# LSM 880

# LSM 880 NLO

Operating Manual (Quick Guide - English)

## ZEN 2 (black edition)

March 2015

We make it visible.

## Introduction

This Quick Guide describes the basic operation of the LSM 880, LSM 880 NLO Laser Scanning microscopes with the ZEN 2 software.
The purpose of this document is to guide the user to get started with the system as quick as possible in order to obtain some first images from their samples.
This Quick Guide does NOT replace the detailed information available in the full user manual or in the manual of the respective microscopes (Axio Imager, Axio Observer, Axio Examiner).
Also, this Quick Guide is written for users who are familiar with the basics of Laser Scanning Microscopy.

## Contents



## For your safety

**1. Notes on Handling the Laser Components and Illumination Systems**

・The LSM systems are laser hazard class 3B instruments. If equipped with a Ti:Sa Laser, the LSM systems are devices that belong to laser hazard class 4.These moderate and high-risk classes embrace medium-power and high power lasers. You must take care not to expose yourself to the radiation of such lasers. In particular, never look into the laser beam!

・Do not remove cables and optical fibers connected to microscope, scan module and laser module.

**2. Notes on Care, Maintenance**

・If spilt some water-soluble buffer (culture medium, etc), remove it by wiping with a dust-free cotton cloth or a moistened cloth immediately. Before cleaning, switch off the microscope and disconnect it from the main power supply.

・Remove oily or greasy dirt (immersion oils, finger prints) using cotton wool buds or a dust-free cotton cloth and Optical Cleaning Mixture L. (This cleaning mixture is manufactured from 85% vol n-hexane and 15 % vol isopropanol)

・Do not touch lamp housing when it is hot. Always check that the instrument is switched off and cooled down before covering.

**3. Observe the following instructions:**

・In the Operating Manual, read the chapter *Safety Instructions* carefully before starting operation.

・Follow the safety instructions described in the operating manual of the microscope and X-Cite 120 lamp / HBO 100 mercury lamp.

Work flow to acquire image

**Starting the system, and turning on the lasers (P. 2~6)**

↓

**Microscope observation via eye pieces (P. 7)**

↓

**Configuring the LSM beam path for dye you use (P. 8~11)**

↓

**Scanning an image (P. 12~17)**

# 1. Starting the System

## 1-1. Starting the System

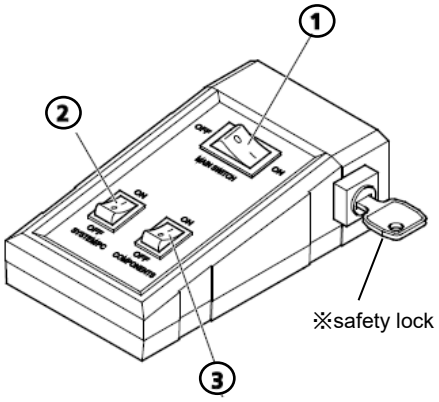



Fig. 1 Power remote switch

### 1-1-1. Switching on the LSM system

① Switch on the **Main Switch** of the Power remote switch.

※ Check the safety lock is ON.

② Switch on the **System/PC**, and log on as '**LSM user**'

③ Switch on the **Components**.

- **SYSTEM/PC** provides power to the computer. This allows use of the computer and ZEN software offline
- **COMPONENTS** starts the other components and the complete system is ready to be initialized by the ZEN software.

### 1-1-2. Switching on the mercury lamp (X-Cite 120 or HBO 100)

- Switch on the main switch of the X-Cite 120 / HBO 100 lamp for reflected light illumination via the power supply as described in the respective operating manual.

### 1-1-3. Switching on the NLO laser (Optional)

- If the Chameleon (Coherent) laser is required, turn the key in front of the power supply from **Stand by** to **ON**.

## 1-2. Starting ZEN software

Double click the **ZEN** icon  on the desktop to start the Carl Zeiss LSM software. The ZEN Main Application window and the **Startup** window appear on the screen. Choose **Start system** to start the system hardware for acquiring new images.


Fig. 2 Startup window of ZEN

**Start System** : initializes the whole microscope system and activates the entire software package for new image acquisition and analysis.

**Image Processing** : ignores all hardware and activates only data handling and image processing functionality for already acquired images.

※ Click  symbol to show the Boot Status display.

## 《 Introduction to ZEN – Zeiss Efficient Navigation 》

ZEN 2 interface is clearly structured and follows the typical workflow of the experiments performed with confocal microscopy systems:

**Left Tool Area**(Fig.3-①,②,③)

The user finds the tools for sample observation, image acquisition, FCS, image processing and system maintenance, easily accessible via four Main Tabs.

**① Main Tabs** **② Action Tabs** **③ Tool Group**

**Center Screen Area**(Fig.3 - ④,⑤,⑥)

This area is for viewing. Each displayed image can be displayed and/or analyzed with many view options available through view tabs which can be found on the left side of the image.

**④ Image Window** **⑤ View Tabs** **⑥ View Controller**

**Right Tool Area**

File management and data handling tools.

**⑦ Open Images list (Thumbnail view)**

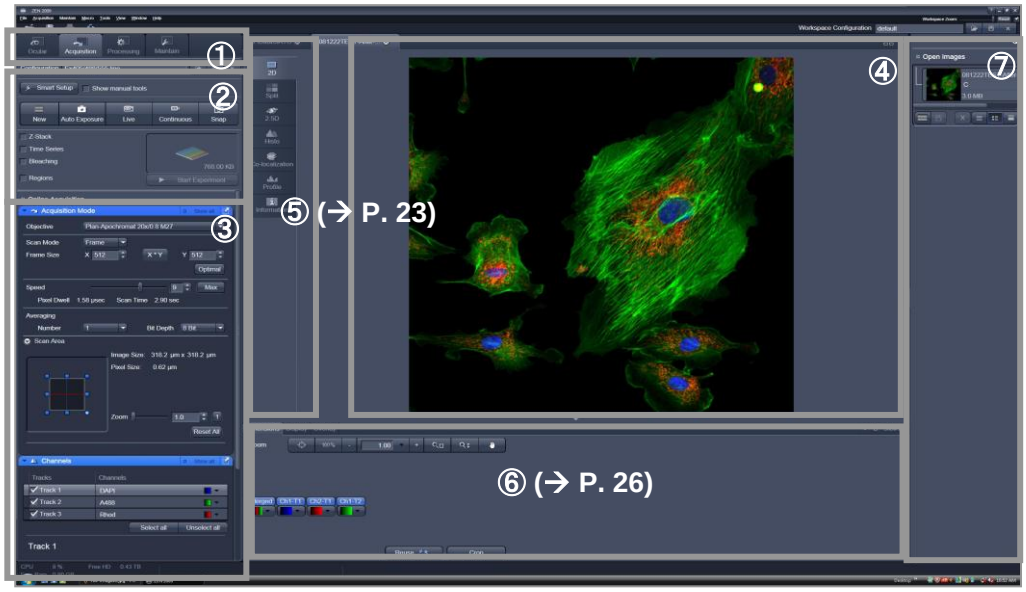



Fig.3 ZEN main application window after starting up

**Show all tools**

With **Show all tools** activated, the **Setup Manager** tools are displayed. Users can edit the configuration from **Light Path** tool.

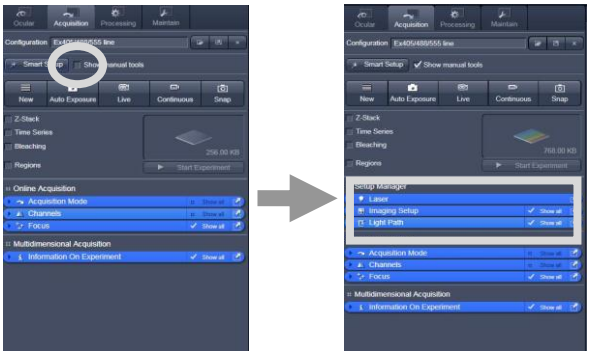


Fig.4 Show all tools

F

## Show all

With **Show all** de-activated, the most commonly used tools are displayed. For each tool, the user can activate **Show all** mode to display and use additional functionality


Fig.5 Show all

## More features of ZEN 2 include:

– User can add more columns for tools to the **Left Tool Area** or detach individual tools to position them anywhere on the monitor. To add a column, drag a tool group by the title bar (e.g., Online Acquisition) to the right and a new tool column automatically opens. Alternatively use the context menu "move tool group to next column". To detach a tool, click on the little icon on the right end of the blue tool header bar

– Another unique feature in the Imaging Software is the scalable ZEN interface. This **Workspace Zoom** allows adjustment of the ZEN window size and fonts to the situational needs or your personal preferences

– Setting up conventional confocal software for a specific experiment can take a long time and is often tedious to repeat. With ZEN these adjustments have to be done only once – and may be restored with just two clicks of the mouse. For each type of experiment one can now set-up and save the suitable **Workspace Layout**. These configurations can also be shared among users.

※ These are just some of the most important features of the ZEN interface. For a more detailed description of the functionality for the ZEN 2 software, please refer to the User Manual that is provided with your system.

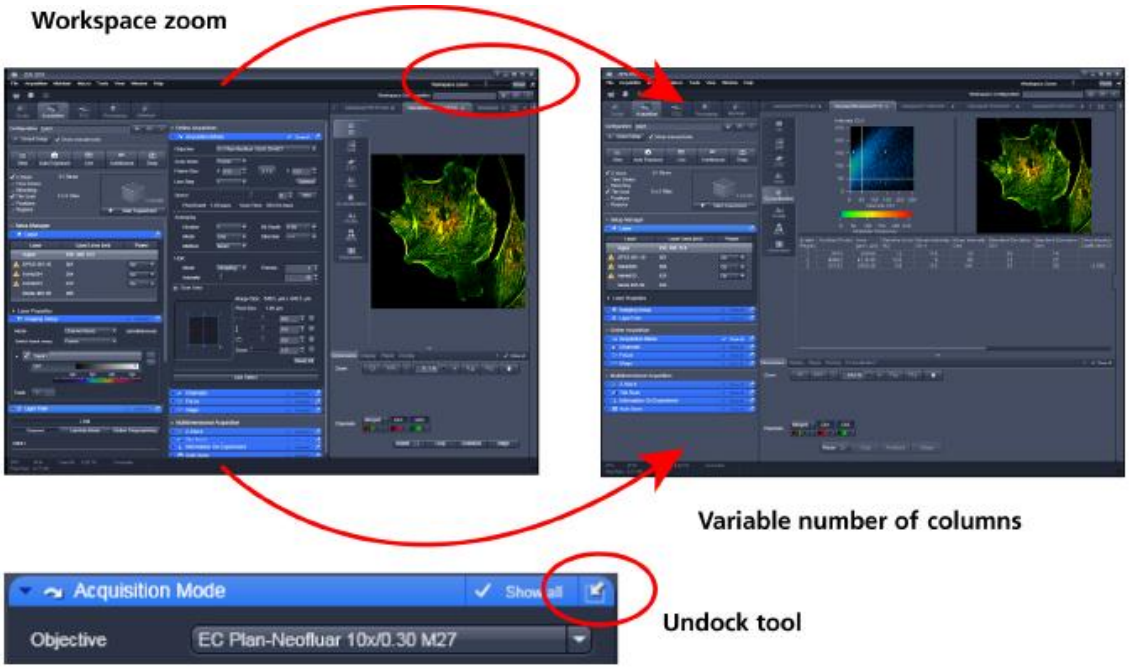



Fig.6 ZEN Window Layout

## 2. Turning on the lasers

ZEN 2 operates all lasers automatically. Whenever they are used (manually or by loading configuration), the lasers are turned on automatically.

To manually switch lasers on or off:

Check the **Show all tools** tick box and open the **Laser** tool. All available lasers can be operated within this tool (Fig. 7), except Ar laser. Select the required Laser, and turn on it from the pull down menu.

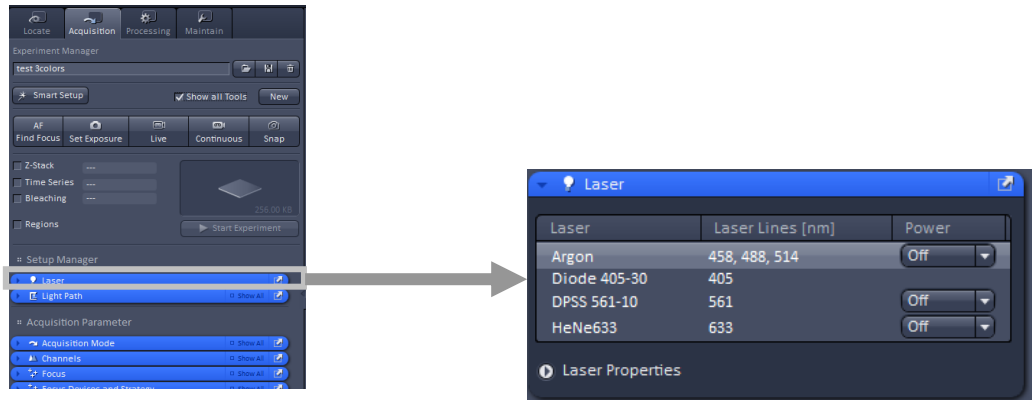



Fig.7 Laser Control tool

- **Blue Diode 405（405nm）**

  The diode lasers is switched on automatically after the software is started.

- **Argon（458/488/514nm）, DPSS (561nm), HeNe (543nm、594nm、633nm)**

  Switch the requested lasers **ON** by using the pull down menus. Ar laser temporary turns into **Standby** and then is switched to **ON** automatically after **5-minute warming up**.

- **MaiTai (Spectra Physics), Chameleon (Coherent) for NLO**

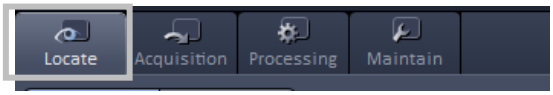
  Switch the requested lasers **ON** by using the pull down menus.

※ Users can check the laser power and status by clicking on **Laser Properties** in Laser Control tool.

# 3. Setting up the microscope

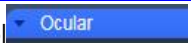
## Changing between direct observation and laser scanning mode

The **Locate** and **Acquisition** buttons switch between the use of the microscope and the LSM:




• Click on the **Locate** tab for the direct observation mode. In this mode, lasers are blocked.

## Setting up the microscope and storing settings

Open the Ocular tool 

to configure the components of your microscope (e.g., filters, shutters and objectives). Place specimen on the microscope stage. The cover slip must be facing the objective lens. Remember the immersion medium if the objective lens chosen requires it!

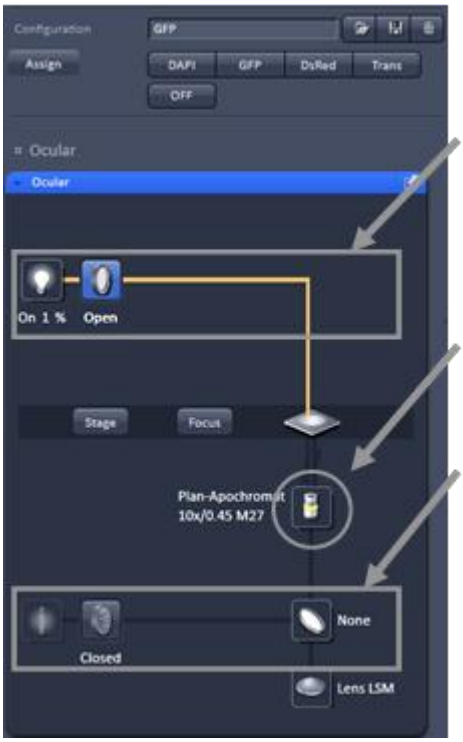



Fig.8 Microscope Control window (Axio Observer. Z1)

**Transmitted light**

- Open the graphical pop-up menu by clicking on the **Transmitted Light** icon. Click on the **On** button. Set the intensity of the Halogen lamp using the slider.

**Selecting an objective**

- Click on the **Objective** icon and select the objective lens for your experiment. The chosen objective lens automatically moves into the beam path.

**Reflected light**

- Click on the **Reflected Light** shutter to open the shutter of the X-Cite 120 lamp / HBO100.
- Click on the **Reflector** button and select the desired filter set by clicking on it.

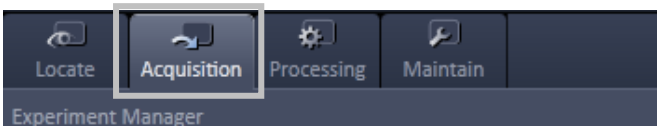
### Storing the microscope settings

Microscope settings can be stored as configurations. These configurations can be assigned to buttons that are easier to set up the microscope.


Fig.9 Microscope Configuration panel

# 4. Configuring the beam path and lasers

Click on the **Acquisition** button to change to the **LSM** mode.




There are 3 ways to configure the beam path and lasers.

We highly recommend to use **'4-1. Experiment Manager'** especially for users who are not familiar with LSM.

## 4-1. Experiment Manager

For loading an existing configuration, click  button in the **Experiment Manager** and select the appropriate one from the list box.

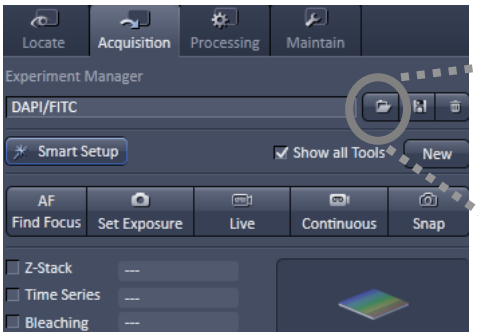



Fig. 10 Experiment Manager

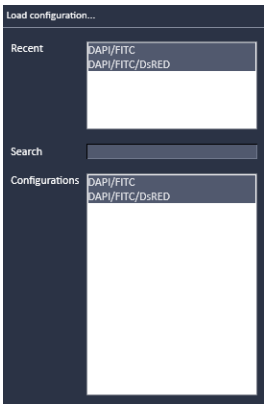



Fig. 11 Configuration List

ex) Single staining -> 'Ex 488 (GFP, FITC, Alexa488)' for Green fluorescence, 'Ex 561 (Cy3, Alexa568, Rhodamine)' for Red fluorescence, etc..

Multi staining -> 'Ex 488/561' for Green and Red double staining, 'Ex488/561/633' for Green, Red, and Far-red triple staining, etc…

**※ Please do not overwrite  / delete  existing configuration!**

## 4-2. Smart Setup

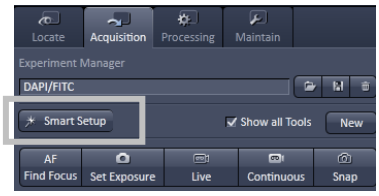



Fig. 12 Smart Setup Button

① Click on the **Smart Setup** button to open the smart setup window.

② Click on the arrow in **Configure your experiment**.

③ Choose the dye(s) you use from the list dialogue. In this dialogue, the candidate dyes can be also searched by typing the name in the search field.

④ Add or delete dye name by click on **+** or **−**.

⑤ Once finished with the input, **Smart Setup** suggests three alternative configurations; **Fastest**, **Best signal** and **Smartest**, the best compromise between speed and signal intensity (see below).

⑥ Click on the **Apply**, and ZEN automatically sets the ideal hardware parameters for the dyes chosen.

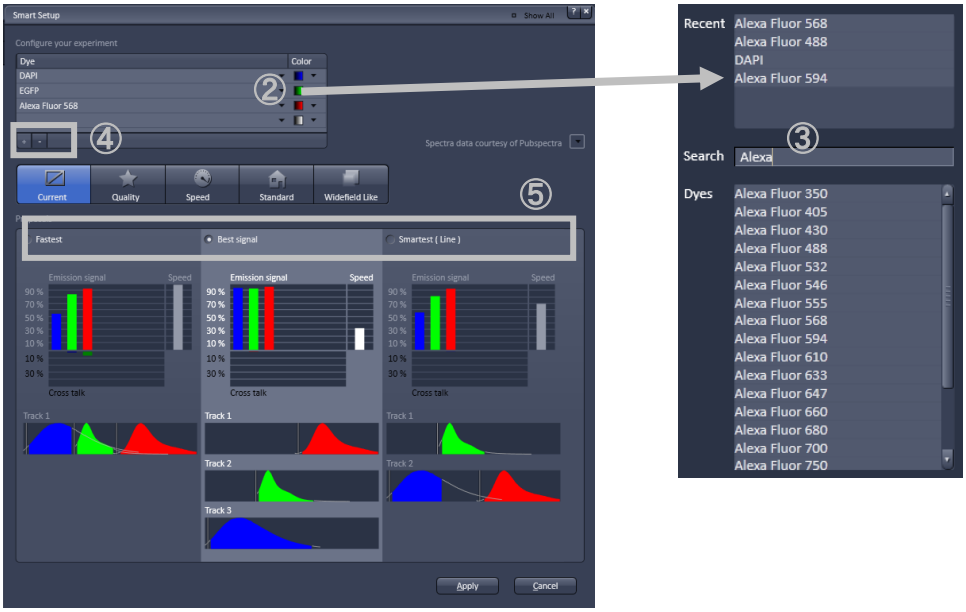



Fig13. Smart Setup tool

**Fastest (Simultaneous)**

– Advantage: faster image acquisition

– Disadvantage: potential cross-talk between channels

**Best Signal (Sequential)**

– Advantage: minimum cross-talk between channels; a single laser and detector are used alone at a time.

– Disadvantage: slower image acquisition

**Smartest (Semi-Sequential)**

– Best compromise between Fastest and Best signal.

## 4-3. Setting up a configuration manually

① Activate '**Show all tools**'.


Fig.14 Show all tools

② Open the **Imaging Setup** tool to set-up the beam path. This tool displays the selected track configuration.

③ Users can change the settings of this panel using the following function elements.

※ Customized configurations can be stored in the list of Experimental Manager.

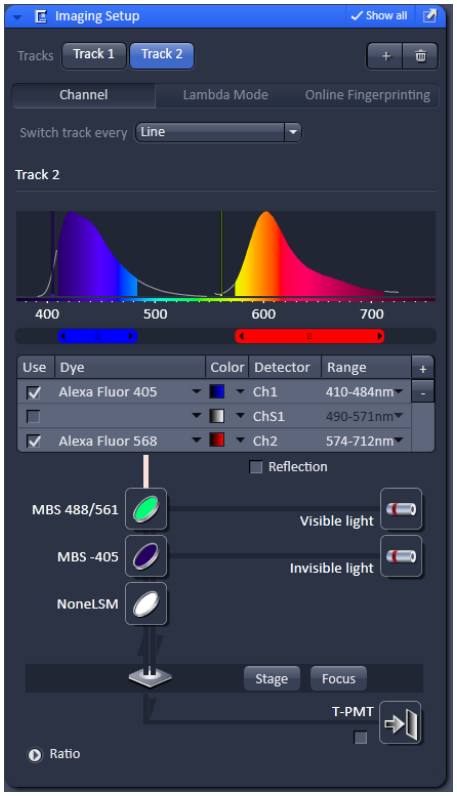
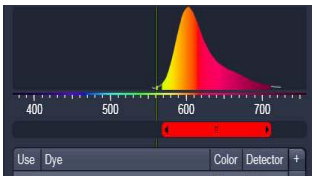


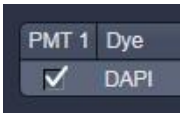

Fig.15 Imaging Setup tool

Activation / deactivation of the excitation wavelengths (check box) and setting of excitation intensities (slider)

Selection of the main dichroic beam splitter (MBS) from the relevant list box




Selection of an emission range for scanning



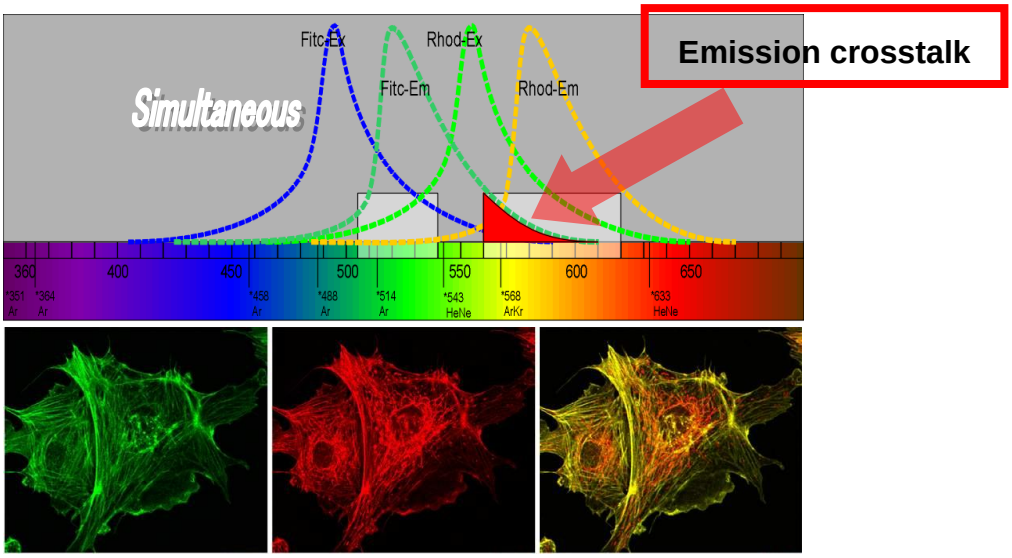

Activation / deactivation (via check box) of the selected channels (Ch 1-2, QUASAR detectors ChS1-8) for scanning


Assigning a color of the channel

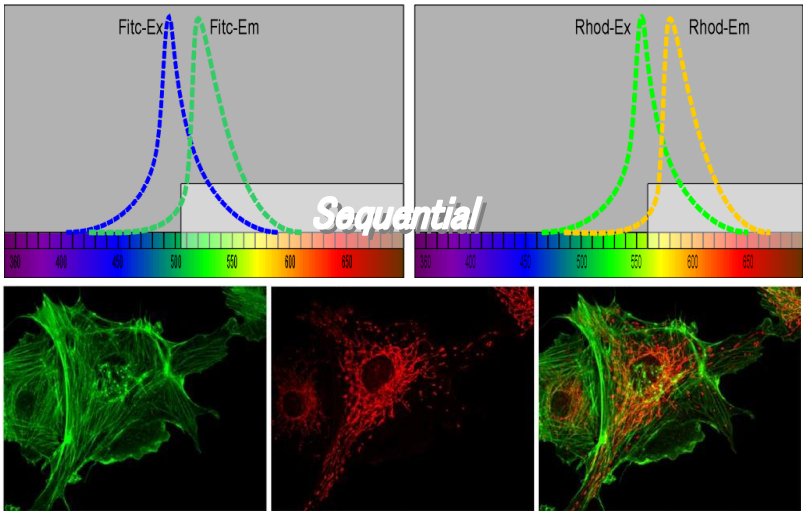

Activation / deactivation (via check box) of the transmission channel

## 《 Methods for multi-color imaging: Simultaneous and Sequential 》

Simultaneous and sequential acquisition are the methods of choice for multi-fluorescence imaging. Both methods have merits and demerits, and the user can select one of the methods according to the purpose of the experiment.




In simultaneous excitation and detection of multiple dyes, although the image acquisition speed is higher, emission crosstalk can occur.




Sequential acquisition (multi-track imaging) allows avoiding artifacts from the emission crosstalk. To sequentially acquire multiple channels, laser lines are switched very fast and channels are recorded quasi-simultaneously.

# 5. Scanning an image

**Workflow to acquire images**

**Adjusting pinhole size**
↓
**Auto Exposure**
↓
**Live scan**
**Adjusting Brightness/Contrast**
↓
**Setting the parameters**
↓
**Snap**

## Adjusting pinhole size

① Select the **Channels** tool in the Left Tool Area. The **Channels** tool provides the control of the parameters for the individual detection channels.

② Set the Pinhole size to **1 AU** (Airy unit) for best compromise between depth discrimination and detection efficiency.

※ Pinhole adjustment changes the Optical Slice thickness. When collecting multi-channel images, adjust the pinholes so that each channel has the same Optical Slice thickness. This is important especially for colocalization studies.

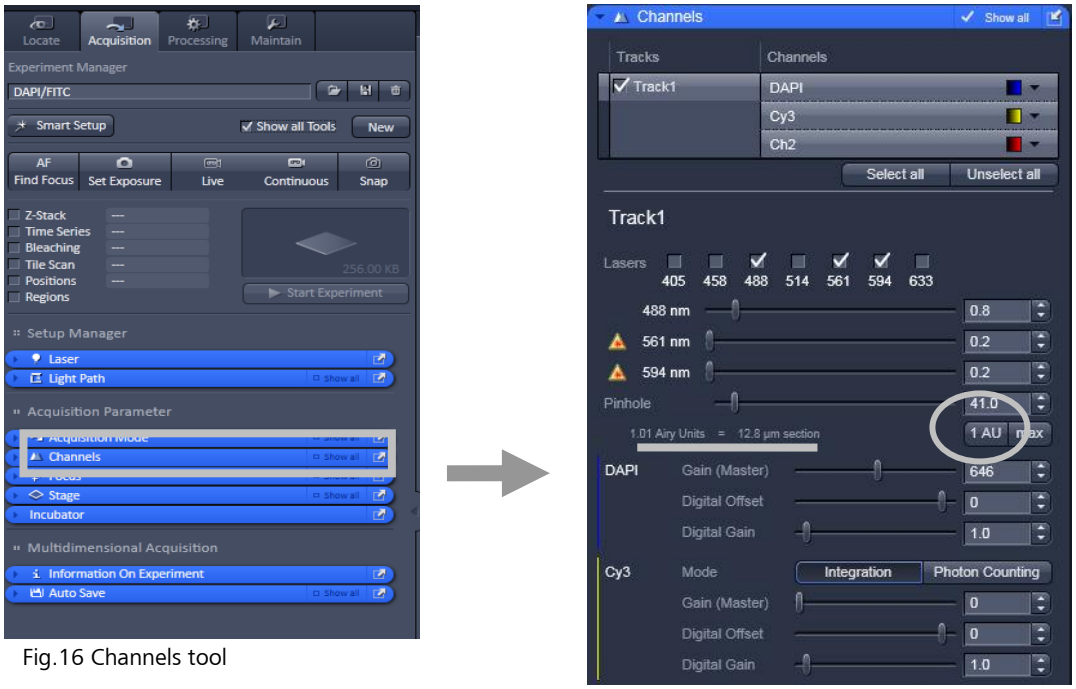
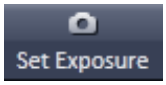



Fig.16 Channels tool

## Image acquisition – Auto Exposure

③ Click **Set Exposure** button, and ZEN optimizes the settings of the Gain (Master) and offset for the given laser power and pinhole size. Users can easily optimize the image further by using this recommended parameters.



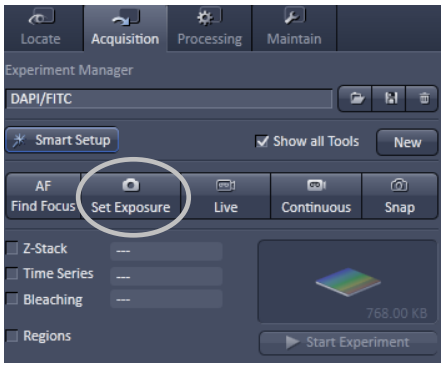




Fig.17 Set Exposure

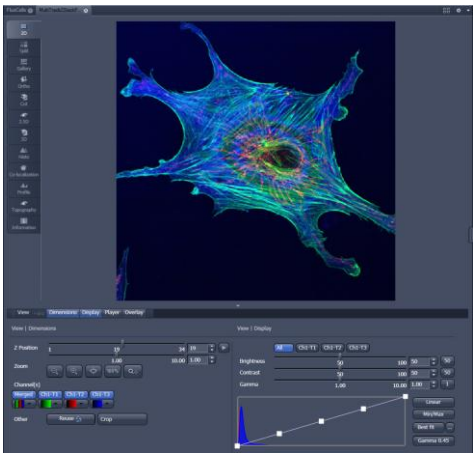
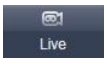


Fig.18 Image Display

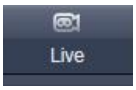
## Image acquisition - Live

④ Click on the Live 

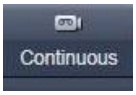
or Continuous 

buttons to start the scanning procedure to continuously acquire an image. Click on the Stop button to stop the current scan if necessary.


Select New button for new empty image window

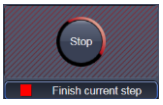



Select Live button for continuous fast scanning – useful for finding and changing the focus.




Select Continuous button for continuous scanning with the conditional scan speed.


Select Snap button for recording a single image.




Select Stop button for stopping the current scan procedure.

**Split View** 

displays the individual channels of a multi-channel image as well as the superimposed image.

※ The **Dimensions** View shows the **Merged** tick box to activate / deactivate the display of the channel overlay.

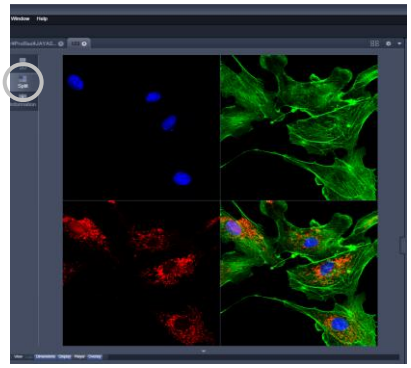


Fig.19 Split View

## Adjust Brightness and Contrast

⑤ Adjust the image intensity of selected channel during continuous scanning.


Fig.20 Channels tool

**Lasers** :

Control of the laser lines and their attenuation power

**Gain (Master)** :

Setting of the high voltage of the PMT photomultiplier - setting of image contrast and brightness

**Digital Offset :**

Setting of the electronic offset –setting of image background

**Digital Gain :**

Amplification factor

⑥ Click on the Stop 

button to stop the current scan procedure when finish the adjustment.

## Image Optimization

### Activating Range Indicator

In the **View** – **Dimensions** View Option Control Block, activate **Range Indicator** tick box (Fig. 21).

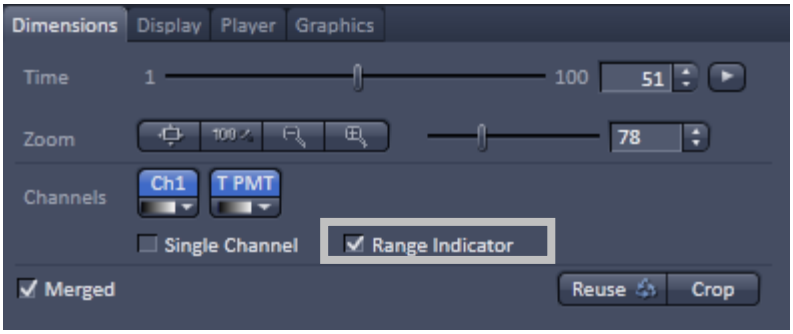
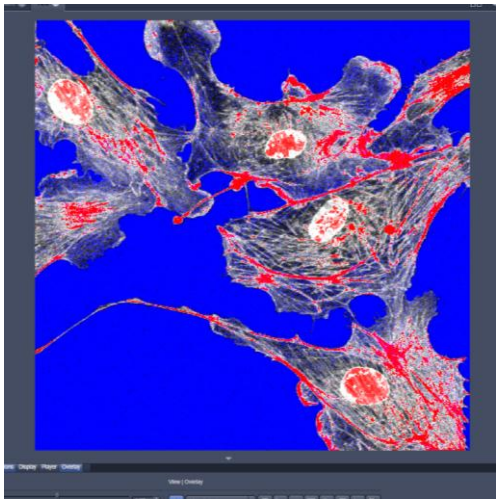



Fig.21 Dimensions Control window



The scanned image appears in a false-color presentation

If the image is too bright, saturated pixels are indicated as **red** color.

If the image is not bright enough, background pixels (the intensity of which is null) are indicated as **blue** color.

Fig.22 Range Indicator

**Adjusting laser intensity**

- Set the **Pinhole** to **1 Airy Unit** (Fig. 16)
- Set the **Gain (Master)** high (applox. 800 ~ 1000).
- When the image is saturated, reduce AOTF transmission in the **Laser** control section of the **Channels** tool (Fig. 20).

**Adjusting gain and offset**

- Increase the **Digital Offset** until all blue pixels disappear, and then make it slightly positive.
- Reduce the **Gain (Master)** until the red pixels only just disappear.

## Setting the parameters for scanning

⑦ Select the **Acquisition Mode** tool from the Left Tool Area.

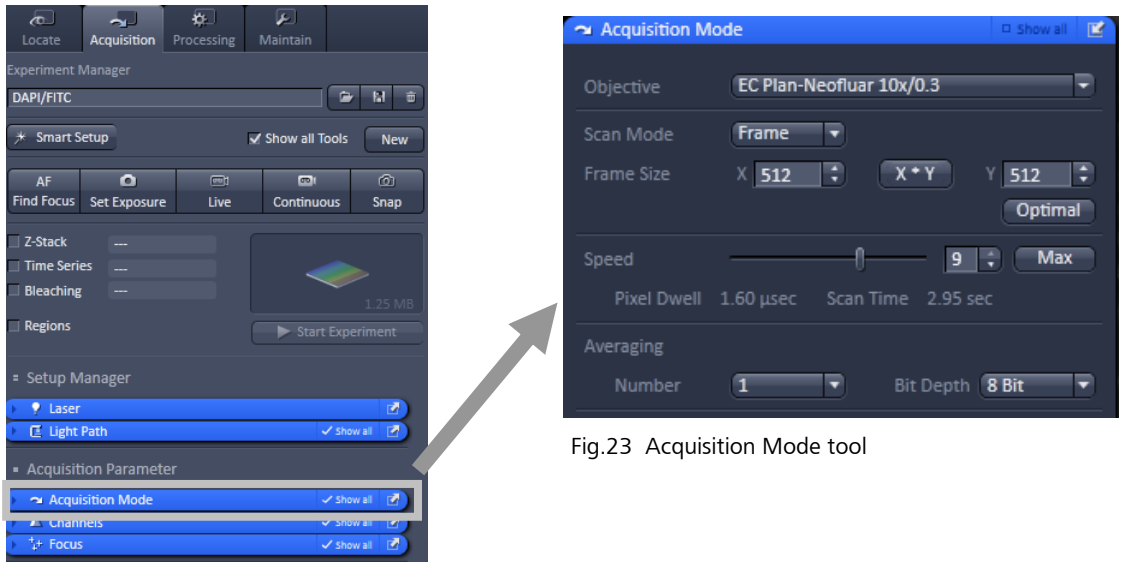



Fig.23 Acquisition Mode tool

### Frame Size

Select the Frame Size as predefined number of pixels or enter any values (default is 512 x 512) in the Acquisition Mode tool. Click on the **Optimal** button for calculation of the appropriate number of pixels depending on an objective N.A. and λ.

※ The number of pixels influences the image resolution!

### Speed

Use the Scan Speed slider in the Acquisition Mode tool to adjust the scan speed.

※ A higher speed with averaging results in the best signal-to-noise ratio. Scan Speed 8 usually produces good results, and try Speed 6 or 7 for superior images.

### Bit Depth

Select the dynamic range 8, 12 or 16 Bit (per pixel) in the Bit Depth pull-down in the Acquisition Mode tool

※ 8 Bit gives 256 gray levels; 12 Bit gives 4,096 gray levels, and 16 Bit gives 65,536 gray levels. Publication quality images should be acquired using 12 or 16 Bit data depth. 12 or 16 Bit is also recommended when doing quantitative measurements or when imaging low fluorescence intensities.

## Averaging

Averaging improves the signal-to-noise ratio of images. Averaging scans can be carried out line-by-line or frame-by-frame; frame averaging helps to reduce photo-bleaching, but does not give quite as smooth as line averaging does.

For averaging, select the number of lines or frames to average from the pull down menu. Averaging number up to 4 usually produces a good result.

## Scan Area

In this panel, the scan field is set for zoom, rotation and offset in relation to the field of view of the microscope. The outer square (dotted line) corresponds to the field of view of the microscope and the inner square ( or rectangle) represents the scan area (arrow heads in Fig. 24).

By clicking on the Reset All 

button the scan zoom is set to 1, the XY offsets are set to the zero position and the ratio angle is set to 0°

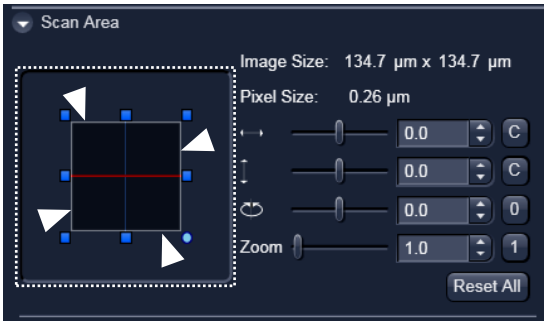
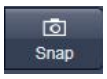



Fig.24 Scan Area

## Snap

⑧ Finally, click on Snap 

for recording an image.

⑨ Before starting next scan, save the image (Chapter 6) or click on New 

to open an empty image window. An unsaved 2D image in the active image tab will be **over-written** by a new scan.

※ Multi-dimensional data or saved images are not over-written and a new scan image will be displayed in a new window created automatically.

※ Acquired data are **not** automatically saved to the disc. Make sure you save your data appropriately and back it up regularly. The ZEN software asks whether users want to save unsaved images when users try to close the application.

# 6. Storing and exporting image data

## 6-1. Save image

**Save Status icon**  appears in the image that is not saved yet.

An unsaved 2D image in the active image tab will be **over-written** by a new scan!

**Please make sure you save your data appropriately and back it up regularly.**

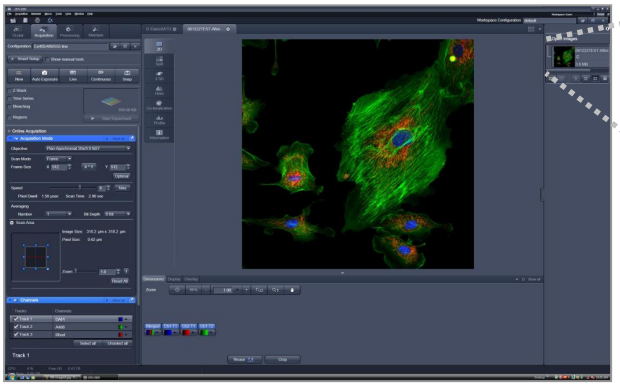



Fig.26 Save status icon

Fig. 25 ZEN main window

There are 2 ways to save images**.**

1) **Save as .czi format (or .lsm format)** (→ page 19)

Merit) ・It stores an image together with the acquisition parameters.

・Reloading configuration from the stored image data is available.

De-merit) ・Other software cannot load CZI images .

※ You can download the free software 'ZEN Light Edition' from Carl Zeiss website. (Windows OS only) **http://www.microimaging.zeiss.co.jp/**

2) **Save as multipurpose format (Export), like TIF, JPEG…etc.** (→ page 20)

Merit) ・Users can save as several formats.

・Exported data can be load in other software.

De-merit) ・It loses the acquisition parameters (hardware setting, scaling, etc.).

## 1) Save as CZI or LSM format

The **Save** function allows to store an image together with the acquisition parameters to be stored in **.czi (or .lsm)** files.

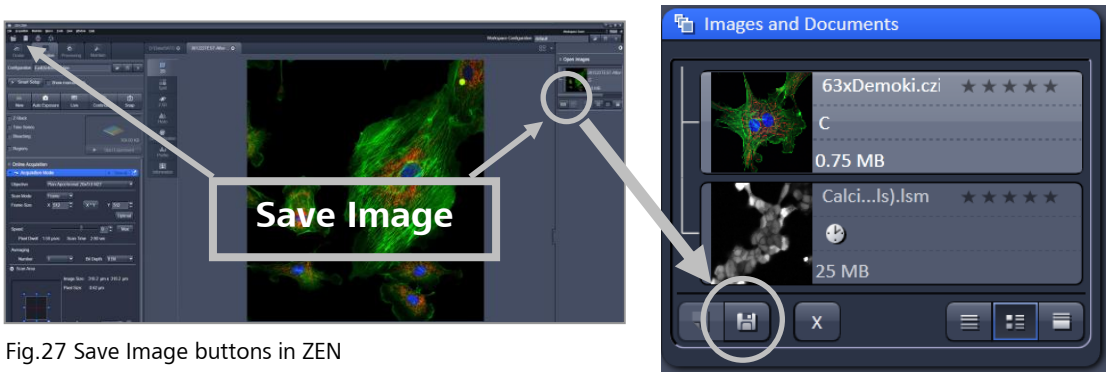



Fig.27 Save Image buttons in ZEN

① To save your acquired images, click on the **File** - **Save** or **Save As** button, or click on the  icon at the bottom of the Right tool Area (Fig. 27).

② The WINDOWS **Save As** window appears.

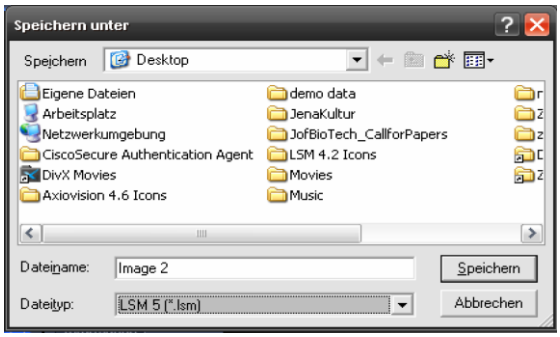



Fig.28 Save as window

③ Enter the file name and choose the appropriate image format.

Note: the CZI and LSM format (.czi, .lsm, respectively) are the native Carl Zeiss LSM image data format and contains all available extra information and hardware settings of your experiment.

④ Click on the **Save** button.

※ If you close the image which has not been saved, a pop-up window will ask you whether you want to save it. Choosing **Yes** will lead you to the WINDOWS Save As window.

## 2) Export of Images as general formats

① Select the image to be exported, and choose **File** - **Export** from the menu.

② Select the **file format**, and the data type which the image is to be exported under **Data**.

③ Click on **Select file name and save…** button.

④ The standard Windows File saving dialog appears. Click **Save** to complete the export process.


Fig.29 Export window

- **Format** : File format (e.g., Tagged Image File (TIFF), JPEG)
- **Data**
  - **Raw data**

    Image is saved as RGB, or monochrome. It does not contain overlaid items (e.g., text, scale bar, arrow).
  - **Contents of image window**

    Image is saved as a display size of monitor like a print screen.
    It includes analysis window and overlaid items.
  - **Full resolution image window**

    Image is saved as a raw data size with overlaid items.

## 6-2. Open images

To open images, select the file from **File** menu – **Open** or **New File Browser**.

### ZEN File Browser

① Advanced data browsing is available through the **New File Browser** (Ctrl + F or from the File Menu).

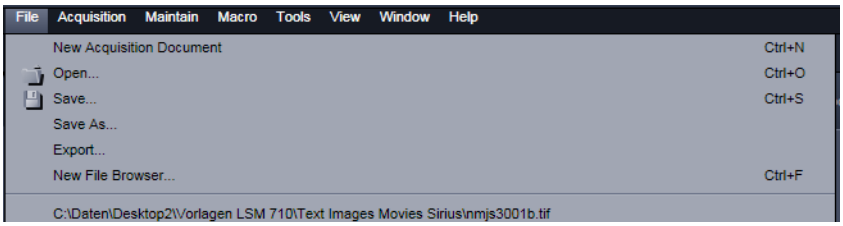



Fig.30 File Menu

② The ZEN File Browser can be used like the WINDOWS program file browser. Images can be opened by double-click and image acquisition parameters are displayed with the thumbnails

Select folder in your HDD

Images are displayed with the thumbnails

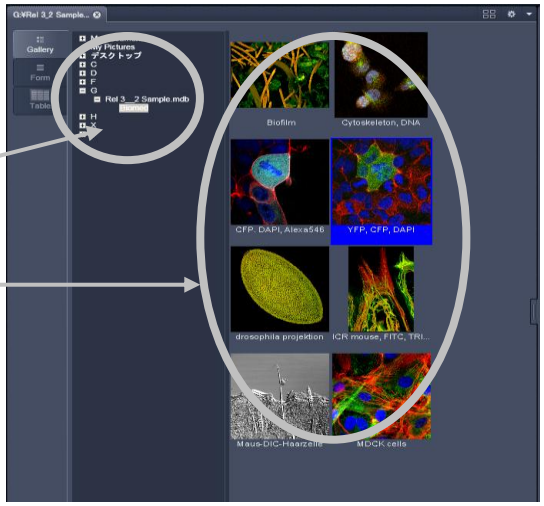



Fig.31 New File Browser

### Reuse (Loading acquisition parameters from existing images)

Clicking the **Reuse** button transfers ALL acquisition parameters from the stored image data to the **Microscope Hardware Settings / Control** tools and applies those parameters directly to the system.

The acquisition parameters of an image are displayed in the **Information** View.

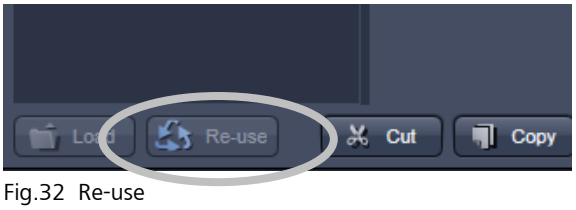



Fig.32 Re-use

# 7. Z stack (3D imaging)

The Z-Stack function permits scanning a series of XY-images in different focus positions resulting in a Z-Stack, thus producing 3 dimensional data from your specimen.

## Scanning a Z stack

① Select **Z-Stack** in the main tools area. Open the Z Stack tool in the Left Tool Area.

② Select **Mode** **First/Last** on the top of the **Z-Stack** tool.

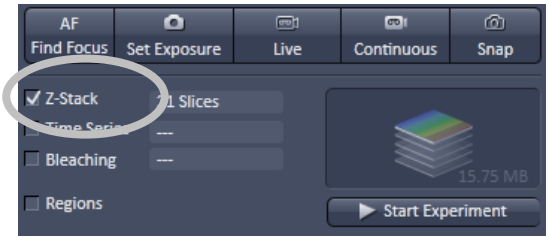
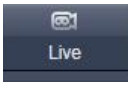



Fig.33 Selecting applications

③ For defining the first and last image of the stack ;

1) Click on the 

button in the **Action Button** area for continuous scanning.

2) Use the focus drive of the microscope to focus on the upper position of the specimen where the Z Stack is to start. Click on the **Set First** button to set the upper end of the Z Stack.

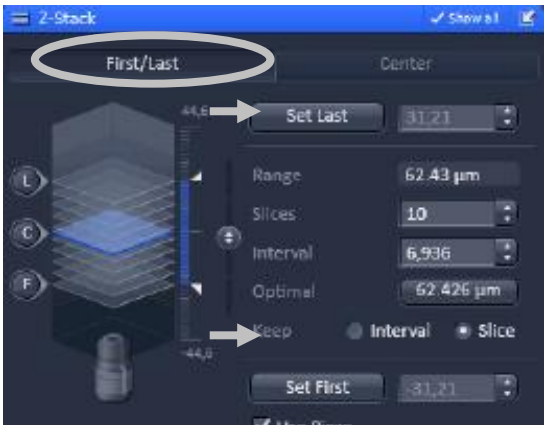
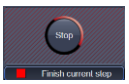



Fig.34 Z-stack tool (for First/Last setting)

3) Then focus on the lower specimen area where the recording of the Z Stack is to end. Click on the **Set Last** button to set this lower end.

4) Click on the 

button to stop scanning.

④ Click on the **Optimal** button to set number of slices to match the optimal Z-interval for the given stack size, objective lens, and the pinhole diameter.

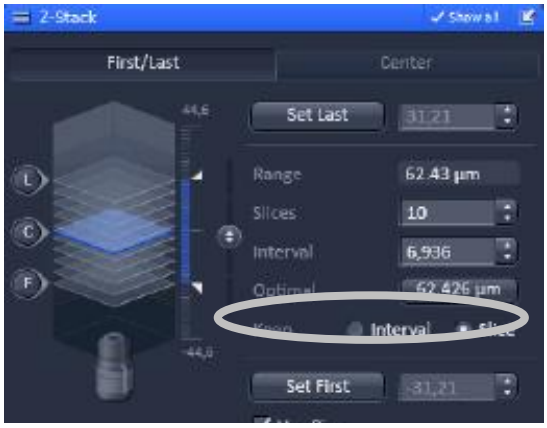



Fig.35 Optimal button

⑤ Click on the **Start Experiment** button to start the recording of the Z-Stack.

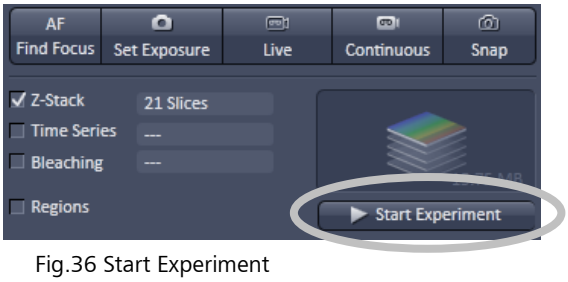



Fig.36 Start Experiment

## Visualization of Z-stack data

### 1) View tabs

For Gallery, Ortho (orthogonal section), Cut and 3D view, please refer next **Chapter 8. View Tabs** (page 24 to 26).

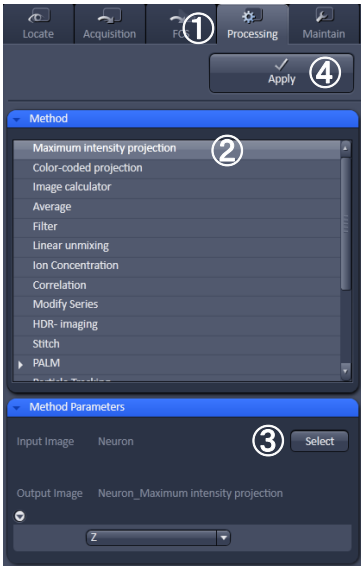
### 2) Maximum intensity projection

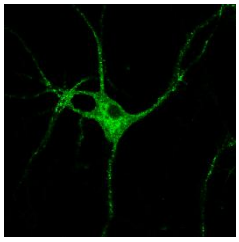
① Click on the **Processing** tab.

② Select **Maximum intensity projection** from **Method** menus.

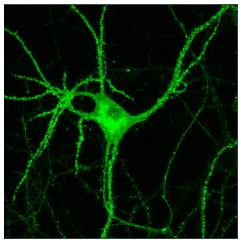
③ Display a Z-stack image to be projected on the center screen area, and click on the **Select**.

④ Click on **Apply**, and a MIP image is generated as a new file.



Single plane image



MIP image

# 8. View Tabs

The View tabs make all viewing options and image analysis functions directly available from the main view. Switching from one View tab to another changes the view type only for the currently activated image, keeping the image in the foreground.

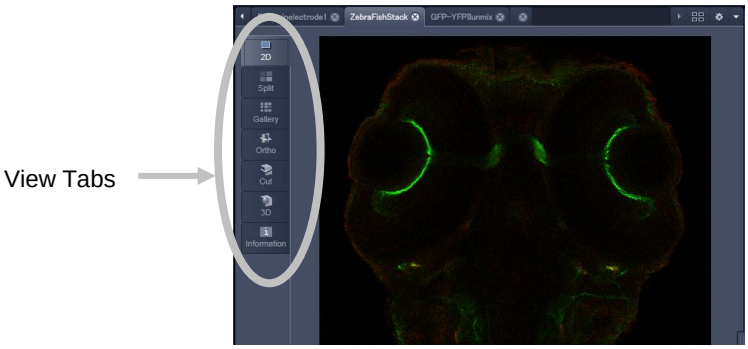



Fig.37 2D View

## 8-1. 2D View


- displays a single image in frame mode,
- displays a multiple channel image in superimposed mode.
- In the case of multi-dimensional image (Z stack, time series, Lambda, etc), users can select a single image from the stack by **Dimensions** or **Player** view controller.

## 8-2. Split View (for multi-channel images)


- displays the individual channels of a multi-channel image as well as the superimposed image.
- The Dimensions View Options control block shows the Merged tick box to activate / deactivate the display of the channels overlay

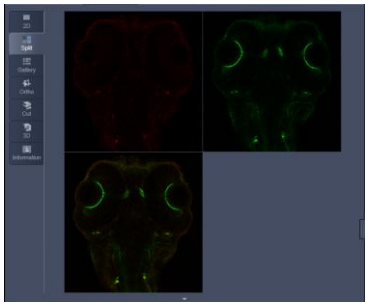


Fig.38 Split View

## 8-3. Gallery View (for Z-stack, time series, λ stack, etc.)


- displays images (Z-Stack, time series, combination of both) side by side in a tiled fashion,
- add data relevant to the displayed images (Z-Stack slice distance, time of acquisition or wavelength)

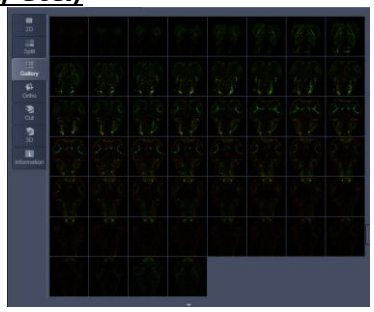
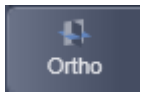


Fig.39 Gallery View

## 8-4. Ortho View (for Z-stack)




- displays a Z-Stack of images in an orthogonal view
- Users can measure distances in three dimensions

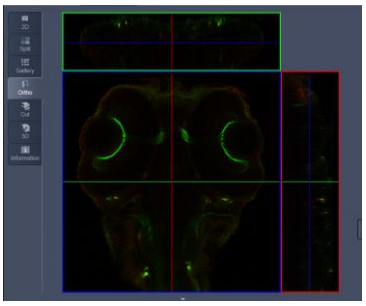


Fig.40 Ortho View

## 8-5. Cut View (for Z-stack)


- displays a user defined section plane (= cut plane) of a Z-Stack.
- By varying the parameters X, Y, Z, Pitch and Yaw, users can position a section plane of any orientation within the stack volume.

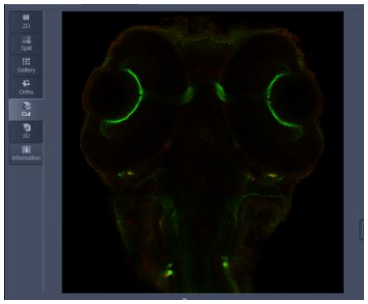


Fig.41 Cut View

## 8-6. 3D View (for Z-stack)


- 3D Data is reconstructed online, and users can grab and turn the data stack with a mouse.
- The **Create image** button opens a new image window and produces a 2D image of the currently used render mode.

※To save as a 3D movie, please refer **'9-5. Series'.**

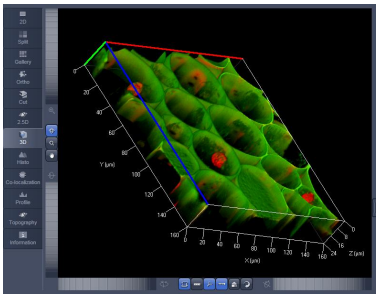


Fig.42 3D View

## 8-7. Histogram View


- displays a histogram (distribution of pixel intensities) of an image or Region of Interest.
- shows the histogram values in table form. Users can copy the table to clipboard or save as text file, measure area and mean gray value and standard distribution.

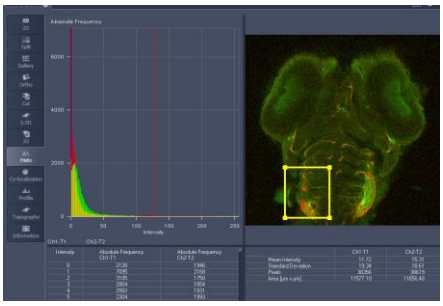
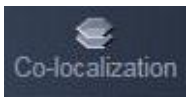


Fig.44 Histogram View

※Tables can be saved by right-mouse clicking on the table display!

## 8-8. Co-localization View




- permits interactive analysis of two channels of an image by computing a scatter diagram (co-localization).
- Quantitative Colocalization Parameters are shown in the Data Table.

※ Tables can be saved by right-mouse clicking on the table display!

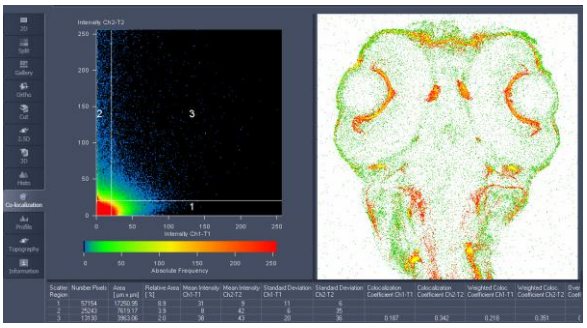
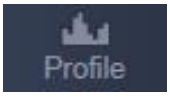


Fig.44 Colocalization View

## 8-9. Profile View




- displays the intensity distribution of an image along a straight or curved line.
- shows the intensity values in table form.
- shows separate profiles for each channel in a multi-channel image.

※ Tables can be saved by right-mouse clicking on the table display!

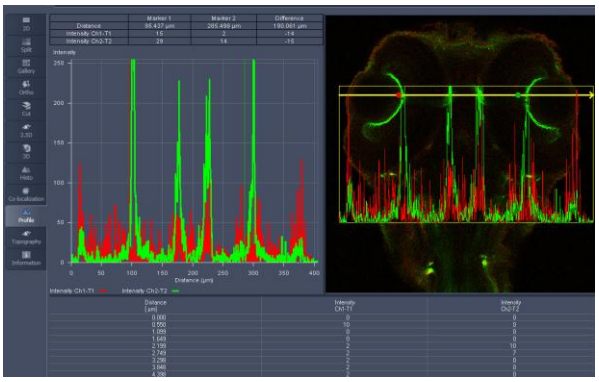


Fig.45 Profile View

## 8-10. Information View


- shows a summary information sheet of all relevant image acquisition parameters.

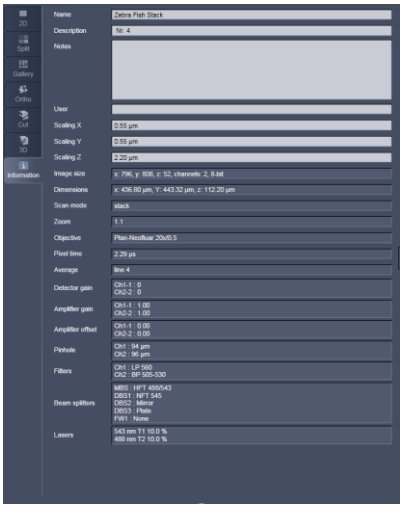
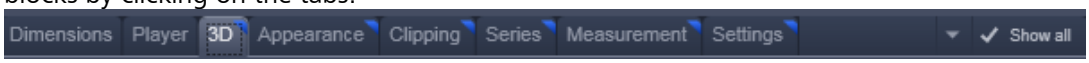


Fig.46 Information View

# 9. View Option Control tab (View Controller)

These tabs allow individual activation / deactivation of the available View Option control blocks by clicking on the tabs.




(The View tab Specific control tabs are marked with a blue triangle on their upper right corner.)

※ When activating the **Show all** mode of the View Options Area, all available view options control tabs are shown.

## 9-1. Dimensions

- Modifying the image display (zoom, color, channel on/off).
- The sections (slices) can be scrolled with sliders (Z-position and Time) and also directly addressed with setting numbers in the spin-boxes next to the sliders.

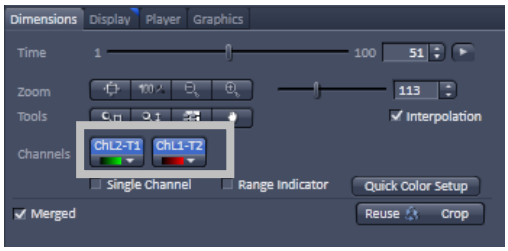



Fig. 47 Dimensions tab

- **Zoom** : allows you to enlarge / reduce the zoom factor of an image.
- **Channel(s)** : are designed to switch on/off channels or the display of the merged image as well as to assign color look-up tables (LUTs) to the individual channels
- **Crop** : allows to interactively define the size and orientation of a rectangular scan area on the image displayed in the Image Display window.
- **Reuse** : transfers ALL acquisition parameters from the stored image data to the Microscope Hardware Settings / Control tools and applies those parameters directly to the system

## 9-2. Display

- Brightness, Contrast and Gamma of the displayed image can be adjusted
- With the Channel buttons, the effect of the slider settings can be restricted to an individual channel.

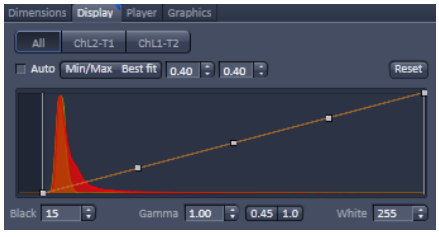



Fig. 48 Display tab

## 9-3. Player

- Operating animations of Z-Stack or time series
- Specifying animation parameters such as range and animation speed


Fig. 49 Player tab

## 9-4. Graphics

- add a scale bar to the image, as well as text annotations,
- use a set of interactive measurement functions for length, angle, area and size,

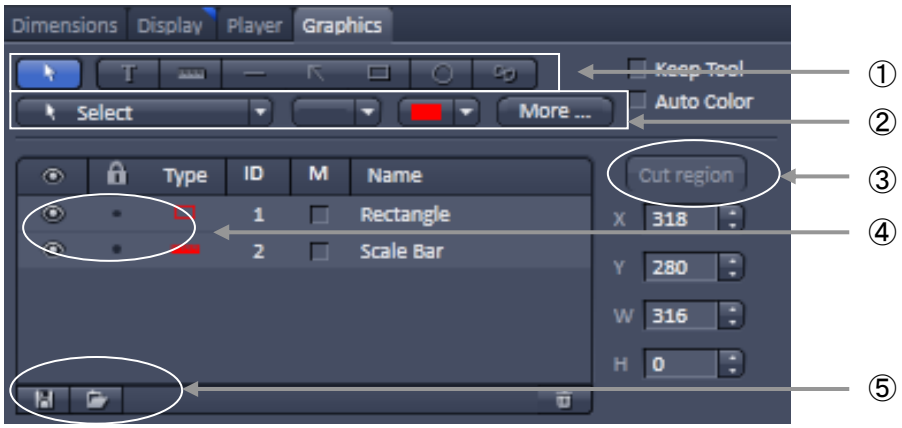



Fig.50 Graphics tab

① Selection from a set of drawing functions such as rectangles, arrows, scale bar, etc..

② Editing the selected overlay element

**More** : allows you to add a text box that displays the coordinate of a hidden dimension (e.g., the z-position in a Z-Stack or the timestamp in a time series).

③ **Cut region** : The region of a Z-Stack or 4D-image surrounded by an Overlay element is extracted and displayed separately in a new Image Display.

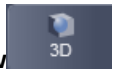
④  : hides the overlay element.


: measures the distance, area, angle of overlay element on the image.

⑤ To load / save overlays from / to a file use the Load / Save buttons in this View Options control block.

## 9-5. Series

This panel allows to set the axis for rotating the 3D reconstructed images.

① Select **3D View** 

Tab

② Select the render mode, and set the position of the image (zoom, angle) in the **Image Display** window

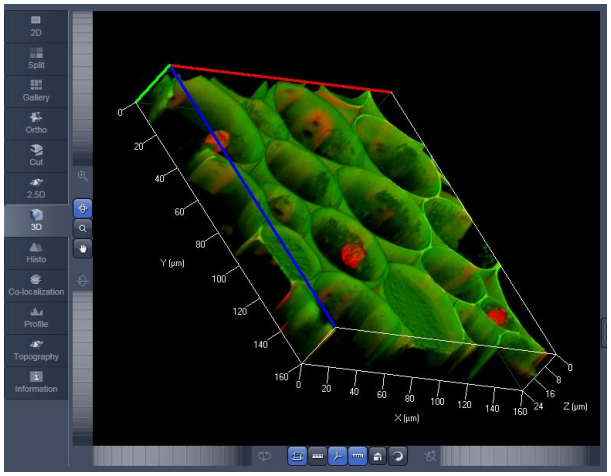



Fig. 51 3D view

③ Set the parameters for animation in the **Series** tab.

(1) Turning axis

Select from the pull down menu of **Render series.** ('Turn around X, Y' or 'Start & end').

(2) Number of views

Set the Total frames for animation.

(3) First angle

To create 360°rotate movie, click on **Difference angle** - **Panorama.**

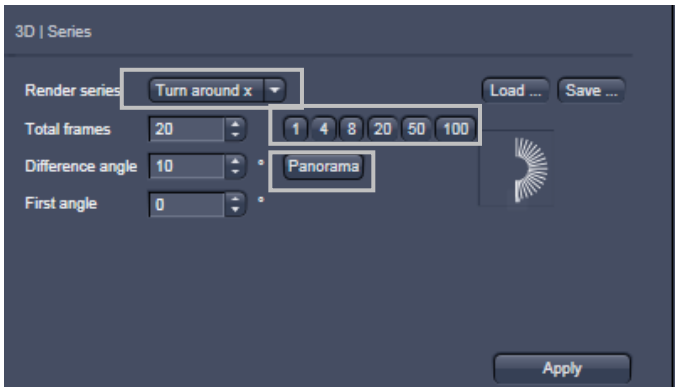


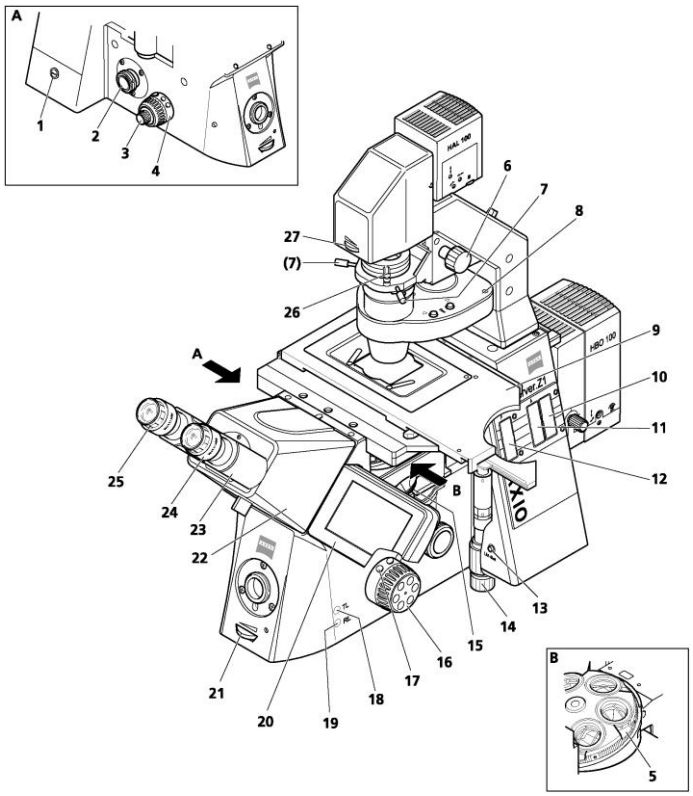

Fig.52 Render Series window

④ Click on **Apply** to create the animation in a separate Image Display window, which permits the animation to be saved afterwards.

⑤ Check the animation in **Player** tab (page 27).

# 10. Operation of Light Microscope (Axio Observer. Z1)

In this system, not only laser scanning microscopy, but also bright field, differential interference contrast, phase contrast and wide field fluorescent microscopy are available, depending on the system specification (e.g., objectives, filters, the type of the condenser).



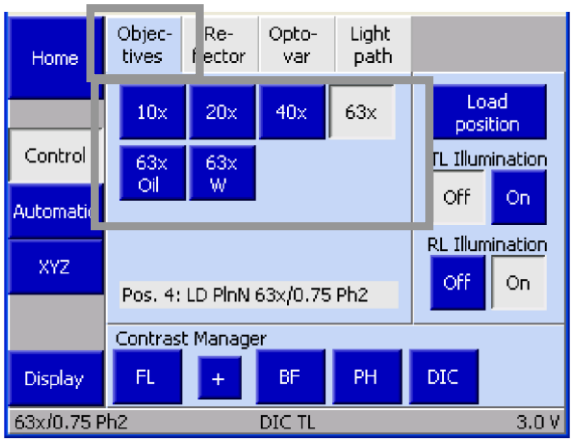

By tilting the transmitted light illuminator carrier, users can easily access the sample holder and set samples. Please hold a support, **do not** hold the halogen lamp house or the detector for transmitted light. During observation, the carrier must be moved back to the original position.

## TFT display touchscreen on the Axio Observer.Z1

On the motorized Axio Observer, the user can operate and configure the microscope and utilize optional functions using the TFT display. The TFT display is designed as a touch-sensitive screen.

- **Objectives**

  For objective positions which have already been configured, the magnification and, where applicable, the following additional information is displayed:




**Oil** Oil immersion objective

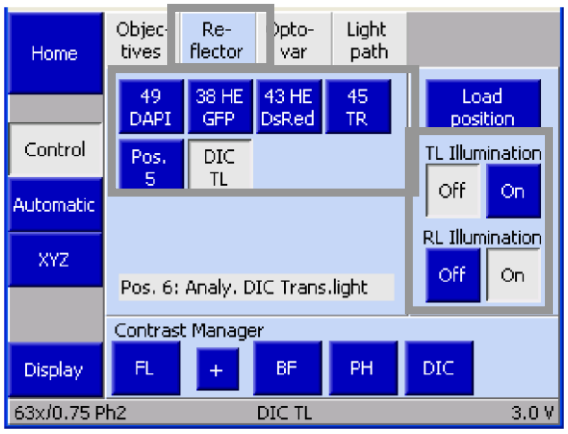
**W** Water immersion objective

**Imm** Immersions

• Touch the button for that **objective**, to move an objective into the optical path.

- **Reflector**

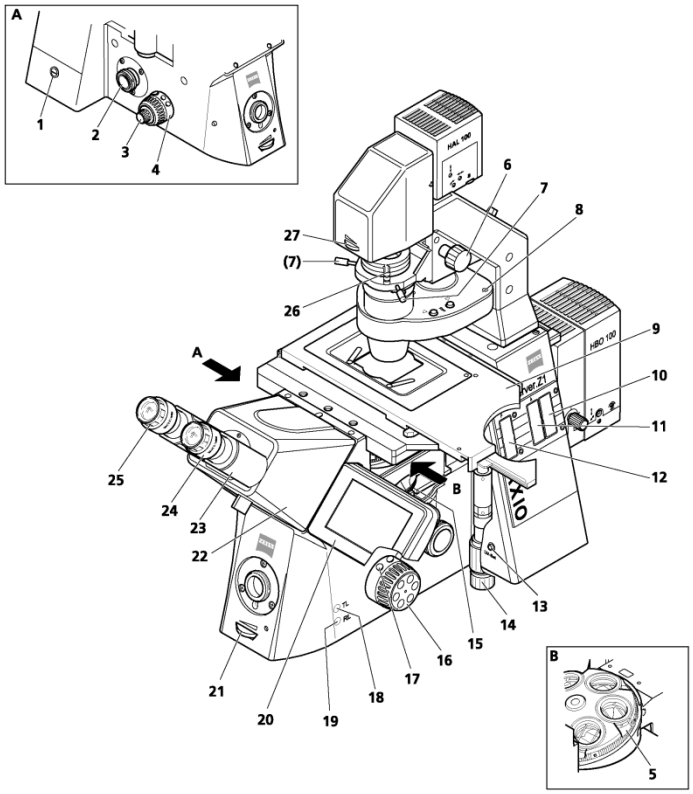
  Depending on the reflector turret installed, six controls for reflector positions 1 to 6 will be displayed. Reflector modules which have already been configured are identified by the description on the button.




• Touch the button for the **reflector** module required to move it into the optical path.

- **TL Illumination** : Control of **Halogen lamp On** / **Off.**
- **RL Illumination** : Control of **Hg lamp On** / **Off .**

## Controls and functional elements for Axio Observer.Z1




1 Standby button
2 Left Sideport
3 Focus drive coarse / fine (left side)
4 Control ring, left
5 Objective nosepiece
6 Vertical adjustment knob for condenser
7 Condenser centering screw
8 Condenser (manual or motorized)
9 Microscope stage
10 3-position filter slider slot (diameter 25 mm)
11 Slot for iris stop slider as reflected light aperture stop (motorized) or FL attenuator (motorized)
12 Slot for iris stop slider as reflected light luminous-field stop (motorized)
13 LM set button
14 Drive knobs for controlling XY positioning of the mechanical stage
15 Reflector turret (coded or motorized)
16 Coarse / fine focus drive (motorized) with fine drive, flat (right side)
17 Control ring, right
18 TL button for switching the transmitted light halogen illuminator on and off or for opening and closing the transmitted light shutter
19 RL button for switching the reflected light shutter (fluorescence) on and off
20 TFT display
21 Halogen illumination intensity control
22 Binocular tube
23 Binocular section of the binocular tube
24 Eyepiece
25 Eyepiece adjustment ring
26 Polarizer D with 2-position filter changer or 3-position filter changer
27 Luminous-field stop control

## Acquiring transmitted-light images

For overlaying fluorescence and transmitted-light images, click on the T-PMT button in the Imaging Setup tool.

All transmitted light applications like

- Phase contrast
- Differential interference contrast (DIC)
- Polarization contrast (Pol)
- Darkfield

can be performed if the hardware of the microscope is equipped with the necessary optics.

Set the high voltage of the T-PMT with the **Gain** slider in the **Channels** tool

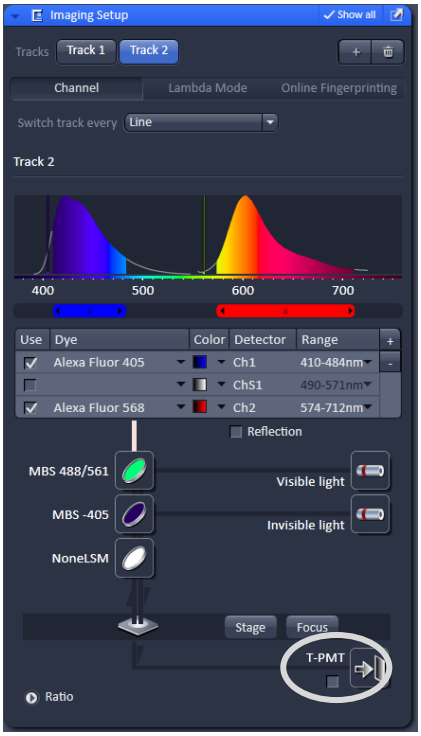
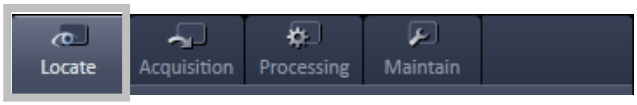



Fig.53 Imaging Setup tool

## 10-1. Bright field observation

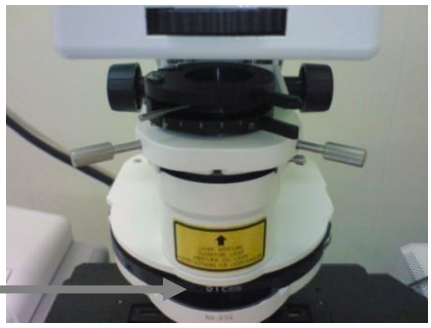
① Switch to the Locate mode on ZEN.




② Turn on the transmitted light in the Ocular tool or the TFT touchscreen.

③ Turn the condenser turret adjustment ring to move the condenser turret to the **H** position for bright field.

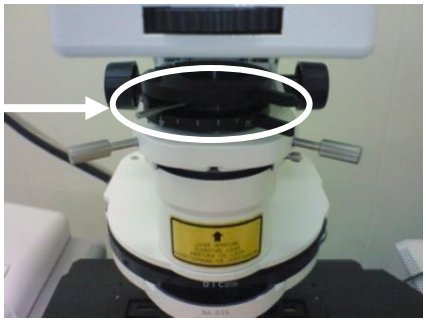
④ Adjust light intensity and set up KOHLER illumination.



## 10-2. Differential interference contrast (DIC) for transmitted light

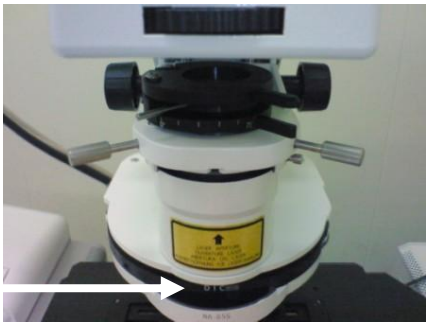
① Move the polarizer on the transmitted light illuminator carrier into position, and load the analyzer in the reflector turret to position from ZEN or TFT touchscreen.

Polarizer



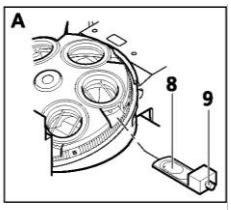
② Turn the condenser turret adjustment ring to move the condenser turret to the **DIC II or III** position for bright field according to the lens to be used.

Condenser turret



**DIC II** : Dry lenses up to 40x
**DIC III** : Immersion lenses over 40x

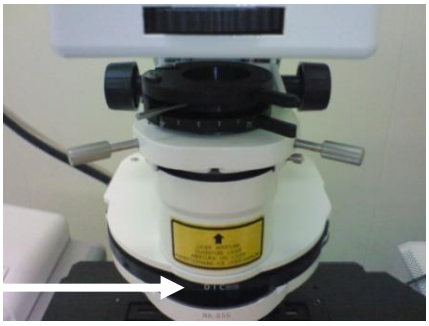
③ Adjust image contrast with the screw head of the DIC slider.



## 10-3. Phase contrast

Turn the condenser turret adjustment ring to move the condenser turret to the **Ph 1 ~ 3** position for bright field according to the lens to be used.

Condenser turret



## 10-4. Epifluorescence contrast

① Block the reflected light path with the fluorescence shutter by pressing the RL button.
② Select the FL reflector module with the required fluorescence filter combination in ZEN or TFT touchscreen.
③ Open the fluorescence shutter by pressing the RL button on TFT.

※ Close the fluorescence shutter immediately after observation to avoid photo bleaching.

# 11. Switching off the system

**11-1.** Turn off the Lasers

- **Argon (458/488/514nm), DPSS (561nm), HeNe (543nm, 594nm, 633nm), MaiTai (Spectra physics) [Optional]**:

  Open the **Setup Manager - Laser** tool. **Turn off** the lasers from pull down menu.

  For Ar laser, **wait until the fan of the Argon laser has automatically switched off (about 5min)**. Don't switch off the main power (11-6②) during cooling the laser.

- **Chameleon (Coherent) [Optional]**:

  Open the **Setup Manager - Laser** tool. **Turn off** the laser from pull down menu.

  After that, turn the **key switch** from On to **Standby.**

**11-2.** Switch off the **X-Cite 120 lamp** or the **HBO 100** mercury burner.

**11-3.** **Clean the Objective lens if you used immersion medium (Oil, ImmersolW)**, and set the position of the objective lens to the lowest magnification for the next user.

**11-4.** Click on the **File** - **Exit** button to leave the ZEN software.

**11-5.** Shut down the computer.

**11-6.** Turn off the Power remote switch.

① Turn off the **Components** switch and the **System/PC** switch.

② Turn off the **Main Switch.**

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Carl Zeiss Microscopy Co., Ltd.