Quick Guide

ZEN (blue edition)

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Effective from: 04 / 2018

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1 Introduction

ZEN (blue edition) is a modular image acquisition, processing and analysis software for digital microscopy. The abbreviation ZEN stands for ZEISS Efficient Navigation and points out that the software can be used to control all microscopes and imaging systems by ZEISS.

In addition to basic functionality for image acquisition, elementary image processing and annotations, image analysis and reporting functions a lot of optional modules for specific microscopy 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.

2 Starting the Software

Prerequisites

- You have installed **ZEN (blue edition)** 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** | **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 (e.g. if you work with an LSM only **ZEN system** and **Image Processing** is available). 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.

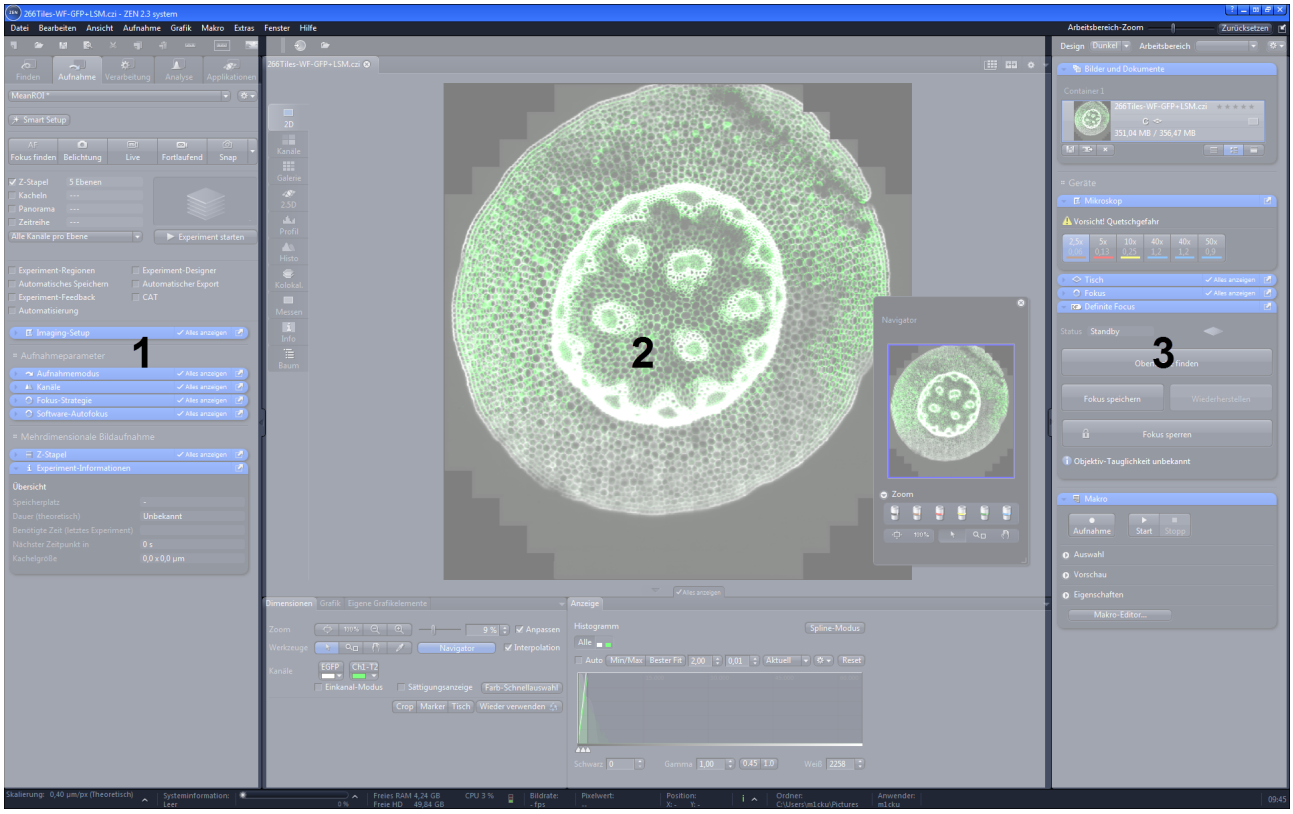
You successfully started the software.

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 User Interface

The software user 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) and image analysis (**Analysis** tab). The **Center Screen Area (2)** is used to display your images with several image views available. In the **Right Tool Area (3)** you find the Images and Documents Gallery, the Objective Selection and the Stage and Focus controls. Additionally system specific tools can be available here (e.g. **Definite Focus.2** controls).



3.1 Title bar

Parameter	Description
Help icon	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.

Parameter	Description
Maximize Over 2 Screens	Maximizes the program window across 2 screens if available. 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.
Restore Down	Reduces the program window to any selected size.
Close	Closes the program window.

3.2 Workspace Configuration

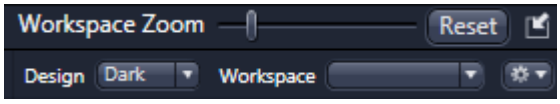


Fig. 3.1: Workspace Configuration

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

3.3 Menu bar

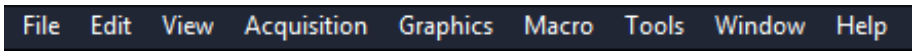


Fig. 3.2: Menu bar

The menu bar contains all the menus you need to manage, edit and view your projects, see Menus.

3.4 Tool bar

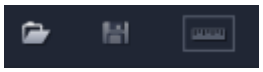


Fig. 3.3: Tool Bar

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.

3.5 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.

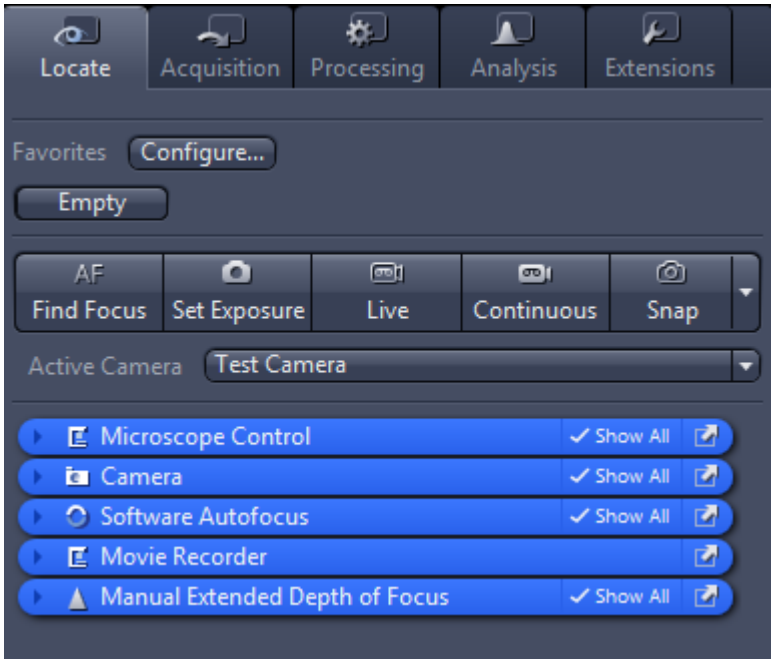


Fig. 3.4: Left Tool Area

3.6 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.

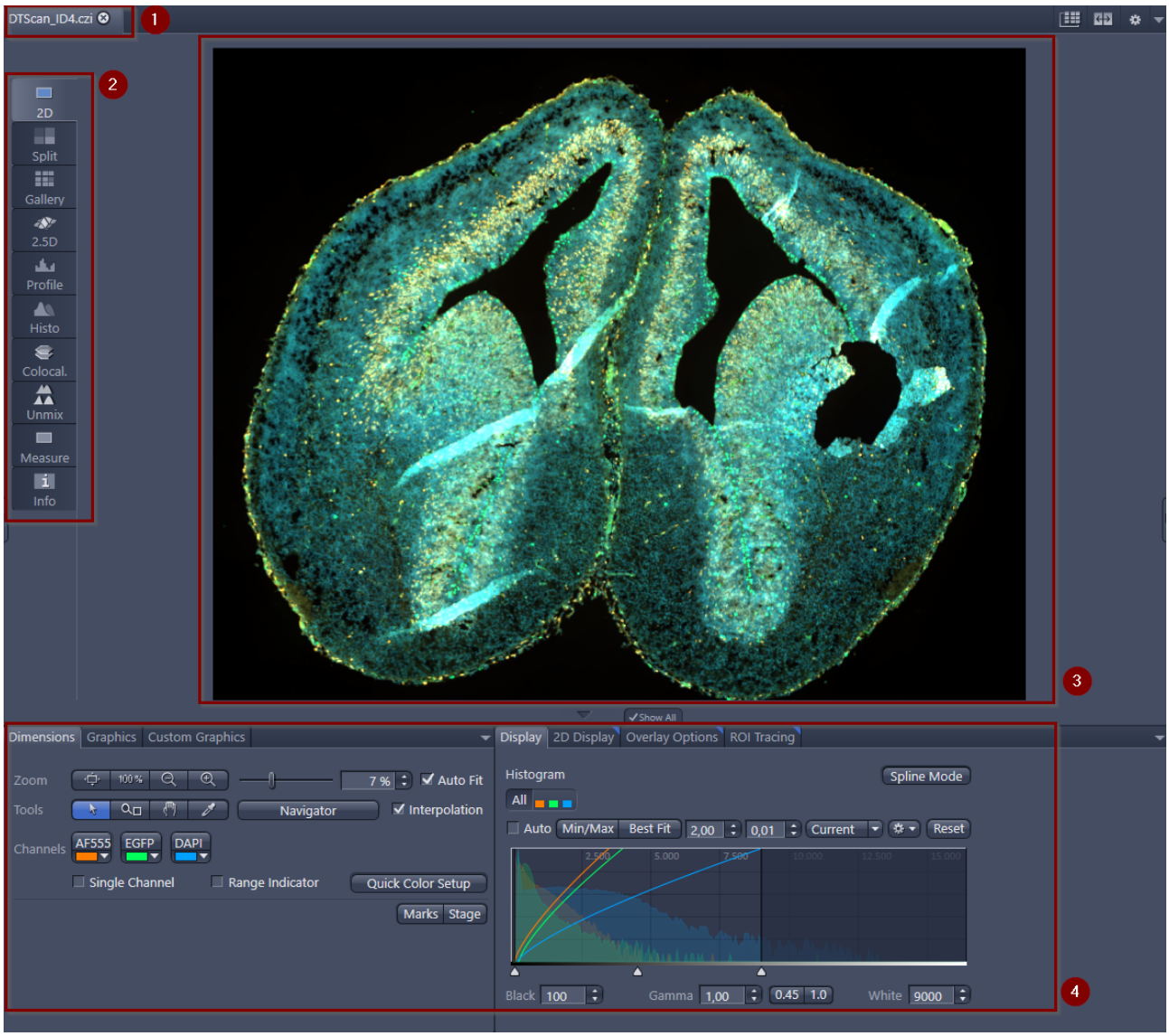


Fig. 3.5: Center Screen Area

1. Document bar, *learn more* [▶ 10].
2. Image Views (organized on tabs), *learn more* [▶ 10].
3. Image Area
4. General and specific view options, *learn more* [▶ 10].

3.6.1 Image views

The software offers a lot of different image views. The **general image views** are visible for each image. The **specific image views** are available only if the image has the appropriate characteristics (eg multiple channels, Z-stack, etc.) or you have licensed a specific module (e.g. for 3D View). Each image view has general and specific controls which you can use to work with the view.

3.6.2 General View Options

The general view options are visible in any view. Some of the view options are only visible when you open a particular file type. E.G. see the **ApoTome** view option only if you have opened a **ApoTome** image.

3.7 Right Tool Area

Here you find mainly the tools for image and file handling (e.g. **Image Gallery**) and hardware control (e.g. **Stage** / **Focus** tool). Depending on your system configuration, other tools can be available. The tools are described in the corresponding chapters of the online help.

3.8 Document bar

Fig. 3.6: 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 from **Expose** to **Splitter** mode and further view options (**View** menu).

INFO

A **asterisk** (*****) next to an image/document title indicates that unsaved changes have been made to this document. Save your pictures/documents from time to time in order to avoid data loss.

3.9 Status bar

Here you will see important information on the system status:

Parameter	Description
Scaling	Displays which lateral scaling is currently being used. If you click on the arrow, the Scaling dialog will be opened. There you have access to advanced scaling settings and the scaling wizard.
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 arrow 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 small **status bar** provides an overall assessment of the system usage. **Info**: Double-clicking in 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	Displays the gray value to 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.

Parameter	Description
Information (i)	If you click on the icon, a window opens with a *System Messages* [▶ 12].
Storage Folder	Displays the location where new images are automatically saved. This path can be changed in the menu **Tools** | **Options** | **Saving**.
User	Shows the Windows user name of the logged in user.
Time	Shows the current Windows system time.

3.9.1 System Messages

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.

Parameter	Description
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.

i 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.

4 Adjusting Workspace Appearance

4.1 Setting the User Language

Prerequisites

- You have successfully started the application.

Procedure

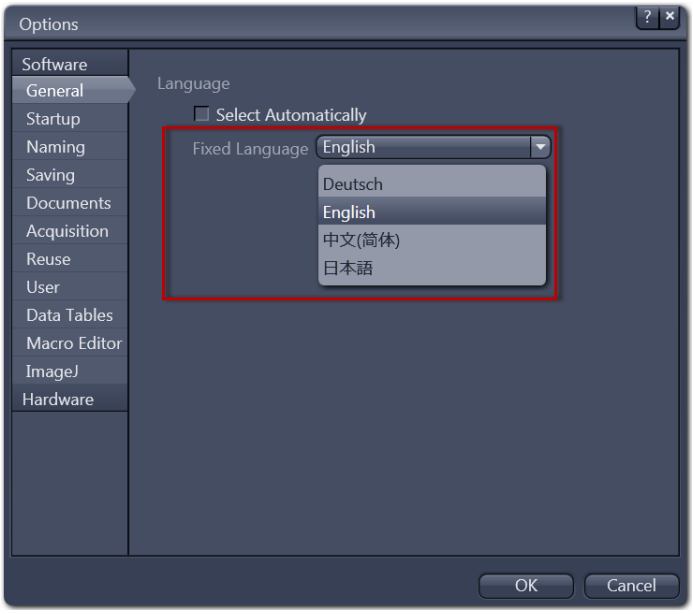
1. Click on menu **Tools** | **Options**.

   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.



   A message appears to restart the application.

   Note, that the availability of additional languages can differ between software versions.

4. Click on **OK**.

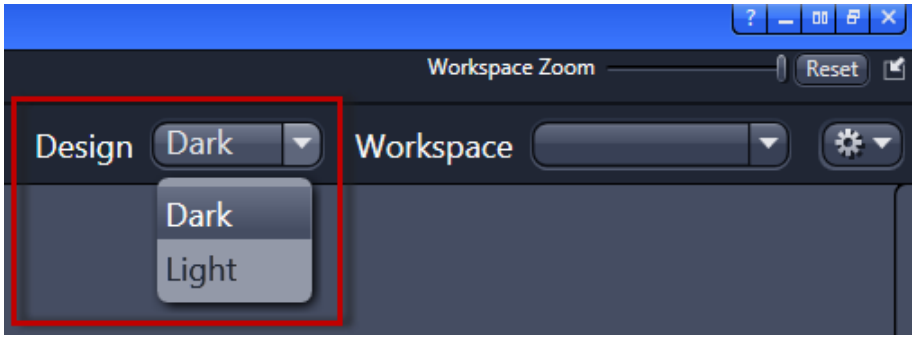
   The **Options** dialog closes.

5. Exit and restart software.

You have successfully set the user language.

4.2 Selecting a Screen Design

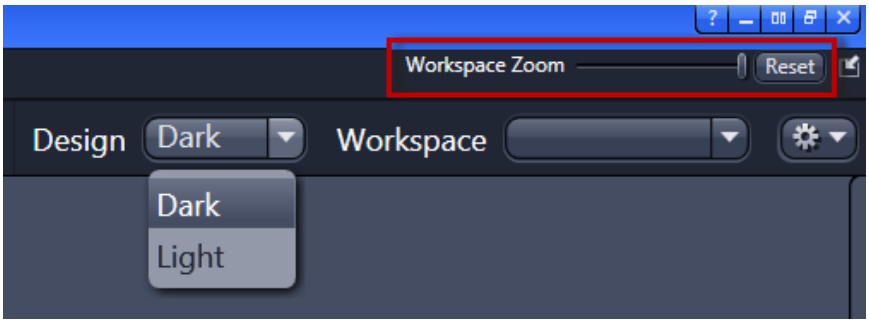
In the upper right corner of the program window under **Design** you can select a **Light** or **Dark** screen layout .



4.3 Adjusting the Workspace Zoom

Procedure

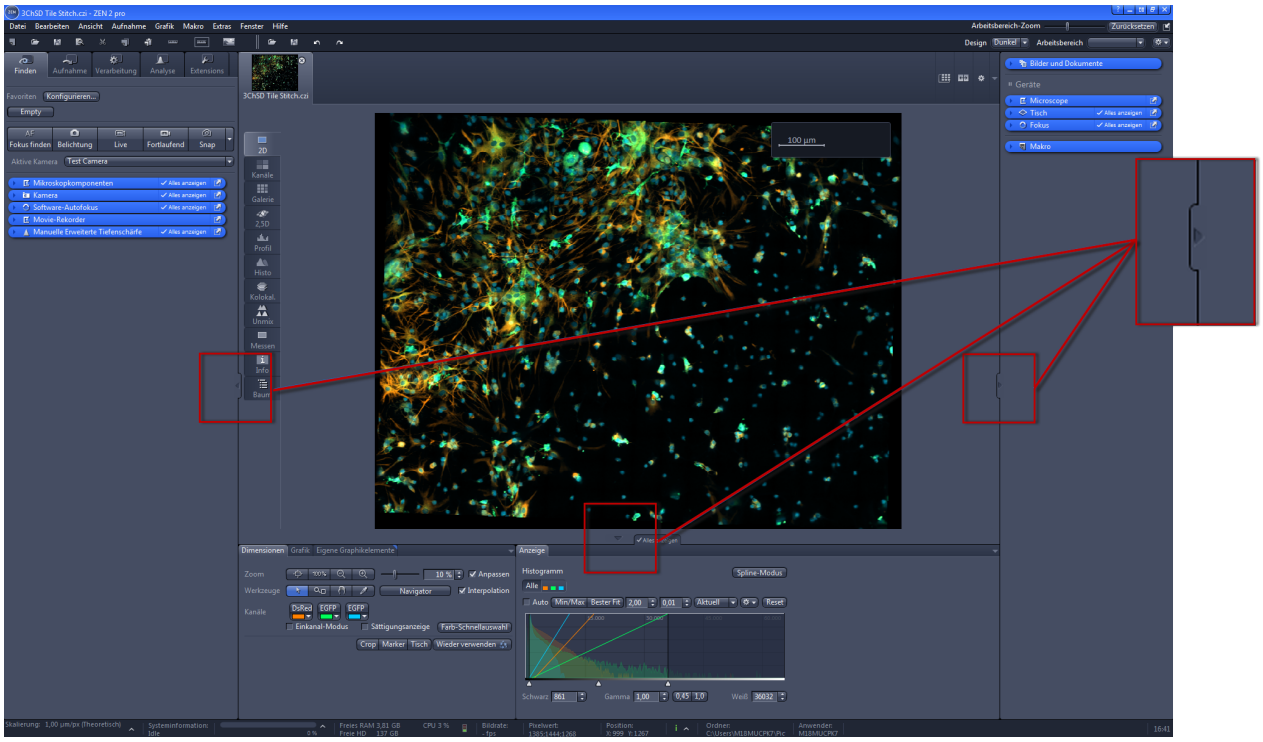
1 To zoom in or out of the workspace move the slider left or right.



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

4.4 Showing/Hiding Workspace Areas

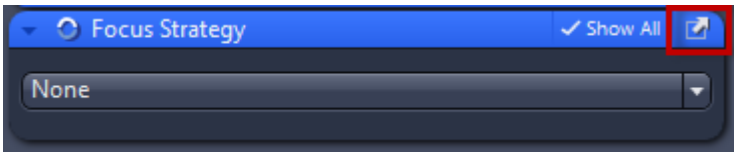
Procedure **1** Click on **show/hide** buttons to show or hide areas.



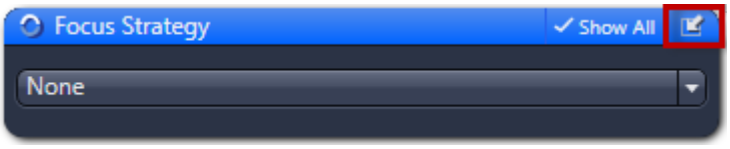
4.5 Undocking/Docking Tool Windows

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.



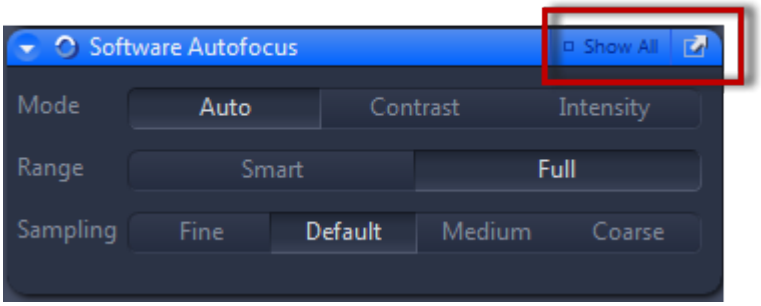
INFO

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

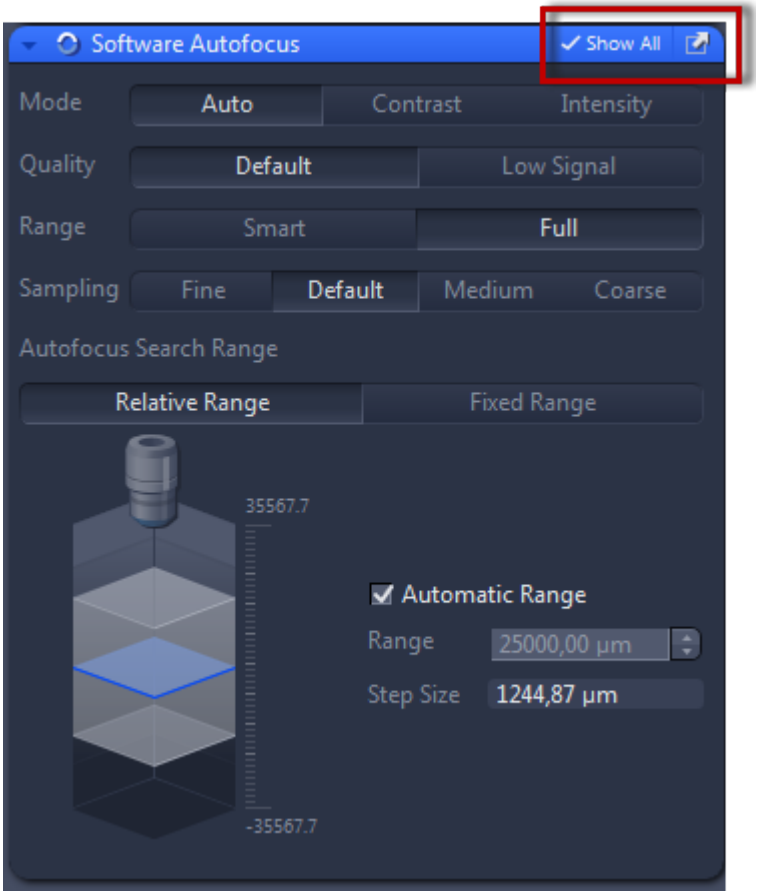
4.6 Acitvating the Show All Mode

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.



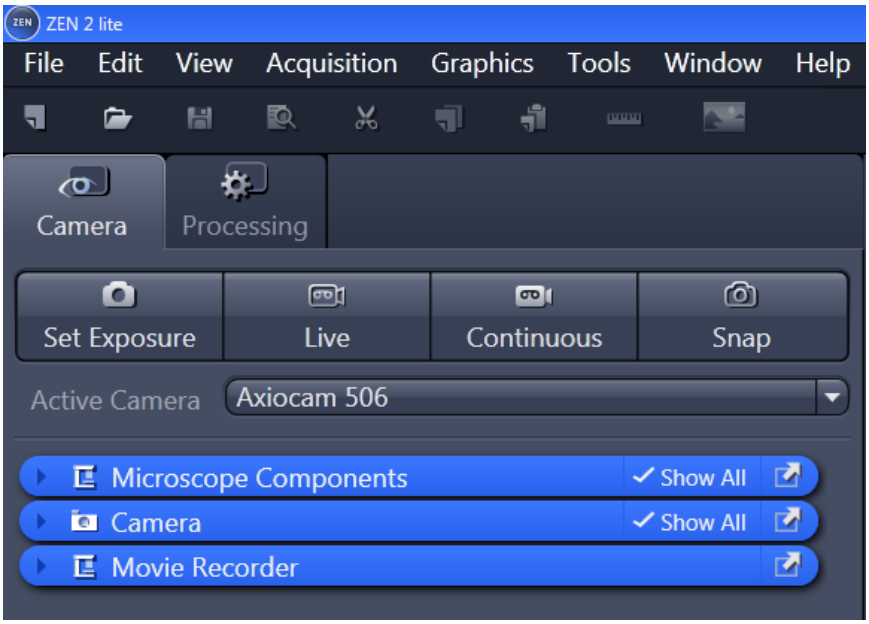
5 Step by step to the first image

5.1 Configuring 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

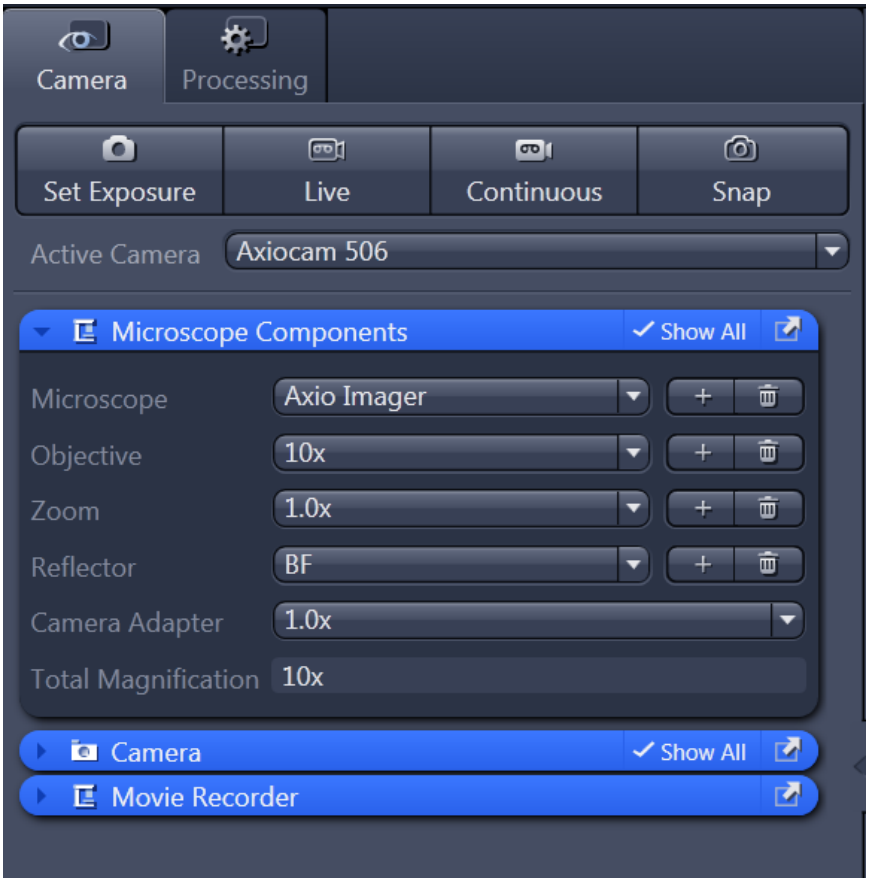
- You have selected the **Camera** tab.



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.).

INFO

If you have activated the **Select automatically** button in the status bar 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 *Creating a Manual Scaling* [▶ 22].

You have successfully configured your microscope components.

5.2 Acquiring a First Camera Image

This topic guides you through acquiring your first camera image with **ZEN (blue edition)** software.

Prerequisites

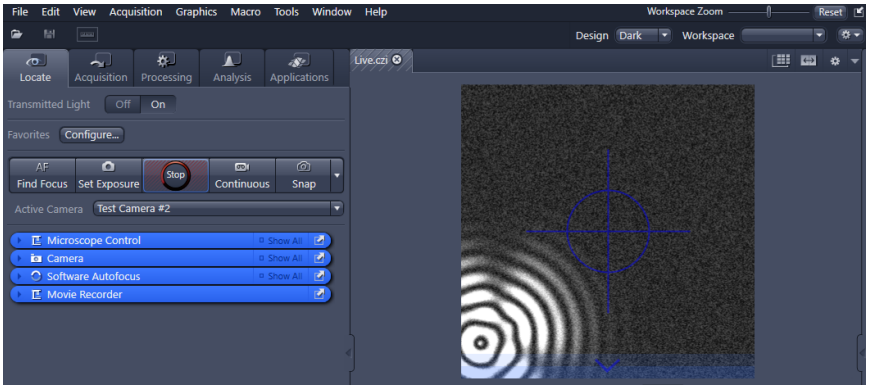
- You have connected and configured a microscope camera (i.e. AxioCam MR) to your system.
- You have started the software.

- 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. 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 *Adjusting Live Image Settings* [▶ 21] 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. Right click in the **Center Screen Area** to open the context menu. Select the entry **Focus Bar**. The focus bar will be shown.

5 Click on **Snap** button.

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

5.3 Adding Annotations to an Image

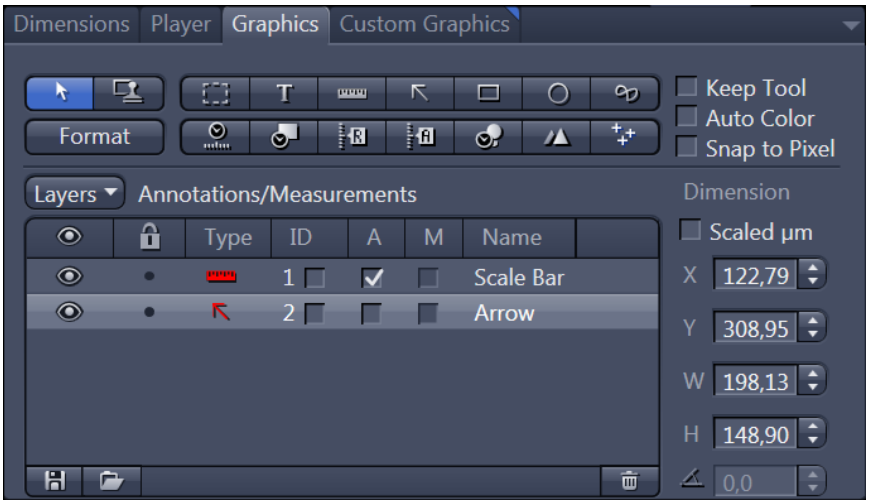
Annotations are the generic term for all the graphics (e.g. rectangle, arrow, scale), measurements, texts or other metadata (e.g. recording time) that you can add to your image.

Prerequisites

- You have acquired or loaded an image.

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

Right click on 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 Element**. 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.

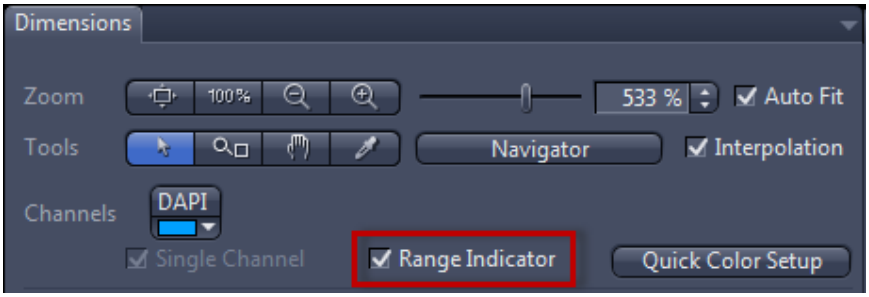
5.4 Adjusting Live Image Settings

Prerequisites

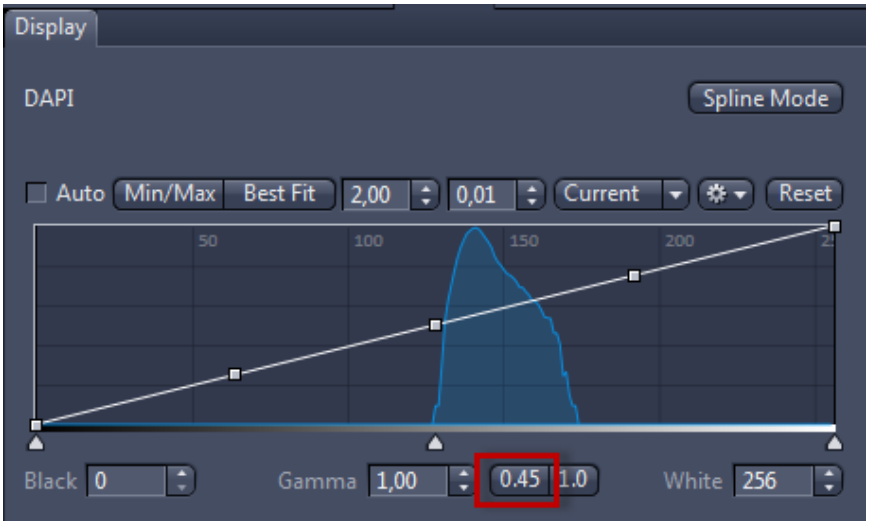
- You have started the **Live** mode via the **Live** button and see the camera's live image in the **Center Screen Area**.
- Under the image area you see the general view options on **Dimensions** tab, **Graphics** tab and **Display** tab.

Procedure

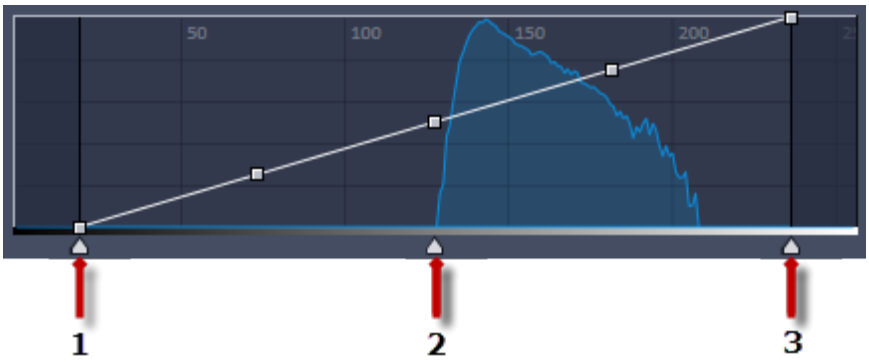
1 In the **Dimensions** tab activate the **Range Indicator** checkbox. This will mark overexposed (too bright) areas in the live image in red and underexposed (too dark) areas in blue.



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

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 Creating a Manual Scaling

Prerequisites

- You have an object micrometer oriented horizontally on the microscope stage.
- You have selected all definitions for your microscope correctly 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 *Acquiring a First Camera Image* [▶ 18]) of the scale in your object micrometer using the objective to be scaled manually.

2 In the bottom status bar click on the arrow in the **Scaling** area. In the **Scaling** dialog deactivate the **Select Automatically** checkbox.

3 In the **Create new scaling** section, click on the **Interactive Calibration...** button.

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 (activated as default).

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 you click in the status bar in the **Scaling** area to open the **Scaling** dialog and to activate the checkbox **Select Automatically** 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.

6 Closing the Software

Procedure **1** Click on **File | Exit**. Alternatively you can use the short cut *ALT+F4* or click on the **Close** icon in the program bar.

i INFO

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

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