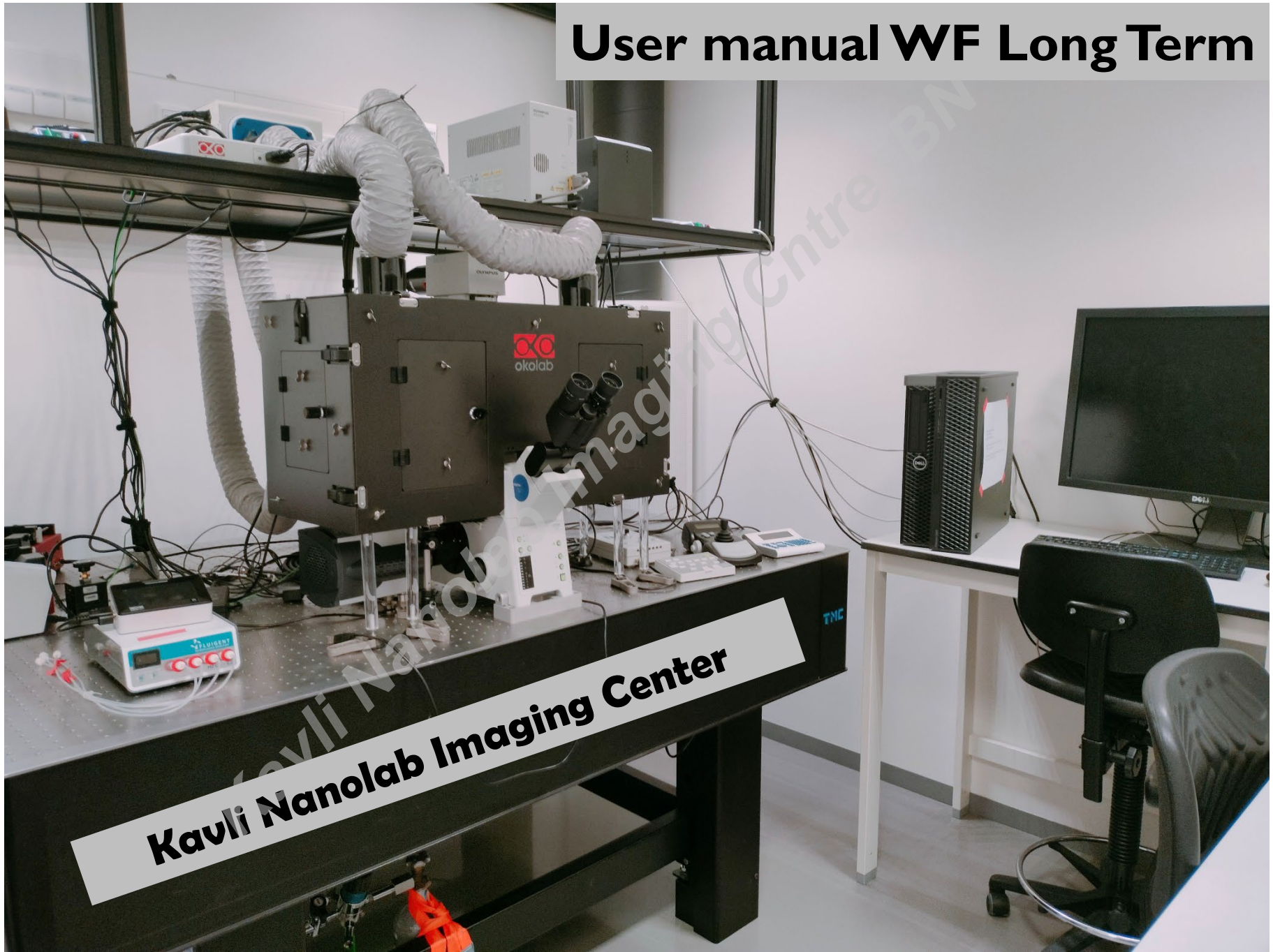
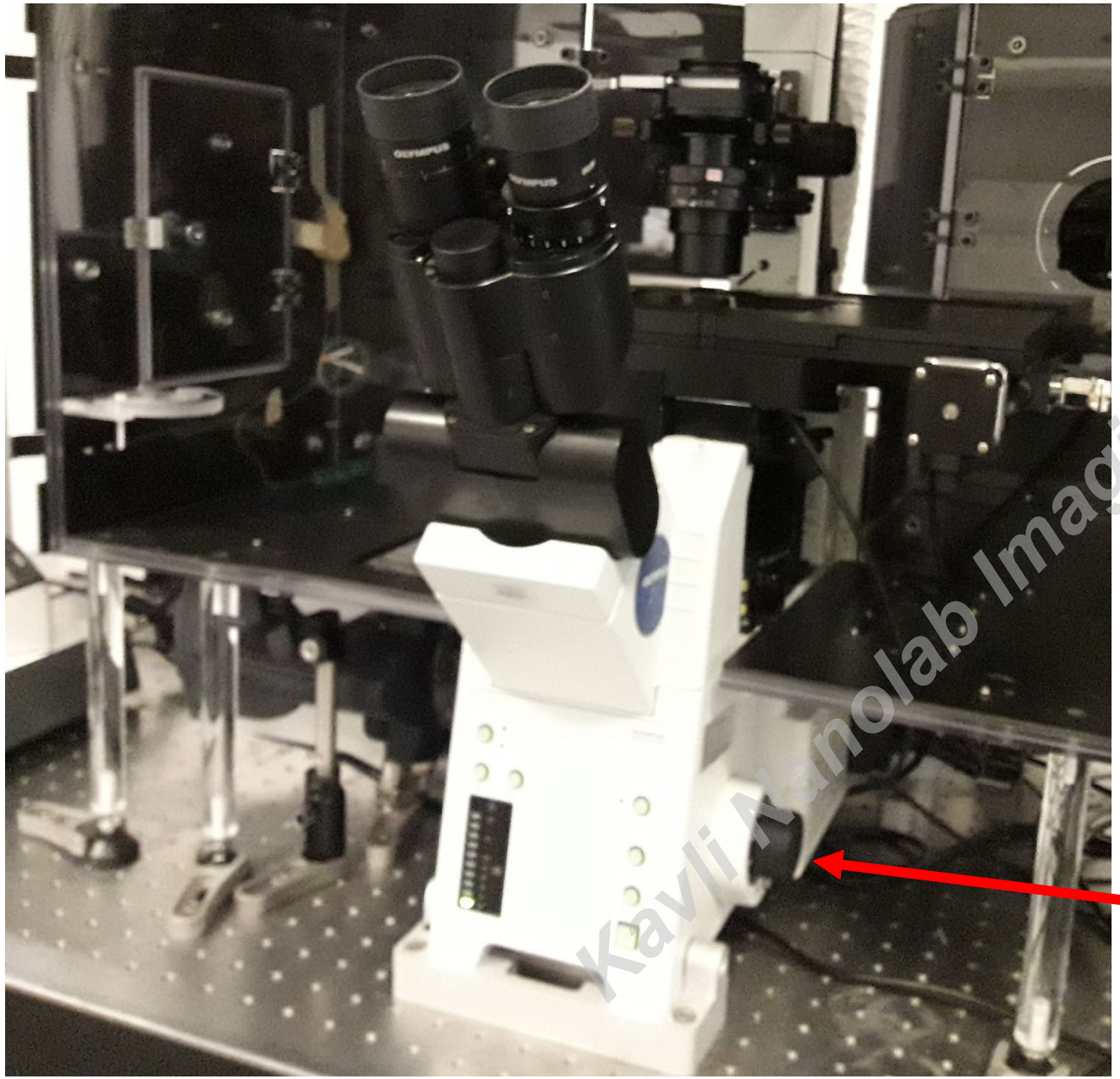
User manual WF Long Term



1, Turn ON 1&2 Main switches

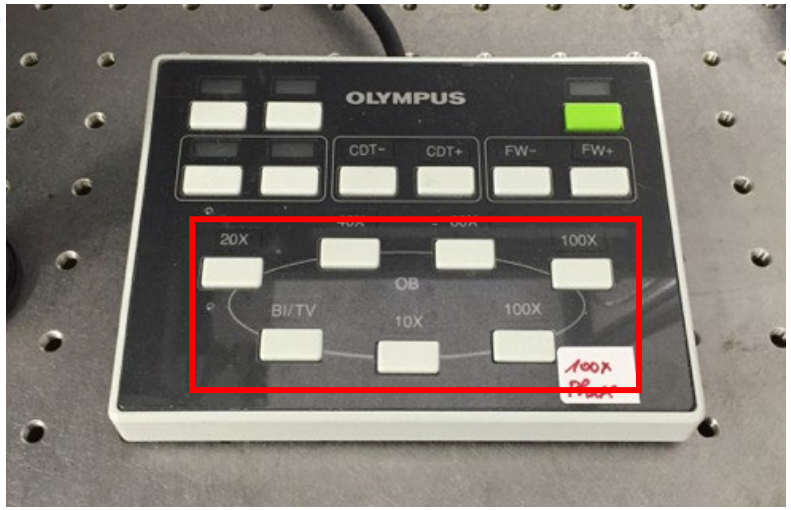


2, Find your sample

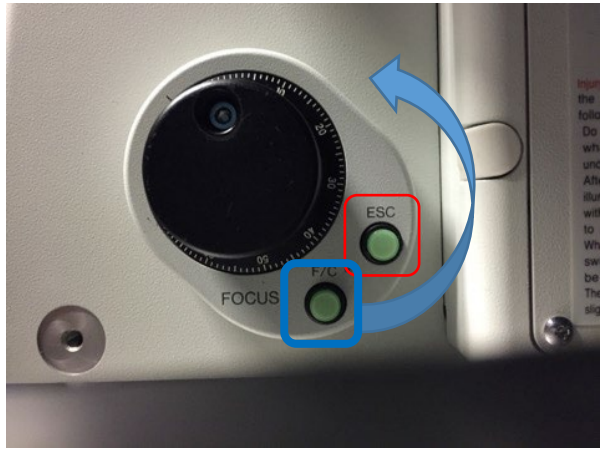
2,1, Choose Objective:

Start with 10X (Dry)

**Ask guidance before using Oil for the first time!*



2,2, Find your sample's focus



Raise the objective with knob- carefully (use *Fine /Coarse*)

Use **ESC** every time you start with new sample

Transmitted light OR Fluorescence:

2,3, Make sure you choose the eye path

Adjustment of Transmitted light:

Choose:
BF (Brightfield)
DIC (Differential Interference contrast)
Ph (Phase contrast)

Passes through your sample.

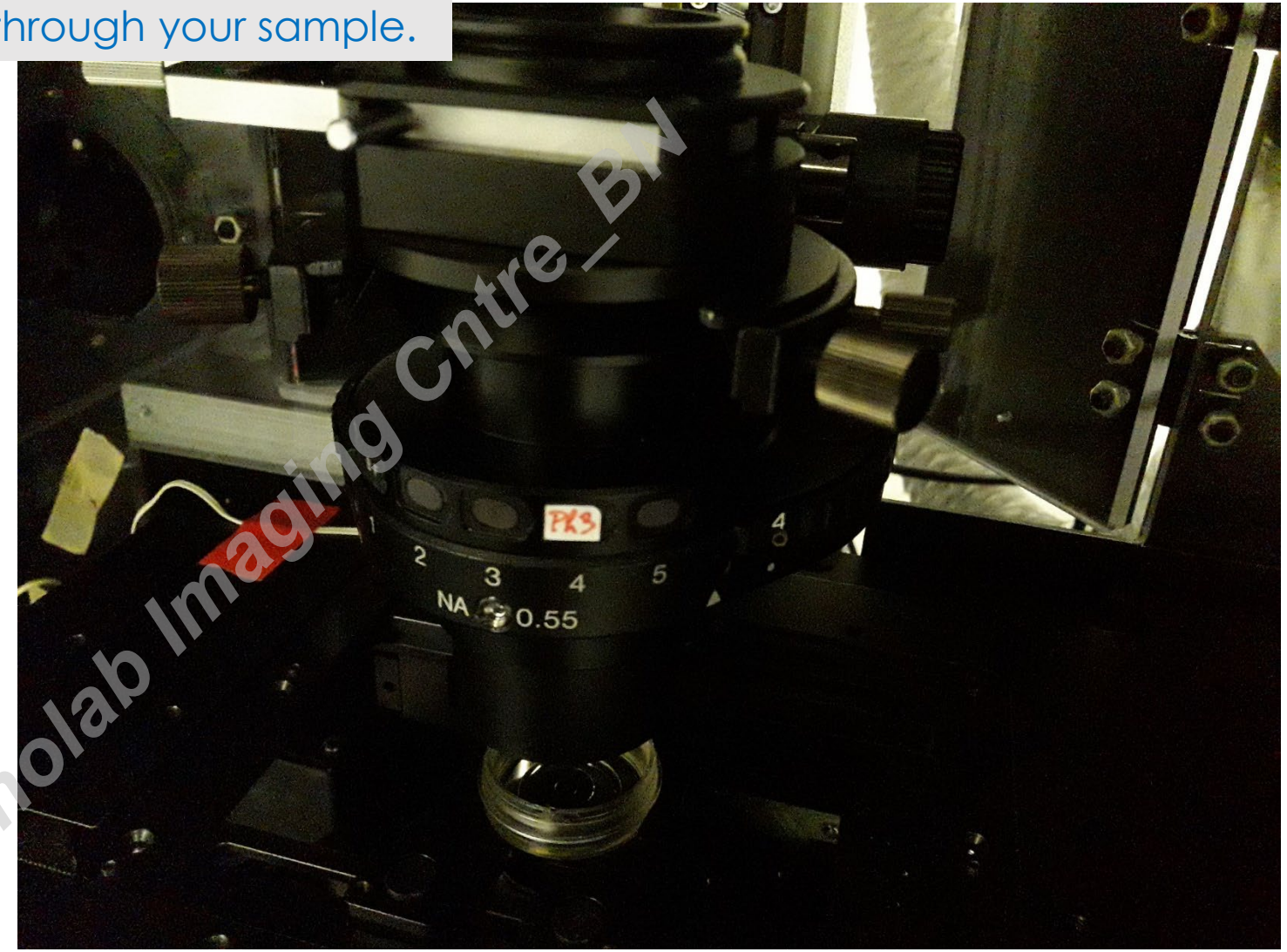
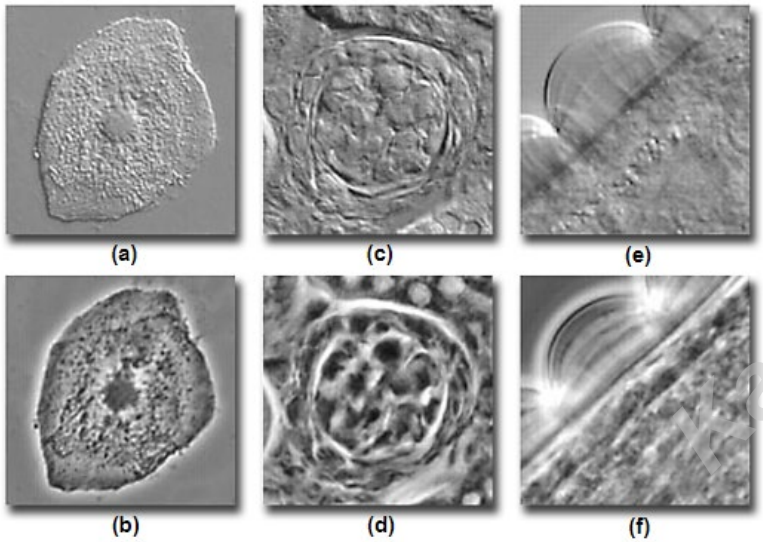


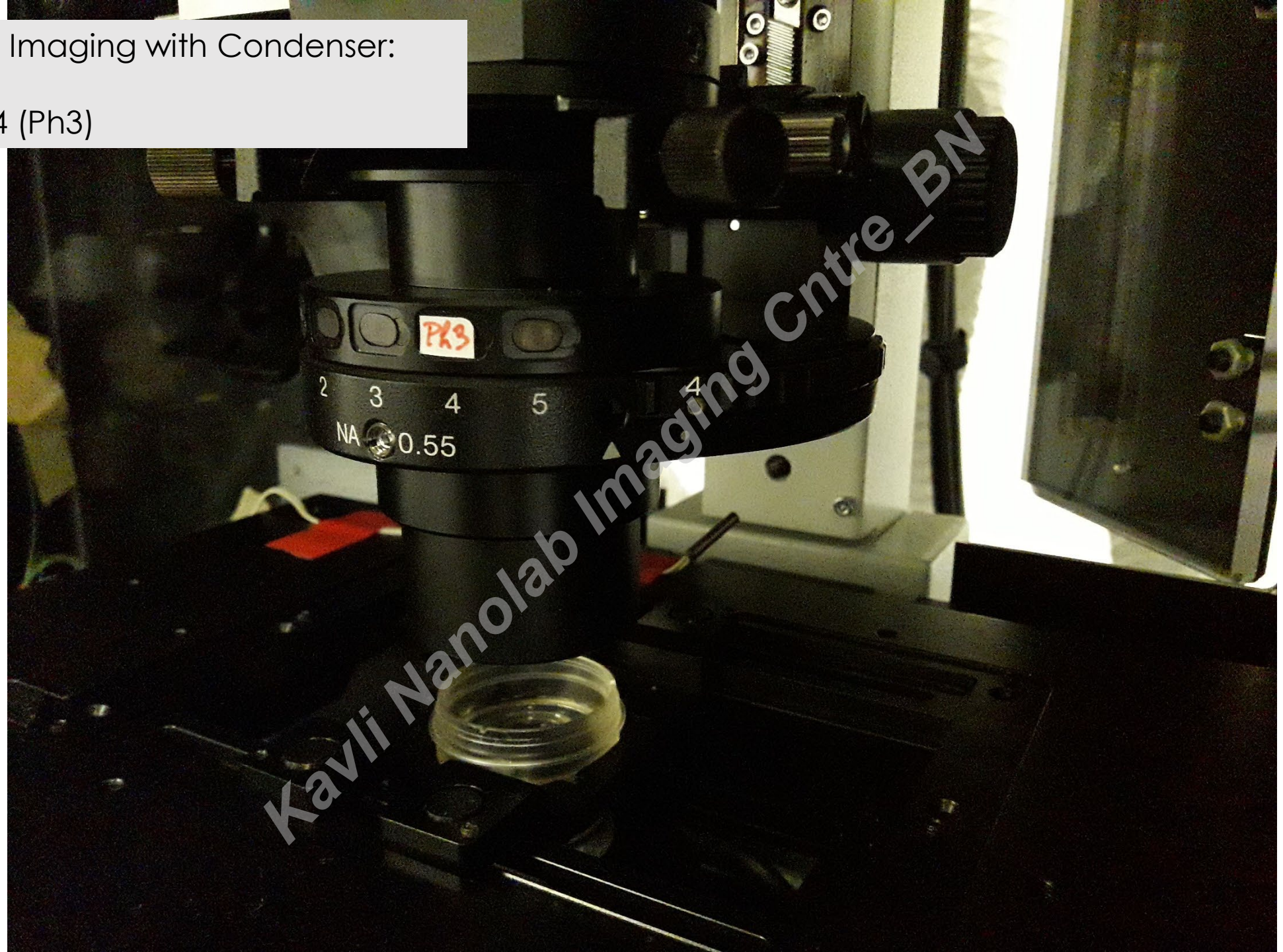
Figure 1 - Transparent Specimens in Phase Contrast and DIC



https://www.microscopyu.com/tutorials/comparison-of-phase-contrast-and-dic-microscopy

To set Phase Imaging with Condenser:

Use Position4 (Ph3)



Focus maintenance for long term imaging- Hardware

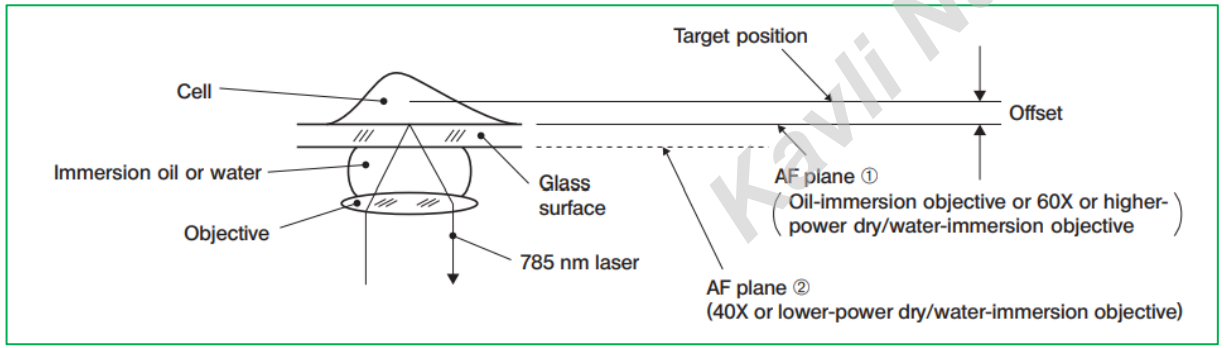
Hardware AF:

On the Left Side of the microscope body you can set the Olympus ZDC Dichroic Mirror (DM):

Shift left (IN) to place mirror in the optical path

Shift right (OUT) to place mirror out of the optical path

This gains more light if AF is not needed



This is a Drift Compensation mechanism- red light (*785 nm*) is focused to the glass surface, and relative positioning to the objective is kept. <u>It is limiting your sample preparation:</u>

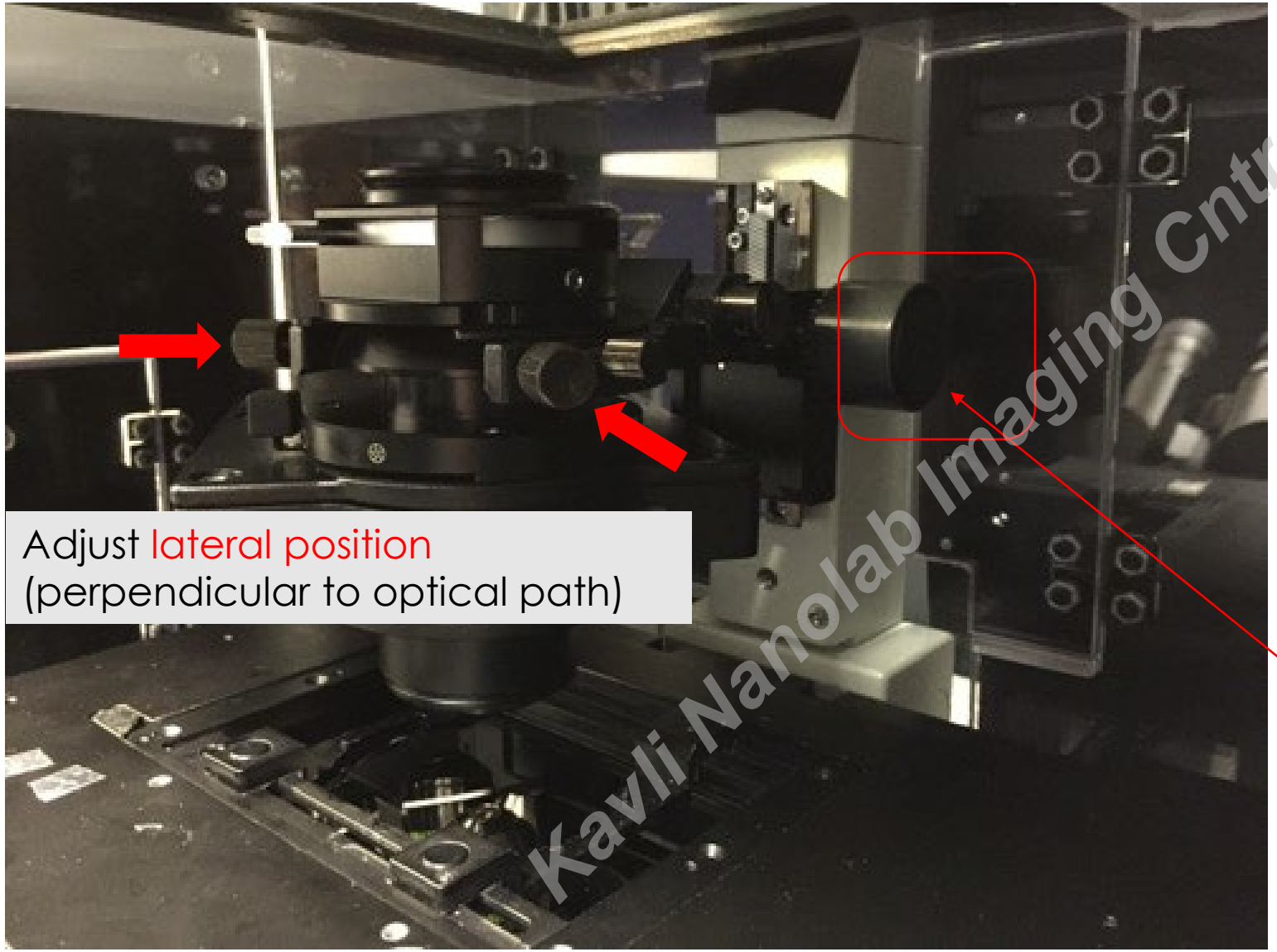
<u>Only glass (#1.5)-</u> could be that one brand works better, check

<u>High magnification objective only>60X</u>

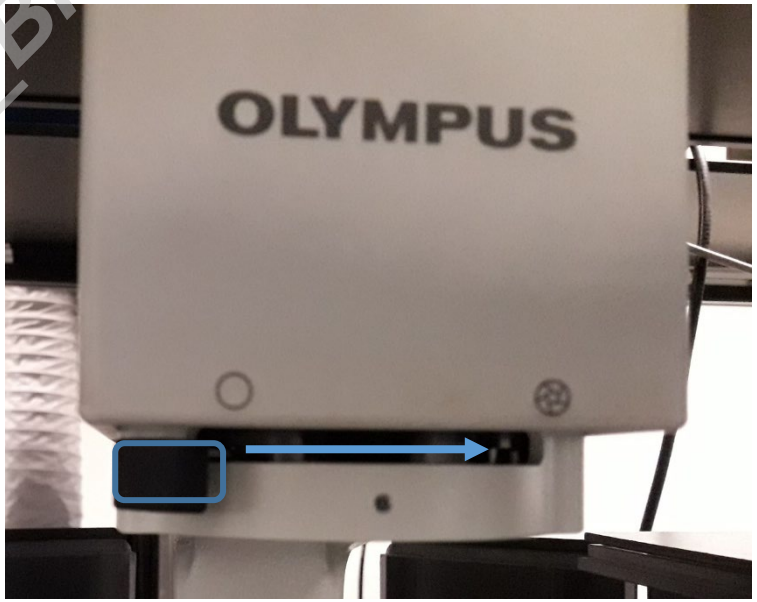
<u>Thin sample</u> (<8µm from coverglass)

<u>best for Flu-</u> Doesn't work well with phase/DIC

Need to align phase contrast?



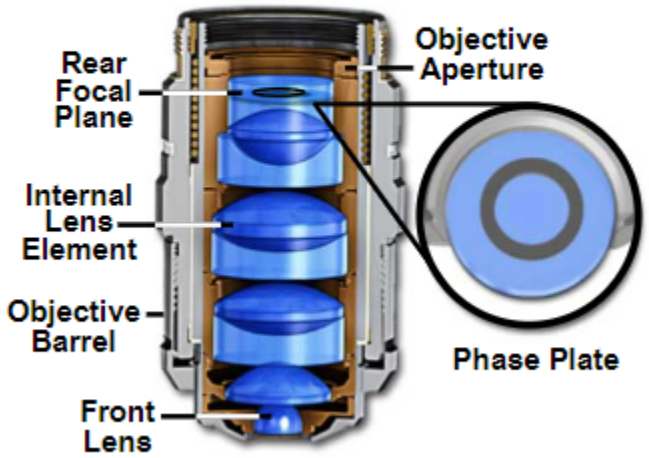
Use 10X
Close the shutter so you see edges



Change **condenser height** until you get best focus
(along the optical path)

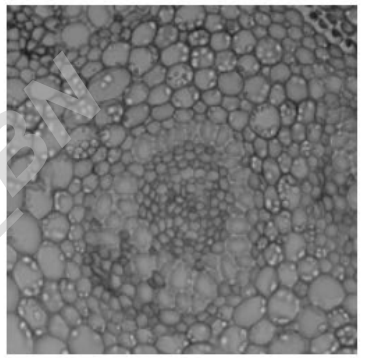
Phase contrast alignment (1/2): Procedure

Figure 4 - Phase Contrast Objective

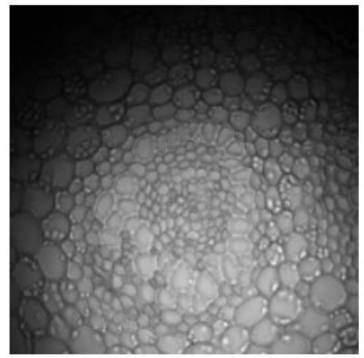


https://www.microscopyu.com/techniques/phase-contrast/phase-contrast-microscope-configuration

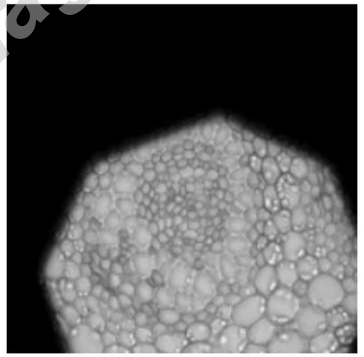
1- Choose either the 10x, 20x or 40x objectives, as this procedure won't work with higher magnification



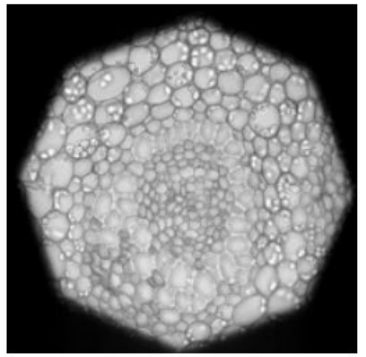
2- Focus on the sample and keep it fixed during the whole procedure



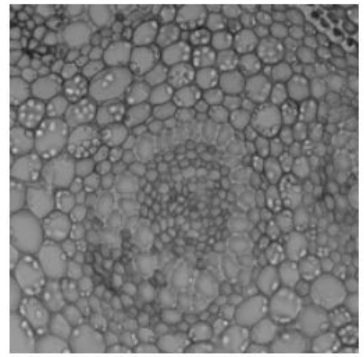
3- Close the stop-field iris



4- Adjust the condenser hight to get a clear image of the iris



5- Centre the iris



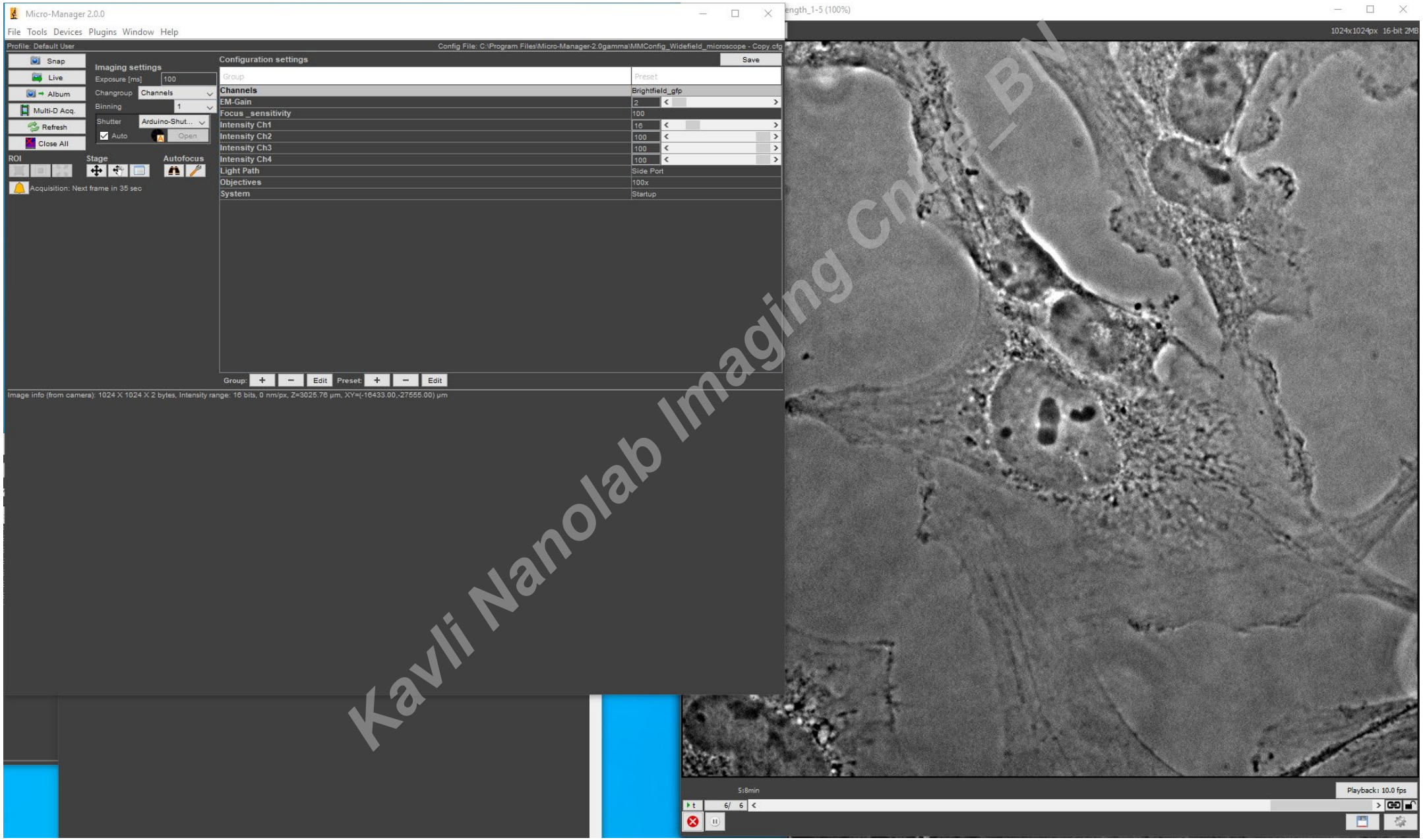
6- Open the iris slightly more than the field-of-view

Jérémie Capoulade

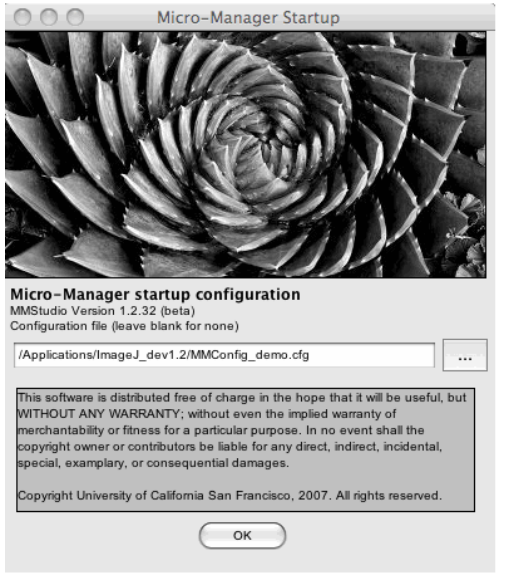
See also:

https://zeiss-campus.magnet.fsu.edu/articles/basics/practical.html

3, Start the PC and software



Log in as Default user
- You can use the general protocol for WF or save your own protocol



Channels: *choose from the list and optimize each. Brightfield needs to be combined with specific filter- if you have fluorescent signal, keep the same filter to avoid redundant filter shifts*

EM gain- *only increase if you have good enough signal*

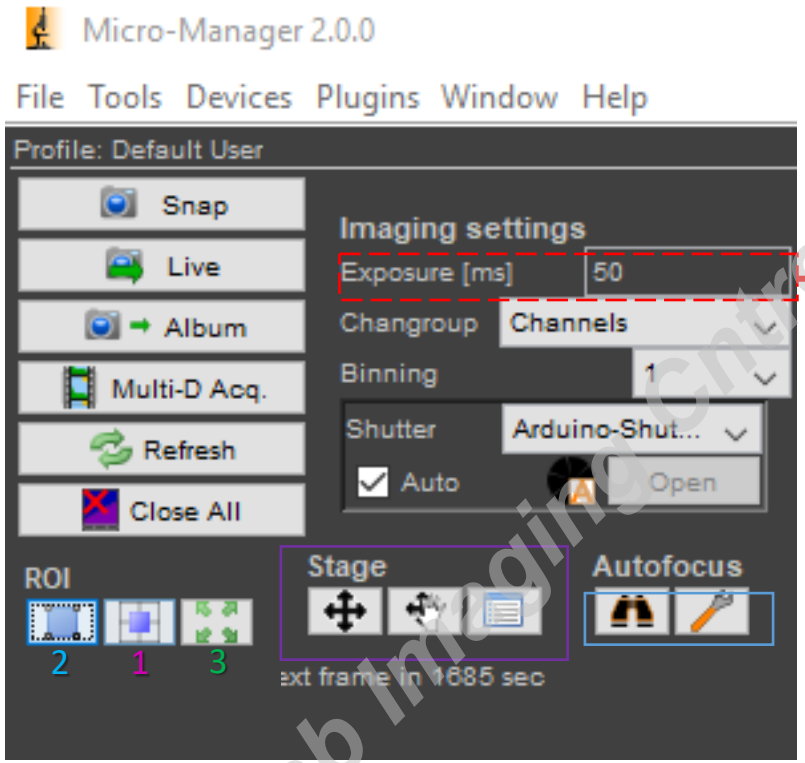
Intensity #1-4: *tune the channels chosen with CoolLed*

Objective: *should fit the chosen; don't move automatically!*

Light path: *side port (camera)*

To learn more about configuration and possibilities, read:
https://micro-manager.org/Micro-Manager_User's_Guide

Snap: take a single image
Live: continuous



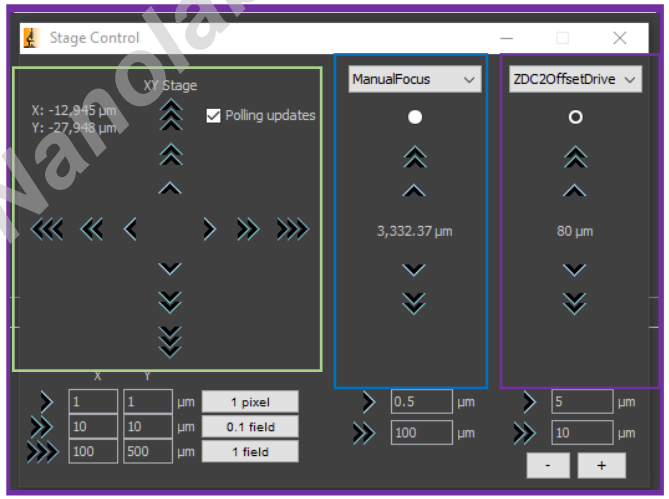
modify light intensity and camera exposer time for minimal bleaching with fast acquisition

*Click on **ROI icon**[1] in µManager to implement*

Click on ROI icon[2] to implement

*Click on **Reset**[3] to re-image full FOV*

Autofocus: can be set to hardware/software based.
See last pages in tutorial for info



XY stage
Move laterally
!If working with high magnification objective-limit your range!

ZDC2OffsetDrive
Changing autofocus relative height

Manual focus
To change objective height

Setting an experiment with Multi-D acquisition

Time: set duration and interval. For fast imaging, set time interval to 0 and keep only 1 channel with low camera acquisition time

Multiple positions: you can set a grid for tile / Independent list of positions, save Z for each

Z stacks: Change objective height to define upper and lower focus planes. Match interval to your objective.
**For Deconvolution Oversample in Z*

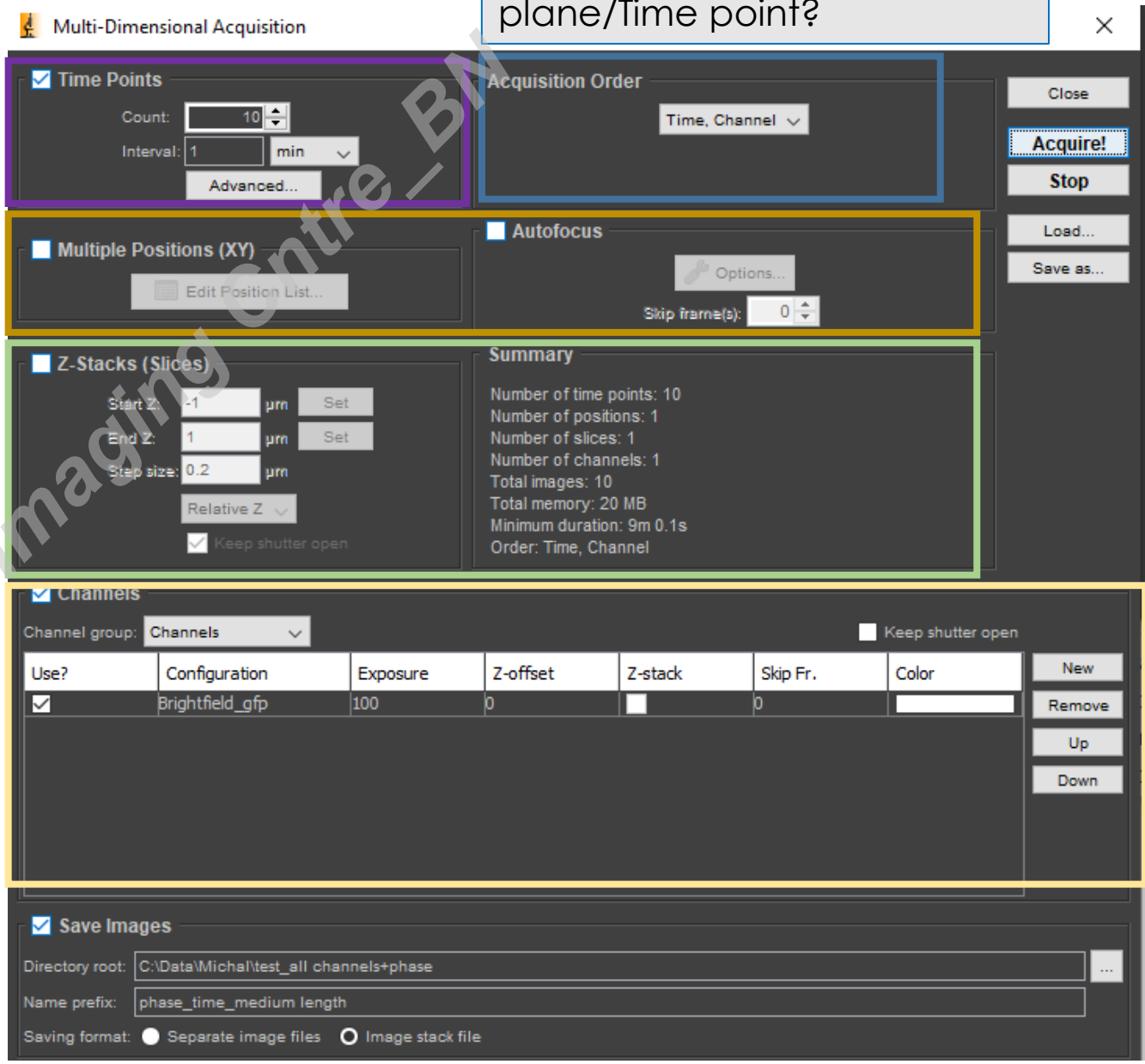
Channels:
"**New**" to add channel, "Remove" to delete
"**Up**" changes acquisition order (sequential)
"**Color**" sets your LUTs (just for presentation)

!! Not always the exposer time syncs with your previous definition for that channel- always verify!!

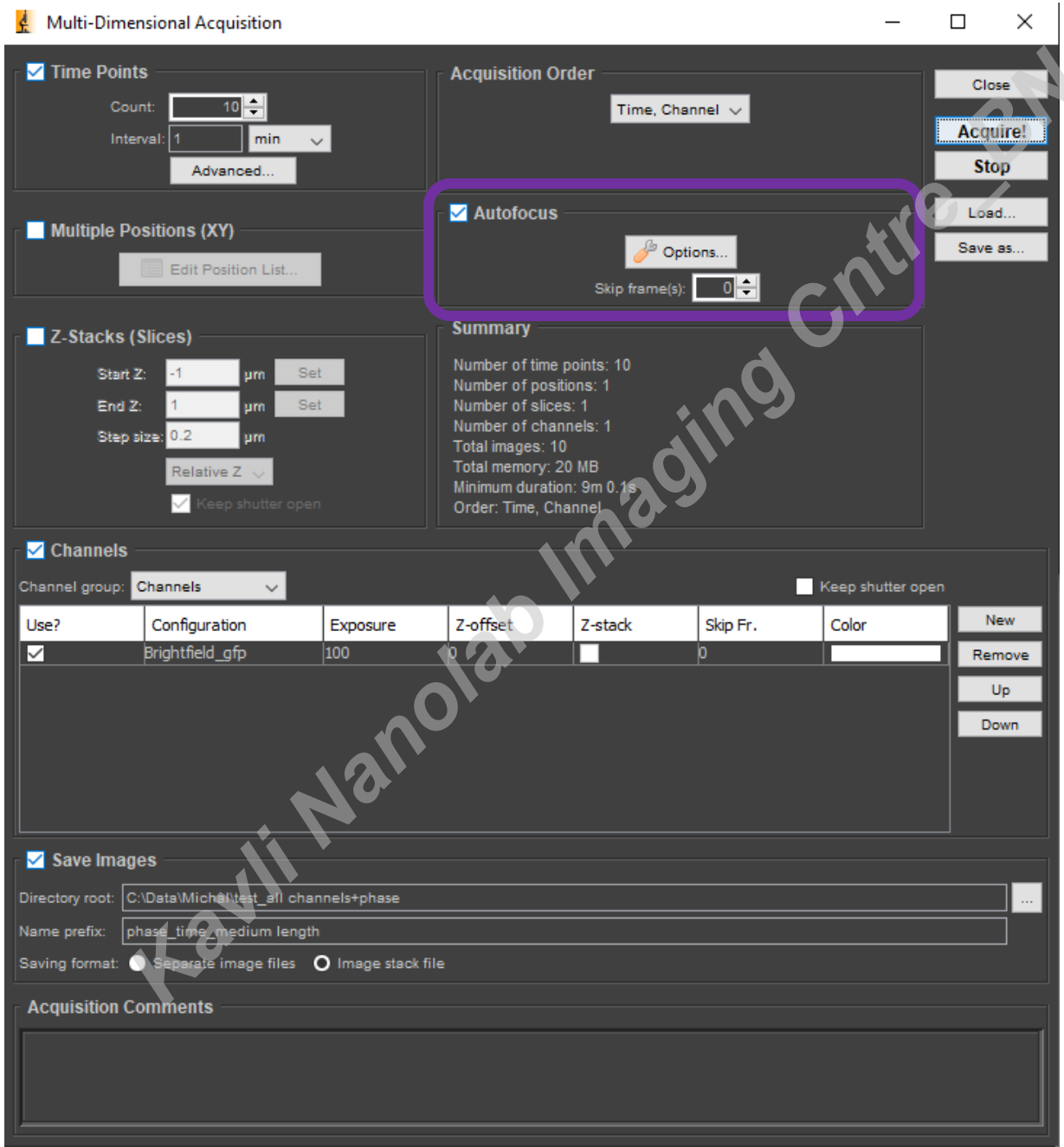
Save Images: Define your folder & File name.

Save locally under "Data".
Always remember to copy to shared folder when done

Order: do you want all channels imaged at a single Z plane/Time point?



Focus maintenance for long term imaging



To set focus maintenance actively during your experiment, **check this box.**
Skip Frames to only run AF once in several time frames

You can also just keep autofocus on **(unchecked in your experiment) for continuous Z drift compensation*

Focus maintenance for long term imaging- Hardware drift compensation

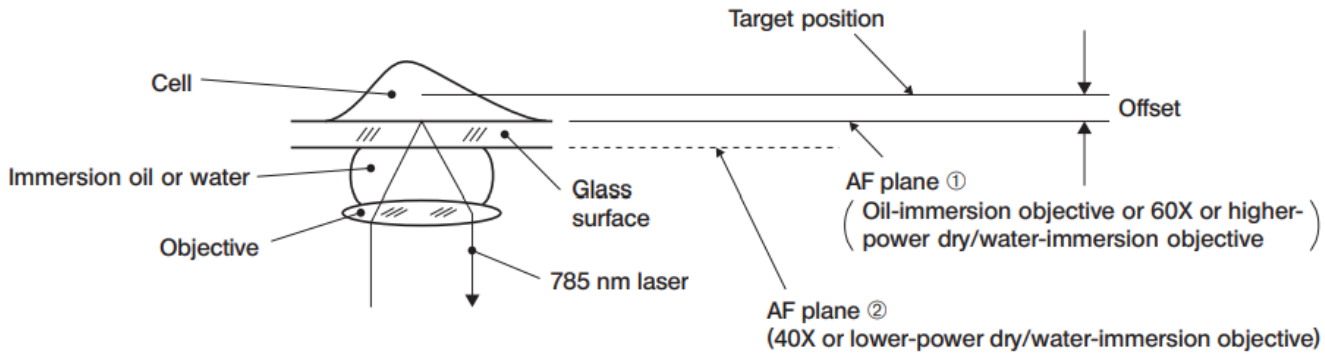
Autofocus properties

Refresh! | AutoFocusZDC | Close

Show read-only properties

Property	Value
AutoFocusZDC-ContinuousMode	On
AutoFocusZDC-Description	ZDC Auto Focus accessory
AutoFocusZDC-Name	AutoFocusZDC
AutoFocusZDC-ObjectiveTypeSetting	UPlanAPo 100XOI3
AutoFocusZDC-Offset	500.0000
AutoFocusZDC-SearchRange	100.0000

Choose "AutoFocusZDC" (Olympus hardware drift compensation module)
Set Objective used
Set searching range:
In principle, setting range<20µm will focus on AF plane 1 **or** 2 selectively (see image)
But you need to test it yourself and try several values



You can also set Software Auto-Focus (based on Image)

Autofocus properties

Refresh! | JAF(H&P) | Close

Show read-only properties

Property	Value
JAF(H&P)-1st step size	2.0
JAF(H&P)-1st step number	1
JAF(H&P)-2nd step size	0.2
JAF(H&P)-2nd step number	5
JAF(H&P)-Threshold	0.02
JAF(H&P)-Crop ratio	0.2
JAF(H&P)-Channel	

Choose "JAF(H&P)" (software based AF)
Start with current default settings.
Adjust and optimize according to time/quality needed

**By using this method, you take images at certain Z thickness (1st step size) and choose how many images you want (2nd step size). The focus for your experiment will be the one that produces highest contrast image*

Shut Down

1, Save your data and exit software, copy/backup and close PC
2, Press ESC to lower objective to lowest position
3, Remove your sample
4, If used oil objective- clean according to instructions (always ask guidance for 1st time)
5, Shift to 10X objective (lowest magnification)
6, close shutters

Always leave your environment clean,
take out the trash and avoid broken glass
Anything freakish? Let us know!

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