Quick Guide

ZEN 2

First steps with ZEN

We make it visible.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

Effective from: October 2014

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Content

1 Concept

1 Concept

ZEN 2 is a modular image-processing and analysis software for digital microscopy. In addition to basic functionality for image acquisition and microscope definitions, elementary image processing and annotations, image analysis and documentation optional modules for specific tasks are available.

With ZEN lite the basic version of the software is available for free. Starting from a basic functionality for image acquisition, simple image processing, image analysis and documentation a lot of optional modules are available for ZEN lite as well. More detailed information is available in the product brochure.

1.1 Image Acquisition

The software ZEN 2 completes all microscopes and cameras from ZEISS to efficient and tailor-made imaging systems. With little training you will interactively control the entire workflow from image acquisition, processing and analysis.

Depending on the system you capture single images, multi-channel fluorescence images or video sequences with up to 16-bit per channel image information. ZEN supports reliably: Smart Setup proposes the optimal dye and wavelength combinations for your experiment.

A range of different camera types can be used with ZEN 2, from simple TV cameras through to high-resolution and high-sensitivity cameras. The seamless integration of cameras into the software allows you to create complex images and image sequences by one mouse click.

1.2 Image Processing

The acquired image is immediately displayed on the monitor. It can then be optimized using a wide range of techniques:

- Contrast, brightness and color adjustment
- Noise suppression, smoothing and contour enhancement
- Sharpness enhancement/emphasizing of details
- Correction of illumination influences and white balance

ZEN 2 can also be used to add any annotations that you may require to the images. All elements, from scale bars and colored markings through to text and graphics, have been integrated into the program.

1.3 Image Analysis

Even with **ZEN lite** you are able to perform simple interactive measurements. The measured values (e.g. lengths, areas and perimeters) are made available in a data table, and can be processed further using spreadsheet programs.

1.4 Documentation

Besides the image itself, the new image format (***.czi**) also saves additional data, such as the image number, date of acquisition, microscope settings, exposure values, size and scale details, contrast procedures used etc. Annotations and measured values are also saved with the image.

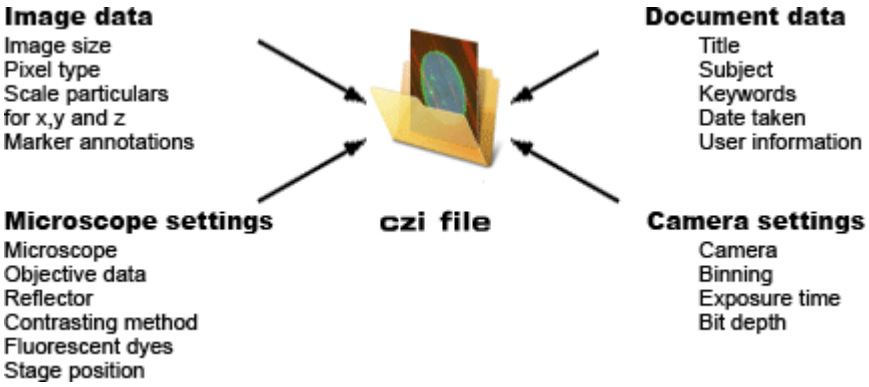


Fig. 1: CZI file format

1.5 Extensions

The extensions concept allows you to extend ZEN blue dynamically in its functionality. From a technical point of view the concept is comparable with plug-in`s or add-on`s. For the extensions we reserved a special area (Extensions tab) within the software so that you can find all loaded extensions at a glance.

2 Start software

2 Start software

Prerequisites

- You have installed ZEN 2 on your computer.

Procedure

1. Double click on the program icon on your desktop.

2. Alternatively click on **Start | All Programs | Carl Zeiss Microscopy | ZEN 2 | ZEN (blue edition)** entry (blue icon).

   The software starts. After a while you see the login screen.

3. Click on the button of the application you want to work with. The available applications depend on your licenses and system. Make sure that the hardware components you use are switched on and are ready for operation.

   The software starts. During the program start the hardware settings will be initialized.

You successfully started the software.

Info

For using pre-recorded images when starting the software, in the menu **Tools | Options | Startup**, the **Reload Last Used Documents** checkbox must be activated.

3 Program interface

3 Program interface

The ZEN 2 program interface is divided into three main areas. Via the tabs in the **Left Tool Area (1)** you can access all the main tools for microscope control (Locate tab), acquisition (Acquisition tab), image processing (Processing tab), image analysis (Analysis tab) and report generation (Reporting tab). The **Center Screen Area (2)** is used to display your images, while the **Right Tool Area (3)** provides you with an overview of all open documents and is used for advanced file management.

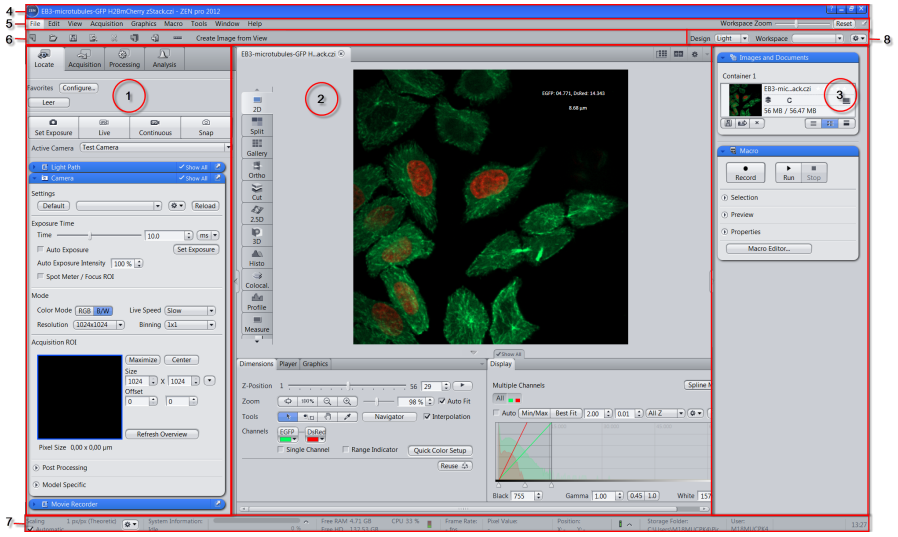


Fig. 2: Application Layout

1 Left Tool Area

2 Center Screen Area

3 Right Tool Area

4 Title bar

5 Menu bar

6 Tool bar

7 Status bar

8 Workspace Configuration

3.1 Left Tool Area

Here you find the Main Tabs for microscope and camera settings (Locate tab), image acquisition (Acquisition tab), image processing (Processing tab), image analysis (Analysis tab) and reporting (Reporting tab).The Main Tabs are organized in an order which follows the typical workflow of experiments in bioscience or material science.

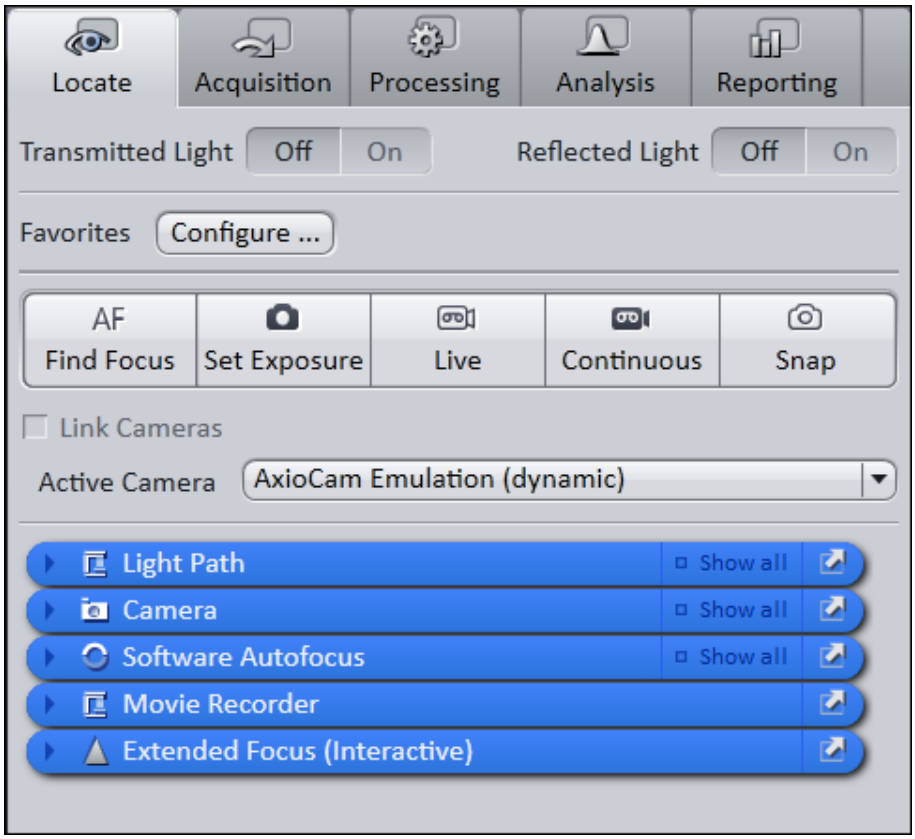


Fig. 3: Left Tool Area (ZEN pro, desk, system)

3.2 Center Screen Area

The Center Screen Area is structured in 4 areas. The **Document bar (1)** is on top. Down the left side of the displayed image you find the tabs for the general and specific **Image Views (2)**. In the middle of Center Screen Area is the **Image Area (3)**, images, reports and tables were shown here. Under the image area you find the **General -** and **Specific View Options (4)** organized on tabs. View specific control tabs are flagged with a blue corner.

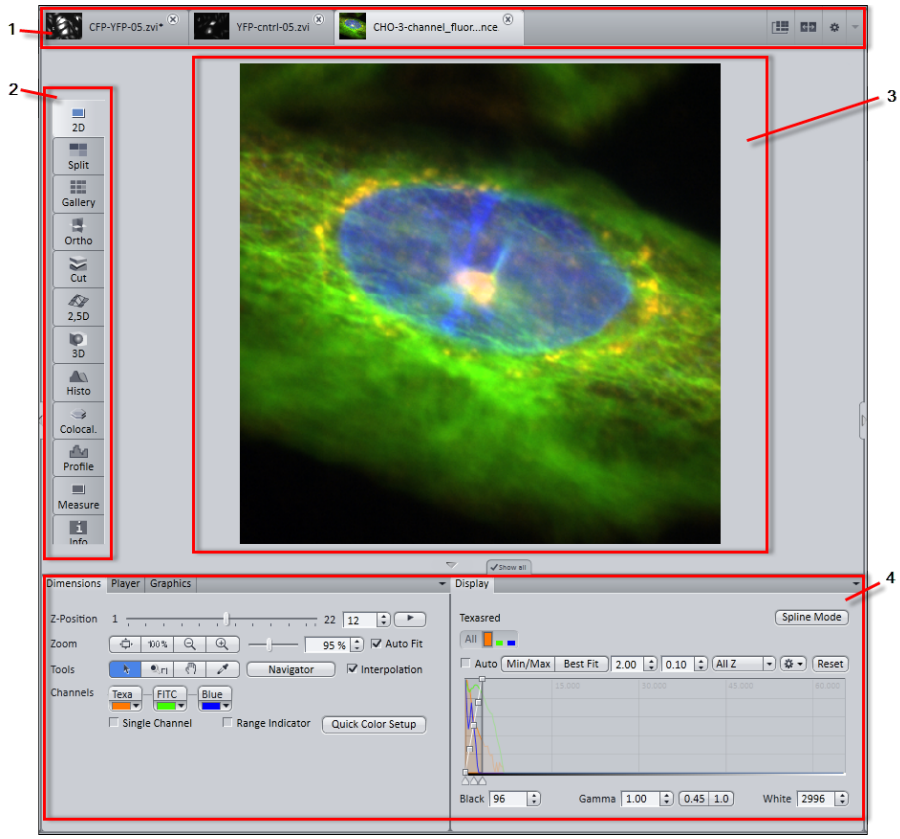


Fig. 4: Overview Center Screen Area

1 Document bar

2 Image Views organized on tabs

3 Image area

4 General and specific view options organized on tabs

3.3 Right Tool Area

Here you find the **Images and Documents** gallery and the **Macro** tool (depending on module/ license options).

3.4 Title bar

Fig. 5: Title bar

Help

Activates the "drag & drop" help function. A question mark appears beside the mouse pointer. Move the mouse pointer to a place in the software where you need help. Left-click on the desired location. The online help opens.

Minimize

Minimizes the program window.

Maximize across 2 screens

Maximizes the program window across 2 screens. This option is only possible if you are working with 2 screens with the same resolution.

Maximize

Maximizes the program window to the main screen.

Reduce

Reduces the program window to any selected size.

Close

Closes the program window.

3.5 Tool bar

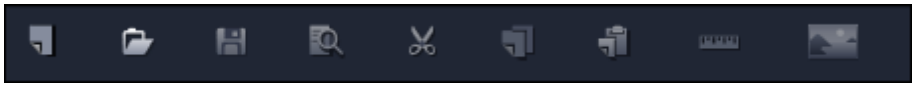


Fig. 6: Tool bar

Here you gain quick access to important functions, e.g. saving or opening files. Further right you find more workspace settings, e.g. **Design** and **Workspace** selection. Read how to customize the Tool bar in chapter Customize toolbar.

3.6 Document bar

Fig. 7: Document bar

Here you see tabs of all opend documents. Click on a tab to view the image/ document. On the right end of document bar you find buttons to switch view mode (**Expose** and **Splitter mode**) and further view options (**View** menu).

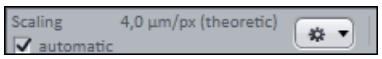
i Info

A **asterisk** (*) next to an image/document title indicates that unsaved changes have been made to this document.

3.7 Status bar

Here you will see important information on the system status:

Scaling options

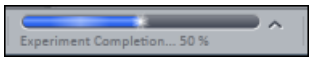


Displays which lateral scaling is currently being used. The **automatic** checkbox is activated by default. The scaling will be calculated automatically based on your hardware settings (i.e. objective, adapters, etc.). If the **automatic** checkbox is deactivated, you can also load/import scalings or start the scaling wizard in the **Options** menu.

System Information

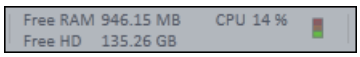
Always shows the latest, currently active process that the system is performing.

Progress bar



Displays the progress of the currently active process. Each new process added supersedes older still active processes. If you click on the up button, a window opens with a list of all processes in chronological order. You can stop a process that is running using the **Stop** button.

Performance indicators



In this group you will see an overview of the performance of individual computer components:

- **Free RAM** indicates how much physical memory is still available
- **Free HD** indicates how much space is still available on the hard drive onto which the next image is to be acquired (see Extras/Options/Save).
- **CPU** indicates the usage of the Central Processing Unit.
- The **status bar** provides an overall assessment of the system usage.

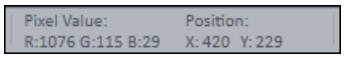
Info

Double click on the performance indicators area opens the Windows task manager.

Frame rate

Indicates the current frame rate in frames per second (fps) used by the active camera for producing new images. Please note in most cases that at speeds greater than 100 frames per second, this value cannot always be accurately determined.

Pixel Value and Position



Pixel Value displays the gray value of the image at the current position of the mouse pointer. In the case of multichannel images the gray value/channel is displayed for up to 4 channels.

Position displays the X/Y position (in pixel coordinates) of the mouse pointer in the image.

Information (i)

If you click on the icon, a window opens with a List of System Messages [▶ 22].

Storage folder

Displays the location where new images are automatically saved. This path can be changed in the menu **Tools** | **Options** | **Saving**.

Info

Double click on the Storage Folder area opens the file where images are saved on your computer.

User

Shows the Windows user name of the logged in user.

Time

Shows the current Windows system time.

3.7.1 List of System Messages

Important system messages are collected here.

Info

If you **right click** on a system message the **Copy** button will appear. Left click on **Copy** button to copy the message to clipboard. Then paste it into a text file or an E-Mail. The idea behind is that you can easily send error messages to your support team for example. This copy/paste function works for all upcoming system messages or error messages within the application as well.

Information

System information that arises during normal operation. This system information does not lead to an interruption of the workflow. The information window is not displayed automatically.

Warnings

Information that requires input from the user, e.g. a prompt to change a manual microscope component. This information leads to the information window being shown briefly. However, it closes again after a few seconds.

Errors

Error messages indicate a malfunction by the system. In this case the information window opens and remains open. The system requires input from the user in order to continue.

Info

Hundreds of messages can accumulate in the course of a session. A maximum of 300 messages are displayed. To display messages for a certain category, activate or deactivate the corresponding checkboxes.

3.8 Workspace configuration

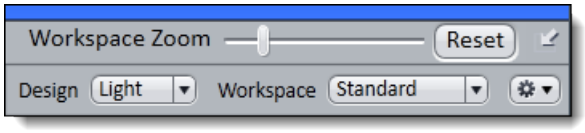


Fig. 8: Workspace Configuration

Here you find settings to adjust your workspace. Select **Light/Dark Design** of the user interface or enlarge the screen with **Workspace Zoom**. Save and reload all your personal settings as a **Workspace** configuration. With the **Dock all tool windows** button in the top right corner you can easily dock all undocked tools back to the **Left Tool Area** by one click.

4 Adjusting workspace appearance

4 Adjusting workspace appearance

4.1 Set user language

Prerequisites

- You have successfully started the application.

Procedure

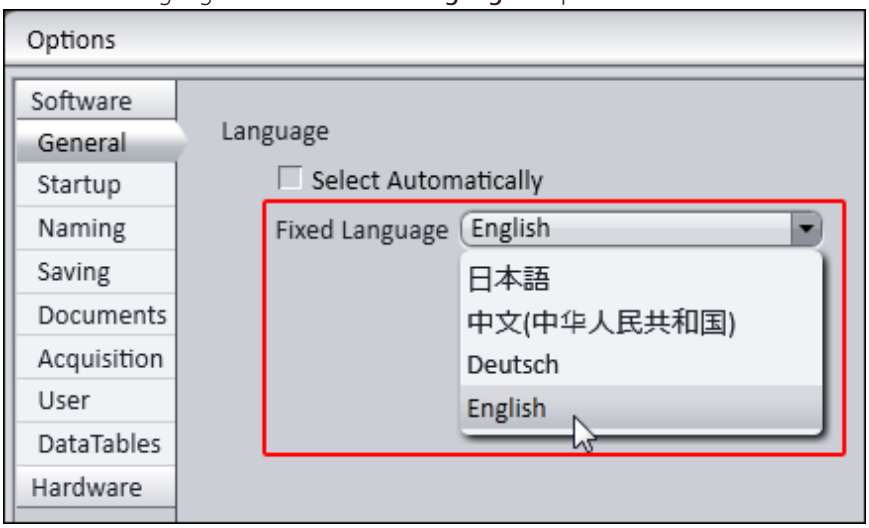
1. Click on menu **Tools** | **Options**.

   The **Options** dialog opens. The **General** entry in the **Software** group is selected.

2. Deactivate the **Select Automatically** checkbox if you want to set the language manually.

> **Info**
>
> If the **Select Automatically** checkbox is activated the software uses the language which is set in the system settings of your computer. This is the default setting.

3. Select user language from the **Fixed Language** dropdown list.



   The message appears to restart the application.

4. Click on **OK**.

   The **Options** dialog closes.

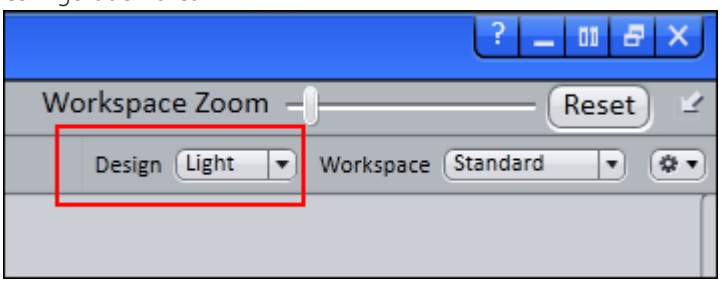
5. Exit and restart software.

You have successfully set the user language.

4.2 Selecting design

Procedure

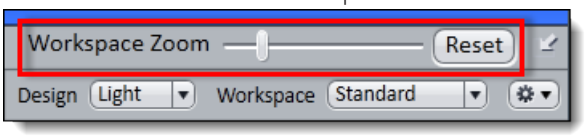
1 Select **Light/Dark** design from **Design** dropdown list in the workspace configuration area.



4.3 Zooming in/out workspace

Procedure

1 To zoom in or out of the workspace move the slider left or right.

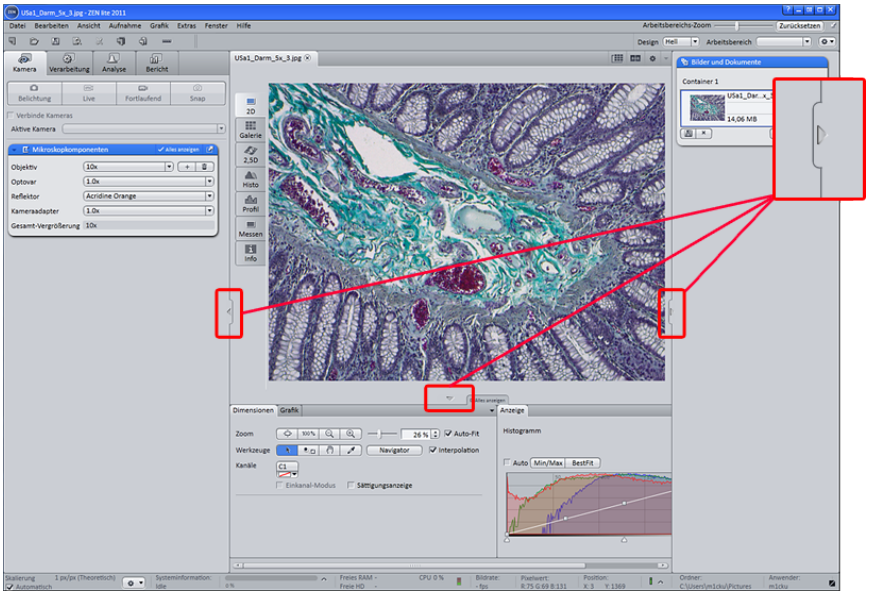


2 To reset workspace zoom to default click on **Reset** button.

4.4 Showing/hiding areas

Procedure

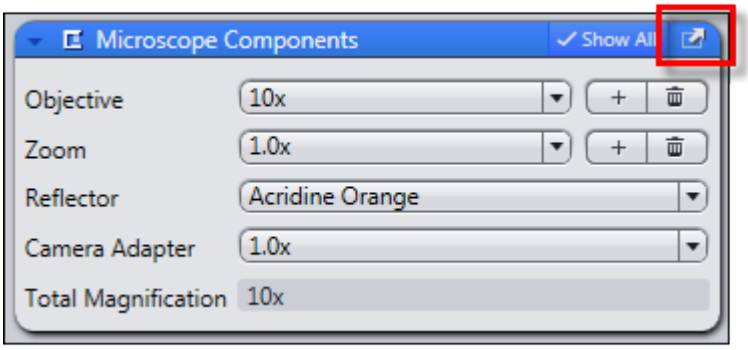
1 Click on **show/hide** buttons to show or hide areas.



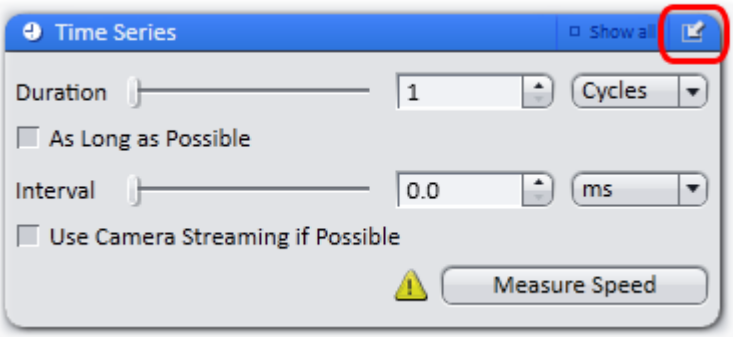
4.5 Undocking/docking tool window

This function allows you to undock/dock a tool window. An undocked tool window can be positioned anywhere on the screen.

Procedure **1** Click the **Undock** button to undock a tool window. Once undocked, the tool window can be moved around by clicking and dragging it on the blue bar.



2 Click the **Dock** button to dock a tool window back to its place in the left tool area.

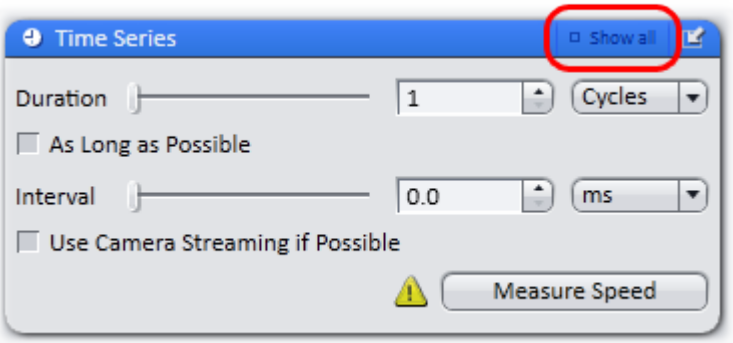


Info

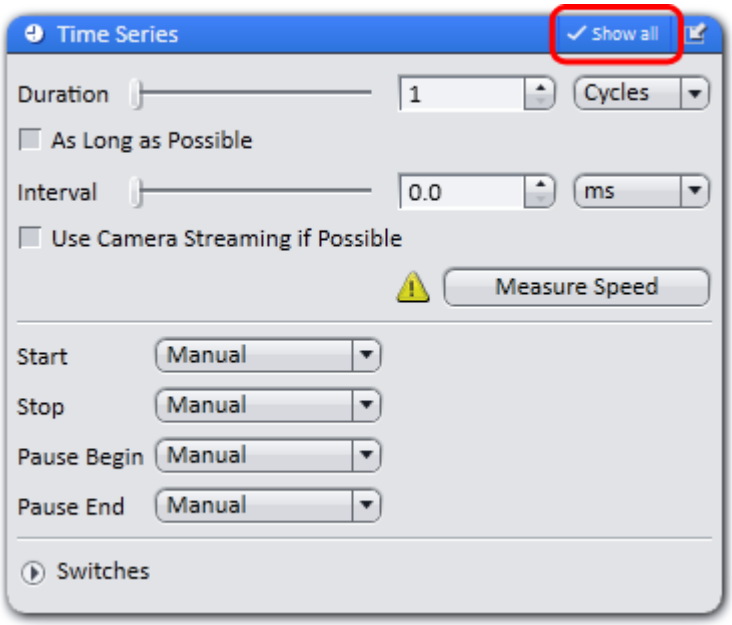
With the **dock all tools** function in the Workspace Configuration [▶ 23] you can globally attach all undocked tool windows back to the **Left Tool Area**.

4.6 Acitvating Show All mode

Procedure **1** With the **Show All** mode deactivated (default setting), only the basic functions of tool windows or view options are shown.



2 To show the advanced settings or expert functions of tool windows or view options, click on the **Show All** button.



5 Step by step to the first image

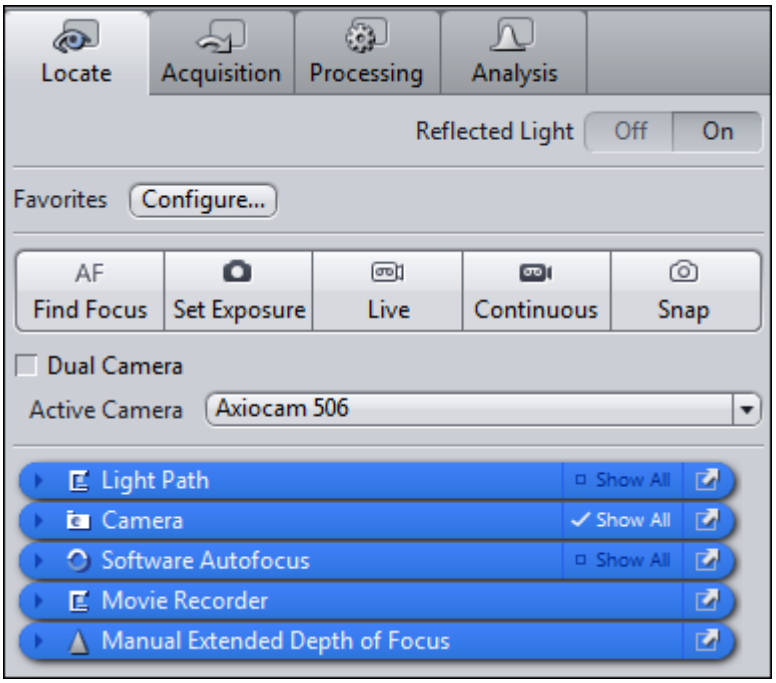
5 Step by step to the first image

5.1 Configure Microscope Components

This chapter refers to the manual configuration of the microscope components in **ZEN lite**. All microscope components definitions will be stored in the meta data of the acquired image.

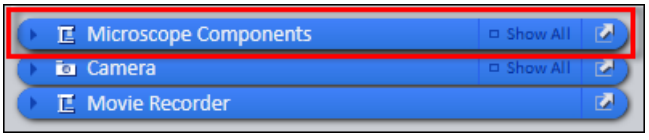
Prerequisites

- You have selected the **Camera** tab.

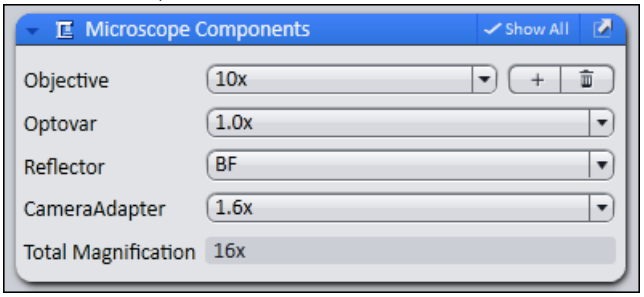


Procedure

1. Click to the blue header of the **Microscope Components** tool.



The tool will open. Consider that the button **Show all** is activated.



2. Under **Objective** select that objective you will use for your acquisitions.
3. Select all other microscope components you eventually will use (i.e. Optovar, Reflector, etc.).

i Info

If you have activated the **automatic** button in the **Statusbar** under **Scaling** (standard settings), the scaling will be calculated on the basis of your definitions. If you want to perform a manual scaling, read the chapter **Create Manual Scaling**.

You have successfully configured your microscope components.

5.2 Create manual scaling

Prerequisites

- You oriented an object micrometer horizontally on the microscope stage.
- You selected correctly all definitions for your microscope in the **Microscope Components** tool (ZEN lite only). In our example we use an objective with a 10x magnification.

Procedure

1. Acquire an image (see Acquire a first image [▶ 35]) of the scale in your object micrometer using the objective to be scaled manually.
2. In the **Status** bar | **Scaling** deactivate the **Automatic** button.
3. Open the **Options** menu and click on the entry **Create New Scaling**.

   The calibration wizard will appear in the image area.
4. Click on single **Reference Line** button (selected as default) and activate the **Automatic Line Detection** button.

i Info

The function **Automatic Line Detection** calculates the theoretical maximum of the reference line's both end points to the closest scale lines in the image. Thus the distance will be calculated with sub-pixel accuracy.

5. Draw in the reference line along the scale.
6. Enter the true distance between both scale lines in the calibration wizard. In our example this is 500 micrometer.
7. Enter a name for the scaling (i.e. Obj 10x) and click the **Save Scaling** button.

You performed a manual scaling for your objective. Repeat this sequence for all objectives you will need a manual scaling for. Always ensure that you did select the correct objective in the tool **Microscope Components** and for this performed and selected the matching scaling in the status bar.

Info

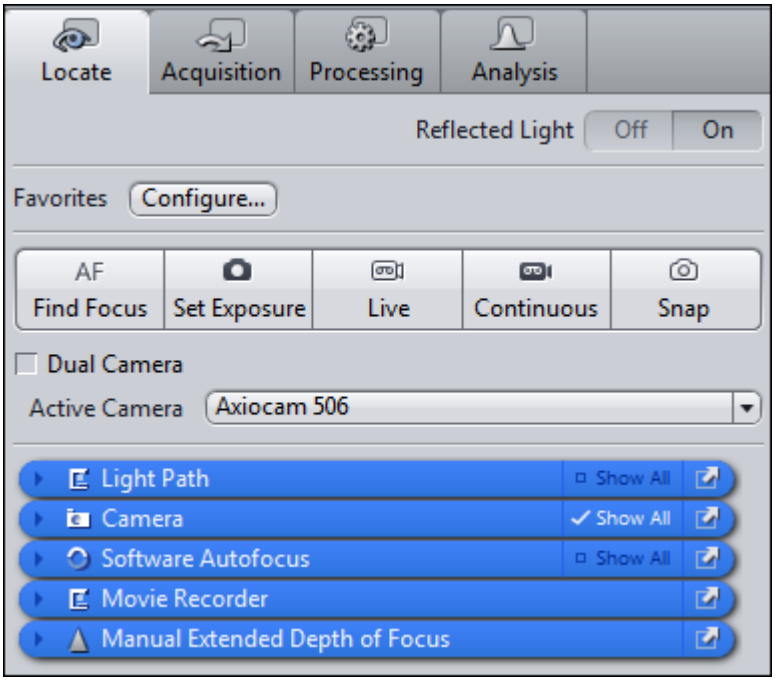
If you defined manual scalings for your available objectives, and if you activate in the **Status bar** under **Scaling** the checkbox **Automatic** again, the system will use the measured scalings instead of the theoretic ones. You will recognize this via the label "**measured**" instead of "**theoretic**" beside the pixel size.

5.3 Acquire a first image

This topic guides you through acquiring your first image with ZEN 2 software.

Prerequisites

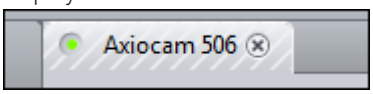
- You have connected and configured a microscope camera (i.e. AxioCam MR) to your system.
- You have started the software.
- You have configured the microscope components (e.g. objective, camera adapter) und you are using the automatic or manual scaling.
- You are on the **Camera** (ZEN light only) or **Locate** tab.
- You see your microscope camera available in the **Active Camera** section. If not, select the camera from the list.



Procedure

1. Position your sample on the microscope and adjust the microscope to see a focused image through the eyepieces.
2. Adjust the tube slider of the microscope to divert the image to the camera (e.g. **50% camera** and **50% eyepieces**).
3. Click on **Live** button.

The **Live Mode** will be activated. You will recognize the **Live Mode** by the green signal and by the hatched tab in the Document Bar [▶ 20]. In the **Center Screen Area** you will see the camera live image. By default the live image shows a cross hair helping to navigate on the specimen. In the chapter Optimize live image settings [▶ 36] you will learn how to optimize live image display.



4 Click on **Set Exposure** button.

The exposure time will be automatically determined and set.

Info

If you do not see a focused image please refocus the specimen on the microscope. You may activate the focus bar as an additional aid. Open the context menu in the **Center Screen Area** via the right mouse key. There select the entry **Focus Bar**. The focus bar will be shown.

5 Click on **Snap** button.

You successfully acquired your first image with **ZEN blue**. Save the image in the file system via the menu **File | Save as**.

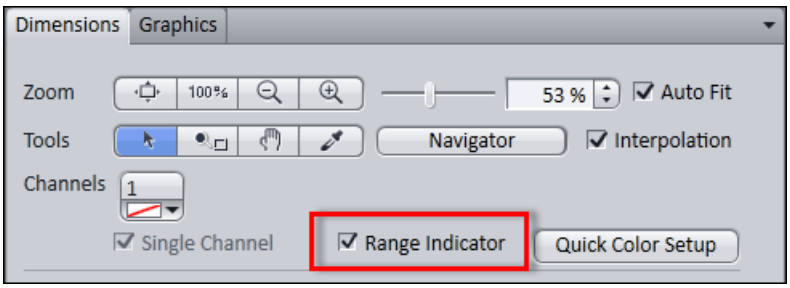
5.4 Optimize live image settings

Prerequisites

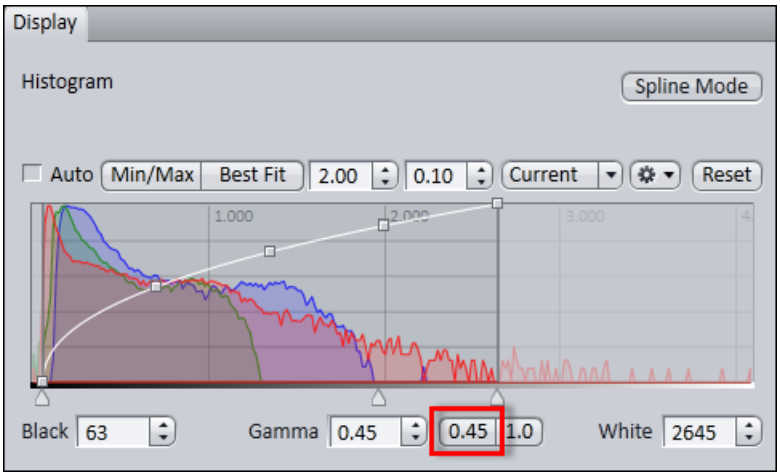
- You have started the **Live** mode via the **Live** button and see the camera's live image in the **Center Screen Area**.
- Under the image area you see the general view options on **Dimensions** tab, **Graphics** tab and **Display** tab.

Procedure

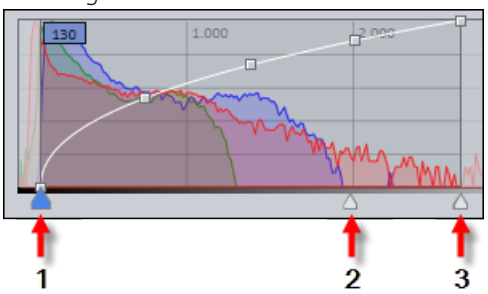
1 In the **Dimensions** tab activate the **Range Indicator** checkbox. This will mark overexposed (too bright) areas in the live image in red and underexposed (too dark) areas in blue.



2 On the **Display** tab click the **0.45** button. The display curve will be adapted to a gamma value of 0.45. This will set the optimum color presentation. If you do not see this button, activate the **Show all** mode.



3 Move the controls under the display curve left and right in order to directly adjust the values for **Brightness** (**White**), **Gamma**, and **Contrast** (**Black**) in the live image.



1 Contrast (black point) control

2 Gamma control

3 Brightness (white point) control

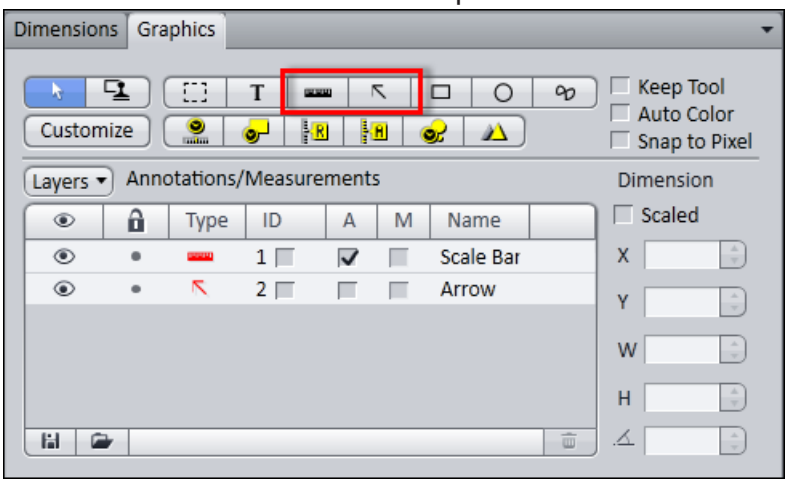
i Info

With the settings above the display of the live image will be adapted. These settings will also be transferred to your acquired image. This will not change the camera settings.

5.5 Add Annotations

Prerequisites ■ You acquired an image with ZEN 2 .

Procedure **1** In the **Center Screen Area** select the **Graphics** tab.



2 Click on the **Scale Bar** button.

The scale bar will appear directly in the image.

Info

Click with the right mouse key to any requested annotation in the image to edit this annotation (e.g. color, line width). This will open the context menu. Select the entry **Format Graphical Elements…** In this dialog you have numerous formatting possibilities.

3 Click on the **Draw Arrow** button.

The button will turn into blue to indicate its activation. Now you may draw an arrow into your image.

You added the annotations **Scale Bar** and **Arrow** from the toolbar to your image.

6 Close software

6 Close software

Prerequisites

- You have acquired or processed an image, created a table or a report with ZEN blue.

Procedure

1. Click on **File | Exit** to end **ZEN blue** software. Alternatively you can press **ALT+ F4** on your keyboard or click on **Close** icon in the **program bar**.

Info

If you haven't saved your files the **Save/Keep Documents** dialog will open before the program closes. Select files you want to save or unselect files you don't want to save.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

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Quick Guide

ZEN 2

Importing and exporting images

We make it visible.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

Effective from: October 2014

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Content

1 Workflow Export/Import

1 Workflow Export/Import

This example describes the workflow for the **Image Export**. The typical workflow is the same for both export and import of images.

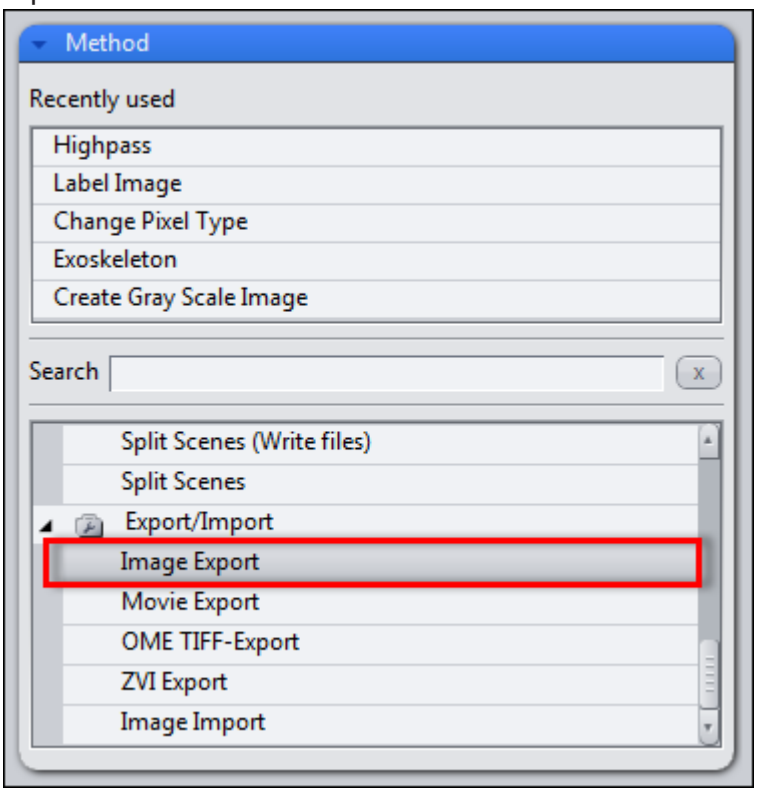
Prerequisites

- You have selected the **Processing** tab.

Procedure

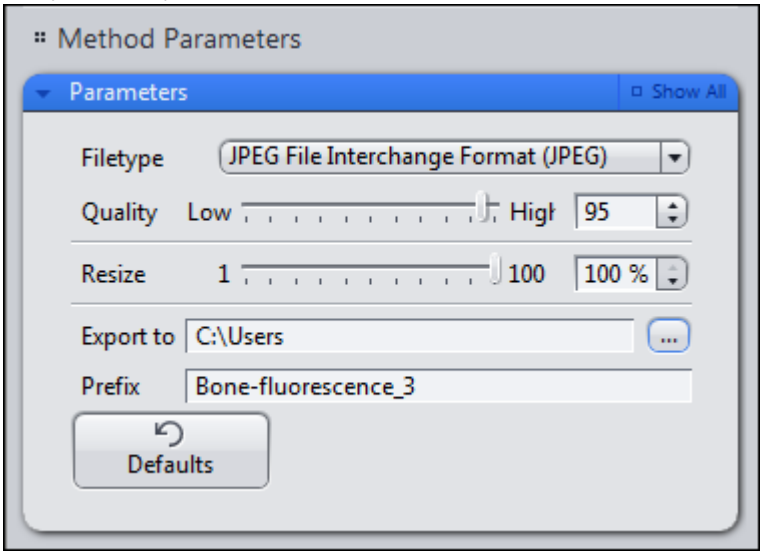
1 **Select a method**

In the **Method** tool open the **Export/Import** group and select the **Image Export** method.



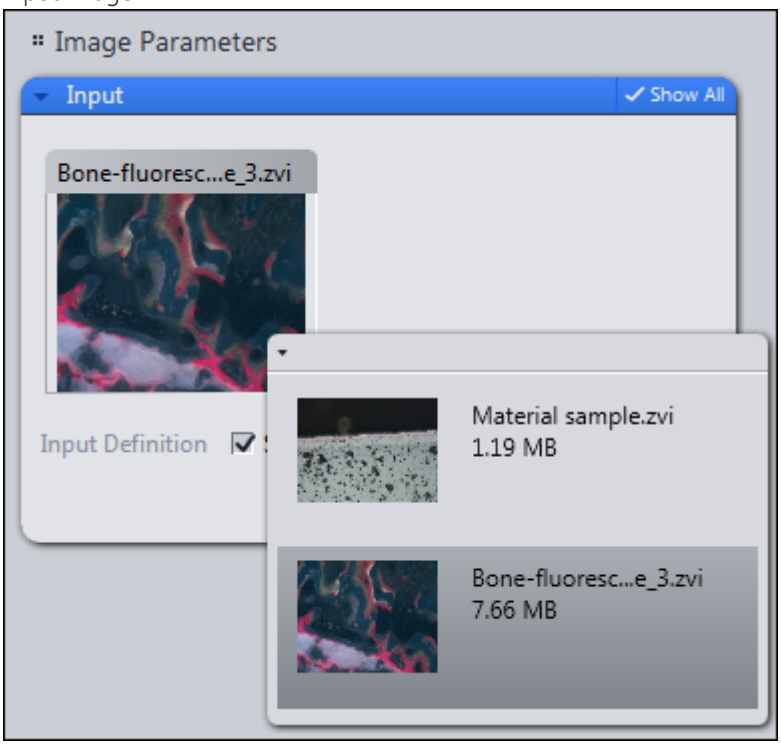
2 Set method parameters

Under **Method Parameters** | **Parameters**, set the desired export settings, e.g. file type, quality, export folder, etc..



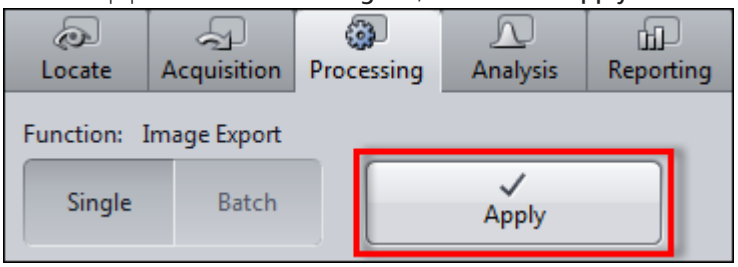
3 Select the image to export

Under **Image Parameters** | **Input**, select the image you want to export. To do this, click on the small preview image within the **Input** tool. You will see a preview of all the open images. To select an image, click on the image that you want to use. This is only necessary if you have several images open simultaneously. By default, the image currently selected is always used as the input image.



4 **Export the image**

On the top part of the **Processing** tab, click on the **Apply** button.



You have successfully exported the selected image.

2 Exporting images with one click

2 Exporting images with one click

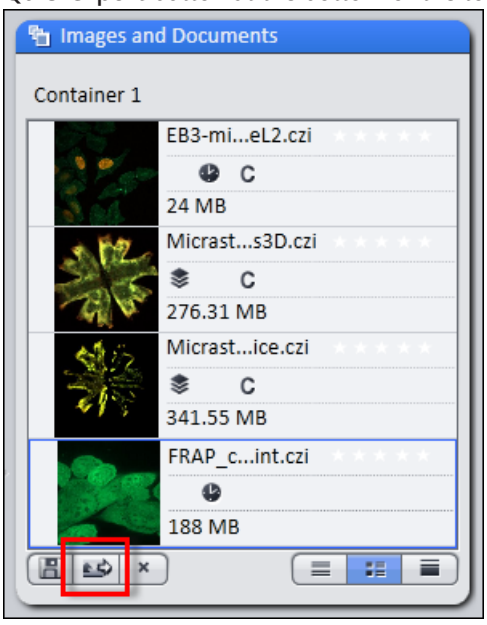
Using the **Quick Export** function you can export images automatically with a single click of the mouse, without setting the method parameters.

Prerequisites

- You have acquired or opened an image.

Procedure

1. In the **Right Tool Area** | **Images and Documents** tool click on the **Quickexport** button at the bottom of the tools window.



Alternatively, you can click on the **Quick Export** entry on the **File** menu | **Import/Export**.

The selected image is automatically exported with the default settings of the **Image Export** method (JPEG, quality 95%, size 100%). The image can then be found in a subfolder within the Windows image folder (**…/User/My Pictures**).

Info

If you export a time lapse image using the Quickexport function, a film is automatically generated using the default values of the **Film Export** method.

3 Exporting movies with one click

3 Exporting movies with one click

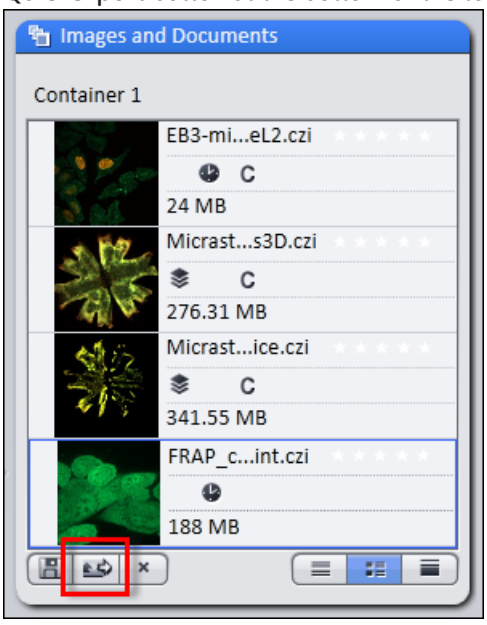
Using the **Quick Export** function you can export films automatically with a single click of the mouse, without setting the method parameters.

Prerequisites

- You have acquired or opened an image from a time series or a Z-stack image.

Procedure

1. In the **Right Tool Area** | **Images and Documents** tool click on the **Quickexport** button at the bottom of the tools window.



Alternatively, you can click in the **File** menu | **Export/Import** on the **Quick Export** entry.

The selected experiment is automatically exported with the default settings of the **Movie Export** method (Mode: **AVI (M-JPEG)**, Format: **Original Size**, Mapping: **1 Frame per image**). The movie will be exported to your default video folder (**.../User/My Videos**).

4 Exporting multichannel images to a ZIP file

4 Exporting multichannel images to a ZIP file

Here you will find out how to export individual images from a multichannel image with three channels and save them automatically in a ZIP archive. You will also discover how to export the whole multichannel image (pseudo color image) as an individual image.

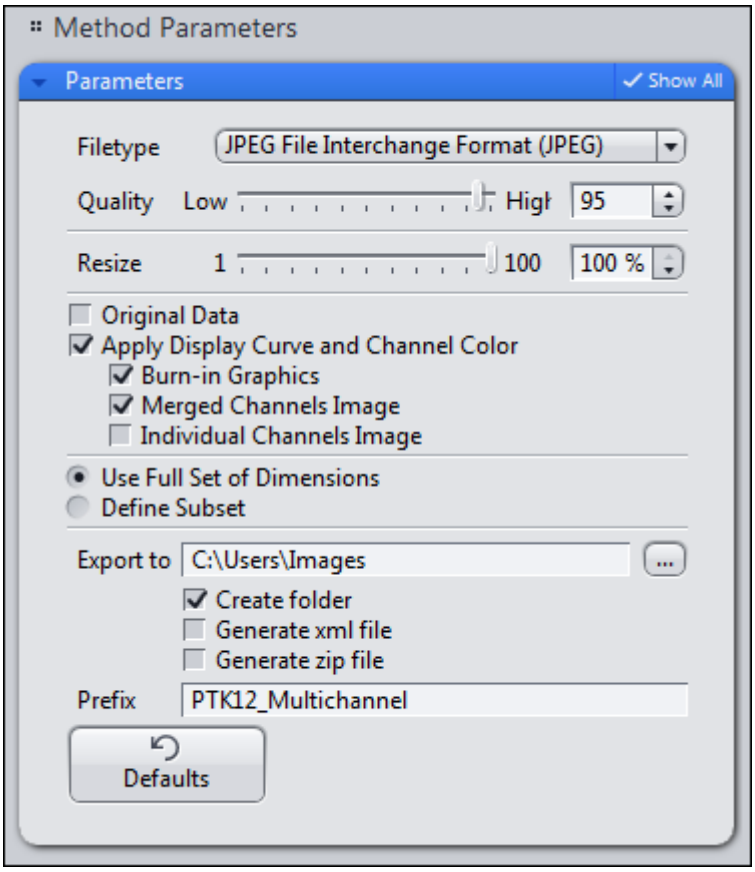
Prerequisites

- You have acquired or opened a multichannel image.

Procedure

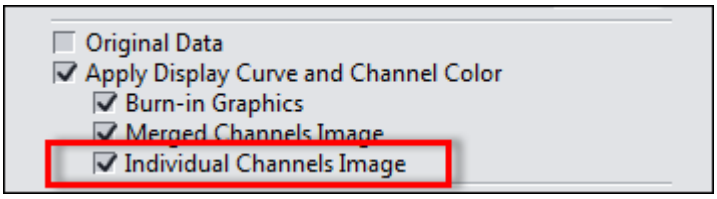
1 In the **Processing** tab open the parameters for **Image Export**.

You will see the default settings of the parameters for image export. Make sure that the **Show All** mode has been activated.

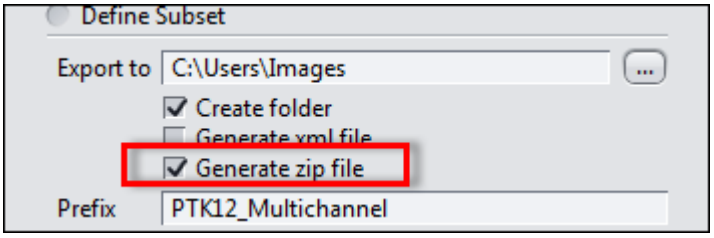


Activate the **Individual Channels Image** checkbox.
This means that an image will be exported from each channel. The **Merged Channels Image** checkbox is activated by default. This means that the pseudo color image (mixed color image from all channels) will also be exported as an individual image.

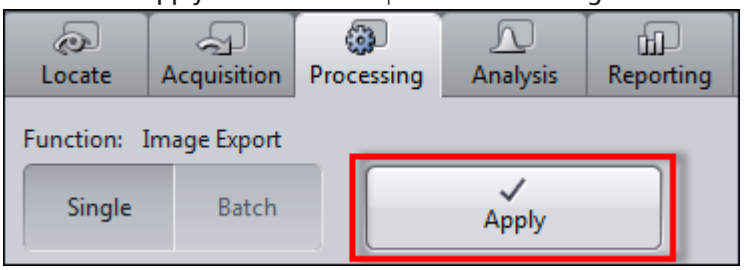
2



3 Under **Export To** activate the **Generate zip file** checkbox. If you have activated the **Create folder** checkbox, a subfolder with the name of the prefix will be created. The ZIP file is then saved in the subfolder.



4 Click on the **Apply** button at the top of the **Processing** tab.



You have exported the images of the individual channels and the pseudo color image of your multichannel image and automatically saved them in a zip file. The file containing the individual images can be found in the export folder indicated.

5 Exporting the individual tiles of a tile image

5 Exporting the individual tiles of a tile image

Prerequisites

- You have acquired or opened a tile image.

Procedure

1. In the **Processing** tab open the parameters for **Image Export**.

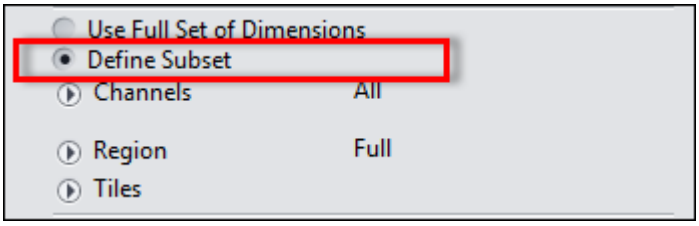
   You will see the settings of the parameters for image export. Make sure that the **Show All** mode has been activated.

2. Select the file type that you want to use. We recommend the **PNG** format, as this is a format that offers lossless compression with an acceptable file size.

> **Info**
>
> In the case of particularly large tile images, we recommend that you reduce the size of the images you want to export. To do this, set a percentage under **Resize** by which you want the images for export to be reduced, e.g. by 25%.

3. Activate the **Apply Display Curve and Channel Color** checkbox. This means that the images will be exported with the settings you have made, e.g. tonal value corrections or contrast. If you activate the **Original Data** checkbox, the images are exported unchanged. In this case, the settings from the display characteristic curve, e.g. tonal value corrections and contrast, are not adopted.

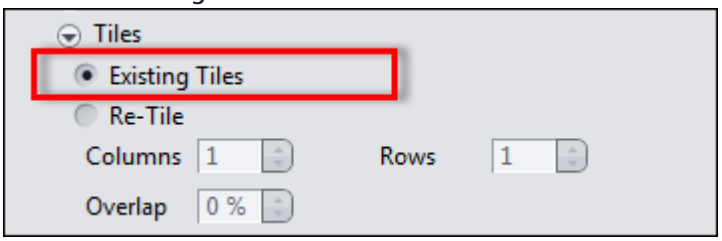
4. Select the **Define Subset** radio button.



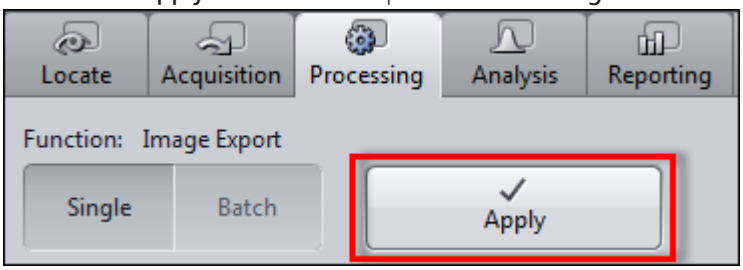
   The settings for the available dimensions will be displayed.

5. Open the settings for the **Tiles** dimension.

6. Select the **Existing Tiles** radio button.



7 Click on the **Apply** button at the top of the **Processing** tab.



You have successfully exported the individual tiles from a tile image. The files can be found in the export folder indicated.

6 Importing a Z-stack image from individual images

6 Importing a Z-stack image from individual images

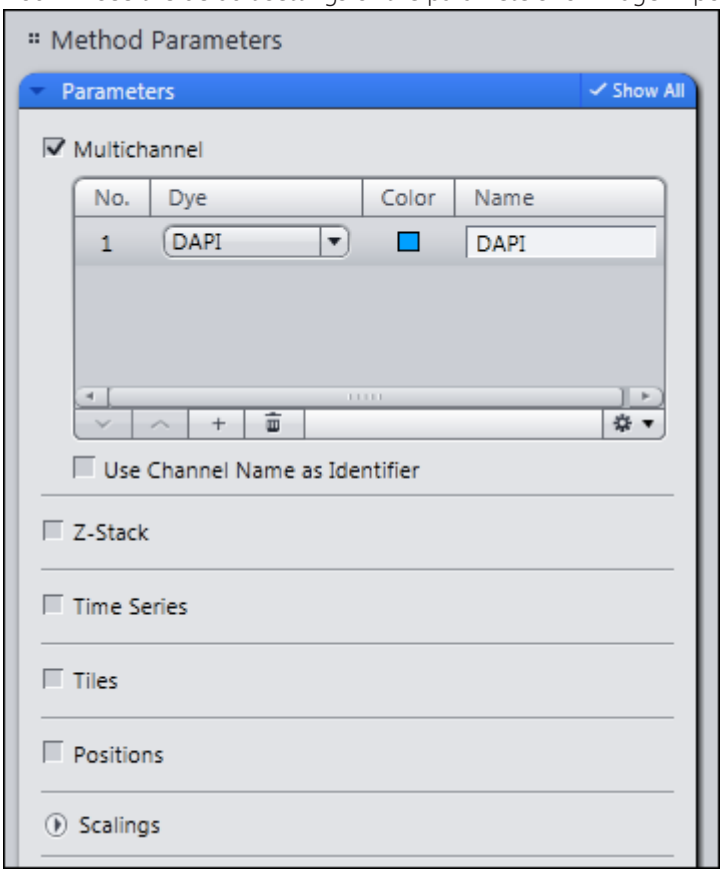
Prerequisites

- You have saved the individual images of a Z-stack in a folder on your computer. The images have been named systematically, e.g. Image_Z0, Image_Z01, etc...

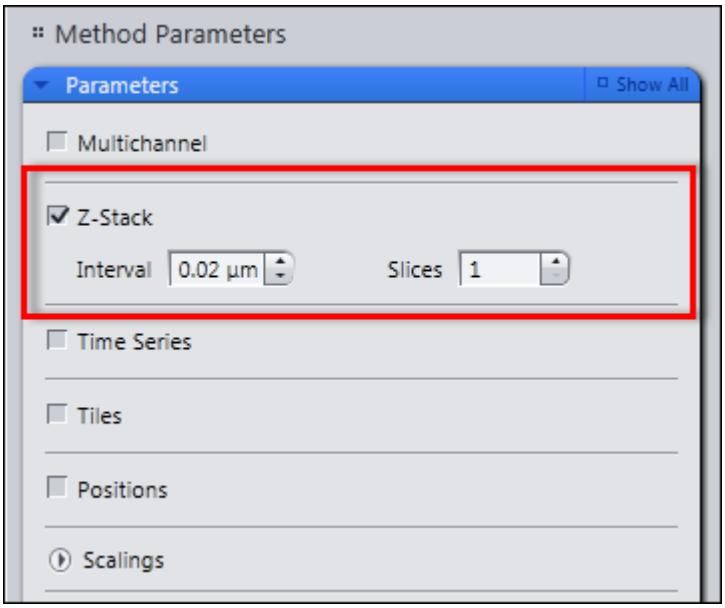
Procedure

1. In the **Processing** tab open the parameters for Image Export.

   You will see the default settings of the parameters for image import.

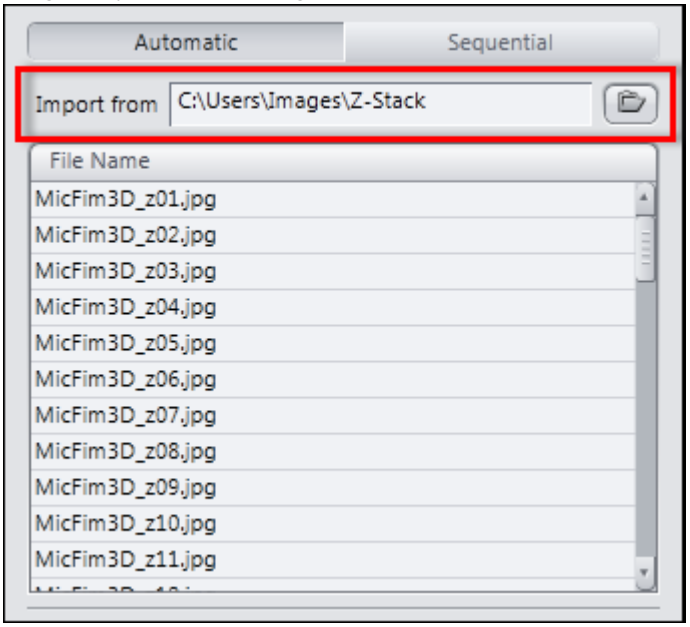


2 Activate the **Z-stack** checkbox. Deactivate all the other dimensions.



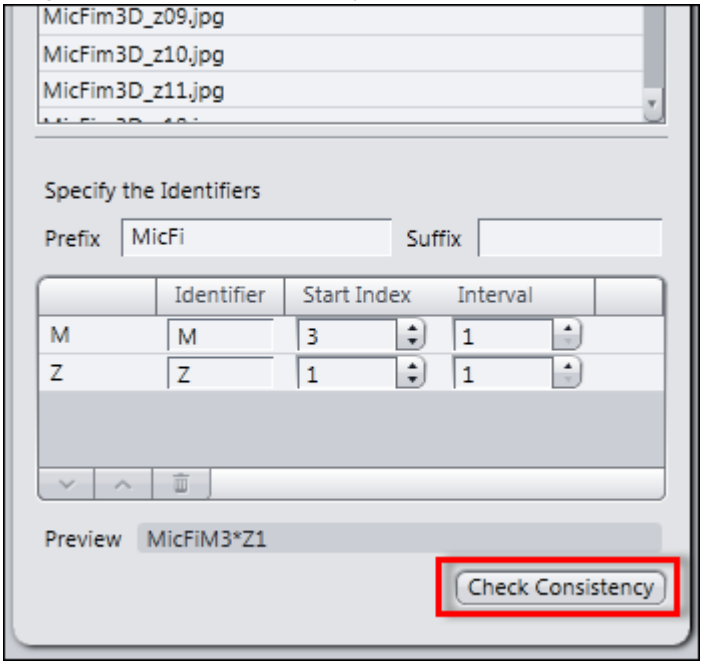
3 Enter the **Interval** for the Z-stack. The number of slices is set automatically if the images have been named systematically (see above).

4 In the **Import From** section, select the folder that contains the individual images of your Z-stack image.

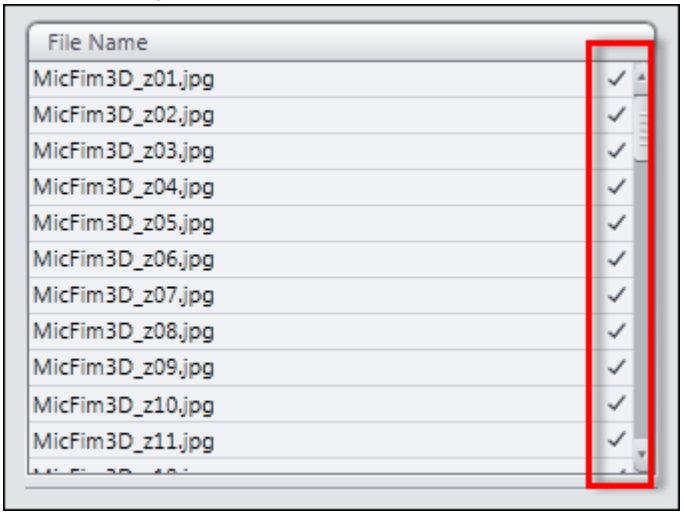


The individual images are displayed automatically in the list under the import directory.

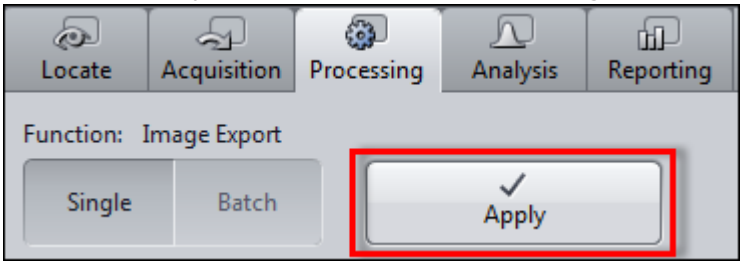
5 Click on the **Check Consistency** button. This allows you to check whether the images can be imported correctly.



A check mark appears after each file name in the list. You can import the individual images now.



6 Click on the **Apply** button at the top of the **Processing** tab.



The individual images are imported and combined to form a Z-stack image. You have successfully imported a Z-stack image from individual images.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

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Quick Guide

ZEN 2

Acquiring multidimensional images

We make it visible.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

Effective from: October 2014

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Content

1 Introduction

1 Introduction

This quick guide shows the most important steps that you must perform within the software to acquire multi-dimensional images. It is primarily concerned with understanding the workflow and get to know the software, not a detailed description of all available options.

We also limit to max. 2 dimensions, e.g. channels (multi-channel experiments), channels + positions (multi-position experiments) channels + time series (time series experiments). The correct configuration of all hardware components (e.g. camera, motorized stage, motorized filter wheels, etc.) in the **MTB 2011** (MicroToolBox) and the correct settings of the light path required in the **Locate** tab are explained in separate instructions. They are not part of this quick guide.

In the first chapter (Setting up multi-channels experiments [▶ 13]), you will learn how to create new experiments in the **Acquisition** tab. After that, the two variants will show you how to configure one or more channels for acquisition. This approach is the basis for all multi-dimensional experiments. To further your knowledge, in addition, the configuration of simple **multi-position** and **time series** experiments is described in the following chapters.

2 Acquiring Multi-Channel images

2 Acquiring Multi-Channel images

In the following chapters you will learn how to set-up and run multi-channel experiments with ZEN 2 quick and easy.

i Info

Make sure that you work with a fully motorized microscope system. In advance all microscope components (e.g. objectives, filters, etc.) must be configured correctly in the Microtoolbox (MTB) software.

In principle there are two variants for setting up multi-channel experiments. The first variant uses Smart Setup, while the second variant uses the **Channels** tool. Both variants have similarities and differences, which are presented in the following overview:

Commonalities

- Fluorescent dyes and transmitted light techniques can be selected from a database.
- Hardware settings for motorized microscopes, which take the properties of the selected dye and the available microscope hardware into account, can be created automatically.
- Bases for experiments can be created using both variants and experienced users can optimize settings further.

Differences

Smart Setup	Channels tool
A maximum of 4 fluorescence channels and 1 transmitted light channel are available	No restriction on the number and type of channels
Offers up to 3 proposals of variants of the experiment (depending on the selected combination of dyes and available hardware)	-
Offers more optimization of experiment settings by using the Motif buttons	-
Graphic overview of the expected signal strength for the selected dyes	-

Smart Setup	Channels tool
Graphic overview of the expected spectral crosstalk with the selected dye combinations	-
Display of the excitation and emission spectra of the selected dyes	-
-	Channels can be configured for dyes that are not supported (or not supported sufficiently well) by the available hardware

2.1 Set up a new experiment

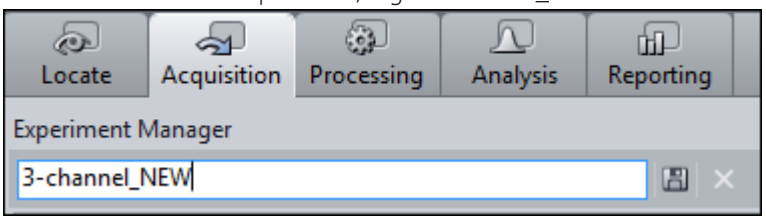
Prerequisites

- You have switched on and configured your microscope system and all components.
- You have successfully started the software.

Procedure

1. In the **Left Tool Area** click on the **Acquisition** tab.
2. In the **Experiment Manager** click on the **Options** button  .

   The **Options** dropdown list opens.
3. To create a new, "empty" experiment, click on the **New** entry.
4. Enter a name for the experiment, e.g. "3-channel_NEW".

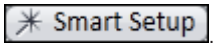


5. To create the experiment, click on the **Save** button  .

You have created a new, blank experiment. All other settings are now stored in this experiment. If you make changes to the experiment, an asterisk (*) after the file name appears. This means that the experiment was modified and not saved. Save your experiments from time to time to ensure that your settings are not lost.

2.2 Variant 1: Configure channels by using Smart Setup

Procedure

1. Click on the **Smart Setup** button  .

   The **Smart Setup** dialog opens.

2 To add a channel, click on the **Add** button in the **Configure your Experiment** section  .

The **Add Dye or Contrasting Method** dialog opens.

3 Select the desired dye or contrast method.

4 Click on the **Add** button. Alternatively you can double-click on the entry in the dye database. The dye is then adopted directly into the experiment.

You have added a channel to your experiment. To add further channels, repeat the last 2 steps.

Info

If you see the error message "**Smart Setup calculation failed**", it was not possible for Smart Setup to calculate any proposal. This may be because the filters and light sources available on the system do not allow an image of the dye to be acquired with a good signal strength or with little crosstalk. The channel for this dye or the contrast method cannot therefore be created. In this case, try selecting another, similar dye.

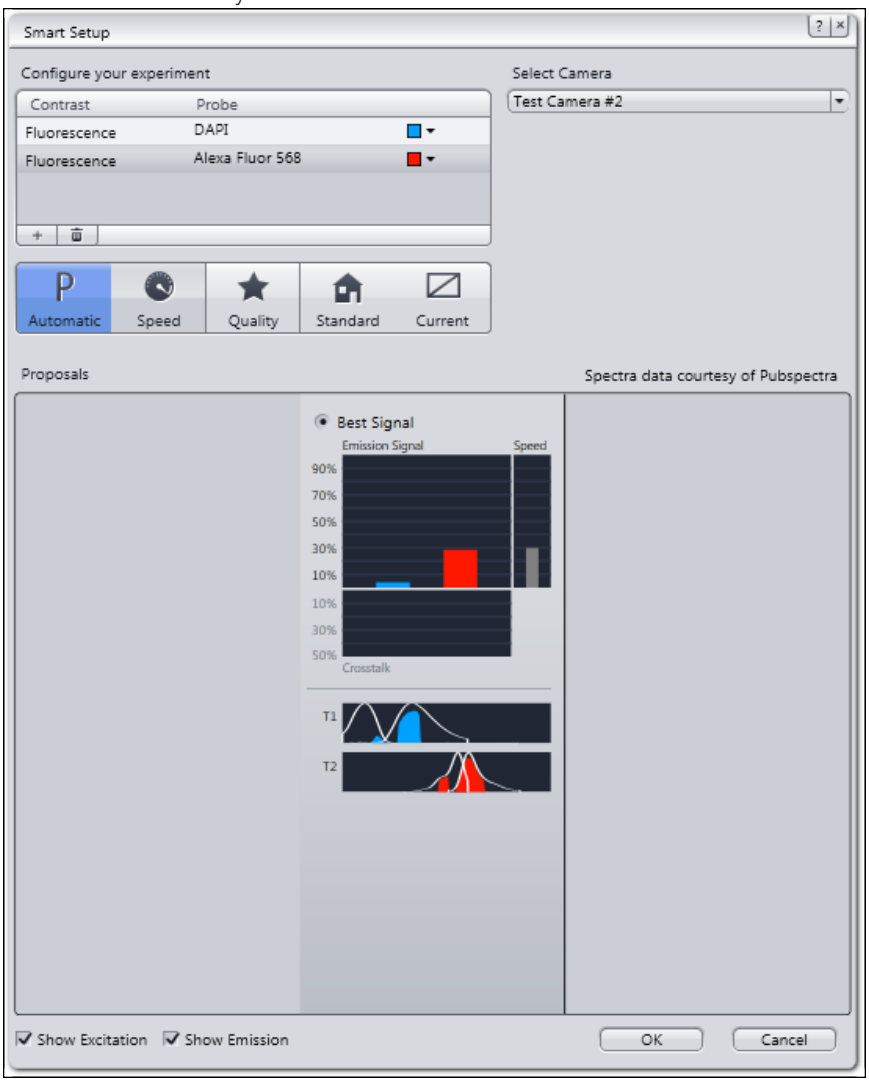
Should the error message be displayed for all dyes that you select, this may be due to one of the following causes:

- no light source has been configured or the light source is switched off

- no camera has been configured on the system, the camera is not connected or (on some models) has been switched off.

5 To return to **Smart Setup**, click on the **Close** button.

You will now see a graphic overview in the **Proposals** section. This displays the spectra of the dyes, the expected signal strengths per dye and the spectral crosstalk schematically.



Info

Depending on which dye you have selected and the microscope hardware available, up to three different proposals (Best Signal, Fastest, Best Compromise) are displayed. These differ in terms of signal strength, crosstalk and speed. Select the proposal that best meets the needs of your experiment.

6 To select a proposal (if there's more than one), activate the **radio** button on top of the proposal.

7 To optimize experiment settings additionally, click on a **Motif** button. Automatic button is set as default setting.

Info

By the **Motif** buttons you can optimize acquisition parameters and camera settings automatically either for a high quality (**Quality** button) image or a faster acquisition but reduced image quality (**Speed** button). Find a more detailed description of **Motif** buttons in Smart Setup dialog.

8 To optimize experiment settings, adopt the suggestion and leave **Smart Setup**, click on the **OK** button.

The added channels are adopted automatically into the **Channels** tool.

9 Click on the **Set Exposure** button in the **Action buttons** bar on top of the **Acquisition** tab.

The exposure time is now measured for all three channels one after the other. This is adopted into the settings for the channels. Following the measurement of the exposure time, the multi-channel image is acquired automatically and displayed in the **Center Screen Area**.

10 To save the experiment together with all the settings, click in the **Experiment Manager** on the **Options** button  .

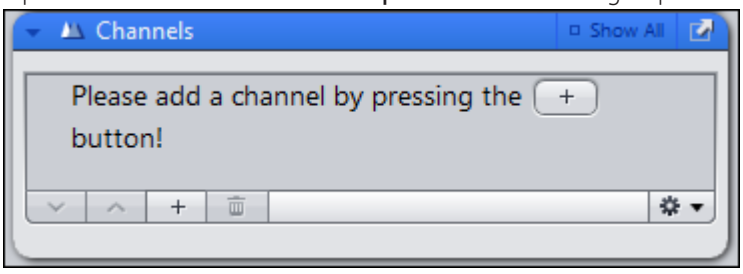
11 In the **Experiment Manager** click on the **Save** entry in the dropdown list.

You have set up the multichannel experiment using **Smart Setup**, executed it and then saved the configuration. This means that you can repeat the experiment as often as you like using the same settings.

2.3 Variant 2: Configure channels by using Channels tool

Procedure

1 Open the **Channels** tool in the **Acquisition Parameter** group.



2 To add a channel, click on the **Add** button  .

The **Add Dye or Contrast Method** dialog opens.

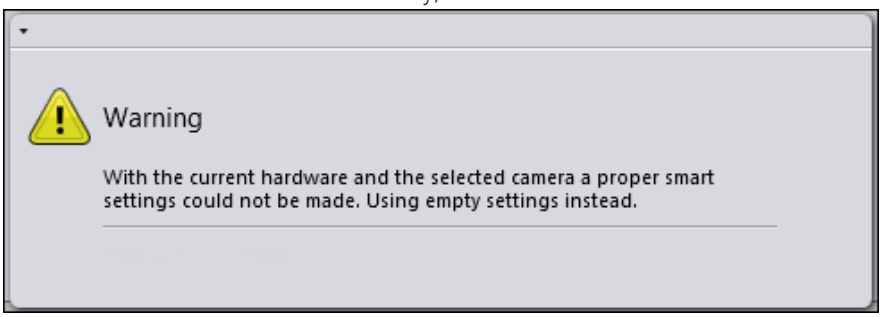
3 Select the desired dye or contrast method.

4 Click on the **Add** button at the bottom of the dialog.

You have added a channel to your experiment. To add more channels, repeat the last 2 steps.

Info

If you see this warning for a few seconds (see below), the software is unable to calculate a suggestion for the acquisition of this dye or contrast method. This may be because the filters and light sources available on the system do not allow an image to be acquired with a good signal strength or with little crosstalk. The channel for this dye or contrast technique is nevertheless created and can be found in the **Channels** tool. The appropriate acquisition settings cannot be created for this automatically, however.



Try selecting another, similar dye or edit the available suggestion manually in the **Acquisition Light Path** tool.

5 Click on the **Close** button.

You will see the added channels in the **Channels** tool.

6 Click on the **Set Exposure** button in the **Action buttons** bar on top of the **Acquisition** tab.

The exposure time is now measured for all three channels one after the other. This is adopted into the settings for the channels. Following the measurement of the exposure time, the multi-channel image is acquired automatically and displayed in the **center screen area**.

7 To save the experiment together with all the settings, click in the **Experiment Manager** on the **Options** button  .

8 Click on the **Save** entry in the dropdown list.

You have set up the multichannel experiment using the **Channels** tool, executed it and then saved the configuration. This means that you can repeat the experiment as often as you like using the same settings.

3 Acquiring Time Series images

3 Acquiring Time Series images

Prerequisites

- To set up **Time Series** experiments you need to license the **Time Series** module.
- You have set up a new experiment [▶ 14], at least defined one channel [▶ 13] and adjusted focus and exposure time correctly.
- You are on **Acquisition** tab.

Procedure

1. Activate the **Time Series** tool by activating the **Time Series** checkbox in the **Acquisition Dimensions** section.

   The **Time Series** tool appears in the **Left Tool Area**.

2. Open the **Time Series** tool.
3. Set length of your time series by the **Duration** slider. You are able to select an interval (days, hours, minutes, seconds) or the cycles (1-n) e.g. 10 cycles.
4. Set interval of your time series by the **Interval slider**, e.g. 5 s.
5. Click on **Start Experiment** button.

The time series experiment will be started. You`ve successfully learned the basics of how to set up time series experiments. In our example in 10 cycles after each 5 seconds an image is acquired. The time Series image also contains 10 single images.

4 Acquiring Multi-Position images

4 Acquiring Multi-Position images

CAUTION

Damage to the device because of missing calibration

A missing or false calibration of stage, focus and sample carrier can lead to damage on your microscope system.

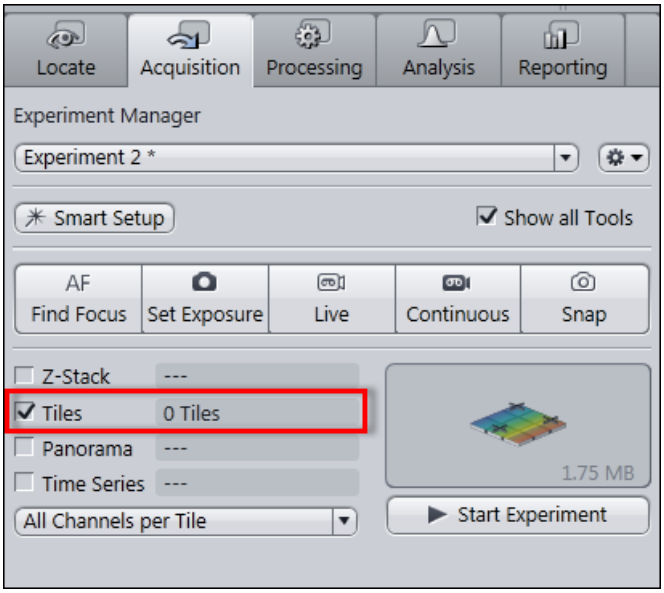
Calibrate **stage**, **focus** and the **sample carrier** before you set up or execute a **Tiles** or **Position** experiment.

Prerequisites

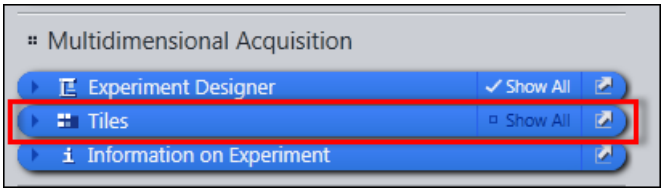
- You are on the **Acquisition** tab.

Procedure

1. Set up a new experiment in the **Experiment Manager**, see Set up a new experiment [▶ 14].
2. Configure at least one channel for image acquisition either by using **Channels** tool or **Smart Setup**.
3. Click on **Set Exposure** button in the **Action buttons** bar on top of the **Acquisition** tab. This will calculate exposure time automatically.
4. Click on **Find Focus** button. This will focus your sample automatically. If necessary adjust the fine focus of the sample manually by moving the z-drive of the microscope via **Live** image.
5. Activate the **Tiles** checkbox in the **Acquisition Dimensions** section.

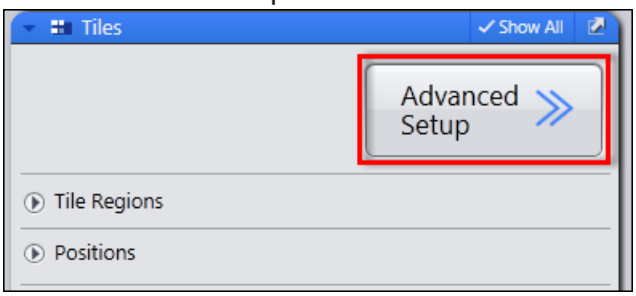


The **Tiles** tool appears in the **Multidimensional Acquisition** tool group.



6 Open the **Tiles** tool window.

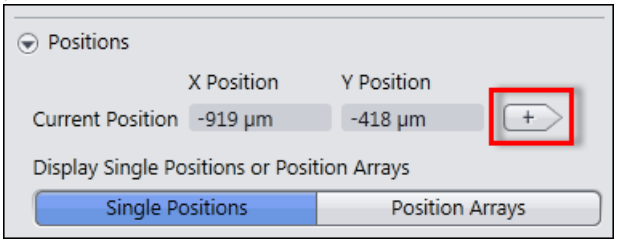
7 Click on **Advanced Setup** button.



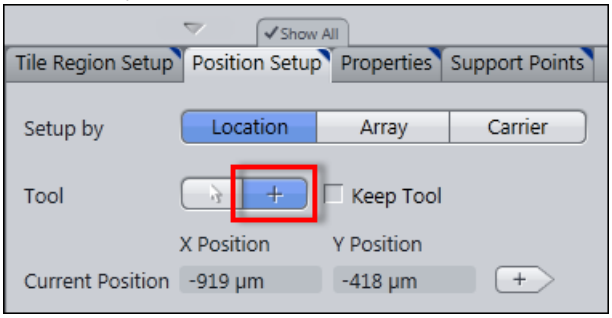
Advanced Tiles Setup (ATS) view opens. You see the **Live** image from the camera in the **Live Navigator** tool.

8 Move **Live Navigator** tool to a position where you want to add a position by simply double clicking at the position.

9 Click on **Add** button in **Tiles** tool | **Positions** section, to add this position to your experiment.



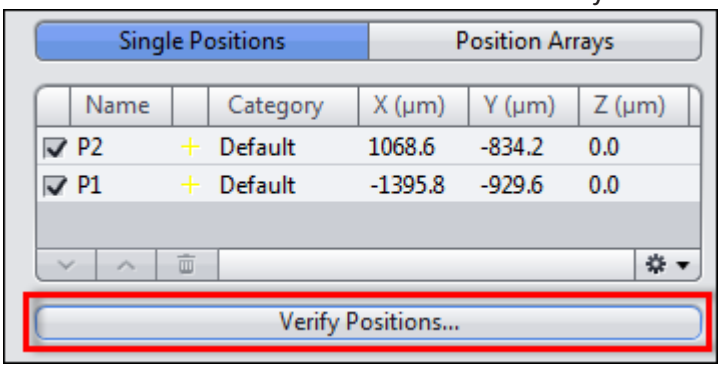
10 Alternatively click on **Add** button in **Position Setup** view control tab.



11 To add further positions, repeat the last 3 steps.

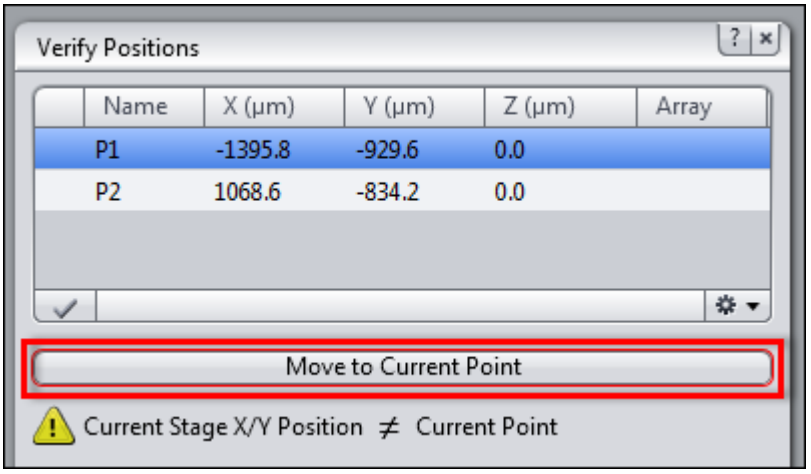
As you have added several positions to your experiment you can go on verify the focus values of each position whether automatically or manually. Proceed as follows:

12 Click in **Tiles** tool in the **Positions** section on the **Verify Positions** button.



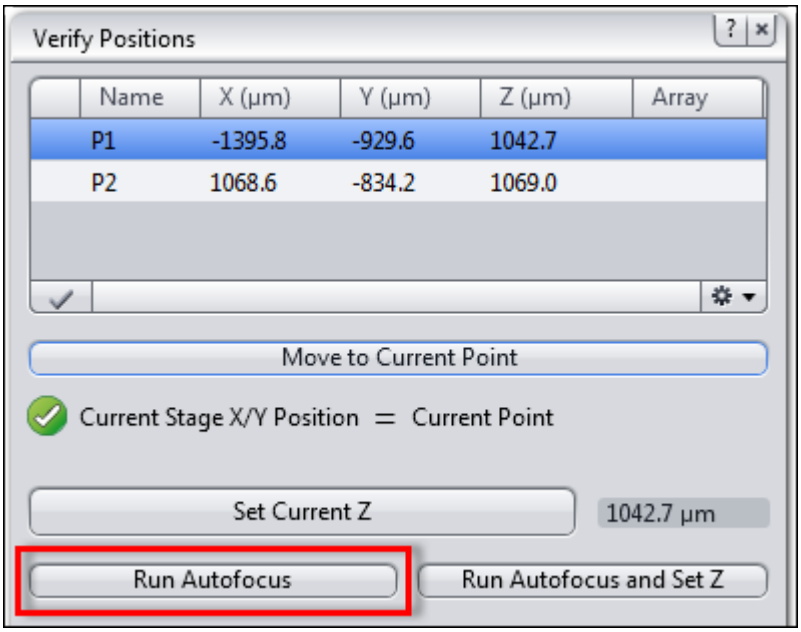
The **Verify Positions** dialog opens.

13 Click on **Move to Current Point** button.



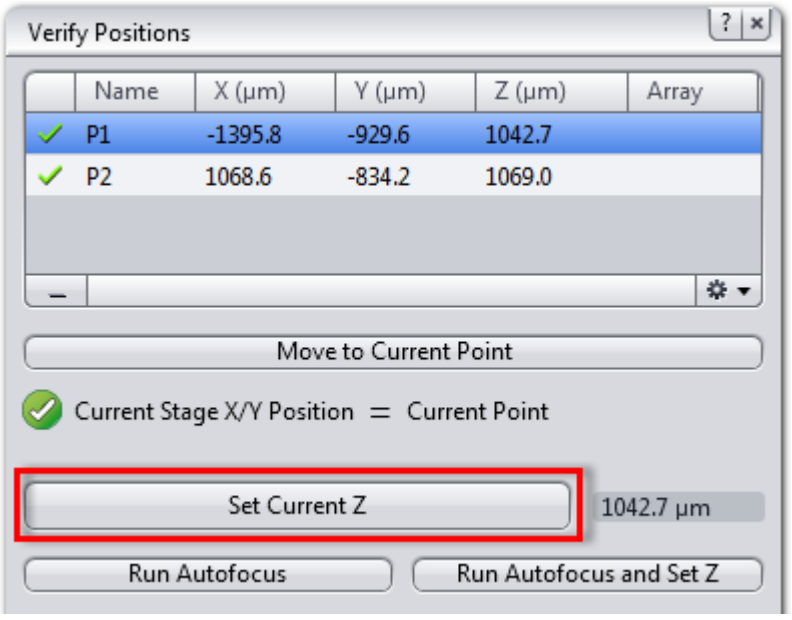
The stage moves automatically to the blue highlighted position (e.g. P1) in the positions list. Alternatively double click on the list entry to move the stage to that position.

14 Click on **Run Autofocus** button.



An autofocus search will be started. You can also set the fine focus manually by moving the z-drive of the microscope. Make sure that the sample is focused now.

15 Click on **Set Current Z** button.



The position will be marked as verified in the list by a checkmark.

16 Click on **Move to Next Point** button.

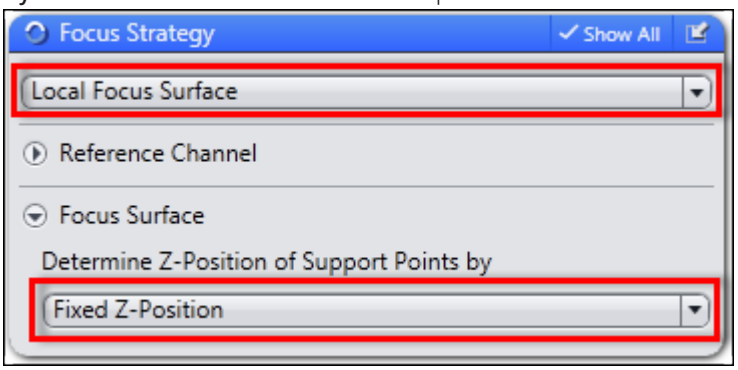
The stage moves to the next position automatically. Repeat the last 3 steps until you have verified all positions in the list. The message **All points have been verified** appears.

17 Close the **Verify Positions** dialog.

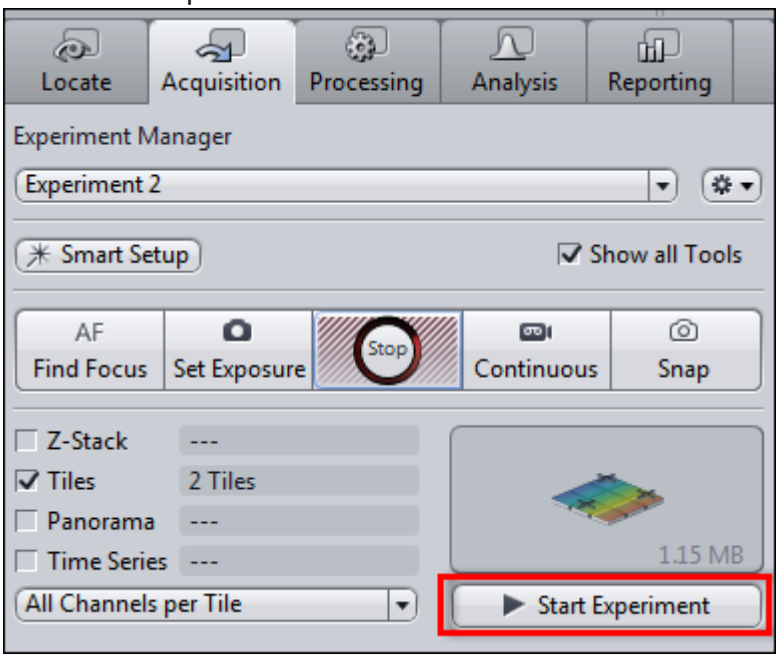
18 In the **Acquisition Parameter** tool group open the **Focus Strategy** tool.

19 Select **Local Focus Surface** as focus strategy from the dropdown list.

20 In the section **Focus Surface** under **Determine Z-position of Support points by** select **Fixed Z-Position** from the dropdown list.



21 Click on **Start Experiment** button.



The multi-positions image will be acquired now.

22 To save the experiment together with all the settings, click in the **Experiment Manager** on the **Options** button  .

23 Click on the **Save** entry in the dropdown list.

You have successfully set up a multi-position experiment, verified the positions focus and acquired the multi-position image.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

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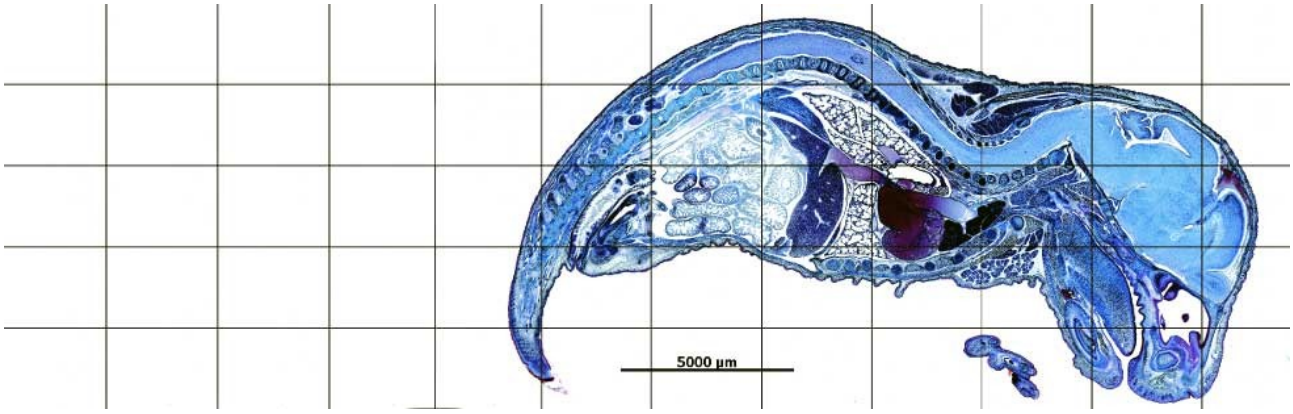

Software Guide

ZEN 2

The Tiles & Positions Module

ZEISS

We make it visible.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

Effective from: June 2015

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1 About this document

1.1 Safety notes conventions

The safety notes in this document follow a system of risk levels, that are defined as follows:

⚠ CAUTION

Risk of personal injury

CAUTION indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate personal injury.

NOTICE

Risk of property damage

NOTICE indicates a property damage message. In addition, NOTICE is used for data loss or corrupt data as well.

i Info

An info indicates useful additional information. Infos help you to make your daily work easier, but they are all optional. There is no risk for personal injury or property damage involved.

1.2 Text formats and conventions

Bold texts

Bold is used for texts within the software like names of GUI elements (e.g. buttons, sections, tools, menus), key commands (e.g. **Crtl + C**), buttons on a device, product names, etc.

Font type "Courier"

`Font type "Couriere" is used for programming code. E.g. macro code as well as for anything that you would type literally when programming, including keywords, data types, constants, method names, variables, class names, and interface names.`

Shortcuts and key commands

Shortcuts do appear like **Crtl+C** , that means you must press **Crtl-Key** and **C-Key** together.

Procedures

Following formats are used for procedures (instructive sequences):

Prerequisites ■ Stands for a condition which must be fulfilled before starting with the action.

Procedure **1** Stands for a single step the user is asked to perform.

Web-Links

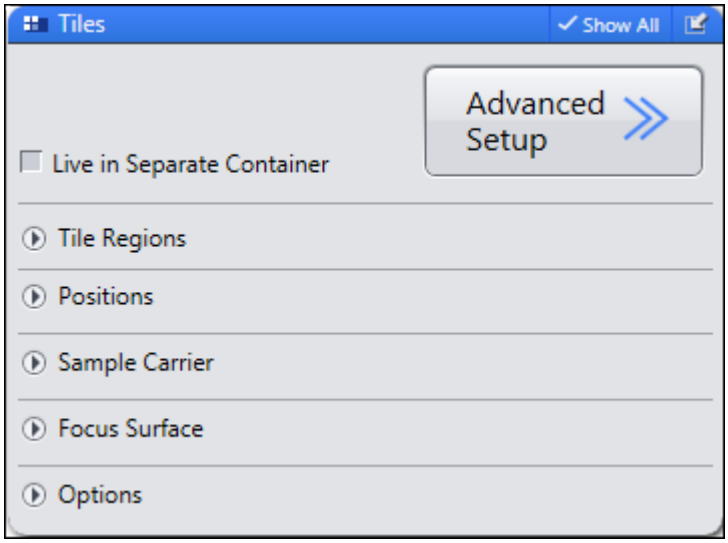
Web links do appear in blue text color. To open the linked website, simply right click on the link. Just in case you are not connected to the internet, make sure you have a internet connection established before opening the web link.

2 User Interface and Functions

2.1 Tiles tool

In the **Tiles** tool you configure the acquisition of images that consist of several image fields. Therefore you define Tile Regions or Positions. In addition you can set up Focus Surfaces and Sample Carrier Templates here.
The **Tiles** tool is only available if you have activated the **Tiles** checkbox in the **Experiment Manager** | **Acquisition Dimensions**.

The Tiles tool is located in the **Left Tool Area** under **Multidimensional Acquisition**.



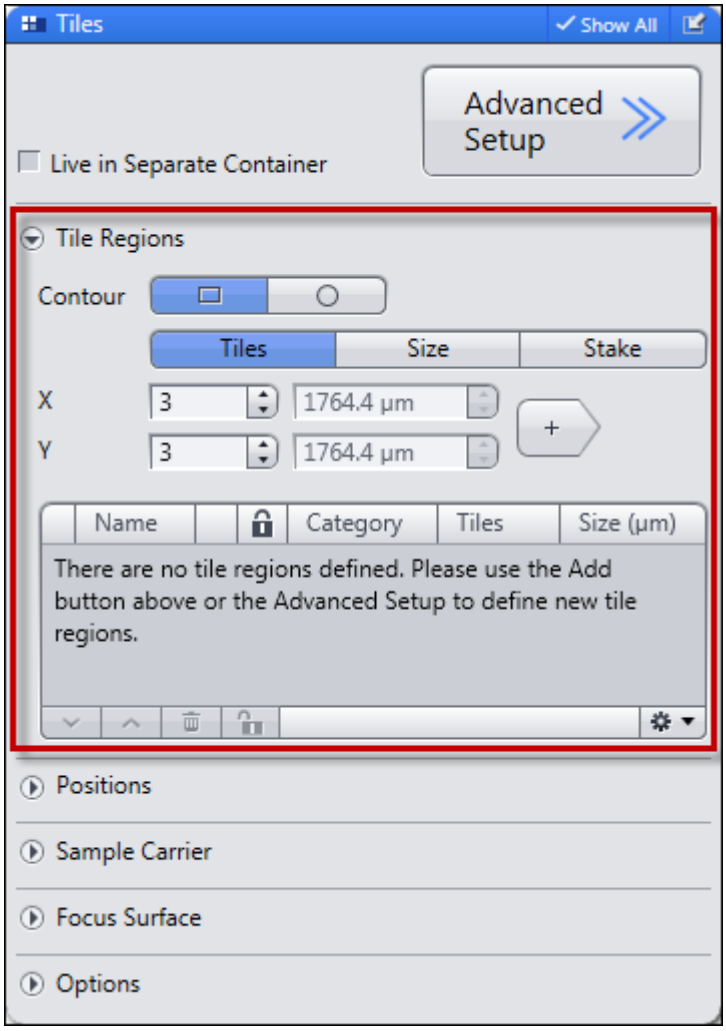
Option	Description
Live in Separate Container checkbox	Opens the **Live** mode in a separated window. Note that the option **Automatic Container Layout** has to be activated.
Advanced Setup button	Opens the *Advanced Tiles Setup* [▶ 32] view in the **Center Screen Area**.

i Info

The **Sample Carrier**, **Focus Surface** and **Options** sections are only visible if the **Show All** mode is activated.
If you have no license for the **Tiles** module you will only find **Tile Regions**, **Positions** and **Options** sections here.

2.1.1 Tile Regions section

To show the section in full, click on the **arrow** button .



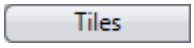
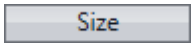
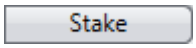
Contour section

Only visible if the **Show All** mode is activated.

Defines the outline of the tile region that you are adding. To learn more about tile regions see glossary "Tile region"

Option	Description
Rectangle button	Adds a rectangular tile region.
Circle button	Adds a circular tile region

Mode section

Option	Description
Tiles button 	Using this mode you have to enter the number of tiles as a reference for the size of the tile region. Enter the number of tiles in the **X / Y** input fields. If you are adding a circular tile region, enter the number of tiles for the diameter in the **Diameter** input field.
Size button 	Using this mode you have to enter the size as a reference for the size of the tile region. Enter the size of the tile region in the **X / Y** input fields. If you are adding a circular tile region, enter the diameter of the tile region in the **Diameter** input field.
Stake button 	This mode allows the definition of a tile region by the placement of at least two markers (user defined X/Y stage coordinates). If you want to modify the tile region (expand/ reduce) you have to adjust the tile region to the desired size. To complete the tile region press **Done**. Circular or rectangular tile region can be created in this manner by selection of the appropriate contour.
Add button 	Adds the tile region to the **Tile Regions List** and activates it for acquisition. Added tile regions are displayed in the form of red grids in the stage view of the **Advanced Tiles Setup**.

Tile Regions list

Displays the added tile regions. The list contains the following columns and buttons:

Option	Description
Checkbox column	Activates the relevant list entry for acquisition.
Name column	Allows you to edit the name of the tile region.
Contour column	Displays the contour of the tile region.
Category column	Displays the category of the tile region. Categories can be defined in the view options of the advanced tiles setup on the properties tab.
Tiles column	Displays the number of tiles of the tile region.
Size column	Displays the size of the tile region.
Z column	Displays the Z-position of the tile region.

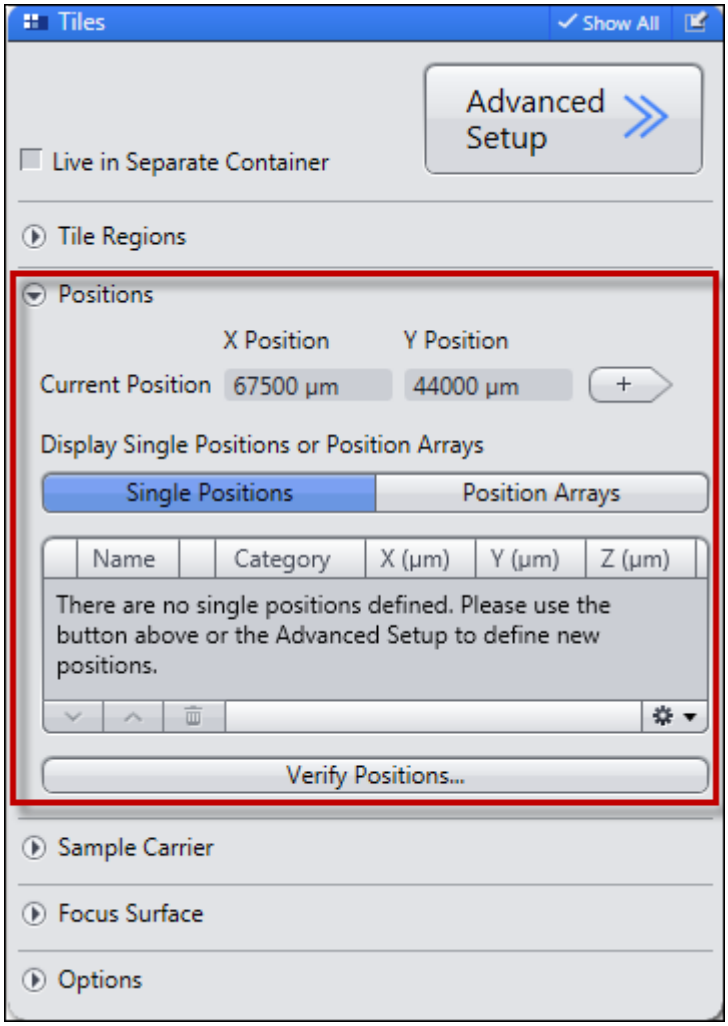
Option	Description
List Navigation buttons 	With the buttons you can shift selected list entry one position up or down in the tile regions list. This allows you to modify the acquisition order.
Delete button 	Deletes the selected list entry.
Lock button 	Unlocks the selected list entries to allow editing.
Options button 	Opens the *Options for editing Tile Regions* [▶ 10].

2.1.1.1 Options for editing Tile Regions

Option	Description
Set Current Z for Selected Tile Regions	Sets the current Z-Position for all selected tile regions.
Delete	Deletes the current tile region.
Delete All	Deletes all tile regions.
Activate	Activates the current tile region for acquisition.
Deactivate	Deactivates the current tile region for acquisition.
Unlock	Unlocks the current tile region.
Unlock All	Unlocks all locked tile region.
Sort	**By Center Position (Y -> X)** sorts all tile regions according to their overall Y position.
	By Center Position (X -> Y) sorts all tile regions according to their overall X position.
	By Category sorts all tile regions according to their category.
Convert to Positions…	Converts a selected tile region into Positions or a Position Array.

2.1.2 Positions section

To show the section in full, click on the **arrow** button .

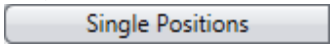
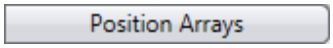


Current Position section

Displays the current stage position.

Option	Description
X Position display field	Displays the X coordinate of the current position.
Y Position display field	Displays the Y coordinate of the current position.
Add button 	Adds the current position to the **Positions List** and activates it for acquisition.

Display mode section

Option	Description
Single Positions button 	Shows the **Single Positions List**. To learn more about single positions see glossary "Position".
Position Arrays button 	Shows the **Position Arrays List** and the **Positions of selected Array List**, that shows a full **Single Positions List** for the selected position array. To learn more about position arrays see glossary "Position".

Single Position List

Displays the added positions. The list contains the following columns and buttons:

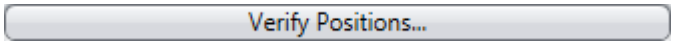
Option	Description
Checkbox column	Activates the relevant list entry for acquisition.
Name column	Displays the name of the single position.
Category column	Displays the category of the single position. Categories can be defined in the view options of the advanced tiles setup on the properties tab.
X column	Displays the X-position of the single position.
Y column	Displays the Y-position of the single position.
Z column	Displays the Z-position of the single position.
List Navigation buttons  and 	With the buttons you can shift selected list entry one position up or down in the tile regions list. This allows you to modify the acquisition order. Note that the **Tile Regions/Positions** checkbox have to be deactivated *Tiles Options* [▶ 52].
Delete button 	Deletes the selected list entry.
Lock button 	Unlocks the selected list entries to allow editing.
Options button 	Opens the *Options for editing Single Positions* [▶ 13].

Position Array List

Displays the added position arrays. The list contains the following columns and buttons:

Option	Description
Checkbox column	Activates the relevant list entry for acquisition.
Name column	Allows you to edit the name of the tile region.
Contour column	Displays the contour of the position array.
Positions column	Displays the number of positions of the position array.
Size column	Displays the size of the position array.
List Navigation buttons  and 	With the buttons you can shift selected list entry one position up or down in the tile regions list. This allows you to modify the acquisition order.
Delete button 	Deletes the selected list entry.
Lock button 	Unlocks the selected list entries to allow editing.
Options button 	Opens the *Options for editing Position Arrays* [▶ 14].

Verify Positions section



Option	Description
Verify Positions... button	Opens the *Verify Z Position dialog* [▶ 22].

2.1.2.1 Options for editing Single Positions

Option	Description
Set Current Z for Selected Positions	Sets the current Z-Position for all selected positions.
Set Current XYZ for Selected Position	Sets the current X-Y-Z-Position for the selected position.
Delete	Deletes the current position.
Delete All	Deletes all positions.

Option	Description
Activate	Activates the current position for acquisition.
Deactivate	Deactivates the current position for acquisition.
Sort	Sorts the list entries according to the chosen parameter.
- **By Center Position (Y -> X)**	Sorts all positions according to their overall Y position.
- **By Center Position (X -> Y)**	Sorts all positions according to their overall X position.
- **By Category**	Sorts all positions according to their category.

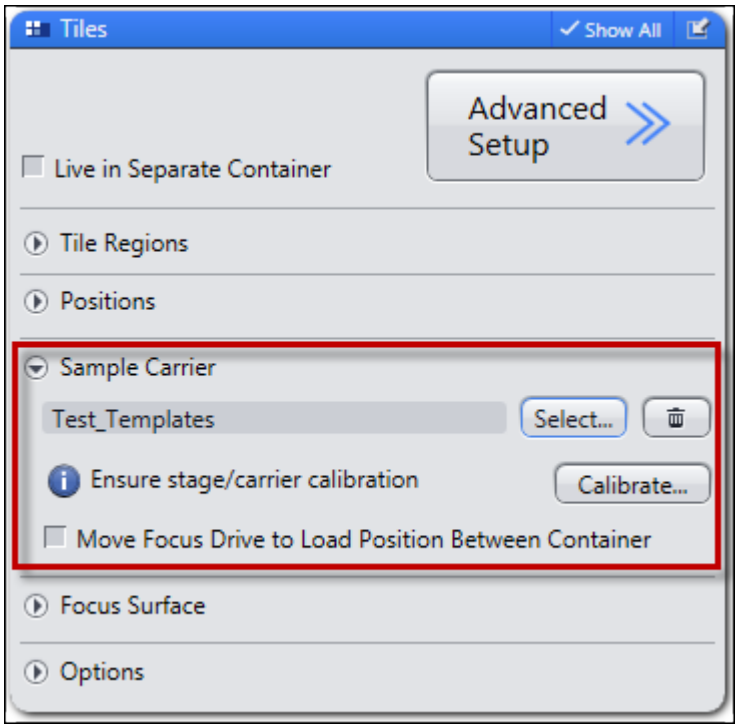
2.1.2.2 Options for editing Position Arrays

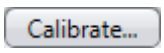
Option	Description
Delete	Deletes the current position array.
Delete All	Deletes all position arrays.
Activate	Activates the current position array for acquisition.
Deactivate	Deactivates the current position array for acquisition.
Unlock	Unlocks the current position array.
Unlock All	Unlocks all locked position arrays.
Sort	Sorts the list entries according to the chosen parameter.
- **By Center Position (Y -> X)**	Sorts all positions according to their overall Y position.
- **By Center Position (X -> Y)**	Sorts all positions according to their overall X position.

2.1.3 Sample Carrier section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button .

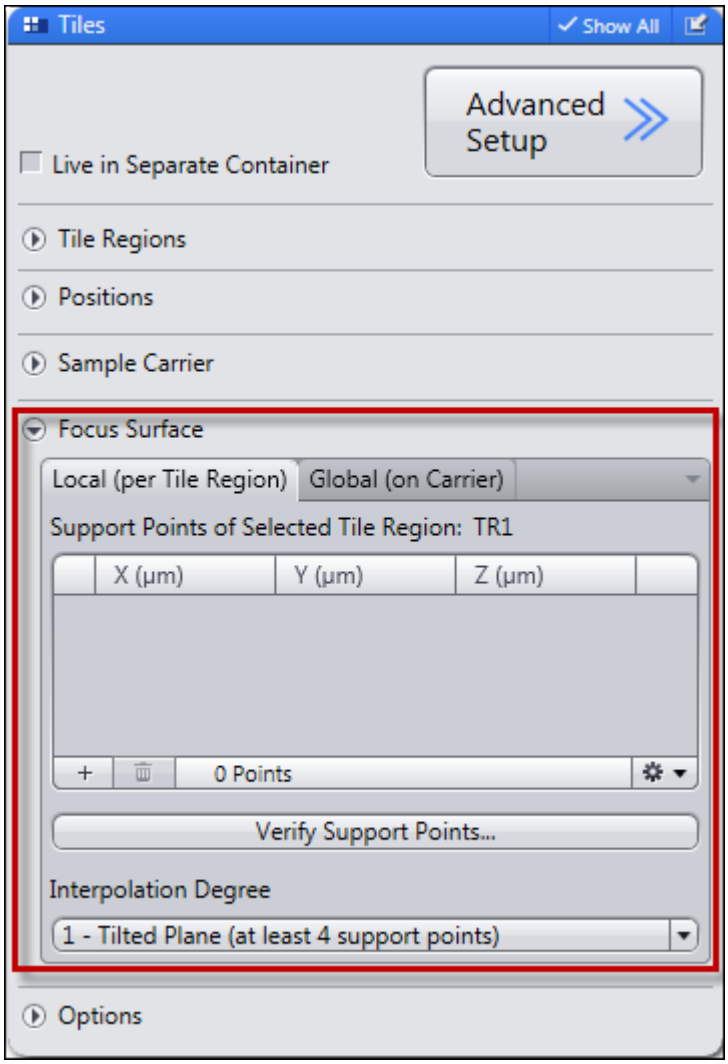


Option	Description
Sample Carrier field	Displays the selected sample carrier template. If no template is selected it will display "None".
Select... button 	Opens the *Select Sample Carrier Template dialog* [▶ 25]. Here you can select the sample carrier template.
Delete button 	Deletes the selected sample carrier from the sample carrier field. The template will still be available in the **Select Sample Carrier Template** dialog.
Calibrate... button 	Opens the **Sample Carrier Calibration Wizard**. Here you will be guided through the sample carrier calibration.
Move Focus Drive to Load Position Between Containers checkbox	**Activated:** Moves the focus drive to the loading position during the movement of the stage to another container of the sample carrier (e.g. a well or slide). This prevents possible damage.

2.1.4 Focus Surface section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button .



Local (per Tile Region) Support Points List

Displays the added local support points of a selected tile region. These can be edited in the view options of the advanced tiles setup on the support points tab. The list contains the following columns and buttons:

Option	Description
X column	Displays the X coordinate of the focus reference point.
Y column	Displays the Y coordinate of the focus reference point.
Z column	Displays the Z coordinate of the focus reference point.

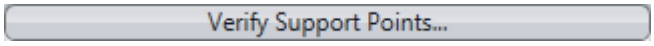
Option	Description
Add button 	Adds a new support point to the selected tile region at the current stage and focus position.
Delete button 	Deletes the selected list entry.
Options button 	Opens the *Options for editing Support Points* [▶ 18].

Global (on Carrier) Support Points List

Displays the added global support points of the selected sample carrier. These can be edited in the Select Sample Carrier Template dialog.
As the list does not differ much from the **Local Support Points List** only the additional columns are explained in the following:

Option	Description
Container column	Allows you to sort the global support points according to their container on the sample carrier.

Verify Support Points section



Option	Description
Verify Support Points... button	Opens the *Verify Z Position dialog* [▶ 22].

Interpolation Degree section

Option	Description
Interpolation Degree dropdown list	Shows the selected degree of interpolation. To select an other degree of interpolation click on  and select it from the dropdown list.

Info

The more variable the surface of your specimen the higher you should choose the interpolation degree. For higher degrees you will need more support points. The minimum number of support points for each interpolation degree is given in the dropdown list. As an overachievement of this minimum number ensures a solid calculation, we recommend minimizing the interpolation degree even if you added more support points. Increase the interpolation degree only that far as the surface condition of your specimen demands for.

2.1.4.1 Options for editing Support Points

Options for editing Local Support Points

Option	Description
Add Support Point at Current Stage and Focus Position	Adds a new support point at the current stage and focus position.
Set Current Z for Selected Support Points	Sets the current Z-Position for all selected support points.
Set Current X/Y/Z for Selected Support Points	Selects the current X-Y-Z-Position for the selected support point.
Delete	Deletes the current support point.
Delete All	Deletes all support points from the current tile region.
Delete all Support Points from Selected Tile Regions	Deletes all support points from the selected tile regions.
Delete all Support Points from all Tile Regions	Deletes all support point from all tile regions.

Options for editing Global Support Points

As the list does not differ much from the **Options for editing Local Support Points** only the additional options are explained in the following:

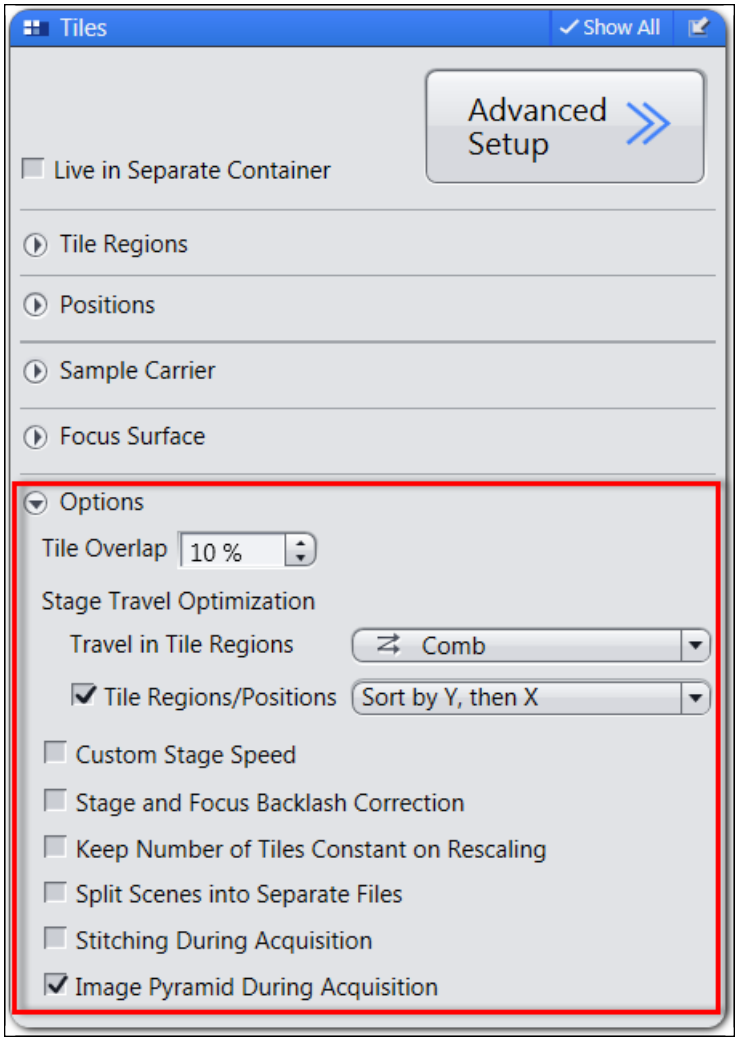
Option	Description
Set Current Z for Selected Support Points	Sets the current Z-Position for all selected support points.

2.1.5 Options section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button .

Here you can determine the acquisition and travel behavior during the experiment. Changes in this section of the tool affect all elements, tile acquisitions, positions and position arrays.



Tile Overlap section

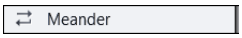
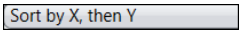
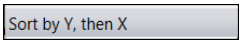
Option	Description
Tile Overlap input field	Defines the overlap in percent of individual tiles of the tile regions here. The value is set to 10 % by default.

i Info

Lower overlap might cause artifacts. No overlap will not allow the images to be stitched correctly.

Stage Travel Optimization section

Adjust settings for stage traveling during an experiment here:

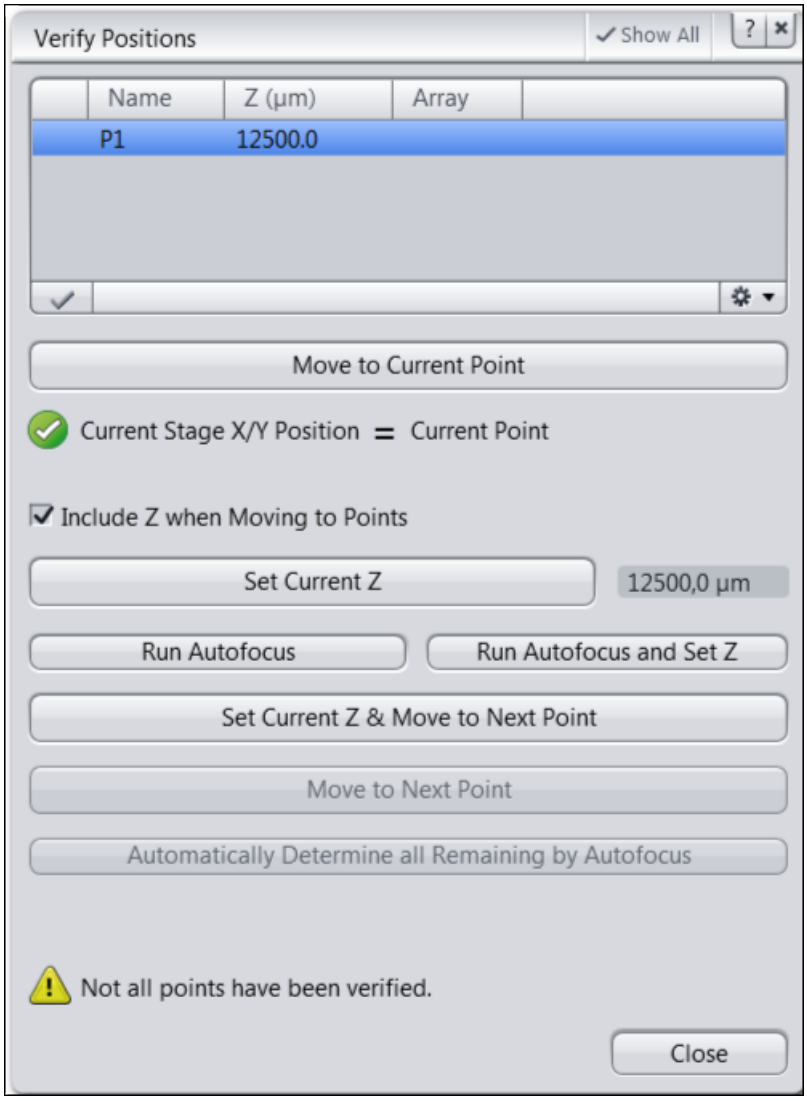
Option	Description
Travel in Tile Regions	Allows you to optimise the stage travel in a tile region.
- **Comb** button 	Acquires tile regions following a comb pattern – always from one travel direction only (left -> right). This scan movement is more precise.
- **Meander** button 	Acquires tile regions following a meander pattern – alternately from both travel directions (left -> right; right -> left). This scan movement is faster.
- **Spiral** button 	Acquires tile regions following a spiral pattern – from the center of the region to the outer bounds in a clockwise motion. This mode works only for regions with rectangular or elliptical contours.
Tile Regions/Positions	**Activated**: Individual positions and tile regions are not acquired in the sequence in which they are defined in the **Tile Regions** list. The stage movement will be automatically adapted to the location of the individual tile regions and positions. If you add or remove tile regions or positions, the sequence of acquisition therefore also changes.
- **Sort by X, then Y** button 	The tile regions and positions are sorted by their absolute position (first x, then y).
- **Sort by Y, then X** button 	The tile regions and positions are sorted by their absolute position (first y, then x).
Carrier Wells/Container	**Activated**: Applies the Comb/Meander patterns (see description above) when acquiring tiles using wells/containers.

Additional Options

Option	Description
Custom Stage Speed checkbox	**Activated**: A custom continual stage speed is used instead of the current possible speed. You can adjust the speed (in percent) if you have activated the checkbox.
Stage and Focus Backlash Correction checkbox	**Activated**: Stage and focus positioning is done with a backlash correction which is more precise but slightly slower.
Keep Number of Tiles Constant on Rescaling checkbox	**Activated**: The number of tiles (columns and rows) remains constant when the scaling changes, e.g. due to an objective change. When this option is activated, the scanned area on the stage does not remain fixed. It depends on the current tile size.
Split Scenes into Separate Files checkbox	**Activated**: The scenes (e.g. tile regions and positions) are stored into separate physical files. They are still combined into one logical image file.
Stitching During Acquisition checkbox	**Activated**: Stitching of tiles is done during the acquisition.
Image pyramid during acquisition checkbox	**Activated**: An image pyramid is generated during the acquisition. This optimizes the image for fast display. If this option is not activated, the acquired image will not be shown and updated in the document area while the acquisition is running. This function is only visible if a carrier has been selected in the sample carrier section.

2.1.6 Verify Z Position dialog

The Z positions of positions, local support points and global support points are verified in a seperate dialog. As the dialog contains the same items and options for verifying the Z positions it is described once.



Z Position List

Displays the added positions. The list contains the following columns and buttons:

Option	Description
Status cloumn	Shows if the Z-Position is already verified.
Name column	Only valid for verifying Z positions of positions. Displays the name of the selected point.
X column	Displays the X-position of the point.
Y column	Displays the Y-position of the point.

Option	Description
Z column	Displays the Z-position of the point.
Array column	Only valid for verifying Z positions of positions. Shows if the position is part of an Position Array.
Tile Region column	Only valid for verifying Z positions of local support points. Shows to which tile region the local support point belongs.
Container column	Only valid for verifying Z positions of global support points. Shows to which container the global support point belongs.
Status button 	Changes the status of the selected point to verified. If the selected position is already verified the button looks different  and will set the status of the selected point back to unverified.
Options button 	Opens the options for verifying Z positions.

Options for verifying Z positions

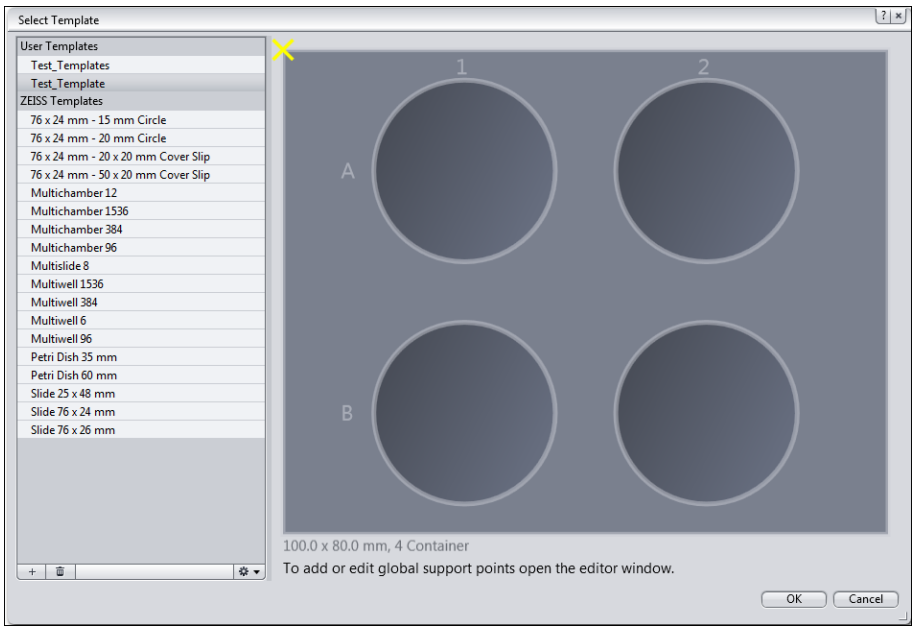
Option	Description
Current Point Verified	Changes the status of the selected point to verified. If the selected point is already verified it will set the status of the selected point back to unverified.
Set all Points as Verified	Changes the status of all points to verified.
Reset the Verification State of all Points	Changes the status of all points to unverified.
Apply Z-Offset...	Opens al dialog to apply a z-offset for all or the selected points.

Verify Z Positions section

Option	Description
Move to Current Point button	Moves the stage to the selected point.

Option	Description
Current Stage position indicator field	Show if the current stage position is the position of the selected point.
Set Current Z button	Sets the current z for the selected point and sets the status to verified.
Run Autofocus button	Runs the software autofocus.
Include Z when Moving to Points checkbox	Runs th software autofocus and sets the determined z value for the point.
Move to Next Point button	Moves the stage to the next point.
Move to Next and Run Autofocus button	Moves the stage to the next point and runs the software autofocus.
Automatically Determine all Remaining by Autofocus button	Automaticcaly moves to the remaining points and determines the z value with the software autofocus for them.
Verification Status idicator field	Shows if all points have been verified.

2.1.7 Select Sample Carrier Template dialog



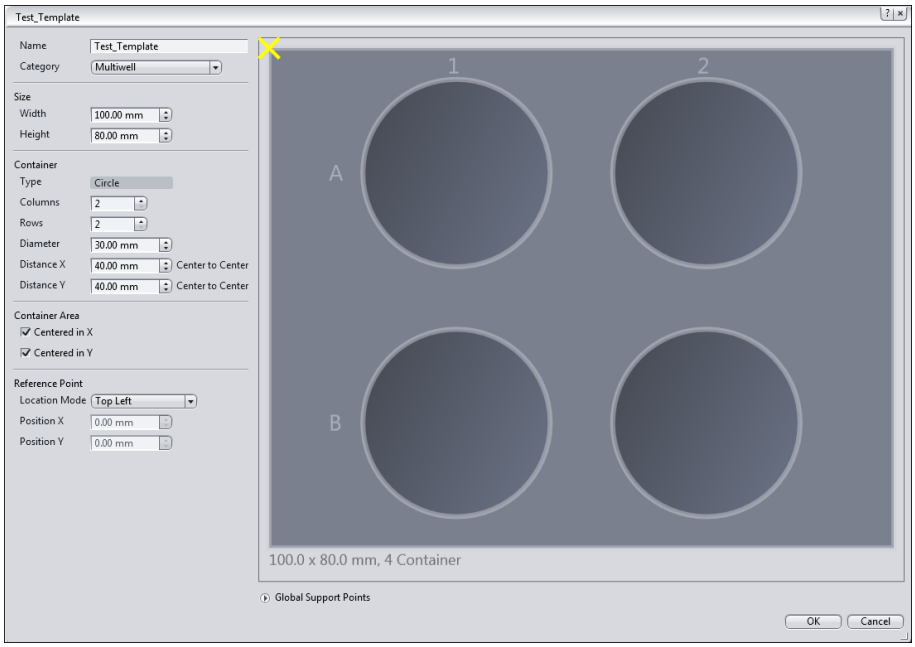
Option	Description
User Templates section	Shows all custom sample carrier templates.
ZEISS Templates section	Shows predefined ZEISS templates for several sample carriers.
Add button 	Opens the *Edit Sample Carrier Template dialog* [▶ 27] to create a new template.
Delete button 	Deletes the selected sample carrier template.
Option button 	Opens the *Options for editing sample carrier templates* [▶ 26].
Preview field	Shows a preview of the selected sample carrier template.

2.1.7.1 Options for editing Sample Carrier Templates

Option	Description
New Template...	Opens the *Edit Sample Carrier Template dialog* [▶ 27] to create a new template.
Show/Edit...	Opens the selected template in the **Edit Sample Carrier Template** dialog and allows editing. ZEISS templates are read only. If you want to edit a ZEISS template you have to use the **Copy and Edit...** option.
Copy	Copies the selected template.
Copy and Edit...	Copies the selected template and opens it in the *Edit Sample Carrier Template dialog* [▶ 27] dialog for editing.
Import	Imports an template.
Export	Exports the selected template.
Delete	Deletes the selected template.
Refresh Templates	Refreshes the list of templates after creating a new one.

2.1.7.2 Edit Sample Carrier Template dialog

The dialog for editing a template and creating a new one are the same. If you create a new template the dialog fields are empty. On the right you see a live **Template Preview** of the setting for the template and global support points.



Name and Catergory section

Option	Description
Name input field	Shows the name of your template. You can enter a new name for the template here as well.
Category drop down list	Shows which sample carrier category the template uses. You can choose between **Slide**, **Multislide**, **Petri Dish**, **Multiwell**, **Multichamber** and **Custom**. The category defines the overall appearance of the template and affects the further editing possibilities of the template.

Size section

Width input field	Determines the width of the sample carrier template.
Height input field	Determines the height of the sample carrier template.

Container section

Depending on the category of the template you have different options for editing in this section.

Option	Description
Type field	Shows if the containers of the template are rectangles or circles. If the category is **Custom** you can manually set the type of containers.
Columns input field	Shows how many columns of containers the template contains. You can not edit this field if the templates category is **Slide** or **Petri Dish**
Rows input field	Shows how many rows of containers the template contains. You can not edit this field if the templates category is **Slide** or **Petri Dish**
Width and **Height** input fields	Only for **rectangular** containers. Determines the width and height of the containers. Not active for **Slide**.
Diameter input field	Only for **circular** containers. Determines the diameter of the containers. Not active for **Slide**.
Distance X input field	Determines the distance in x direction between the containers from center to center. Not active for **Slide** and **Petri Dish**.
Distance Y input field	Determines the distance in x direction between the containers from center to center. Not active for **Slide** and **Petri Dish**.

Container Area section

Option	Description
Centered in X checkbox	**Activated**: The containers will be positioned centered in X direction on the sample carrier template.
Centered in Y checkbox	**Activated**: The containers will be positioned centered in Y direction on the sample carrier template.

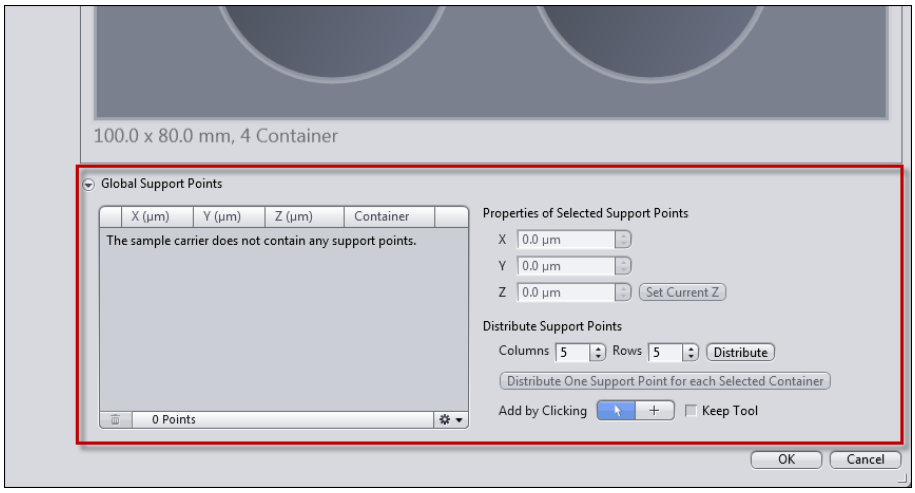
Reference Point section

The reference point is marked by a **Yellow X** .

Option	Description
Location Mode drop down list	Defines the position of the templates reference point. You can choose between **Center**, **Top Left**, **Top Right**, **Bottom Left**, **Bottom Right** and **Custom**. The default position of the reference point varies with the type of carrier.
Position X input field	Only active if you have selected **Custom** location mode. Sets a custom X position for the reference point of the template.
Position Y input field	Only active if you have selected **Custom** location mode. Sets a custom Y position for the reference point of the template.

2.1.7.2.1 Global Support Points section

To show the section in full, click on the **arrow** button .



Global Support Points list

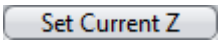
Option	Description
X column	Displays the X coordinate of the support point.
Y column	Displays the Y coordinate of the support point.
Z column	Displays the Z coordinate of the support point.
Container column	Shows the container of the support point.

Option	Description
Delete button 	Deletes the selected list entry.
Options button 	Opens the Options for editing global support points.

Options for editing global support points

Option	Description
Set Current Z for Selected Support Points	Sets the current Z value for the selected support points.
Delete	Deletes the selected support points.
Delete All	Deletes all support points.

Properties of Selected Support Points section

Option	Description
X input field	Sets the X coordinate of the selcted support point.
Y input field	Sets the Y coordinate of the selcted support point.
Z input field	Sets the Z coordinate of the selcted support point.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

Distribute Support Points section

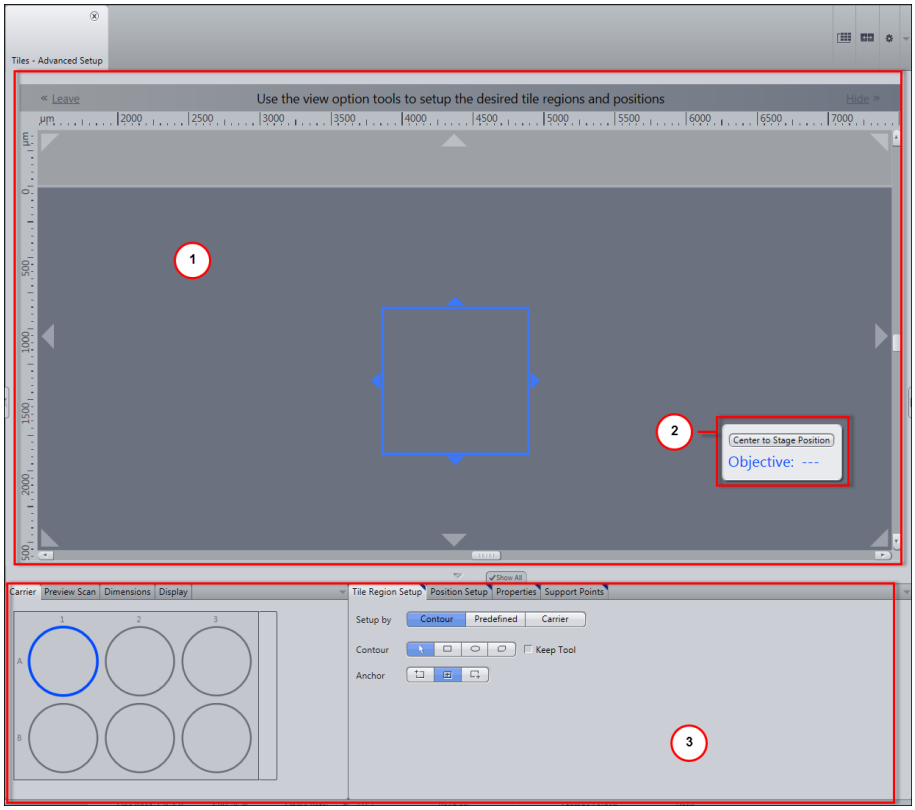
Option	Description
Columns input field	Sets the number of columns of support points within the template.
Rows input field	Sets the number of rows of support points within the template.
Distribute button 	Distributes the entered number of support points defined in the column and row input fields within the template. Previously defined support points will be deleted.
Distribute One Support Point for each Selected Container button	Sets one support point in the center of the selected containers. Previously defined support points will be deleted.

Add by Clicking section

Option	Description
Selection button 	Select a support point to move it. Support points distributed by the **Distribute One Support Point for each Selected Container** button can not be moved.
Add button 	Add a new support point on the template perview.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

2.2 Advanced Tiles Setup

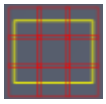
Here you can configure advanced settings and plan your Tiles and Positions experiment. In the Center Screen Area you can see the *Stage view* [▶ 32]. When the Advanced Tiles Setup (ATS) opens the stage view is "zoomed" to a predefined factor. The Zoom can be changed view the Dimensions view control, or by pressing Ctrl + scrolling mouse wheel.
To navigate around one has the following options: In each corner and along each edge the arrowheads can be clicked to move the view in this direction. To re-center the view on the current stage position press the **Center to Stage Position** button located in the lower right-hand corner of the Stage View. Additional settings and tools relating to tile regions or positions can be found under *Specific View options* [▶ 34].



1 *Stage View* [▶ 32] **2** Center to Stage Position
3 *Specific View options* [▶ 34]

2.2.1 Stage View

The image area shows the full travel range of the microscope stage, along with the current stage position, the graphical display of sample carriers and your acquired mosaic images. You can control the stage view using the arrow icons at the edges of the image area. The view can be enlarged, reduced or moved using the general control elements.

Symbol	Name	Description
 / 	**Selected / Active** container/ well	The currently **Selected / Active** container / well is represented by a blue border.
	**Live Navigator** tool	In the Live navigator tool the current stage position including the live image is shown as a frame outlined in blue. To move the frame, double-click on the position to which you want to move it. Alternatively hold the left mouse button on the live navigator tool while dragging the mouse. The frame can also be used to control acquisition. If you click on one of the frame's blue arrow icons, an image is acquired. The Live Navigator tool is moved one frame width in the relevant direction. You can create tile images of your sample easily in this way.
	**Tile Region**	Tiles / tile regions are represented in the stage view by a red grid.
	**Positions**	Positions are represented in the stage view by a yellow plus symbol.
	**Position Array**	Position Arrays are represented in the stage view by the corresponding position symbols surrounded by a dashed line.
	**Local Support Point**	Local Support Points are represented in the stage view by a yellow circle with a dot in the middle.
	**Global Support Point**	Global Support Points are represented in the stage view by a white circle with a dot in the middle.

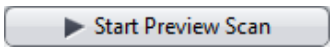
2.2.2 Specific View options

2.2.2.1 Preview scan tab

Here you can define the settings for a preview scan. Typically a low magnification objective is used, especially when a larger tile is to be acquired, to give the user a low resolution overview of the sample in question.

i Info

The objective setting used in the preview scan is not independent of that found in the experiment settings, but is the same as that set in locate or on the microscope´s TFT.

Option	Description
Use Existing Experiment Settings checkbox	**Activated:** Uses the existing experiment Settings. That is the default setting. **Deactivated:** Additional options **Camera** and **Channels** appear. That allow independent activation/ deactivation of channels and **Use Binning from Experiment** versus the defined experiment settings. If binning is used then the exposure time is automatically compensated to avoid saturation. Changing these parameters does not effect the settings that will be used for the experiment.
Delete Existing Preview Images checkbox	**Activated:** Deletes all existing preview images when the next preview is acquired.
Objective dropdown list	Here you can select the objective which will be used for the preview scan. To acquire an overview of all active tiles and positions images, switch to an objective with a low magnification. Set the channel exposure time and start the acquisition of the overview image. To select an other objective click on  and select it from the dropdown list.
Start Preview Scan button 	Starts the preview scan to acquire the overview images.

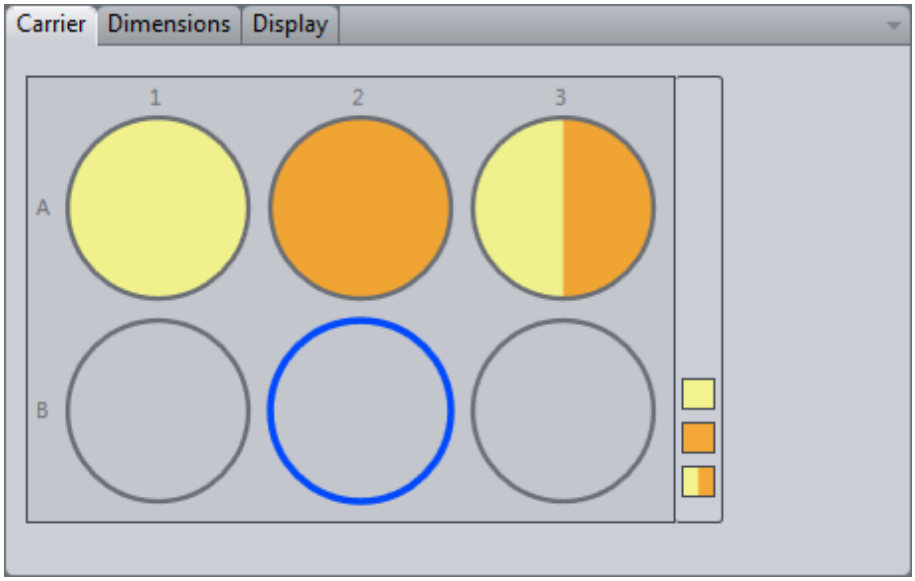
i Info

Note that the selected objective will now be used for any subsequent activities with Locate or Acquisition tab. Thus, you must actively change the objective after the preview scan if you want to use another objective for your experiment.

2.2.2.2 Carrier tab

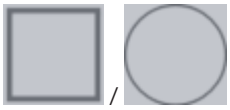
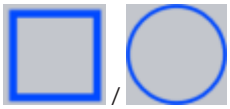
Only visible if a sample carrier was selected.

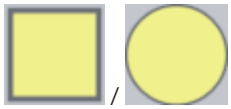
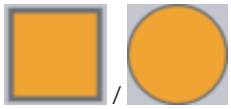
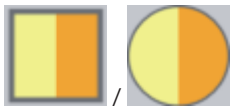
Here you can see a graphical preview of the sample carrier being used. Please note the following features of the display:



i Info

Only the containers / wells whose tile regions and positions were set up with the *Setup by Carrier* [▶ 40] of the **Tile Regions Setup** tab or the *Setup by Carrier* [▶ 44] of the **Positions Setup** tab will be taken into account.

Symbol	Description
	**Empty** containers / wells, meaning that no tile regions or positions were set up with the Carrier option, are represented by a grey conatiner / well.
	The currently **Active** container / well is represented by a blue border.

Symbol	Description
	A container / well only filled with **Tile Regions** is represented by a yellow filled conatiner / well.
	A conatiner / well only filled with **Positions** is represented by a orange filled conatiner / well.
	A container / well filled with **Tile Regions** and **Positions** is represented ba a half yellow, half orange filled conatiner / well.

i Info

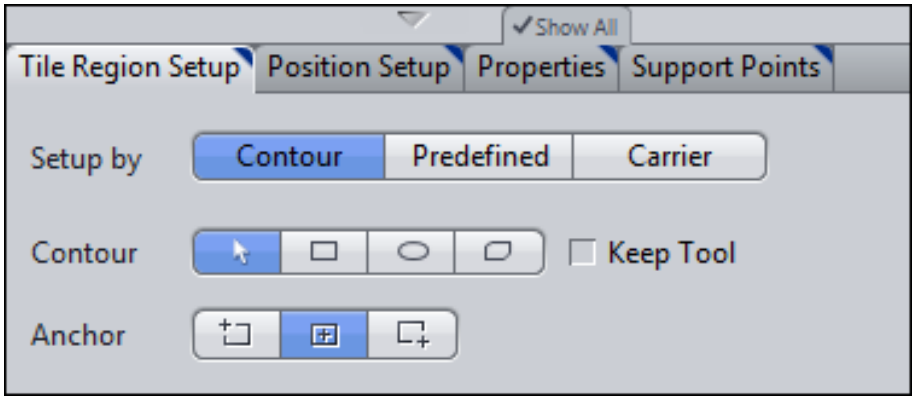
Right click opens a small context menu. Here you can copy the contents of the selected well, or paste the contents to the selected or all wells.

2.2.2.3 Tile Region Setup tab

Here you can select which setup you want to be used for the settings of the tile regions. Three setups with different setting options are available:

2.2.2.3.1 Setup by Contour

Here you can define the tile regions by means of the contour.



Contour section

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Selection button 	With this tool you can select an already created tile region by clicking on it to move or edit it.
Rectangle button 	With this tool you can draw a rectangle tile region.
Ellipse button 	With this tool you can draw a elliptical tile region.
Polygon button 	With this tool you can draw a polygonal tile region.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

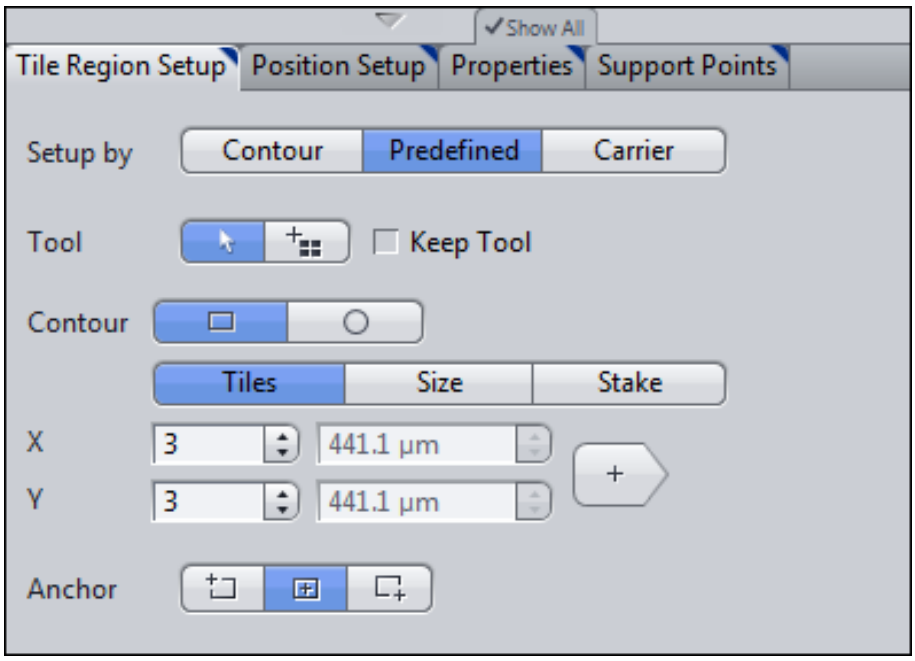
Anchor section

Here you can select the anchor position of the new tile region. The following tools are available:

Option	Description
Anchor Top Left button 	The anchor of the defined shape is at the top left.
Anchor Centered button 	The anchor of the defined shape is centered.
Anchor Bottom Right button 	The anchor of the defined shape is at the bottom right.

2.2.2.3.2 Setup by Predefined

Here you can define the tile regions by means of the number or size.



Tool section

Here you can select a tool to work with. The following tools are available:

Option	Description
Selection button	Select an element in the stage view to edit or move it.
Add Tile Region button	Adds the current tile definition in the image area.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

Contour section

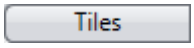
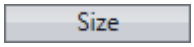
Only visible if the **Show All** mode is activated.

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Rectangle button	Adds a rectangular tile region.

Option	Description
Circle button 	Adds a circular tile region

Mode section

Option	Description
Tiles button 	Using this mode you have to enter the number of tiles as a reference for the size of the tile region. Enter the number of tiles in the **X / Y** input fields. If you are adding a circular tile region, enter the number of tiles for the diameter in the **Diameter** input field.
Size button 	Using this mode you have to enter the size as a reference for the size of the tile region. Enter the size of the tile region in the **X / Y** input fields. If you are adding a circular tile region, enter the diameter of the tile region in the **Diameter** input field.
Stake button 	This mode allows the definition of a tile region by the placement of at least two markers (user defined X/Y stage coordinates). If you want to modify the tile region (expand/ reduce) you have to adjust the tile region to the desired size. To complete the tile region press **Done**. Circular or rectangular tile region can be created in this manner by selection of the appropriate contour.
Add button 	Adds the tile region to the **Tile Regions List** and activates it for acquisition. Added tile regions are displayed in the form of red grids in the stage view of the **Advanced Tiles Setup**.

Anchor section

Only visible if the **Show All** mode is activated.

Here you can select the anchor position of the new tile region. The following tools are available:

Option	Description
Anchor Top Left button 	The anchor of the defined shape is at the top left.

Option	Description
Anchor Centered button 	The anchor of the defined shape is centered.
Anchor Bottom Right button 	The anchor of the defined shape is at the bottom right.

2.2.2.3.3 Setup by Carrier

Here you can define the tile regions automatically by means of the fill factor of the sample carrier.

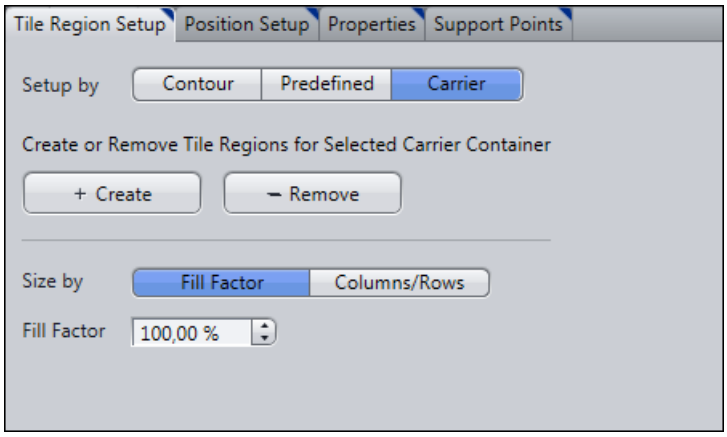
Info

A sample carrier must have been selected in the *Sample Carrier section* [▶ 15] of the **Tiles** tool.

Info

Manually created tile regions and positions (setup by **Contour** and setup by **Predefined**) will be deleted, if you switch to the setup by **Carrier**. If you want to combine manual and automatic setup, first use setup by **Carrier** and then switch to a manual setup.

Tile regions that are created automatically by setup by **Carrier**, are defined to a container and permanently assigned and locked by default, against manual edits. You can unlock the tile regions in the **Tiles** tool by selecting the desired tile region and click on the unlock button.



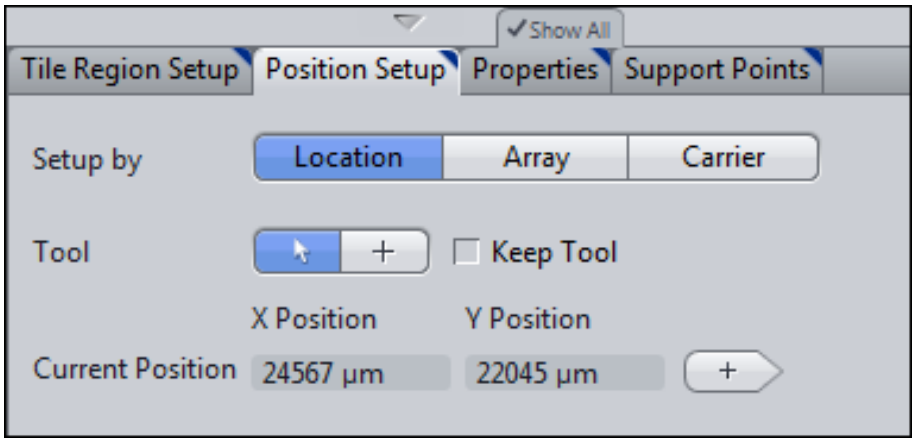
Option	Description
Create button + Create	Only active if you have selected a container on the *Carrier tab* [▶ 35] or in the *Stage View* [▶ 32]. Automatically creates the tile regions with the set fill factor in the selected container of the sample carrier.
Remove button – Remove	Removes all tile regions in the selected container.
Fill Factor input field Fill Factor	Here you can enter the fill factor used to fill the selected container.
Columns/Rows input field Columns/Rows	Here you can add single tile regions to a container by defining the number of columns and rows of the tile. The tile region is always placed at the center of the well container.

2.2.2.4 Position Setup tab

Here you can select which setup you want to be used for the settings of the positions. Three setups with different setting options are available:

2.2.2.4.1 Setup by Location

Here you can define the positions by means of the location. You can add various positions in the stage view using the mouse.



Tool section

Here you can select a tool to work with. The following tools are available:

Option	Description
Selection button 	Select an element in the stage view to edit or move it.
Add button 	Add a new position on the stage view.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

Current Position section

Displays the current stage position (X/Y).

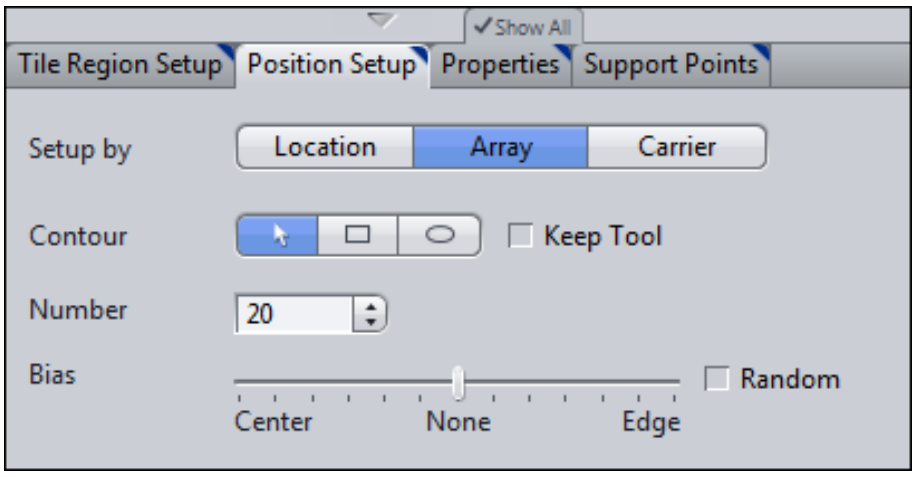
Option	Description
Add button 	Adds a new position at the current stage position.

2.2.2.4.2 Setup by Array

Here you can define the positions by means of position arrays. You can add various contours for position arrays in the stage view.

Info

Position arrays are groups made up of a number of individual positions. Typically, position arrays contain several hundred individual positions. They make your work easier if you work with regular or evenly distributed samples.



Contour section

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Selection button 	With this tool you can select an already created position array by clicking on it to move or edit it.
Rectangle button 	With this tool you can draw a rectangle position array.
Ellipse button 	With this tool you can draw a elliptical position array.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

Number section

Option	Description
Number input field	Shows the current number of positions that are distributed to newly created position array. Change the number to increase or decrease the number of single positions obtained by a position array.

Bias section

Only visible if the **Show All** mode is activated.

Here you can set the distribution bias of the single positions created for a new position array.

Option	Description
Bias slider	Adjusts the overall position of the single positions in the position array.
- **None**	The single positions of the position array will be distributed evenly within the array.
- **Center**	The single positions of the position array will mainly be distributed near to the center of the position array. Less positions will be at the edges of the array.
- **Edge**	The positions of the position array will be distributed to the edges of the array. Less positions will be in the center of the array.
Random checkbox	**Activated**: The single positions will mainly be distributed randomly within the position array. The overall bias will still be taken into account.

2.2.2.4.3 Setup by Carrier

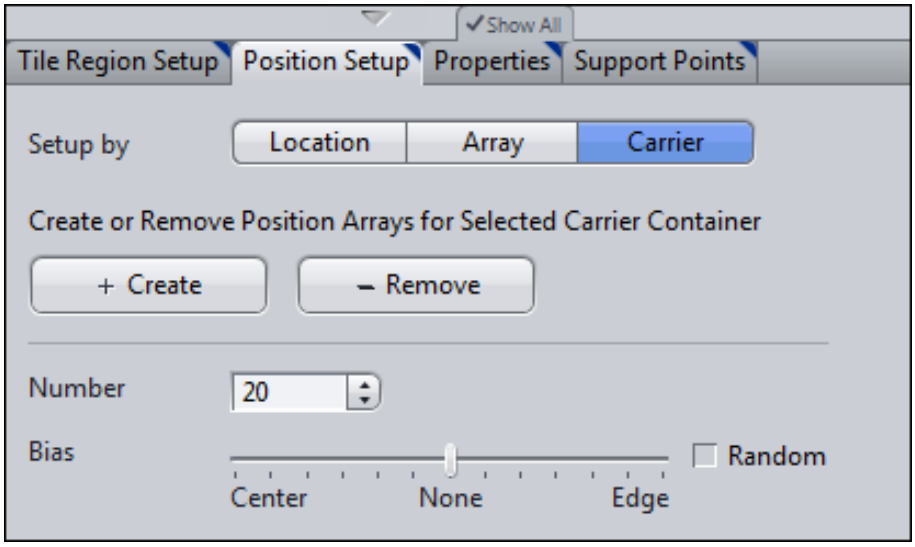
Here you can define the positions automatically by means of the relevant sample carrier.

Info

A sample carrier must have been selected in the *Sample Carrier section* [▶ 15] of the **Tiles** tool.

Info

Manually created tile regions and positions (setup by **Contour** and setup by **Predefined**) will be deleted, if you switch to the setup by **Carrier**. If you want to combine manual and automatic setup, first use setup by **Carrier** and then switch to a manual setup.



Option	Description
Create button + Create	Only active if you have selected a container on the *Carrier tab* [▶ 35] or in the *Stage View* [▶ 32]. Automatically creates the tile regions with the set fill factor in the selected container of the sample carrier.
Remove button – Remove	Removes all tile regions in the selected container.

Number section

Option	Description
Number input field	Shows the current number of positions that are distributed to newly created position array. Change the number to increase or decrease the number of single positions obtained by a position array.

Bias section

Only visible if the **Show All** mode is activated.

Here you can set the distribution bias of the single positions created for a new position array.

Option	Description
Bias slider	Adjusts the overall position of the single positions in the position array.
- **None**	The single positions of the position array will be distributed evenly within the array.
- **Center**	The single positions of the position array will mainly be distributed near to the center of the position array. Less positions will be at the edges of the array.
- **Edge**	The positions of the position array will be distributed to the edges of the array. Less positions will be in the center of the array.
Random checkbox	**Activated**: The single positions will mainly be distributed randomly within the position array. The overall bias will still be taken into account.

2.2.2.5 Properties tab

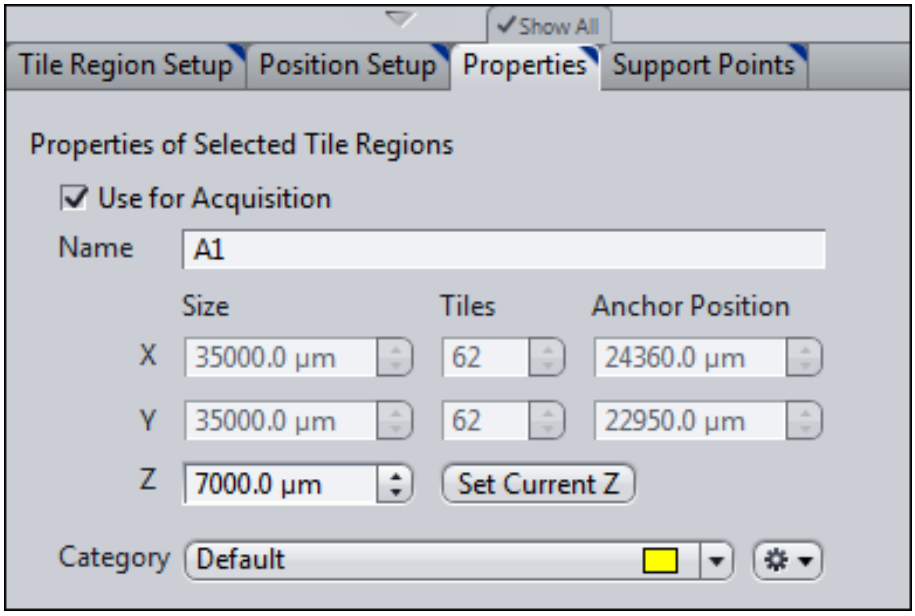
Here you can adjust the properties of a selected tile region or position.

i Info

You have to select a tile region or single position to see the parameters available on this tab. It is not possible to see and adjust the parameters for a whole position array.

2.2.2.5.1 Properties of selected Tile Regions

Here you can adjust the properties of the selected tile region.



Option	Description
Use for Acquisition checkbox	**Activated:** Uses the selected tile region for acquisition.
Name input field	Here you can enter a name for the selected tile region.

Properties section

Only visible if the **Show All** mode is activated.

The properties section contains the following columns and buttons:

Option	Description
Size column	Here you can see and edit the size of the tile region in the **X / Y / Z** dimensions. The **X / Y** dimensions of tile regions created with the *Setup by Carrier* [▶ 40] can not be edited as they are fixed by the container / well size.
Tiles column	Here you can see the number of tiles in the **X / Y** dimensions. You can not edit the number of tiles as it is fixed by the size of the tile region.

Option	Description
Anchor Position column	Here you can enter the anchor position of the selected tile region in **X / Y** dimensions. The anchor position of tile regions created with the *Setup by Carrier* [▶ 40] can not be edited as they are fixed by the container / well.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

Category section

Only visible if the **Show All** mode is activated.

Here you can assign categories to tile regions. Category definitions will be displayed in the appropriate column of the table in the Tiles tool. This value is also written in the image meta-date. Thus, well definition patterns or variables can be created and stored as part of a experiment template.

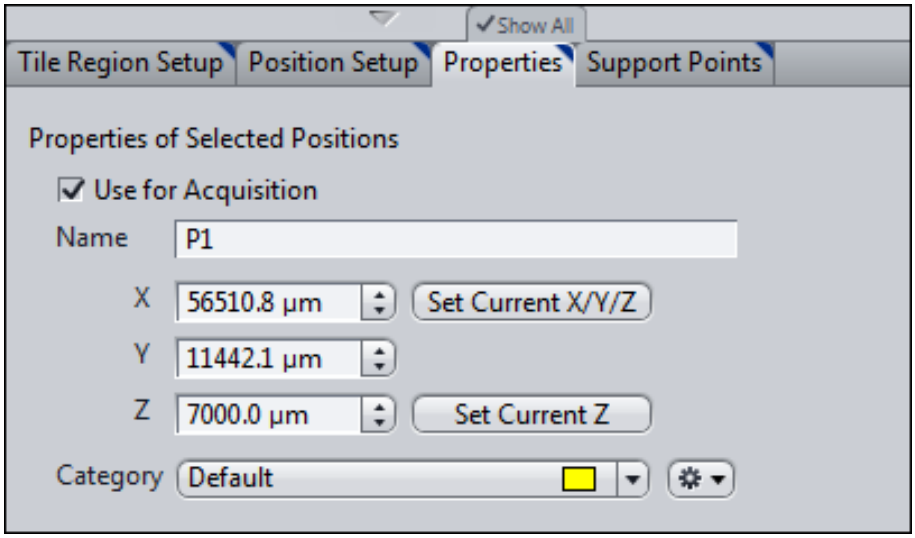
Option	Description
Category dropdown list	Shows the currently assigned category of the selected tile region. The **Default** category is set for all new tile regions. Click on  assign an other category.
Options button 	Opens the options for editing categories.

Options for editing Categories

Option	Description
New...	Opens the **New Category** dialog to create a new category.
Edit…	Opens the **Edit Category** dialog to edit the selected category.
Delete	Deletes the selected category and sets the category of the tile region to **Default**.

2.2.2.5.2 Properties of selected Positions

Here you can adjust the properties of the selected position.

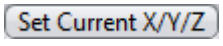
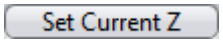


Option	Description
Use for Acquisition checkbox	**Activated:** Uses the selected position for acquisition.
Name input field	Here you can enter a name for the selected position.

Properties section

Only visible if the **Show All** mode is activated.

The properties section contains the following columns and buttons:

Option	Description
Position column	Here you can see and edit the position of the selected position on the stage in **X / Y / Z** dimensions.
Set Current X/Y/Z button 	Sets the **X / Y / Z** dimension at the current **X / Y / Z** position of the stage.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

Category section

Only visible if the **Show All** mode is activated.

Here you can assign categories to tile regions. Category definitions will be displayed in the appropriate column of the table in the Tiles tool. This value is also written in the image meta-date. Thus, well definition patterns or variables can be created and stored as part of a experiment template.

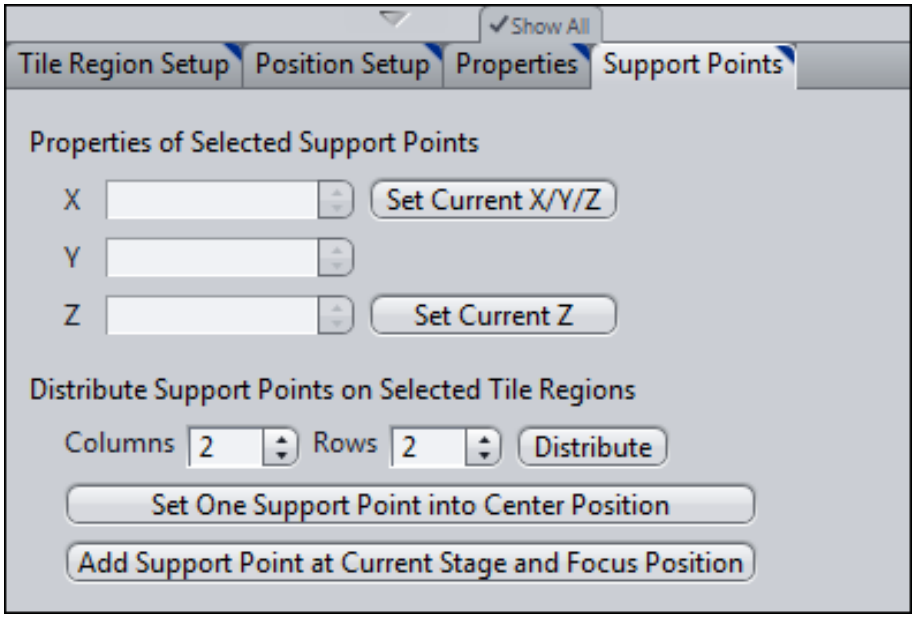
Option	Description
Category dropdown list	Shows the currently assigned category of the selected tile region. The **Default** category is set for all new tile regions. Click on assign an other category.
Options button	Opens the options for editing categories.

Options for editing Categories

Option	Description
New...	Opens the **New Category** dialog to create a new category.
Edit...	Opens the **Edit Category** dialog to edit the selected category.
Delete	Deletes the selected category and sets the category of the tile region to **Default**.

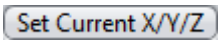
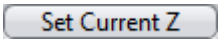
2.2.2.6 Support Points tab

Here you can adjust the properties of selected local and global support points.



Properties of Selected Support Point section

The properties of selected support point section contains the following columns and buttons:

Option	Description
Position column	**X / Y** dimensions are only visible if the **Show All** mode is activated. Here you can see and edit the position of the selected position on the stage in **X / Y / Z** dimensions.
Set Current X/Y/Z button 	Only visible if the **Show All** mode is activated. Sets the **X / Y / Z** dimension at the current **X / Y / Z** position of the stage.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

i Info

Properties of Global Support Points

The properties of a selected global support point slightly differ from those of a local one as you can not edit the **X / Y** dimensions because they are fixed by the sample carrier template you have selected. Therefore there is no **Set Current X/ Y/Z** button for global support points.
Activating / Deactivating the **Show All** mode will not show / hide any additional options for global support points not even the options that are shown / hidden for local support points.

Distribute Support Points on Selected Tile Regions section

i Info

You can see this section only if you have selected a tile region or a local support point of a tile region. Selecting a local support point will prevent editing the section.

The distribute support points on selected tile regions section contains the following input fields and buttons:

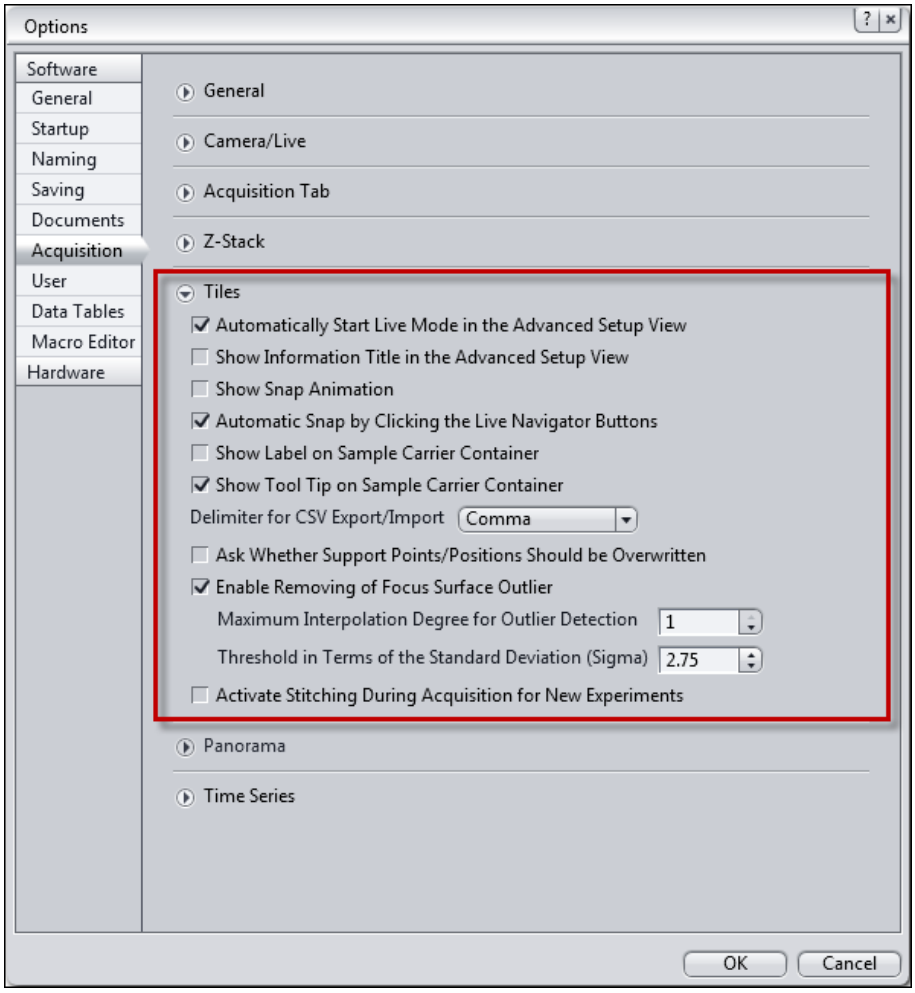
Option	Description
Columns input field	Sets the number of columns of support points within the selected tile region.

Option	Description
Rows input field	Sets the number of rows of support points within the selected tile region.
Distribute button 	Distributes the entered number of support points defined in the column and row input fields within the tile region. Previously defined support points will be deleted.
Set One Support Point into Center Position button	Only visible if the **Show All** mode is activated. Sets one support point in the center of the selected tile region. Previously defined support points will be deleted.
Add Support Point at Current Stage and Focus Position button	Only visible if the **Show All** mode is activated. Adds a support point at the current stage and focus position. Does not affect previously defined support points.

2.3 Tiles Options

The additional options for the tiles module allow to set up several options for image acquisition and additional information. The tiles options dialog can be found in the menu bar under **Tools** | **Options...** | **Acquisition** | **Tiles**.

To show the section in full, click on the **arrow** button .



Option	Description
Automatically Start Live Mode in the Advanced Setup View checkbox	**Activated**: Automatically starts the **Live** mode in the live navigator tool when you open the advanced setup. Uncheck this option to prevent unnecessary specimen bleaching.

Option	Description
Additionally Open Snap Images as Separate Documents checkbox	**Activated**: Snap images created in the advanced tiles setup are opened additionally in a separate image containers, not just as a thumb nail in the preview area of the advanced tiles setup.
Show Information Title in the Advanced Setup View checkbox	**Activated**: Displays a bar abbove the Advanced Setup view containing additional information.
Show Snap Animation checkbox	**Activated**: Shows the snap animated when snapping a new image in Advanced Setup.
Automatic Snap by Clicking the Live Navigator Buttons checkbox	**Activated**: A snap will be taken every time the live navigator tool is moved with its navigation buttons.
Enable Stage Moving with Live Navigator Handle checkbox	In the Live navigator tool the current stage position including the live image is shown as a frame outlined in blue. To move the frame, double-click on the position to which you want to move it. The frame can also be used to control acquisition. **Activated**: If you click on one of the frame's blue arrow icons, an image is acquired. The Live Navigator tool is moved one frame width in the relevant direction. You can create tile images of your sample easily in this way.
Show Label on Sample Carrier Container checkbox	**Activated**: Shows a label on every container / well of a selected sample carrier.
Show Tool Tip on Sample Carrier Container checkbox	**Activated**: Shows a tool tip with the name of the container / well when the mouse is over it in the **Carrier** tab.
Delimiter for CSV Export / Import dropdown list	Specifies the delimiter for a CSV export or import. You can choose between **Comma** (default), **Semicolon** and **Tab**.
Ask Whether Support Points / Positions Should be Overwritten checkbox	When the support points and/ or positions are determined by a software autofocus run the existing points can be overwritten with the new **Z** values. **Activated**: Shows a message box asking if the points should be overwritten if there is a autofocus **Z** value.

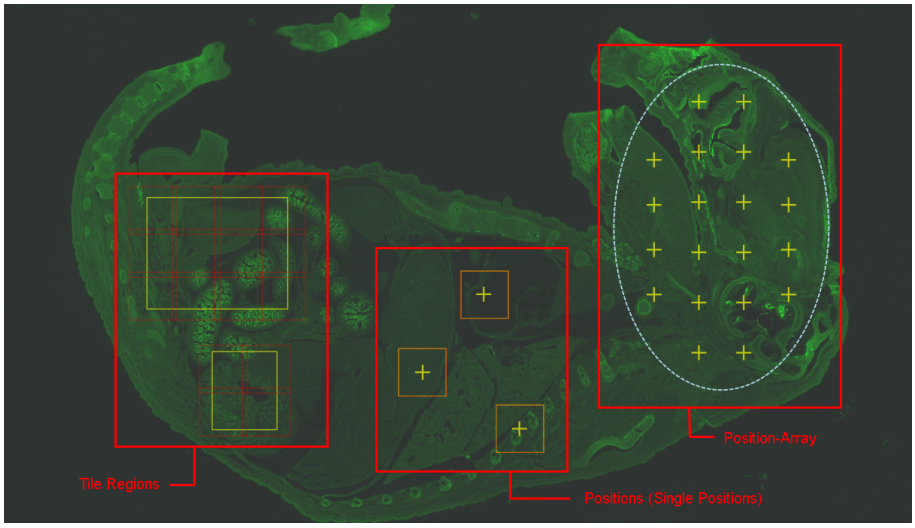
Option	Description
Enable Removing of Focus Surface Outlier checkbox	**Activated**: Support points that are significantly outside the interpolated focus surface are ignored. You have the following setting options available:
- **Maximum Interpolation Degree for Outlier Detection** input field	This value can be 0 or 1. If 1 then a linear fit is used to detect the outlier support points. This is the default. If 0 a simple average value is used to detect outliers.
- **Treshold in Terms of the Standard Deviation (Sigma)** input field	This parameter defines a threshold value to determine which of the support points are outliers from the fitting process. This is defined by the standard deviation (sigma value) set in the spin box. Support points not meeting this criteria are subsequently ignored when the focus surface is determined.
Activate Stitching During Acquisition for New Experiments checkbox	**Activated**: Stitching during acquisition is active by default for all new experiments. This value is overwritten by the corresponding option in the **Tiles** setup for a new experiment.
Use Local Focus Surface for Preview Scans checkbox	**Activated**: Local focus surface values (z-values of positions, tile regions and if defined interpolated focal surfaces defined by support points) will be used for the acquisition of preview scan images. Note that on activation of the Tiles dimension the appropriate strategy **Use Focus Surface Defined by Tiles Setup** is pre-selected.
Binning Compensation of Exposure Time in Preview Scans input field	Defines the power to which the binning ratio is modified to automatically determine the exposure time value used for a preview scan were the binning setting between the experiment and preview scan differs. The default value is 2.0 i.e. quadratic. Thus, for example the exposure time would be reduced by a factor of four if the experiment binning is 1x1 and the preview scan binning is 2x2. The value can be varied between 1.0 and 2.0 in steps of 0.1.

Option	Description
Live Image in Sample Carrier Calibration Wizard (relevant for systems with cameras)	
- **Use Imaging Device from Selected Channel with "Acquisition" Settings** radio button	**Activated**: Default setting for the live image that allows navigation and focus interaction during the carrier calibration wizard.
- **Use Active Camera with "Locate" Settings** radio button	**Activated**: Allows the user to alternatively apply locate camera settings for use in the carrier calibration wizard (live image). By default the experiment settings for the currently selected channel/ Track will be used. This option is only relevant for systems with a wide field (camera based) detector.

3 Workings with Tiles and Positions

3.1 Introduction

Using the **Tiles** tool you can acquire images that are made up of a number of individual images (tiles). To do this, it is possible to define tile regions and positions. The **Tiles** module supplements the functions of the **Tiles** tool with the **Advanced Setup** feature. This allows you to set up **Tiles** experiments more easily and also to use sample carriers and focus surfaces.



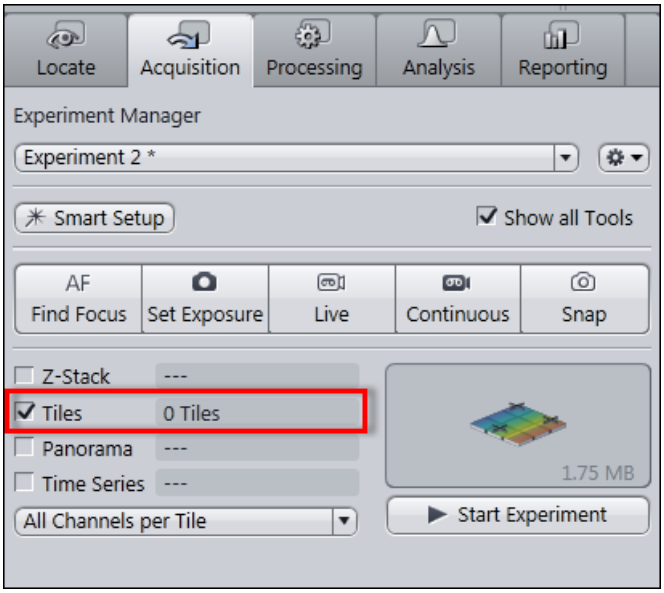
Info

If you want to acquire tile regions or positions with different Z-positions, you need to use a suitable focus strategy. To do this, please first read: Using focus strategies.

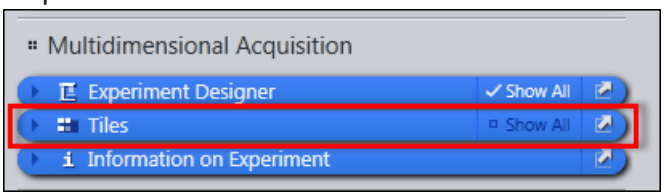
Prerequisites

- To set up **Tiles** experiments, you require a motorized stage. This must be configured and calibrated correctly in accordance with the camera orientation. For more information read *Calibrating Stage and Adjusting Camera Orientation* [▶ 57].
- You have created a new experiment, defined at least one channel and correctly set the focus and exposure time.
- You are on the **Acquisition** tab.

Procedure **1** Activate the **Tiles** tool by activating the **Tiles** checkbox in the **Acquisition Dimensions** section.



In the **Left Tool Area** the **Tiles** tool appears under **Multidimensional Acquisition**.



You have successfully completed the general preparations. You can now set up **Tiles** or **Positions** experiments (see *Setting up a simple tiles experiment* [▶ 60].

3.1.1 Calibrating Stage and Adjusting Camera Orientation

On start up of a system with motorized stage and/or focus a request will appear asking if the components should be driven to the end switches **calibrated**. This ensures that you beginning working with absolute coordinates in this session with the microscope. If the microscope power is cycled then this process should be repeated. This function is of particular use if you continually work with a sample carrier e.g. 96 well plate, of the same format mounted in the same manner repeatedly with a given experiment template. If you perform a carrier calibration *Sample Carrier section* [▶ 15] once with a calibrated stage then the carrier calibration is essentially always valid.

i Info

The request to calibrate stage and focus on Startup can be activated/deactivated under **Options** | **Startup** | **Stage/Focus Calibration**.

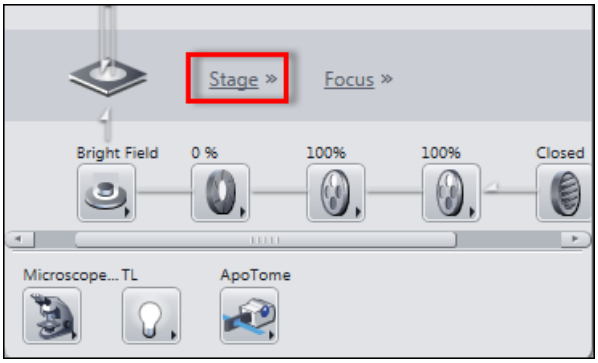
Procedure **1** Put your Sample Carrier on the stage.

2 Go to the **Locate** tab.

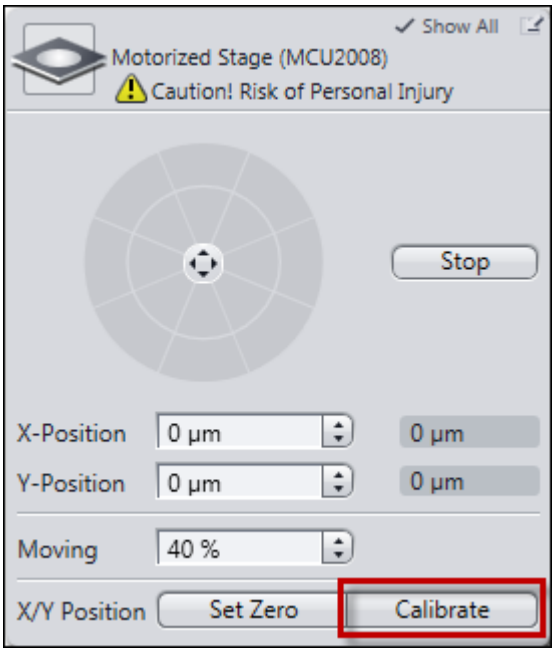
3 Choose a low magnification objective (e.g. 10x).

4 Click on the **Live** button and find your focus area either using transmission or fluorescence light.

5 In the **Microscope Control** tool click on the **Stage** button.



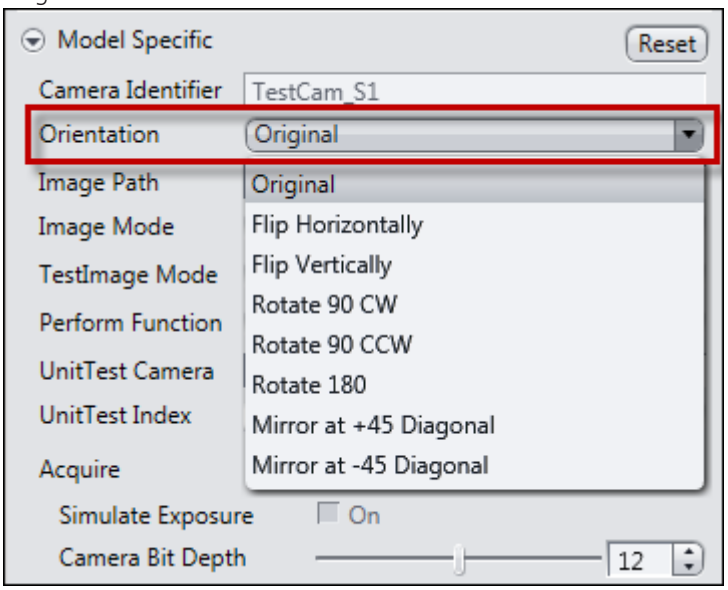
6 Activate the **Show all** mode and then click on the **Calibrate** button.



7 Check if the alignment of your camera and joystick is correct by dragging the software joystick up, down, left and right and observe whether the movement of your image corresponds to movement of the circle. In addition, check whether the image movement also corresponds accordingly when you move the joystick.

8 If the alignment is incorrect, go to the **Camera** tab activate the **Show all** mode and click **Model Specific**.

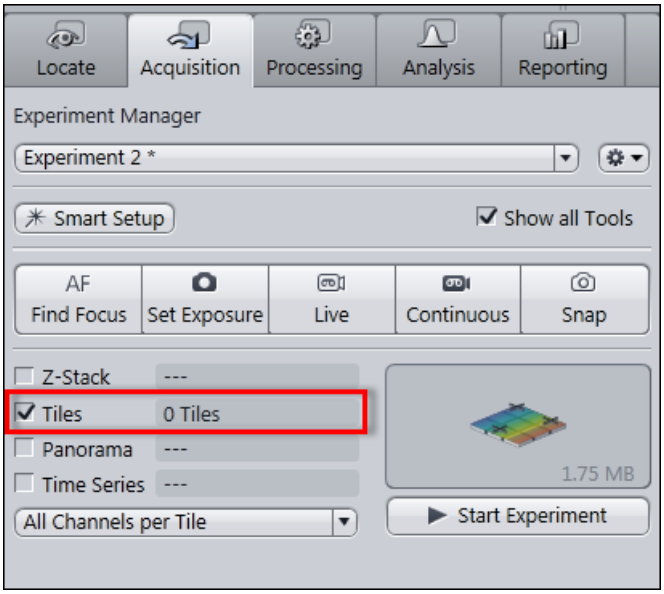
9 In **Orientation** you can now adjust the camera orientation to the joystick orientation. Alternatively, you can and may also invert X- and Y-axis of your stage in the **MTB** in order to align the joystick and the software-controlled stage movement.



10 Go to the **Acquisition tab**.

11 Do all the prerequisites (e.g. channel and camera settings) for a **Tiles & Positions experiment** on your sample. For that use Smart Setup and, if needed, Experiment Designer (not advisable for beginners).

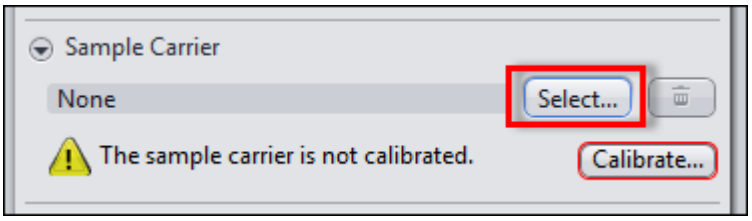
12 After you have defined at least one channel (e.g. EGFP), activate the **Tiles** module checkbox.



13 Open the **Tiles** tool in the **Multidimensional Acquisition** module and activate the **Show all** mode.

14 In the **Tiles** tool open the **Sample Carrier** section.

15 Click on the **Select...** button.



16 Choose a predefined **Sample Carrier template** and click **OK**.

3.2 Setting up a simple tiles experiment

Prerequisites

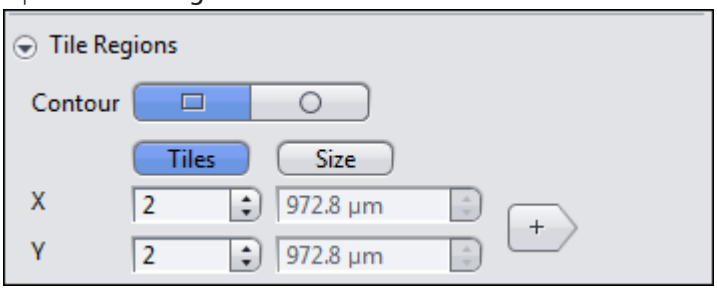
- You have read the chapter *Introduction* [▶ 56].
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1 Start the **Live** mode to use the stage to locate a point that you want to be at the center of your tile region.

2 Bring the specimen into focus using the focus drive.

3 Open the **Tile Regions** section.



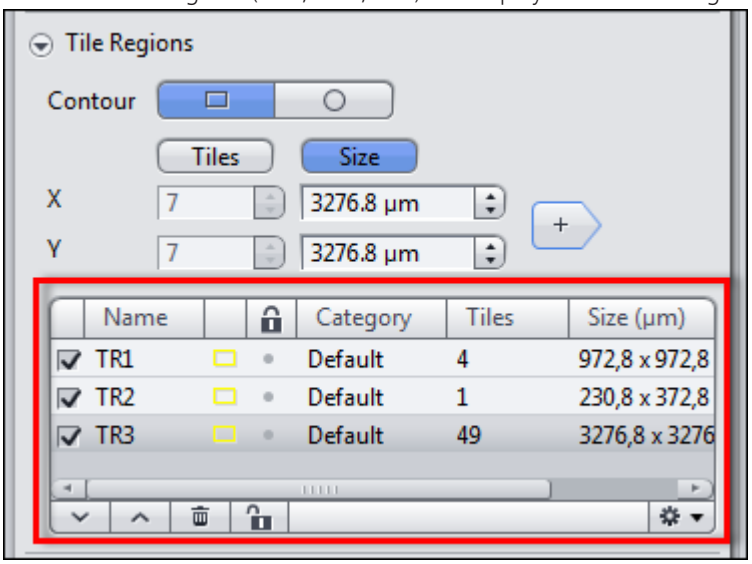
4 The **Tiles** mode is activated by default. In this mode enter the number of tiles you want in the **X** and **Y** input fields, e.g. **X** = 3, **Y** = 3 equals a tiles region containing 9 tiles.
Alternatively, you can enter the size of the tile region that you want to add. To do this, activate the **Size** mode.

5 Click on the **Add** button  .

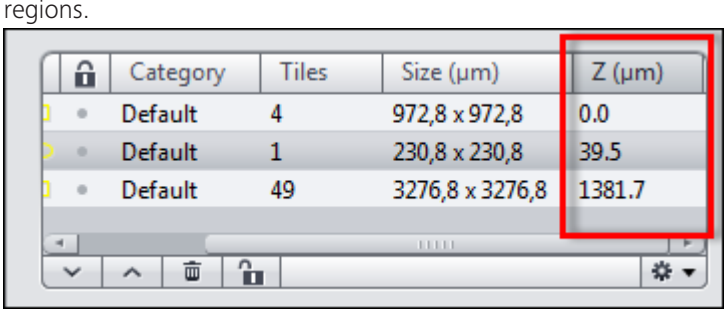
The tile region is added to your experiment. The current stage position determines the center and the Z-position of the tile region.

6 To add further tile regions, move the stage to another position on the sample and repeat the previous steps.

The added tile regions (**TR1**, **TR2**, etc.) are displayed in the tile regions list.



If you scroll to the right in the table, you can read the Z-position of the tile regions.



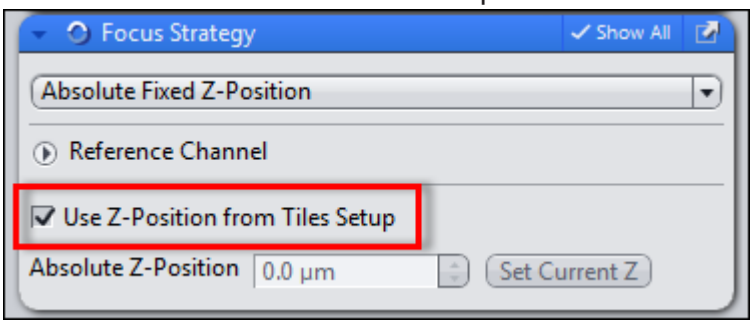
i Info

To ensure that the individual Z-positions of the tile regions are taken into account ZEN automatically selects the most appropriate focus strategy when the checkbox **Tiles** is activated. For the experiment described here no further modification needs to be made. If you want to acquire all tile regions at the same Z-position then you must select **None** from the dropdown list in the **Focus Strategy** tool. The individual Z-positions are then ignored and the current Z-position at the time the experiment is started is used for all tile regions.
The steps 7-9 are not necessary then.

7 In the **Left Tool Area** open the **Focus Strategy** tool on the **Acquisition** tab.

8 Select the **Absolute Fixed Z-Position** entry as the focus strategy from the dropdown list.

9 Activate the **Use Z-Position From Tile Setup** checkbox.



10 Save the experiment. To do this, in the **Experiment Manager** click on the **Options** button and select the **Save As** entry. Enter a name for the experiment in the input field (e.g. Simple Tile Experiment).

11 Click on the **Start Experiment** button.

The **Tile Region** experiment is acquired.

The individual tile regions are displayed in the acquired file as scenes and can be selected using the **Scene** slider on the **Dimensions** tab. If you deactivate the **Scene** checkbox, all tile regions are displayed as an overview.

You have successfully set up and acquired a simple **Tile Region** experiment.

3.3 Setting up a simple positions experiment

Prerequisites

- You have read the chapter *Introduction* [▶ 56].
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1 Open the **Positions** section.

2 Start the **Live** mode to use the stage to locate a position that you want to acquire.

The X and Y coordinates of the current position are displayed in the **Current Position** display fields.

3 Bring the specimen into focus using the focus drive.

4 Click on the **Add** button .

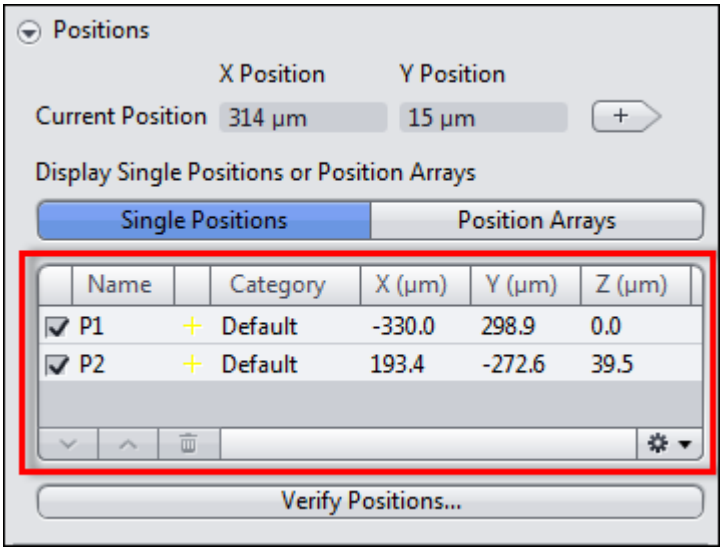
The current position is added to your experiment.

Info

If you are about at the same position in the sample which has been added, then a message appears if you really want to add the last selected position.

5 To add further positions, move the stage to another position on the sample and repeat the previous steps.

The added positions are shown in the list in the **Single Positions** section with their X, Y and Z-coordinates.



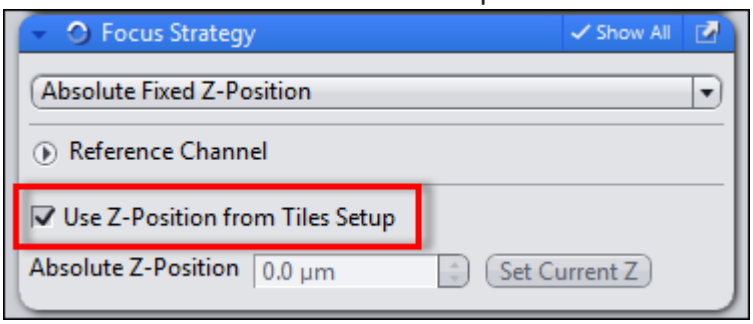
Info

To ensure that the individual Z-positions of the tile regions are taken into account ZEN automatically selects the most appropriate focus strategy when the checkbox **Tiles** is activated. For the experiment described here no further modification needs to be made. If you want to acquire all tile regions at the same Z-position then you must select **None** from the dropdown list in the **Focus Strategy** tool. The individual Z-positions are then ignored and the current Z-position at the time the experiment is started is used for all tile regions.
The steps 6-8 are not necessary then.

6 In the **Left Tool Area** open the **Focus Strategy** tool under **Acquisition Parameters**.

7 Select the **Absolute Fixed Z-Position** entry as the focus strategy from the dropdown list.

8 Activate the **Use Z-Position From Tile Setup** checkbox.



9 Save the experiment. To do this, in the **Experiment Manager** click on the **Options** button and select the **Save As** entry. Enter a name for the experiment in the input field (e.g. Simple Tile Experiment).

10 Click on the **Start Experiment** button.

The Positions experiment is acquired.

The individual positions are displayed in the acquired file as scenes and can be selected using the **Scene** slider on the **Dimensions** tab. If you deactivate the **Scene** checkbox, all positions are displayed simultaneously as an overview.

You have successfully set up and acquired a Positions experiment.

3.4 Tiles & Positions with Advanced Setup

Advanced Setup makes it easier for you to create tile regions and positions by displaying the distribution and dimensions of tile regions and positions in the travel range of the stage. You can generate a **Preview Scan** and drawn in tile regions or positions precisely on the basis of this template. For the preview scan you have the option of using an objective with a lower magnification and/or a different channel (e.g. transmitted light).

i Info

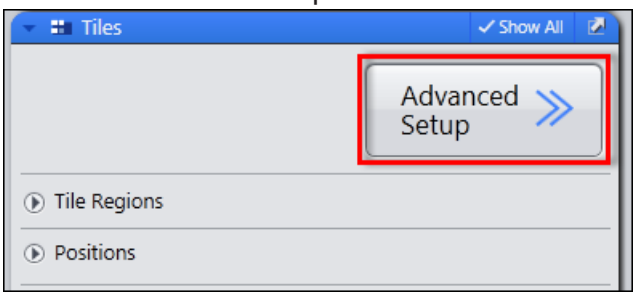
To ensure that the individual Z-positions of the tile regions are taken into account ZEN automatically selects the most appropriate focus strategy when the checkbox **Tiles** is activated. For the experiment described here no further modification needs to be made. If you want to acquire all tile regions at the same Z-position then you must select **None** from the dropdown list in the **Focus Strategy** tool. The individual Z-positions are then ignored and the current Z-position at the time the experiment is started is used for all tile regions.

Prerequisites

- To set up tiles experiments in **Advanced Setup**, you need the **Tiles** module.
- You have read the chapter *Introduction* [▶ 56].
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1 Click on the **Advanced Setup** button.



The **Advanced Tiles Setup** view opens.

The Live mode is activated automatically. Deactivate the live mode if you do not need it to prevent bleaching of the sample. To do this, click on the active **Stop** button in the **Left Tool Area**.

3.4.1 Generating a preview scan

Prerequisites

- You are in **Advanced Setup** in the **Tiles** tool.

Procedure

1 In the **Specific View options** area open the **Preview Scan** tab.

2 Select an objective with a relatively low magnification.

3 In the **Left Tool Area** select a channel in the Channels tool that you want to use for the preview scan. Deactivate the other configured channels.

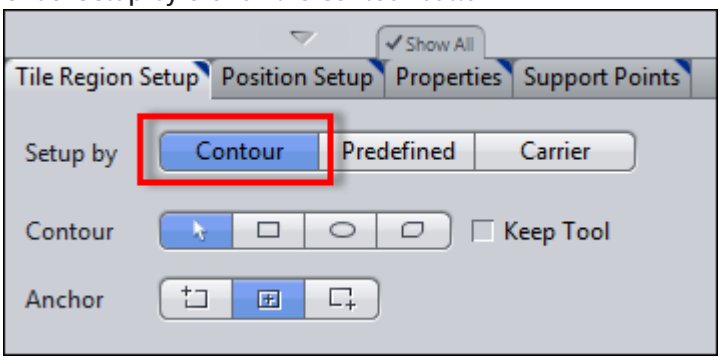
4 If necessary, use the Live mode to adjust the focus area and exposure following a change of objective or channel.

5 To obtain a better overview, zoom out of the **Advanced Setup** view slightly.

6 Start the **Live** mode to use the stage to locate approximately the center of the region for which you want to generate a preview scan.

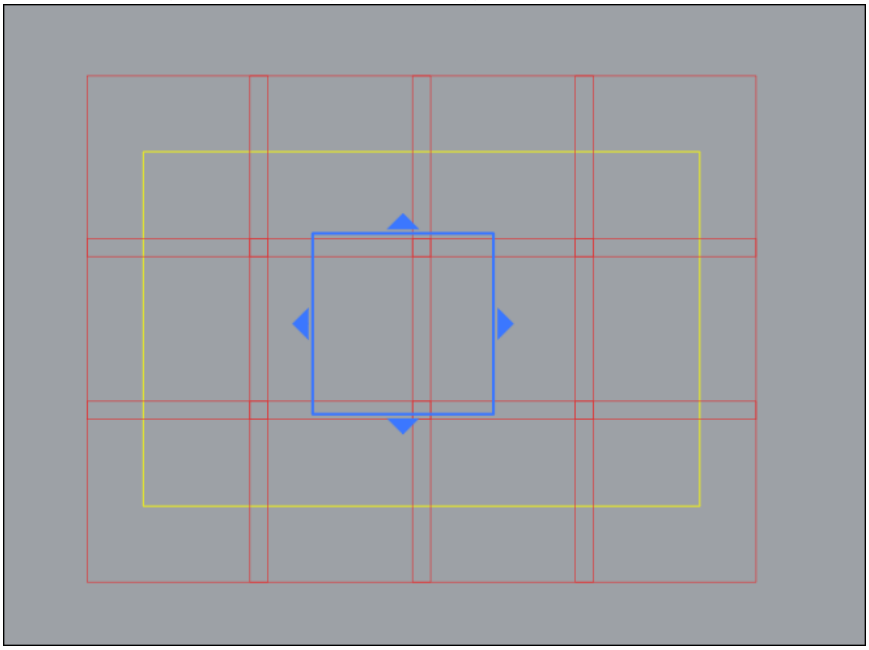
7 Select the **Tile Region Setup** tab from the **Advanced Setup** view options.

8 Under **Setup by** click on the **Contour** button.



9 Under **Contour** select the **Rectangular Contour** tool.

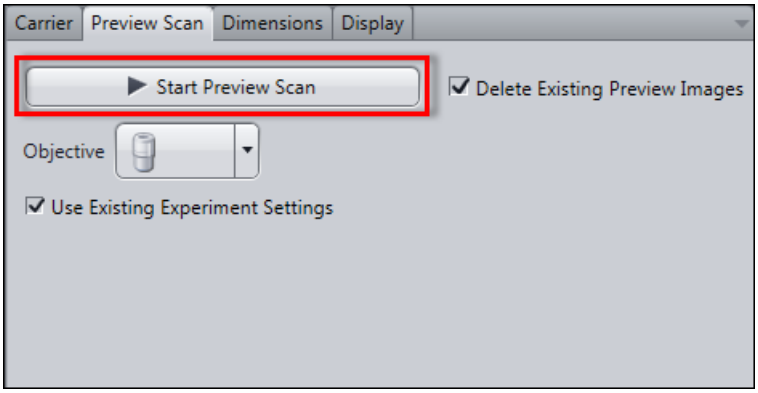
10 In the stage view, use the tool to drag out a rectangle that approximately encloses the region for which you want to generate a preview scan.



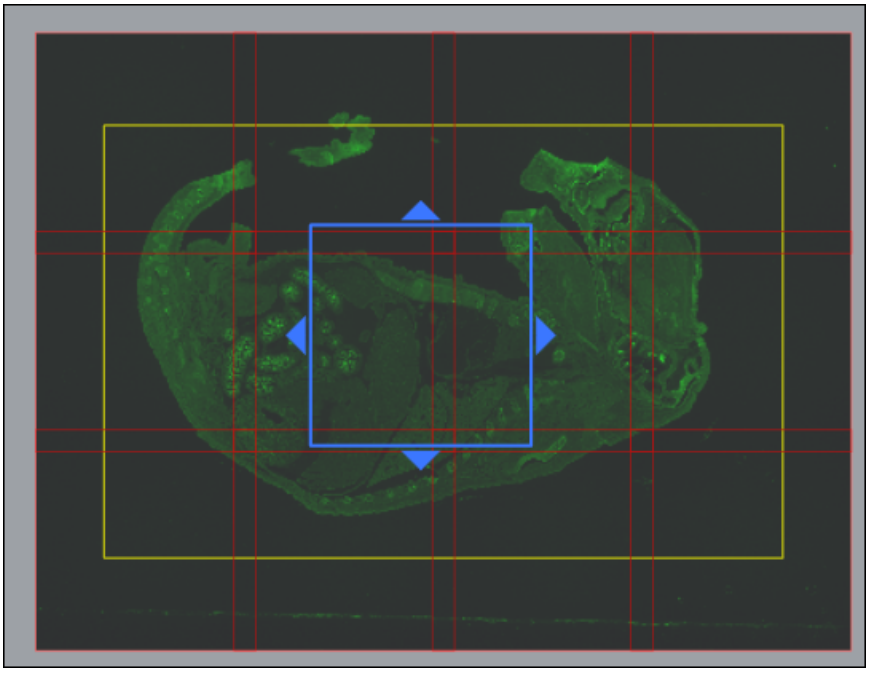
A tile region is created for the marked region and displayed in the list in the **Tile Regions** section of the **Tiles** tool.

11 With the help of the **Live** mode, check whether the desired image region is covered by the tile region. To do this, use the stage to locate the corners and edges of the tile region and increase or reduce the yellow selection frame as necessary.

12 In the **Preview Scan** tab click on the **Start Preview Scan** button.



A series of snap images is acquired to generate a preview of the marked region.

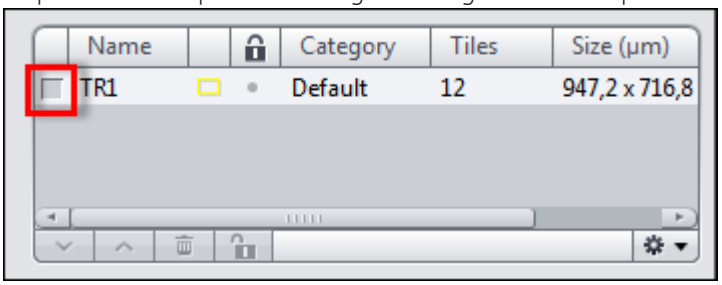


You have successfully generated a preview scan.

Before you continue with the actual experiment, carry out the following steps:

Procedure

1. In the **Tiles Regions** section deactivate the preview tile region (TR 1) by deactivating the checkbox of the corresponding list entry. This prevents the acquisition of the preview tile region during the actual experiment.



2. In the **Preview Scan** tool select the objective you want to use for final acquisition.
3. In the **Channels** tool activate the channels for actual acquisition.
4. Use the **Live** mode to adjust the **focus area** and **exposure** accordingly.

You can now continue setting up the tile experiment.

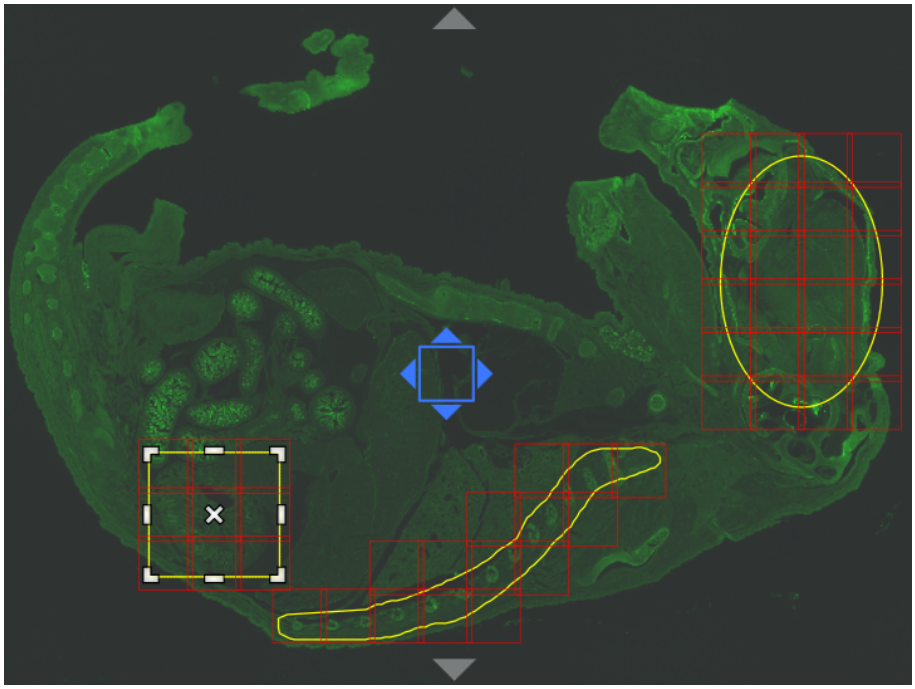
3.4.2 Creating tile regions by Contour

Prerequisites

- You have generated a *preview scan* [▶ 65] that will help you to position the tile regions more easily.

Procedure

1. Select the **Tile Region Setup** tab from the **Advanced Setup** view options.
2. Under **Setup by** select **Contour**.
3. In the **Contour** section select the desired contour tool.
4. Use the **Contour** tool in the stage view to draw in the tile regions you want to acquire.



Tile regions are created for each marked region. They are added to the list in the **Tile Regions** section of the **Tiles** tool.

You have successfully created tile regions in **Advanced Setup**.

3.4.3 Creating tile regions by Predefined

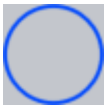
Prerequisites

- You are in **Advanced Setup** in the **Tiles** tool.

Procedure

1. In the **Carrier** tab (lower left side, below the Center Screen area) select the well(s) of interest by holding **Ctrl-key** and clicking on the desired wells.

   The selected wells are now bordered by a blue circle.

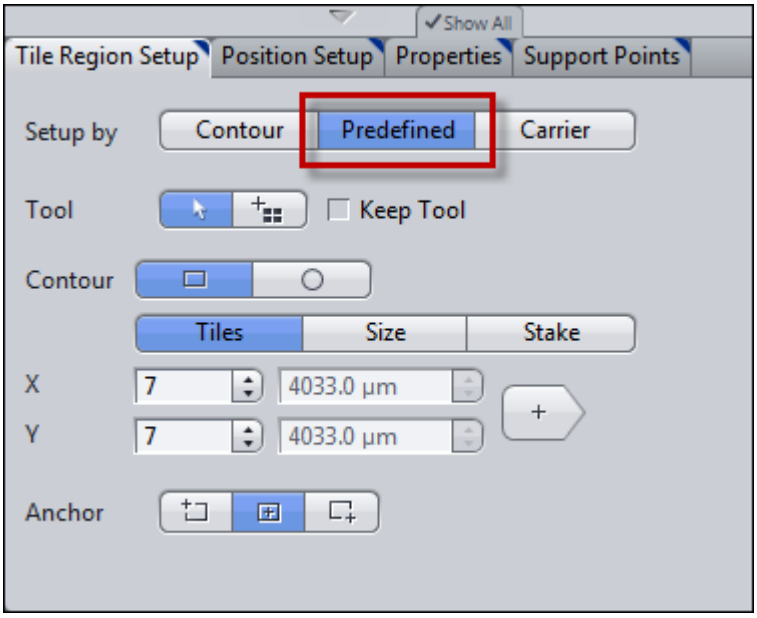


Info

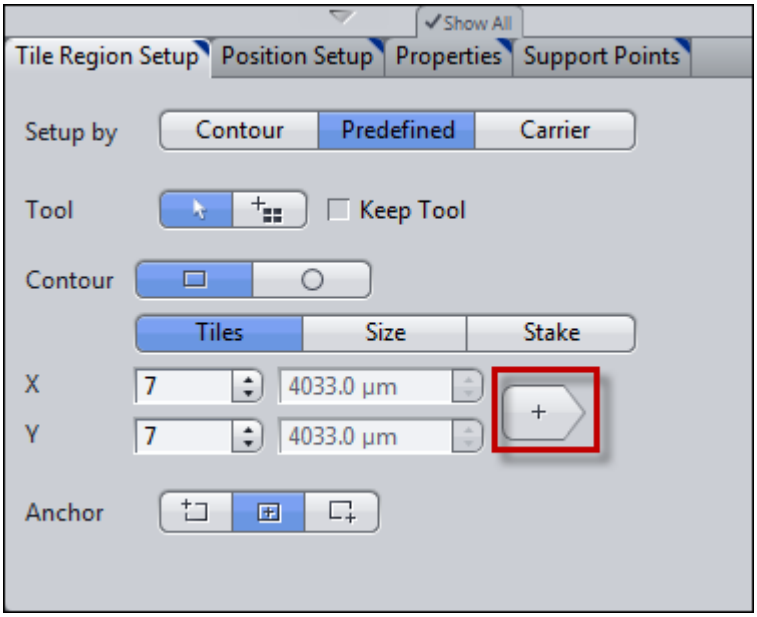
If only one well is double-clicked, the stage will move to the center of that well.

2 Open the **Tile Region Setup** tab below the Center Screen Area and activate the **Show All** mode.

3 In the **Tile Region Setup** tab under **Setup by** select **Predefined**.



4 Choose how many tiles in x and y dimension you want to add and click on the **Add** button + to confirm.



The predefined Tile Region is now created top left, centered or bottom right in the chosen wells depending on the anchor position .

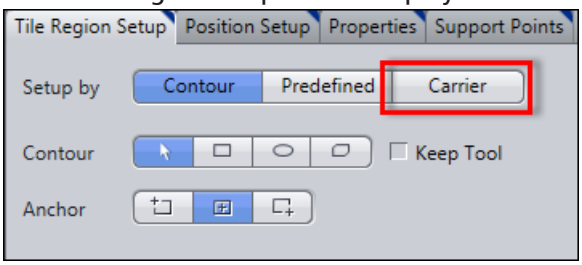
3.4.4 Creating tile regions by Carrier

Prerequisites

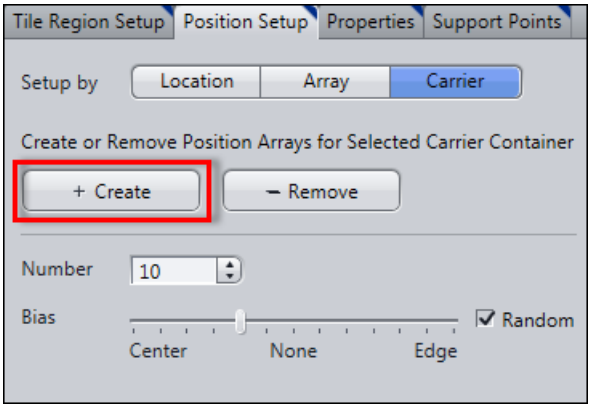
- You are in **Advanced Setup** in the **Tiles** tool.

Procedure

1. Open the **Tile Region Setup** tab below the Center Screen Area and activate the **Show All** mode.
2. In the **Tile Region Setup** under **Setup by** select **Carrier**.



3. In the **Carrier** tab select the individual wells for which you want to create Tile regions by holding **Ctrl-key** and clicking the desired wells.
4. In the **Position Setup** tab enter the size of your desired area per well in the **Fill Factor** input field and click on the **Create** button.



According to the selected **Fill Factor**, the wells will be filled with a calculated number of tiles that are located around the center.

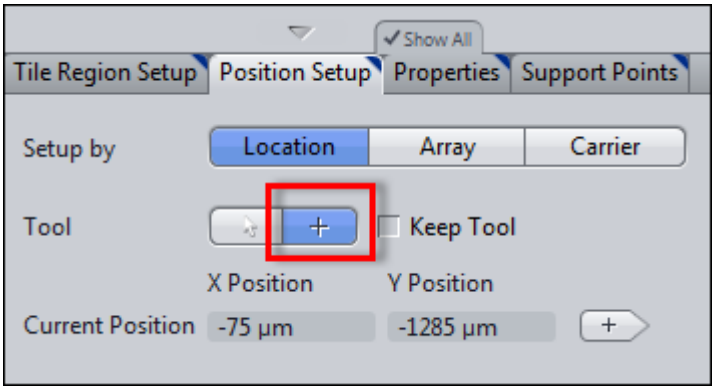
3.4.5 Creating positions by Location

Prerequisites

- You are in **Advanced Setup** in the **Tiles** tool.

Procedure

1. Select the **Position Setup** tab from the **Advanced Setup** view options.
2. Under **Setup by** click on the **Location** button.
3. In the **Tool** section select the **Add** tool.



4. In the stage view click on the location at which you want to add a position.

   The added positions are displayed in the **Single Positions** list in the **Positions** section of the **Tiles** tool.

You have successfully created positions in **Advanced Setup**.

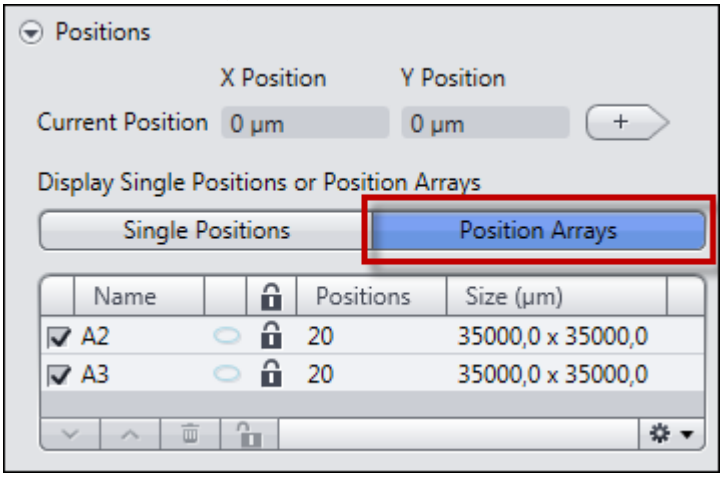
3.4.6 Creating positions by Array

Prerequisites

- You are in **Advanced Setup** in the **Tiles** tool.

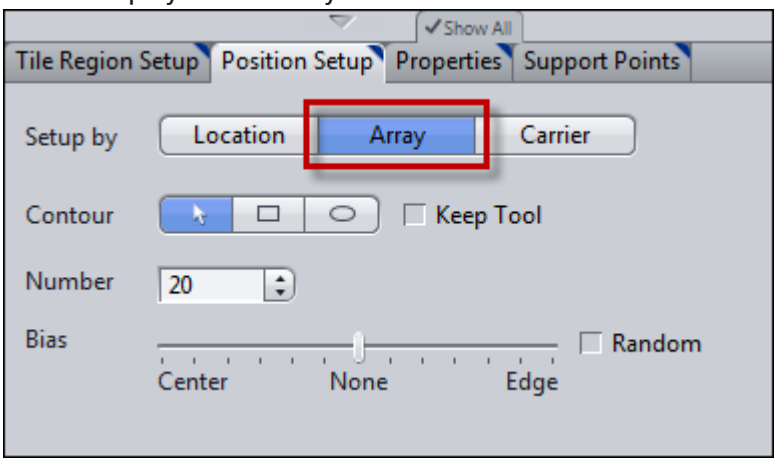
Procedure

1. In the **Tiles** module go to the **Positions** section.
2. Select **Position Arrays**.



3. Go to the **Position Setup** tab (below the Center Screen Area) and activate the **Show All** mode.

4 Under **Setup by** click on **Array**.



5 Either choose the rectangular or circular **Contour**, adjust the **Number** of required positions and the **Bias** where the positions should be located.

i Info

If the **Random** checkbox is activated the chosen number of positions for the array will be determined randomly within the arrays space.

6 Subsequently, mark the interesting area of the carrier in the Center Screen Area by keeping the left mouse button clicked.

The positions will be automatically generated.

3.4.7 Creating positions by Carrier

Prerequisites

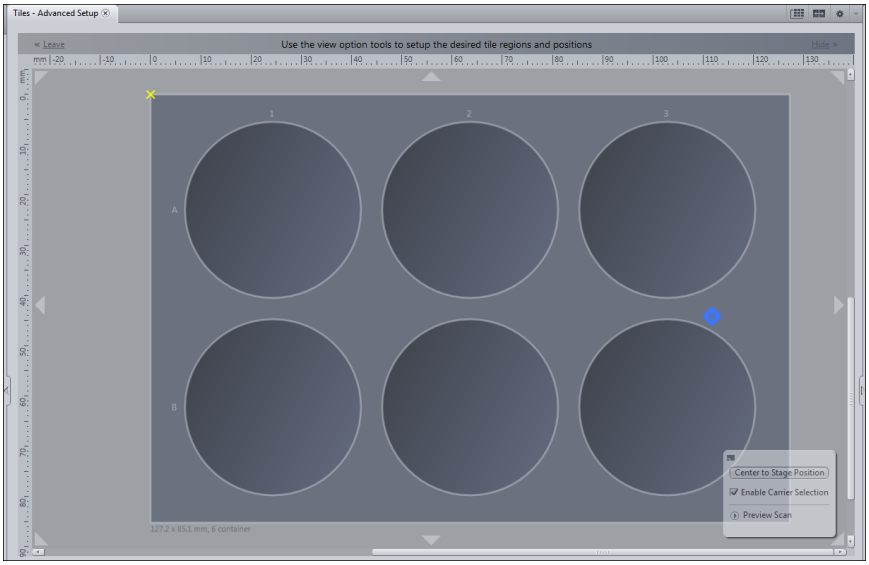
- You have selected and calibrated a *sample carrier template* [▶ 96].
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

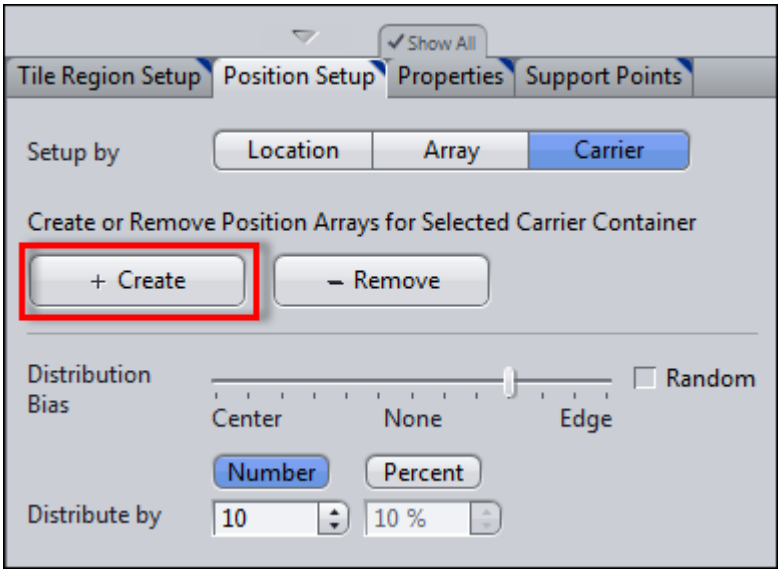
1. Click on the **Advanced Setup** button.

   Advanced setup is opened.

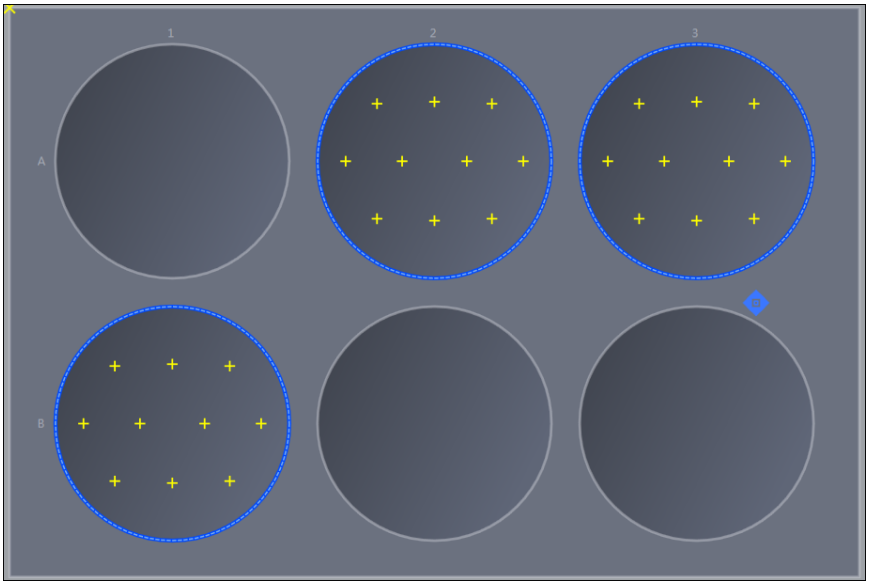
2. To obtain a complete overview of the sample carrier, zoom out of the view (**Crtl** + Mouse wheel).



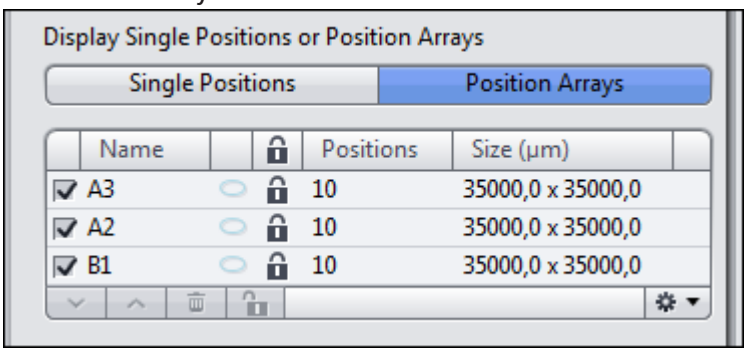
3. In the view options select the **Position Setup** tab.
4. In the **Setup by section** click on the **Carrier** button.
5. Select the **containers** in which you want to distribute **positions** by holding down the **Ctrl** key and clicking on the relevant containers.
6. Click on the **Create** button.



The selected **containers** are each filled with a **Position Array** (group of positions).



In the **Positions** section of the **Tiles** tool the **Position Arrays** are displayed in the **Position Arrays** list.



You have successfully used a **sample carrier** and the **Setup by Carrier** to create positions.

Info

Analogous to the **Position Arrays** tile regions can also be created on the**Tile Region Setup** tab by using the **Carrier** button. In both cases, you can use the additional functions of the carrier setups to make other useful settings. For example, the patch surface of containers or the number and distribution of positions can be set, see *Setup by carrier (tile region)* [▶ 40] and *Setup by carrier (position)* [▶ 44].

3.5 Copying a Tile Region or Position

When you want to copy and paste a Tile Region or Position setting (e.g. a certain arrangement of tiles, positions or local support points) from one well to other wells or even to all containers of a carrier, apply the following workflow.

Procedure

1. Select the well from where the Tile Region/ Position setting should be copied.

   The selected well is now highlighted by a blue border.

2. Right click within the selected well in the Center Screen Area (outside the tile region) to open the context menu.

3. Select **Copy Container for replication**.

4. If you want to choose specific wells and not all use the left mouse button to select the wells into which you want to paste the copied Tile Region/ Position setting.

> **Info**
>
> You can select multiple wells in combination with the **Ctrl**-key.

5. Right click in the Center Screen Area and select the context menu entry **Paste Replication to** and either choose **Selected Container** or **All Container**.

The copied Tile Region/ Position setting is pasted into the selected wells or all the wells of the carrier with the same relative coordinates to the center of each well.

3.6 Adjusting Z-positions

If you add positions or tile regions, the current Z-position is automatically adopted for the tile region or position.

- You can read about how to check and change the Z-positions of positions under *Adjusting Z-positions of positions* [▶ 77].
- You can read about how to check and change the Z-positions of tile regions under *Adjusting Z-positions of tile regions* [▶ 76]. Please bear in mind that the Z-positions defined here are valid for all tiles in the tile region in question.

To acquire large tile regions on tilted or uneven specimens, you need to assign individual Z-values to the individual tiles of a tile region. You do this by *Creating a local focus surface* [▶ 79]. Please bear in mind that a **Local Focus Surface** is always associated with precisely one tile region. You therefore need to create a focus area separately for each tile region.

To create a focus area covering the entire sample, create a Global Focus Surface. **Global Focus Surfaces** are based on a sample carrier template (e.g. for slides or multiwell plates) and result in a focus surface that is valid for the entire sample carrier and therefore for all the tile regions and positions it contains. This allows you to compensate for any tilting and bending of the sample carrier.

Info

To ensure that the individual Z-positions of the tile regions are taken into account ZEN automatically selects the most appropriate focus strategy when the checkbox **Tiles** is activated. For the experiment described here no further modification needs to be made. If you want to acquire all tile regions at the same Z-position then you must select **None** from the dropdown list in the **Focus Strategy** tool. The individual Z-positions are then ignored and the current Z-position at the time the experiment is started is used for all tile regions.

3.6.1 Adjusting Z-positions of tile regions

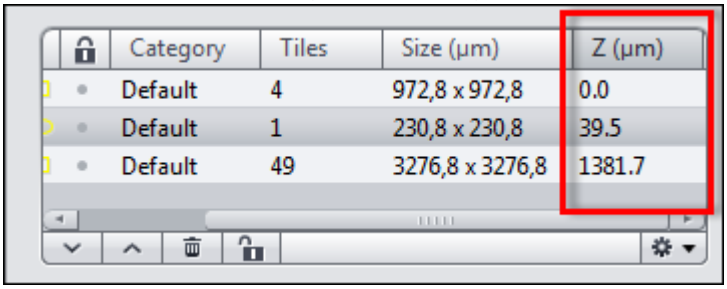
Prerequisites

- You have set up a **Tiles** experiment with at least one tile region. Further information on this can be found under: Set up tiles and positions experiments.

Procedure

1. To check the Z-position of tile regions, open the **Tile Regions** section in the **Tiles** tool.

   The Z-positions of the tile regions are displayed in the last column of the list.



2. Double-click on the list entry of the tile region that you want to check.

   The stage automatically locates the center of the tile region and the associated Z-position.

3. Use the Live mode to check the Z-position of the tile region.
4. To adjust the Z-position, set the new Z-position using the focus drive.
5. In the **Tile Regions** list click in the bottom right on the **Options** button and select **Set Current Z For Selected Tile Regions**.
6. To check further tile regions, repeat steps 2 to 4.

You have successfully checked and adjusted the individual Z-positions for **tile regions**.

3.6.2 Adjusting Z-positions of positions

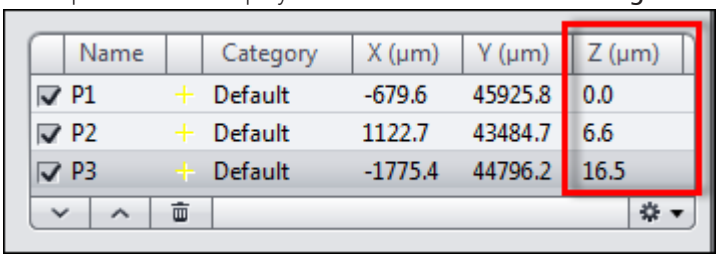
Prerequisites

- You have set up a tile experiment with at least one position. Further information on this can be found under: Set up tiles and positions experiments.
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1. To check and adjust the Z-position of positions, open the **Positions** section.

   The Z-positions are displayed in the last column of the **Single Positions** list.



2. Double-click on the list entry of the position that you want to check.

   The stage automatically locates the position.

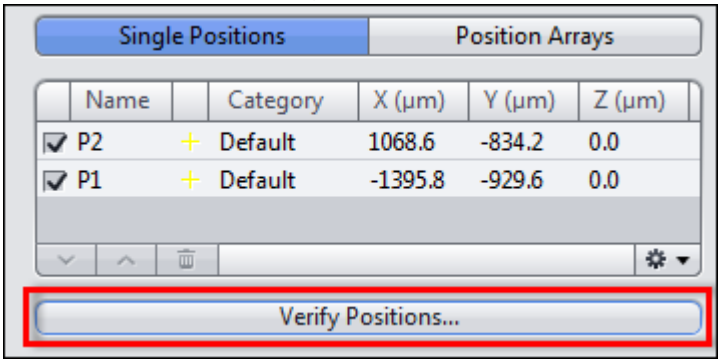
3. Use the Live mode to check the Z-position of the position.

4. To adjust the Z-position, set the desired Z-position using the focus drive.

5. In the **Single Positions** list click in the bottom right on the **Options** button  and select **Set Current Z For Selected Positions**.

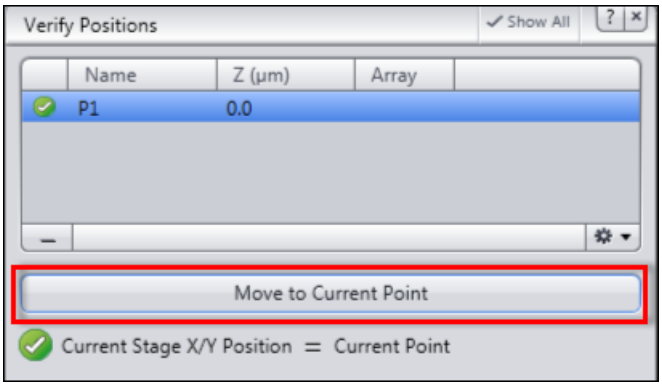
6. To check and adjust a large number of **positions**, use the **Verify Positions** dialog.

7. To do this, click on the **VerifyPositions…** button in the **Positions** section.



   The **Verify Positions** dialog opens.

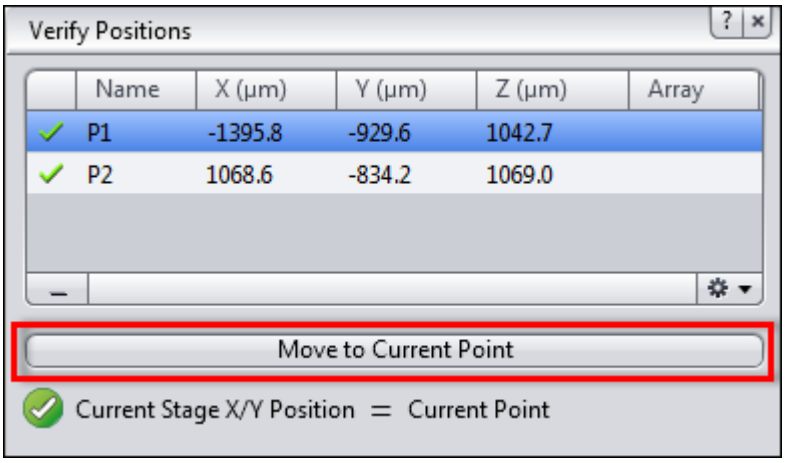
8 Click on the **Move to Current Point** button.



The stage moves automatically to the position in the list that is highlighted in blue. Alternatively, you can double-click on the position in the list that you want to check.

9 Use the **Live** mode to set the desired Z-position using the focus drive. Alternatively, you can have the Z-position of the focal plane determined automatically by clicking on the **Start Autofocus** button.

10 Click on the **Set Current Z** button.



The position is marked with a check mark.

11 Click on the **Move to Next Point** button.

The stage moves automatically to the next position in the list.

12 Repeat the last 3 steps until you have checked all the points in the list.

The message **All points have been verified** appears.

13 Close the **Verify Positions** dialog.

You have successfully verified and adjusted the individual Z-positions for positions.

3.6.3 Creating a local focus surface

To create local focus surfaces, you must distribute support points across your tile regions and assign their focus position. Tile-region-specific focus areas are then interpolated from the values of these support points.

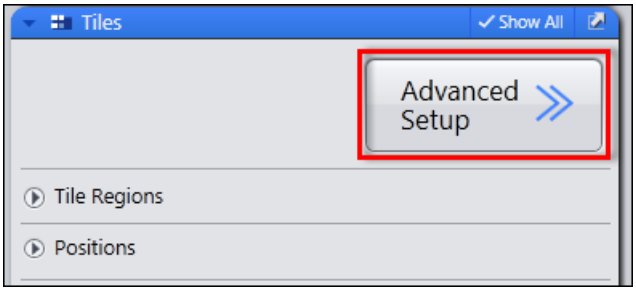
3.6.3.1 Distributing Support Points

Prerequisites

- To create a local focus surface you will need the **Tiles** module.
- You have set up a **Tiles** experiment with at least one tile region. Further information on this can be found under: Set up tiles and positions experiments.
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1 Click on the **Advanced Setup** button.



Advanced tile setup is opened.

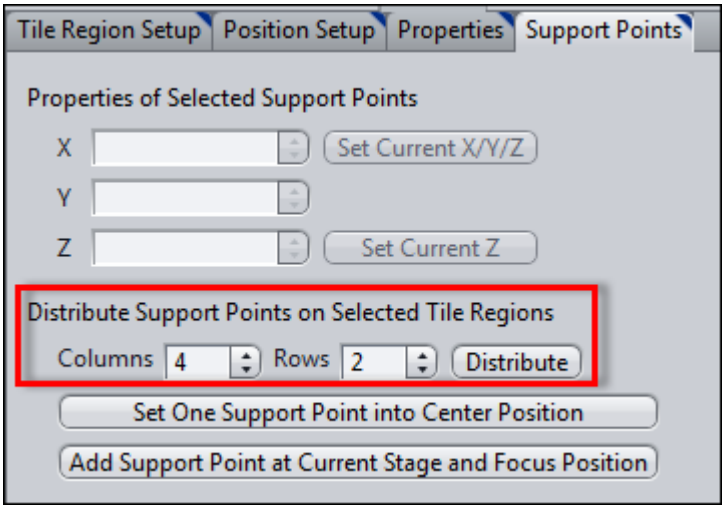
2 Select a tile region for which you want to create support points. To do this, click on the corresponding tile region in the list in the **Tile Regions** section of the **Tiles** tool.

Info

Alternatively, you can select tile regions by clicking directly on the desired tile region in the **Advanced Setup** view. Both methods allow you to select several tile regions simultaneously by holding down the **Ctrl** key.

3 Select the **Support Points** tab from the **Tiles - Advanced Setup** view options.

4 Under **Distribute Support Points on Selected Tile Regions**, indicate the number of columns and rows for the distribution of the reference points.



5 Click on the **Distribute** button.

The support points are distributed within the tile region selected and shown as yellow points in the  stage view.

The support points of the selected tile region are displayed with their coordinates in the **Local (per Tile Region)** list in the **Focus Surface** section of the **Tiles** tool.

6 If necessary, you can adjust the distribution of the support points manually in the **Advanced Setup**. You can change the position of the support points using drag & drop.

7 Additional, individual support points can be added by using the stage to locate the desired position and clicking on the **Add Support Points At Current Stage and Focus Position** button on the **Support Points** tab.

i Info

Distribute the support points evenly across your **tile region**. The more irregular the surface of your specimen, the more reference points you should set. An even but tilted surface requires at least 4 reference points for a solid calculation, while a simple saddle surface requires at least 9 reference points. A high reference-point density leads to a more precise result, although the maximum useful density is one reference point per tile.

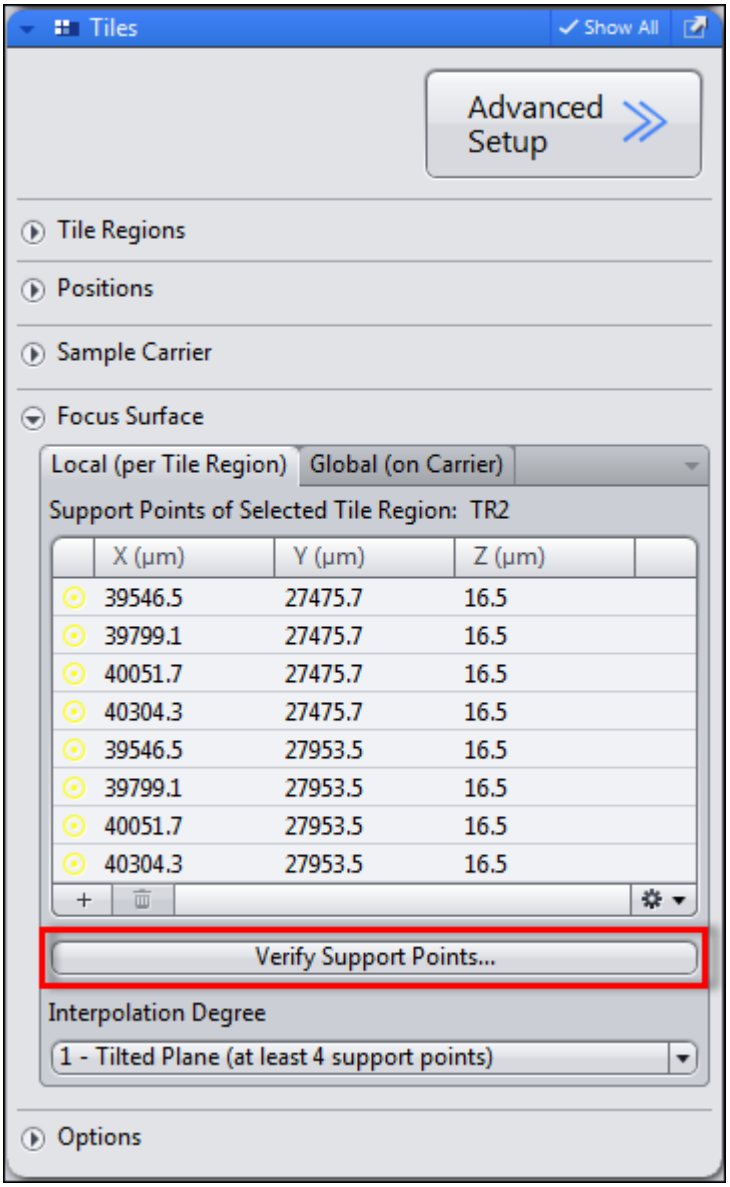
8 Repeat steps 2 to 6 until you have distributed reference points across all desired tile regions.

You have successfully distributed support points across the tile regions.

3.6.3.2 Verifying Z-Position of Support Points

Procedure

1 Click on the **Verify Support Points...** button in the **Focus Surface** section of the **Tiles** tool.



The **Verify Local Support Points** dialog opens.

2 Click on the **Move To Current Point** button.

The stage moves automatically to the support point that is highlighted in blue in the reference point list. Alternatively, you can also double-click on the support point in the list that you want to check.

3 Use the Live mode to set the Z-position using the focus drive. Alternatively, you can have the Z-position of the focal plane determined automatically by clicking on the **Run Autofocus** button.

4 Click on the **Set Current Z** button.

The checked reference point is marked with a green check mark.

5 Click on the **Move To Next Point** button.

The stage moves automatically to the next support point in the list.

6 Repeat the last 3 steps until you have checked all the support points.

The message **All points have been verified** appears.

7 Close the **Verify Local Support Points** dialog.

You have successfully verified the Z-positions of the support points.

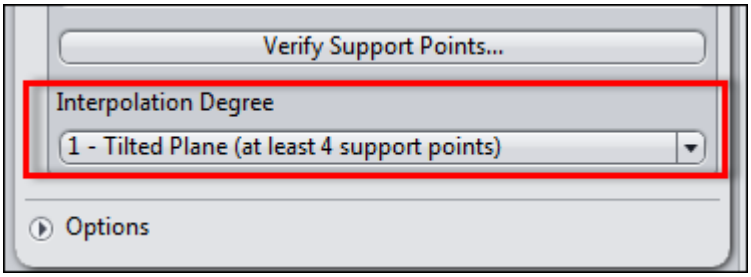
i Info

Positions always have a horizontal local focus surface, which is determined by the Z-value of the position. If you use positions in addition to tile regions, you can verify the Z-values of the positions with the help of a similar dialog. Open this dialog by clicking on the **Verify Positions...** button in the **Positions** section of the **Tiles** tool.

3.6.3.3 Selecting Interpolation Degree

Procedure

1 Select the interpolation level in the **Interpolation Degree** dropdown list in the **Focus Surface** section.



i Info

The minimum number of support points necessary per tile region is indicated in the **Interpolation Degree** dropdown list for each entry. The calculation is more solid if the number of support points exceeds this minimum number. We therefore recommend that you only increase the interpolation degree as far as the surface of the sample demands, even if you have set more support points. If the number of support points does not correspond to the minimum number for the selected interpolation degree, the interpolation degree will be reduced automatically.

You have successfully created a local focus surface.

Info

To ensure the Tiles are acquired along the focus surface during the experiment ZEN automatically selects the most appropraite focus strategy in the **Focus Strategy** tool. For information on this please read: Use focus strategies.

3.6.4 Creating a global focus surface

To create a global focus surface, you must distribute support points across your sample carrier and indicate their focus position. A focus area across the sample carrier is then interpolated from the values of these reference points.

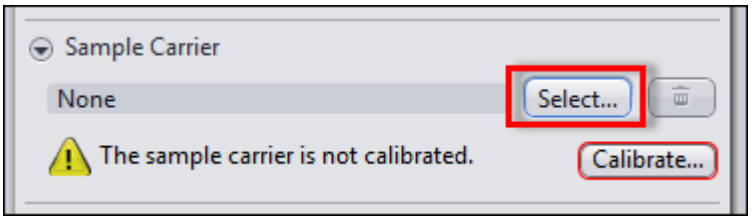
3.6.4.1 Distributing Support Points

Prerequisites

- You have configured the general settings for setting up a tile experiment (experiment created, at least one channel defined, Tiles dimension activated).
- To create a global focus surface, you will need the **Tiles** module.
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1. Open the **Sample Carrier** section.
2. Click on the **Select...** button.

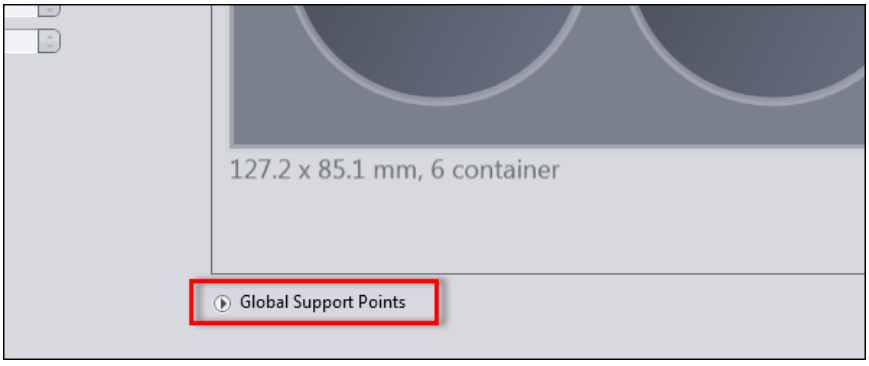


   The **Select Template** dialog opens.

3. Select the sample carrier template that you want to use.
4. Click on the **Options** button and select the **Copy And Edit...** entry.

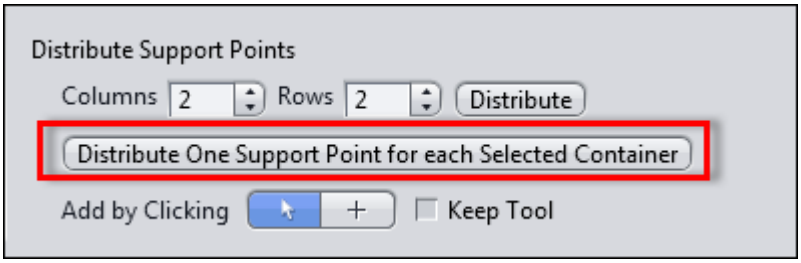
   A copy of the existing template is generated and opened in the Sample Carrier Editor.

5. To distribute support points across the sample carrier template, open the **Global Support Points** section.



6 Select the containers in which you wish to create support points. To do this, hold down the **Ctrl** key and click on the containers.

7 Click on the **Distribute One Support Point For Each Selected Container** button.



One support point is assigned to each container selected.

Info

If you use a **sample carrier** without containers (e.g. slide), use the **Distribute** button instead, to distribute support points on the basis of columns and rows.

The support points are distributed automatically across the sample carrier.

You can add further support points manually using the **Add** button  .

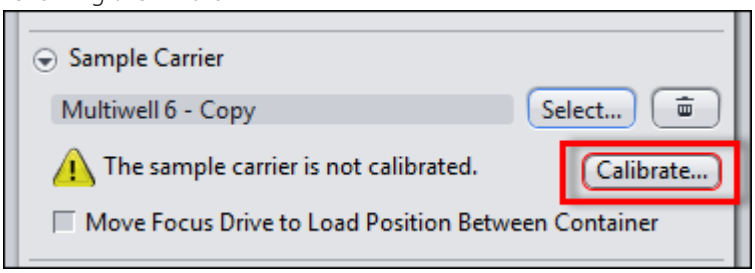
Info

Only create support points where you can bring the sample into focus (within the containers). This is the only way that you can indicate the Z-position of the support points later. The assignment of container-based support points to the center of the container is fixed and these cannot be moved. If the surface of your **sample carrier** is tilted but even, you will need at least 4 support points for a solid calculation. The more irregular the surface, the more support points you should distribute.

8 To close the **Editor** window, click on the **OK** button.

9 To select the edited **sample carrier template**, click on the **OK** button.

10 Calibrate the sample carrier by clicking on the **Calibrate…** button and following the wizard.



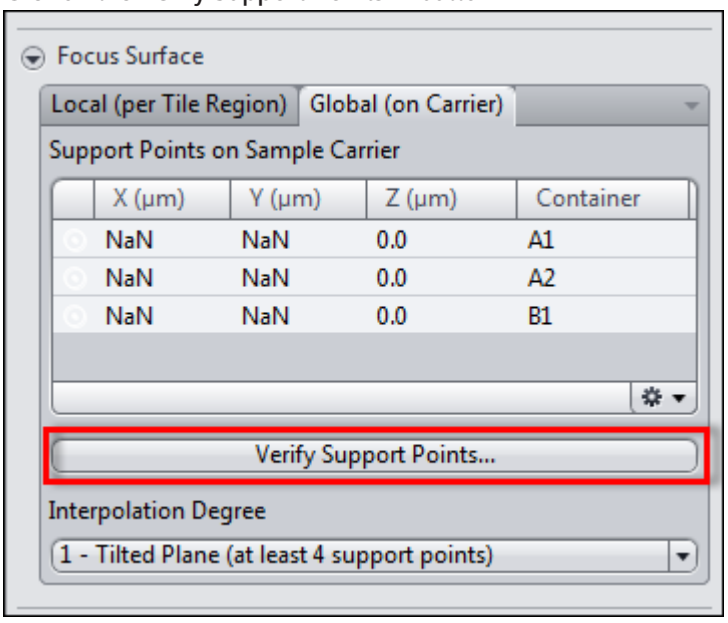
You have successfully distributed support points across a sample carrier template and have selected and calibrated it.

3.6.4.2 Verifying Z-Position of Support Points

Procedure

1. In the **Tiles** tool open the **Focus Surface** section.
2. Go to the **Global (on Carrier)** tab.

   All the support points of the selected sample carrier template are displayed in the **Support Points on Sample Carrier** list.
3. Click on the **Verify Support Points…** button.



   The **Verify Global Support Points** dialog opens.
4. Click on the **Move To Current Point** button.

   The stage moves automatically to the support point that is highlighted in blue in the list. Alternatively, you can also double-click on the support point in the list that you want to check.
5. Use the Live mode to set the Z-position using the focus drive. Alternatively, you can have the Z-position of the focus surface determined automatically by clicking on the **Start Autofocus** button.
6. Click on the **Set Current Z** button.

   The support point is marked with a check mark.
7. Click on the **Move To Next Point** button.

   The stage moves automatically to the next support point in the list.
8. Repeat the last 3 steps until you have checked all the support points.

   The message **All points have been verified** appears.

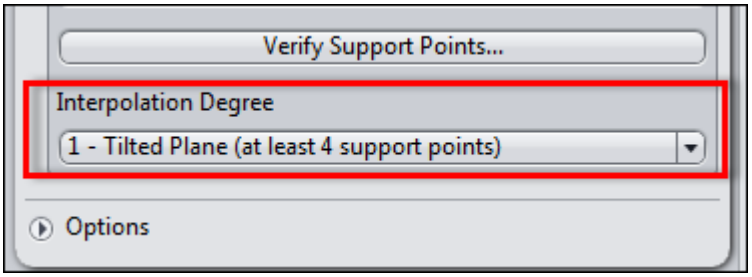
9 Close the **Verify Global Support Points** dialog.

You have assigned a Z-position to all support points.

3.6.4.3 Selecting Interpolation Degree

Procedure

1 Select the interpolation degree in the **Interpolation Degree** dropdown list in the **Focus Area** section.



Info

The minimum number of support points necessary is indicated in the **Interpolation Degree** dropdown list for each entry. The calculation is more solid if the number of support points exceeds this minimum number. We therefore recommend that you only increase the interpolation degree as far as the surface of the carrier demands, even if you have created more support points. If the number of support points does not correspond to the minimum number for the selected interpolation degree, the interpolation degree will be reduced automatically. Interpolation degree **1 – Tilted Plane (at least 4 support points)** is sufficient to compensate for any tilting of the sample carrier.

You have successfully created a global focus surface.

You can now set up your tile experiment using the sample carrier. Further information on this can be found under: *Using sample carriers* [▶ 96].

Info

To ensure the Tiles are acquired along the focus surface during the experiment ZEN automatically selects the most appropriate focus strategy in the **Focus Strategy** tool. Make sure that you select the **Global (on Carrier)** tab in the **Focus Surface** section. For information on this please read: Use focus strategies.

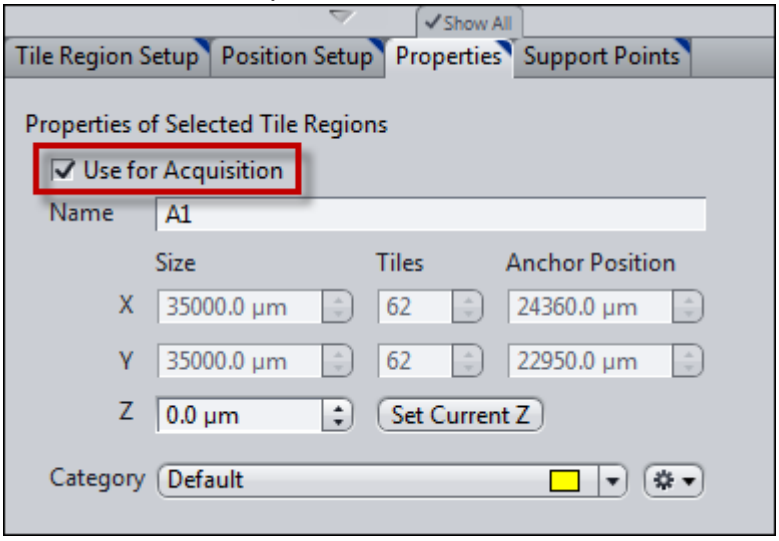
3.7 Assigning categories to Tile Regions and Positions

For some customers it could be important to not only display the well number together with the acquired images (Path: Graphics -> Frequent Annotations -> Carrier Container Name) but also create certain additional annotations for different tile regions or positions, e.g. "control condition" or "experimental condition 1". For that purpose, allows you to add/ edit names and categories to the different Tiles Regions/ Positions that have been generated.

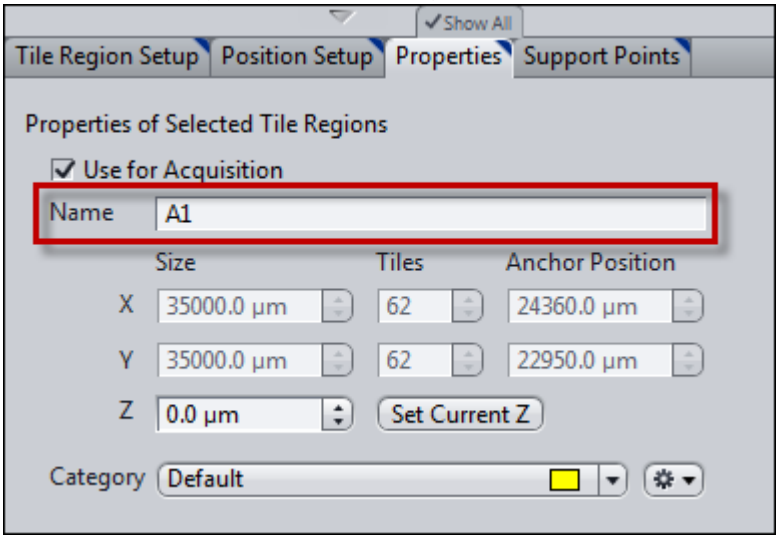
Procedure

1. In order to assign individual names to different individual positions and/or tile regions in a well plate experiment, click on the respective Tile Region or Position and open the **Properties** tab in the Center Screen Area (Tiles – Advanced Setup view).

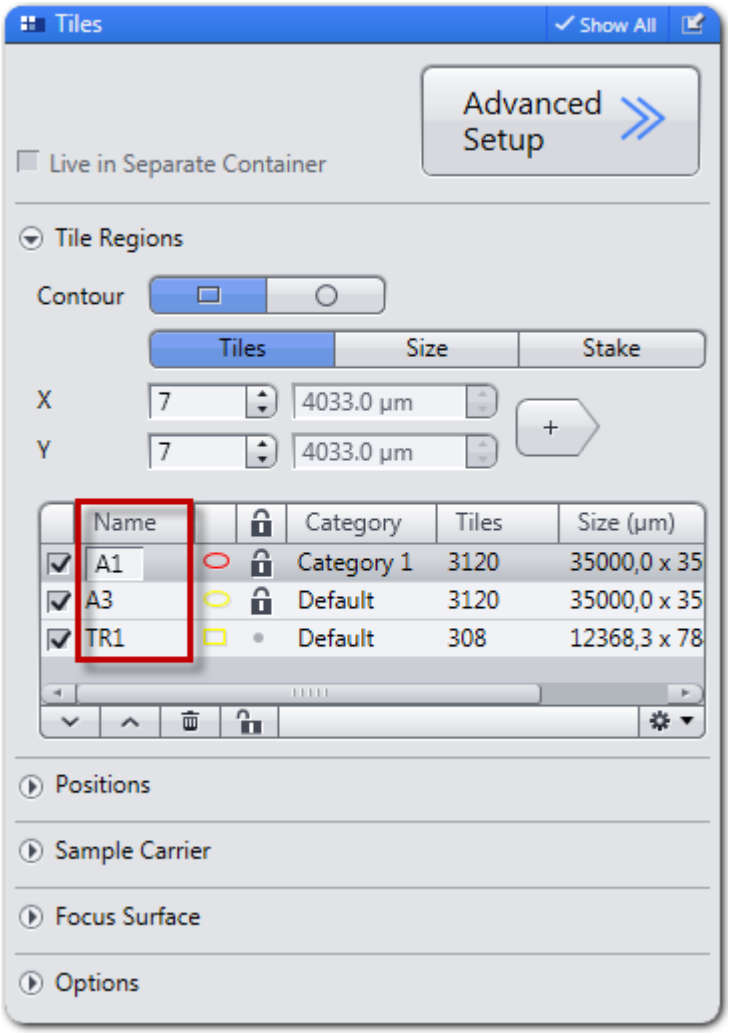
2. Activate the **Use for Acquisition** checkbox.



3. Edit the **Name** for your selected Tile Region/ Position.



4 Alternatively edit the **Name** of a Tile Region/ Position by clicking in **Tiles** module on the respective name under **Tile Regions** or **Positions**.



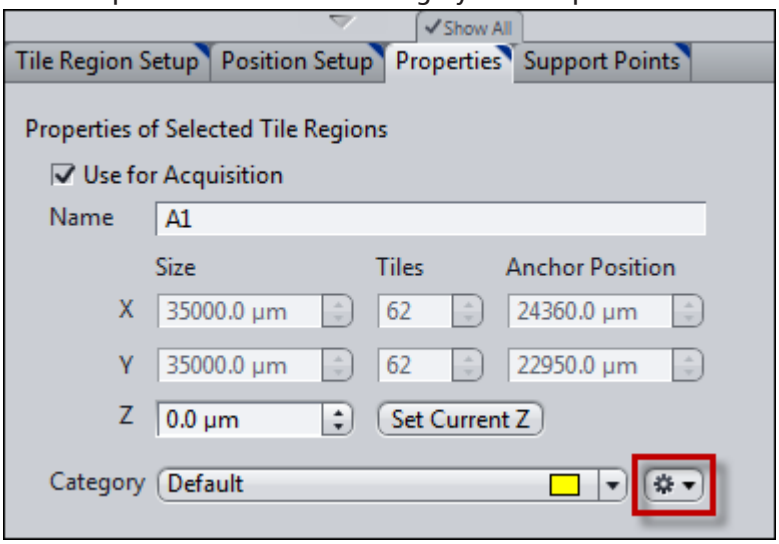
5 Repeat this step when you want to rename different Tile Regions or Positions.

Info

To display the name of your Tile Region/ Position later in your acquired image(s), go to **Graphics**, **Frequent Annotations**, **More…** and select **Image.Scene.Name** from the Metadata list.

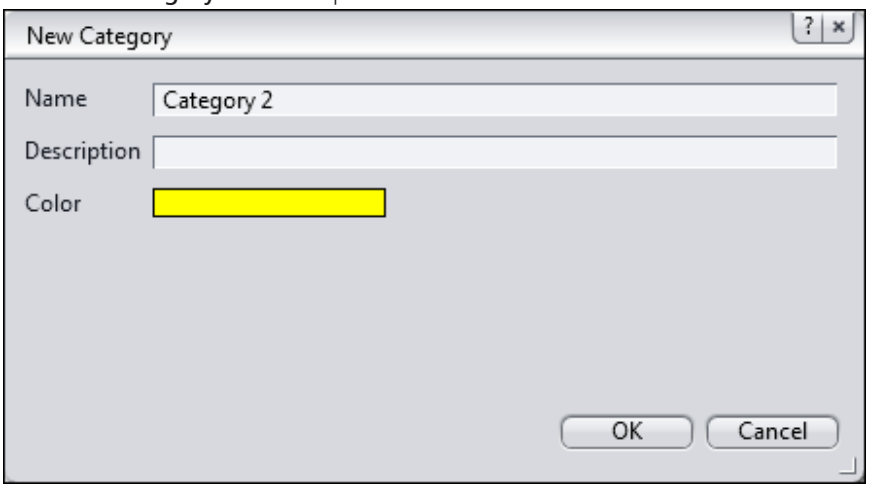
6 To assign or edit categories of your Tile Regions/ Positions, first activate the checkbox of all desired Tile Regions/ Positions that should be grouped in the same category.

7 In the **Properties** tab click under **Category** on the **Options** button.



8 Select **New…** from the dropdown list

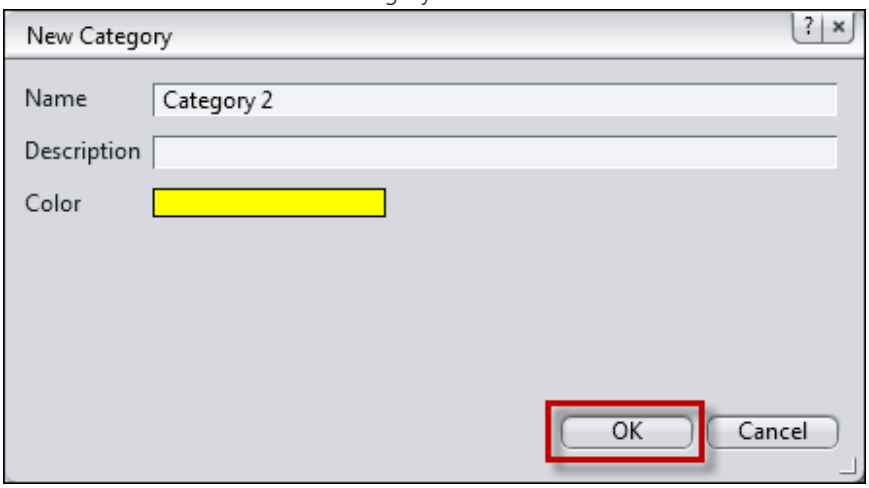
The **New Category** window opens.



9 Enter a **Name** and add a **Description** for the selected Tile Regions/ Positions.

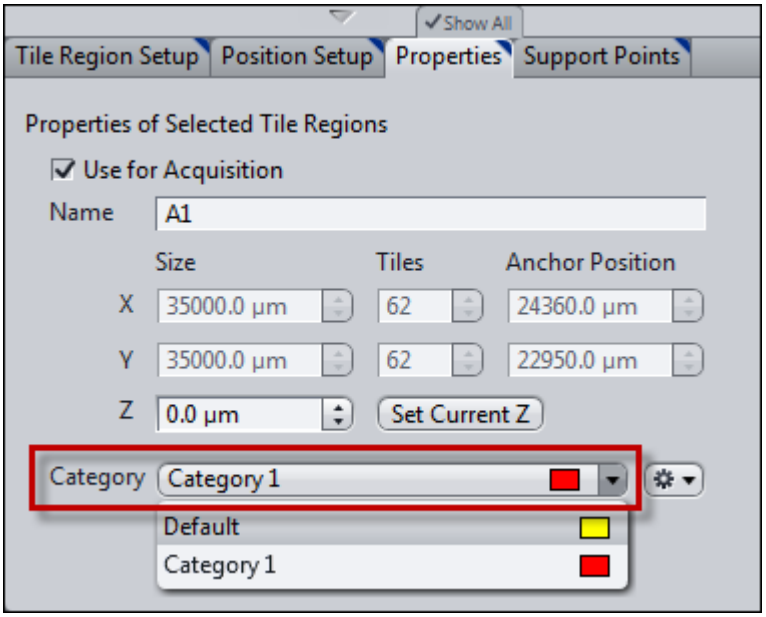
10 Assign a **Color** for the new category by clicking on the color bar and choosing a preferred color.

11 Click on **OK** to create the new category.



The **New Category** window closes and the new categroy is created.

12 Under **Category** choose the desired category for the selected Tile regions/ Positions from the drop down list.



The chosen category is now assigned to the selected Tile Regions/ Positions.

i Info

Note that a predefined category can also be applied to a differentiated selection of Tile Regions/ Positions from more than one well.
Note also, that the assigned color is only used as a feature in the Tiles tab (Left Tool Bar Area).

Info

To display a Tile Region/ Position Category feature (**Name** and/ or **Description**) in your acquired image, you go to **Graphics**, **Frequent Annotations**, **More…**.

Type “category” in the search bar and select the desired feature to be displayed. (Although the option "Color" is given, no reasonable element will be displayed by the software)

Info

To adjust parameters of your annotations (e.g. font size), right-click on it and go to **Format**, **Graphical Elements**.

Displaying categories in the Tiles/ Positions List (Left Tool Bar Area)

Prerequisites

- You have selected several different positions or tile regions and assigned different categories.

Procedure

1. Under **Positions** or **Tiles** of the Tiles module (Left Tool Bar Area) select a position or tile region.
2. Right-click on the selected position/ tile, choose **Sort** and select **By Category**

The positions/ tiles will be sorted alphabetically according to the assigned categories.

3.8 Re-positioning of your sample carrier after incubation

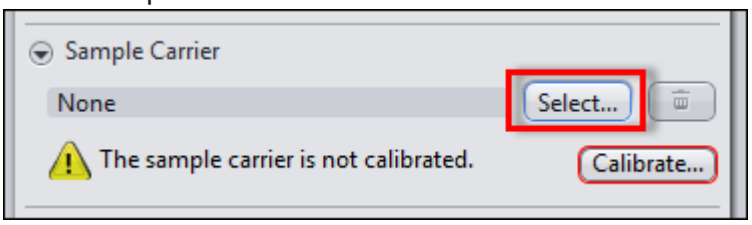
When you want to take images of positions/ tile regions on a sample carrier, that had to be taken off the stage, e.g. for incubation purposes or changes of the immersion medium, proceed as follows to re-position your sample carrier..

Starting the Experiment

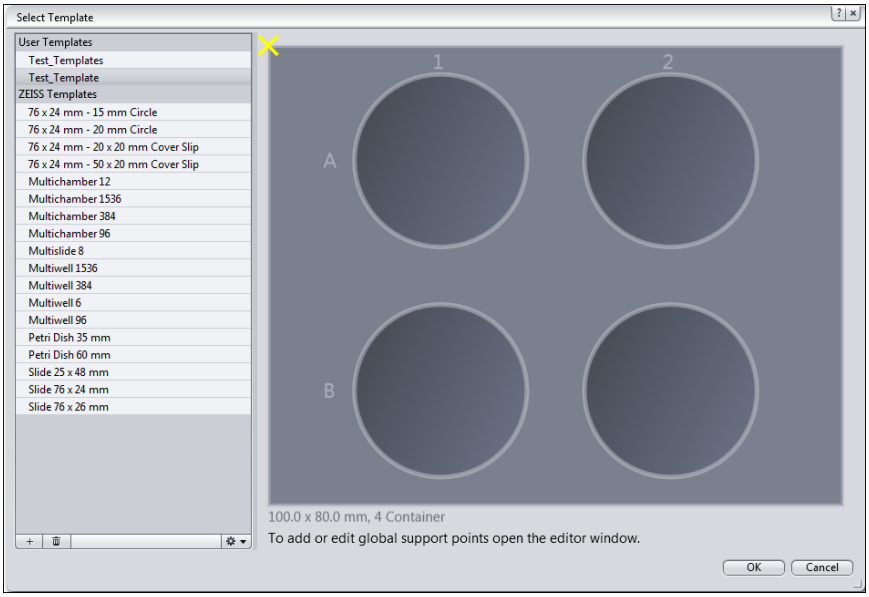
Prerequisites

- You have located you sample under **Locate** and run the **Stage Calibration** see also the chapter *Calibrating Stage and Adjusting Camera Orientation* [▶ 57].
- You have set up at least one channel and adjusted the light/ camera exposure time.
- You have activated the **Tiles** checkbox and the **Show All** mode

Procedure **1** Click on **Sample Carrier** and then click **Select**.



The **Select Template** dialog opens.



2 Select the template of choice.

i Info

For demo purposes, select a standard slide that can mimic your test sample.

i Info

Regarding calibration of your template, you can customize your own carrier see the chapter *Customizing your Sample Carrier* [▶ 97], but for slides with one coverslip or well, there is only the option for Single Reference Point Calibration. For Multi-well plates, you will have the option for 7-point, 4-point, 3-point or 1-point calibration. This becomes important for adjusting for the rotation of the sample.

3 Adjust the surface of the sample carrier. Refer to the chapter *Creating a global focus surface* [▶ 83].

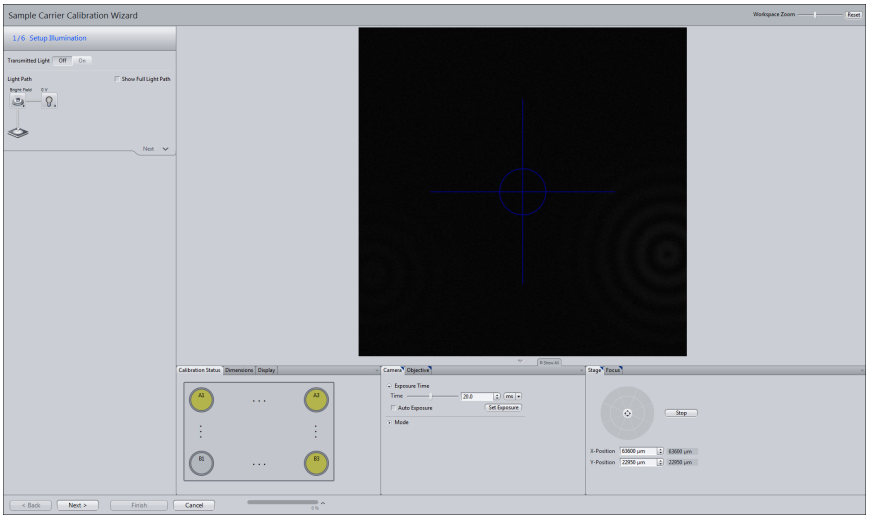
The **Select Template** dialog closes.

Info

In the following it is assumed that you just use a conventional glass slide with some cells or tissue that is positioned in the center

4 Press **Calibrate**.

The **Sample Carrier Callibration Wizard** opens.

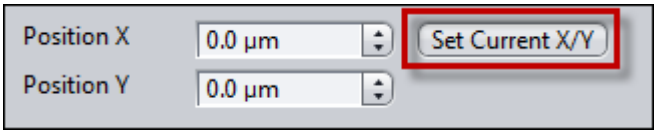


5 Right-click to activate **Crosshairs** in the live image and move a sample reference point into the middle of the crosshairs. This reference point can be any unique identifiable point on the slide and does not have to in the middle of the slide.

6 Under **X/Y Position** click on **Set Zero**.

7 Click **Next** and go to the step **Search Reference Point**.

8 Click on **Set Current X/Y**.



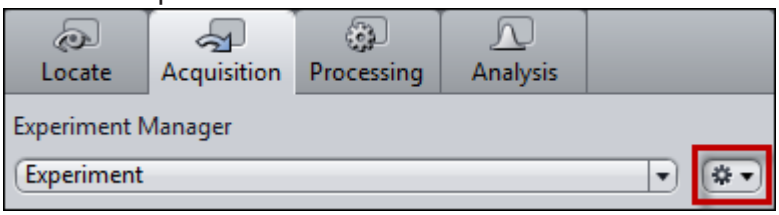
9 In the **Tiles** tool click on **Advanced Setup** and add positions/ tile regions at your locations of interest.

Info

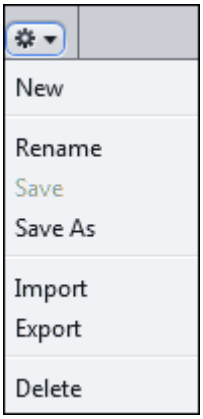
You can zoom in and out using the mouse scroller, and move the stage in the Center Screen Area to a point of interest with a double-click on the sample carrier.

10 Once you have defined all your positions/ tile regions, go in the **Acquisition** tab to the **Experiment Manager** tool.

11 Click on the **Options** button.



12 Save your experimental settings, including the lists of positions/ tile regions, by using either **Save As** or **Export**.



Info

With **Save As** the settings will be saved directly in the Experiment Manager.
With **Export** the settings will be saved in a folder of your choice.

13 Start your experiment and record images from your selected positions/ tile regions.

14 Remove your sample off the stage and e.g. put it back into the incubation chamber.

15 Close software.

You have done all settings for a successfull re-positioning of your sample carrier after the experiment.

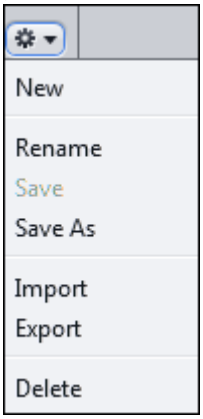
Re-Positioning of the Sample Carrier after the experiment

Info

If you cycle the power on the microscope ZEN will prompt you to calibrate the stage and/or focus drives. Thus, if the calibration of the multi-well plate was performed under the same conditions then the sample carrier calibration will still be valid. You must however, ensure that other parameters like plate orientation and placement on the microscope have not changed.

Procedure

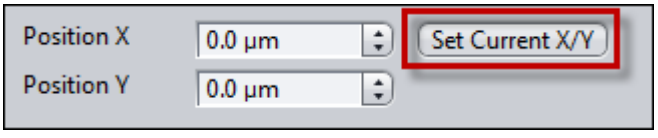
1. Restart the software.
2. In the **Acquisition** tab go to the **Experiment Manager** and Reload or Import your experimental settings including your list of positions/ tile regions.



3. In **Tiles** under **Sample Carrier** click on **Calibrate**.

   The **Sample Carrier Calibration Wizard** opens.
4. Right-click to activate **Crosshairs** in the live image and move your previously chosen sample reference point into the middle of the crosshairs.
5. Under **X/Y Position** click on **Set Zero**.

6. Click **Next** and go to the step **Search Reference Point**.
7. Click on **Set Current X/Y**.



8. Now, you still need to verify the Z-offset of your positions. Therefore, follow the corresponding instructions given in the chapters *Adjusting Z-positions of tile regions* [▶ 76] and *Adjusting Z-positions of positions* [▶ 77].

All of your selected positions/ tile regions are now re-assigned to the correct X/Y/Z-values in relation to your (unique identifiable) reference point.

You can re-start your experiment and record images from your selected positions/ tile regions.

3.9 Using sample carriers

Use a sample carrier template to display the size and appearance of your sample carrier (e.g. slide or multiwell plate) in **Advanced Setup**. This allows you to distribute tile regions or positions easily across your sample carrier.

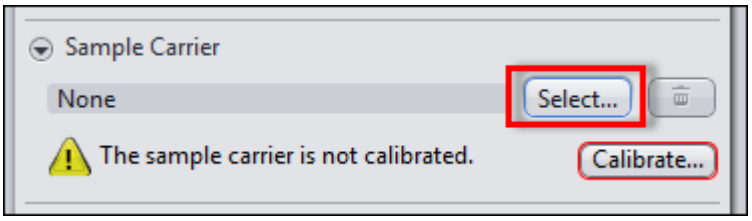
3.9.1 Selecting the sample carrier template

Prerequisites

- You have configured the general settings for setting up a tile experiment (experiment created, at least one channel defined, Tiles dimension activated).
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1. Open the **Sample Carrier** section.
2. Click on the **Select...** button.

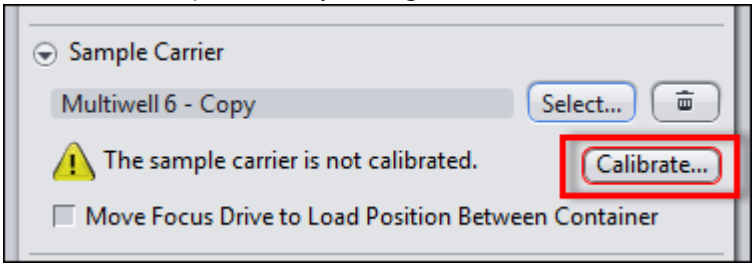


   The **Select Sample Carrier Template** dialog opens.

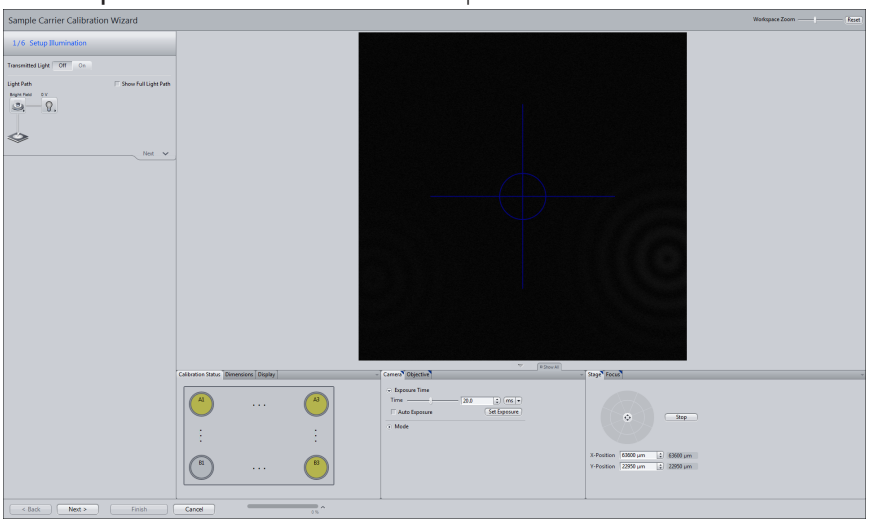
3. Select an existing **sample carrier template** or generate a new template by clicking on the + button.

> **Info**
>
> You can display or edit the **user templates** by clicking on the button and selecting the **Display/Edit…** entry. **Zeiss templates** cannot be edited. To create an editable user copy, select the **Copy And Edit…** entry.

4. To close the dialog, click on the **OK** button.
5. Calibrate the sample carrier by clicking on the **Calibrate…** button.



The **Sample Carrier Calibration Wizard** opens.



6 Follow the wizard until you have fully calibrated the sample carrier.

The information **The sample carrier is calibrated** appears in the **Sample Carrier** section.

Info

Note that the calibration values are stored in the experiment and can be re-used if you work with absolute coordinates by calibrating the end stops of the stage and focus drives and no other changes are made to the hardware set-up. This can save considerable time when you want to work repeatedly with the same sample carrier model and acquisition regime.

You have successfully selected a **sample carrier**.

3.9.2 Customizing your Sample Carrier

As some customers work with sample carrier that are not listed in the template database of ZEN, you need to apply the following workflow in order to create such a template for the software.

Prerequisites

- You have done all prerequisites for a Tiles & Positions experiment
- You have defined at least one channel.
- You have activated the **Tiles** checkbox.

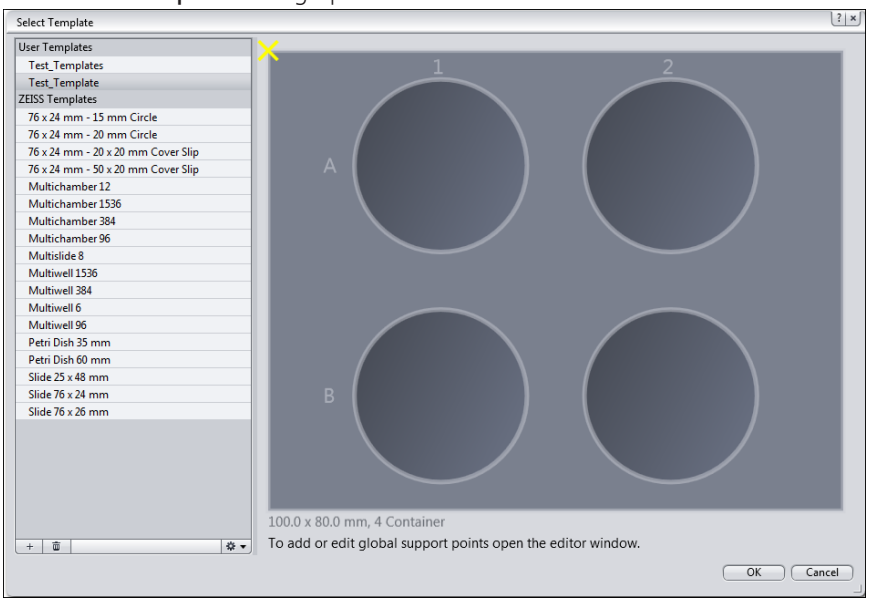
Procedure

1 Go to the **Acquisition** tab.

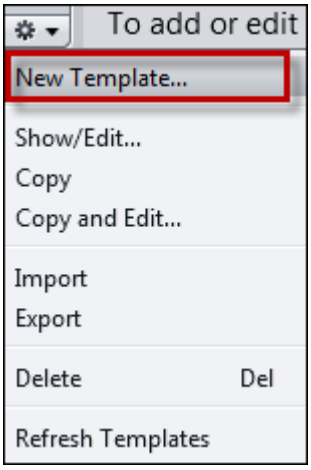
2 Open the **Tiles** tool and activate the **Show All** mode.

3 Open the **Sample Carrier** section and click on the **Select…** button.

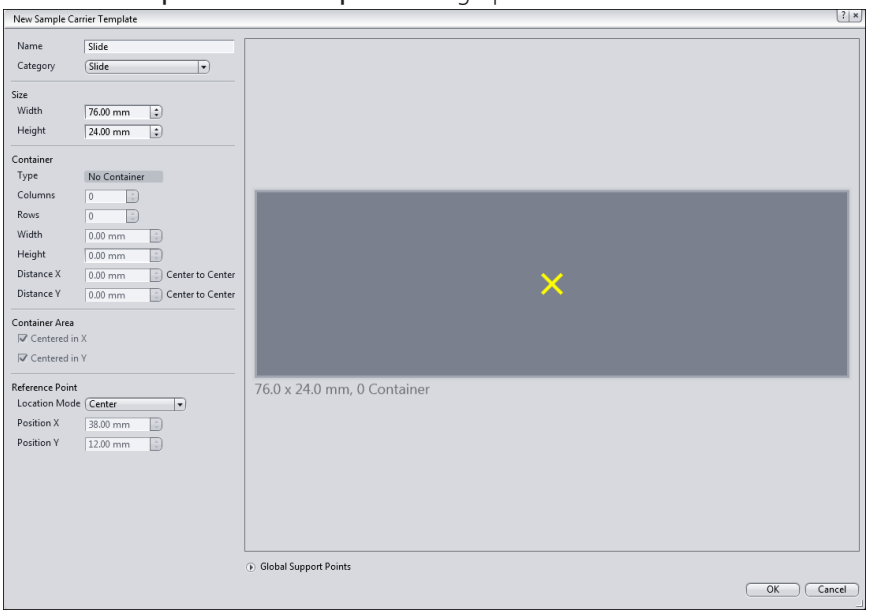
The **Select Template** dialog opens.



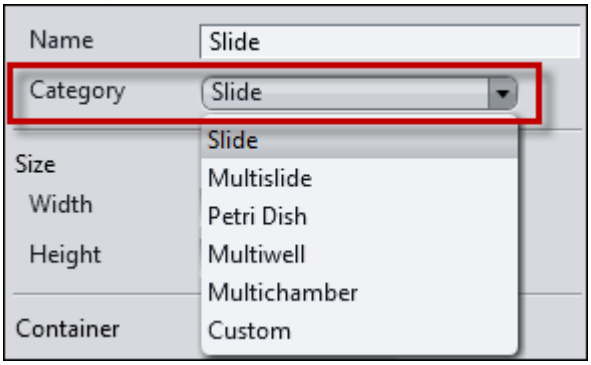
4 Click on the **Options** button and choose **New Template**….



The **New Sample Carrier Template** dialog opens.



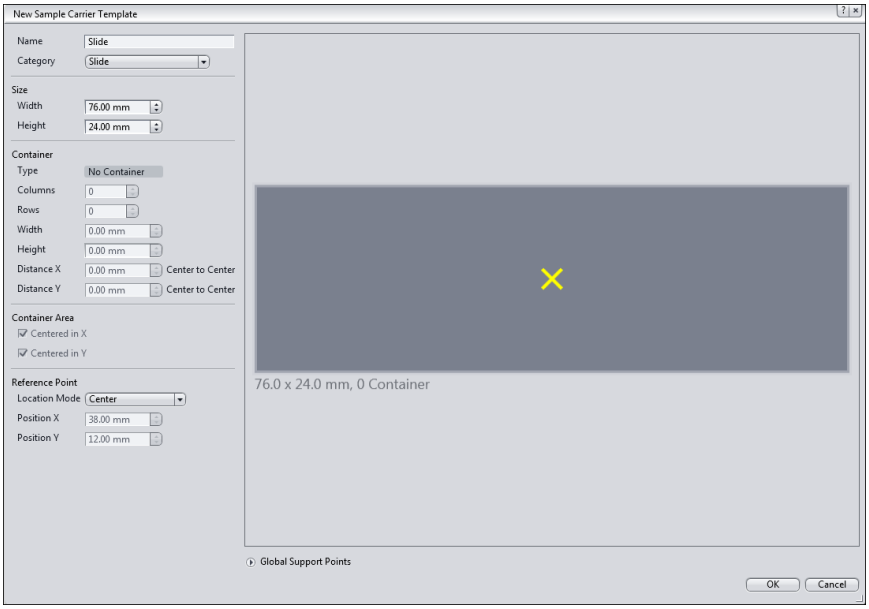
5 Choose a **Category** that corresponds to the type of your carrier and assign a **Name** to your template.



Info

Corresponding to the **Category** you choose xou can define different parameters for the template.

6 For example if you select **Slide**, you can now configure the **Width**and **Height** of the slide and adjust the location of a **Reference Point**.



7 If you select a **Multislide**, **Petri Dish**, **Multiwell** or **Multichamber** template, you can configure and adjust additional parameters of your carrier.

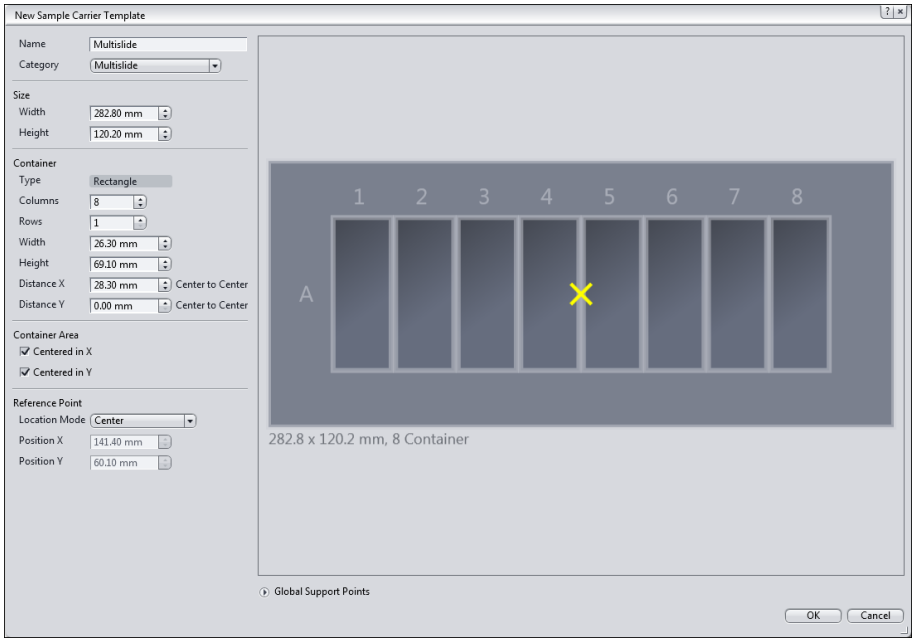


Illustration 1: Multislide template

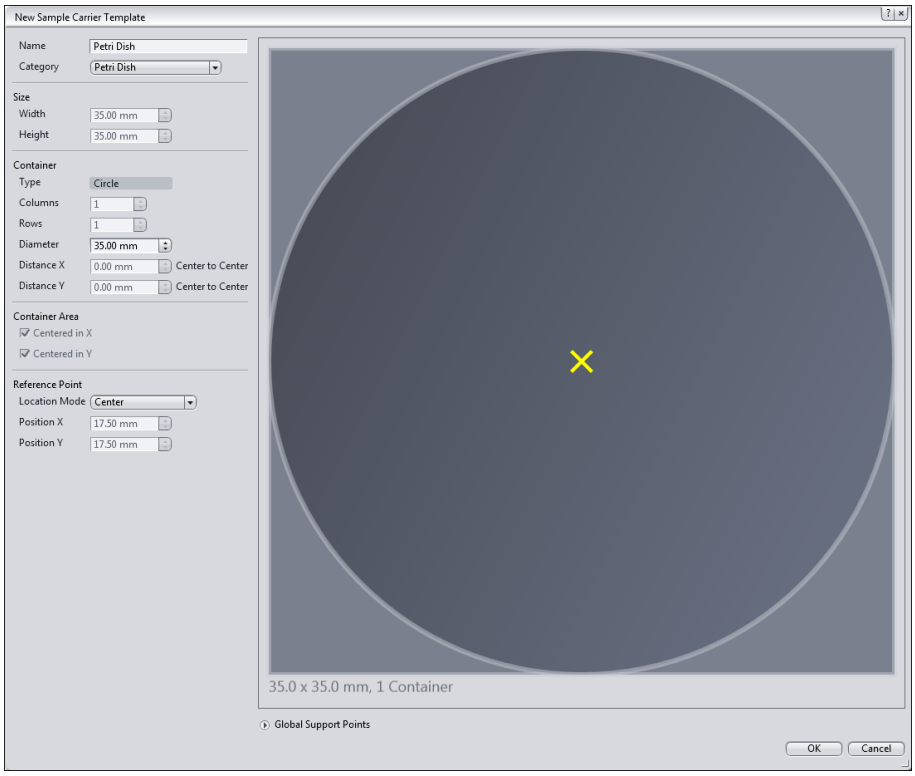


Illustration 2: Petri Dish template

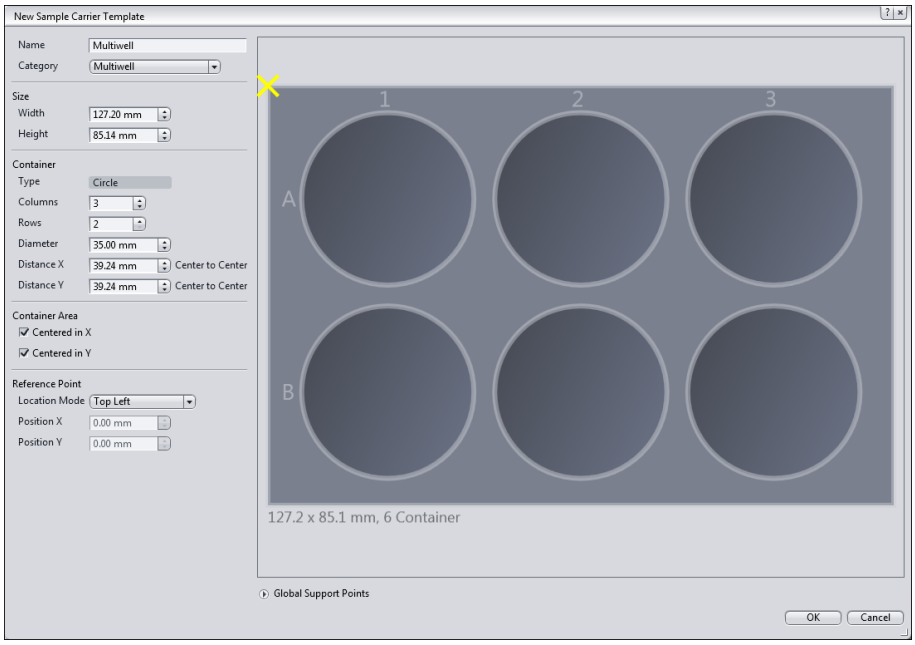


Illustration 3: Multiwell template

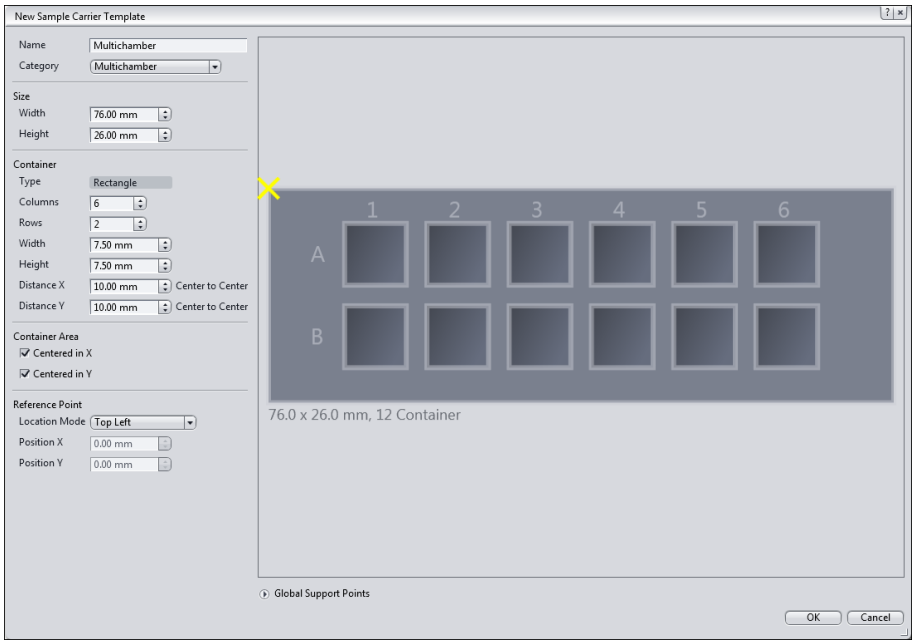


Illustration 4: Multichamber template

8 In case you need to modify one of the above depicted templates even further, first select the **Category** that appears closest to your carrier, go again to the **Category** tab and then choose **Custom**.

i Info

By that, some predefined options of the template will be made accessible for further modifications.

You have customized a sample carrier template or set up a custom one.

A

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V

P

Position In a tile experiment positions refer to independent individual image fields (tiles) that are localized at various places on the sample. A position corresponds to a tile region consisting of just one tile. Each position is based on an X and Y coordinate of the stage and a Z coordinate of the focus drive. Individual positions or position arrays (grouped individual positions) are defined using the Tiles tool. After acquisition the individual positions are displayed as scenes.

T

Tile region In a tile experiment a tile region refers to a group of individual image fields (tiles) that belong together and are arranged in the form of a grid. With the help of tile regions it is possible to acquire areas with dimensions that exceed the size of an individual image field. Within an experiment a number of tile regions can be acquired at various positions on the sample. Each tile region is based on an X and Y coordinate of the stage and a Z coordinate of the focus drive. Tile regions are defined using the Tiles tool. After acquisition the individual tile regions are displayed as scenes.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

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Quick Guide

ZEN 2

The Physiology Module

We make it visible.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

Effective from: October 2014

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Content

1 Working with MeanROI View (Offline)

1 Working with MeanROI View (Offline)

1.1 Introduction

Using the **MeanROI** offline functions you can specify user-defined measurement regions (ROIs) following acquisition of your time lapse experiment and analyze their time-dependent changes in intensity. You can display the intensity curves in diagrams or export the values in the form of tables. This basic functionality is available for time series images opend in ZEN 2 (excluded ZEN lite).

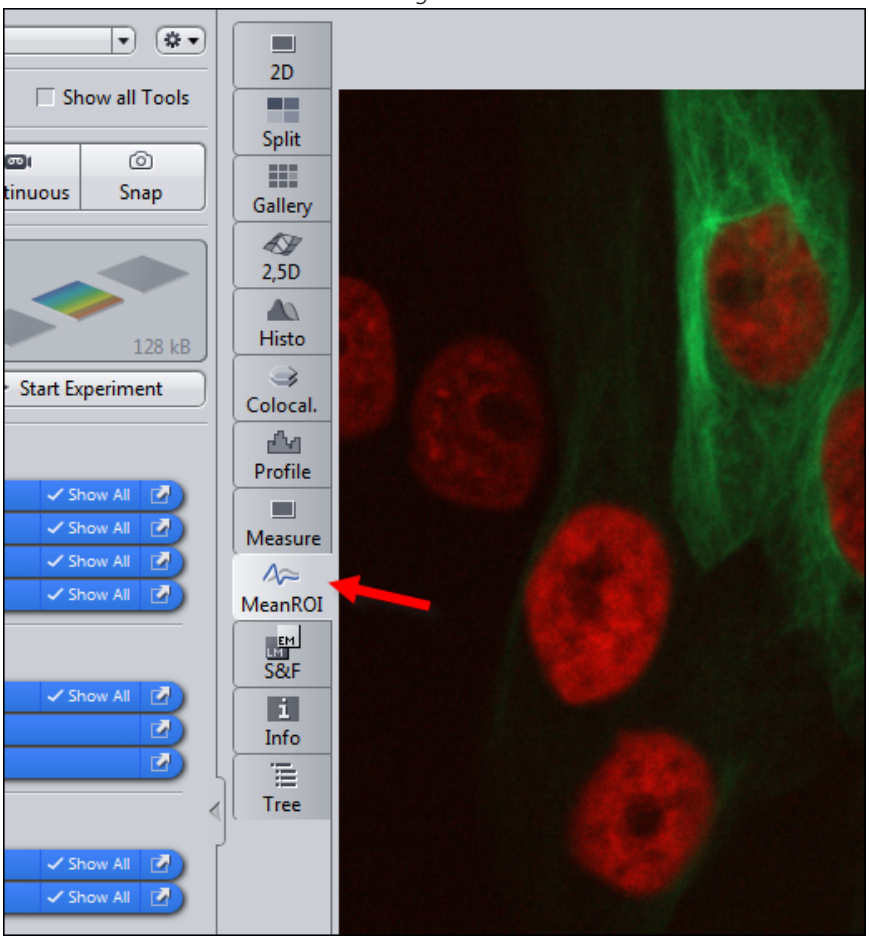
The **Physiology** module expands the MeanROI offline functions to give you the option of calculating online ratios and makes additional display options available for this purpose (**Timeline** view, etc.).

Prerequisites

- You have acquired a time series experiment. The experiment is open and the first time point is displayed in the **2D view**.

Procedure

1 Select the **MeanROI** tab from the image view tabs in the **Center Screen Area**.



The **MeanROI view** opens.

You`re now prepared to start working with the **MeanROI** view. The following chapters will show you the first steps.

1.2 Drawing in and adjusting ROIs

Here you will find out how to draw in and edit measurement regions (ROIs) for intensity measurements and how to adjust them for individual time points.

Info

If you save the experiment, the **ROIs** are automatically saved with the experiment. They are available to you once again in the **MeanROI View** the next time it is opened. Click on the **Recalculate** button on the **ROI Tools** tab to perform and display the intensity calculations again for the saved ROIs.

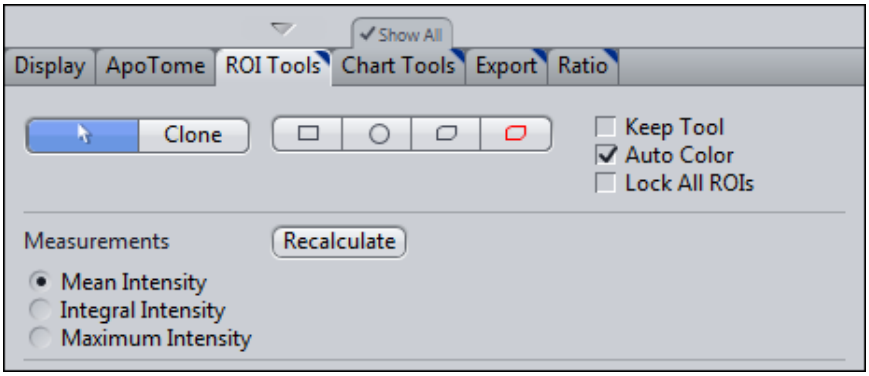
1.2.1 Drawing in ROIs

Prerequisites

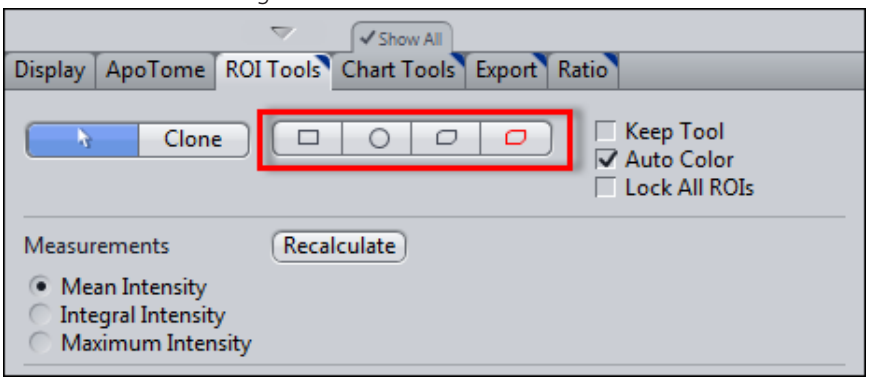
- You are in the **MeanROI View** or **Physiology Setup**.

Procedure

1 Select the **ROI Tools** tab in the **View Options**.



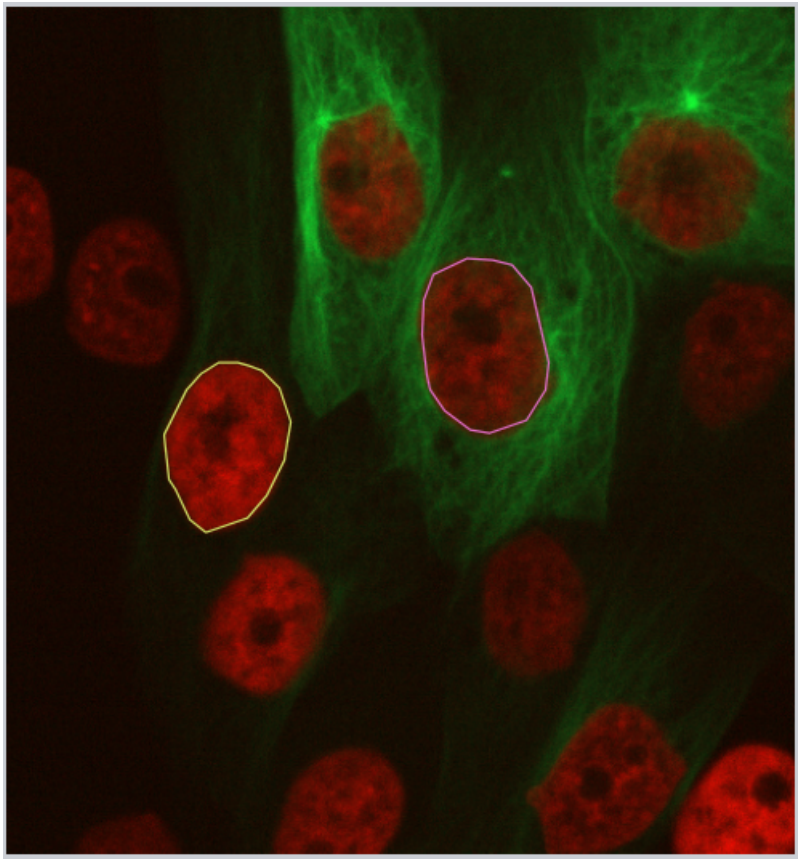
2 Select a tool for drawing in **ROIs**.



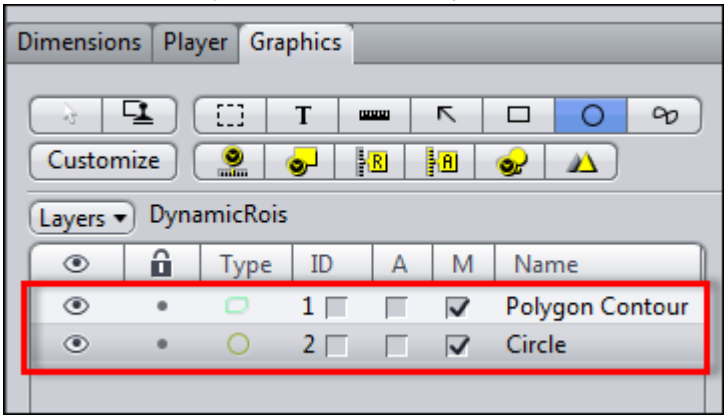
3 Activate the **Keep tool** checkbox.

The selected tool remains active after you have drawn in an **ROI**. This means you can draw in several ROIs without having to re-select the tool.

4 Using the selected tool, in the image view draw in the objects or regions (ROIs) for which intensity measurements are required.



The **ROIs** are displayed in the list under **DynamicROIs** on the **Graphics** tab.



Intensity measurements are performed for each **ROI** and displayed in the diagram area to the right of the image view.

You have successfully defined measurement regions for the intensity measurement.

1.2.2 Adjusting ROIs for time points

If objects move laterally in the course of the time series, you can adjust the **ROIs** at each **Time Point** in order to follow the objects.

Prerequisites

- You have defined at least one ROI.
- You are in the **MeanROI view**.

Procedure

1. Open the **Dimensions** tab in the general view options.
2. Use the **Time slider** to scroll through the time points. Stop at the first time point at which you want to adjust an **ROI.**
3. Adjust the position of the **ROI** using drag & drop. To do this, select the ROI in the **image area** by left-clicking and hold the mouse button down. Then move the ROI to the new position and release the mouse button.
4. To change the shape of an **ROI**, right-click on an ROI and select the **Edit Points** entry (e.g. for polygon contours).
5. Adjust the shape of the **ROI**, by drag & drop the contour points.

   Changes to the position and shape of the **ROIs** are adopted for all subsequent time points.
6. Repeat the previous steps for all other time points for which you want to adjust an ROI.

You have successfully adjusted the measurement regions to the course of the experiment.

1.3 Adjusting the display

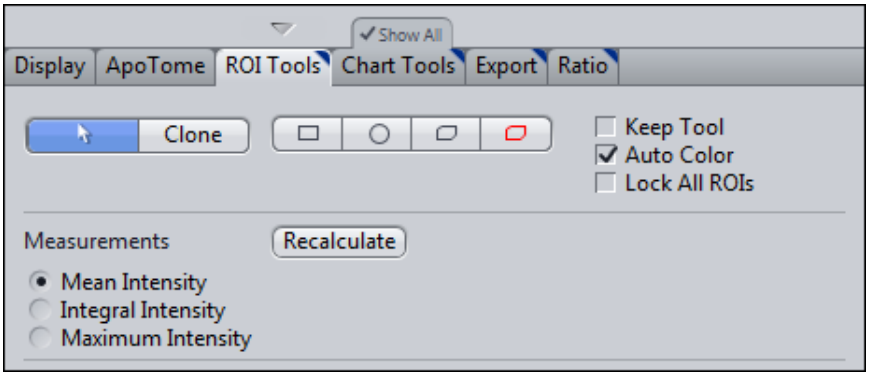
Here you will find out how to adjust the display of the measured intensity values in diagrams and tables according to your wishes.

Prerequisites

- You are in the **MeanROI** view.
- You have defined at least one ROI.

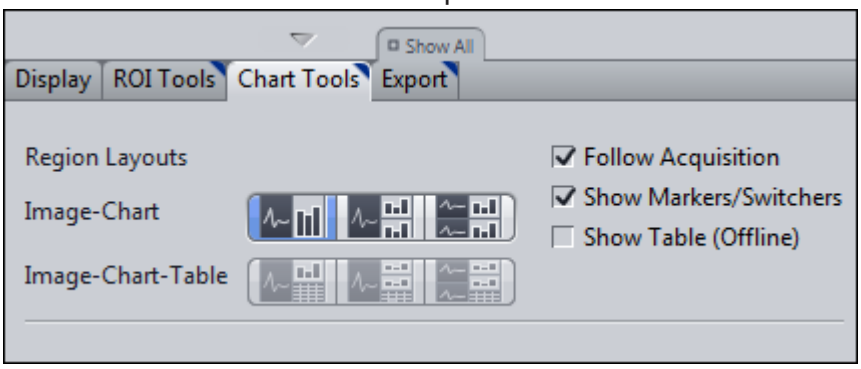
Procedure

1. Select the **ROI Tools** tab in the view options.



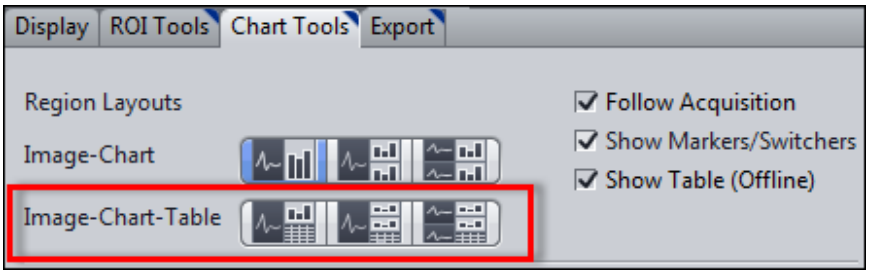
2. In the **Measurements** section select the intensity type (Mean, Integral or Maximum Intensity) that you want to be displayed in the diagrams.

3 Select the **Chart Tools** tab in the **view options**.



4 To adjust the layout of the image and diagram display, select the desired display mode under **Image-Chart**.

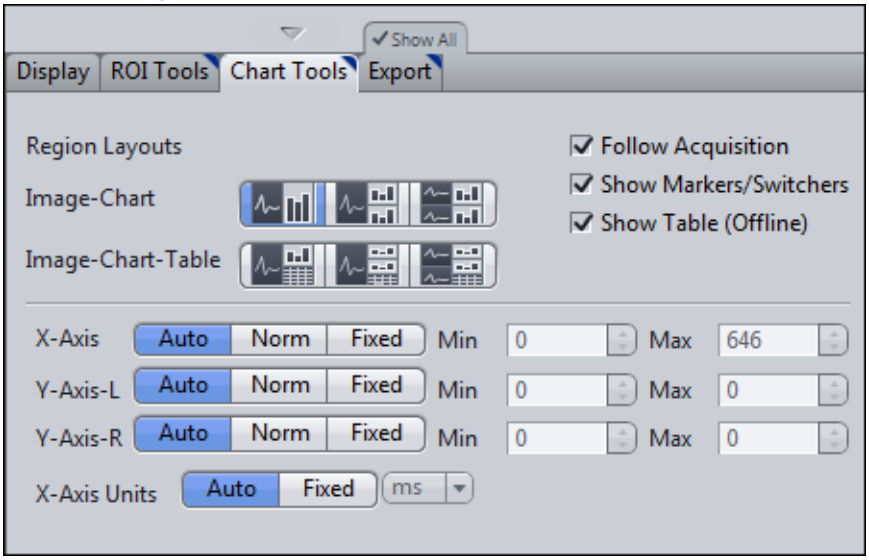
5 If you also want your data to be displayed in table form, activate the **Show Table (Offline)** checkbox.



The Image-Chart-Table modes are activated. You can now select a suitable layout for the image, diagram and table display.

6 If you want to adjust the axis scaling, activate the **Show All** option.

The axis scaling functions become visible.



7 To define the minimum and maximum values of the axes manually, click on the **Fixed** button under **X-/Y-Axis**.

The **Min** and **Max** input fields for the axis are activated.

8 Enter the desired values into the **Min** and **Max** input fields.

The minimum and maximum axis values of the diagrams are adjusted.

9 To change the unit of the X axis, click on the **Fixed** button under **X-Axis Units**.

The dropdown menu for the units is activated. You can now select the desired unit.

You have successfully adjusted the display of the intensity values.

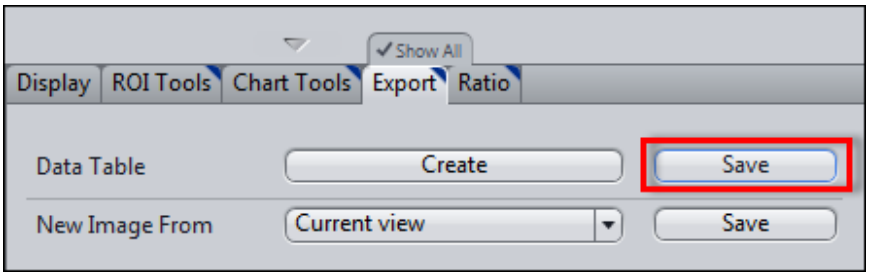
1.4 Exporting a data table

Prerequisites
- You are in the **MeanROI View**.
- You have defined at least one ROI.

Procedure

1 Select the **Export** tab in the View Options.

2 In the **Data Table** section click on the **Save** button.



The **Save As** dialog opens.

3 Enter a suitable file name, navigate to the desired folder and click on **Save**.

All the measurement data are saved as comma-separated values in a csv file. This contains the time information, the area of the ROIs and the values for three types of intensity measurements (Mean, Sum, Maximum) for each channel and each ROI.

1.5 Using background correction

Use this function to subtract background values from the measurement values. A background correction will allow you to make a better comparison in the magnitude of any fluorescent intensity changes observed over the time course of an experiment. Determine the background value with the help of a Background ROI or define a fixed value.

Defining a Background ROI

Prerequisites
- You are in the **MeanROI** View or **Physiology Setup**.

Procedure

1 At the first time point of the time series draw an **ROI** into a region of the image that contains only background signal in all channels.

2 Right-click on the **ROI.**

The shortcut menu opens.

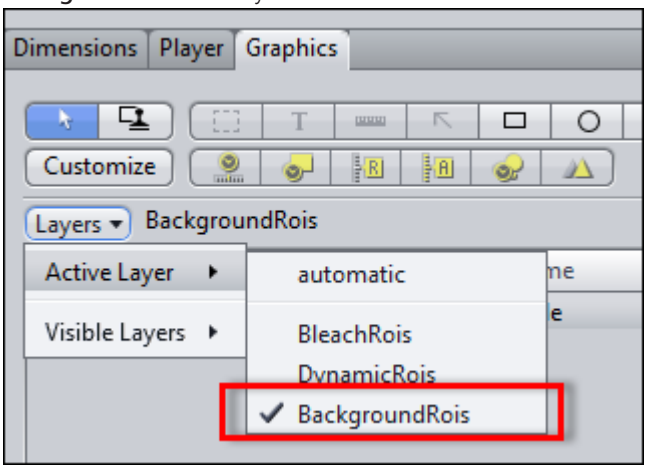
3 Select the **Use as Background ROI** entry.

The **ROI** is defined as a **Background ROI** and displayed with cross-hatching.

The **Background ROI** is assigned to the **Background ROI** layer. This is inactive by default and therefore cannot be edited.

4 To edit the **Background ROI**, activate the **Background ROI** layer. To do this, open the **Graphics** tab.

5 From the **Layers** dropdown menu under **Active Layers** activate the **Background ROIs** entry.



The background ROI is displayed in the list and can be selected and edited there and in the image area.

6 On the **ROI Tools** tab in the **Background Correction** section select the **ROI** option.

7 Click on the **Recalculate** button.

You have successfully defined a **Background ROI**. The mean intensity of the background ROI is subtracted from the measured values of the **ROIs** in a channel-specific and time-point-specific way. The corrected values are adopted into all diagrams and tables.

Defining a fixed background value

Prerequisites

- You are in the **MeanROI View** or Physiology Setup.

Procedure

1 On the **ROI Tools** tab in the **Background Correction** section select the **Constant** option.

The associated input field is activated.

2 Enter a fixed background value into the **Constant** input field.

3 Click on the **Recalculate** button.

The defined background value is subtracted from all measured values of the **ROIs**.

1.6 Calculating ratios

1.6.1 Calculating a ratio for one wavelength

Prerequisites

- To calculate ratios (quotient of two fluorescence intensities) and display ratio images, you need the **Physiology** module.
- You are in the **MeanROI View** on the **Ratio** tab (view option).

Procedure

1. In the **Method** dropdown list select the **Single Wavelength (F/F_0)** entry.
2. In the **Calculation** dropdown list select the channel for calculating the ratio.
3. In the **Reference image (Ft_0) Set-up**, define the frames of the time series image from which you want the reference value Ft_0 to be calculated.
4. Click on the **Recalculate** button.

   The ratio values are calculated. The ratio image and a diagram for the ratio values are displayed in the MeanROI View.

You have successfully calculated a ratio for a channel.

1.6.2 Calculating a ratio for two wavelengths

Prerequisites

- To calculate ratios (quotient of two fluorescence intensities) and display ratio images, you need the **Physiology** module.
- You are in the **MeanROI View** on the **Ratio** tab (view option).

Procedure

1. In the **Method** dropdown list select the **Dual Wavelength** entry.
2. In the **Calculation** dropdown lists select the channels for calculating the ratio.
3. Click on the **Recalculate** button.

   The ratio values are calculated. The ratio image and a ratio diagram are added to the MeanROI View.

You have successfully calculated a ratio for two channels.

2 Setting up Physiology Experiments

2 Setting up Physiology Experiments

2.1 Introduction

Using the **Physiology** tool you can specify user-defined measurement regions (ROIs) before the acquisition of your time lapse experiment and analyze their time--dependent changes in intensity online during acquisition. Ratios can also be calculated and displayed online.

Before the experiment

A precondition for a physiology experiment is a **Time Series experiment,** which is set up in the **Time Series** tool. In the **Physiology** tool you must activate the physiology functions (online measurements) first. Here you can also activate and configure settings for the calculation of the **Online Ratio**. In addition, the tool contains the button for opening **Physiology Setup**. There you can draw in ROIs and adjust the display of the measurement results. When Setup is opened a snap is automatically acquired, on the basis of which you can configure the settings for the subsequent experiment. The structure of Physiology Setup is based on the MeanROI View.

During the experiment

After being started, physiology experiments are displayed in the online mode of the **MeanROI View**. This allows you to analyze and influence the experiment during acquisition. The structure and options largely correspond to the offline mode of the **MeanROI View**. We therefore recommend that you familiarize yourself with the MeanROI View [▶ 9] (offline) before performing your Physiology experiment.

After the experiment

After you have performed your Physiology experiment the data are displayed in the offline mode of the MeanROI View and can be analyzed, processed and exported there. Further information on this can be found under: Working with MeanROI View (Offline) [▶ 9].

Prerequisites

Prerequisites

- To perform **physiology experiments**, you need the **Physiology** module.
- You have created a new experiment, defined at least one channel and adjusted the focus and exposure time.
- You are on the central **Acquisition** tab.

Procedure

1 Activate the **Time Series** tool in the **Acquisition Dimensions** section.

The **Time Series** tool appears in the **Left Tool Area** under **Multidimensional Acquisition**.

The **Physiology** tool appears in the **Left Tool Area** under **Applications**.

Info

The Physiology tool is not available if the **Z-Stack**, **Tiles** or **Panorama** dimensions are activated. Deactivate these dimensions to make the **Physiology** tool available.

2 Set up a time series experiment.

3 Open the **Physiology** tool.

4 Activate the **Enable Physiology** checkbox.

You have completed the general prerequisites for physiology experiments.

2.2 Activating online ratio calculation

Prerequisites

- You have read the Introduction [▶ 19] chapter.

Procedure

1 In the **Physiology** tool open the **Online Ratio** section.

2 Activate the **Enable** checkbox.

3 If you want to save the ratio images, activate the **Save Ratio Images** check box. Otherwise only the ratio values and not the calculated ratio images are available after the experiment.

4 In the **Method** dropdown list select a method for the ratio calculation.

Info

If you select the **Single wavelength (F/F0)** method for the calculation of the online ratio, you will be informed that no reference image has been defined. To calculate a reference image, indicate the number of images from which the reference image should be calculated in the input field of **Reference Image Set-up** in the **Online Ratio** section. Then click on the **Define** button to acquire the images and calculate a reference image from them.

5 If you want to use background correction for the calculation of the online ratio, activate the desired entry under **Background Correction.**

To allow you to indicate a constant background value (**Constant** entry), an input field, in which you can enter the desired value, appears under **Calculate** in the formula for the ratio calculation.

The **ROI** entry can only be selected once you have defined a background ROI in Physiology Setup.

6 Under **Calculate** complete the formula for calculating the online ratio by selecting the desired entries from the dropdown lists and indicating values in the input fields.

7 Under **Color** define an LUT (Look-Up-Tabelle) for the display of the ratio image.

8 Activate the **Threshold** checkbox, if you want to set a threshold in your experiment.

9 Click on the **Physiology Setup** button.

Physiology Setup is opened. Snaps of the activated channels are acquired automatically. The ratio image is calculated and displayed with the associated diagram.

10 You can adjust the settings for the ratio calculation in the **Physiology** tool. Then click on the **Snap** acquisition button to update the ratio image.

You have successfully activated the calculation of the online ratio.

2.3 Setting up an experiment in Physiology Setup

Prerequisites

- You have read the Introduction [▶ 19] chapter.
- You are in the **Physiology** tool.

Procedure

1 Click on the **Physiology Setup** button.

Physiology Setup is opened.

An image is acquired automatically, on the basis of which you can configure your settings. You can click on **Snap** at any time to update the image.

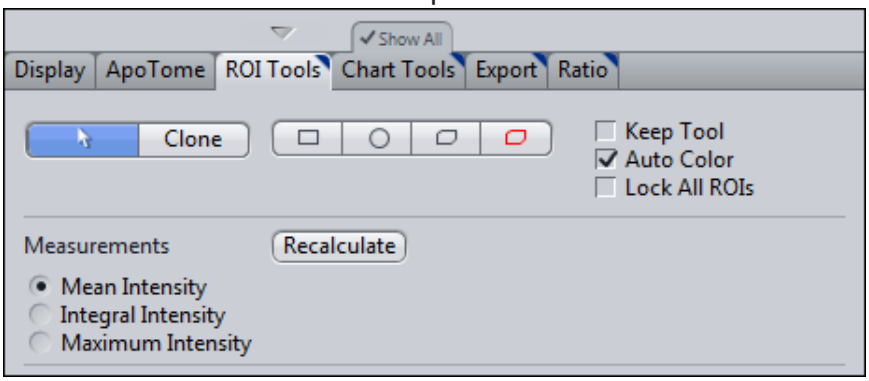
2.3.1 Drawing in ROIs

Prerequisites

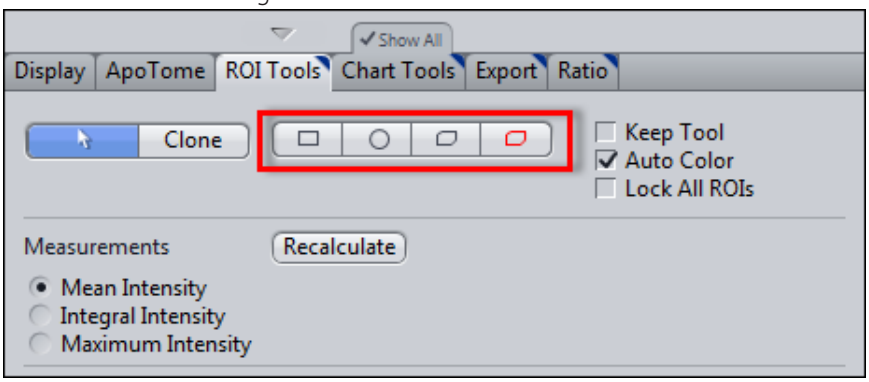
- You are in the **MeanROI View** or **Physiology Setup**.

Procedure

1 Select the **ROI Tools** tab in the **View Options**.



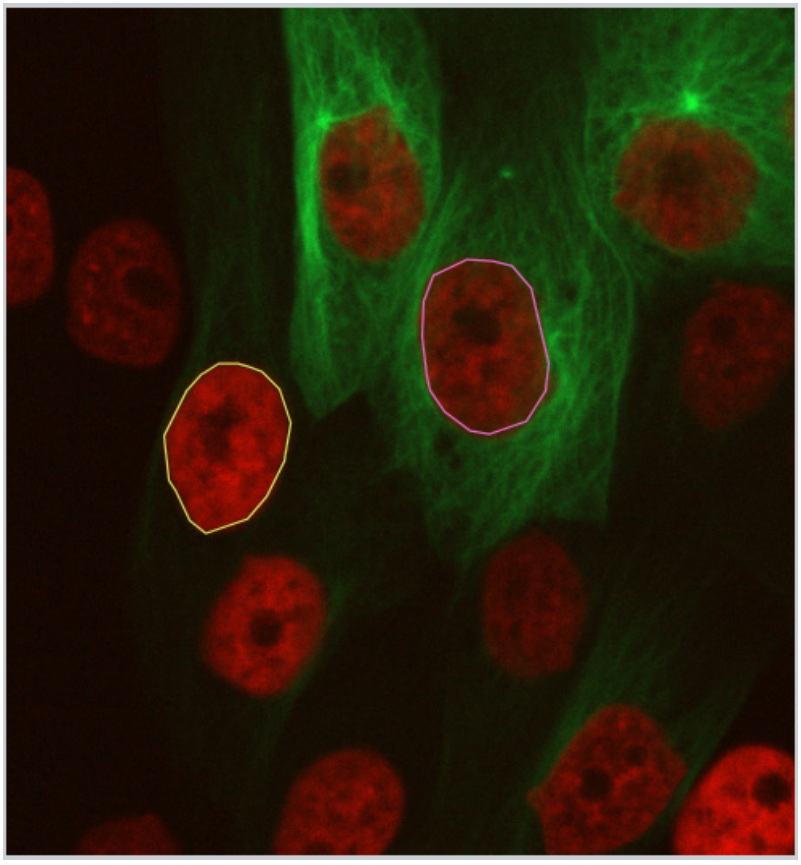
2 Select a tool for drawing in **ROIs**.



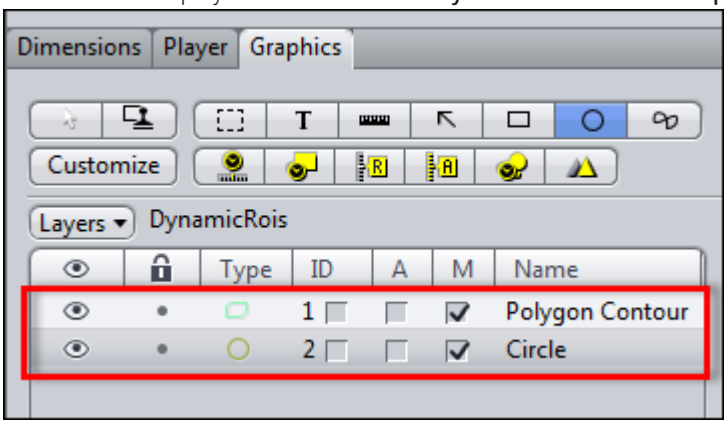
3 Activate the **Keep tool** checkbox.

The selected tool remains active after you have drawn in an **ROI**. This means you can draw in several ROIs without having to re-select the tool.

4 Using the selected tool, in the image view draw in the objects or regions (ROIs) for which intensity measurements are required.



The **ROIs** are displayed in the list under **DynamicROIs** on the **Graphics** tab.



Intensity measurements are performed for each **ROI** and displayed in the diagram area to the right of the image view.

You have successfully defined measurement regions for the intensity measurement.

2.3.2 Adjusting the display

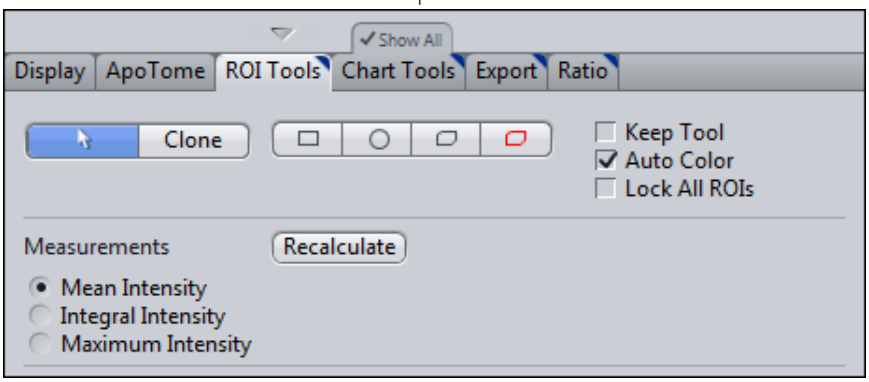
Here you will find out how to adjust the display of the measured intensity values in diagrams and tables according to your wishes.

Prerequisites

- You are in the **MeanROI** view.
- You have defined at least one ROI.

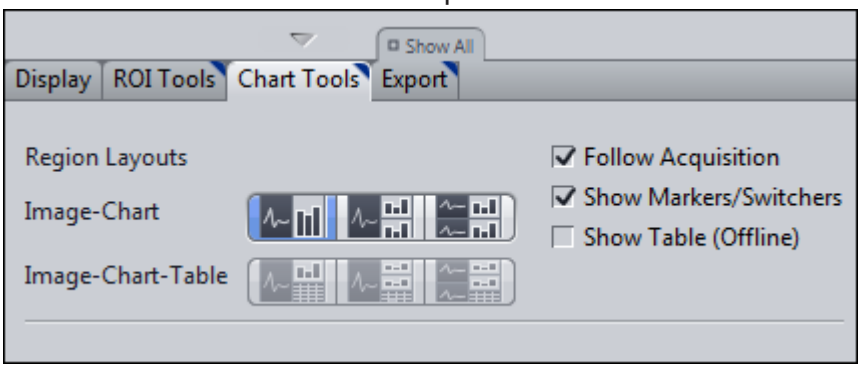
Procedure

1 Select the **ROI Tools** tab in the view options.



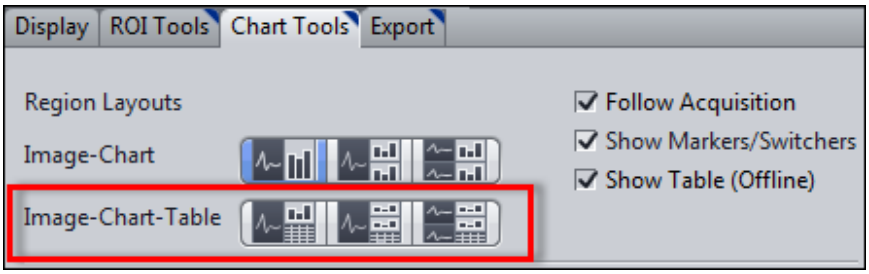
2 In the **Measurements** section select the intensity type (Mean, Integral or Maximum Intensity) that you want to be displayed in the diagrams.

3 Select the **Chart Tools** tab in the **view options**.



4 To adjust the layout of the image and diagram display, select the desired display mode under **Image-Chart**.

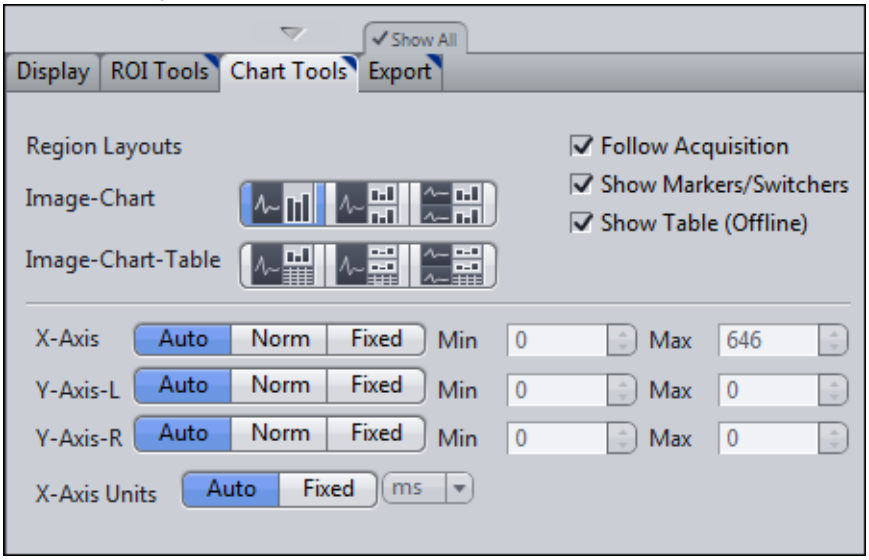
5 If you also want your data to be displayed in table form, activate the **Show Table (Offline)** checkbox.



The Image-Chart-Table modes are activated. You can now select a suitable layout for the image, diagram and table display.

6 If you want to adjust the axis scaling, activate the **Show All** option.

The axis scaling functions become visible.



7 To define the minimum and maximum values of the axes manually, click on the **Fixed** button under **X-/Y-Axis**.

The **Min** and **Max** input fields for the axis are activated.

8 Enter the desired values into the **Min** and **Max** input fields.

The minimum and maximum axis values of the diagrams are adjusted.

9 To change the unit of the X axis, click on the **Fixed** button under **X-Axis Units**.

The dropdown menu for the units is activated. You can now select the desired unit.

You have successfully adjusted the display of the intensity values.

2.3.3 Using background correction

Use this function to subtract background values from the measurement values. A background correction will allow you to make a better comparison in the magnitude of any fluorescent intensity changes observed over the time course of an experiment. Determine the background value with the help of a Background ROI or define a fixed value.

Defining a Background ROI

Prerequisites

- You are in the **MeanROI** View or **Physiology Setup**.

Procedure

1 At the first time point of the time series draw an **ROI** into a region of the image that contains only background signal in all channels.

2 Right-click on the **ROI.**

The shortcut menu opens.

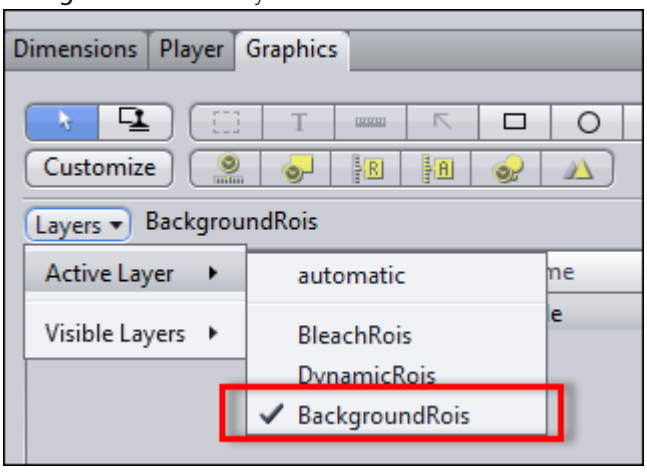
3 Select the **Use as Background ROI** entry.

The **ROI** is defined as a **Background ROI** and displayed with cross-hatching.

The **Background ROI** is assigned to the **Background ROI** layer. This is inactive by default and therefore cannot be edited.

4 To edit the **Background ROI**, activate the **Background ROI** layer. To do this, open the **Graphics** tab.

5 From the **Layers** dropdown menu under **Active Layers** activate the **Background ROIs** entry.



The background ROI is displayed in the list and can be selected and edited there and in the image area.

6 On the **ROI Tools** tab in the **Background Correction** section select the **ROI** option.

7 Click on the **Recalculate** button.

You have successfully defined a **Background ROI**. The mean intensity of the background ROI is subtracted from the measured values of the **ROIs** in a channel-specific and time-point-specific way. The corrected values are adopted into all diagrams and tables.

Defining a fixed background value

Prerequisites
- You are in the **MeanROI View** or Physiology Setup.

Procedure

1 On the **ROI Tools** tab in the **Background Correction** section select the **Constant** option.

The associated input field is activated.

2 Enter a fixed background value into the **Constant** input field.

3 Click on the **Recalculate** button.

The defined background value is subtracted from all measured values of the **ROIs**.

2.4 Starting and influencing an experiment

Prerequisites
- You have read the Introduction [▶ 19] chapter and set up an experiment in **Physiology Setup**.
- You are on the **Acquisition** tab.

Procedure

1 Start your Physiology experiment by clicking on the **Start Experiment** button.

The time lapse experiment is started. The MeanROI View (online) opens and displays the current images and the intensity curves for each ROI measured online. The intensity curves are displayed in the Time Line View and in the diagrams.

2 You can pause the experiment at any time by clicking on the **Pause Experiment** button and continue it again by clicking on the **Continue Experiment** button.

3 The **Focus** can be adjusted during the experiment. To prevent images that are not sharp being acquired, pause your experiment and use the **Live** acquisition button to adjust the focus. Then continue the experiment.

4 Adjust the display of the intensity values during the experiment by changing the settings on the **Chart Tools** tab. The unit of the X-axis cannot be changed during the experiment.

5 You can move and change ROIs during acquisition. The changes are adopted for all subsequent time points, see Drawing in and adjusting ROIs [▶ 10].

6 Activate **Switches** in the **Time Series** tool during the experiment to perform the corresponding actions.

i Info

Various events, such as the activation of switches or the pausing of the experiment, are labeled in the Time Line view by markers.

7 On the **Chart Tools** tab deactivate the **Follow Acquisition** checkbox to analyze the data acquired up to that point. To do this, select the corresponding time points using the **Time** slider on **Graphics** tab, the diagram sliders or the Time Line view slider in the **MeanROI** view.

8 Change the size of the area marked in blue in the Time Line View to adjust the section displayed in the diagrams.

You have successfully started the experiment, analyzed it online and influenced it.

2.4.1 Adjusting ROIs during experiments

If objects move laterally in the course of the experiment, you can adjust the **ROIs** at any time during the experiment in order to follow the objects.

Prerequisites

- You have defined at least one ROI.
- You have started your Physiology experiment.

Procedure

1 In the **Experiment Manger** click on **Pause experiment** button.

2 Adjust the position of the **ROI** using drag & drop. To do this, select the ROI in the **image area** by left-clicking and hold the mouse button down. Then move the ROI to the new position and release the mouse button.

3 To change the shape of an **ROI**, right-click on an ROI and select the **Edit Points** entry (e.g. for polygon contours).

4 Adjust the shape of the **ROI** by dragging and dropping the contour points.

Changes to the position and shape of the **ROIs** are adopted for all subsequent time points.

5 Repeat the previous steps for all subsequent ROIs that you wish to adjust.

You have successfully adjusted the measurement regions (ROIs) to the course of the experiment.

2.5 Sample experiment Fura-2 with DG4/5

2.5.1 Step 1: Creating channels

Prerequisites

- To perform the experiment, you need the **Physiology** module.
- You have a **Sutter DG4/5** with appropriate excitation filters for **Fura-2** and a **Fura-2 filter set** in the microscope's reflector wheel.
- You are on the **Acquisition** tab.

Procedure

1. Create a new experiment in the **Experiment Manager**, e.g. "**Physiology Fura-2**".
2. Add the channel Fura-2 using **Smart Setup**.
3. Activate the **Time Series** checkbox in the acquisition dimensions.
4. Open the **Channels** tool.
5. Select the Fura-2 channel from the list.
6. Click on the **Options** button  and select the **Copy** entry.
7. Select the first Fura-2 channel from the list.
8. Click on the **Options** button  and select the **Rename** entry.

   You can now rename the channel, e.g. **Fura-2 340 nm**.
9. Repeat steps 7 and 8 to rename the second channel, e.g. **Fura-2 380 nm**.
10. Select the **Fura-2 380 nm** channel.
11. Select another LUT from the dropdown list, e.g. red.
12. Select the entry **21 HE Ex. FURA 380** from the Excitation dropdown list.

    The excitation filter is used for this channel.
13. Adjust the exposure time and focus for both channels.

You have created the channels for your experiment.

2.5.2 Step 2: Setting up a time series and creating switches

Procedure

1. Open the **Time Series** tool.
2. Using the **Duration** slider and the dropdown list for the unit, specify the duration of the experiment, e.g. 10 min.
3. Using the **Interval** slider and the dropdown list for the unit, specify the length of the interval between acquisitions, e.g. 1 second.
4. To create buttons open the **Switches** section in the **Time Series** tool. This section is visible only if the **Show All** mode is activated.
5. Click on the **Add** button  .

A new switch is added.

6 Edit the switch by clicking on the black arrow to the right of the switch.

The switch properties are visible.

7 Enter a name, e.g. **Fast**. Activate the **Color** checkbox and select a color, e.g. blue. Define an action to be performed when you activate the button, e.g. **As fast as possible**.

You have successfully set up a time series and created a switch.

2.5.3 Step 3: Setting up an online ratio

Procedure

1 Open the **Physiology** tool.

2 Activate the **Enable Physiology** checkbox.

3 Open the **Online Ratio** section.

4 Under **Method** select the **Dual Wavelength** entry from the dropdown list.

5 Under **Calculation** select the **Fura-2 340 nm** entry from the dropdown list in the numerator of the formula.

6 Under **Calculation** select the **Fura-2 380 nm** entry from the dropdown list in the denominator of the formula.

7 Activate the **Activate** checkbox.

You have successfully activated the ratio functions and specified the calculation of the ratio.

2.5.4 Step 4: Physiology Setup

Procedure

1 In the **Physiology** tool click on the **Physiology Setup** button.

Physiology Setup is opened. Snaps of the configured channels are acquired automatically and displayed in the Center Screen Area. A preview of the ratio image, which is calculated according to the ratio settings, is also displayed. The diagrams for each image are displayed to the right of this.

2 On the **ROI Tools** tab, select a tool for drawing in ROIs, e.g. Circle.

3 Activate the **Keep Tool** checkbox.

4 Draw your ROIs into one of the images.

5 Deactivate the **Keep Tool** checkbox and select the selection tool (**arrow**) again.

6 Under **Measure** on the **ROI Tools** tab select the type of intensity measurement to be displayed, e.g. Mean Intensity.

7 Under **Region Layouts** on the **Chart Tools** tab select a layout for the image and diagram display, e.g. Multi-Image Multi-Chart.

8 Click on the **Fixed** button under **X-Axis Unit** and select a unit from the dropdown list, e.g. seconds.

9 Click on **Finish** at the top left of **Physiology Setup** to leave Physiology Setup.

You have successfully configured and adjusted the Physiology Setup.

2.5.5 Step 5: Starting, analyzing and influencing an experiment

Procedure

1 Start the experiment by clicking on the **Start Experiment** button.

The experiment is started. In our example an image is acquired every second for a period of 10 minutes. The experiment opens in the online mode of the MeanROI View, which displays the current images and measurements.

2 Activate the created switch at the desired time point. To do this, open the **Switches** section in the **Time Series** tool. Click on a switch as soon as you want its action to be performed, e.g. click on the "**Fast**" switch to acquire the subsequent images as quickly as possible one after the other. A marker will mark the time point at which the switch was activated on the X axis in the color of the switch (e.g. blue).

3 Once the time series has been completed you can analyze the experiment in the offline mode of the **MeanROI** view, process it and export its values.

You have successfully performed the experiment.

Carl Zeiss Microscopy GmbH
Carl-Zeiss-Promenade 10
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy

Carl Zeiss Microscopy GmbH
Königsallee 9-21
37081 Göttingen
Germany

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