Quick Guide (EN_CZJ)

ZEN 3 (blue edition)

LSM900/ 980/ 980NLO

with Axio Observer

2019.11

Introduction

This Quick Guide describes the basic operation of the LSM 900, 980, 980NLO Laser Scanning microscopes with the ZEN blue 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). 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.

・For systems with a Z Piezo stage or incubation device, do not raise the focus above the height of the bottom of the sample holder. Since the sample holder is fixed, forcibly raising the focus may cause damage or breakdown of the holder or lens as well as the sample.

3. Observe the following instructions:

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

Work flow to acquire image

Starting the system, and turning on the lasers（P. 2~5）

↓

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 LSM980 system

① Switch on the **Main switch** on the laser bench.

② Switch on **PC** and log on as 'LSM User'.
*if you only use a PC, switch on only ① and ②.

③ Switch on the **Laser Key Switch** on the laser bench.

④ Switch on the **Components Switch.**

⑤ If Chameleon laser (Coherent) is required, turn the key in the front of the power supply from **Standby to ON.**
*please always keep the power switch of Chameleon laser and Chiller for cooling **ON**.

*when you switch on the reflected light illumination (HXP or HBO) manually, please switch on after 1-1②.

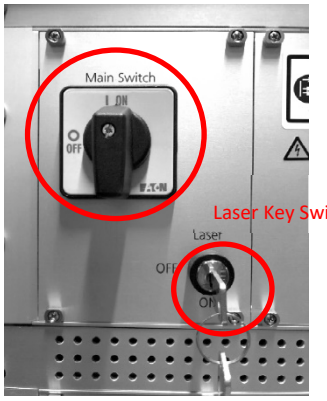


Fig 1. Power supplies (LSM980)

1-2. Starting LSM900 system

① Switch on the **Main switch** of the power tap.

② Switch on the **SYSTEM**, **COMPONENTS** of PSU.

③ Turn the laser key switch from (O) to (I) direction. After waiting 30 s to 1 min until 'Laser On" indicator lamp turns OFF, you can start up software.

④ Switch on **PC** and log on as "**LSM User**".
*if you only use a PC, switch on only ① and ④.

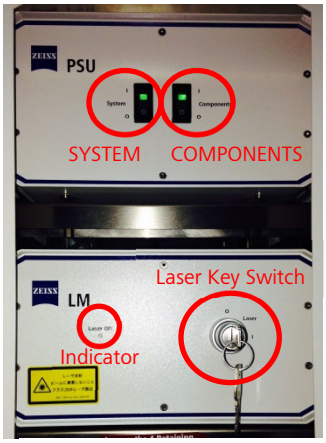


Fig 2. Power supply (LSM900)

*when you switch on the reflected light illumination (HXP or HBO) manually, please switch on after 1-1②.

1-3. Starting ZEN software

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

※If you only need to use the image processing and analysis function, select **ZEN Image Processing**.

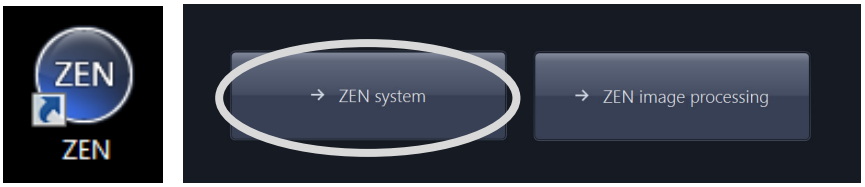


Fig.3-1 ZEN icon and startup window of ZEN

For systems with a motorized stage*, perform stage calibration. (*option)

Click **Calibrate Now** to perform calibration automatically.

※ The stage will move during calibration, so please be careful not to pinch your fingers.

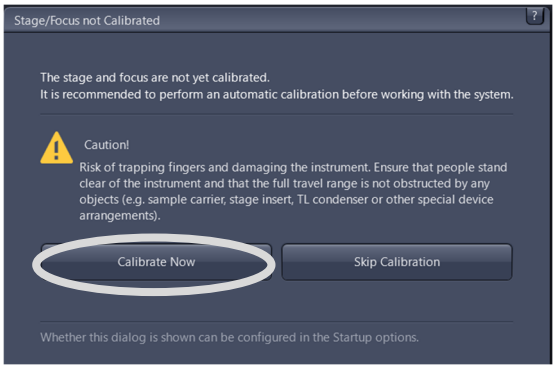


Fig.3-2 Stage/Focus calibration window

《 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.4-①,②,③)

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**

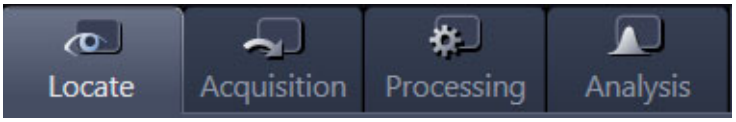


Fig.4 Main tab

Center Screen Area(Fig.4 - 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 (Fig.4 -⑦, ⑧)

File management and data handling tools.

⑦ Open Images list (Thumbnail view)

⑧ Device tool list: Stage (in the case of Motorized stage) & Focus control

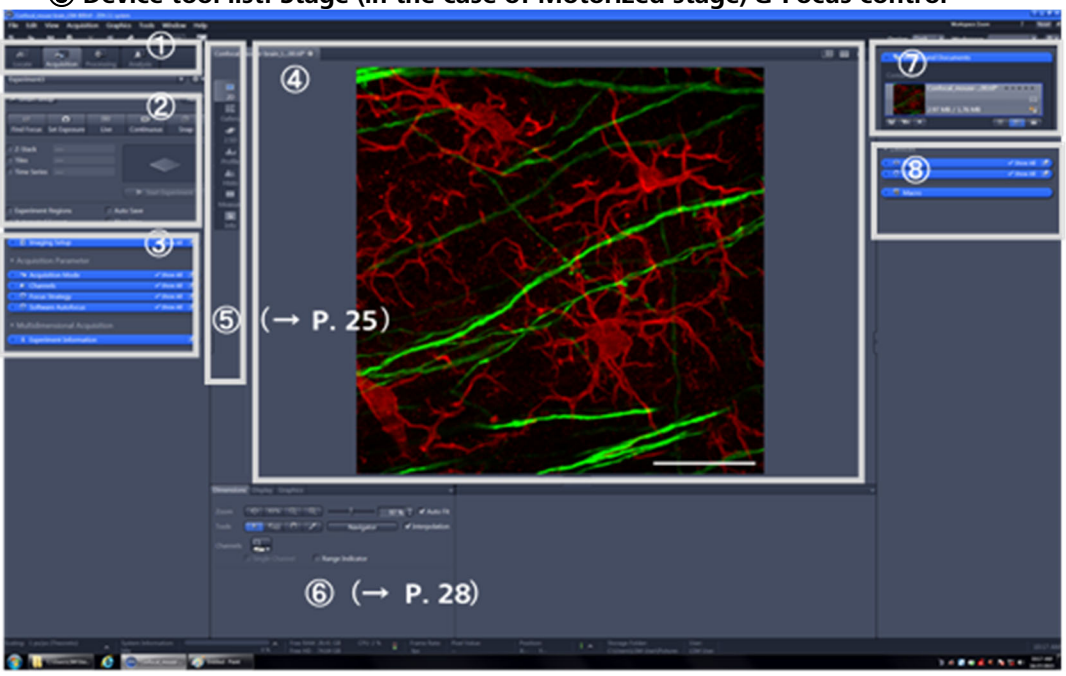


Fig.5 ZEN main application window after starting up window

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.

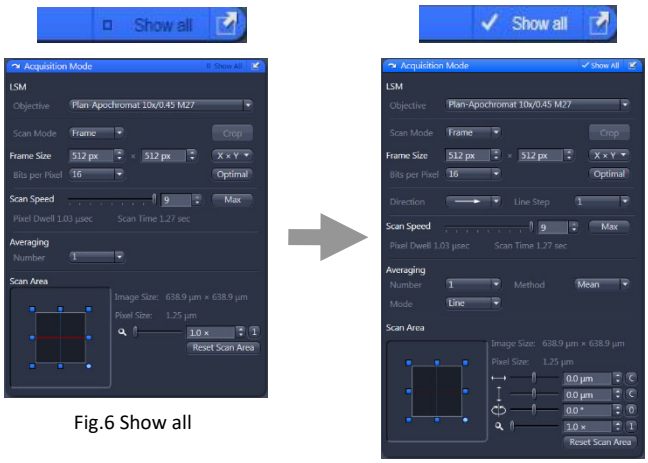


Fig.6 Show all

More features of ZEN 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.

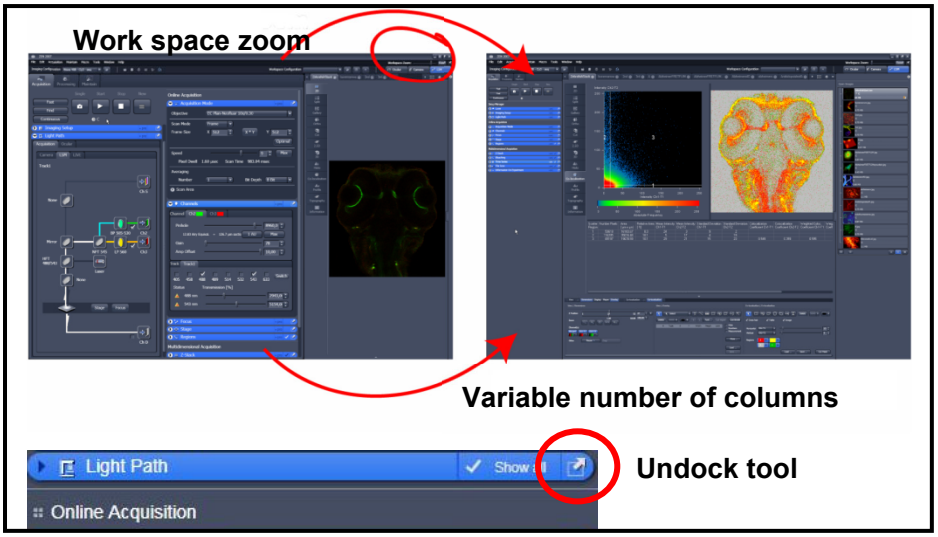


Fig.7 ZEN window layout

2. Turning on the lasers (in the case of LSM980)

ZEN3 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:

Open the Laser window. All available lasers can be operated within this window (Fig. 7). Select the required Laser and turn on it from the pulldown menu.

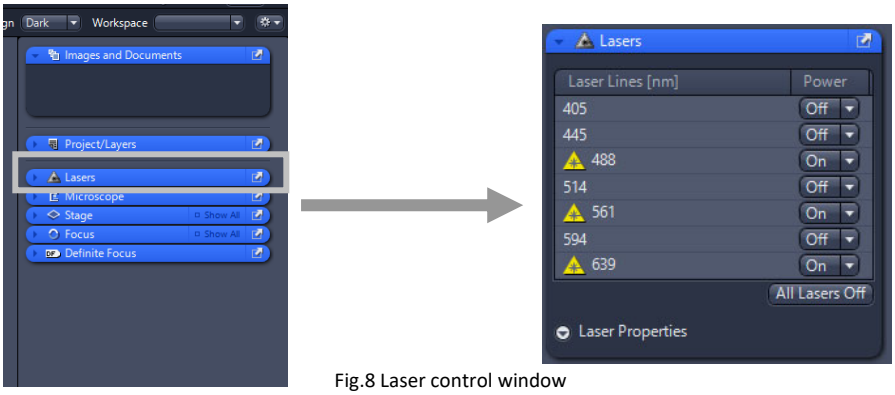


Fig.8 Laser control window

- **Diode laser (405nm, 445nm, 488nm, 514nm, 561nm, 594nm, 639nm)**

  Switch the required lases **ON** by using the pulldown menu.

  ※ A kind of displayed lasers varies depending on the system configuration.

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

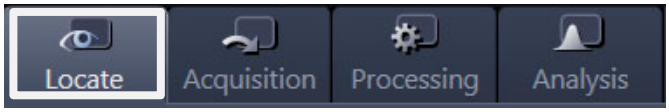
  Switch the requested lasers **ON** by using the pulldown menu.

※ Users can check the laser power and status by clicking on **Laser properties** in Laser window.

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



Locate: Ocular observation

Acquisition: LSM

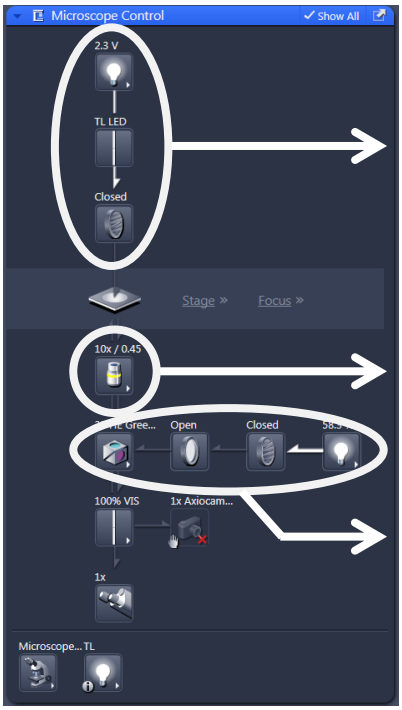
Fig.9 Locate Tab

▪ 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 Microscope control 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!



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 HXP lamp.
- Click on the **Reflector** button and select the desired filter set by clicking on it.

Fig.10 Microscope control window (Axio Observer 7）

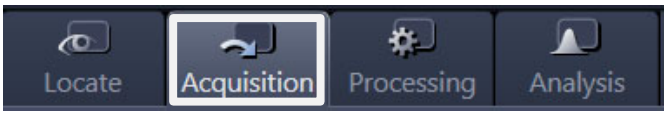
Storing the microscope settings

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

Fig.11 Microscope configuration panel

4. Configuring the Beam path and lasers

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



There are 2 ways to configure the beam path and lasers automatically.

4-1. Experiment Manager
4-2. Smart Setup

We highly recommend using **'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.

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.

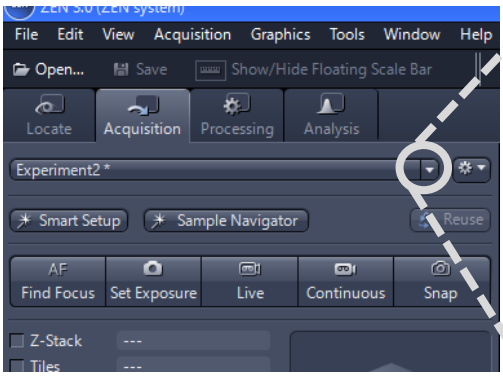


Fig.12 Experiment Manager

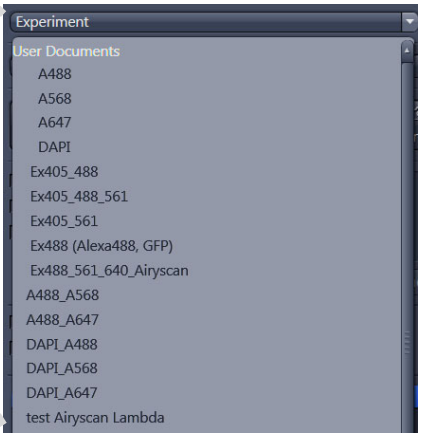
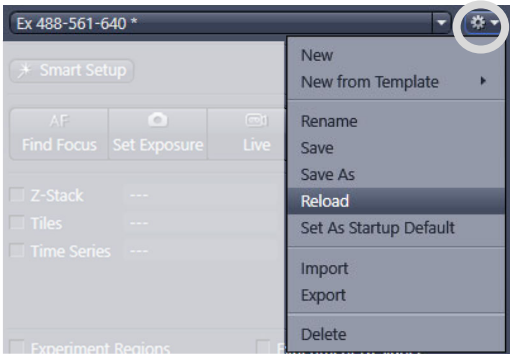


Fig.13 Experiment Manager



※  **: setting**

You can Save (Overwrite), Rename, Reload and Delete the configuration from here.

When the configuration is changed, the '*' mark is displayed.

To restore the default setting, select '**Reload**' from the **setting menu**.

4-2. Smart Setup

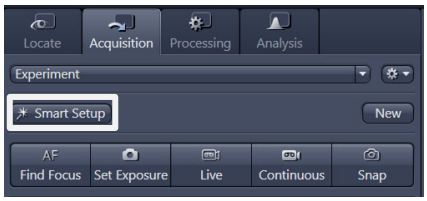


Fig.14 Acquisition

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

② Select **LSM**.

③ Click on the + in **Configure your experiment**.

④ 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. Add dye by double-clicking.

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

Fig.15 Smart setup

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.

※ **Airyscan (Optional)**
- Light path of using Airyscan. In case of multi-channels, Sequential setting is loaded.

4-3. Setting up a configuration manually

① Open the **Imaging Setup** tool to set-up the beam path. This tool displays the selected track configuration.

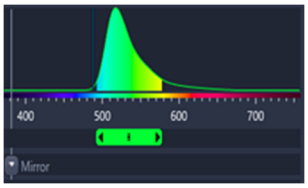
② Users can change the settings of this panel using the following function elements.

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

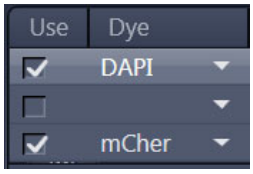
Fig.16 Imaging Setup (in the case of LSM980)

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

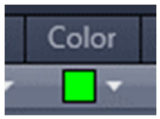
Selection of the main dichroic beam splitter (MBS) from the relevant list box.



Selection of an emission range for scanning.



Activation/ deactivation (via check box) of the selected channels (Ch1,2 QUASER detectors Ch S1-8) for scanning.cv



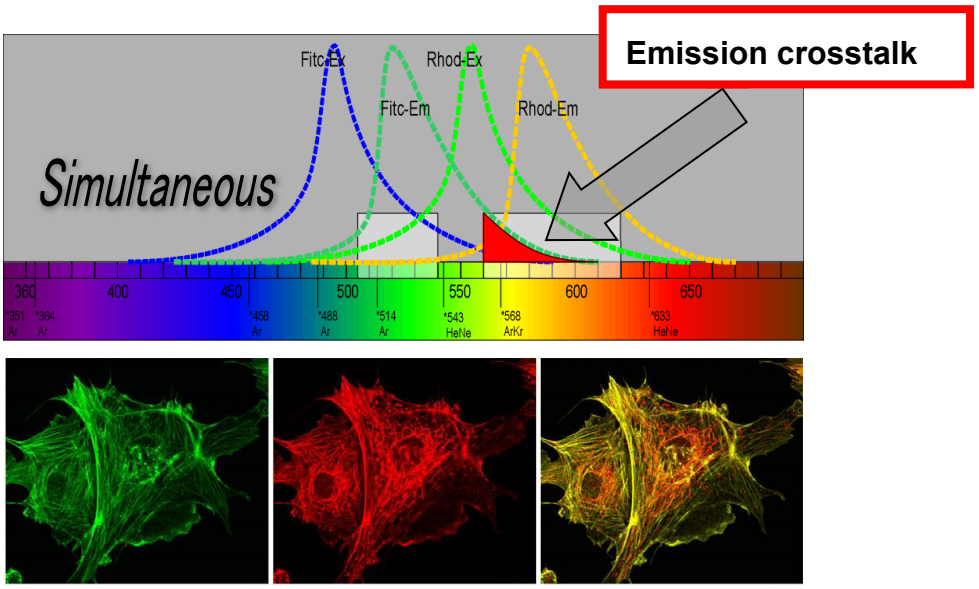
Assigning a color of the channel

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

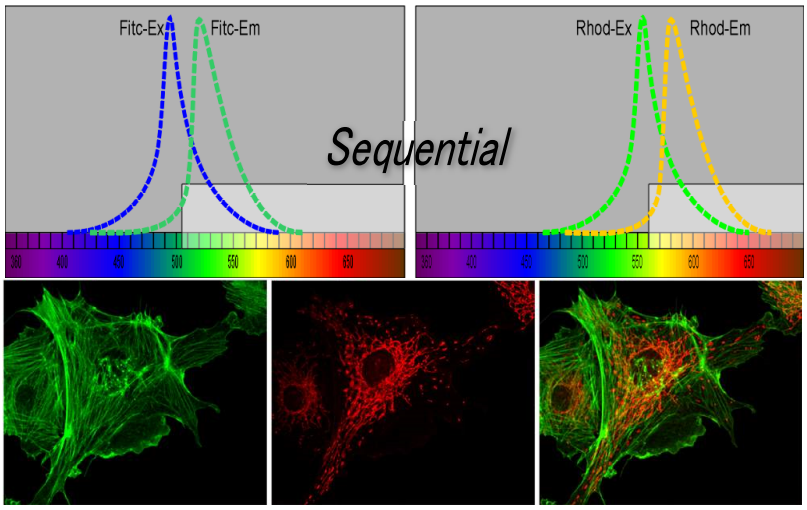
※ A kind of displayed information varies depending on the system configuration.

《 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
↓
Set 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.

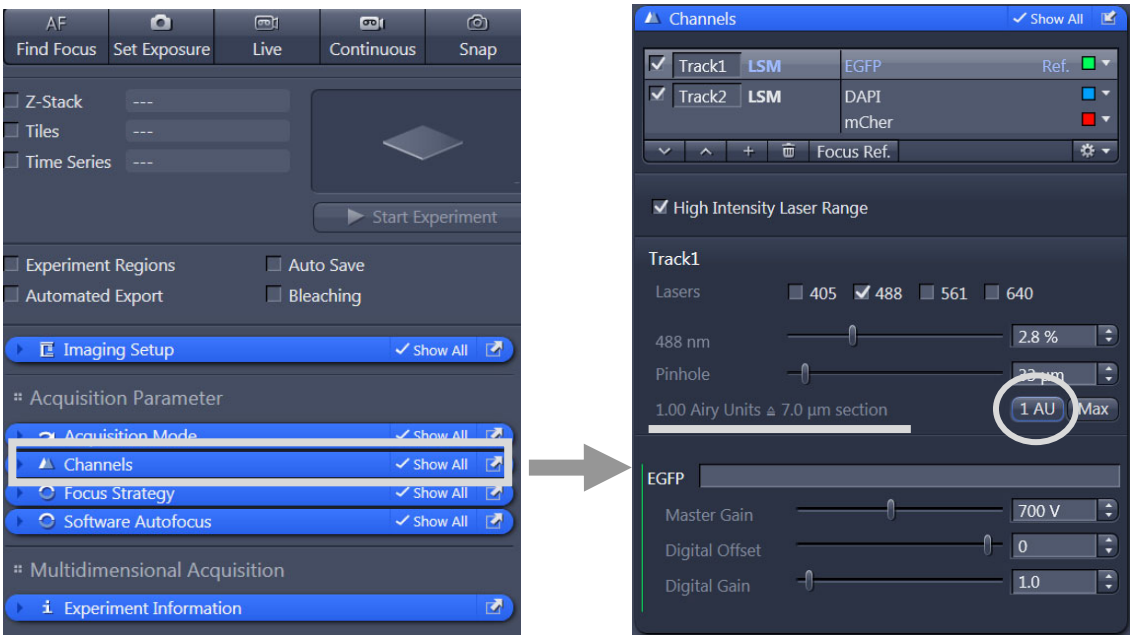


Fig.17 Channels tool

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 these recommended parameters.

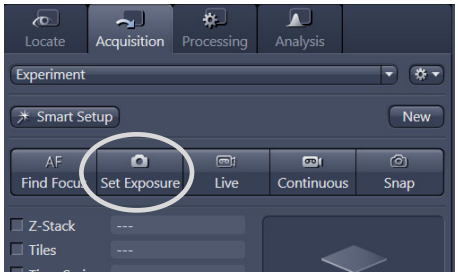


Fig.18 Set Exposure

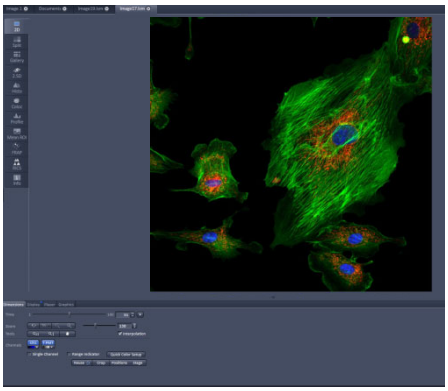
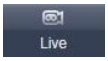


Fig.19 Image display

Image acquisition - Live

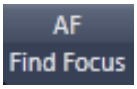
④ Click on the Live 

or Continuous 

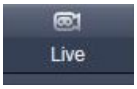
buttons to start the scanning procedure to continuously acquire an image. "Live" is only scanning the channel of highlighted in "Channels" window when multi channels imaging.

Click on the Stop button to stop the current scan if necessary.

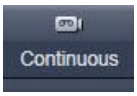
To Display image, use these buttons.



Display the image after finding brightest focus

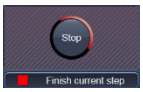


Select Live button for continuous fast scanning – useful for finding and changing the focus. (for changing focus position, adjusts via focus knob or push **Ctrl** button + Scrolling mouse)

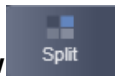


Select Continuous button for continuous scanning with the conditional scan speed.

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

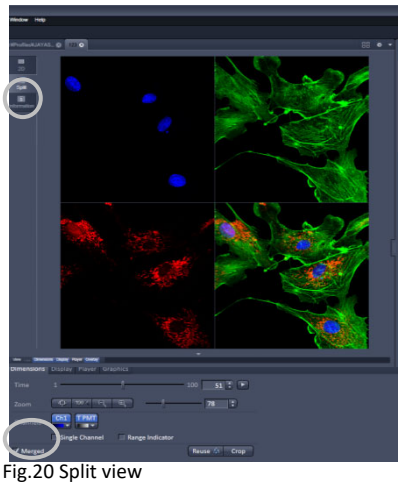


Fig.20 Split view

Adjust Brightness and Contrast

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

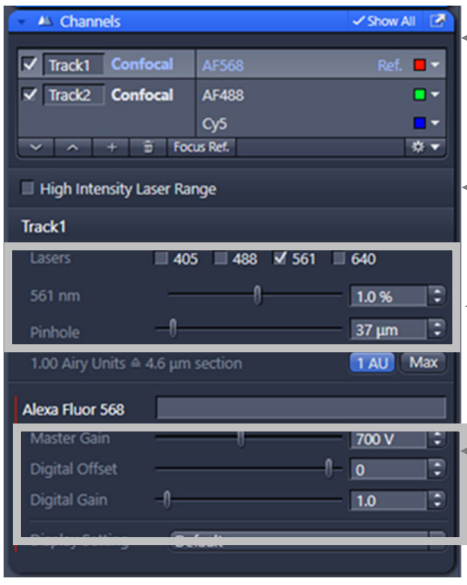


Fig.21 Channels tool

Select the channel in the **LIVE view**

In the case of LSM900, Decide the laser power range (all laser)
ON : **0.2 ~ 100**%
OFF: **0.01 ~ 5.0**% (depend on the laser wavelength)

Lasers : Control of the laser lines and their attenuation power

Master Gain : 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).

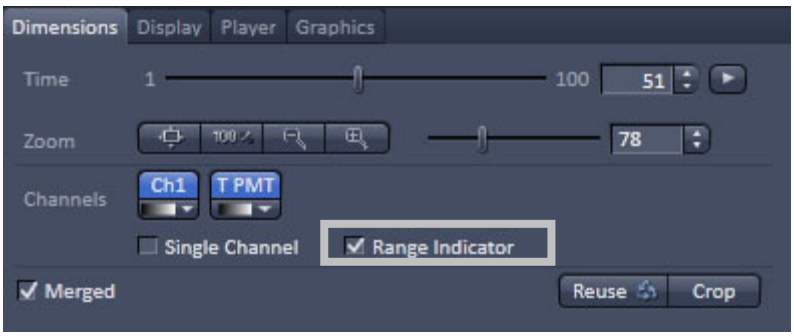


Fig.22 Dimensions Control window

※  add your own pseudo color if Click ▽

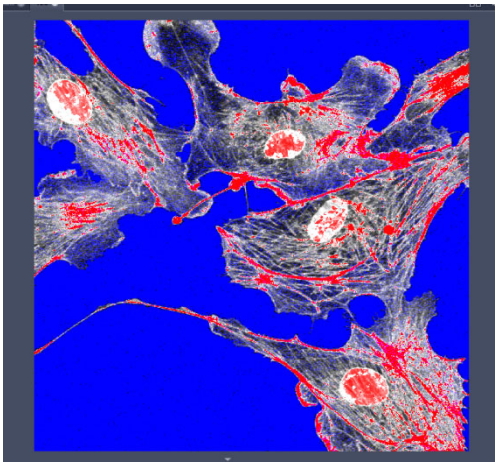


Fig.23 Range Indicator

The scanned image appears in a pseudo-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.

Adjusting laser intensity

- Set the **Pinhole** to **1 Airy Unit** (Fig. 17)
- Set the **Gain (Master)** high (approx. 800 ~ 1000).
- When the image is saturated, reduce AOTF transmission in the **Laser** control section of the **Channels** tool (Fig. 21).

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

⑥ Select the **Acquisition Mode** tool from the Left Tool Area.

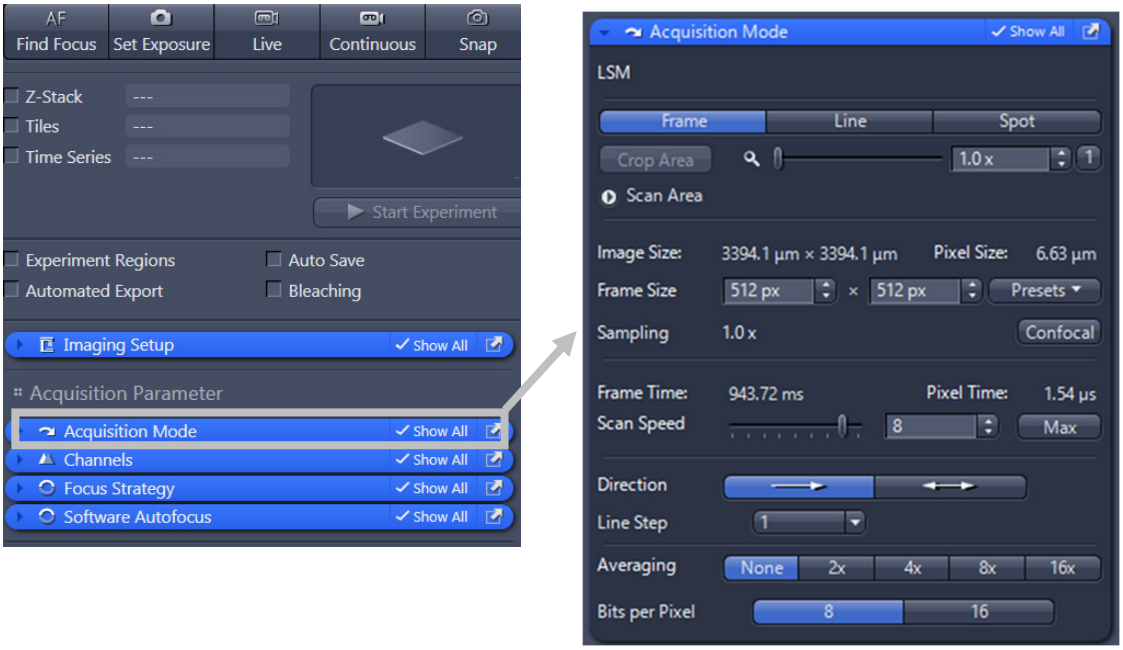
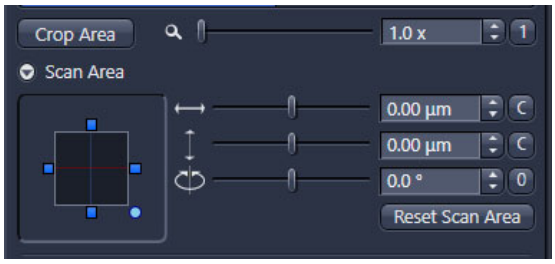


Fig.24 Acquisition Mode tool

Scan Area

Crop Area button makes square shape on the image, this square indicates the scan area. By dragging the corner of square the Zoom factor can be changed and dragging the cross-line defines rotation angle.

Scan Area function define the same type of function to Crop Area by clicking



X position Y position
Scan rotation Zoom factor
Right side of slider C, 0, 1 buttons reset the parameter, Reset Scan Area all reset to default.

Fig.25 Scan Area

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 **Confocal** 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!

Scan 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.

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 menu. Averaging number up to 4 usually produces a good result.

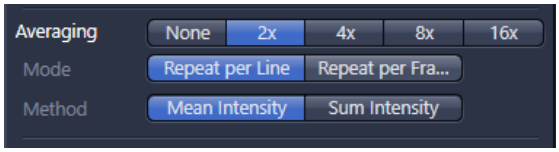


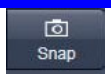
Fig.26 Averaging

Bit Depth

Select the dynamic range 8 or 16 Bit (per pixel) in the Bit per depth in the Acquisition Mode tool

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

Snap

⑧ Finally, click on Snap 

for recording an image.

※ 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. Airyscan imaging (Optional)

6-1. Light path of Airyscan

If there are already recorded the Airyscan setting, load the configuration from Experiment manager (Fig. 10).
If not, there are two method to make new setting.

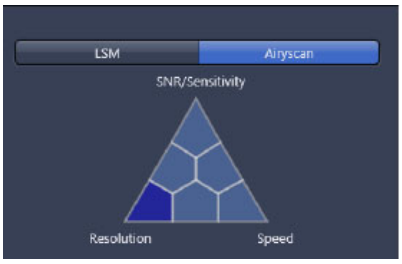


Fig. 27 Smart setup (Airyscan)

Smart Setup

Select Airyscan from **Proposal** of the **Smart Setup** window (Fig. 13②).

Imaging Setup tool

Open the **Imaging Setup** Window and select Laser, emission filter from Airyscan tab.(Fig. 25)。

Fig. 28 Imaging Setup (Airyscan)

6-2. Acquisition mode of Airyscan

There are some modes in Airyscan2.

[LSM980]

-**SR Airyscan**	: Superresolution mode. You can get Maximum resolution image.
-**Multiplex 4Y, 8Y**※	: Image acquisition can be parallelized from 4x up to 8x fold while still providing superresolution imaging with superior SNR at speed. (Multiplex 8Y※ Option)
-**CO 8Y**※	: Image acquisition can be parallelized 2x while keeping improvement in SNR with a confocal resolution. (optional)

[LSM900]

-**SR Airyscan**	: Superresolution mode. You can get Maximum resolution image.
-**Multiplex 2Y, 4Y**※	: Image acquisition can be parallelized from 2x up to 4x fold while still providing superresolution imaging with superior SNR at speed. (Multiplex 4Y※ Option)
-**CO 2Y**	: Image acquisition can be parallelized 2x while keeping improvement in SNR with a confocal resolution.

6-3. Acquisition of Airyscan imaging

Set the brightness/ contrast on Channels tool and scanning parameter on Acquisition mode tool, and **Snap**. (same as usual confocal imaging.)

ATTENSION: In order to take best performance of Airyscan, please check the following.

① Objective lens

please remove DIC slider from the objective revolver. (please check the recommended objective lens while an instruction)

② Scan Zoom in "Scan Area" on "Acquisition mode" window

LSM980: Set more than 1.7x zoom factor

LSM900: Set more than 1.3x zoom factor

③ Image Intensity（Laser power, Gain）

Please ignore saturation of pixels for post Airyscan processing, using **Range indicator** (Fig.23) .

During the scan, the Airyscan detector will be adjusted automatically. When the adjustment is completed, the Airyscan detector adjustment mark at the bottom of the window will turn green.

Fig. 29-1 Airyscan detector adjustment

To perform detector alignment properly you need a sample focussed.

If the detector adjustment is not OK (red mark) check the focus and intensity again with Live or Continuous scan.

④ Frame Size

Select SR Optimal resolution from **Acquisition Mode**. And if use the Multiplex mode, select the mode button respectively.

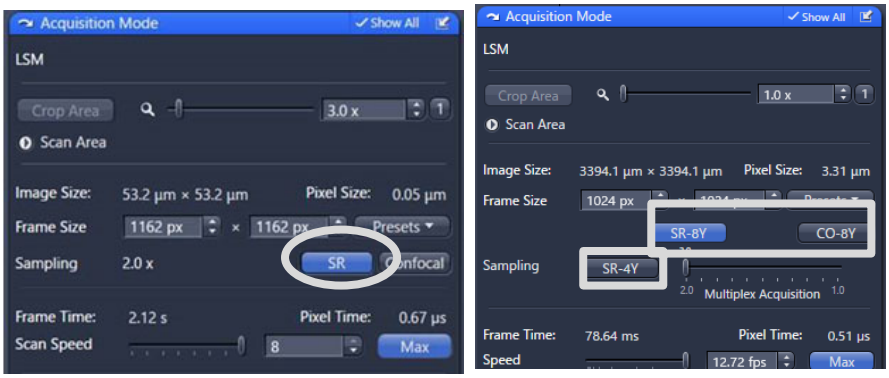


Fig. 29 Frame size and Zoom

Fig. 30 message of attention of Airyscan

※if setting is not optimally, display the attention message（Fig. 30）.

5-4. Processing of Airyscan image (SR image)

View controller

Check the Super resolution (SR) image in the **Airyscan view controller tab.**

Change the processing parameter on the Airyscan view controller (Fig. 26).

※optimize the display curve using **Min/Max** or **Best Fit,** because processing image has different dynamic range from the original data.

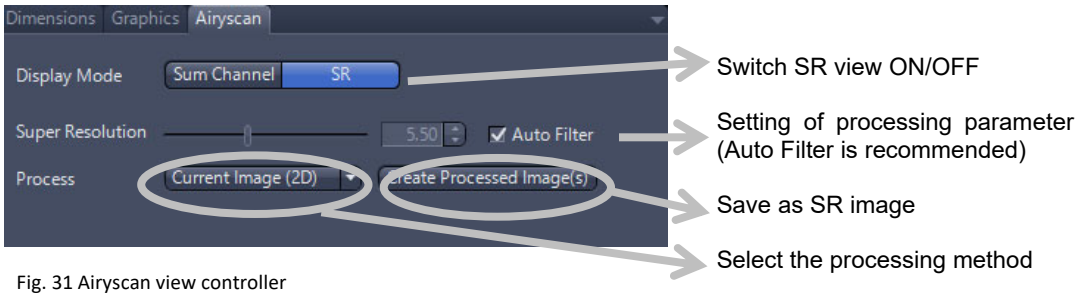


Fig. 31 Airyscan view controller

Processing tab

in 3D or timeseries data, Process from the Processing tab.

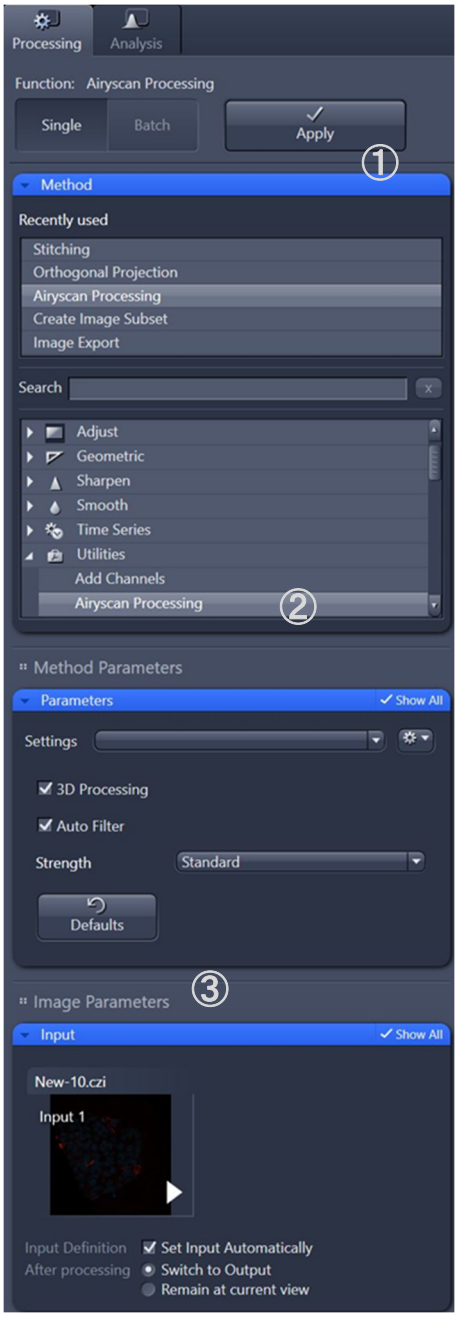


Fig. 32 Processing tab of Airyscan processing

① Select the **Airyscan processing** from Method on Processing tab.

② Select the Image in Input window

③ Set the processing parameter from parameters.
3D image: 3D processing
2D image: 2D SR processing is recommended

Activation/ deactivation (via check box) of the Auto filter and select of the strength from relevant list (Low, Standard and High).

⑤ click **Apply,** new window (SR image) open.
*Strength value of Auto filter is written in Airyscan mode on "Info" tab of the new window, if you need.

*to manually adjust the parameter, deactivate the Auto filter checkbox. 'Adjust per Channel' and 'strength' can be activated (Fig. 32-2)

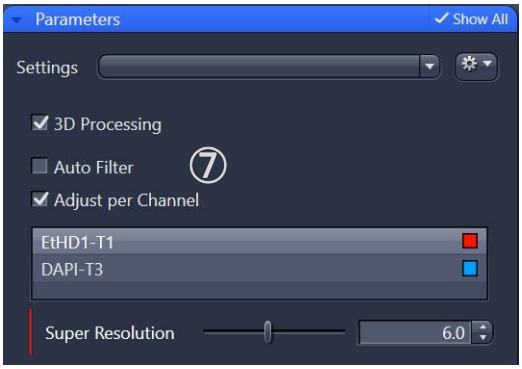


Fig. 32-2 Airyscan processing (manual strength)

7. Storing and exporting image data

7-1. Save image

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

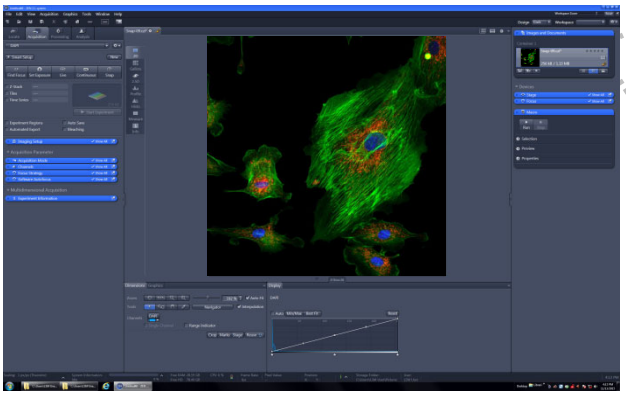


Fig.33 ZEN main window

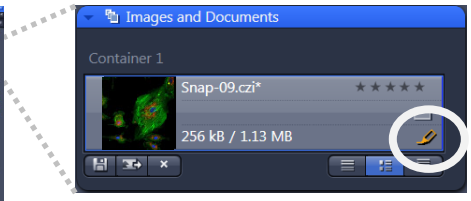


Fig. 34 Auto Save (unsaved)

There are 2 ways to save images.

1) **Save as .czi format** (→ page 23)

Merit) ・It stores an image together with the acquisition parameters.
・ Reloading configuration from the stored image data is available.
(Reuse function, page 25)

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.zeiss.co.jp/microscopy

2) **Save as multipurpose format (Export), like TIF, JPEG…etc.** (→ page 24)

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 format

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

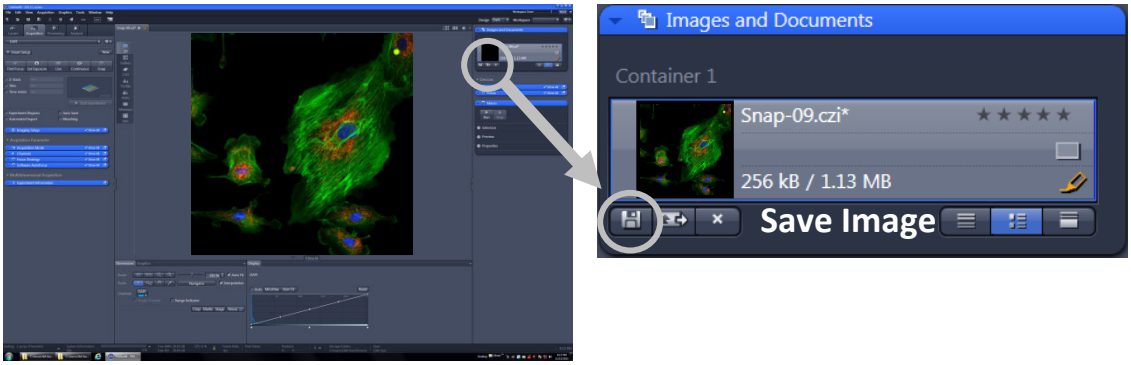


Fig.35 Save image

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. 35).

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

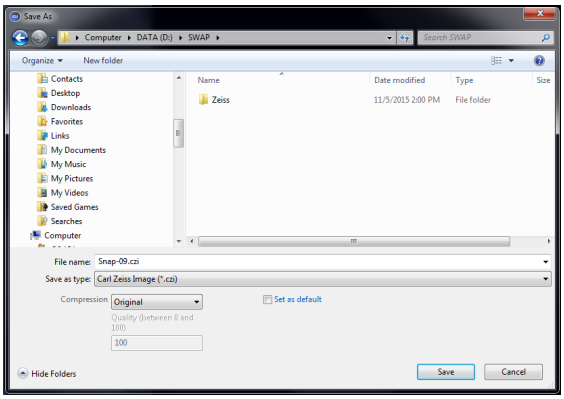


Fig.36 Save as window

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

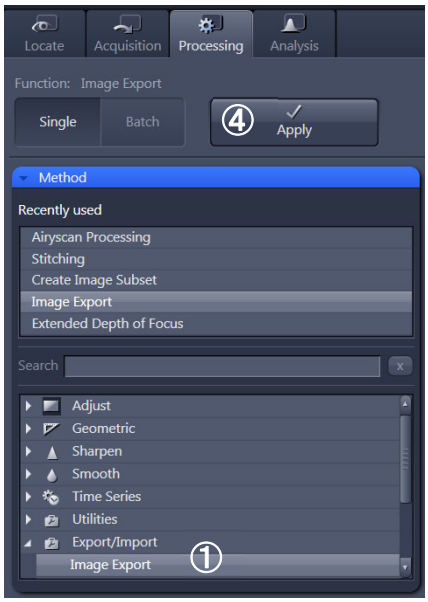


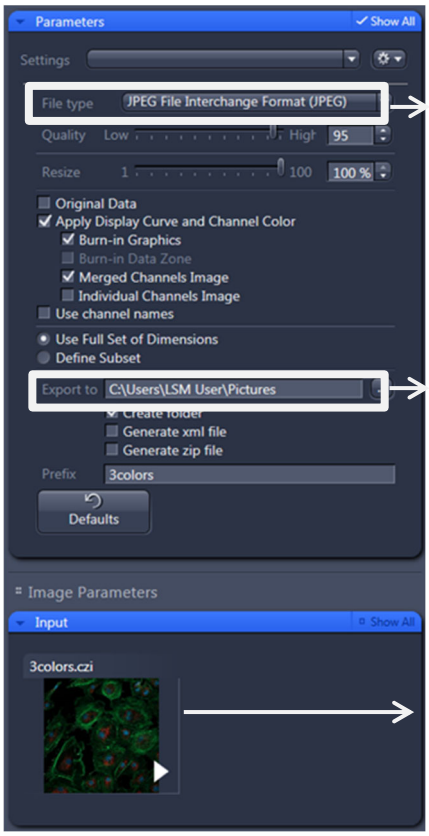
Fig.37 Processing – Image Export

① Open Processing tab and select **Export/Import** – **Image Export** from Method (Fig. 37).

② Select file from **Input** window (Fig. 38).

③ Set the export format from **Parameters** window (Fig. 38).

④ Click **Apply,** export the file to the select file folder (Fig. 37).



Select the export file format

Original Data : Original intensity and grey image

Apply Display Curve and Channel Color :
Displayed color and intensity
Burn in Graphics :
Add the graphics (scale bar etc.)
Merged… : merged image in multi-channel image
Individual… individually channel image saving

Select the file path.

Select the file for export

Fig.38 Export parameter

7-2. 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. 39)。

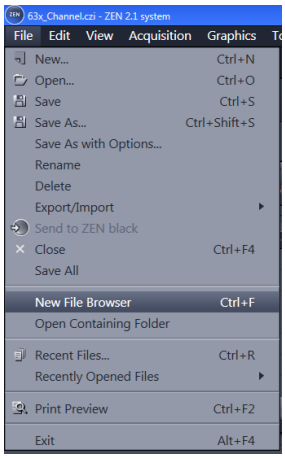


Fig.39 File Menu

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 (Fig. 40).

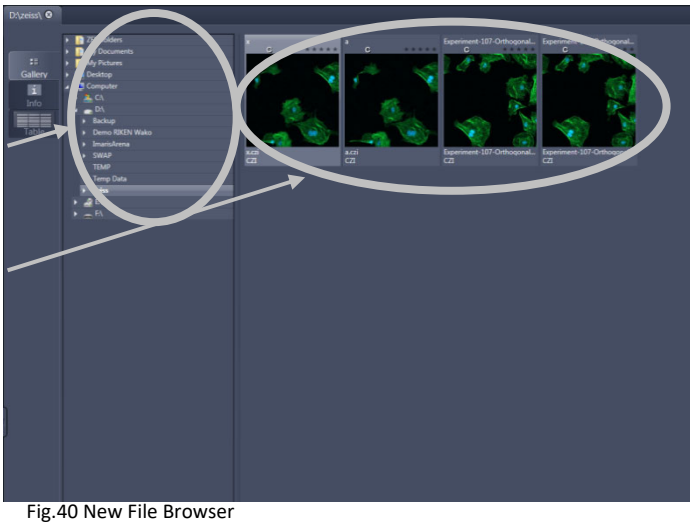


Fig.40 New File Browser

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.

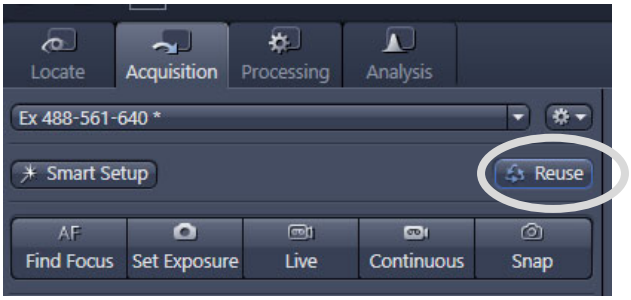


Fig.41 Reuse function

8. 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.

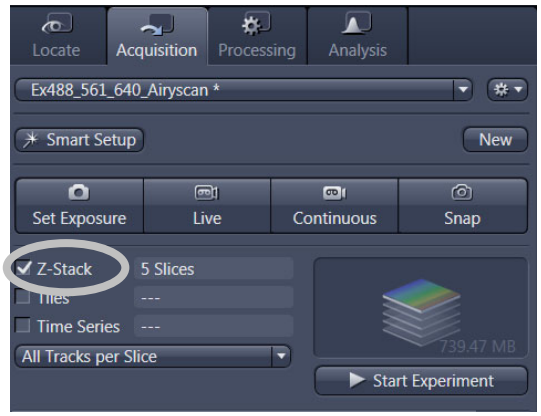
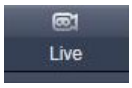


Fig.42 select Application

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

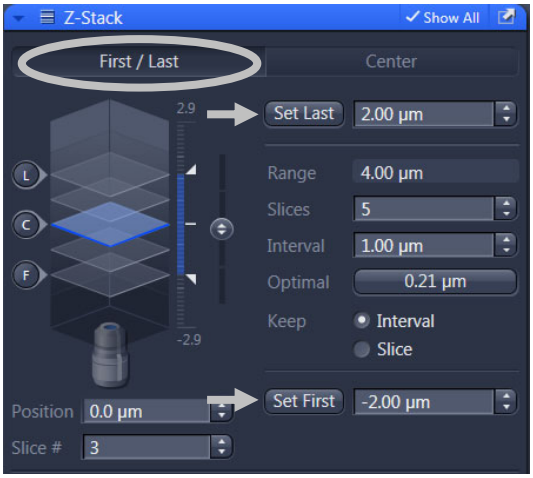


Fig. 43 First / Last

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.

3) 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.

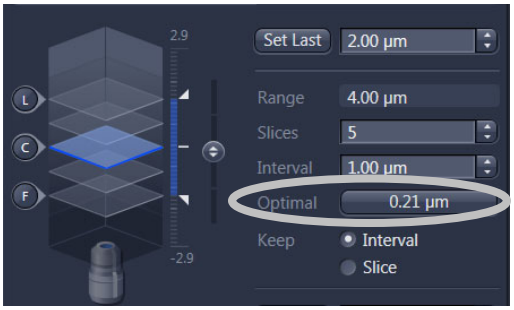


Fig.40 Optimal button

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

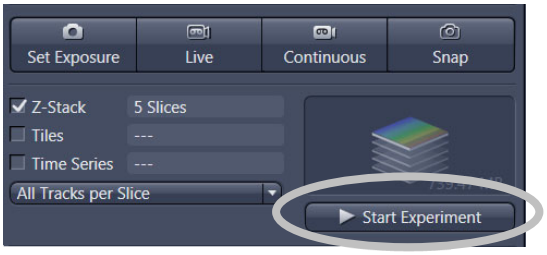


Fig.45 Application start

Visualization of Z-stack data

View tabs

Please refer next **Chapter 8. View Tabs,** for Gallery, Ortho (orthogonal section), Cut and 3D view.

Maximum intensity projection (MIP)

Go to **Processing** tab

① Select **Geometric** → **Orthogonal Projection** from method.

② Select Z-stack image on Input window.

③ Set the Projection Plane (usually **Frontal**), Method (**Maximum**), Start position and Thickness, Parameter

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

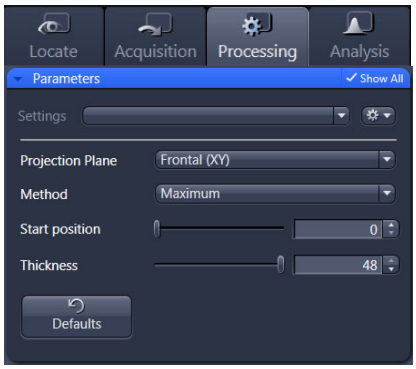
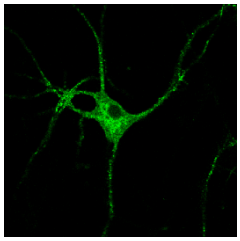
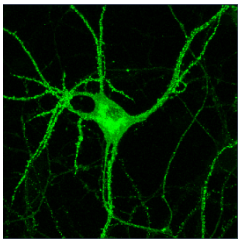


Fig.46 Orthogonal Projection



Single plane



MIP

9. 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.

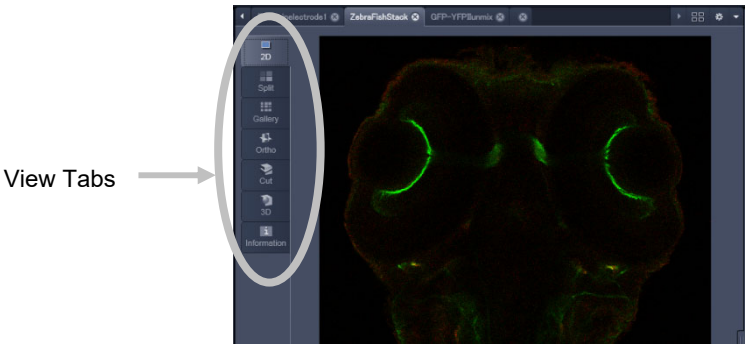
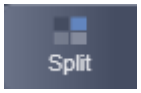


Fig.47 2D View

9-1. 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**.**

9-2. 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

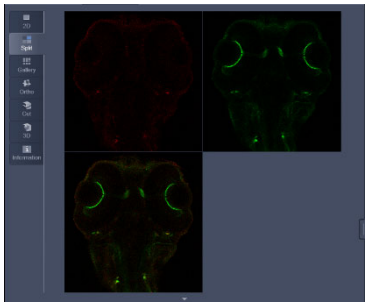


Fig.48 Split View

9-3. 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)

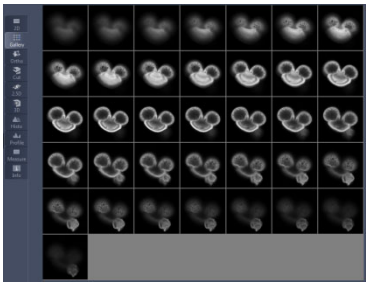
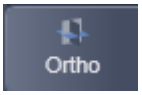


Fig.49 Gallery View

9-4. Ortho View (for Z-stack)



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

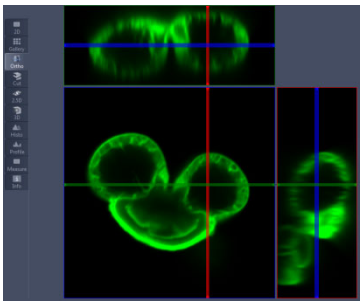


Fig.50 Ortho view

9-5. 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.

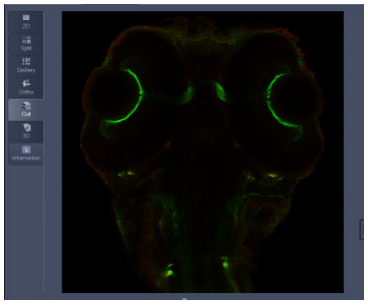


Fig.51 Cut View

9-6. 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'.**

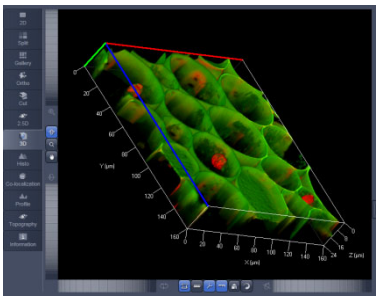
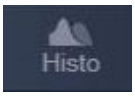


Fig.52 3D View

9-7. 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.

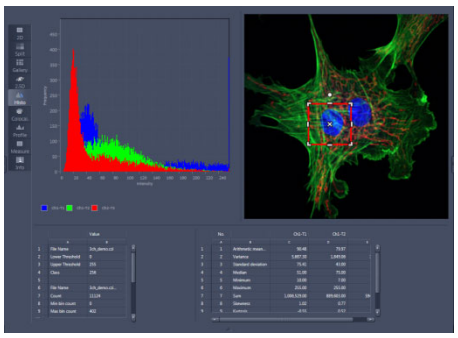
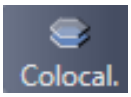


Fig.53 Histogram view

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

9-8. 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!

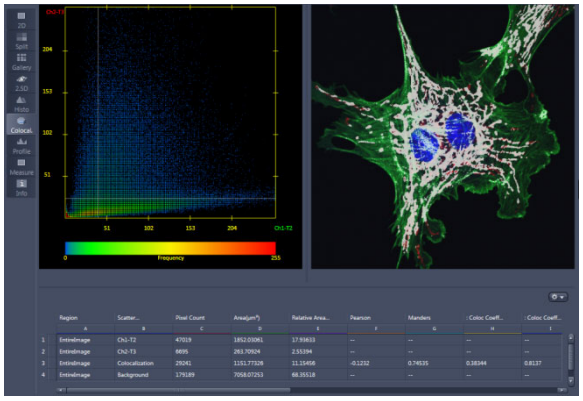
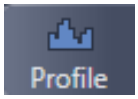


Fig.54 Co-localization view

9-9. 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!

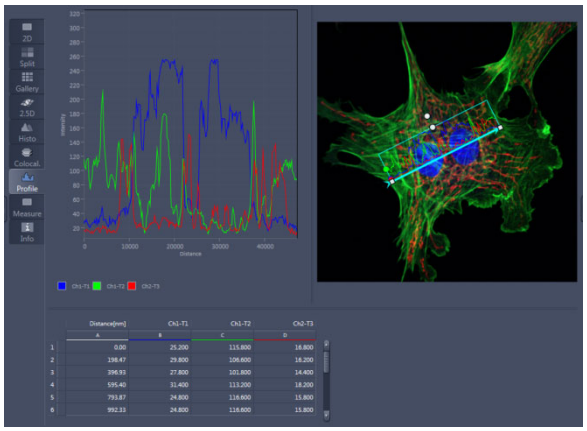


Fig.55 Profile view

9-10. Information View

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

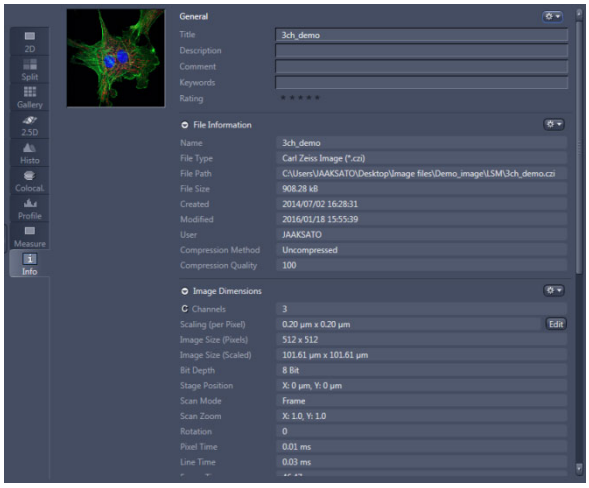
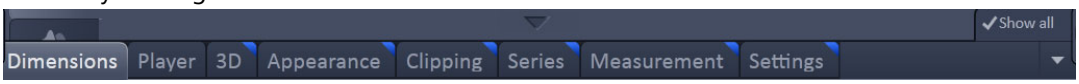


Fig.56 Information view

10. 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.

10-1. 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.

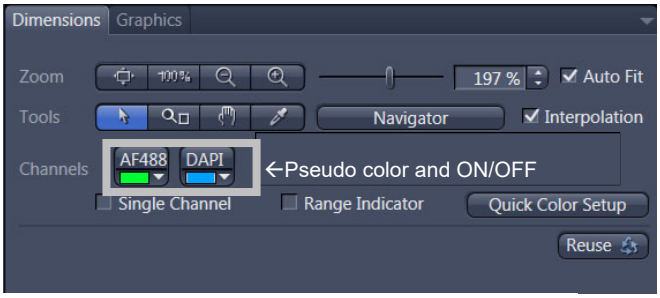


Fig.57 Dimensions

・**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

10-2. 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.58 Display

10-3. Player

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

Fig.59 Player

10-4. 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,

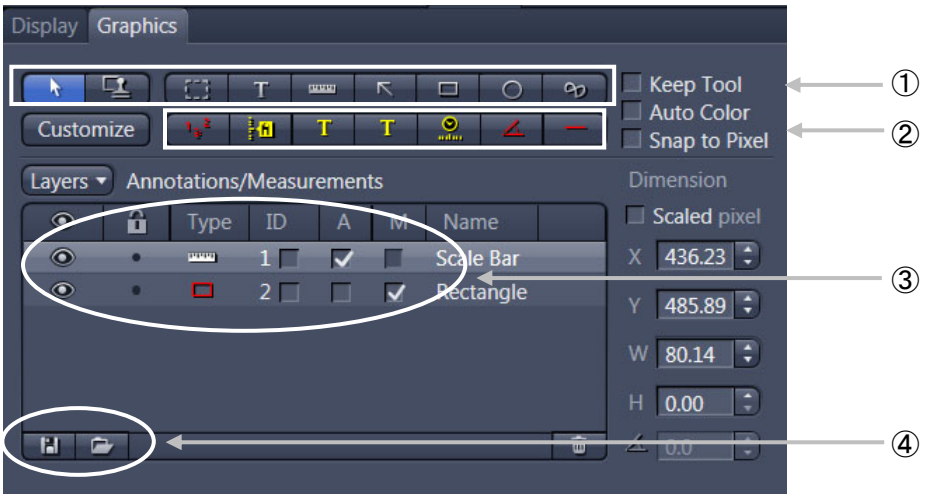


Fig.60 Graphics

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

② Editing the selected overlay element

③ 

: hides the overlay element.

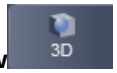
: measures the distance, area, angle of overlay element on the image.

④ To load / save overlays from / to a file use the Load / Save buttons in this View Options control block.

Change the properties of the object color, line width, font etc., from right-click the object itself.

10-5. 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

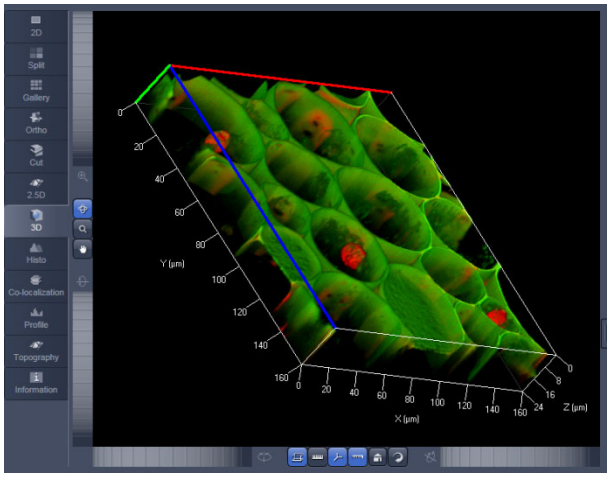


Fig.61 3D view

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**.

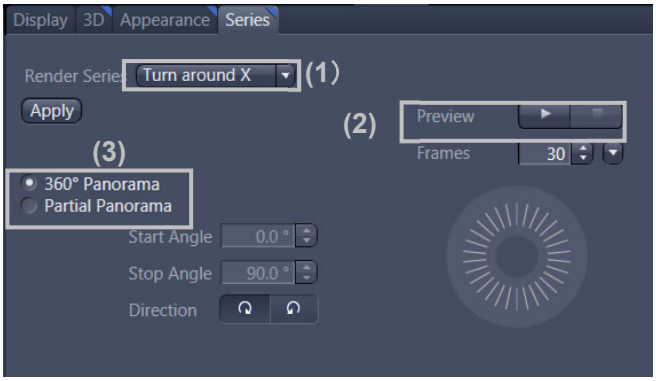


Fig.62 setting for rotation movie

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 31, 9-3).

11. Operation of Light Microscope (Axio Observer. 7)

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).

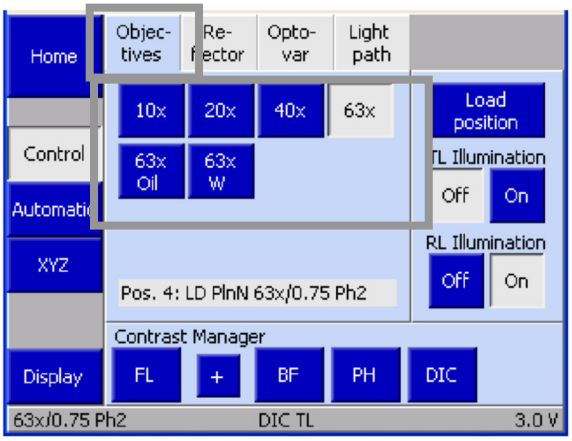
By tilting the transmitted light illuminator carrier, users can easily access the sample holder and set samples. Please hold a support, **do not** hold the halogen lamp house or the detector for transmitted light. During observation, the carrier must be moved back to the original position.

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

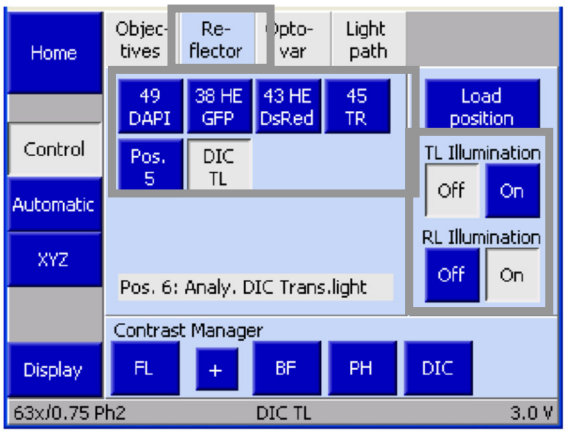
W Water immersion objective

Imm Immersions

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

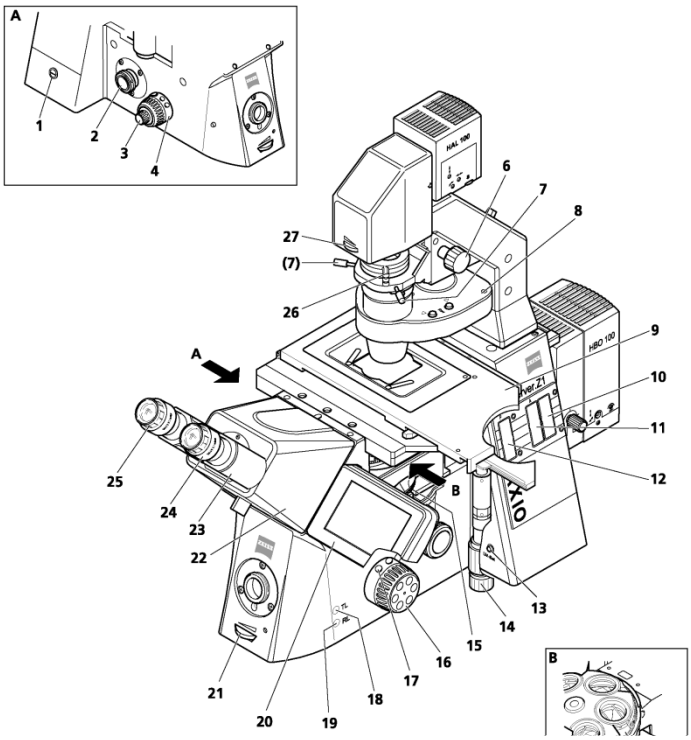
- **Reflector**

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.

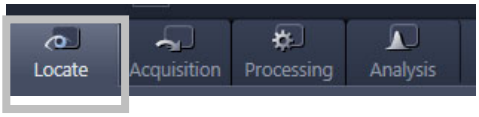
- **TL Illumination** : Control of **Halogen lamp On** / **Off.**
- **RL Illumination** : Control of **Hg lamp On** / **Off .**



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

11-1. Bright field observation

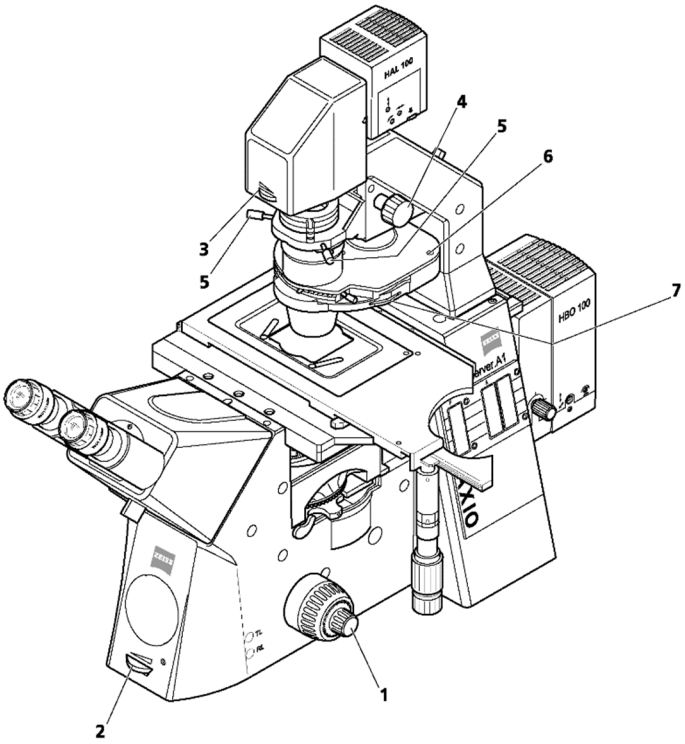
① Switch to the Locate mode on ZEN.



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.

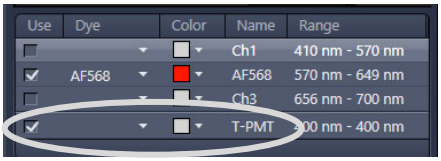
④ Adjust light intensity and set up KOHLER illumination.



1 Focus knob
2 Transmitted light brightness
3 Field stop
4 Condenser handle
5 Condenser centering knob
6 Condenser
7 Aperture

Acquisition of Transmitted image

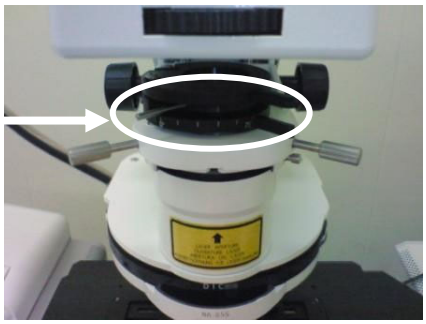
1) Load the FL configuration from **Smart Setup** or **Experiment Manager**
2) Open the **Imaging Setup** tool, and activate the **T-PMT.**
3) Adjust the Gain of the corresponding channel.



11-2. Differential interference contrast (DIC) for transmitted light

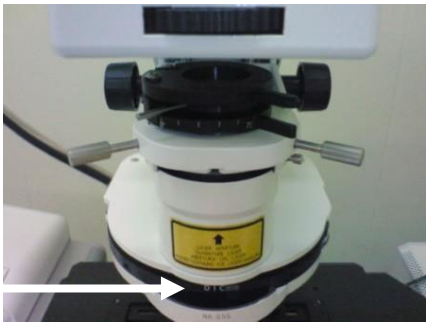
① Move the polarizer on the transmitted light illuminator carrier into position and load the analyzer in the reflector turret to position from ZEN or TFT touchscreen.

Polarizer



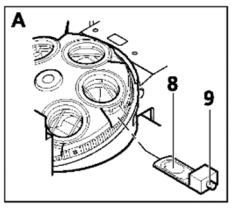
① Turn the condenser turret adjustment ring to move the condenser turret to the **DIC II or III** position for bright field according to the lens to be used.

Condenser turret



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

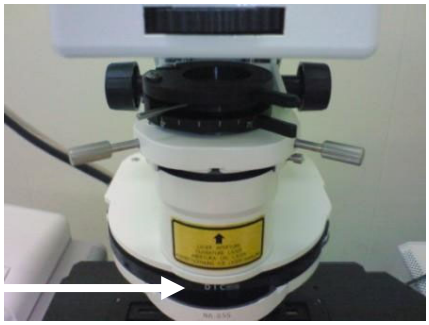
② Adjust image contrast with the screw head of the DIC slider.



11-3. 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



11-4. Epifluorescence

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.

12. Switching off the system

12-1. In the case of LSM980, switch off all lasers on.

Select **OFF** the laser from [Laser] window on the right tool area,

12-2. Clean the Objective lens if you used immersion medium (Oil, Immersol W), and set the position of the objective lens to the lowest magnification for the next user.

Cleaning solution contents : 85% n-hexane and 15% isopropanol

12-3. Click on **the File – Exit** button to leave the ZEN software.

If there are unsaved images, appear the check window.

12-4. After ZEN icon (microscope mark  on tool bar**) disappeared**, shut down the Computer.

12-5. Turn off the System Power

[LSM980]

① Turn off the switch of COMPONENTS.

② Turn off the laser key switch

③ Turn off Main Switch.

[LSM900]

① Turn off the laser key switch to (O) direction.

② Turn off the switch of "SYSTEM" and "COMPONENTS".

③ Turn off main switch of power tap.

*Waiting for more than 15 min, before switch on again.

Feb. 2022 (ZEN3.5)

Carl Zeiss Co., Ltd.