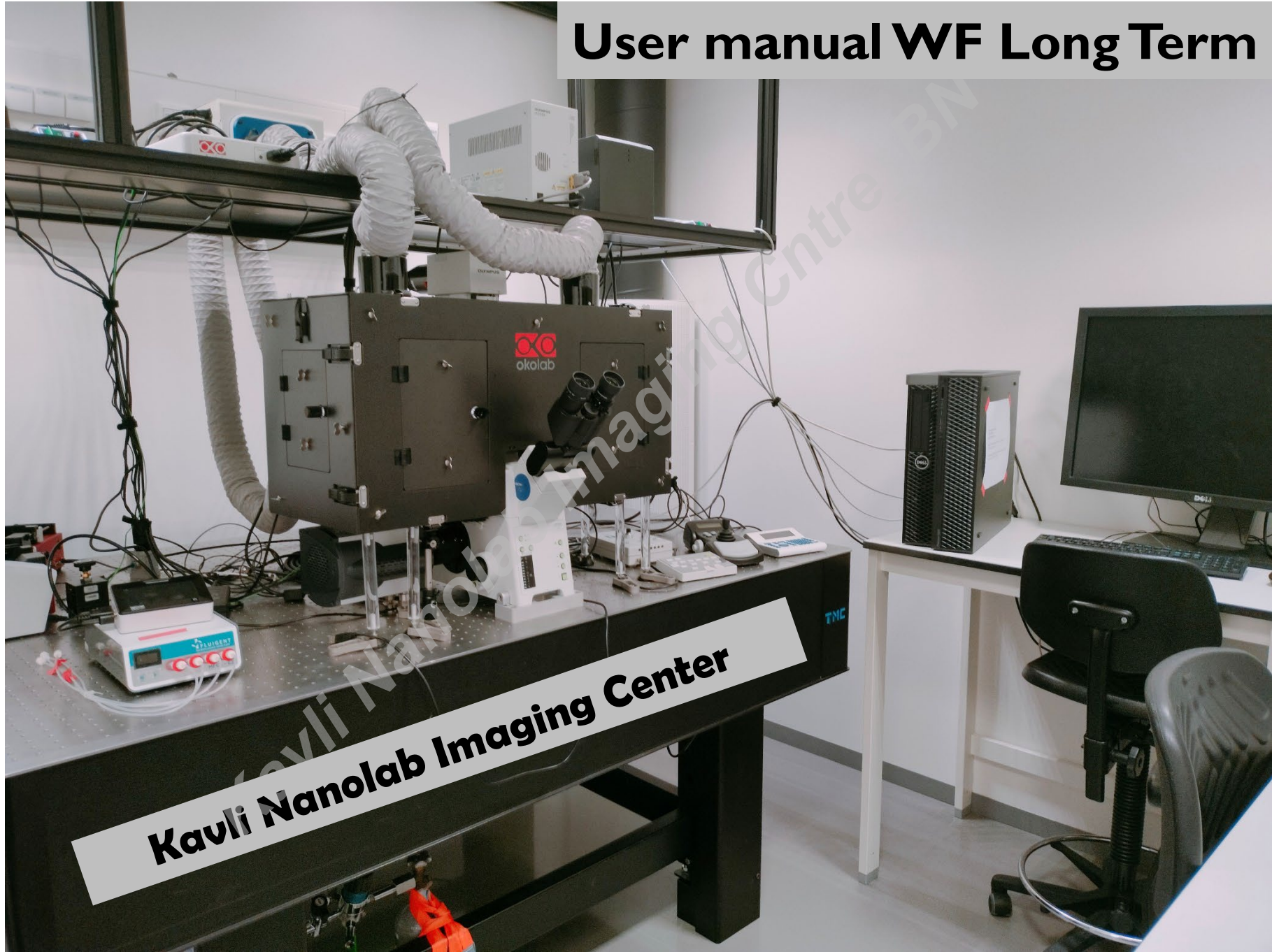


User manual WF Long Term

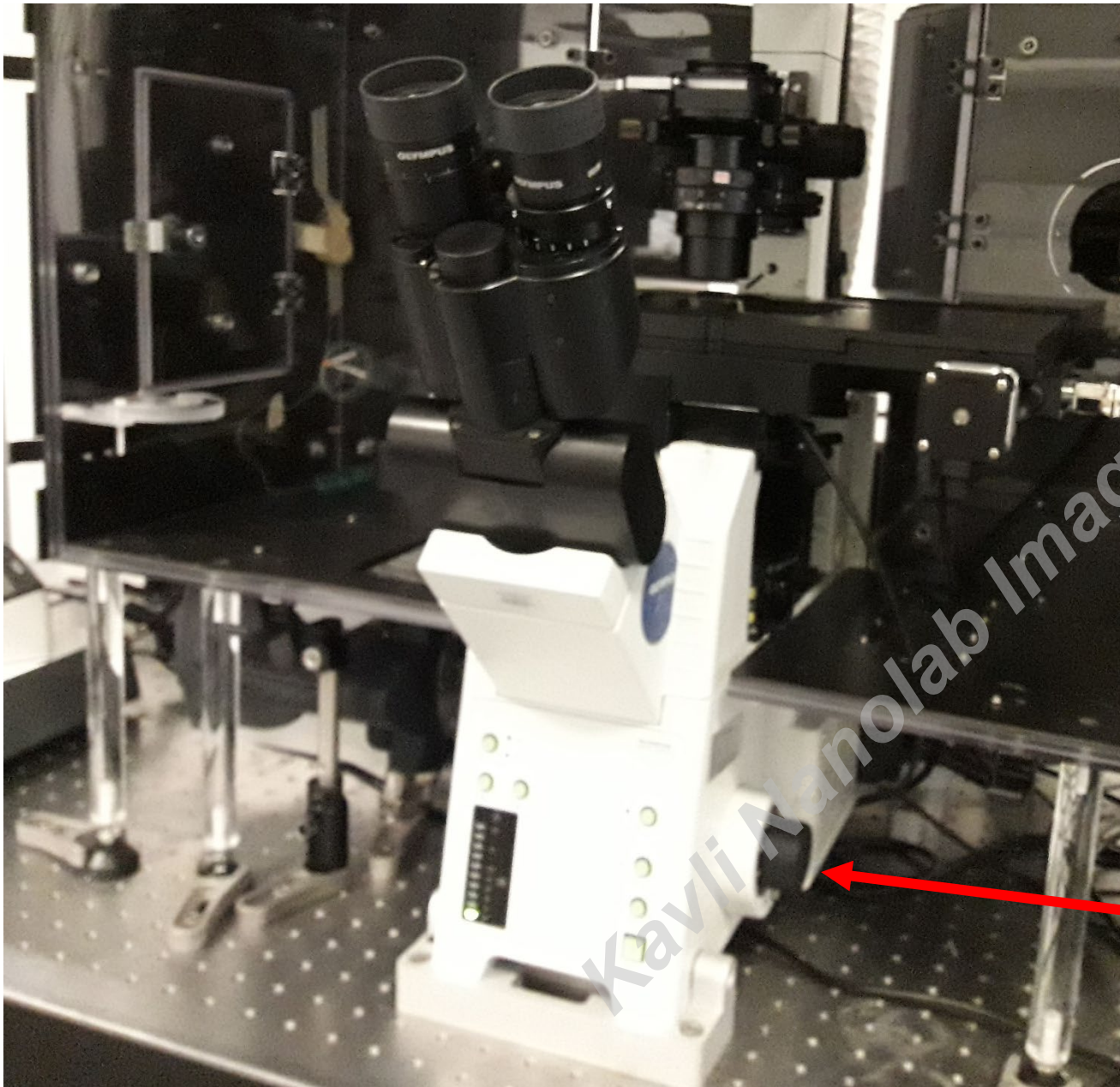


Kavli Nanolab Imaging Center

TYC

1, Turn **ON** 1&2 Main switches



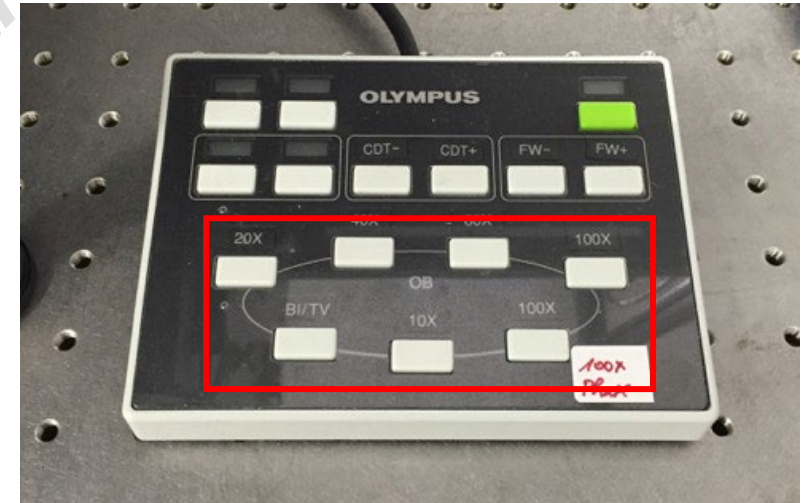


2, Find your sample

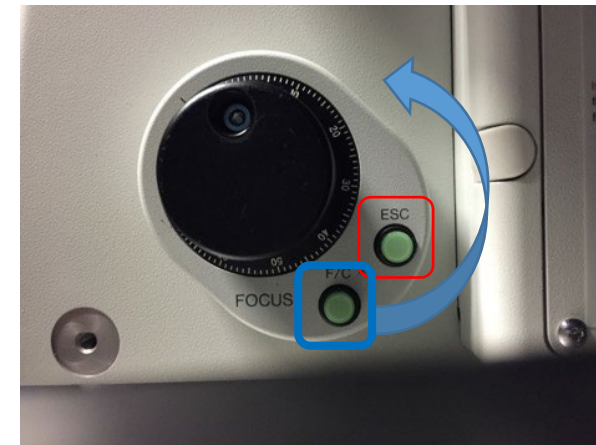
2,1, Choose Objective:

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:

TTL- Computer Controlled

Use **Continuous** for eye

Choose **Advanced**

Set your **wavelength**

and **Intensity**



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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.

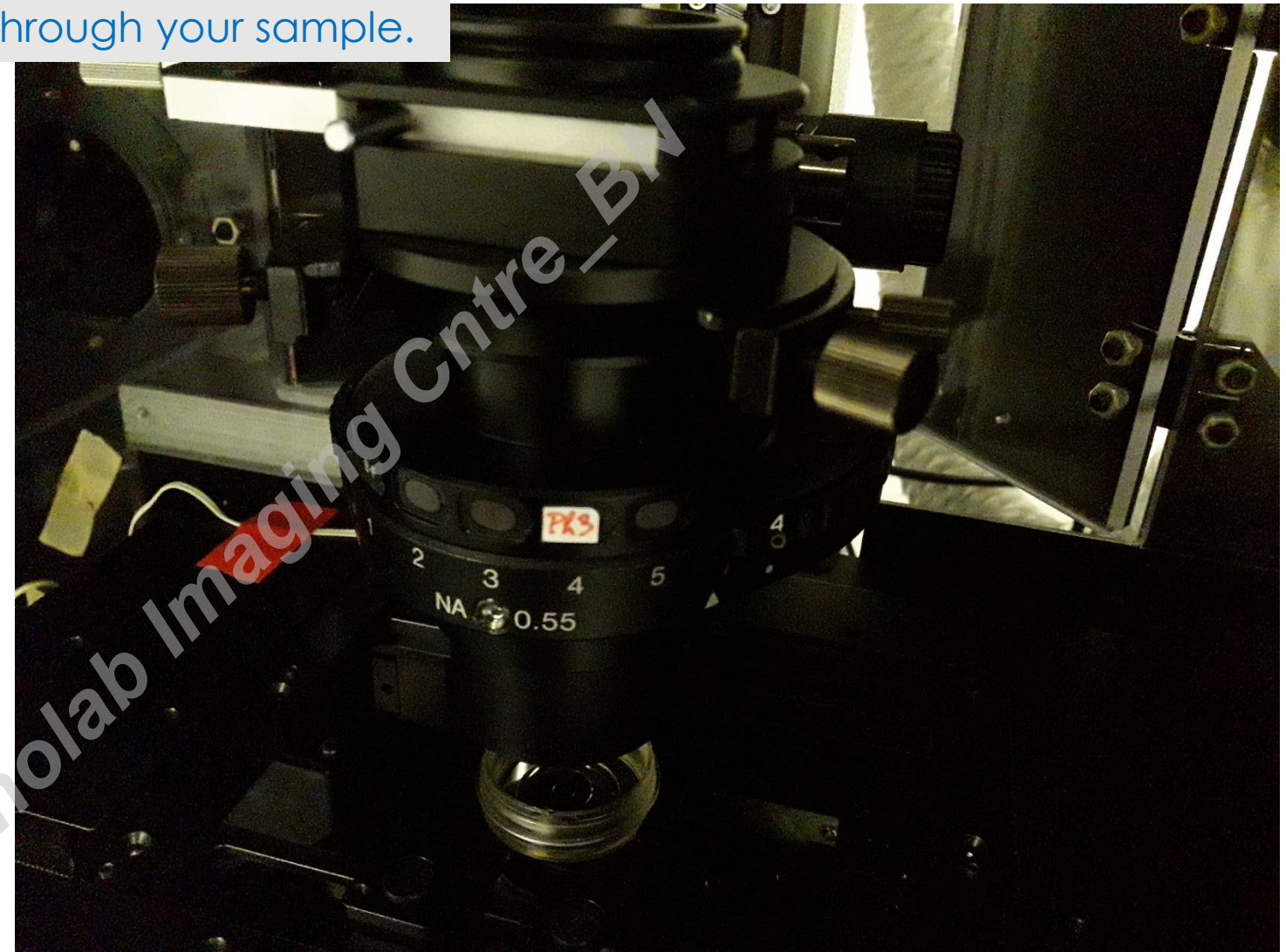
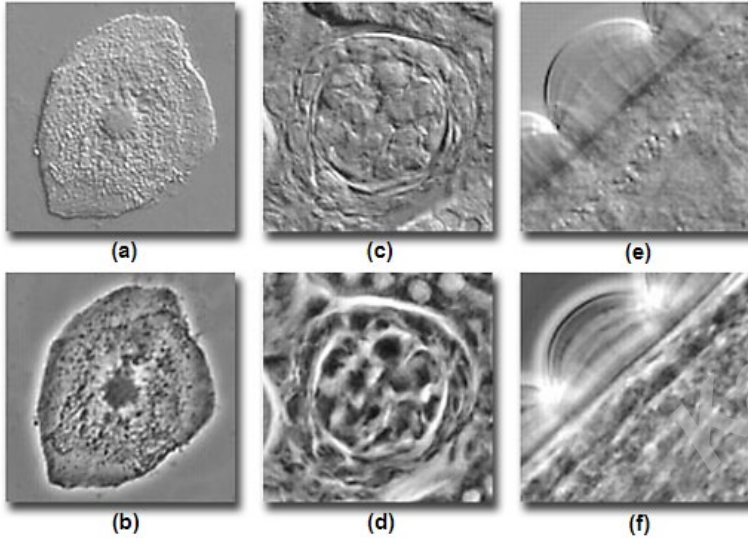
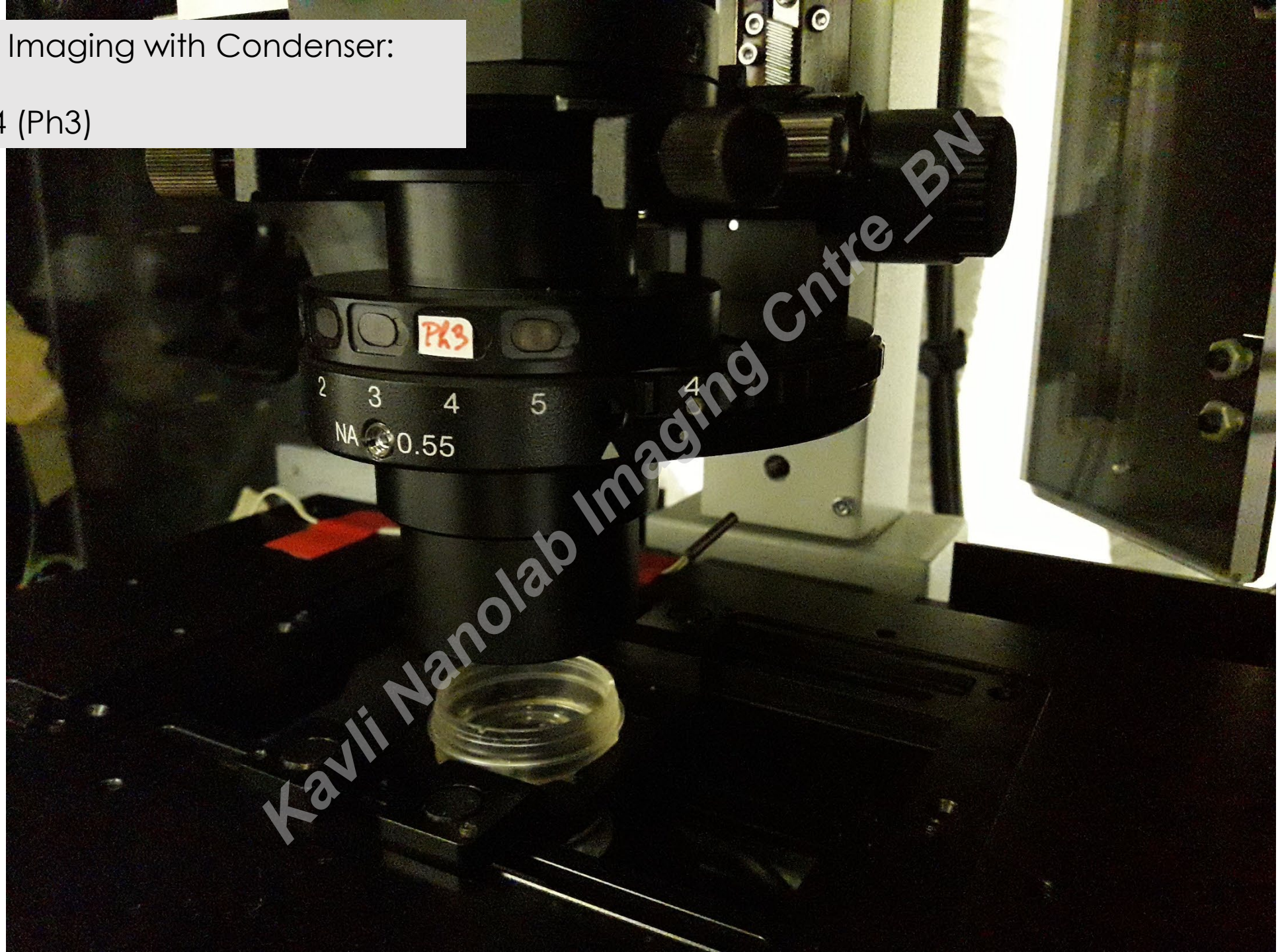


Figure 1 - Transparent Specimens in Phase Contrast and DIC



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):

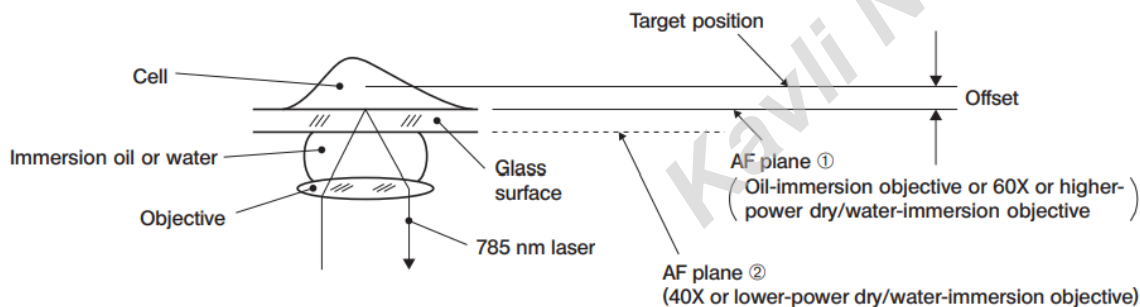
Shift left (IN) to place mirror in the optical path



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



This gains more light if AF is not needed



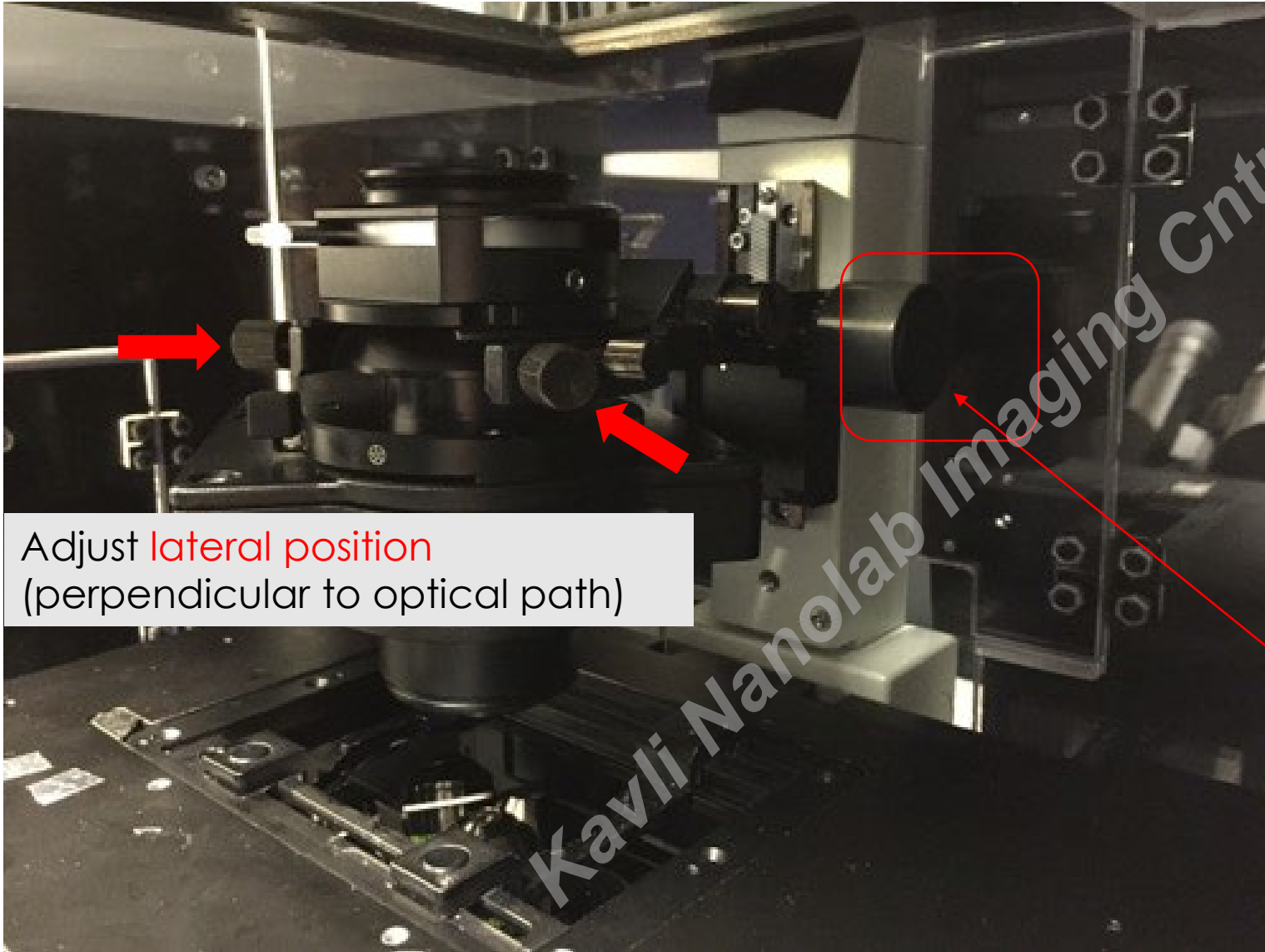
This is a Drift Compensation mechanism- red light (785 nm) is focused to the glass surface, and relative positioning to the objective is kept. It is limiting your sample preparation:

Only glass (#1.5)- could be that one brand works better, check
High magnification objective only >60X

Thin sample (<8 μm from coverglass)

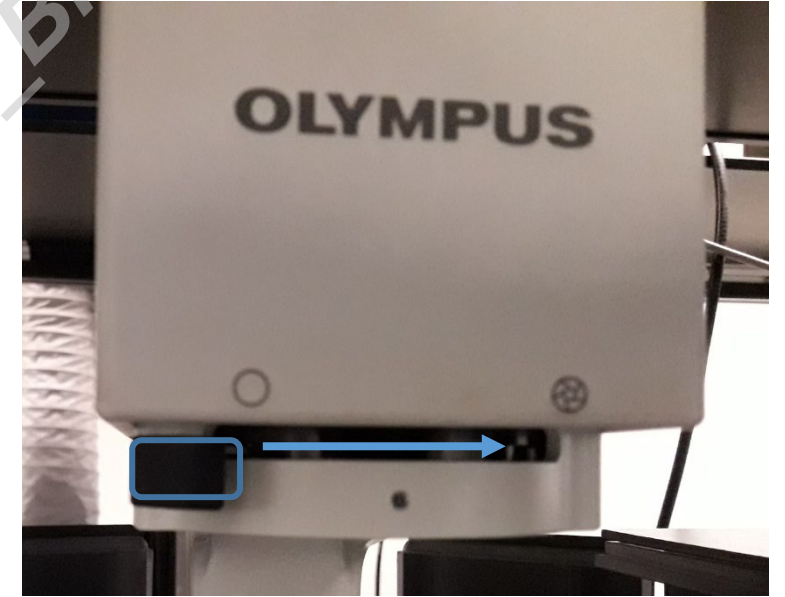
best for Flu- Doesn't work well with phase/DIC

Need to align phase contrast?



Adjust **lateral position**
(perpendicular to optical path)

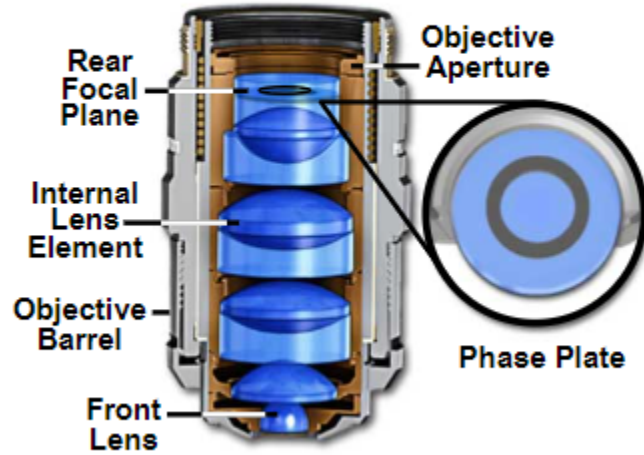
Use 10X
Close the shutter so you see
edges



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

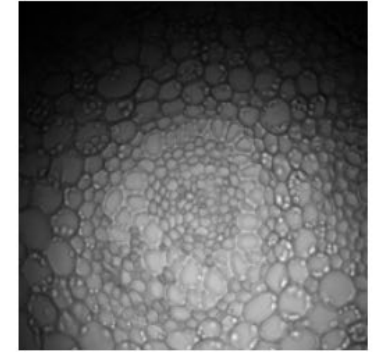
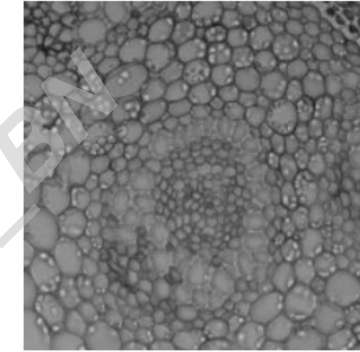
Phase contrast alignment (1/2): Procedure

Figure 4 - Phase Contrast Objective



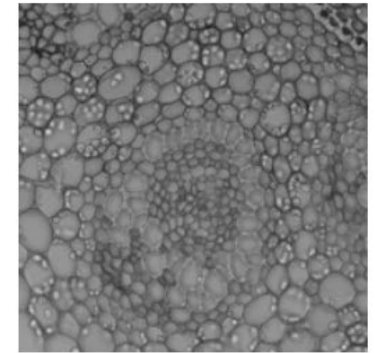
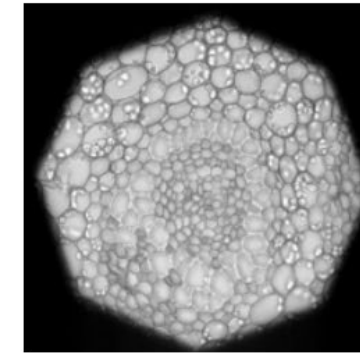
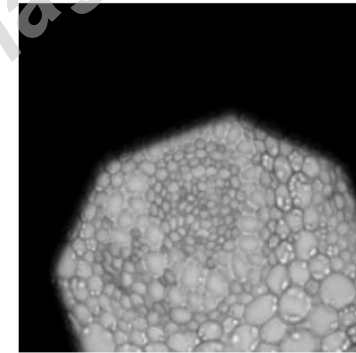
<https://www.microscopyu.com/techniques/phase-contrast/phase-contrast-microscope-configuration>

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 height to get a clear image of the iris

5- Centre the iris

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

See also:

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

Jérémie Capoulade

3, Start the PC and software

Micro-Manager 2.0.0

File Tools Devices Plugins Window Help

Profile: Default User Config File: C:\Program Files\Micro-Manager-2.0\gamma\MMConfig_Widefield_microscope - Copy.cfg

Imaging settings

- Exposure (ms): 100
- Changroup: Channels
- Binning: 1
- Shutter: Arduino-Shut...
- Auto

Configuration settings

Group	Preset
Channels	Brightfield_gfp
EM-Gain	2
Focus_sensitivity	100
Intensity Ch1	16
Intensity Ch2	100
Intensity Ch3	100
Intensity Ch4	100
Light Path	Side Port
Objectives	100x
System	Startup

Image info (from camera): 1024 X 1024 X 2 bytes, Intensity range: 16 bits, 0 nns/px, Z=3026.76 μm, XY=(-16433.00,-27658.00) μm

5:8min Playback: 10.0 fps

6/6

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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

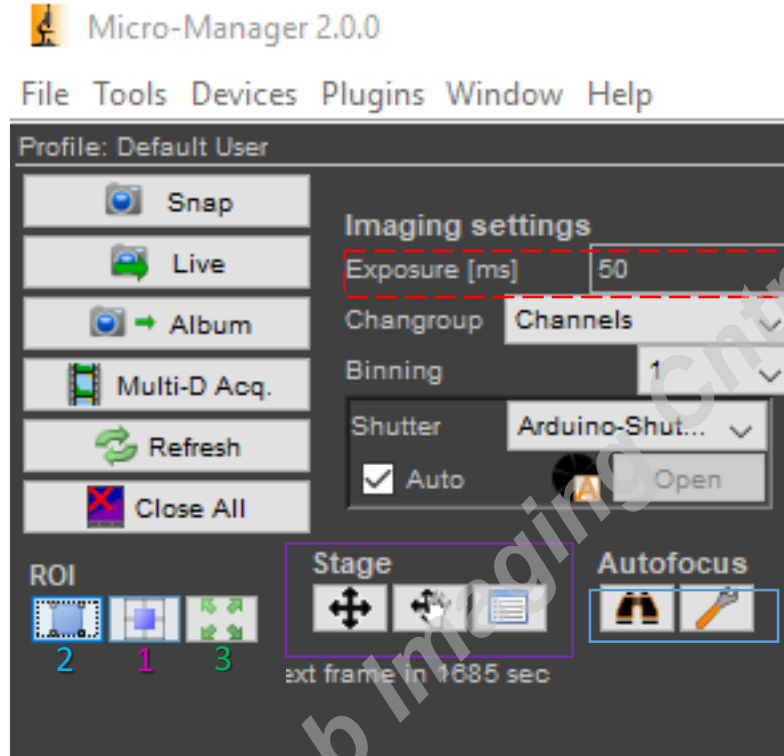
Intensity #1-4: tune the channels chosen with CoolLed

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

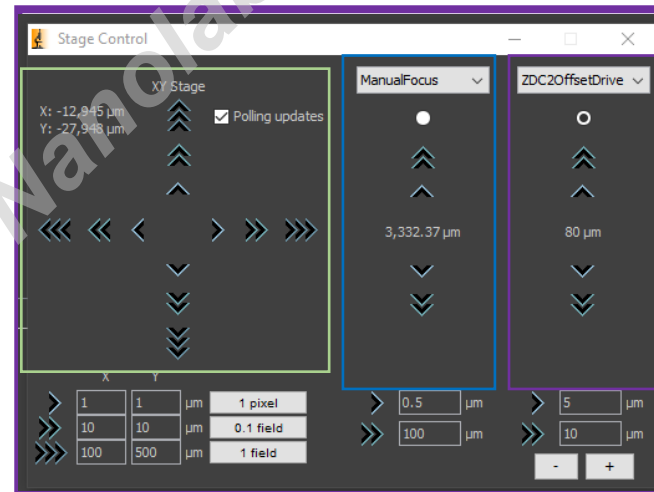
Click on **ROI icon¹** in μ Manager to implement

Click on **ROI icon²** to implement

Click on **Reset³** 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

Multi-Dimensional Acquisition

Time Points
Count: 10
Interval: 1 min
Advanced...

Acquisition Order
Time, Channel

Multiple Positions (XY)
Edit Position List...

Autofocus
Options...
Skip frame(s): 0

Z-Stacks (Slices)
Start Z: -1 µm Set
End Z: 1 µm Set
Step size: 0.2 µm
Relative Z
 Keep shutter open

Summary
Number of time points: 10
Number of positions: 1
Number of slices: 1
Number of channels: 1
Total images: 10
Total memory: 20 MB
Minimum duration: 9m 0.1s
Order: Time, Channel

Channels
Channel group: Channels Keep shutter open

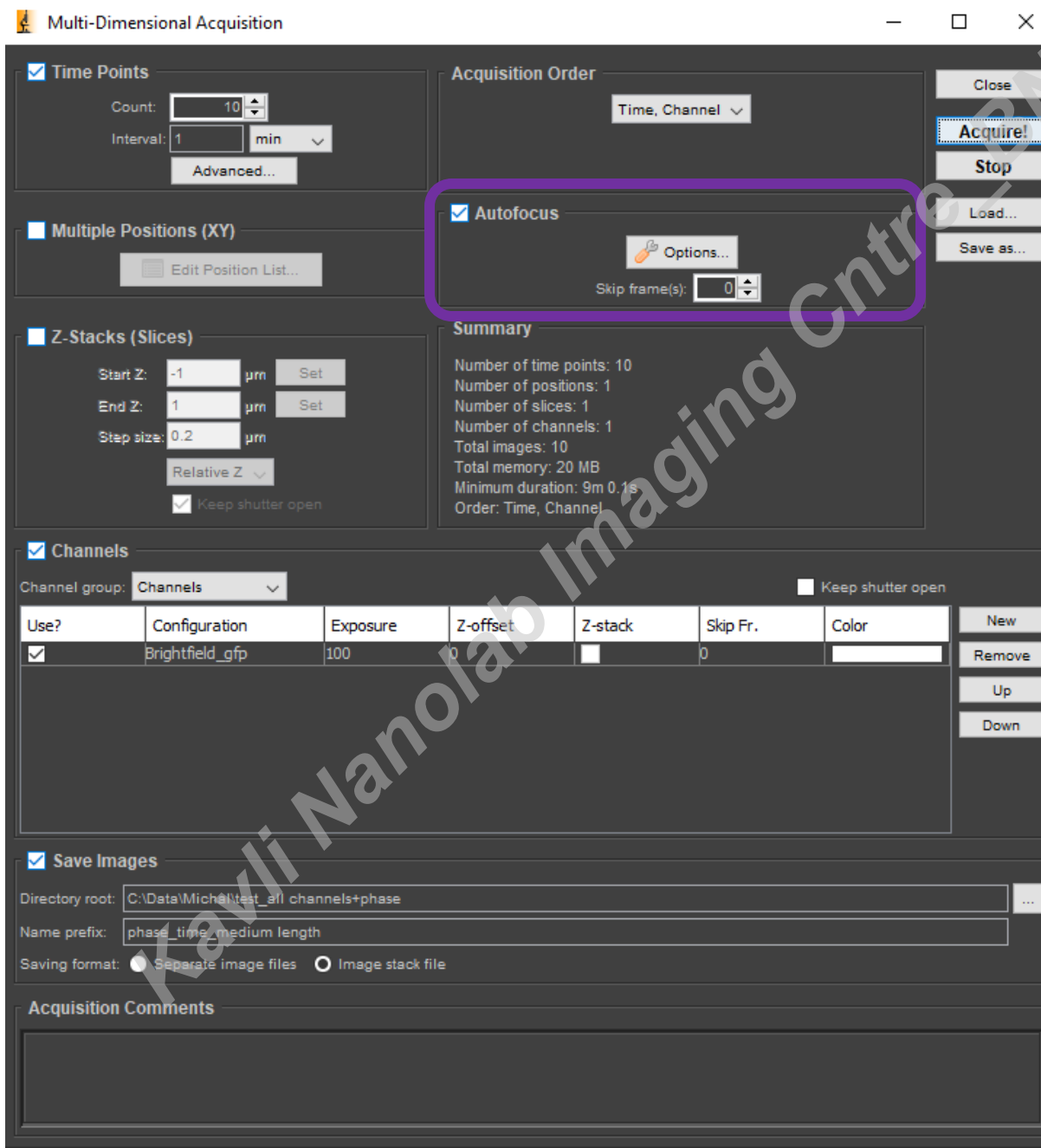
Use?	Configuration	Exposure	Z-offset	Z-stack	Skip Fr.	Color	
<input checked="" type="checkbox"/>	Brightfield_gfp	100	0	<input type="checkbox"/>	0		New Remove Up Down

Save Images
Directory root: C:\Data\Michal\test_all channels+phase
Name prefix: phase_time_medium length
Saving format: Separate image files Image stack file

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

Close
Acquire!
Stop
Load...
Save as...

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

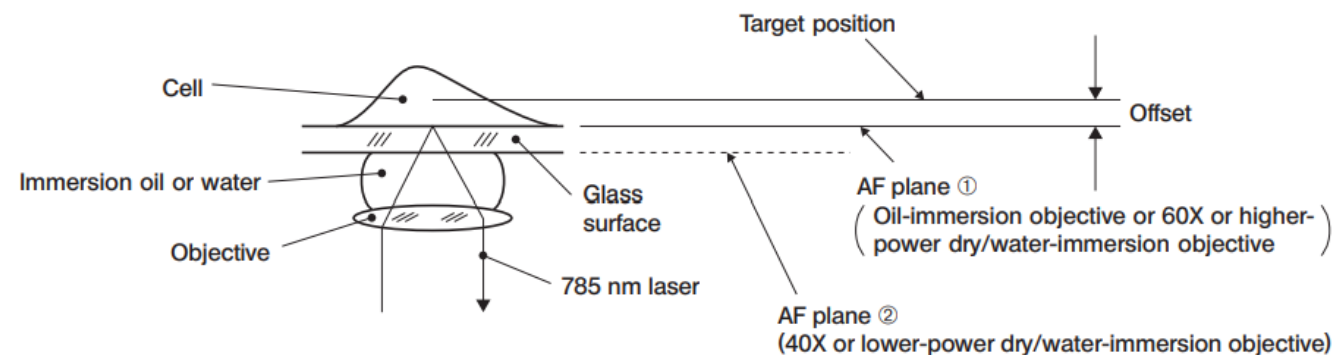
 Autofocus properties ✕

 Refresh! AutoFocusZDC Close

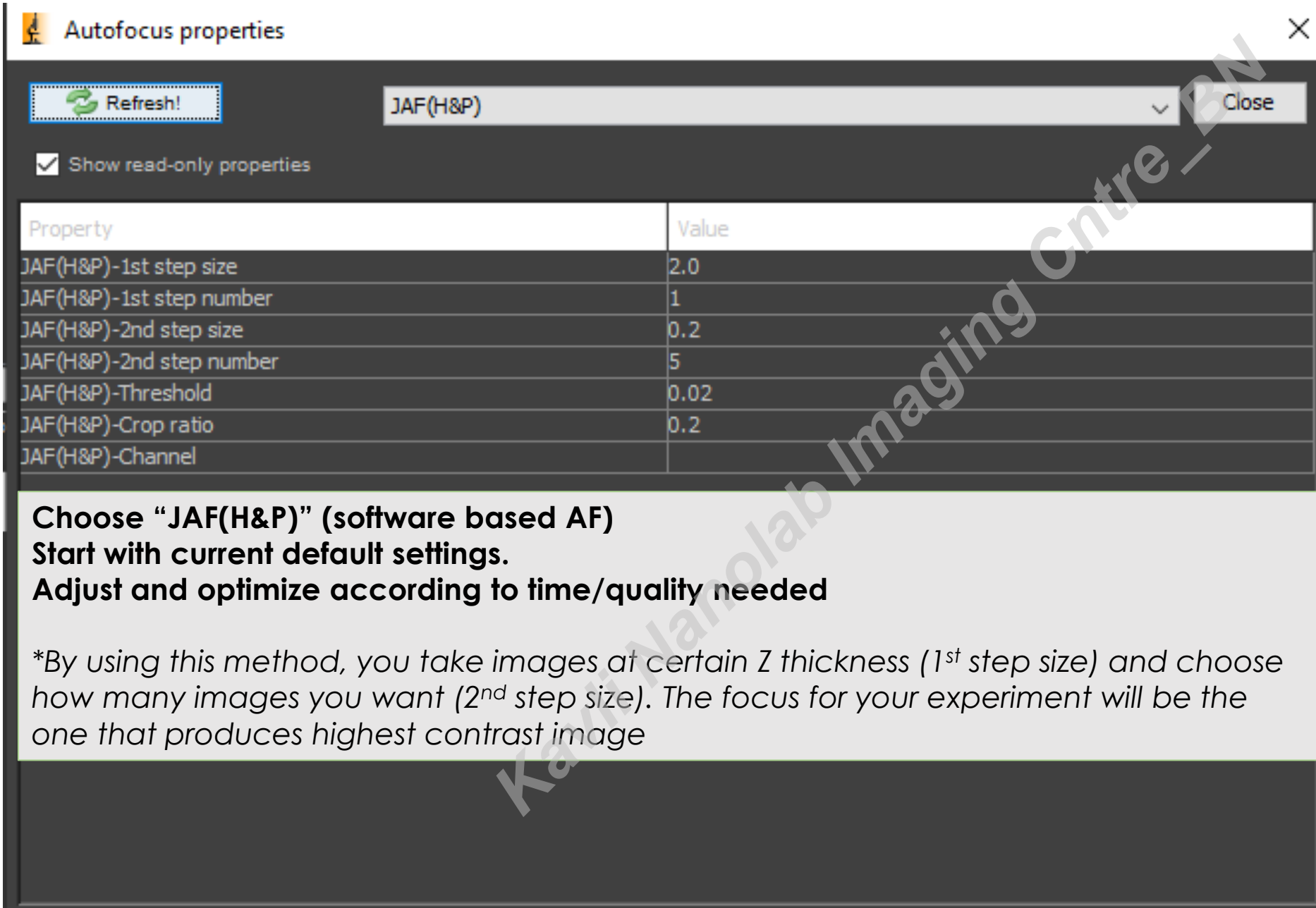
Show read-only properties

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 <math>< 20\mu\text{m}</math> 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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Wiel: W.H.Evers@tudelft.nl

