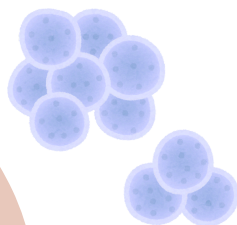
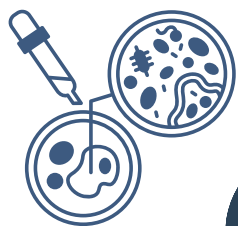


Olympus IX83

Inverted Fluorescence Microscope

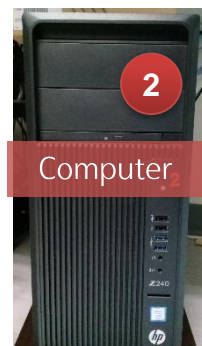
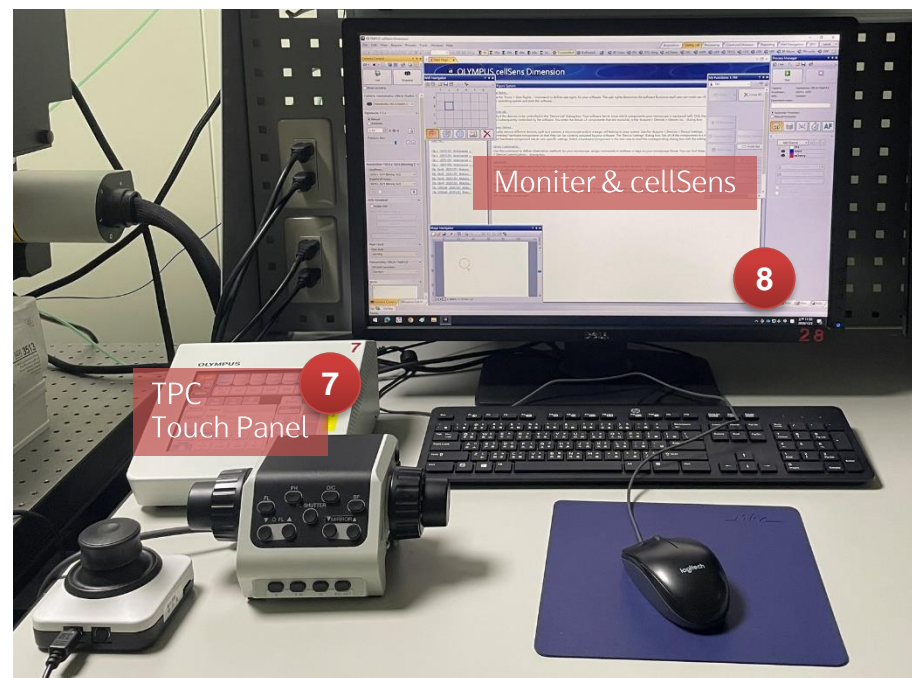


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Inverted Microscope Olympus IX83

System Startup (1)

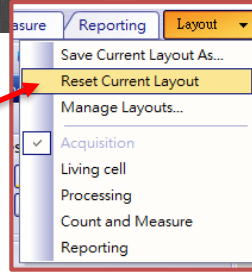
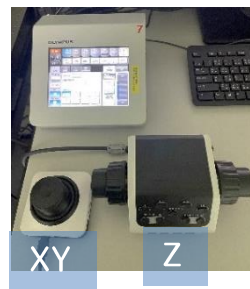


Inverted Microscope Olympus IX83

System Startup (2)

- ① EMCCD (Hamamatsu ORCA-Flash4.0 V3 sCMOS)
- ② Computer
- ③ Automatic Stage
- ④ U-CBF (Microscope control)
- ⑤ CBH (Microscope control)
- ⑥ LED (Fluorescent light source)
- ⑦ Touch Panel Control
- ⑧ Software: cellSens [Layout ▼] YM2017/ Reset Current Layout

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



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

- ⑨ CO₂
- ⑩ Stage top incubator
- ⑪ Thermo Box

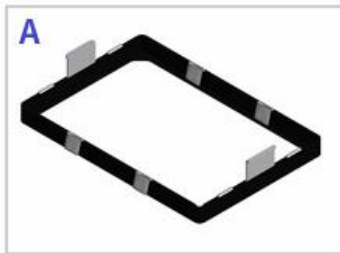
Please rotate the black switch and check the pressure value.

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.



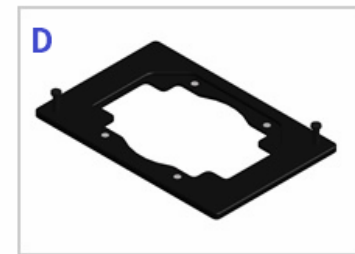
A
ATX-W
For Well Plate



B
ATX-CSG
Chamber slide, slide glass and
Chambered coverglass



C
ATX-D
For 35/50/60mm dish



D
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

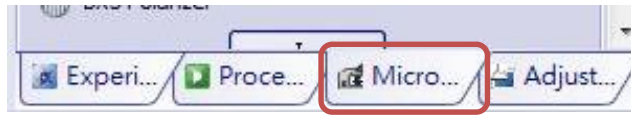
After replacing the inner fixture, the following specifications can be accommodated:

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

Microscope Control

Objectives (4x)

4x 10x 20x 40x
60x 2x

Shutters

Transmitted **Bright field** Close
Reflected **Fluorescence** Open

Observation Methods

BF Color PH BF Mono
Deck-1
FITC-lo... mCherry DIC DAPI
GFP TRITC CY5 CFP
YFP

Stage Control

IX3 XY Stage

X: 0.1 μm
Y: -0 μm
Set 0
Stop Limits...

IX3 Z Stage

Z: 181.9 μm
Set 0
Escape
Stop Limits...

Escape/Return to current Z position

My Functions

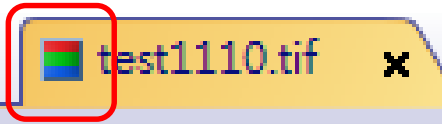


	Button	Details
1	Multi Channel	
2	Separate Channels	To separate a multi-channel image into 16-bit grayscale images
3	Combine Channels	To combine grayscale images to get an overlay 24-bit color image (details in the appendix).
4	RGB Color	To convert a grayscale image to a 24-bit color image
5	Scale Bar	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

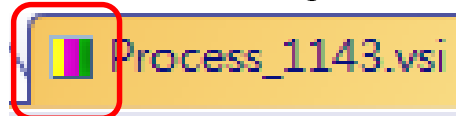
➤ Color Image (24-bit)

RGB color image → [Save As] .tif



➤ Grayscale Image (16-bit)

Fluorescence snapshot or Multi-Channel Images

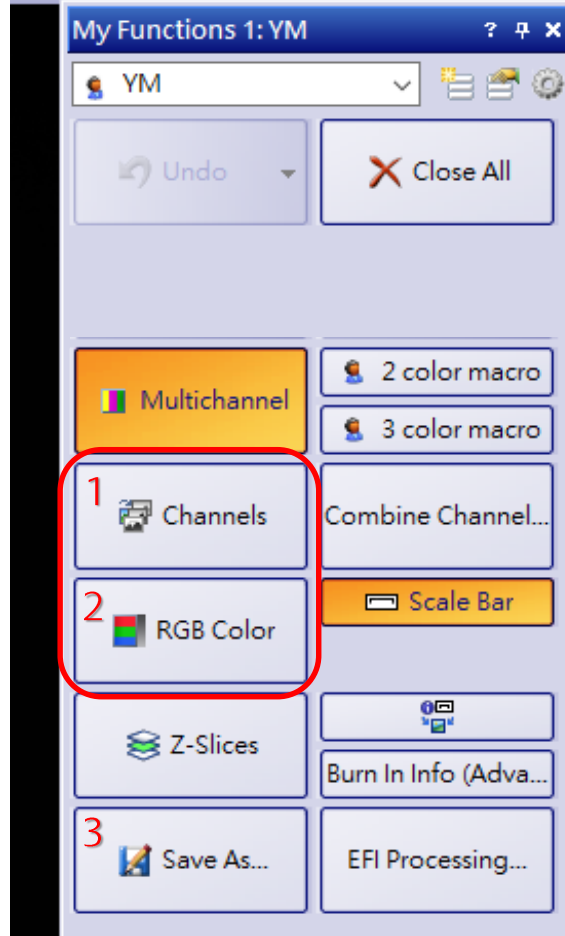
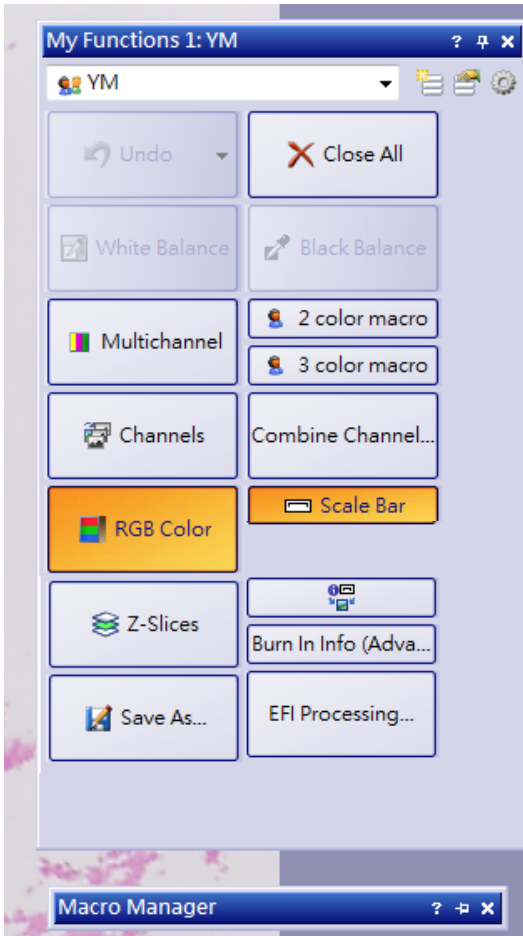


→ [Save As] .vsi

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



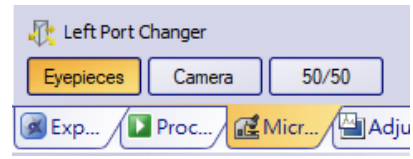
➤ 1. Channels:
Separate Multichannel
→ Grayscale images

➤ 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

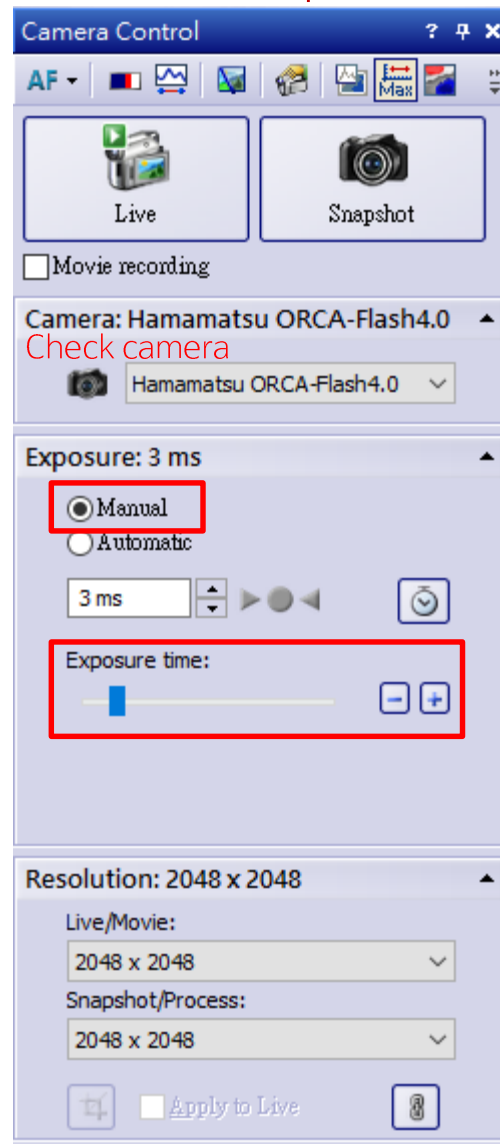


Microscope Control →→

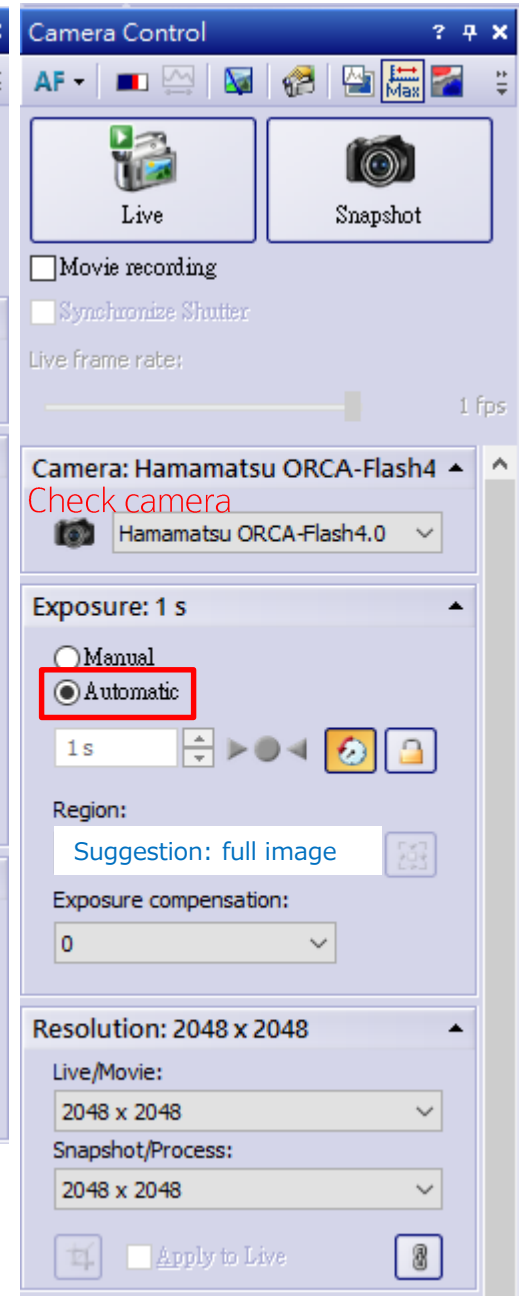
Observation Methods	Left Port Changer	Camera
BF color, PH color	Eyepieces or 50/50	SC180
BF mono, PH, DAPI, GFP, etc.	Camera	Hamamatsu

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

The screenshot shows the 'Process Manager' window with several steps highlighted by red boxes and numbers:

- 5.** The 'Start' button is highlighted in a red box.
- 2.** The 'Multi Channel' icon (a circle with colored dots) is highlighted in a red box.
- 3.** The 'Add Channel' dropdown menu is highlighted in a red box, showing a list of channels: TRITC, FITC, and DAPI. Each channel has its own settings table.
- 4.** The 'Read Settings' and 'Read Z-Offset' buttons are highlighted in a red box.
- 1.** The 'Process Manager' button in the bottom right corner is highlighted in a red box.

Channel Settings Tables:



Channel	Acquire	Exposure Time	Sensitivity	Z-offset reference	Custom Grayscale
TRITC	<input checked="" type="checkbox"/>	200 ms	ISO200	0.0 μm	off
FITC	<input checked="" type="checkbox"/>	200 ms	ISO400	+0.4 μm	off
DAPI	<input checked="" type="checkbox"/>	50 ms	ISO200	+0.4 μm	off




Additional settings shown at the bottom:


- Auto Exposure:
- Read Settings:
- Read Z-Offset:
- Use Z-Offset:
- Z before channel:

Multi Position(1): Stage Navigator

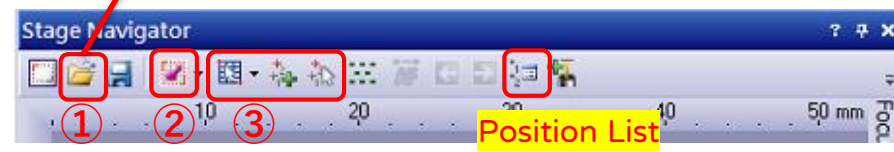
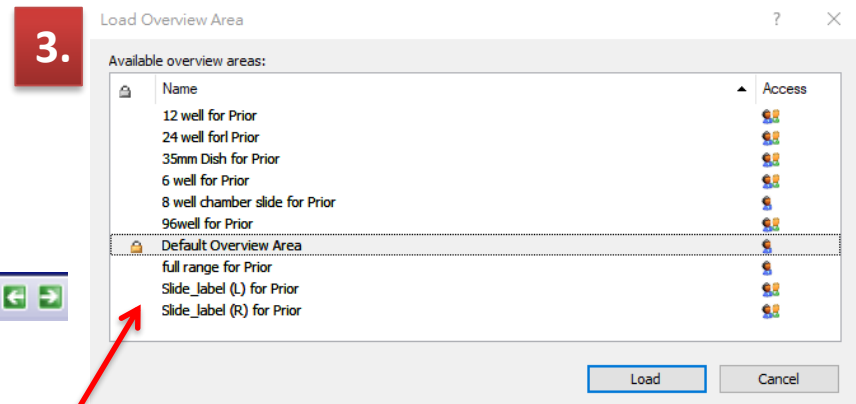
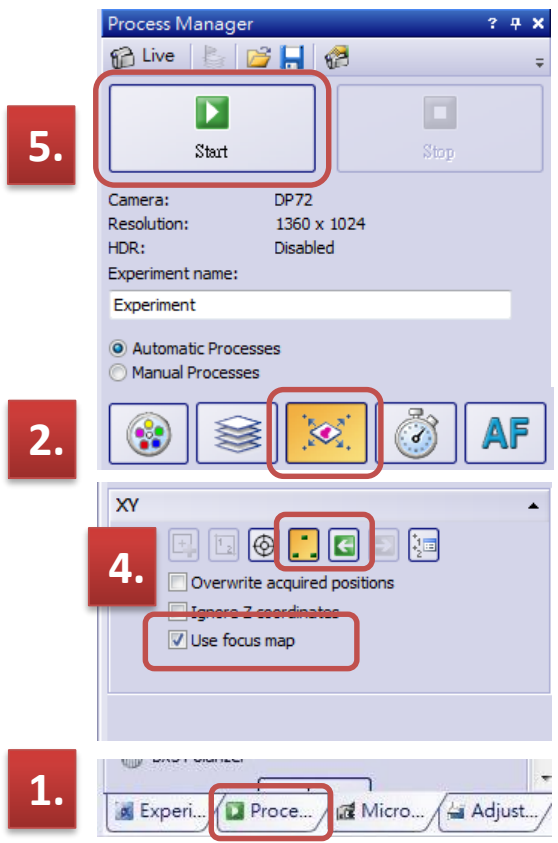
Sample type: slide or 35mm Dish

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

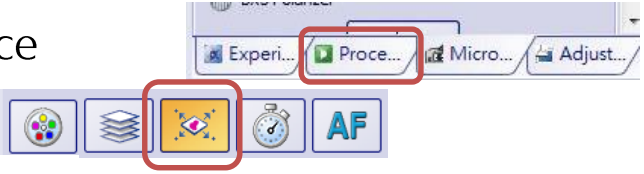


Multi Position(2): Well Navigator

Sample type: well plate or chamber slide

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

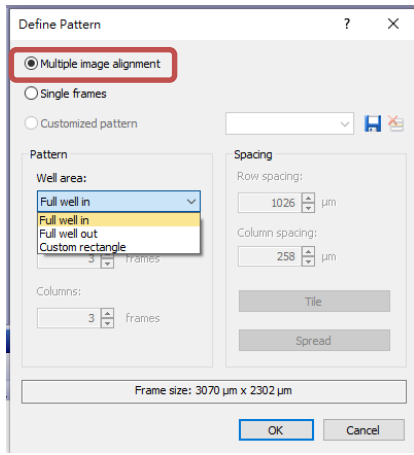
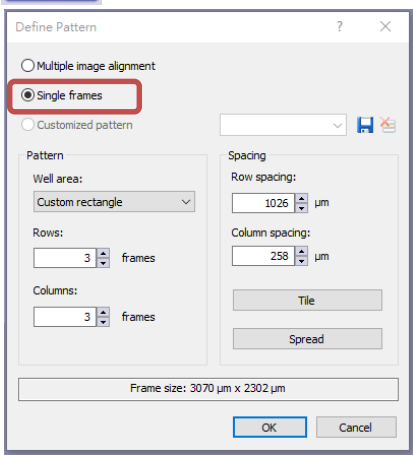
2. Select [MIA] for Multi Position



3. Well Navigator

① Load Overview Area

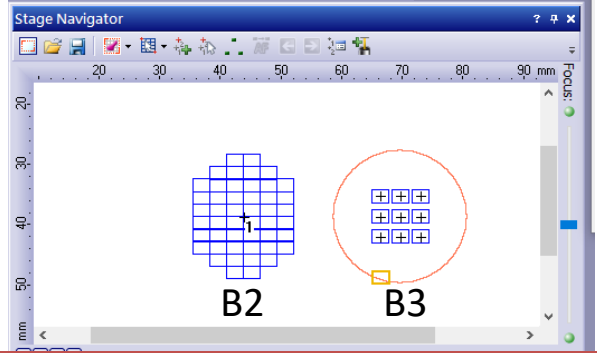
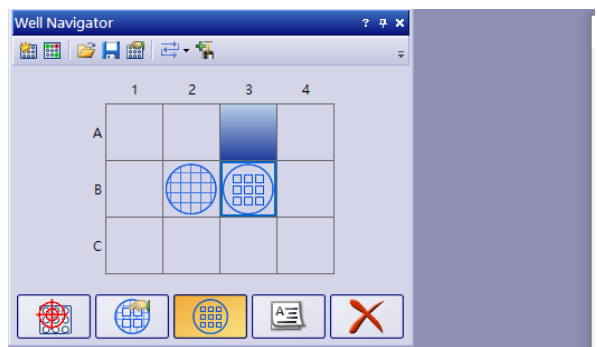
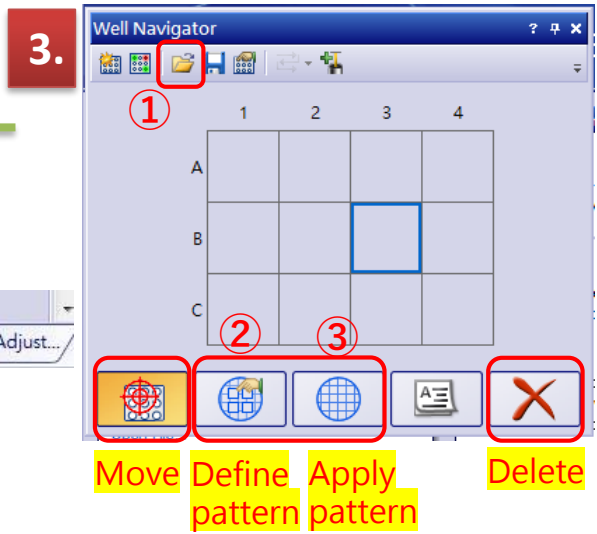
② Define Pattern: choose between a single frame or an area



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



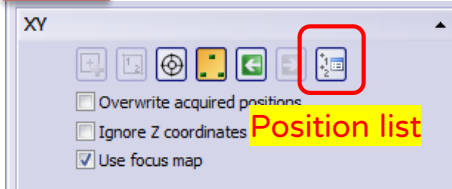
Example:
B2: Multiple image alignment + Well-in
B3: single images + 3x3 with spacing

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

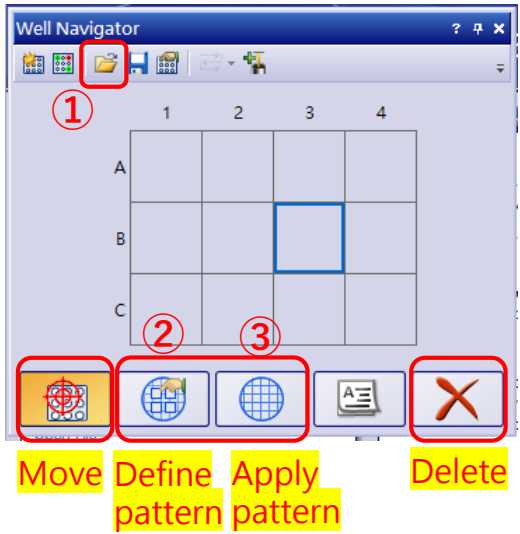
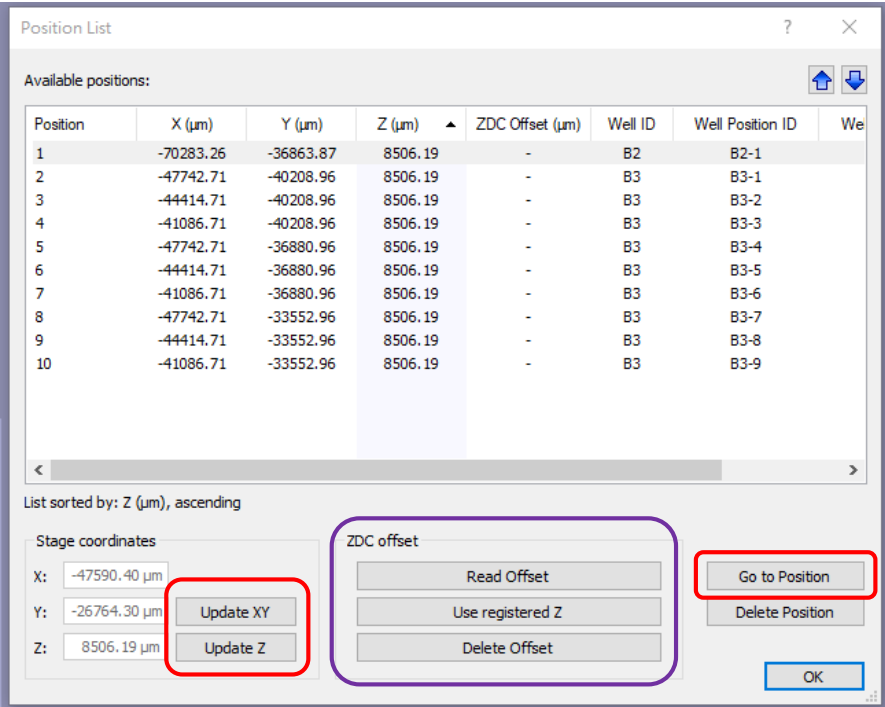
4-1.



→ 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

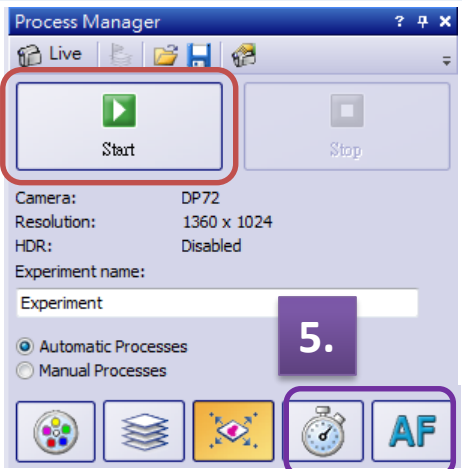
4-2.



6. Click [Start] to acquire MIA images

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

6.



5.

[Time-lapse] & [AF]

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

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

5. Start

Camera: DP72
Resolution: 1360 x 1024
HDR: Disabled
Experiment name: Experiment

Automatic Processes
Manual Processes

2. Z-Stack

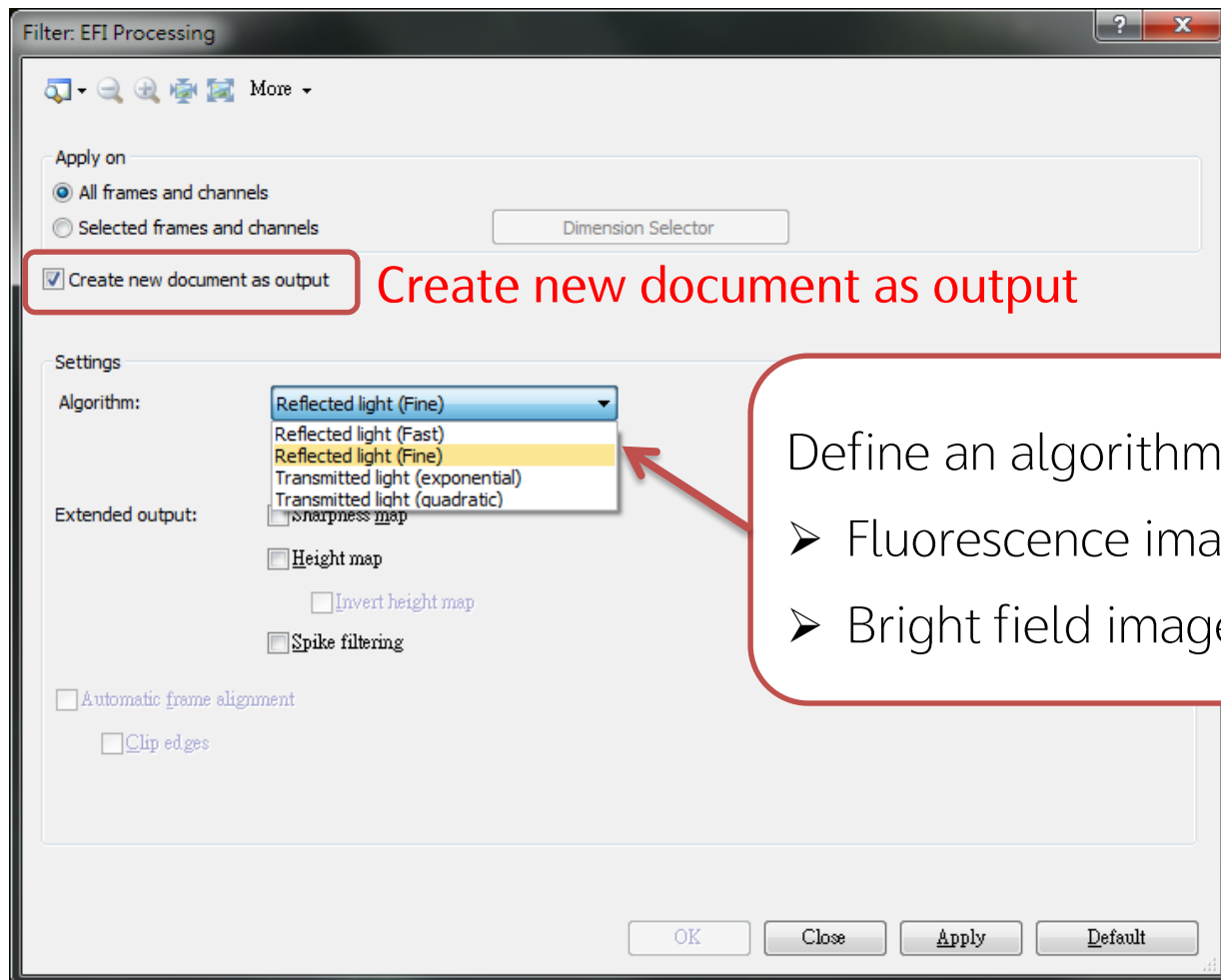
3. Start: 19168.22 μ m Set Go
Center: 19171.8 μ m 10 μ m
End: 19175.42 μ m Set Go

4. Recommended Step Size: 18.33 μ m Apply Step size
Step Size: 0.4 μ m Position: -89.2 μ m
Z-Slices: 19 Total Z slices
Set 0
Stop
Escape
 Extended Focal Imaging

1. Process

EFI Processing

(Extended Focal Image)



Create new document as output

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 (ddH₂O)
- The evaporation rate within the water bath is approximately 0.5 to 1 mL per hour.



Shut down

① Remove water and use a Kimwipe with ethanol to clean it

Use soft cloth to remove remaining water & use a small amount of ethanol with soft cloth (Kimwipe) to wipe after



Shut down

② Keep the water bath completely dry

Finally wipe it again with dry cloth. Keep the Water Bath completely dry condition. CAUTION!! the remaining water can result in molt, breeding of bacteria



Shut down

③ Clean the top heater with Kimwipe and ethanol

Hold the frame and clean Top Heater with soft close + ethanol. NOTE: clean it gently to prevent breakage (Top Heater is made of glass).



⑩ Stage top incubator Temperature and CO₂ control, typically set to 37°C, 5% CO₂

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

Process Manager - XYT (Time-Lapse)

The screenshot displays the 'Process Manager' window in the OLYMPUS cellSens Dimension software. The window is titled 'Process Manager' and contains several tabs: 'Acquisition', 'Well Navigation', 'Processing', 'Count and Measure', 'Reporting', and 'Layout'. The 'Acquisition' tab is active, showing various channel names like FITC-long, mCherry, DIC, DAPI, GFP, TRITC, CY5, CFP, YFP, and BF Mono. The 'Process Manager' window is divided into several sections:

- Recording time:** 00000:00:00.321 (highlighted with a red box)
- Interval:** 00:00:00.321 (highlighted with a red box)
- Cycles:** 2 (highlighted with a red box)
- Start delay:** 00:00:00
- As fast as possible:** (checkbox)
- Duration:** 0:00:01 (highlighted with a red box)

On the right side of the window, there are 'Start' and 'Stop' buttons, and a 'Live' button. Below these, there are camera settings: Camera: Hamamatsu ORCA-Flash4.0, Resolution: 2048 x 2048, HDR: Disabled, Experiment name: Experiment. There are also radio buttons for 'Automatic Processes' and 'Manual Processes', and a 'Live' button. At the bottom right, there are buttons for 'Ex...', 'Pro...', 'Mi...', and 'Adj...'. The status bar at the bottom shows 'Ready' and '50.3 % (10.1x)'.

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

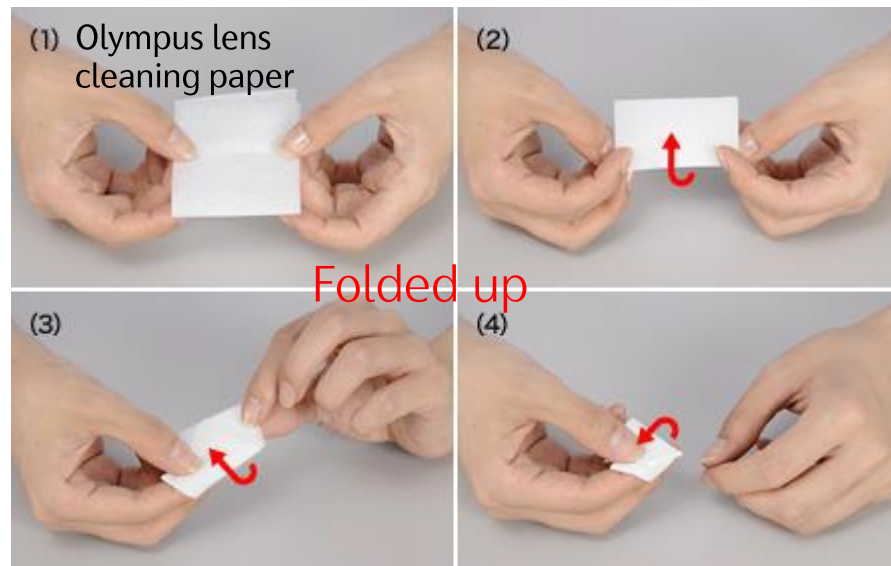
3. Total time duration will be calculated

How to clean the silicone immersion objective

These are not Kimwipes!

60X

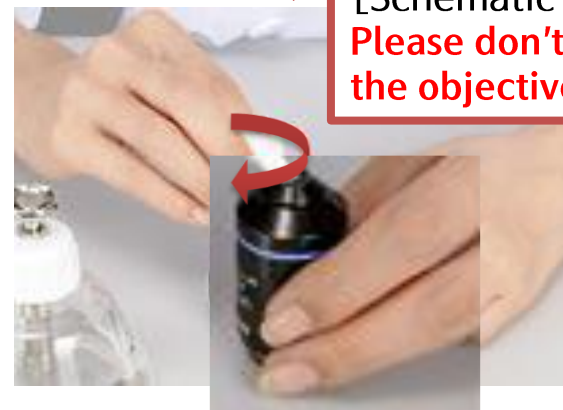
- (1) Wipe the excess immersion oil off with a clean **lens cleaning paper**
- (2) Take a new one and fold it up
- (3) Rinse the corner of folded lens cleaning paper with **95% Ethanol**, then gently clean the objective with a spiral motion from the center to the rim
- (4) Check with another new lens cleaning paper



Folded up



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



Olympus
Silicone
Immersion Oil

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

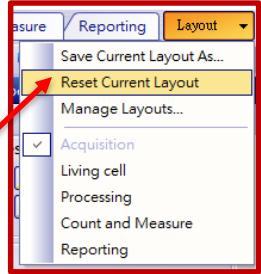
Inverted Microscope Olympus IX83

System Shutdown

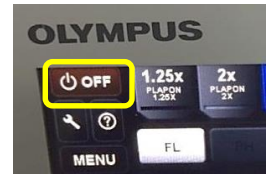


⑧ Exit cellSens

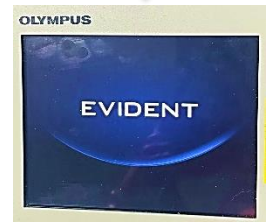
- a. Before exiting the software → YM2019 [Layout ▼] → Reset Current Layout
- b. Change to 4X objective, and lower it down to Z position = 0



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)

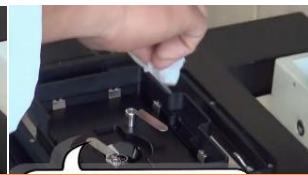
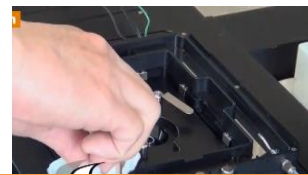


Shut down the live-cell incubation system if used.

⑨ CO₂



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



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

Output folder

The screenshot shows the 'Export' dialog box with the following elements:

- Output folder: C:\Users\BX63\Desktop
- File list table:

Name	Date modified	Type	Size(KB)
20171206 10x error	2017/12/06 17:01:59		
20171207	2017/12/07 22:27:19		
20171214 test	2017/12/14 11:40:43		
BX63 scale bar	2017/09/04 13:56:06		
crop	2017/11/24 12:10:31		

- File name: Process_1143G1L1.tif
- Save as type: TIFF (*.tif;*.tiff)

Suggested format: *.tif

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

The screenshot shows the 'ROI overlay' section with the following options:

- No overlay
- All ROI
- Selected ROI
- Overlay Color scale

The screenshot shows the 'OutputFormat' section with the following settings:

- Procedure: RGB Color with Merge
- 24bit Full Color
- Amount

Save to export

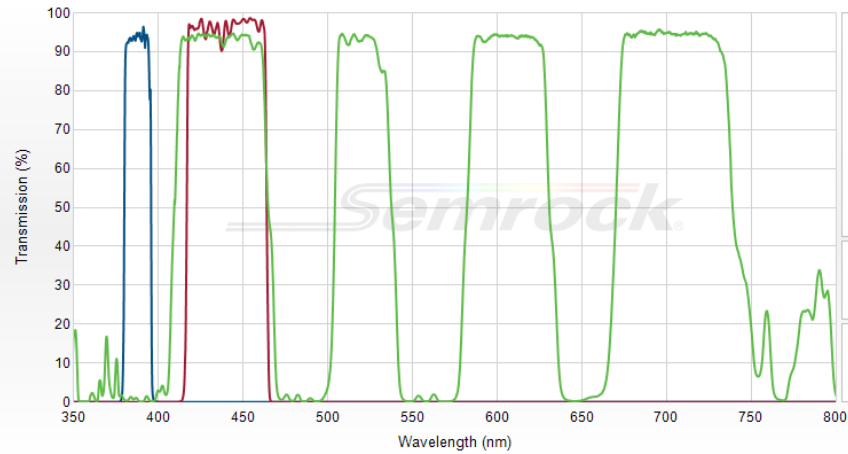
The screenshot shows the bottom of the dialog box with the following buttons:

- Save
- Cancel

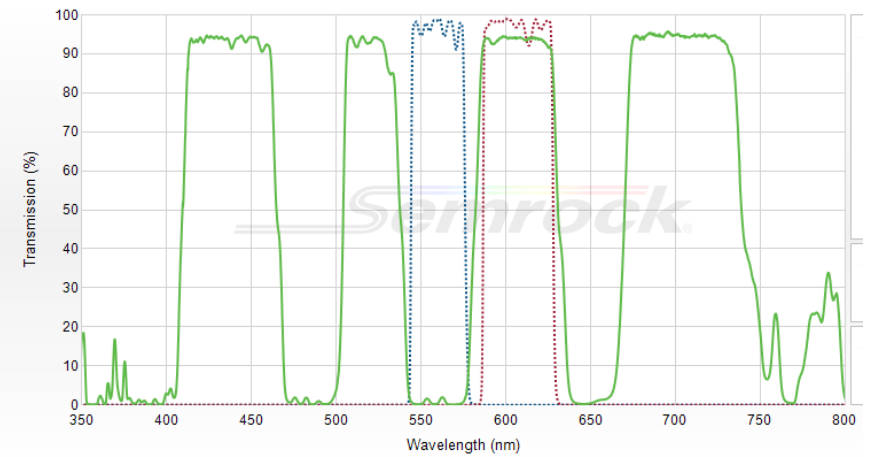
Fluorescence Filter Set (1)

● Excitation
● Emission

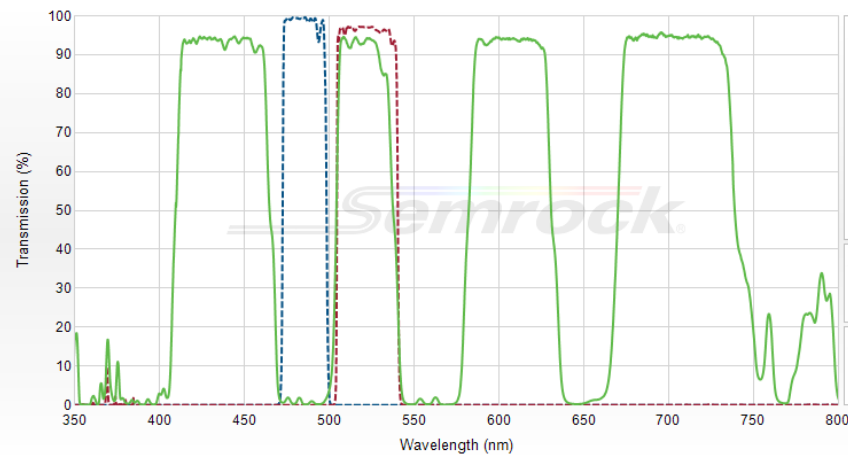
DAPI



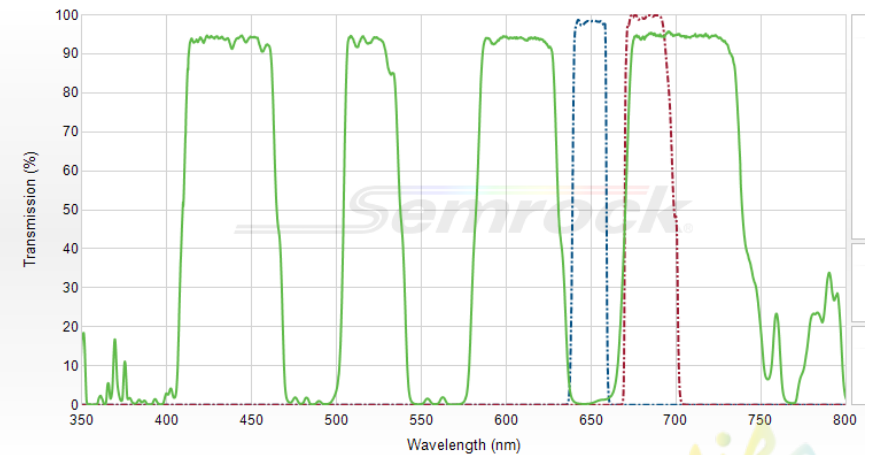
TRITC



GFP



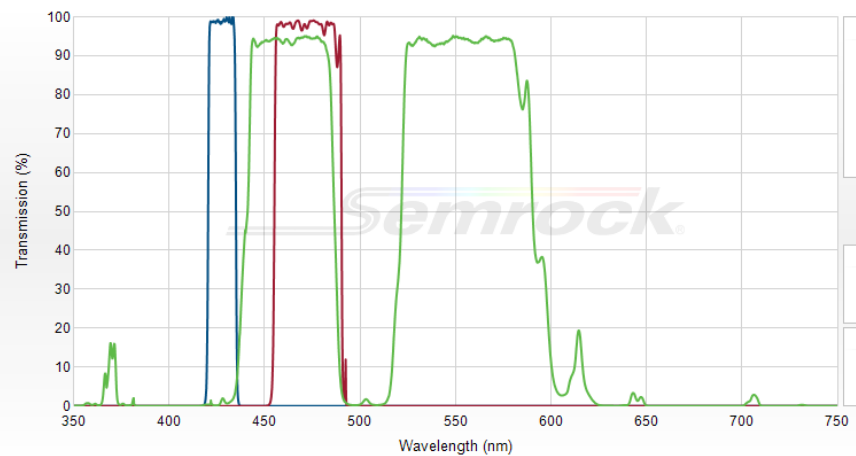
Cy5



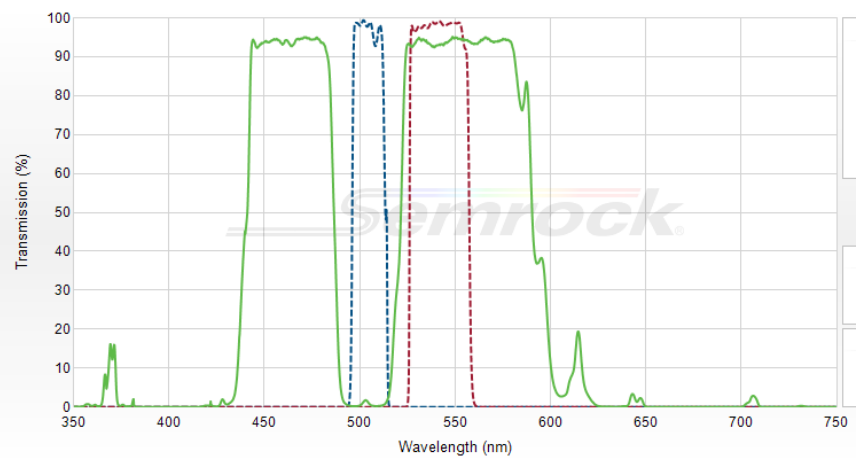
Fluorescence Filter Set (2)

● Excitation
● Emission

CFP



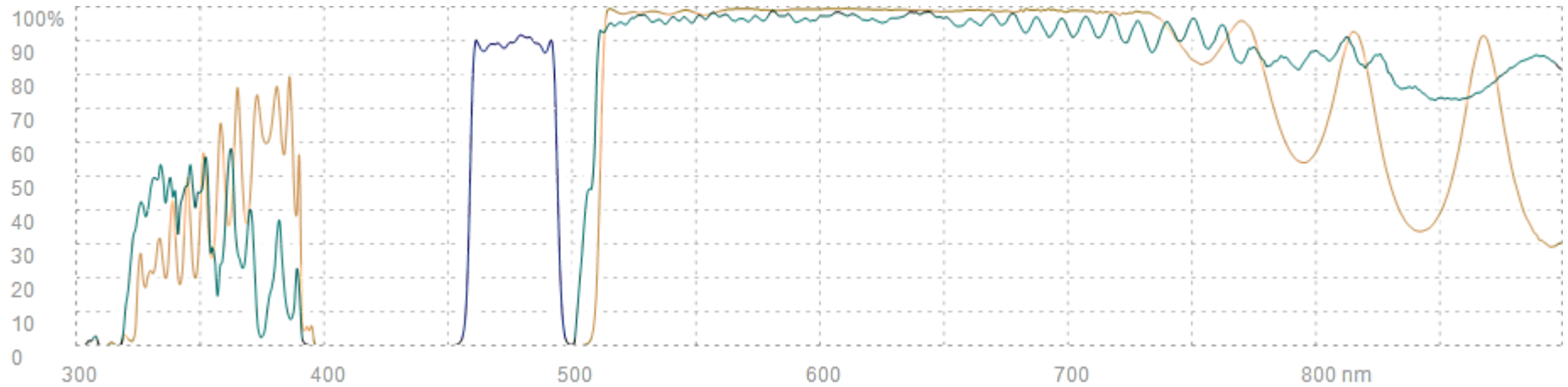
YFP



Fluorescence Filter Set (3)

● Excitation
● Emission

➤ FITC-long

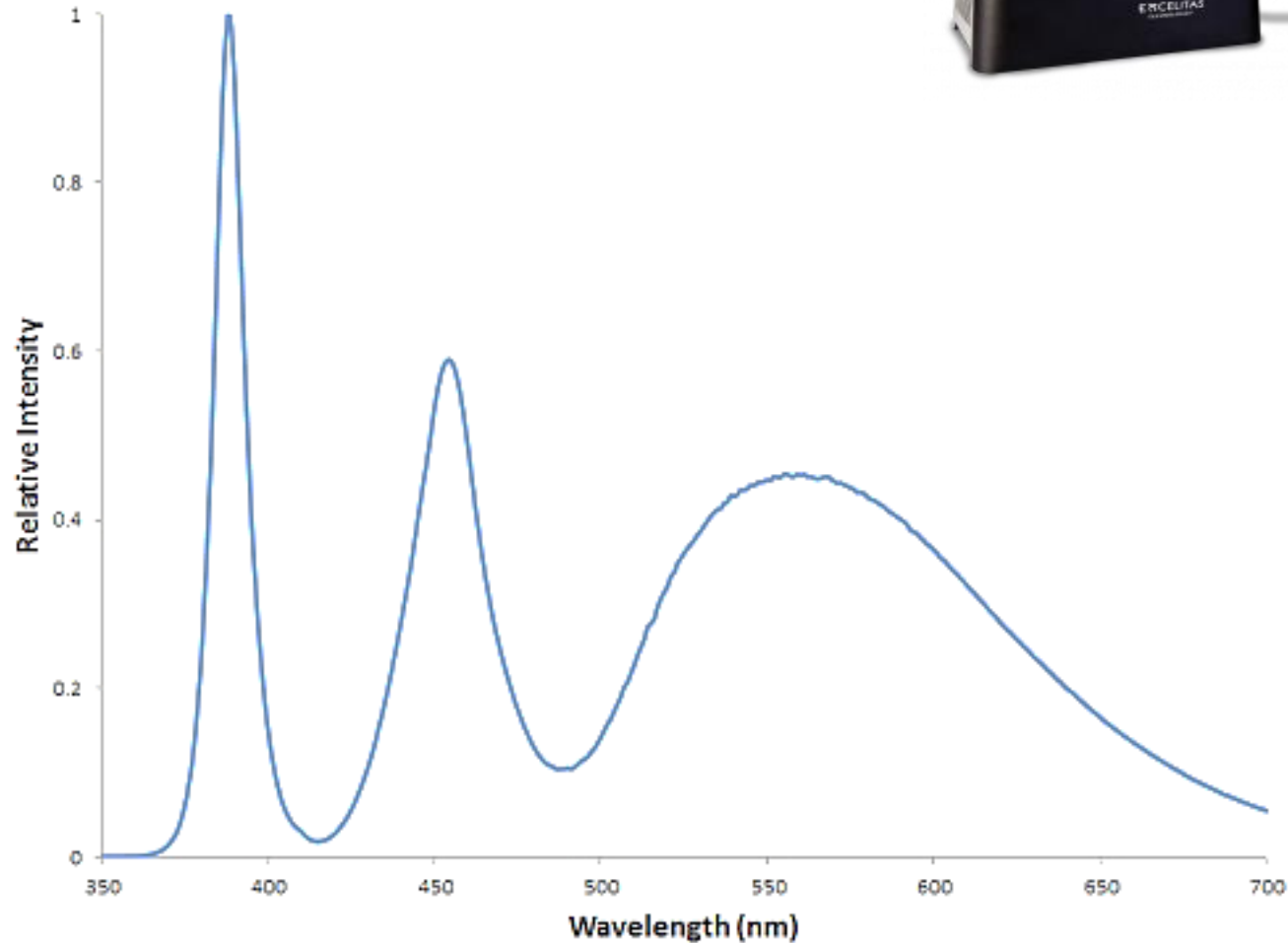


➤ mCherry



Fluorescence Light Source

X-cite[®] 120 LED *Boost*



Combine Channels

Please first open grayscale images in the software 24
they must have the same resolution and bit depth
to enable image overlay.

The screenshot shows the 'cellSens Standard' software interface. The 'Image' menu is open, and 'Combine Channels...' is selected. The 'Combine Channels' dialog box is displayed, showing a preview of the combined image and various settings. Annotations include:

- 1. Choose images to combine:** A red box highlights the 'Available images' list, which contains four entries: 'HER2 (3x16-bit) - DAPI.tif', 'HER2 (3x16-bit) - DAPI.tif', 'HER2 (3x16-bit) - FITC.tif', and 'HER2 (3x16-bit) - Texas Red (Sulfonyl chloride).tif'. A red arrow points from the 'Combine Channels...' menu item to this list.
- 2. Choose color for each image:** A red box highlights the 'Color' column in the table, which has a dropdown menu set to 'DAPI'. A red arrow points from this box to the 'Color' column header.
- 3. Convert the result image to a color image:** A red box highlights the 'Convert to RGB' checkbox, which is checked. A red arrow points from this box to the checkbox.
- 4. OK:** A red box highlights the 'OK' button at the bottom right of the dialog. A red arrow points from this box to the text '4. OK' below the dialog.

Additional annotations include:

- A red box around the 'Image' menu item, with a red arrow pointing to the 'Combine Channels...' option.
- A red box around the 'Combine Channels...' menu item, with a red arrow pointing to the dialog box.
- A red box around the 'Adjust XY shift (optional)' text, with a red arrow pointing to the 'X Shift' and 'Y Shift' fields in the table.
- A red box around the 'Transmission:' dropdown menu, which is set to 'No transmission'.
- A red box around the 'OK' button.

Images	Name	Color	X Shift	Y Shift	Intensity
HER2 (3x16-bit) - DAPI.tif	DAPI	...	0.0	0.0	50
HER2 (3x16-bit) - DAPI.tif					
HER2 (3x16-bit) - FITC.tif					
HER2 (3x16-bit) - Texas Red (Sulfonyl chloride).tif					

Adjust XY shift (optional)

Pixel shift

X: 0

Y: 0

Clip edges

OK Cancel Apply

(optional) combine with one transmission image IC P Fmono)

4. OK