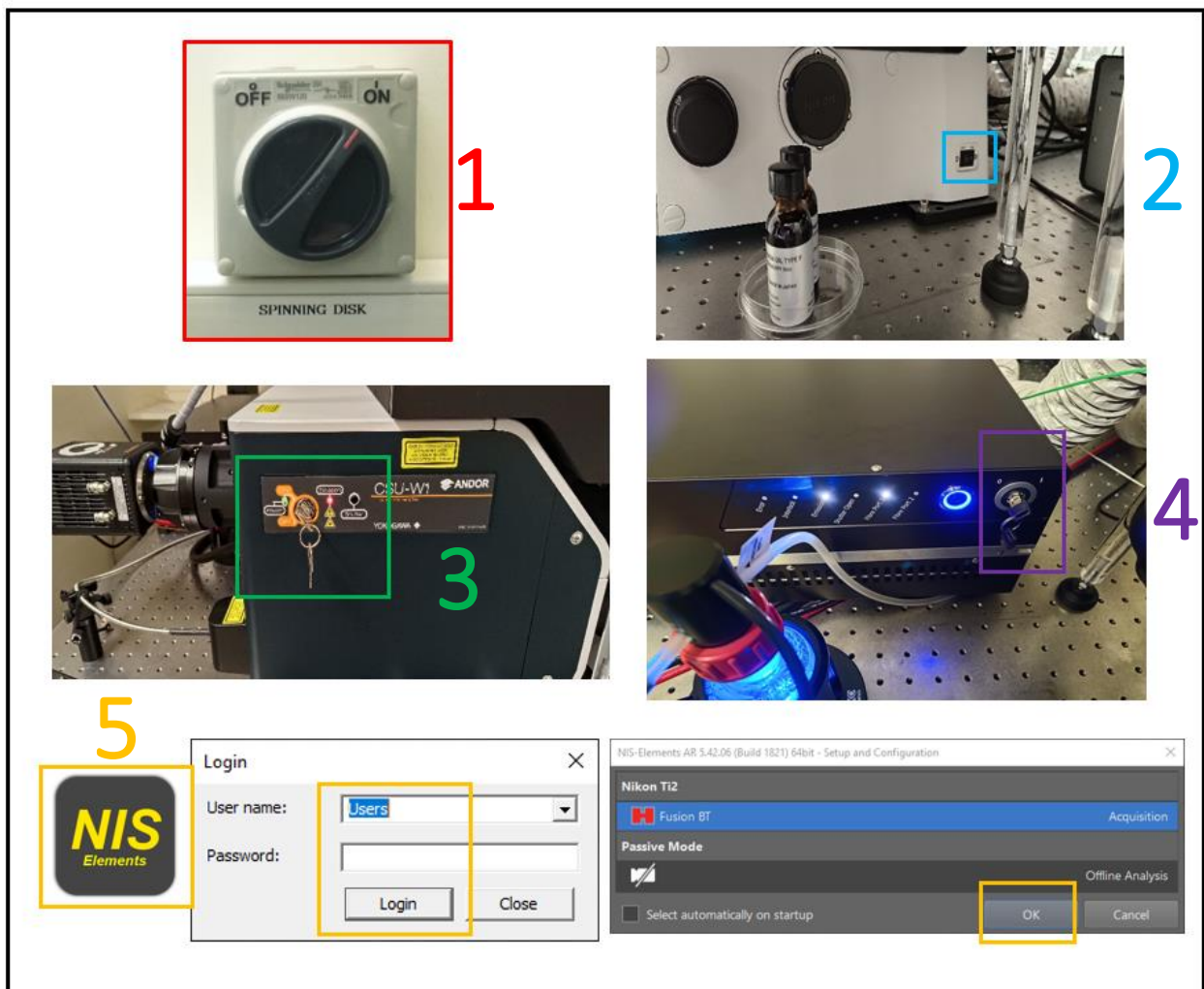


SWITCH ON THE SPINNING DISK	2
SWITCH ON THE TEMPERATURE CONTROLLER.....	3
MICROSCOPE FRAME CONTROLS.....	4
EYEPIECE OBSERVATION	5
SPINNING DISK ACQUISITION	6
ND ACQUISITION	8
ACQUISITION SAVING.....	17
SWITCH OFF THE SPINNING DISK.....	18
SWITCH OFF THE TEMPERATURE CONTROLLER	19

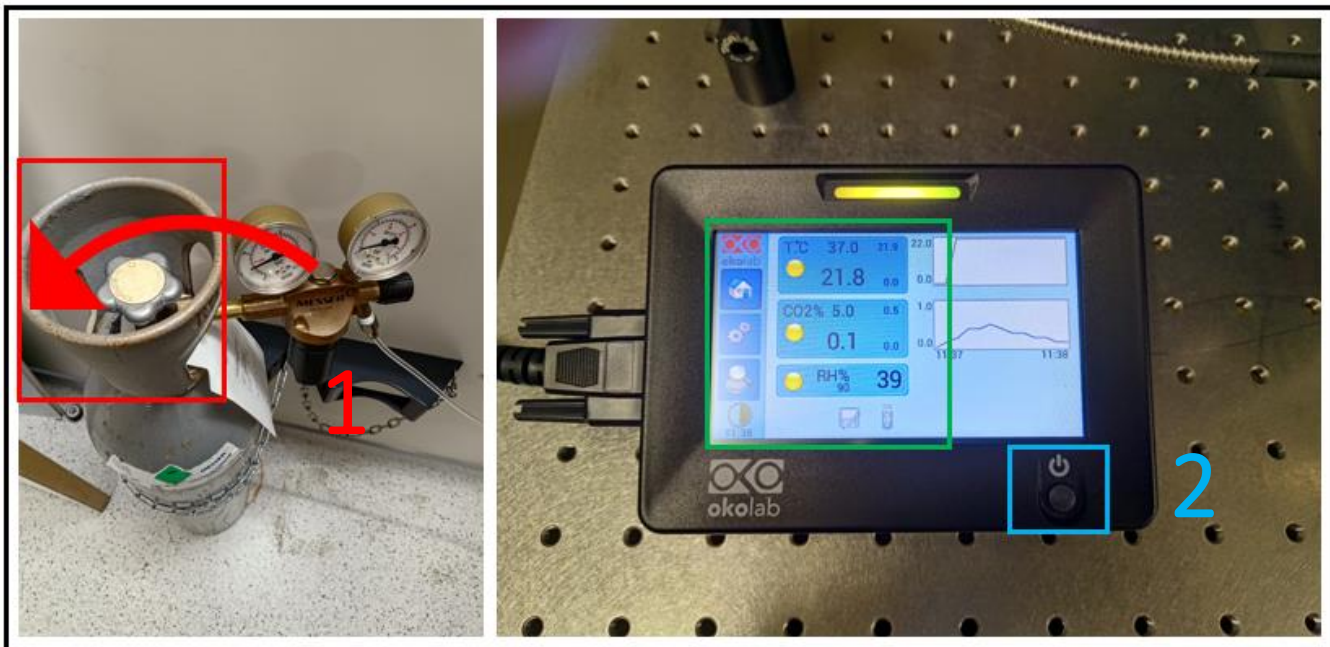
SWITCH ON THE SPINNING DISK

- 1- The main switch on the wall must remain in the « on » position
- 2- Switch on the frame of the microscope. This switch is located on the right of the microscope
- 3- Turn the key of spinning disk CSU-W1
- 4- Turn the key of the laser
- 5- Once you turn on the computer, start the "NIS Elements" software, choose the user session and click on « login ». Then choose the acquisition mode and click on « ok »



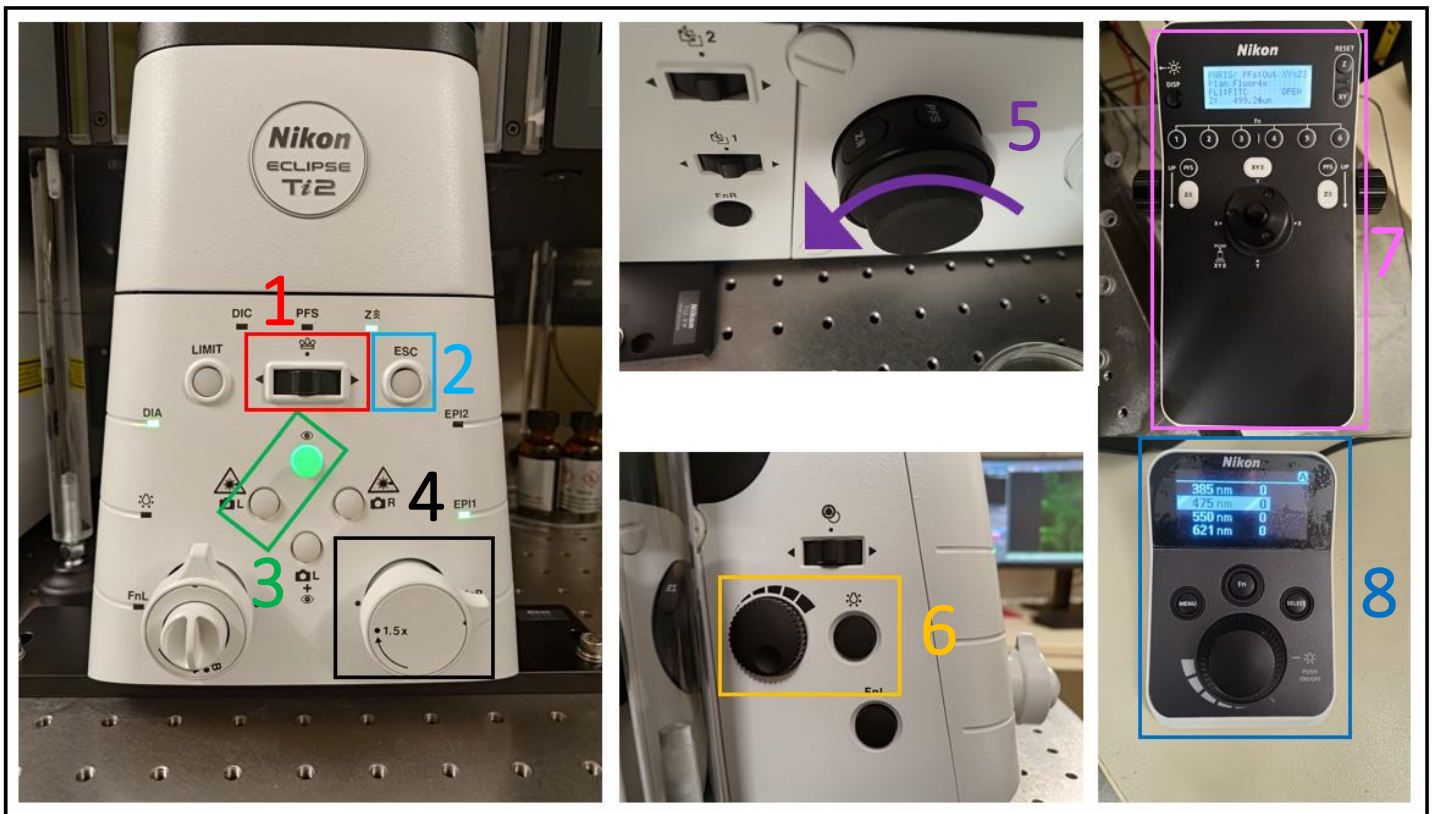
SWITCH ON THE TEMPERATURE CONTROLLER

- 1- Open the CO2 cylinder
- 2- Switch on the temperature/CO2 controller display (push and maintain the button)
- 3- Adjust various parameters (temperature ; CO2 percentage ; fan speed etc)



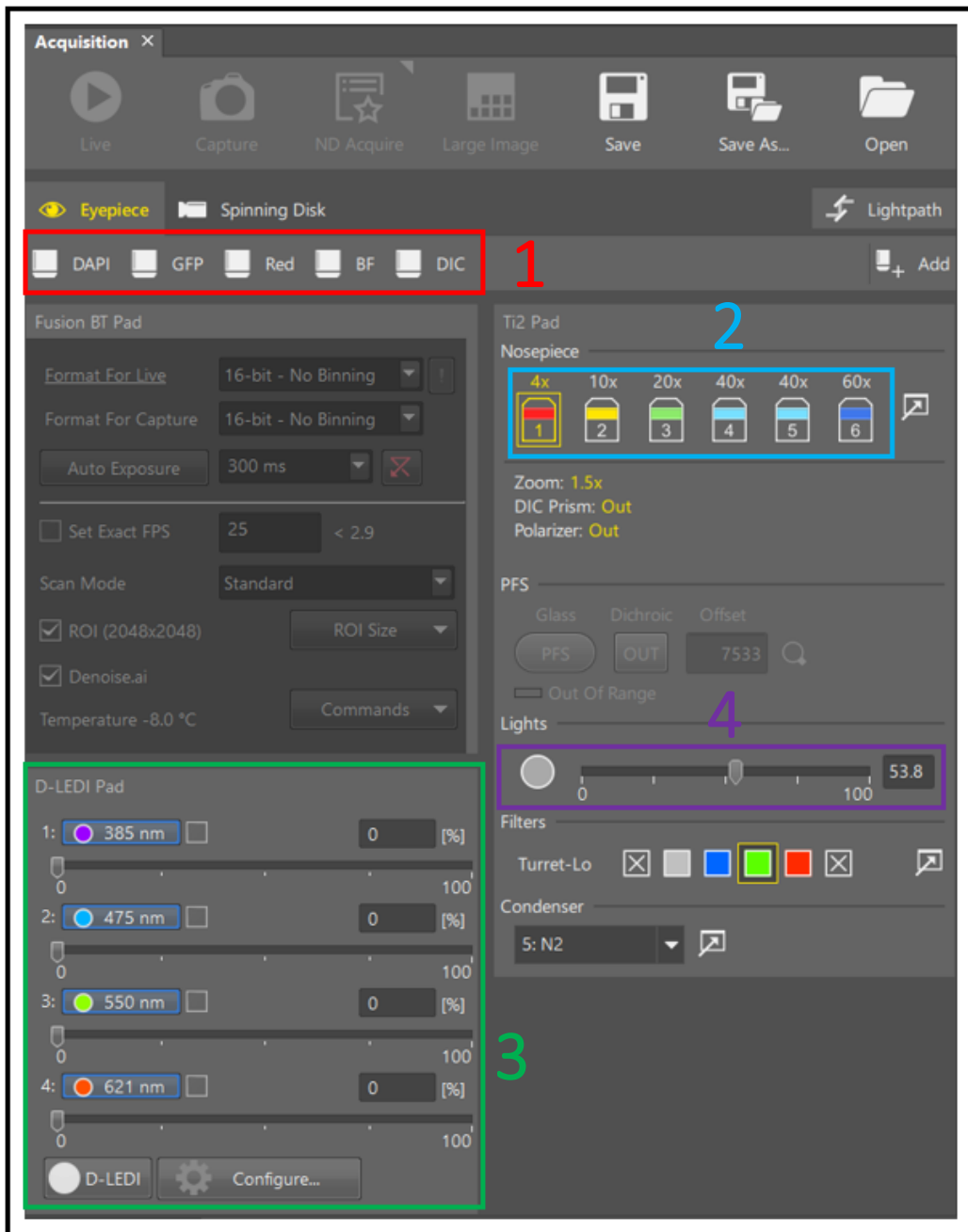
MICROSCOPE FRAME CONTROLS

- 1- Select the objective
- 2- ESC allows you to lower the objective. Use it before changing an objective with an immersion. Then, you can push this button again to upper the objective
- 3- Choose how you want to observe your sample. Here, green light is on eyepieces
- 4- Optovar lens up to 1.5x
- 5- There is no coarse or fine focus. The wheel is affected by the speed. Furthermore, to raise the objective, turn the wheel towards you
- 6- The left side of the frame is for the brightfield observation. You can adjust the intensity of the light
- 7- Use the joystick for the x and y position. You can adjust the speed by pushing the button and the joystick (3 different levels). You can also adjust the speed of the z (3 different levels)
- 8- Use the wheel to adjust the power of led for the eyepiece



EYEPIECE OBSERVATION

- 1- Select the illumination mode
- 2- Select the objective
- 3- Adjust the power of each LED
- 4- Adjust the power of the white lamp



SPINNING DISK ACQUISITION

I. Acquisition settings

1- Select the illumination mode (Triggered allows you to observe multiple dye at the same time)

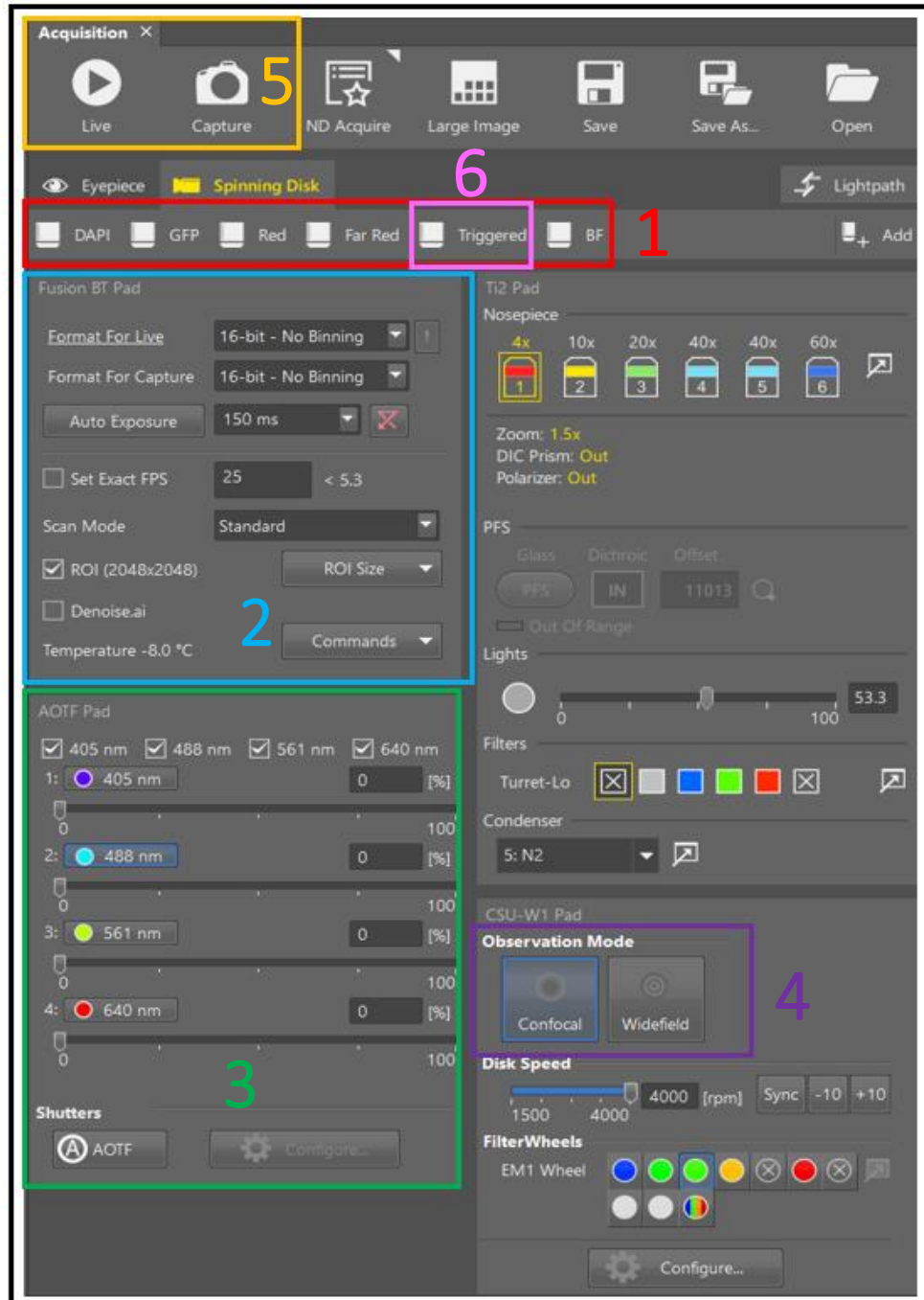
2- Camera settings (binning; exposure time; ROI)

3- Select the number of laser line you want and adjust the power of each

4- Choose the observation mode

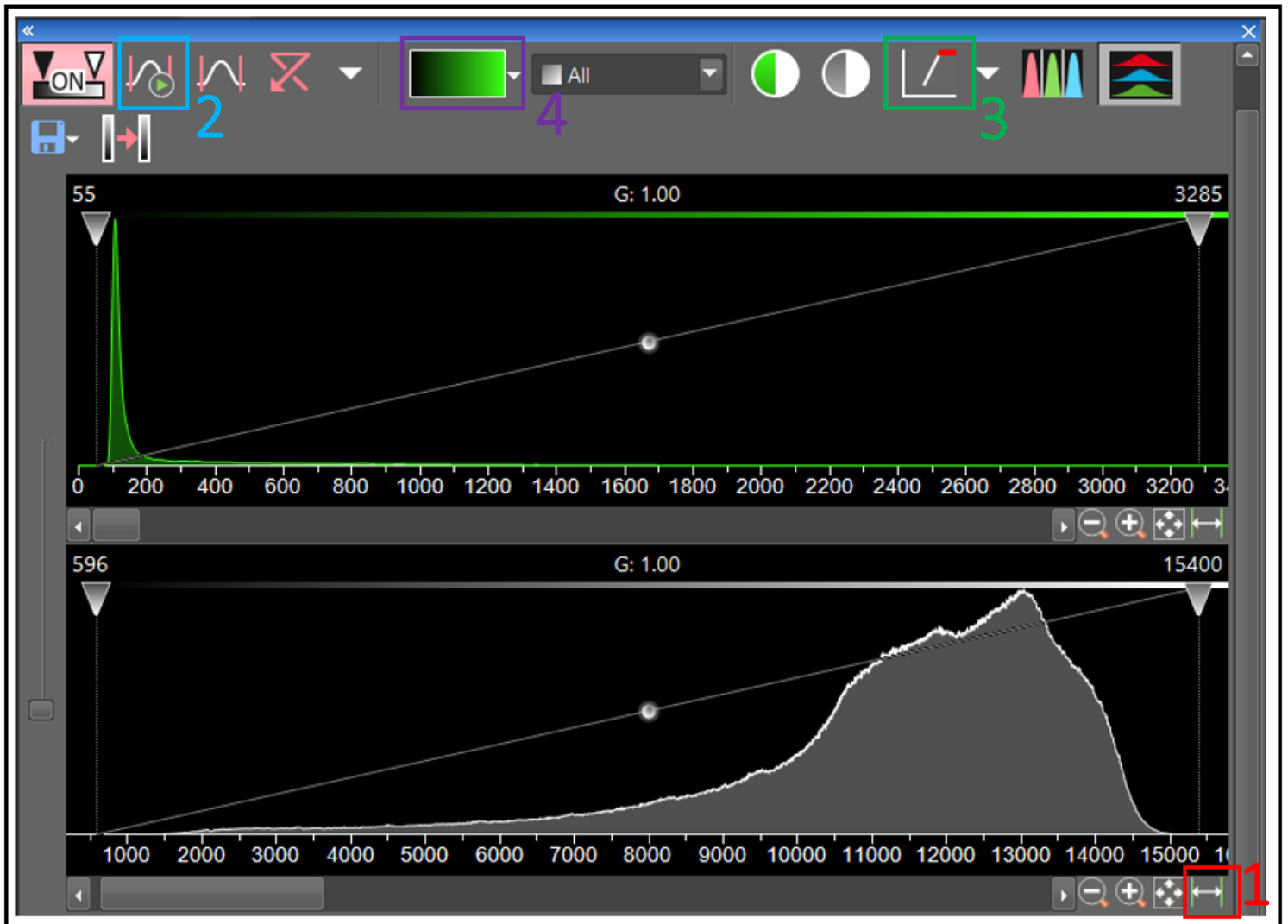
5- Click on live to make the focus and click on capture to acquire an image

6- Triggered mode allows you to observe multiple colors at the time



II. Contrast

- 1- Adjust the gate of your contrast
- 2- Auto-contrast
- 3- Check if there is any saturated pixels
- 4- Choose the color for your image



ND ACQUISITION

I. Time acquisition

1- Select « Time »

2- Add as many phase as you want and set the parameters (interval ; duration)

The screenshot displays the 'ND Acquisition' software interface. At the top, there are two tabs: 'ND Acquisition' (active) and 'XYZ Overview'. Below the tabs, the 'Experiment:' field is set to 'ND Acquisition'. A progress bar labeled 'T:' is visible. There are checkboxes for 'Save to File' and 'Custom Metadata', and a 'Record Data...' button. A dropdown menu for 'Order of Experiment' and a 'Timing...' button are also present. A red box highlights the 'Time' icon in the 'Order of Experiment' dropdown, with a red '1' next to it. Below this, the 'Time Schedule' section is highlighted with a blue box and a blue '2'. It contains a table with columns for 'Phase', 'Interval', 'Duration', and 'Loops'. The first row is checked and contains '#1', '1 sec', '1 min', and '61'. Below the table, there are checkboxes for 'Close Active Shutter when idle', 'Perform Time Measurement (0 ROIs)', 'Switch Transmitted Illuminator off when Idle (0.01 s)', and 'Use PFS'.

Experiment: ND Acquisition

T: [Progress Bar]

Save to File Custom Metadata

Order of Experiment: [Dropdown] Timing...

Time XY λ Z Large Image

Time Schedule

Phase	Interval	Duration	Loops
<input checked="" type="checkbox"/> #1	1 sec	1 min	61
<input type="checkbox"/>			
<input type="checkbox"/>			

Close Active Shutter when idle Use PFS

Perform Time Measurement (0 ROIs)

Switch Transmitted Illuminator off when Idle (0.01 s)

II. Multiples positions acquisition

- 1- Select « XY »
- 2- Add as many position as you want
- 3- Click on « include z » to add the third coordinate

The screenshot shows the 'ND Acquisition' software interface. The 'Order of Experiment' dropdown is set to 'Timing...'. The 'Points' section is active, showing a table with the following data:

Point Name	X [mm]	Y [mm]	Z [µm]	
<input checked="" type="checkbox"/> #1	-> 3.453	-22.720	3625.443	<- Offset All X,Y,Z
<input checked="" type="checkbox"/> #2	-0.934	-23.320	3625.643	
<input type="checkbox"/>				
<input type="checkbox"/>				

The 'Include Z' checkbox is checked, and the 'Z Device' is set to 'Ti2 ZDrive'.

III. Use Autofocus Hardware (PFS : Perfect Focus System)

- 1- Select « Use PFS ». By selecting « Use PFS », you will be able to change the offset value.
- 2- Unclick « Include Z »
- 3- Click on the arrow to set the PFS value Offset

The screenshot shows the ND Acquisition software interface. The left panel is titled "ND Acquisition" and contains the following elements:

- Experiment: ND Acquisition
- M: []
- λ: []
- Z: []
- Save to File
- Path: E:\User ImagoSeine\2024\July\29\20240729_Mitochondria_slides\HeLa-Mock
- Filename: HeLa_Mock001.nd2
- Custom Metadata
- Order of Experiment: Timing...
- Time, XY, λ, Z, Large Image
- Points: Move Stage to Selected Point

Point Name	X [mm]	Y [mm]	PFS
#1	3.526	-18.096	7672
#2	-2.432	-16.699	7622
#3	-4.029	-16.230	7622
#4	2.885	-16.818	7616

- Include Z, Relative XY
- Close Active Shutter during Stage Movement
- Use PFS
- Buttons: Load, Save, Remove, 1 time loop, Run now

The right panel is titled "Ti2 Pad" and contains the following elements:

- Nosepiece: 4x, 10x, 20x, 40x, 40x, 60x
- Zoom: 1x
- DIC Prism: Out
- Polarizer: Out
- PFS: Glass (PFS), Dichroic (IN), Offset (7622)
- Lights: 0 to 100 slider
- Filters: Turret-Lo (selected)
- Condenser: 5: N2

Annotations in the image include:

- A blue box around the "Include Z" checkbox with a "2" next to it.
- A red box around the "Use PFS" checkbox with a "1" next to it.
- A green box around the "PFS" column in the points table with a "3" next to it.

IV. Lambda

1- Select « λ »

2- Add as many illumination as you want. Click or unclick on an illumination to acquire it or not

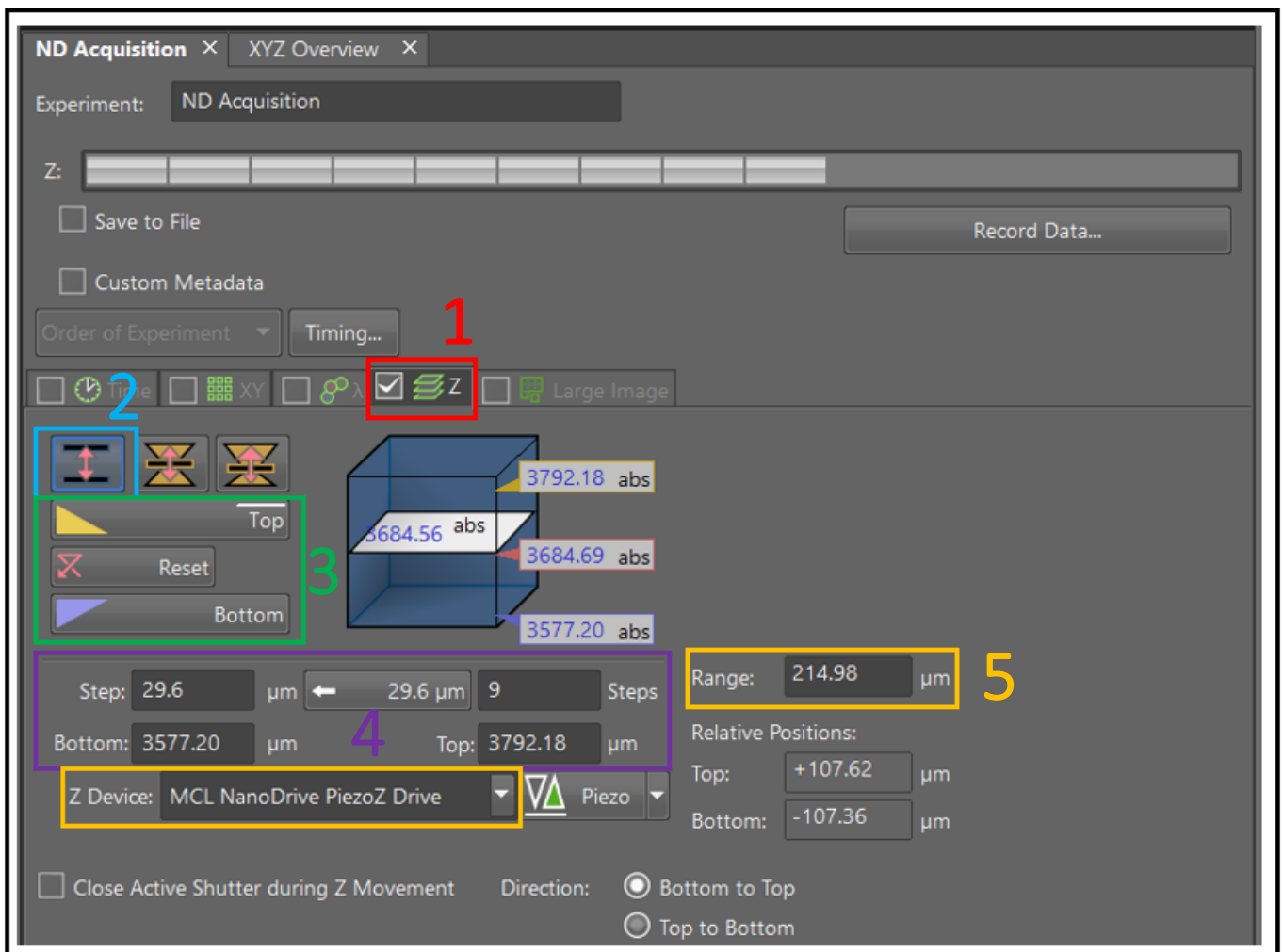
The screenshot shows the 'ND Acquisition' software interface. At the top, there are tabs for 'ND Acquisition' and 'XYZ Overview'. Below the tabs, the 'Experiment' is set to 'ND Acquisition'. A wavelength selection bar is visible, followed by checkboxes for 'Save to File' and 'Custom Metadata', and a 'Record Data...' button. A 'Timing...' button is highlighted with a red '1'. Below this, there are icons for 'Time', 'XY', 'λ', 'Z', and 'Large Image'. The 'λ' icon is highlighted with a red box. A blue '2' is placed to the right of the 'λ' icon. Below the icons is a 'Setup' table with columns for 'Opt. Conf.', 'Name', 'Comp. Color', and 'Focus Offset'. The table contains five rows of illumination configurations. The 'Spinning Disk:BF' row is highlighted in blue. At the bottom, there are checkboxes for 'Close Active Shutter during Filter Change', 'Use Ratio', 'Use PFS', and 'Use Trig. Acq.', along with a 'Define Ratio...' button.

Opt. Conf.	Name	Comp. Color	Focus Offset
<input checked="" type="checkbox"/> Spinning Disk:DAPI	DAPI	Blue	
<input checked="" type="checkbox"/> Spinning Disk:GFP	GFP	Green	0
<input checked="" type="checkbox"/> Spinning Disk:Red	Red	Red	0
<input checked="" type="checkbox"/> Spinning Disk:Far Red	Far Red	Magenta	0
<input checked="" type="checkbox"/> Spinning Disk:BF	BF	Brightfield	0

V. Z-stack

a) Top and Bottom

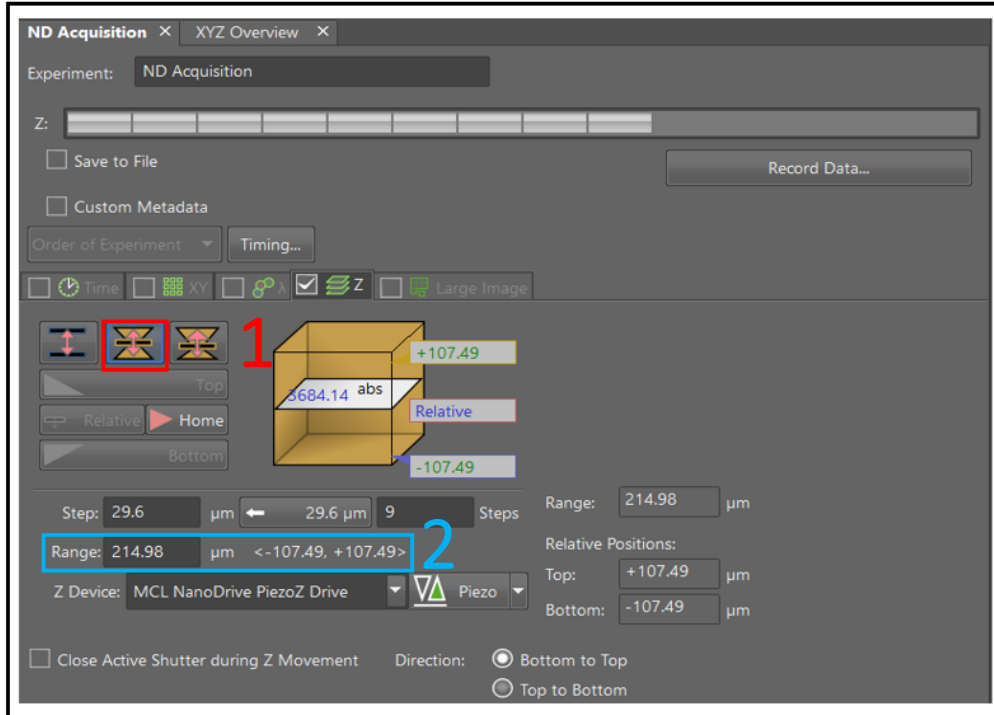
- 1- Select « Z »
- 2- Select top and bottom
- 3- Check on live the bottom and the top value
- 4- Choose the recommended step size
- 5- If you use the PiezoZ for the z-stack, the range limit is 500 μm . Above this value, you as to change for the Ti2



b) Symmetric

1- Select « symmetric »

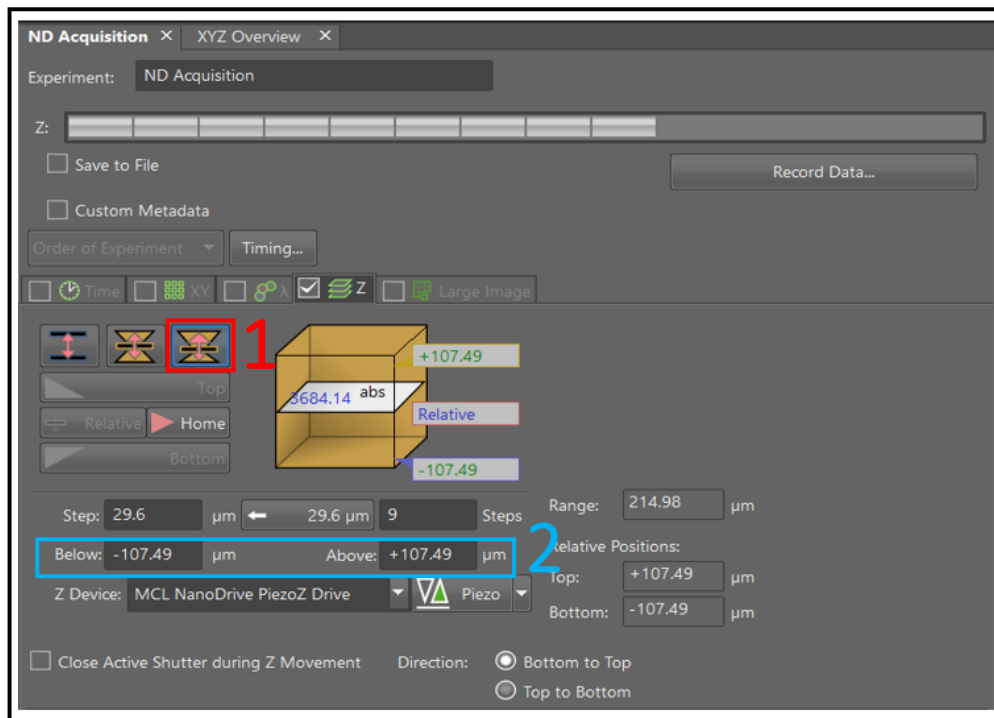
2- The software will do the half of the range you entered above and below the actual z. Click on « Home » to set the center and on « Relative » if you are using multiple positions.



c) Asymmetric

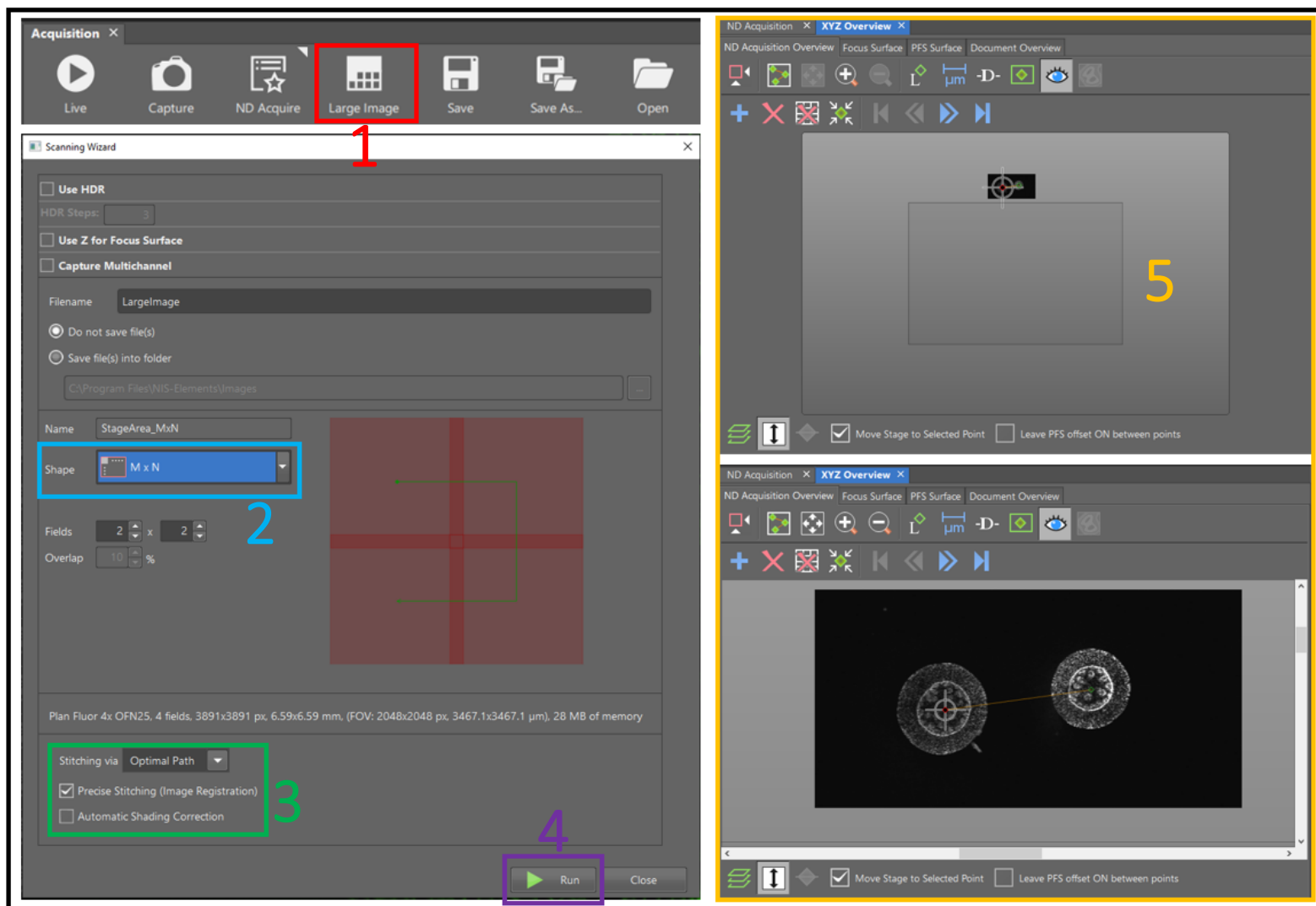
1- Select « asymmetric »

2- The software allows you to enter the desired value above and below the actual Z. Click on « Home » to set the center and on « Relative » if you are using multiple positions.



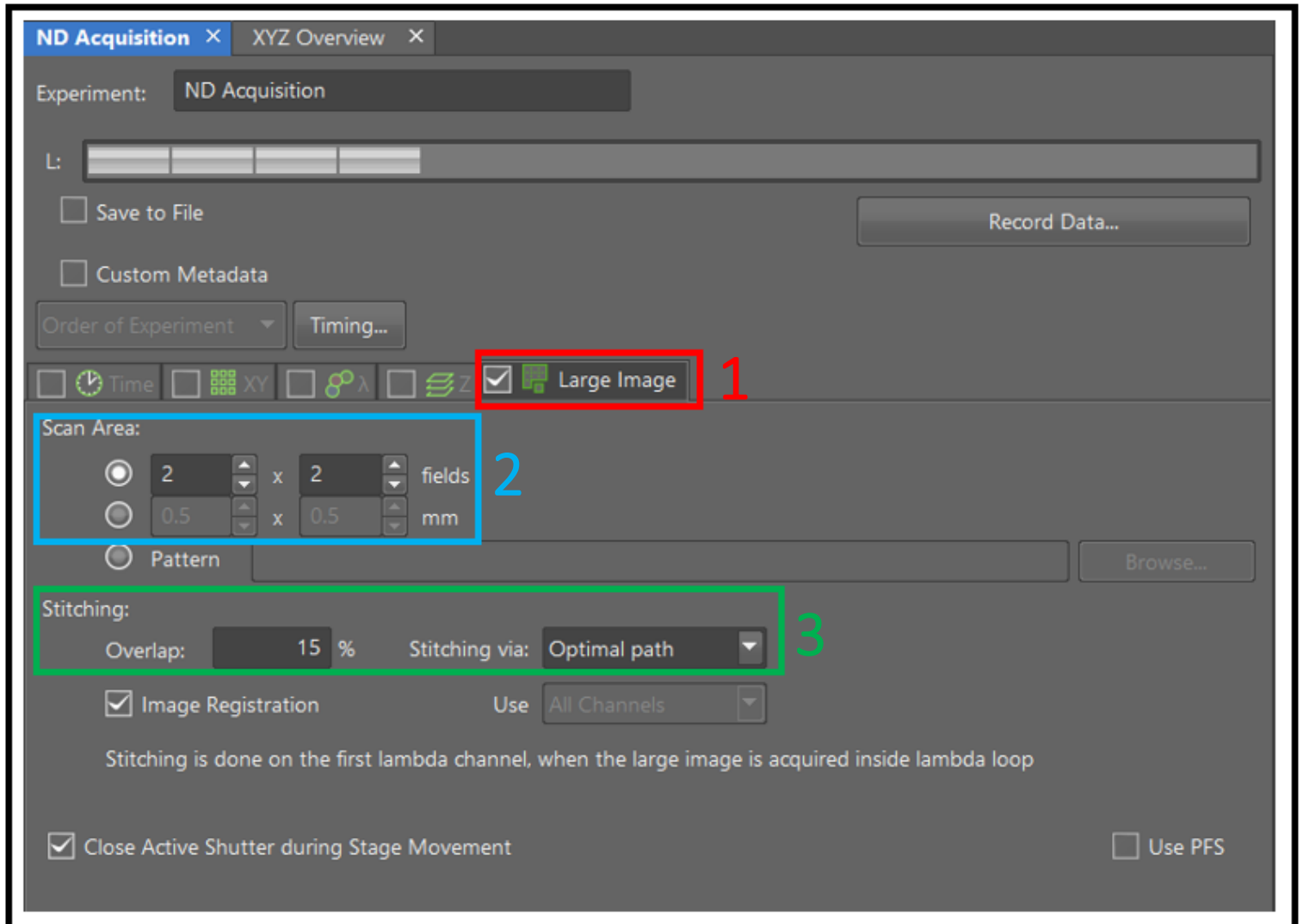
VI. Large Image

- 1- Choose a large image
- 2- Choose the shape of the tile according to the form of your sample
- 3- Let « Optimal Path »
- 4- Click on « Run »
- 5- When the acquisition is finished, you can make a right click on the image and choose « Use as preview in XYZ Overview ». Check it in the tab « XYZ Overview »



You can also do it in the « ND Acquisition tab »:

- 1- Click on « Large Image »
- 2- Choose the size of the field. Here, you can't choose the shape as the first method
- 3- Let « Overlap » to 15% and the Stitching on « Optimal path »



VII. Run experiment

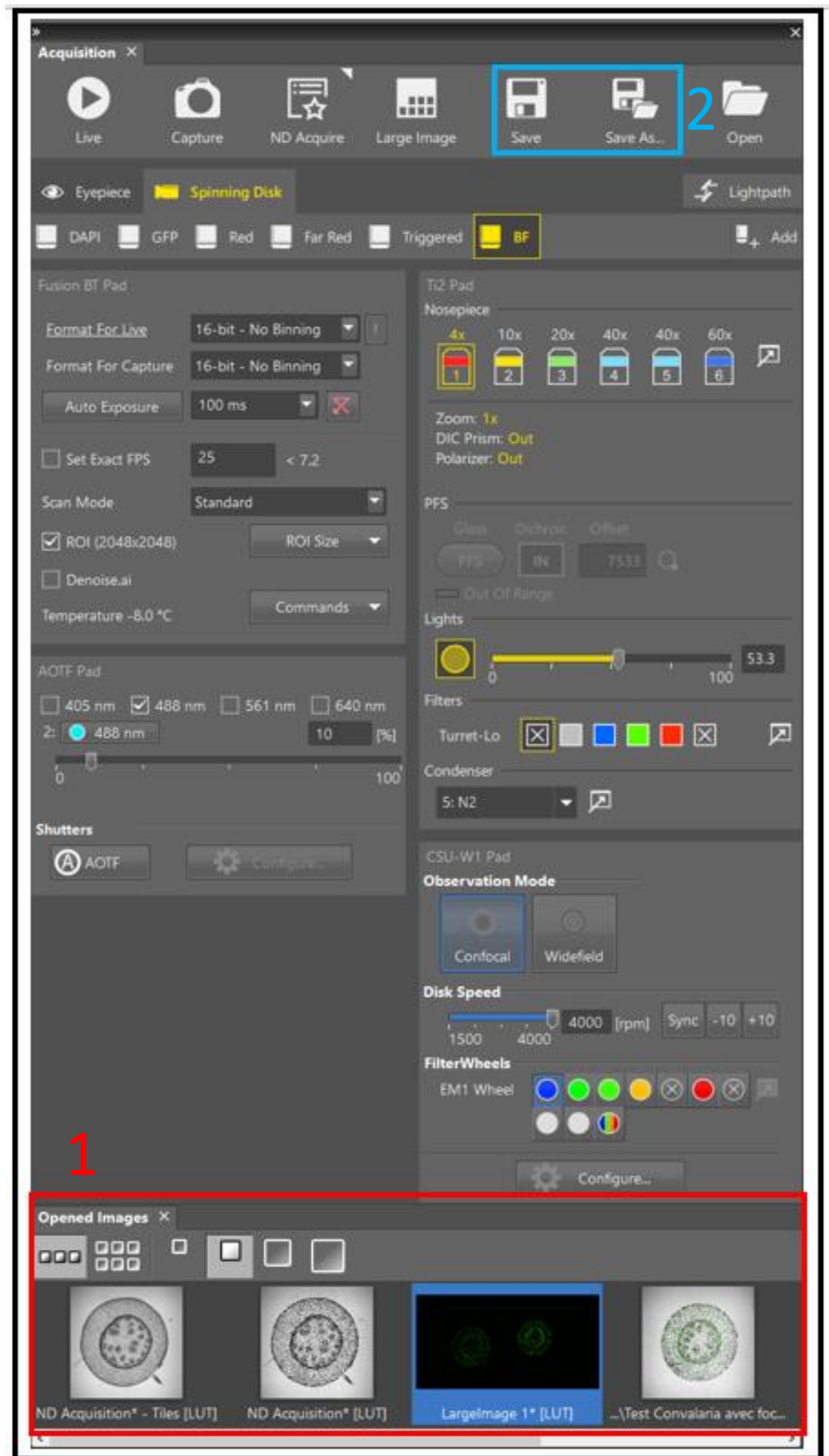
- 1- Select the order of experiment, this window can be used until two acquisition modes are selected. This step allows you to choose an order in the acquisition to be faster
- 2- Check the order according to the model chosen
- 3- Click on « run »

The screenshot shows the 'ND Acquisition' software interface. The window title is 'ND Acquisition' and 'XYZ Overview'. The 'Experiment:' field is set to 'ND Acquisition'. The 'Order of Experiment' section is highlighted with a blue box and contains a list of acquisition modes: Z series, Lambda, and Large Images. A red box highlights the 'Run now' button at the bottom right. A green box highlights the 'Run now' button. A blue '2' is in the top right, and a red '1' is near the acquisition mode list.

X [μm]	Z [μm]	Offset All X,Y,Z
720	3625.443	<-
320	3625.643	

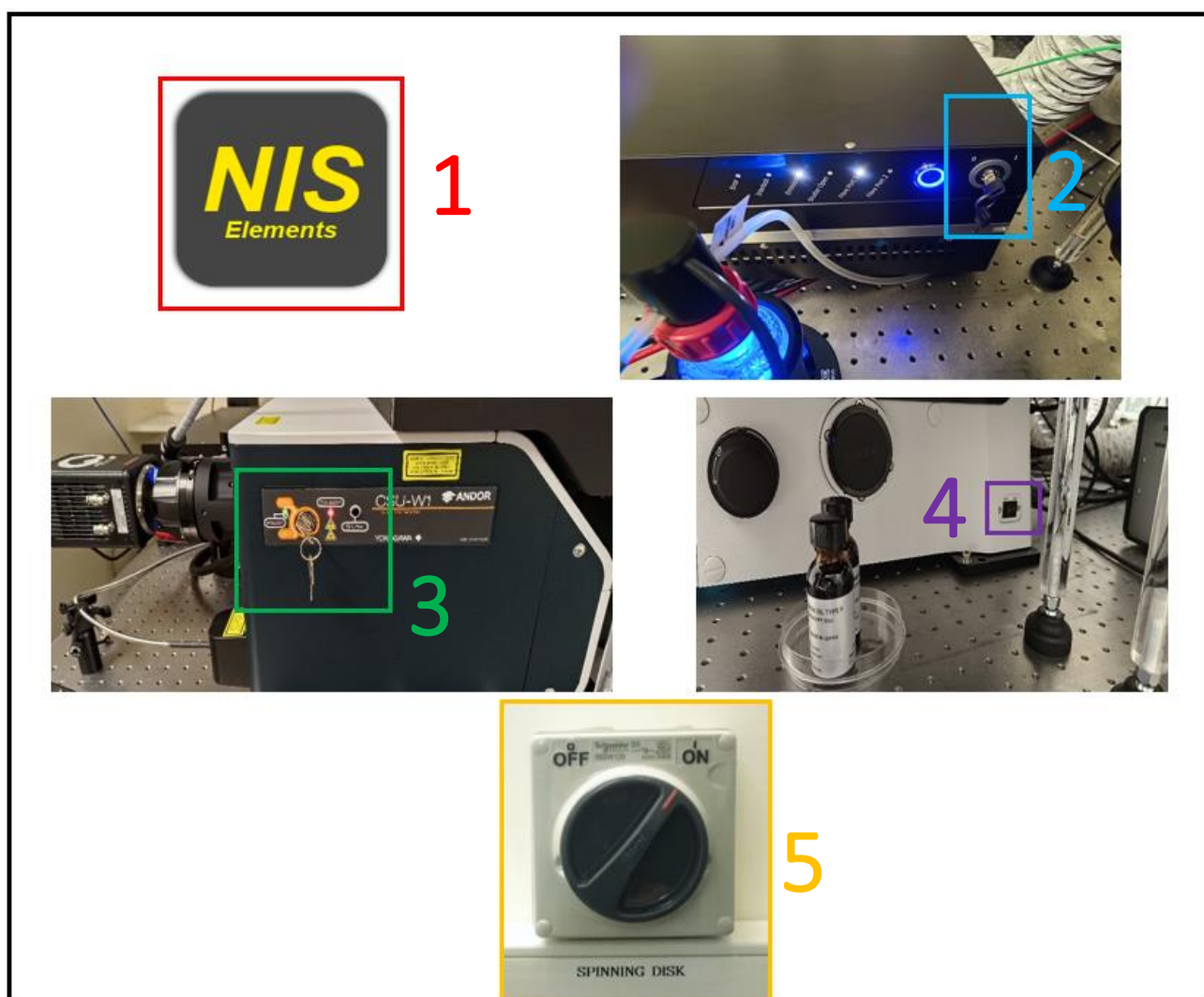
ACQUISITION SAVING

- 1- Select the image
- 2- Click on « Save As... »



SWITCH OFF THE SPINNING DISK

- 1- Close NIS and turn off the computer
- 2- Turn the key of the laser
- 3- Turn the key of spinning disk CSU-W1
- 4- Switch off the frame of the microscope. This switch is located on the right of the microscope
- 5- The main switch on the wall must remain in the « on » position



SWITCH OFF THE TEMPERATURE CONTROLLER

1- Switch off the temperature/CO2 controller display (push and maintain the button). Switch it off before turning off the microscope (fourth step of « switch off the spinning disk »)

2- Close the CO2 cylinder

