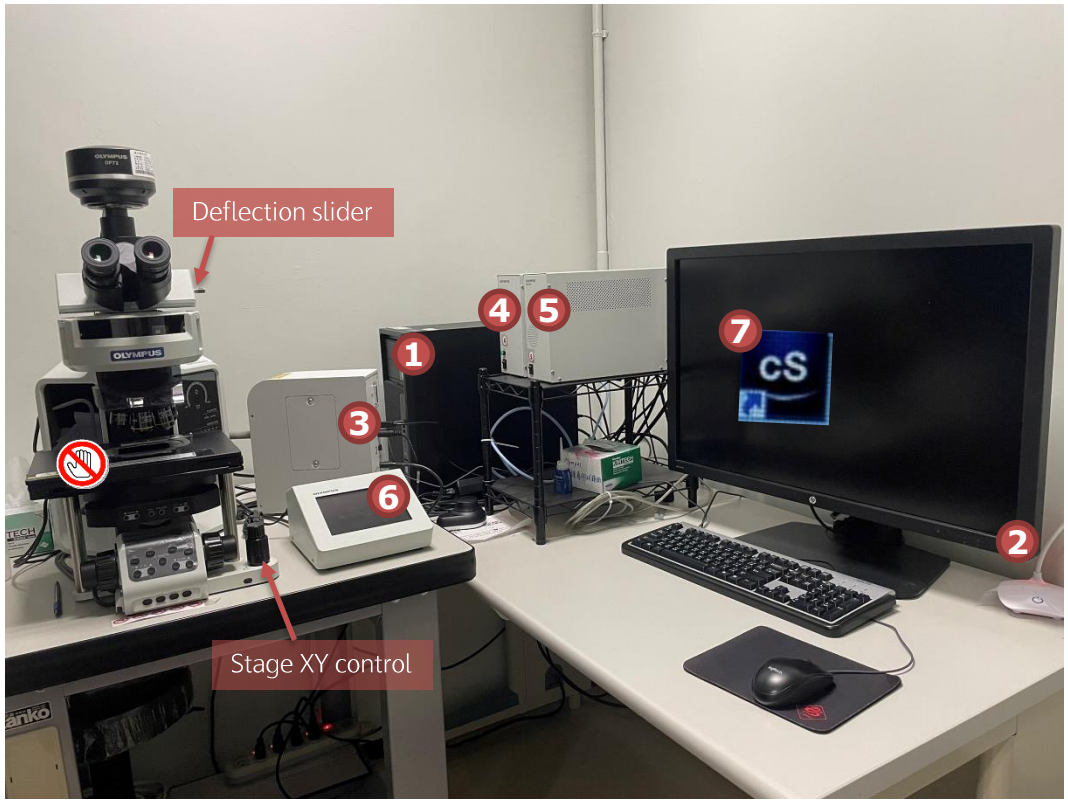
Index

Upright Microscope Olympus BX63

System Startup



① Computer

② Monitor

③ Fluorescence light source

④ Supersonic Stage

Do not push it! Use the XY control only!

⑤ CBH (Microscope control)

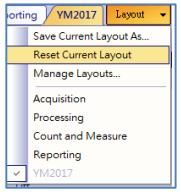
⑥ Touch Panel Control

Press the switch in the back once, and wait until it shows [Start Operation].

⑦ Software: cellSens

→ The upper right of the window

→ YM2017/Reset Current Layout



Microscope control

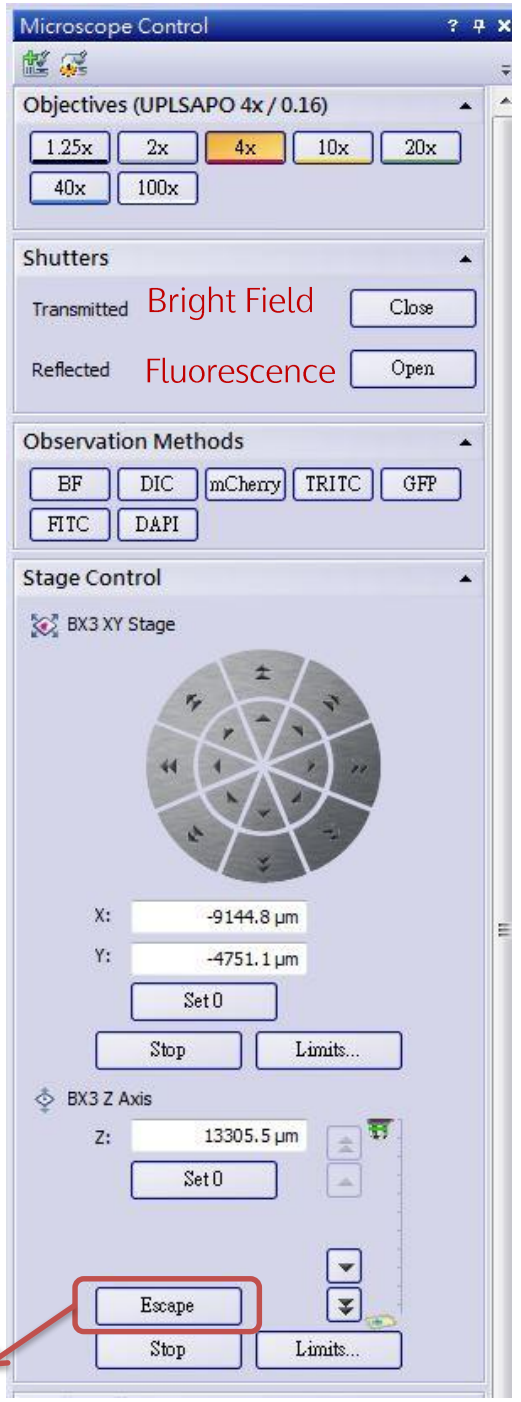
➢ Objectives

Switch to 4x before shutting down

100x is an oil immersion objective!

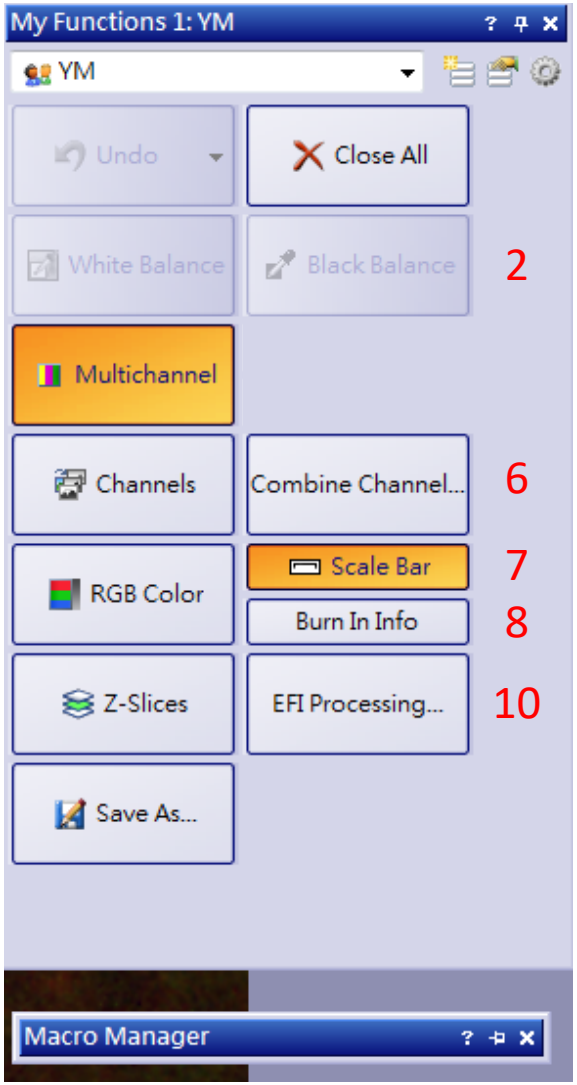
➢ Observation Methods

Method	LED	Mirror Unit	Excitation filter	Dichromatic Mirror	Emission filter	Applicable Fluorochrome
DAPI	385	89402 ET –Multi LED set This filter is preferred for multi-channel images with faster acquisition.	391/32	410-458	418-450	DAPI, Hoechst, etc.
GFP	475		479/33	497-540	505-530	eGFP, Alexa488, FITC, etc.
TRITC	525		554/24	570-615	577-610	Alexa546/555/568, DsRed, Cy3, PI, etc.
CY5	630		638/31	655-730	663-725	Cy5, Alexa633, etc.
FITC-long	475	U-FBW	BP460-495	DM505	BA510IF	eGFP, eYFP, Alexa488, etc.
mCherry	575	U-FYW	BP545-585	DM595	BA600IF	Alexa594, DsRed, Texas Red, Cy3.5, etc.
PO		--	Transmitted light, Polarization microscopy			Picro Sirius red
BF		--	Transmitted light, Bright Field image			--
DIC		--	Transmitted light, Differentiated Interference Contrast image			--



Stage Escape /Return

My Functions

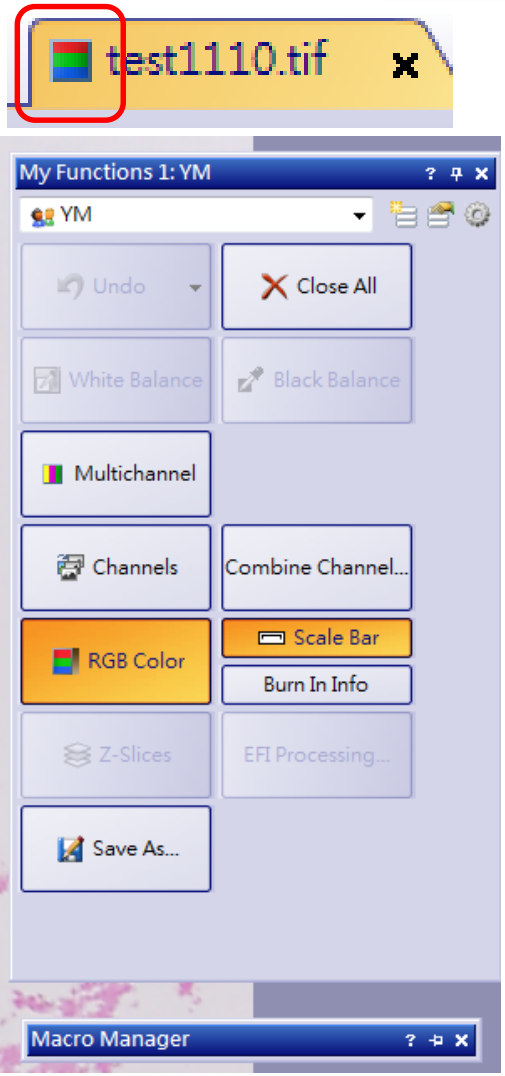


	Button	Details
1	White Balance	Set white balance for bright field image
2	Black Balance	(Optional, not necessary) Set black balance for fluorescent observation Please RESET it with right-click after use!
3	Multi Channel	
4	Channels	Separate a multi-channel image to 10-bit grayscale images
5	RGB Color	Convert a grayscale image to a 24-bit color image
6	Combine Channels	Combine channels to get an overlay 24-bit color image (details in appendix.)
7	Scale Bar	To show the scale bar
8	Burn In Info	To burn in the scale bar on the image. Once you burn in info on an image, you cannot change or remove it.
9	Z-Slices	Separate a z-stack image into different z-slices
10	EFI Processing..	Extended Focal Image processing results in an image that is focused throughout all of its segments.
11	Save As⋯	Recommend format: *.tif or *.vsi

Image Format: Color Image and Grayscale Image

➢ Color Image (24-bit)

RGB color image → [Save As] .tif

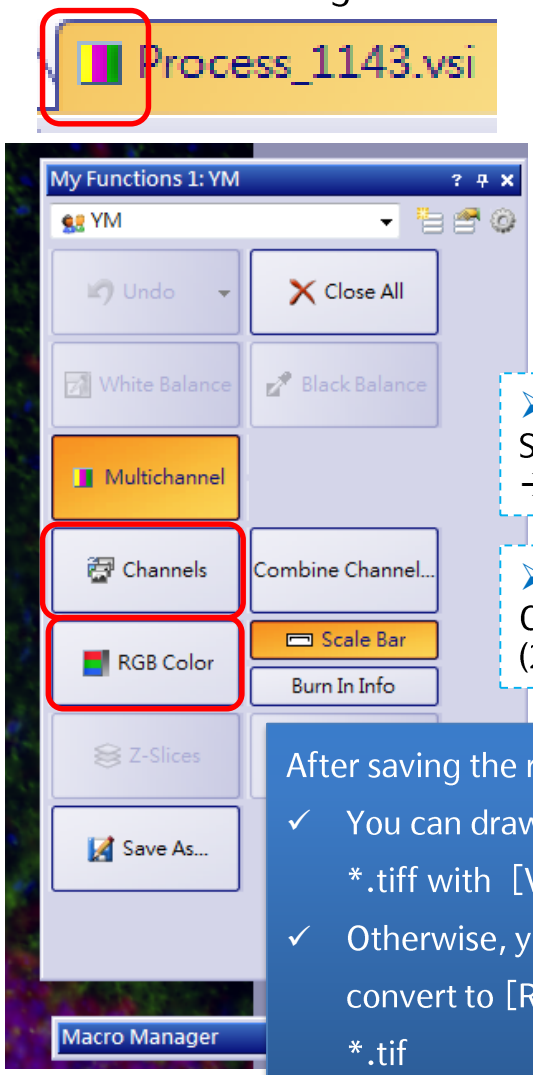


➢ Grayscale Image (10-bit)

Fluorescence snapshot or Multi-Channel Images

→ [Save As] *.vsi

It's recommended to save the raw data as *.vsi first.



➢ Channels:

Separate Channels
→ grayscale raw image

➢ RGB color:

Convert raw image to color images (24-bit)

After saving the raw data (*.vsi)

- ✓ You can draw the scale bar and export it into *.tiff with [Viewer] software
- ✓ Otherwise, you can [separate channels] and convert to [RGB color images] to [save as] *.tif

Acquiring **Single** Images

1. Choose objective
2. Choose the observation method
3. Find the required view from the eyepiece
4. Pull out the deflection slider
5. Click [Live] to adjust focus

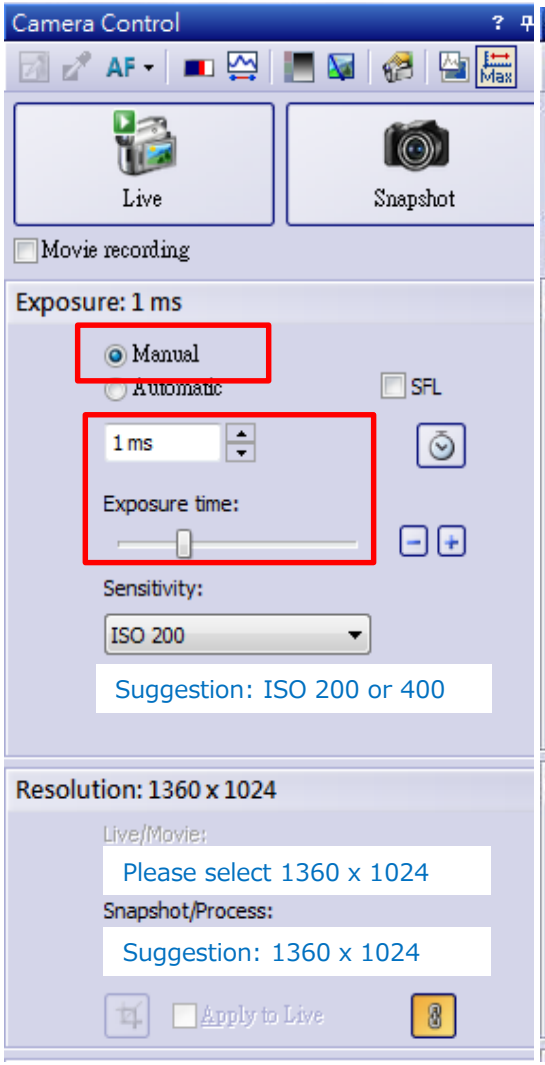
   [Ctrl + H]: Switch to range indicator mode to help set exposure time
6. Set exposure time

   It suggests using the automatic exposure for bright field images and entering the exposure time manually for fluorescence images.

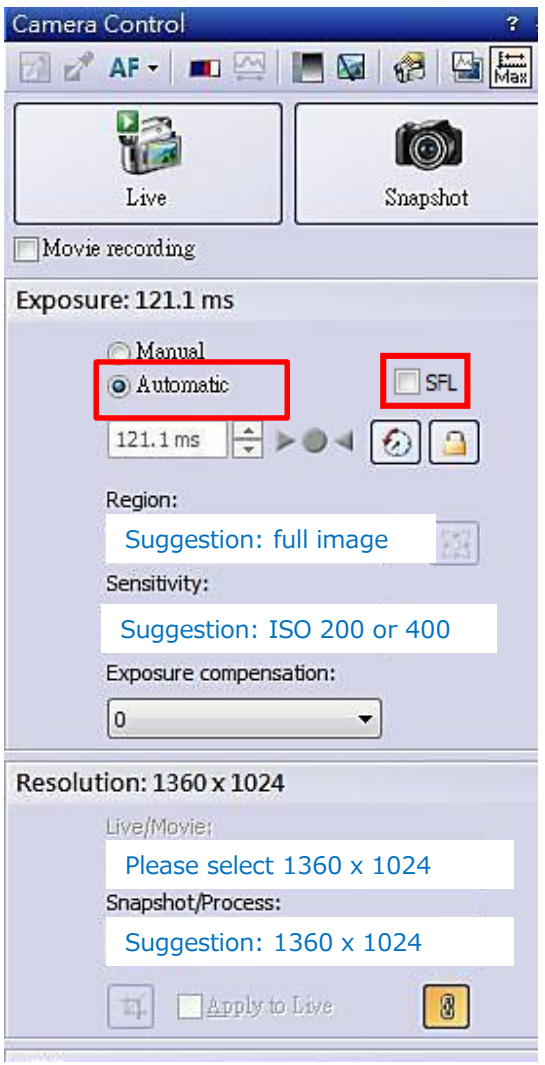
   Set white/black balance if necessary

   White balance is for bright field images; Black balance is for fluorescence image (MUST reset it before and after use!)
7. Set snapshot/process resolution
8. [Snapshot] to acquire the image
9. [Save As]

Manual Exposure



Automatic Exposure



SFL: Automatically enhance contrast of fluorescence image

✧ Do not select it if you are acquiring images that require keeping the same imaging conditions

Acquiring **Multi-Channel** Images

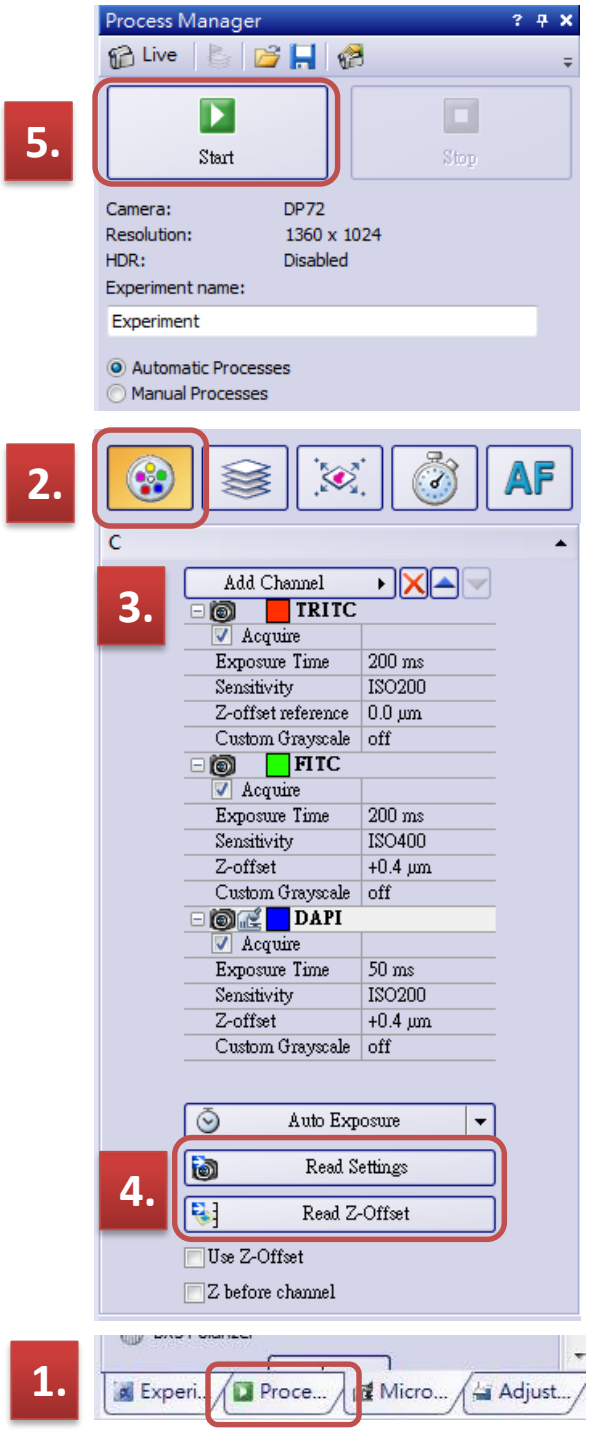
After you find the field of interest and set the focus, …

1. Go to the [Process Manager] tool window,
   located on the bottom right of the user interface
2. Select [Multi-Channel]
3. Add Channel
4. Click [Live] to set exposure conditions for each of the channels added
   → [Read Settings] of every channel respectively

✧ Read the Z-offset of every channel if necessary
(1) MUST start with the first channel
(2) Click [Live] and adjust Z focus → Read Z-offset
(3) Adjust and read 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 or Z-stack for multi-dimensional images



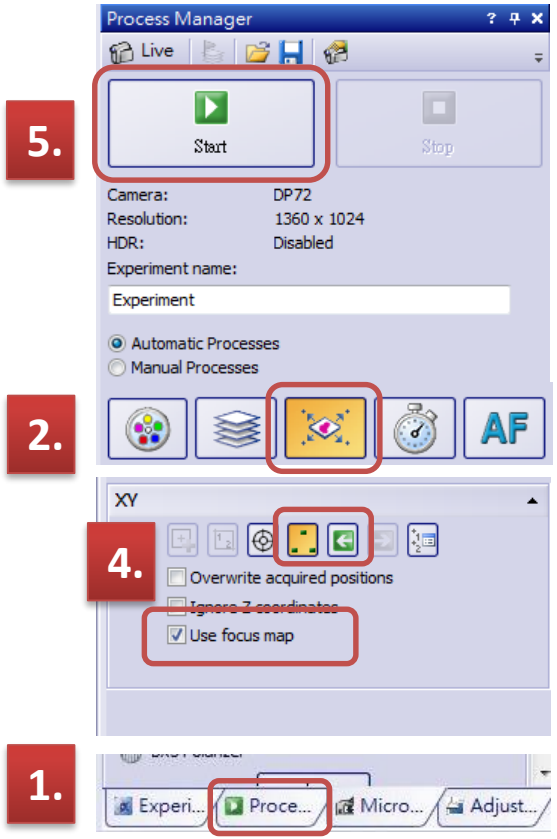
Acquiring **Multi-position** Images

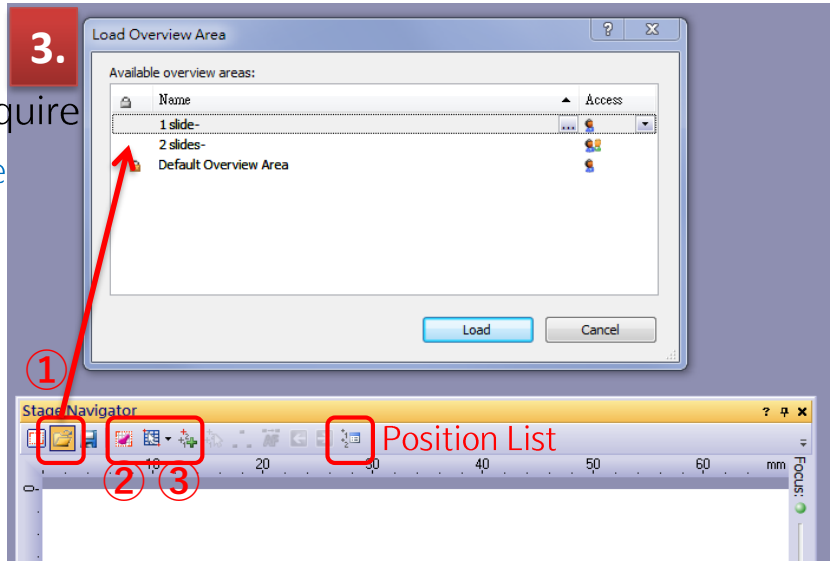
After choosing the objective, observation method, focusing, [Live] to set exposure time, …

* Suggestion: you can use BF + automatic exposure.

1. To the [Process Manager] tool window,
   located on the bottom right of the user interface
2. Select [MIA] for Multi-position
3. To the [Stage Navigator] tool window
   ① Load Overview Area
   ② Acquire Overview
   Automatically acquire an overview image with the lowest objective
   ③ Add multi-position or define rectangle scan area to acquire
   Define a rectangular area to acquire a stitched image
   Add single positions
4. (Optional) Set up a focus map if necessary
5. Click [Start] to acquire multi-position images

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





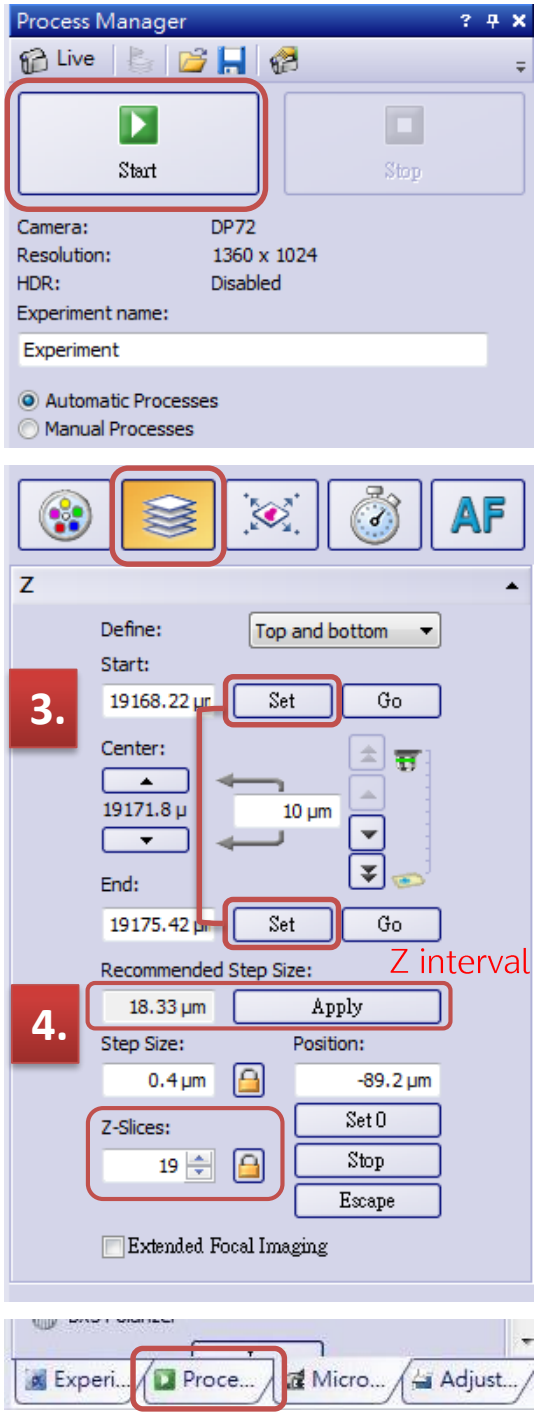
Acquiring Z-stack Images

After choosing the objective and observation method, find a view of interest and set exposure time…

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

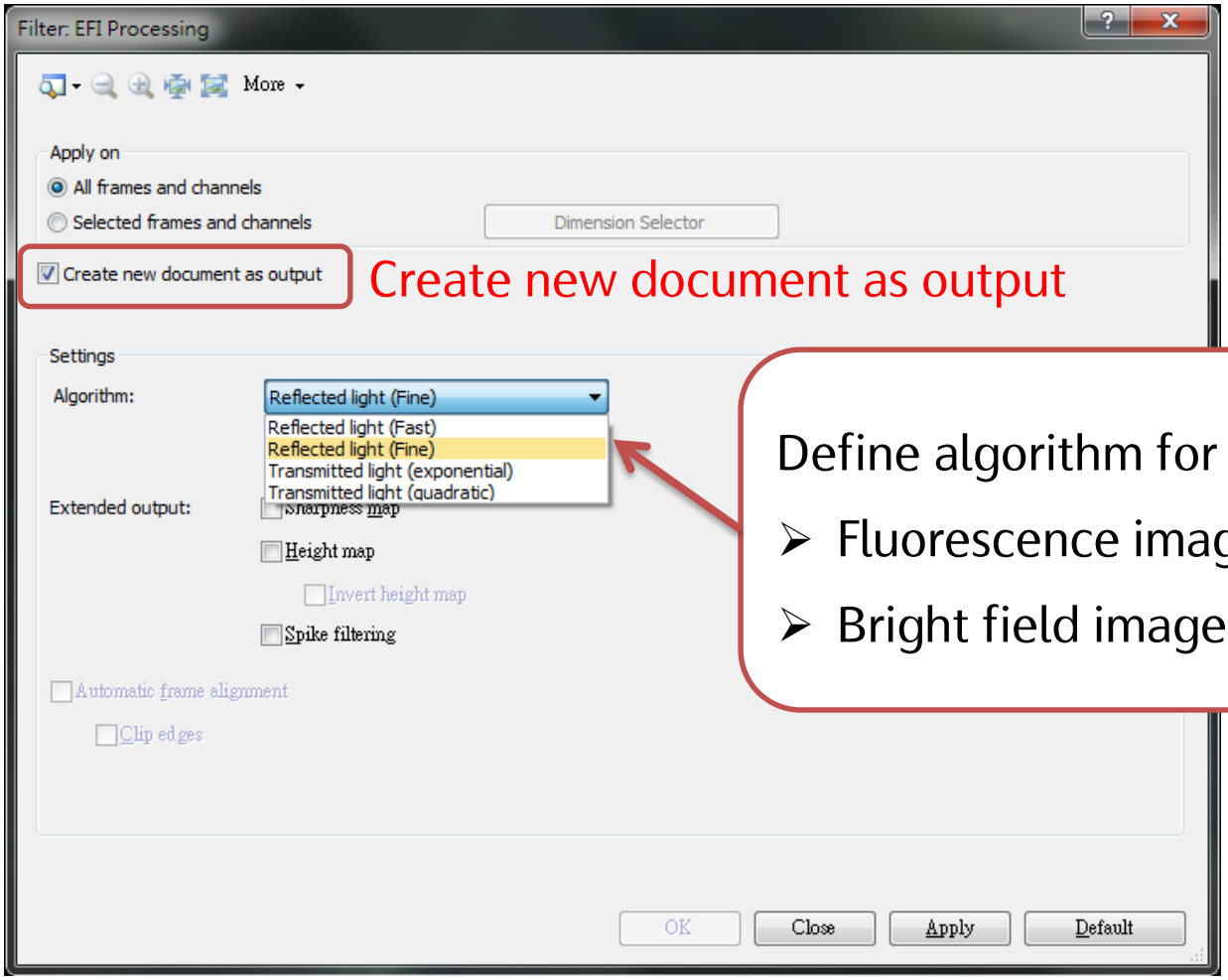
   The Z-stack image can be processed into EFI afterward if needed.
   However, if ☑ EFI is selected, it will result in only one image focused throughout its segments.
5. Click [Start] to acquire z-stack images

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



EFI Processing

(Extended focal image)



Define algorithm for EFI processing

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

How to clean the Oil immersion objective

These are not Kimwipes!

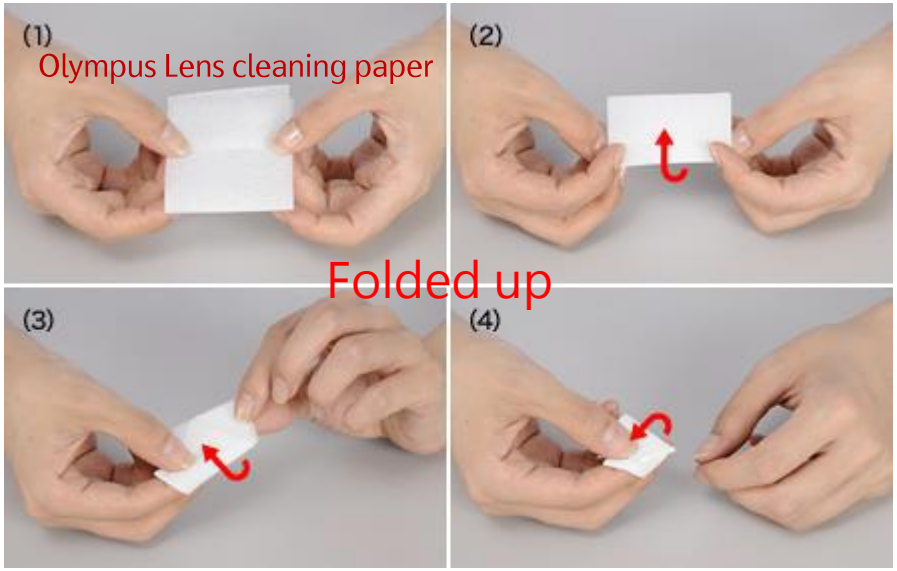
100X

(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!

Olympus immersion oil

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

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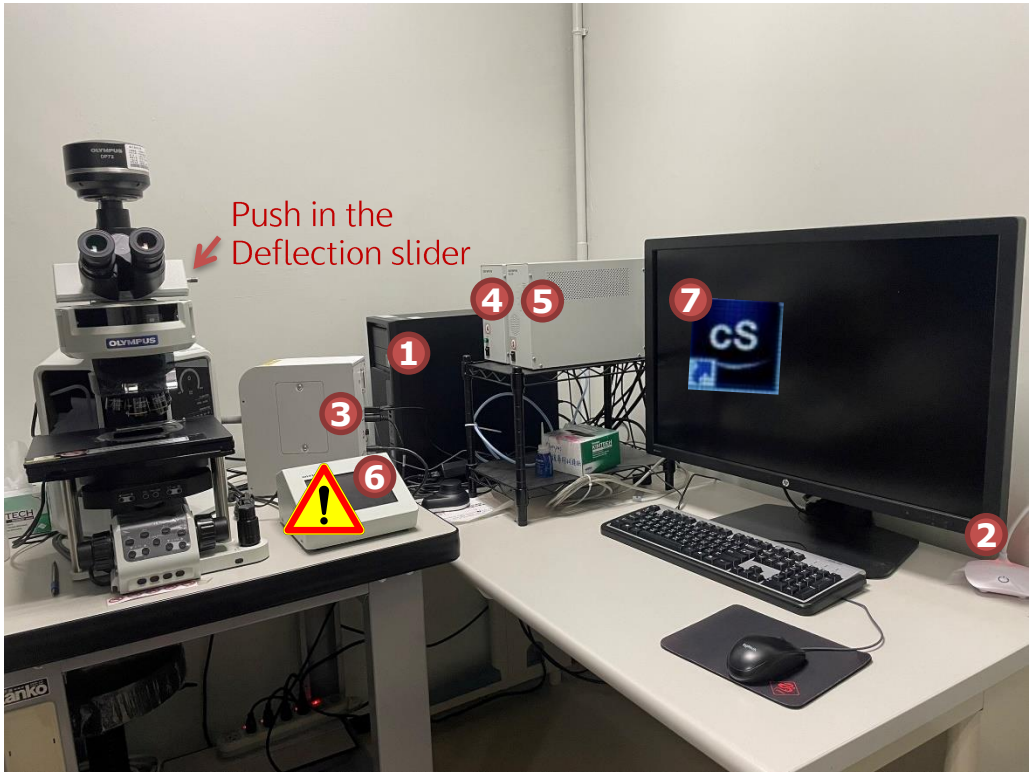
System Shutdown



Make sure everything is turned off properly before you leave, and MUST write down the objectives used!

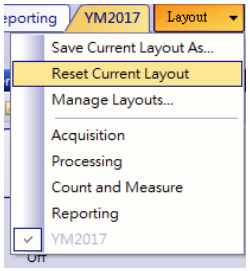
- Change back to 4X, move up to 0
- Push in the deflection slider

⑦ Exit cellSens

Before you exit the software

→ YM2017 Layout

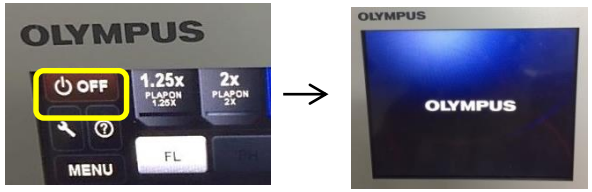
→ Reset Current Layout



⑥ Touch Panel Control

Tap on [Off] to shut down the touch panel

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



⑤ Turn off CBH control

④ Turn off the Supersonic Stage

③ Turn off the fluorescence light source

①② Turn off the computer

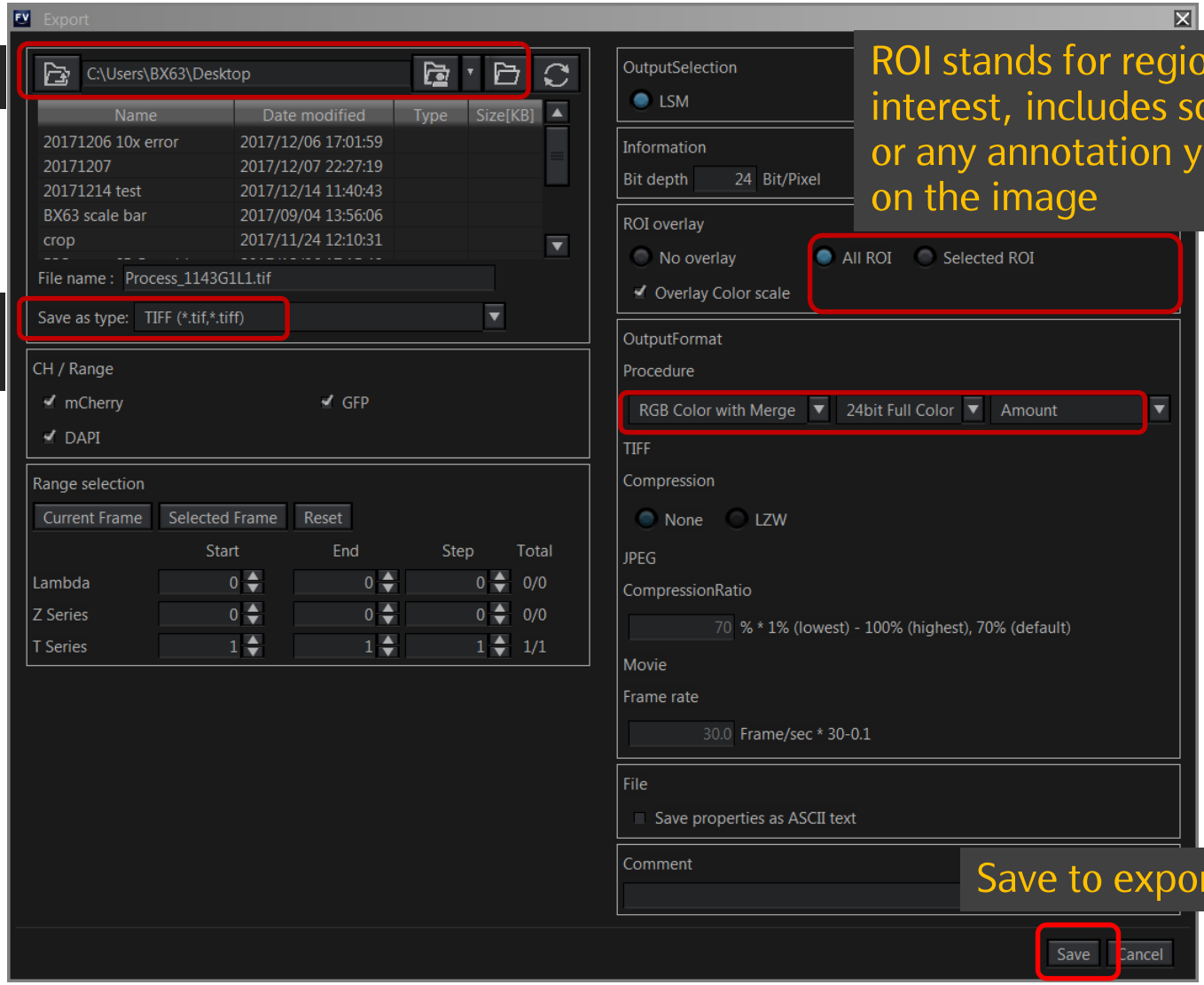
Image export by FV31S-DT viewer

(64-bit)

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

Output folder

Suggested format: *.tif

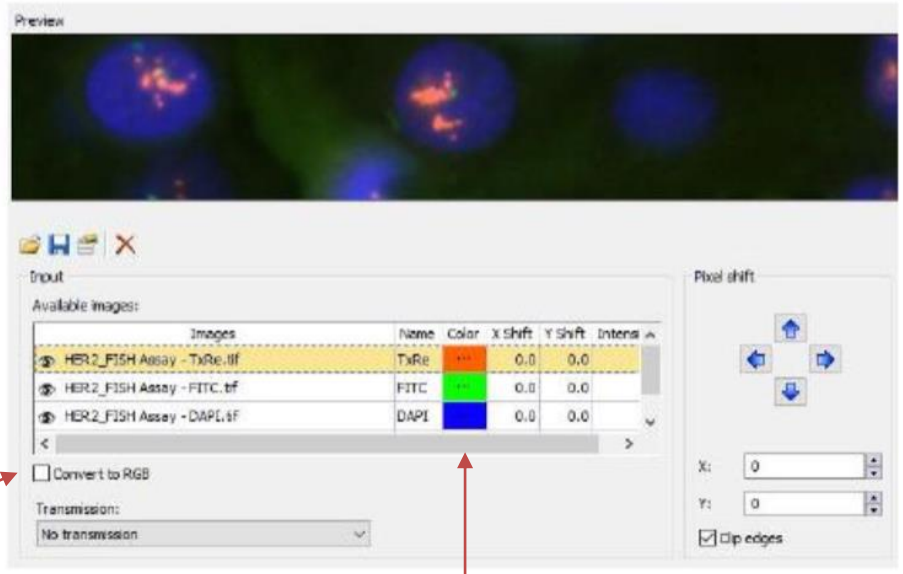


ROI stands for region of interest, includes scale bar or any annotation you draw on the image

Save to export

Combining multi-channel images

- **Image > Combine Channels**
- Select the desired images, add transmission image if available
- Correct for pixel shift if necessary

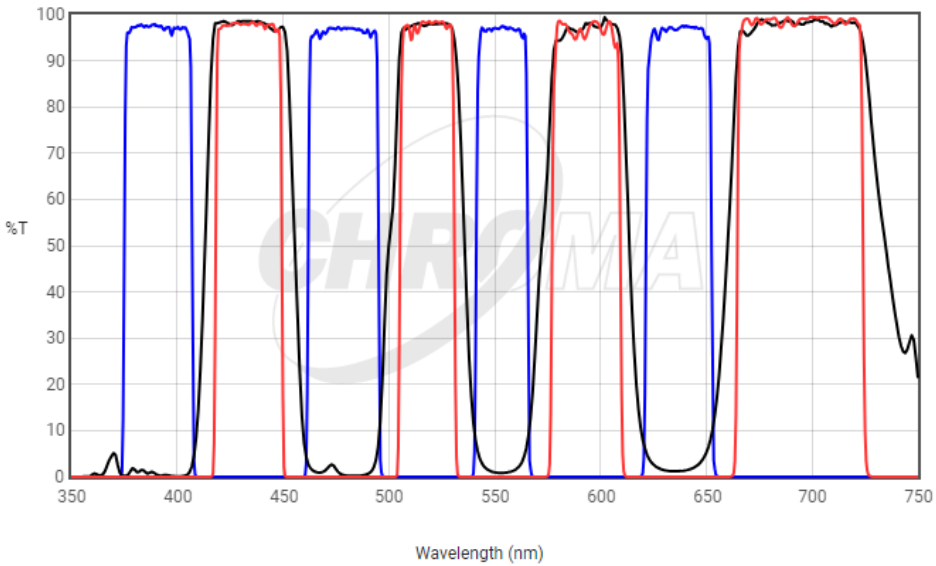


Convert to RGB color image if needed

Assign display color for each channel

Fluorescence Filter Set and light source

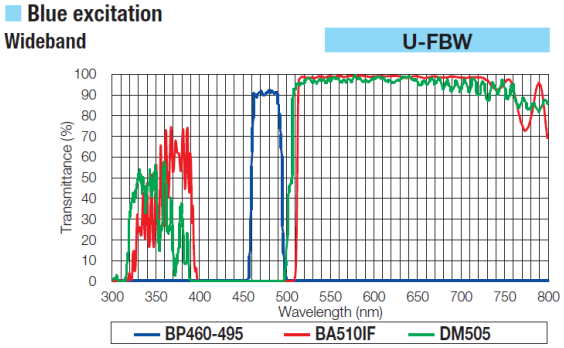
- DAPI/eGFP/TRITC/Cy5
- 89402 ET –Multi LED set



X-Cite TURBO unit contains the following LED Wavelengths:

LED Position	LED Center Wavelength	Useable Wavelength Range
1	385nm	375nm-400nm
2	430nm	410nm-450nm
3	475nm	460nm-495nm
4	525nm	505nm-550nm
5	575nm	555nm-610nm
6	630nm	615nm-660nm

- FITC-long



- mCherry