

# **User Manual**

# Basler Microscopy Software

## **Contents**

1.	About yo	ur software	3	
2.	Working	with the Basler Microscopy Software	4	
	2.1. Overvie	w - User interface	4	
	2.2. Working	g with documents	5	
3.	Acquiring	g images	7	
	3.1. Acquiring a single image			
	3.2. Camera Control			
	3.2.1. 3.2.2. 3.2.3. 3.2.4. 3.2.5.	Maximizing the live-image to screen  Automatic exposure time  Manual exposure time  Setting the camera resolution  Toggle Subarray	14 16 17	
	3.3. Acquisit	20		
	3.3.1. 3.3.2. 3.3.3. 3.3.4. 3.3.5. 3.3.6. 3.3.7. 3.3.8.	Acquisition Settings - Acquisition - General Acquisition Settings - Document Name Acquisition Settings - Saving Acquisition Settings - Camera - General Acquisition Settings - Camera - Adjustment Acquisition Settings - Camera - Color Acquisition Settings - Camera - Exposure Acquisition Settings - Camera - Information		
4.	Working with images			
	4.1. Changing the way an image is displayed			
	4.2. Processing images			
	4.3. Perform	ning a white balance on an image	33	
	4.4. Working with drawing objects			
5.	Measuring images			
	5.1. Overview			
	5.2. Measuring images			
6.	Configuri	ing the system	43	
	6.1. Overvie	w	43	
	6.2. Configu	ring the system	44	
	6.3 Manual Magnification Calibration			

# About your software

### **Acquiring images**

Note: Different camera and acquisition settings are available depending on the camera you are using.

Your system enables you to acquire high resolution images of a sample in just a few steps. Your system is comprised of your software, a Basler camera, and your microscope. With the image acquisition process, data is read out from the camera that is attached to the microscope and is displayed on the computer's monitor.

You can first observe the live-image and make good settings for it. The live-image is constantly refreshed. This means that if you move the stage, the live-image changes correspondingly. You can switch the live-image on and off and acquire an image of the parts of the sample that interest you. When you do this, you create a digital image that you can save and process or measure with a variety of your software's functions.

### Measuring images

You can make various measurements on images. You can measure the length of a line for example. The measurement objects are displayed in the image's drawing layer. All of the image information is therefore retained. The measurement results are shown in a table and are saved together with the image.

### **Processing images**

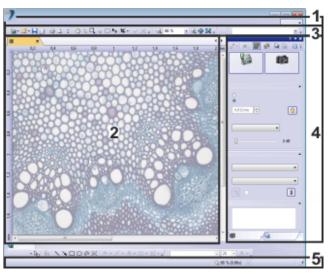
You can process the images you acquired and optimize the image quality for your purposes. Sharpen filters and contrast optimization functions are available for this.

# 2. Working with the Basler Microscopy Software

### 2.1. Overview - User interface

The graphical user interface determines your software's appearance. It determines which menus are shown, how the individual functions are called up, how and where images are displayed, and much more. In the following, the basic elements of the user interface are described.

Appearance of the user interface



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

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

(1) Menu bar

You can call up many commands by using the corresponding menu. You can find a variety of commands in the *View* menu with which you can change the appearance of the user interface.

(2) Document group

The document group contains all of the loaded images.

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, there are many functions which are only accessible via a toolbar, the drawing functions required for annotating an image for example.

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

(5) Status bar

The status bar contains information, a brief description of each function for example. Position the mouse pointer on the name of a command or on a button to display a brief description of a function.

# 2.2. Working with documents

There are a number of ways to open, activate, save, or close images.

### Saving images

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

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

- 1. To save an individual image, activate it in the document group. Then use the *File > Save As...* command, or the [Ctrl + S] keyboard shortcut.
- 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.
- 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.

Automatic save

- 1. When you exit your software, all of the images that have not yet been saved will be listed in the *Unsaved Documents* dialog box. This gives you the chance to decide which images you still want to save.
- 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 images

There are a number of ways in which you can close images in your software.

- Use the *Documents* tool window. Select the desired images and use the *Close* command in the context menu. The standard MS-Windows conventions for multiple selection apply for the selection of images.
- 2. To close a single image, activate the image in the document group and use the *File* > *Close* command. Alternatively, you can click the button with the cross [x]. You can find this button at the top right of the image's tab's header next to the name of the image.
- 3. Use the *Gallery* tool window. Select the desired images and use the *Close* command in the context menu. The standard MS-Windows conventions for multiple selection apply for the selection of images.

Closing all images

To close all of the images that are loaded, use the *Close All* command or the [Ctrl + Alt + W] keyboard shortcut. You can find this command in the *File* menu, and in both the *Documents* and the *Gallery* tool windows' context menu.

Closing an image immediately To close an image immediately without being prompted to save it, press the [Shift] key while closing it. Data you have not saved will be lost.

### **Opening images**

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

- 1. Use the File > Open... command.
- 2. Drag the images you want directly out of the MS-Windows Explorer into your software's document group.

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

Generating a test image

If you want to get used to your software, then sometimes any image suffices to try out a function.

Press [Ctrl + Shift + Alt + T] to generate a color test image.

With the [Ctrl + Alt + T] shortcut, you can generate a test image that is made up of 256 gray values.

### Activating images in the document group

There are several ways to activate one of the images in the document group and display it on your monitor.

- 1. Use the *Documents* tool window. Click the desired image there.
- 2. Use the *Gallery* tool window. Click the desired image there.
- 3. Click the title of the desired image in the document group.
- 4. Click the small arrow at the top right of the document group to open a list of all of the loaded images. Left click the image that you want to have displayed on your monitor.

Note: You can choose whether you want to activate the images in the *Documents* tool window and in the *Gallery* with a single or a double mouse click. Use the *Tools > Options...* command. Select the *Environment > General* entry in the tree view.

Select the *Activate documents with single click* check box. Then it's sufficient to click once with the left mouse button to have the image you have chosen displayed in the document group.

Clear the check box. Now you activate the image with a double click.

### Attaching images to an e-mail

- 1. Load the images you want to attach to your e-mail.
- 2. Use the File > Send E-mail... command.
- 3. Check whether all images you want to attach are selected.
- Click the Send button to generate an e-mail with the selected images included as attachments.
  - You will receive a warning message if the sum of file sizes of all images exceeds the maximum permitted size.
  - A new e-mail form will be opened by your e-mail program. Your e-mail program does not have to be already running for this to happen.
     The e-mail contains all of the selected image files as attachments.
- Enter the recipient's address and your message and then send off your e-mail.

# 3. Acquiring images

# 3.1. Acquiring a single image

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

- 1. Use the *View > Layout > Reset Current Layout* command.
  - You can find the *Microscope Control* (1) toolbar at the upper edge of the user interface, right below the menu bar.

    To the right of the document group, you can find the *Camera Control* (2) tool window.



Setting the magnification

Switching on the liveimage



- 2. On the *Microscope Control* toolbar, click the button with the objective that you use for the image acquisition.
- 3. In the Camera Control tool window, click the Live button.
  - The live-image (3) will now be shown in the document group. A new image document will automatically be created for the live-image.
- 4. Go to the position on the sample you are interested in.

### **Preferences in the Acquisition Settings**

Use the acquisition settings to make default settings for the image acquisition.

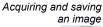


- 5. Open the *Acquisition Settings* dialog box. To do so, click the *Acquisition Settings* button in the *Camera Control* tool window's toolbar.
- Select the Camera > General entry in the tree view.
   From the Color temperature list, select a predefined color setting to acquire the sample in particular light conditions. Select the color temperature you want.
- Carry out a Gamma correction.
   Select the Camera > Adjustment entry in the tree view.
   Move the Gamma slide control to increase the image contrast either in bright image areas or only in dark image areas.
- 8. Close the Acquisition Settings dialog box with OK.

### **Settings in the Camera Control**

Use the functions of the Camera Control tool window to set the image quality.

- 9. Bring the sample into focus. The *Focus Indicator* toolbar is there for you to use when you are focusing on your sample.
- 10. Check the color reproduction. If necessary, carry out a white balance.
- 11. Check the exposure time. You can either use the automatic exposure time function, or enter the exposure time manually.
- 12. Select the resolution you want.
- 13. In the *Camera Control* tool window, click the *Snap* button.
  - The acquired image will be shown in the document group.
- 14. Use the *File* > *Save As...* command to save the image. Use the TIF file format.





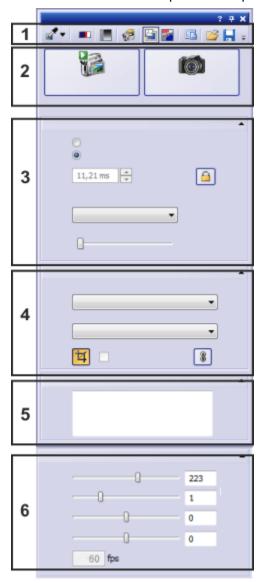
## 3.2. Camera Control

Use the *Camera Control* tool window to acquire images. This tool window is displayed on the right side of the user interface by default. Should this tool window not be visible, use the *View > Tool Windows > Camera Control* command to show it.

Note: Some of the settings in the *Camera Control* tool window are camera specific (e.g., the available resolutions). For this reason, your camera's functionality can differ from what is described here.

Structure of the tool window

The tool window is made up of several parts.



- (1) Toolbar of the tool window
- (2) Acquiring images
- (3) Adjusting the exposure time
- (4) Setting the resolution
- (5) Entering an image comment
- (6) Changing the camera settings

### (1) Toolbar of the tool window

In the *Camera Control* tool window, you can find the following buttons. You can find these buttons in a toolbar, at the top of the tool window.



White Balance

In live mode, click the small arrow next to the button to open a list of available white balance functions and then select the function you want. The active function is indicated by the icon on the button. The selected function is also identified with a check mark in the menu.

#### Prerequisite: Your camera is operating in RGB mode.

	One Touch White Balance	Select the <i>One Touch White Balance</i> command to perform a white balance on the active live image. The color settings that were determined are used until you click the <i>One Touch White Balance</i> button again.
	Automatic White Balance	The color settings are continually checked and adjusted in this mode.
ď	White Balance on ROI	Use the White Balance on ROI command to define a particular image segment that you want to use for the white balance.

Toggle RGB/Grayscale mode

Some cameras can also be used to acquire gray-value images.



The *Toggle RGB/Grayscale mode* button looks like this when your camera is in RGB mode and you are acquiring true-color images. Release this button to acquire gray-value images with your camera.



The *Toggle RGB/Grayscale mode* button looks like this when your camera is in grayscale mode and you are acquiring gray-value images. Click the button to acquire color images with your camera.

Toggle Saturation Indicator Prerequisite: Your camera is operating in grayscale mode.



The saturation indicator tells you whether an image (or a part of an image) is underexposed or overexposed. When the saturation indicator is switched on, all of the pixels whose intensity value is too low are shown in blue and all pixels whose intensity value is too high are shown in red in the live-image. If too many blue pixels can be seen, the image is underexposed. If too many red pixels can be seen, the image is overexposed.

The color table is only applied to the live-image. The image you acquire will be a normal gray-value image.

### (2) Acquiring images

Acquiring images





Click the *Live* button to switch your camera to the live mode. The live-image will then be displayed in the image window. The button will change its appearance. This enables you to immediately recognize that you are in the live mode.

When you are in the live mode, click the *Live* button once more to end this mode without acquiring an image. You can change this behavior. To do so, use the *Acquisition Settings > Acquisition > General* dialog box.



Click the *Snapshot* button to acquire a snapshot. The live mode will then, as a rule, be exited. The acquired image will be shown in the document group.

Image acquisition - several wavs

You have several ways of switching to the live mode to acquire snapshots:

- Use the Camera Control tool window.
- Use either the *Acquire > Live* or the *Acquire > Snap* menu commands.
- Use the [F7] key to switch to the live mode. Use the [F8] key to acquire an image.

### (3) Adjusting the exposure time

Use the *Exposure* group, to set the exposure time.

The functions in the exposure time control depend on whether you choose to use the automatic exposure time option or to set the exposure time manually. The *Automatic* option is always set by default.



- (1) Automatic exposure time
- (2) Manual exposure time

Exposure time in the image properties The exposure time is adopted in the *Properties* tool window after each image acquisition. You can find this information in the *Camera* group. This information will be retained if the image is saved in the TIF file format.

### (4) Setting the resolution

Use the *Resolution* group to set the camera resolution.

Note: Different resolutions are available, depending on the camera being used.

You can set different resolutions for the live-image and the acquired image. Select the resolution you want from the *Live* list or the *Snap* list (1).

Another way of reducing your camera's resolution, is to reduce the size of the image area (2) by using a subarray.



- (1) Setting the camera resolution
- (2) Toggle Subarray

### (5) Entering an image comment

In the *Camera Control* tool window, you can enter text in the *Note* field. This text will then be saved along with the image as a comment for every image acquisition.

Note: The text in the *Note* field isn't saved when you save the current camera settings. The text in the *Note* field is deleted when you close your software.

Viewing a comment

After the image has been acquired, you can view and edit the image comment in the *Properties* tool window's *Document > Note* field. To do so, load the image, and use, e.g., the *View > Tool Windows > Properties* command to make the tool window appear.

Displaying the comment in the image window

You can have the image comment displayed in the image window, together with the info stamp.

Deleting or editing comments for individual images

- 1. Go to the *Documents* group, located in the *Properties* tool window.
  - You will see that the comment you entered in the Camera Control tool window has been adopted in the Note field.
- 2. Click in the Note field.
  - When the Note field is active, a small button with three dots will appear.
- Click this button.
- 4. Delete or change the comment in the *Note* dialog box.

### (6) Changing the camera settings

Use the *Camera Settings* group to change brightness, contrast and the color settings.

#### Brightness and Contrast

Use the *Brightness* slide control, if the whole image is too bright or too dark. Values smaller than 0 lower the image brightness. Values greater than 0 increase the image brightness.

Move the *Contrast* slide control to change the contrast values of the image. Values smaller than 1 lower the image contrast. Values greater than 1 enhance the image contrast.

When changing the image contrast the brightest part of the image always remains the same.

After changing the image contrast it may be that dark or bright image areas are not displayed optimally anymore. In this case use the *Brightness* slide control to make the whole image brighter or darker.

You can also change the *Contrast* and *Brightness* values in the *Acquisition Settings* > *Camera* > *Adjustment* dialog box.

The values are automatically saved together with the image. You can view the values in the *Properties* tool window.

Hue and Saturation

Increase the *Saturation* value when there is a preponderance of one color in your images. In this way you will attain a better reproduction of the other colors in your image.

To change the parameter, move the slide control. A change made in the Saturation will have an effect on all of the color channels.

Use the *Hue* value to alienate the color impression of the acquired images. Certain structures in the image can thus be better recognized, for example. A value of 180 means that the color in the image corresponds to the color of the sample.

You can also change the *Saturation* and *Hue* values in the *Acquisition Settings* > *Camera* > *Color* dialog box.

The values are automatically saved together with the image. You can view the values in the *Properties* tool window.

Live frame rate

The *Live frame rate* field shows the current frame rate (images per seconds) in the live mode.

In the live mode, a huge amount of data is transferred from the camera to your PC. It may occur that the USB connection to your system is not sufficiently high to support the full data rate. This may lead to certain errors in the live-image, for example, stuttering or color distortion.

In this case, use the *Data rate* slider to reduce the amount of data that is transferred. You can find this slider in the *Acquisition Settings > Camera > Adjustment* dialog box.

### 3.2.1. Maximizing the live-image to screen

To see only the live image on your monitor, click the *Maximize to Screen* button. The user interface will be completely faded out. You can find this button in the *Camera Control* tool window.

Size of the live-image

The live-image's zoom factor will not be changed when you switch to the Maximize mode. If you want to see the whole of the live-image in Maximize mode, click the *Fit to Window* button before you switch to the Maximize mode.

Switching off the Maximize mode To switch off the mode and return to the normal user interface, use the [Esc] key or click the *Exit Maximized Screen* button.

Setting the image background

In the Maximize mode, the live-image does not necessarily take up all of your monitor's screen. You can set a different color for the background. When you are working with a fluorescence acquisition, for example, the diffused light can cause a disturbance when you are looking at an image with a gray background. In this case you can set a black background.

To do this, use the *Tools > Options...* command. Select the *Images > General* entry in the tree view. Select the background you want from the *Background* list.

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### 3.2.2. Automatic exposure time

Some of the exposure time correction settings depend on the model of camera that you are using. For this reason, the *Exposure* group might have a somewhat different appearance for your camera.

Switching on the automatic exposure time

The functions for setting the exposure time can be found in the *Camera Control* tool window.

Select the *Automatic* option located in the *Exposure* group to choose an automatic exposure time.

In the automatic exposure time mode, your system will continuously calculate the optimum exposure time for the live-image. The snapshot acquisition always uses the last exposure time that was calculated in the automatic exposure time mode.

Functions in the "Exposure" group

The *Exposure* group in the *Camera Control* tool window offers options for effecting the functioning of the automatic exposure time mode.



- (1) Displaying the exposure time
- (2) Locking the current exposure time
- (3) Setting the region
- (4) Setting the gain

### (1) Displaying the exposure time

The current exposure time is displayed in the *Camera Control* tool window and on the *Camera Control* toolbar.

The current exposure time is also shown in the function group's header in the *Camera Control* tool window. This ensures that the exposure time is always displayed, even if you minimize the function group and only the header remains visible.

### (2) Locking the current exposure time

Click the Lock Current Exposure Time button to have the currently calculated exposure time used for the complete duration of the live-acquisition. In this way you will switch off the automatic adjustment for the duration of the acquisition. That means that even when you move to a completely different place on your sample, the exposure time will not be adjusted accordingly. The Lock Current Exposure Time button remains active (also for later live-images) until you switch it off again.

### (3) Setting the region

The exposure time is always calculated on the basis of the histogram, that is, the intensity distribution in the image. From the *Region* list, select the image segment that is to be used for the calculation of the histogram and thus of the automatic exposure time.

Full Image

Select the *Full Image* option so that the histogram of the entire image goes into the calculation of the exposure time. This method is recommended if the structures to be observed are equally dispersed throughout the entire image.

Spot

Select the *Spot* option to have the histogram of only a rectangular image segment calculated. The sizes shown in the *Region* list depend on the camera model being used. As soon as you switch to the live-image you will see a white dashed rectangle that identifies the relevant segment of the image. With some cameras you can move the rectangle to any position in the image by dragging it there with your left mouse button pressed, and in this way you will then influence the exposure time. You can use this method if relevant details are only visible in a part of the image. The appearance can thus be optimized for precisely this segment of the image.

You can also change the image segment you've selected, however you want to, at a later date.

### (4) Setting the gain

Use the *Gain* slide control when you want to decrease the exposure time without making the image darker. The more you increase the gain, the shorter the exposure time becomes. This increases the image noise, however. So, only use the *Gain* slide control if it's especially important to have a short exposure time.

### 3.2.3. Manual exposure time

Switching on the manual exposure

The functions for setting the exposure time can be found in the *Camera Control* tool window.

Select the *Manual* option. Then you can freely set the exposure time.

Functions in the "Exposure" group



- (1) Setting and displaying the exposure time
- (2) Setting the gain

### (1) Setting and displaying the exposure time

Adjusting the exposure time

There are several different ways of manually setting the exposure time.

- To do this, move the slide controls.
- Click the [-] and [+] buttons to alter the exposure time in small steps.
- Enter an exposure time in the edit field, then press the [Enter] key. Or change the exposure time by using the arrow buttons next to the edit field.

Displaying the exposure time

The current exposure time is displayed in the *Camera Control* tool window and on the *Camera Control* toolbar.

The current exposure time is also shown in the function group's header in the *Camera Control* tool window. This ensures that the exposure time is always displayed, even if you minimize the function group and only the header remains visible.

### (2) Setting the gain

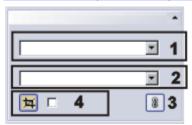
Use the *Gain* slide control when you want to increase the sensitivity without changing the microscope's exposure time or illumination intensity. The more you increase the gain, the brighter the image becomes. It makes sense to use this setting when the fluorescence is weak, for example.

### 3.2.4. Setting the camera resolution

In the *Camera Control* tool window, you can use the *Resolution* group to set the camera's resolution. Should this group be hidden, just double click on the *Resolution* area.

Note: Most cameras can acquire images in various resolutions. Which resolutions are possible, depends on the camera being used.

Functions in the "Resolution" group



- (1) Setting the resolution for a live image
- (2) Setting the resolution for a snapshot
- (3) Using the same resolution for live and snapshot
- (4) Toggle Subarray

### (1) Setting the resolution for a live image

Select the value you want for the live-image's resolution, in the *Live* list. A resolution of 1920x1080 means, for example, that the image you acquire will be made up of 1920x1080=2073600 pixels.

With most cameras you can reduce the resolution in the live-image. The method will be specified in brackets behind the resolution. The smaller the resolution you select is, the quicker the live-image will react. Inversely, the spatial resolution in the image will improve with the increase in resolution.

Binnina

With binning, pixels on the camera will be combined when they are read out. Then less data will be transferred from the camera into the software, and the live-image will be updated more quickly. At the same time, the noise in the image will decrease.

When you select a resolution using the binning mode the term 'binning' will be added in brackets after the resolution.

Toggle Subarray

When you reduce the size of the image area the speed in the live-image will be increased, just as it is with binning, and you reduce the size of the file. In contrast to binning, however, when you reduce the image area, the image segment displayed in the live-image will be displayed in the same spacial resolution, that's to say, without loss of quality.

You can find the functions for reducing the image area, in the *Resolution* group.

### (2) Setting the resolution for a snapshot

Select the value you want for the snapshot's resolution, in the *Snapshot* list.

If you acquire images with a lower resolution, it will provide you with files that are smaller. When you use a camera resolution of 1920x1080 an image with the highest resolution will need approx. 6000 KB of storage capacity, and the same image with the lowest resolution around 500 KB.

After the image has been saved, its size will be shown in the *Size on Disk* field in the *Documents* group in the *Properties* tool window.

Display of the resolution

The current resolution for the image acquisition is also shown in the function group's header in the *Camera Control* tool window. This ensures that the resolution is always displayed, even if you minimize the function group and only the header remains visible.

### (3) Using the same resolution for live and snapshot



To use the same resolution for the live-image and the snapshot acquisition, click the button with the chain icon. Then you only need to set another resolution in one place.

From that moment on, the *Live* list will be grayed out. Then you can't set anything there. The value shown will, however, be updated if you set another value in the *Snapshot* field.

### (4) Toggle Subarray

Trim the image that your camera acquires.

### When does which resolution make sense?

High resolution

In the highest resolution even sample structures that are very near to each other are still shown separately in an image. This image contains the most information on the sample. The disadvantage is, that a high resolution reduces the speed at which the live-image is acquired.

Low resolution for liveimage If you want to increase the live-image's reaction speed, it can make sense to set a lower resolution. Then less data will be transferred from the camera into the software, and the live-image will be updated more quickly. Admittedly, a reduction of the resolution will also always result in less image information being acquired.

Different resolution for live-image and snapshot

Note: You can set a different (lower) resolution for the live-image than that which you use for the snapshot. That makes sense, for example, when you wish to have a live-image that reacts quickly, but do not want to accept a loss of image quality when you acquire a snapshot.

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### 3.2.5. Toggle Subarray

The *Toggle Subarray* button is located in the *Camera Control* tool window. You can find the button in the *Resolution* group. With it you trim the image that your camera acquires.

### Why use the "Toggle Subarray" mode?

You can use the *Toggle Subarray* mode for a variety of purposes.

Quick live-image

With cameras that support this function, the *Toggle Subarray* mode will lead to a quicker live-image acquisition. You define a rectangular image segment and the readout for the live acquisition limits itself to this area. A higher frame rate is attained because less data needs to be transferred. Please note that you have to select the *Apply to Live* check box before you can use the *Toggle Subarray* mode on the live-image.

Toggle Subarray -Binning Like with the Toggle Subarray mode, binning (decreasing the resolution) increases the speed of the live-image.

As opposed to binning, with the *Toggle Subarray* mode you will in fact see only a small sample segment, this sample segment can, however, be displayed with the greatest possible camera resolution. Use the *Toggle Subarray* mode when you need an active-image that reacts quickly for focusing, and you only want to reproduce very small sample structures.

The binning functions can also be found in the Resolution group.

You can also combine binning with the *Toggle Subarray* mode. In this way you will increase the speed in the live-image still further, and also reduce the size of your files even more.

Trimming the image

Sometimes it can be useful to trim an image you have acquired. By using the *Toggle Subarray* mode, you can already trim uninteresting borders from your images when you acquire them, and in this way considerably reduce the size of your files.

Should you not have set an area for the Toggle Subarray mode before you acquired an image, but are only interested in a part of this image, you can trim it at any time by using the *Image* > *Crop* command.

### **Description of the function**

Switching "Toggle Subarray" mode on or



Switch to the live mode. Click the *Toggle Subarray* button to switch on the *Toggle Subarray* mode. The button will retain its clicked appearance. You can then immediately see whether the *Toggle Subarray* mode is switched on or off.

Note: You can only switch the *Toggle Subarray* mode on and off in the live-image.

Using the "Toggle Subarray" mode for the live-image too The *Toggle Subarray* mode will, in every case, have an effect on the snapshot acquisition. Should you also want to use the *Toggle Subarray* mode with the live-image, select the *Apply to live* check box.

Specifying the image segment for the "Toggle Subarray" mode When the *Toggle Subarray* mode is switched on, a blue frame in the image window will show the active image segment.

You can at any time change the image segment that has been selected in the live-image with the help of your mouse.

Note: The image segment for the *Toggle Subarray* mode can only be changed when the *Apply to live* check box has **not** been selected. This is because, when the *Toggle Subarray* mode has been activated, only a part of the image will be on display, which means it would only be possible to define a new image segment within the segment on display, but not outside this segment.

# Specifying the image segment for the "Toggle Subarray" mode

- 1. Make the *Camera Control* tool window appear. To do this, you can use the *View > Tool Windows > Camera Control* command.
- 2. Switch to the live mode. To do this, click the *Live* button.

Subarray button.



3. If necessary, clear the *Apply to Live* check box in the *Resolution* group.



- Switch on the *Toggle Subarray* mode. To do so, click the *Toggle*
  - In the image window a blue frame will then identify the active image segment for the *Toggle Subarray* mode. If you can't see the frame, click the *Toggle Subarray* button once more.
- 5. To change its position, with the left mouse button depressed, take the frame and drag it into the position you want. Position the image segment for the *Toggle Subarray* method over an important image detail. To change its size, move your mouse pointer over one of its handles. By dragging the marker with the mouse button depressed, you can adjust the frame's size as wished.



- 6. Click the *Snap* button to stop the live mode and thus acquire a new image.
  - The acquired image will now only show the image segment you have chosen.

## 3.3. Acquisition Settings

Use the *Acquisition Settings* dialog box to change a variety of settings for the image acquisition.

Opening the dialog box

To open this dialog box, click the *Acquisition Settings* button. You can find this button on the *Camera Control* toolbar and on the *Camera Control* tool window's toolbar.

The dialog box's tree view offers you access to dialog boxes with a variety of setting options. Select an entry in the tree view to display the respective dialog box.

Note: The exact functionality in the camera settings depends on the camera used. For this reason, the functionality in your software may differ from that described here.

3987 20112015

### 3.3.1. Acquisition Settings - Acquisition - General

This dialog box offers you several general options for working with live images.

### Live

The live-image will be allocated its own window in the document group. This window's title will be *Live* (active). The behavior of this live window depends on the settings in the *Live* group.

Closing the live window when stopping the liveimage Select the *Close document when Live is stopped* option to have the live window closed every time you exit the live mode.



When you exit the live mode by clicking the *Live* button, in this case, no image will be acquired.



To acquire an image, exit the live mode by clicking the *Snap* button.

Keeping the live window when stopping the live-image



Select the *Keep document when Live is stopped* option to keep the live-image when you exit the live mode.

When you exit the live mode by clicking the *Snap* button, the live-image will be stopped and additionally an image acquired. You will find the stopped live-image in the image window with the title *Live* (*stopped*). You can save the stopped live-image just as you can every other image.

Note: A stopped live-image is not the same image as the acquired snapshot. This means that you can set different resolutions for the live-image and the snapshot acquisition. In that case, a stopped live-image and an acquired individual image each have different resolutions.



When you exit the live mode by clicking the *Live* button, the behavior in this mode depends on the status of the *Create new document when Live is started* check box.

Creating a new document when the live mode is started

The Create new document when Live is started check box is only relevant when you've selected the Keep document when Live is stopped option.

Clear the *Create new document when Live is started* check box to have only one single live window.

When you exit the live mode by clicking the *Live* button, you will find the stopped live-image in the *Live* (*stopped*) live window. The next time you start the live mode, the contents of the live window will be overwritten.

Select the *Create new document when Live is started* check box, if you want to have a new window opened every time you start the live mode. In this way you'll

prevent the overwriting of the last live window's contents.

If you finish the live mode now, the stopped live-image is kept in all cases. A new image document will be created for the stopped live-image.

You can use this setting (*Keep document when Live is stopped* together with *Create new document when live is started*) when you want to make a measurement in the live-image. When you have finished making your measurements, stop the live-image. You can then save the image you have measured along with the measurements. When you start the next live-image, you can perform a new measurement.

Continuing the live mode after a snapshot





Select the *Continue live after acquisition* check box to only make a pause in the live mode while you acquire a snapshot. A snapshot acquisition will then create a new image window, but the window for the live-image will remain active and will immediately switch back into the live mode.

To exit the live mode, click the *Live* button, located in the *Camera Control* tool window.

### Calibration

Choosing the basic unit

You can set the basic unit for the X/Y-calibration that is to be used for the image acquisition. To do so, select the unit you want to use from the *Basic Unit* list. As basic units *Meters* [m] and *Inches* [in] are available.

When you select another basic unit, all of the images that you from that moment on acquire will be automatically calibrated in this new basic unit. Now, all values that apply to the X/Y-calibration will be specified in this new basic unit. These could be:

- the labeling of the scale bar
- the calibration data in the Properties tool window
- the measurement results when you make measurements on an image
- the label for the ruler that you can shown in the image window. Use the *Options > Images > View* dialog box to show or hide rulers. The ruler's label doesn't change until you restart your software.

Note: The basic unit for the X/Y-calibration of images you have already acquired will not be changed, when you alter the basic unit. If you have acquired an image with the basic unit *Meters* it will remain calibrated in meters or in a unit derived therefrom, such as mm or  $\mu$ m. If you wish to measure this image in another basic unit, use the *Image* > *Calibrate Image...* command to alter the basic unit.

### Confirm after acquisition

Select the *Confirm magnification after acquisition* check box to have the *Calibrate Image* dialog box automatically opened after every image acquisition.

Use this feature whenever you want to have the possibility to still change the objective magnification for the image acquisition after switching to the live-image. In the *Calibrate Image > XY-Calibration* dialog box, select the *Magnification* option. Now, you can choose the objective magnification you have acquired the image with from the *Set magnification > Objective* list. This ensures that the image is properly calibrated.

When you clear the check box you have to select the current objective magnification, e.g., by clicking the corresponding button on the *Microscope Control* toolbar, before you start the image acquisition. Only then is the image you acquire correctly calibrated.

### 3.3.2. Acquisition Settings - Document Name

When an image is acquired, your software allocates it a default name. For instance, the first image that your software acquires will be named *Image\_01*, by default. You can change this name in the *Acquisition Settings > Document Name* dialog box.

### **Preview**

The *Preview* field shows the name of the next image that you acquire. This preview will be updated as soon as you change the image's name.

### **Customize**

An automatically created name is made up of different parts. In the *Customize* group, you define a prefix and specify the numbering system.

Defining a prefix

Enter the first part of the image's name in the *Text* field. *Image* is the default prefix. By default, the acquired images are called Image01, Image02 ....

If possible don't use any special characters for the image name. Certain special characters, e.g. ? are not accepted when you enter them.

Shifting the prefix

You can also move the prefix that you defined in the *Text* field to any other position in the name of the image.

- 1. To do this, click the All Options... button.
  - A dialog box will open in which you can assemble an automatically created name from various placeholders.
  - You can find the prefix defined in the Text field in the top position of the Selected properties column.
- 2. Select the prefix and use the *Up* and *Down* buttons.

Defining counters

In the *Counter digits* field, define how many digits the numbering should consist of, e.g., 3 for the number 001. Please note that the value entered in the *Counter digits* field will not place an upwards limit on the numbering. This means that if you have entered a value of 2, for example, and the last image you acquired was Image 99, the next image will be called Image 100.

If you want to start the numbering from a certain value, change the value in the *Counter start* field. You can, for example, return the numbering to 1 if you have acquired a great many images. Or you can continue the numbering of a series of images from the previous day. If you change the value in the *Counter start* field then the next image will always start with the number that has been set. All additional images will be counted up by the value 1.

Each time your software restarts, the numbering of the images will start with the value set in the *Counter start* field.

You can only change the value in the *Counter start* field if you have cleared the *Reset automatically* check box.

Note: The value in the *Counter start* field may be ignored if automatic saving is active. In this case, your software checks whether a file with the name wanted already exists in the current directory. Should this file exist, the next higher available number will automatically be used. To switch the automatic storage process on or off, use the *Acquisition Settings > Saving* dialog box.

Automatically resetting the counter

Select the *Reset automatically* check box when you want to be sure that images that belong together will be consecutively, serially numbered without any breaks. You can then no longer change the numbering of the images manually.

Customizing document names

Your software supplies you with a number of placeholders that you can use in image names. Click the *All Options...* button to choose the placeholders that you want.

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### 3.3.3. Acquisition Settings - Saving

By default, when you make an image acquisition, a new image document will be created and displayed in the document group. You can rename and save this image. If you have not already saved it when you end your software, you will be asked if you want to do so. In the *Acquisition Settings > Saving* dialog box, you can also have the image automatically saved after the acquisition.

### **Automatic save**

You can automatically save all images after acquisition.

Automatically saving images after acquisition Select the *File system* entry from the *Destination* list if you want to save your images as files. Now, in the *Directory* group, define the destination path for saving your documents.

Note: At the same time, up to 150 documents can be loaded in the document group. When you're acquiring an image, you receive an error message if the maximum possible number of documents is already loaded. When you have switched on automatic saving, the image is acquired and saved to the current directory even though it can no longer be displayed in the document window.

Deactivating automatic saving

Select the *No automatic save* entry to switch off the automatic saving of images. You will then have to save the images yourself by using e.g., the *File > Save As...* command, when you have acquired an image and want to keep the image file. In this case, no other functions will be available in this dialog box.

File type

In the *File type* list, select the file format in which the images should be saved after the acquisition. For the image file formats TIF and JPEG, there are also additional settings that should be taken into account when you save images. Click the *Options...* button if you want to see these settings or change them.

Note: Images acquired with your software always contain a range of additional information which can be seen in the *Properties* tool window. This additional information will only be retained if the images are saved in the TIF format.

Close after save

Select the *Close after save* check box to have the image document close immediately after the image has been saved. The images will then be saved as files. Please note that you then can't see the images in your software after the image acquisition. Use this possibility to avoid taking up too much of your PC's memory capacity when you acquire images.

### **Directory**

Prerequisite: The *Directory* group is only available if you select the *File system* entry from the *Automatic save* > *Destination* list.

Selecting a path

The *Path* field shows the directory that will currently be used when your images are automatically saved.

Click the [...] button next to the *Path* field to choose a different directory into which the images are to be saved after their acquisition.

Creating a subdirectory

When you save images automatically after the image acquisition, you have the possibility of saving images that belong together in their own individual directory. By default, all of the images that you acquire in one day will then be saved in one separate directory. On the following day, a new directory will be automatically opened. This enables you to always have a clear overview, even when you acquire a great number of images.

Select the *Create Subdirectory* check box to have the acquired images saved in their own subdirectory.

Click the *Customize*... button if you want to change the name that has been suggested for the subdirectory. The *Customize Subdirectory Name* dialog box opens.

Your software supplies you with a number of placeholders that you can use in directory names. With your choice of the placeholder you also determine the criterion with which your directory tree structure will be organized. This enables you to set up a subdirectory for each user, for instance.

Checking the preview

The *Preview* field shows you the current subdirectory for the next image acquisition. This preview will be updated as soon as you change the subdirectory's name.

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### 3.3.4. Acquisition Settings - Camera - General

Use this dialog box to make a variety of general camera settings. For example, you can mirror the acquired image, use a color camera in black & white mode, or select a color table.

Note: Some of the settings are camera specific. For this reason, your camera's functionality can differ from what is described here.

### Image type

Setting the bit depth

Some cameras can acquire images of different image types. From the *Bit depth* list, select the type of image that you want to acquire with your camera. The image types differ basically in their depth of bit, i.e., the amount of intensity values that each pixel can adopt.

Which entries are available in the *Bit depth* list depends on your camera.

Select the 8-bit RGB color entry to acquire 24-bit true-color images.

You can use some cameras in a black & white mode. Select the *8-bit grayscale* entry to acquire 8-bit gray-value images.

Switching between the color and grayscale



Choosing the color temperature

If you have to frequently switch between the color and the grayscale mode, use the *Toggle RGB/Gray Scale Mode* button. You can find this button on the *Camera Control* tool window's toolbar. You can then switch from one mode to the other with a single mouse click.

A camera can only reproduce correctly colored images when it knows the prevailing color temperature. When you use this process, the individual colors in the image (Red, Green, Blue) will be scaled in such a way that the white area of the image is correctly reproduced as white.

From the *Color temperature* list, select a predefined color setting to acquire the sample in particular light conditions. This automatically chooses the suitable color setting for your camera.

5000 K corresponds to morning or evening sunlight.

6500 K corresponds to overcast daylight.

2800 K corresponds to tungsten light.

Select the *Off* entry if you do not need to adjust the color settings to particular light conditions. That makes sense, for example, when you wish to quantitatively compare images that were acquired under different light conditions. The color impression becomes considerably paler.

Note: You can change the settings for the color temperature directly in the live mode. You can thus see what effect a change has on the color reproduction straight away.

Use the white balance to adjust these default settings, if necessary. Different methods for determining the white balance are available in the *Camera Control* tool window, depending on the camera that you are using.

### **Mirror**

Horizontal

Select the *Horizontal* check box to mirror the image horizontally during acquisition. The illustration shows left the source image and right the horizontally mirrored image.





Vertical

Select the *Vertical* check box to mirror the image vertically during acquisition. The illustration shows left the source image and right the vertically mirrored image.





Horizontal and Vertical

The two forms of mirroring can be combined with each other. The illustration shows left the source image and right the horizontally and vertically mirrored image.





### **Pseudo Color**

Prerequisite: The *Pseudo color* group is only available if you selected the *8-bit grayscale* entry in the *Bit depth* list.

You can use color tables to have images displayed in color on your monitor. Then every intensity value in an image will be allotted a hue.

Using no pseudo colors

By default, when you acquire images, no pseudo color tables will be used. In this case, the *Pseudo Color > Off* option will be selected.

Using the saturation indicator

Select the *Use saturation indicator* option to have all of the pixels with a low intensity value displayed in blue, and all of those with a high intensity value displayed in red. This color table being used now only applies to the live-image. When you acquire this image, it will show all of the pixels back in their actual color, in the document group.

With this pseudo color table you can immediately see if the image is well illuminated. When there are both blue and red pixels in the image, the camera's complete dynamic range will be in use. If too many blue pixels can be seen, the image is underexposed. If too many red pixels can be seen, the image is overexposed.



If you switch the *Use saturation indicator* option on and off frequently, use the *Toggle Saturation Indicator* button. You can find this button on the *Camera Control* tool window's toolbar. This allows you to switch the option on and off with a single click of the mouse.

Using a pseudo color table for gray-value images You can apply a color table at as early as the image acquisition. To do this, select the *Use pseudo color table for grayscale images* option.

You can color only the live-image, or only the acquired image, or both, with the pseudo color table you choose. To do so, select the one of the following entries *For live only*, *For snapshot only* or *For live and snapshot* from the list.

Selecting the pseudo color table

Click the *Pseudo color...* button to change the pseudo color table you have chosen, or to define a new one.

### 3.3.5. Acquisition Settings - Camera - Adjustment

Use this dialog box to manually change settings on the camera that affect the image quality.

Note: Some of the settings are camera specific. For this reason, your camera's functionality can differ from what is described here.

Changing the image contrast in the liveimage

Before you change the settings, you should switch to the live-image, so that you can immediately see how it changes. To do this, use the *Acquire > Live* command.

### **Enhancement**

Gamma

Move the *Gamma* slide control to increase the image contrast either in bright image areas or only in dark image areas.

When each image acquisition is made, the Gamma value will be adopted in the *Properties* tool window. This information will be retained if the image is saved in the TIF file format.

You will change the parameters for all color channels simultaneously. If you want to accentuate or tone down one particular color, use the functions in the *Acquisition Settings > Camera > Color* dialog box.

Contrast and Brightness Move the *Contrast* slide control to change the contrast values of the image. Values smaller than 1 lower the image contrast. Values greater than 1 enhance the image contrast.

When changing the image contrast the brightest part of the image always remains the same.

After changing the image contrast it may be that dark or bright image areas are not displayed optimally anymore. In this case use the *Brightness* slide control to make the whole image brighter or darker.

Use the *Brightness* slide control, if the whole image is too bright or too dark. Values smaller than 0 lower the image brightness. Values greater than 0 increase the image brightness.

You can also view the *Contrast* and *Brightness* values in the *Camera Control* tool window and you can also change them there.

The values are automatically saved together with the image. You can view the values in the *Properties* tool window.

### **Limit throughput**

In the live mode, a huge amount of data is transferred from the camera to your PC. It may occur that the USB connection to your system is not sufficiently high to support the full data rate. This may lead to certain errors in the live-image, for example, stuttering or color distortion.

Note: You should only reduce the data rate if errors in the live mode occur on your system.

In this case, use the *Data rate* slider to reduce the amount of data that is transferred. In the *Camera Control* tool window, a correspondingly smaller frame rate is now shown in the live mode.

### 3.3.6. Acquisition Settings - Camera - Color

Use this dialog box to change the camera's color settings manually.

Note: Some of the settings are camera specific. For this reason, your camera's functionality can differ from what is described here.

Changing the color settings in the liveimage Before you change the color settings, you should switch to the live-image, so that you can immediately see how it changes. To do this, use the *Acquire > Live* command.

### **Description of dialog box**

White Balance

Prerequisite: You can only change the color settings when you are working in your camera's color mode.

With the gain value, you can weight the individual color channels separately. Increase, e.g. the R value, to increase the amount of red in the image.

You shouldn't usually change the gain value manually. Instead, use your camera's white balance functions. The result of the white balance will be automatically adopted in the fields of this dialog box.

You can change the gain individually for each color channel. To change a parameter, move the slide control. *R*, *G* and *B* stand for the Red, Green and Blue color channels.

The gain values being used are transferred to the *Properties* tool window with every image acquisition. You will find the *Gain (White Balance)* entry in the *Camera* group. This information will be retained if the image is saved in the TIF file format.

Device dependent white balance

The white balance is influenced by every optical component within the light path. In particular, the objectives can affect the color impression of an image.

Select the *Device dependent white balance* check box to automatically save each white balance performed. When you acquire images later on using an objective with a valid white balance, this white balance will automatically be loaded and applied.

If you have cleared the *Device dependent white balance* check box the latest white balance will be used, regardless of the objective used.

Saturation and Hue

The sliders *Saturation* and *Hue* offer additional possibilities to influence the color settings of your camera. Switch your system to the live mode before you open the *Acquisition Settings* dialog box. When you change the saturation and hue value now, you can observe directly in the image window how the color reproduction is influenced.

Increase the *Saturation* value when there is a preponderance of one color in your images. In this way you will attain a better reproduction of the other colors in your image. To change the parameter, move the slide control. A change made in the Saturation will have an effect on all of the color channels.

Use the *Hue* value to alienate the color impression of the acquired images. Certain structures in the image can thus be better recognized, for example. A value of 180 means that the color in the image corresponds to the color of the sample.

You can also view the *Saturation* and *Hue* values in the *Camera Control* tool window and you can also change them there. The values are automatically saved together with the image. You can view the values in the *Properties* tool window.

Default

Click the *Default* button to select the preset color settings. The current settings will then be lost.

### 3.3.7. Acquisition Settings - Camera - Exposure

Use this dialog box to set the maximum possible exposure times for the image acquisition. By doing this you can prevent the exposure times from becoming unnecessarily long.

### Maximum exposure time

You can set the maximum possible exposure time for the live-image and for the snap. To do this, use the slide controls. Alternatively, you can also enter the value you want directly in the text field. The maximum value that you can enter here depends on the camera you use.

The *Manual* and *Automatic* slide controls correspond to the two possible modes for determining the exposure time, the automatic and the manual mode. You can determine which mode you want to use in the *Camera Control* tool window, for example.

3039 20112015

### 3.3.8. Acquisition Settings - Camera - Information

Use this dialog box to look at data pertaining to your camera.

Displayed information

Camera name	The type of camera being used
Serial number	The camera's serial number
API version Firmware version Driver version	Information about the camera driver being used

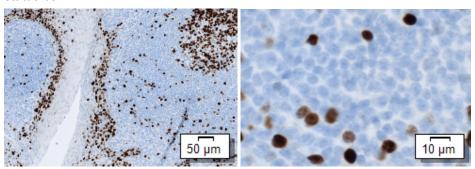
# 4. Working with images

# 4.1. Changing the way an image is displayed

An image can be displayed in very different ways in your software's image window. There are several ways in which you can change the way your image is displayed.

# Enlarging or reducing the size of the image in the image window

There are several different ways to change your image's zoom factor in the image window. The current zoom factor is displayed on the bottom right in the status bar.



On the **left**, the whole image is displayed in the image window. On the **right**, the zoom factor has enlarged the image segment so that it can be viewed in higher resolution. The scale bar relates to the magnification of the image in the image window and is adjusted accordingly.

The different zoom options are described in the text that follows.

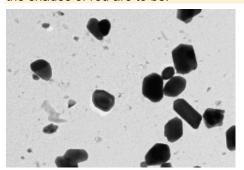
- 1. Use the buttons on the *Zoom* toolbar.
- **(**
- 2. Use the Zoom Tool button on the Toolbox toolbar.
- 3. If you are not in zoom mode: Right click on an image window. In the context menu you can find several commands with which you can alter the image's zoom factor.
- 4. Rotate the mouse wheel to change the zoom factor.
  - You can set whether you want to enlarge or reduce the size of the image in the image window when you move the mouse wheel forwards. To do this, check or clear the Zoom in the image by scrolling the mouse wheel away from you (forward motion) check box. You can find this check box in the Tools > Options > Images > Zoom dialog box.
- 5. Use the *Image Navigator* tool window.
  - In the *Image Navigator* tool window, while keeping the left mouse button depressed, drag the navigation frame to a smaller size. As soon as you release the mouse button, only the image segment you have selected will be shown in the image window.
  - Enter the zoom factor you want directly in the edit field below the image area in the *Image Navigator* tool window and press the [Enter] key, or use the slide control.

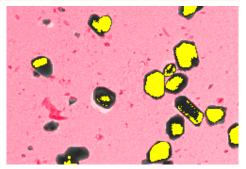
### Changing an image's color mapping

You can display gray-value images in color. You can do this using color tables.

Task

Create a color table which displays dark gray-values in yellow, and light gray-values in different shades of red. And, the lighter the gray values are; the lighter the shades of red are to be.

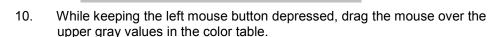




On the **left** is the gray-value image. On the **right** the image has been colored with a color table. The coloring allows you to see more details in the dark and light gray-value range.

Creating a color table

- 1. Load a gray-value image. You can use the [Ctrl + Alt + T] shortcut to create a test image.
- 2. Use the *Image > Pseudo color...* command to open the *Pseudo color* dialog box.
  - The current color table is shown in the *Current Color Table* group.
- 3. Move your mouse pointer onto the color table in the dialog box.
  - Your mouse pointer then takes on the form of a hand.
- 4. While keeping the left mouse button depressed, drag the mouse over the lower gray values in the color table.
  - A blue bar under the color table indicates the gray-value range that has been selected. You can now assign this gray-value range a color.
- 5. Click the left color field in the *Edit Color Table* group once.
- 6. Select the required color, yellow for example, in the *Colors* dialog box and confirm it with *OK*.
- 7. Click the right color field in the *Edit Color Table* group once.
- 8. Select the required color, yellow for example, in the *Colors* dialog box and confirm it with *OK*.
- 9. Click the *Apply* button in the *Edit Color Table* group.
  - All of the current image's gray values that are within the selected gray-value range will then be displayed in yellow.
  - The current color table will be correspondingly updated.



- A blue bar under the color table indicates the gray-value range that has been selected. You can now assign this gray-value range a color.
- 11. Click the left color field in the *Edit Color Table* group once.

- 12. Select the required color, dark red for example, in the *Colors* dialog box and confirm it with *OK*.
- 13. Click the right color field in the *Edit Color Table* group once.
- 14. Select the required color, white for example, in the *Colors* dialog box and confirm it with *OK*.
- 15. Click the *Apply* button in the *Edit Color Table* group.
  - All of the current image's gray values that are within the selected gray-value range will now be shown in either yellow or red. In contrast to the yellow pixels, the red pixels become constantly brighter, the higher their intensity value is.
  - The current color table will be correspondingly updated.



Saving a color table

- 16. Click the *Save Color Table* button. You can find this button at the top of the *Pseudo Color* tool window.
  - The Save Color Table As dialog box opens.
- 17. Enter a descriptive name for the new color table in the *Name* field, *Yellow-Red* for example.
- 18. Click the *Save* button to save the color table and to return to the *Pseudo Color* dialog box.
  - You can apply the Yellow-Red color table to additional gray-value images from now on.
- 19. Close the *Pseudo Color* dialog box with *OK*.
  - The active gray-value image is now displayed in color.

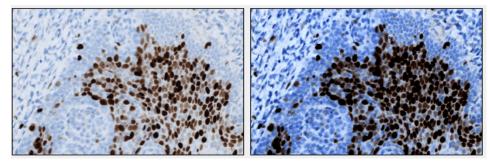
# 4.2. Processing images

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

- Load the image you want to process, or activate the image in the document group.
  - Please note that the *Process* menu will only be visible when an image is loaded and active in the document group.
- 2. Use one of the commands in the *Process* menu, e.g., *Process* > *Enhancements* > *Adjust Intensity...* .
  - The image processing dialog box opens. The image processing operation that is active will be shown in the dialog boxes header.



- 3. Click the small arrow next to the *Preview* button to open a list of all of the preview functions. Select the *Original and Preview* entry.
  - This preview function displays the same image segment twice in the dialog box. The first one shown is the source image. The second is the image that results when the current parameters are used.
  - Most of the image processing operations need one or two of the parameters that are shown in the Settings group.
- 4. Change the image processing operation's parameters. Decrease the gamma value and increase the brightness, for example.
  - 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.
- 5. Click the *Default* button, to readopt the preset parameters in the *Settings* group, when the current parameter doesn't make sense to you.
- 6. 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.
  - The new image is not automatically saved. The fact that a change hasn't been saved is indicated by an asterisk shown behind the image name in the document group.



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

## 4.3. Performing a white balance on an image

Should an area in your image that ought to appear white look reddish, or is another color, this means your image has a tinge. In such cases, use the *Process > Enhancement > White Balance...* command to perform a white balance.

- 1. Load the image you want to process, or activate the image in the document group.
- 2. Use the *Process > Enhancements > White Balance...* command.
  - The Filter: White Balance dialog box opens.
- 3. For the start, define a white point in the image. This should be a point or surface that is actually white, but isn't displayed in white in the image.



- 4. For example, click the Select white point using the pipette button.
  - The dialog box disappears. The mouse pointer has turned into a pipette.
  - The size of the pipette's point is predefined at 3x3 pixels. Alternatively, you can also use the ellipse or the rectangle to define the white point, and by doing so, define an arbitrarily large area as the white point.
- 5. Click a position in the image that you want to define as the white point.



- The position is then marked with a red cross. When that has
  occurred, you can still move the point. To do this, move your mouse
  over the red cross so that it takes on this shape. Then, while
  pressing the left mouse button, you can move the white point
  wherever you want to.
- 6. Confirm your choice by clicking your right mouse button, then using the *Confirm Input* command in the context menu.
  - The Filter: White Balance dialog box reopens.
  - In the *Interactive white-point definition* group, the color values of the three colors for the defined white point are displayed.
  - In the White-point correction factors group, the correction values determined for each color, are displayed.
  - In the dialog box's preview image, you can see what the corrected image will look like, should you adopt these values.
  - If necessary, you can still manually change the white point correction factors that have been determined. The preview window will then be immediately, correspondingly updated.
- 7. When you are satisfied with the preview image, click the *Apply* button, then click *OK*.
  - The white balance will be performed immediately. This means that
    the colors in the image are adjusted to make the selected white point
    and all of the areas with the same color values appear as white. You
    can see the results immediately in the image.
  - 8. If you are not satisfied with the results: Please note that you can undo the white balance by using the *Edit* > *Undo* command.

# 4.4. Working with drawing objects

Various drawing functions are available that you can label your images with.

### Inserting drawing objects

Task

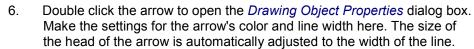
You want to insert an arrow into an image and label it.

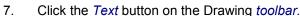
- 1. Load the image that you want to label.
- 2. Set the zoom factor of the image window to display the image in a size that makes the label is easy to read. You can rotate the mouse wheel to change the zoom factor in the image window.
- Use the View > Toolbars > Drawing command to have the Drawing toolbar displayed.

Inserting an arrow



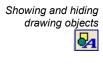
- 4. Click the *Arrow* button in the *Drawing* toolbar.
  - There is a button for each drawing object on the *Drawing* toolbar.
     Click the button for the drawing object that you want to insert and then define the drawing object on the image.
- 5. Keep the left mouse button pressed to draw the arrow.





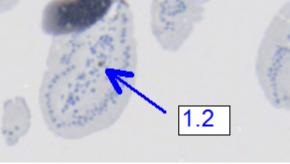
- 8. Keep the left mouse button pressed draw a rectangle. Enter the required text and format it.
- 9. Use the [Esc] key to leave text-entry mode.

10. You can display the image and the drawing layer together, on your monitor, or alternatively the image without the drawing layer. Click the *Show Drawings* button in the *Drawing* toolbar, to switch between



Inserting a text object





Two drawing objects have been defined on the image: an arrow and a label for the arrow.

### Selecting drawing objects

Selecting several objects

For certain actions it is useful to select more than one drawing object. This enables you to copy several drawing objects from one image to another at the same time, or to format several drawing objects simultaneously.

1. Use the buttons in the *Drawing* toolbar to create several drawing objects.



2. If necessary, switch to the edit-object mode. To do so, click the *Select Drawing Objects* button on the *Drawing* toolbar. The button will appear clicked when the edit-object mode is active. You can recognize this

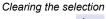
status by the button's colored background.

When the button already appears clicked, you will already be working in the edit-object mode.

- 3. Use one of the following methods to select a collection of drawing objects.
  - Left click an object.
    - While keeping the [Shift] key depressed, click other objects you want
    - Objects you have selected will be identified by white or gray markers.
  - While keeping the left mouse button depressed, drag a rectangle to encompass several objects.
    - All objects that are completely inside this rectangle will be selected. All of the other existing selections will be deleted.
  - To select all of the drawing objects on the image, use the [Ctrl + A]
  - shortcut.
- 4. If necessary, switch to the edit-object mode. To do so, click the Select Drawing Objects button on the Drawing toolbar. If the button already appears clicked, this means that you are already in the edit-object mode.
- 5. There are several ways of clearing a selection of drawing objects:
  - Use the *Edit* > *Deselect* command.
  - Use the [Ctrl + D] keyboard shortcut.
  - Click any place in the background of a document with your left mouse button. The complete current selection will be cleared.
  - To deselect individual objects, click an object that has already been selected.

### Determining the default formatting for drawing obiects

- 1. Insert a drawing object and assign the properties to it that you want to use as default settings. For example, change the line color from black to red if you want the lines in your images to be red by default.
- 2. If necessary, select the drawing object that you want to use as a template for the formatting.
- 3. Use the *Draw > Set As Default For Drawings* command. You can find the Draw menu on the Drawing toolbar.
  - The drawing object that has been selected is now used as a template for all new drawing objects.
- 4. Insert another drawing object, e.g., an ellipse.
  - The new drawing object will then be formatted in exactly the way you determined that the first drawing object should be.





# 5. Measuring images

## 5.1. Overview

You can measure distances and areas with your software. All of the results are saved together with the image.

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

Use the *View > Tool Windows > Measurement* command. In the *Measurement* tool window, you have fast access to the measurement functions and to the settings which effect measurements. This tool window is at the same time the measurement display and contains all of the values that have been measured on the active image.

### Starting a measurement

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

Working in the default measurement mode

As soon as you have clicked a measurement function, your software will automatically switch to measurement mode. You can measure image structures in measurement mode. When you have finished making your measurements you automatically leave measurement mode.

You can now carry out another measurement, or use any other software functions that you want.

Working in continuous measurement mode

Double click a measurement function to switch to continuous measurement mode. In continuous measurement mode, your mouse pointer turns into a cross. In the continuous measurement mode, you can immediately make further measurements with these measurement functions. The continuous measurement mode is valid for all loaded images. You can, therefore, easily measure numerous images one after the other.

The button that has been selected in the *Measurement* toolbar remains active to identify the current measurement function.



You will remain in this measurement mode until you explicitly switch it off. To do so, click the *Select Measurement Objects* button on the *Measurement* toolbar.

### Displaying and saving measurement results

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

Saving the measurement results

The measurements will be saved along with the image, if you save the image in TIF format.

### **Editing measurements**

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

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

Selecting measurement objects



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

Moving measurement objects

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

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



Change the measurement object by moving the handles.

Deleting measurement objects

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

Displaying helper lines



You can display helper lines for the *Arbitrary Line* measurement function. Select a measurement object in an image and right click to open a context menu that contains the *Helper Lines* command.

## Measuring in the live mode

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

Note: The measurements that you perform in the live-image aren't transferred to the image when it is acquired.

## 5.2. Measuring images

You can measure distances and areas with your software.

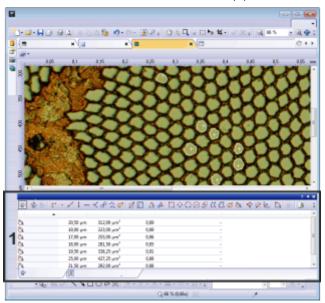
### Measuring image objects interactively

Task

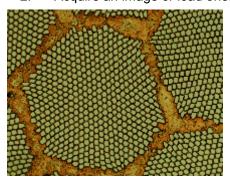
You want to measure the filaments in a superconductor.

To do this, load a suitable image, or acquire one. Measure the diameter of some of the hexagonal filaments, between opposite corners. Then edit the measurement and delete some of the measurements that have been performed.

- Use the View > Tool Windows > Measurement command to display the Measurement tool window.
  - You'll find the tool window (1) at the lower edge of the user interface.



2. Acquire an image or load one.



Setting the labeling color

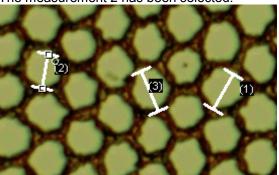
The measurement results will be written into the image according to the default settings, in a red font and without a background. This can make the measurement results hard to read on some images. Change the labeling settings.

- 3. Use the *Tools > Options...* command.
- 4. Click the *Measurement > Measurement Display* entry in the tree view.
- 5. Click in the *Background Color* field and choose a color, black for example.

- 6. Select the *Text color > Fixed colors* option and select a suitable color from the palette. Select the color white to display the measurements in white and the labels in white on a black background.
- 7. Close the dialog box with *OK*.

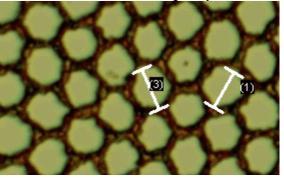
### Measuring lengths

- 8. Click the *Arbitrary Line* button. You can find the button on the toolbar at the top of the tool window.
- 9. Click with your left mouse button at the starting point and end point of the reference distance.
- 10. Repeat the measurement several times.
- 11. 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

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



#### Closing the image

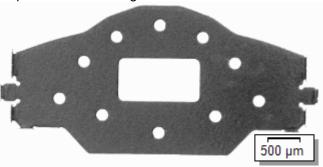
- 14. Click the button with the cross [x] to the right of the image name in the document group.
  - You have changed the image because you've added interactive measurements. For this reason, you'll receive a query whether you wish to save the image or not.
- 15. Save the image in the TIF file format. 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 area of a workpiece. Have a variety of measurement parameters, such as the area, the perimeter and the diameter, output. Have the diameter shown in the image.

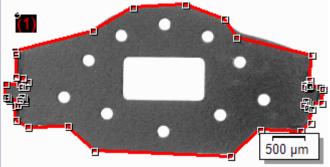
1. Acquire or load an image.



Measuring areas



- 2. In the *Measurement* tool window, click the *Closed Polygon* button.
- 3. Left click to define individual points on the edge of the object. Right click to finish the measurement.
- 4. Take a look at the result in the *Measurement* tool window.
  - The illustration shows the image with a measurement object.



Viewing the list of measurement parameters



Outputting additional measurement parameters

- 5. In the *Measurement* tool window, click the *Select Measurements* button.
  - In the dialog box you'll see a list with all of the available
    measurement parameters. At the bottom of the dialog box you'll see
    a list of the measurement parameters that are currently calculated for
    all objects.
- 6. Go to the list of all of the available parameters, then click the *Diameter* measurement parameter.
  - On the right, an illustration shows you how the parameter is calculated.



You can see that there are different ways in which the diameter of a 2D object can be calculated.

- 7. Click the *Max* entry in the list under the illustration, to select the *Max* (*Diameter*) measurement parameter. This determines the workpiece's largest span.
- 8. Click the Add 'Max (Diameter)' button.

- This measurement parameter will be added to the list of measurement parameters to be calculated. All of these measurement parameters will be displayed in the tool window.
- 9. Close the dialog box with *OK*.
- Take a look at the result for the diameter in the *Measurement* tool window.

Outputting measurement parameters in the image

- 11. Use the *Tools > Options...* command and select the *Measurement > Measurement Display* entry in the tree view.
- 12. Select the *Text* > *Show value* check box and close the dialog box with *OK*.
  - Now the measurement values will be displayed in the image.
- 13. Open the Select Measurements dialog box.
- 14. At the bottom of the list of all of the calculated measurement parameters, click the *Max. (Diameter)* measurement parameter.



- 15. To the right of this list you'll see a button with a blue arrow. Click this button to move the measurement parameter to the top of the list.
- 16. Close the dialog box with OK.
- 17. Take a look at the result for the diameter in the image.

Note: The measurement display in the image has to be updated before the new settings are taken into account. You can update the measurement display by adding another measurement or by selecting an existing measurement in the image.

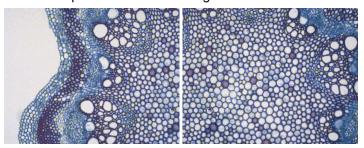
### Measuring several images

Task

You want to measure cells on multiple images. To do so, acquire some images and measure them one after another. Have the results from all images displayed simultaneously. Take a look at the mean value for all of the measurements.

Loading images

1. Acquire or load some images.



Measuring cells

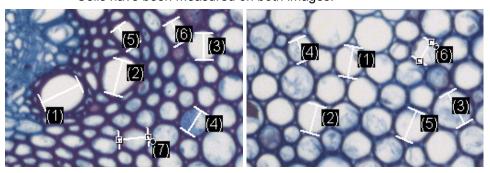
2. Activate the first image in the document group.



- 3. Double click the *Arbitrary Line* button, located on the toolbar at the top of the *Measurement* tool window.
  - You will then switch to continuous measurement mode. In the continuous measurement mode, you can immediately make further measurements.
- 4. Measure the diameter of several cells.
- 5. Activate the next image. Measure the diameter of several cells on this image, too.



- 6. Click the *Arbitrary Line* button again, and switch off the length measurement.
  - Cells have been measured on both images.



Displaying the measurement results of all of the images

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

# 6. Configuring the system

## 6.1. Overview

Prerequisite: Your software has been installed and the camera is connected to the computer. The camera driver is installed in MS-Windows. When starting, your software now recognizes the camera that is connected.

### Process flow of the configuration

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

Configure the hardware



Calibrate the system

Configure the hardware

Every microscope is equipped with a different objective. In addition, your microscope can also have a magnification changer or a camera adapter with a particular magnification. Use the *Acquire > Devices > Device Settings* dialog box to enter your microscope components.

You can also find all of the camera settings in the *Device Settings* dialog box.

Calibrating the system

When you have entered the objectives being used and, if necessary, the camera adapter's and the magnification changer's magnifications, all of the images that you acquire with your software are automatically X/Y-calibrated. Use the *Manual Magnification Calibration* calibration process if this calibration is not precise enough.

## About the system configuration

When do you have to configure the system?

You should configure and calibrate your system when you start the computer after installing the software for the first time. If you later change the way your microscope is equipped, you will need to change the configuration of certain hardware components, and possibly also recalibrate them.

## 6.2. Configuring the system

Your software requires information about your camera, the objectives, and the magnification of the microscope's camera adapter in order to be able to acquire correctly calibrated images. That's why you need to configure the system.

Preconditions

Your software has been installed and the camera is connected to the computer. The camera driver is installed in MS-Windows.

### Configuring the specified hardware

- 1. Use the Acquire > Devices > Device Settings... command.
  - In the tree view on the left side, you can find the hardware components that are required for the correct calibration of the images that are acquired.
- 2. Select the Lightpath entry in the Sort by list.

Configuring the camera

- 3. In the tree view on the left-hand side, expand the *Camera* > <*camera* name> entry (*Basler USB3 Vision* for example).
- 4. Select the *Camera Adapter* entry.
- 5. Select your camera adapter's magnification in the *Magnification* list on the right. The magnification is imprinted on your camera adapter. Typical values would be 1.00 or 0.63.

Saving the hardware configuration

- 6. For your software, a typical hardware configuration is predefined. The name of this hardware configuration is *BaslerDefault*. In the *Configuration* group, click the *Copy Device Configuration* button.
- 7. Enter a name for the new hardware configuration in the *Copy Device Configuration* dialog box.
  - The copied hardware configuration is added to the list of the hardware configurations.

Configuring the objective nosepiece

- 8. Select the *General > Manual Nosepiece* entry in the tree view.
  - The current configuration of the nosepiece is shown on the right side of the dialog box. When you configure the software for the first time, several typical objectives are preset.
- 9. Check the preset objectives and adjust the values if necessary.
- 10. Select the objectives with which your nosepiece is currently equipped from the *Magnification* lists on the right. Start with the smallest magnification, then a higher magnification, and so on. The magnification is printed on the objective itself.
  - 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.
- 11. 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 zoom/magnification changer

- 12. Select the *General > Manual Zoom / Magnification Changer* entry in the tree view.
- 13. Enter one of your magnification changer's magnifications in the *Magnification* field and click the *Add* button.
  - All of the magnifications that have been entered will be available on the Microscope Control toolbar.

Finishing the configuration of the system

- If your microscope doesn't have a magnification changer, make the default settings yourself.
- 14. Close the *Device Settings* dialog box with *OK*.
  - You may get a message asking you to check the calibrations. You can perform the calibration either now or later.
- Use the View > Toolbars > Microscope Control command to display the toolbar.
  - The Microscope Control toolbar contains buttons with all of your objectives, correctly color coded.

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## 6.3. Manual Magnification Calibration

All images you acquire using your software are automatically X/Y-calibrated. Use the *Manual Magnification Calibration* calibration process if this calibration is not precise enough. You need a calibration standard to perform the calibration process.

How is the preset magnification calibration determined? The X/Y-calibration of an image you have acquired with your software is calculated from the size of a pixel and from the total magnification. The total magnification at the time of acquisition is usually a combination of the objective magnification and the camera adapter's magnification. If you are using a magnification changer, this will also have an effect on the total magnification.

The preset magnification calibration uses the hardware components from the *Device Settings* dialog box and the camera's pixel size, which can be read out from the camera driver.

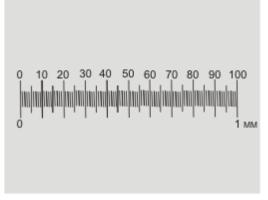
Starting the manual magnification calibration Returning to the preset magnification calibration

Use the *Acquire > Calibrations...* command. Select the *Manual Magnification Calibration* entry in the *Calibrations* dialog box. Then click the *Calibrate...* button.

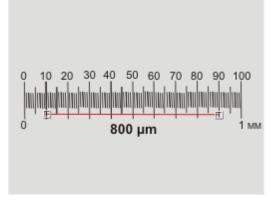
When you have manually calibrated your objectives, you can at any time return to the preset values. Select the *Manual Magnification Calibration* calibration process in the *Calibrations* dialog box. Then click the *Edit...* button. Clear the *Use manual calibration* check box in the *Manual Objective Calibration* dialog box.

### Calibrating the objective manually

1. Lay a calibration standard under your light microscope.



- 2. If you are using a magnification changer, set the magnification changer to a magnification of 1 and select the magnification 1x on the *Microscope Control* toolbar.
- 3. Start the *Manual Magnification Calibration* calibration process.
  - In the *Calibrate objective* list all of the objectives that you have registered with your software are listed.
  - All of the objectives that have already once been manually calibrated are identified by a check-mark .
- 4. Select the check box in front of each objective that you want to calibrate.
- 5. Click the *Next* > button.
  - Your software will automatically switch to the live mode.
- 6. Bring the objective you want to calibrate into the light path. The dialog box shows the name of the objective.
- 7. Focus on the calibration standard.
- 8. Click the *Next* > button.
- 9. Click the Set Reference Distance button.
  - The image is acquired and live mode is closed.
  - The mouse pointer appears in the image window.
- 10. Determine the starting and end point of the calibration reference distance with your mouse pointer, for example, a distance of 800 µm. You define these two points by clicking with your left mouse button.



11. Click the right mouse button and select the *Confirm Input* command in the context menu.

- The Set Reference Distance dialog box opens.
- 12. First select an appropriate unit μm .

  If, for example, you are using a calibration standard with a length of 1 mm and 100 scale units, select the unit μm.
  - Note: If you select the unit in here, this image will be calibrated in inches. That doesn't mean, though, that all of the images will now be acquired with in as the basic unit. You specify the basic unit for the image acquisition in the camera settings, in the Acquisition Settings > Acquisition > General dialog box.
- 13. In the *Length* field, you enter the length of the calibration standard that you want to use for the calibration. With a calibration standard that is 1 mm long for example, you can enter 800 in this field if you have defined the reference distance between the scale divisions 10 and 90.
- 14. Click the *OK* button to confirm the calibration.
- 15. Click the *Next* > button if you want to calibrate further objectives, or the *Finish* button when you don't have any more objectives to calibrate.
- 16. Repeat the manual calibration for the other objectives that you've chosen.
  - When the last magnification calibration has been carried out, the Manual Magnification Calibration dialog box opens.
- 17. Check the results and close the *Manual Magnification Calibration* dialog box with *OK*.
  - In the Calibrations dialog box, the Manual Magnification Calibration calibration process will then have the Calibrated status.
- 18. Close the *Calibrations* dialog box.
  - When you then acquire an image, the image's X/Y-calibration will be calculated with the new magnification values. Please note that before you make the image acquisition, you will have to select the objective you want to use, in the *Microscope Control* toolbar. If you are using a magnification changer, you will also have to select the magnification level used.
- 19. You can view the actual objective magnifications resulting from the manual magnification calibration at any time.

  Select the *Manual Magnification Calibration* calibration process in the *Calibrations* dialog box. Then click the *Edit...* button.