Living up to Life

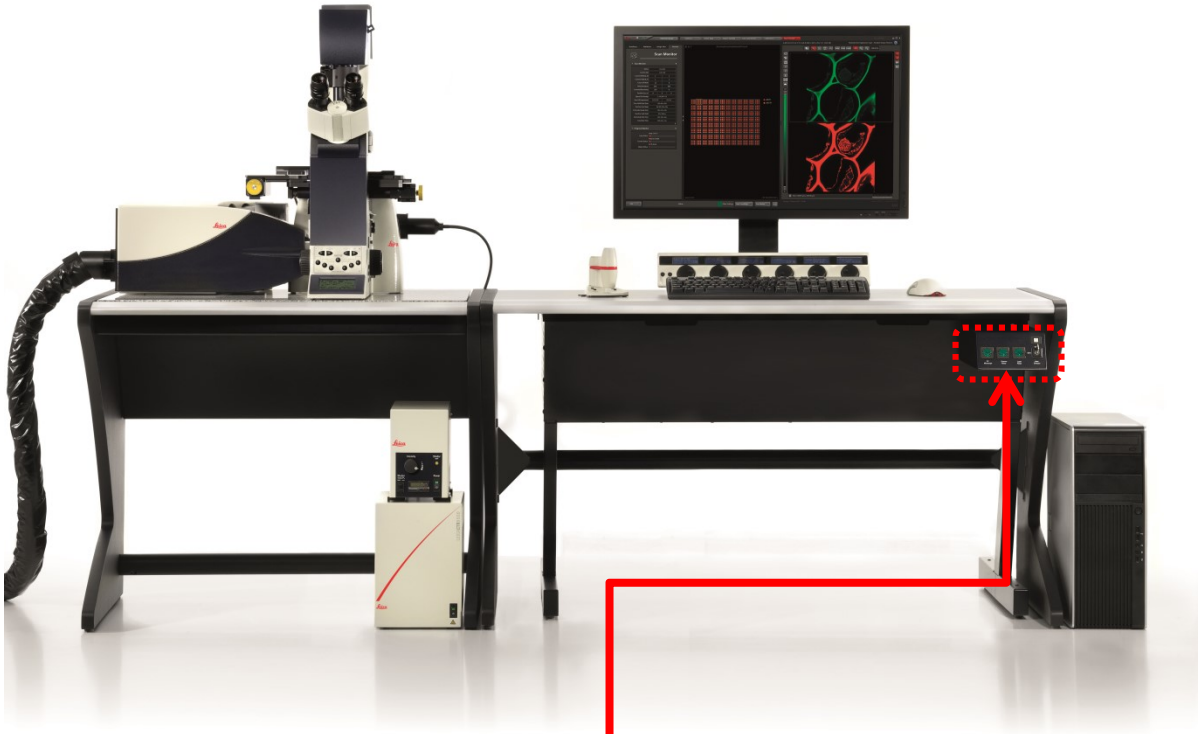
Leica
MICROSYSTEMS

TCS SP8
FSU
Summery Manual
LAS X 3.1

20170703

Contents

I. Start up



Main Switches

Indicator lamp of imaging lasers

④Key switch of imaging laser

①Switch for PC

②Power switch for the Scanner

③Main power supply of imaging lasers and cooling

① **Switch on the PC.**

② **Switch on the Scanner.**

③ **Switch on the main power supply of imaging lasers.**

④ **Switch on the key switch of the imaging lasers (yellow indicator lamp will be on)**

⑤ **Switch on the HG lamp.**
Note) Do not switch on/off frequently and wait about 5min when restart.

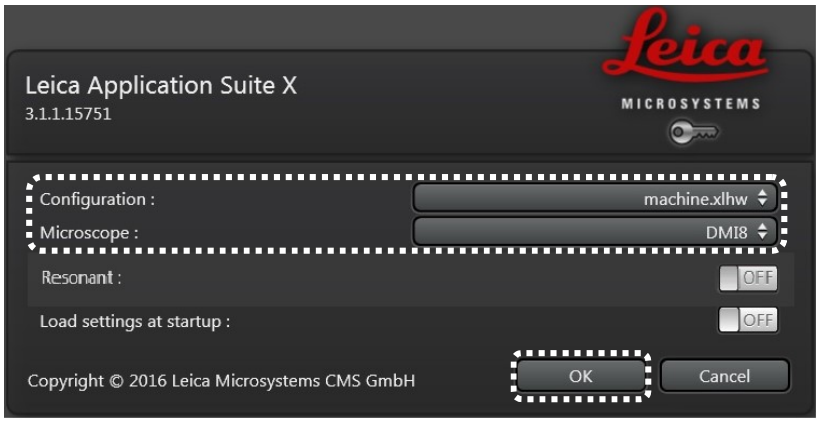
⑥ **Switch on the microscope.**

⑦ **Log in as “TCS User”**

⑧ **Double click on the “LAS X” on the desktop.**

⑨ **The following window comes up.**
Select modes/switches and click "OK" and wait until the interface opens without operation including microscope.

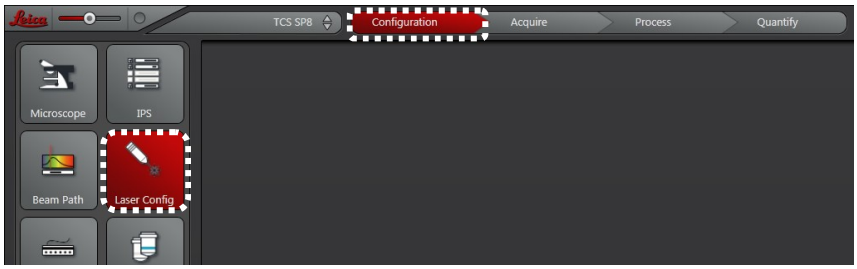
Configuration: **machine.xlhw** → Imaging through the microscope.
SimulatorSP8.xlhw → Starting up without connected hardware(Simulator mode). Turn on PC only.
Microscope: **DMi8**



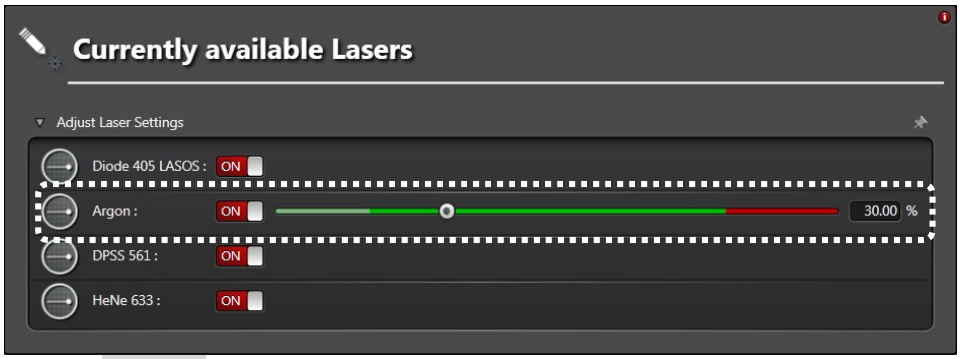
Select the Scanner
OFF:
FOV scanner;High resolution

ON:Option
Resonant scanner;High speed

⑩ **Switch on the lasers**
Open the Configuration tab, click the Laser icon.

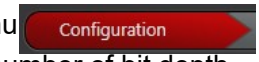


Activate each laser by single click (<u>Do not double click</u>.).



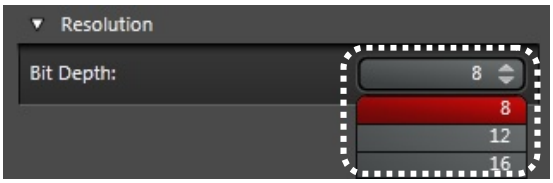
- Set **Ar laser** power as 20-30%.
- **WLL** laser power will be set as 70% automatically, do not change the power.

⑪ Change the bit depth

Click the Configutation menu 

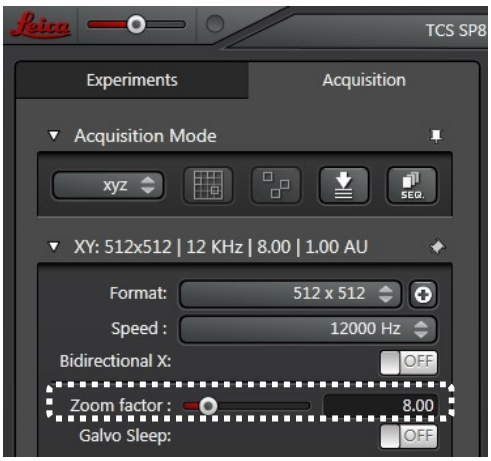
, and click the Hardware 

,
And select an appropriate number of bit depth.

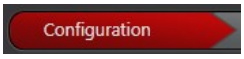


⑫ If using Resonant mode; Define the zoom factor

With Resonant mode, the zoom factor set as bigger when the software opened (zoom factor depends on the 8/12kHz.). Define the zoom factor to minimum before starting acquisition.
（Default zoom factor with FOV scanner is 1.0 and minimum is 0.75）



⑬ if using Resonant mode; set the Live averaging

In order to get higher image quality with resonant mode under Live scan, set the Live" Line Average during Live Acquisition" in the "Hardware" in the Configuration tab 

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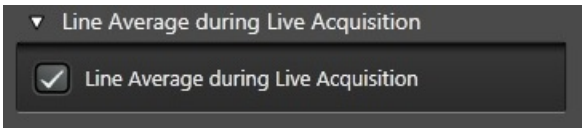
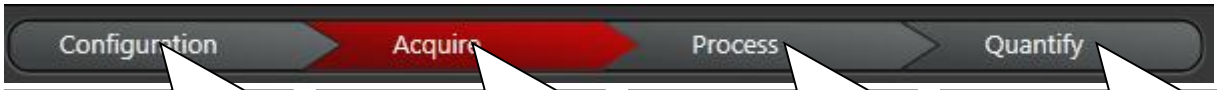
The live image will be averaged by setting number for Line average factor. (See II. Image Acquisition)



II. Image Acquisition



Configuration:
Set up menu for microscope, lasers and systems

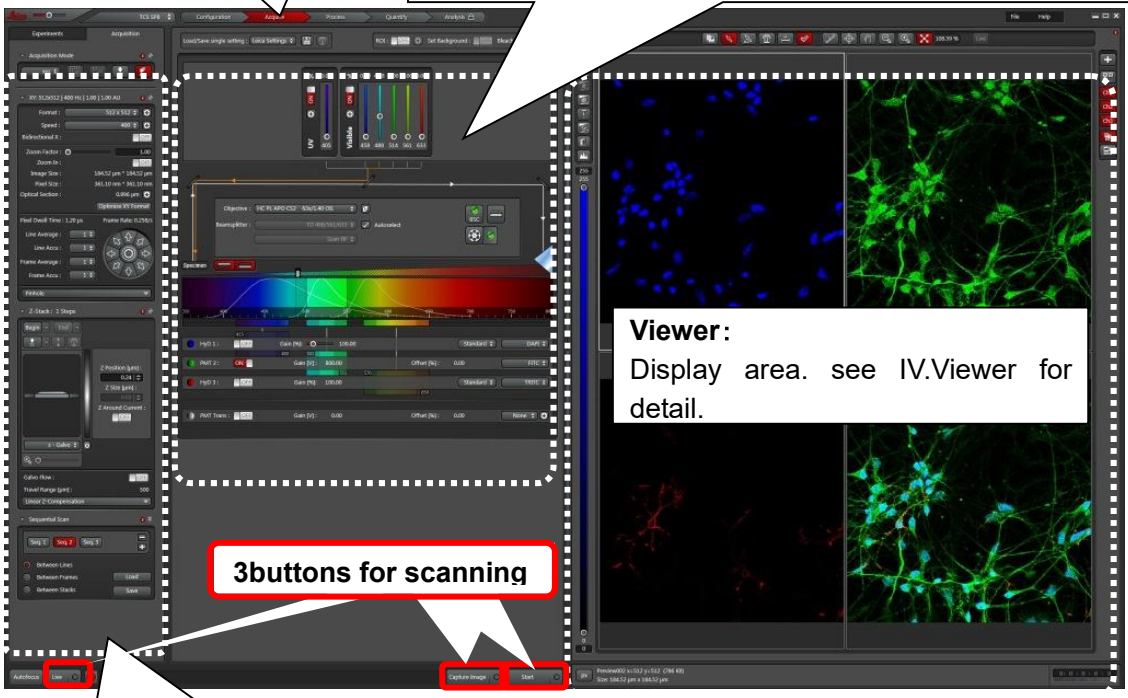
Acquire:
Main menu for imaging

Process:
Image processing menu

Quantify:
Quantify menu for measuring and analyses

Open Acquire Menu

Wavelength settings:
Selecting lasers, detectors, emission wavelength, objective lenses. To simplify the operation, load the defined settings.



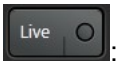
Viewer:
Display area. see IV.Viewer for detail.

Switching Acquisition/Projects tab

Acquisition : Definition of acquire parameters.
Pixels, zoom factor, Z stack settings, etc.

Projects: Data container
Acquired image data using by Capture Image button or Start button are

3 buttons for scanning

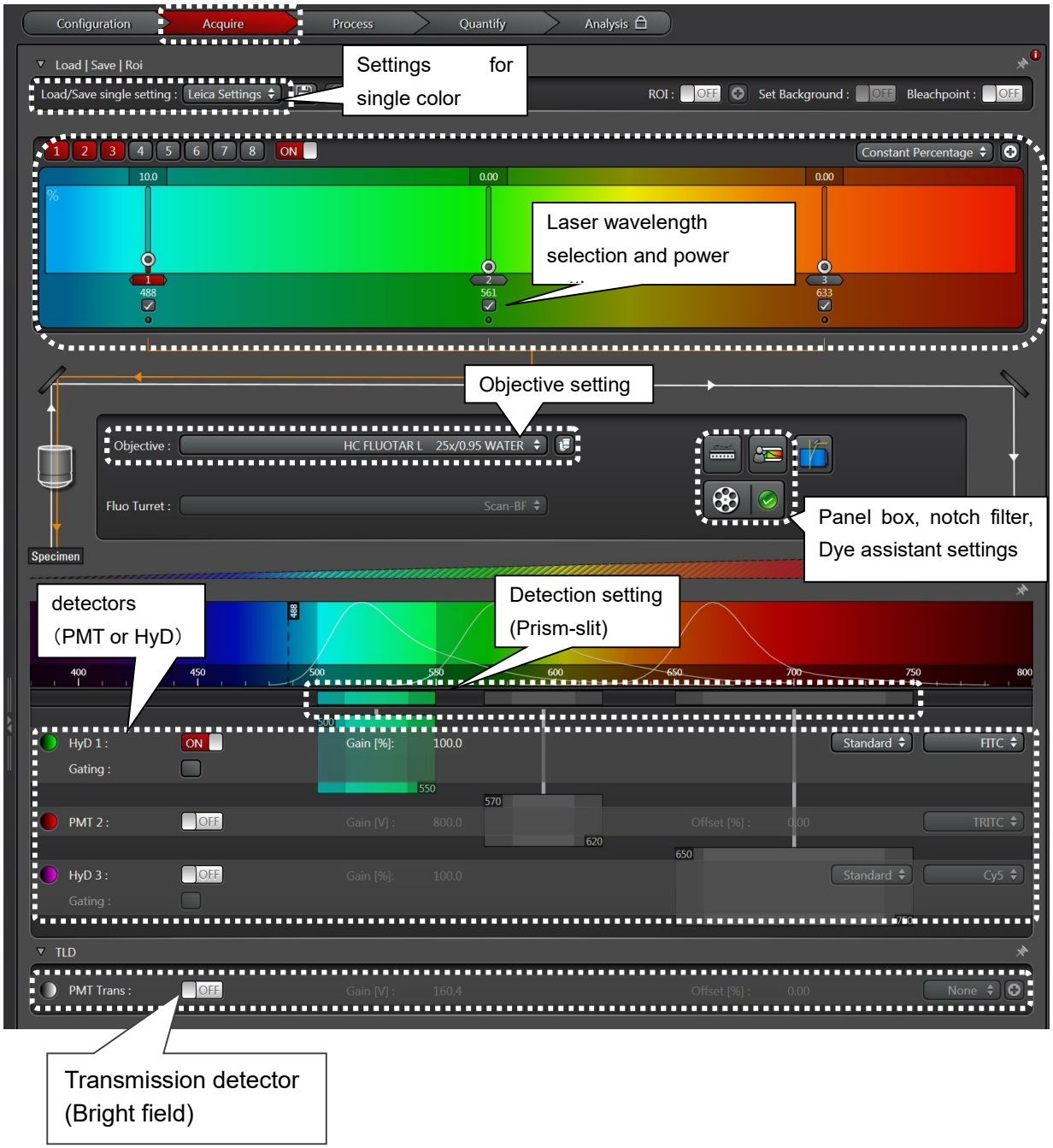


: for preview/adjust button. Data will not be stocked.

: for single image acquisition.

: for multiple image acquisition.

Wavelength settings



①Select objective

Select objective and observe specimen, find the position and the focus.

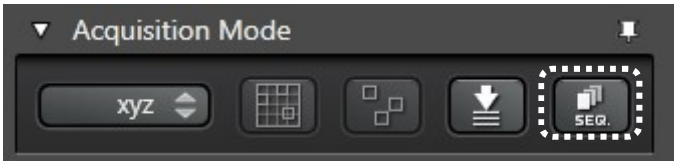
① **Load the fluorescence setting** **For Single staining observation**

Select the light path setting from the pull-down menu of “Leica Settings” of “Load/Save single settings”. *For the multi staining, go to ③.

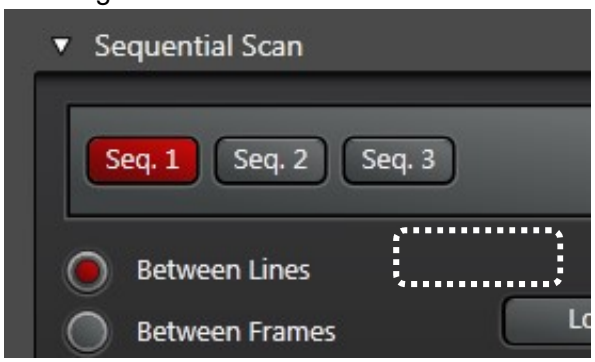
DAPI: UV ex. Blue fluorescence observation
FITC: Blue ex. Green fluorescence observation
(Alexa488, Cy2, GFP etc)
TRITC: Green ex. Red fluorescence observation
(Alexa555,568, Cy3, DsRed etc)
Cy5: Red ex. Far Red fluorescence observation
(Alexa633,647, TOTO3, TOPRO3 etc)
Transmission: bright field

② **Load the fluorescence setting** **For multiple staining observation**

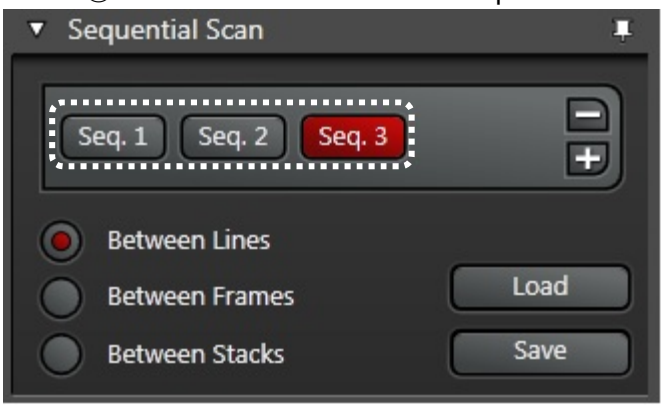
③ －1 Activate “SEQ”button in the “Acquisition mode” panel. “Sequential Scan” panel will open.



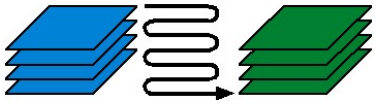
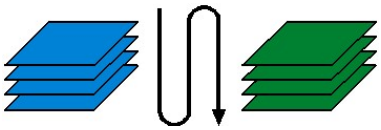
③－2 Click the “Load”button on the “Sequential scan” panel and select the sequential setting for the multiple staining.



③－3 Several “Seq.” buttons are displayed depend on the number of fluorophores.
Activate each “Seq”button and define for the each staining (laser power, gain).
Refer ④ and after how to define these parameters.



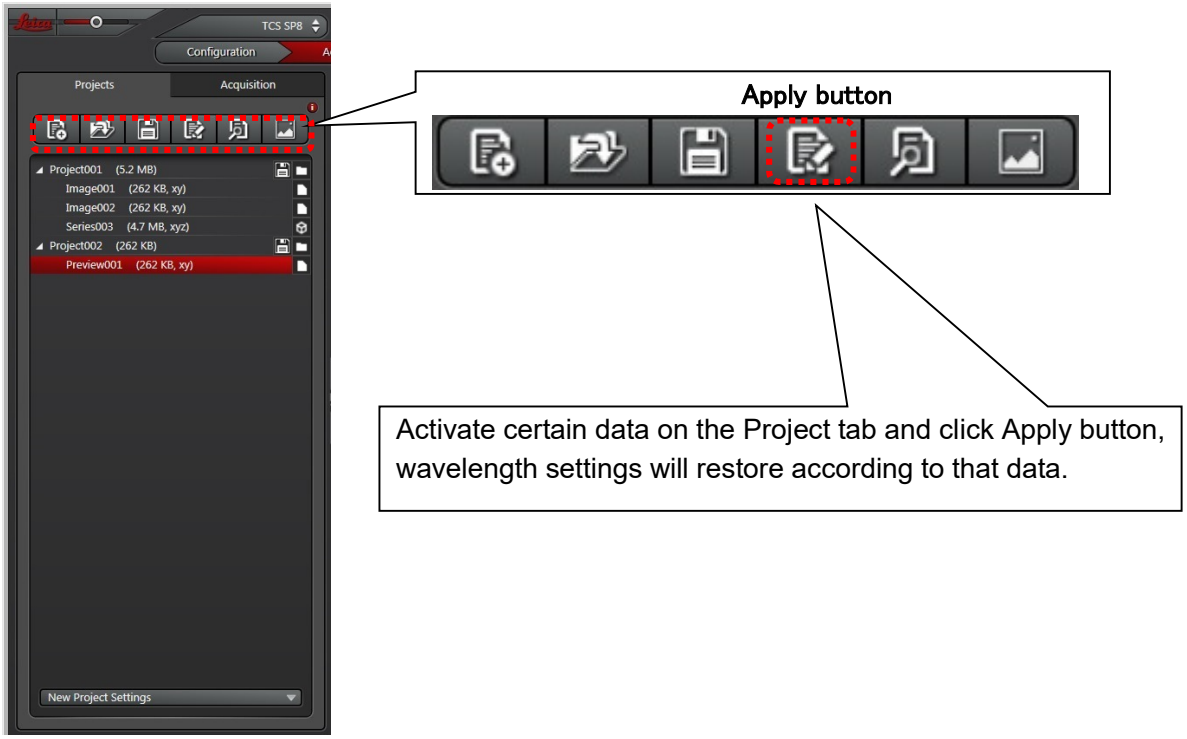
＊Sequential mode

between lines		Each excitation changes in line by line. Suitable for live imaging since it is almost simultaneous scanning, time lag is only a line period.Necessary to have same number of detectors with dyes.
between frames		Image acquisition executes each frame by frame in different excitation. It means there is a time lag of a frame period.
between stacks		Each excitation changes in stack by stack. This method achieves the fastest completion time even the time lag between the dyes becomes bigger

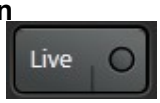
Loading the settings from the acquired/saved data

By using the Apply button on the Experiments tab, the settings of the acquired/saved data can be loading (reproducible).Activate the raw image data on the Projects tab and click Apply.

All the parameters of wavelength(Excitation, emission), detectors will be reproduced.



④Image acquisition

Click the Live button 

at the lower left on the monitor.

Scan starts and align the parameters as follows.

Notes: Live button expose lasers your specimens. So it should be stopped often in order to avoid the bleaching.

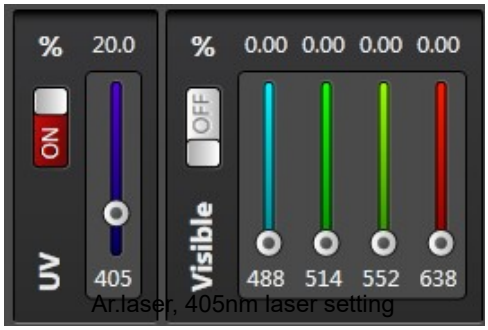
■ Defining the laser output

Define the laser output so that it may be optimized to the specimen by using slider or imputing the number on the laser panel.

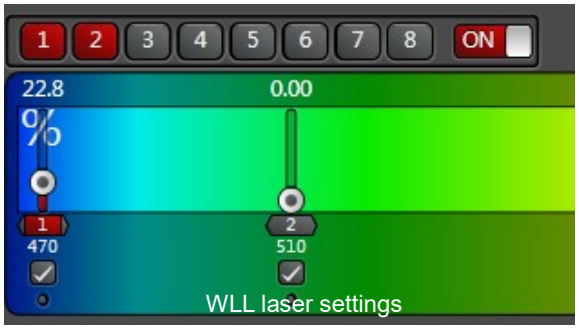
Switching WLL and Ar laser, 405nm laser.

Displays switch by clicking the vertical buttons “Switch to Whitelight”, ”Switch to Conventional”

Defining laser power by using slider or inputting numbers.

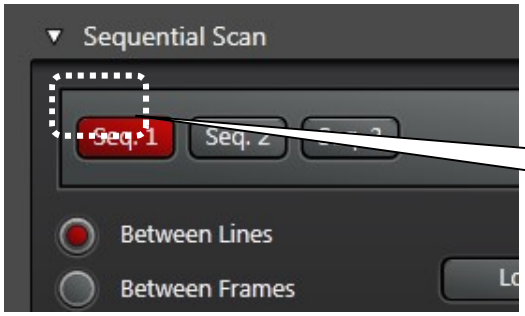


Ar.laser, 405nm laser setting



WLL laser settings

* Activate corresponding Seq button whenever define each laser power.



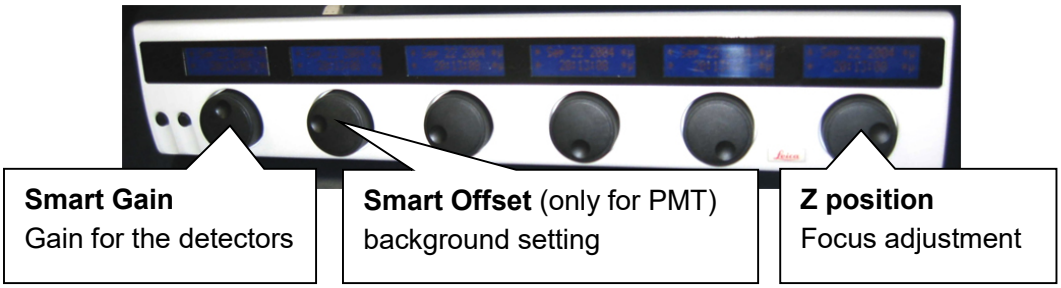
First activate corresponding Seq button and change the laser power.

Defining the focus, and the detector gains

Adjust focus and the detector gains and offsets by using each dial of the control panel (option) as below.

For the multistaining, the “Smart Gain” and the “Smart Offset”dials can adjust each staining by activating channel image respectively on the monitor.

- default settings of gains as a standard → PMT：800V HyD：100%

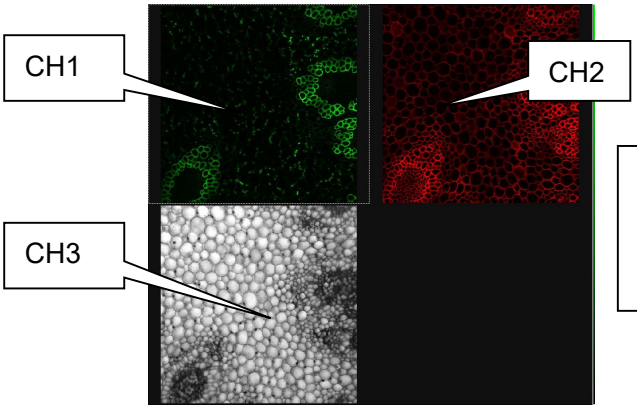


- point

The less Gain achieves the higher S/N. If higher gain is used, use “Average” function in order to reduce noises.

	PMT（conventional）	HyD（Hybrid detector）
Gain（the voltage of detector） **Default Gain as a standard**	0-1250V **800V**	10-500% **100%**
Offset（background setting）	Possible	-
Photoncounting mode	-	possible

For multicolor imaging, with Panel Box, you can change each channel Gain/Offset by using Smart Gain/Smart Offset dial, just click certain (singlecolor) image on the monitor,



Click single color image on the monitor and operate Smart Gain/Smart Offset dial.

Quick Look Up Table (Q LUT) button

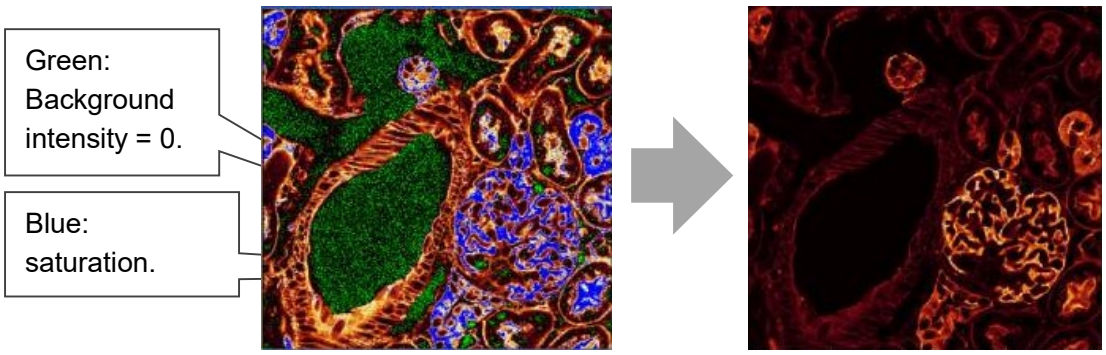
For easy tuning of signal intensity, click Q LUT button and the look up table (image display color) changes to “Grow Over/Under”.
Image display color changes like follows by clicking this button.

Original（pseudo color like green, red, etc.）
→Grow Over/Under（for checking）
→Gray（black white）
→Original（pseudo color like green, red, etc.）

Image can be taken with 8/12/16bit. For instance 8bit; it has 256 intensity levels (grey scale/gradation), and “Grow Over/Under” color shows this 256 level in different colors like follows.

>255	blue（saturation = overflow）
255	blue
・	white
・	brown
・	black
0	green
0 <	green

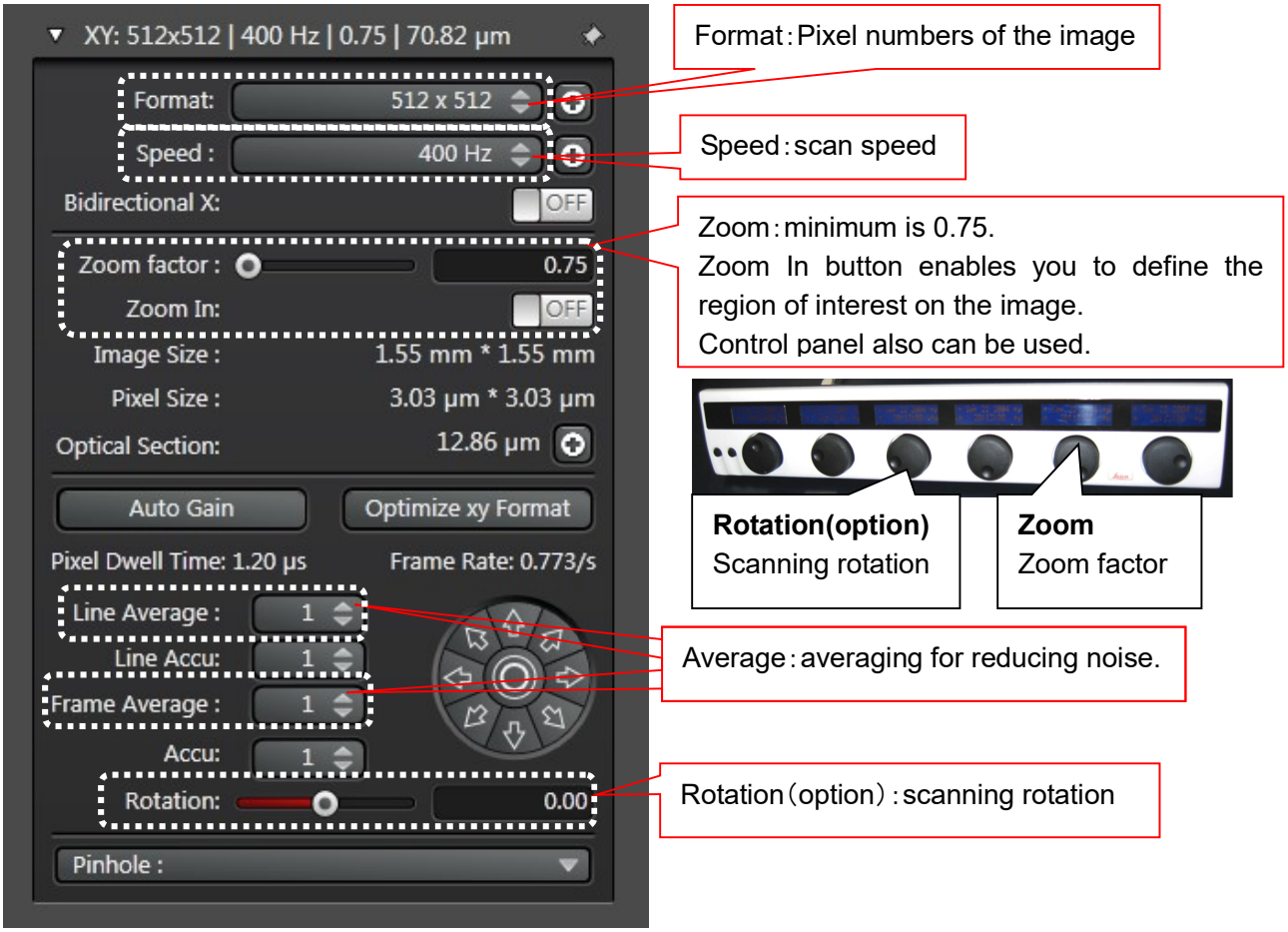
Blue color means the signal saturation. Readjust laser power and/or gain to avoid saturation.
Green color means the darkest signal. Too low offset makes enlarged green area and some weak signals may be failed to be detected.



⑤ Other parameters（format, scan speed, zoom, etc）

If necessary, Format (pixel numbers of the acquired image), Speed(scan speed), Zoom factor(optical zoom) can be also changed. The slower the scan speed, the smoother the image quality.

Please note, the bleaching effects becomes bigger with the higher the zoom factor.

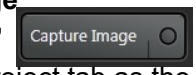


⑥ Average function

“Average” enables to get higher signal to noise (image quality) ratio of the image.

- Set higher number of average for higher detector Gain.
- Higher number of average does not necessary for lower Gain.

⑦ Acquisition of the Single image

Click “Capture Image button” 

for single slice image.

Image data will be kept in the Project tab as the name of “Image001” temporarily.

The number of Average will be activated by these buttons during the acquisition.

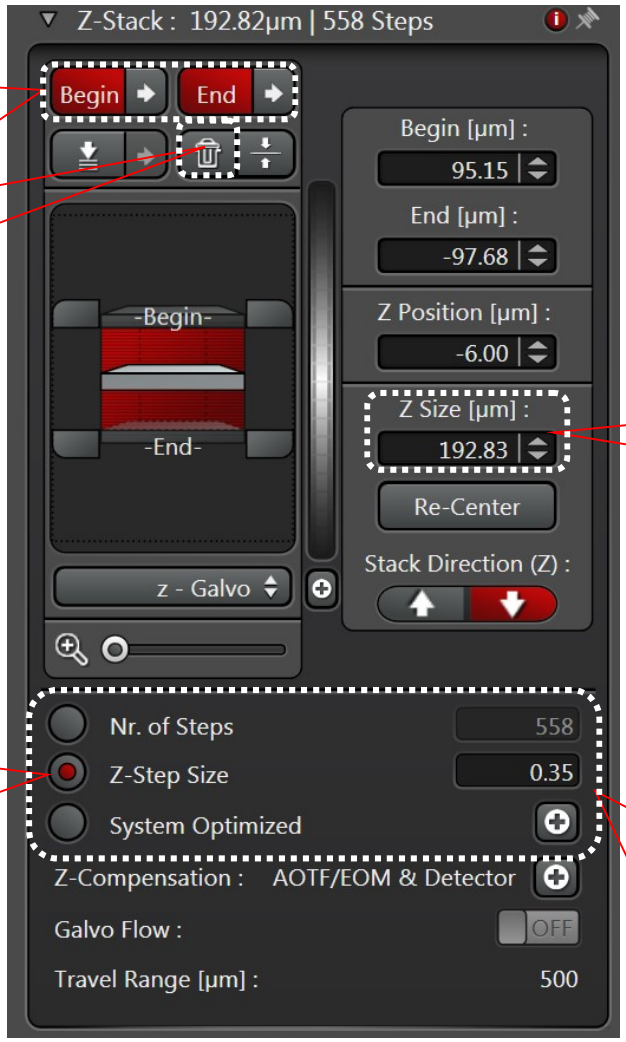
⑧ Z stack acquisition

Define start position with “Begin” button and end position with “End” button while scanning by “Live”button. Focus can be moved by Z position dial of the Control panel.

Z position
Adjust z position/focus

⑧－1
Define Begin/End position

Delete defined Begin/End position



Thickness by defined Begin/End

Activate either of them

⑧－2
Define number of frames or step size.

Guideline for z-step size:
About the twice of the number of “System Optimized”

⑨ Acquisition of the Zstack

Data acquisition starts by clicking following button.

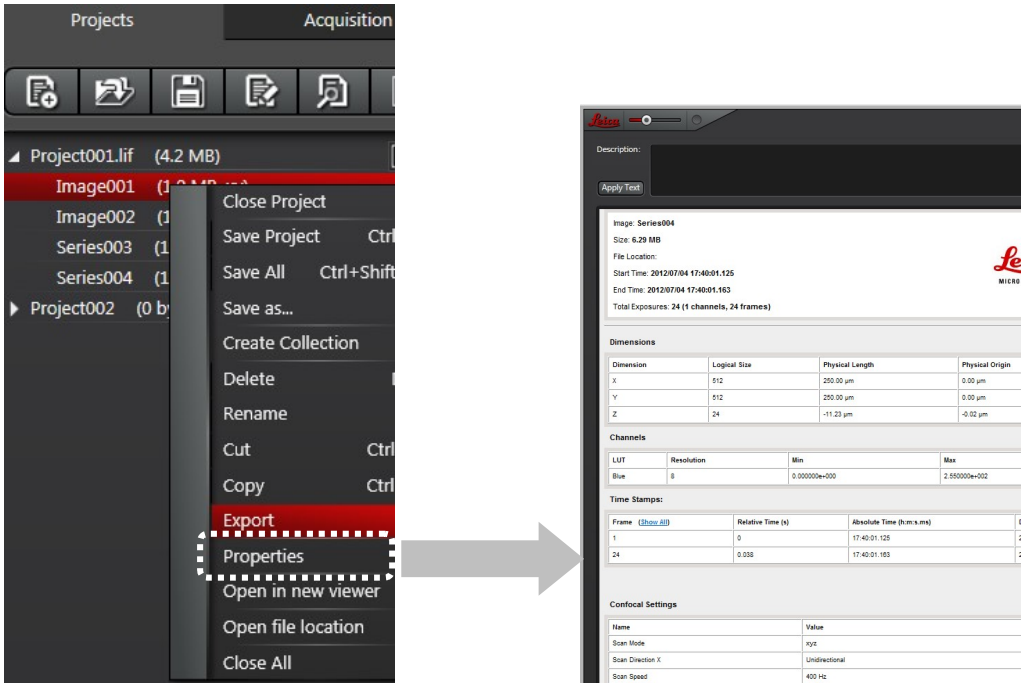
XYZ (or for several continuous image acquisition: 

click “Start” button.
Image data will be kept in the Project tab as a name of “Series001”temporarily.

Note: keep away from the microscope and the anti-vibration table while scanning in order to avoid the vibration noise to the images.

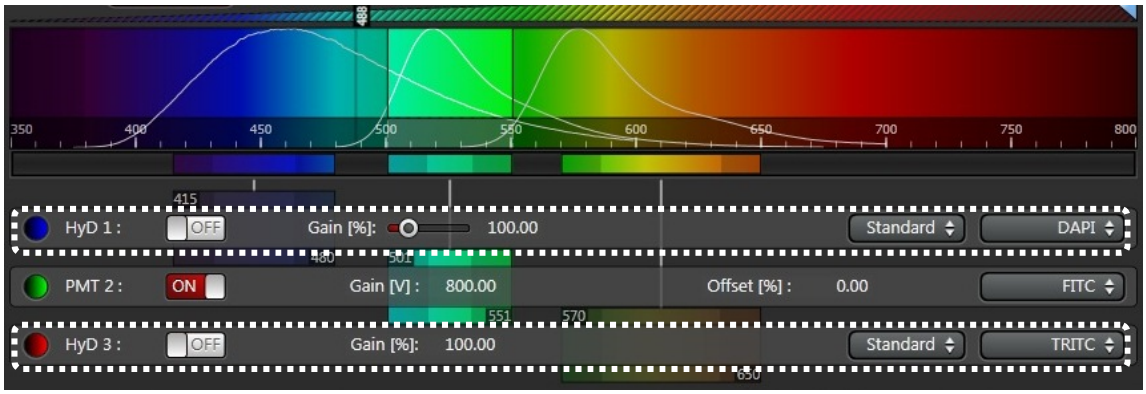
Parameters of the acquired image data

Right mouse click the data on the Experiments tab and select “Properties”.
“Experiment Data” will open and able to confirm the parameters of the image data.



HyD (Hybrid Detector *option)

HyD has higher sensitivity, higher S/N, and extremely low noises. Photoncounting mode can be selected.



Caution:

- Please separate mobile items (cellar phone and other items which send electric wave) from HyD about 1m. If they are close to locate each other, HyD would detect noises from them.

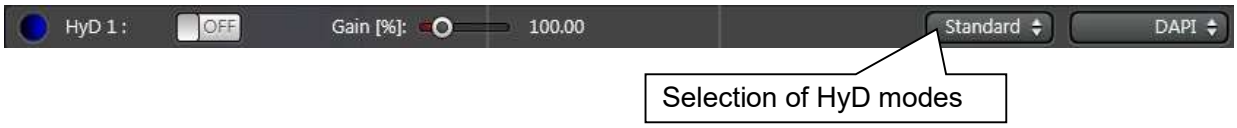
- If HyD detects extremely high intensity, its shutter closes automatically. It means too much emission fluorescence by high excitation power. Redefine lower laser power.

The difference from PMT

- HyD Gain is variable from 10 to 500% whose PMT is variable from 0V to 1250V.
- NO Offset function for HyD.
- There are three kinds of modes with HyD.

Detection mode for HyD

- **Standard:** Normal mode as PMT.
- **Counting:** Please refer next page.
- **BrightR:** Mode for imaging with wider dynamic range a little bit.



Counting mode　(Photon Counting mode)

This is a mode to count and accumulate photons in each pixel. In this case, the intensity means the number of photons. Detector noises are almost zero and the S/N and the quantitativity improves quite a lot.

Theoretically it is not possible to use Gain with Counting mode, the image shows less bright than Standard mode, to get brighter image with Counting mode, please refer following settings.

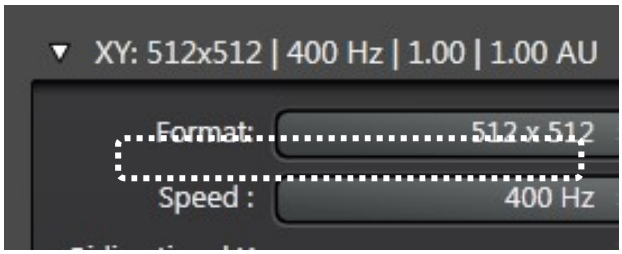
＜points of using Counting mode＞

■ Gain cannot be used in theoretically. Only the number of photons are shown as a image.

■ brightness adjustment can be done with following method.

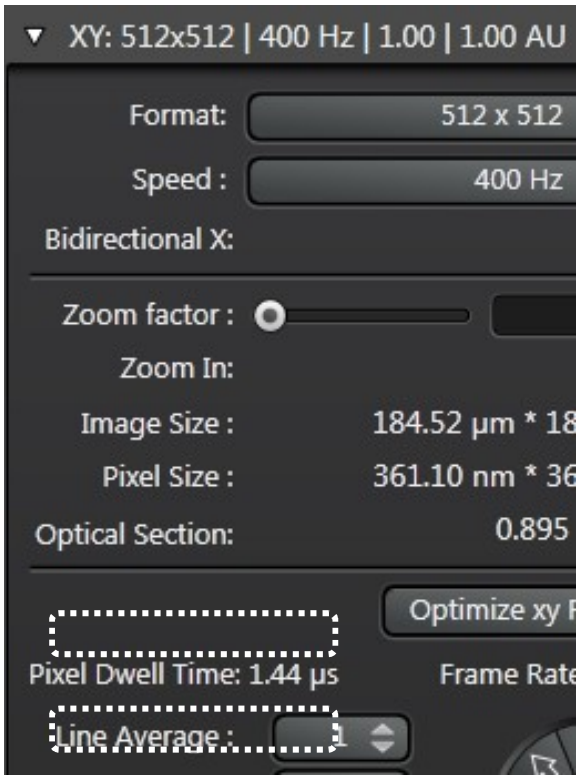
① take slower scanning speed.（only with FOV scanner）

slower scanning speed can get more photons per pixel which means brighter image.



② take Acculmulation

For example, 2 times Accumulation made about twice as bright image.



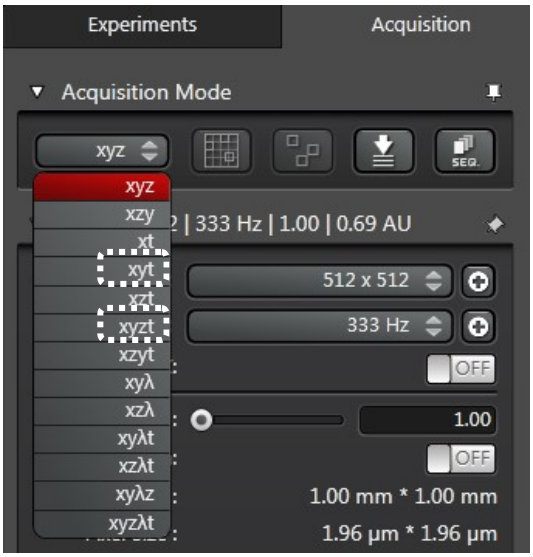
⑩ timelapse

⑩－1. Select mode

Select “xyt” or “xyzt” from the Acquisition Mode.

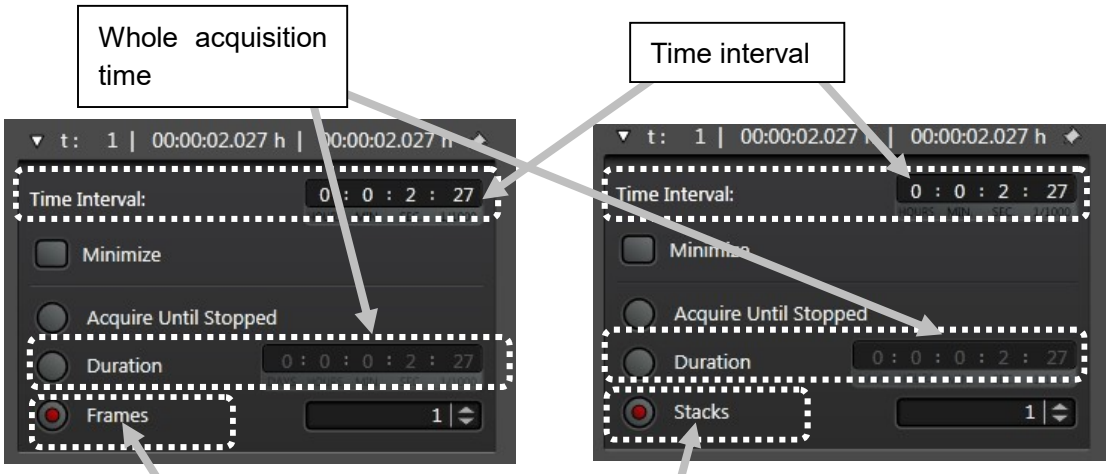
xyt ： XY timelapse imaging without Z stack

xyzt ： XYZ timelapse imaging with Z stack.



⑩－2. Following panel will open according to the selected mode.

Define the “Duration”(whole acquisition time) and “Time interval”



Frames/Stacks will show depend on the mode(xyt/xyzt). If the xyzt mode is selected, this column will be Stacks and Z settings should be defined on the Z stack panel.

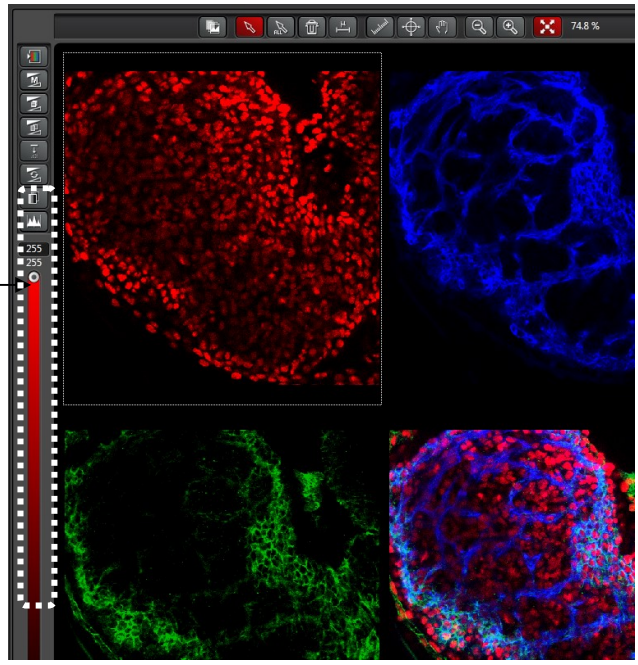
⑩－3. Timelapse acquisition

Define other settings（image adjustment, zoom, Z stack, etc.） and click “Start” button 

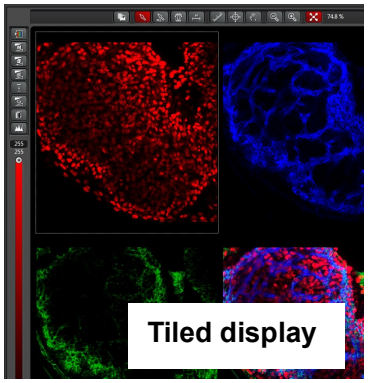
for scanning.

III. Viewer

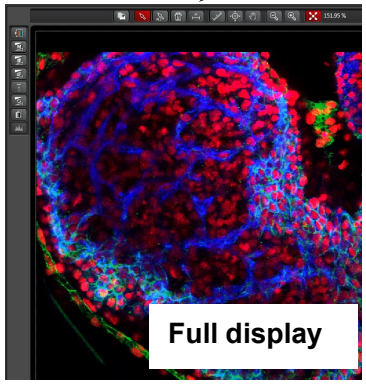
Viewer：Window which is shown the acquired images. There are function buttons around the images.



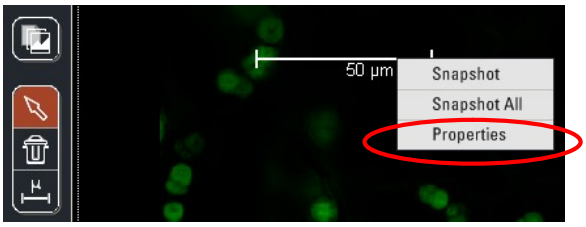
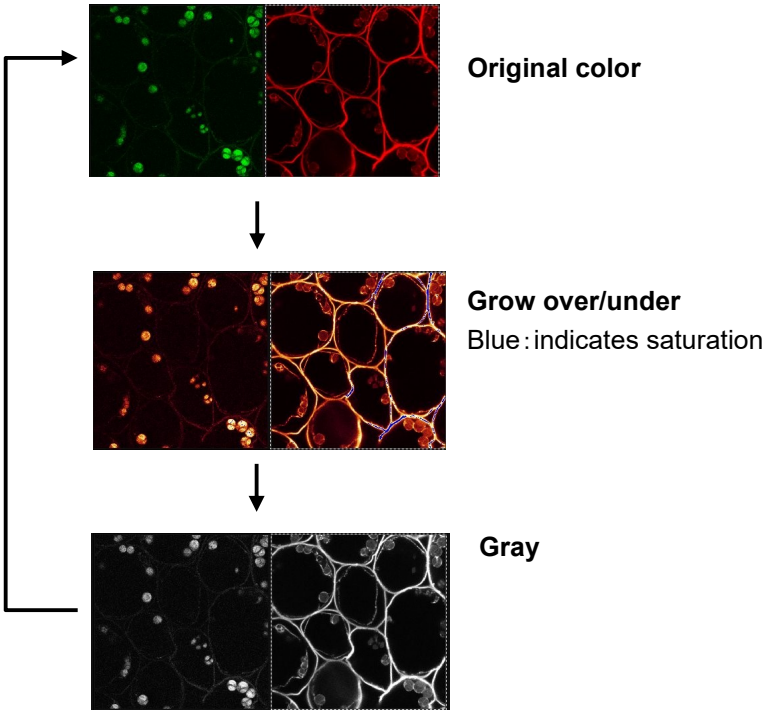
The colors of the image can be changed by clicking this LUT bar.

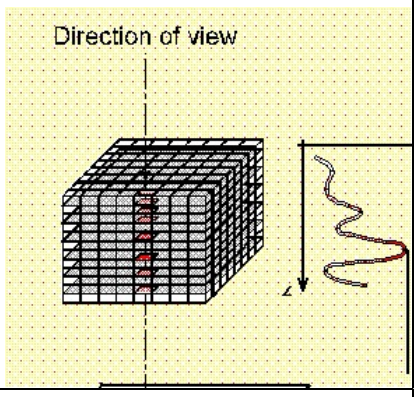
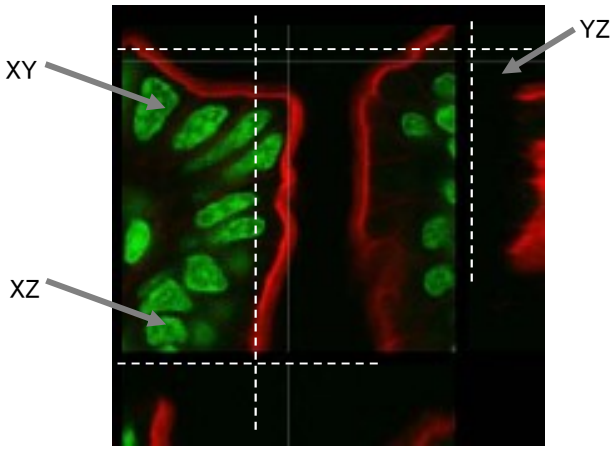


Tiled/full display can be changed by double clicking the image.



Icons of the viewer

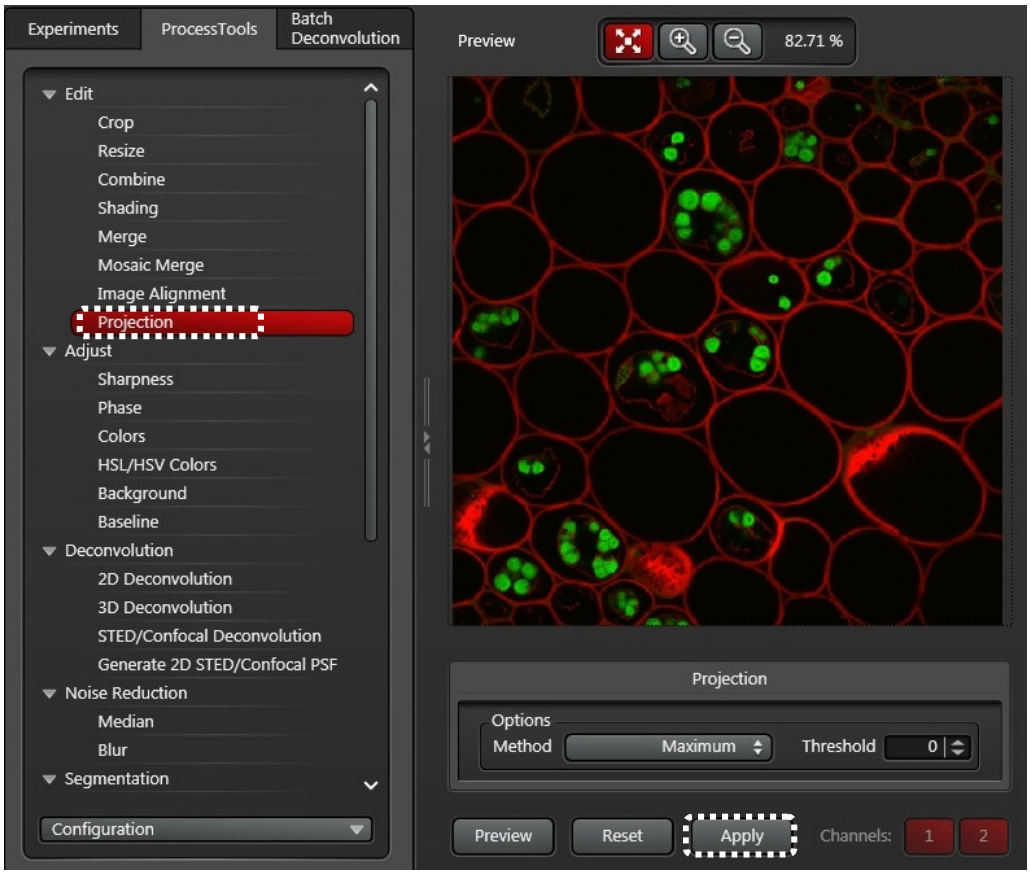
	Select tool
	Delete selected/activated item
	Insert scale. Activate the scale by using  button and right mouse click, select “Properties” then define the length, angle, etc. 
	Display scale. On/Off this button and the image changes 100% display or shrink or zoom depend on the image resolution and the display resolution.
	Quick Look Up Table The color of the image changes by clicking this button each time, Grow Over/Under→Gray→Original. 

	Overlay Display or not display “overlay image” of the multiple channels by activating/not activating this button.
	Maximum projection Create Maximum projection image from the XYZ stack data temporarily. The maximum intensity in the z stack for each pixel will be shown as a single 2D image. 
	Orthogonal Sectioning Show XZ and YZ section images from the XYZ image data. 
	3D 3D Viewer launches with this button. 3D view will be shown from the XYZ data.

IV. MaxProjection

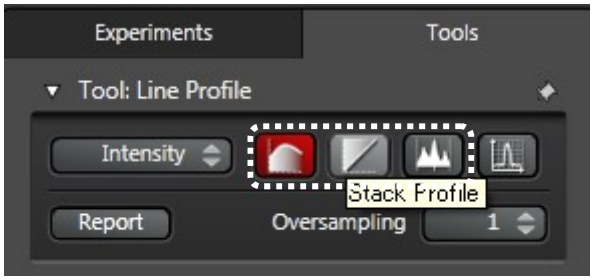
Create Maximum Projection image data from the Z stack image.

Open the “Process” menu and select the “Projection” in the Process Tools.
Activate the Z stack data on the Experiments tab and click “Apply” button.
Maximum Projection image data will create/add in the Experiment tab.



V. Quantify menu

Functions to measure lengths, intensities, areas, histograms, ratio, etc.
There are three mode as follows.



 Line	Lengths and intensities and other statistical data are shown by drawing line on the images. This function is supposed to use for single image.
 Stack	Intensities, areas and other statistical data of defined ROIs（Region of Interest） are shown. This function is supposed to use for XYZ or timelapse data.
 Histogram	Histogram and the other statistical data are shown by defined ROIs(Region of interest). This function is supposed to use for single image.

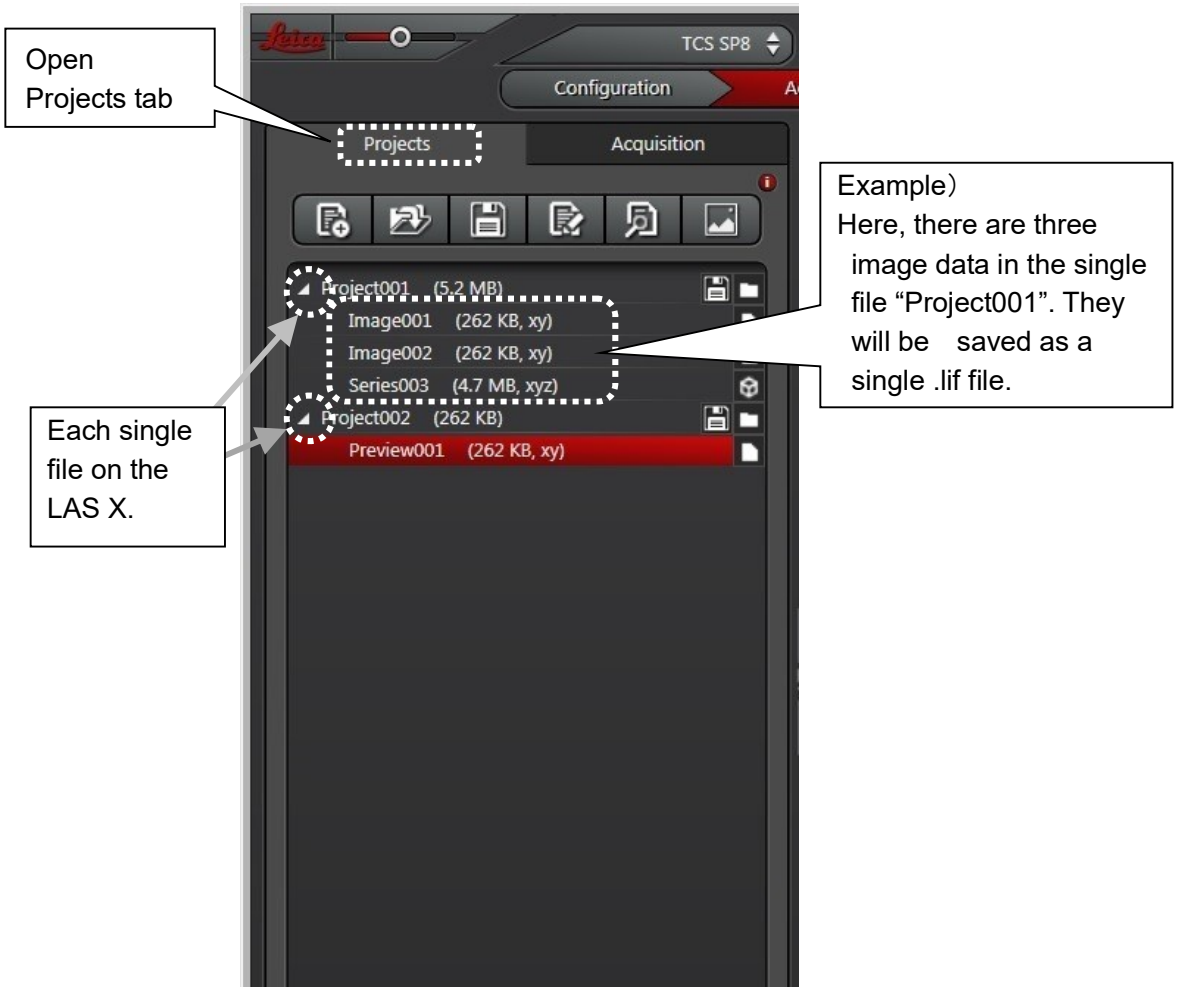
Result export by “Report” button

Result sheet can be export by “Report” button.

Saved data by this button will be .xml, jpg, csv files. Graph data will be imported by Excel, as well.

VI. Data saving

All image data in the single file will be saved as a .lif file.



Project Tool bar

New: Create new Project file.

Open: Open saved data.

Save: Save all data on the Projects tab.

Apply: Restore parameters of which activated data. Selection of applying parameters can be defined from following mene.
Configuration > Instrument Parameter Settings (IPS)

Browse: Browsing saved data.

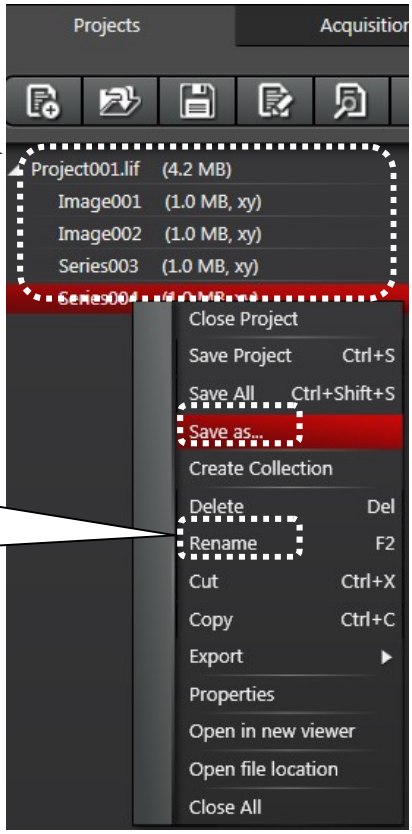
Shows: Show the first image data on the Projects tab.

①Right mouse click the data and select"Save As".

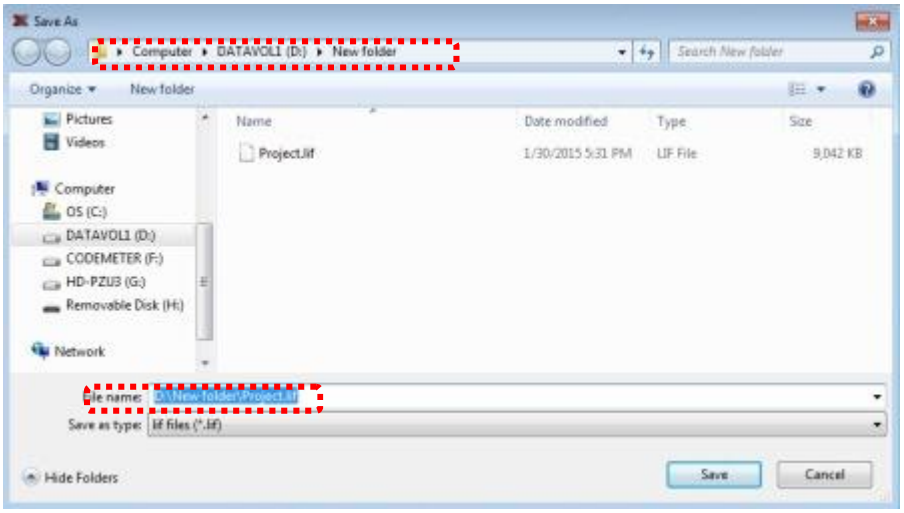
Image data in the same file will be saved as a single file as explained above.

All image data in the same file will be saved as a single .lif file.

To rename each data (Series001,Image001, etc), right mouse click and select "Rename" or just double clicking the data.

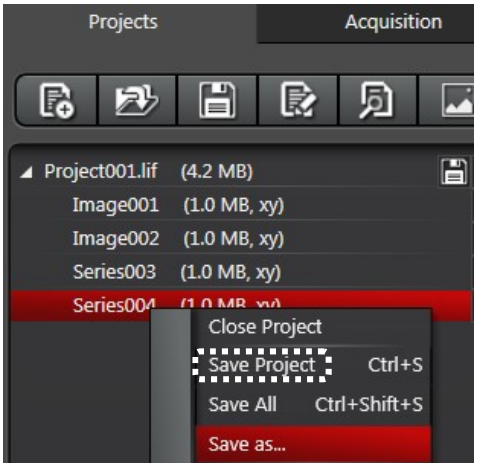


② Select the path to save the data.



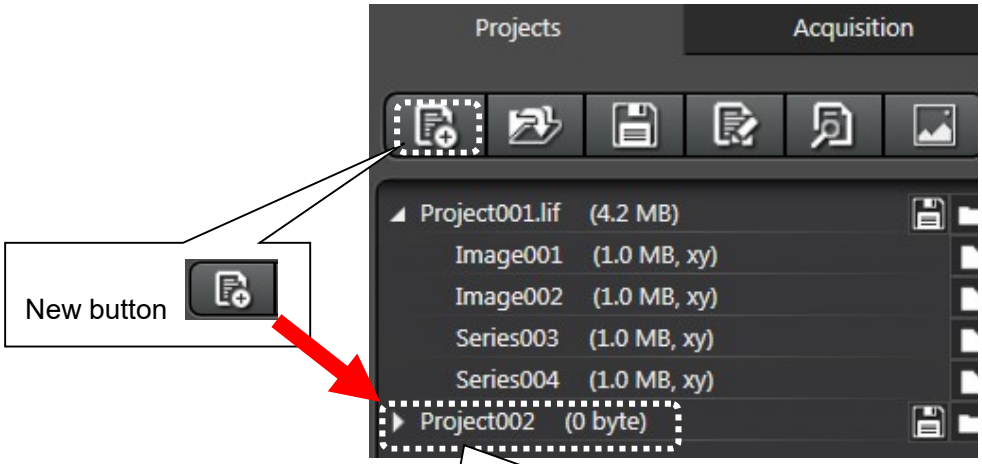
③ **Overwrite saving (refresh saving)**

Adding/editing data after saving .lif file, data should be overwritten by executing “Save Project”



④ **Acquiring new data in the new file**

To create new file, click “New” button, new data will be acquired in the activated new file.

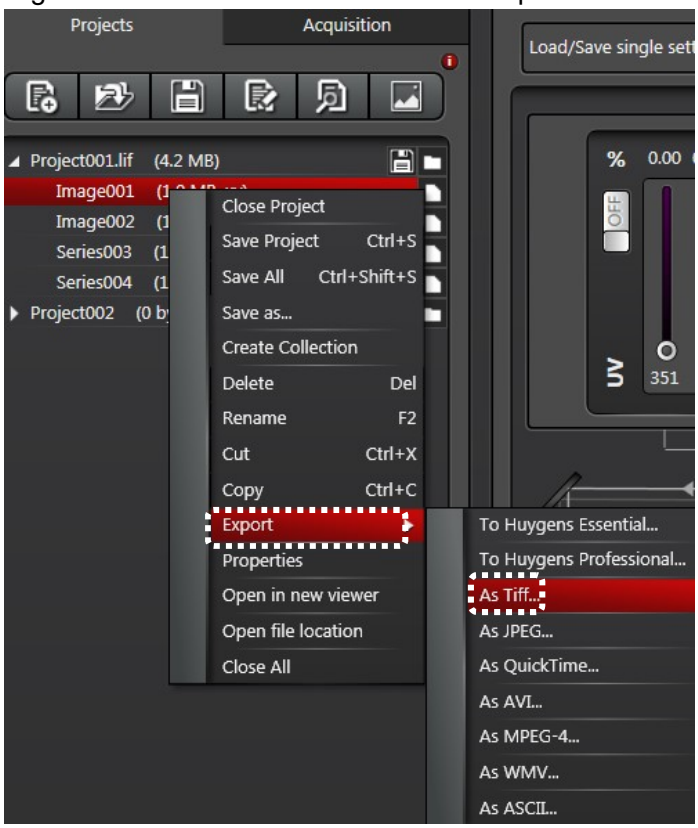


Created new file
Activate this file and acquire new data, the data will be input in the new file.
＊data will be able to copy/move by click and drop/copy and paste to the other file.

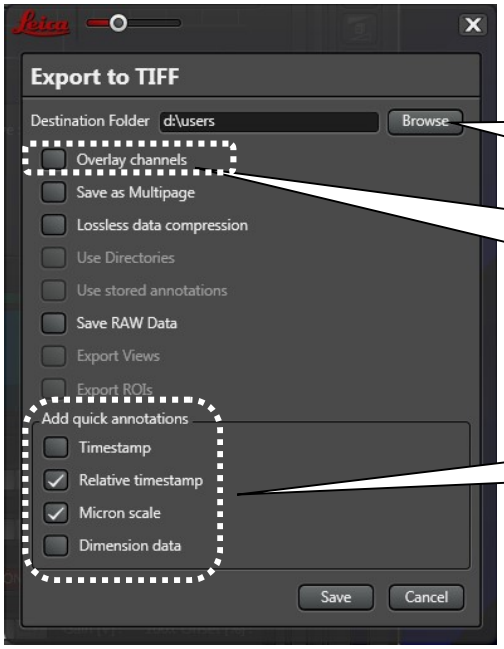
Note)
If the new data are acquired without activating new file, it will be acquired in the (another) activated file.

⑤ Exporting file as “.Tiff”, “.Jpg” format

Right mouse click the data and select ”Export” and select ”As Tiff...” or ”As JPEG..”.



Then following dialog will open.
Select the path and click save.



Destination Folder
by clicking Browse button to select the path

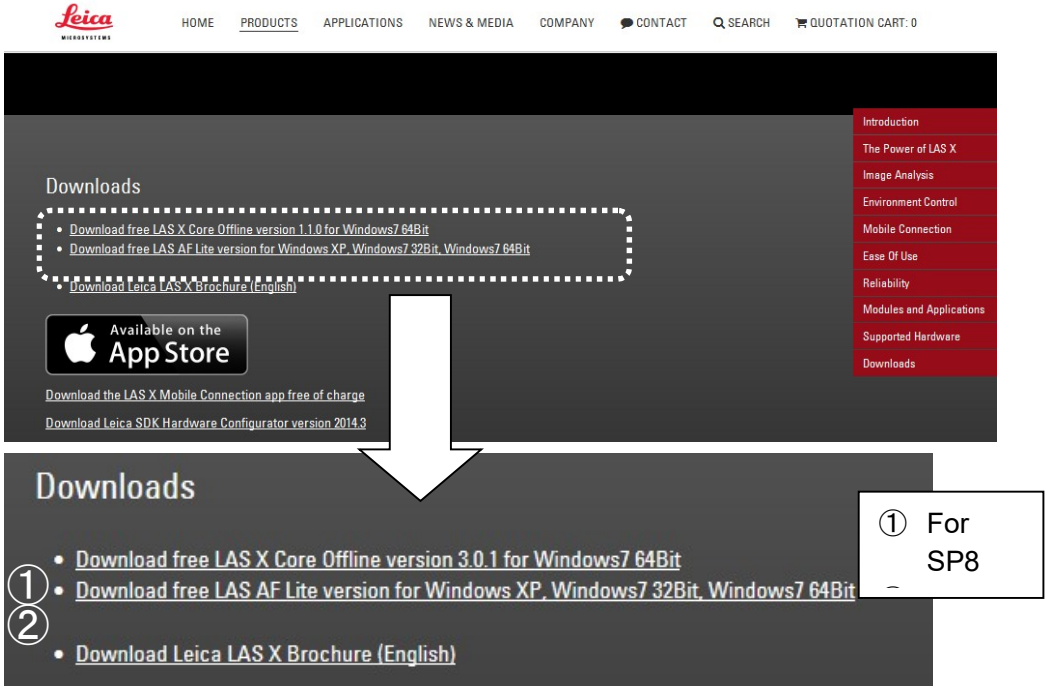
Overlay Channels
For multicolor staining, select “Overlay” or not for saving.

Select to inputting scale bar, timestamp, etc.

LAS X Download site

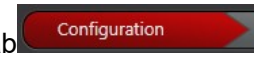
1. Access to following site.
http://www.leica-microsystems.com/products/microscope-software/software-for-life-science-research/las-x/

2. There is a link at the most end of the page, click to download.

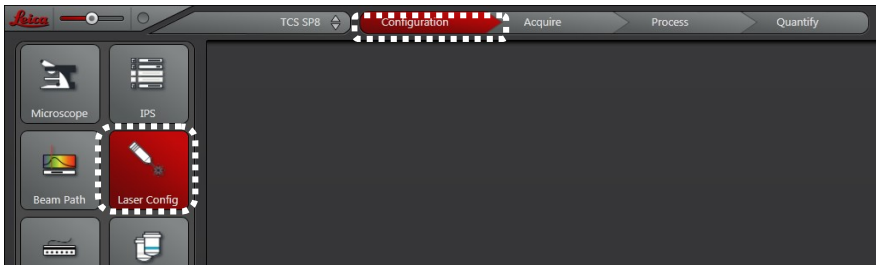


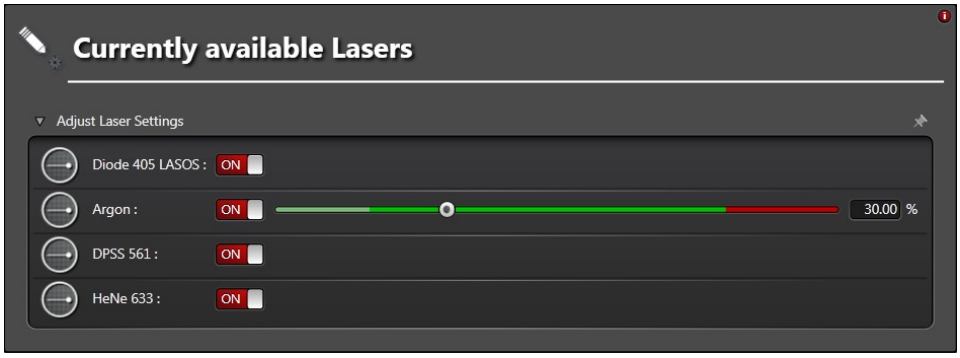
VII.Shut down

1. Switch off the lasers on the software.

Open the Configuration tab 

and click “Laser Config”.





- Ar laser: Decrease the laser power down to 0% and switch off.
- WLL laser: Switch off while keeping laser power 70%

2. Lens cleaning

Using lens paper/ cotton-tipped stick and lens cleaner, clean up liquid immersion lens.
Note) Do not use Kim wipe or other papers for lens cleaning.

3. Close LAS X and Windows.

Save all data and close the LAS X and shut down the Windows.

4. Switch off the mercury lamp .

Note; Wait about 5min. for restart.

5.Switch off the Microscope.

6.Switch off main switches

① **If the Windows is already finished, switch off the PC.**

② **Switch off the scanner**

③ **(If Ar laser is equipped) <u>after the cooling fan was stopped (about 5min after deactivate Ar laser on the software), switch off the key switch</u>.**

④ **Switch off the laser power supply.**

③ Key switch of imaging lasers.

① Switch for PC

② Power switch for the Scanner

④ Main power supply of imaging lasers and cooling fan of Ar.laser

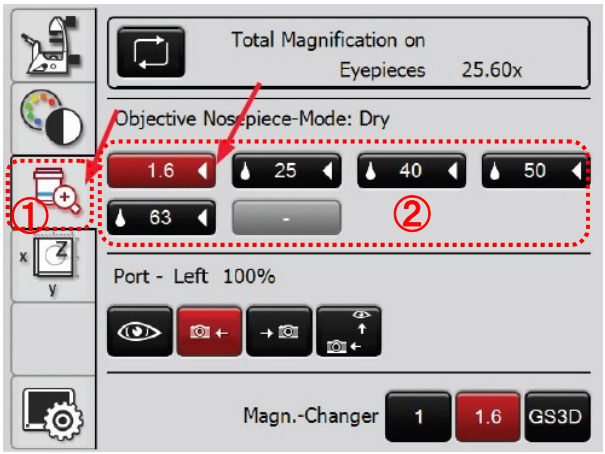
VIII. Microscope operation

1. Focusing and XY Stage control

Focus the image by turning the focus dials on the left and right sides of the stand. Alternatively, rear rotatory knob on the Leica Smart Move control element can also be used. XY stage can be controlled by Leica Smart Move control element.

2. Changing objective nose pieces

2－1 Changing objective by using the Touch Screen



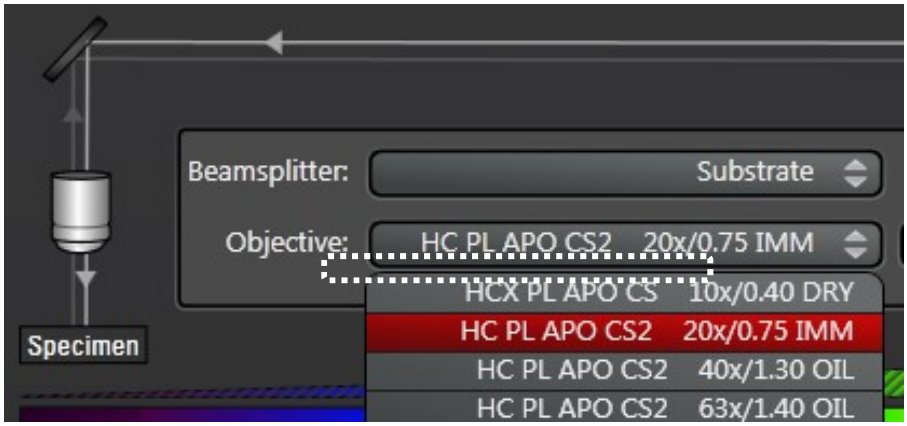
① Use the  tab to switch to the Magnification memu. Currently selected objective is displayed in red.

② To switch between objectives, press the corresponding objective key from Objective Nosepiece Mode. Immersion objectives are marked with drop symbol  . When you change the objective between IMM and Dry, you need to touch the objective key two times. The objective key will brink by the first touch and the objective will be changed by the second touch.

Notre) If the immersion liquid is attached to dry lens, clean the dry lens certainly. Otherwise it causes the decrease of image quality.

2－2 Changing objective on the software.

Click 'Objective' in the software and select the appropriate lens from the pull-down menu.



※ The message is shown when changing between dry lens and immersion lens.

"Immersion of current objective(OIL/IMM/DRY) is different from selected objective(OIL/IMM/DRY).
Do you want to turret automatically? Yes No "

→ **Crick Yes** to change the objective lens automatically

In changing from Immersion lens to Dry lens, wipe off the Immersion from the specimen to prevent Immersion forming on the objective lens.

※ Immersion (IMM) Objectives

For immersion objectives use the appropriate immersion medium.

OIL: Use optical immersion oil from Leica only

W: Water immersion

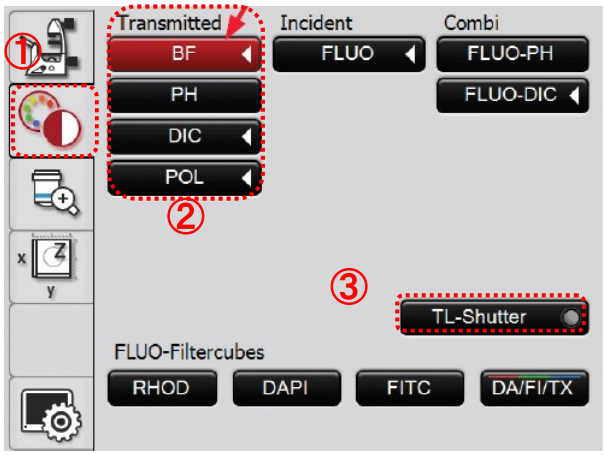
Gly: glycerin

IMM: Universal objective for water, glycerin, Oil immersion. (Require setting of the appropriate correction circle to use each IMM)

Notre) If the immersion liquid is attached to dry lens, clean the dry lens certainly. Otherwise it causes the decrease of image quality.

3. Microscope observation method (by your eyes.)

3－1. Transmitted method(Including DIC)

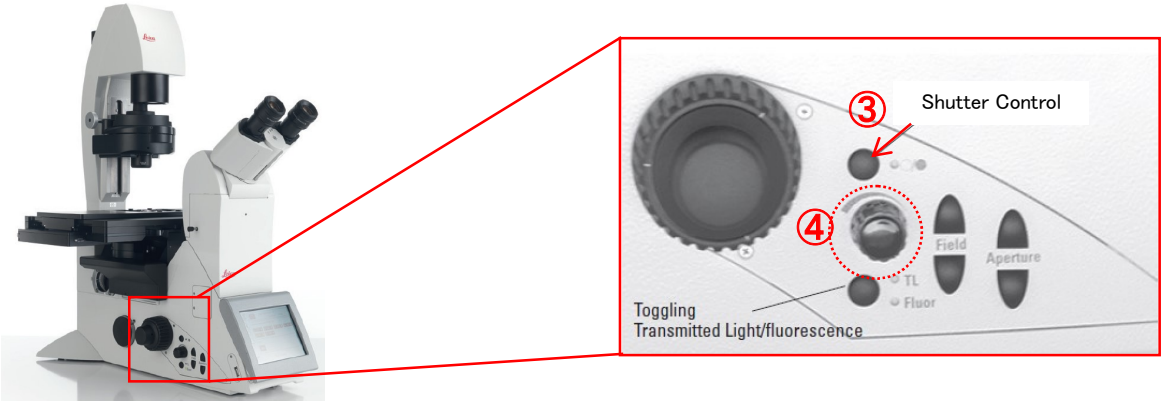


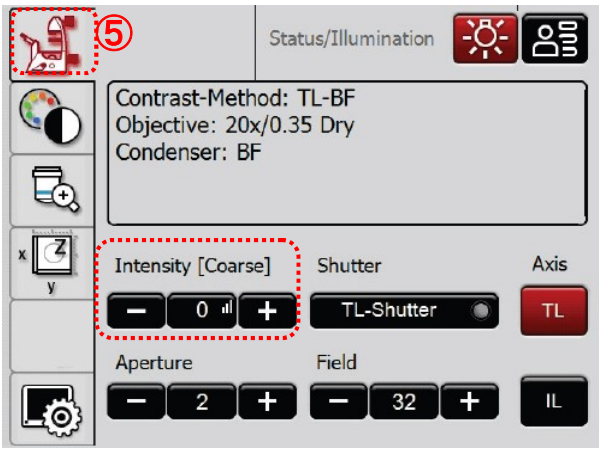
① On the Touch Screen use the tab to configure the contrast method.

② Select the contrast method from Transmitted method list.
BF: Brightfield Transmitted Light
DIC: Differential Interference Contrast

③Touch the TL-Shutter key on the Touch Screen to open or close the Shutter (active illumination axis on or off). Alternatively, Shutter control button on the left side of the stand can also be used.

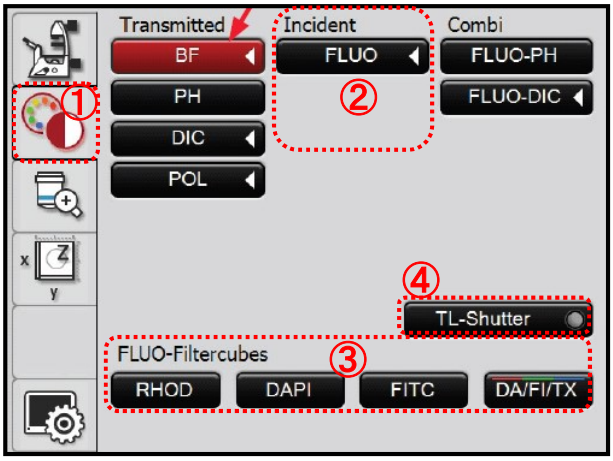
④The brightness is adjusted by the knob on the left side of the stand.





⑤ On the Touch Screen use the tab to confirm the current intensity.

3－2. Operating the fluorescence



① On the Touch Screen use the tab to configure the contrast method.

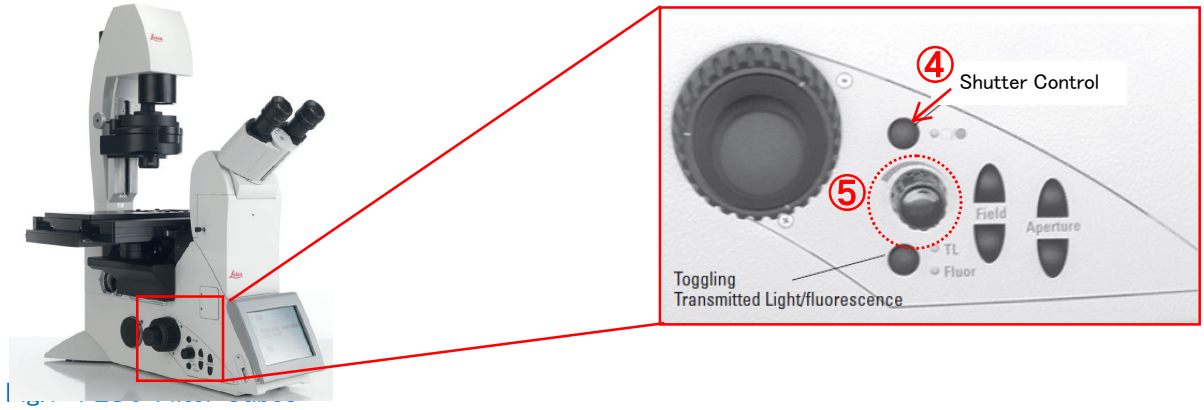
②Select FLUO

③ Select the desired cube using the corresponding key from FLUO-Filtercubes.

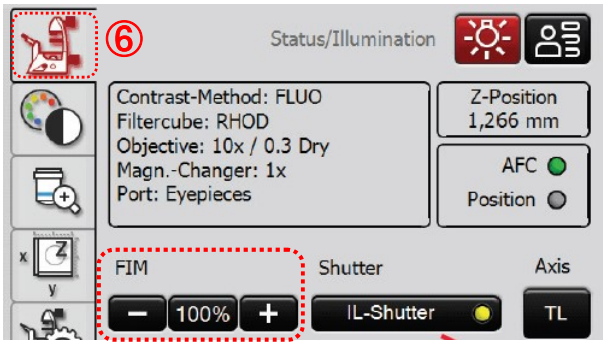
※Set of Filter cubes are depending on the system. Please refer the Fig1.1about the spec of each FLUO filter cubes.

④Touch the IL-Shutter key on the Touch Screen to open or close the Shutter (active illumination axis on or off). Alternatively, Shutter control button on the left side of the stand can also be used.

⑤The brightness is adjusted by the knob on the left side of the stand.



Filter	Typical fluorophore	Excitation	Excitation filter	Dichroic	Fluorescent filter
DAPI	DAPI	UV	BP350/50	400	BP 460/50
FITC	FITC (Band Pass)	Blue	BP480/40	505	BP527/30
FITC LP	FITC (Long Pass)	Blue	BP470/40	510	LP515
RHOD	Rhodamine	Green	BP515-560	580	LP590



⑥ On the Touch Screen use the tab to confirm the current FIM (Fluorescence Intensity Manager). The brightness is adjusted in 5 defined increments.

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