2017-06-21
INSTRUCTIONS OLYMPUS BX63 UPRIGHT 2017-05-24.DOCX

Instructions for Olympus-Upright

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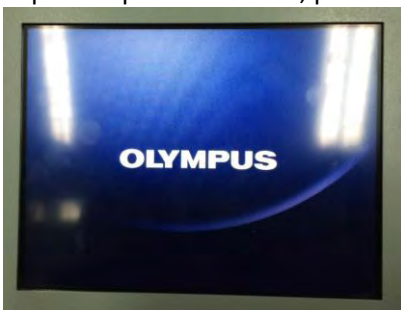
Start

In this order:
(1) X-cite lamp
Ordinarily the computer, controller and touch pad are on in standby mode;
to start tap the touch pad if it is on.

Otherwise turn on:
(2) computer
(3) BX3-CBH (after power failure it turns itself on to standby)
(4) touch pad (after power failure it does not turn on)

Tap touchpad to activate, press on “full operation” after it becomes active



→


Open the X-cite lamp shutter when it becomes active

→


Microscope:

Upright Olympus BX63.
Stage: manual xy
Nosepiece: mechanical z
Condensor NA
Objectives are changed manually.
Filterset cubes are changed mechanically.

Camera:

DP73 is a 17.28 megapixel color CCD (2 megapixel with pixel shifting), 12bit
1600x1200 pixel images at 15 fps, 800x600 at 27fps

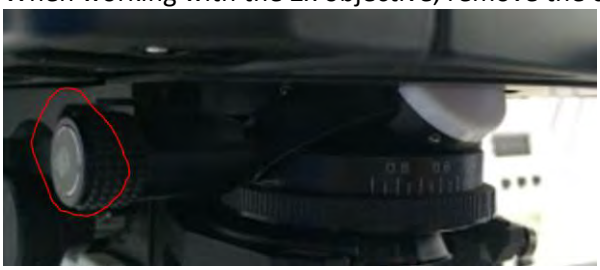
Light path:

Halfway for ocular, all the way out for camera

Objectives in nosepiece:

	Magnification	Immersion	NA	Color correction	Working distance
PlanApoN	2x		0.08	∞	-
UPlanFLN	10x		0.30	∞	-
UPlanFLN	20x		0.50	∞	0.17
UPlanFLN	40x		0.75	∞	0.17
UPlanSApo	60x	oil	1.35	∞	0.17
UPlanSApo	100x	oil	1.4	∞	0.17

When working with the 2x objective, remove the conderser **dry top lens** from the path.



On the Touchpad

To **focus** use the up and down arrows.

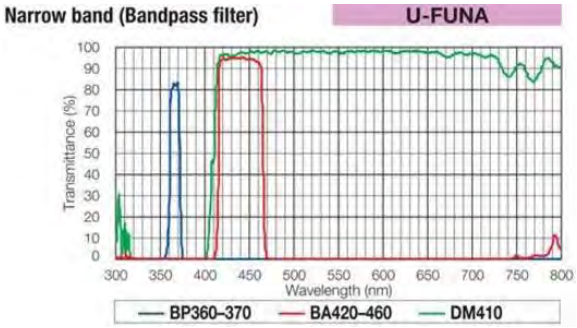
Range from 0-20,000, focus is at about 19,100.

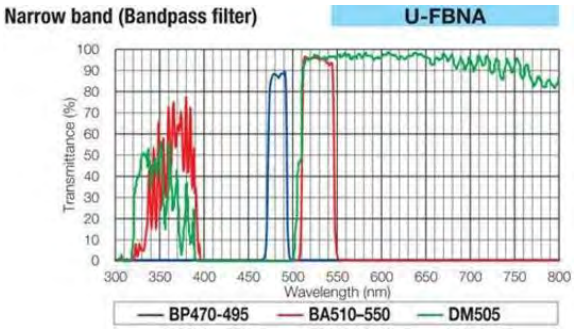
On the microscope

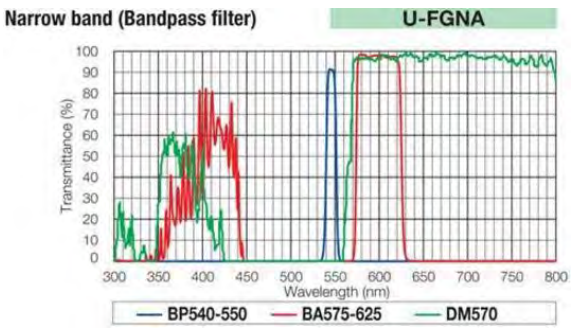
Focus knob

Mirrors (filtersets):

Position	Button	Filter set	Excitation	Dichroic	Emission	Fluorophore family
1	DAPI	U-FUNA	BP 360-370	DM 410	BA 420-460	DAPI
2	GFP	U-FBNA	BP 470-495	DM 505	BA 510-550	GFP, Cy2, Alexa 488
3	mCherry	U-FGNA	BP 540-550	DM 570	BA 575-625	Cy3, Alexa 555, mCherry
4	Brightfield	-				
5	Analyzer					







On the Touchpad

On the microscope

TRANSMITTED LIGHT

DIA tab (diascopic = transmitted)

DIA→Brightness tab:

Use the arrows to adjust lamp voltage:

Halogen brightness

DIA→Iris tab:

FS, field stop, use for Kohler

EPIFLUORESCENCE

EPI tab (epifluorescence)

Use the buttons to navigate the mirrors (filter cubes)

Change filter cubes

and open/close shutter

Open/close FL shutter

To alternate transmitted/reflected use these buttons:

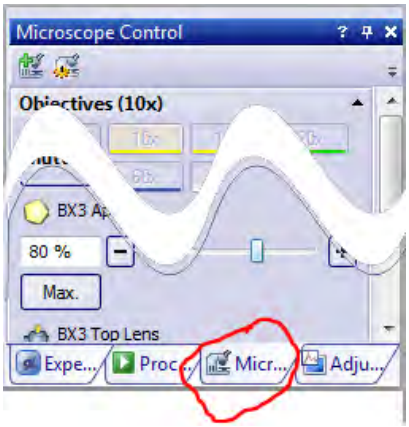
Software

Use the Bookitlab dialogue to activate your reservation; this will open the Cell Sens Dimension software

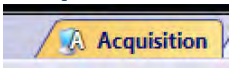
In **start page → Device list** choose BX63

If you do not see tabs **Camera control** and **Microscope control** do
layout → reset layout

Settings (shutters, condenser, focus, turret filter cube, lamp voltage, aperture stops) can also be adjusted on **[Microscope control]** panel (right panel)
[microscope manager] tab



Acquisition



top tab

In the left panel [**Camera control**]:
Pull out the light path rod
Use **Live** to start

RGB/monochrome.

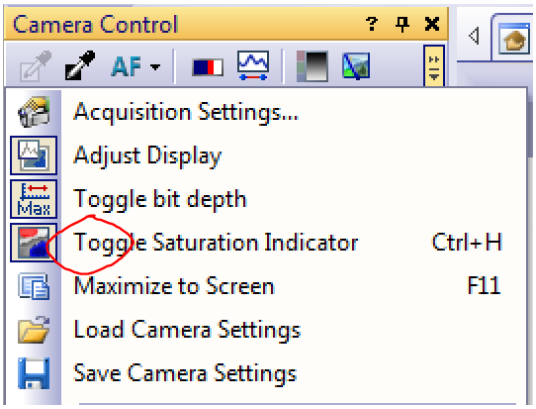
Press to toggle.
(For fluorescence and fluorescence+transmitted choose monochrome)

When using brightfield in RGB mode, adjust

White balance

Saturation/black level

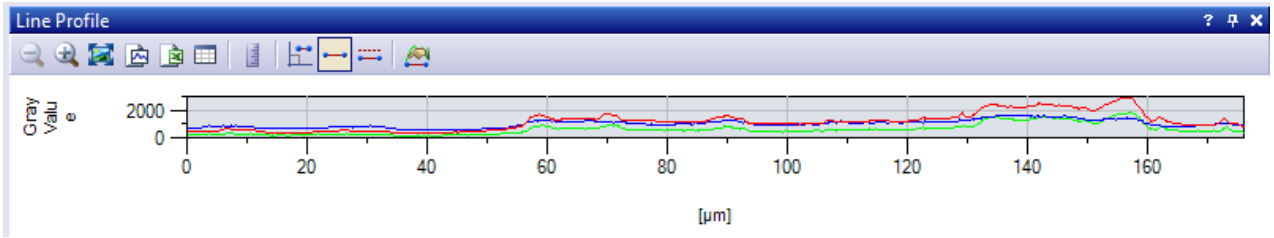
Monitor by toggling <ctrl-H> or



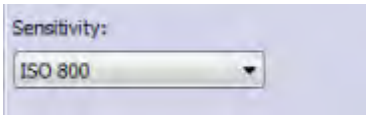
Line profile

Or use **line profile** to monitor saturation

(appears below the image)



Set **ISO** to 100



For weak signal you can change to a higher ISO, however the noise will be higher

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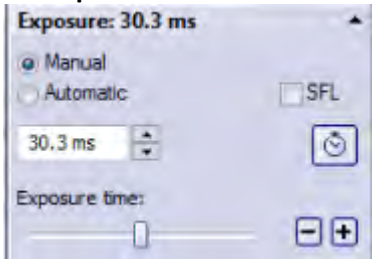
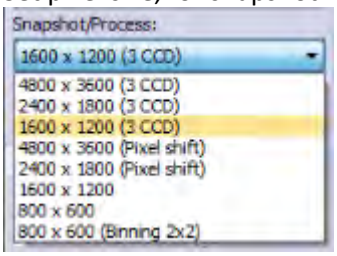
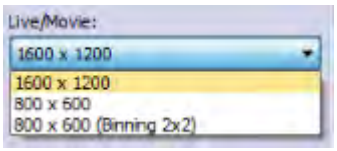
Set **exposure** time



Set pixel size, for snapshot 4800 x 3600 (pixel shift) or 1600 x 1200



For movie 1600 x 1200



Saving options

In [Process Manager] tab

Set saving options, name and location of new images (USERS DATA→YYYY→MM-YYYY→PI-name).

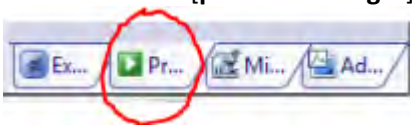
For single channel

Press Snapshot

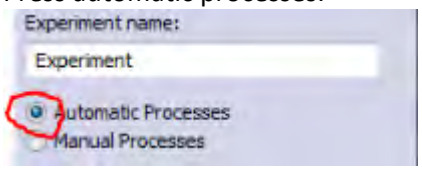
Besides saving the file (**File→Save**) in the native VSI format you can also export the image to tiff:
do **File → Save as → Tiff** for further processing in image analysis programs.

For multichannel/multidimensional images:

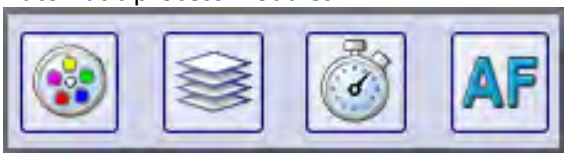
In the right panel - bottom:
Choose the tab [**process manager**]



Press automatic processes.

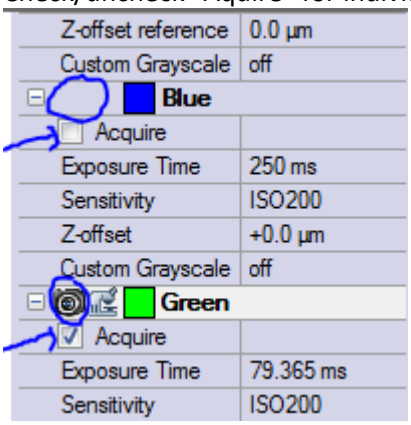


Automatic process modules:



Channels - Z sections - Time lapse - Autofocus

In Channels:
Check/uncheck “Aquire” for individual channels



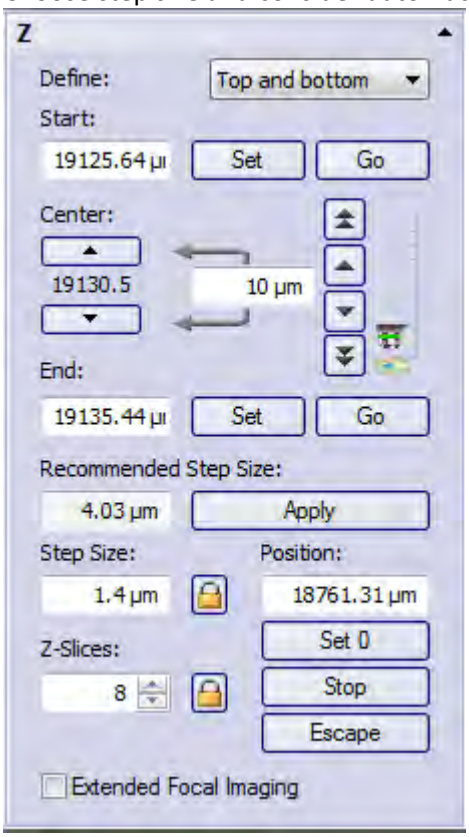
For each channel, after setting all parameters in Camera Control (right panel), in the channels tab, press [**Read Settings**].

Z sections

In **[Process manager]** panel

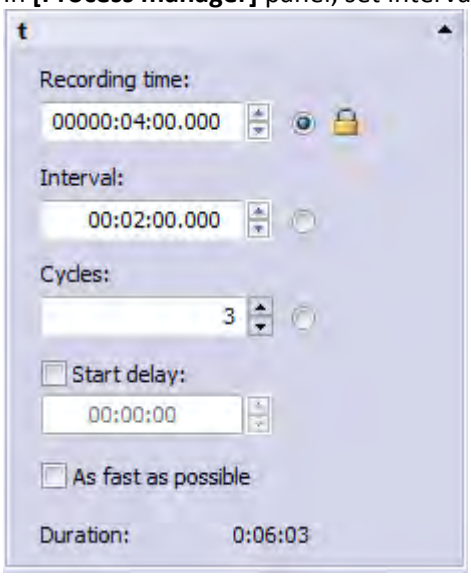
Choose [**Top and Bottom**], focus to the desired top or bottom and press [**Set**].

Choose step size and consider automatically generating "Extended Focal Imaging"

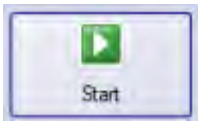


Time lapse

In **[Process manager]** panel, set interval and number of cycles.

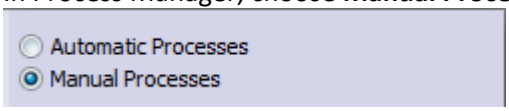


Press Start

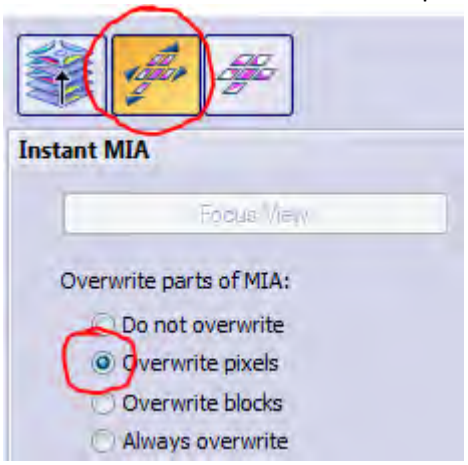


Tiling

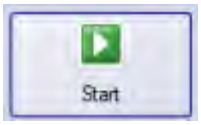
In Process Manager, choose **Manual Process**



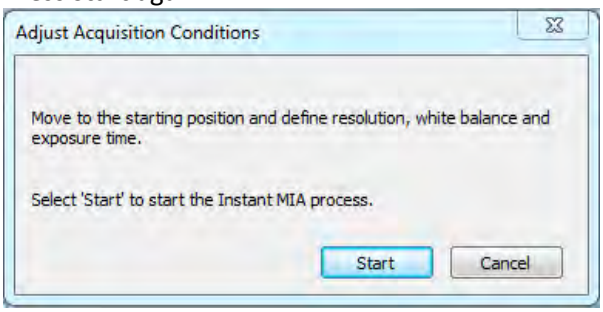
Choose Instant MIA and Overwrite pixels



Press **start**

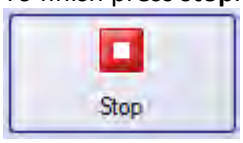


Press **start** again



Move the stage slowly to cover the desired area. As long as the frame is turquoise you are doing fine. If you pass an area twice the software will determine which iteration was better quality and overwrite.
If you go over an area with little data or too fast the frame will become fuchsia and you there is a chance you will not be able to recover your path. You can always finish.

To finish press **stop**.



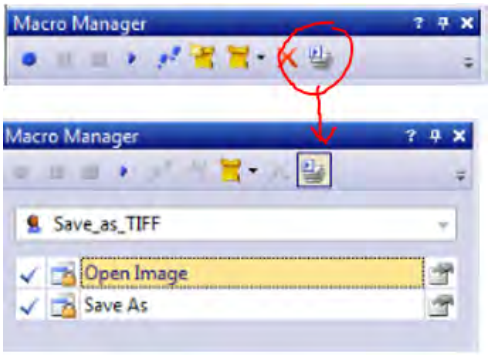
Save/Export/Batch

The multidimensional file you save will consist of a native VSI file and a folder of the same name. These should stay in the same folder and their names should be identical and not changed later, even if to identical.

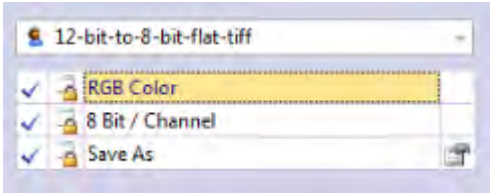
To open in FIJI download the Olympus Viewer Plugin
http://imagej.net/OlympusImageJPlugin

Besides saving the file (**File→Save**) in the native VSI format you can also export the image to tiff:
do **File → Export → Tiff** for further processing in image analysis programs.

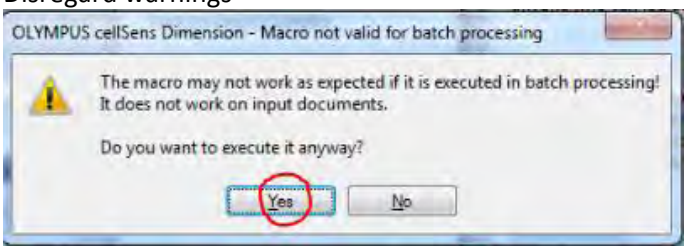
For batch export to tiff,
If files are RGB, 8 bit use the **Save_as_TIFF** macro:
View→Tool Windows→Macro Manager
Select batch mode:



Convert to 8 bit flat TIFF
If files are 12 bit channel stacks use the **12-bit-to-8-bit-flat-tiff** macro:

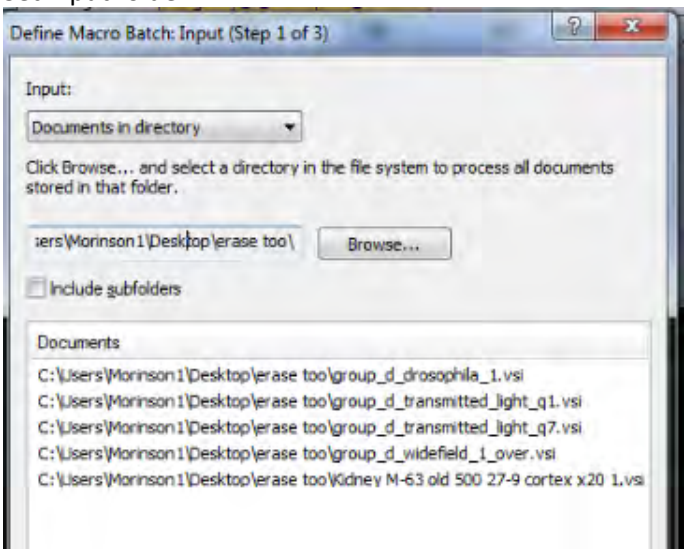


Disregard warnings



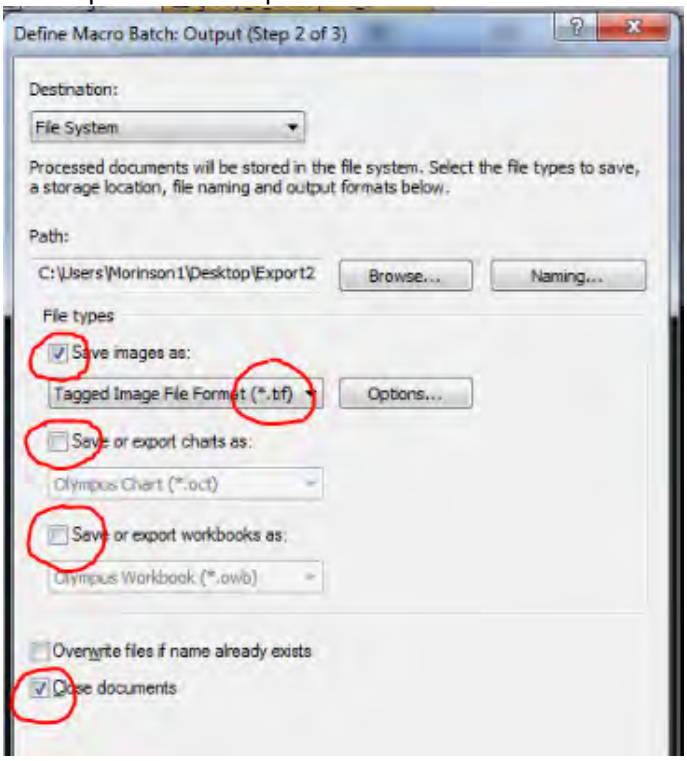
Set input folder and output folders.
If the file names are the same, including subfolders will not help!!!
If file names are the same you will have to convert each folder separately!

Set input folder:



Press **Next**

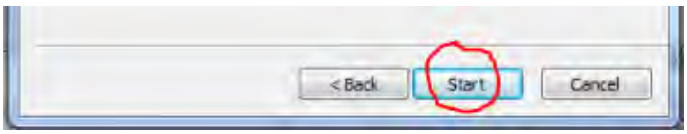
Set output folder and parameters:



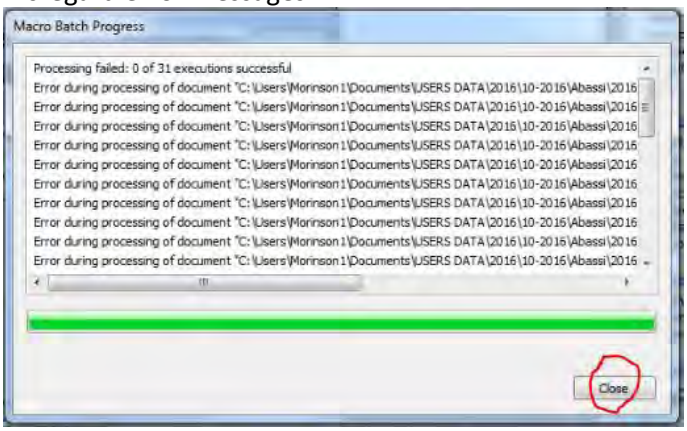
You can define a new output folder on the fly.

Press **Next**

Press **Start**

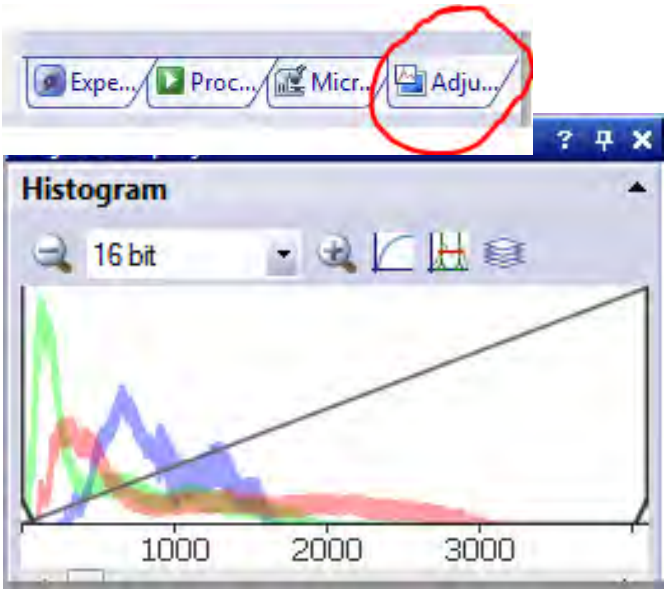


Disregard error messages



Histogram

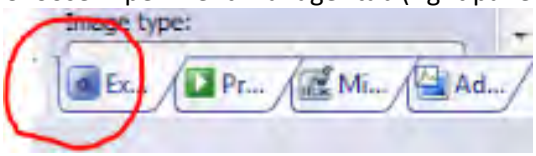
select the tab **[Adjustments]**:



Experiment Manager

(Intelligent way to save a multidimensional imaging configuration)

To make a new experiment:
Choose Experiment Manager tab (right panel, below)

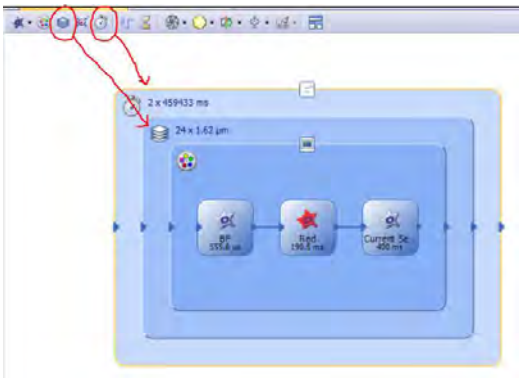


Click on [New]

In the upper ruler click on **Multichannel Group** ( ) and in the central panel draw a container (rectangle).

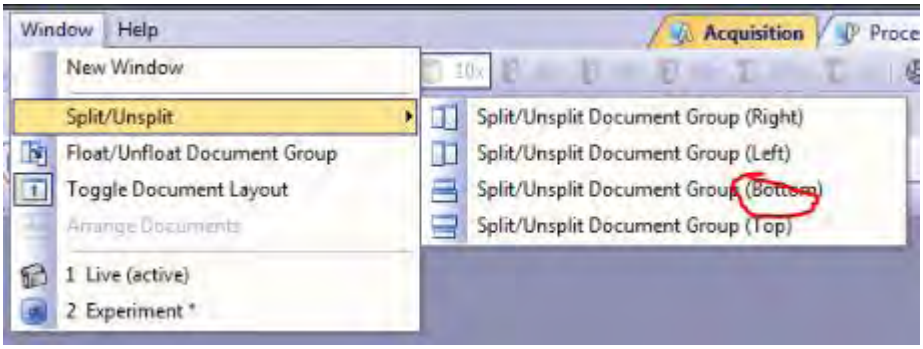
Click on **Image Acquisition** ( ) and click inside the container.
Repeat for the number of remaining channels you need.

You can add Z-sections and Time Lapse by clicking on the appropriate sign on the ruler and drawing around the channel container.

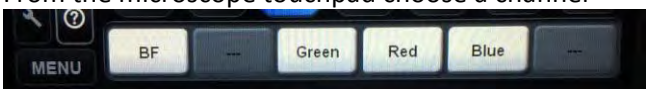


Click on Live either on the right panel or the left panel:

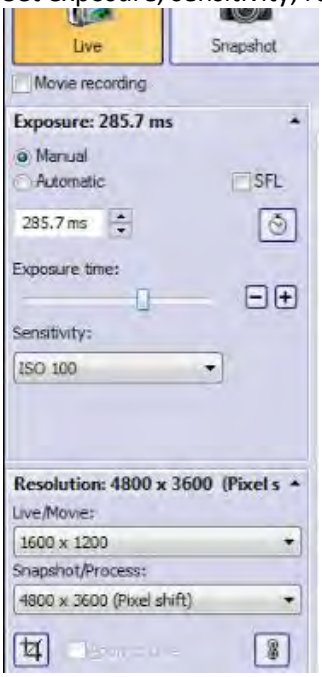
A new tab will appear behind the experiment in design with the Live window.
You can see both windows simultaneously by using Windown→Split/Unsplit



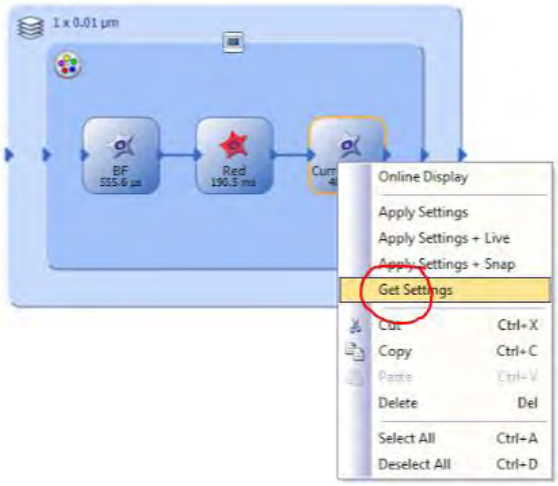
From the microscope touchpad choose a channel



Set exposure, sensitivity, resolution…



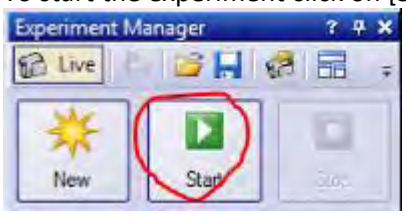
Select a channel (yellow perimeter=selected), right click → [**Get settings**] to apply the current settings to the selected channel.



The option [**Apply settings**] will change the settings of the Live window to the settings stored on the channel.

A number of such experimental configurations can be set up consecutively.
Save the experimental setup to an .oex file

To start the experiment click on [**Start**]



Scale bar

To add scale bar: **View→ scalebar**
To burn scalebar, take snapshot and then **image → burn info**

Polarized light microscopy

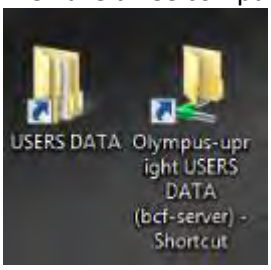
In the turret, choose the analyzer (no. 5) (EPI tab)

Turn the halogen lamp brightness to 5 (DIA tab → brightness)

Rotate the polarizer manually to exclude directly transmitted light

Backup files

Move your files from the local USERS DATA folder to the server to access from any computer in the building
Please do not use a USB flash disk on this computer.
We have three computer in the Analysis room that have active virus protection for this purpose.



Shut down

(1) Quit CellSens
(2) Log off from Bookitlab
(3) On the touchpad press **Off** and again **Off** and then **OK**
(4) Turn off the X-cite lamp
(5) Cover microscope