Measurement and ROI* tool window.
    - The corresponding line will be selected in the image.
14. Press the [Del] key.
    - The measurement will be deleted both in the image and in the tool window.
    - When a measurement has been deleted, the image and the tool window contain one measurement less. The IDs of the remaining measurements won't be changed by the deletion of a measurement.



Note: When you've finished making the measurement, you should switch off the measurement mode, since you could otherwise accidentally select your measurements and move them.

15. Check whether one of the buttons on the *Measurement and ROI* tool window's toolbar appears clicked. Release this button

Exporting results to MS-Excel

16. Click the *Export to Excel* button.
17. In the In/Output dialog box you set up the directory in which the data is to be saved, and enter the name of the MS-Excel sheet. Adopt the *Excel-Sheet (*.xls)* file type.
18. Click the *Save* button to have the MS-Excel sheet with the measurement results saved.

Closing the image

19. Click the small button showing a cross [x], located at the right of the image name in the document group.

- You have changed the image because you've added interactive measurements. For this reason, you'll receive a query whether you wish to save the image or not.

20. Save the image in the TIF or VSI file format. The measurements will then also be saved in the image file. They can at any time, be edited deleted or augmented.

Outputting various measurement parameters

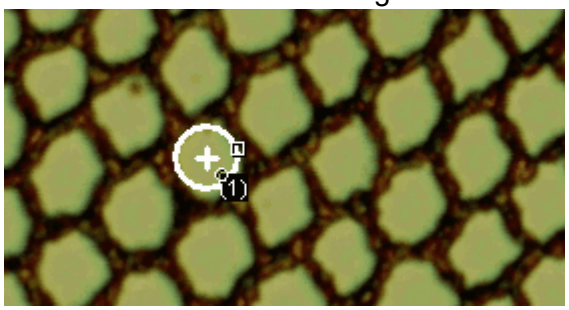
Example: You want to measure the filaments in a supraconductor.
Measure the hexagonal structure as a circular surface. Have a variety of measurement parameters, such as the area, the perimeter and the diameter, output. Have the diameter shown in the image.

1. Acquire an image or load an image, the Supraconductor.tif example image, for example.

Measuring areas

2. In the *Measurement and ROI* tool window, click the *2 Point Circle* button.
3. Left click the center point of the hexagonal structure that you want to measure.
4. Move your mouse, and in the process drag out the circle. Match the circular object as well as possible to the hexagonal structure. Click the left mouse button.

5. Click the *2 Point Circle* button again, and switch off the measurement mode.
6. Take a look at the result in the *Measurement and ROI* tool window.
   - The illustration shows the image with a circle measured.

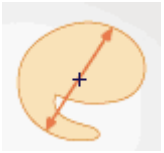


Viewing the list of measurement parameters

7. In the *Measurement and ROI* tool window, click the *Select Measurements* button.
   - In the dialog box you'll see a list with all of the available measurement parameters. At the bottom of the dialog box you'll see a list of the measurement parameters that are currently calculated for all objects.

Outputting additional measurement parameters

8. Go to the list of all of the available parameters, then click the *Diameter* measurement parameter.
   - On the right, an illustration shows you how the parameter is calculated.

     You can see that there are different ways in which the diameter of a 2D object can be calculated.
9. Click the *Mean* entry in the list under the illustration, to select the *Mean (Diameter)* measurement parameter. When you do this, the mean value of all of the possible diameters is determined.
10. Click the *Add 'Mean (Diameter)'* button.
    - This measurement parameter will be added to the list of measurement parameters to be calculated. All of these measurement parameters will be displayed in the tool window.
11. Close the dialog box with *OK*.
12. Take a look at the result for the circle's diameter in the *Measurement and ROI* tool window.

Outputting measurement parameters in the image

13. Open the *Select Measurements* dialog box.
14. At the bottom of the list of all of the calculated measurement parameters, click the *Mean (Diameter)* measurement parameter.
15.  To the right of this list you'll see a button with a blue arrow . Click this button to move the measurement parameter to the top of the list.
16. Close the dialog box with *OK*.
17. Take a look at the result for the circle's diameter in the image.

Note: The measurement display in the image has to be updated once, so that the settings that have been changed are also taken into account. You update the measurement display, for instance, by adding another measurement, or by once selecting an existing measurement in the image.

Measuring several images

Example: You want to measure the thickness of a spray coating. To do so, you acquire several images of the coating. Have the results from all images displayed simultaneously. Take a look at the mean value for all of the measurements.

Loading images

1. Acquire or load some images.

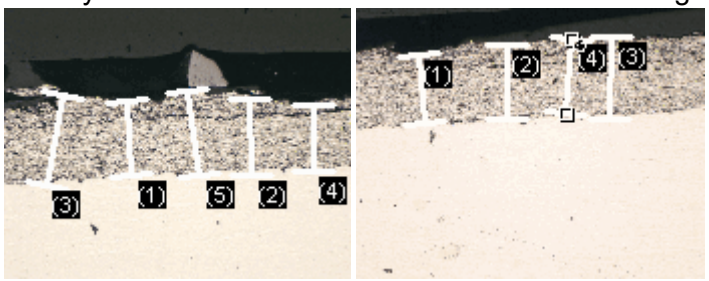
- During the installation of your software some sample images have been installed, too. You can carry these step-by-step instructions out directly with the example images SprayCoating2.tif and SprayCoating4.tif.

Measuring the layer thickness

2. Activate the first image in the document group.

3. Click the *Arbitrary Line* button, located on the toolbar at the top of the *Measurement and ROI* tool window. Measure the thickness of the layer at several different places.
4. Activate the next image. Measure the thickness of the layer at several different places, here also.

5. Click the *Arbitrary Line* button again, and switch off the length measurement.
   - The layer's thickness has been measured on both images.

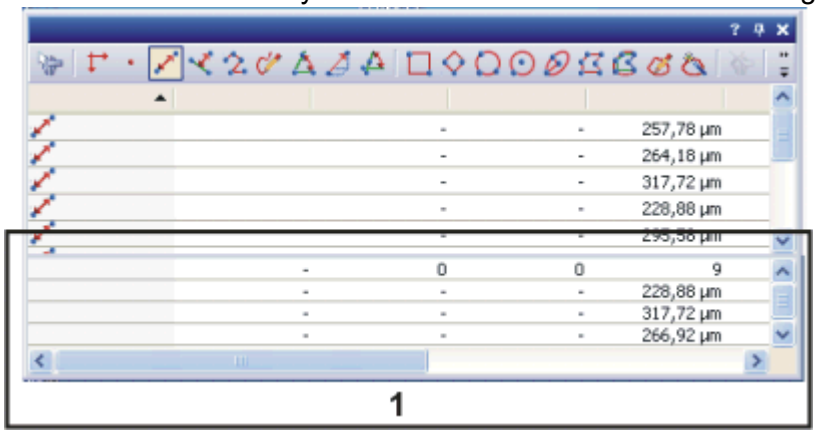


Displaying the measurement results of all of the images

6. In the *Measurement and ROI* tool window, click the *Measurement and ROI Options* button.
7. In the tree view, select the *Measurement > Results* entry.
8. Clear the *Show measurement objects > Only of the active image* check box.
9. Close the dialog box with *OK*.
   - Now, the results for both images will be shown simultaneously in the tool window.
   - Use the *Document* measurement parameter to display the name of the image with which the measurement results are associated in the results sheet. Now you can match the measurement results unambiguously to an image, even if all measurement results are displayed together in the tool window.

Viewing statistical parameters

10. In the *Measurement and ROI* tool window, click the *Measurement and ROI Options* button.
11. Select the *Measurement and ROI > Results* entry in the tree view.
    - In the *Statistics* group, you can find various statistical parameters.
12. Select the *Mean* check box.
13. Close the dialog box with *OK*.
    - Now, in the *Measurement and ROI* tool window under the measurement results, the chosen statistical parameter (**1**) will by shown. You can see there the mean value of the layer thickness for all of the measured images.



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7.3. Measuring welds

Measuring the cross section of a weld is a method commonly used for judging a weld's quality. With the *Weld Measurement* solution you can interactively measure microscope images of weld cross-sections and can output the results on the image and in a table. The following measurement functions are available:

	Multiple perpendicular lines	Use this measurement function, to determine the distance from several measurement points to a reference line.
	Asymmetry Lines	Use this measurement function, to construct the perpendicular bisector of the connection between two reference points, and to determine the distance of a measurement point from the perpendicular bisector.
	Throat thickness	Use this measurement function, to determine the thickness of a fillet weld's throat.

Starting a measurement

You will find the weld measurement functions in the *Measure* menu, or as a button on the *Measurement and ROI* tool window or toolbar. Start a measurement, e.g., by clicking the corresponding button.

Interactive measurement functions and weld measurements
The functions which you can use to measure welds, behave just like the other interactive measurement functions offered by your software, e.g. the *Arbitrary Line* measurement function. All of the information on the interactive measurement functions also applies for the measurement of welds.

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7.3.1. Measuring a throat thickness

Use the *Throat thickness* measurement function to determine the thickness of a fillet weld's throat. You will find the measurement function in the *Measure* menu, or as a button on the *Measurement and ROI* tool window or toolbar.

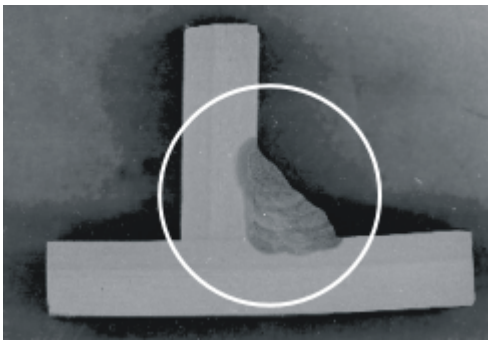
Prerequisite: The *Throat Thickness* measurement function will only be available if you purchased the *Weld Measurement* solution together with your software.

Measuring a throat thickness

1. If necessary, use the *View > Tool Windows > Measurement and ROI* command to have the *Measurement and ROI* tool window displayed.

Loading an image

2. Acquire an image or load one.

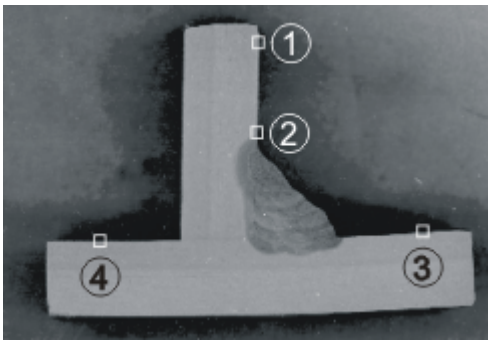


The illustration shows a cross section of two welded pieces of metal. The weld is circled.
How thick is the weld's throat?

3. Set a zoom factor for your image window that will make the image segment that is to be measured clearly visible. You will achieve the most precise measurements if you set the zoom factor at 100%.

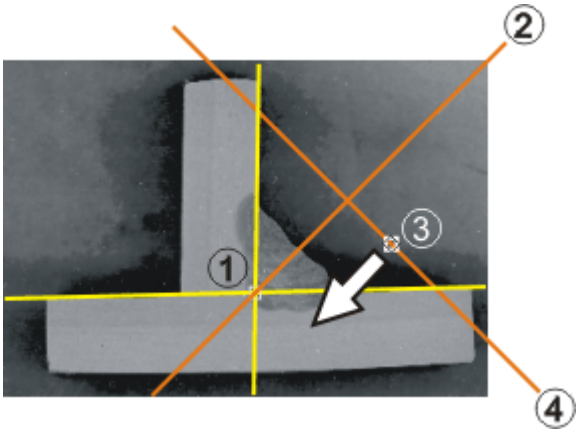
Measuring the throat thickness (with a welded root)

4. Start the measurement. To do so, click the *Throat Thickness* button, on the toolbar at the top of the tool window.



With four mouse clicks (**1**-**4**), define two lines along the inner surfaces of the metal pieces which are welded together.

5. Click on a point on the inner surface of the first piece of metal (**1**). This point should be a far as possible from the weld's root. You can put the measurement point before or after the weld.
   - The point which you define will be shown in the image with a handle.
   - The mouse pointer's shape on the image window shows which measurement mode you are in.
6. With three more mouse clicks (**2**-**4**), define two lines along the inner surfaces of the metal pieces which are welded together.
   - Your software will now automatically display some lines and handles in the image window.
   - The mouse pointer is now linked to an auxiliary line, which is perpendicular to the bisector of the angle. When you move the mouse you will move this line at the same time.



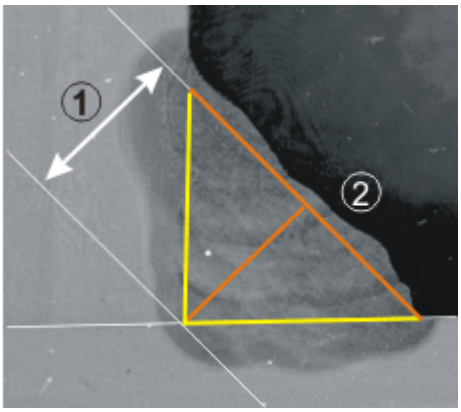
Once the inner surfaces are defined (yellow lines), the position of the root (**1**) will automatically be drawn in and your software will calculate the bisector of the angle (**2**). Together with the handle (**3**), move the line (**4**) which is perpendicular to the bisector of the angle to determine the throat thickness.

7. Move the auxiliary line (**4**) to the outer surface of the weld. The part between the two lines shown in yellow above must be just inside the cross-section of the weld along the entire length.
   - The measurement of the throat thickness is thus complete. The *Throat Thickness* measurement object (an equilateral triangle) is completely defined.

- The throat thickness (the height of the triangle) will be shown in the image. In the *Measurement and ROI* tool window table, a new measurement value with a type of *Throat Thickness* will be entered.

Note: If the measurement results are not shown, check the measurement parameters currently displayed. You will find step-by-step instructions to modify the measurement parameters further down.

8. Take a look at the result in the *Measurement and ROI* tool window and in the image.

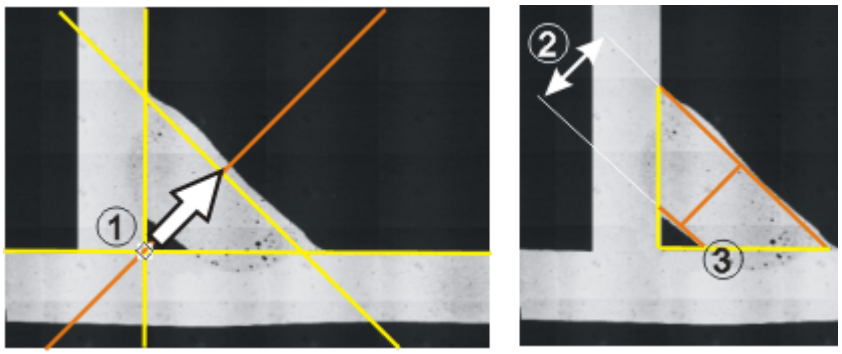


The illustration shows an enlargement of the weld with the measurement object (an equilateral triangle). The result of the measurement is the throat thickness (**1**) (the height of the triangle). The auxiliary line (**2**) (the base of the triangle) must be positioned so that it just lies completely within the weld.

Measuring the throat thickness (when the root is exposed)

If the root of the weld is exposed, another step will be required to measure the throat thickness.

9. Select the measurement object.
   To do so, click the *Select Measurement Objects* button to switch to a selection mode and then click on the measurement object in the image window. You can find this button, e.g., on the *Measurement and ROI* tool window's toolbar. Directly after a throat thickness measurement, the measurement object will automatically be selected.
10. Click on the vertex.
11. Keep the left mouse button pressed and drag the vertex towards the outer seam of the weld towards the base of the triangle). In this way you are moving a second auxiliary line. This auxiliary line must also lie with its full length just within the weld's cross section.



If the root of the weld is exposed, drag another auxiliary line (**3**) from the vertex (**1**). The throat thickness is now the distance (**2**) between the two auxiliary lines which are perpendicular to the bisector of the angle.

Saving the image

12. Save the image in the TIF or VSI file format. The measurements will then also be saved in the image file. They can at any time, be edited deleted or augmented.

Finishing the measurement

13. You can now measure other images.

14. If the *Throat Thickness* button is still active, click on the button again to finish measurement mode.

Changing settings for a throat thickness measurement

Adjusting measurement parameters

During each interactive measurement, significantly more values are measured than can be shown in the image or in the *Measurement and ROI* tool window. To alter the measurement parameters shown, follow these step-by-step instructions.
In particular, ensure that at least the *Length* and *Angle* measurement parameters are displayed, as they are both used for the throat thickness measurement.

1. In the *Measurement and ROI* tool window, click the *Select Measurements* button.
   - The *Select Measurements* dialog box opens. In the dialog box, at the top left, you'll see a list with all of the available measurement parameters. At the bottom of the dialog box, you'll see a list of the measurement parameters that are currently calculated and displayed for all objects.
1. In the *Available measurements* list, click on the *Measurement* column title to alphabetically sort all of the parameters.
2. Select the *Length* measurement parameter in the *Available measurements* list. This measurement parameter corresponds to the throat thickness.

4. Click the *Add 'Length'* button to have the *Length* measurement parameter added to the list of calculated measurement parameters.
5. Also add the *Angle* parameter to the list of calculated measurement parameters.
6. You can now further modify the display of the measurement parameters for a throat thickness measurement. You can for instance delete all other measurement parameters currently shown, so that the list of the measurement results becomes clearer.

7. Close the dialog box with *OK*.
8. Do a throat thickness measurement and examine the result in the *Measurement and ROI* tool window.

Displaying measured angles in addition to the throat thickness in the image

By default, for a throat thickness measurement, the measurement will be shown in the image. You can also output the angle between the two pieces of metal which have been welded together in the image.

1. Carry out a throat thickness measurement or load an image which contains a throat thickness measurement.
2. Select the measurement object on the image. For example, select the corresponding measurement in the *Measurement and ROI* tool window.
3.  Click the right mouse button and select the *Create Angle* command in the context menu.
   - In addition to the throat thickness, the angle measured will also be shown in the image.
   - This command creates another measurement object of type *Angle*. In the *Measurement and ROI* tool window you will thus see two entries for the measured weld.

Note: The measurements on a screen will automatically be numbered in sequence. The angle measurement will thus always have a different measurement ID from the associated throat thickness measurement. You can switch off the display of the measurement IDs, if you find their display distracting. To do so, open the *Tools > Options > Measurement and ROI > Measurement Display* dialog box, and clear the *Show ID* check box.

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7.3.2.  Measurement object - Asymmetry Lines

Use the *Asymmetry Lines* measurement function to construct the perpendicular bisector of the connection between two reference points and to determine the distance of a measurement point from the perpendicular bisector. You will find the measurement function in the *Measure* menu, or as a button on the *Measurement and ROI* tool window or toolbar.

Prerequisite: The *Asymmetry Lines* measurement function will only be available if you purchased the *Weld Measurement* solution together with your software.

1. If necessary, use the *View > Tool Windows > Measurement and ROI* command to have the *Measurement and ROI* tool window displayed.

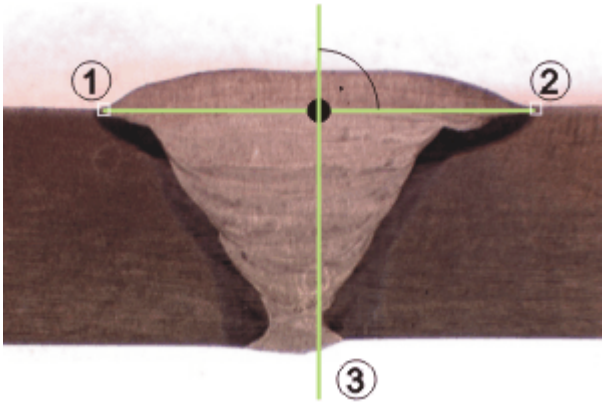
Loading an image

2. Acquire an image or load one.

3. Set a zoom factor for your image window that will make the image segment that is to be measured clearly visible. You will achieve the most precise measurements if you set the zoom factor at 100%.

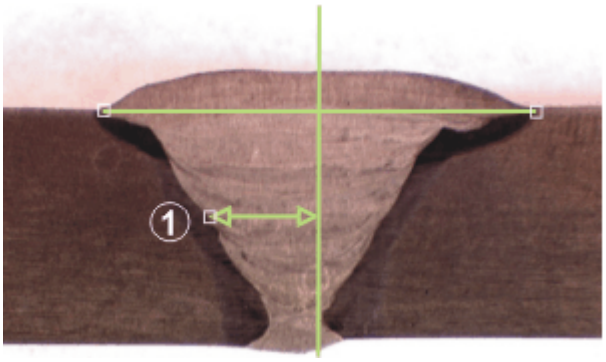
Measuring asymmetry

4. Start the measurement. To do so, click the *Asymmetry Lines* button, on the toolbar at the top of the tool window.



The illustration shows a cross section of two welded pieces of metal. How asymmetric is this weld? Click on the two reference points (**1**) and (**2**) in turn. Your software will automatically calculate the perpendicular bisector as a reference line for the measurement of the asymmetry (**3**).

5. With the left mouse button, click on two reference points in turn.
   The perpendicular bisector of the connecting line between these two reference points is the reference line for the measurement of the asymmetry.
   In the example shown, the reference points define the width of a weld. The reference points in the example shown lie horizontally next to one another. They could just as well have any orientation in the image.
   - The points which you define will each be marked in the image.
   - The mouse pointer's shape on the image window shows which measurement mode you are in.
   - The mouse pointer is now linked to an auxiliary line, parallel to the perpendicular bisector. When you move the mouse you will move this line at the same time.
6. Left click a measurement point to measure its distance from the reference line.
   - The result of the measurement will be shown in the image.



Define a measurement point (**1**). The distance between the point and the reference line will be measured.

7. If required, you can also define other measurement points. For each measurement point defined, the distance to the reference line will be measured.

Undoing measurement points

8. As long as the measurement is not yet finished, you can undo individual measurement points, if you have made a mistake in the measurement. To do that, press [backspace] on your keyboard.

Note: If the measurement results are not shown, check the measurement parameters currently displayed.

Finishing the measurement

9. Click the right mouse button to end the measurement.
   - In the *Measurement and ROI* tool window table, a new entry with a type of *Asymmetry Lines* will be shown.
     Please note that all of the measured distances belong to a single measurement object. In the *Measurement and ROI* tool window table there may, under certain circumstances, be several length measurements assigned to a single entry in the *Type* or *Name* column.
10. You can now measure other images.
11.  If the *Asymmetry Lines* button is still active, click on the button again to finish measurement mode.

Saving the image

12. Save the image in the TIF or VSI file format. The measurements will then also be saved in the image file. They can at any time, be edited deleted or augmented.

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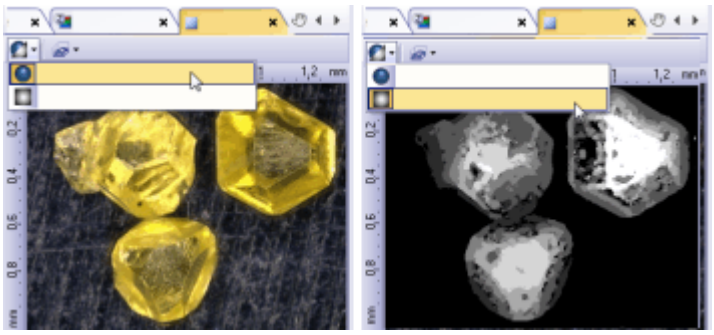
8. Images containing height information

What is a height map?

Your software can use a series of images focused at different depths (Focus series) to calculate a resulting image (EFI image). This resulting image will be in sharp focus throughout. In addition to an EFI image, you can also create a height map. The height map shows you your sample's topography. It shows, for every pixel, from which frame in the Z-stack the pixel has been taken. The Z-position thereby determines the pixel's intensity value. A dark pixel comes, e.g., from a frame with a low Z-value. A bright pixel comes from a frame with a high Z-value.



The image on the left shows an EFI image of diamonds. The image on the right shows the corresponding height map. Low-lying structures can be recognized by their dark gray values, structures that lie higher, by their light gray values.

Height maps

- Creating an EFI image and a height map from a Z-stack
- Creating a height map while acquiring an EFI image
- Displaying a height map in the image window

3D-surfaces

- Creating the 3D-surface
- Changing the appearance of the 3D-surface
- Creating an image of the 3D-surface

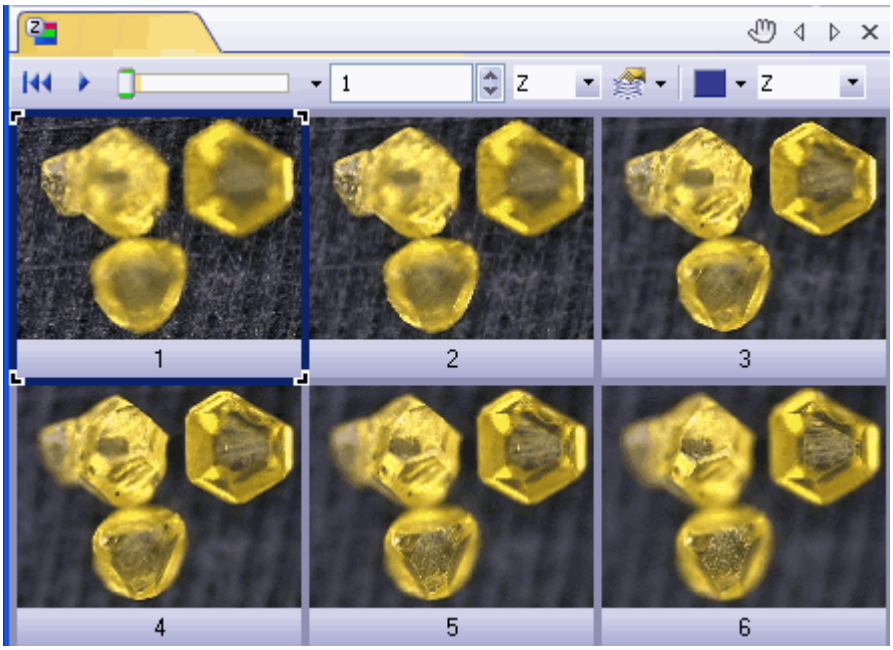
Measuring heights

- Creating and measuring height profiles
- Measuring heights interactively

8.1. Creating an EFI image and a height map from a Z-stack

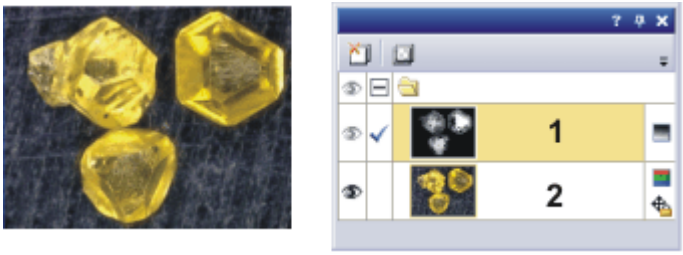
Example: From a Z-Stack that shows diamonds at different focus positions, calculate the EFI-image including a height map.

1. Load the Z-stack from which you want to calculate an EFI image.



The image shows three diamonds, seen through a reflected light microscope. In the process, images were acquired at different focus positions. In the illustration, the Z-stack is displayed in the tile view. You can easily recognize how the focal plane travels from the bottom upwards. In image 1 the background is sharply reproduced. In image 6 the surface of the diamonds is sharply reproduced.

2. Use the *Process > Enhancements > EFI Processing…* command.
3. Select the *Apply on > All frames and channels* option.
4. From the *Algorithm* field select the *Reflected light* entry.
5. Select the *Height map* check box.
6. Select the *Create new document as output* check box.
7. Close the dialog box with *OK*.
   - A new image document will be created in the document window. You can see the EFI image with the diamond's texture. In the EFI image, the image background as well as the upper surface of the diamonds are in focus.
   - The resulting image is a multi-layer image and is therefore accompanied by this icon in the image window's title.
   - The height map is a layer of the EFI image. The texture image makes up the second layer. Use the *Layers* tool window to view the structure of the image.



The illustration shows, on the left, the EFI image of the diamonds. On the right, you can see the *Layers* tool window with both of the image layers *Height map* (**1**) and *Texture map* (**2**).

8.2. Creating a height map while acquiring an EFI image

Example: Use the *Instant EFI* acquisition process to acquire a height map together with the EFI image.

Prerequisite: Your stage must have a motorized Z-drive or a Z encoder.

Selecting the acquisition process

1. Use the *View > Tool Windows > Process Manager* command to make the *Process Manager* tool window appear.
2. Select the *Manual Processes* option.

3. Click the *Instant EFI* button.
   - The button will appear clicked. You can recognize this status by the button's colored background.
   - The *Instant EFI* group will be automatically displayed in the tool window.

Setting the acquisition parameters

4. From the *Algorithm* list, select the *Reflected light* entry, when you use your light, or stereo microscope in the reflected light mode.
5. If you work with a stereo microscope, select the *Automatic frame alignment* check box.
   If you don't work with a stereo microscope, clear the *Automatic frame alignment* check box.
6. Select the *Height map* check box.
   - Now a height map is automatically computed together with the EFI image.

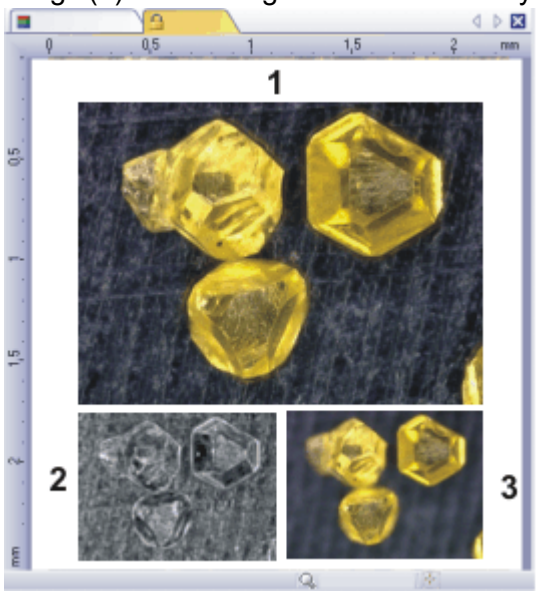
Preparing EFI acquisition

7. Use the *View > Tool Windows > Camera Control* command to make the *Camera Control* tool window appear.

8. In the *Camera Control* tool window, click the *Live* button.
9. In the live-image, move the microscope focus to the Z-position where either the lowest or the highest position on the sample is only just out of focus.
10. Check the exposure time, and correct it if necessary. When the *Instant EFI* acquisition process has been started, the exposure time will be kept constant during the whole of the acquisition.

Acquiring an EFI image

11. In the *Process Manager* tool window, click the *Start* button.
    - The live-image in the document group will divide itself into 3 images. On the bottom right, you'll still see the live-image (**3**). On the bottom left, you'll see the sharpness map (**2**). The large image above them is the composite resulting image (**1**). The 3 images will be continually updated.



12. Use your microscope's Z-drive to move your stage slowly through the height range of the sample's surface.
    - Your software will acquire images at the various focal planes, then it will set them together. While this is being done, the camera will acquire the images as quickly as possible. The sharpness value of individual pixels will be calculated for every image. If the sharpness values are higher than in the previous images, the pixels in the composite EFI image will be adopted. The EFI image contains the pixels with the highest sharpness values from all of the images acquired up till then.
    - The sharpness map at the bottom left will show you which image areas will be sharply reproduced in the EFI image. The brighter a pixel is in the sharpness map, the higher is its sharpness value in the EFI image.
    - Once the acquisition process has been started, the sharpness map should only be bright at the deepest or highest parts of the sample, the rest of the map is dark.
13. Focus on the sample slowly once through all the focal planes.
    After each change of the focus position, wait until you see that further areas become brighter in the sharpness map.
    - As the process continues, more and more areas in the sharpness map should become brighter. At the same time the EFI image will also get better and better.
14. Check the EFI image and the sharpness map. Are all areas of the image now sharp? Are there any areas in the sharpness map that are still dark?
    Focus on these areas and have additional images calculated into the EFI image.
    Continue acquiring additional images until the whole sample has been sharply

reproduced.

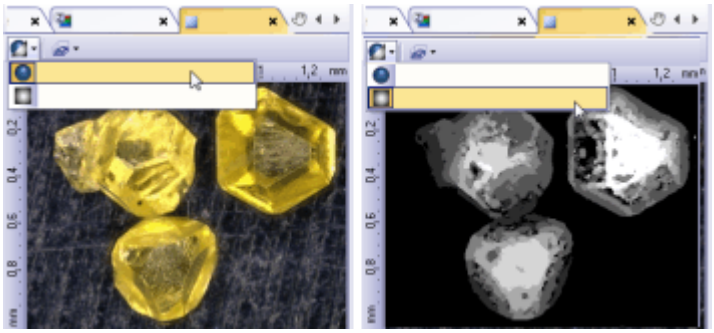
15. In the *Process Manager* tool window, click the *Stop* button.
    - The resulting image is a multi-layer image and is therefore accompanied by this icon in the image window's title.
    - The EFI image will be automatically saved. You can set the storage directory in the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.
16. In the *Camera Control* tool window, click the *Live* button again to release it.

8.3. Displaying a height map in the image window

Switching between the EFI image and the height map

Prerequisite: The navigation bar is displayed in the image window. This is the default setting.

1. Load an EFI image with a height map.
    - An additional button is now shown in the image window's navigation bar.
2. In the image window's navigation bar, click the *Show texture layer or show height map layer* button to switch between the EFI image and the height map in the image window.



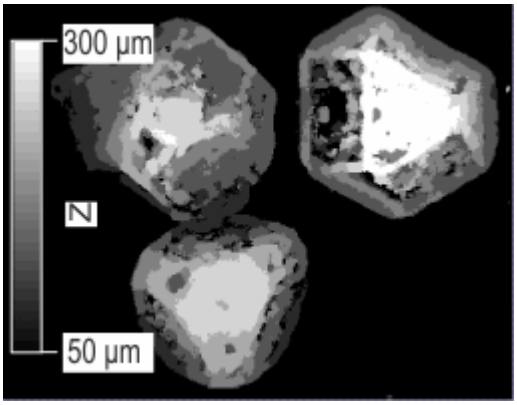
There is a multi-layer image composed of two layers in the document window. The image on the left shows the texture image. The image on the right shows the corresponding height map. Low-lying structures can be recognized by their dark gray values, structures that lie higher, by their light gray values. Use the button on the image window's navigation bar to switch between the two images.

Displaying the intensity calibration in the image

The height map is calibrated in Z-direction. You can display a color bar showing the Z-calibration in the image.

1. Display a height map in the image window.
2. Use the *Tools > Options* command, and select the *Color Bar > General* entry in the tree view.
3. Select the *Apply intensity calibration* check box.
   Clear the *Show for pseudo color mode only* check box.
4. In the *Position* group, select where in the image window you want the bar with the calibrated intensity values to be shown. Click this button to show the bar on the left of the image, for example.

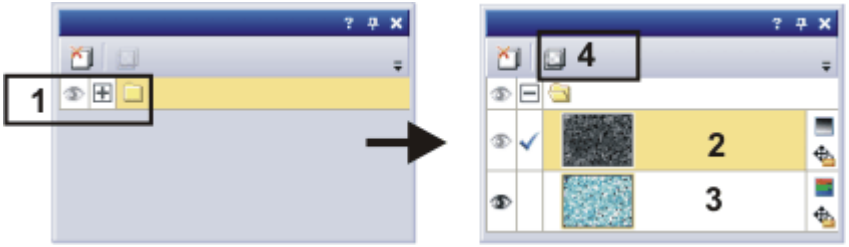
5. Close the *Options* dialog box with *OK*.
6. Use the *View > Color Bar* command to display a bar with the intensity calibration in the image window.



A bar with the intensity calibration is displayed in the height map. The color bar tells you which height a gray value represents. For example, the white areas in the image have a height of 300 µm.

Switching between the EFI image and the height map when the navigation bar isn't shown.

1. Use the *View > Tool Windows > Layers* command to make the *Layers* tool window appear.
2. In the *Layers* tool window, click the [+] sign (**1**) and open the image's layers.
   - You can now see the image's individual layers: height map (**2**) and texture image (**3**). The height map can't be seen in the image window, because it's absolutely transparent at the moment.

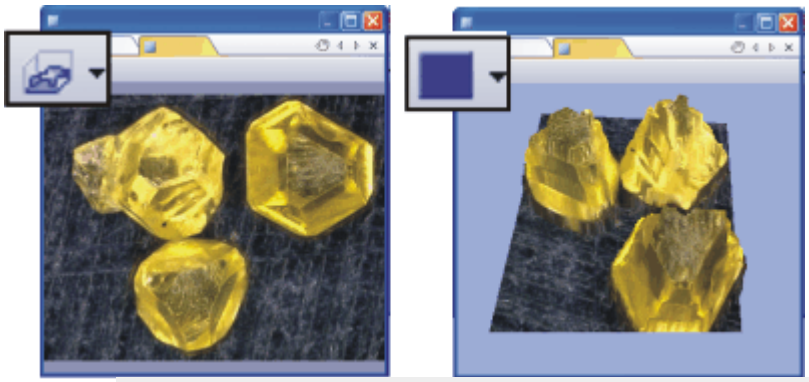


3. Select the height map in the *Layers* tool window.
4. Click the *Set Layer Opacity* (**4**) button, located on the toolbar at the top of the tool window.
5. Drag the slider all the way to the right, to an opacity of 100%, then take a look at the height map.
6. Activate a layer and click its eye icon to make it disappear. By doing this, you can, e.g., view only the height map or the EFI-image.
7. Click an empty cell without an eye icon to make the corresponding layer reappear.

8.4. Creating the 3D-surface

Your software enables you to display the height map three-dimensionally. To do so, use the *Surface View* image window view.

1. Click the small arrow next to the last button on this navigation bar to open a menu with commands you can use with image window views.
2. Use the *Surface View* command to switch to that image window view.
   - In the image window, the height map is now displayed as a 3D-surface.
3. Use the *View > Tool Windows > Surface View* command to make the *Surface View* tool window appear. Use this tool window, to configure the Surface View.



The illustration shows, on the left, the height map, and on the right, the 3D-surface. Pay attention to the navigation bar in the image window. There, you can find the buttons for switching between the image window views. When the height map can be seen in the image window, you'll see the button for switching to the Surface View. When the Surface View is shown, you'll see the button for switching to the Single Frame View.

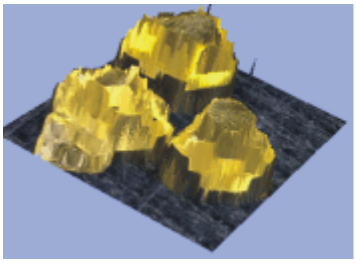
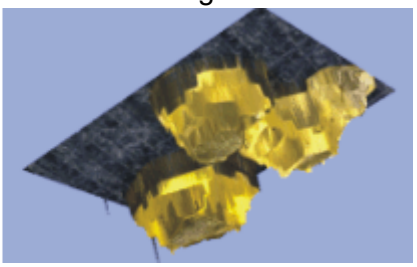
Note: The functions in the *Surface View* tool window are only available for the *Surface View* image window view. When another image window view is active, for example, the Single Frame View, this tool window will be empty.

8.5. Changing the appearance of the 3D-surface

There are several ways in which you can change the way a 3D-surface is displayed. To do so, use the *Surface View* tool window.

Moving the 3D-surface

1. In the *Surface View* tool window, you can find the *Navigation* group. Use the slide controls in this group to rotate, or tilt the 3D-surface, or to change its size.
   Take a look at the diamonds from different angles of vision.

2. Alternatively, you can, while keeping the left mouse button depressed, also tilt and rotate the 3D-surface in the image window.
   To do this, right click on the image window, and use the context menu's *Zoom with Mouse* and *Rotate with Mouse* commands.

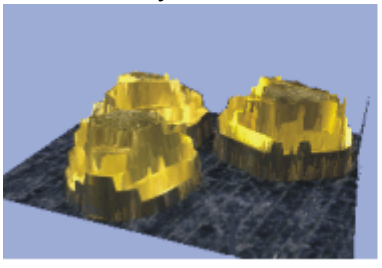
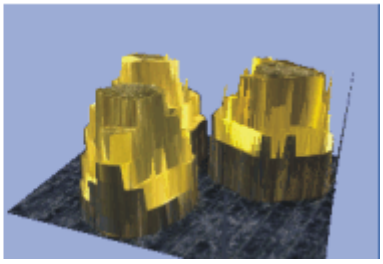
Smoothing the 3D-surface

1. Right click on the image window, then use the *Global Surface View Options...* command.
   - The *Options > Surface View > Filter* dialog box opens.
2. Select the *Perform Gaussian blur filter* check box in the *Smooth data* group.
3. In the *Radius* field, enter 5. The larger the value entered here is, the greater is the smoothing effect.
4. Close the dialog box with *OK*.

Changing the appearance of the 3D-surface

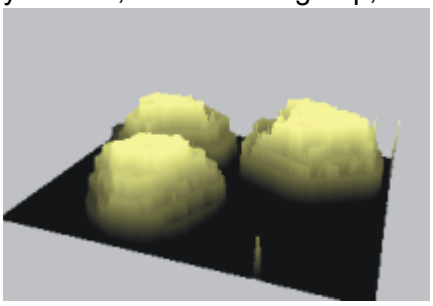
The *Surface View* tool window offers several possibilities for changing the appearance of the 3D-surface.

1. In the *Intensity Processing* group, you can alter the surface's relative height. Reduce, e.g., the value in the *Stretch height by factor* field. Set the relative height in such a way that the diamonds look as realistic as possible.

2. You can change the 3D-surface's texture and color. To do so, click this button on the *Surface View* tool window's toolbar.
   - The *Surface Color Settings* dialog box opens.

3. From the *Color mode* list, choose, e.g., the *Single color height-shaded* entry to have the surface displayed in one color. In the process, the height-shading takes care that the surface still has a three-dimensional appearance.
   Then select the *Single color selection > Arbitrary color* option. Select the color you want from the color palette.
   Watch how the display of the 3D-surface changes in the image window.
4. Close the *Surface Color Settings* dialog box.
5. Change the background color in the image window. Select the background color you want, in the *Colors* group, located in the *Surface View* tool window.

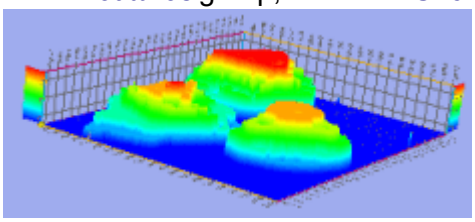


6. You can show and hide the coordinate system and also change the appearance of the coordinate system. To do so, click this button, located on the *Surface View* tool window's toolbar.

   - The coordinate system is always displayed behind the 3D-object. When you rotate 3D-objects, the position of the coordinate system automatically changes too.
   - A small yellow point indicates the origin of the coordinate system, independently of the current orientation of the coordinate system.

Displaying the color bar on the 3D-surface

1. Click this button on the *Surface View* tool window's toolbar.
   - The *Surface Color Settings* dialog box opens.
2. Select the *Lookup table* entry from the *Color mode* list to select a pseudo color scheme for the 3D-surface.
3. Select the *Color ramp* option in the *Lookup table selection* group.
4. In the *Features* group, select the *Show color bar* check box.



   - Every value on the height map is now allocated a color value.
   - The color bar tells you which colors represent which heights. The color bar is automatically positioned on opposite sides of the coordinate system.

8.6. Creating an image of the 3D-surface

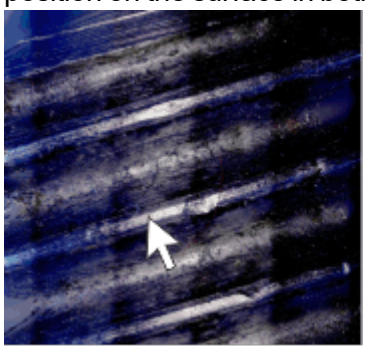
1. Create a 3D-surface and adjust its appearance until you're happy with it.
2. Right click on the image window, then use the *Create Image from View…* command from the context menu.
3. Select the settings you want.
4. Click the *Create* button to create, in the image window, an image with a similar appearance from the current reproduction of the 3D-surface. Use this command, if you require an image of the 3D-surface for a presentation or for documenting your work.
5. Activate the image window with the Surface View. Close the *Create Image From View* dialog box.

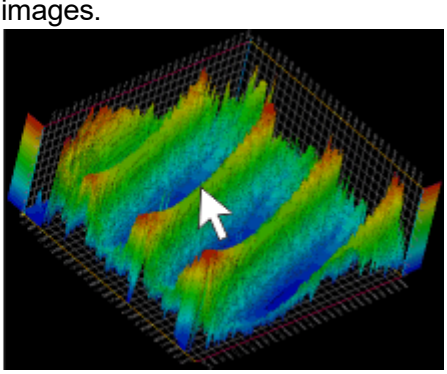
8.7. Creating and measuring height profiles

Example: You have created an EFI image and a height map of the surface of a screw. Measure the distance between the ridges of the thread at different positions on the screw.

Note: If you want to measure a height map that wasn't computed using the EFI algorithm, you can use the *3D Line* interactive measurement function. You can find step-by-step instructions further below.

1. Load the EFI image with its height map.
   - The illustration on the left shows the EFI image of the screw. On the right is the 3D-surface displayed with a pseudo color scheme. You can see that three threads are shown in the image. The white arrow indicates roughly the same position on the surface in both images.





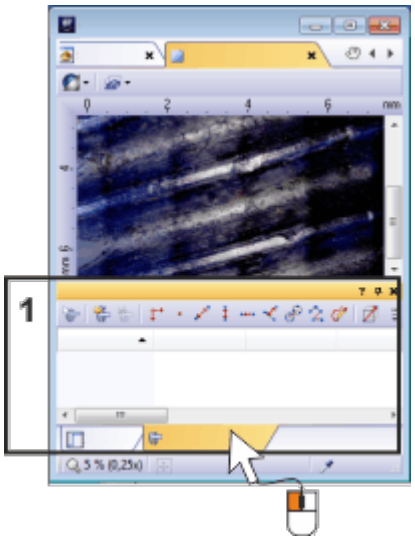
Setting up the user interface

2. If necessary, use the *View > Tool Windows > Measurement and ROI* command to have the *Measurement and ROI* tool window displayed.

3. In the *Measurement and ROI* tool window, click the *3D Profile Measurement* button.
   - The *3D Profile* tool window is appears. The tool window is empty.
   - By default, the *3D Profile* tool window is placed on top of the *Measurement and ROI* tool window.

- The *3D Profile Measurement* button in the *Measurement and ROI* tool window appears clicked, indicating that the *3D Profile* tool window is shown. You can recognize this status by the button's colored background.

4. Tool windows can only be moved in the expert mode. Therefore, switch to the expert mode.
   To do this, use the *Tools > Options* command. In the tree view, select the *Environment > General* entry. Select the *Expert mode* option in the *User interface* group.
   Close the dialog box with *OK*.
5. Arrange the *3D Profile* and *Measurement and ROI* tool windows side by side. You can do this by dragging the *Measurement and ROI* tool window to a different position. To do this, you have to click on the tab's header located below the tool windows.



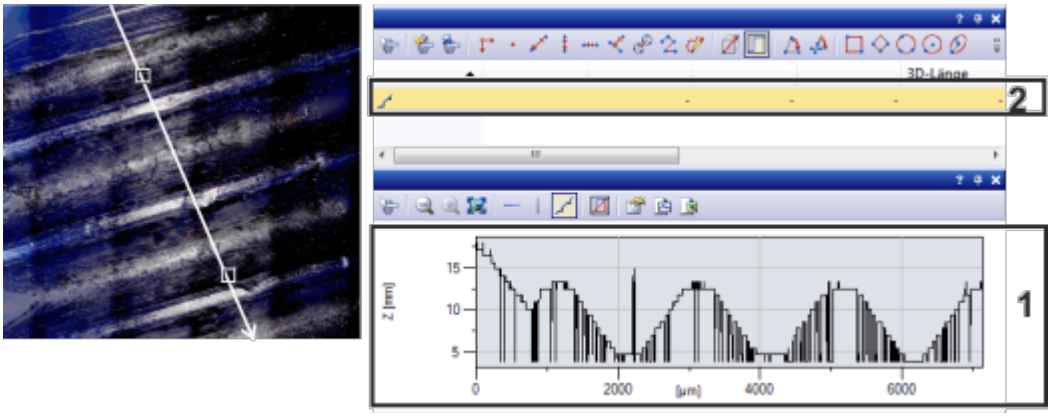
The *3D Profile* and *Measurement and ROI* tool windows are both positioned at the lower edge of the user interface (**1**). Grab one of the tool windows by its header and drag it to a different position, above the other tool window for example.

Creating a 3D profile

6. Click the *Arbitrary 3D Profile Line* button in the *3D Profile* tool window.
7. In the image window, specify the position of the 3D profile line with two clicks of the mouse. Define the 3D profile line in this image to run perpendicular to the threads.
   - You can see the 3D profile line in the image window. The 3D profile line has two control points that you can use to change the position of the 3D profile line, if required. The arrow indicates the orientation of the 3D profile's X-axis. The 3D profile's origin is at the end of the 3D profile line opposite to the direction of the arrow.
   - The 3D profile is now displayed in the *3D Profile* tool window. In this example, you can clearly see the three threads.

- In the *3D Profile* tool window, the *3D Profile Line Measurement* button becomes active-

- An *Arbitrary Profile Line* measurement object is automatically created in the *Measurement and ROI* tool window. The actual measurement results, however, are not yet displayed.

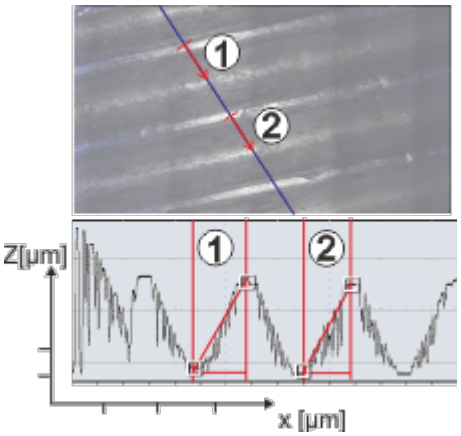


The 3D profile line is shown in white in the image on the left. The *3D Profile* tool window displays the corresponding 3D profile (**1**). In the *Measurement and ROI* tool window, a measurement object (**2**) has been created for the 3D line.

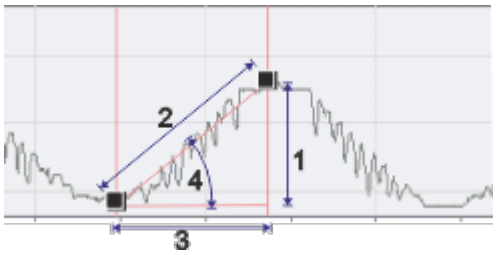
Measuring a 3D profile

8. Click the *3D Profile Line Measurement* button in the *3D Profile* tool window.
   - You are now in measurement mode.
   - The button becomes active, indicating that you are now in measurement mode.
9. Left click on two points on the 3D profile. The order of the clicks determines the orientation. In this example, click on one of the lowest points and the nearest highest point to measure both the depth and half of the distance between two ridges of the thread.
10. Repeat this measurement for all of the distances that you want to measurement on the 3D profile.
    - The measurements are now displayed in the 3D profile in the *3D Profile* tool window.
    - In the image window, the distances that were measured are displayed on the 3D profile line. Each measured distance has two control points that you can use to change its length, if required.
    - Measurement values for each measured distance are now displayed in the *Measurement and ROI* tool window. All of the measurement values belong to one measurement object.

11. In the *Measurement and ROI* tool window, click the *Select Measurements* button to display the measurement parameters you want.



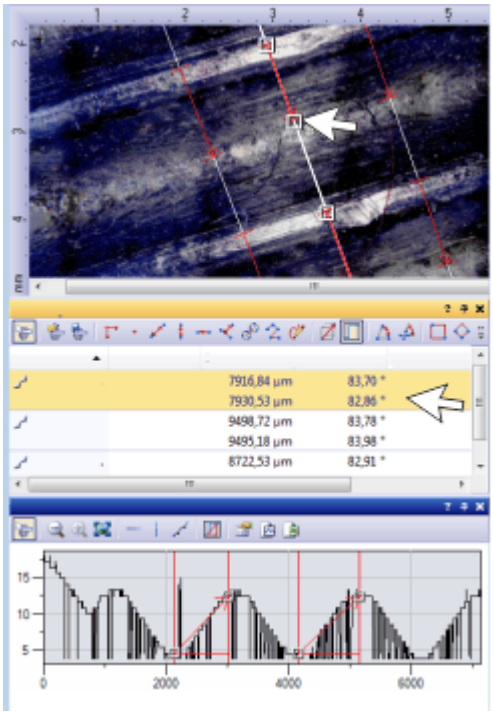
The image shows a height map on which a 3D profile line has been defined (**the blue line**). The chart in the *3D Profile* tool window (**below**) shows the 3D profile. Two measurements were performed on the 3D profile (**1**) and (**2**). The measurement is displayed in the image and in the tool window's chart.



The illustration shows a 3D profile on which two points have been defined. The measurement parameters that you can measure are shown: (**1**) *3D Intensity Projection*, (**2**) *3D Length*, (**3**) *3D Image Plane Projection*, (**4**) *3D Angle*.

Performing additional 3D measurements

12. If you want, define additional 3D profile lines on the image. You can measure several distances on each new 3D profile line.
    - In the *3D Profile* tool window, only the 3D profile and the measured values of the active 3D profile line are displayed.
    - The control points indicate which is the active 3D profile line. In the *Measurement and ROI* tool window, the measurement object belonging to the active 3D profile line is highlighted.
13. If you want to view a different 3D profile, click the *Select Measurement Objects* button. Then select the corresponding 3D profile line in the image window. Alternatively, you can also select the measurement object belonging to the 3D profile line that you want to view in the *Measurement and ROI* tool window
    - The selected 3D profile is displayed in the *3D Profile* tool window.



Three 3D profile lines have been defined on the height map. That's why three measurement objects are displayed in the *Measurement and ROI* tool window. Because two distances were measured on each 3D profile line, the measurement object contains two sets of measurement values.
The *3D Profile* tool window displays the active 3D profile (the middle line).

Displaying and saving measurement results

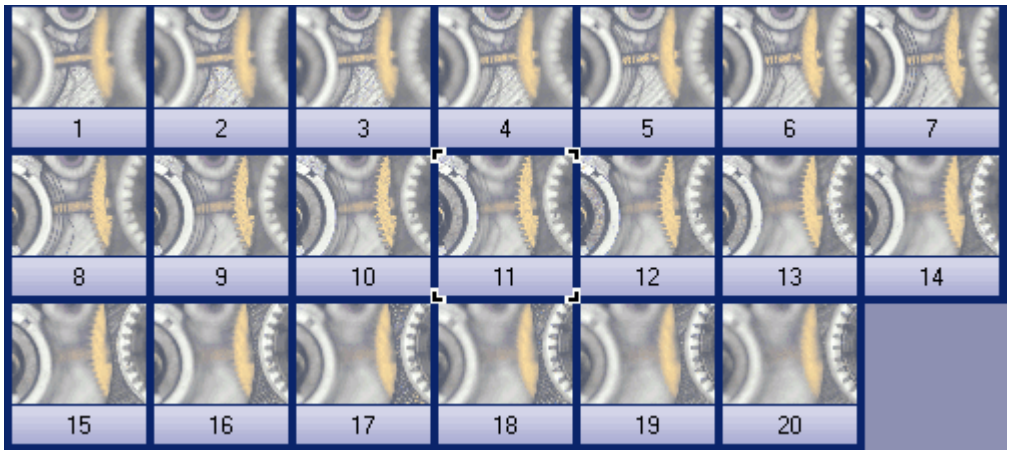
14. To export the measurement values, click the *Export to Excel* button in the *Measurement and ROI* tool window.
15. To export the 3D profile, click the *Export to Excel* button or the *Export to chart* button in the *3D Profile* tool window.
16. Save the image in the TIF or VSI file format. The measurements will then also be saved in the image file.

8.8. Measuring heights interactively

Example: To be able to make height measurements, you need to have an image whose intensity values have been calibrated. This could be an EFI image with a height map or a gray-value image that you have calibrated using the *Image > Calibrate Intensity* command.
Use the Clockwork.tif multi-dimensional image and use the EFI algorithm to calculate the height map. Measure the height difference between the brass-colored gear wheel in the middle image segment, and the silver-colored gear wheel on the right-hand side of the image.

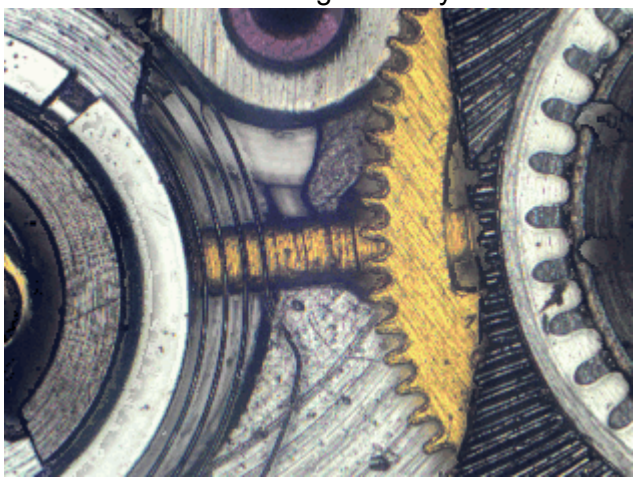
Note: Use the *3D Profile* tool window if you want to perform extensive 3D profile measurements on a height map. You can find step-by-step instructions above.

1. Load the Clockwork.tif image.
   - The Clockwork.tif image is a Z-stack. The works of a clock was analyzed under a reflected light microscope. In the process, images of the works were acquired at different focus positions.
     In the illustration, the Z-stack is displayed in the tile view. Note the brass gear wheel that is only sharply reproduced in the middle of the Z-stack.



Creating a height map

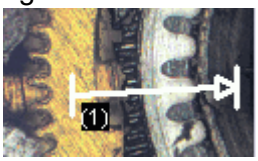
2. Use the *Process > Enhancements > EFI Processing* command to compute an EFI image with a height map.
   - You can see the EFI image with the clockwork's texture. The resulting image is a multi-layer image and is accompanied by this icon in the image window's title. The icon indicates that the individual image layers in the multi-layer image aren't of the same image type.
   - The height map is a layer of the EFI image. The texture image makes up the second layer. The height information is therefore also present in the EFI image. You can measure the height directly on the texture image.



Measuring height

3. If necessary, use the *View > Tool Windows > Measurement and ROI* command to have the *Measurement and ROI* tool window displayed.

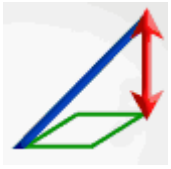
4. In the *Measurement and ROI* tool window, click the *3D Line* button.
5. Now, measure the height difference between two image objects. Click, for example, the brass-colored gear wheel, and the silver-colored gear wheel on the right-hand side of the image.



6. Click the *3D Line* button in the *Measurement and ROI* tool window again, then switch off the 3D measurement.
   - In the *Measurement and ROI* tool window, and in the image, the *3D Length* measurement parameter is output with the line's complete length. If the *3D Length* measurement parameter isn't shown, follow the step-by-step instruction and display the measurement parameter as well.
     The *3D Intensity Projection* measurement parameter measures the height difference between two points.

Displaying additional measurement parameters

7. In the *Measurement and ROI* tool window, click the *Select Measurements* button.
8. In the list of all of the available measurement parameters, take a look at the parameter of the *3D Line* object type. All of these measurement parameters apply to 3D measurements.
   - You can find a short description and an illustration of each measurement parameter in the *Select Measurements* dialog box.
9. Select the *3D Intensity Projection* measurement parameter.



10. Insert this measurement parameter into the list of the displayed measurements.
11. Close the dialog box with *OK*.
    - In the tool window, you'll now see the *3D Intensity Projection* measurement parameter. It tells you how far in height the two gear wheels are from each other.

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9. Materials Science Analysis

9.1. Tool Window - Materials Solutions

Use this tool window to measure an image, or several images at the same time, according to different material science analysis processes.

The *Materials Solutions* tool window works similarly to a software wizard. As soon as you've started an analysis process you'll be guided step by step through the measurement.

Note: Which of theses analysis processes are available to you, depends on the software license you've acquired. Maybe you will only see one or two analysis processes.

Overview of the supported analysis processes
(**1**) Chart Comparison
(**2**) Grains Intercept
(**3**) Grains Planimetric
(**4**) Layer Thickness
(**5**) Cast Iron
(**6**) Inclusions Worst Field
(**7**) Inclusion Content
(**8**) Throwing Power
(**9**) Porosity
(**10**) Phase Analysis
(**11**) Particle Distribution
(**12**) Automatic Measurement
(**13**) Coating Thickness
(**14**) Dendrite Arm Spacing

Starting an analysis process

You start an analysis process by clicking the corresponding button.

Note: A lot of your software's other functions aren't available while an analysis process is running. For example, you can't open the program options then.

Stopping an analysis process

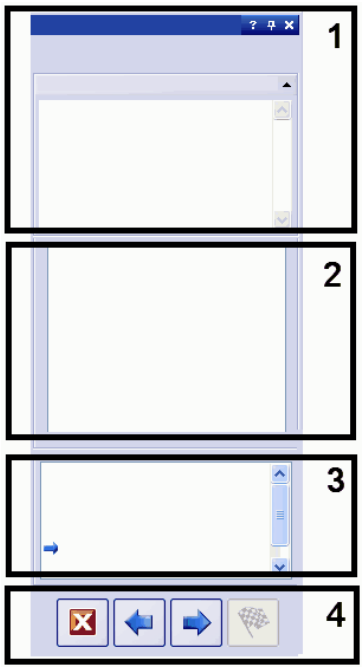
You can use the *Cancel* button at the bottom of the tool window to do this.

Alternatively, you can click the appropriate button in the small message box that is by default displayed at the top left edge of the monitor when an analysis process is in progress.

Note: If you are in a measurement mode, you must leave it before you can cancel the analysis process.

Independent of which analysis process has been currently selected, the tool window is always configured in the same way. It comprises static and dynamic areas.

Structure of the tool window



The static areas (**1**), (**3**) and (**4**) are located at the top and bottom edges of the tool window. The content of these areas is always largely similar.

The dynamic area (**2**) is located in the middle part of the tool window. Its appearance differs according to which step and which analysis process has been chosen.

(1) Name of the analysis and "Instructions" group

You'll find the name of the current acquisition process right at the top of the tool window. In the *Instructions* group, you will find an instruction of what to do in this step and, if available, additional information.

(2) Dynamic area
The contents of this area changes completely for each analysis process and for each step in the analysis. It is therefore described each time one of the different analysis process is presented.

(3) Current step in the analysis
Here, you can see at which step in the analysis you are at this moment. The current step is indicated by a blue arrow.

(4) Buttons
Here, you find the buttons you use to proceed to the next step in the analysis, or to return to the previous step. You can also cancel an analysis here. Depending on the current step in the analysis, not all of the buttons are active.

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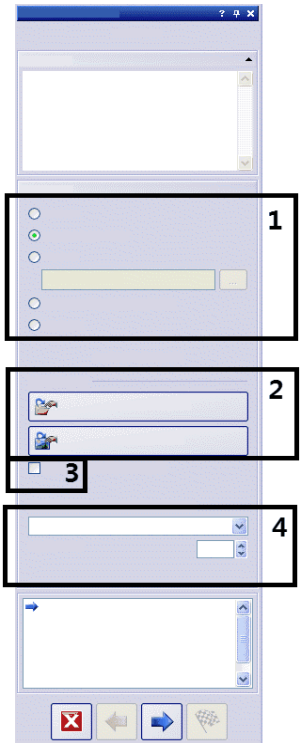
9.1.1. Materials Solutions - Image source

The *Materials Solutions* tool window leads you step-by-step through a materials science measurement. In the *Image source* step, the following options are available:



(1) "Image source" group
In this group, you select the image that you want to analyze. You can also analyze several images at the same time. The following options are available:

- *Live image* option: With this option, the additional step *Image acquisition* will be shown. In this step, a live image will be acquired, which will then be analyzed in the following steps. When the *Image results* step has been completed, a new

image of the live image will be automatically acquired, then analyzed. This enables you to analyze as many images as you would like during the same measurement. You can then either save the analyzed images or reject them.
- *Selected images* option: Loaded images that are currently selected in the *Gallery* tool window. Loaded images that are not selected in the *Gallery* tool window, will be ignored for the analysis.
- *Folder* option: All of the images in a specific directory. You can choose the directory as you wish.
- *Selected database images* option: All of the images you have currently selected in your software's database.
- *Stage Path* option: All images which you would like to acquire with the saved stage path. This option is only visible if your microscope stage has a motorized XY drive.

Not all materials science analysis processes support the use of stage paths. This is why the *Stage Path* option is only available for these analysis processes: *Grains Intercept*, *Grains Planimetric*, *Inclusions Worst Field*, *Porosity*, *Phase Analysis*, *Particle Distribution*.

(2) Buttons to load saved settings

Here, you can load the settings that you want to use for the analysis. Click on the *Load from file...* button, if you want to use settings that have been saved. For example, you can in this way load the comments from a sample that has already been analyzed, and adapt them for the current sample. As well as that, with some materials science analysis processes, the slide controls that are available in the *Settings* step will also be set to the saved position.

Click on the *Get from image...* button, if you want to use the settings used for an already analyzed image for the current analysis. To make this possible, the image that has already been analyzed must be opened in your software.

(3) "Skip 'Sample Information'" check box

Select the *Skip 'Sample information'* check box, to skip the *Sample information* step. As soon as you click the *Next* button, you'll go straight to the *Settings* step. This makes sense if you analyze numerous images of the same sample, and you only want to enter the information on the sample with the first image.

Note: When you analyze images of numerous samples, make sure the *Skip 'Sample information'* check box is not selected, because otherwise you won't see the *New Sample* button.

(4) "Check settings and results" list and "Image interval" field

This list is only of significance if you are analyzing several images. If you are only analyzing one image, leave the preset *All images* entry as it is.

If you select several images, you can choose how frequently you would like to check the settings with which the images are analyzed. If you would like to analyze a lot of images with the same settings, you can automate the analysis.

The following entries are available in the *Check settings and results* list:

- *All images*: Select this entry if you want to check the settings for each image. This option is preset. The *Settings* step is then displayed for each new image. This makes sense, for example, if the images that are to be analyzed are very different in their image qualities.
- *Never*: Select this entry, if the settings are never to be checked. With this option, the system will jump over some steps in the analysis and the *Image results* step will be displayed. In general, this setting is only sensible if you have saved the settings to be used as a parameter set and you load them before starting the analysis.
- *First image*: Choose this entry, if the settings are only to be checked for the first image and are then to be used for all other images (even from other samples).
- *First image per sample*: Choose this entry, if you have several samples (with several images per sample) and the settings are to be checked for the first image of each sample.
- *First image per scan area*: You'll only see this entry when you have chosen the *Stage Path* option. Select this entry, if the settings are only to be checked for the first image in each scan area and if the same scan area is to be used for other images.
- *Image interval*: Select this entry, if you would like to analyze several images and would like to check the settings at regular intervals. If this entry is selected, the *Image interval* field will become active. In this field you could, for instance, enter 10 to check the settings for every tenth image.

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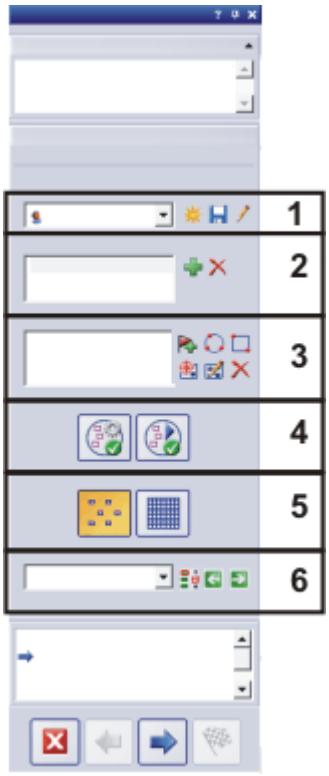
9.1.2. Materials Solutions - Stage path settings

The *Materials Solutions* tool window leads you step-by-step through a materials science inspection. In the *Stage path settings* step, you define a stage path on your sample.

What is a stage path?

For most materials science analysis processes, you can define several stage positions on each sample and can save them as a stage path. Here, stage positions can either be entire scan areas or individual XY positions. The stage path contains the number of samples to be analyzed, and information about which scan areas and/or XY positions are defined on each sample. For the materials science inspection, the stage will move to the defined positions one after the other. At each XY position, an image will be automatically acquired. For a scan area, several images will automatically be acquired and will be assembled into a single image. Each image acquired will be analyzed with the selected materials science analysis process.

The following stage path settings are available.



(**1**) Choosing a stage path
(**2**) Defining samples
(**3**) Defining scan areas and/or XY-positions
(**4**) Aligning the sample
(**5**) Selecting inspection mode
(**6**) Selecting focus mode

(1) Choosing a stage path

To be able to take a materials science inspection at different positions on one or more samples, you have to define a stage path. You can use a saved stage path or you can define a new one.

Defining a new stage path

1. Click the *Creates a new stage path* button to define a new stage path.
   - If there is more than one sample on a slide, you can define the inspection for more than one sample. You can enter different information for each sample. After the inspection is finished, you get the results for each sample separately.
   - A stage path is always linked to at least one sample. With the new stage path, a new entry in the *Samples* list will always also be produced. If you click on the *Creates a new stage path* button, the *Sample information* dialog box will be opened first.
2. In the *Sample information* dialog box, you enter information about the sample. By default, the *Reference*, *Group* and *Comment* fields are available to enter details for the sample.
   - If you have changed the default settings, the *Reference* and *Group* fields can also have another name. You can change the default settings in the *Tools > Options > Materials Solutions <Name of the analysis process>* dialog box.
   - You'll see this information when you create a workbook or a report at the end of the analysis.
3. Close the *Sample information* dialog box with *OK* to create the new stage path.
   - The new stage path is added to the *Stage path* list. Once created, the stage path is empty and still has to be completely defined.
   - Now, define scan areas and/or XY-positions on your sample.

Note: There can only ever be one stage path active. If you define a new stage path, you will automatically remove all of the currently defined samples and stage positions. You should thus save a stage path which you would like to use again before defining a new stage path.

Saving a stage path

Click the *Saves the current stage path* button, if you would like to use a stage path for several inspections. The following information will be saved:

- The number of samples
- The data entered about the sample
- All of the defined stage positions, i.e. the position markers for individual XY-positions and all defined scan areas
- Inspection mode and Focus mode

Using an existing stage path

In the *Stage path* list you will find all of the stage paths that already exist.

1. Select a stage path from the list to load the sample information and stage positions defined in the stage path.
   - If one of the positions in the stage path is outside of the currently defined stage area, you will be presented with an error message. In this case you will not be able to load the stage path.

Note: The *Stage path* list contains the stage paths saved by you as well as those saved by any other user with *Public* access rights. You will not see stage paths saved by other users with *Private* access rights.

You can edit the stage path and thus adapt it to the current sample.

1. Double click on an entry in the *Samples* list to open the *Sample information* dialog box. Here, you can change all of the loaded sample information.
2. Define new stage positions for individual samples, or delete individual stage positions from the *Scan Areas* list.
3.  Click this button next to the *Stage path* list to save the altered stage path under a new name or to overwrite the existing stage path.

Managing existing stage paths

Click this button next to the *Stage path* list to open the *Manage Stage Paths* dialog box. Here, you can copy an existing stage path, rename, or delete it.

Note: Public stage paths can be edited, and even deleted by every user of your software.

(2) Defining samples

Prerequisite: The *Samples* list isn't available for all materials science analysis processes.

If there is more than one sample on a slide, you can define the inspection for more than one sample. You can enter different information for each sample. After the inspection is finished, you get the results for each sample separately. The results also contain the information that was entered about the sample.

The *Samples* field lists all samples which are defined in the current stage path. After the name of the sample, in brackets you will find the number of stage positions currently defined for this sample.

Adding and deleting samples

Click this button to add a new sample to the current stage path. The *Sample information* dialog box automatically opens. Here, you can enter information about the sample.

Select one of the samples listed. Click this button to delete the selected sample. All scan areas and XY-positions which were defined for this sample will also be deleted.

Viewing and changing the sample data

Double click on a sample to open the *Sample information* dialog box with the current sample information and, if necessary, to edit it.

(3) Defining scan areas and/or XY-positions

Use the *Scan Areas* group to define stage positions on the selected sample, to edit existing stage positions, and to move the XY-microscope stage.

The following buttons are available:

	Adding XY-positions
	Adding scan areas
	Moving the XY stage to the selected stage position
	Editing stage positions
	Deleting stage positions

Adding XY-positions

You can mark several positions on your samples. At each XY-position, an image will be acquired and will be analyzed with the selected materials science procedure.

1. Select a sample from the *Samples* list.
2. Move the XY stage to a position on the sample that you would like to inspect using the current analysis process.
   - To navigate the XY stage, you can for example use the *Microscope Control* or the *Stage Navigator* tool window. Both tool windows will automatically be displayed in the *Stage path settings* step in the analysis.
   - In the *Stage path settings* step, your system will automatically switch to live mode, so that you can examine the live image to check whether the position on the sample is suitable for analysis.
3.  Click this button , located next to the *Scan Areas* list.
   - The current position of the XY stage will now be saved and assigned to the selected sample.
   - The defined XY-position will be marked by a position marker in the *Stage Navigator* tool window.
4. Move the XY stage to the next position on the sample, where you would like to take a measurement.
   - The stage will later be moved to the positions specified and in the sequence specified in the *Scan Areas* list. Take this into account when defining the stage positions.
5.  Click the button again.
6. Repeat the two last steps until you have defined all of the positions on the sample.

Adding scan areas

Instead of individual positions, you can also define a whole area on your sample for materials science analysis. This area can be rectangular or circular.

1. Click this button to define a rectangular scan area. To do so, you move on the sample with the motorized XY stage to the rectangular area's top left-hand corner, then to its bottom right-hand corner.
2. Click this button to define a circular scan area by moving the XY stage. You define the scan area by moving your XY stage to three points, which are on the edge of the round scan area. Your software will help here with corresponding message boxes.
   - Your software will automatically calculate how many individual images are required to completely acquire and analyze the defined sample area. The number of the individual images depends on the current magnification. If you change the magnification, the number of images will be recalculated. You do not have to redefine the scan area.
   - The scan area is displayed in the *Stage Navigator* tool window. In the stage navigator's image display area, you can directly see how many individual images are needed for the defined area at the current objective magnification. If you change the magnification, the display will be updated.
   - The stage will later be moved to the positions specified and in the sequence specified in the *Scan Areas* list. Take this into account when defining the stage positions.
3. In the *Inspection Mode* group, select how the scan areas are to be analyzed. You can find more information further below.

Editing stage positions

You can redefine scan areas and XY-positions which have already been defined. In contrast to deleting a stage position and then adding a new one, the name of the stage position will not be changed.
You can for instance use this option to adjust an existing stage path for a different sample.

1. From the *Scan Areas* list, select one of the stage positions shown e.g. *Rectangle 2*.
2. Move the XY stage to the position on the sample, to which you would like to move the selected stage position.
3. Click this button to redefine the selected *Rectangle 2* stage position. For a scan area, you will also have to redefine the size in this case.
   - The name of the new stage position will remain unchanged *Rectangle 2*.

(4) Aligning the sample

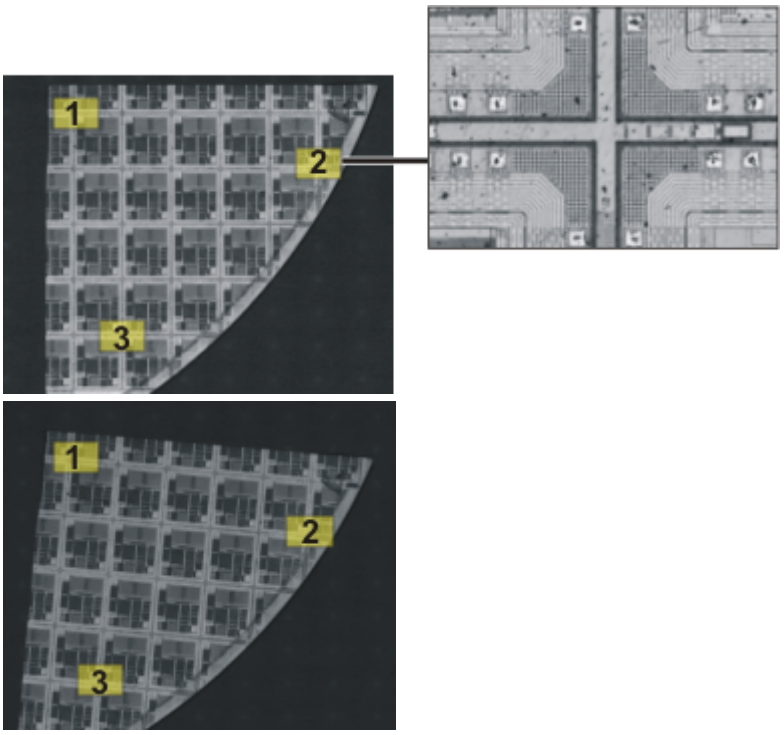
With some materials science analysis processes, the inspections have to be carried out at certain positions on the sample. In this case, all samples on the stage have to be positioned the same way so that the stage path can go to the correct positions on the

sample. Use the functions in the *Sample alignment* group to compensate for differing alignments of the samples on the stage.

Example: You can use the *Automatic Measurement* solution to measure test structures on a wafer. On the wafer, define three positions that are located on every wafer to be measured. If you now put a new wafer to be measured on the stage, at the beginning of the measurement, move the stage to the three reference positions. This allows your software to recalculate the stage path.

Defining the reference position

1. Click this button to start the definition of the reference position.
   - A yellow triangle on the button indicates that no reference positions have been defined yet for this stage path.
   - The *Acquire Reference Images for Sample Alignment* dialog box opens. It guides you step-by-step through the definition of the reference position.
2. Move the stage to reference position 1 and focus.
   In order for the sample alignment to work well, the reference positions should meet the following conditions.
   - The reference positions should be unambiguous.
   - The reference positions should be as easy to find on the sample as possible.
   - The reference positions should be as far away from each other as possible.
3. Click the *Next >* button to move to the next reference position.
   - Your software now acquires an image at the first reference position. This image is saved as a reference image together with the stage path.
4. Define reference positions 2 and 3.
5. Click the *Finish* button to finalize the definition of the reference positions.
   - The button in the *Sample alignment* group changes its appearance. A green check on the button shows that reference positions have been defined for this stage path.
6. Click this button next to the *Stage path* list to save the stage path along with the reference positions and the reference images.



On the **left** is an overview of a whole sample. Define three reference positions (**1**-**3**) on the sample. A reference image is acquired at each reference position. The illustration shows the reference image at position 2. The reference image is displayed in the live-image during the alignment of the sample to assist you with positioning.
On the **right** is a similar sample that is positioned differently on the stage. The same stage path can be used on both samples with the aid of the reference positions.

Aligning a sample

1. Begin a materials science analysis process that contains a stage path. Reference positions for the stage path are already defined.
   - Your software automatically starts a wizard in the *Define Stage Path* step in the analysis. You can cancel the wizard if you don't want to align the sample yet.
2. Click the *Yes* button in the message box or click the *Align images for sample alignment* button, shown above, to align the current sample with the aid of saved reference images and reference positions.
   - The *Align images for sample alignment* button is only available if reference positions have been defined for the selected stage path.
   - A yellow triangle on the button indicates that the current sample isn't aligned yet.
   - The *Align images for sample alignment* dialog box opens.
3. Decide how the reference image should be displayed. You have the following options in the *Align images for sample alignment* dialog box:
   - Select the *Show reference image as thumbnail* option. Now the reference image for the current position will be displayed as a small image on the top left of the live-image.

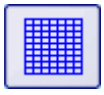
- Select the *Show reference image in overlay* option. Now the reference image is superimposed in full size on the live-image. Use the *Display opacity* slide control to change the transparency of the reference image. The smaller the value, the more transparent the reference image is. Select the value 0 if you don't want to see the reference image for orientation.

4. Move the stage to the required reference positions one after another. Orientate yourself using the displayed reference image.
5. When you've moved to the third reference position, click the *Finish* button.
   - Your software now compares the positions saved in the stage path with the current positions you moved the stage path to and positions the stage path accordingly.
   - The button in the *Sample alignment* group changes its appearance. A green check on the button shows that the sample is aligned.

(5) Selecting inspection mode

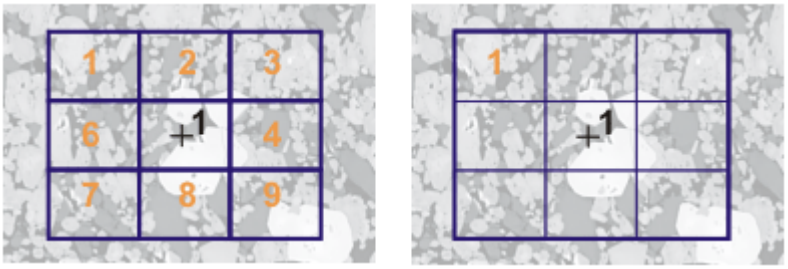
Prerequisite: The options in the *Inspection Mode* group are only relevant for scan areas, not for XY positions.

Select the *Single frame inspection* option. Now, all of the images from a scan area will be individually analyzed with the selected materials science method.



Select the *MIA image inspection* option. Now, all of the images acquired from a scan area will be assembled directly as they are acquired, like a puzzle, into a stitched image, to be analyzed with the selected materials science method.

With MIA image inspections, the individual images are acquired with a certain overlap area. Your software will then use pattern recognition to look for two images with the same image information, in the overlap area.
You determine the size of the overlap area in the *Acquisition Settings > Acquisition > Automatic MIA* dialog box. You can open this dialog box, for example, via the *Process Manager* tool window. In the tool window's toolbar, click the *Acquisition Settings* button. Select the *Acquisition > Automatic MIA* option in the tree view.



The illustration shows a sample on which one scan area (**1**) is defined. 9 individual images are needed to fully acquire the scan area.
On the left, the *Single frame inspection* option is selected. If, for example, you do a phase analysis and output a workbook as a result, you will now find the results for 9

images on the sample's worksheet.
On the right, the *MIA image inspection* option is selected. On the sample's worksheet you will now find only one result for the same scan area, as the individual images will be assembled to a single image before the analysis.

(6) Selecting focus mode

When you use a stage path, the various positions that the stage moves to during the inspection may be at some distance from each other. In this case it will generally be necessary to refocus several times during the inspection, so that each individual image is ideally focused and can be analyzed successfully.

From the *Focus Mode* list, select one of the following options:

- Not refocusing on samples
- Manually refocusing on samples
- Using a focus map
- Using the software autofocus

The selected focus mode applies for the entire stage path, which means for all samples and all stage positions.

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9.2. Chart Comparison

9.2.1. What are chart comparisons?

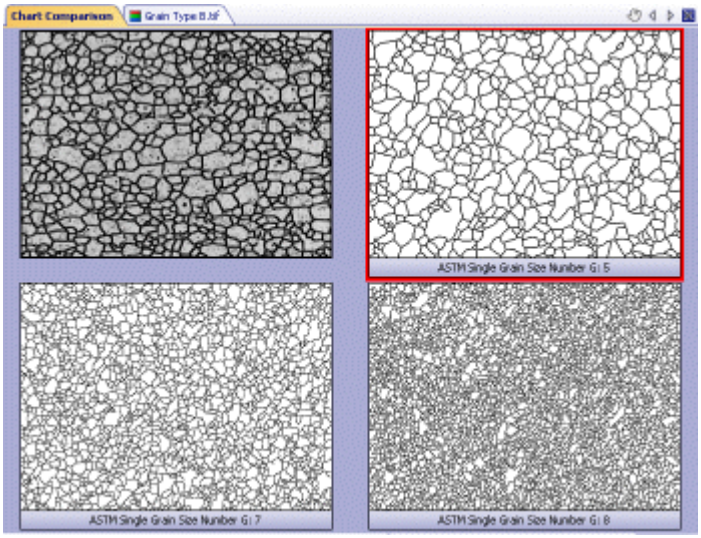
In metallography, chart comparisons are used as a means of quality control. They make it possible to compare an image with numerous reference images. The reference images are a part of the industry standards (which have to be purchased) by which the chart comparisons are carried out.

Example 1:
During a qualitative grain size analysis, you determine the grain size of metallic samples. You compare the images that are to be checked with the reference images. You assign the reference image with grains of the same size to each of the images that is to be checked.

Example 2:
During a quality control, you check various components to see if they are free of defects. To do so, you compare the components with images of various components that are either defective or free of defects. You assign the appropriate reference image to the object that is to be checked.

General procedure for a chart comparison

The image that is to be checked, and all or some of the reference images, are displayed simultaneously on the screen. Your software makes sure that all of the images are always shown on the same scale. By making a visual comparison, the user finds out which of the reference images is the most similar to the image that is to be checked. Saved along with every reference image is the value that it was assigned by the industry standard. By the selection of a reference image, the image that is to be checked is assigned this value, too.



The above image shows the document group during a chart comparison. The image that is to be checked is located at the top left, the reference images are arranged either next to it or beneath it. The selected reference image is framed in red.

Results

The results of a chart comparison can be output in a workbook. As well as that, when you carry out chart comparisons on live-images, you can immediately reject the samples that don't meet the required values.

If the Chart Comparison analysis process isn't displayed in the Materials Solutions tool window

To be able to carry out chart comparisons with the image analysis program, the charts from at least one industry standard have to be installed. Only then will the *Chart Comparison* analysis process be displayed in the *Materials Solutions* tool window. The industry standards that are to be used for the chart comparison have to be purchased. They can be purchased through Olympus Soft Imaging Solutions. You will receive a DVD for each industry standard that you purchase. Use the Quick Setup Guide which accompanies the DVD to install the industry standard's charts.

Note: Even if you haven't purchased an industry standard yet, you can still view the *Chart Comparison* analysis process. To do this, install a demo plate. Using this, you can get an impression of how this analysis process works. Real analyses (complying with industry standards) are, however, not possible using these demo plates.

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9.2.2. Performing a chart comparison

Preconditions

The *Chart Comparison* analysis process is only displayed in the *Materials Solutions* tool window when you have purchased at least one industry standard and have installed its' charts.

Even if you haven't purchased an industry standard yet, you can still view the *Chart Comparison* analysis process. To do this, install a demo plate. You can carry these step-by-step instructions out with the *Demo single grain size* demo plate.

Note: Real analyses (complying with industry standards) are, however, not possible using these demo plates.

Example image FerriteGrains.tif
During the installation of your software some sample images have been installed, too. You can follow these step-by-step instructions when you use the example image FerriteGrains.tif. Open this image and make sure that it has been selected in the document group.

Image source step

1. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

2. Click the *Chart Comparison* button.
   - As soon as you've started this analysis process you'll be guided step by step through the measurement. A lot of your software's other functions will not be

available while an analysis process is running.

- The *Materials Solutions* tool window displays the *Image Source* step.

3. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
4. Select the *Skip 'Sample information'* check box if you don't want to add any details about the sample or about an image of the sample. If you want to add details, make sure the check box is not selected.

Note: If you want to analyze images from more than one sample in the same analysis process, the *Skip 'Sample information'* check box must be cleared. Only then will the *New Sample* button be displayed. With this button, you can specify when an image to be analyzed belongs to a new sample.

5. Select the *All images* entry in the *Check settings and results* list.
   - If you would like to analyze your own images later on, you can also select another entry from this list, e.g. if you would no longer like to check the settings for every image.
6. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Sample information step

Note: You will only see this step in the analysis if, in the previous step, the *Skip 'Sample information'* check box wasn't selected.

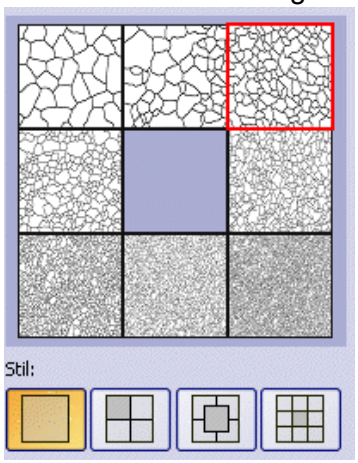
1. Enter information on your sample. By default, these fields are called *Reference* and *Group*.
2. If you want to, enter a comment about the sample. This comment is valid for all of the images of this sample.
3. If you want to, enter a comment about the current image, too.
4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Settings step

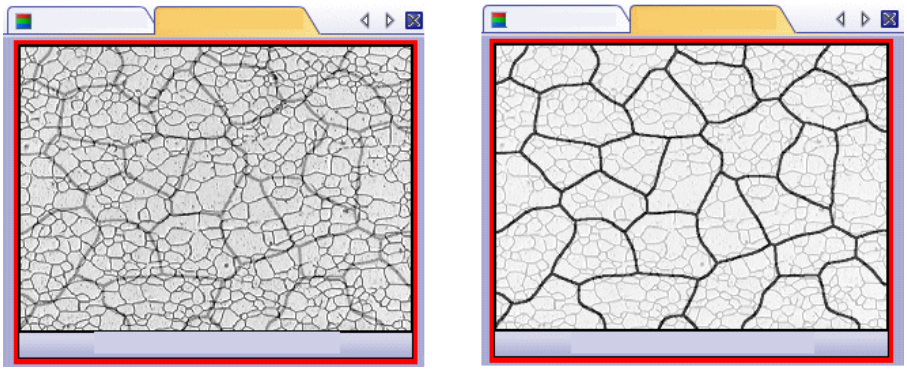
1. Select the chart by which you want to analyze the image. If you've installed a demo plate, select that.
   - For the FerriteGrains.tif image, you can select the *Single grain size* entry in these step-by-step instructions to specify the grain size. You'll only see this entry if you've chosen the *Demo single grain size* demo plate.
2. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.
   - In the document group, the new *Chart Comparison* document will be displayed.

Comparison step

1. In the *Style* group, choose how the images for the chart comparison are to be arranged in the document group. Choose an arrangement in which the FerriteGrains.tif image and the selected reference image are superimposed. To do so, click this button.
   - In the document group, the *Chart Comparison* document will now be displayed. It contains exactly one image.
   - In the *Overview* field, you see the arrangement that has been chosen. The selected reference image is framed in red.



2. Compare the structures of the current image with those of the reference image. Move the slide control below the *Style* field towards the *Opaque* position, if the image that is to be checked is to superimpose the reference image. Alternatively, move the slide control towards the *Transparent* position, if the image that is to be checked is to be superimposed by the reference image.



The illustration on the left shows the image that is to be checked. Because the slide control is located in near the *Opaque* position, the reference image's structures can only be faintly recognized. For the illustration on the right, the slide control has been moved towards the *Transparent* position. Now, the reference image can be clearly recognized, and the image that is to be checked can be only faintly recognized.

3. If you want to choose another reference image, in the *Comparison* group, click that image with your left mouse button.
4. When the reference image that is the most similar to the image that is to be checked, has been chosen: Click the *Accept* button.

- The chosen image's data will be accepted in the *Results* field.
- It's possible to accept several reference images, for example, with samples that have very different structures.

5. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Note: When you carry out analyses on the live-image: Click the *Get Results* button. You will then see the *Results* step. Otherwise, when you've finished analyzing one live-image, the next live-image will always then automatically be offered for analysis.

Results step

1. Select the *Generate workbook* check box to have a document of the *Workbook* type automatically created at the end of the analysis.
2. Click the *Finish* button.
   - The *Materials Solutions* tool window switches back to the start position. You can now use all of your software's functions again.

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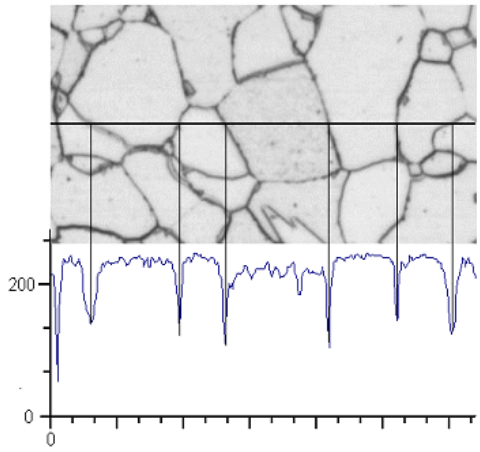
9.3. Intercept Analysis

9.3.1. What is an intercept analysis?

The intercept analysis is used to measure grain sizes and to document them. It is often used in material analyses, for example, when the quality of steel or other metals is being tested.

When an intercept analysis is made, measuring lines are placed in an image. Along these measuring lines, your software searches for abrupt deviations in the pixels' intensity (gray value). An intensity deviation occurs, for example, if dark pixels are present in an image made up of mainly light pixels. When an intensity deviation exceeds the parameters that have been set, an intercept point will be plotted at this position on the measuring line.

The intercept points are counted. The distance between two intercept points is also measured. From this measurement, the mean intercept length is calculated.



Description of the above illustration
The intensity profile is determined along the horizontal measuring line. Whenever the measuring line crosses a grain boundary, this leads to a distinctive minimum in the intensity profile. When an intercept analysis is made, these minima in the profile are used to determine the intercept points. In the illustration shown, the grain boundaries are dark, the process can, however, also be used on images with light grain boundaries. The analysis of cascaded grain boundaries (with multi-phase materials) is also possible.

Results of an intercept analysis
An intercept analysis provides the so-called G-value, which is defined as a characteristic grain size in the corresponding industry standards. G is calculated from the number of intercept points and the mean intercept length. The grain sizes are measured in accordance with the industry standards:

- ASTM E 112-13
- GB/T 6394-2002
- GOST 5639-82
- EN ISO 643:2012
- DIN 50601:1985
- JIS G 0551:2013
- JIS G 0552:1998
- ASTM E1382-97 (2015)

The results of an analysis can be displayed in a workbook. Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.

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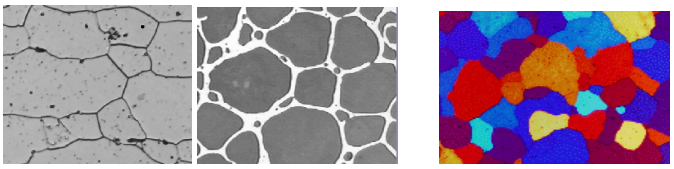
9.3.2. Settings

In this step, you make important settings for the analysis. You have the following options:

(1) Buttons for selecting the grain boundary type

Here, you specify which criteria are used to detect the grain boundaries. Depending on the image that is to be analyzed, the grain boundary type can be dark (left illustration) or light (middle illustration). Where images that don't have any intensity

deviations, but only show different gray values, are concerned, select the *Step* setting (right illustration).



(2) Pattern of test lines

The line pattern determines along which lines the intercept points are looked for. At every position along the line, intensity deviations will be searched for in the intensity profile. As soon as an intensity deviation fulfills the definition criteria set, it will be displayed as an intercept point in the image. Which line pattern is suitable for a specific task, depends on the type of structures that are to be measured, and their position in the image.

The following line patterns are available:

Circles

Three circles are placed in the center of the image. The size of the measurement pattern corresponds to the diameter of the largest circle. This line pattern is appropriate for images with structures distributed equally throughout the image or structures which progress from the middle of the image to the edges.

Cross

The cross consists of two diagonally crossed lines, as well as a line each below and to the left of this cross. The size of the measurement pattern corresponds to the length of the horizontal line below the cross.

Cross and Circles

The *Cross and Circles* line pattern combines the two line patterns *Cross* and *Circles*.

Vertical Lines

With this line pattern, vertical lines are distributed evenly across the measurement pattern.

Horizontal Lines

With this line pattern, horizontal lines are distributed evenly across the measurement pattern.

Horizontal and vertical lines

With this line pattern, horizontal and vertical lines are distributed evenly across the measurement pattern, forming a grid.

(3) Slide controls for changing the results displayed

You can change the position of the slide controls however you want to in this step. This has an effect on the number of intercept points that will be found. Therefore you should keep an eye on the display in the image.

Grain boundary width
Here, you set the necessary width for the detection of a grain boundary. When a small grain boundary width is set, your software finds considerably more intercept points than with a wider grain boundary.

Noise reduction
Use this slide control to apply a smoothing filter to the image. The smoothing filter reduces the image noise. You should therefore apply a smoothing filter to images that are very noisy before the intercept analysis is made. Move the slide control from the left to the right, to increase the strength of the smoothing filter in small steps. This will lead to a reduction of the detected intercept points.

(4) Number of test lines

These fields are only active if you selected a pattern of test lines that contains horizontal or vertical lines. In this case, you specify here the number of lines to be used for the intercept analysis.

(5) Industry standard used

In the *Standard* field, select the industry standard that is to be used for the measurement.

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9.3.3. Performing an intercept analysis

Step - Image source

1. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.
2. Click the *Grains Intercept* button.
3. In the *Image source* group, choose the image or the images that you want to analyze. When you do this, pay attention to the information as to how many images have been selected This information is shown in bold font at the bottom of the group.
4. Decide whether you want to load settings that you have saved while you were analyzing another image. Then you can, if necessary, adapt these settings and apply them to this image. Click the *Load from file...* button to load the settings that have been saved.
5. Decide whether or not you want to add data about the sample or about individual images while the analysis process is in progress. If you don't want to do so, select the *Skip 'Sample information'* check box.
   Should you want to add data, (e.g., because you are analyzing images of several samples in the same analysis), leave the check box deselected.
6. Select the *All images* entry in the *Check settings and results* list.
   - If you analyze your own images later on, you can also select another entry from

this list, for example, if you don't want to check the settings for every image anymore.

7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.
   - Should you be analyzing the live-image and a database is open, you'll be asked whether you want to save the acquired individual image in the database.

Step - Sample information

Note: You will only see this step in the analysis if, in the previous step, the *Skip 'Sample information'* check box wasn't selected.

1. Enter information on your sample. By default, these fields are called *Reference* and *Group*.
   - If you have changed the default settings, these fields can also have another name.
2. If you want to, enter a comment about the sample. This comment is valid for all of the images of this sample.
3. If you want to, enter a comment about the current image, too.
4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Settings

1. Select a suitable grain boundary type.
2. Choose a pattern of test lines that is appropriate for the structures in the image that is to be analyzed. You can choose between various patterns.
   - The pattern of test lines determines along which lines intercept points in the image are looked for.
3. Take a look at the intercept points that have been found in the image. If necessary, change the settings to optimize the results shown.
4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Image results

1. Check the results shown. You can see the results of the current image, and the overall results of all of the images that have already been analyzed for this sample.
2. Should you not be satisfied with the results for the current image: Click the *Back* button to switch back to the *Settings* step. Then you can try to improve the results for this image by choosing another line type or by moving the slide controls to another position.
3. Should you want to correct the intercept points that have been automatically found, click the *Add Intercepts...* or *Delete Intercepts...* buttons. This will enable you to add intercept points manually, or to delete superfluous intercept points.

4. When you analyze images that you selected before the analysis began: Click the *Next* button.
   - Should you analyze images from the database, you will then be asked whether you want to save the changed images, or not. You can either insert the analyzed images as new images into the database, or overwrite the existing database images with them. As well as that, you can either save the images in the file system or reject them.
   - The *Materials Solutions* tool window will display the next step.
   - Only when you carry out an analysis on the live-image, or you want to leave out the analysis of all of the remaining images: Click the *Get Results* button instead of the *Next* button. You will then see the *Results* step. Otherwise, when you've finished analyzing one live-image, the next live-image will always then automatically be offered for analysis.

Step - Results

1. Check the results shown. You can see the overall results for all of the images that have already been analyzed for this sample.
2. Select the *Generate report* check box and then select either the *Word* option or the *Excel* option to automatically create a report in the corresponding application program when the analysis finishes.
   - The additional step *Reporting* will be added to the current analysis. In the lower part of the dialog box, the *Finish* button will change into the *Next* button.
3. Select the *Generate workbook* check box to have a document of the "Workbook" type automatically created at the end of the analysis.
4. If you want to save the current settings to a file, click the *Save settings...* button. Then assign a descriptive name in the next dialog box.
   - You can load these settings (parameters) when you analyze further images. To do so you must load the image to be analyzed and, in the *Image source* step, click the *Load from file...* button. The sample and image comments, the line pattern used, and the position of the slide controls in the *Settings* step will be saved.
5. Click the *Next* button.
   - This button is only active if you have selected the *Generate report* check box as described above.

Step - Reporting

1. Select the *Default* option to use the template that has been defined as the default template. If you would like to select another template, select the *User defined* option. Click the button with the three points and select the new template in the *Open* dialog box.
2. When you want to create an MS-Word report: In the *Content* group, select the check box for the pages the report should contain.
   - Select the *Summary page* check box, if the first page of the report is to contain a summary of all of the results of the current analysis. The creation of a

summary page can, e.g., be useful, when you have analyzed a large number of images of a variety of different samples.

- Select the *One page per sample* check box, if the report should contain one page for every sample. This page displays the overall results for all of the images belonging to that sample. Using this setting is a good idea, for example, when you have analyzed images of different samples.
- Select the *One page per image* check box, if the report should contain a page of its own for every image. Should only this check box have been selected, and you have analyzed three images, your report will contain exactly three pages.
- Select the *Show results in overlay* check box if the image layer that contains the results is to be displayed along with the images.

3. When you want to create an MS-Excel report: Click the *Save settings* button to save the current settings in a file.
   - These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.
4. Click the *Finish* button.
   - The report will be generated and displayed in the application program that you selected.
   - The workbook will be created. It always contains a minimum of two worksheets. On the first worksheet, you'll see a summary of the results. On the second worksheet you'll see the details concerning the sample used. Should you have analyzed several samples, the workbook will contain additional worksheets.
   - The *Materials Solutions* tool window switches back to the start position. You can now use all of your software's functions again.
5. Through the materials analysis measurement, the image has collected one or more additional layers (can be seen in the *Layers* tool window). If required, save the image in TIF or VSI format to retain these newly created image layers.

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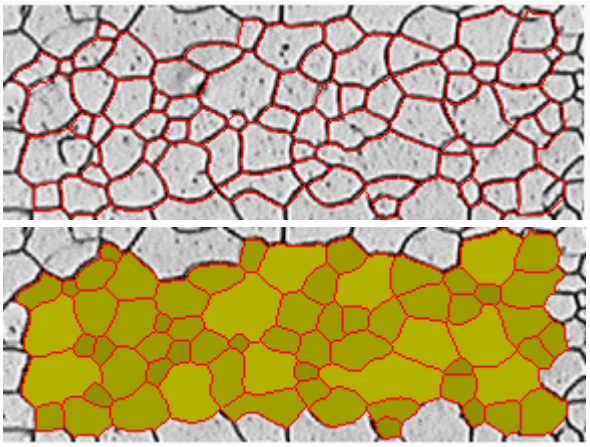
9.4. Grains Planimetric

9.4.1. What is Grains Planimetric?

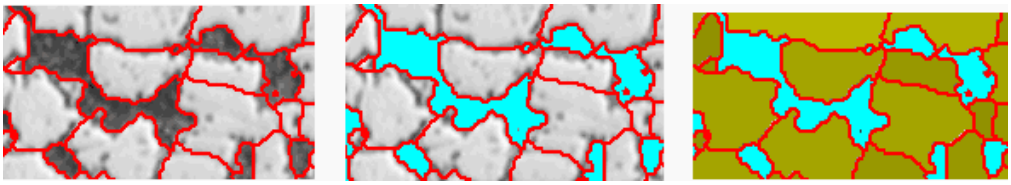
The grains planimetric analysis is used to measure grain sizes and to document them. It is often used in material analyses, for example, when the quality of steel or other metals is being tested. The grains planimetric analysis determines the grain size by means of the grains' area. In this way, it differs from the intercept analysis that determines the grain size by means of the number of intercept points. Samples with either dark or bright grains can be used. The analysis of cascaded grain boundaries (with multi-phase materials) is also possible.



The image shown above shows the results of an automatic detection of the grain boundaries. By default, the grain boundaries that have been detected are displayed in red (first illustration). Additionally, it's possible to have the grains that have been found displayed in color (second illustration). Small grains are displayed in darker shades than big grains.

Measuring the second phase

Samples that have a second phase can also be measured. Ferrite pearlite microstructures, which are important in the material analysis of steel, have two phases; the dark pearlite and the light ferrite. For these kind of samples, your software can determine the area of all of the second phase objects and subtract them from the area of the first phase.



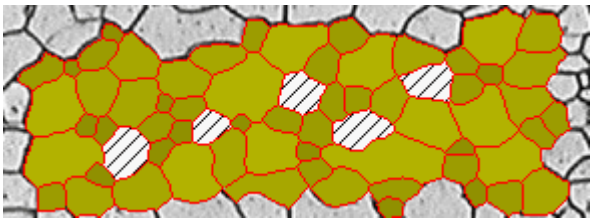
The above images display a ferrite pearlite microstructure. In the first image you can see (in red) the grain boundaries that have been detected. In the second image, all of the image areas that belong to the second phase are shown (in turquoise). The third image additionally shows (in green) the grains that were detected.

Editing grain boundaries

You can manually edit the grain boundaries that your software found automatically. You can delete unnecessary grain boundaries and add boundaries that are missing.

Validating detected grains

You can correct the way that the software detected the grains by selecting the grains and deleting them manually. If you inadvertently delete any grains, you can restore them.



The above image shows the result of an automatic detection of the grain boundaries after a number of grains have been manually deleted. The deleted grains are no longer taken into account when the measurement results are determined. They are cross hatched in the image.

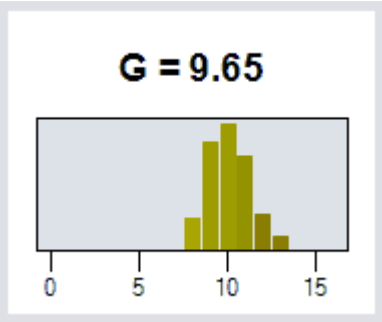
The results of a grains planimetric analysis

A grains planimetric analysis provides the so-called G-value, which is defined as a characteristic grain size in the corresponding industry standards. The following standards are available for the measurement:

- ASTM E 112-13
- GB/T 6394-2002
- GOST 5639-82
- EN ISO 643:2012
- DIN 50601:1985
- JIS G 0551:2013
- JIS G 0552:1998
- ASTM E1382-97 (2015)

In addition to this, other measurement results are determined; the total number of grains, the mean grain area and the sum of grain areas for example.

Documenting the results
The results of an analysis can be displayed in a workbook and in a chart. Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.



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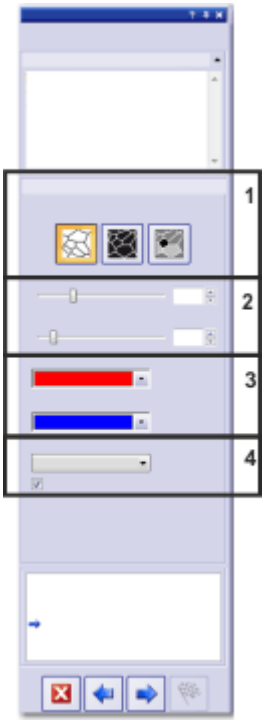
9.4.2. Settings

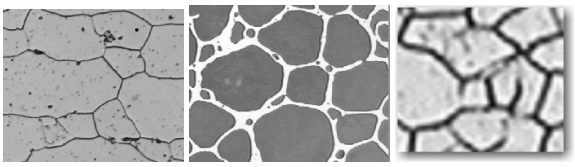
In this step, you make important settings for the analysis. You'll only see some of the setting options described below. Which of them you see depends on the image type you chose in the previous *Sample type* step.



(1) Buttons for selecting the grain boundary type

Prerequisite: You'll only see these buttons if you chose the *Bright or dark grains* type in the *Sample type* step.

Here, you specify which criteria are used to detect the grain boundaries. Depending on the image that is to be analyzed, the grain boundary type can be bright or dark. If the image you want to analyze contains both bright and the dark grain boundaries, click the *Bright and dark grain boundaries on gray background* button.



In the illustration on the left, the grain boundaries are dark. In the illustration in the middle, the grain boundaries are bright. In the illustration on the right, the grain boundaries are mainly dark, but there are some bright boundaries as well.

(2) Slide controls

The positioning of the slide controls influences the detection of the grain boundaries. While you are positioning the slide controls, observe which grain boundaries are found. The preview is updated after every change in the settings.

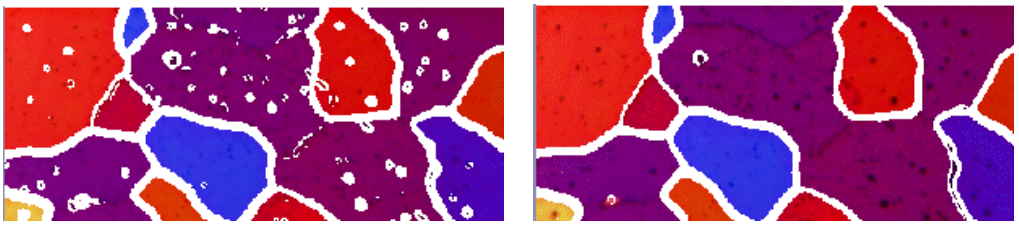
Position the slide controls in such a way that the grain boundaries are detected as completely as possible. It doesn't matter if the grain boundaries are interrupted somewhere in between. The algorithm that calculates the G-value will automatically close small interruptions in the boundaries.

Note: If you are not sure whether or not a slide control is positioned correctly, click the *Next* button and have a look at the results in the *Image results* step. With the *Back* button, you can always return to the *Grain boundaries* step.

Smoothness

With the help of this slide control you can specify that small structures or patterns that are located within the grains are to be ignored for the analysis. These structures have nothing to do with grains. Therefore, it is important to exclude them from the detection. If this is not done, these small structures are taken for grains and will thus affect the result of a planimetric measurement negatively.

Set the smoothness as exactly as possible, so that small structures or patterns only just stop being detected. Don't choose a larger value than necessary. If the image smoothness chosen is unnecessarily great, real small grains won't be detected.

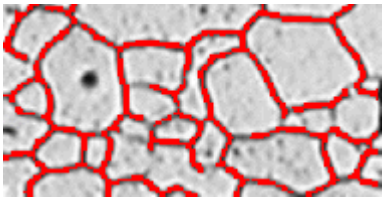
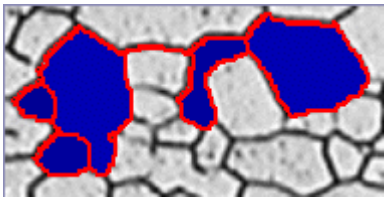


In the first illustration, the selected image smoothness is too small. With this setting, numerous structures (e.g., patterns) within the grains are detected, and this negatively affects the results of the planimetric measurement. In the second illustration, a higher value for the image smoothness has been chosen. You can clearly see that only a few structures were still detected within the grains. Therefore, the result of the planimetric measurement is more exact.

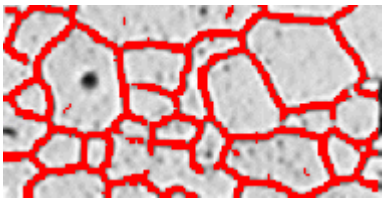
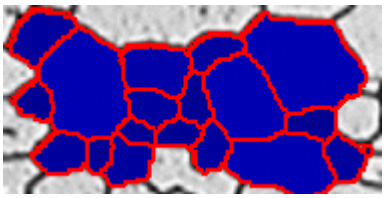
Threshold

Choose whether a smaller intensity range is sufficient for the detection of a grain boundary. This is, the case, when all of the grain boundaries stand out clearly against the background, for example. In this case, you can move the slide control to the far right.

If not all of the grain boundaries stand out clearly against the background, e.g., because some grain boundaries are brighter than others, a larger intensity range has to be defined for the detection of the grain boundaries. In this case, move the slide control to the far left.

In the first illustration, the selected threshold value is too high. In the *Image results* step, you can see that not all of the grain boundaries have been detected.

In this illustration, a lower value for the threshold values has been given. In the *Image results* step, you can see that all of the grain boundaries have now been detected.

(3) Selecting the grain boundary color and the grain fill color

Here, you specify in which color the grain boundaries that have been detected are to be shown. To do so, click the arrow button that is located at the right border of the field and select a color. The grain boundaries should be clearly distinguishable from the sample's color. By default, the color red is selected.

In the *Grain fill color* field, select the color in which the detected grains will be shown. To do so, click the arrow button that is located at the right border of the field and select a color.

(4) Selecting the industry standard

In the *Standard* field, select the industry standard that is to be used for the measurement. The following standards are available:

- ASTM E 112-13
- GB/T 6394-2002

- GOST 5639-82
- EN ISO 643:2012
- DIN 50601:1985
- JIS G 0551:2013
- JIS G 0552:1998
- ASTM E1382-97 (2015)

Select the *Show grain boundaries* check box to display the grain boundaries in the image window.

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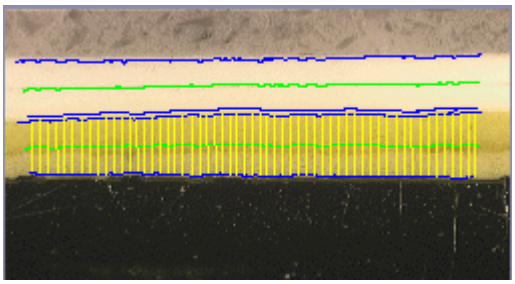
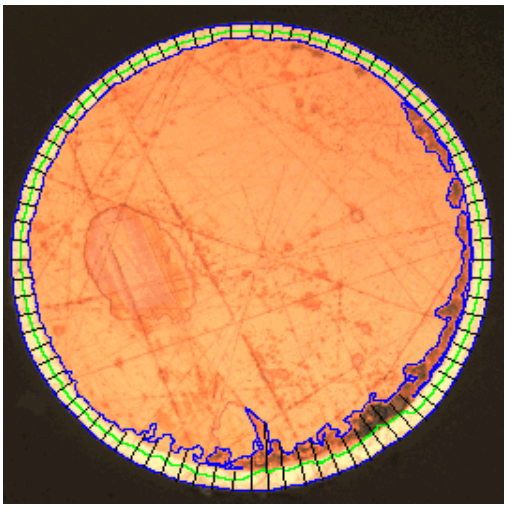
9.5. Layer Thickness Measurement

9.5.1. What are layer thickness measurements?

By using layer thickness measurements you can measure layers on calibrated images automatically or interactively. The object that is to be measured is the thickness of one layer or of several layers. Each layer is defined by two borders and a neutral fiber. The neutral fiber is a reference line which is there to specify the layer's course. The neutral fiber is automatically defined by the program. You can define either open or closed layer types. When you have a closed layer type, you can measure circular layer structures. In this mode, the measurement line's first point is automatically connected to its last point.



Measuring an open layer: In the image, two layers have been measured. You can see 4 layer borders (blue lines) and two neutral fibers (green lines). The measurement lines (yellow lines) are shown for the currently selected layer.



Measuring a closed layer: In the image, the outer layer has been measured. You can see the layer borders (blue lines), the neutral fiber (green line) and the measurement lines (black lines).

Results of a layer thickness measurement

The results of an analysis can be displayed in a workbook. Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.

The borders that have been found, the neutral fibers and the measurement lines will be saved together with the image, if you save it in TIF or VSI format. This information will be saved in a separate image layer that you can show and hide via the *Layers* tool window.

General procedure for a layer thickness measurement

1. Selecting the analysis process
Click the *Layer Thickness* button, located in the *Materials Solutions* tool window.

2. "Image source" step
Choose the image you want to measure.

3. “Settings” step
Choose the definition method you want to use (Automatic, Manual, Magic Wand).

4. “Magic wand” step
If you have selected the *Magic wand* definition method: Define the contours.

5. "Define borders" step
If you have selected the *Automatic* or *Magic wand* definition method: Define the borders.

6. “Define layers" step
Define the layers.

7. "Image results" step
Take a look at the measurement results.

8. "Results" step
Document the results (report or workbook).

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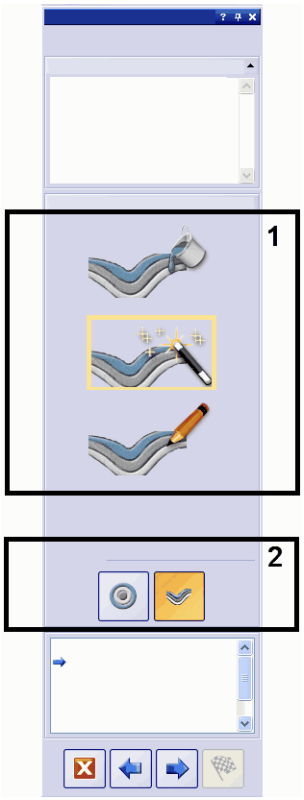
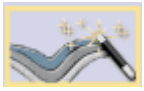
9.5.2. Settings

In this step, the following possibilities are available:



(1) "Settings" group

In the *Settings* group, choose how the contours are to be defined. To do this, click the corresponding icon. You can choose between the following definition methods. The current definition method is outlined in yellow.

-  automatic definition
-  manual definition
-  a definition with the magic wand

An automatic definition is suitable for samples whose layers feature distinct intensity differences (e.g., light layers in front of a dark background). With these samples, as a rule, the automatic threshold value setting used for this definition method functions well.

A definition by magic wand is suitable for samples that have irregular borders that would be very difficult to trace manually.

A manual definition is suitable for samples in which there are only very small intensity differences, which means that the automatic definition would not provide you with satisfactory results. Also when only a small part of a layer interests you, you can easily set it with the manual definition.

Please note: You can change the definition method during a measurement: For example, you can first have a contour determined by using the magic wand, then add an additional border manually.

(2) "Layer type" group

In the *Layer type* group, you choose whether open or closed layers are to be defined. To do this, click the corresponding icon.

With an open layer type, you can, e.g., measure layer structures that continue all through the image. When you have a closed layer type, you can measure circular layer structures. In this mode, the measurement line's first point is automatically connected to its last point.

Please note: The layer type can only be specified at the beginning of a measurement. In contrast to the definition method, the layer type can't be changed during the measurement.

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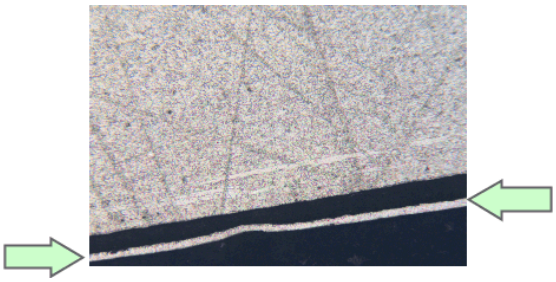
9.5.3. Performing an automatic layer thickness measurement

Note: You can follow these step-by-step-instructions on your PC. They describe a layer thickness measurement on an example image.

Step - Image source

1. Load the Coating.tif example image.



   - On this image, the thin light layer is to be measured.
2. Activate the *Materials Solutions* tool window.

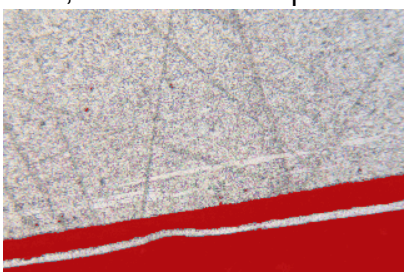
3. Click the *Layer Thickness* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
6. Select the *All images* entry in the *Check settings and results* list.
7. Click the *Next* button.

Step - Settings

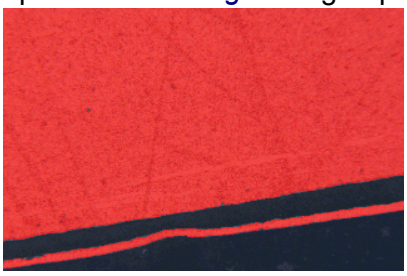
1. Click the *Automatic* button.
2. In the *Layer type* group, click the icon for an open layer.
3. Click the *Next* button.

Step - Automatic

1. You see the image on which some of the image structures are now shown in color, because the first phase was automatically set up.



2. Since the required image structures are not yet shown in color, select the *Dark* option in the *Background* group.

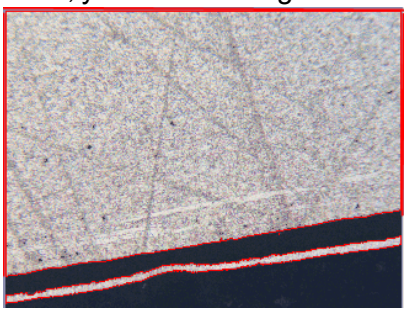


- Now, the required image structures are shown in color.

3. Click the *Next* button.

Step - Define borders

1. Here, you see the image in which the contours are outlined in red.



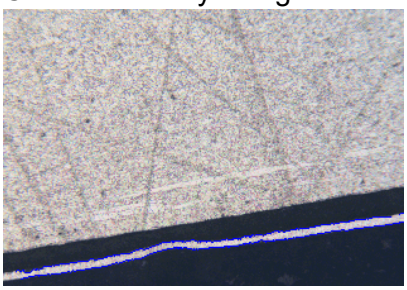
2. Click the *Define borders...* button.
3. Now, specify which part of the contour represents a border. Click the contour once with your left mouse button, to activate the mode.

   Then click with your left mouse button at the position in the contour where the first border is to begin.

Then click with your left mouse button at the position in the contour where the first border is to end.

- The beginning and the end of this border will be indicated by two green crosses.

4. Now, define the second border. To do so, click with your left mouse button again at the position where this border is to begin. Then click with your left mouse button again at the position where this border is to end.
   - The beginning and the end of this second border will be indicated by two blue crosses.
5. Click once with your right mouse button in the image.



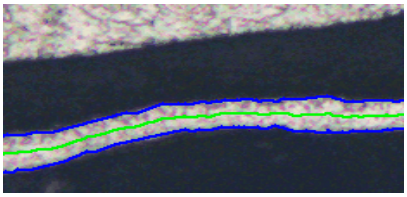
   - The borders that have been defined will be plotted in blue.
6. Since you don't want to define any additional borders: Then click once more with your right mouse button in the image, to switch off the mode for defining the borders.
7. Click the *Next* button.

Step - Edit borders

1. Since you have already defined both of the borders, and don't want to change them: Click the *Next* button.

Step - Define layers

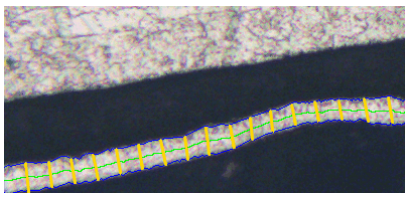
1. Click the *Add layers...* button.
2. Click the first border.
3. Click the second border.



   - The layer has now been defined. The neutral fiber is plotted in green. It always lies in the middle of the layer.
4. Click your right mouse button to finish the definition of the layer.
5. Click the *Next* button.

Step - Image results

1. Take a look at the results of the current image, shown in the *Image results* group. This group contains a table with the measurement results.
   - The values in the *Steps*, *Distance* and *Type* fields can be edited when you double click in the cell you want to edit.
   - The lower part of the group contains several buttons, with which you can change the way the layer thickness measurement is displayed.
2. Check the results shown in the image.



   - The measurement lines are shown in yellow in the image.
3. Click the *Next* button.

Step - Results

1. Select the *Generate report* check box and then select either the *Word* option or the *Excel* option to automatically create a report in the corresponding application program when the analysis finishes.
2. Select the *Generate workbook* check box to have a document of the "Workbook" type automatically created at the end of the analysis.

- You can load these settings (parameters) when you analyze further images. To do that for the new image in the *Image Source* step, click the *Load from file...* button.

Step - Reporting

Define the report that contains the measurement results.

- These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.
- The *Materials Solutions* tool window switches back to the start position. You can now use all of your software's functions again.
- Through the materials analysis measurement, the image has collected one or more additional layers (can be seen in the *Layers* tool window). If required, save the image in TIF or VSI format to retain these newly created image layers.

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9.5.4. Performing a layer thickness measurement with the magic wand (closed layer)

You can follow these step-by-step-instructions on your PC. They describe a layer thickness measurement on an example image.

Step - Image source

1. Load the "Copper Wire Section.tif" example image.
   - The image shows a cross section through a copper wire. The outermost layer is to be measured.
2. Activate the *Materials Solutions* tool window.

3. Click the *Layer Thickness* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
6. Select the *All images* entry in the *Check settings and results* list.
7. Click the *Next* button.

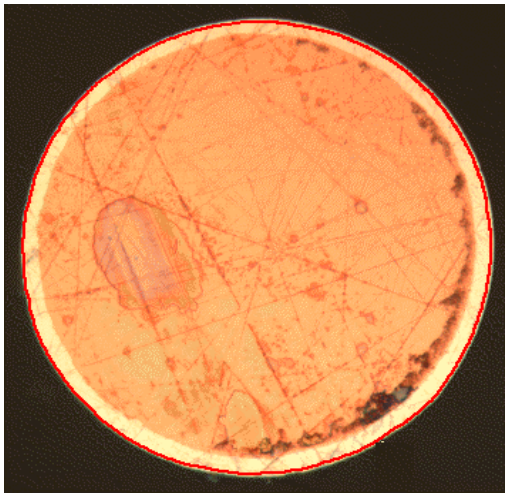
Step - Settings

1. Click the *Magic Wand* button.
2. In the *Layer type* group, click the icon for a closed layer .
3. Click the *Next* button.

Step - Magic wand

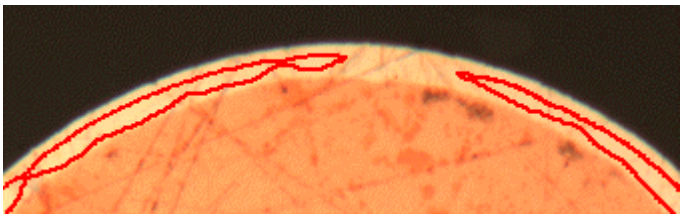
1. Click the *Add contours...* button.

2. Click the button for the *HSV* color space.
3. Then define the first contour. To do this, click once with your left mouse button on a position in the image that lies within the outermost layer.
   - The contour will be shown by a red line.

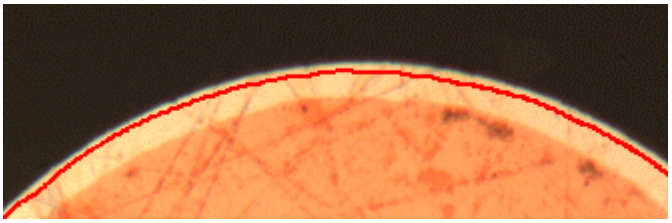


Note: Make sure that the contour completely includes the outer layer. And that the contour's outline isn't discontinued at any point on the outer layer. Change the position

of the slide control in the *Tolerance* field, until the contour completely includes the layer that is to be measured.



Wrong: The contour's outline is noncontinuous.



Correct: The contour completely contains the layer that is to be measured.

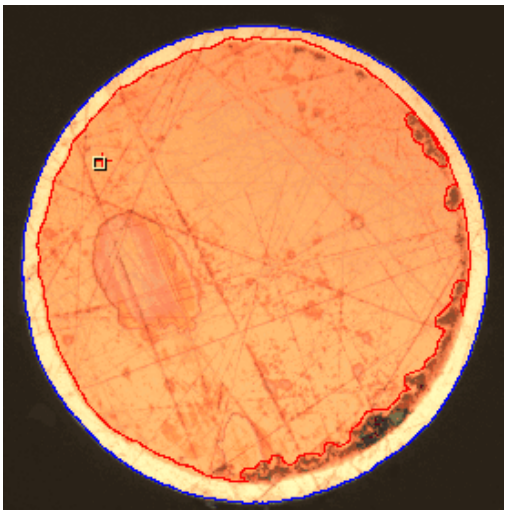
4. Click your right mouse button to finish the definition of the contour.
   - Now, the first border has been defined. It will be plotted in blue.
5. Click the *Next* button.
   - The *Edit borders* step in the analysis will be shown.

Step - Edit borders

1. Click the top *Add contours...* button.
2. Click the *Next* button.

Step - Magic wand

1. Then define the second contour. To do so, click the *Add contours...* button again.
2. Then click a position inside the copper wire.
3. Take care again that the contour contains the inside of the copper wire as completely as possible, and that its outline isn't discontinued anywhere. At the same time, this new contour mustn't touch the contour that has already been defined. Change the position of the slide control in the *Tolerance* field, until the second contour looks roughly as shown below:



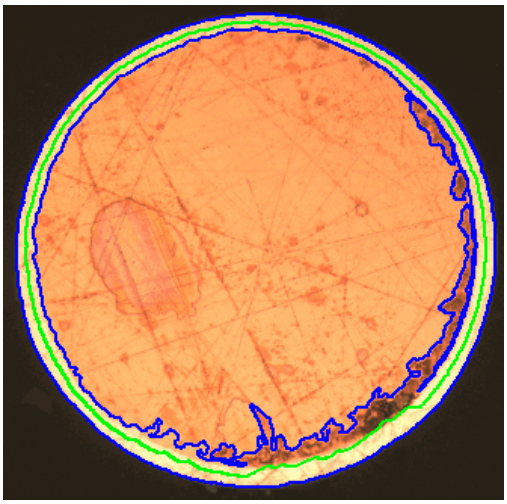
4. Click your right mouse button to finish the definition of the contour.
5. Click the *Next* button.

Step - Edit borders

1. Since you have already defined both of the borders, and don't want to change them: Click the *Next* button.

Step - Define layers

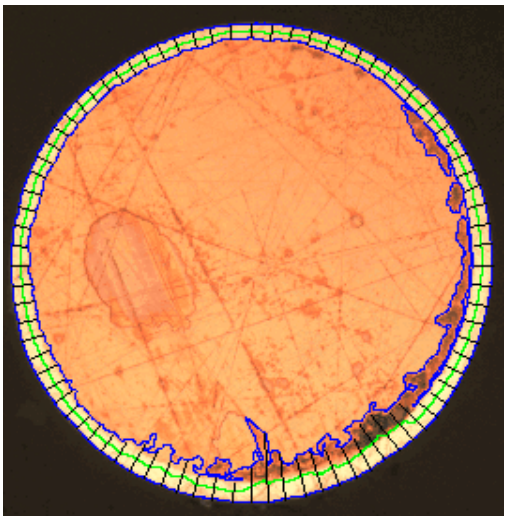
1. Click the *Add layers...* button.
2. Click the first border.
3. Click the second border.
   - The layer has now been defined. The neutral fiber is plotted in green. It always lies in the middle of the layer.



4. Click your right mouse button to finish the definition of the layer.
5. Click the *Next* button.

Step - Image results

1. Take a look at the results of the current image, shown in the *Image results* group.
   - The values in the *Steps*, *Distance* and *Type* fields can be edited when you double click in the cell you want to edit.
   - The lower part of the group contains several buttons, with which you can change the way the layer thickness measurement is displayed.
2. Check the results shown in the image.
   - The measurement lines are shown in the image. To make them contrast better, the color of the measurement lines was set to black before the measurement took place.



3. Click the *Next* button.

Step - Results

1. Select the *Generate report* check box and then select either the *Word* option or the *Excel* option to automatically create a report in the corresponding application program when the analysis finishes.
   - The additional step *Reporting* will be added to the current analysis. In the lower part of the dialog box, the *Finish* button will change into the *Next* button.
2. Select the *Generate workbook* check box to have a document of the "Workbook" type automatically created at the end of the analysis.
3. If you want to save the current settings to a file, click the *Save settings...* button. Then assign a descriptive name in the next dialog box.
   - You can load these settings (parameters) when you analyze further images. To do that for the new image in the *Image Source* step, click the *Load from file...* button.
4. Click the *Next* button.

Step - Reporting

Define the report that contains the measurement results.

- These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.

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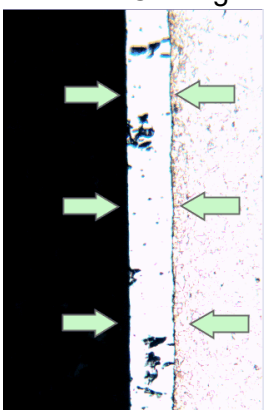
9.5.5. Performing a manual layer thickness measurement

Note: You can follow these step-by-step-instructions on your PC. They describe a layer thickness measurement on an example image.

Step - Image source

1. Load the "Coating with porosity.tif" example image.



- On this image, the middle layer is to be measured.

2. Activate the *Materials Solutions* tool window.

3. Click the *Layer Thickness* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
6. Select the *All images* entry in the *Check settings and results* list.
7. Click the *Next* button.

Step - Settings

1. Click the *Manual* button.
2. In the *Layer type* group, click the icon for an open layer .
3. Click the *Next* button.

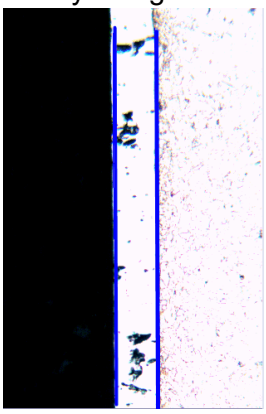
Step - Manual

1. Click the *Add borders...* button.
2. Define the first border. To do so, first click with your left mouse button at the position in the image where the border is to begin. Mark the course of the border with further left mouse clicks. Then click with your right mouse button at the

position in the image where the border is to end.

- The border will be shown in red.

3. Define the second border. To do this, proceed exactly as you did when you defined the first border.
4. Click your right mouse button to finish the definition of the two borders.



- The borders will be shown in blue.

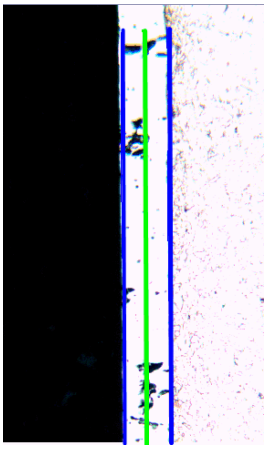
5. Click the *Next* button.
   - The *Materials Solutions* tool window displays the *Edit borders* step.

Step - Edit borders

1. Since you have already defined both of the borders, and don't want to change them: Click the *Next* button.

Step - Define layers

1. Click the *Add layers...* button.
2. Click the first border.
3. Click the second border.

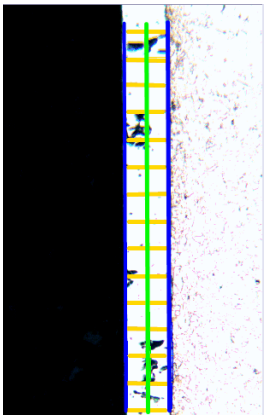


- The layer has now been defined. The neutral fiber is plotted in green. It always lies in the middle of the layer.

4. Click your right mouse button to finish the definition of the layer.
5. Click the *Next* button.

Step - Image results

1. Take a look at the results of the current image, shown in the *Image results* group. This group contains a table with the measurement results.
   - The values in the *Steps*, *Distance* and *Type* fields can be edited when you double click in the cell you want to edit.
   - The lower part of the group contains several buttons, with which you can change the way the layer thickness measurement is displayed.
2. Check the results shown in the image.



   - The measurement lines are shown in yellow in the image.
3. Click the *Next* button.

Step - Results

Select the results you want.

Step - Reporting

Define the report that contains the measurement results.

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9.6. Cast Iron analysis

9.6.1. What is a cast iron analysis?

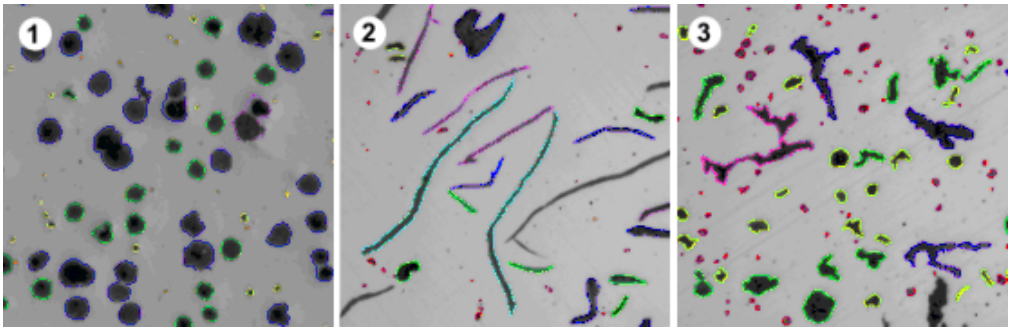
The quality and consistency of cast iron depends on the distribution and the morphology of its carbon content. By using a cast iron analysis you can determine the cast iron's graphite fraction with the help of unetched samples. As well as that, with the help of etched samples you can determine the ferrite/pearlite ratio.

The classification of the detected particles is performed according to the industrial standard that is selected in the program options. Each standard requires a different classification of the detected particles. These classifications are included in the software package purchased, and are automatically installed with it. The following standards are supported:

- EN ISO 945-1:2018
- ASTM A247-17
- JIS G 5502:2001
- KS D 4302:2006
- GB/T 9441-2009
- ISO 16112:2017
- JIS G 5505:2013
- NF A04-197:2017

Determination of the graphite fraction

By using your software's *Cast Iron* Solution, you can measure the graphite fraction and classify the detected particles. For this purpose, the sample must not be etched. How the classes are defined, depends on the standard according to which the cast iron analysis is carried out.



You see the results of a cast iron analysis made of different forms of graphite. The color coding of the particles indicates their belonging to a specific size class (**1**), form class (**2**), and a form factor (**3**).

Results of a cast iron analysis made to determine the graphite fraction

The results of an analysis can be displayed in a workbook. Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.

While you are performing a cast iron analysis, you can create a chart showing the graphite size, the graphite form or the graphite nodularity. You can also save these charts as files.

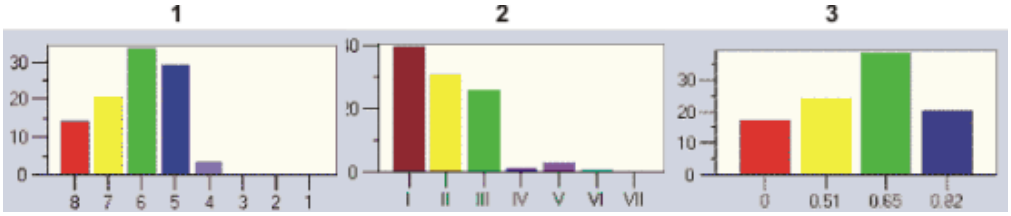


Figure (1) shows a chart of the graphite size. Along the X-axis the size classes are shown, along the Y-axis the number of detected particles in % is shown.
Figure (2) shows a chart of the graphite form. Along the X-axis the form classes are shown, along the Y-axis the number of detected particles in % is shown.
Figure (3) shows a chart of the graphite nodularity. Along the X-axis the form factor is shown, along the Y-axis the number of detected particles in % is shown.

General procedure for a cast iron analysis made to determine the graphite fraction

1. Selecting the analysis process
Click the *Cast Iron* button, located in the *Materials Solutions* tool window.

2. "Image source" step
Choose the image you want to measure.

3. “Settings” step
Choose the sample type (unetched).
Set the graphite parameters.

4. "Graphite distribution" step
If chosen: Determine the distribution of the graphite particles.

5. "Image results" step
Take a look at your image results.
If necessary, Delete or separate detected particles, or add new ones.

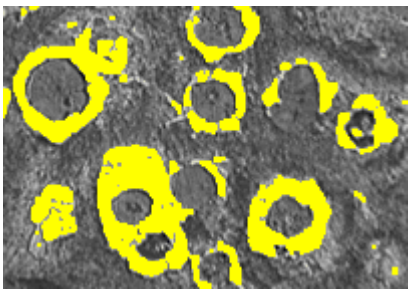
6. "Results" step
Document the results (report or workbook).

Determination of the ferrite/pearlite-ratio

By using your software's *Cast Iron* solution, you can also measure the ferrite/pearlite ratio. For this purpose, the sample must have been etched. Since graphite and pearlite have very similar gray values, it's difficult to differentiate between these two fractions in

a sample during the same analysis. For this reason, determining the ferrite/pearlite ratio is done as follows:

To begin with, your software determines, by means of the definition of phases, the ratio of the bright ferrite areas to the dark (graphite and ferrite) areas. During the analysis, the graphite fraction is entered, and is then subtracted from the dark areas. This graphite fraction has either been determined in an earlier measurement (this value can then be imported), or it can alternatively be estimated. Using the pearlite area that has in this way been corrected, the ferrite/pearlite ratio is calculated.



You see a step in the analysis during the determining of the ferrite/pearlite ratio. The bright ferrite phase has been determined by your software (shown in yellow here).

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9.6.2. Performing a cast iron analysis (unetched sample)

Note: You can follow these step-by-step-instructions on your PC. They describe how the graphite fraction is determined.

Step - Image source

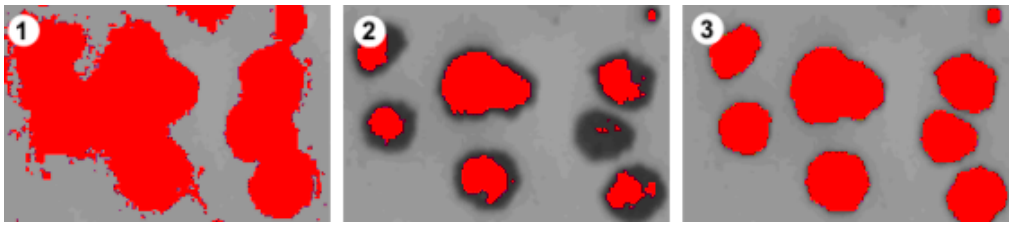
1. Load the GlobularGraphite.tif example image.
   - The graphite fraction is to be measured.
2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Cast Iron* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
   - By doing so, you skip the *Sample information* step which is not relevant for this example image. However, it is quite possible that, when performing your own analyses, you might want to load sample results (e.g., the result of a previous cast iron analysis that determined the graphite fraction). In this case, make sure the *Skip 'Sample information'* check box is cleared, which will enable you to use the *Load results* button, in the *Sample information* step.
6. Select the *All images* entry in the *Check settings and results* list.

7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Settings

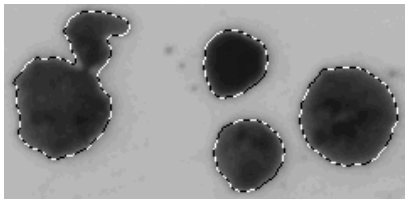
1. Click this button to set that you want to determine the graphite fraction in an unetched sample.
   - If the button for etched samples has been active before, the setting options in this window will now change.
2. Use the slide control to define the threshold value for the graphite detection. Observe the sample. The threshold value has been correctly set when the graphite particles can be completely detected.



In the illustration (**1**), the threshold value has been set too high, the detected particles are too coarse. In the illustration (**2**), the threshold value has been set too low, the particles are not detected completely. The illustration (**3**) shows a correctly set threshold value.

3. In the *Standard* pick list, select the industry standard according to which the cast iron analysis is to be carried out.
   - Some standards contain either rules regarding the measurement of the nodularity or references to secondary standards. For this reason, additional fields may now be shown or hidden in the lower area of the tool window.
4. Select the graphite parameter that is to be determined. To do so, select the corresponding check box. The possibilities listed below are available: Which size classes, form classes and form factors are used for the classification, depends on the industry standard according to which the cast iron analysis is performed.
   - *Graphite size:* Sorts the detected particles into specific classes, according to their size.
   - *Graphite form:* Sorts the detected particles into specific classes, according to their form.
   - *Graphite nodularity:* Sorts the detected particles into specific classes, according to their nodularity. The nodularity is a unit of measure for the sphericity of the graphite.
   - *Graphite distribution:* Makes it possible to compare the distribution of the particles in the current image with the distribution in specific reference images. When this check box has been selected, the additional step, the *Graphite distribution* will be added to the cast iron analysis. The graphite distribution (types A-E) can only be determined for lamellar graphite.
5. In the *Minimum size for graphite particle* field, specify the minimum size that a particle must have if it is to be taken into account during the cast iron analysis.

- All particles that fall short of the value entered will be ignored when the analysis is carried out.
- Particles that have been detected, but that aren't used for the analysis, (e.g., because they don't come up to the minimum size that has been set here) are shown with a dashed line in the image.



- The calculation of the sample's graphite fraction is not affected by this setting, as the smaller particles are also used to calculate the area fraction.

6. In the *Standard for size* or *Standard for nodularity* picklist, select the industry standard that you want to use to measure the nodularity.

Note: Whether this picklist is displayed and whether it is called *Standard for size* or *Standard for nodularity* depends on the entry that is selected above in the *Standard* picklist.

7. When the *EN ISO 945-1:2010* entry is selected in the *Standard* picklist, the *Classify form IV particles as nodular particles* check box becomes active. Select this check box if all particles that belong to the form IV class should be considered for the detection of the graphite nodularity. That means that the graphite nodularity increases and that the number of nodular particles per mm^2 is also higher.
8. If the *ASTM A247-17* entry has been selected in the *Standard* picklist: In the *Shape factor threshold* field, set the threshold value to be used for counting a detected graphite particle as nodular graphite.
   - You can enter values between 0 and 1, the default value is 0.6. If you enter a smaller value (e.g. 0.4), a larger proportion of the graphite particles found will be counted as nodular graphite than for example with a value of 0.6.
9. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Graphite Distribution

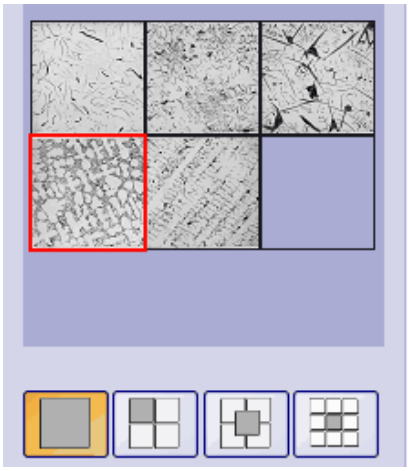
Prerequisite: You will only see this step if, in the previous step, you selected the *Graphite distribution* check box.

In this step, you can compare the particles that have been detected with reference images that show different distributions of graphite particles. You can then determine which of the reference images shows a distribution that is most similar to that of the current image. The reference images correspond to images that the chosen standard contains.

1. In the *Style* group, choose how the images are to be arranged in the document group for the comparison. Choose an arrangement in which the GlobularGraphite.tif image and the selected reference image are superimposed. To do so, click this button

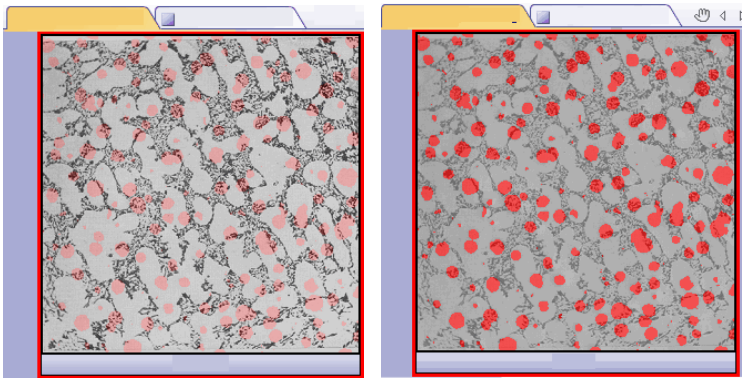
.

- In the *Overview* field, you see the arrangement that has been chosen. The selected reference image is framed in red.



- The *Cast iron analysis* document will now be displayed in the document group. It contains exactly one image.

2. Compare the graphite distribution of the current image with that of the reference image. Move the slide control below the *Style* field towards the *Opaque* position, if the image that is to be checked is to superimpose the reference image. Alternatively, move the slide control towards the *Transparent* position, if the image that is to be checked is to be superimposed by the reference image. If you want to choose another reference image, in the *Overview* field, click that image with your left mouse button.



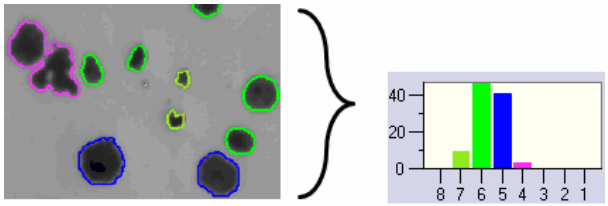
The illustration on the left shows the image that is to be checked. Because the slide control is located in near the *Opaque* position, the reference image's structures can only be faintly recognized. For the illustration on the right, the slide control has been moved towards the *Transparent* position. Now, the reference image can be clearly recognized, and the image that is to be checked can be only faintly recognized.

3. When you have selected the reference image that is the most similar to the image that is to be checked: Click the *Accept* button.
   - The chosen image's data will be accepted in the *Results* field.
   - It's possible to accept several reference images, for example, with samples that have very different structures.

4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

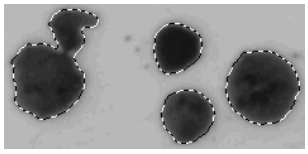
Step - Image results

1. Take a look at the results that are shown in the table and also in the image. Select the *Show graphite detection* check box, in the *Validation* group.
   - Every particle that has been detected will then be outlined with a colored line. The color with which the particle is outlined, shows you to which class it belongs. The same colors will be used in the chart.



On the left, you see the colored identification of the particles in the image. On the right, you see the chart of graphite sizes that uses the same colors.

   - Particles that have been detected, but that aren't used for the analysis (e.g., because they don't come up to the minimum size that has been set for the program options), have a dashed line.



2. If you selected several graphite parameters in the *Settings* step, toggle between the different charts.
3. If you want to correct the automatically found particles, use the buttons in the *Validation* group.
4. Click the *Next* button.

Step - Results

1. Take a look at the results that are shown in the table. Among other things, the number of particles is shown here.
2. Select the *Generate report* check box and then select either the *Word* option or the *Excel* option to automatically create a report in the corresponding application program when the analysis finishes.
3. Select the *Generate workbook* check box to have a document of the *Workbook* type automatically created at the end of the analysis.
   - Leave the *Generate chart* check box cleared for these step-by-step instructions.
4. Click the *Save results* button, if you want to also determine the ferrite/pearlite-ratio in another cast iron analysis, on the basis of the etched sample. You can then load the graphite fraction determined here, and won't need to enter it manually.

5. Click the *Next* button.

Step - Reporting

Define the report that contains the measurement results.

- These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.
- The *Materials Solutions* tool window switches back to the start position. You can now use all of your software's functions again.
- Save the image in the TIF or VSI file format.

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9.6.3. Performing a cast iron analysis (etched sample)

Note: You can follow these step-by-step-instructions on your PC. They describe how to measure the ferrite/pearlite-ratio.

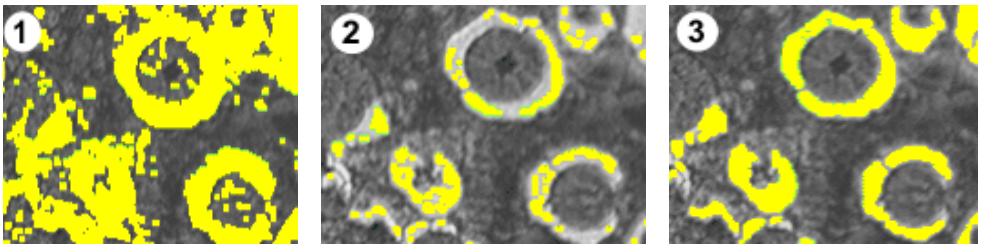
Step - Image source

1. Load the "Ferrite Pearlite.tif" example image.
   - The ferrite/pearlite-ratio is to be measured.
2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Cast Iron* button.
   - As soon as you've started this analysis process you'll be guided step by step through the measurement. A lot of your software's other functions will not be available while an analysis process is running.
   - The *Materials Solutions* tool window displays the *Image Source* step.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
6. Select the *All images* entry in the *Check settings and results* list.
7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

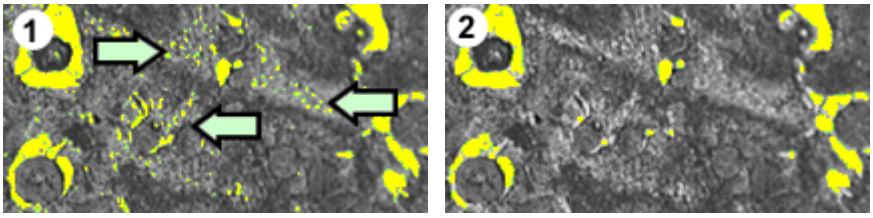
Step - Settings

1. Click this button to set that you want to determine the ferrite/pearlite-ratio, using an etched sample.
   - If the button for unetched samples has been active before, the setting options in this window will now change.
2. Use the *Threshold for ferrite* slider to define the ferrite phase. By doing so, you set the range of intensity values (the phase) that is valid for the ferrite detection. If the slide control is closer to the *Low* position, the phase contains a larger part of the intensities that are present in the image. If the slide control is closer to the *High* position, the phase contains a smaller part of the intensities. This means that only a smaller part of the intensity values is detected as ferrite. All of the pixels that have been detected as ferrite will be highlighted in yellow in the image.
   - The threshold value has been correctly set when the ferrite is completely detected.



In the illustration (**1**), the threshold value has been set too low, too many particles are detected as ferrite. In the illustration (**2**), the threshold value has been set too high, the ferrite is not detected completely. The illustration (**3**) shows a correctly set threshold value.

3. Use the *Close gaps in pearlite phase* slide control to define how rigid the voids that the pearlite contains, are to be closed. In this context, a void in the pearlite is an area within the pearlite that has so bright intensity values that it is assigned to the ferrite. In the image, voids are visualized as an accumulation of small yellow points within the pearlite.
   Using the *Close gaps in pearlite phase* slide control is a means of correcting these voids. To do so, a morphological filter is applied. Morphological filters are often used in image analysis to optimize the results of an automatic object analysis.

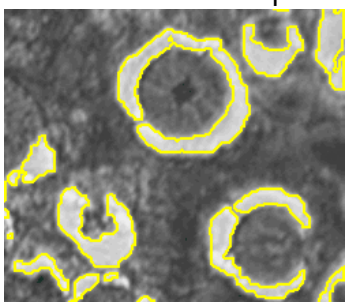


In the illustration (**1**), the pearlite phase is little closed. This is why many voids have been detected within the pearlite (see arrows). The illustration (**2**) shows a pearlite phase that is more closed.

4. In the *Graphite fraction* group, select how this sample's graphite fraction is to be entered. The graphite fraction will be subtracted from the detected pearlite fraction. Using the pearlite area that has in this way been corrected, the ferrite/pearlite ratio is calculated. This step is necessary because graphite and pearlite have very similar gray values and can therefore not be detected separately by the software. There are two possibilities how to enter the graphite fraction:
   - You select the *Enter manually* option and enter the value. This option is always active. You can have e.g., made a note of this value, or have saved it in a report.
   - You select the *Result of unetched sample analysis* option. This option is only active if, in the same analysis, you have already measured the graphite fraction, using an unetched part of the sample. This option is also active if, you measured the graphite fraction in a previous analysis, saved these values in a parameter set and loaded them in the current analysis' *Sample information* step.
5. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Image results

1. Take a look at the results that are shown in the table. Among other things, here you will find the ferrite/pearlite-ratio that has been measured.
2. Take a look at the displayed results in the image as well. To do so, select the *Show ferrite detection* check box, in the *Validation* group.
   - Each detected ferrite particle will now be outlined in yellow.



3. Click the *Next* button.

Step - Results

Select the results you want.

Step - Reporting

Define the report that contains the measurement results.

- Select the *One page per sample* check box, if the report should contain one page for every sample. This page displays the overall results for all of the images belonging to that sample. Using this setting is a good idea, for example, when you have analyzed images of different samples.
- These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.

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9.7. Non-metallic inclusions

9.7.1. Overview

There are two analysis processes available in your software for the analysis of non-metallic inclusions in metal samples:

1. an analysis of the inclusion content
2. an inclusions worst field analysis

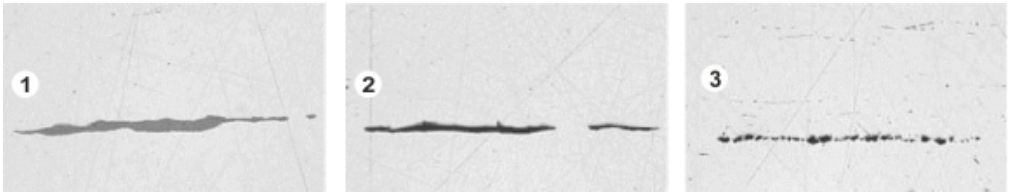
What is an inclusions worst field analysis?

An inclusions worst field analysis is a procedure used to check whether there are non-metallic inclusions in metal samples. This analysis can be used to measure the size and distribution of non-metallic inclusions in steel samples and to determine what type they are. Non-metallic inclusions can be sulfides and oxides, for example.

With the measurement results, different production processes can be compared, or the quality of a product determined.

What exactly is a non-metallic inclusion?

During the production processes, non-metallic inclusions can accrue within steel alloys. Inclusions affect the chemical and mechanical properties of the steel. The fewer inclusions there are in a steel, and the smaller and homogeneous these are, the better is its quality.

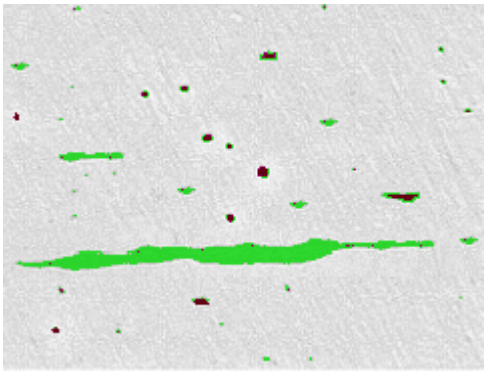


Microscope image of different inclusions in a polished steel sample. The inclusions differ in their color and form. The images show a sulfide inclusion (**1**), a silicate inclusion (**2**), and an aluminum oxide inclusion (**3**).

The nature and appearance of the non-metallic inclusions depend on a variety of factors, such as, e.g., the steel type, or the production process. The inclusions are divided into different classes according to their appearance (color, form, and size). The classification is made according to different industry standards.

Since all inclusions are darker than the color of the steel, they can easily be detected by means of an automatic image analysis. When detecting the inclusions, the inclusions worst field analysis searches for particles. For the image analysis software, a particle is a cohesive number of pixels, that all lie within a defined intensity range. For this reason, you first have to define the intensity range.

You can also define two intensity ranges. This is necessary when the sample contains both gray (sulfide) and black (oxide) inclusions, for example.



Particle detection during an inclusions worst field analysis. When a suitable definition of the gray value ranges has been made, the sulfides (green) and the oxides (red) will be detected.

Editing inclusions

You can manually edit the inclusions that your software found automatically. You have the possibility of deleting, splitting, or joining up inclusions, and you can also change their type.

Results of an inclusions worst field analysis

If the sample is suitable and the thresholds are set correctly, this analysis finds either the largest non-metallic inclusion in the sample being analyzed, or the field with the most inclusions (sorted into inclusion types). The classification and naming convention of the inclusions differs from industry standard to industry standard. The sizes are measured in accordance with the industry standards:

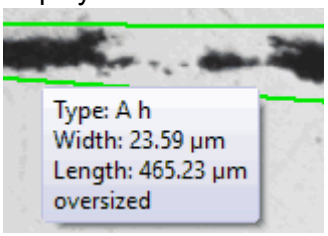
- ASTM E 45-18 method A
- DIN 50602:1985 method M
- ISO 4967:2013 method A
- GB/T 10561:2005 method A
- JIS G 0555:2003 method A
- UNI 3244:1980 method M
- EN 10247:2017 method M(L/n)
- EN 10247:2017 method M(L/d)
- EN 10247:2017 method M(a)
- EN 10247:2017 method M(a/n)
- EN 10247:2017 method P(a)
- EN 10247:2017 method P(L/d)
- SEP 1571:2017 method M

Note: It is also possible to perform an inclusions worst field analysis with the EN 10247 standards' version from 2007. To do so, select this version of the standard in the program options. You have to do this before starting the analysis process.

Viewing inclusions on the image

If you want to view detailed results for individual particles while the analysis is still in progress, use the *Show Inclusion Results* button in the *Image results* step.

When this button is active, the details for an inclusion are displayed when you hover the mouse pointer over it in the image window. Which details these are depends on the standard that has been selected. Usually, the type, the length, and the width are indicated. For some standards, the area is also shown. For some standards, if the length or width of an inclusion exceeds the specified limit *oversized* will also be displayed.



Displaying the results in the workbook

The results of an analysis can be displayed in a workbook. If the *Display inclusion results in the workbook* check box has been selected in the program options, the workbook will also contain the individual results for each inclusion that was detected, in addition to the overall results. If oversized inclusions were detected, they will be identified with a plus sign *(+)* in the *Type* column.

Displaying the results in the report

Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.

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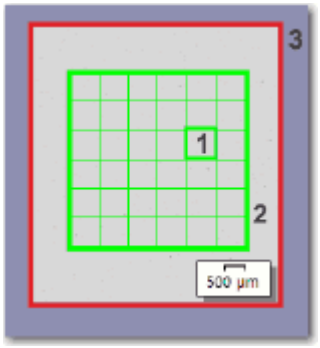
What is an analysis of the inclusion content?

An analysis of the inclusion content is a procedure used to check whether there are non-metallic inclusions in metal samples. This analysis can be used to measure the size and distribution of non-metallic inclusions in steel samples and to determine what type they are. Non-metallic inclusions can be sulfides and oxides, for example.

With the measurement results, different production processes can be compared, or the quality of a product determined.

If the sample is suitable and the thresholds are set correctly, the **analysis of the inclusion content** detects all of the non-metallic inclusions in the sample being analyzed. The prerequisite is that the inclusions must be within the area of the fields. Because of the complexity, an analysis of the inclusion content can currently only be performed on monochrome 8 bit images.

The images to be analyzed are usually stitched images of a polished steel sample. The whole image is divided into fields by default. All of the fields are called the *Area of fields*. Each *field* has a size of 710 µm x 710 µm as specified by the standard. This corresponds to an area of the sample of 0.5 mm^2 per field. The supported standards recommend a minimum sample area of 10 mm x 16 mm. This amounts to 320 fields.



The illustration shows an image (**3**) with the area of fields (**2**). The area of fields is made up of individual fields (**1**).

Observing ambiguous inclusions at the microscope

If you want to observe an inclusion in detail, click it in the image window. Your stage will then move to the corresponding part of the sample and you can observe this inclusion in detail at the microscope.

This option is only available if your microscope has a motorized stage and if you made all the necessary settings for the stage path and the scan area.

The results of an analysis of the inclusion content

If the sample is suitable and the thresholds are set correctly, the analysis of the inclusion content detects all of the non-metallic inclusions in the sample being analyzed. This is done for each inclusion type separately. The classification and naming convention of the inclusions differs from industry standard to industry standard. The analysis is performed in accordance with the selected standard and the selected method. The following standards are available:

- *ASTM E 45-18 method D*
- *ISO 4967:2013 method B*
- *EN 10247:2017 method K*
- *SEP 1571:2017 method K*

Note: The results of the different standards are displayed differently in the image window and in the *Image Navigator* tool window. With the first two standards, a colored outline is displayed around each field (and around each inclusion) that contains a detected inclusion of the currently selected type. With the *EN 10247:2017* and *SEP 1571:2017* standards, a colored outline is displayed around each inclusion that is detected. The fields don't have a colored outline.

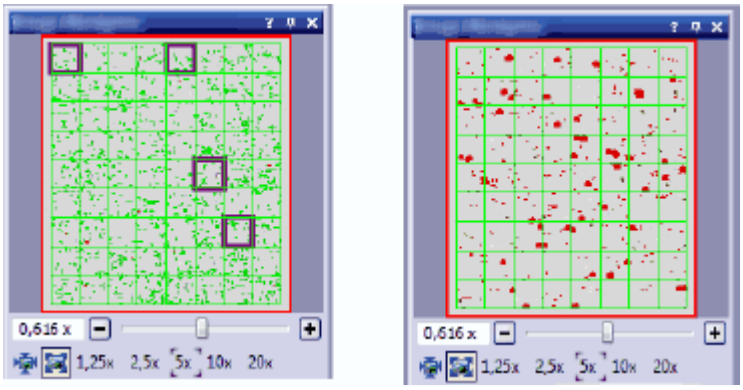


Illustration on the left: Display of the results in the *Image Navigator* tool window when the analysis is performed in accordance with the *ASTM E 45-18 method D* or the *ISO 4967:2013 method B* standard. In the example shown 4 fields are outlined.
Illustration on the right: Display of the results in the *Image Navigator* tool window when the analysis is performed in accordance with the *EN 10247:2017 method K* or the *SEP 1571:2017 method K* standard. The inclusions of the currently selected type are outlined.

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9.7.2. Performing an inclusions worst field analysis

Note: You can follow these step-by-step-instructions on your PC. It describes how you can detect the worst inclusion in a sample.

Prerequisite: In order to analyze images with the inclusions worst field analysis, make sure that the sample is placed on the stage in a way that the inclusions have a horizontal orientation in the image.

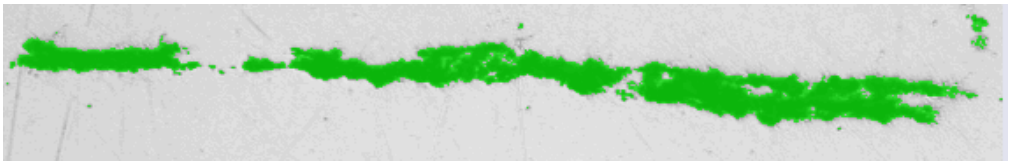
Image source step

1. Load the NMI0_0.tif example image.
   - The largest non-metallic inclusion is to be measured.
2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Inclusions Worst Field* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
6. Select the *All images* entry in the *Check settings and results* list.
   - If you analyze your own images later on, you can also select another entry from this list, for example, if you don't want to check the settings for every image anymore.
7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Settings step

1. In the *Evaluation method* field, set the industry standard you are going to use for the analysis.
2. Use the *All inclusions* slide control to define the threshold value for all of the inclusions. You can find this slide control in the *Thresholds* group. Observe the sample. The threshold value has been correctly set when the inclusions are completely recognized.

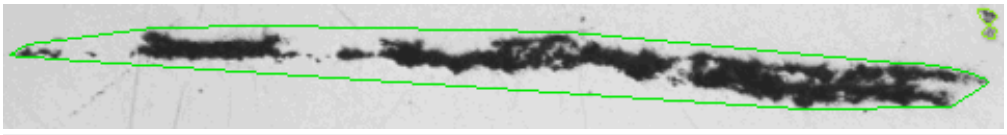


The illustration shows a correctly set threshold value.

3. Since in this sample there are no oxide inclusions, set the *Oxide inclusions* slide control at the position *Low*.
4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Image results step

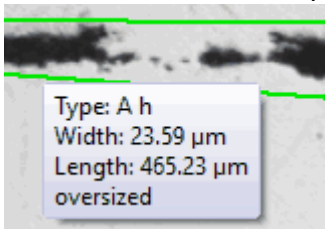
1. Take a look at the results that are shown in the table. Should you have analyzed several images of the same sample, you can switch between a display of the image results for the current image and the results for all of the images. To do this, select either the *Image* option, or the *Sample* option, located below the table.
   - The table with the measurement results contains a classification of the inclusions that have been detected. How this classification looks like, depends on the standard by which the analysis was performed.
     For example, the "ASTM E 45 Method A" standard uses the classification A (Sulfide), B (Alumina), C (Silicate) and D (Globular Oxide). Furthermore, this standard groups the inclusions into "t" (thin) and "h" (heavy), according to their mean width (inclusions type A, B, C) or to their diameter (inclusions type D). Other standards use another classification of the inclusions, and don't further divide them up into groups.
2. Take a look at the displayed results in the image as well.
   - In the image, every detected inclusion will now be outlined with a colored line.



The illustration shows a detected particle. The entire inclusion is outlined with a colored line.

   - Particles that have been detected, but that aren't used for the analysis (e.g., because they don't come up to the minimum size that has been set in the industry standard), are shown with a yellow line.

3. If you want to see detailed results for an inclusion: Click the *Show Inclusion Results* button and move the mouse pointer in the image window to the required inclusion.
   - The details for the selected inclusion will be shown. Which details these are depends on the standard that has been selected. Usually, the type, the length, and the width are indicated. For some standards, the area is also shown. For some standards, if the length or width of an inclusion exceeds the specified limit *oversized* will also be displayed.



3. If you want to correct the automatically found inclusions, use the buttons in the *Edit inclusions* group.
5. Click the *Next* button.

Results step

1. Take a look at the results that are shown in the table. Here, you see, for each inclusion type separately, the worst inclusion found in any of the analyzed images.
2. Select the *Generate report* check box and then select either the *Word* option or the *Excel* option to automatically create a report in the corresponding application program when the analysis finishes.
3. Select the *Generate workbook* check box to have a document of the "Workbook" type automatically created at the end of the analysis.
4. If you want to save the current settings to a file, click the *Save settings* button. Then assign a descriptive name in the next dialog box.

Reporting step

Define the report that contains the measurement results.

- These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.

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9.7.3. Performing an analysis of the inclusion content

The following step-by-step instruction describes, in a simplified way, how you can detect the inclusion content in a sample.

Preparation
When working with stitched images, it is useful to have the *Image Navigator* tool window always displayed. Then you can easily zoom in and out of the stitched image

without losing orientation. Therefore, click the *Disable Auto Hide* button once in the *Image Navigator* tool window before starting the analysis process.

Preconditions that must be met by the image to be analyzed:
The following prerequisites must be met so that an analysis of the inclusion content can be successfully performed on a stitched image.

- the steel sample must be optimally prepared for analysis of the inclusion content (cleaned and polished)
- the steel sample must be Illuminated appropriately for the acquisition (no overexposure)
- the steel sample must show inclusions that are suitable for an automatic analysis
- the inclusions must be aligned horizontally in the image

Image source step

1. Load the image that you want to analyze. You want to measure all non-metallic inclusions on this image.

Note: As a rule, stitched images are saved in VSI file format. When you load images, the default file format is TIF. If you don't see the image that you want to analyze in the *Open Image* dialog box, select *All* file format.

2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Inclusion Content* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.

Note: If your microscope has a motorized stage, you can select the *Stage Path* option here. When you make all the necessary settings for the stage path and the scan area, then you have the following possibility: In the *Image results* step, you can click on an inclusion in the image window . Your stage will then move to the corresponding part of the sample and you can observe this inclusion in detail at the microscope.

5. Select the *Skip 'Sample information'* check box.
6. Select the *All images* entry in the *Check settings and results* list.
7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Area of fields step

1. Decide whether you want the area of fields to be a rectangle or a polygon. For these step-by-step instructions, select the *Rectangle* option.
   - By default, the area of fields is rectangular and covers the whole image.
2. Use the mouse to reduce the size of the area of fields and then place it at a suitable position on the sample (see illustrations).

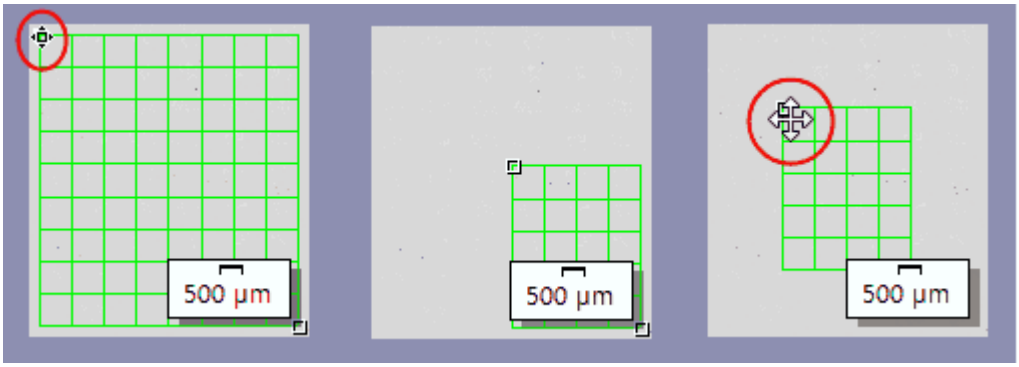
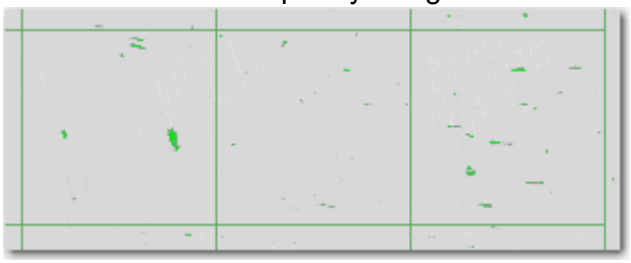


Illustration on the left: Position the pointer on a marker in the image window. The mouse pointer will change shape (see the red ellipse). Drag the handle in the required direction.
Illustration in the center: The size of the area of fields has been reduced. The values in the *Area* and *Number of fields* fields are automatically updated.
Illustration on the right: To change the area of fields, position your mouse pointer over one of its handles again. The mouse pointer turns into a four headed arrow (see the red circle). While pressing the left mouse button, drag the area of fields to the required position.

3. If required, change the line color that is used to show the area of fields.
4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Settings step

1. In the *Evaluation method* field, set the industry standard you are going to use for the analysis. For these step-by-step instructions, the *EN 10247:2017 method K* industry standard is selected.
2. Set the slide control for *All inclusions* more to the position *High* (for example, to the value *200*). Observe the sample. The threshold value has been correctly set when the inclusions are completely recognized.

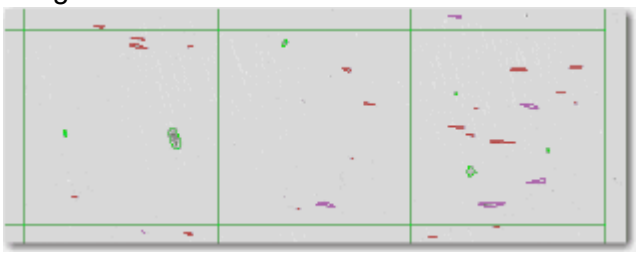


3. Set the slide control for *Oxide inclusions* more to the position *Low* (for example, to the value *50*).
4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Image results step

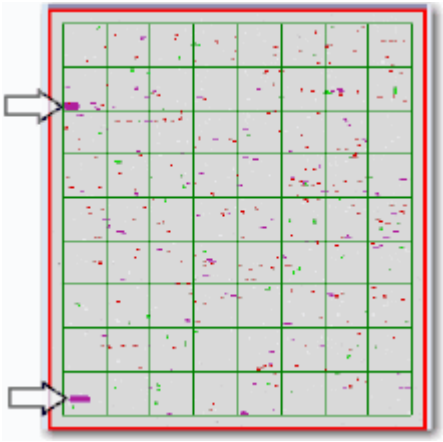
1. First, take a look at the displayed results in the image. Each inclusion that has been detected is now outlined in the color of the inclusion type it has been

assigned to.



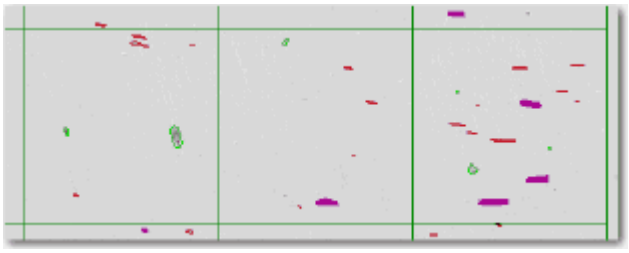
2. Then take a look at the results that are shown in the table *Inclusions results*. The table with the measurement results contains a classification of the inclusions that have been detected.
3. Click on a cell in the *Number* column to have all inclusions displayed in bold that correspond to the selected inclusion type and that have been assigned to the selected length class. The length classes can be found in the left column in the *Inclusions results* table.

	Number	Total Length [µm]
5.5	0	0
11.0	0	0
22.0	4	88
44.0	20	880
89.0	41	3649
178.0	12	2136
355.0	2	710
710.0	0	0
1420.0	0	0



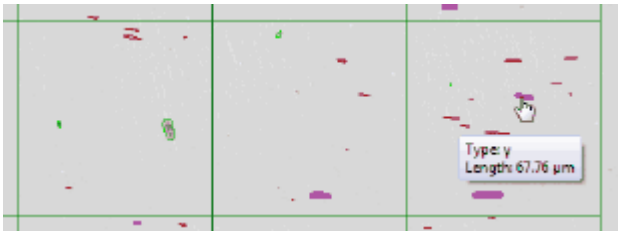
The illustration on the left shows an example where the 2 inclusions are selected in the table that have been assigned to the 355 µm length class.
The illustration on the right shows the *Image Navigator* tool window. The two inclusions have been highlighted which makes them easy to find (see the two arrows).

4. Select the *Show all inclusions of selected type* check box to display all inclusions of the currently selected type in bold in the image window, no matter what their size is.



5. If you want to see detailed results for an inclusion: Click the *Show Inclusion Results* button and move the mouse pointer in the image window to the required

inclusion.



- The details for the selected inclusion will be shown. Which details these are depends on the standard that has been selected. Usually, the type as well as the exact length and width are displayed. For some standards, the area is also shown. For some standards, if the length or width of an inclusion exceeds the specified limit *oversized* will also be displayed.

6. If you want to correct the automatically found inclusions, use the buttons in the *Edit inclusions* group.
7. Click the *Next* button.

Results step

1. Take a look at the results that are shown in the table. The following information is given:
    - Industry standard and method used
    - Sample name
    - Number of images
    - Total area of fields
    - Detailed sample results, sorted by inclusion types
2. Select the *Generate workbook* check box to have a document of the *Workbook* type automatically created at the end of the analysis.
3. If you want to save the current settings to a file, click the *Save settings* button. Then assign a descriptive name in the next dialog box.
4. Click the *Finish* button.

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9.7.4. Editing inclusions

There are two analysis processes available in your software for the analysis of non-metallic inclusions in metal samples:

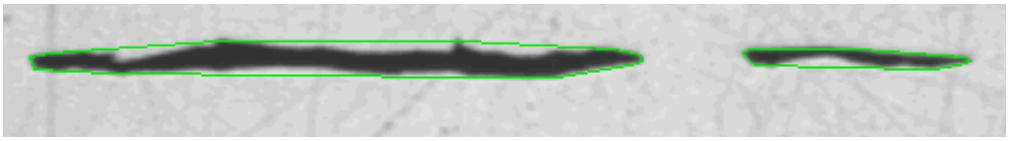
1. an analysis of the inclusion content
2. an inclusions worst field analysis

In both analysis processes, you can manually edit the inclusions that your software found automatically.

Note: If you have manually corrected inclusions and return to the *Settings* step (e.g., to change the settings of the slide controls), your manual corrections will be deleted.

Merging inclusions

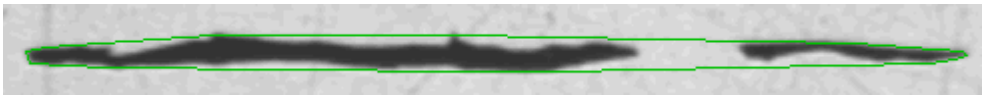
1. Enlarge the display of the image until you can easily recognize the two inclusions that you want to join.
   - In this example, these two inclusions are to be joined.



2. In the *Edit inclusions* group, click the *Merge inclusions* button.
   - The mouse pointer will change its form. You will then be in edit mode. The only thing you can do now is to merge inclusions. In this mode, other work with your software isn't possible.
3. With your left mouse button, click the two inclusions.

Note: Should you join two inclusions that belong to different inclusion types, the inclusion type of the first inclusion you selected will be used for the new joint inclusion. In this case, take care that you click the two inclusions in the right order.

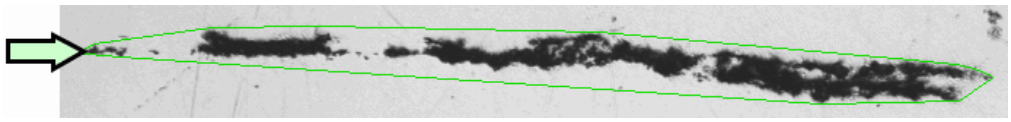
   - The inclusions will be joined. The results will be updated.



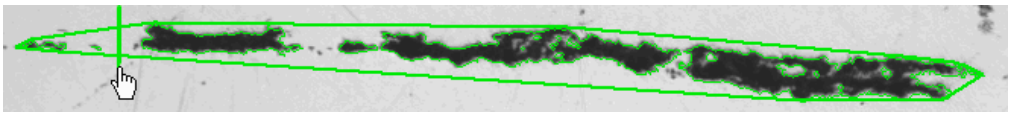
4. If you want to, you can merge further inclusions.
5. Click your right mouse button to leave the edit mode, and to confirm the changes.

Splitting an inclusion

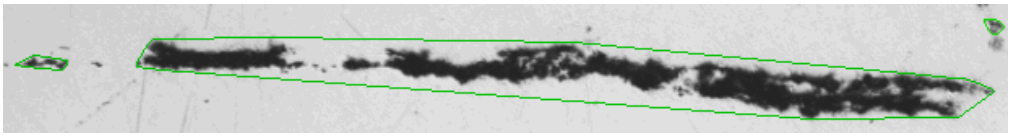
1. Enlarge the display of the image until you can easily recognize the inclusions that are to be separated.
   - In this example, the particle on the far left-hand side that is indicated by an arrow is to be split.



2. In the *Edit inclusions* group, click the *Split inclusion* button.
   - The mouse pointer will change its form. You will then be in edit mode. The only thing you can do now is to split inclusions. In this mode, other work with your software isn't possible.
3. To do so, click once with your left mouse button at an arbitrary position on the line surrounding the inclusion.
   - The surrounding line and all of the particles that belong to this inclusion will be displayed bold.
4. Click with your left mouse button at the position in the image where the separation line is to begin. This defines the line's start point.
5. Move the mouse pointer to draw a separation line through the object.



6. Click the left mouse button to confirm the separation.
   - The inclusion will be split.

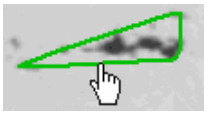


7. If you want to, you can split further inclusions.
8. Click your right mouse button to leave the edit mode, and to confirm the changes.
   - The results will be updated.

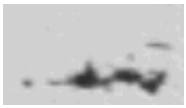
Deleting an inclusion

1. Enlarge the display of the image until you can easily recognize the inclusion that is to be deleted.

2. In the *Edit inclusions* group, click the *Delete inclusion* button.
   - The mouse pointer will change its form. You will then be in edit mode. The only thing you can do now is to delete inclusions. In this mode, other work with your software isn't possible.
3. Position your mouse pointer on the inclusion that is to be deleted.
   - The line surrounding the inclusion will be displayed bold.



4. Click the left mouse button.
   - The inclusion will be deleted.

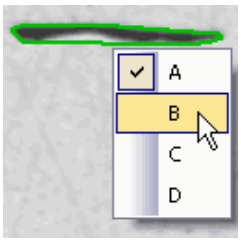


5. If you want to, you can delete further inclusions.
6. Click your right mouse button to leave the edit mode, and to confirm the changes.
   - The results will be updated.

Changing the inclusion type

1. Enlarge the display of the image until you can easily recognize the inclusion that is to be changed.

2. In the *Edit inclusions* group, click the *Change inclusion type* button.
   - The mouse pointer will change its form. You will then be in edit mode. The only thing you can do now is to change an inclusion's type. In this mode, other work with your software isn't possible.
3. Left click once on the inclusion that you want to assign to a different inclusions type.
   - A picklist will drop down. It shows all of the inclusion types that the currently chosen standard contains. A check marks the currently chosen inclusion type.



An example of what the picklist can look like. Depending on the standard that has been chosen, the picklist can contain other entries.

4. Select the new type of inclusion you want.
   - The new inclusion type will be assigned. In the image, the inclusion will now be displayed with a surrounding line in another color.

5. Click your right mouse button to leave the edit mode, and to confirm the changes.
   - The results will be updated.

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9.8. Throwing Power Measurement

9.8.1. What is a throwing power measurement?

Use the *Throwing Power* solution to determine the quality of the copper plating on an HDI panel. You can measure through-holes, microvias and filled microvias.

The *Throwing Power* solution is completely integrated into the *Materials Solutions* tool window. The tool window works in a similar way to a software wizard. As soon as you've started an analysis process you'll be guided step by step through the measurement.

Before you start a throwing power measurement

The following conditions must be met before you start a throwing power measurement.

1. Prepare suitable cross-sections through the panels. To measure a through-hole, you will also need a flat section of it.
2. The results of a throwing power measurement is usually saved in a database. You should thus open the required database.
   If no database exists yet, create one using the database template supplied.

   The flexible measurement mode is an exception. In this mode, the table with the measurement results isn't automatically saved.
3. Align your microscope.
4. Make sure that your software is correctly configured.
5. Start your software. Switch to live mode and select the best settings to acquire an image. While a throwing power measurement is in progress, you may no longer alter all settings for the image acquisition.
   - Check the white balance If necessary, carry out a white balance.
   - Select the live-image's resolution in the *Camera Control* tool window.

General procedure for a throwing power measurement

1. Selecting the analysis process
Click the *Throwing Power* button, located in the *Materials Solutions* tool window.

2. Step - Settings
Select one of the following measurement methods: *Through-holes*, *Microvias*, *Filled through-holes*, *Filled microvias*.
When you select the *Flexible mode* entry, the final two steps in the analysis disappear.

3. Step - Diameter
If you have selected the *Through-holes* measurement method: Place a flat section of the through-hole under your microscope. Measure the diameter of the through-hole.

4. Step - Measurements
Place a cross section through the panel under your microscope. Measure all of the parameters specified in the *Measurements* list.
Repeat this step in the analysis and measure other cross sections.

5. Step - Report images
Acquire three images for the final report.

6. Step (MS-Word, MS-Excel)
Produce a report with the results of the throwing power measurement. The procedure is slightly different, depending on whether you want to create the report in MS-Word or MS-Excel.

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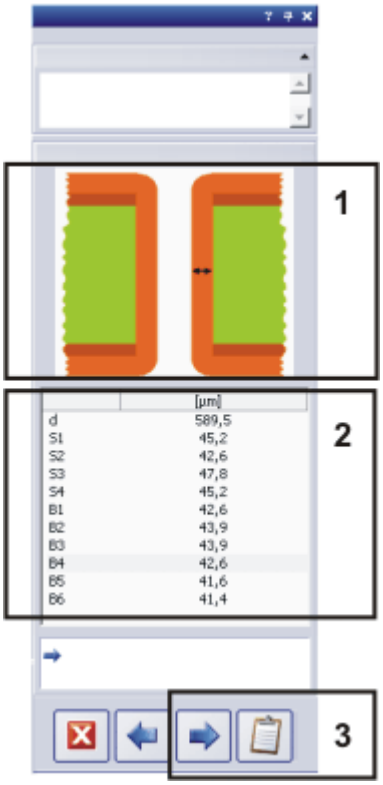
9.8.2. Measurements

In this step you carry out the actual throwing power measurement. The sequence and the measurement parameters are specified. Your software will show all of the required measurement parameters in a diagram.

The following options are available:



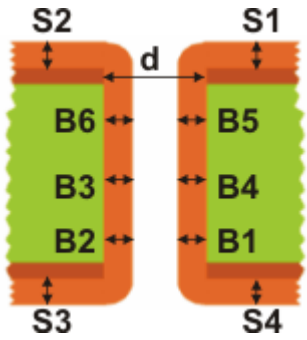
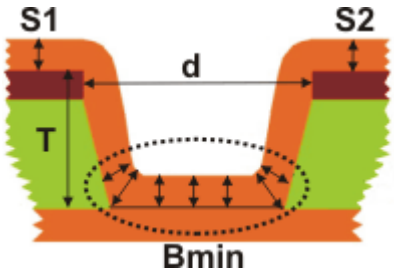
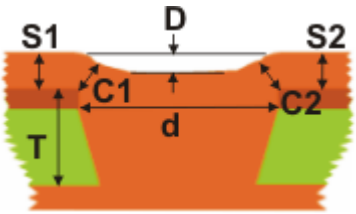
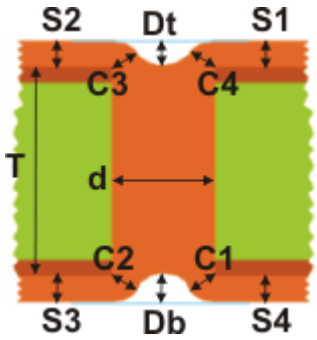
(**1**) Displaying the parameters to be measured
(**2**) Performing the throwing power measurement
(**3**) Continuing the throwing power measurement

(1) Displaying the parameters to be measured

Your software will provide you with visual support when performing the throwing power measurement. The distance which is to be measured next will be drawn in the schematic illustration at the top of the *Materials Solutions* tool window. The copper plating is shown in orange, the panel in green.

From the *Measurements* list, choose any measurement parameter to show the distance to be measured in the schematic illustration.

Required measurement parameters

Through-holes		**d** = diameter of the through-hole (additional remarks further below) **S1-4** = thickness of the surface plating **B1-6** = thickness of the plating inside the through-hole
Microvias		**d** = diameter of the microvia **T**= thickness of the panel **S1**, **S2** = thickness of the surface plating **Bmin** = minimum thickness at the bottom of the microvia (additional remarks further below)
Filled microvias		**d** = diameter of the microvia **T**= thickness of the panel **S1**, **S2** = thickness of the surface plating **C1**, **C2** = minimum corner plating thickness **D** = the dimple's height or depth (additional remarks further below)
Filled through-holes		**d** = diameter of the microvia **T**= thickness of the panel **S1-4** = thickness of the surface plating **C1-4** = minimum corner plating thickness at the top and bottom of the microvia **Dt** =the dimple's height or depth at the upper side **Db** = the dimple's height or depth at the lower side (additional remarks further below)

Remarks about the required measurement parameters

1. You can't precisely measure the actual **through-hole diameter** on the panel's cross section if the cross section doesn't run exactly through the center of the hole. Therefore, a separate measurement of the hole's diameter is needed on a flat section of the through hole.
2. Look for the minimum plating thickness in the area that is indicated in the illustration. Measure this thickness to get the **Bmin** parameter.
3. A **Dimple** represents the difference in heights between the plated copper within a microvia or through-hole and around the perimeter of that microvia or through-hole. When the filled microvia or through-hole is not completely filled, the measurement value is positive.
   When the filled microvia or through-hole is overfilled, the measurement value is negative.

Instruction images for flexible measurement mode

You can also use the *Throwing Power* Solution to define your own rules for measuring lengths on similar samples. You can provide the user with visual support when performing the throwing power measurement by providing instruction images.

(2) Performing the throwing power measurement

Measure the distance which is shown in the diagram. To do that, click with the left mouse button on the start and end points of the distance to be measured.

(3) Continuing the throwing power measurement

As soon as you have measured all of the parameters which are required for the selected measurement method, the *Next* and *Get Results* button will become active in the lower part of the dialog box.

Performing additional measurements

Click the *Next* button to measure more structures on your panel. All of the measurement parameters will be reset for the new measurement. The previously measured parameters will be saved and will be included in the analysis, which will be issued in a report at the end of the throwing power measurement. If you would like to examine the individual measurements again later on, save a workbook in the database in the *Reporting* step.

Finishing the throwing power measurement

Click the *Get Results* button. With this, the actual throwing power measurement is finished. Acquire pictures for the report now and then create the report.

If you are using a user-defined measurement method, click the *Finish* button to end the measurement.

Possible warnings once the measurement is complete

To get statistically reliable measurement results, you will have to measure several though-holes or microvia. You can set a required minimum number of between 1 and 10. To do this, use the *Tools > Options > Materials Solutions > Throwing Power* dialog box. Three measurements is preset as the minimum number.
If you finish the measurement before that, a corresponding warning message will be produced.

During the analysis, the arithmetic mean of all of the measurements carried out will be calculated. If the standard deviation for a measurement parameter exceeds 5%, you will also get a corresponding warning. In this case, measure another five structures on the panel, to increase the statistical reliability.

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9.8.3. Measuring the throwing power of a microvia on a circuit board

Example: These step-by-step instructions describe the *Microvias* measurement method as an example of a throwing power measurement. The other measurement methods which are available work in a similar way.

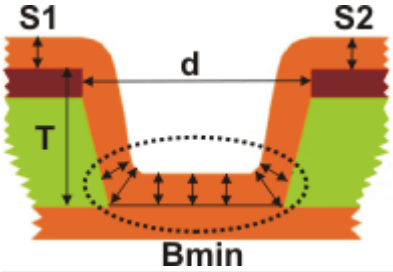
1. Prepare the throwing power measurement.
2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Throwing Power* button.
   - The *Materials Solutions* tool window displays the *Settings* step.

Selecting the measurement method

4. From the *Measurement method* list, select the *Microvias* entry.
   - In the *Materials Solutions* tool window you will now see a schematic illustration, which shows the cross section through a microvia.

5. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.
   - Your software will automatically switch to the live mode.
   - The *Camera Control* and *Microscope Control* toolbars will be displayed so that you can set the exposure time and the current magnification.
   - The *Measurements* table in the *Materials Solutions* tool window contains the required parameters to measure the throwing power of a microvia. The first measurement parameter, **d**, will automatically be selected and will be shown in the schematic illustration in a tool window.



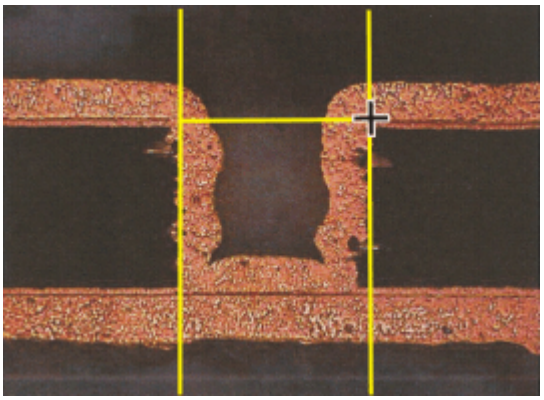
The *Microvias* measurement method contains the following parameters: *d* = diameter of the microvia, **T** = thickness of the circuit board, **S1** and **S2** = thickness of the surface coating of the track, **Bmin** = minimum thickness of the coating at the base of the microvia

Measuring the first microvia

6. Place one of the circuit board cross sections to be measured under your microscope. Refer to the cross section as shown in the schematic illustration.
7. Move the stage so that the first distance to be measured can be clearly seen in the live image and adjust the focus.
8. Select the best magnification. To do that, in the *Microscope Control* toolbar, click the required objective's button.

Note: The images which you acquire with your software will only be correctly calibrated if you specify the current objective magnification before acquiring the image. Correctly calibrating the image is a requirement for a correct measurement.

9. Set a zoom factor for your image window that will make the microvia that is to be measured clearly visible. You can rotate the mouse wheel to change the zoom factor of the live image in the image window.
   You will achieve the most precise measurements if you set the zoom factor at 100%.
10. If necessary, adjust the exposure time.
11. Measure the distance which is shown in the diagram. To do that, click with the left mouse button on the start and end points of the distance to be measured.
    - The line you have measured will be displayed in the image.
    - The result will be displayed in the *Materials Solutions* tool window in the *Measurements* table.
    - Your software will now automatically activate the next parameter to be measured in the *Measurements* table and will also show this in the diagram.



In the live image, measure the diameter of the microvia. The two helper lines stand vertically on the measured distance and help you to align the measured distance exactly with the microvia's borders.

12. Move the stage so that the next distance to be measured can be clearly seen in the live image and adjust the focus.
    If necessary, select another objective magnification to be able to measure the distance with the best accuracy.
13. Measure the required distance.
14. Repeat the last steps until you have measured all of the required parameters.
    For the last measurement parameter, Bmin, measure the lowest thickness of the coating within a certain area. This area is circled in the schematic illustration.
    - As soon as you have measured all of the parameters which are required for the selected measurement method, the *Next* and *Get Results* buttons will become active in the lower part of the dialog box.

Measuring the other microvias

15. Click the *Next* button to conclude the measurement for the current microvia.
    - The *Materials Solutions* tool window displays the *Measurements* step.
    - As an intermediate step, your software will save all of the values measured so far.
    - All values from the last measurement will be deleted from the *Measurements* table.
16. Now, measure the next microvia. To get statistically reliable measurement results, you will have to measure several microvia.

Finishing the throwing power measurement

17. Click the *Get Results* button when you have measured the required number of microvias.
    - The *Materials Solutions* tool window displays the *Report images* step.
18. Acquire three images to document the measurement. You could for example, acquire three different cross sections at a low magnification. Or you acquire an overview image of a microvia and then acquire two images at a higher magnification showing interesting details.
    If necessary, change the sample for this. Move the stage to the required location.

Select a suitable magnification and exposure time and focus on the sample. Click on this button to acquire the image.

- The images acquired will be displayed in the *Materials Solutions* tool window.

Acquire three images to finish the throwing power measurement.

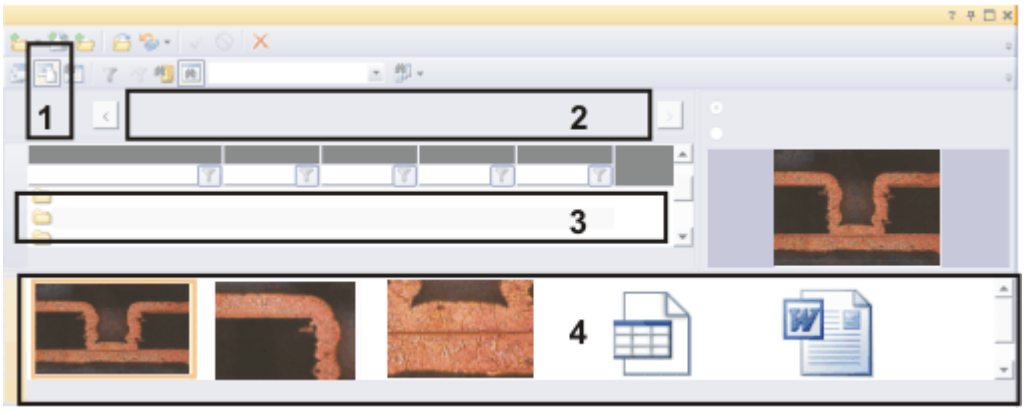
19. Open the database where you would like to save the measurement results. In the database, select the folder where the measurement results are to be saved, or create a new record.

20. Click the *Next* button.
    - The *Materials Solutions* tool window displays the *Reporting* step. In the *Template* group, you'll see a preview of the document template that has currently been chosen.
21. Select the *Add workbook to the database* check box.
22. Start the MS Word application.

23. Click this button .
    - This finishes the throwing power measurement.
    - The *Materials Solutions* tool window switches back to the start position.
    - The three images that are acquired will be saved in the database. The name of the images in the database is determined by the current default value for the *Image name* database field. The database administrator can set up this default value.
    - A workbook with the measurement results will be created and saved in the database.
    - The concluding report will be generated and displayed in MS-Word.

Editing the report and saving it

1. Check the report in MS Word. If necessary, add more text.
2. When you are satisfied with the report, in MS Word, use the *Olympus > Save to Database* command to also insert the report into the database. Before doing that, make sure that the correct database folder has been selected.



The results of a throwing power measurement will be saved in the database. You can for example access the data in the document view of the database (**1**). The project header view (**2**) shows the higher level database folder. In the sample list view (**3**) the database folder will be selected which contains the data. The gallery view (**4**) shows the three images acquired, the workbook with the measurement results and the saved MS Word report.

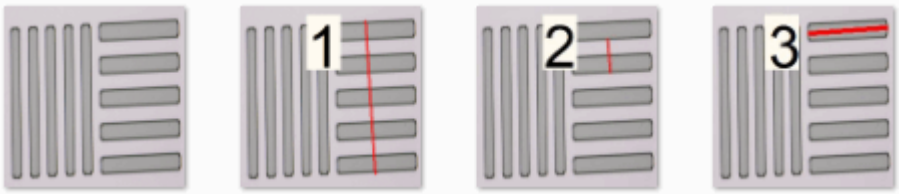
Loading the measurement results

3. In your software, open the database where the measurement results are saved.
4. Switch to your database's document view. Select the database folder which contains the measurement results. If your database is based on the supplied database template, then the database folder is a record of type *Sample*.
5. In the gallery view, double click, for instance, on the symbol for the workbook to view the measurement results.
   - The workbook contains the values for all of the microvias measured.
   - Statistical values e.g. standard deviation, can also be displayed in the workbook. You can specify exactly which statistical values will be shown. To do this, open the *Tools > Options > Measurement and ROI > Results* dialog box.

9.8.4. Performing a flexible number of length measurements on more than one sample

You can also use the *Throwing Power* Solution to define your own rules for measuring lengths on similar samples.

Example: You want to measure several distances on different wafers. Create a user-defined measurement method and perform the measurement on 10 wafers.



The first image shows a part of a wafer. You want to measure the same three distances on different wafers (**1**-**3**).

Creating a preview image and an instruction image.

When you are making measurements on several samples, the distances always have to be measured in the same order. You can provide the user with visual support when performing the throwing power measurement by providing instruction images.

The image must meet the following conditions:

- All of the files must be saved in PNG format.
- All of the files must be in the same directory.
- The file names must have the following syntax:
  00.png - Preview image
  01.png - Instruction image for the measurement of the first distance
  02.png - Instruction image for the measurement of the second distance
- There must be an image in the folder for each distance that you want to measure.
- The image resolution must be 200x200 pixels.

1. Create an image that shows the sample that you want to measure.
2. Create an image for each measurement that you want to perform. The image should show the distance that you want to measure as well as the name of the measurement. You can either simply number the measurements, or you can name the measurement parameters.

Defining your own measurement method

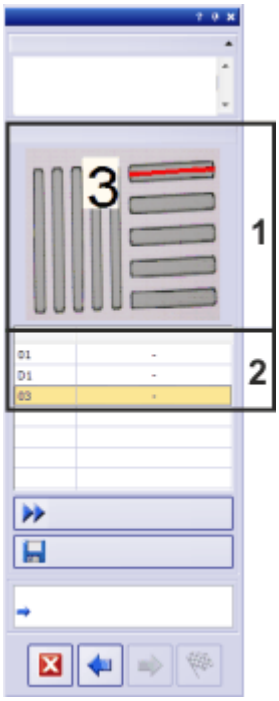
3. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

4. Click the *Throwing Power* button.
   - The *Materials Solutions* tool window displays the *Settings* step.
5. From the *Measurement method* list, select the *Flexible mode* entry.
   - Additional setting options now appear in the *Materials Solutions* tool window.

6. This example measures three distances. Therefore, enter a value of 3 in the *Number of measurements* field.
7. This example measures only distances. Therefore, clear the *Additional parameters* check box.
8. For this example, we want to create a report in the MS-Excel application program at the end of the analysis. Select the *Generate report in Excel* check box and leave the *Generate workbook* check box clear.

9. Click the *Next* button.
   - The *Materials Solutions* tool window displays the *Measurements* step.
   - The table contains three entries, with the measurement parameters *01*, *02* and *03*. Each parameter represents one of the distances that will be measured on the sample.
   - If necessary, you can rename the measurement parameters now. To do this, double click the name of the parameter and enter the required name.

10. Click the *Save Settings...* button.
    - A dialog box for saving parameter sets opens.
11. Enter a descriptive name for the user-defined measurement method in the *Name* field, *Wafer-3d* for example.
12. In the *Description* field, enter a comprehensive description of the measurement method. This description will be shown in the *Materials Solutions* tool window later when you perform the measurement.
13. Select the *Public* option. The measurement method can now also be used by other users. You will be able to recognize this by the small icon () next to the parameter set's name.
14. Click the *Save* button to close the dialog box.
    - A message box appears.
15. Click the *Yes* button and go to the folder that contains the preview and instruction images for the user-defined measurement method.



In the *Materials Solutions* tool window, the instruction image (**1**) that belongs to the selected measurement parameter (**2**) is displayed.

- If the images in the folder that you selected don't meet the requirements, an error message appears.
- The images are copied and saved together with the parameter set.

16. You can now start with the measurement itself. If you only wanted to define the measurement method, click this button in the *Materials Solutions* tool window's navigation area.

Performing a measurement with a measurement method that has already been defined

1. Click the *Throwing Power* button, located in the *Materials Solutions* tool window.
   - In the *Measurement method* list, you find all of the user-defined measurement methods that have been saved.
2. Select the *Wafer-3d* entry from the *Measurement method* list.
   - In the *Materials Solutions* tool window, you can now see the description and the preview image.

3. Click the *Next* button.
   - Your software will automatically switch to the live mode.
   - The *Camera Control* and *Microscope Control* toolbars will be displayed so that you can set the exposure time and the current magnification.
   - The *Measurements* table in the *Materials Solutions* tool window contains the required measurement parameters. The first measurement parameter is selected automatically and the first instruction image is displayed in the tool window.

4. Place one of the samples that you want to measure under your microscope. Move to the position on the sample that is shown in the preview image.
5. Measure the distance that is shown in the instruction image. To do that, click with the left mouse button on the start and end points of the distance to be measured.
   - The line you have measured will be displayed in the image.
   - The result will be displayed in the *Materials Solutions* tool window in the *Measurements* table.
   - Your software now automatically activates the next parameter to be measured in the *Measurements* table. The instruction image is updated automatically.
6. Measure the required distance.
   - As soon as you have measured all of the parameters which are defined in the measurement method, the *Next* and *Finish* buttons in the lower part of the dialog box become active.

7. Click the *Next* button to conclude the measurement for the current sample.
   - The *Materials Solutions* tool window displays the *Measurements* step.
   - As an intermediate step, your software will save all of the values measured so far.
   - All values from the last measurement will be deleted from the *Measurements* table.
8. Now, measure the next sample or the next position on the sample. To get statistically reliable measurement results, you have to perform several measurements.

9. Click the *Next* button to show the next step *Reporting*.
   - You will only see this step if you selected the *Generate report in Excel* check box in the previous step *Settings*.

10. Decide whether you want to use a default report template or a user-defined report template. If you would like to select a user-defined report template, select the *User defined* option. Click the button with the three points and select the new report template in the *Open* dialog box.

11. Click the *Finish* button to complete the measurement.
    - The MS-Excel application program opens and the report is displayed.
12. Use the *File > Save* command to save the report. Give it a descriptive name.

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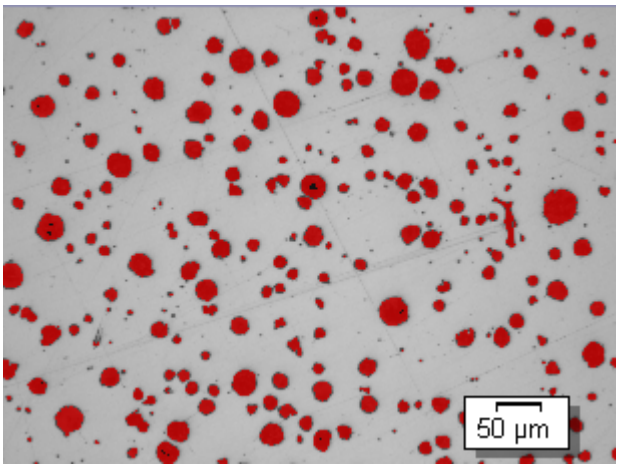
9.9. Porosity Measurement

9.9.1. What is a porosity measurement?

With a porosity measurement, you measure the percentage of the surface of your sample which is made up of pores as well as determining the number and density of the pores. If the size of the pores is also being determined, all of the pores that exceed a defined maximum pore size can be displayed in color in the image. In this case, the largest pore can be displayed in color in the image as well.

Note: The samples are usually metallographic sections that have been prepared especially for porosity measurements. The porosity that is measured only applies to the exposed cross-sectional plane of the sample. Therefore the porosity of other parts of the sample that are above or below the cross-sectional plane may be different.

It is a precondition for a porosity measurement that the pores differ from the rest of the sample, for example, because they are darker. The pores thus have differing intensity values to the rest of the sample, making automatic analysis of the image possible. For the image analysis, so-called phases are defined which cover a certain range of intensity values.



Porosity measurement on one image. All of the pixels which lie within the defined intensity range, will be shown in color during this step in the analysis. In the example shown, red has been selected for the phase.

Selecting the industry standard

One of the following standards can be used for the measurement if required.

- VW 50093/P 6093:2012
- VDG P 201-2002
- VDG P 202-2010
- VDG P 211-2010

Manually processing the image porosity value

You can manually adjust the result of the automatic image analysis. You do this interactively on the image. Note that you are not changing the image itself, but the

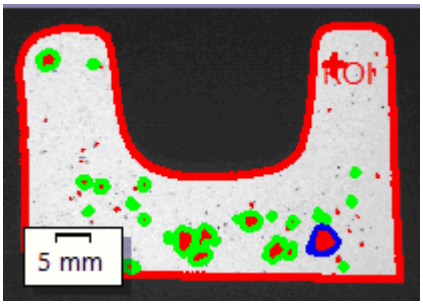
image's measurement layer.

You can manually delete parts of the image which have been recognized as pores (in image analysis, one speaks of detected objects). This can be necessary, if for example artifacts in the image are recognized as pores because they have similar intensity values. By manually deleting these objects, the artifacts will be excluded from the analysis.

In addition, you can also manually add other image segments which were not detected as such but which are actually pores. With the manual addition and deletion of objects, you always change the percentage porosity value of the image.

Measuring on ROIs

You can choose whether you would like to measure the entire image or if the measurement should only be carried out on a part of the image, a so called ROI (Region Of Interest). You can also define several ROIs.

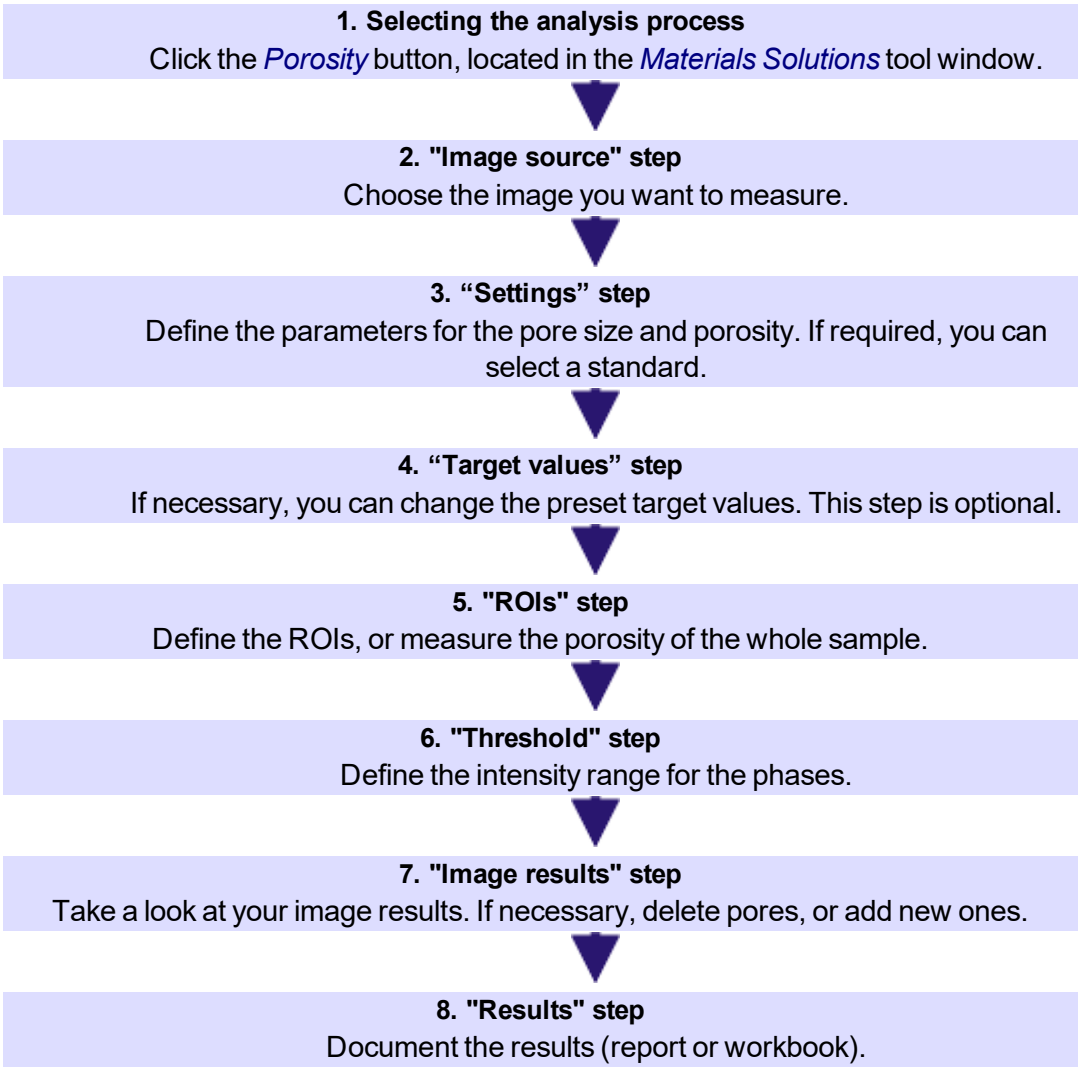


On the image, the porosity of an ROI is measured.

Results of a porosity measurement

The results of an analysis can be displayed in a workbook. Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.

General procedure for a porosity measurement



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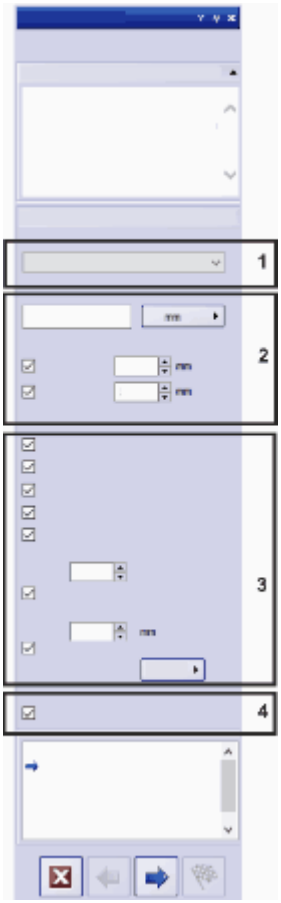
9.9.2. Settings

In this step in the analysis, you make all of the settings that you want to apply to the porosity measurement. First of all, at the top of the tool window select which standard you want to use, if any.

If you are analyzing more than one image at the same time, the *Settings* step is only displayed for the first image. The settings that you make here are automatically applied to all of the other images.



(1) Selecting the industry standard

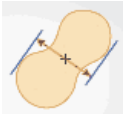
Decide whether you want to use one of the following standards for the porosity measurement. The *None* entry is selected by default. This means that no standard will be used.

- VW 50093/P 6093:2012
- VDG P 201-2002
- VDG P 202-2010
- VDG P 211-2010

When a standard is selected, some of the fields in the tool window change. For example, the *Pore accumulations* and *Pore nests* check boxes are only displayed when you have selected a standard.

(2) Pore size parameter

In the *Pore size parameter* field, choose how the pore size is calculated.



Choose the *Max. (Feret)* setting to use the maximum spacing of parallel tangents on opposing sides of particles.

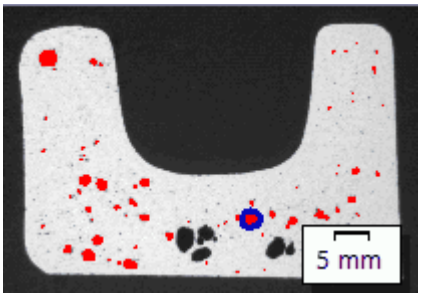
Choose the *Equivalent Circle Diameter* setting, to use the diameter of a circle which has the same area as the particle.

If necessary, click on the button which shows the units and select the units in which the image to be analyzed has been calibrated.

Disregarded pores

In the *Lower limit* field, enter the minimum size that an object must have to be considered when determining the number of pores. In the *Upper limit* field, enter the maximum size that an object may have to be considered when determining the number of pores.

Note: In the *Image results* step, the disregarded pores are shown as not having been detected. They don't have a colored overlay.



Example of the way that disregarded pores are displayed in the *Image results* step. The pores that are displayed without a colored overlay exceed the value specified in the *Upper limit* field.

(3) Porosity parameters

Select which parameters you want to use to determine the porosity.

Parameters	Description
Porosity	When this check box is selected, the porosity will be determined. Which algorithm will be used depends on the standard that has been selected and on the settings in the *Settings* and *Target values* steps. If necessary, you can view and change the value in the *Permissible porosity* field in the *Target values* step. The porosity is expressed in %.
Pore size	When this check box is selected, the pore size will be determined. Pores that exceed the maximum permissible pore size are displayed with a colored edge in the *Image results* step. If you haven't changed the color in the program options (*Tools > Options > Materials Solutions > Porosity*) the color *Green* will be used for this. When the pore size is determined, the **largest** pore is also displayed with a colored edge by default in the *Image results* step. If you haven't changed the color in the program options, the color *Blue* will be used for this. If required, the maximum permissible pore size is defined by the standard being used. If necessary, you can view and change the pore size in the *Max. permissible pore size* field in the *Target values* step.

Number of pores	When this check box is selected, the number of pores will be determined. If you have defined ROIs, only the number of pores that are inside the ROIs will be determined. You can view and change the number of pores in the *Permissible number of pores* field in the *Target values* step.
Distance of adjacent pores	When this check box is selected, the distance between two adjacent pores will be determined. Pores that are at **less than** the permissible distance will not be included in the results. If necessary, you can view and change the distance in the *Permissible distance factor* field in the *Target values* step.
Pore accumulations > *Distance factor*	When this check box is selected, your software will search for what are called pore accumulations. The definition of a pore accumulation is that the distance between two pores is smaller than the diameter of the smaller of the two pores (when the value is set to 1 in the *Distance factor* field). If necessary, you can view and change the value in the *Pore accumulations* field in the *Target values* step.
Pore nests > *Max. permissible pore size*	When this check box is selected, your software will search for what are called pore nests. These are groups of pores that have an even larger area than pore accumulations. Pore nests will only be searched for if the value in the *Max. permissible pore size* field is larger than 0. If necessary, you can view and change the value in the *Pore nests* field in the *Target values* step.
Pore density > *Unit*	When this check box is selected, your software will calculate how densely the found objects are spaced in the defined area. If required, in the *Unit* field, change the units used to show the pore density in the results. The unit is always a unit of area (for example, 1 mm² or 1 µm²). The sample density selected in the *Unit* field must match the unit in which the image that you want to analyze is calibrated. If necessary, you can view and change the value in the *Permissible pore density* field in the *Target values* step.

(4) Displaying the “Target values” step

The *Target values* step is optional. The check box is not selected by default. Select the *Define target values* check box if you want to view or change the default settings, or if you want to view the *Target key* field.

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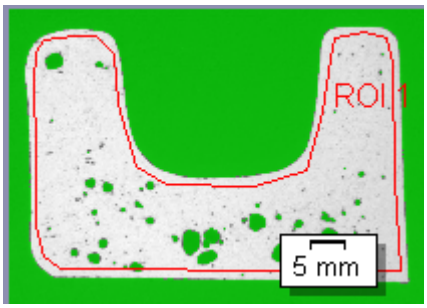
9.9.3. Threshold

All of the pixels which lie within an automatically defined intensity range, will be shown in color during this step in the analysis. This intensity range is called a "phase".

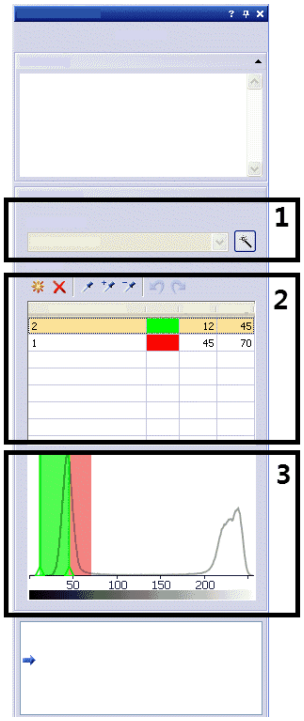
The intensity value range is limited by a top and a bottom intensity value. These are the so-called threshold values.

In this step in the analysis you can change the threshold values. You can also create another phase.



On the left you can see a sample for which only one phase has been defined.

Please note that defined ROIs will not be considered in this step in the analysis, but only in the next step.



(1) "Component" field

Click the *Automatic Threshold Computation* button to have the threshold values initially calculated automatically. Then you can manually process them, if necessary. The *Automatic Threshold Computation* dialog box opens. Make the necessary settings here.

If you measure the porosity in a color image, in the *Component* list you can select whether the threshold value is to be determined on the intensity value or on the red, green or blue part of the image. The threshold value setting in color images is more complex than it is in gray-value images.

(2) Defining threshold values

Note: If you would like to set threshold values for several phases in a gray-value image, you will have to begin by setting the threshold value for the darkest phase. Then set the threshold value for the next phase, and so on.

Click the *New Threshold* button to set an initial value for the selected phase's threshold value range. As soon as you move your mouse pointer onto the image it will change its shape to that of a pipette.
Click on one pixel or on the image area whose intensity value is to be utilized as the initial value for the threshold range. All of the pixels that have the same intensity value will be colored in the image, and displayed in the histogram. The threshold value range initially contains only this one intensity value. As a rule, you will still need to expand this threshold value range. Continue clicking relevant pixels or threshold value ranges, until all of the required structures in the image are a part of the phase.

Click the *Add Threshold* button to select additional pixels that are to belong to the threshold value range. The image segments will be colored and displayed in the histogram. The current threshold value range will continue to be expanded until it contains the intensity values of all of the selected pixels.

Click the *Shrink Threshold* button to select pixels that aren't to belong to the threshold value range. The threshold value range will continue to be reduced until it no longer contains the pixels you have selected.

Click the *Undo Pipet* button to undo the last selections step by step. Click the *Redo Pipet* button to restore the last selections that were undone, step by step.

Adding, changing, and deleting phases

Click on the *Add Phase* button to add a phase for which the threshold values are to be calculated automatically. Double click on the field in the *Phase Name* column, to enter a name for the phase.

Double click on the field in the *Color* column to choose a color. The phase will be displayed in the color you have assigned it, in the image window and in the histogram. The intensity range for the phase will be automatically calculated. In the *[Min.* field, the lower threshold value will be specified. In the *Max.[* field the higher value will be specified. You can change the values here or you can change them interactively in the histogram.

Click the *Remove Phase* button to delete a phase. It's only possible to remove a phase when at least two phases have been defined.

(3) Changing threshold values interactively in the histogram

The histogram shows the intensity distribution of the active image. If the image mainly consists of light and dark areas, the histogram will show two peaks. A peak is an intensity value (or an intensity range) which occurs particularly frequently in the image.

The intensity range which was defined for a phase will be shown in color in the histogram. You can move the edges of the range in the histogram. To do that, move

the mouse pointer to the edge of the slide. If you have more than one phase, the phase that you would like to change must be selected in the table.

When the mouse pointer changes shape, click and drag the edge of the range in the required direction. The values in the *[Min.* and *Max.[* fields in the table will change. In the image, there will now be more or less pixels in the color of the phase.

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9.9.4. Performing a porosity measurement

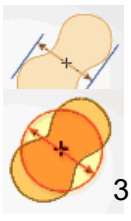
Step - Image source

1. Load the MacroscopicComponent.tif example image.
   - The porosity is to be measured in this image.
2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Porosity* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
   - By doing so, you skip the *Sample information* step which is not relevant for this example image.
6. Select the *All images* entry in the *Check settings and results* list.
7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Settings

1. Decide whether you want to perform the porosity measurement according to a particular standard. For these step-by-step instructions, use the *VDG P 202-2010* standard.
2. In the *Pore size parameter* field, choose how the pore size is calculated.



   - Choose the *Max. (Feret)* setting to use the maximum spacing of parallel tangents on opposing sides of particles.
   - Choose the *Equivalent Circle Diameter* setting, to use the diameter of a circle which has the same area as the particle.
3. The example image is calibrated in millimeters. You should thus click on the button which shows the units (at the right, next to the *Minimum size for counting* field), and select mm as the units.
4. For these step-by-step instructions, leave the *Lower limit* check box and the *Upper limit* check box in the *Disregarded pores* group clear.
5. In the *Porosity parameters* group, select the following check boxes. *Porosity*, *Pore*

size, *Number of pores*. Leave the other check boxes clear for these step-by-step instructions.

6. Leave the *Define target values* check box selected.
   - Then the *Target values* step, which is optional, will be shown. In this step in the process you can view and change the porosity values that the sample you are analyzing must attain.
7. Click the *Next* button.

Step - Target values

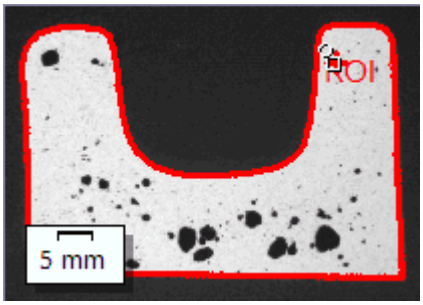
1. The values that the sample you are analyzing must attain to pass the porosity measurement are displayed at the top of the *Target values* step. These values are shown in the *Target key* field in the lower part of the tool window.
   - The target key formats the values that you entered in a way specified by the standard you are using. Some of the values in the target key are rounded up or down. The more parameters that are analyzed for the porosity measurement, the longer the target key will be.

Example: The target key **VDG P202-%10/Ø1** means: The **VDG P202** standard was used. The permissible porosity is 10% (written **%10**). The maximum permissible pore size is 1mm (written **Ø1**).

   - At the end of the measurement, the *Porosity key* field is displayed in the *Image results* step. The porosity key shows the results of the measurement. All of these values have also been rounded up or down. The format of the porosity key and the target key are identical. This enables you to quickly compare the required measurement results with the measurement results that were attained.
2. Because no adjustments are required for the MacroscopicComponent.tif example image: Click the *Next >* button.
   - For your own samples you will later need to enter suitable values here. You can save these and use them for subsequent measurements.

Step - ROIs

1. For the MacroscopicComponent.tif example image, define a ROI which encompasses the shape of the object. To do this click the *Create magic wand ROIs* button and click a bright point in the image inside the component whose porosity you are measuring.

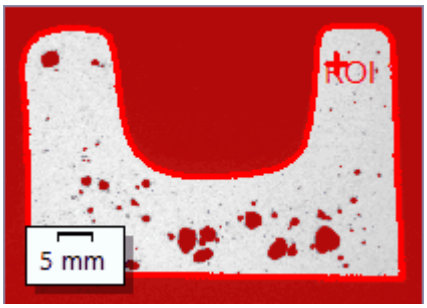


- The ROI will be displayed. If required, you can change the size and shape of the ROI by changing the parameters in the *Magic wand properties* group.
- It is not absolutely necessary to define ROIs. So you can't make any settings in the *ROIs* step.

2. Click the *Next* button.

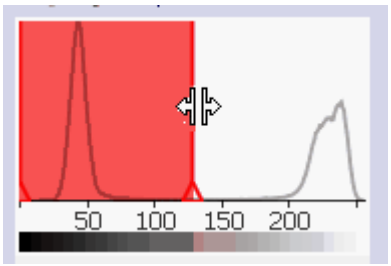
Step - Threshold

All of the pixels which lie within a defined intensity range, will be shown in color during this step in the analysis. This intensity range is called a "phase". The intensity value range is limited by a top and a bottom intensity value. These are the so-called threshold values.



Please note that the defined ROI will not be considered in this step in the analysis, but only in the next step. This is why the background color in this step is also shown in color.

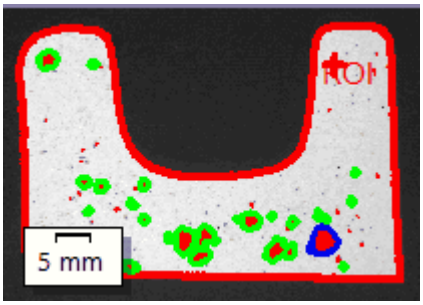
1. If necessary, reduce or increase the intensity range of the phase. In the image, watch how the object areas found become larger and more objects are found.
   - To do this, change the values in the *Min.* and *Max.* fields in the table in the tool window. Alternatively, interactively change the lower and upper threshold values in the histogram shown at the bottom of the tool window. Move the mouse pointer over the edge of the phase, until the pointer changes and, with the left mouse button pressed, drag the edge in the required direction.



2. Click the *Next* button.

Step - Image results

1. Take a look at the results in the overlay. All of the objects used to determine the porosity value will, in this step in the analysis, be shown in the color that has been selected for the phase.



- When the *Show largest pore* check box is selected, the largest pore that was detected will be displayed with a colored edge in the overlay. In the program options, the color *Blue* is the default color.
- When the *Show pores exceeding max. permissible pore size* check box is selected, the pores that exceed the maximum pore size will also be displayed with a colored edge. In the program options, the color *Green* is the default color.

2. Select the *Image* option and take a look at the results displayed in the table.
   - The porosity value is displayed. You can also compare the target key with the porosity key here.
3. If necessary, manually add objects or delete objects that have been detected. To do this, use the two buttons in the lower part of the tool window.

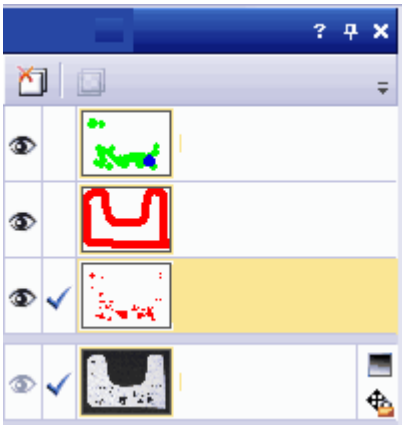
   - The results that are shown in the table are refreshed immediately.
4. Click the *Next* button.

Step - Results

Select the results you want.

Step - Reporting

1. Select the *Default* option to use the template that has been defined as the default template. If you would like to select another template, select the *User defined* option. Click the button with the three points and select the new template in the *Open* dialog box.
2. When you want to create an MS-Word report: In the *Content* group, select the check box for the pages the report should contain.
3. When you want to create an MS-Excel report: You can click the *Save settings* button to save the current settings in a file.
   - These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.
4. Click the *Finish* button.
5. Through the materials analysis measurement, the image has collected one or more additional layers (can be seen in the *Layers* tool window). Save the image in TIF or VSI format to retain these newly created image layers.



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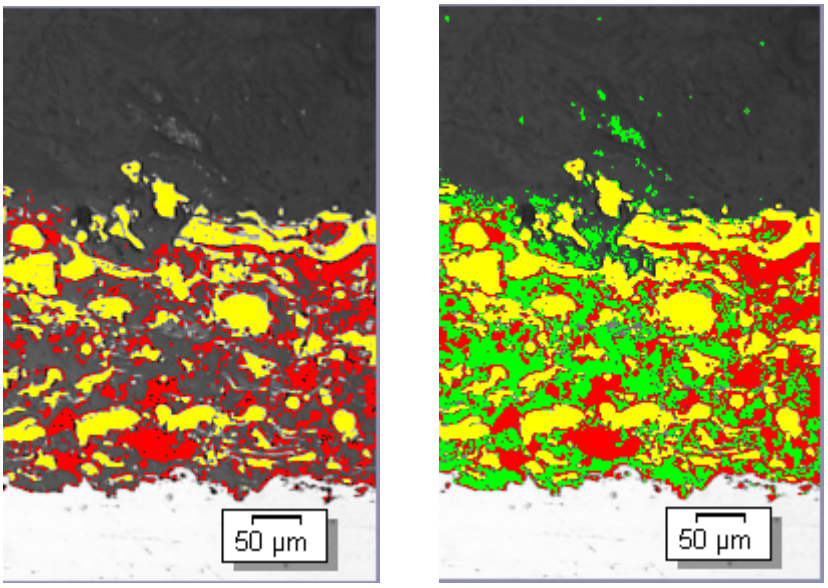
9.10. Phase Analysis

9.10.1. What is a phase analysis?

With a phase analysis, you measure the percentage of the area fraction that the phase covers in your samples. A phase is a number of pixels which lie within a defined intensity range. The intensity value range is limited by a top and a bottom intensity value. These are the so-called threshold values.

It is a precondition for the phase analysis, that the phases differ from the rest of the sample e.g. because they are darker or lighter. You can define one or more phases. If the parts (objects) of the sample whose area fraction you would like to measure mostly have the same intensity values, then one phase is enough. If the objects have very different intensity values, then several phases will have to be set up.

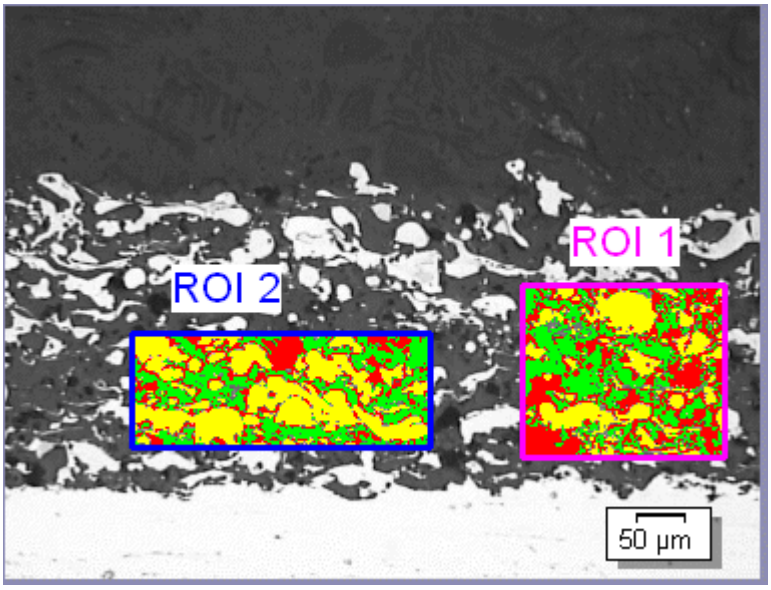


With a phase analysis you can define phases and measure the percentage of the area fraction that a particular phase covers. To the left, you can see an example for a phase analysis with two phases (light and dark). In the right example, a third phase was created for the same sample which covers the pixels lying between the dark and the light phase.

The result of the automatic image analysis can be restricted by an object filter. Objects which do not reach the minimum object size will not be included when determining the area fraction covered by the phase in percent. In this way you can, for example, prevent dust particles being assigned to a phase and distorting the result.

Measuring on ROIs

You can choose whether you would like to measure the entire image or if the measurement should only be carried out on a part of the image, a so called ROI (Region Of Interest). You can also define several ROIs.



On this image, the area fraction covered by the phases is measured on two ROIs.

Manually adjusting the result of the automatic image analysis

You can manually adjust the result of the automatic image analysis. You do this interactively on the image. Note that you are not changing the image itself, but the image's measurement layer.

You can manually delete parts of the image which were detected as objects. This can be necessary, if for example artifacts in the image are detected as objects because they have intensity values similar to the defined phase. By manually deleting these objects, the artifacts will no longer be included when determining the area fraction covered by this phase.

In addition, you can also manually add other image segments which were not detected as such but which are actually objects. When you manually add and delete objects, you always change the area fraction covered by the corresponding phase.

Results of a phase analysis

The results of an analysis can be displayed in a workbook. Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.

General procedure for a phase analysis

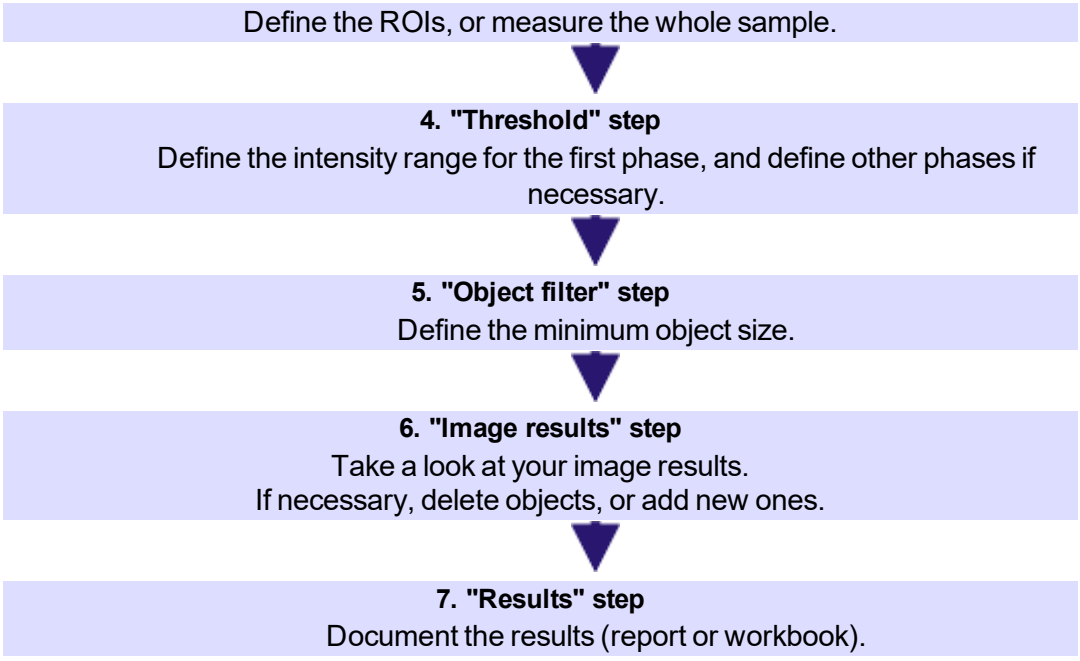
1. Selecting the analysis process
Click the *Phase Analysis* button, located in the *Materials Solutions* tool window.

▼

2. "Image source" step
Choose the image you want to measure.

▼

3. "ROIs" step



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9.10.2. Performing a phase analysis

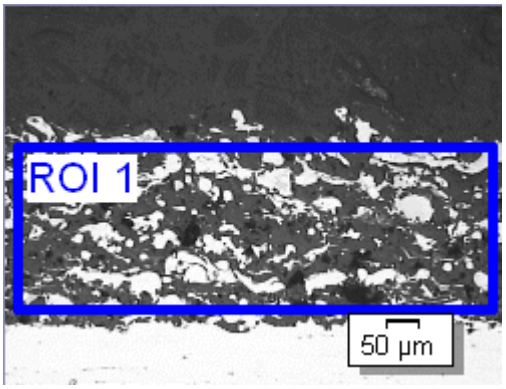
Step - Image source

1. Load the SprayCoating.tif example image.
   - In this image, the percentage area of the bright and the dark phases are to be measured within an ROI.
2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Phase Analysis* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
   - By doing so, you skip the *Sample information* step which is not relevant for this example image.
6. Select the *All images* entry in the *Check settings and results* list.
   - If you analyze your own images later on, you can also select another entry from this list, for example, if you don't want to check the settings for every image anymore.
7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - ROIs

1. For the SprayCoatingt.tif example image, define a rectangular ROI which covers the part of the sample that you would like to analyze. To do so, click the *Create Rectangular ROIs* button, and define the rectangle on the image by two mouse clicks.



Note: It is not absolutely necessary to define ROIs. If you want to measure the entire image, in the *ROIs* step in the analysis, click directly on the *Next* button without defining a ROI.

2. Leave the *Use for next images* check box clear, because these instructions only analyze one image. Later on, when you are using your own images and want to analyze more than one image at the same time, you can select this check box to use the same ROI on all of the images that are selected in the current analysis process.

Note: The ROI that is defined only applies for the current analysis process. When you start a new analysis process, you have to define new ROIs. If you want to use the same ROIs for more than one analysis process, save them and reload them later.

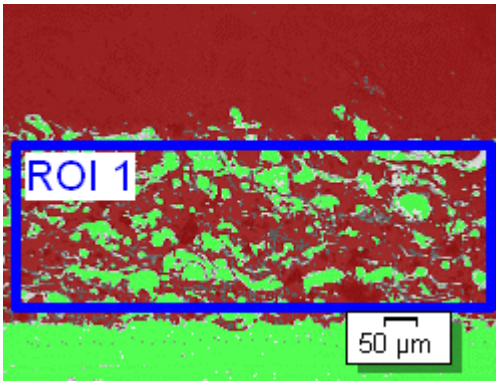
3. If you want to set a fixed size for the rectangular ROI, select the *Use discrete size* check box. You will find this check box in the *Rectangle properties* group. This group is only displayed when the *Create Rectangular ROIs* button is selected or when a rectangular ROI is selected in the image. With the help of the *Use discrete size* check box, you can create several rectangular ROIs that all have a size (or a multiple of a size) defined by you.
4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Threshold

Prerequisite: These step-by-step instructions assume that the *Simplified color space (I/R/G/B)* option has been selected. You can find this option in the *Options > Materials Solutions > Phase Analysis* dialog box.

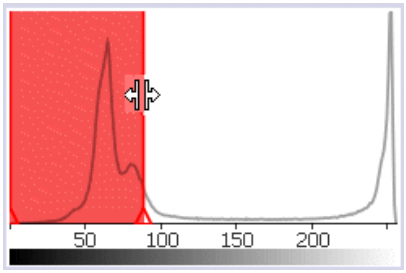
1. When you are performing the phase analysis on color images, select the *Intensity Value* entry in the *Component* list. If you are analyzing gray-value images, the *Intensity Value* entry is preset and can't be changed.

- All of the pixels which lie within a defined intensity range, will be shown in color during this step in the analysis. This intensity range is called a phase. The intensity value range is limited by a top and a bottom intensity value. These are the so-called threshold values.

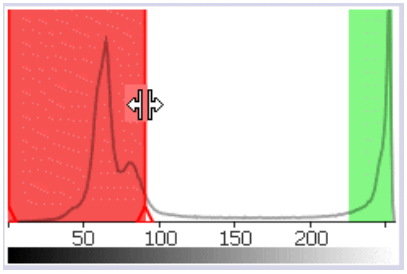


- Please note that the defined ROI will not be considered in this step in the analysis, but only in the next step. In this step in the analysis, pixels which are outside of the ROI will thus also be shown in color.

2. If necessary, reduce or increase the intensity range of the first automatically created phase. Make sure that the first phase covers the dark pixels. (You can only define the phase for the bright pixels in the next step.) In the image, watch how the object areas found become larger and more objects are found.
   - To reduce or increase the intensity range, in the tool window's table change the values in the *Min.* and *Max.* fields. Alternatively, interactively change the lower and upper threshold values in the histogram shown at the bottom of the tool window. Move the mouse pointer over the edge of the phase, until the pointer changes and, with the left mouse button pressed, drag the edge in the required direction.



3. Now, define the second phase. To do so, click the *Add Phase* button, and click the *New Threshold* button. Now click so long in the bright areas within the ROI, until they are shown in the color of the phase.
4. If necessary, change the two phases which have already been defined. To do that, select the phase which you want to change in the table in the tool window.

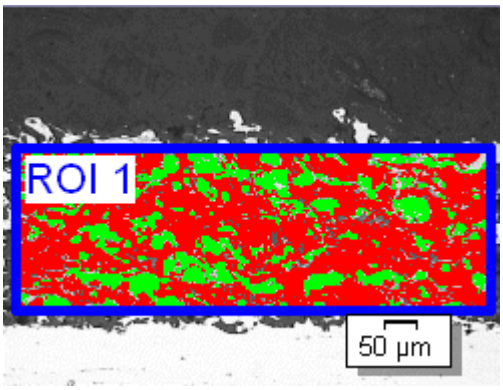


5. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Object filter

In this step in the analysis, only the pixels within the defined ROI are considered. All objects which meet the conditions defined in the object filter, will be shown in the color of the phases.

All objects which do not meet the conditions defined in the object filter, will be shown in this step in the analysis in red hatching. This means that these objects will not be taken into account when determining the percentage area of the phase.



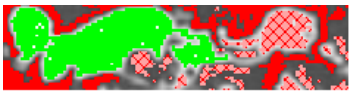
1. If necessary, change the conditions for the object filter. First adjust the units. As the SprayCoating.tif image was calibrated in micrometers, click on the button showing the units (to the right next to the *Minimum object area* field), and select µm as unit.
2. In the *Minimum object area* field, enter the minimum size that an object must have to be considered when determining the area fraction covered by the phase. You can thus exclude small objects such as dust particles from the determination of the percentage area of the phase. Watch how more or less object areas are found as the hatched objects in the image increase or decrease.

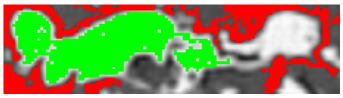
During the analysis process, you can use your software's zoom function as usual. Move your mouse pointer onto the appropriate position in the image, then use the mouse wheel to zoom into or out of the image.

3. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Image results

In this step in the analysis, all of the objects used to determine the phase fraction will be shown in the color of the phase. Objects which do not come up to the minimum area and which were thus shown hatched in the previous step in the analysis, will now be shown with no color.





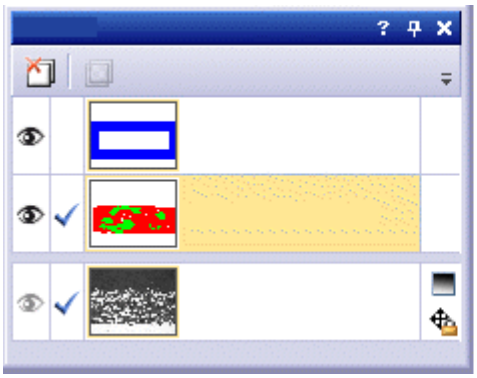
1. Take a look at the results that are shown in the table. In the *Image results* field, you will see the area fraction covered by each phase.
2. If necessary, manually change which objects your software uses to determine the area fraction of the phase. You can delete, or add objects.
3. Click the *Next* button.

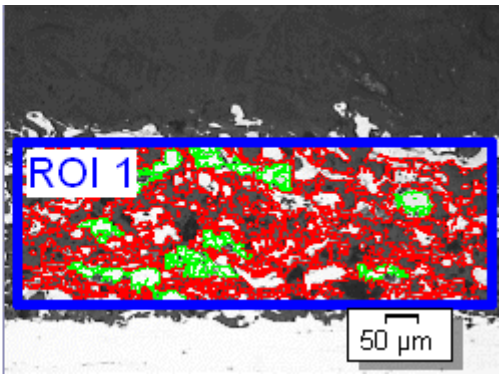
Step - Results

Select the results you want.

Step - Reporting

1. Select the *Default* option to use the template that has been defined as the default template. If you would like to select another template, select the *User defined* option. Click the button with the three points and select the new template in the *Open* dialog box.
2. When you want to create an MS-Word report: In the *Content* group, select the check box for the pages the report should contain.
3. When you want to create an MS-Excel report: Click the *Save settings* button to save the current settings in a file.
   - These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.
4. Click the *Finish* button.
5. Through the materials analysis measurement, the image has collected one or more additional layers (can be seen in the *Layers* tool window). If required, save the image in TIF or VSI format to retain these newly created image layers.





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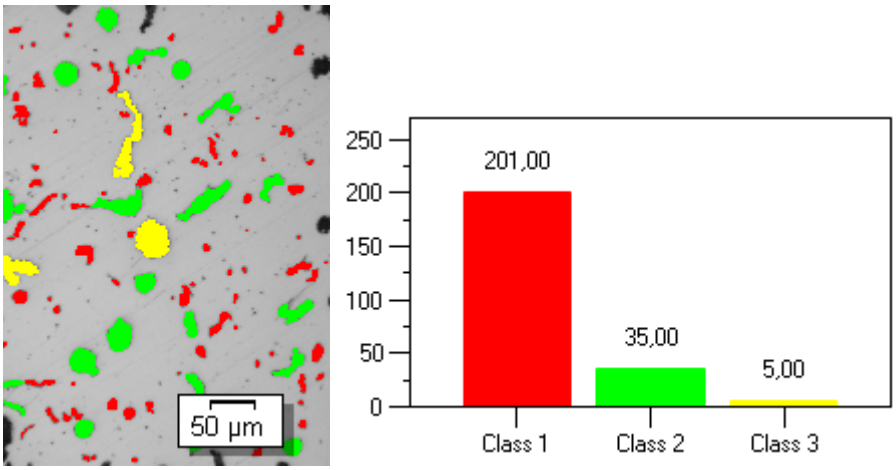
9.11. Particle Distribution

9.11.1. What is a particle distribution?

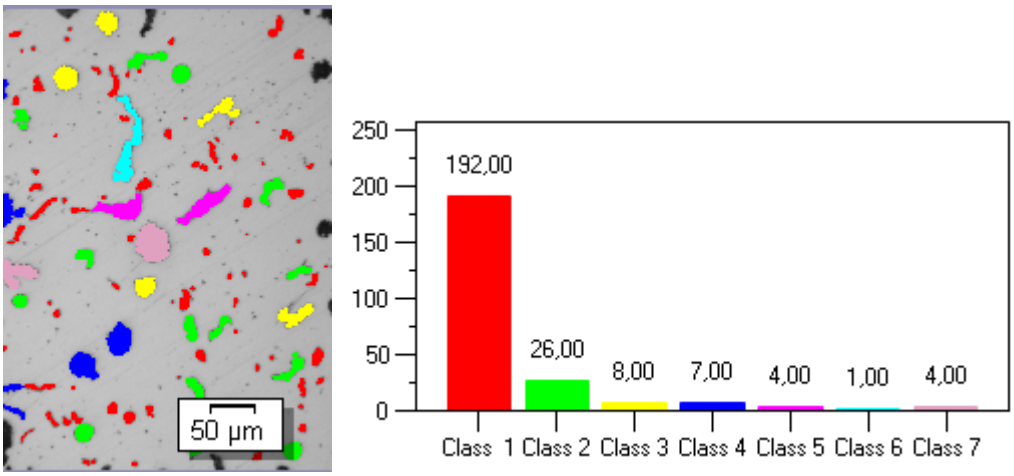
With a particle distribution measurement, the software counts how many particles are in an image and classifies them, for example, according to their size or their form.

It is a precondition that the particles can be detected by the software. Therefore, the particles must differ from the rest of the sample, for example, because they are darker or lighter. In this case, you can define a phase with an intensity range that covers the intensity values of all of the particles. If the particles that you want to measure largely have the same intensity values, then one phase is enough. If you want to measure light and dark particles, you will need a second phase.

All detected particles are measured according to a measurement parameter that you selected (for example, *Area*). The results can be classified automatically. For this, you define a classification with up to 16 classes. For some samples, a coarse classification with only 2 classes is sufficient, whereas other samples require a more detailed classification with 10 classes for example.



Example of a particle distribution measurement. In the image, the particles have been detected and measured according to the *Area* measurement parameter. The results are shown according to the defined classification. In the example shown, the particles were assigned to three size classes. The diagram shows how many particles each size class has.



You see the same particle distribution measurement as in the example above, but now with a more detailed classification. Now the particles were assigned to seven size classes.

Measuring on ROIs

You can choose whether you would like to measure the entire image or if the measurement should only be carried out on a part of the image, a so called ROI (Region Of Interest). You can also define several ROIs. The particle distribution will always be measured over all ROIs and the results do not differentiate between ROIs.

Filtering and editing particles

Define one or more filters to specify which particles to include in the analysis.

You can edit the particles manually. You do this interactively on the image. Note that you are not changing the image itself, but the image's measurement layer.

You can manually delete parts of the image which were detected as particles. This can be necessary, if for example artifacts in the image are recognized as particles because they have intensity values similar to the defined phase. By manually deleting these particles, the artifacts will no longer be considered when measuring the particle distribution. In addition, you can also manually add other image segments which were not detected as such but which are actually particles.

Additionally, you can split particles manually and merge several small particles into one big particle. To do so, in the image first click on the particles which you want to merge.

Results of a particle distribution measurement

The results of an analysis can be displayed in a workbook and in a chart. Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.

General procedure for a particle distribution measurement

1. Selecting the analysis process
In the *Materials Solutions* tool window, click the *Particle Distribution* button.

2. "Image source" step
Choose the image you want to measure.

3. "ROIs" step
Define the ROIs, or measure the whole sample.

4. "Threshold" step
Define the intensity range of the phase.

5. “Auto split particles” step
Configure a morphological filter to split particles.

6. "Edit Particles” step
Define filters and edit the particles.

If necessary, delete particles or add new ones. You can also manually split particles and merge separate particles into one.

7. "Classification" step
Check and change the classification.

8. "Image results" step
Take a look at your image results.

9. "Results" step
Document the results (report, workbook, or chart).

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9.11.2. Measuring the particle distribution

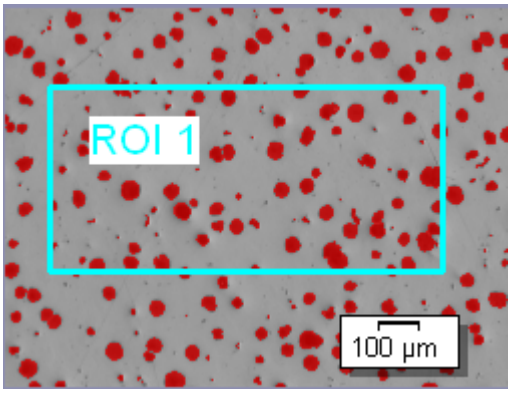
Step - Image source

1. Load the "GlobularGraphite.tif" example image.
   - In this image, the dark nodular graphite particles are to counted and the particles are to be classified according to their size.
2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Particle Distribution* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
   - By doing so, you skip the *Sample information* step which is not relevant for this example image.
6. Select the *All images* entry in the *Check settings and results* list.
   - If you analyze your own images later on, you can also select another entry from this list, for example, if you don't want to check the settings for every image anymore.
7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

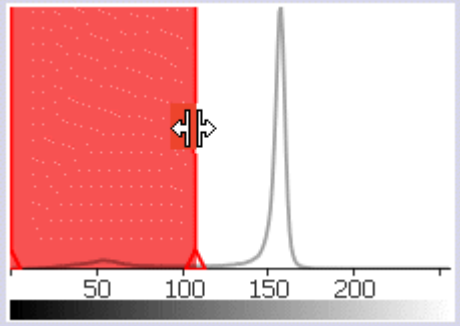
Step - Threshold

All of the pixels which lie within a defined intensity range, will be shown in color during this step in the analysis. This intensity range is called a "phase". The intensity value range is limited by a top and a bottom intensity value. These are the so-called threshold values.



Please note that the defined ROI will not be considered in this step in the analysis, but only in the next step. In this step in the analysis, pixels which are outside of the ROI will thus also be shown in color.

1. If necessary, reduce or increase the intensity range of the phase. In the image, watch how the particle areas that are found become larger or smaller and how more or less particles are found.
   - To reduce or increase the intensity range, in the tool window's table, change the values in the *Min.* and *Max.* fields. Alternatively, interactively change the lower and upper threshold values in the histogram shown at the bottom of the tool window. Move the mouse pointer over the edge of the phase, until the pointer changes and, with the left mouse button pressed, drag the edge in the required direction.



2. Select the *Auto split particles* check box, which is displayed below the histogram.
   - The additional step *Auto split particles* will be added to the current analysis.
3. Select the *Check classification* check box, which is displayed below the histogram.
   - The additional *Classification* step will be added to the current analysis.

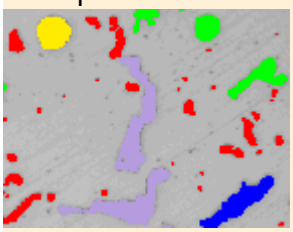
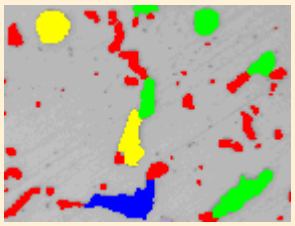
Note: If you analyze several images of a sample in the same analysis process, you can only check the classification for the first image of the sample. The selected classification will be adopted for all other images of this sample. This is why the *Check classification* check box is not available from the second image of the sample onwards.

4. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Auto split particles

In this step, you configure the morphological filter. It is used for splitting objects. To do so, use the *Fine / Coarse* slider. The settings that you specify can have a big effect on the image results.

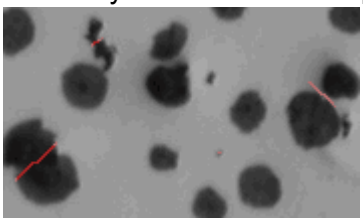
Example:

On the left is the image where the morphological filter for separating objects has been set relatively high. The tendency is for fewer and larger objects to be found. On the right is the same image where the morphological filter for separating objects has been set low. Here, more and smaller objects are found.

Note: This step is only shown if the *Auto split particles* check box in the *Threshold* step is selected.

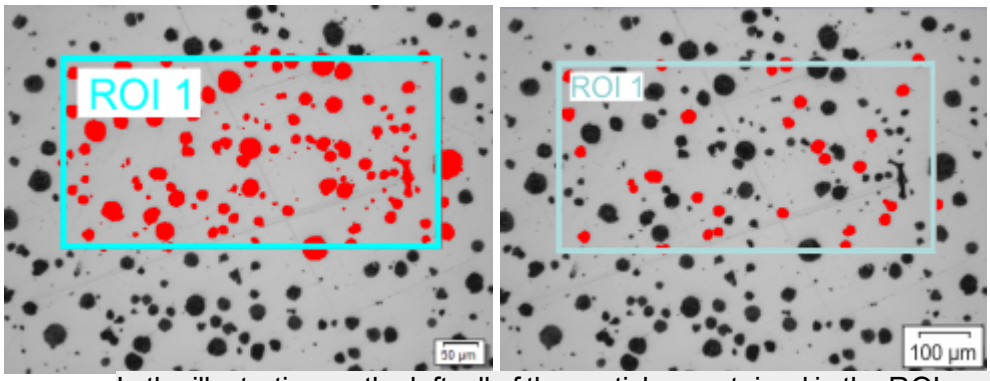
1. Click the *Preview* button to see how many borders are found when using the preset value of 1.
   - This setting tends to find more borders where objects can be split.
2. Take a look at the borders that have been found in the image. Each border is shown by a red line across a particle.



3. If required, move the *Fine / Coarse* slider slightly or enter the required value in the edit field and press the [Enter] key.
4. Click the *Preview* button again to view the changes in the image.
   - The higher the value you enter, the fewer borders are found.
5. Reset the value for the "GlobularGraphite.tif" example image back to the preset value of 1, and click the *Preview* button.
6. Click the *Next* button.
   - The objects are split.
   - The *Materials Solutions* tool window will display the next step.

Step - Edit Particles

You can define one or more filters in this step. You can use these filters to limit the particles that are included in the analysis.



In the illustration on the left, all of the particles contained in the ROI are colored red. A filter has been applied to the image on the right. Only the particles that are within the defined measurement range have been colored red.

1. In the table, click the measurement parameter for which you want to define a filter range. You can click the *Area* measurement parameter, for example.

- If the required measurement parameter doesn't appear in the table, click the *Select particle measurements* button. Select the measurement parameter you want in the *Select Particle Measurements* dialog box.

2. Define the upper and the lower value for the filter range for the measurement parameter. You can either enter the filter range directly in the list or you can interactively determine it, by selecting particles in the image.

Entering the filter range directly

1. Double click in the *[Min.* field, located next to the measurement parameter to enter the lower value for the filter range.
2. Either enter the required measurement value, or use the arrow keys.
3. Double click in the *Max.[* field, then enter the higher value for the filter range.
   - Particles that will be included in the analysis are colored red.

Defining the filter range interactively

1. In the table, click the measurement parameter for which you want to define a filter range.
2. Click the *Select maximum value* button to define the filter range's upper value.
3. In the image, click a particle whose measurement value is to be used as the lower value for the filter range. When you, for example, define a filter range for the *Area* parameter, click the smallest particle that you still want to measure.
   - The measurement value will then be automatically adopted in the *[Min.* field.
   - If you want to undo the selection you've made, click the *Clear minimum value* button.
4. Click the *Select maximum value* button to define the filter range's upper value.
5. Click a particle whose measurement value is to be used as the upper value for the filter range. Click the largest particle that you still wish to measure.

- The measurement value will then be automatically adopted in the *Max.[* field.
- If you want to undo the selection you've made, click the *Clear maximum value* button.
- The particles that will be included in the analysis are colored red.

Saving and loading filter settings

1. Click the *Save Filter* button to save your filter settings as a parameter set. A parameter set that has been saved can be exported and imported.
2. Use the *Load Filter* button to load it at a later date.

Editing particles

1. If necessary, you can delete and add particles. Additionally, you can split particles manually and merge several (previously selected) small particles into one big particle.

- Delete particles by first clicking on the particle to be deleted in the image and then clicking on the *Delete selected particles* button. If you say yes to the warning message, the particle will be deleted. The display below the table with the measurement parameters is updated. You can also delete several particles at once, by holding the [Ctrl] key pressed, while clicking on the particles.

- Add particles by first clicking on this button . Then draw a freehand polygon around the particle to be added. Make sure that the freehand polygon lies as exactly as possible on the edge of the particle to be added. End the definition of the polygon with the right mouse button. The number in the display below the table with the measurement parameters increases.

- Merge particles by first clicking in the image on the particles which you want to merge. Keep the [Ctrl] key pressed while you click on the particles. Then click the *Merge selected particles* button.

- Split particles by first clicking on the *Draw a line that will split particles* button, and then define a line between the particles you want to split. Click the right mouse button and confirm the input.

Note: If you have edited particles and return to the *Threshold* step in the analysis (e.g. to change the threshold values) your manual corrections will be deleted. If necessary, you will then again have to manually edit particles in the *Edit Particles* step in the analysis.

2. Click the *Next* button.

Step - Classification

In this step in the analysis, only the pixels within or on the edge of the defined ROI will be considered. If a filter was defined in the previous step, only the particles that fall within the filter's measurement value range will be included in the analysis. All particles that will be used for the particle distribution measurement are colored in this step.

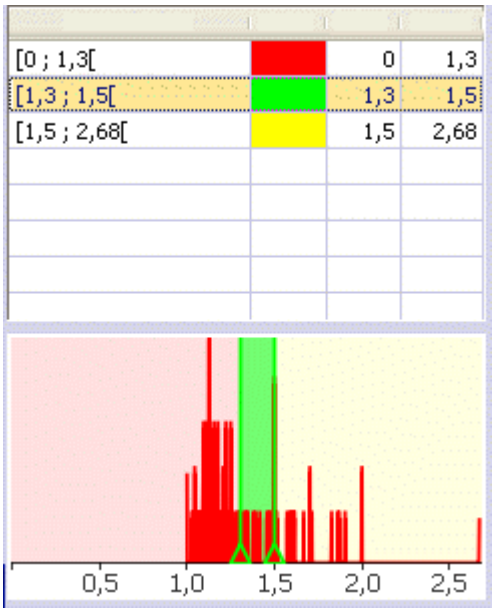
Note: This step is only displayed if the *Check classification* check box in the *Threshold* step is selected.

1. As the nodular graphite particles on the "GlobularGraphite.tif" image are to be classified by their size, in the *Measurement* list, select the *Area* parameter.
   - The particle distribution always uses exactly one measurement parameter. The three most frequently used parameters are: *Area*, *Max. (Feret)*, and *Equivalent Circle Diameter*. These parameters are always shown in the *Measurement* list and can be selected quickly.

   - If you analyze your own images later on, you may want to measure the particles according to another parameter, for example, according to the shape. To select another measurement parameter, click the *Select Particle Measurement* button, located at the right hand side of the *Measurement* list. Then select the measurement parameter you want in the *Select Particle Measurement* dialog box.
2. If necessary, adjust the measurement units. As the "GlobularGraphite.tif" image is calibrated in micrometers, the *µm²* measurement unit must be selected.

Note: The preset measurement unit depends on the parameter selected in the *Measurement* field. Some parameters don't require a measurement unit. The button will thus not be shown.

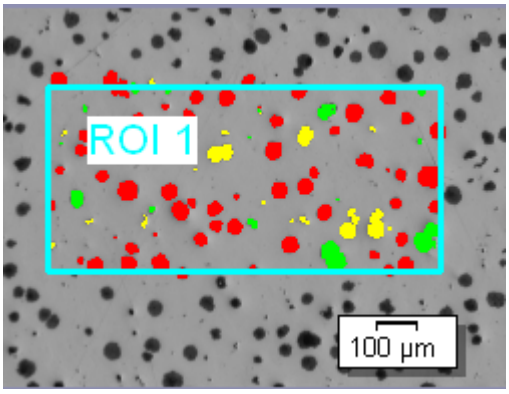
3. Click the *Automatic Classification* button. You will find this button in the toolbar above the table.
   - The *Automatic Classification* dialog box opens.
4. In the *Automatic Classification* dialog box, click the *Get Min./Max. from Image* button. In the *Minimum* and *Maximum* fields, the area of the smallest and biggest particle is entered. The kind of value that is read out from the image and entered in the *Minimum* and *Maximum* fields depends on the selected measurement parameter. In the *Number of classes* field, enter how many classes are to be used for the classification of particles. For the "GlobularGraphite.tif" image, enter the value 3. Close the dialog box with *OK*.
5. Look at the table in the tool window. It contains the classification with the three classes. Also look at the diagram below the table. It displays how many particles are in each class.



6. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

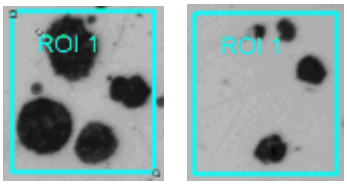
Step - Image results

In this step in the analysis, all of the particles will be shown in the color of the class to which they belong. All particles that do not belong to any of the defined classes are shown hatched in this step.



1. Take a look at the displayed results in the *Image results* field. You see how many particles each class contains.
2. The *Particle area fraction* field displays the particle area fraction as a percentage. This value informs you about the percentage the sum of the area of all particles found in this analysis has in comparison to the total area being analyzed (the detection area).

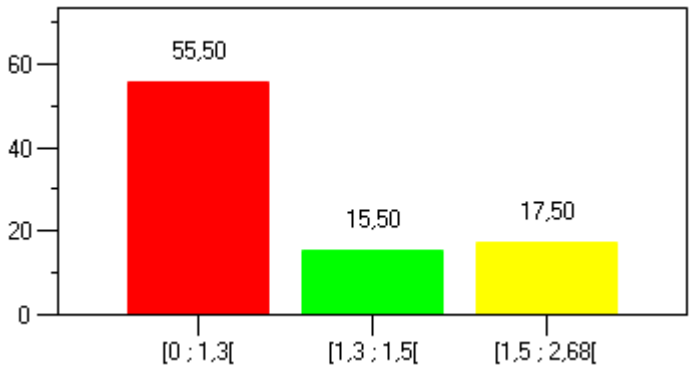
   The particle area fraction is determined by dividing the area of all found particles by the detection area. It doesn't matter whether the found particles have been assigned to a class or not. The detection area can either be the whole image, or one or more ROIs. With particles that are on the border of the detection area, only the part that is inside the detection area is included in the calculation.



On the left is a ROI with a particle area fraction of 40%. On the right is a ROI with a particle area fraction of 10%.

3. In the diagram below the *Image results* field, the classification of the particles is shown graphically. If many classes have been defined, a look at the chart is the quickest way to know which class contains most particles.

Note: You can also select a different way of classifying the results. Then the chart can look very different. Use the *Tools > Options...* command and select the *Materials Solutions > Particle Distribution* entry in the tree view. This command is not available while an analysis is running.



Note: You will get this chart as a file in OCT format, if in the *Results* step in the analysis, you select the *Generate chart* check box.

4. Click the *Next* button.

Step - Results

Note: If you analyze several images of a sample in the same analysis process, this step is only shown after the last image has been analyzed.

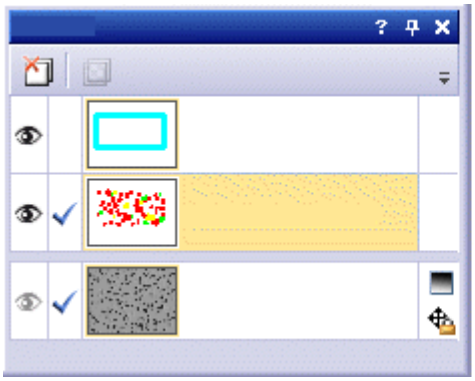
1. Select the *Generate report* check box and then select either the *Word* option or the *Excel* option to automatically create a report in the corresponding application program when the analysis finishes.
2. Select the *Generate workbook* check box to have a document of the "Workbook" type automatically created at the end of the analysis.
3. Select the *Generate chart* check box, so that, at the end of the evaluation, the system will automatically create the diagram shown in the *Image results* step as a separate document, of type "workbook".
4. If you want to save the current settings to a file, click the *Save settings...* button. Then assign a descriptive name in the next dialog box.
   - You can load these settings (parameters) when you analyze further images. To do that for the new image in the *Image Source* step, click the *Load from file...* button. The sample and image comments are saved, as are the phases used and the settings in the *Classification* step in the analysis.
6. Click the *Next* button.

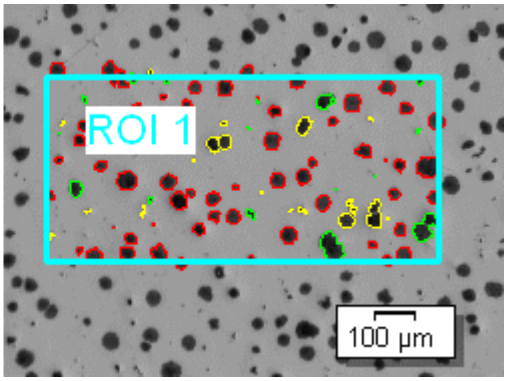
Step - Reporting

1. Select the *Default* option to use the template that has been defined as the default template. If you would like to select another template, select the *User defined* option. Click the button with the three points and select the new template in the *Open* dialog box.
2. When you want to create an MS-Word report: In the *Content* group, select the check box for the pages the report should contain.
3. When you want to create an MS-Excel report: Click the *Save settings* button to save the current settings in a file.

- These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.

4. Click the *Finish* button.
5. Through the materials analysis measurement, the image has collected one or more additional layers (can be seen in the *Layers* tool window). If required, save the image in TIF or VSI format to retain these newly created image layers.





Note: Use the *Tools > Options > Count and Measure > Display* dialog box to specify whether the found particles should be displayed in outline or whether they should be filled. You can change these settings at any time, before or after the analysis, for example, and also for images that have already been saved in TIF or VSI format.

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9.12. Automatic Measurement

9.12.1. What exactly are automatic measurements?

Use automatic measurements when you want to repeatedly carry out the same measurement on similar images. You use a measurement routine defined by the software administrator for the measurements. You only have to specify the position on the sample when carrying out the measurement. The actual measurement is automatically carried out by your software.

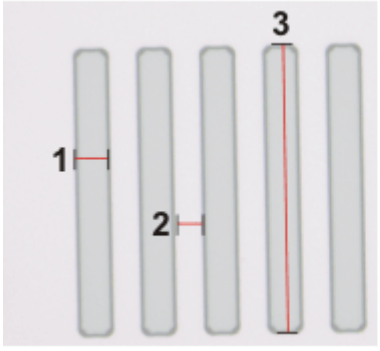
Prerequisites for automatic measurements

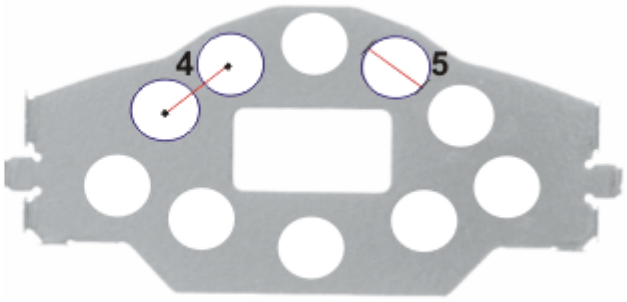
The measurement tasks that can be carried out using the *Automatic Measurement* solution have to fulfill the following requirements:

1. Simple geometric structures can be measured, for example, the distance between two lines or the diameter of a circle.
2. The measurement object must be displayed on one image. The measurement can't analyze any structures that are spread over more than one image.
3. The imaging conditions for the image acquisition should be comparable for all samples that are to be measured with one measurement routine. The average image brightness and the image contrast in particular should be comparable.
4. The samples to be measured should be aligned the same. The measurement routine will not deliver any results if the samples are aligned differently. For example, wafers that can be positioned precisely on the stage are suitable.

Examples of measurement tasks

The following examples are structures that can be measured with the *Automatic Measurement* solution.





You can measure line structures like those illustrated in the image on the left with the *Automatic Measurement* solution. You can for example measure the width of a line (**1**), the distance between two lines (**2**) or the length of a line (**3**).
On the right is an example of a workpiece with holes. You can measure the distance between two holes (**4**) or the diameter of a hole (**5**), for example.

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9.12.2. Performing an automatic measurement

Prerequisite: A measurement routine is required for an automatic measurement. You can only create and manage a measurement routine if you've started your software as Administrator or Power User.

9.12.3. Defining a measurement routine

Overview of the procedure for defining a measurement routine

The following steps are necessary if you want to define a measurement routine.

Define Routine
In this step you create a new measurement routine or delete an existing measurement routine.

Define Reference Image
In this step, you make important settings for the measurement routine. You select the measurement parameters, acquire a reference image, and define a matching pattern.

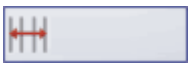
Define Inspections
In this step, you select one or several measurement parameters that are to be used in this measurement routine.

Define Measure
In this step, you define the actual measurement routine on the reference image. To do this, you define one or more scanners, depending on the inspection mode you chose. You specify some parameters for the analysis of the scanner.

Define Tolerance
In this step, you define the tolerance range for the measurement routine's measurement results.

Define Workbook
In this step, you configure the workbook that is created at the end of an automatic measurement.

1. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.



2. Click the *Automatic Measurement* button.
   - The *Materials Solutions* tool window displays the *Start Page* group.
3. In the *Start Page* group, select the *Import Routines* button and import the "WAFER-500x.amr" example measurement routine. If you create your own

measurement routines at a later point in time, you can use these example measurement routines as a basis and adapt them to fit your needs.

4. Click the *Manage Routines* button.
   - You can now define the imported measurement routine.

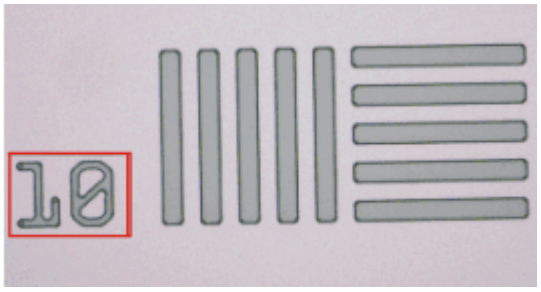
Define Routine step

1. Select the "Wafer-500x" measurement routine from the *Measurement routine* list.
2. Select the *Live image* option.
3. Click the *Next* button.

Define Reference Image step

For every measurement routine, a reference image is saved showing the structure to be automatically measured by the measurement routine.

1. Select a reference image. To do this, select the *From disk* option and load the "Wafer-500x.tif" example image.
2. Select the *Use a matching pattern* check box.
3. Click the *Define pattern area* button to define a pattern area on your sample. In the reference image, keep your left mouse button pressed and drag a rectangle around the matching pattern.
4. Right click twice to confirm the matching pattern
   - The matching pattern is shown in the image.
   - The structure can be automatically found by your software using pattern recognition. The scanners are then automatically positioned correctly. A scanner is the segment of the image that is analyzed by the automatic measurement.



In the illustration the matching pattern is framed in red.

5. Click the *Next* button.

Define Inspections step

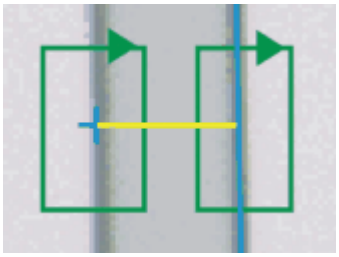
In the *Define Inspections* group, all of the inspections that are defined in the "Wafer-500x.amr" measurement routine are listed. In this example, measurement parameters are selected that measure the width of a line, the distance between two lines and an angle.

1. Click the *Next* button.

Define Measure step

In the *Define Measure* group, the scan area for each measurement parameter is defined on the reference image. Which scanners and how many scanners are required depends on the inspection that was selected in the *Define Inspections* step. For the *Point to line distance* inspection, for example, two rectangular scanners have to be defined.

1. Click the *Define scanner area* button located in the *Scanner 1* tab.
   - The scan area that has already been defined in the example measurement routine is displayed. The measurement point for the *Point to line distance* inspection is defined with this scan area.
   - The arrow indicates the scanner's orientation.
2. Finish the definition of the scan area.
   To do so, either right click the image window or click the *Confirm Input* button. You can find this button on the *Toolbox* toolbar.
   - Your software will now analyze the scan area. The analysis can take a moment, depending on the size of the defined scan area.
   - The result is a measurement point that is displayed with a small blue cross in the image.
3. Switch to the *Scanner 2* tab.
4. Click the *Define scanner area* button.
5. The scan area that has already been defined in the example measurement routine is displayed. The measurement line for the *Point to line distance* inspection is defined with this scan area.
   - The arrow indicates the scanner's orientation.
6. Finish the definition of the scan area.
   To do so, either right click the image window or click the *Confirm Input* button. You can find this button on the *Toolbox* toolbar.
   - Your software will now analyze the scan area. The analysis can take a moment, depending on the size of the defined scan area.
   - The result is a measurement line that is displayed in blue in the image. The distance is calculated between this measurement line and the measurement point from Scanner 1. The distance is shown with a yellow line.



The illustration shows the *Point to line distance* inspection in which two scan areas (green) are defined. In this example, the distance between the small blue cross and the blue line is measured. The yellow line shows the measured area.

7. After all the scan areas have been defined and analyzed, the result of the measurement is displayed at the bottom of the *Materials Solutions* tool window.
   - If you want, you can change the units of measurement for the measurement results.
8. Click the *Next* button.

Define Tolerance step
In this step, you define the tolerance range for the measurement routine's measurement results. The *Measurement result* field displays the results of the *Point to line distance* measurement method on the "Wafer-500x.tif" reference image. This measurement result is the reference value for all automatic measurements carried out with this measurement routine. The values saved in the example measurement routine are displayed in the *Minimum allowed* and *Maximum allowed* fields.

1. Adopt the values and click the *Next* button.
   - You automatically go back to the *Define Inspections* step.

Note: You have to define the position of the scanners separately for each inspection.

2. Repeat the last steps and define the scan areas for all of the inspections.

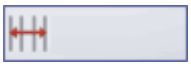
Define Workbook step
After you define the scan areas for all of the inspections, the *Finish* button becomes active.

1. Click the *Finish* button to save the settings in the measurement routine.
   - You can now use the measurement routine for automatic measurements.
2. If you haven't defined a workbook in the *Define Inspections* step, you cannot complete the steps. The *Finish* button isn't active. You automatically go back to the *Define Inspections* step.
3. Click the *Define Workbook* button in the *Define Inspections* step.
4. In the *Define Workbook* group, select the properties that you want the workbook's header to contain.
5. Click the *Finish* button to save the settings in the measurement routine.

9.12.4. Performing an automatic measurement

Prerequisite: You don't need any Administrator or Power User rights to perform an automatic measurement. You do, however, need a measurement routine that was created by an Administrator or a Power User.

1. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.



2. Click the *Automatic Measurement* button.
   - As soon as you've started this analysis process you'll be guided step by step through the measurement. A lot of your software's other functions will not be

available while an analysis process is running.

- The *Materials Solutions* tool window displays the *Start Page* group.

3. Select the *Start Routine* button in the *Start Page* group.

Select Routine step

1. Select the "Wafer-500x" measurement routine from the *Measurement routine* list.
2. Select the *Folder* option and load the five "Wafer-500x.tif" example images. The example images are numbered from 01 to 05 in the file name.
3. Click the *Next* button.
   - The first "Wafer-500x.tif" example image is measured.
   - The results of the automatic measurement are displayed in the *Materials Solutions* tool window.

4. In the *Measure* group, click the *Measure* button.
   - The next "Wafer-500x.tif" example image is measured.
5. Repeat this procedure until all five of the example images have been measured.
6. Click the *Finish* button.
   - The results are automatically exported to a *Workbook* document.

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9.13. Coating Thickness

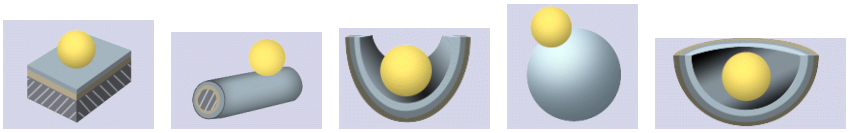
9.13.1. What is a coating thickness measurement?

Using the *Coating Thickness* analysis process you can analyze ball indentation cuts of thin coatings and determine their coating thickness. The sample under test should be a substrate, which has one or more coatings that were applied using different coating methods (PVD, CVD, VPS, APS etc.).

To determine the coating thickness, a ball indentation is ground into the sample. This is done using a rotating grinding ball, which has a diameter between about 10 and 50 mm. The ball indentation must have the minimum thickness of the sum of all coatings.

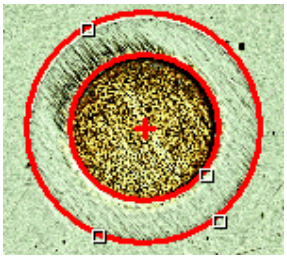
In case of a flat or spherical sample surface, the grinding ball's indentation is round. If the sample surface is curved in one direction, the grinding ball's indentation is ellipse-shaped.

You can choose between the following sample surfaces:
Flat, *Cylindrical convex*, *Cylindrical concave*, *Spherical convex*, or *Spherical concave*.



Measurement order
You can carry out the measurements in any the order you want. You can measure the coatings from the outside to the inside, for example. To do this, the coating's outer border is defined first on the image and, following that, all of the remaining borders. Alternatively, you can also measure the coatings in the reverse order, from the inside to the outside. It's also possible, for example, to specify a border on a middle coating to begin with and to first measure from this border towards the inside and then towards the outside.

The borderlines that have been defined are shown in color. They are located in an additional image layer (can be seen in the *Layers* tool window). By default, the borderlines are shown in red. In the program options, you can set a different color or thickness for the borderline. In the program options, you can also specify for each measurement line to be displayed in a different color.



You can see a coating thickness measurement on the flat sample surface. One coating has been measured.

Number of measurements per image

Every image is only measured once by default. However, you can set the program options to measure an image several times. Then the results of the last measurement will constantly be compared to the results of the previous measurement. The average value of all of the measurements performed to date is always displayed.

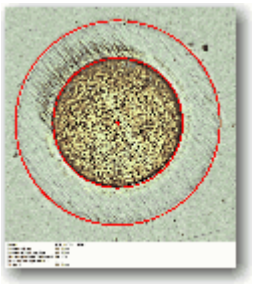
Results of a coating thickness measurement

The coating thickness is measured in accordance with the industry standard that is set in the program options. The following industry standards are available:

- EN 1071-2:2002
- VDI 3824:2001
- EN ISO 26423:2016

The results of an analysis can be displayed in a workbook. Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format.

If the *Create image with results shown in information bar* check box has been marked in the program options, a new image document will be created additionally during the measurement. This image document shows the measured image with the borderlines and an information bar (under the image). You can determine the contents of the information bar. You can save the image document as a TIF file and send it to others who don't have access to the image analysis program, for example.



General procedure for a coating thickness measurement

1. Selecting the analysis process
Click the *Coating Thickness* button, located in the *Materials Solutions* tool window.

▼

2. "Image source" step
Choose the image you want to measure.

▼

3. “Settings” step
Select the sample surface type (*Flat* for example).
Specify further measurement parameters.

▼

4. "Measurement" step
Define the outer and inner borders of each coating.

▼

5. "Results" step
Document the results (report or workbook).

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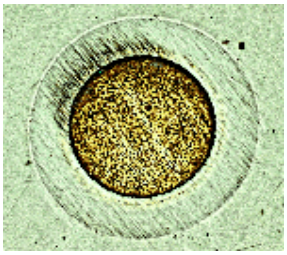
9.13.2. Measuring the coating thickness

This step-by-step instruction describes how you can measure the thickness of a coating. An image of the flat sample surface on which 1 coating is to be measured once has been selected as an example. If you selected an image with a different surface in the *Settings* step, there will be small differences in the procedure.

CoatingThickness2_GrindingBallDiameter_40mm.tif example image
During the installation of your software some sample images have been installed, too. You can follow this step-by-step instruction using the CoatingThickness2_GrindingBallDiameter_40mm.tif example image. Open this image and make sure that it has been selected in the document group.

Step - Image source

1. Load the CoatingThickness2_GrindingBallDiameter_40mm.tif example image, or alternatively, the image that you want to measure.



2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

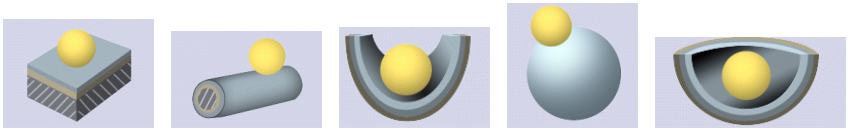
3. Click the *Coating Thickness* button.
4. In the *Image source* group, choose the *Selected images* option to analyze the loaded image. For this to work, this image must be selected in the document group.
5. Select the *Skip 'Sample information'* check box, to skip the *Sample information* step.
   - As soon as you click the *Next* button, you'll go straight to the *Settings* step. You can do this if you don't want to enter any information about the sample, which is the case here.

Note: If you want to analyze images from more than one sample in the same analysis process, the *Skip 'Sample information'* check box must be cleared. Only then will the *New Sample* button be displayed. With this button, you can specify when an image to be analyzed belongs to a new sample.

6. Select the *First image* entry in the *Check settings and results* list.
   - If you select the *First image per sample* entry, you can check the settings for each new sample.
7. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Settings

1. Select the sample surface type. For the CoatingThickness2_GrindingBallDiameter_40mm.tif example image, select the *Flat* sample surface.
   - You can choose between the following sample surfaces: *Flat*, *Cylindrical convex*, *Cylindrical concave*, *Spherical convex*, or *Spherical concave*.



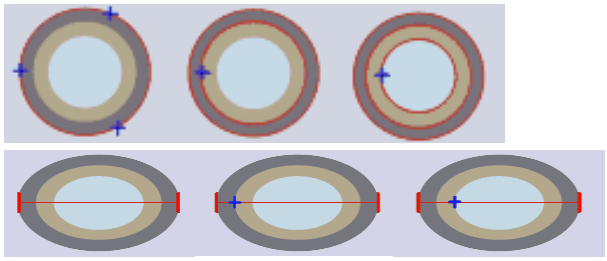
2. Select the crater shape. The indentation that the grinding ball makes in the sample's surface is called a "crater".
   - If the *Flat*, *Spherical convex* or *Spherical concave* sample surfaces are selected, this indentation is round. The indentation is elliptical when the *Cylindrical convex* or *Cylindrical concave* sample surface types are selected.
3. If you selected the *Cylindrical convex* or *Cylindrical concave* sample surface types: Select the direction of the ellipse's long axis. This information is taken into account when calculating the coating thickness.
4. Specify how many coatings you want to measure in the *Number of coatings* field. A maximum of 20 coatings can be measured.
5. Enter the diameter of the grinding ball used in the *Grinding ball diameter* field. The grinding ball's diameter must be known in order to produce an accurate coating thickness measurement. If necessary, change the suggested unit.
6. If you selected the *Spherical convex* or *Spherical concave* sample surface types: Enter the curvature radius of the surface used in the *Curvature radius of surface* field. This value must be known because it's needed for the calculation of the

coating thickness.

- The curvature radius of the surface is only important for the measurement of the coating of spherical sample surfaces. That's why this field isn't displayed when you've selected a different sample surface type.

Step - Measurement

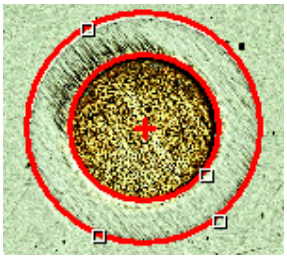
1. Move the mouse pointer onto the image window. All other areas of your software can't be used in this step.
   - The mouse pointer turns into a cross .
2. You can carry out the measurements in any the order you want. When you want to measure the coating from the outside to the inside, proceed as follows: Define the first coating's outer borderline by clicking three points on its outer border. With cylindrical sample surfaces, the outer borderline is defined by clicking twice on the ellipse's outer border (taking the direction of the selected long axis into account).



The illustration in the *Measurement* step shows how the borders of a coating have to be defined.

   - The outer borderline is displayed. It's has the color red by default. You can set a different color or thickness for the borderline. Make these settings before you start the analysis process.
3. Define the first coating's inner borderline by clicking once three times on its inner border. Whether you define the second border with one or with three clicks of the mouse depends on whether the *Measure using multiple points* check box was selected in the *Settings* step.

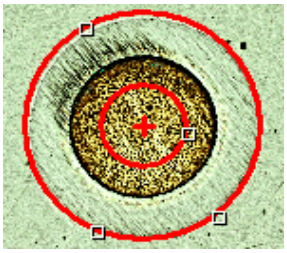
   - The inner borderline is displayed. If you only want to measure one coating, the mouse pointer turns into an arrow.



4. If you want to measure more than one coating: Define all further coatings to be measured, each with one additional mouse click.

   - As soon as you've defined the inner border of the last coating, the mouse pointer turns into an arrow .
5. Check the values in the *Measurements* table.

6. If you want, you can correct a borderline. To do this, move the mouse pointer to the small handle on the borderline so that it takes on this shape . Now click the left mouse button and move the borderline to where you want it.
   - The borderline is corrected and the values in the *Measurements* table are updated.



7. If you want, you can change the coating's name. Normally, the coatings are numbered serially. If, for example, you prefer to specify the coating material, click once on the number in the *Coating* field in the *Measurements* table to select the entry. Then click on the entry one more time to overwrite it. Enter the desired text.
8. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step. If you specified that an image should be measured more than once in the program options, you now remain in the *Measurement* step and perform the next measurement.

Step - Results

The *Materials Solutions* tool window displays the measurement results. You can view the results for all of the currently analyzed images, sorted by sample. The average values are displayed in the *Coating thickness*, *Total Thickness*, *Total Penetration Depth*, and *Penetration Depth in Substrate* fields. This means that the results of all of the measurements of the same type are added together and then divided by the number of measurements.

Step - Reporting

1. Select the *Default* option to use the template that has been defined as the default template. If you would like to select another template, select the *User defined* option. Click the button with the three points and select the new template in the *Open* dialog box.
2. When you want to create an MS-Word report: In the *Content* group, select the check box for the pages the report should contain.
3. When you want to create an MS-Excel report: Click the *Save settings* button to save the current settings in a file.
   - These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.
4. Click the *Finish* button.
5. Through the materials analysis measurement, the image has collected one additional layer (can be seen in the *Layers* tool window). If required, save the image in TIF or VSI format to retain this newly created image layer.

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9.14. Dendrite Arm Spacing

9.14.1. What is a dendrite arm spacing measurement?

Dendrites are formed in metal alloys when they solidify. They are branching, tree-like structures. A dendrite arm spacing measurement, to put it simply, measures the distance between the individual branches of the tree.

From the dendrite arm spacing an expert can tell whether a metal alloy solidified quickly or slowly, among other things.

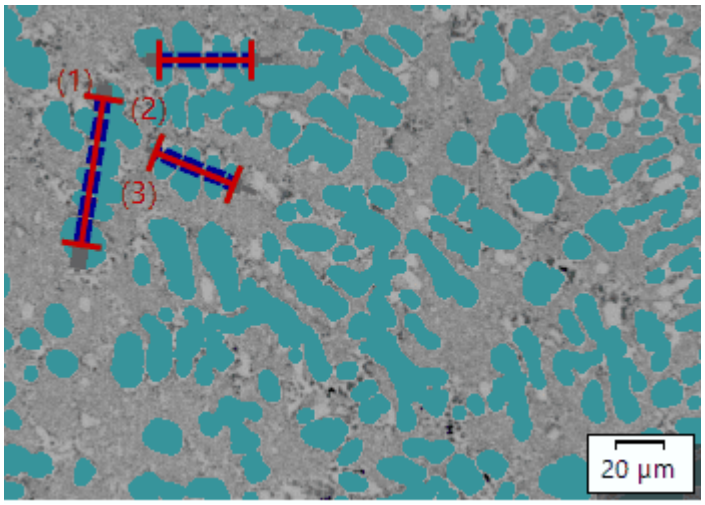
The samples are usually metallographic sections that have been prepared especially for the measurement of dendrite arm spacing. In order for your results to be valid, the dendrite arms that you measure must lie entirely within the cross-sectional plane. The measurement lines should be positioned in the image so that they intersect several neighboring dendrite arms at a right angle.

Schematic diagram of a measurement line that intersects four dendrite arms. The black double arrow shows the dendrite arm spacing between the second and third dendrite arm.

It is a precondition for a dendrite arm spacing measurement that the dendrites differ in some way from the rest of the sample, because they are brighter for example. In this case, the dendrites will have different intensity values from the rest of the sample, making automatic analysis of the image possible. For the image analysis, so-called phases are defined which cover a certain range of intensity values.

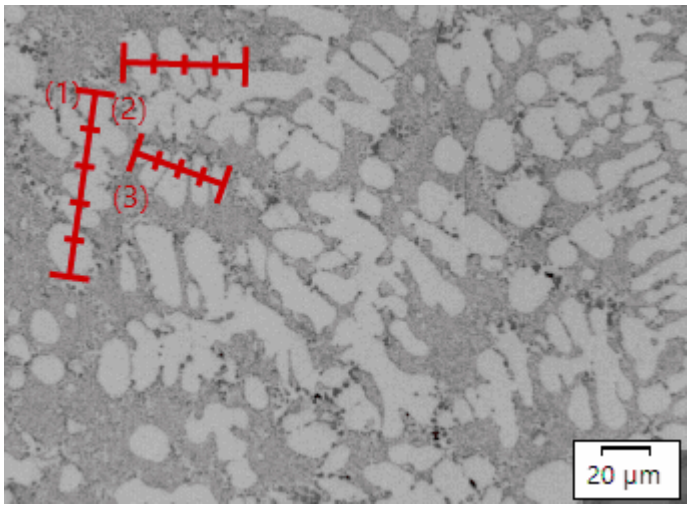
Different methods for detecting dendrites

If your images are suitable, the dendrites can be detected using automatic threshold setting. The image's foreground is separated from its background by using the threshold value setting method. All of the objects that are to be analyzed have to belong to the image's foreground. The number of dendrite arms that are on the measurement line that you drew will then be detected by the software.



Three dendrite arm spacing measurements using the automatic method of dendrite detection. All of the pixels that have been classed as part of a dendrite are displayed in the color *Dark Cyan* in the image.

If using automatic threshold detection doesn't produce adequate results, manually enter the number of dendrite arms that intersect the measurement line that you drew.



Three dendrite arm spacing measurements using the manual method of detecting of dendrites.

Displaying the results

The results of an analysis can be displayed in a workbook. The following information is given:

- Sample name
- *Number of Measurement Lines*
- *Total Length*
- *Dendrite arms*
- *Average DAS*
- *Median DAS*
- *Variance of average DAS*

Additionally, the results can be displayed in a report in either MS-Word or MS-Excel format. The user can specify the structure of the report. The reports can also contain images and the measurement lines that were used.

Example of a page in a report in MS-Word that shows the image that was measured and the positions of the measurement lines.

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9.14.2. Measuring dendrite arm spacing

Note: You can follow these step-by-step-instructions on your PC. It describes how to measure dendrite arm spacing.

Step - Image source

1. Load the *DAS1.tif* example image.
   - Let's assume you want to perform two dendrite arm spacing measurements.
2. Activate the *Materials Solutions* tool window. Should this tool window not be visible, use the *View > Tool Windows > Materials Solutions* command to have it displayed.

3. Click the *Dendrite Arm Spacing* button.
   - As soon as you've started this analysis process you'll be guided step by step through the measurement. A lot of your software's other functions will not be available while an analysis process is running.
   - The *Materials Solutions* tool window displays the *Image Source* step.
4. In the *Image source* group, choose the *Selected images* option to analyze the example image. This image must have been opened for this purpose, and have been selected in the document group.
5. Select the *Skip 'Sample information'* check box.
   - By doing so, you skip the *Sample information* step which is not relevant for this example image.

6. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Settings

1. Because the *DAS1.tif* example image is suited for automatic threshold setting: Select the *Automatic* option.
2. Use the *Threshold for dendrites* slide control to define a suitable threshold for the detection of the dendrites.
   - All of the pixels that have been classed as part of a dendrite are displayed in *Dark Cyan* in the image. This is only the case if the *Show dendrite detection* check box is selected.
3. Use the *Improve dendrite detection* slide control to optimize the threshold for the detection of the dendrites.
   - The *Improve dendrite detection* slide control specifies a second phase. This phase only includes gray values from 0-100.
4. With the *DAS1.tif* example image, leave the *Material-dependent constant* field clear.
5. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Measurement

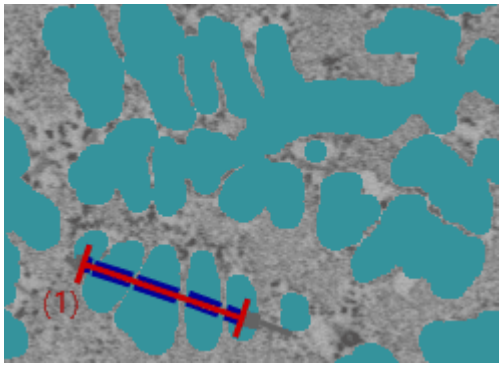
1. When you go to this step in the analysis, your software automatically switches into a measurement mode.

   - The mouse pointer turns into a cross on the image. The measurement function icon appears at the bottom right of the mouse pointer .
   - You will remain in this measurement mode until you explicitly switch it off.
2. Draw a measurement line through the first dendrites that you want to measure. To do this, left click once on the image to mark the start of the measurement line. Then move the mouse pointer to the end of the measuring line and left click again.

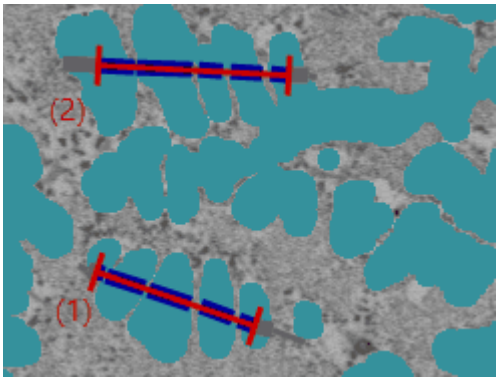
Note: The dendrite arms that you want to measure must lie in the sample's cross-sectional plane in order to deliver reliable results.

   - The measurement line is shown in red. The measurement line is also shown in blue where it runs through parts of the sample that belong to the detected

phase.



3. Draw more measurement lines through dendrites whose arm spacing you want to measure.



4. Right click or press the [Esc] key on your keyboard to leave the measurement mode.
   - Now you can move the mouse pointer freely again.
   - You can now still move the existing measurement lines if necessary. A measurement line must be selected before you can move it.
5. Take a look at the measurement results in the *Measurement results* table. You can still make the following changes. Use the buttons below the table for this.

   - Adding measurement lines
   - Deleting measurement lines
   - Changing the number of dendrite arms in a measurement line
6. Take a look at the measurement results in the *Results* field. What you see here is the overall results for all of the measurement lines. When you measure more than one image or more than one sample in an analysis, the *Results* field shows the overall results for all of the measuring lines.

Note: If you aren't satisfied with the results and go back to the *Settings* step to change the settings, this deletes all of the measuring lines. You will then have to redraw all of the measurement lines in the *Measurement* step.

7. Leave the *Show DAS on measurement line* check box cleared for these step-by-step instructions.

8. Click the *Next* button.
   - The *Materials Solutions* tool window will display the next step.

Step - Results

Select the results you want.

Step - Reporting

1. Select the *Default* option to use the template that has been defined as the default template. If you would like to select another template, select the *User defined* option. Click the button with the three points and select the new template in the *Open* dialog box.
2. When you want to create an MS-Word report: In the *Content* group, select the check box for the pages the report should contain.
3. When you want to create an MS-Excel report: You can click the *Save settings* button to save the current settings in a file.
   - These are largely the same settings that you could already save in the previous step, the *Results* step. You can, however, additionally specify which Excel template you want to use for the report creation here.
4. Click the *Finish* button.
5. Through the materials analysis measurement, the image has collected one or more additional layers (can be seen in the *Layers* tool window). Save the image in TIF or VSI format to retain these newly created image layers.

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10. Counting and measuring objects

10.1. Overview

You can detect and analyze objects in images with your software. You can find an overview of the process flow of an automatic image analysis here.

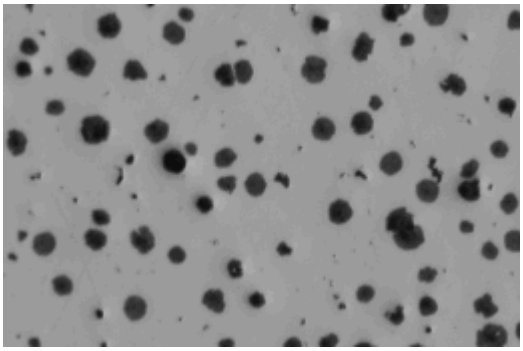
Prerequisite: The automatic object analysis functions are only available when the *Count and Measure* software solution has been purchased and is active.

10.1.1. The schematic process flow of a sample analysis

A complete sample analysis is, as a rule, made up of several steps. In the following, a simplified, schematic process flow is described. In this example graphite particles are counted and classified. After each individual step in the analysis, the resulting image is shown.

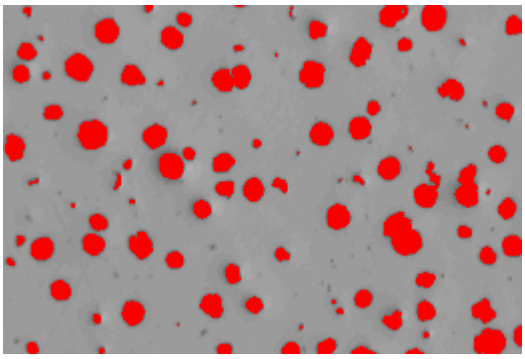
A sample analysis typically proceeds in three steps.

(**1**) Segmenting → (**2**) Counting and Measuring → (**3**) Classifying



The source image: How many graphite particles are in the image and how large are they?

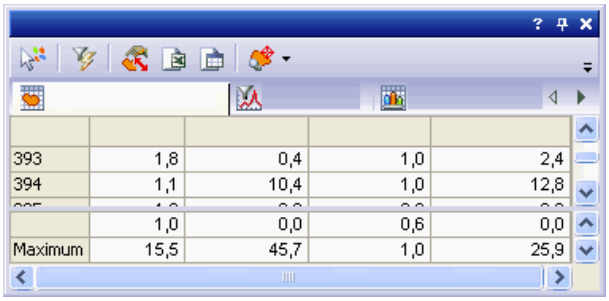
(1) Segmenting
To begin with, the image has to be segmented. The image's foreground is separated from its background by using the threshold value setting method. All of the objects that are to be analyzed have to belong to the image's foreground. This is a prerequisite for the next step, in which the objects are measured and counted.



The segmented image: The graphite particles are colored. This distinguishes them clearly from the background.

(2) Counting and measuring

The objects are detected, counted, and measured. For the measurement of the objects, numerous measurement parameters are available. Select the measurement parameters that interest you.

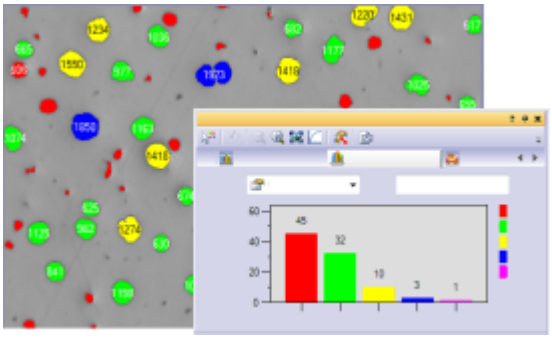


393	1,8	0,4	1,0	2,4
394	1,1	10,4	1,0	12,8
	1,0	0,0	0,6	0,0
Maximum	15,5	45,7	1,0	25,9

The *Object Measurements* results view shows the results in a table.

(3) Classifying

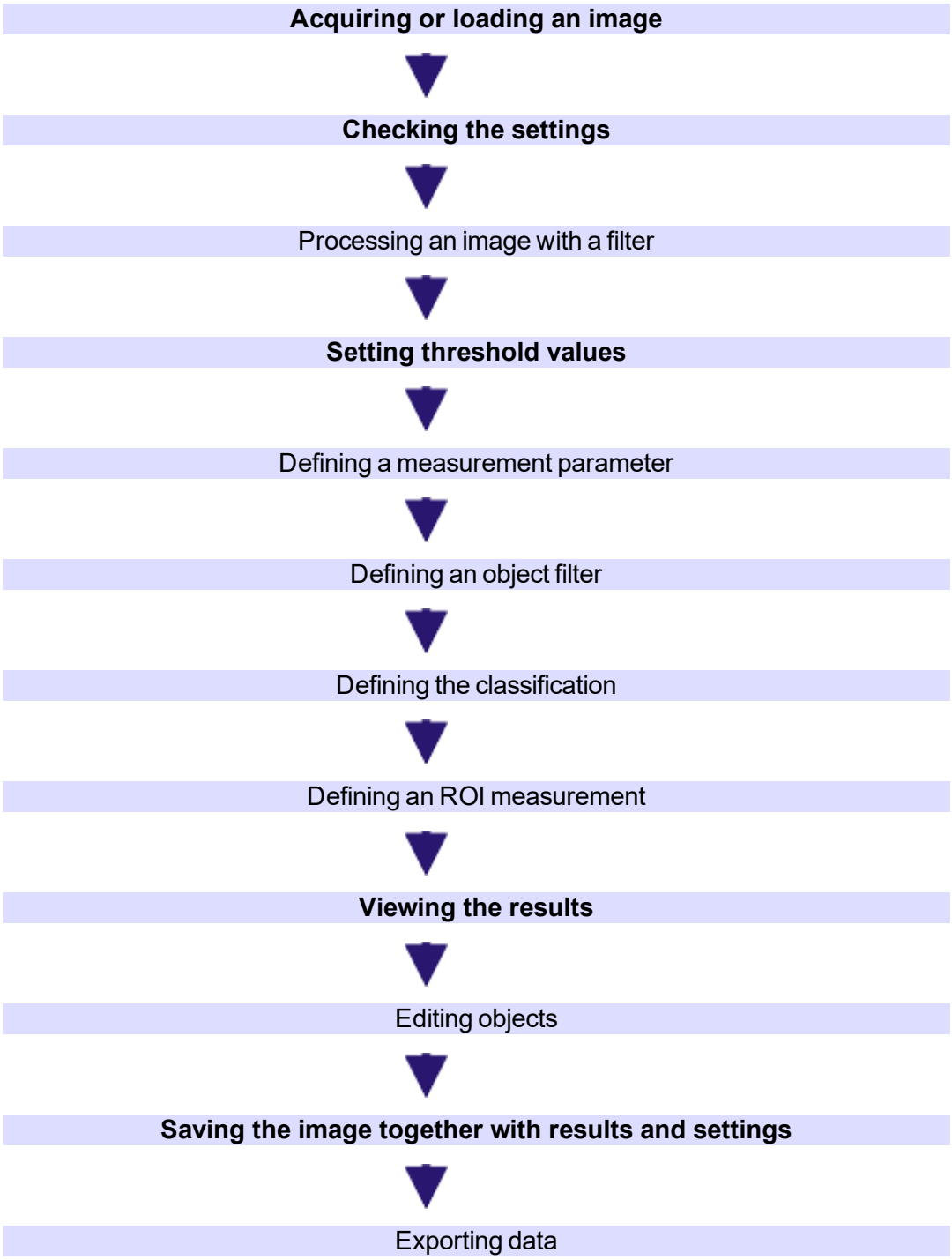
When the objects have been measured, they can be classified. For this purpose, a classification, in which the number and definition of the individual object classes is specified, has to be defined. The size distribution of the graphite particles is going to be determined in this example. You need to define a classification scheme that sorts all of the objects into different classes according to size.



Left: The classified objects in the image, made recognizable by the allocation of different colors.
Right: The *Class Histogram* results view shows the results in a diagram.

10.1.2. The detailed process flow of a sample analysis

Not all of the steps listed below are essential. Some steps are optional, and can be carried out additionally. In the example that follows, an object analysis is described in which many of the possible steps are carried out. Normally, however, you would not carry out all of these steps, only certain ones. Steps that always have to be carried out, that's to say, those that are not optional, appear in bold format.



Acquiring or loading an image
Acquire an image or load one. The current image is shown in the document window. All of the steps are carried out on this image.

Checking the settings
Check all of the current settings. In the *Tools > Options > Count and Measure > Detection* dialog box, there are some settings that have a significant influence on the results. For this reason, you should always check the settings before you begin an object analysis.

Processing an image with a filter
You can process the image with a number of filters to improve on the requirements for the automatic object analysis. Use, for example, the *Separate Objects* morphological filter, to better separate the objects in the image.

Setting threshold values
The threshold values can be set automatically or manually. Select a suitable threshold values method, for example, *Manual threshold value*. The image's foreground is separated from its background by using the threshold value setting method. All of the objects that are to be analyzed have to belong to the image's foreground.

Defining a measurement parameter
In the *Select Object Measurements* dialog box, select the required measurement parameters for objects. Only the selected measurement parameters are output in the results views.

Defining an object filter
Define which objects are to be excluded from your analysis. With the help of the object filter, you can define an individual filter range for each object parameter. Objects that don't fall within this filter range won't be shown in the results. The results only relate to the objects that lie within the defined filter range.

Defining the classification
First, define or select a suitable classification scheme. To do this, use the *Options > Count and Measure > Classification* dialog box. The classification scheme specifies the number of object classes and the way that they are defined.

In the *Select Class Measurements* dialog box, select all of the class measurement parameters that interest you. A typical measurement parameter for classes is, for example, the number of objects per class. You can naturally also display other measurement parameters for classes, such as the area of all of the objects in a specific class.

Non classified objects are objects that can't be categorized by the classification process. These are displayed hatched. This can, for example, happen, when a classification is used for the first time, or has to be further customized.

Defining an ROI measurement
You can limit the object analysis to certain image segments. These image segments are called ROIs (Region Of Interest). To make it possible for the object analysis to be

carried out on one or more ROIs, the ROIs first have to be defined on the image.

In the *Select ROI Measurements* dialog box, select all of the ROI measurement parameters that interest you. A typical ROI measurement parameter is, for example, the number of objects per ROI. You can naturally, also calculate other measurement parameters for ROIs, such as, the area of all of the objects on a specific ROI.

Viewing the results

Click the *Count and Measure* button, located in the *Count and Measure* tool window, to have the object analysis carried out.

The objects are detected and measured in one step. The objects are classified, and displayed in the corresponding class color in the image. Objects that don't fall within any class are cross hatched.
The default setting is the *Phase* classification scheme. You can define phases in the threshold dialog box. The settings that you specify there, the color of individual phases for example, are automatically adopted by the classification scheme.
You can define other classification schemes and select them. For example, classify the objects according to their size or their color.

The *Object Count* group in the *Count and Measure* tool window shows how many objects there are altogether, and how many of the objects lie within the filter range.

Results views

In the *Count and Measure Results* tool window, you can select between various results views for the display of the data. The *Object Measurement* results view shows the results sheet with the individual results of all of the detected objects and the statistical values.

The *Object Filter* results view, offers the possibility of having the histogram for a chosen object parameter displayed. This enables you, for example, to output a size distribution of the detected objects. In the size distribution you can see how many objects have a specific area. As well as that, you can view the applied filter range and the statistics for each object measurement.

The *Class Measurements* results view, shows the results for all of the defined classes, for example, the number of objects per class. In the *Class Histogram* results view, you see the class results as a histogram, for example, along the X-axis, the classes, and along the Y-axis, the area ratio per class.

The *ROI Measurements* results view, shows the results for all, of the defined ROIs, for example, the number of objects per ROI. Select the *ROI Histogram* results view, to view the same results as a histogram.

Displaying the measurement results

The measurement results will be shown in the image in a special data layer, the *Detected Objects* layer. Try and picture the layer as a transparency which is placed over the image. When you measure an image, the image data will not be changed by having the measurement results displayed on it.

You can, at any time, hide or show the *Detected Objects* layer. To do so, use the *Layers* tool window. There you have access to all of an image's layers. The eye icon  identifies all of the layers that are currently on display on your monitor. Click the eye

icon in front of the measurement layer, to display the *Detected Objects* layer. Click an empty cell without an eye icon to make the corresponding layer reappear. You can configure the display and the output of the measurement results.

Editing objects

In the *Count and Measure* tool window, you can find a toolbar with which you can deal with individual objects. You can select one or more objects, add new objects, or delete objects. As well as this, it's possible to manually or automatically separate objects that are joined together.

Saving the image together with results and settings

The image is automatically saved together with the all of the results and settings. It isn't necessary to save the results separately.

Note: Always use the TIF or VSI file format when saving an image. Otherwise you will lose most of the image information and the results during saving.

When you have analyzed and saved an image, you can restore all of the settings from the original image analysis with the *Restore Options* button. You can use the settings again, for the analysis of another image for example. This applies to all of the settings of the threshold value setting, detection, and classification.

This doesn't apply to the filter settings. These can be saved and loaded separately, in the *Object Filter* results view. Excluded are also the object, class, and ROI, parameters. These can also be saved and loaded separately.

This button is located on the *Count and Measure* tool window's toolbar.

Exporting data

Data can be exported as an MS-Excel table, or as an internal workbook. They can also be exported as a diagram from the *Class Histogram* and *ROI Histogram* results views. This makes it possible to save the results independently of the image and the object analysis's settings.

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10.2. Carrying out an automatic image analysis

You can use an automatic image analysis to carry out numerous measurement tasks. Several typical tasks and their process flow are described here.

Prerequisite: The automatic object analysis functions are only available when the *Count and Measure* software solution has been purchased and is active.

Basic functions for automatic image analysis

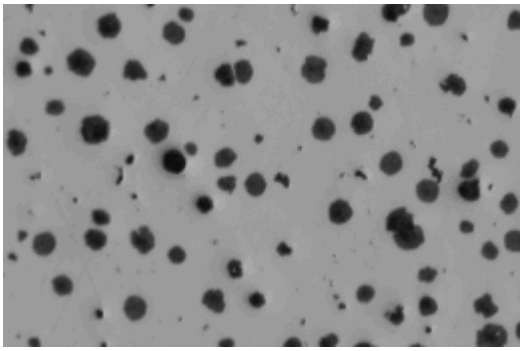
Counting objects
Measuring objects (selecting and outputting measurement parameters)
Filtering objects
Classifying objects

Performing a phase analysis

Performing a phase analysis

10.2.1. Counting objects

Example: You have an image with objects that interest you. You want to know how many of these objects there are in the image.



You want to detect and count graphite particles on the example image.

Preconditions

The objects that you want to count must not be connected, but must be clearly separated from one another. The objects in the foreground should be optically clearly separated from the image's background. In the example image shown, the background is bright. The objects are in the foreground and are dark in color.

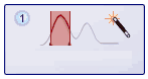
Preparations

1. Use the *View > Tool Windows > Count and Measure* command to have the *Count and Measure* tool window displayed.
2. Acquire an image or load one.
   - During the installation of your software some sample images have been installed, too. You can follow these step-by-step instructions using the *GlobularGraphite.tif* example image.

Setting options

3. Open the *Options* dialog box by clicking the *Count and Measure Options* button, located in the *Count and Measure* tool window.
4. Select the *Count and Measure > Detection* entry in the tree view.
5. In the *Options* group, enter the value 5 in the *Minimum object size* field. An object must now be at least 5 pixels large in order to be counted as an object. By doing that, you will rule out the possibility that individual pixels, that may well have the same color or intensity as the objects, but don't belong to an object, are counted as objects, which would then falsify the results. This way you can exclude noise and dust particles.
6. Click *OK* to exit the *Options* dialog box.

Setting threshold values



7. In the *Count and Measure* tool window, click the *Automatic Threshold...* button to open the *Automatic Threshold* dialog box.

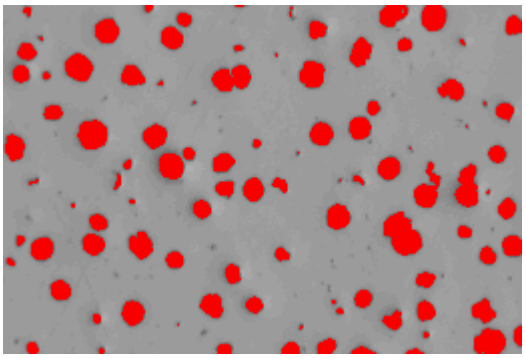
- If the *Automatic Threshold* button is not yet active, activate it first. To do so, select the *Automatic Threshold...* entry in the *Threshold* button's menu. You open this menu by clicking the small arrow next to the button.
- The threshold values are set automatically in the *Automatic Threshold* dialog box.
- All of the objects that have been detected will be displayed in color.

8. Check whether the objects have been correctly recognized.
   Should the objects not have been correctly recognized, go to the *Background* group and enter whether the background is bright or dark.
   For the image shown above, select the *Background > Bright* option, since the image shows dark objects against a bright background.

9. Only when the *Remove Phase* button in the *Channel thresholds for phase ...* group is active:
   Delete all but one of the phases by continuing to click the *Remove Phase* button until the button becomes inactive.
   - By doing that, you will make certain that no phases from earlier analyses are still defined.

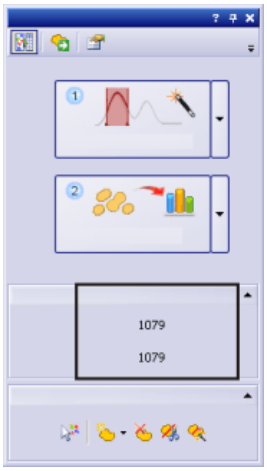
Viewing the results

10. To obtain the results, click the *Count and Measure* button in the *Automatic Threshold* dialog box.



All the objects that are found are displayed in color in the image.

- The *Automatic Threshold* dialog box will be closed.
- The number of objects found is displayed in the *Object Count* group in the *Count and Measure* tool window.
- The objects that have been analyzed are then displayed in color, on their own image layer. This image layer is called *Detected Objects*. Use the *Layers* tool window to make these image layers appear or disappear, or to delete them.

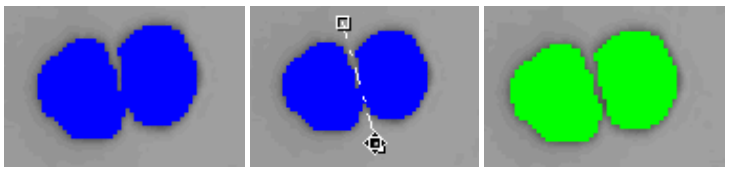


The number of objects detected will be shown below, in the *Count and Measure* tool window, in the *Object Count* group. Should you not be able to see this number, click the small black arrow to make it visible.

Separating objects

It is sometimes the case that two objects that are next to each other are not detected separately because, as far as the software is concerned, they are joined together. These sorts of objects can be separated manually.

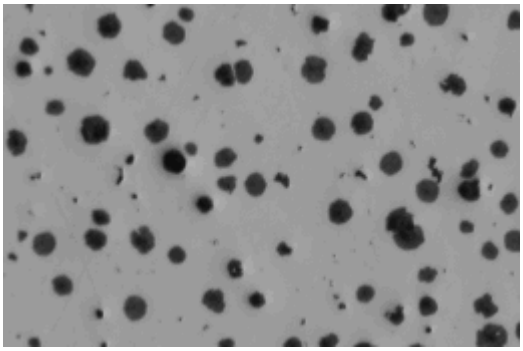
1. Zoom into the image to enable you to better process the object.
2.  Then click the *Manually Split Objects* button, located in the *Edit Objects* group, then move your mouse pointer onto the image.
3. Now define a separation line through the object by clicking the left mouse button. Make sure, when you do this, that you drag the line over the object's outside edge, since otherwise it won't be separated.
4. Right click to confirm the separation line.
   - The object will then be divided up into two independent objects. The results will be updated.
5.  Then click the *Manually Split Objects* button again, located in the *Edit Objects* group, to leave the mode for splitting objects.



Left: Two objects are touching each other and thus are counted as a single object.
Middle: Draw a separation line through the object.
Right: The joined up object has been separated, there are now two independent objects. Separating the object puts it in a different size class and it is therefore allotted a different color.

10.2.2. Measuring objects (selecting and outputting measurement parameters)

Example: You have an image with objects of different sizes. You want to know the area of the largest object and to have a close look at that object in the image. In addition to that, you want to export the results into a sheet.



Preparations

1. Acquire or load an image.
2. Carry out an automatic object analysis on the image.

Selecting a measurement parameter

3. Open the *Options* dialog box by clicking the *Count and Measure Options* button, located in the *Count and Measure* tool window.
4. In the tree view, select the *Count and Measure > Measurements* entry, then click the *Select Object Measurements* button, located in the *Measurements* group.
5. In the *Select Object Measurements* dialog box, add the *Area* and *Object ID* measurement parameters and close any open dialog boxes.
   - From some measurement parameters other, more complex, measurement parameters can be derived. In this case, you will find the basic measurement parameters in the list of measurement parameters. Select the basis measurement parameter from the list and define which measurement parameters are to be derived from it in the area of the dialog box to the right of the list.
     There are, for example, many different ways of determining the inner extent of objects. In this case, you can select between the minimal, maximal and mean inner extent.
6. Next, in the *Count and Measure* tool window, click the *Count and Measure* button to output the results.

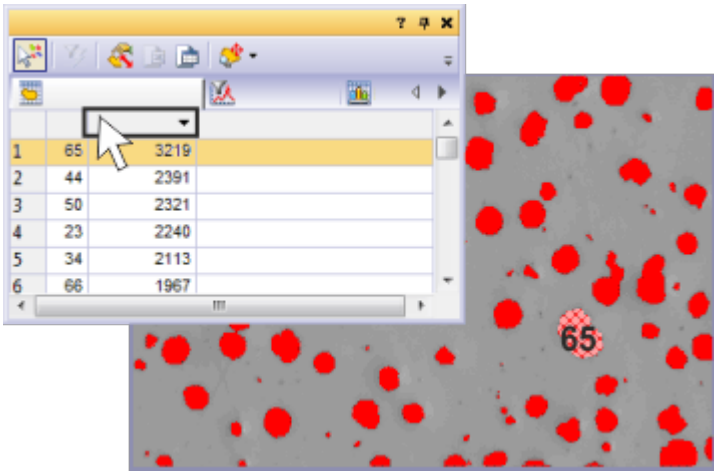
Viewing and sorting the results

7. In the *Count and Measure Results* tool window, select the *Object Measurements* results view.
   - The measurement values for the objects' areas are displayed in the *Area* column.

8. Sort the *Area* column to find out which value is the smallest or the largest. To do so, double click on the header of the *Area* column.
   - This column's measurement values will then be sorted in ascending or descending order.
9. Double click the header of the column again to sort the measurement values in the reverse order.
   - An arrow in the header will show you the direction in which they are sorted.

Object - sheet link

10. Select the largest value in the *Area* column.
    - The corresponding object will likewise be selected in the image window. In this way, you can easily find an object that belongs to a specific value, and view it.



On the top left you can see the *Object Measurements* results view. The area of every object that was detected is listed. The object with ID number 65 is the largest and comes first in the table after sorting has taken place. The object with ID number 65 has been selected in the results view and is therefore cross hatched in the image window.

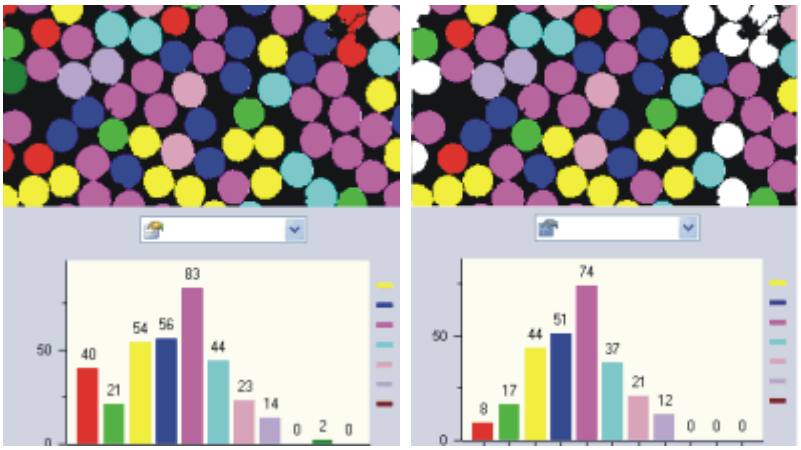
Exporting the results to a sheet

11. In the *Object Measurements* results view, click the *Export to Workbook* button.

10.2.3. Filtering objects

Objects that disturb you, or that don't interest you, can be excluded from the measurement results. All of the measurement values that lie outside the defined measurement value area, won't be displayed, nor taken into account in any of the results views.

Example: On an image with spheres of different sizes, 9 size classes are defined. You want to know how many spheres fall into which size class. When the analysis has been carried out, you discover that the number of the small spheres has been overestimated, because spheres that weren't correctly separated, were also taken into account (image on the left). Define an object filter that only counts roughly circular objects.



Left: At the top right of the image, you can see some spheres that weren't divided properly. They have been sorted into the class of small spheres and are displayed in red.
Right: After the definition of an object filter, the number of objects in each class has changed. In particular, the red class of small spheres now has fewer objects.

Preparations

1. Load the image you want to analyze or acquire one.
2. Carry out an automatic object analysis on the image.
3. In the *Count and Measure Results* tool window, switch to the *Object Filter* results view.
   - In the table you will see a list of all of the selected measurement parameters and their corresponding filter ranges. There will always only be one measurement parameter active.
   - If the measurement parameter that you want to use for the object filter doesn't appear in the list, click the *Select Object Measurements* button. You can find the button on the *Count and Measure Results* tool window's toolbar.

     If you only want to evaluate the almost spherical objects, you can select the *Sphericity* object parameter.

Entering the filter range directly

4. In the table in the *Object Filter* results view, click the measurement parameter for which you want to define a filter range.
5. Double click in the *[Min.* field, located next to the measurement parameter to enter the lower value for the filter range.
6. Either enter the required measurement value directly, or use the arrow keys.
7. Double click in the *Max.[* field, then enter the higher value for the filter range.
   - The higher value itself no longer belongs to the filter range.
   - You can delete individual values by double clicking the value, then pressing the [Del] key.

Defining the filter range interactively

8. In the table, click the measurement parameter for which you want to define a filter range.

9. Click the *Select minimum value* button, above the *Measurement* list to define the filter range's lower value.
   - The mouse pointer will change its form.
10. Click an object whose measurement value is to be used as the lower value for the filter range.
    - The measurement value will then be automatically adopted in the *[Min.* field. When you, for example, define a filter range for the *Area* parameter, click the smallest object that you still want to measure.
    - In the image window, the result of the filtering of the objects can be seen straight away. All of the values that are outside the defined filter range will be excluded from the results.
    - The filter range contains precisely those values that are to appear in the measurement results. All of the values that are outside the defined filter range will be excluded from the results.
    - The *Toggle Object Filter* button appears clicked, thereby showing you that the object filter is active.
11. If you want to undo the selection you've made, click the *Clear minimum value* button.
12. Click the *Select maximum value* button to define the filter range's upper value.
13. Click an object whose measurement value is to be used as the upper value for the filter range. Click the largest object that you still want to measure.
    - The measurement value is rounded up and automatically adopted in the *Max.[* field. The object is still within the filter range.

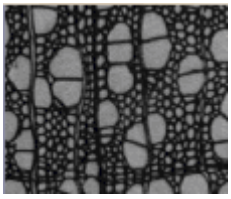
Switching off the object filter

14. Release the *Toggle Object Filter* button.

Note: A defined object filter is not automatically deactivated when you load another image. If, for example, no objects are shown, make sure that the object filter is deactivated.

10.2.4. Classifying objects

Example: You have an image with two object classes, e.g., large and small cells. You want to know how many objects fall into which size class.



Preparations

1. Acquire an image or load one. You can follow these step-by-step instructions using the *WoodVessels.tif* example image.

2. Perform an automatic object analysis on the image.
3. Select the *Area* object measurement.

Selecting measurement parameters for the object classes

4. In the *Count and Measure Results* tool window, select the *Class Measurements* results view.

5. Click the *Select Class Measurements* button, then in the *Select Class Measurements* dialog box, add the *Mean (Area)*, *Object Class* and *Object Count* measurement parameters.
   - With the *Mean (Area)* parameter, the mean area of all of the objects in a class will be calculated. That's to say, the parameter give you a measured value for how large the objects in this class are, on average.
   - With the *Object Class* parameter, you write the name and the color of the class in the results sheet, as well. You should, without fail, adopt this parameter in the results sheet to make it possible to assign the measurement results correctly to the individual classes. You can also adopt this parameter in the *Object Measurements* results sheet. Then, in the results sheet, you'll be able to immediately recognize to which class each of the individual objects belongs.
   - At the end, the *Object Count* parameter delivers the values you are looking for in the task: the number of objects found in each class.
6. Close the *Select Class Measurements* dialog box.

Defining classes

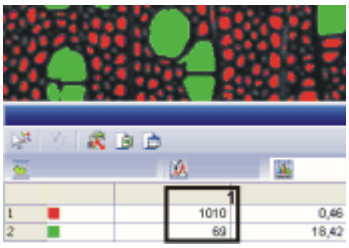
7. Open the *Options* dialog box by clicking the *Count and Measure Options* button, located in the *Count and Measure* tool window.
8. Select the *Count and Measure > Classification* entry in the tree view.

9. In the *Current Classification* group, click the *New Classification* button, then select the *New 'One parameter Classification'* entry.
   - The *Define 'One parameter' Classification* dialog box opens.
10. Enter a descriptive name for the new classification in the *Name* field, *size class* for example.
11. Select the *Area* entry in the *Measurement* list.
    - Only the measurement parameters that have been selected for the object analysis are shown in the list.

12. Click the *Automatic Classification* button to switch to the *Automatic Classification* dialog box.
13. In the *Automatic Classification* dialog box, click the *Get Min./Max. from Image* button. Then the smallest and largest value of the selected parameter that has been entered in the *Minimum* and *Maximum* fields, will be used.
    - In this way, you'll be certain that all of the objects in the image can be assigned to one of the classes that have been defined.
14. Enter the value 2 in the *Number of classes* field, and in the *Scale* field, select the *Logarithmic* entry.
    - By doing this, you have defined two size classes.

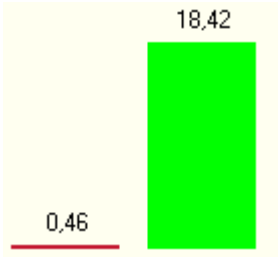
Viewing the results

15. Click *OK* and then the *Count and Measure* button, located in the *Define 'One parameter' Classification* dialog box.
    - The classes will be displayed in the image in color. The measurement parameters that have been selected for the classes will be output in the *Class Measurements* results view.



In the illustration, you can see the image with both of the size classes. The column (**1**) shows the number of large (green) and small (red) cells that was being looked for.

16. Close the *Define 'One parameter' Classification* dialog box.
    - In the *Options > Count and Measure > Classification* dialog box, the new classification is active in the list. You can now use this classification for other analyses as well.
17. Close the *Options* dialog box with *OK*.
18. Then in the *Count and Measure Results* tool window, activate the *Class Histogram* results view to have the class results displayed as a bar chart.
19. Select the *Mean (Area)* entry in the *Measurement* picklist, and the *Class* entry in the *Grouped by* picklist.
    - Now the histogram displays the mean area of the objects for each class.



In the illustration, you see the results for the object classes in the *Class Histogram* results view. The mean area ratio for the object classes is displayed as a diagram. You can clearly see that the green objects are significantly larger than the red objects.

10.2.5. Performing a phase analysis

Example: You have an image with several phases. You want to know how large the area ratio of the individual phases is.

Setting options

1. Open the *Options* dialog box by clicking the *Count and Measure Options* button, located in the *Count and Measure* tool window.
2. Click the *Count and Measure > Detection* entry in the tree view. Enter a value of 1 in the *Minimum object size* field. This ensures that the whole image will be analyzed.

Selecting measurement parameters

3. Select the *Area* object parameter.
4. Select the *Object Class*, *Sum (Area)* and *Relative Object Count* class parameters.

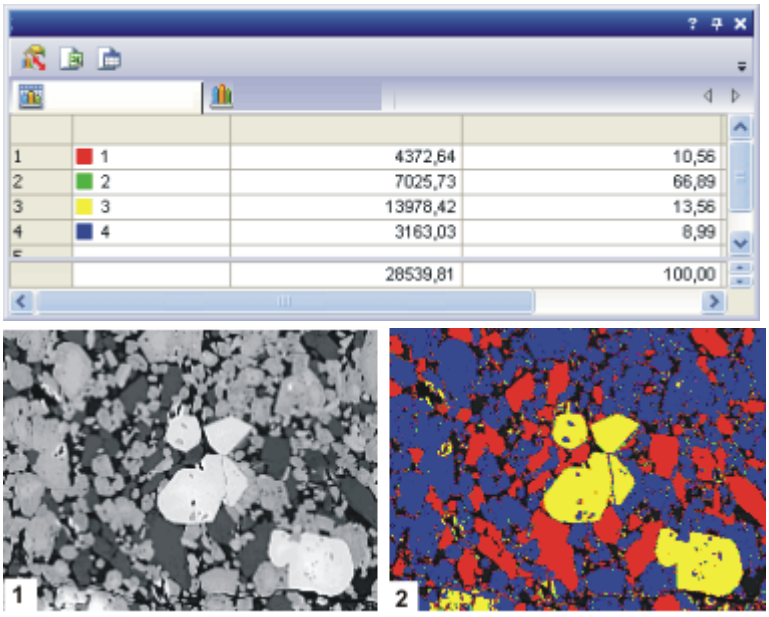
Setting threshold values

5. In the *Count and Measure* tool window, click the *Automatic Threshold...* button to open the *Automatic Threshold* dialog box.
6. Select the *None* option in the *Background* group. This means that no area of the image is defined as background. In this case, the complete image will be evaluated when the automatic analysis is carried out.

7. Keep clicking the *Add Phase* button to add new phases until thresholds have been set for all of the phases in the image.
   - The threshold values are automatically set.
   - The phases that have been defined are shown in the histogram.
   - You can also observe the definition of the phases in the image window. The defined phases have the same color that they were allocated in the dialog box.

Viewing the results

8. Then click the *Count and Measure* button to get the results.
   - The results are shown in the *Count and Measure Results* tool window in the *Class Measurements* results view. For each phase, you see the area that this phase takes up in the image.



The result of a phase analysis: The results sheet shows the area ratio of each phase. The sum of the percentage area ratio is 100%, because the whole image was analyzed.
Image (**1**) has four phases; one black, one light gray, one dark gray and one white.
Image (**2**) shows the image resulting from the phase analysis.

Note: The phase analysis is also available in the analysis processes in the *Materials Solutions* tool window. You can use it to very simply apply the phase analysis to several images consecutively and to display the results in a report.
This analysis process is only available if you have bought the corresponding solution.

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10.3. Carrying out an automatic image analysis on ROIs

An ROI (Region Of Interest) is a certain area of an image. You can limit an automatic image analysis to a certain segment of the image. The analysis will then only be performed on this image segment. You can also define several ROIs and compare the results with each other.

Defining ROIs
Performing a phase analysis on a ROI
Analyzing object classes on ROIs

10.3.1. Defining ROIs

There are several ways of defining ROIs.

- Use the functions in the *Count and Measure* tool window.
- Convert a detected object into an ROI.

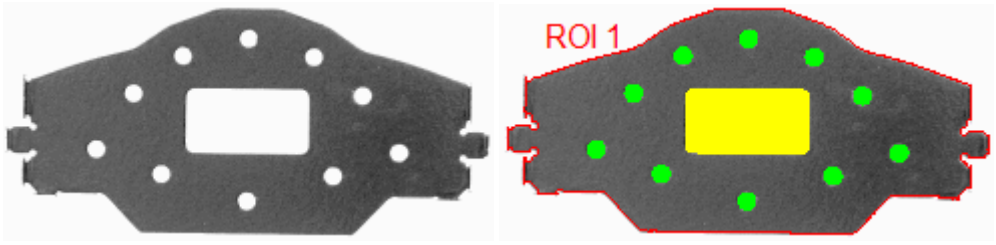
Using the "Count and Measure" tool window

1. Load the image you want to analyze or acquire one.
2. Use the *View > Tool Windows > Count and Measure* command to have the *Count and Measure* tool window displayed.
3. Carry out an automatic object analysis on the image.
4. In the *Count and Measure* tool window, click the small black arrow next to the *Count and Measure* button.
5. Select the *Count and Measure on ROI* entry from the button's context menu.
   - The *Count and Measure* button is now called *Count and Measure on ROI*.
6. From the *Count and Measure on ROI* button's context menu, select the *New ROI* command.
   - A context menu that offers you 3 tools for the definition of ROIs, will open. You can define an ROI as a rectangle, circle or polygon. It is also possible to define several ROIs on an image using different tools.
7. Click on a tool to select it, the *Rectangle* button for example, then move your mouse pointer onto the image.
   - The pointer will change its shape to a cross. The selected tool appears under the mouse pointer.
8. With your left mouse button, define the segment in the image that is to be used for the analysis. If needed, right click to confirm the ROI.
   If necessary, define further ROIs.
9. When all of the ROIs have been defined, click the *Count and Measure on ROI* button to obtain the results.

Note: If the *Count and Measure on ROI* button has been activated, but no ROI have been defined, the automatic analysis will by performed on the complete image.

Converting an object into an ROI

You can use this method of defining an ROI when you want to analyze objects within an object.



The image on the left shows workpiece with holes. The automatic object analysis (**right**) has turned the workpiece into a ROI.

1. Load the image you want to analyze or acquire one.
2. Define the threshold values to include the object that you want to turn into an ROI.
   - In the example shown, you can use automatic threshold value setting and select a light background.
     Please note that the holes should not be filled by the image analysis. To do this,

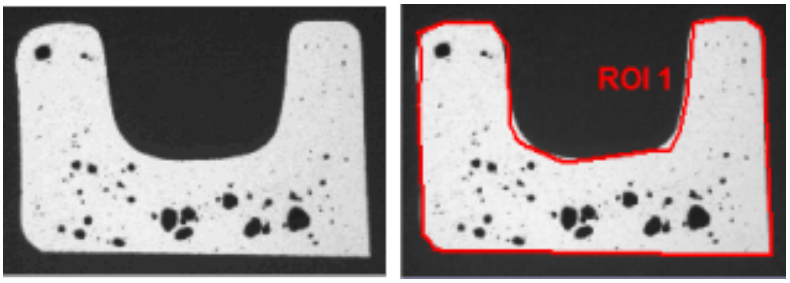
clear the *Fill holes* check box in the *Tools > Options > Count and Measure > Detection* dialog box.

3. Carry out an automatic object analysis on the image.
4. Click the *Select Detected Objects* button to switch to the selection mode. You can find the button in the *Count and Measure* tool window, in the *Edit Objects* group.
5. Select the object that you want to turn into an ROI.
6. Click the right mouse button to open a context menu.
7. Select the *Create ROIs from Selected Objects* command from the context menu.
   - The object will now be converted into an ROI.
   - You can find the ROI in the *Measurement and ROI* tool window. You can rename the ROI here. You can also save and delete the ROI.
8. Now define suitable threshold values for the objects inside the defined ROI.
   - In the example shown, you can use automatic threshold value setting and select a dark background.

10.3.2. Performing a phase analysis on a ROI

Task

You have a light object. The light object contains several smaller areas belonging to a dark phase. You want to know what percentage of the whole area of the light object is taken up by the dark phase. This problem can be resolved by performing a phase analysis on a ROI.



On the second image (right), the ROI has been defined as a light object. This enables the percentage area ratio to be computed.

1. Load the image you want to analyze or acquire one. You can follow these step-by-step instructions using the *MacroscopicComponent.tif* example image.

Selecting measurement parameters

2. Select the *ROI* and *Area Fraction ROI* class parameters. To do this, you can click the *Select Class Measurements* button located in the *Class Measurements* results view.

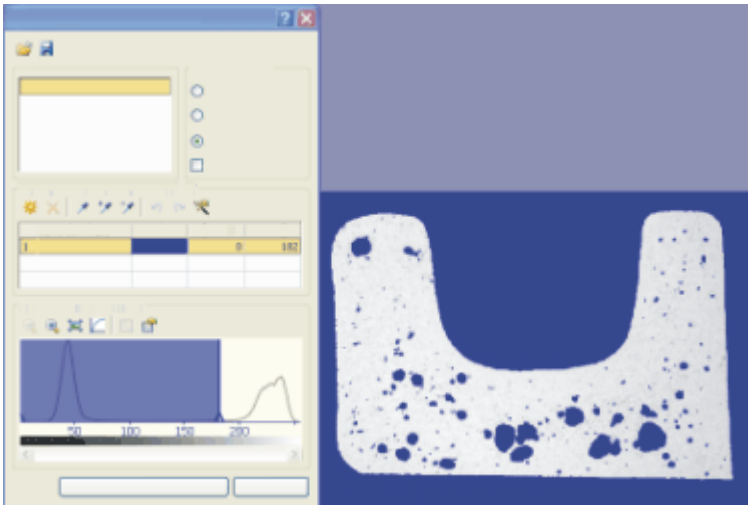
Defining an ROI

3. In the *Count and Measure* tool window, click the *Count and Measure* button's small black arrow to open a context menu. In the context menu, select the *New ROI > Polygon* command.

4. Move your mouse pointer onto the image.
   - The pointer will change its shape to a cross.
5. With your left mouse button, define the segment in the image that is to be used for the analysis. To do this, click on pixels on the edge of the light object.
6. Right click to confirm the ROI.

Setting threshold values

7. Open the *Manual Threshold* dialog box.
8. Set suitable threshold values for the phase.



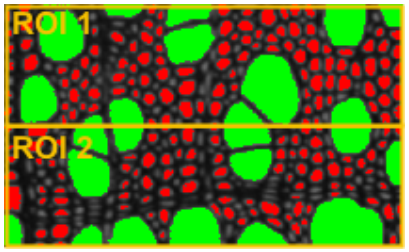
Viewing the results

9. In the *Count and Measure* tool window, click the *Count and Measure* button's small black arrow to open a context menu. In the context menu, select the *Count and Measure on ROI* command.
   - The results for the ROI are shown in the *Class Measurements* results view. The *Area Fraction ROI* column shows what percentage of the ROI's area is taken up by the phase.

10.3.3. Analyzing object classes on ROIs

Task

You are interested in two segments and two object classes on an image.



Two ROIs have been defined on the image. You want to calculate the number of large and small cells in the upper and lower area of the image and compare them to each other.

Preparations

1. Acquire an image or load one.
   - You can follow these step-by-step instructions using the *WoodVessels.tif* example image.
2. Perform an automatic object analysis on the image.
3. Select the *Area*, *Object Class* and *ROI* object measurements.
4. Select the *Mean (Area)*, *Object Class*, *Object Count* and *ROI* class measurements.
5. Select a classification that groups all objects into two size classes.

Defining an ROI

6. Define two rectangular ROIs on the image.

Setting options

7. Open the *Options* dialog box by clicking the *Count and Measure Options* button, located in the *Count and Measure* tool window.
8. Select the *Count and Measure > Detection* entry in the tree view.
9. In the *Borders - ROI* group, select the *Truncate* option. By doing this, you will make sure that objects that lie on the edge of the ROI are counted as well. Observe though, that the objects will be cropped. The area of the objects on the edge won't, therefore, be correctly measured. You should use this option especially when you're mainly interested in finding out how many objects are present, and are not interested in the area.

Selecting measurement parameters for the ROIs

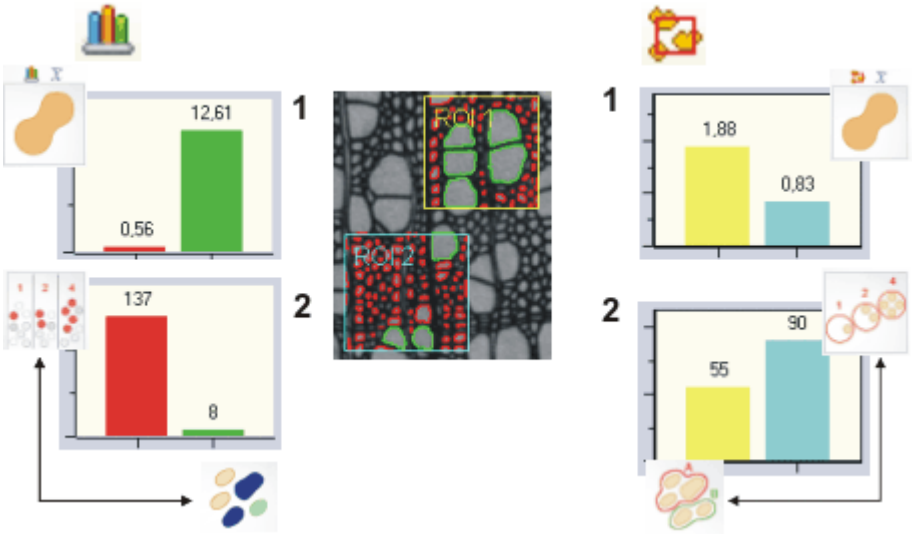
10. Select the *Count and Measure > Measurements* entry in the tree view.
11. Click the *Select ROI Measurements* button, then in the *Select ROI Measurements* dialog box, add the *Mean (Area)*, *ROI* and *Object Count* measurement parameters.

12. Close all open dialog boxes.

Viewing the results

13. In the *Count and Measure* tool window, click the *Count and Measure* button's small black arrow to open a context menu. Select the *Count and Measure on ROI* entry there.
    - The button is now called *Count and Measure on ROI*. The results will be automatically output.
    - The classes will be displayed in the image in color. The selected measurement parameters for the classes and ROIs will be output in the *Class Measurements* and *ROI Measurements* results views.



The analysis performed above, supplied numerous different results. This illustration explains some of the possible results of the analysis that was performed above.

In the image in the middle, you can see that the analysis was carried out on two ROIs (blue and yellow). In both ROIs, objects were recognized that were assigned to two size classes. The small objects are shown in red, the large objects in green.

Class measurements

The results of the class measurement are shown to the left of the image. You can find these results in both the *Class Measurements* and the *Class Histogram* results view.
In diagram (**1**), you can see the mean area of an object for each of the size classes defined.
As can be expected, the green objects are on average, much larger than the red ones.

In diagram (**2**) the number of objects that fall into the green class and the red class, are shown. Clearly, there are far more small, red objects, than there are large, green ones. The class results take all of the objects into account, regardless of in which ROI they were found.
You can, however also output the class results per ROI. In this case, select the *ROI* entry, in the *Grouped by* list.

ROI measurement

The results of the ROI measurement are shown to the right of the image. You can find these results in the *ROI Measurements* and the *ROI Histogram* results view.
In the diagram (**1**) you can see, for each ROI, the mean area of all of the objects found in that ROI. More large, green objects were found in the yellow ROI, than in the blue ROI. For this reason, the mean area of an object in the yellow ROI is considerably larger than that in the blue ROI. The difference is, however, not so extreme as the ratio of small to large objects is.

In diagram (**2**) the number of objects per ROI has been plotted. There are more objects in the blue ROI than there are in the yellow one.

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10.4. Editing objects

In the *Count and Measure* tool window, you can find a toolbar with which you can deal with individual objects. You can select one or more objects, add new objects, or delete objects. As well as this, it's possible to manually or automatically separate objects that are joined together. Should you not be able to see the *Edit Objects* group in the *Count and Measure* tool window, click the small black arrow to make it visible.

Editing objects

The buttons in the *Edit Objects* group aren't available until the image has been analyzed. Some of the editing functions require you to first select the objects you want to work on.

Select one or more objects that you want to edit and then click, for example, the *Delete Selected Objects* button to delete all of the selected objects simultaneously. The results will be immediately updated. If you have deleted or added objects, the total will be correspondingly adjusted.

Note: When you have edited objects in an image, then analyze the image again, the changes you've made will be lost.

Selecting detected objects

1. Carry out an object analysis on the current image or load an image with an existing object analysis.
2.  Click the *Select Detected Objects* button in the *Count and Measure* tool window to switch to the selection mode.
   - Now, as soon as you move the mouse onto the image, the shape of the mouse pointer will change. The shape of the mouse pointer is different depending on whether the mouse pointer is on an object or not.
3. Click on any object to select it.
   - The selected object will be displayed hatched, and can now be edited.
   - The corresponding row in the *Object Measurement* results view will be selected. Thus, selecting, is one more way in which you can examine measurement values of individual objects.
   -  Alternatively, you can also use the *Selection Tool* button, located on the *Toolbox* toolbar.

Selecting several objects

4. To select several objects simultaneously, in the selection mode, while keeping the [Ctrl] key depressed, click the objects you want to select, one after the other.
   - Every object you click will be added to the selection.
   - Alternatively, you can also keep the left mouse button depressed, and while doing so, drag out a frame. All of the objects that are fully, or only partly, within this frame, will be added to the selection.

Undoing the selection

5. To undo a selection, in the selection mode, while keeping the [Ctrl] key depressed, click the selected object again.

Selecting all of the objects

6. To select all of the objects simultaneously, use the keyboard shortcut [Ctrl + A], in the selection mode.

Selecting objects via the results sheet

You can also select objects using the results sheet in the *Count and Measure Results* tool window. This method can be useful if you only want to select objects with specific numerical values, for example.

1. In the *Count and Measure Results* tool window, click any row in the *Object Measurement* results sheet.
   - The row will be selected. The corresponding object in the image will also be selected, and will appear hatched. You can always recognize which object belongs to which measurement result.
   - You will then automatically switch into the selection mode.
2. You can also select several rows simultaneously here, or undo selections. To do this, use the same keyboard shortcuts that you use for the selection of objects in the image.
3.  Then click the *Select Detected Objects* button, located in the *Edit Objects* group, again to leave the selection mode.

Adding new objects

1. Carry out an object analysis on the current image or load an image with an existing object analysis.
2.  To add new objects to an image, use the *New Object* button in the *Count and Measure* tool window. Click the arrow next to the *New Object* button.
   - A context menu opens that offers you 2 tools for adding objects. You can add an object as a polygon or as a circle. It's also possible to use both tools on one image.
3.  Click on a tool, e.g., the *New Circle . Object* button, then move your mouse pointer onto the image.
   - The shape of the mouse pointer on the image indicates the current mode.
4. While keeping your left mouse button depressed, drag out a circle on the image, that is to be added as a new object.
5. Confirm your selection with the right mouse button. Add, if necessary, further objects.
   - The results shown in the *Object Measurements* table are updated and the object count is increased accordingly.

Deleting objects

1. Carry out an object analysis on the current image or load an image with an existing object analysis.
2.  Click the *Select Detected Objects* button in the *Count and Measure* tool window to switch to the selection mode.
3. Click on an object to select it. If you want, expand the selection and select more objects to be deleted.
4.  Click the *Delete Selected Objects* button to delete the selected object.
   - All of the selected objects will be deleted from the image. The corresponding data in the *Object Measurement* sheet will be deleted.
5. You can also select objects using the results sheet in the *Count and Measure Results* tool window. Select one or more rows there. Then right click and select the *Delete All Selected Objects* command in the context menu.

Separating objects manually

1. Carry out an object analysis on the current image or load an image with an existing object analysis.
2.  Click the *Manually Split Objects* button, then move your mouse pointer onto the image.
   - The shape of the mouse pointer on the image indicates the current mode.
3. Next, while keeping your left mouse button depressed, drag a line through the object that you want to separate into two. Make sure, when you do this, that you drag the line over the object's outside edge, since otherwise it won't be separated. You can also separate several objects with a separation line.
4. Right click to confirm the separation line.
   - The object will then be divided up into two independent objects.
   - The classification is updated. As a consequence, some objects may now be assigned to other classes. The object count is increased, and a new line is added to the results sheet.

Separating objects automatically

1. Carry out an object analysis on the current image or load an image with an existing object analysis.
2.  Click the *Select Detected Objects* button in the *Count and Measure* tool window to switch to the selection mode.
3. Click one or more objects to select them.
4.  Click the *Auto Split Selected Objects* button to have the touching objects automatically separated.
   - Objects that fulfill the morphological criteria for a separation, will be separated.
   - If a lot of objects are selected, the automatic object separation can take a long time. If this is the case, a progress bar is displayed in the status bar. You can

abort the process at any time by clicking the *Cancel* button.

- The results will be correspondingly updated.

Left: A joined up object that is to be separated.
Middle: The joined up object has been selected.
Right: The joined up object has been separated, there are now two independent objects.

Note: The automatic separation of objects only functions in cases that are very clear-cut. For this reason, the recommendation; apply a morphological filter before the actual object analysis is performed, to generally improve the separation of objects.

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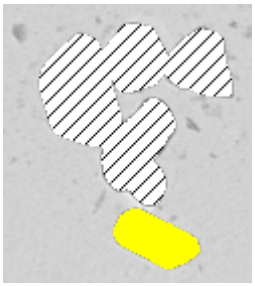
10.5. Enhancing the segmentation

It may well occur, that after the segmentation has taken place, objects in an image have not yet been correctly separated. To improve the segmentation, you can, for example, use a morphological filter.

Separating touching objects

Task: Use the *Separate Objects* morphological filter to separate touching objects before performing an image analysis.



In this image, several objects are touching. This means that the detection process doesn't recognize them as separate objects. It counts them as a single object.

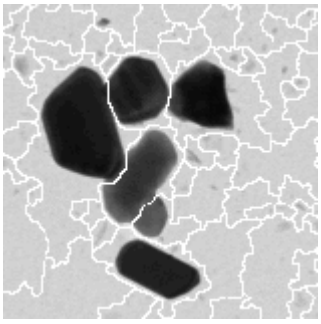
Preparation

1. Load the image that you want to analyze.
2. The *Separate Objects* filter creates separation lines on the image. This changes the image information. Therefore, save the source image under a different name if you want to keep the original data.

Separating objects

3. Use the *Process > Morphological Filter > Separate Objects...* command.
   - The *Filter: Separate Objects* dialog box opens.
4. Zoom into the source image to make it possible to easily recognize a typical object that has not been separated.
5. Select the *Original and Preview* preview function. Now, the same image segment will be displayed twice in the preview area of the image processing dialog box. The one shown on the left is the source image. The one on the right is the image that results when the current parameters are used.
6. For this example, select the *Step* option in the *Settings* group.
7. Move the *Fine / Coarse* and *Smoothness* slide controls and observe the effect in the dialog box's preview. Start with the small values. Small values generally result in a lot of separation lines.
   In this example, the objects are separated well using the *Fine / Coarse* = 1 and *Smoothness* = 3 parameters.
8. Select whether the filter is to take 4 or 8 neighboring pixels into account in each case, then observe the effect this has in the dialog box's preview. Select the parameter that does the best job of separating the touching objects.

9. In this example, select the *Burn white* option. The separation lines are now white and so don't interfere with the setting of the threshold values for the dark objects.

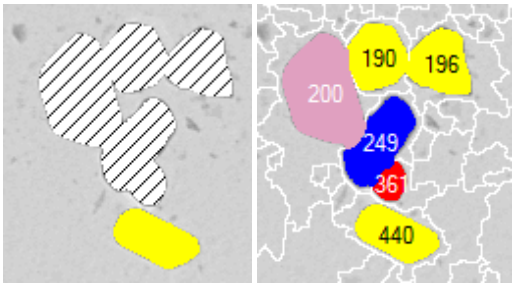


After the *Separate Objects* filter is applied white separation lines separate the touching objects.

10. In the *Filter: Separate Objects* dialog box, click the *OK* button to apply the filter.
    - Note: The contents of the image will be changed. You may have to check the threshold value settings for the next object analysis.
    - Depending on the size of the image, applying the *Separate Objects* filter can take a long time. Watch the progress bar located in the status bar.

Carrying out an object analysis

11. In the *Count and Measure* tool window, click the *Count and Measure* button to perform the object analysis and output the results.



The image on the **left** shows the original image before the objects were separated. The image on the **right** shows the separated objects after the *Separate Objects* filter has been applied. The numbers are the objects' IDs. All objects with the same color belong to the same size class. Before the object was separated, it was so large that it couldn't be assigned to a size class. That's why it was cross hatched.

Separating touching objects without changing the original image

Task: In this example, objects that haven't been correctly separated by the segmentation process, are to be separated by using the *Separate Objects* morphological filter. This doesn't change the original image.

Showing the segmentation image

Usually, the segmentation image is not shown in the *Dimension Selector* tool window. You have to activate it first.

1. In the *Count and Measure* tool window, click the *Count and Measure Options* button, and select the *Count and Measure > Segmentation* entry in the tree view.
2. Select the *Show 'Segment' button* check box, and clear the *Delete segmentation after detection* check box.
3. Close the *Options* dialog box with *OK*.

Starting an object analysis

4. Load the image you want to analyze and set the thresholds.
5. Then click the *Segment* button in the *Count and Measure* tool window to create the segmentation image.
   - You can now see the segmentation image in the image window. The segmentation image is a binary image in which all of the objects that are defined by the thresholds that have been set are displayed in red. The segmentation image belongs to the original image and is added to the original image in its own image layer. Use the *Dimension Selector* tool window to toggle between the source image and the segmentation image.
   - The subsequent separation of the objects takes place in the segmentation image.

Separating objects in the segmentation image

6. Use the *Process > Morphological Filters > Separate Objects* command to separate objects in the segmentation image.
   - The *Filter: Separate Objects* dialog box opens.
7. Select the *Boundary shape > Dark* option, if you want to separate bright objects that have a dark background.
8. Select the *Burn black* option, if you want to separate bright objects that have a dark background.
9. Set a value between 1 and 10 with the *Fine / Coarse* slide control and observe the effect in the dialog box's preview. Select the parameter that does the best job of separating the touching objects.
10. Choose the *Apply on > Selected frames and channels* option. Now, the *Separate Objects* filter works exclusively on the segmentation image. The source image remains unchanged.
11. In the *Filter: Separate Objects* dialog box, click the *OK* button to apply the filter.

- The object analysis that now follows is performed exclusively on the changed segmentation image.

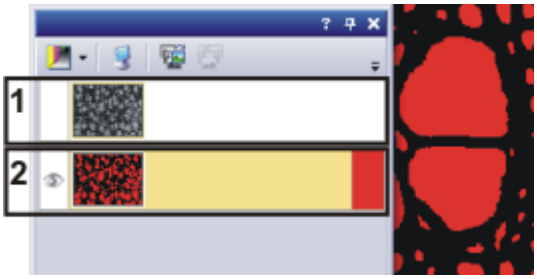
The image on the left shows an object when the segmentation has taken place. The object was falsely detected as joined up, although there are actually two touching objects. In the image on the right, one can see the correctly separated objects, after the *Separate Objects* filter has been applied.

Carrying out an object analysis

12. In the *Count and Measure* tool window, click the *Count and Measure* button to perform the object analysis and output the results.
    - After you have performed the object analysis, take a look at the unchanged source image with the analysis results in the image window.

Displaying the segmentation image

13. Use the *View > Tool Windows > Dimension Selector* command to show the *Dimension Selector* tool window.
    - You can toggle between the source image and the segmentation image there.



The source image (**1**) and the segmentation image (**2**) are displayed in the *Dimension Selector* tool window. Click the eye icon in front of one of the images to hide it in the image window. Click on the empty cell without an eye icon to make the corresponding image reappear.

Note: Use the *Dimension Selector* tool window to show or hide the segmentation image.
Use the *Layers* tool window to show or hide the results of the object analysis in the image window.

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11. Reports

11.1. Overview

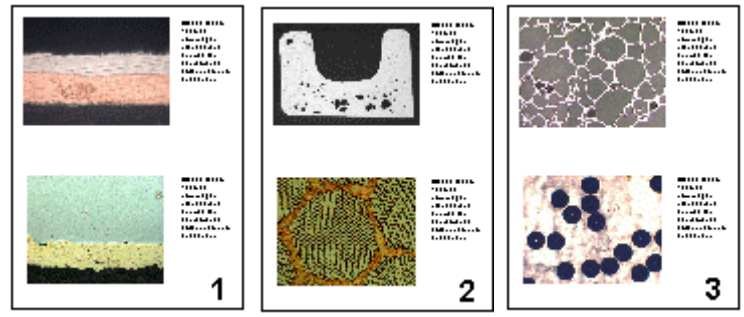
You can create reports with your software to document the results of your work and to make them available to third parties. You can share reports as files or as printed documents.

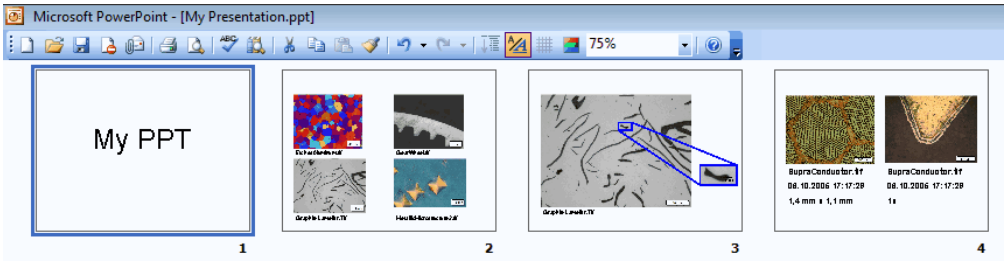
Two programs are always involved in the creation of reports: Your image analysis software and a Microsoft Office application program.

You can use the following Microsoft Office application programs to create reports:

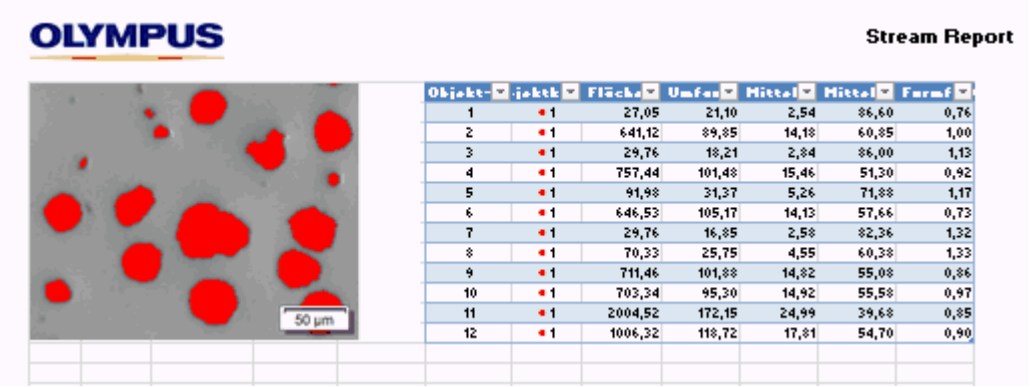
- Microsoft Word 2010, 2013, 2016, 2019 or the Office 365 Word Desktop app
- Microsoft Excel 2010, 2013, 2016, 2019 or the Office 365 Excel Desktop app
- Microsoft PowerPoint 2010, 2013, 2016, 2019 or the Office 365 PowerPoint Desktop app

Examples of reports in different file formats





The illustration displays one report in MS-Word format and one in MS-PowerPoint format.



Objekt-	jektk	Fläche	Umfan	Mittel	Mittel	Formf
1	1	27,05	21,10	2,54	86,60	0,76
2	1	641,12	89,85	14,18	60,85	1,00
3	1	29,76	18,21	2,84	86,00	1,13
4	1	757,44	101,48	15,46	51,30	0,92
5	1	91,98	31,37	5,26	71,88	1,17
6	1	646,53	105,17	14,13	57,66	0,73
7	1	29,76	16,85	2,58	82,36	1,32
8	1	70,33	25,75	4,55	60,38	1,33
9	1	711,46	101,88	14,82	55,08	0,86
10	1	703,34	95,30	14,92	55,58	0,97
11	1	2004,52	172,15	24,99	39,68	0,85
12	1	1006,32	118,72	17,81	54,70	0,90

This illustration shows a report in MS-Excel format. The report displays an image with measurements on it and an Excel sheet with the measurement results.

Different ways of generating reports

The requirements for working with reports are very different depending on the user and the way you are working. There are different procedures for creating reports.

1) Creating MS-Word reports using the "Report Composer" tool window

For users who regularly create reports that are made up in the same way with a lot of images and who require these in MS-Word format.

For this, your image analysis program should be open in the foreground. In the *Report Composer* tool window, open or create a report instruction (RCI file) in which you specify which images and which page layout the report should contain. Then you create a report which is displayed in MS-Word at the touch of a button. In MS-Word you now only undertake small corrections of the report.

Note: With the *Report Composer* tool window, it is **only** possible to create reports that can be opened with the MS-Word application program.

2) Creating and editing reports using the Olympus MS-Office Add-in

For users who require reports in MS-PowerPoint format.

For users that want to insert images or documents that were created with the image analysis program into new or existing MS-Excel documents.

For users that want to insert images or documents that were created with the image analysis program into new or existing MS-Word documents. Also for users who want to process MS-Word reports that were created using the *Report Composer* tool window.

When you use the Olympus MS-Office add in, your image analysis program opens in the background. You can use the Olympus MS-Office add-in to insert images, workbooks or charts from your software into an MS-Word, MS-Excel, or MS-PowerPoint document. You use what are called templates to do this. With MS-Word reports, you define **Page Templates** in the DOC or DOCX file format. With MS-PowerPoint reports, you define **Slide Templates** in the PPT or PPTX file format. With MS-Excel reports, you define **Excel templates** in the XLTX file format.

3) Creating MS-Excel reports from the software

For users who require reports in MS-Excel format, for example, because they want to analyze the data and measurement results gained in the image analysis program further in MS-Excel. The table with the measurement results is inserted into the MS-Excel document as what is known as an Excel sheet.

For this, your image analysis program should be open in the foreground. Let's assume you've made some length measurements on an image and now click the *Create Excel Report* button in the *Measurement and ROI* tool window. In the *Create Excel Report* dialog box, you select the Excel template to be used for the report. Clicking the *OK* button now starts the MS-Excel application program where the report is displayed.

Procedure 1: Report generation using the "Report Composer" tool window

1. New report instruction
Switch to the *Reporting* layout and create or open a report instruction. Place the documents you want in it.

2. Creating a report
Create a report.

3. Working with the Olympus MS-Word add-in
In MS-Word: Have a look at the report and make small changes, if necessary.

4. Saving the report and the report instruction
If you want, print the report or create a PDF file. Save the report instruction (and the report as well, if needed).

Procedure 2: Report generation using the Olympus MS-Office add-in

1. Creating a new PPTX, XLSX or DOCX file
Create a new file or open an existing document.

2. Working with the Olympus MS-Office add-in
In MS-Word, MS-Excel, or MS-PowerPoint: Use the functions on the *Olympus* tab to add documents to the report.

3. Working with the Olympus MS-Office add-in
Use the functions on the *Olympus* tab to layout the report (info stamp, detail zoom and border, for example).

4. Saving the report
Save the report. If you want, print it or create a PDF file.

Procedure 3: Creating MS-Excel reports from the software

1. Measuring and saving an image
Open or create an image and carry out several measurements on it. Save the image.

2. Selecting the Excel template and the data to be used for the report and starting the report creation
Click the *Create Excel Report* button and make the necessary settings in the *Create Excel Report* dialog box. Click the *OK* button to start MS-Excel.

3. Working with the Olympus MS-Office add-in
Use the functions on the *Olympus* tab to layout the report (info stamp, detail zoom and border, for example).

4. Saving the report
Save the report. If you want, print it or create a PDF file.

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11.2. Working with the report composer

The *Report Composer* tool window supports you when you are creating and updating report instructions. In this tool window, you also find the *Create* button that is used to start the report creation.

Note: Two programs are involved in the creation of reports using the *Report Composer* tool window: Your software and the MS-Word application program. You can use the following versions for working with reports: Microsoft Word 2010, 2013, 2016, 2019 or the Word Desktop app of Office 365.

Note: With the *Report Composer* tool window, it is **not** possible to create a report that can be opened with the MS-PowerPoint or MS-Excel application programs.

Should the *Report Composer* tool window be hidden, use the *View > Tool Windows > Report Composer* command to make it appear.

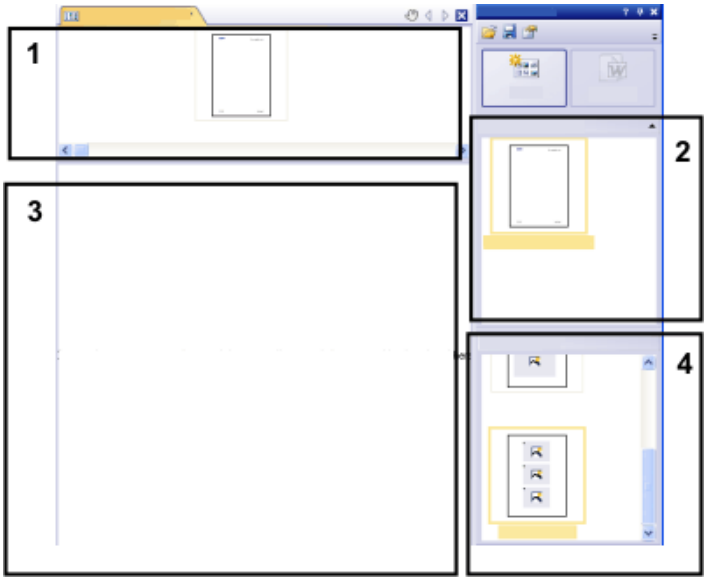
Creating a new report instruction

To create a report, first create a new report instruction in your software. You can also use a saved report instruction.

Note: The report instruction has to contain at least one registered page template.

1. Switch to the *Reporting* layout.

2. Click the *New Report Instruction* button. You find this button in the *Report Composer* tool window.
   - A new document of the *report instruction* type will be created in the document group. This document is at the same time the workspace in which you put the report together.



3. If no default document template has been defined: Drag the document template you want onto the upper part (**1**) of the report instruction. You find a list of the available document templates in the upper part (**2**) of the *Report Composer* tool

window.

- If a default document template has been defined, it will be automatically inserted in the upper part of the new report instruction.
- Creating a report is also possible when you leave the upper part of the report instruction empty. In this case, the default MS-Word document template is used.

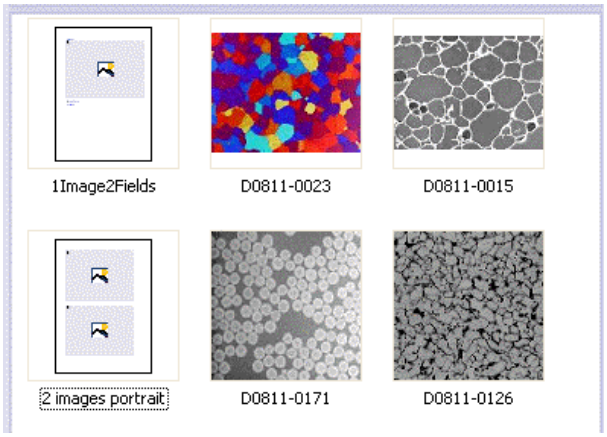
4. Drag the page templates you want onto the lower part of the report instruction (**3**). You find a list of the available page templates in the lower part (**4**) of the *Report Composer* tool window.
   - Every report has to contain at least one page template.
   - Make sure that the page templates contain the correct placeholders for the document types that you want to drag onto the report instruction. Accordingly, if your report is to contain an image and a chart, select a page template that contains one placeholder for an image and another for a chart.
   - If you want to use workbooks in your reports, MS-Excel must be installed on your PC. The minimum MS-Excel version required is MS-Excel 2010.
   - The placeholder for a workbook can also be used for an MS-Excel file. To do so, select the MS-Excel file in the *File Explorer* tool window and drag it onto the report instruction. In the report instruction, MS-Excel files are shown with this icon:

5. Drag the documents you want onto the lower part of the report instruction (**3**).
   - In the *Reporting* layout, the *Database*, *Gallery* and *File Explorer* tool windows are arranged to the left of the document window. In each of the tool windows you can select one or more documents and drag them onto the report instruction. If you use the *File Explorer* tool window, the documents do not need to be open for this. If you use the *Database* tool window, the documents don't have to be open. It is sufficient to open the database. However, the *Gallery* tool window only allows you to select documents that are currently open in your software.
   - You can also integrate MS-Word files (e.g., background information regarding the project) into your MS-Word reports. MS-Word file don't need a placeholder in the report instruction. Select the MS-Word file in the *File Explorer* tool window and drag it directly onto the report instruction. In the report instruction, MS-Word files are shown with this icon:

   - The documents must have been saved, because unsaved documents cannot be included in a report.

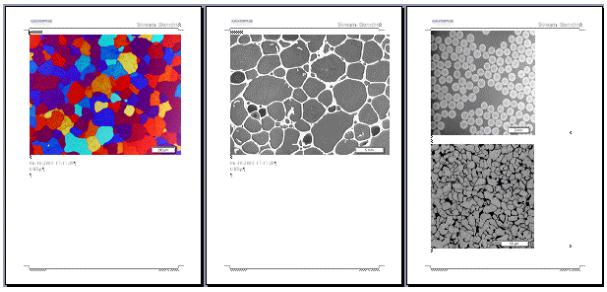


The illustration shows an example of a report instruction. In the report, two different page templates are to be used. The first page template contains a single placeholder for an image, the second page template contains two placeholders for an image. After the page template, the images that are to be inserted in the report page are displayed.

6. Check the report instruction now. You may still edit it and, e.g., delete or shift documents or select another page template.

Creating a report

1. Click the *Create* button. You find this button in the *Report Composer* tool window.
   - The report will be created. Creating a report can take some time when large reports with many images and documents are involved. Pay attention to the progress bar that is shown. The MS-Word application program will open automatically and display the new report. In the example shown below, the report has three pages. (The fact that the first page template only contains one image placeholder and two images have been added to the report instruction, automatically leads to the creation of two report pages.)



2. If you want to, you can still make additional changes in the MS-Word application program. To do so, use the add-in from Olympus.
3. If you want to, save the report instruction and the report.

Editing a report instruction

You can make the changes described below to a report instruction. These changes do not apply to reports the have already been created on the basis of this report instruction. Therefore you must create a new report in order to see the changes you made. This will generate a new MS-Word document. Any changes that you may have

made in the first version of the report is not be contained in the newly created MS-Word file.

Exchanging the document template

1. Load the report instruction that you want to edit.
   - Report instructions have the file extension RCI.
2. To delete a document template, select it and press the [Del] key on your keyboard.
3. Drag the new document template onto the upper part of the report instruction.
   - By doing so, the document template is exchanged. Please note that a report instruction can only contain one document template.
   - A report instruction must not contain a document template at all. When you leave the upper part of the report instruction empty, the MS-Word default document template will be taken.

Changing the page templates

1. Load the report instruction that you want to edit.
2. In the report instruction, select the page template you want to exchange.
3. Use the [Del] key on your keyboard to delete the selected page template from the report instruction.
   - By doing so, you only deselect the page template, no file will be deleted.
4. Drag the new page template to the position in the report instruction, where the deleted page template had been located.
   - Every report has to contain at least one page template.

Shifting the page templates

1. To shift a page template to another place in the report instruction, select it and, with the left mouse button depressed, drag it to a new position (Drag&Drop).
   - In certain cases, this may change the appearance of the report considerably. All documents that come after this page template in the report instruction will use this page template in the report.

Deleting documents

1. Load the report instruction that you want to edit.
2. In the report instruction, select the documents that you want to delete.
3. Use the [Del] key on your keyboard to delete all of the selected documents in the report instruction.
   - By doing so, you only undo the document selection, no file will be deleted.

Adding documents

You can add new documents to an existing report instruction at any time.

1. Load the report instruction that you want to edit.
2. Simply drag the new documents onto the position you want in the report instruction.

- Dragging & dropping images onto the report instruction is possible from the *Database*, *Documents*, *File Explorer* and *Gallery* tool windows.
- Please note the page templates must be placed before the images.

Moving documents
You can change the order in which the selected documents are arranged in the report instruction at any time.

1. Load the report instruction that you want to edit.
2. Select an image, and with the left mouse button depressed, drag it to another position (Drag&Drop).

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11.3. Working with the Olympus MS-Office add-in

Note: The language on the *Olympus* tab corresponds to the language set in your image analysis software. This language can differ from the language in which the MS-Word, MS-Excel or MS-PowerPoint application program is shown.

11.3.1. The add-ins' functions

This add-in assists you with very different tasks:

1. Inserting a document that is currently open in your image analysis program, into an MS-Word, MS-Excel, or MS-PowerPoint document. In order for this to work, the document must have been saved. Unsaved documents can’t be inserted.
2. Inserting a document that is saved locally, or is in your image analysis program's database, into an MS-Word, MS-Excel, or MS-PowerPoint document.
3. Inserting a field that contains information that is saved in your image analysis program into your MS-Word, MS-Excel, or MS-PowerPoint document. This makes sense, for example, when you want to see the acquisition date of a certain image.
4. You add one or more detail zooms to an image.
5. You change the image properties and set, for example, whether or not the info stamp and the scale bar should be shown.
6. You change the resolution of one or all images of the report. If you want to share the report, it may be sensible to reduce the resolution, thereby also reducing the file size.
7. You update all placeholders in your report. This makes sense, for example, when you've made changes to the documents in your image analysis software that the report doesn't contain yet.
8. Inserting an MS-Word, MS-Excel, or MS-PowerPoint document into the software's database. This command is only available if your software supports the database functionality.
9. Defining templates that you want to use for your work with reports. With MS-Word reports, you define page templates in the DOC or DOCX file format. With MS-PowerPoint reports, you define slide templates in the PPT or PPTX file format.

With MS-Excel reports, you define Excel templates in the XLTX file format.

10. You can insert an additional table placeholder into the MS-Excel report to view the contents of an additional worksheet.

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11.3.2. Creating MS-Excel reports

When your image analysis software is installed, an Olympus add-in is added to the MS-Excel application program. When you open MS-Excel and see the *Olympus* tab, this indicates that the add-in has been installed. With the help of this add-in, you can create reports in MS-Excel that contain images, workbooks and charts from your image analysis program.

Creating reports using the MS-Excel application program is an alternative to creating reports using the MS-Word application program or the MS-PowerPoint application program.

MS-Excel reports are particularly useful for users who want to analyze the data and measurement results gained in the image analysis program further in MS-Excel.

Creating an MS-Excel report that contains an image and measurement results
Editing the data in an MS-Excel report
Displaying the contents of an additional worksheet in an MS-Excel report

Creating an MS-Excel report that contains an image and measurement results

Example: Let's say that you want to make measurements on the **Seal.tif** example image and to output the measurement results together with the image into a simple MS-Excel report. The MS-Excel report will be created using the predefined "1 Image 1 Table.xltx" Excel template.

To do so, proceed as follows:

1. Load the **Seal.tif** example image.
2. Switch to the *Processing* layout and make a measurement on the image. You can measure the diameter of a circle and the area of the rectangle, for example.
   - The measurement results will be shown in the *Measurement and ROI* tool window.
3. Save the image. Unsaved images cannot be inserted into a report.
4.  Click the *Create Excel Report* button, located in the *Measurement and ROI* tool window's toolbar.

Note: This toolbar also has a button called *Export to Excel*. This button saves the measurement results, but not the image, straight away to an MS-Excel file. But because these step-by-step instructions are for creating an MS-Excel report that uses a default Excel template, the *Export to Excel* button is not relevant for this example.

   - The *Create Excel Report* dialog box opens. All of the Excel templates in the directory that is currently selected in the *Path* field are displayed in the left-hand part of this dialog box. By default, the right-hand part displays the current image

along with one or more tables with the current measurement results from this image.

Note: If you changed the default settings, all of the images with measurements that are open in the document group can be displayed in the right-hand part of the dialog box. In this case, you must first select the images and documents that you want the MS-Excel report to contain.

5. For this example, select the default Excel template "1 Image 1 Table.xltx" from the left-hand part of the dialog box. Select the **Seal.tif** image and its associated table with the measurement results from the right-hand part of the dialog box.
6. If the *Use only data of selected images in tables* check box is displayed, leave it clear for this example. This check box is only relevant when you want to output the results of a measurement on more than one image to a single MS-Excel sheet.
7. Click the *OK* button.
   - The MS-Excel application program opens. The MS-Excel report is displayed.
   - The table with the measurement results is inserted into the MS-Excel document as what is known as an Excel sheet. Excel sheets enable you to manage the data that they contain independently of all of the other data in the worksheet.
8. If required, you can change the width of the columns or the height of the rows in the Excel sheet. You can also hide rows or columns. You can do this using MS-Excel's standard functions.
9. Save the MS-Excel report.

Editing the data in an MS-Excel report

The Olympus MS-Office add-in gives you different ways of editing the report. For example, you can edit the image properties, change the image resolution, or insert a detail zoom.

In addition, you can analyze the data in the Excel sheet using all of MS-Excel's functions. You can find more information about this in the MS-Excel documentation.

Technical restrictions when using the Olympus MS-Office add-in in MS-Excel

Please note the following two technical restrictions when you use the Olympus MS-Office add-in to edit an MS-Excel report.

Note: MS-Excel's *Undo* and *Redo* functions cannot be used for all of the Olympus MS-Office add-in's commands. These buttons therefore become inactive as soon as you use any of the Olympus MS-Office add-in commands.

Note: Cutting and pasting or copying data only works properly within the same worksheet. Therefore, don't copy data from one MS-Excel worksheet to another worksheet (or from one MS-Excel workbook to another workbook).

Displaying the contents of an additional worksheet in an MS-Excel report

Example: You have made measurements on an image in your software and have created a workbook that has two worksheets. The first worksheet contains a summary of the measurement results and the second worksheet contains the individual results for all of the measurements. You now want to change an existing MS-Excel report so that the content of the second worksheet is shown as well.

To do so, proceed as follows:

1. Open the MS-Excel report that contains the image with measurements on it and an Excel sheet that displays the contents of the first worksheet.
2. Use the *Insert Table Placeholder* command to insert a second table placeholder. You can find this command in the *Templates* group on the *Olympus* tab.
3. In the *Insert Document* dialog box, select the workbook and click the *Replace* button. Workbooks have the OWB file format.
   - The workbook is inserted into the MS-Excel report as an Excel sheet. The contents of the first worksheet is displayed again.
4. Place the cursor anywhere in the second Excel sheet and open the *Table Properties* dialog box. To do this, click the *Table Properties* button. This button is on the *Olympus* tab.
5. Set the value in the *Select the worksheet to be displayed* field to 2.
   - The contents of the second worksheet is now displayed in the second Excel sheet.
   - The report now contains the image with measurements on it and two Excel sheets. The first Excel sheet shows the summary of the measurement results and the second Excel sheet shows the individual results for all of the measurements.

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11.4. Editing a report

There are several options for editing reports that contain images and data from your image analysis program. To do so, use the add-in from Olympus.

This add-in always offers the same functionality, no matter whether you use the MS-Word, MS-Excel, or MS-PowerPoint application program for editing the report.

Activate the *Olympus* tab to view all of the buttons that you can use when working with reports.

Changing the image properties
Adjusting documents
Changing image resolution
Update Placeholders
Inserting a document
Inserting a field

Considerations for users who created the MS-Word report using the "Report Composer" tool window

If you want to make some changes to a report you created using the *Report Composer* tool window, before doing so, you should decide whether it will be better to make the changes in the report (i.e., in MS-Word) or in the report instruction (i.e., in your software).

Often, it is advisable to change the report instruction first and then create a new report. Changes you make in the report instruction are valid for every subsequent report that you create with this report instruction. There are numerous changes that you can anyway, only make in the report instruction, for example, the selection of other page templates. However, changes that you make in a report are only valid for that particular report.

Changing the image properties

When images are transferred to a report, the image link is transferred as well. This makes it possible to change the image display in a report (for example, to scroll the image segment).

1. Double click the image in the report to open the *Image Properties* dialog box.
2. In the *Display* group, select the check boxes for the elements that you want the report to contain. The following elements are there to choose from: *Scale bar if calibrated*, *Color bar if available*, *Info stamp* and *Border*.
   - The properties of these elements can be defined in the *Options > Image Information* dialog box. Click the *Options* button to open this dialog box.
3. In the *Size* group, select one of the options that specify how large the image is to be displayed in the report.
4. If your settings should apply for all future images, click the *Set as Default* button.
5. Click the *OK* button.
   - The *Image Properties* dialog box closes. The changed image properties will be shown in the report now.

Adjusting documents

In the report, you can select a document of the "image" or "chart" type and select the *Adjust Document* button on the *Olympus* tab. You will then change over to the image analysis software, where you can edit the document and then automatically change back to the report.

Example: You are editing a report that contains a lot of images in the MS-Word, MS-Excel, or MS-PowerPoint application program. With a certain image you notice that an important measurement is missing. With the *Adjust Document* button, you switch over to the image analysis software, add the missing measurement and then switch back to MS-Word, MS-Excel, or MS-PowerPoint in order to continue editing the report.

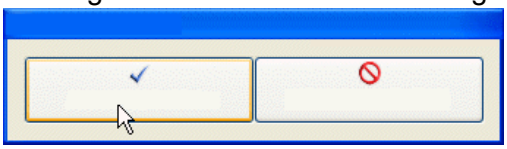
Adjusting an image

1. Open the report and select the image that you want to adjust.
2. On the *Olympus* tab, click the *Adjust Document* button.

- You switch to the image analysis software. If it was closed, it will be started and displayed in the foreground.
- The image that you want to adjust is also opened. In case it is from a database that is currently closed, the database will be opened in the background.

Note: The image analysis software is now in a special "adjust-document" mode. In this mode, you can only make certain adjustments to the image. This is why a lot of other functions are hidden.

3. Make the required change.
4. If the image information was changed: Save the image in the image analysis software.
   - Some changes made to an image don't have to be saved, e.g., when you select another frame in a multi-dimensional image. Other changes have to be saved, e.g., adding a measurement. The fact that a change has to be saved will be indicated by an asterisk shown behind the file name in the document group.
5. Click the *Update Report* button. You find this button in the *Adjust Document* message box that is shown in the foreground.



   - MS-Word, MS-Excel or MS-PowerPoint will now be shown in the foreground again. The edited image will be displayed. You can now continue to edit the report.
   - If your image analysis software was closed before you clicked the *Adjust Document* button, it is closed again. If any images or databases had to be opened for this command, they will be closed as well.

Editing a workbook

Editing a workbook in MS-Word or MS-PowerPoint

Your software supports the handling of workbooks. A workbook is created, for example, when you open the *Measurement and ROI* tool window and export a results sheet.

Note: If you want to use workbooks in MS-Word or MS-PowerPoint reports, MS-Excel must be installed on your PC. You require Microsoft Excel 2010, 2013, 2016, 2019 or the Office 365 Excel Desktop app.

Apart from the *Image* and *Chart* document type, reports can also contain workbooks. A workbook is imported as an MS-Excel object into MS-Word or MS-PowerPoint. You can further edit it in the report.

1. In the report, double click on the workbook.
   - You will change into the edit mode. You can recognize it by the fact that now the column headers and the row numbers are shown. In edit mode, you also can see all worksheets, if several worksheets are available.

2. If need be, select the worksheet that you want to edit.
3. Make the required change.
   - When you want to format individual cells differently, select the cell and use the *Format Cells* command in the context menu.
   - When you want to format the complete worksheet differently, (e.g., other font or other background color), select the complete worksheet (e.g., with the keyboard shortcut [Ctrl + A]), then select the *Format Cells* command in the context menu.
   - When you want to hide a column, click on the column's header, then select the *Hide* command in the context menu.
4. Exit edit mode by clicking on any point in the report, outside the workbook.

Editing a workbook in an MS-Excel report

You can also insert the same workbook that you have created in your image analysis program into an MS-Excel report instead of an MS-Word or an MS-PowerPoint report.
If the same worksheet is selected, the same data is displayed in MS-Excel and in MS-Word or MS-PowerPoint. Because the data is inserted into MS-Excel as what is called an Excel sheet (and not as a linked MS-Excel object like in MS-Word or MS-PowerPoint), significantly more functions for filtering, sorting, laying out, and analyzing the data in the sheet are available in MS-Excel.

For this reason, MS-Excel reports are particularly useful for users who want to analyze the data and measurement results gained in the image analysis program further in MS-Excel.

Changing image resolution

By default, all images in a report are transferred to reports with a resolution of 192 dpi. In certain cases, it can make sense to change the resolution of individual or all images in a report For example, if you want to print the report, you can raise the resolution. Alternatively, if you want to publish the report on the Internet, you can reduce the resolution.

1. Open the report in MS-Word, MS-Excel or MS-PowerPoint. Decide whether you want to change the resolution of all images or just certain images
2. If you only want to change the resolution of one individual image, select that image. If you want to change the resolution of all images, you don't have to select any.
3. On the *Olympus* tab, click the *Change Image Resolution* button.
   - The *Change Image Resolution* dialog box opens.
4. Select the option you want in the *Apply to* group. You can choose between *Selected images* and *All images in report*.
   - The *Selected images* option is inactive if no images were selected when the button was clicked.
5. Specify in the *Image Resolution* group how you want to change the image resolution. If you choose the *User-defined* option, you can enter any resolution of

your choice between 96 and 600 dpi into the *DPI* field.

6. Click the *OK* button to change the image resolution.
7. Check whether you are satisfied with the changed image resolution. If not, change the image resolution anew.
   - You can first reduce the image resolution, then save the report and then increase the image resolution again. This is possible because each time that you click the *Change Image Resolution* button, the image is transferred from your software to MS-Word or MS-PowerPoint again.
8. Once you are satisfied with the changes to the image resolution, save the report. Take a look at the new file size in the Windows Explorer.

Updating placeholders

The *Update Placeholder* button makes it easy to have any changes made to the images after the report has been created also shown in the report. Please note that all the changes made in your software have to be saved if they are to be displayed when the *Update Placeholder* button is clicked.

Example: In MS-Word, MS-Excel, or MS-PowerPoint, you open a report that you created some time ago. In the meantime, you had changed a lot of images in your image analysis software (e.g., added measurements). Now, the report is to be updated so that it shows the newest version of all of the images.

1. If you only want to update one placeholder, select just that one.
2. On the *Olympus* tab, click the *Update Placeholder* button.
   - The *Update Placeholders* dialog box opens.
3. In the *Update Placeholders* dialog box, specify whether or not all placeholders should be updated.
4. Select the *Update fields linked with placeholder(s)* check box if your report contains fields which should also be updated.
5. Click the *OK* button.
   - The placeholders will be updated.

Inserting a document

You can insert a document at any position in a report. If you have, for example, created a report using the *Report Composer* tool window, and while you are viewing it, notice that you've forgotten an image, you can retroactively insert it into the report.

1. Position the mouse pointer on the location in the report where you want to insert a document.
2. On the *Olympus* tab, click the *Insert Document* button.
   - The *Insert Document* dialog box opens.
3. In the area on the left, select the source the document comes from. You have the following possibilities:
   - Select the *Open Documents* entry if you want to insert a document that is currently opened in your software.

- Select the *Database* entry if you want to insert a document that is part of the currently selected database folder. For this purpose, the database must be opened in your software. Should you work with a version of the software that doesn't support databases, the *Database* entry is hidden.
- Select the *File Explorer* entry if you want to insert a document that is stored on your PC or in your network.

4. Select the required document in the document preview. Click the *Insert* button.
   - The required document will be inserted into the report.
   - The *Insert Document* dialog box remains open.
5. Insert further documents now or close the dialog box.
   - The path of all documents that you inserted will be saved. That enables you to later update the inserted documents by using the *Update Placeholder* button (in case the documents were changed after they have been inserted into the report).

Inserting a field

You can insert a field into a report that describes the image in more detail. All of the values that have been saved in your image analysis software for this image can be displayed in this field.

1. Select the image in the report to which you want to insert a field.
2. On the *Olympus* tab, click the *Insert Field* button.
   - The *Insert Field* dialog box opens.
   - In the *Placeholder* list, the name of the image into which you want to insert a field appears.
3. In the *Available fields* list, select the field that is to be inserted. The entries in this list are arranged hierarchically. Click the plus sign to expand the list.
   - Two types of field are available.
     The *Document Properties* list contains fields that are, by default, in your software, managed for this document type.
     The *Database fields* list contains all of the fields that are available in the database for the selected placeholder. For this purpose, a database must have been opened.
4. Keep the *Insert Field* dialog box open. Position the mouse pointer on the location in the report where you want to insert the field.
5. In the *Insert Field* dialog box, click the *Insert* button.
   - The field contents will be displayed in the report.
6. If necessary, add further fields. To do this, repeat the last 3 steps.
7. Close the *Insert Field* dialog box.
8. Save the report.

Note: Should you want to have the contents of a specific field regularly shown in your reports, you can already insert this field, (that is to say a placeholder for this field) into the page or slide template. Then this field will be automatically filled out in every report.

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11.5. Creating and editing a new template

Creating a template and adding a placeholder for a document
Adjusting the insertion order
Inserting a placeholder for a field

During the installation of your image analysis software, some predefined templates were installed too. In addition to this, you can define your own templates too.

With MS-Word reports, you define **Page Templates** in the DOC or DOCX file format.
With MS-Excel reports, you define **Excel templates** in the XLTX file format.
With MS-PowerPoint reports, you define **Slide Templates** in the PPT or PPTX file format.

Note: You can also create a template from an existing report that suits your requirements. To do this, place the mouse pointer in each document in the report and select the *Remove Document from Placeholder* command. Then save the file under a different name and, if required, in a different file format.

The contents of a template

In a template, placeholders are set up for the documents that the report is to contain. There are placeholders for images, charts, fields and workbooks (for MS-Word reports and MS-PowerPoint reports) or tables (for MS-Excel reports). If, for instance, you want the report to contain pages that have an image at the top and a chart below the image, you should set up a template which has a placeholder for an image and a placeholder for a chart.

Note: For technical reasons, a template must consist of precisely one page. For this reason, create several separate files if you require several self-defined template pages.

Creating a template and adding a placeholder for a document

Note: The procedure for creating a template is largely the same, regardless of whether you are creating a page template, an Excel template, or a slide template. For this reason, you can carry out these step-by-step instructions with either MS-Word, MS-Excel, or MS-PowerPoint open.

1. In the MS-Word, MS-Excel, or MS-PowerPoint application program, select the *File* tab and select the *New* entry.
2. Select the *Blank document* option (MS-Word), *Blank workbook* option (MS-Excel) or *Blank Presentation* option (MS-PowerPoint).
3. Activate the *Olympus* tab.
4. Decide whether to insert an image placeholder, a chart, or a workbook (for MS-Word reports and MS-PowerPoint reports) or a table (for MS-Excel reports). On the *Olympus* tab, click one of these buttons: *Insert Image Placeholder*, *Insert Chart Placeholder*, *Insert Workbook Placeholder*, *Insert Table Placeholder*. These buttons are part of the *Templates* group.
   - The placeholder you've selected will be inserted.

5. If necessary, you can change the size of the placeholder. To do so, grab a handle with the mouse and drag it in the required direction. The length/width ratio remains unchanged, so that the objects won't be distorted by this action.
6. Double click a placeholder for an image, to change the default settings for its appearance.
7. If required, insert additional placeholders for images, charts, tables, or workbooks. Make sure that your template isn't longer than a page.
8. If you want to, you can insert a placeholder for a field. Additional information about a placeholder can be shown in this field, for example, the name, or the date it was set up. You will find additional information on inserting placeholders for fields further down.
9. Save your template under a descriptive name. If you want to view a thumbnail of the template to make it easier to select the right template, activate the thumbnail preview. The procedure for activating the thumbnail preview is a little different for different file types. For this reason it's best if you look up the precise procedure in the online help for the Microsoft Office package.

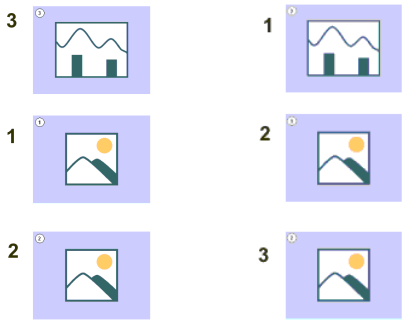
   For MS-Word reports and MS-PowerPoint reports: As a storage location, select the same directory that is set for your user templates or workgroup template in the software.

   For MS-Excel reports: You can save the file anywhere you want. If you want to create a report later on that is based on your new template, open the *Templates Locations* dialog box from the *Create Report from Template* dialog box and navigate to this storage location.
10. Close the file.

Adjusting the insertion order

The placeholders are numbered in the order in which they were inserted. Should you have initially set up placeholders for two images, have then decided to put a placeholder for a chart right at the top of the page, the insertion order would be that shown in the example on the left.

1. In this case, click the *Adjust Insertion Order* button on the *Olympus* tab, to have the insertion order numbered serially from top to bottom (see example).



Inserting a placeholder for a field

1. In the template, select the placeholder into which you want to insert a field.
2. On the *Olympus* tab, click the *Insert Field Placeholder* button. You can find this button in the *Templates* group.
   - The *Insert Field* dialog box opens.
   - In the *Placeholder* list, the name of the placeholder into which you want to insert a field appears.
3. In the *Available fields* list, select the field that is to be inserted. The entries in this list are arranged hierarchically. Click the plus sign to expand the list.
   - Two types of field are available.

     The *Document Properties* list contains fields that are, by default, in your software, managed for this document type.

     The *Database fields* list contains all of the fields that are available in the database for the selected placeholder. For this purpose, a database must have been opened.
4. Keep the *Insert Field* dialog box open. Position the mouse pointer on the location in the report where you want to insert the field.
5. In the *Insert Field* dialog box, click the *Insert* button.
   - The placeholder for a field will then be displayed. You can recognize it by the curly bracket, and by the field name shown.
6. If necessary, add placeholders for further fields. To do this, repeat the last 3 steps.
7. Close the *Insert Field* dialog box.
8. Save the template.

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