OLYMPUS

Manual

OLYMPUS Stream

IMAGING SOFTWARE

Printed in Germany

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Contents

1. Before you start

1.1. About this manual

The documentation for your software consists of three parts: the installation manual, the online help, and this manual.

Where do you find which information?

The installation manual is delivered with your software. There, you can find the system requirements. Additionally, you can find out how to install and configure your software.

In the 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.

In the online help, you will find detailed help for all elements of the program. 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.

Example images that are automatically installed

During the installation of your software some sample images have been installed, too. These example images might be of help to you when you familiarize yourself with the software. Regarding the information as to where the example images are located, please refer to the online help.

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1.2. Online help for your software

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

When you use the online help you'll have access to most online topics. As soon as you access the online 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 will find this button on the *Standard* toolbar.
- Use the*Help > Context Help* command.
- Use the [Shift + F1] shortcut.

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1.3. About your software

Please note: Not every software package contains all of the features!

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

Main features of your software

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 PC 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 Focus Image". 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 format. If 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.

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:

- Intercept analysis
- Grains Planimetric
- Chart comparison
- Layer thickness measurement
- Cast Iron Analysis
- Inclusion worst field analysis

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 have the possibility to document the results of your work in a report. To do this, select the required page templates and images in the Report Composer, and generate an MS-Word report.

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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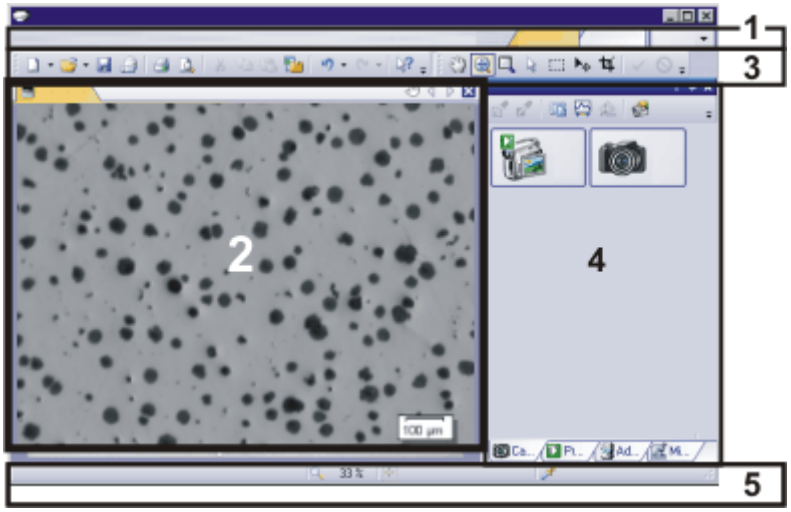
2. User interface

2.1. Overview - 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.

Tip: 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

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. Further information is available in the online help.

(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 will 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 any time.

(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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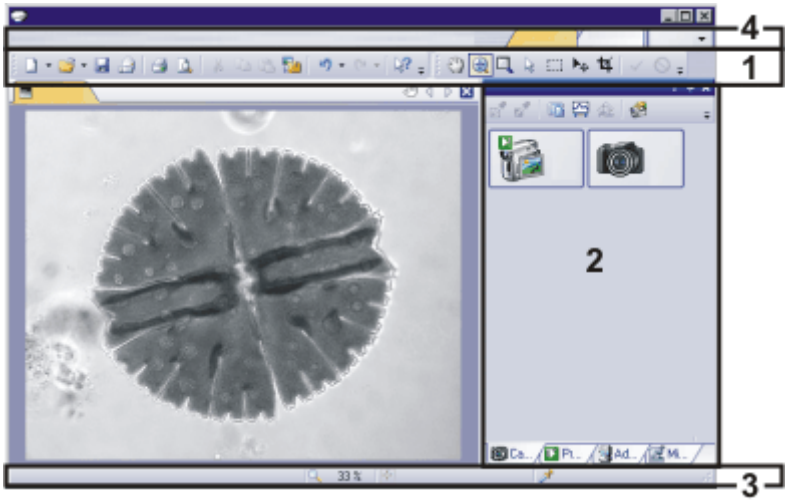
2.2. Overview - Layouts

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:

- Work with a database ("Database" layout)
- Acquire images ("Acquisition" layout)
- View and process images ("Processing" layout)
- Generate a report ("Reporting" layout)

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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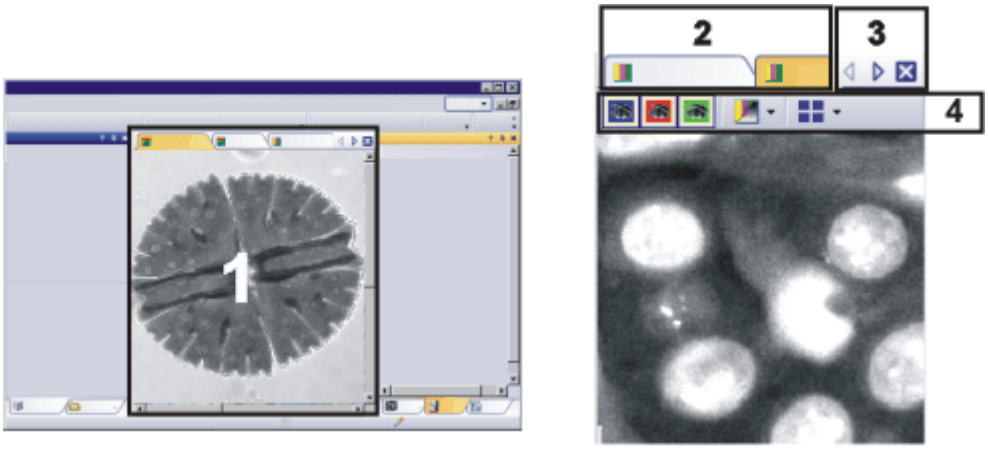
2.3. Document group

The document group contains all loaded documents. These can be of all supported document types.

Appearance of the document group



On the left, the illustration shows a schematic representation of a user interface. On the right, the document group is shown enlarged.

(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 naturally, all of the images that have been acquired also. Also the live-image and the images resulting from, e.g., any image processing function, will be displayed there.

(2) Document bar in the document group

The document bar is the document group's header.

For every loaded document, an individual field 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.

(3) Buttons in the document bar

At the top right of the document bar you will see several buttons.

Button with a hand

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.

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

The arrow buttons located at the top right of the document group are, to begin with, inactive when you start your software. 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. Then you can click one of the two

arrows to make the fields with the document names scroll to the left or the right. That will enable you to see the documents that were previously not shown.

Button with a cross

Click the button with a cross to close the active 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.

(4) Navigation bar in the image window

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

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2.4. 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 will 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 any time.

Showing and hiding tool windows

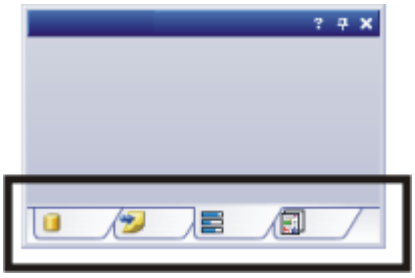
Which tool windows are shown by default depends on the layout you have chosen. You can, naturally, at any time, make specific tool windows appear and disappear manually. To do so, use the *View > Tool Windows* command.

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 keeping the left mouse button depressed, 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. Resetting the layout using the *View > Layout > Reset Current Layout* command will have the result that only the tool windows that are defined by default will be displayed.

Buttons in the header

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

Click the *Help* button to open the online help for the tool window.
Click the *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, rightclick 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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2.5. 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 some other document types. You will find a list of supported documents in the online help.

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 and use the *File > Save As...* command.
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. Detailed information on inserting documents into a database can be found in the online help.

Autosave and close

1. When you exit the program, all of the 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. With some acquisition processes, the acquired images will be automatically saved after the acquisition has finished. You will find an overview of the acquisition processes that are supported in the online help.

3. 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 desired document 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  . You will find this button on the top right in the document group.
3. Use the *Gallery* tool window.
   Select the desired document 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. You will find this command in the *File* menu, and in both the *Documents* and the *Gallery* tool window's 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, doubleclick 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. Use the *Database > Load Documents* command to load documents from the database into your software. Further information is available in the online help.

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.

Using sample images

During the installation of your software some sample images have been installed, too. Regarding the information as to where the example images are located, please refer to the online help.

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. To open a list with all currently loaded documents, use the [Ctrl + Tab] shortcut. Leftclick the document that you want to have displayed on your monitor.
5. Use the keyboard shortcut [Ctrl + F6] respectively [Ctrl + Shift + F6], to have the next document in the document group displayed. With this keyboard shortcut you can display all of the loaded documents one after the other.
6. 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.

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3. Configuring the system

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.

Process flow of the configuration

To set up your software, the following steps will be necessary:

Specifying which hardware is available

Configuring the interfaces

Configuring the specified hardware

Calibrating the system

Specifying which hardware is available

Your software needs 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.
Further information on this dialog box is available in the online help.

Configuring the interfaces

Use the *Acquire > Devices > Interfaces* command to configure the interface between your microscope or other motorized components, and the PC on which your software runs.
Further information on this dialog box is available in the online help.

Configuring the specified hardware
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.
Further information on this dialog box is available in the online help.

Notes on 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 who also wish to work with the software will only have user rights as a *Standard User*. With these rights, the system configuration cannot be changed or viewed, i.e., the *Device List* and *Device Settings* dialog boxes cannot be opened.

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

Further information on user rights is available in the online help.

Switching off your operating system's hibernation mode

When you use the MS-Windows Vista operating system: Switch the hibernation mode off.
To do so, click the Start button located at the bottom left of the operating system's task bar.
Use the *Control Panel* command.
Open the *System and Maintenance > Power Options > Change when the computer sleeps* window. Here, you can switch off your PC's hibernation mode.

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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.

Prerequisite

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 Hardware Configuration*  button.
   - The *Create New Hardware Configuration* dialog box will open.
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_DP25".
   - Under this name, you can later reload this hardware configuration in the *Device Settings* dialog box.
5. Select the *Copy current hardware configuration* option if you have previously chosen your camera and microscope. Otherwise, choose the *Empty hardware configuration* option.
6. Close the *Create New Hardware 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 a hardware configuration

Define the new hardware configuration in the *Device List* dialog box. A description of the dialog box can be found in the online help. Begin with the specifications for the camera and the microscope.

7. Select your camera (e.g. "DP25") 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 (such as IX71), 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 tries to create a connection to the specified devices. You can see whether the devices are able to 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. "DP25“).
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 structure, select the *General > Manual Nosepiece* entry (if you have a manual microscope), or *General > Nosepiece <Name of the nosepiece>* (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, you receive a message 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.

00156

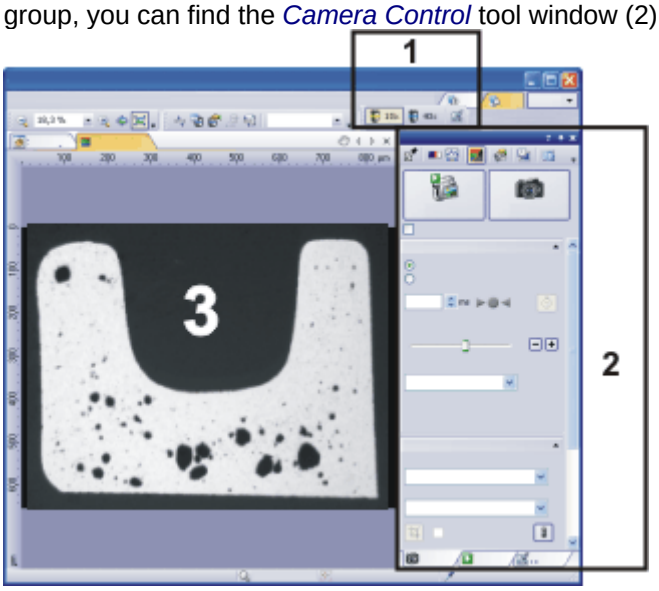
4. Acquiring images

4.1. Acquiring a single image

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.

Acquiring a single image

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* tool window (2).



Selecting 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.
4. Go to the required specimen position in the live-image.

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.
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 file format.

00027

4.2. Acquiring movies and time stacks

With your software you can acquire movies and time stacks.

Acquire 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. The movie will be saved as a file in the AVI format. You can use your software to play it back.

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

Selecting the objective

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 will open.
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 *Filesystem* entry in the *Automatic save > Destination* list to automatically save the movies you have acquired.
   - The *Base* 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 *Base* field to alter the directory.

Selecting the compression method

   - The AVI file format is preset in the *File type* list. This is a fixed setting that cannot be changed.
7. Click the *Options...* button if you want to compress the AVI file in order to reduce the movie's file size.
8. From the *Compression* list, select the *M-JPEG* entry and confirm with *OK*.

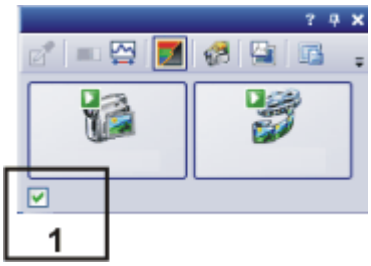
   Please note: Compressing the movie is only possible if the selected compression method (codec) has already been installed on your PC. If the compression method has not been installed the AVI file will be saved uncompressed.
   The selected compression method must also be available on the PC that is used for playing back the AVI. Otherwise the quality of the AVI may be considerably worse when the AVI is played back.
9. Close the *Acquisition Settings* dialog box with *OK*.

Setting the image quality

10. Switch to the live mode, and select the optimal settings for movie recording, 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.
11. Find the segment of the sample that interests you and focus on it.

Switching to the "Movie recording" mode

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



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

Starting movie recording

13. 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 2GB.

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

Stopping movie recording

14. 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 once more.

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.

Task

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, e.g., the *View > Layout > Acquisition* command.

Selecting 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 time stack.
4. Choose the resolution you want for the time stack's frames, from the *Resolution > Snap/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., *Multicolor*, click the button to switch off the acquisition process.
   - The group with the various acquisition processes should now look like this:

Selecting the acquisition parameters

10. Clear the check boxes *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" (for 10 seconds) in the *Recording time* field. You can directly edit every number in the field. To do so, simply click in front of the number you want to edit.
12. Click the button with the lock  located to the right of the field, to specify that the acquisition time is no longer to be changed.
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 Process* 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 informs you about the number of images that are still to be acquired.
    - The acquisition has been completed when you can once more see the *Start*  button in the *Process Manager* tool window, and the progress bar has been faded out.
    - 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. Further information on the navigation bar is available in the online help.
    - The time stack that has been acquired will be automatically saved. The storage directory is shown in the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.

Tip: 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.

00304

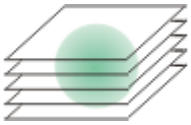
4.3. Acquiring a Z-stack

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.

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

Acquiring a Z-stack

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 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.

Selecting 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 you enter 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.
9. 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

10. 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 *Start Process* 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 Z-stack as acquired up till then, will be preserved.
- At the bottom left, in the status bar, the progress bar will appear. It informs you about the number of images that are still to be acquired.
- The acquisition has been completed when you can once more see the *Start*  button in the *Process Manager* tool window, and the progress bar has been faded out.
- 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. Further information on the navigation bar is available in the online help.
- 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.

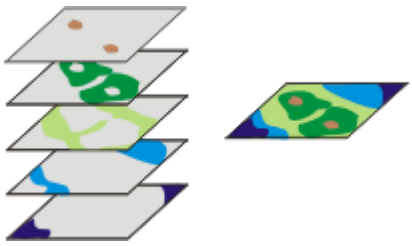
Tip: When other programs are running in the background on your PC, for instance a virus scanning program, it can interfere with the performance when a Z-stack is being acquired.

00367

4.4. Acquiring an EFI image

What is EFI?

EFI is the abbreviation for "Extended Focus Image". 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 separate images ("Focus series") to calculate a resulting image ("EFI image"), that is focused in all of its parts.



The illustration shows left, 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

Acquiring an EFI image without a motorized Z-drive

Task

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, resp. 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 through the complete thickness resp. 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.

Tip: You can use the *Instant EFI* acquisition process with every microscope. When your microscope stage is equipped with a motorized Z-drive, use the *Z-Stack* acquisition process to acquire an EFI image.

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 acquisition parameters

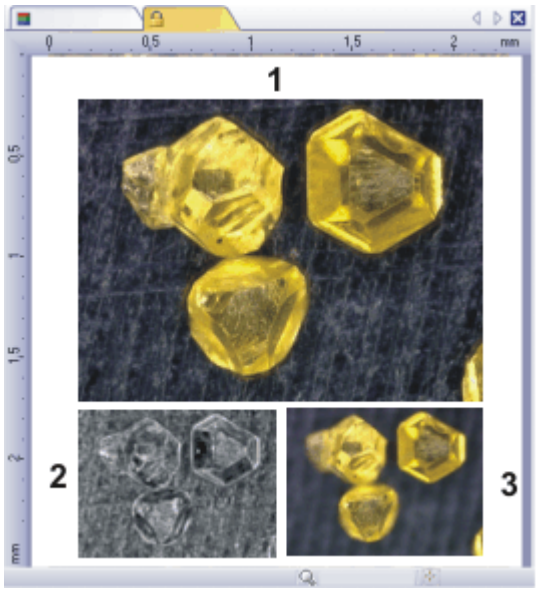
4. From the *Algorithm* list, select the *Reflected light* entry, when you use your light, or stereo microscope in the reflected light mode.
5. If you work with a stereo microscope, select the *Automatic frame alignment* check box.
   If you don't work with a stereo microscope, clear the *Automatic frame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

Preparing an 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. Move the microscope focus to the Z-position where either the lowest or the highest place on the object is only just no longer sharply focused. Use the live-mode for a visual control.
9. Check the exposure time, and correct it if necessary. When the *InstantEFI* 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.
    - The 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, resp. 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 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.

Acquiring an EFI image with a motorized Z-drive

Task

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, resp. 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 through the complete thickness resp. height, of the sample, and have the EFI image calculated for you.

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

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 (exp)* entry, if you're working in the transmitted light mode, and the *Reflected light* entry if you're working in the reflected light mode.
5. Select the *Automatic frame shift* 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 for the acquisition of a Z-stack

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

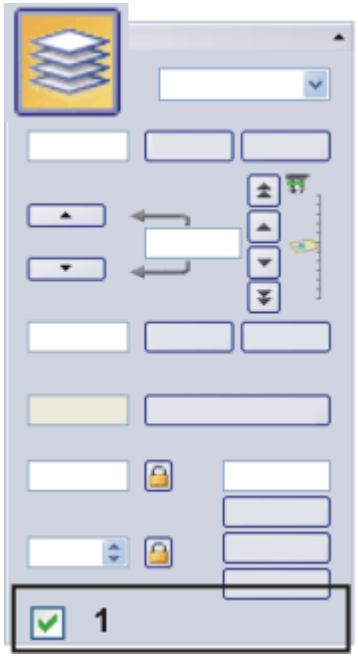
Setting the Z-stack parameters

1. Activate the *Process Manager* tool window.
2. Select the *Z-Stack*  acquisition process.
3. Select the *Top & Bottom* entry in the *Define* list.
4. 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 by steps of 2 µm resp. of 20 µm.
   - The stage's current position will be shown to you in the *Pos.* field.
5. 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.

6. 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.
7. 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 *Start* field.
8. 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.
9. Use the [Enterl] 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 an EFI acquisition

1. 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.



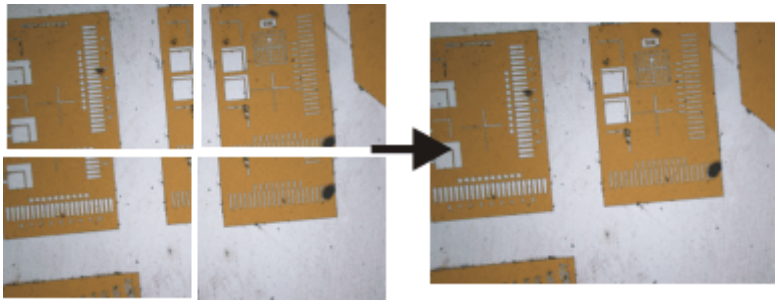
2. Finish the live mode.
3. Click the *Start*  button.
   - The EFI acquisition begins immediately.
   - The acquisition will begin. After the acquisition has been completed the EFI image will be shown in the document group. This image was calculated from the variously focused separate images.

00372

4.5. 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 individual 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 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

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

Task

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, e.g., the *View > Layout > Acquisition* command.

Selecting objective

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

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.
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.

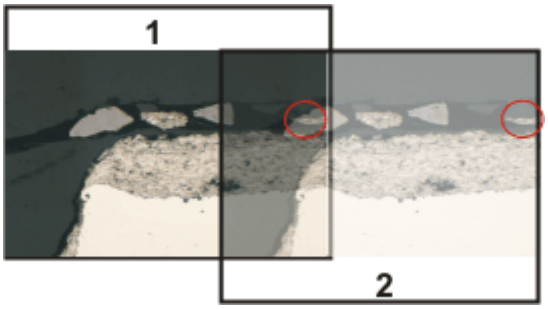
- The button will appear clicked. You can recognize this status by the button's colored background.
- The *Manual MIA* group will be automatically displayed in the tool window.
- Should the *Instant EFI* acquisition process have been active, it will be automatically switched off. You can, however, use 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.

Selecting the acquisition parameters

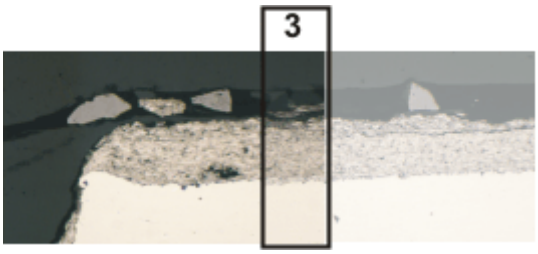
9. Make quite certain that the *Auto Align* button appears clicked. It should then look like this: .
   - Then, your software will search for the same image structures in neighboring individual images. The stitched image will be put together in such a way that image areas that are the same will be superimposed.

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.



    Since you haven't moved the sample, the live-image still shows the current sample position, too, which means that you now see the current image twice.
    The two images overlap. Since the live-image is shown transparent, you see both images in the overlap area simultaneously.
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.

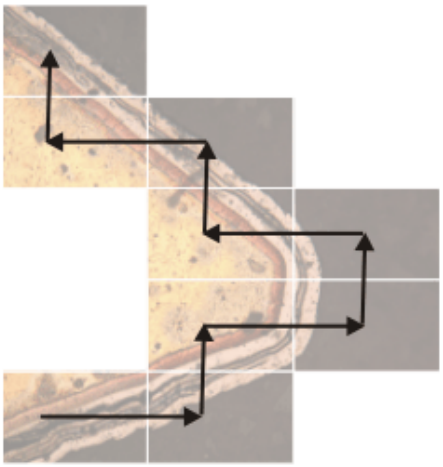


- You can reverse the direction in which your stage moves, in the *Device Settings > Stage* dialog box. Depending on how you can best orient yourself, the live-image will then move to the left or to the right, when you move your stage to the right.

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.

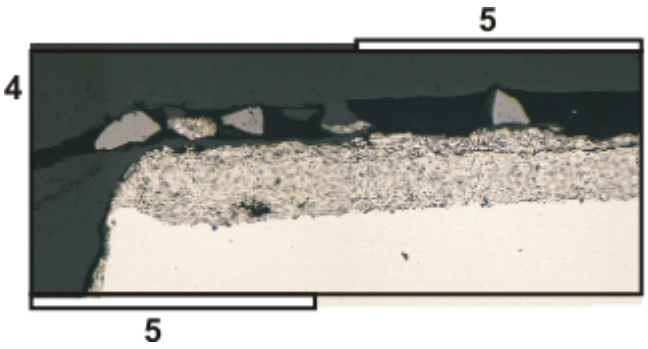
    - During the acquisition, you can change the current stitched image's zoom factor, e.g., to see certain parts in the overlap area better. You will find an overview on the possibilities of changing an image's zoom factor in the online help.

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.



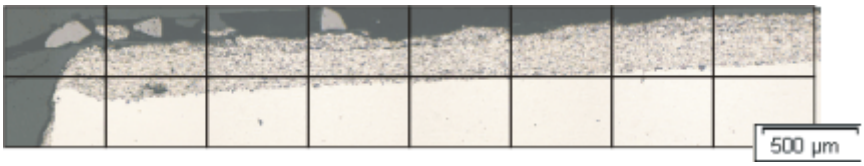
- The stitched image will, by default, be automatically saved. The storage directory is shown in the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.

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.

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

Task



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

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

Prerequisite

- 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, e.g., the *View > Layout > Acquisition* command.

Selecting objective

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

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.

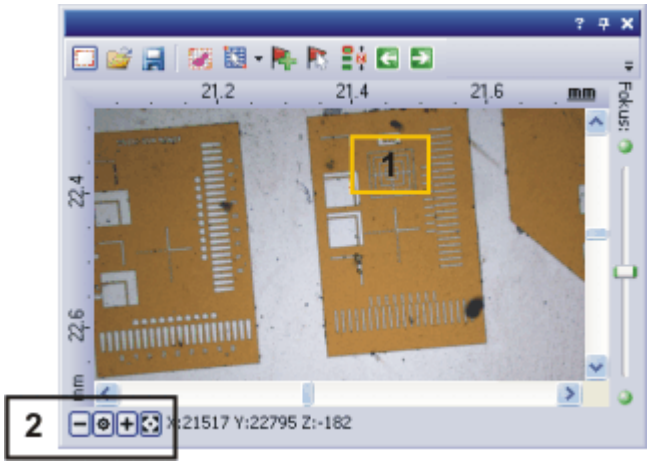
Using the software autofocus

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 *Software Autofocus* button.

   - The *Software Autofocus* group will be automatically displayed in the tool window.
7. In the *Software Autofocus* group, select the *Multiposition / MIA* check box.
8. 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

9. 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.
10. 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.

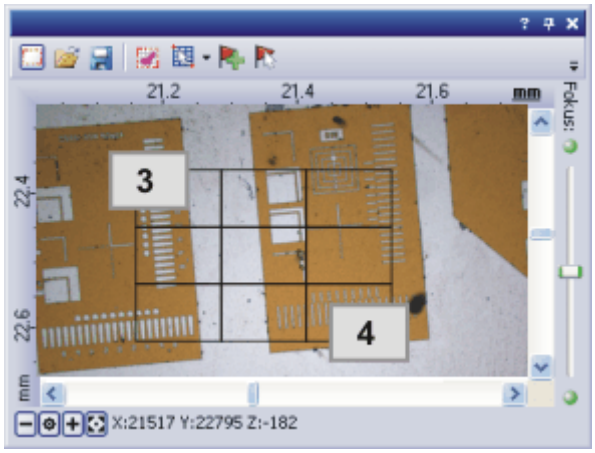


   - Further information on the *Stage Navigator* tool window is available in the online help.

Defining the MIA scan area

11. 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 will open.
12. Move the XY-stage to the top left-hand corner of the MIA scan area you want (3).
13. 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.
14. Confirm the starting position in the *Define MIA Scanning Area* dialog box, with *OK*.

15. 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

16. 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.
    - The *Stop* button will become active. A click on this button will stop the acquisition process. The stitched image acquired up till then, will be preserved.
    - In the status bar at the bottom left of the user interface, you will 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 once more see the *Start* button in the *Process Manager* tool window, and the progress bar has been faded out.
    - 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. The storage directory is shown in the *Acquisition Settings > Saving > Process Manager* dialog box. The preset file format is VSI.

Acquiring a stitched image with extended depth of focus

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.

   You will find a step-by-step instruction for doing this further above.

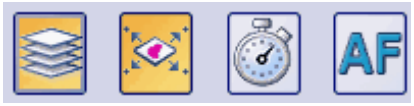
2. Click the *Instant EFI*  button, in the *Manual MIA* group.
   - The *Instant EFI* acquisition process will start at once. Instead of the live-image, you now see the EFI image.
3. Now move your microscope's Z-drive slowly and change the focusing of the image. Observe how the EFI image builds itself 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.

   Tip: 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

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 will 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.

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 will 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.

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

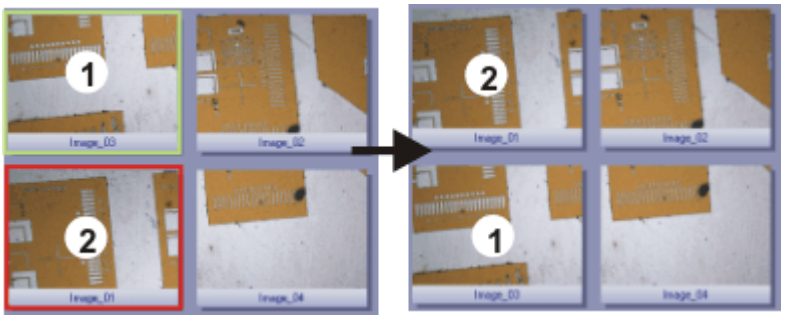
1. Load the images you want to combine, or acquire a suitable set of images.
   - All of the images you want to combine must be of the same image type. You can't, e.g., have a gray-value image combined with a true-color image.
   - When you acquire the images, number their names sequentially, e.g., "Image001", "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, use, e.g., 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 *Multiple Image Alignment* dialog box will open.
   - 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, doubleclick 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 by Drag&Drop.



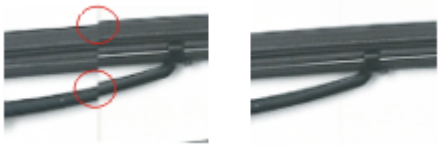
   - The illustration shows the stitching area with four individual images. On the left, the images 1 and 2 are not in the correct position. Image 1 (green frame) will therefore be dragged onto image 2 (red frame). On the right, you see the stitching area after the two images have been interchanged.
7. When the individual images overlap, select the *Correlation* option 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 *OK*.
    - A new image with the name "Image_<consecutive No.>" will be created.

00383

5. Processing images

5.1. Processing images

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

Processing images

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 window is active in the document group.
2. Use one of the commands in the *Process* menu, e.g., *Process > Enhancement > Adjust Intensity...* .
   - The image processing dialog box will open. 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. Change the image processing operation's parameters. After every change that is made in a parameter, the operation will be immediately applied to the source image, and the resulting image will be shown in the preview window. Click the *Default* button, to readopt the preset parameters in the *Settings* group, when the current parameter doesn't make sense to you.
5. 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 will closed.
   - Please note that the image processing operation changes the source image. No new image document will be created. You can, however use the *Edit > Undo* command to restore the source image.

00175

6. Measuring images

6.1. Overview - Measuring images

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

Selecting the measurement environment

Measuring with help of the tool window

Switch to the "Processing" layout if you want to measure images. You will 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 relate to the measurement. 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.

Tip: If there are two tool windows, one under the other, at the bottom of the user interface: Activate the *Measurement and ROI* tool window, by clicking the *Measurement and ROI* tab's title below the tool window.

Starting a measurement

Begin a measurement by selecting the measurement function you want. You will 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. You can make as many measurements as you like with 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 will remain in this measurement mode until you explicitly switch it off. To do this, click the *Select Measurement Objects* button. You can find the button either in the *Measurement and ROI* tool window or on the toolbar.

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' mode after creation* 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. Use the *View > Tool Windows > Measurement and ROI* command to have the tool window displayed.

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. To do this, use the *Export to Excel* or *Export to Workbook* command. You find both of the commands, e.g., in the *Measure* menu.

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 layer to hide the measurements. Click an empty cell without an eye icon to make the corresponding layer reappear.

Setting the unit for the measurement results

The unit in which the measurement results will be issued, is determined by the measurement function that has been selected and the image's calibration. But you can choose whether the length e.g., is shown in mm or µm.
Use the *Tools > Options...* command. Select the *Measurement and ROI > Results* entry in the tree view. Select the unit you want to use from the *Prefix of the unit* list.

Outputting measurement results in a sheet

You can export the measurement results from the *Measurement and ROI* tool window as a sheet, for example, to be able to save the measurement results in their own file, independently of the image. You will find the functions, e.g., as a button next to the measurement functions on the *Measurement and ROI* tool window's toolbar.

Click the *Export to Workbook* button to export measurement results from the *Measurement and ROI* tool window's results sheet to a workbook. Use this export possibility to save the measurement results in a file format that you can at any time load and edit with your software.

Click the *Export to Excel* button to export the results to an MS-Excel sheet. Use this export possibility, for example, when you want to evaluate the measurement results still further. This will also enable you to supply the results to other users who don't have your software.

Editing measurements

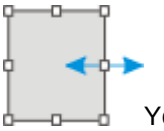
You can edit existing measurement objects at any time. To do this, click the *Select Measurement Objects* button. You can find the button either in the *Measurement and ROI* tool window or on the toolbar.

Please 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.

Moving measurement objects

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

Increasing/decreasing the size of measurement objects



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

Deleting measurement objects

Click the [Del] key on your keyboard in order to delete the selected measurement object.

What happens to the measurement values?

The measurement values in the *Measurement and ROI* tool window will be correspondingly updated.

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 several commands with which you can change the appearance of the selected measurement objects.
From the context menu, select, e.g., the *Change Color...* command. Select the color you want from the color palette, then close the dialog box with *OK*.

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.

Measuring on different image types

Measuring on multi-dimensional images

You can combine a series of separate images into one image. What results is e.g., a time stack in which all of the frames will have been acquired at different times. Further information on multi-dimensional images can be found in the online help.

You can make measurements on every separate image. 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. To do so, use, e.g., the measurement parameter "Index t" for time stacks.

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. Further information on multi-layer images is available in the online help.

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.

Measurement precision

How precise the measurement is, depends on the X/Y-calibration and the image's current zoom factor.

Influence of the X/Y-calibration

The X/Y-calibration defines the width and height of the sample area that is represented by one pixel. For example, it could be that one pixel displays a sample area of 10 µm x 10 µm. A pixel is the smallest image structure that can be measured. For this reason, the maximum measurement precision where this example is concerned, is 10 µm.

Influence of the zoom factor

The zoom factor tells you how large the image will be displayed on your monitor. With a zoom factor of 100%, one pixel on the monitor equals exactly one pixel in the image. With a zoom factor of 50%, one pixel on the monitor equals 2 x 2 pixels in the image. When you make a measurement, you should use the zoom factor 100% whenever possible. Then you will achieve a maximum of measurement precision. Should the zoom factor 100% not be possible, because the image area you want to measure can't then be completely seen, choose the largest possible zoom factor under 100%.

00150

6.2. Measuring images

Your software offers a wide range of measurement functions. They enable you to quickly count objects and measure segments and areas.

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

Measuring image objects interactively

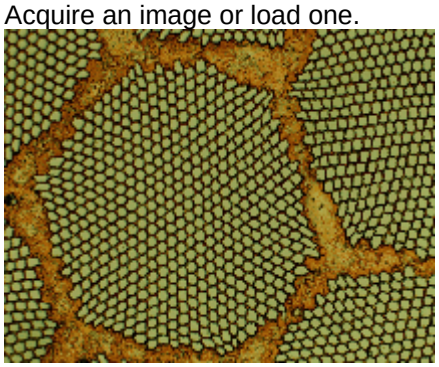
Task

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 > Measurement and ROI* command, to have the *Measurement and ROI* tool window displayed.
   - 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 when you use the exemplary image "SupraConductor.tif". Regarding the information as to where the example images are located, please refer to the online help.

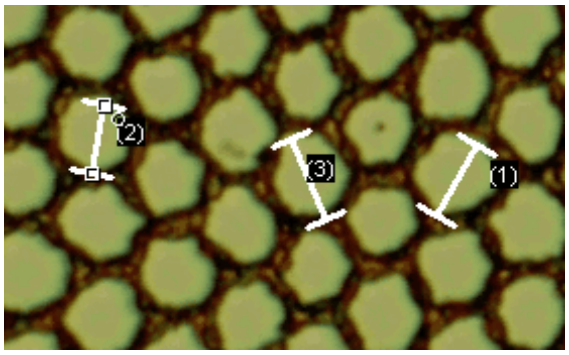
Setting the labeling color

The measurement results will be written into the image according to the default settings, in red font color and without a background. This can't be easily read against the superconductor's structure. Change the labeling settings.

1. Use the *Tools > Options...* command.
2. Click the *Measurement and ROI > Measurement Display* entry in the tree view.
3. Click in the *Background Color* field, and choose, e.g., the color "Black".
4. Select the *Text color > Fixed colors* option, then select the color "White" from the palette to see the measurements in white and the labeling in black in the image.
5. Close the dialog box with *OK*.

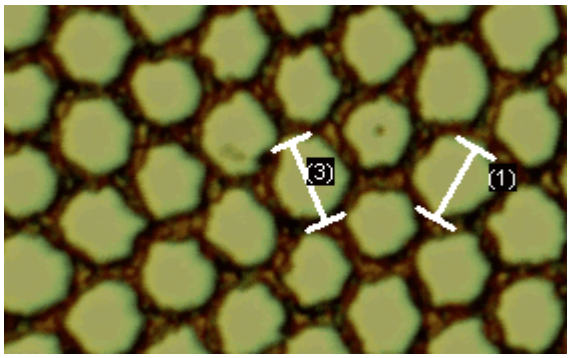
Measuring lengths

1. Click the *Arbitrary Line* button, located on the toolbar at the top of the tool window.
2. Click with your left mouse button at the starting point and end point of the reference distance.
3. If you have measured a reference distance, you can immediately proceed with the next measurement.
4. Click the *Arbitrary Line* button again to end the length measurement.
5. Take a look at the results in the tool window and in the image.
   - The illustration shows the image with three executed measurements. The measurement 2 has been selected



Deleting measurements

1. Click one of the measurement results in the *Measurement and ROI* tool window.
   - The corresponding line will be marked in the image.
2. 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 tool window and the image contain one measurement less. The IDs of the remaining measurements won't be changed by the deletion of a measurement.



When you've completed the measurements, you should switch off the measurement mode, since otherwise, you might inadvertently select your measurements and move them.

3. 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

1. To do this, click the *Export to Excel* button.
2. 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 file type "Excel-Sheet (*.xls)“.
3. Click the *Save* button to have the MS-Excel sheet with the measurement results saved.

Closing the image

1. Click the *Close* button, located at the top right of 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.
2. 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

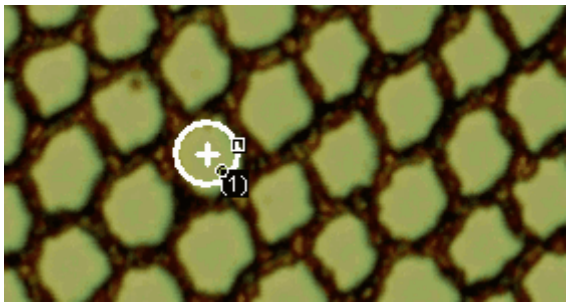
Task

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 the "Supraconductor.tif" example image. Regarding the information as to where the example images are located, please refer to the online help.

Measuring areas

2. In the *Measurement and ROI* tool window, click the *2 Points Circle* button.
3. With your left mouse button, click in the center point of the hexagonal structure.
4. Move your mouse, and in the process drag out the circle. Match the circular object as well as possible to the structure. Click your left mouse button.
5. Click the *2 Points 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 of the superconductor with a circular measurement.

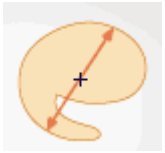


Viewing the list of measurement parameters

1. 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 calculated for all objects.
   - A detailed description of the dialog box can be found in the online help.

Outputting additional measurement parameters

2. Go to the list of all of the available parameters, then click the "Diameter" measurement parameter.
   - On the right-hand side of the dialog box, 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.
3. 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.
4. Click the *Add 'Mean (Diameter)'* button.
   - This measurement parameter will be adopted in the list. All of these measurement parameters will be displayed in the tool window.
5. Close the dialog box with *OK*.
6. Take a look at the result for the circle's diameter in the *Measurement and ROI* tool window.

Outputting measurement parameters in the image

1. Open the *Select Measurements* dialog box.
2. At the bottom of the list of all of the calculated measurement parameters, click the "Mean (Diameter)" measurement parameter.
3. 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.
4. Close the dialog box with *OK*.
5. Take a look at the result for the circle's diameter in the image.

Tip: 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.

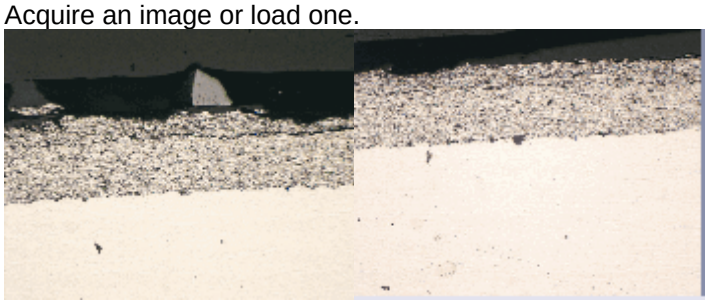
Measuring several images

Task

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 an image

1. Acquire an image or load one.

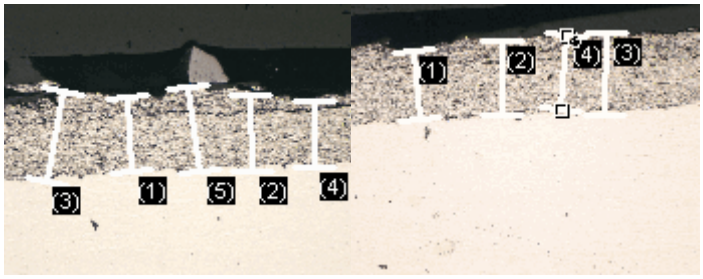


- During the installation of your software some sample images have been installed, too. You can carry these step-by-step instructions out directly with the example images "SprayCoating2.tif" and "SprayCoating4.tif". Regarding the information as to where the example images are located, please refer to the online help.

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

1. In the *Measurement and ROI* tool window, click the *Measurement and ROI Options* button.
2. Select the *Measurement and ROI > Results* entry in the tree view.
3. Clear the *Show measurement objects: Only of the active image* check box.
4. Close the dialog box with *OK*.

- Now the results for both images will be shown simultaneously in the tool window.

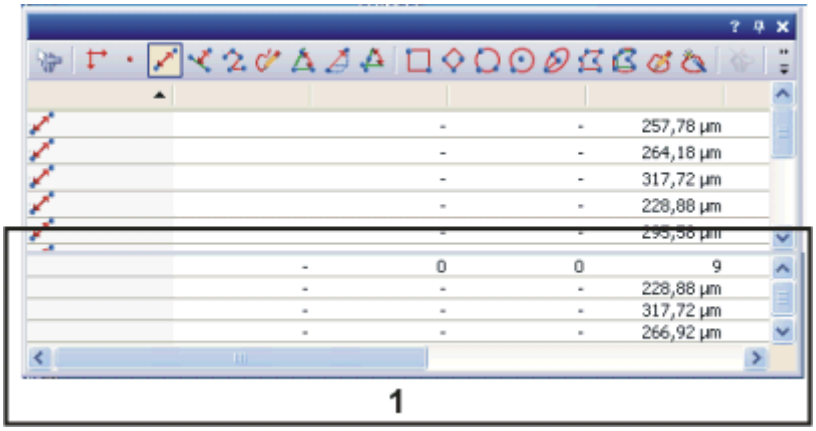
Viewing the statistical parameter

1. In the *Measurement and ROI* tool window, click the *Measurement and ROI Options* button.
2. Select the *Measurement and ROI > Results* entry in the tree view.

- In the *Statistic* group you'll find various statistical parameters.

3. Select the *Mean* check box.

4. 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.

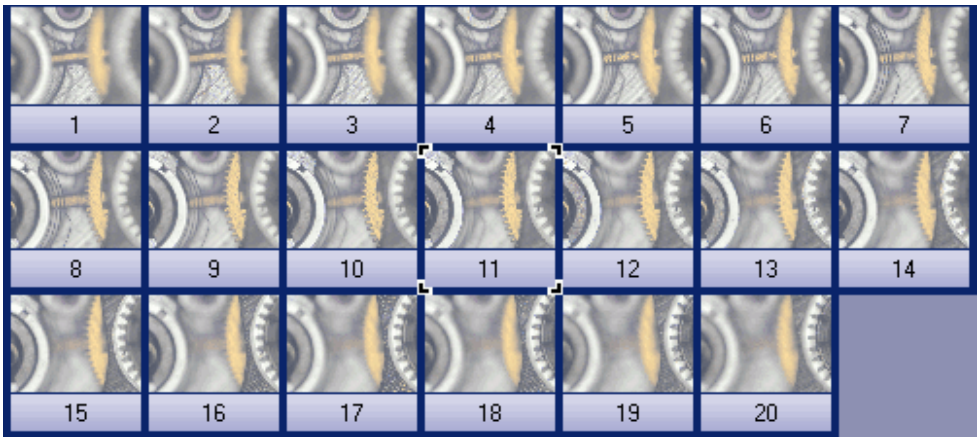


Measuring heights

Task

To be able to make height measurements, you need to have a height map. A height map is a gray-value image whose gray values contain height information. The multi-dimensional image "Clockwork" is a Z-stack. 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.

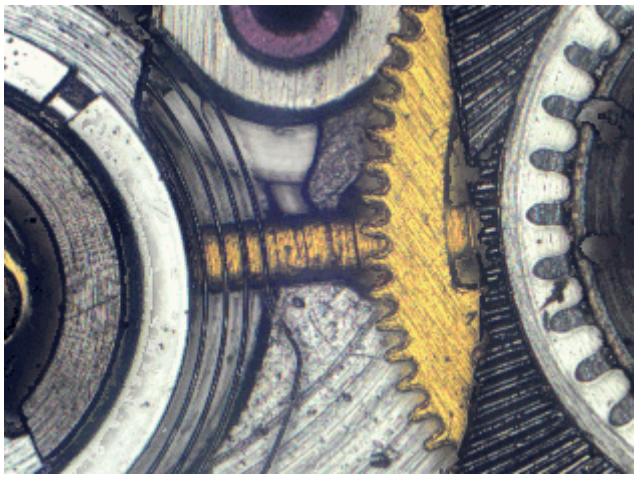
1. Load the "Clockwork.tif" image. Regarding the information as to where the example images are located, please refer to the online help.
   - The "Clockwork.tif" image is a Z-stack. The works of a clock was analyzed under a reflected light microscope. In the process, images of the works were acquired at different focus positions.
     In the illustration, the Z-stack is displayed in the tile view. Pay attention to the gear wheel that is only sharply reproduced in the middle of the Z-stack.



Creating a height map

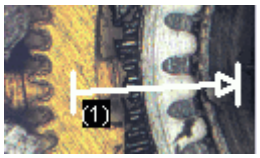
2. Use the *Process > Enhancement > EFI Processing…* command.
3. Select the *Apply on > All frames and channels* option.
4. From the *Algorithm* field select the *Reflected light* entry.
5. Select the *Height map* check box.
6. Select the *Create new document as output* check box.

7. Close the dialog box with *OK*.
   - You can see the EFI image with the clockwork's texture. 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. The height information is therefore also present in the EFI image. You can measure the height directly on the texture image.



Measuring height

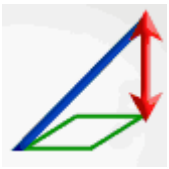
1. In the *Measurement and ROI* tool window, click the *3D Line* button.
2. Now measure the height between two image objects.
   Click, for example, the brass-colored gear wheel, and the silver-colored gear wheel on the right-hand side of the image.



3. 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.
     The "3D Intensity Projection" measurement parameter measures the height difference between two points.

Issuing the height difference

1. In the *Measurement and ROI* tool window, click the *Select Measurements* button.
2. In the list of all of the available measurement parameters, take a look at the parameter of the "3D Line" object type.
3. Select the measurement parameter „3D Intensity Projection“.

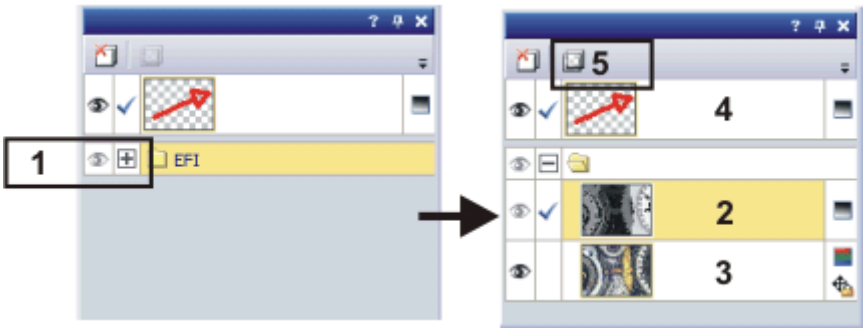


4. Insert this measurement parameter into the list of the displayed measurements.

5. 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.

Viewing the height map

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, because it's absolutely transparent at the moment.
   - The measurements are on another layer (4).



3. Select the height map in the *Layers* tool window.
4. Click the *Set Layer Opacity* (5) 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.
   - Low-lying structures can be recognized by their dark gray values, structures that lie higher, by their bright gray values.

Showing/hiding layers

1. Click its eye icon to make the corresponding layer disappear. By doing this, you can, e.g., for a time, remove the measurements from the image.
2. Click an empty cell without an eye icon to make the corresponding layer reappear.

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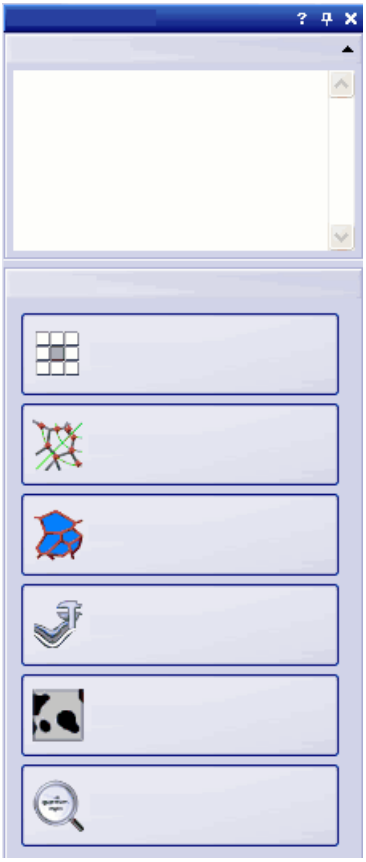
7. Performing a materials science analysis

7.1. 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.



The top illustration shows the *Materials Solutions* tool window before an analysis process has been chosen. In the example shown, several analysis processes are available, as you can see from the three large buttons.

Overview of the supported analysis processes

- Chart comparison
- Intercept analysis
- Grains Planimetric
- Layer Thickness
- Cast Iron Analysis
- Inclusion worst field

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.

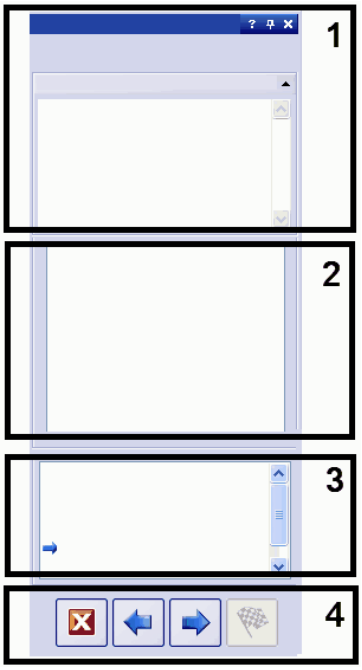
Starting an analysis process

You start an analysis process by clicking the corresponding button.

Tip: 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.

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 contents 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 shown in bold font, and 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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7.2. What are chart comparisons?

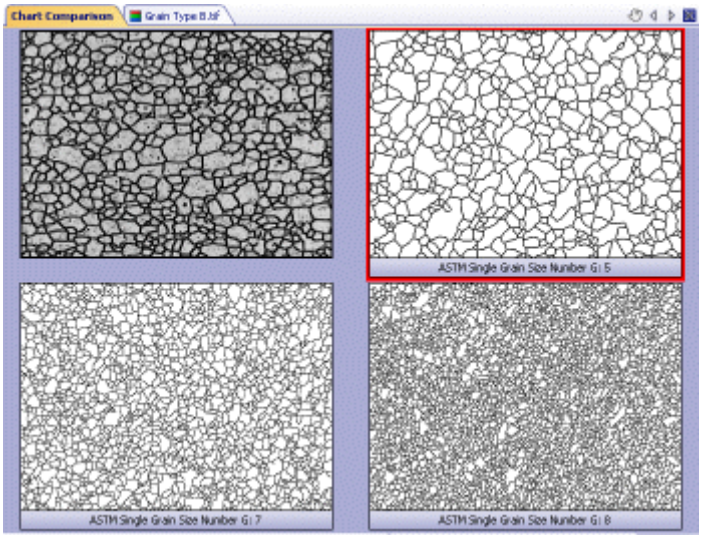
In metallography, chart comparisons are used as a means of quality control. They make it possible to compare an image with numerous reference images. The reference images are, as a rule, a part of the industry standards by which the chart comparisons are carried out.

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 .

The process flow of 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 reference image you've selected will then be 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.

Predefined charts

Several predefined charts are supplied together with your software. The *Single grain size* and *Austenitic grain size* charts contain images with which the grain sizes can be determined.

The *Inclusions* chart contains numerous images of various non-metallic inclusions (NMI). The NMIs show non-metallic inclusions of various types, and of various sizes. You can use the images to determine the type and size of the NMIs in your sample, by comparison.

As well as that, the reference images of numerous international industry standards for material analysis can be incorporated.

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Performing a chart comparison

Tip: 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.

Example image "FerriteGrains.tif"

When your software was installed, several example images have been copied automatically. You can immediately 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. Regarding the information as to where the example images are located, please refer to the online help.

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.
3. In the *Image source* group, choose the *Selected images* option to analyze the "FerriteGrains.tif" 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. Should you want to add data, (e.g., because you are analyzing images of several samples in the same analysis), leave the check box unselected.
5. Click the *Next* button.
   - The *Materials Solutions* tool window displays the next step.

Sample information step

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

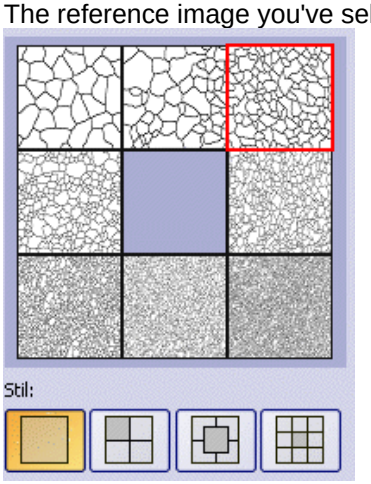
1. Enter information on your sample. By default, these fields are called *Reference* and *Group*.
2. If you want to, enter a comment about the sample. This comment is valid for all of the images of this sample.
3. If you want to, enter a comment about the current image, too.
4. Click the *Next* button.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

Settings step

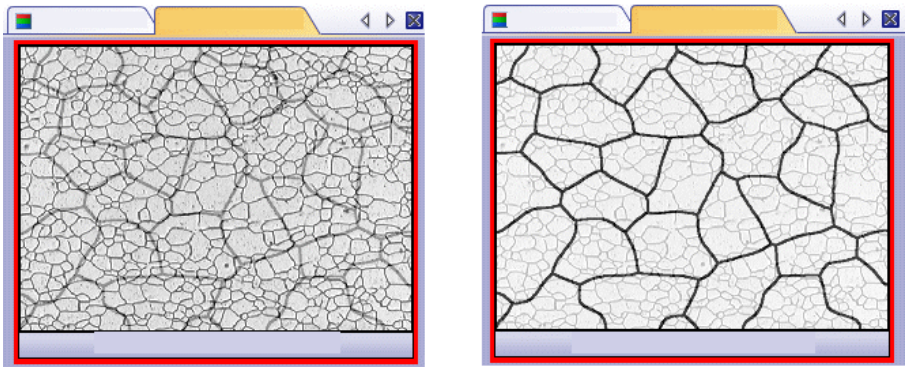
1. Select the chart by which you want to analyze the image. The *Chart* field contains some entries that are, by default, supplied with your software. As well as these, this group can contain additional or totally different entries, depending on which charged-for industry standards have been incorporated for your company.
   - Should no further industry standards have been incorporated in your case: Select, for the "FerriteGrains.tif" image, the *Single grain size* entry, to determine the grain size.
2. Click the *Next* button.
   - The *Materials Solutions* tool window displays 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 this, 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 reference image you've selected will then be 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 in the *Opaque* direction, if the image that is to be checked is to superimpose the reference image. Alternatively, move the slide control in the *Transparent* direction, 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 the direction of 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 in the direction of 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 *Chart 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 displays the next step.

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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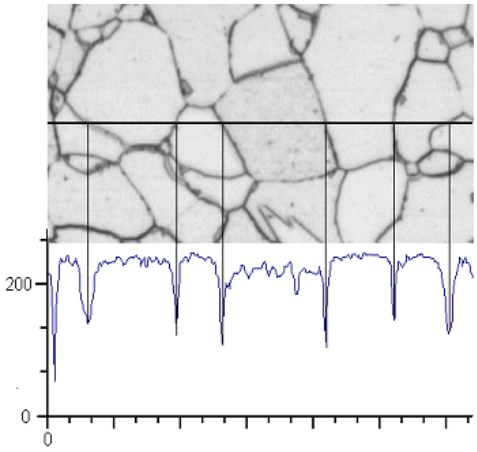
7.3. What is an intercept analysis?

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:

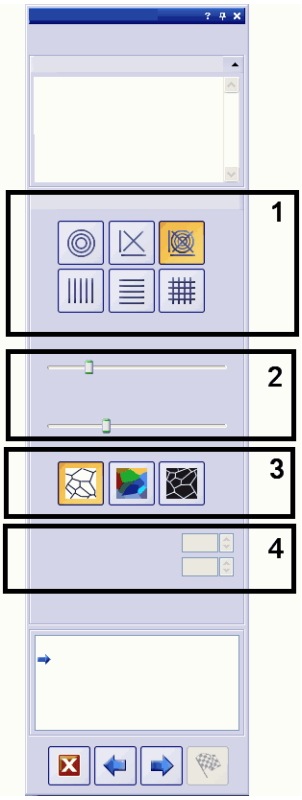
- ASTM E112
- GB/T 6394
- GOST 5639
- ISO 643
- DIN 50601
- JIS G 0551
- JIS G 0552

The results of an analysis can be displayed in a workbook. Additionally, or alternatively to that, the results can be displayed in a MS-Word report.

00700

Materials Solutions - Intercept analysis - Settings

In this step, you make important settings for the analysis. The following possibilities are available:



(1) Selection of the line pattern

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 & Circles

The *Cross & Circles* line pattern combines the two line patterns *Cross* and *Circles*.

Horizontal Lines

With this line pattern, horizontal lines are distributed evenly across the measurement pattern.

Vertical Lines

With this line pattern, vertical 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.

(2) Slide controls for changing the results displayed

Two slide controls are available. 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

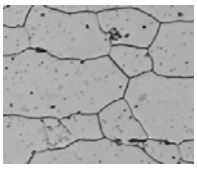
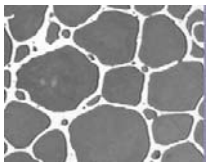
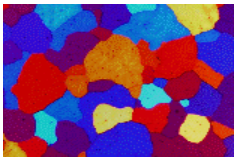
Here you set the necessary width for the detection of a grain boundary. If the grain boundaries can be clearly recognized, a small grain boundary width is sufficient. The slide control can stand well over to the left, in this case. If the grain boundaries are difficult to recognize, you'll need to increase the grain border width. To do this, move the slide control to the right. When you are positioning the slide control, keep a constant eye on the number and quality of the intercept points found.

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. When you do this, keep an eye on the number and quality of the intercept points found.

(3) 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).

(4) Number of test lines

These fields are only active if you selected a line pattern that contains horizontal or vertical lines. In this case, you specify here the number of lines to be used for the intercept analysis.

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Performing an intercept analysis

Tip: 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.

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 *Grains Intercept*  button.
3. In the *Image source* group, choose the image resp. 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. The following options are available:
   - *Live image* option: With this option, the additional step *Image acquisition* will be shown. In this step, an image of the live-image is acquired, that is then analyzed in the steps that follow. 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 want 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 haven't been selected in the *Gallery* tool window will be ignored for the analysis.
- *System 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.

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 Info* 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 unselected.
6. Click the *Next* button.
   - The *Materials Solutions* tool window displays 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.

Sample information step

You will only see this step in the analysis if, in the previous step, the *Skip Sample Info* 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. Further information on how to change the default settings is available in the online help.
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 displays the next step.

Settings step

1. Choose a line pattern that is appropriate for the structures in the image that is to be analyzed. You can choose between various line patterns.
   - The pattern determines along which lines, intercept points in the image are looked for.
2. Take a look at the intercept points that have been found in the image. If necessary, change the settings to optimize the results shown.
3. Click the *Next* button.
   - The *Materials Solutions* tool window displays the next step.

Image results step

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...* or *Delete...* buttons. This will enable you to add intercept points manually, or to delete superfluous intercept points. You will find step-by-step instructions on how to correct intercept points in the online help.
4. Select the *Check settings* check box, to have the *Settings* step displayed with every image.
   - This enables you to individually change the slide control's setting before every individual image is analyzed. This makes sense, for example, if the images that are to be analyzed are of greatly different quality, and you need to adjust the noise reduction individually for each image.
   - The *Check settings* button is only active if you analyze several images of a sample at the same time.
5. 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 displays 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. 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. 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, if you want to have a report automatically generated in the MS-Word application program once the analysis is completed.
   - 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 to file....* 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, load the image to be analyzed, then click the *Load from file...* button, in the *Image source* step. 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.

Reporting step

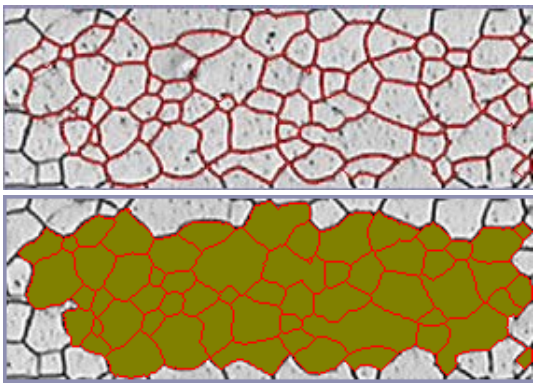
1. Select the *Default* option, to use the document template that has been defined as default document template. The document template determines, e.g., the appearance of the report's header and footer.
   - Should you want to change the default document template, use the *Tools > Options > Report Composer > Document Templates* command. Add the document template you want to the *Templates* list, select it and click the *Set as Default* button.
2. 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.
3. Click the *Finish* button.
   - The report will be generated and displayed in the MS-Word application program.
   - 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.

00701

7.4. 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 dark grain boundaries or samples with bright grain boundaries 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. The grain boundaries that have been detected are plotted in red (first illustration).
Additionally, it's possible to have the grains that have been found displayed in color (second illustration). When this is done, the original image isn't changed, because this information is written to another image layer.

Editing grain boundaries

You can manually edit the grain boundaries that your software found automatically. When you do this, you have the possibility of deleting superfluous grain boundaries and adding boundaries that are missing.

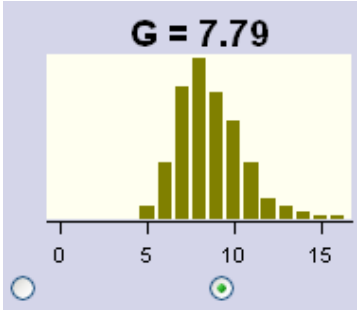
The results of a grains planimetric analysis

A grains planimetric analysis provides the so-called G-value, which is defined as a characteristic grain size in the corresponding industry standards. The grain sizes are measured in accordance with the industry standards:

- ASTM E112
- GB/T 6394
- GOST 5639
- ISO 643
- DIN 50601
- JIS G 0551
- JIS G 0552

Documenting the results

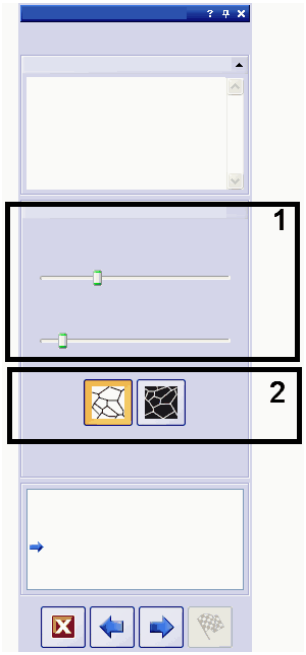
The results of an analysis can be displayed in a workbook and in a chart. Additionally, the results can be displayed in a MS-Word report.



00720

Materials Solutions - Grains planimetric - 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 *Image type information* step.



(1) 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.

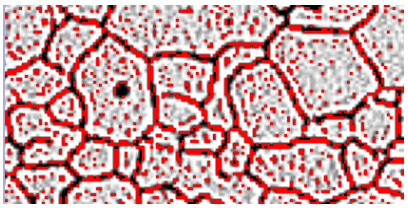
Position the slide controls in such a way that the grain boundaries are detected as completely as possible. It doesn't matter if pixels are detected within a grain as well. The algorithm that calculates the G-value ignores all of the pixels within a grain.

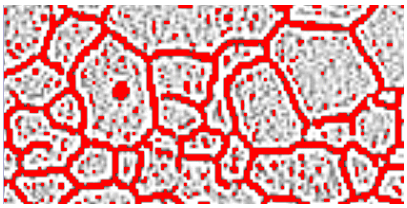
Make sure that the correct grain boundary type has been set, before you adjust the positions of the other slide controls.

Grain boundary width

You'll only see this slide control when you chose the "Flat etched grains" image type in the previous step.

With this slide control you set the necessary width for the detection of a grain boundary. If the grain boundaries can be clearly recognized, a small grain boundary width is sufficient. The slide control can, in this case, stand on the far left (in the direction of the *Small* position). If the grain boundaries are difficult to recognize, you'll need to increase the grain border width. To do so, move the slide control to the right (in the direction of the *Large* position).





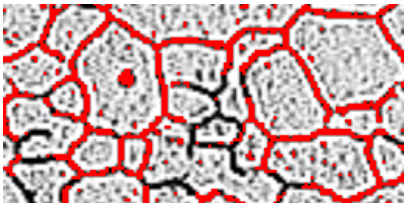
In first illustration, the selected grain boundary width is too small. The grain boundaries haven't been completely detected.
In the second illustration, the grain boundary width has been increased. Now the grain boundaries have been completely detected.

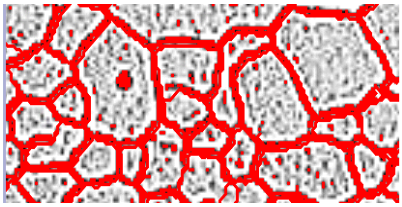
You can use the program options to change the color in which the detected grain boundaries are shown. Further information is available in the online help.

Threshold

Choose whether a smaller intensity value range is sufficient for the detection of a grain boundary. This is, e.g., the case, when all of the grain boundaries stand out clearly against the background. In this case, you can move the slide control to the far right (in the direction of the *High* position).

If all of the grain boundaries don't stand out clearly against the background, e.g., because some grain boundaries are brighter than others, a larger intensity value 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 direction of the *Low* position).





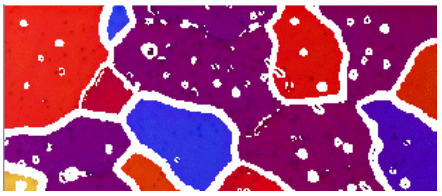
In the first illustration, the selected threshold value is too high. Some of the grain boundaries haven't been detected.
In the second illustration, a lower value for the threshold values has been given. The grain boundaries have now been correctly detected.

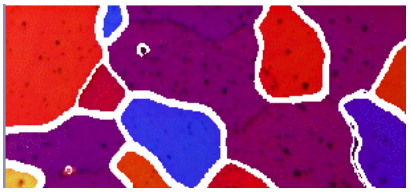
Smoothness

You'll only see this slide control if you chose the "Color etched grains" image type in the previous step.

With the help of this slide control you can specify to which size artifacts that are located within the grains are to be ignored for the analysis. Detected artifacts are taken for small grains by the software, and therefore influence the results of the planimetric measurement.

Set the degree of image smoothness in such a way that artifacts are just no longer detected. Don't choose a greater value than necessary. When the image smoothness chosen is unnecessarily great, "real" small grains won't be detected otherwise.





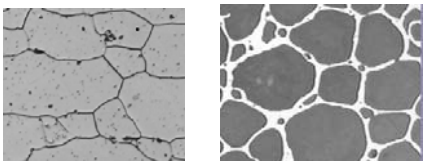
In the first illustration, the selected image smoothness is too small. With this setting, numerous artifacts within the grains are detected, which negatively affects the results of the planimetric measurement.

In the second illustration, a higher value for the image smoothness has been chosen. You can clearly see that only a few artifacts were still detected within the grains. These artifacts therefore hardly influence the planimetric measurement at all.

(2) Buttons for selecting the grain boundary type

You'll only see these buttons when you chose the "Flat etched grains" or "Ferritic grain size with pearlite" image type, in the previous 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. Therefore, this slide control can only stand at the position *Dark* or *Bright*.



In the illustration on the left, the grain boundaries are dark. In the illustration on the right, the grain boundaries are bright.

10284

Performing a planimetric measurement

Tip: 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.

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 *Grains Planimetric*  button.
3. In the *Image source* group, choose the image resp. 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. The following options are available:
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 Info* 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 unselected.
6. Click the *Next* button.
   - The *Materials Solutions* tool window displays the next step.

Sample information step

You will only see this step in the analysis if, in the previous step, the *Skip Sample Info* 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. Further information on how to change the default settings is available in the online help.
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 displays the next step.

Image type information step

1. Click the image type that the image that is to be analyzed most resembles. The current image type is framed in yellow.
   - The image type determines the algorithm that is to be used for the determination of the grain sizes.
2. Click the *Next* button.
   - The *Materials Solutions* tool window displays the next step.

Settings step

1. Click the button that is equivalent to the grain boundaries in your sample (e.g., dark grain boundaries against a bright background).
2. With the *Grain boundary width* slide control, you set the necessary width for the detection of a grain boundary. If the grain boundaries can be clearly recognized, a small grain boundary width is sufficient. The slide control can, in this case, stand on the far left (in the direction of the *Small* position). If the grain boundaries are difficult to recognize, you'll need to increase the grain border width. To do so, move the slide control to the right (in the direction of the *Large* position).
   - You'll only see this slide control when you chose the "Flat etched grains" image type in the previous step.
   - You can use the program options to change the color in which the detected grain boundaries are shown. Further information is available in the online help.
3. With the *Threshold* slide control, you set the intensity value range for the detection of a grain boundary. If all of the grain boundaries can be clearly made out against the background, move the slide control to the far right (in the direction of the *High* position).
   If all of the grain boundaries can't be clearly made out against the background, e.g., because some grain boundaries are brighter than others, move the slide control to the far left (in the direction of the *Low* position).
4. With the *Smoothness* slide control, set the degree of the image smoothness in a way that artifacts located within the grains are just not detected. Don't choose a greater value than necessary.
   - Detected artifacts are taken for small grains by the software, and therefore influence the results of the planimetric measurement. When the image smoothness chosen is unnecessarily great, "real" small grains won't be detected otherwise.
   - You'll only see these slide controls when you chose the "Color etched grains" image type in the previous step.
5. Click the *Next* button.
   - The *Materials Solutions* tool window displays the next step.

Image results step

1. Check the results that are displayed in the image and in the *Materials Solutions* tool window.
   - In the image, the grains that have been detected are now displayed in color. Only the colored grains will be taken into account when the G-value is calculated. If you want to have the grain boundaries shown together with the image, clear the *Show grains* check box, in the *Display* group.
   - In the *Materials Solutions* tool window, you'll see the mean grain size number G for the current sample or for the current image. As well as that, the allocation of the detected grain sizes to the different size classes will be displayed graphically.
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 the images by using another position of the slide controls.
3. If you want to correct the automatically found grain boundaries, click the *Add grain boundaries in free-hand mode...*  , *Add grain boundaries in guided mode...*  or *Delete grain boundaries...*  button. You will find step-by-step instructions on how to correct grain sizes in the online help.
4. Select the *Check settings* check box, to have the *Settings* step displayed with every image. This button is only active if you analyze several images of a sample at the same time.
   - This enables you to individually change the slide control's setting before every individual image is analyzed. This makes sense, for example, if the images that are to be analyzed are of greatly different quality, and you need to adjust the noise reduction individually for each image.
   - You can select or clear the *Check settings* check box during a running analysis. This change will then be valid from the next image onwards.
5. 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 displays 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. 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. 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, if you want to have a report automatically generated in the MS-Word application program once the analysis is completed.
   - 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. Select the *Generate Chart* check box, to save the graphic display of the allocation of the detected grain sizes to the different size classes. It is the same chart that was displayed in the previous *Image results* step in the analysis.
5. If you want to save the current settings to a file, click the *Save to file....* button. Then assign a descriptive name in the next dialog box.
6. Click the *Next* button.

Reporting step

1. If you want to, click the button with the three points  , located to the right of the *Template* field, to change the document template. The document template determines, e.g., the appearance of the report's header and footer. Then select the new document template in the *Open* dialog box.
   - Even if no document template has been registered, you can still generate a report. In this case, the same document template will be used as Microsoft Word uses when you set up a new Word file. This default document template is, as a rule, called "normal.dot".
2. In the *Content* group, select the check box for the pages the report should contain.
3. Click the *Finish* button.
   - The report will be generated and displayed in the MS-Word application program.

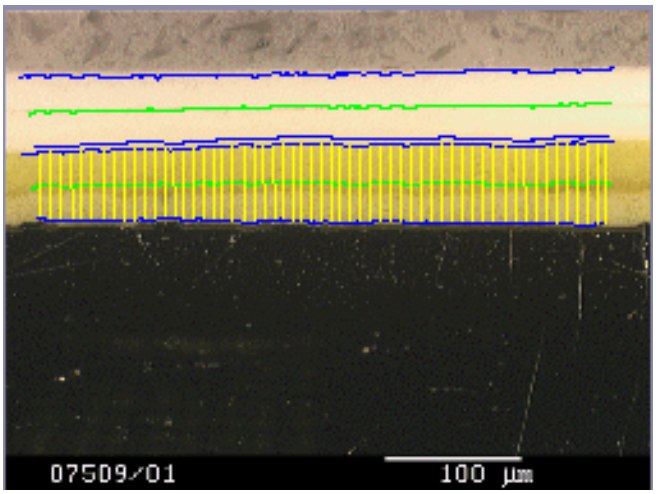
00721

7.5. What are layer thickness measurements?

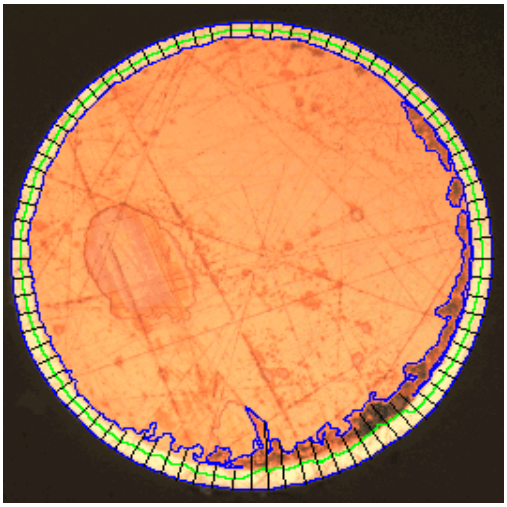
By using layer thickness measurements you can measure layers on calibrated images automatically or interactively. The object that is to be measured is the thickness of one layer or of several layers.

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, or alternatively to that, the results can be displayed in a MS-Word report.

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 process flow of a layer thickness measurement

1. In the *Materials Solutions* tool window, click the *Layer Thickness* button.

2. Choose the image you want to measure.

3. Choose the definition method you want to use (Automatic, Manual, Magic Wand).

4. Define the contours.

5. Define the borders.

6. Define the layers.

7. Take a look at the measurement results.

8. Document the results (report or workbook).

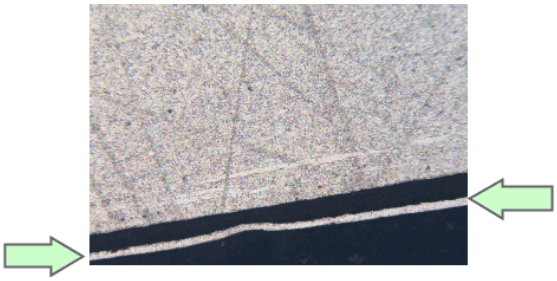
00725

Performing an automatic layer thickness measurement

You can follow the following step-by-step instructions on your PC. They describe a layer thickness measurement on an example image.

"Image source" step

1. Load the "Coating.tif" example image. Regarding the information as to where the example images are located, please refer to the online help.
   - On this image, the thin light layer is to be measured.



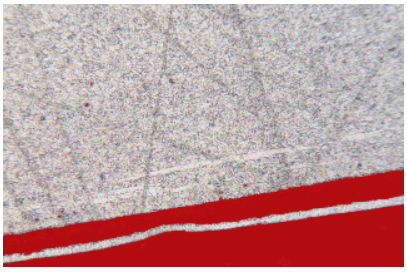
2. Activate the *Materials Solutions* tool window.
3. Click the *Layer Thickness*  button.
4. Select the *Skip 'Sample information'* check box.
5. Click the *Next* button.

"Settings"step

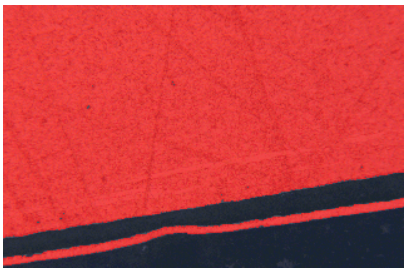
1. Click the *Automatic*  button.
2. In the *Layer type* group, click the icon for an open layer  .
3. Click the *Next* button.

"Automatic" step

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.



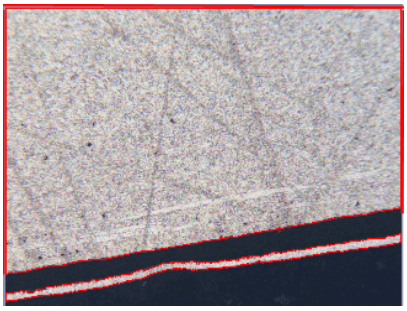
2. Since the required image structures are not yet shown in color, select the *Dark* option in the *Background* group.
   - Now the required image structures are shown in color.



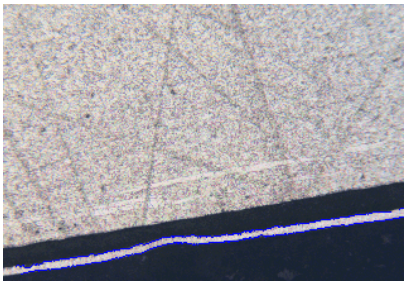
3. Click the *Next* button.

"Define borders" step

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.



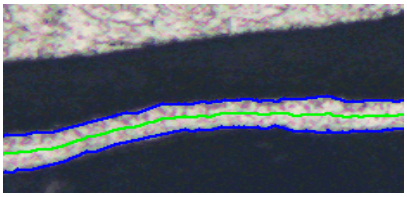
6. Since you don't want to define any additional borders: Then click once more with your right mouse button in the image, to switch off the mode for defining the borders.
7. Click the *Next* button.

"Edit borders" step

1. Since you have already defined both of the borders, and don't want to change them: Click the *Next* button.

"Define layers" step

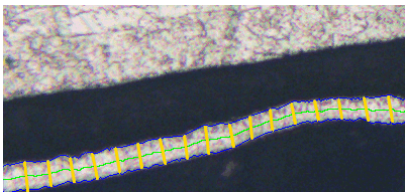
1. Click the *Add layers...*  button.
2. Click the first border.
3. Click the second border, then click your right mouse button.
   - The layer has now been defined. The neutral fiber is plotted in green. It always lies in the middle of the layer.



4. Click the *Next* button.

"Image results" step

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 doubleclick 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. Further information is available in the online help.
2. Check the results shown in the image.
   - The measurement lines are shown in yellow in the image.



3. Click the *Next* button.

"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.
   - Leave the *Create report* check box cleared for these step-by-step instructions.
2. Click the  *Finish* button.
   - The workbook will be created.
   - The analysis has now been completed. The *Materials Solutions* tool window switches back to the start position. You can now use all of your software's functions again.

00732

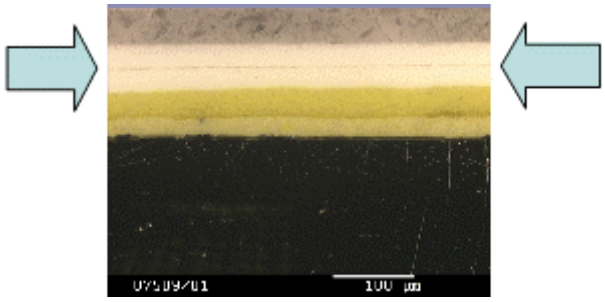
Performing a layer thickness measurement with the magic wand (open layer)

You can follow the following step-by-step instructions on your PC. They describe a layer thickness measurement on an example image. The example image shows an open layer.

Please note: The procedure is slightly different when you measure a closed layer. You will find separate step-by-step instructions on how to measure closed layers in the online help.

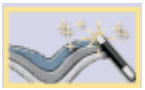
"Image source" step

1. Load the "Painting Coat.tif" example image. Regarding the information as to where the example images are located, please refer to the online help.
   - On this image, the topmost layer is to be measured.



2. Activate the *Materials Solutions* tool window.
3. Click the *Layer Thickness*  button.
4. Select the *Skip 'Sample information'* check box.
5. Click the *Next* button.

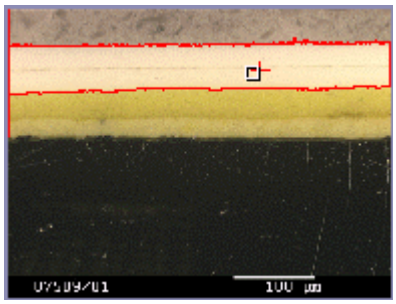
"Settings" step

1. Click the *Magic Wand*  button.
2. In the *Layer type* group, click the icon for an open layer  .
3. Click the *Next* button.

"Magic wand" step

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 within the layer that is to be measured.

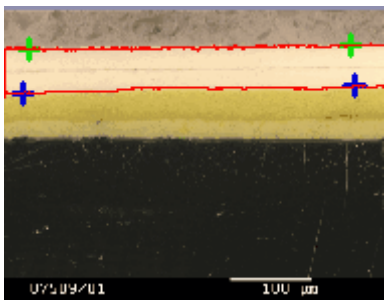
- The contour will be shown by a red line.



4. It's possible that the contour won't completely contain the image structure you want. Alternatively, it can happen that too much space has been detected around the image structure. In this case, increase the value in the *Tolerance* field, until the contour roughly includes the layer that is to be measured.
5. Click your right mouse button to finish the definition of the contour.
6. Click the *Next* button.
   - The *Define borders* step in the analysis will be shown.

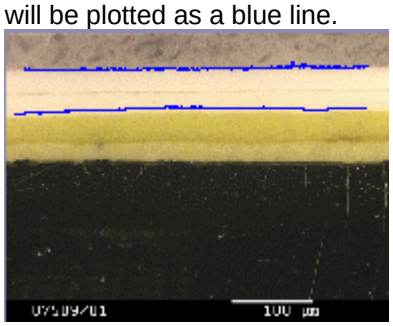
"Define borders" step

1. Click the *Define borders...*  button.
2. With your left mouse button, click once at any position in the contour to activate the mode.
3. Then click with your left mouse button at the position in the contour where the first border is to begin.
   - A green cross indicates the border's starting point.
4. Then click with your left mouse button at the position in the contour where the first border is to end.
   - A second green cross indicates the border's end point.
5. Define the second border. To do this, click again at the two positions in the contour at which this border is to begin and end.
   - The beginning and the end of this second border will be indicated by two blue crosses.



6. Then click with your right mouse button at any place in the image, to switch off the mode.

- The detected contour will disappear. The borders that have been defined will be plotted as a blue line.



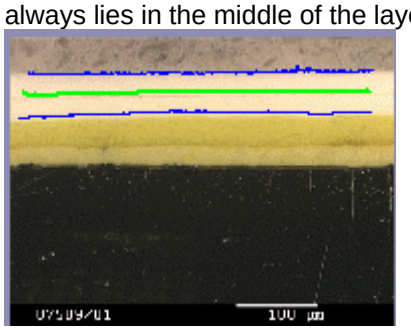
7. Click the *Next* button.

"Edit borders" step

1. Since you have already defined both of the borders, and don't want to change them: Click the *Next* button.

"Define layers" step

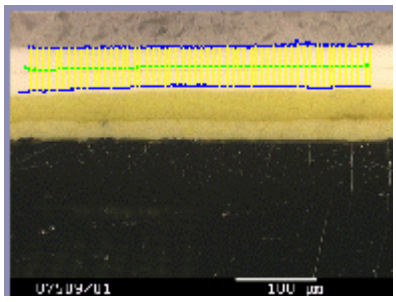
1. Click the *Add layers...*  button.
2. Click the first border.
3. Click the second border.
   - The layer has now been defined. The neutral fiber is plotted in green. It always lies in the middle of the layer.



4. Click your right mouse button to finish the definition of the layer.
5. Click the *Next* button.

"Image results" step

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 doubleclick in the cell you want to edit. Further information is available in the online help.
   - The lower part of the group contains several buttons, with which you can change the way the layer thickness measurement is displayed. Further information is available in the online help.
2. Check the results shown in the image.
   - The measurement lines are shown in yellow in the image.



3. Click the *Next* button.

"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.
   - Leave the *Create report* check box cleared for these step-by-step instructions.
2. Click the  *Finish* button.
   - The workbook will be created.
   - The analysis has now been completed. The *Materials Solutions* tool window switches back to the start position. You can now use all of your software's functions again.

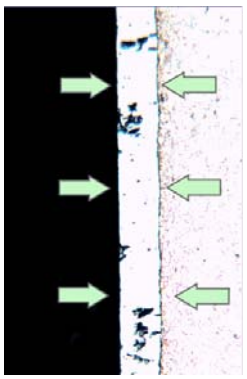
00729

Performing a manual layer thickness measurement

You can follow the following step-by-step instructions on your PC. They describe a layer thickness measurement on an example image.

"Image source" step

1. Load the "Coating with porosity.tif" example image. Regarding the information as to where the example images are located, please refer to the online help.
   - On this image, the middle layer is to be measured.



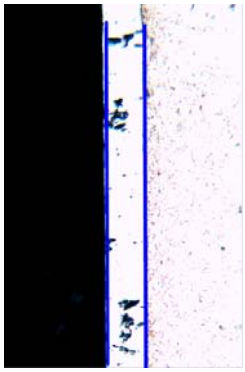
2. Activate the *Materials Solutions* tool window.
3. Click the *Layer Thickness*  button.
4. Select the *Skip 'Sample information'* check box.
5. Click the *Next* button.

"Settings" step

1. Click the *Manual*  button.
2. In the *Layer type* group, click the icon for an open layer  .
3. Click the *Next* button.

"Manual" step

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.

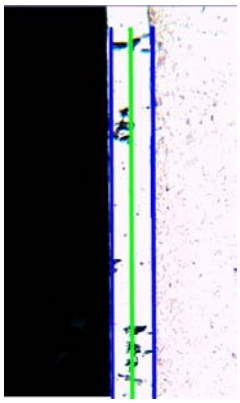
"Edit borders" step

1. Since you have already defined both of the borders, and don't want to change them: Click the *Next* button.

"Define layers" step

1. Click the *Add layers...*  button.
2. Click the first border.
3. Click the second border, then click your right mouse button.

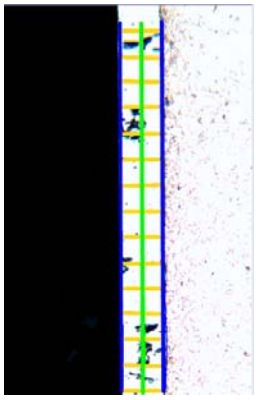
- The layer has now been defined. The neutral fiber is plotted in green. It always lies in the middle of the layer.



4. Click the *Next* button.

"Image results" step

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 doubleclick in the cell you want to edit. Further information is available in the online help.
   - The lower part of the group contains several buttons, with which you can change the way the layer thickness measurement is displayed. Further information is available in the online help.
2. Check the results shown in the image.
   - The measurement lines are shown in yellow in the image.



3. Click the *Next* button.

"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.
   - Leave the *Create report* check box cleared for these step-by-step instructions.
2. Click the  *Finish* button.
   - The workbook will be created.
   - The analysis has now been completed. The *Materials Solutions* tool window switches back to the start position. You can now use all of your software's functions again.

00731

7.6. What is a cast iron analysis?

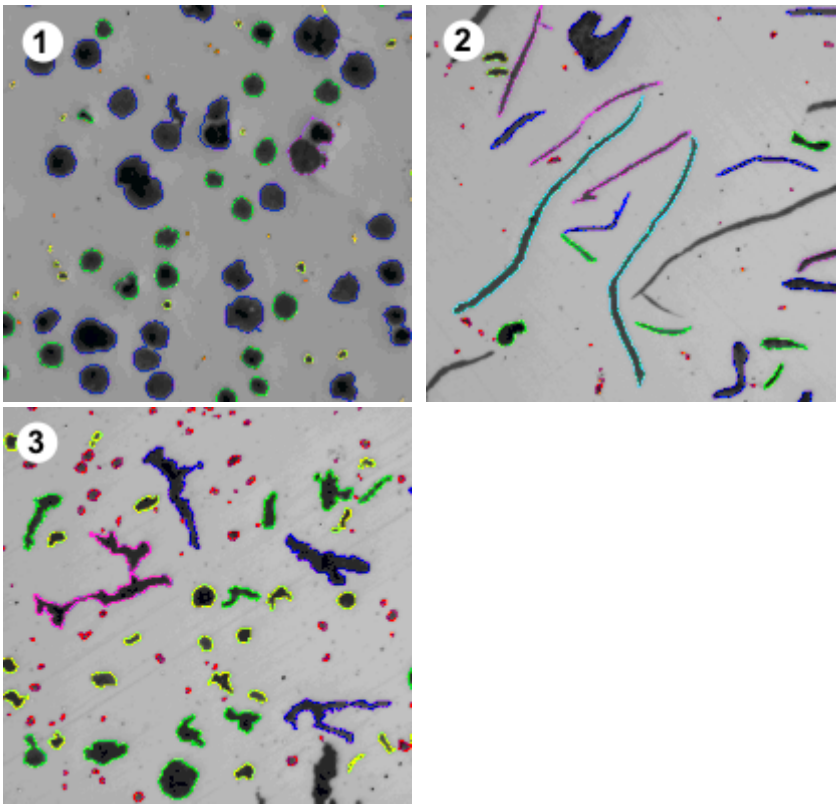
The quality and consistency of cast iron depends on the distribution and the morphology of its carbon content. By using a cast iron analysis you can determine the cast iron's graphite fraction with the help of unetched samples. As well as that, with the help of etched samples you can determine the ferrite/pearlite 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:

- ASTM A 247 2006
- EN ISO 945
- EN ISO 945-1
- KS D 4302-2006
- JIS G 5502-2001
- GBT 9441

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 have been etched. How the classes are defined, depends on the standard according to which the cast iron analysis is carried out. The standard is set in the program options.



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, or alternatively to that, the results can be displayed in a MS-Word report.

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.

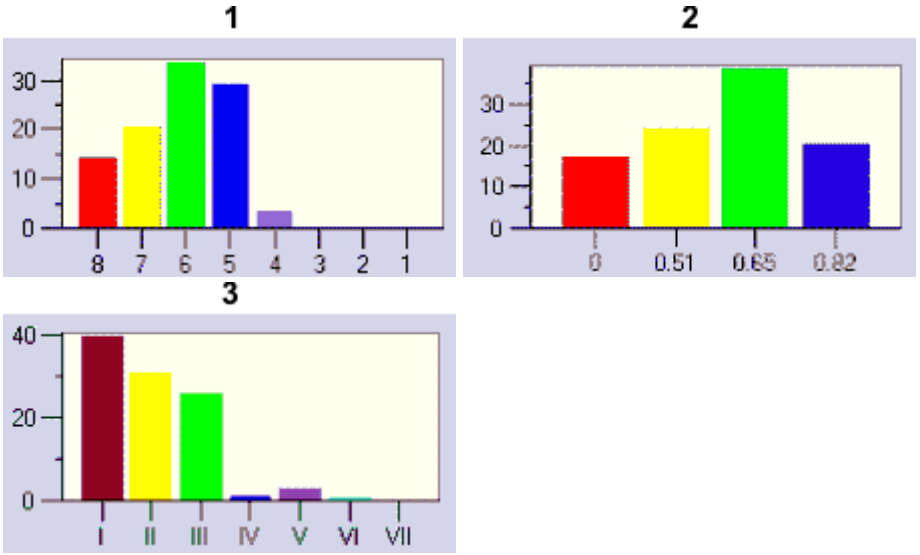


Illustration (1) shows a chart of the graphite size. Along the X-axis the size classes are shown, along the Y-axis the number of detected particles

Illustration (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.

Illustration (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.

Usual process flow of a cast iron analysis made to determine the graphite fraction

1. In the *Materials Solutions* tool window, click the *Cast iron* button.

2. Choose the image you want to measure.

3. Choose the sample type (etched).

4. Set the graphite parameters.

5. If chosen: Determine the distribution of the graphite particles.

6. Take a look at the image results.

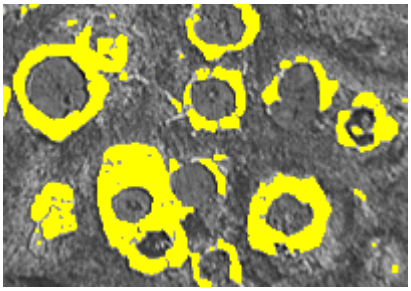
7. If necessary: Delete or separate detected particles, or add new ones.

8. 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).

00734

Performing a cast iron analysis (unetched sample)

You can follow the following step-by-step instructions on your PC. They describe how the graphite fraction is determined.

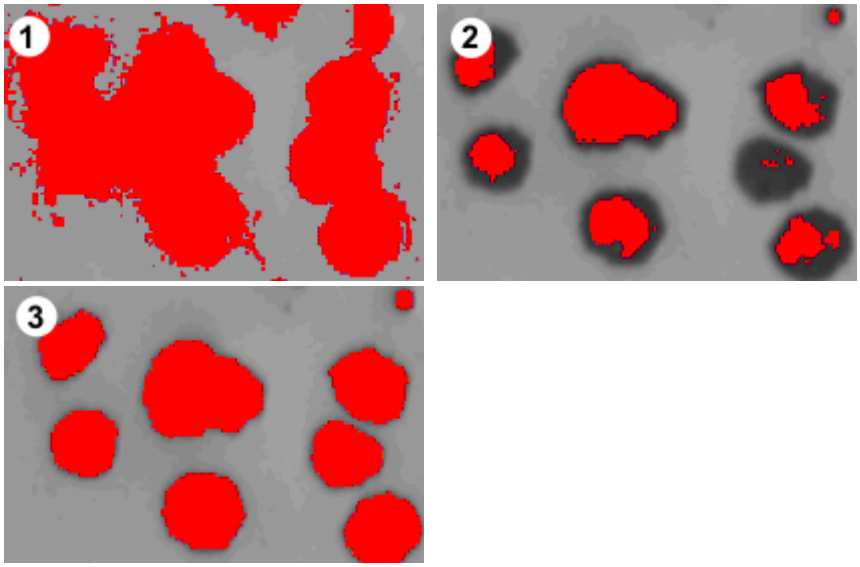
"Image source" step

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 Analysis*  button.
4. Select the *Skip 'Sample information'* check box.
5. Click the *Next* button.
   - The *Materials Solutions* tool window displays the next step.

"Settings" step

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 settings possibilities 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.

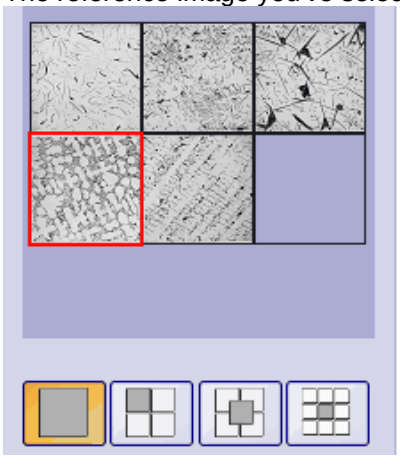
3. Select the graphite parameter that is to be determined. To do so, select the corresponding check box. The possibilities listed below are available: Which size classes, form classes and form factors are used for the classification, depends on the industry standard according to which the cast iron analysis 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.
4. Click the *Next* button.
    - The *Materials Solutions* tool window displays the next step.

"Graphite Distribution" step

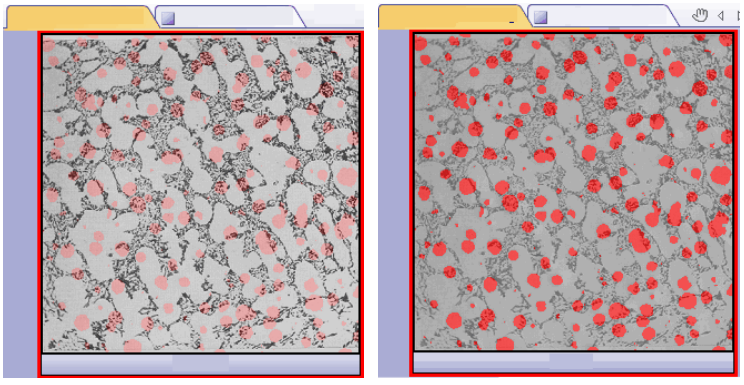
You will only see this step if, in the previous step, you selected the *Graphite distribution* check box.

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 this, click this  button.
   - In the *Overview* field, you see the arrangement that has been chosen. The reference image you've selected will then be framed in red.



   - In the document group, the "Cast Iron Distribution" document will now be displayed. 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 in the *Opaque* direction, if the image that is to be checked is to superimpose the reference image. Alternatively, move the slide control in the *Transparent* direction, 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.

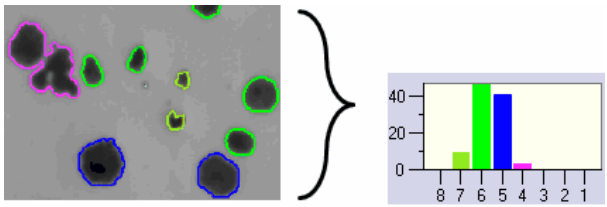


   The illustration on the left shows the image that is to be checked. Because the slide control is located in the direction of the *Opaque* position, the reference image's structures can only be faintly recognized. For the illustration on the right, the slide control has been moved in the direction of 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 displays the next step.

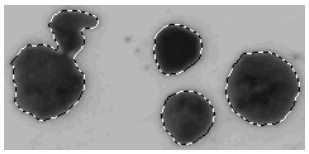
"Image results" step

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), are shown with 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. You will find step-by-step instructions on how to correct particles in the online help.
4. Click the *Next* button.

"Results" step

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 Workbook* check box to have a document of the "workbook" type automatically created at the end of the analysis.
   - Leave the *Generate Report* and *Generate Chart* check boxes cleared for these step-by-step instructions.
3. Click the *Save sample 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.
4. Click the *Finish* button.
   - The workbook will be created.
   - The analysis has now been completed. 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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Performing a cast iron analysis (etched sample)

You can follow the following step-by-step instructions on your PC. They describe how to measure the ferrite/pearlite-ratio.

"Image source" step

1. Load the "Ferrite Pearlite.tif" example image. Regarding the information as to where the example images are located, please refer to the online help.
   - 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 Analysis*  button.
4. 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).

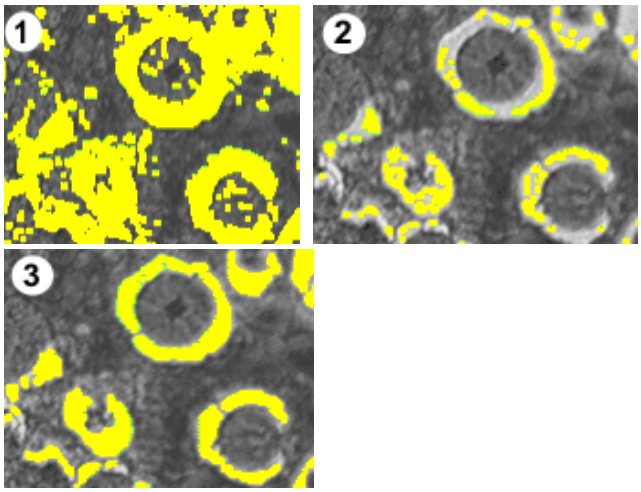
     In this case, make sure the *Skip 'Sample information* check box is cleared, which will enable you to use the *Load sample results* button, in the *Sample information* step.
5. Click the *Next* button.
   - The *Materials Solutions* tool window displays the next step.

"Settings" step

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 settings possibilities 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.

   3. The threshold value has been correctly set when the ferrite is completely detected.

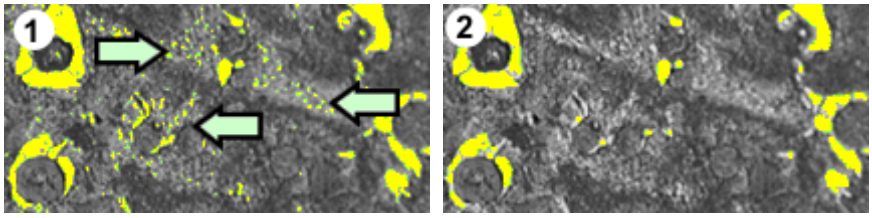


In the illustration (1), the threshold value has been set too high, too many particles are detected as ferrite.

In the illustration (2), the threshold value has been set too low, the ferrite is not detected completely. The illustration (3) shows a correctly set threshold value.

3. Use the *Closing 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 *Closing 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:

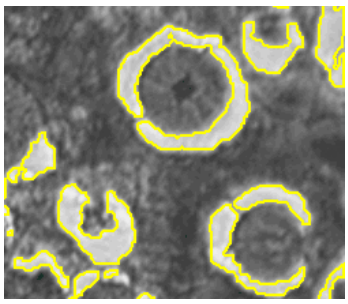
   - You select the *Enter manually* option and enter the value. This option is always active. You can have e.g., made a note of this value, or have saved it in a report.

- You select the *Results 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 displays the next step.

"Image results" step

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.

"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.
   - Leave the *Create report* check box cleared for these step-by-step instructions.
   - Leave the *Create chart* check box cleared for these step-by-step instructions.
2. Click the *Finish* button.
   - The workbook will be created.
   - The analysis has now been completed. 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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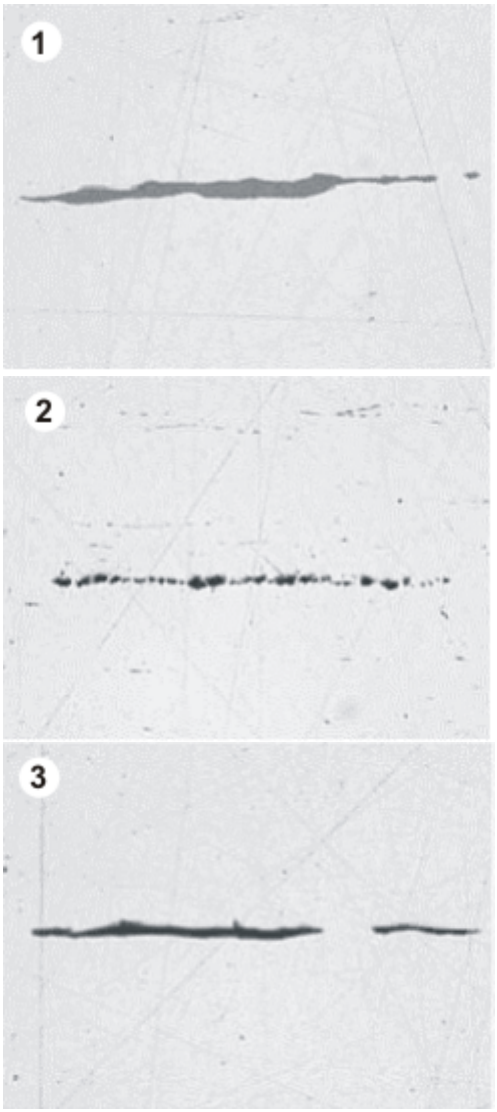
7.7. What is an inclusion worst field analysis?

An inclusion worst field analysis is one of several possible procedures used to detect non-metallic inclusions in metal samples. This analysis is, e.g., used to measure the amount, size and distribution of sulfides and oxides in steel. 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.

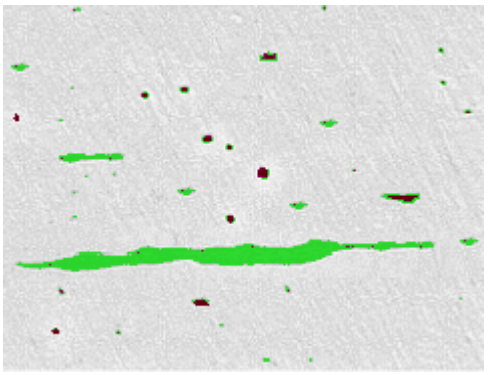


Microscope image of different inclusions in a polished steel sample. The inclusions differ in their color and form. The images show a sulfide inclusion (1), a silicate inclusion (2), and an aluminum inclusion (3).

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 inclusion 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. Since between the different inclusions there are also intensity differences, (sulfides are, e.g., brighter than oxides), you can also define two intensity ranges.



Particle detection during an inclusion 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 inclusion worst field analysis

The inclusion worst field analysis determines which is the largest non-metallic inclusion within the sample under investigation. This is done for each inclusion type separately.
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 Method A
- DIN 50602 Method M
- ISO 4967 Method A
- GB/T 10561 Method A
- JIS G 0555 Method A
- UNI 3244 Method M

The results of an analysis can be displayed in a workbook. Additionally, or alternatively to that, the results can be displayed in a MS-Word report.

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Performing an inclusion worst field analysis

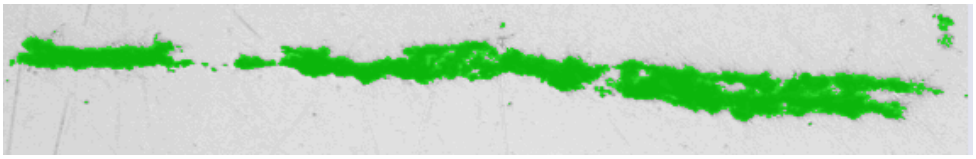
You can follow the following step-by-step instructions on your PC. It describes how you can detect the worst inclusion in a sample.

"Image source" step

1. Load the "NMI0_0.tif" example image. Regarding the information as to where the example images are located, please refer to the online help.
   - 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 *Inclusion Worst Field*  button.
4. Select the *Skip 'Sample information'* check box.
5. Click the *Next* button.
   - The *Materials Solutions* tool window displays the next step.

"Settings" step

1. In the *Evaluation method* field, set the standard you are going to use for the analysis.
2. Use the slide control to define the threshold value for all of the inclusions. Observe the sample. The threshold value has been correctly set when the inclusions are completely recognized.



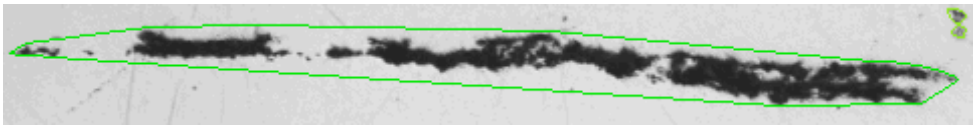
The illustration shows a correctly set threshold value.

3. Since in this sample there are no oxide inclusions, set the *Threshold oxide inclusions* slide control at the position *Low*.
4. Select the *Show ignored particles* check box, when you want to also have the particles that weren't included in the analysis shown.
   - Particles will be ignored when they don't fulfill the requirements that were specified in the program options. Information on the program options is available in the online help.
5. Click the *Next* button.
   - The *Materials Solutions* tool window displays 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 correct the automatically found inclusions, use the buttons in the *Edit inclusions* group. You will find step-by-step instructions on how to correct inclusions in the online help.
4. 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 Workbook* check box to have a document of the "workbook" type automatically created at the end of the analysis.
   - Leave the *Create report* check box cleared for these step-by-step instructions.
3. If you want to save the current settings to a file, click the *Save settings to file...* button. Then assign a descriptive name in the next dialog box.
4. Click the *Finish* button.
   - The workbook will be created.
   - The analysis has now been completed. 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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8. Working with the database

8.1. Overview - Database

If you acquire a large number of images with your software, it makes sense to save them in a database. All of the file management functions, such as searching for images, can be more quickly and easily used in a database, than with files that have been saved separately. Your software's database doesn't only save images, it also saves all other types of file, for example, text files and results sheets.

Please note: The database isn't available in all OLYMPUS Stream software packages.

This documentation is for the users of a database. Therefore, it is assumed that a database has already been set up and configured.

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8.2. "Database" layout

When you work with the database you switch to the "Database" layout.

Distinctive features of the "Database" layout

- Only in this layout is the *Database* tool window maximized by default. Maximizing the tool window has the advantage that it provides you with a clearer overview of the database's records, which will help you use all of the functions for searching, grouping or editing records optimally.
- Only in this layout will the document group not be displayed, since, as a rule, you won't need to resort to the document group when you are working with a database.
- In the "Database" layout, only those of your image analysis program's commands are available that you require when you are working with a database. Commands that are not available will be shown in gray. Should you want to use one of the commands shown in gray, simply switch to another layout.

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8.3. Tool Window - Database

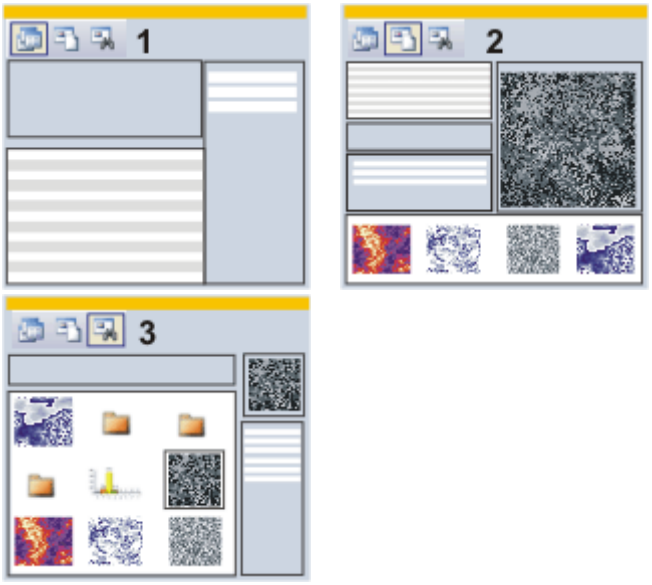
The database will be displayed in the *Database* tool window. This tool window gives you access to all of the records that are in the database.

Toolbars in the database window

The *Database* tool window contains two toolbars. These toolbars provide you with access to the most important functions when you're working with a database.

Main Views in the database window

In the *Database* tool window you can choose between different main views. The tool window is differently divided up in every view, and also displays different information. Use the first buttons  on the *Database* tool window's lower toolbar to toggle between the main views. The button for the active view will appear clicked. You can recognize this status by the button's colored background.



The illustration shows the schematic makeup of the *Database* tool window in the various main views (1 = Project View, 2 = Document View, 3 = Search Results View).

(1) Project View: In the Project View you set up new projects or edit already existing ones. There, you select the project that you can view and work on in the Document View.

(2) Document View: In the Document View you insert new samples and documents into a specific project. There, you can view, work on, or load, already existing documents.

(3) Search Results View: In the Search Results View the results of a database search will be displayed.

Maximizing the database window

The *Database* window can, as the only tool window, be maximized. To do this, click the *Maximize* button. You will find this button in the tool window's header. This will make almost the whole of your software's user interface available for displaying the database.

In the "Database" layout, the *Database* tool window is maximized by default. Therefore, use the "Database" layout when you are working mainly within a database.

Please note: When the *Database* tool window is maximized, the document group will be covered by the database window. Therefore, many of the commands that apply to the document group will, in this case, not be available (e.g., the *File > Open...* command). Should you want to use one of these commands, simply switch to another layout.

Commands in an empty database window

When no database has been opened, the *Database* tool window, offers you the commands with which you can open a database, and set up a new database. There, you will also find a list of the databases that were last opened. Simply click an entry to open the corresponding database.

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8.4. Overview - Inserting data into a database

You can insert two different types of data into a database.

Structuring records

For the better structuring of a database, there are structuring record types, that are indicated within the database by a folder icon. A record that belongs to this record type isn't referenced to a document, but instead contains only the information that is entered in the database fields.
Structuring records are, e.g., "Projects". They are displayed in the your database's Project View.

Documents

Documents are, for example, images, workbooks or diagrams.
You can of course insert all of the documents that you create with your software, or load into it, into a database. You will find an overview of the document types that are supported in the online help.

As well as that, you can, however, also insert any other files that have already been saved on a data medium. That applies also to document types that are not supported by your image analysis program, e.g., MS-Word documents with the file extension DOC.

Overview - Inserting data into a database

There are several ways in which you can insert data into a database. Use the commands in the *Database* menu, or from various context menus, or click the buttons in the *Database* tool window. You can also insert documents by Drag&Drop.
All of these possibilities will be described in the text that follows.

Menu commands

Use the *Database > Insert* menu commands. The following commands are available:

- Insert Open Documents
- Insert All Open Documents
- Insert From Files

Buttons

Use one of the buttons located on the *Database* tool window's upper toolbar.

Commands from the context menu

Use the *Insert* menu in the context menu. You open this context menu by rightclicking on one of the following views in the *Database* tool window.

- Project List View
- Sample List View

You can also insert a single document, for example, an image, directly out of the document group into the database. Richtclick the document's title in the document bar to open a context menu. The context menu will contain the Insert into Database command.

Drag&Drop

Drag a document from the MS Windows Explorer into the database gallery.
To do this, switch to the database's document view. Then select the record into which you want to insert the document, in the database. Then drag the document from the MS Windows Explorer onto the database gallery.

Acquiring images

When a database has been opened, every image you acquire with your software will be automatically saved in the active database.

Notes on how to insert data into a database

When you insert records into a database, please pay attention to the points listed below:

The database is structured hierarchically

You cannot insert records into every database level.

Data can't be overwritten

The documents in the database can't be overwritten. If you want to insert a new version of a document into the database, you will have to insert the new document into the database under a new name, then delete the old document.

Each insertion creates a new record

Images that you want to process after the acquisition (e.g., measure) shouldn't be inserted into the database until after you have finished working on them. Only then will a record be set up exclusively for this image. If you insert image into the database immediately after the acquisition, you will have to set up a new record when you insert the processed image into the database.

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8.5. Insert Open Documents

Use the *Database > Insert > Open Documents...* command to insert documents into the database that have already been opened in your software. A document can be an image, but also a workbook or another document.

When is the command available?

The commands are only available when it is at all possible to insert a document. Should the command or the button be displayed in gray, check the following points.

- Is a database open?
- Has, in the database, a record been selected into which you can insert documents?
- Have documents been loaded?

Inserting documents that have been opened into a database

1. Load, or create, documents that you want to insert into the current database. It doesn't matter whether the documents have already been locally saved or not.
   Switch to e.g., the "Acquisition" layout, and acquire an image, or load an image from your hard disk.
2. First select the record into which you want to insert the document, in the database. Please note that you cannot insert documents at every database level.
3. Use the *Database > Insert > Open Documents...* command.
   - The *Insert Open Documents* dialog box will open. This dialog box lists all documents that have been opened. The icon in front of the document shows you what type of document it is.
4. Here, you have to decide whether you want to insert all of the opened documents into the database, or only some of them. Select the check box in front of each of the documents you want to insert into the database.
5. Click *OK*.
   - The *Insert <Document Type>* dialog box will open successively for each of the files you want to insert. The dialog box's appearance depends on which type of document you insert into the database.
   - The document will be automatically allotted a name by the database, you don't have to take care of that.
6. Enter the required information in the database fields. Database fields that are displayed in bold letters must be filled out.

7. Click the *Insert* button to save the document together with the information displayed, in the database.
   - The documents you have inserted will be displayed in the database gallery. This process can, especially where large images are concerned, take some time. The process is in progress as long as the mouse pointer remains in the form of an hourglass.

Inserting opened documents by Drag&Drop

1. First select the sample into which you want to insert the document, in the database. Please note that you cannot insert documents at every database level.
2. Switch to the "Processing" layout.
3. Use the *View > Tool Windows > Documents* command to make the *Documents* tool window appear.
4. In the *Documents* tool window, select the documents you want to insert into the database.
5. Drag the documents you've selected onto the database gallery.

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8.6. Searching for records

You can search for data in your database at any time you want to. To do so, use the *Database > Define Search...* command. When you search through a database, please pay attention to the following points.

- The database search will always be conducted through the whole database.
- You will find the results of a database search in a special database view, the Search Results View. A detailed description of this view can be found in the online help.
- Each new database search overwrites the results of the previous search.

Different search possibilities

There are always two ways to search for records.

- Searching directly for a record
  When you only use a search definition once, specify the search conditions with the *Database > Define Search...* command, and apply it directly.
- Searching in the Search View
  When you want to use the same search definition repeatedly, save the search definition and used these saved search definition in the Search View. A detailed description of this view can be found in the online help.

Before you start your search

Before you start a search it's advisable to update the view. By updating you will make sure that all of the images will be found that at the time of the search are located in the database.
To update the view, use the *Database > Views > Update Views* command.

Searching directly for a record

1. Use the *Database > Define Search...* command.
   - The *Search Definition* dialog box will open. You define the search conditions here.
2. In the *Search for* group you can limit the search to a few record types. You can, for instance, only search for images or for projects.
3. Define one or more search conditions in the *Conditions* group. Examples for search definitions are available in the online help.
4. Click the *Search* button to start the database search.
   - Your software will switch automatically into the database's Search Results View. Here only the records that fulfill the current search conditions will be displayed.

Exiting the Search Results View

There are several ways to leave the Search Results View.

1. Select a record in the Search Results View.
   Click your right mouse button and select the *Go To Record* command in the context menu.
   - Your image analysis program will then automatically display the sample or the project to which the record you've selected belongs. To do this, your software will automatically switch to the appropriate database view.
2. Click the *Show Projects*  button to switch to the Project View of the database. You will find this button on the *Database* tool window's lower toolbar.
3. Click the *Show Documents*  button to switch to the Document View of the database. You will find this button on the *Database* tool window's lower toolbar.

Searching in the Search View

The *Database* tool window is divided into several areas. In each of these areas, a special database view is displayed. In the Search View you can select predefined search definitions with which you can carry out a database search.

In the step-by-step instructions that follow, it will be assumed that search definitions that have been saved already exist. Should this not be the case, you will find step-by-step instructions on how to create and save a search definition further below.

1. Click the *Show Search Results*  button to switch to the Search Results View of the database. You will find this button on the *Database* tool window's lower toolbar.
2. Make sure that the Search View is on display. That is the case when the *Show/Hide Search View*  button appears clicked. You will find this button on the *Database* tool window's lower toolbar.
3. Select the search definition you want from the list next to the *Search* button.
   - In the Search View, the search conditions will then appear.
   4. Make the necessary entries for the variable search conditions. These are, as a rule, different for every database search.
   - The way you have to make the entries can also vary. You may be offered a picklist, or a calendar field will appear in which you can select a date. Should you be searching for a note, you may in certain cases, have to enter the note you're looking for (resp. a part of it), manually.

5. Click the *Search* button.
   - The search will be carried out. As a result of the database search, all the records that fulfill the search conditions you've set will be displayed.
   - When a database search has been carried out, the number of records that have been found will be shown at the bottom left-hand side of the database window.
     The message "26 of 12000 records" means, for example, in the Project View, that the database contains a total of 12,000 projects, of which, 26 fulfill the search conditions.

Specifying the search conditions for a database search

1. Use the *Database > Define Search...* command.
   - The *Search Definition* dialog box will open.
2. Specify in the *Search for* group, which type of record you want to look for.
3. Define the first search condition in the first line of the *Conditions* group.
   Examples for possible search definitions are available in the online help.
   - As soon as you have completely defined the first search condition, an additional line will appear for an additional search condition.
4. If necessary, define an additional search condition in the second line. In this case you will have to specify the logical link between the two search conditions.
   To do this, select one of the logical operators AND or OR in the line's first field.
   - With the logical operator AND, both of the search conditions must be fulfilled simultaneously, in order for a record to be found with this database search.
     With the logical operator OR, only one of the two search conditions has to be fulfilled in order for a record to be found.
5. Save the search definition. To do so, click the *Save Search Definition*  button.
   - The *Save Search Definition* dialog box will open.
6. Give it a descriptive name, so that you and other users will be able to easily recognize the search definition.
7. Close the open dialog boxes with.
8. Switch to the Search View and try out the database search.

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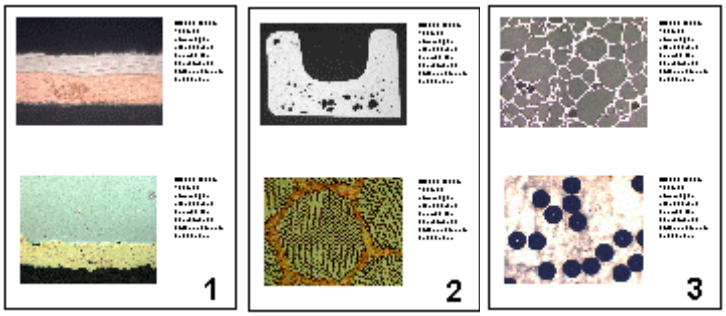
9. Working with reports

9.1. Overview - Report

The report functionality enables you to document the results of your work and to make them available to third parties. You can publish reports as a DOC-file or as a printout. When a corresponding printer driver is available on your system, you can also print reports to a PDF, and send them to other users.

The illustration shows a report example that is made up of three pages, and contains six images.



You have to switch to this layout when working with reports. In this layout, the *Report Composer* tool window, that is required when working with reports, is by default displayed.

The first step when working with reports is always opening or creating a new report instruction in your software. In the report instruction, you specify which images and which page layout are to be taken for the report. The display and any further editing of the created report are done in the MS-Word application program. Therefore, this program has to be installed on your PC when you work with reports.

Please note: Two programs are involved in the creation of reports:
Your software and the MS-Word application program.

Process flow when you create a report

1. Create or open a report instruction and fill it with the documents you want.
New report instruction

▼

2. Create a report.
Generate Report

▼

3. In Microsoft Word: Finish the report.
Working with the Olympus add-In

▼

4. Save the report instruction and the report.
Saving the report and the report instruction

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9.2. 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 will also find the *Create* button that is used to start the report creation.

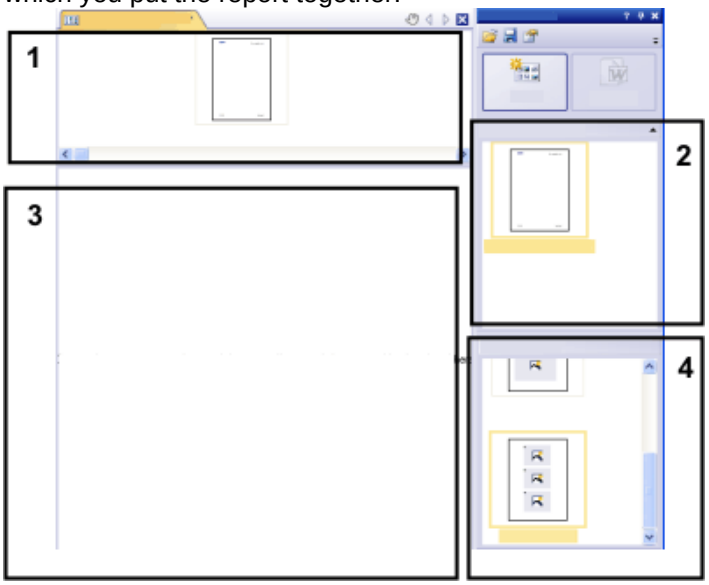
Should the *Report Composer* tool window be hidden, use the *View > Tool Windows > Report Composer* command to make it appear.

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.

The report instruction has to contain at least one registered page template. Further information on registering page templates is available in the online help.

1. Switch to the "Reporting" layout.
2. Click the *New Report Instruction*  button. You will 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 will 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. Normally, this is the "normal.DOT".
4. Drag the page templates you want onto the lower part of the report instruction (3). You will 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. Further information on page templates is available in the online help.

- 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 2003.
- The placeholder for a workbook can also be used for a MS-Excel file. In this case, you drag a MS-Excel file onto the report instruction, instead of dragging a "workbook" file onto it. 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, you will find the *Database*, *Gallery* and *File Explorer* tool windows arranged to the left to 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 either. 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. As MS-Word files have the *.DOC file extension, just as page templates have, they do not require a placeholder. If you want to integrate a MS-Word file into the report, just drag it onto the report instruction. In the report instruction, MS-Word files are shown with this icon:

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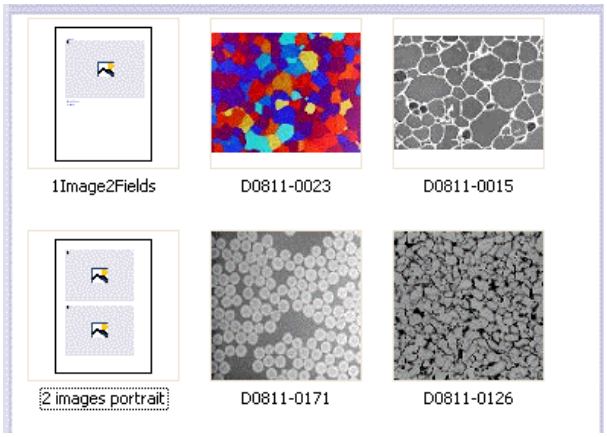
   - The documents must have been saved, because unsaved documents cannot be included in a report.

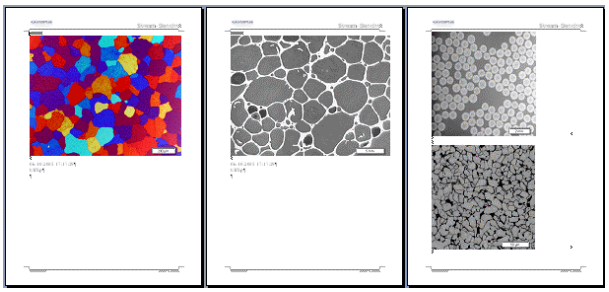


The illustration shows an example of a report instruction. In the report, two different page templates are to be used.
The first page template contains a single placeholder for an image, the second page template contains two placeholders for an image. After the page template, the images that are to be 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.

Generating a report

1. Click the *Create*  button. You will 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 selected in 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 Olympus add-in or the *Olympus* toolbar.
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 will not be contained in the newly created DOC-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. Normally, this is the "normal.DOT".

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.

- The standard MS-Windows conventions are valid for the Multiple Selection.

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*, *Gallery* and *File Explorer* 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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9.3. Working with the Olympus MS-Word add-in

During the installation of your software an add-in from Olympus is added to the MS-Word application program (via the global document template "OlympusWordReport.dot"). When you start MS-Word you will recognize this because the *Olympus* menu and the *Olympus* toolbar will be displayed.

This add-in will help you to carry out important tasks that are necessary when working with reports. It will support you with tasks that prepare the report creation as well as tasks that are for editing the already created reports.

Preparatory tasks

1. You define page templates that you want to use for your reports. For each page template you define placeholders for the document types you want.

During the installation of your software some predefined page templates have been installed, too. Please check first whether these predefined page templates are already sufficient for your needs before creating your own page templates.

2. In case you did not insert the placeholders in a chronological order: You adjust the insertion order of the placeholders, so that they are numbered consecutively, from the first placeholder to the last one.

Tasks for editing the already created reports in MS-Word

1. You add a document that is currently open in your software, to a report (or to any other DOC-file).
2. You add a field to your report, so that a certain information that is saved in your software, is also displayed in MS-Word. This makes sense, for example, when, exceptionally, you want to see the acquisition date of a certain image in the report.
3. You convert all placeholders in your report. This makes sense, for example, when you want to give the report to third party persons.
4. You update all placeholders in your report. This makes sense, for example, when you change the documents in your software after the report creation and when you now want to see them in the report.
5. You insert a MS-Word report into the database of your software This command is only available if your software supports the database functionality.

Should the Olympus-Word add-in not be on display on your PC, select the *Tools > Templates and Add-Ins* command in MS-Word and, on the *Templates* tab, check whether the "OlympusWordReport.dot" entry has been selected, in the *Global templates and add-Ins* group.

Inserting documents and fields from the software in any MS-Word document you want

With the commands from the *Olympus* Add-In you can also insert documents and fields from your software in any MS-Word document that hasn't been set up via the *Report Composer* tool window. In this case, you work mainly in the MS-Word application program and you switch to your software only when you want to open the documents that you want to insert into the MS-Word document.

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9.4. Creating and editing a page template

In a page template placeholders are set up for the documents that the report is to contain. There are placeholders for images, charts, workbooks and fields.

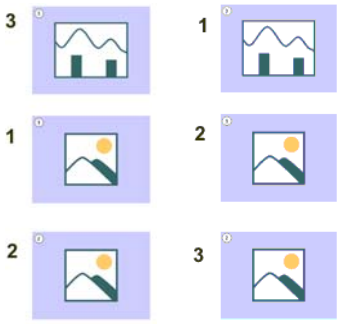
When, for instance, the report is to contain pages that have an image at the top, and below it, a chart, you should then set up a page template, which has a placeholder for an image and a placeholder for a chart.

Creating a page template and inserting placeholders

1. In the MS-Word application program, select the *File > New...* command.
2. Select the *Empty Document* option, if you don't want to use an existing page template as template, but instead want to start from scratch.
3. Select the *Olympus > Insert Placeholder* command. Decide whether you want to insert a placeholder for an image, a chart, or a workbook. Further information on workbooks is available in the online help.
   - The placeholder you've selected will be inserted.
4. If necessary, you can change the size of the placeholder. To do so, move your mouse over a handle, then with the left mouse button depressed, drag it in the required direction. The length/width ratio remains unchanged, so that the objects won't be distorted by this action.
5. Doubleclick a placeholder for an image, to change the default settings for its appearance.
   - The *Image properties* dialog box opens. Further information is available in the online help.
6. If required, insert additional placeholders for images, charts or workbooks.
   - Make sure that your page template isn't longer than a page. It's better to set up two page templates, each of one page, than it is to set up one page template that is made up of two pages.
7. 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 fields, resp. placeholders for fields in the online help.
8. Save your page templates in the DOC file format. You can choose any storage place you like. Don't close the file yet.
9. In the MS-Word application program, use the *File > Properties* command, and switch to the *Summary* tab.
10. Enter a descriptive document title in the *Title* field.
    - In your software, the page template's document title will be shown in the *Report Composer* tool window, and not the file name.
11. Select the *Save preview picture* check box that is located at the bottom of the *Properties* dialog box.
    - Only when this check box has been selected, can your software display a preview of the page template.
12. Close the *Properties* dialog box with *OK*, then save, and close the file.
13. Register the page template in your software. Further information is available in the online help.

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. When you have used the *Olympus > Adjust Insertion Order* command, the insertion order will be numbered serially from top to bottom (see the example on the right).



Inserting a placeholder for a field

1. In the page template, select the placeholder into which you want to insert a field.
2. Use the *Olympus > Insert Placeholder > Field...* menu command.
   The *Insert Field* dialog box will open.
3. Click the *Use Selected Placeholder* button.
   - In the *Placeholder* list, the name of the placeholder into which you want to insert a field will appear.
4. 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.
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 blue bracket, and by the field name shown.
6. If required, move the field to another position in the MS-Word file. By default, field values are shown to the left of, or above, the selected placeholder.
7. If necessary, add placeholders for further fields.
8. Save the page template.

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9.5. Editing a report

Preliminary considerations

When you have created a report and want to make some changes in it, 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.

Changes that you make in a report, are only valid for that particular report. There is also no possible way in which changes that are made in a report can be automatically adopted in the report instruction.

However, there are some cases when it makes sense to make a change only in the report, for example when you've created a very large report with a large number of documents, and only want to change the order of two images in it. If you didn't use a report instruction, you can only edit the report anyway.

Updating placeholders

As long as the placeholders have not been converted, the documents that are shown in the MS-Word report are linked to the images stored in your image analysis software. In MS-Word, use the *Olympus > Update Placeholders* command, to update the report's linked documents and field values. You can update all placeholders or only the currently selected placeholder.

The *Update Placeholders* command makes it easy to have any changes made to the images after the report has been created also shown in the report. Please take note that all changes made in your software have to be saved, if they are to be displayed when the *Update Placeholders* command is used.

Example:
In MS-Word, 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. To do so, you select the *Olympus > Update Placeholders* command and, in the *Update Placeholders* dialog box, you set that all placeholders are to be updated. The edited images will be shown in the report now.

Changing the image display

When images are transferred to a MS-Word report, the image link is transferred as well. This makes it possible to change the image display in a MS-Word report (e.g., to scroll the image segment). This is no longer possible with converted images.

Doubleclick the placeholder for the image, to open the *Image Properties* dialog box. Further information on this dialog box is available in the online help.

Adjusting documents

In the MS-Word report, you can select a document from the "image" or "chart" type and select the *Olympus > Adjust Document* command. You will then change over to the image analysis software, where you can edit the document and then automatically change back to the report. This is no longer possible with converted placeholders.

Basically, the *Adjust Document* command is similar to the *Update Placeholders* command. The difference is that the *Adjust Document* command is meant for users who mainly use MS-Word for creating reports and who only change over to the image analysis software in order to make certain changes to some documents.

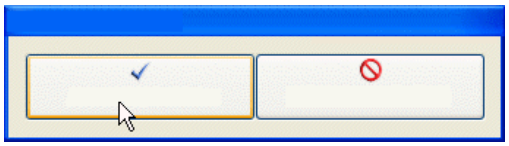
Example:
In the MS-Word application program, you edit a report that contains a lot of images. With a certain image you notice that an important measurement is missing. Using the *Adjust Document* command, you change over to the image analysis software, add the missing measurement and then change back to MS-Word in order to continue editing the report.

Adjusting an image

1. Open the image in MS-Word and select the image that you want to adjust.
2. Select the *Olympus > Adjust Document* menu command.
   - 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.

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 will find this button in the *Adjust Document* message box that is shown in the foreground.



   - The MS-Word application program 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 selected the *Adjust Document* command, it will be closed again. If any images or databases had to be opened for this command, they will be closed as well.

Converting a placeholder

You can convert images (and other documents) in a MS-Word report. When you do this, a new MS-Word document is created. In this document, the link to the saved images does no longer exist. These images cannot be updated any longer. You recognize a converted report by the fact that the *Format Picture* dialog box opens when you doubleclick on an image (and no longer the *Image properties* dialog box that opens when you doubleclick on a linked image).

The conversion reduces the file size of the DOC file significantly. A conversion makes sense when you wish to send the DOC data to a third person who doesn't have access to your image analysis software.

Should a corresponding printer driver be available on your PC, you can alternatively print a report as a PDF file, then send this file to a third person. The PDF file is in any case even smaller in size than the DOC file with converted images.

Converting a placeholder in a report

1. In MS-Word, open or create the report in which you want to convert the placeholders.
   - If you created a new report or if you changed an existing one: Save the report. Only saved reports can be converted.
2. Select the *Olympus > Convert Placeholders* menu command.
3. In the *Convert Placeholders* message box, click the *Convert* button.
   - The report with the converted images will be displayed as a new MS-Word document. You can now save it under a new name and give it to other persons.
   - The report with the linked placeholders remains opened.

Editing a workbook in the report

Your software supports the handling of workbooks. To create a workbook, open, for example, the *Measurement and ROI* tool window and export a results sheet. Further information on workbooks is available in the online help.

Please note: 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 2003.

Apart from the "image" and "chart" document type, reports can also contain workbooks. A workbook is imported as an Excel object in MS-Word. You can further edit it in the report.

1. In the report, doubleclick 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, as well as that, you can see all of the workbook's worksheets.
2. If need be, select the worksheet that you want to edit.
3. Doubleclick the workbook in order to switch to edit mode. 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 in the MS-Word report, outside the workbook.

Inserting a document

In a report (or in any other MS-Word document), you can insert a document at any position. When you have, for example, created a report, and while you are viewing it, notice that you've forgotten an image, you can retroactively insert it into the report.

1. Use the *Olympus > Insert Document...* command.
   - The *Insert Document* dialog box will open.
2. 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 will be hidden.
   - Select the *File Explorer* entry if you want to insert a document that is stored on your PC or in your network.
3. Select the required document in the document preview. Click the *Insert* button.
   - The required document will be inserted into the MS-Word report.
   - The *Insert Document* dialog box remains open.
4. 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 *Olympus > Update Placeholders* command (in case the documents were changed after they have been inserted into the report).

Inserting a field

You can insert a field into a MS-Word report that describes the image in more detail. All of the values that have been saved in your software for this document can be displayed in this field.

1. Select the image in the report to which you want to insert a field.
2. Use the *Olympus > Insert Field...* menu command.
   - The *Insert Field* dialog box will open.
3. Click the *Use Selected Placeholder* button.
   - In the *Placeholder* list, the name of the placeholder into which you want to insert a field will appear.
4. 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 document-specific fields, just as they are shown in your software's *Properties* tool window.
     The *Database fields* list contains all of the fields that are available in the database for the selected image. For this purpose, a database must have been opened.

5. Check at the bottom of the dialog box, whether a field value is displayed in the *Field value* group. Should the *Field value* group remain empty, this means that the field hasn't been filled out in your software. In this case, select another field that has been filled out, instead.
6. Click the *Insert* button.
   - The field contents will be displayed in the MS-Word document.
7. If required, move the field to another position in the MS-Word file. By default, field values are shown to the left of, or above, the selected placeholder.
8. If necessary, add further fields.
9. Close the *Insert Field* dialog box.
10. Save the MS-Word report.

Should you want to have the contents of a specific field regularly shown in your reports, you can already insert this field, (resp. a placeholder for this field) into the page template. Then this field will be automatically filled out in every report.

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