儀器資源中心
Instrumentation Resource Center

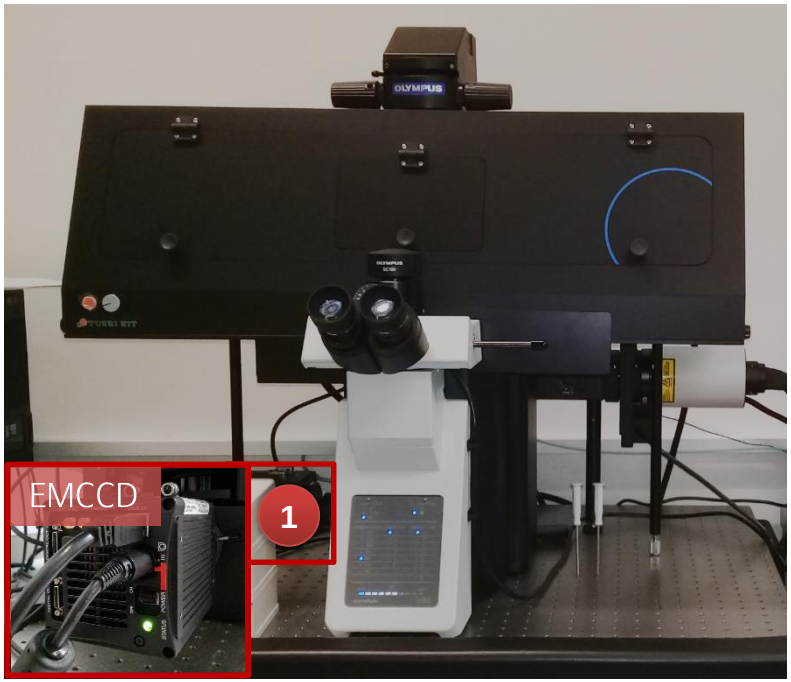
Olympus IX83

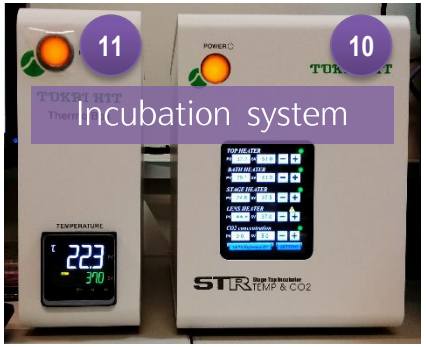
Inverted Fluorescence Microscope

Index

Inverted Microscope Olympus IX83

System Startup (1)





Inverted Microscope Olympus IX83

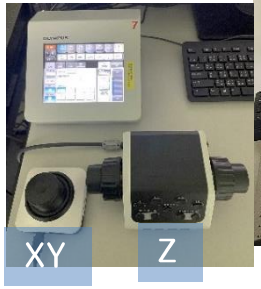
System Startup (2)

① EMCCD (Hamamatsu ORCA-Flash4.0 V3 sCMOS)

② Computer

③ Automatic Stage

The switch is located behind the box. Use the joystick to control the directions.



④ U-CBF (Microscope control)

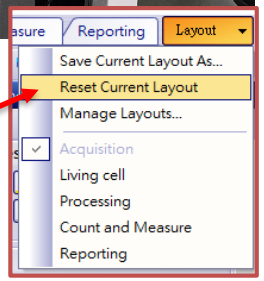
⑤ CBH (Microscope control)

⑥ LED (Fluorescent light source)

⑦ Touch Panel Control

Press the switch on the back once and wait until it displays [Start Operation].

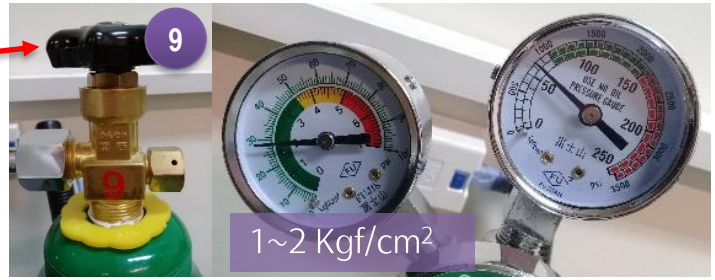
⑧ Software: cellSens [Layout ▼] YM2017/ Reset Current Layout



For optimal performance with the Live-Cell Incubation System, start it 30 minutes beforehand.

⑨ CO_2

Please rotate the black switch and check the pressure value.



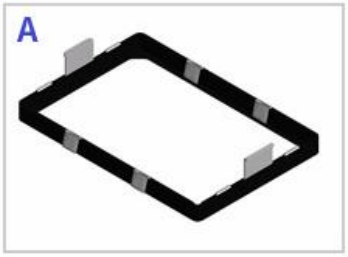
⑩ Stage top incubator

⑪ Thermo Box

There is no need to activate if the filming time is less than 72 hours.

Sample Holders

Please verify with the administrator that the sample carrier is compatible with the IX83 to prevent image acquisition failure and instrument damage.



ATX-W

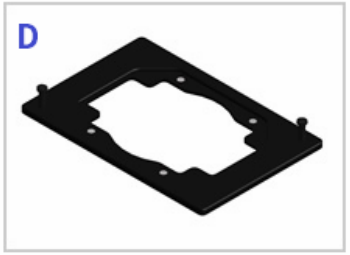
For Well Plate

ATX-CSG

Chamber slide, slide glass and Chambered coverglass

ATX-D

For 35/50/60mm dish



ATX-A

For installation ATX-D,ATX-CSG

It is recommended to use either a 0.17mm glass bottom or a 1.0mm plastic bottom.

A. Well Plate: 6/12/24/96-well cell culture plate

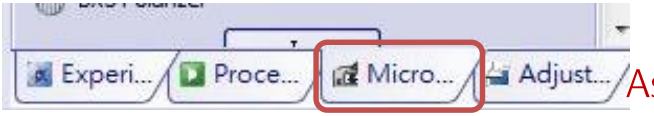
   After replacing the inner fixture, the following specifications can be accommodated:

B. Universal: chamber slide/slide glass/chambered cover glass

C. Petri-dish: 35/50/60mm culture dish

D. Slide: standard 76mm*26mm

Microscope Control



➢ Objectives

Switch to 4X before shutting down

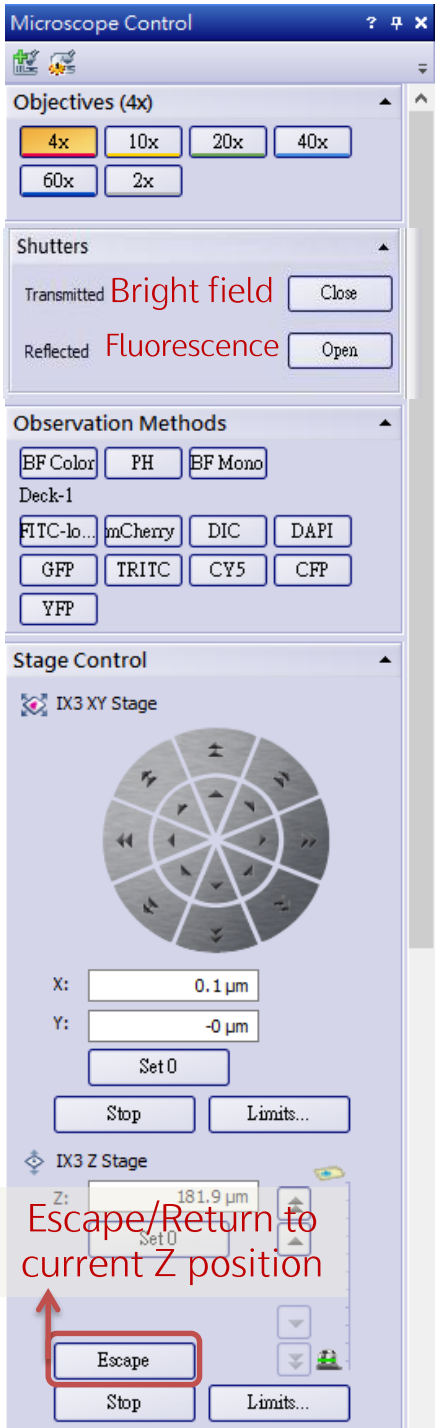
60X is a silicon oil immersion objective!

Ask the administrator first if you need 60X.

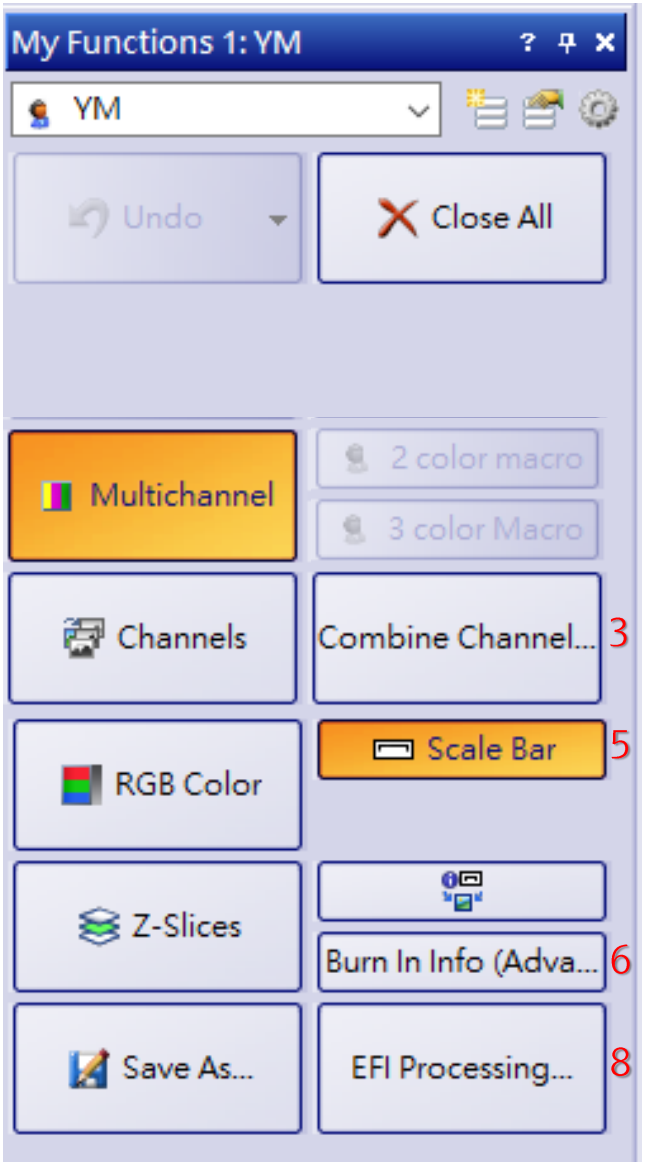
➢ Observation Methods

Method	Excitation filter	Dichromatic Mirror	Emission filter
BF color	Color bright field image, it uses the SC180 camera		
PH color	Color phase contrast image, it uses the SC180 camera		
PH	A grayscale phase contrast image, which can be combined with fluorescent images		
BF Mono	A grayscale bright field image, which can be combined with fluorescent images		
FITC-long	480/40	505	510IF
mCherry	575/20	595	645/90
DIC	A grayscale differentiated interference contrast image, which can be combined with fluorescent images		
DAPI	387/11	410	440/40
GFP	485/20	504	525/30
TRITC	560/20	582	607/36
CY5	650/13	669	684/24
CFP	427/10	440	472/30
YFP	504/12	520	542/27

* Recommend for multi-channel acquisition



My Functions

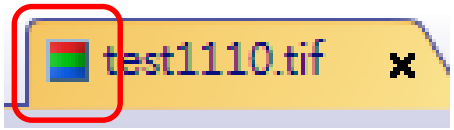


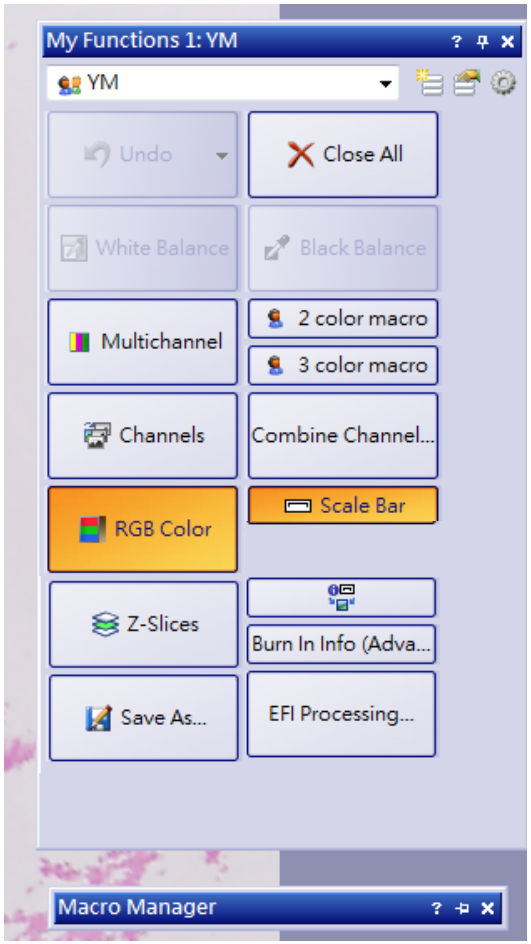
	Button	Details
1	Multi Channel	
2	Separate Channels	To separate a multi-channel image into 16-bit grayscale images
3	Combine Channels	To combine grayscale images to get an overlay 24-bit color image (details in the appendix).
4	RGB Color	To convert a grayscale image to a 24-bit color image
5	Scale Bar	To show the scale bar
6	Burn In Info	To burn in scale bar on image, Once you burn in info on an image, you cannot change or remove it! DO NOT click on resample!
7	Z-Slices	To separate a z-stack image into different z-slices
8	EFI Processing..	Extended Focal Image processing results in an image which is focused throughout all of its segments.
9	Save As…	Recommend format: *.tiff or *.vsi

Image Format: Color Image and Grayscale Image

➢ Color Image (24-bit)

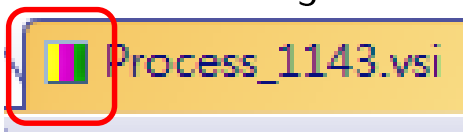
RGB color image → [Save As] .tif

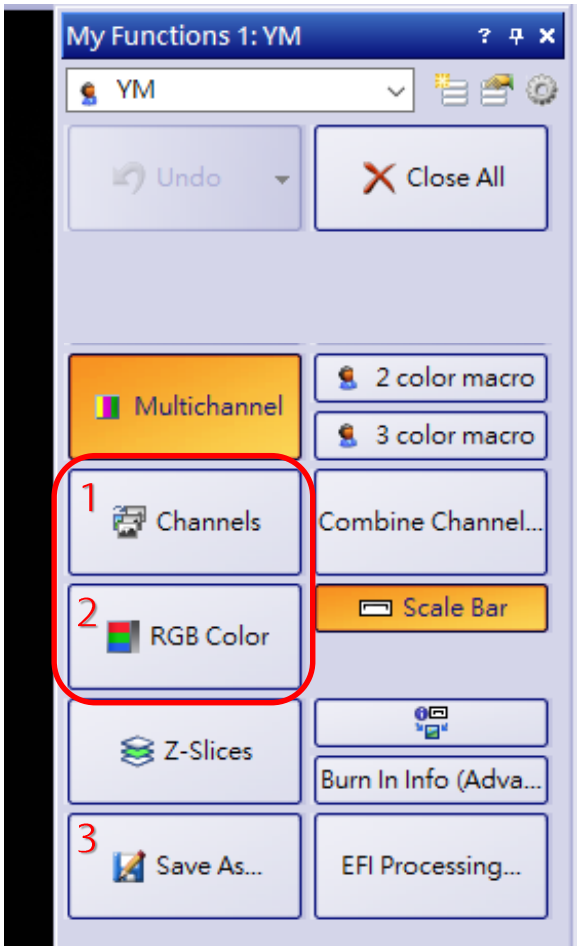




➢ Grayscale Image (16-bit)

Fluorescence snapshot or Multi-Channel Images





→ [Save As] .vsi
Then use the Viewer to draw a scale bar or export to .tif

or

→ Use "My Functions" to separate channels, to convert to RGB color images and to save as *.tif

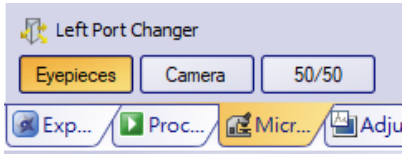
➢ 1. Channels:
Separate Multichannel → Grayscale images

➢ 2. RGB color:
Convert grayscale to a color image (24-bit)

➢ 3. Save As⋯ (.tif)

Acquiring **Single** Snapshot

1. Choose an objective
2. Choose an observation method
3. Left Port Changer

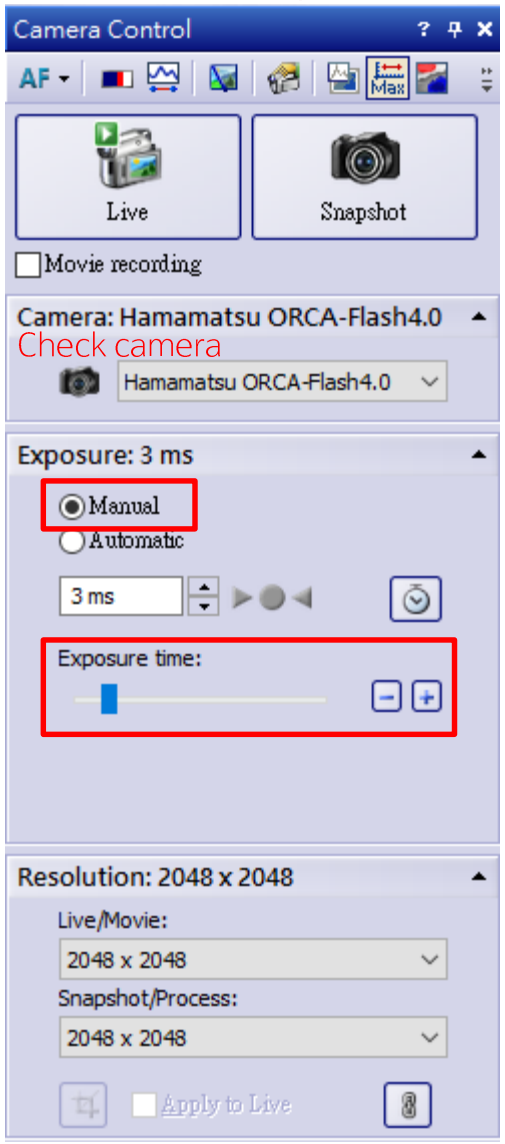


Microscope Control →→

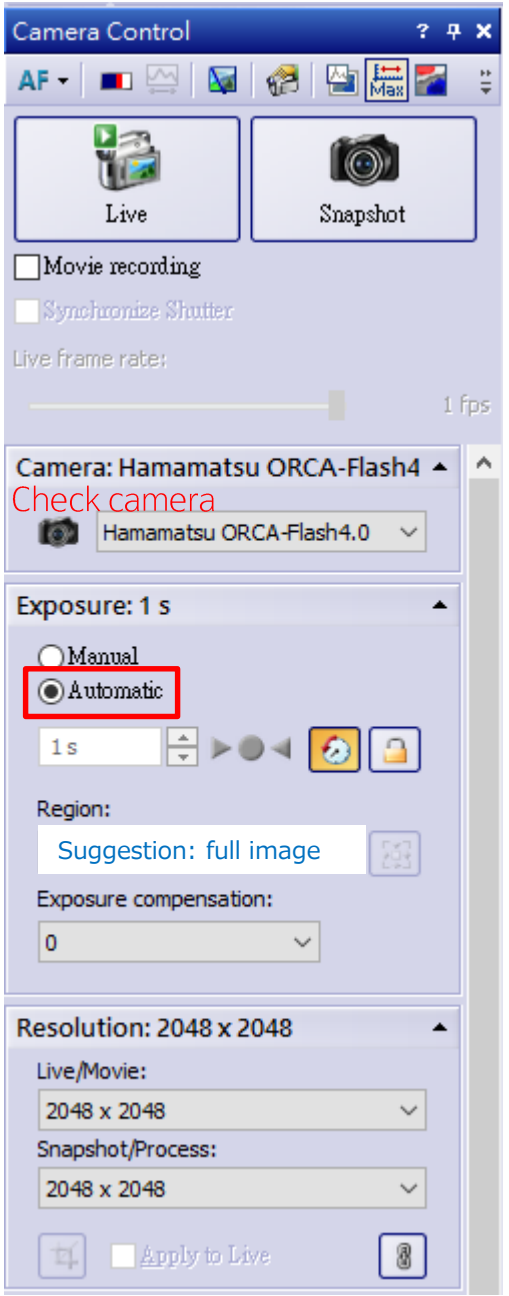
Observation Methods	Left Port Changer	Camera
BF color, PH color	Eyepieces or 50/50	SC180
BF mono, PH, DAPI, GFP, etc.	Camera	Hamamatsu

4. Find a required view from the eyepieces
5. Click [Live] to adjust focus
   - [Ctrl + H]: to switch to the range indicator mode to help set exposure time
6. Set exposure time:
   - It suggests using automatic exposure for bright-field images and entering the exposure time manually for fluorescence images.
7. Adjust the field of view and its focus
8. Click [Snapshot] to acquire a single image
9. [Save As]

Manual Exposure



Automatic Exposure



Acquiring a **Multi-Channel** Image

1. Go to the [Process Manager] tool window, which is located on the bottom right of the user interface
2. Select [Multi Channel]
3. Add Channels
4. Choose one channel

   → [Live] to set exposure conditions

   → [Read Settings] to save the current exposure time

   Set the exposure time for every channel, respectively

   ✧ Read Z-offset of every channel if necessary

   (1) Start with the first channel

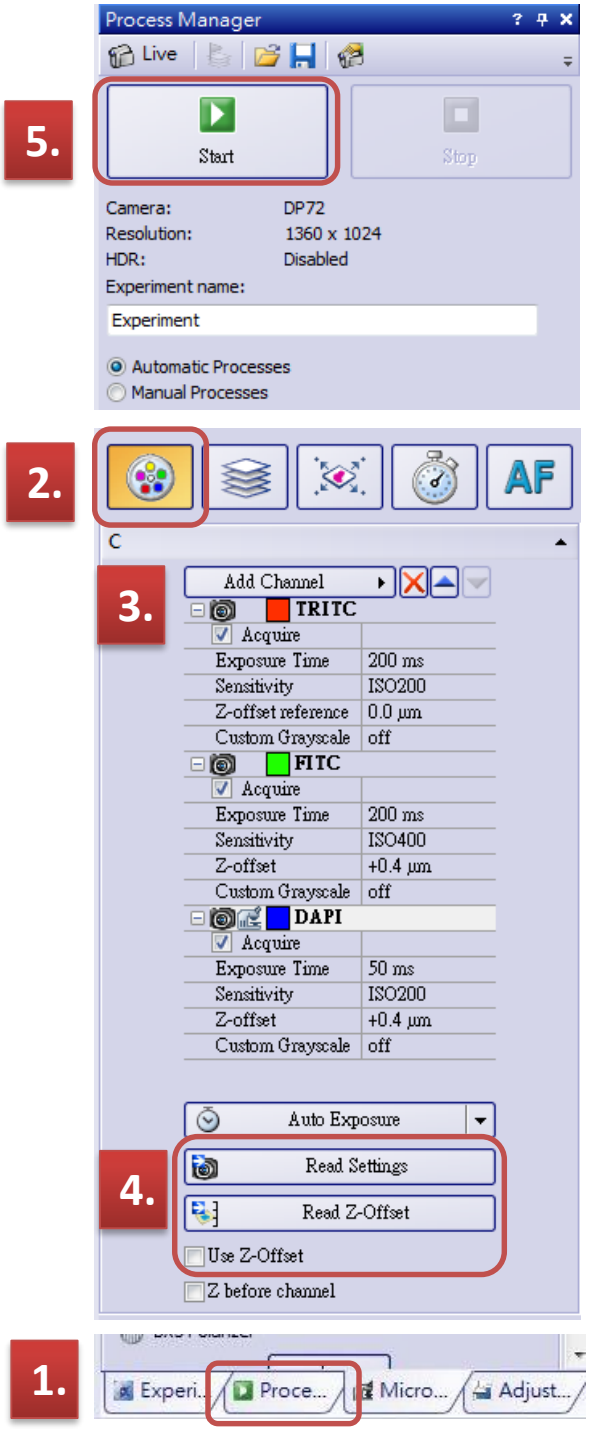
   (2) Click [Live] and adjust Z focus → Read Z-offset

   (3) Adjust and read the Z-offset for each of the channels

   (4) Select ☑ Use Z-offset

5. Click [Start] to acquire multi-channel images

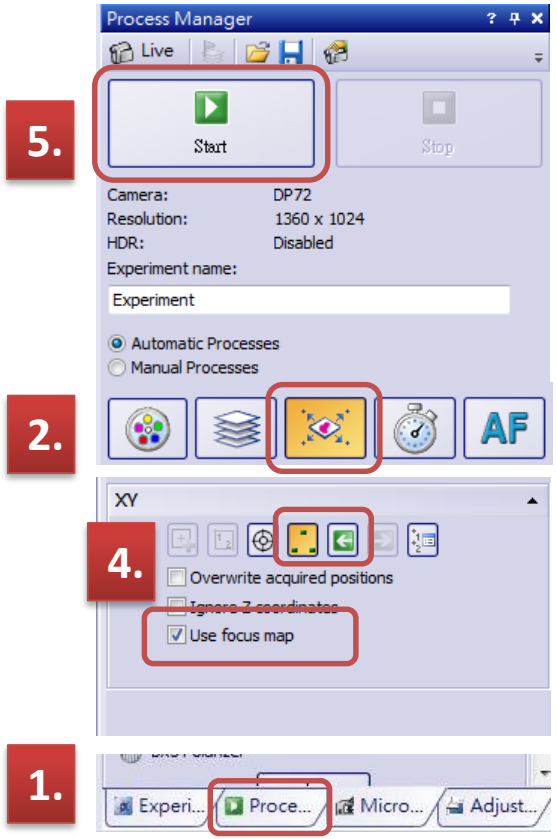
➢ Can be combined with multi-position, Z-stack, or time-lapse for multi-dimensional images

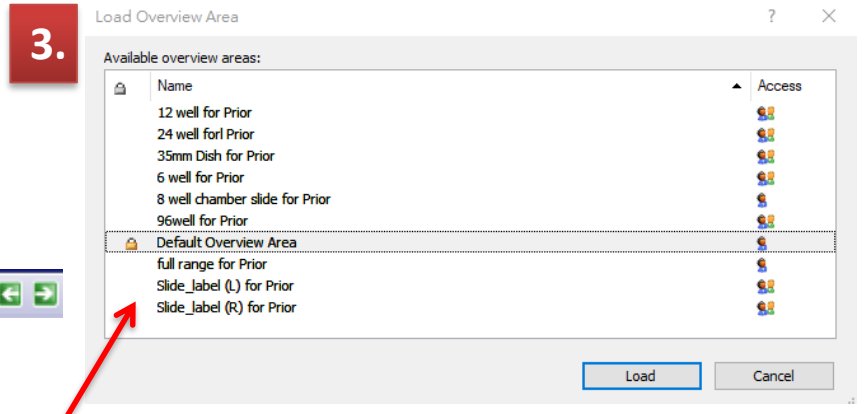


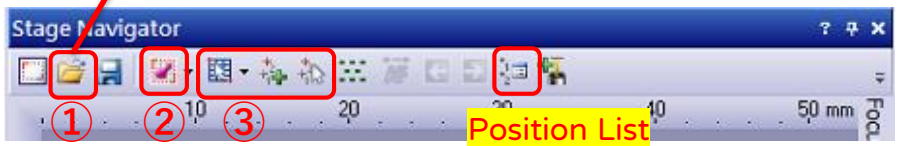
Multi Position(1): Stage Navigator

Sample type: slide or 35mm Dish

1. Got to the [Process Manager] tool window, which is located on the bottom right of the user interface
2. Select [MIA] for Multi Position
3. Go to the [Stage Navigator] tool window
   - ①  Load Overview Area
   - ② Adjust focus and exposure time, then Acquire Overview
      Automatically acquire an overview image with the lowest objective
   - ③ Add multiple positions or define a rectangle/polygon area to scan
      Define a rectangle/circle/polygon area
      Add single positions
      Select positions
4. (Optional) Set up focus map if necessary 
5. Click [Start] to acquire multi-position images





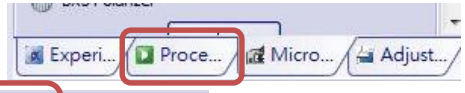


➢ Can be combined with multi-position, Z-stack, or time-lapse for multi-dimensional images

Multi Position(2): Well Navigator

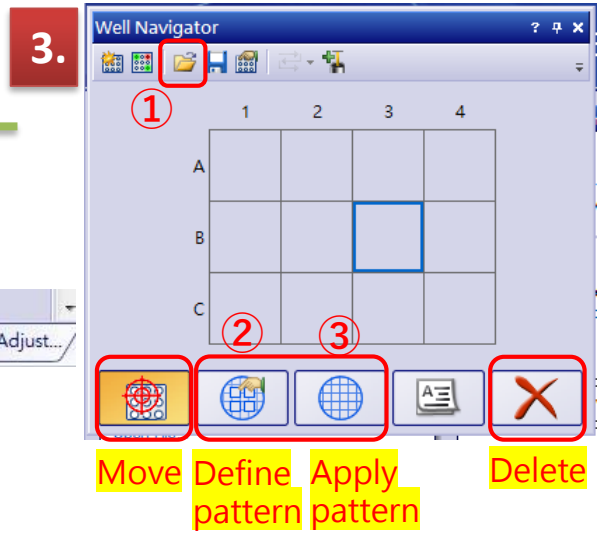
Sample type: well plate or chamber slide

1. Open the [Process Manager] tool window, located at the bottom right of the user interface



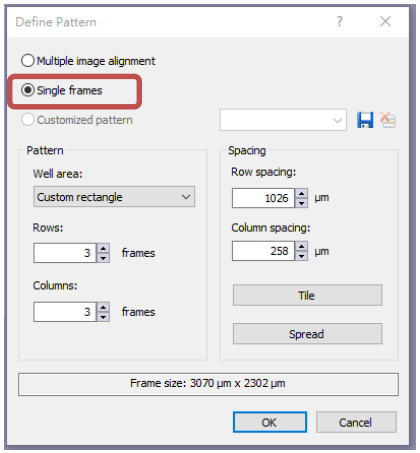
2. Select [MIA] for Multi Position

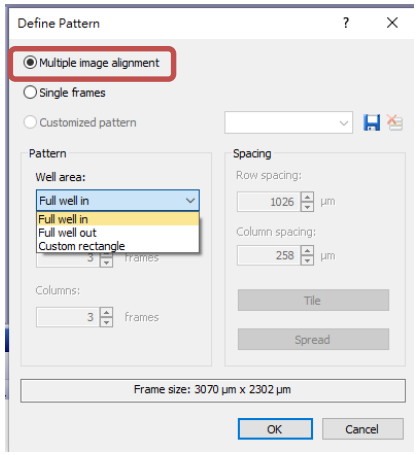
3. Well Navigator



① Load Overview Area

② Define Pattern: choose between a single frame or an area

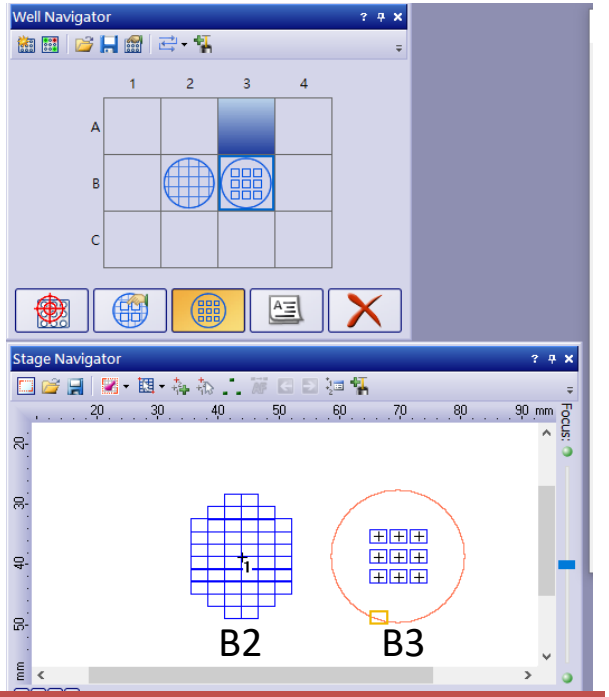




Single frame:
one or more positions arranged in rows and columns with customizable spacing.

Multiple image alignment:
Result in a stitched area, which could be well in/out or custom rows x columns

③ Apply Pattern



Example:
B2: Multiple image alignment + Well-in
B3: single images + 3x3 with spacing

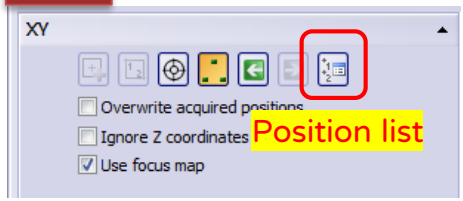
Multi Position(2): Well Navigator

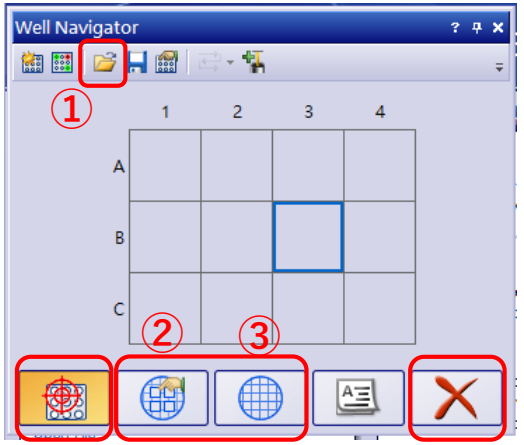
Sample type: well plate or chamber slide

4. Use [Position List] to move to each position, click [Live], and adjust its x/y/z positions
   → Update XY / Update Z
5. (Optional) For Live-cell Time-lapse Imaging, it is recommended to use [AF] with [ZDC] to maintain the focus stability
6. Click [Start] to acquire MIA images

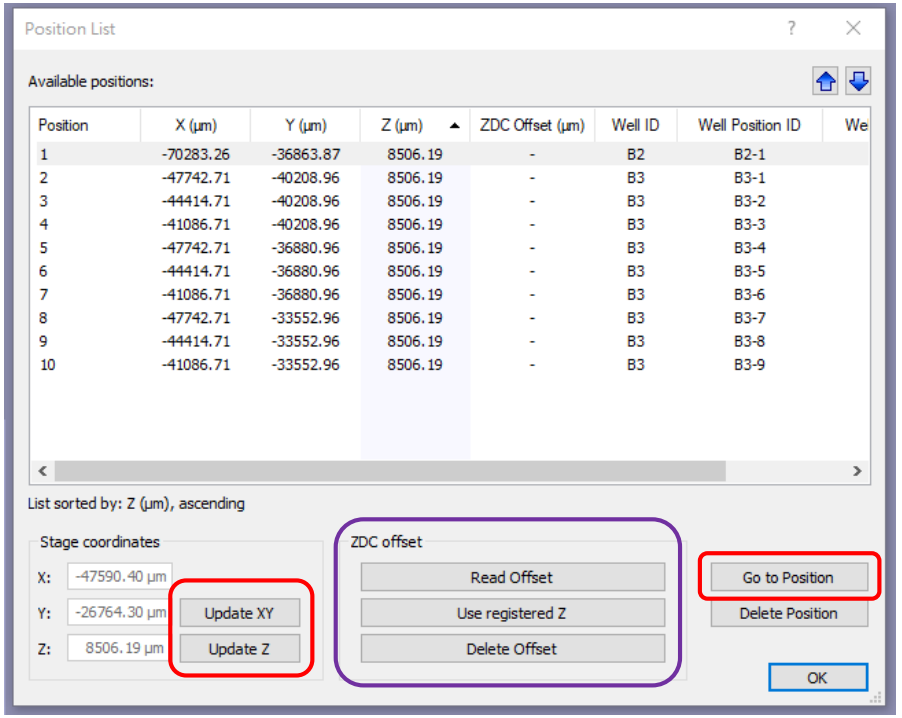
➢ Can be combined with multi-channel, time-lapse & AF

4-1.



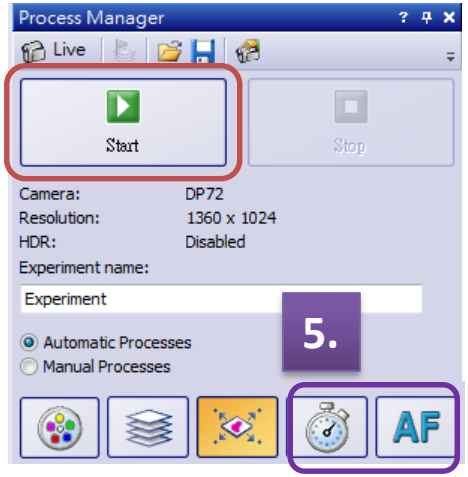


4-2.



Set [ZDC offset] to maintain focus stability for live-cell imaging

6.



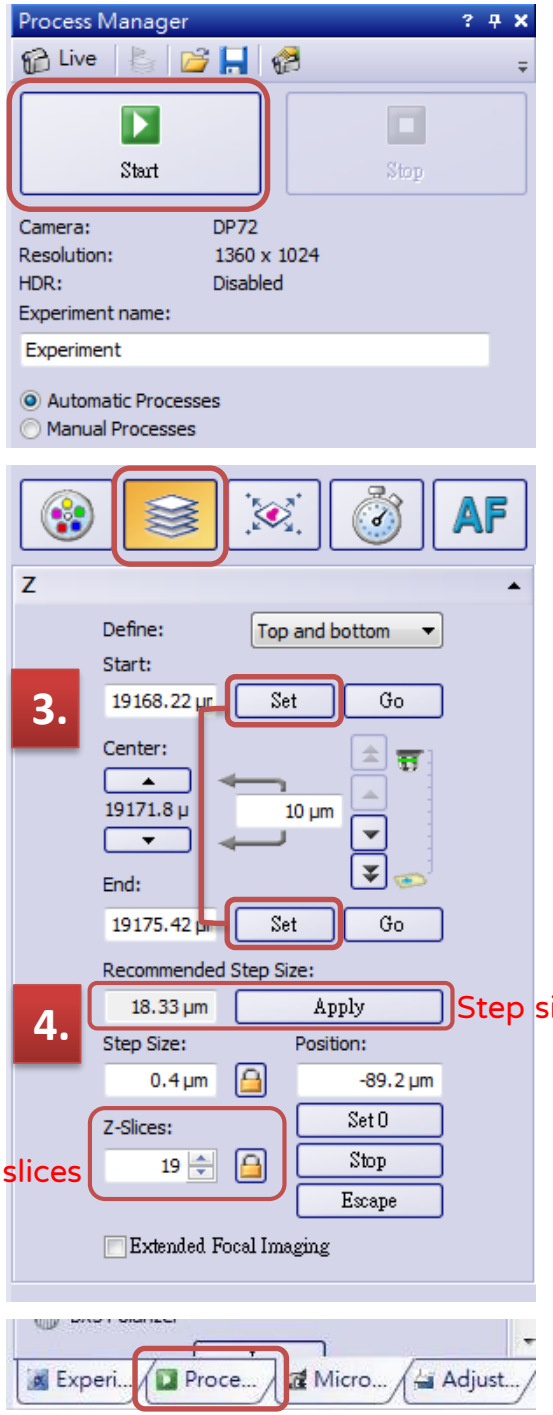
[Time-lapse] & [AF]

Acquiring a Z-stack Image

1. Go to the [Process Manager] tool window, which is located on the bottom right of the user interface
2. Select [Z-Stack]
3. Click [Live] and move focus upwards/downwards to set the upper/lower focus position, respectively
4. Apply the recommended step size or set the distance between two frames manually
   - ✧ Click [Go] to the top/bottom position for confirmation
   - ✧ Suggestion: DO NOT select ☐ Extended Focal Imaging

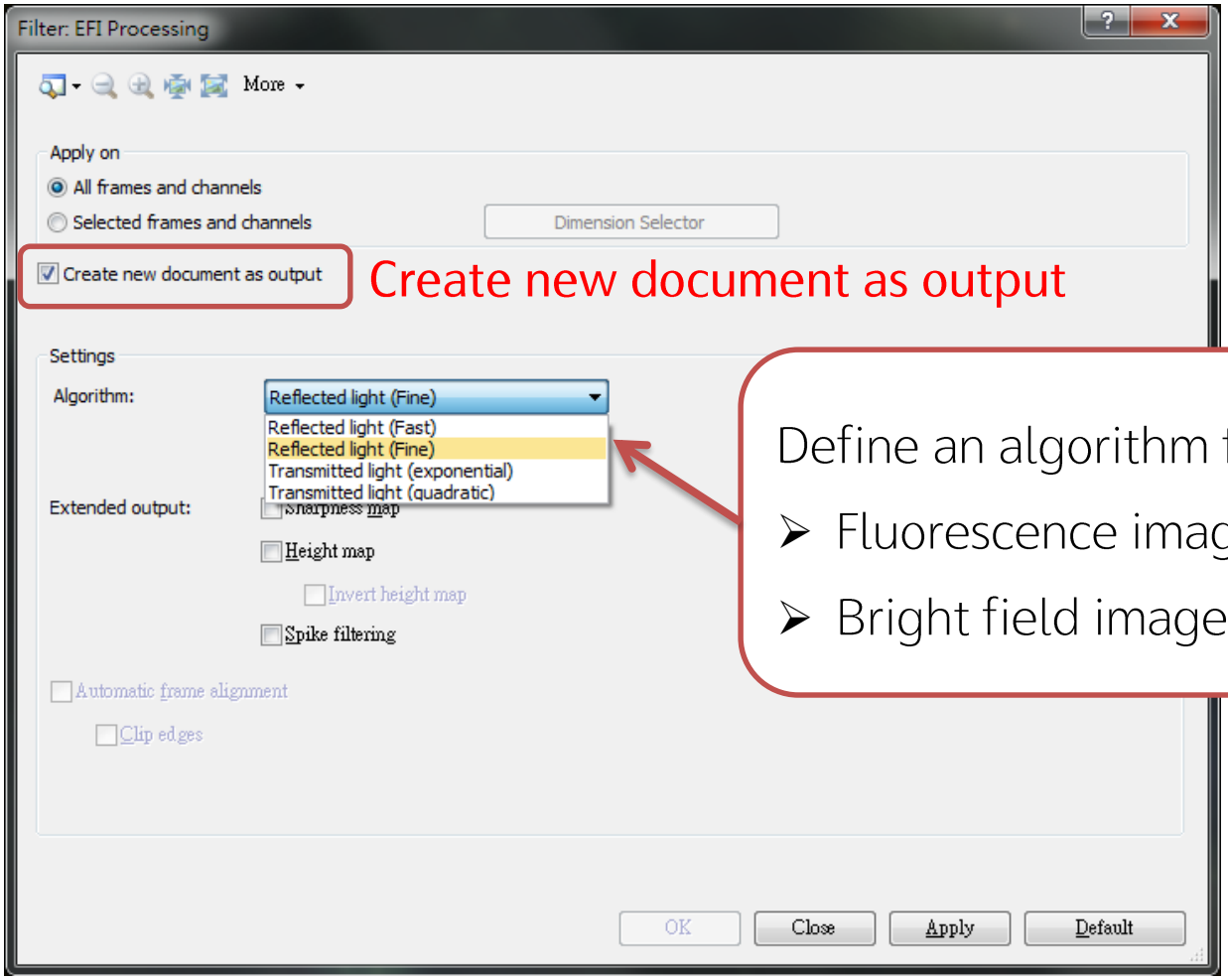
   The Z-stack image can be processed into EFI afterwards if needed. However, if EFI is selected, only one image will be generated, with focus maintained throughout all its segments.
5. Click [Start] to acquire Z-stack images

➢ Can be combined with multi-channel or multi-position for multi-dimensional images



EFI Processing

(Extended Focal Image)



Define an algorithm for EFI processing:

- Fluorescence image → Reflected light
- Bright field image → Transmitted light

Stage-top incubator

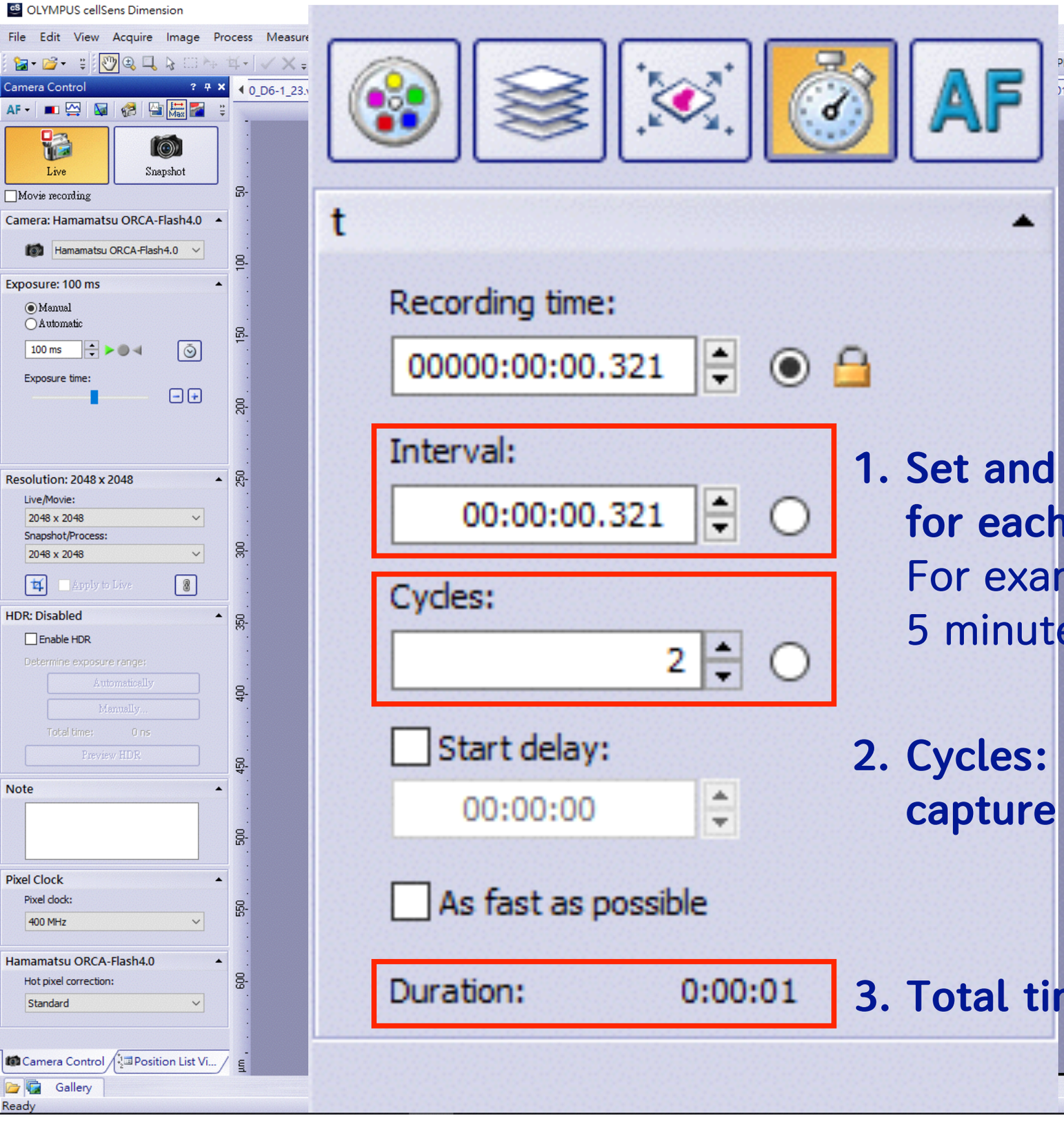
Carefully rotate clockwise to remove the glass cover for 35mm dish after use

- The water bath volume is 40mL.
- It is suggested to add a maximum of 20mL in case the liquid spills out during stage movement
- Please use only TYPE III water (ddH_2O)
- The evaporation rate within the water bath is approximately 0.5 to 1 mL per hour.

⑩ Stage top incubator
Temperature and CO_2 control, typically set to 37℃, 5% CO_2

⑪ Thermo Box
Activate only if the image acquisition time exceeds 72 hours.

Process Manager – XYT (Time-Lapse)



1. Set and lock the time "Interval" for each cycle.
   For example, enter "300" to set 5 minutes time interval.
2. Cycles: the number of images to capture
3. Total time duration will be calculated

How to clean the silicone immersion objective

These are not Kimwipes !

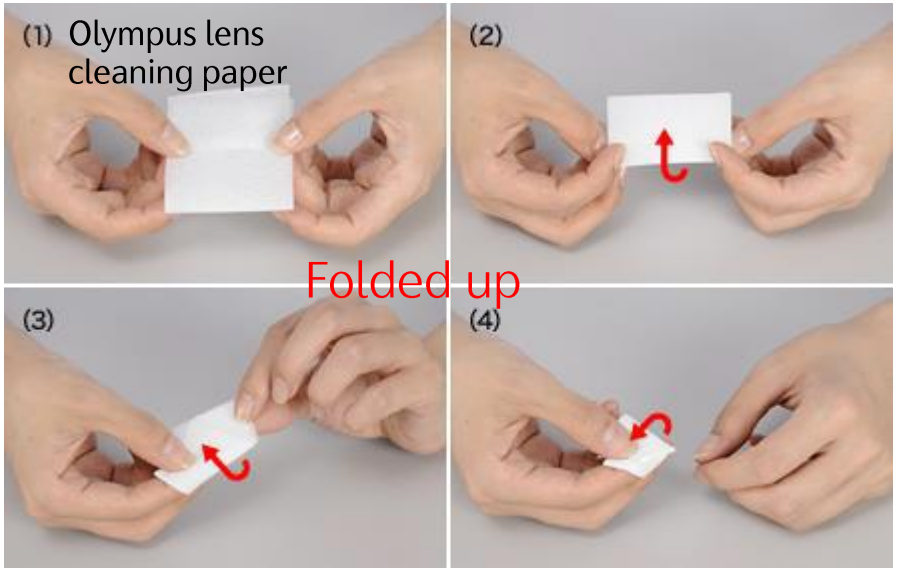
60X

(1) Wipe the excess immersion oil off with a clean lens cleaning paper

(2) Take a new one and fold it up

(3) Rinse the corner of folded lens cleaning paper with 95% Ethanol, then gently clean the objective with a spiral motion from the center to the rim

(4) Check with another new lens cleaning paper



[Schematic Diagram]
Please don't remove the objective!

Don't add too much immersion oil to the sample; a small drop would be enough!

Olympus Silicone Immersion Oil

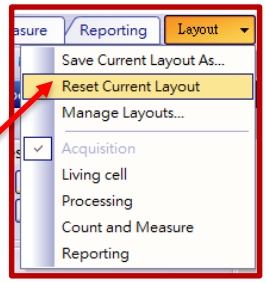
Inverted Microscope Olympus IX83

System Shutdown

⑧ Exit cellSens

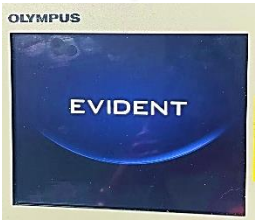
a. Before exiting the software → YM2019 [Layout ▼] → Reset Current Layout

b. Change to 4X objective, and lower it down to Z position = 0



⑦ Touch Panel Control Tap on [OFF] to shut down TPC.

Wait until it shows [Evident] on the screen, then press the switch in the back once.



⑥ Turn off the LED light source

⑤ ④ Turn off CBH and U-CBF (microscope control)

③ Turn off the Automatic stage

② Shut down the computer

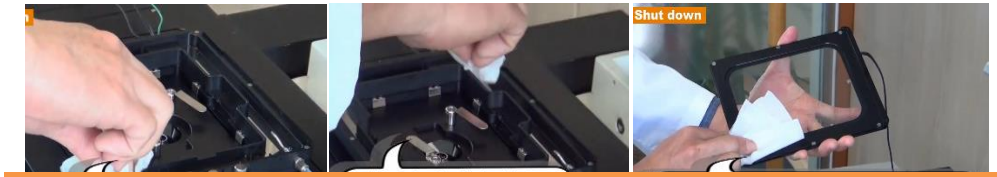
① Turn off the EMCCD (Hamamatsu ORCA-Flash4.0 V3 sCMOS)

Shut down the live-cell incubation system if used.

⑨ CO_2

⑩ Stage top incubator

⑪ Thermo box



Clean the water bath with 95% EtOH and wipe dry with Kimwipes if used.

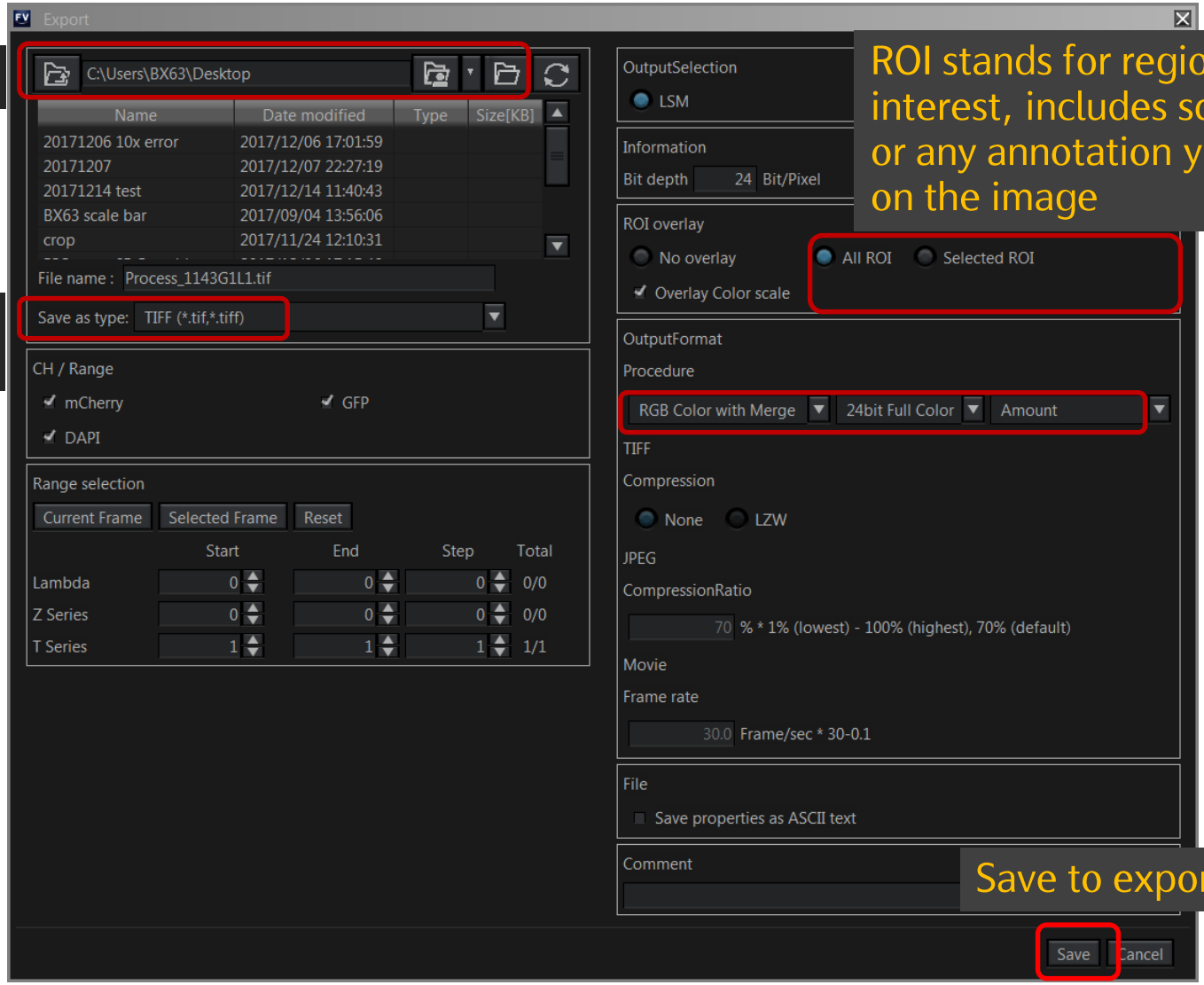
Ensure all devices are properly turned off before leaving, and remember to sign the usage record.

Image export by FV31S-DT viewer (64-bit)

- Open Multi-dimensional images (*.vsi or *.tif) → right click and choose Export

Output folder

Suggested format: *.tif



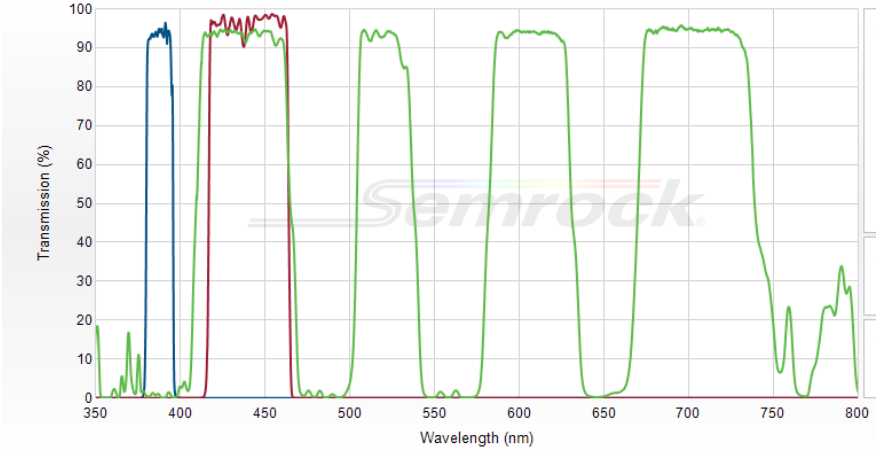
ROI stands for region of interest, includes scale bar or any annotation you draw on the image

Save to export

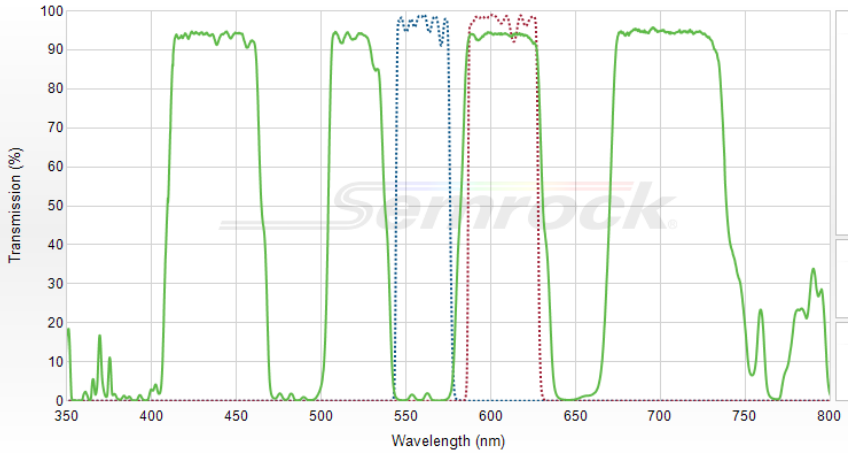
Fluorescence Filter Set (1)

Excitation
Emission

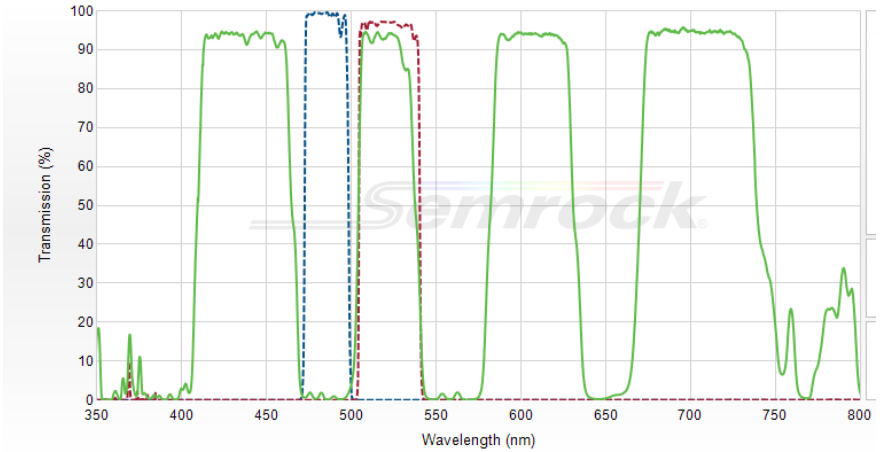
DAPI



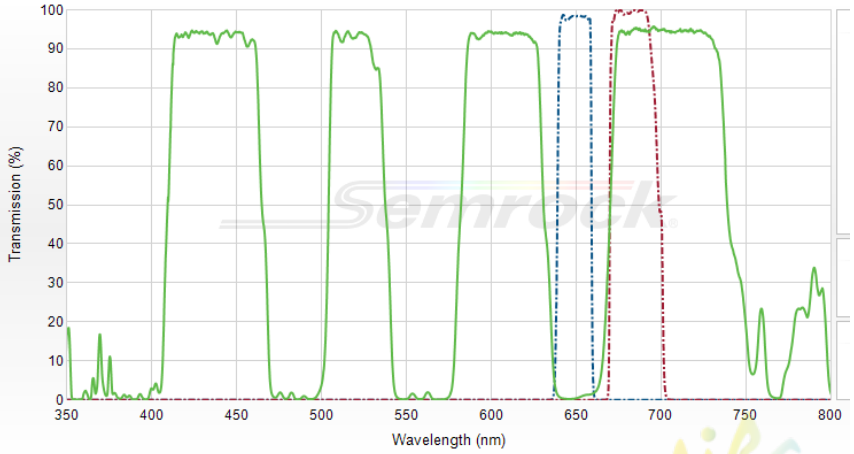
TRITC



GFP



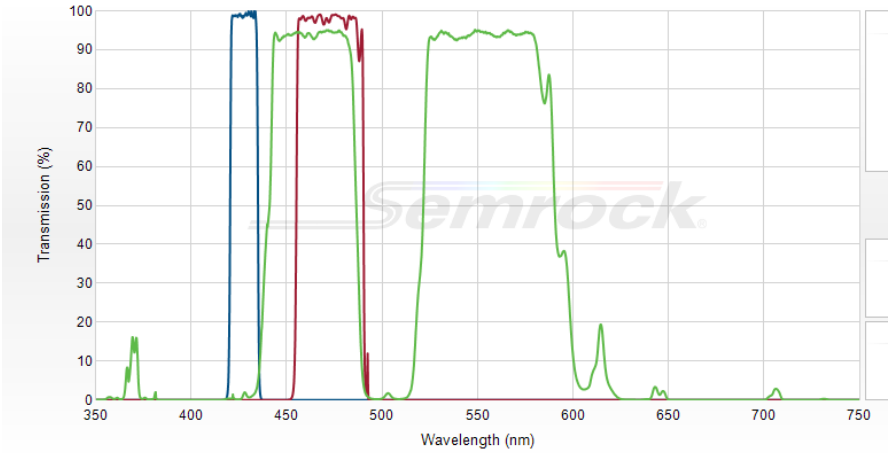
Cy5



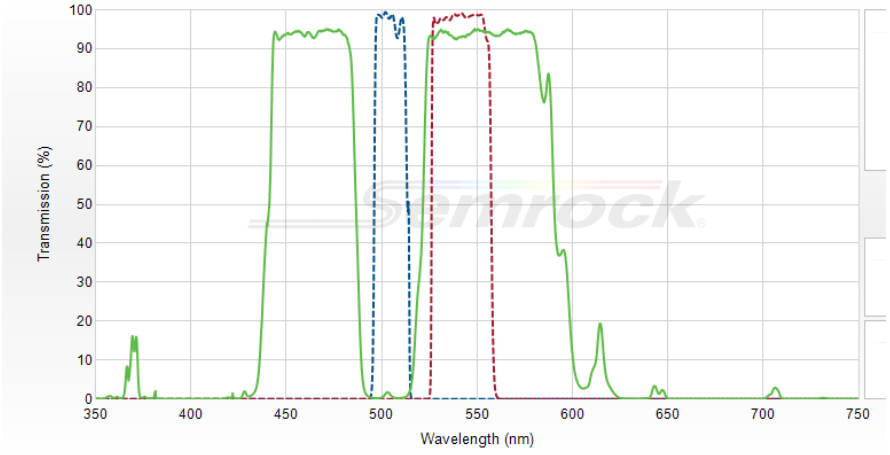
Fluorescence Filter Set (2)

Excitation
Emission

CFP



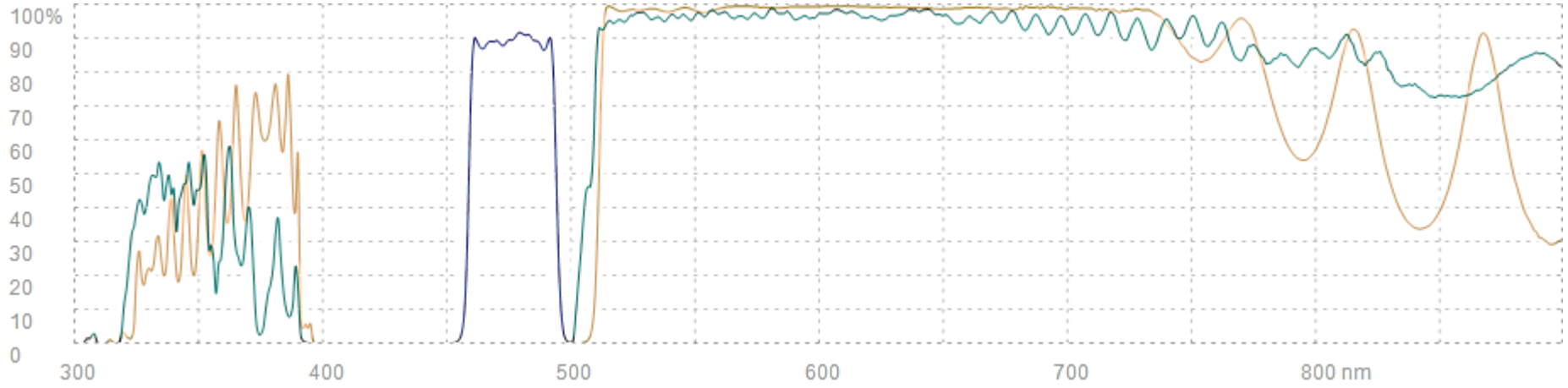
YFP



Fluorescence Filter Set (3)

Excitation
Emission

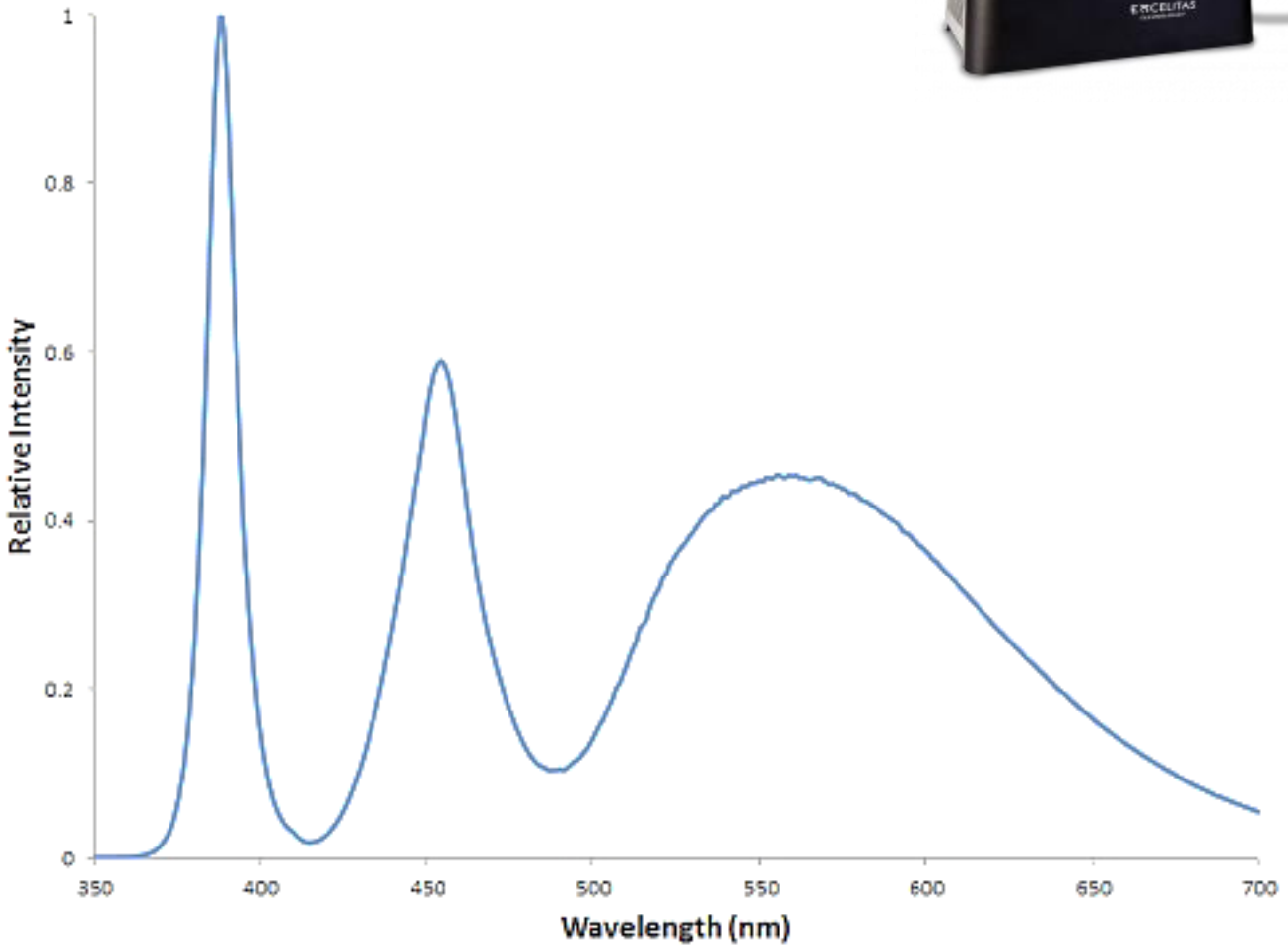
FITC-long



mCherry

Fluorescence Light Source

X-cite® 120 LED *Boost*



Combine Channels

Please first open grayscale images in the software they must have the same resolution and bit depth to enable image overlay.