Nikon A1R. II Confocal Microscopy

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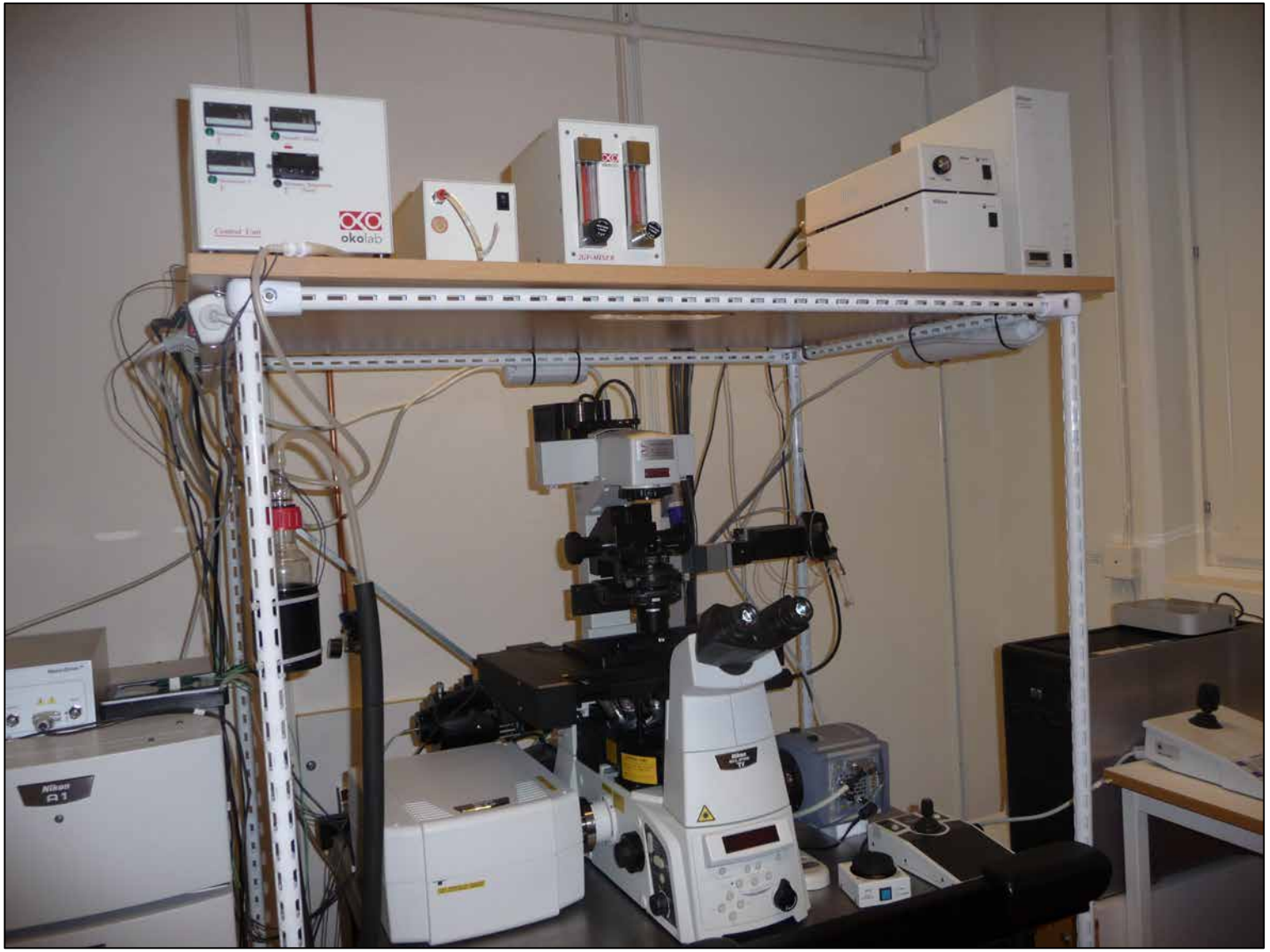


Nikon A1R. II Confocal Microscopy

System	Nikon A1R confocal
Location	KBC Building 6th floor Department of Medical Biochemistry and Biophysics
Room	A6 44 15
contact	irene.martinez@umu.se
Price	200kr/hr. Driver License 1000kr

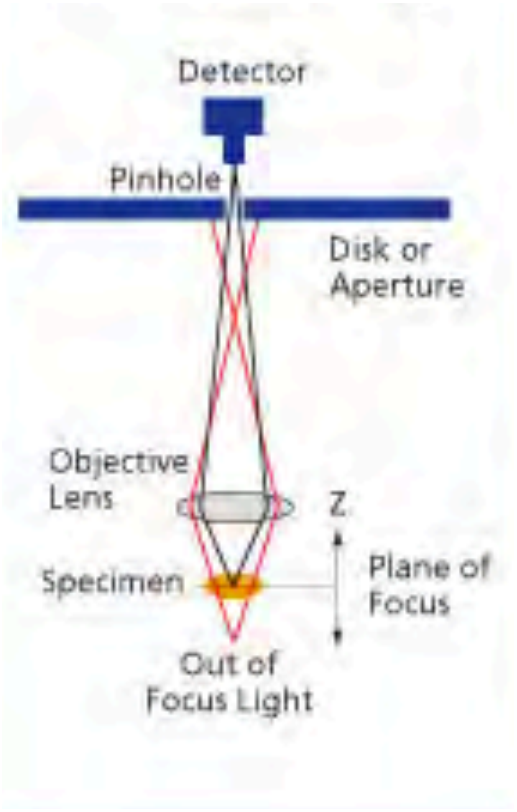
Use this phrase to mention the microscope for publications: Nikon A1R confocal (LSM) controlled by Nikon NIS Elements interface with a Nikon Eclipse Ti-E inverted microscope, equipped with:

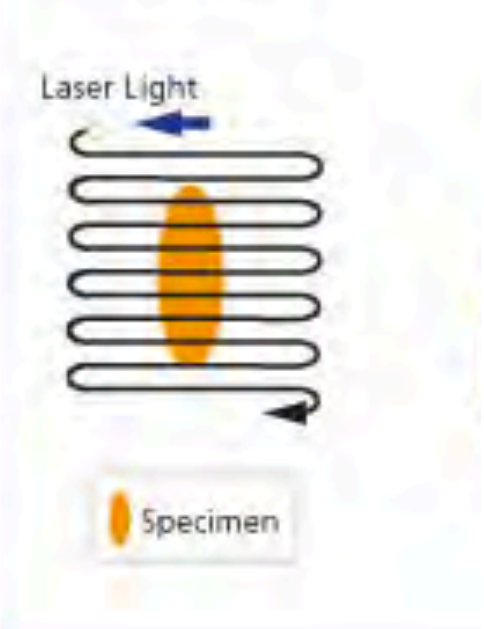
Objectives
Nikon CFI Plan Apochromat 10x (N.A 0.45) DIC objective
Nikon CFI Plan Apochromat 20x (N.A 0.75) DIC objective
Nikon CFI Plan Apochromat 60x oil (N.A 1.40) DIC objective
Nikon CFI Plan Apochromat TIRF 100x oil (N.A 1.49) DIC objective

Laser Lines
Violet diode laser 405nm
Blue argon laser (457, 477, 488, 514nm)
Sapphire laser 561nm
Red diode laser 647nm

Filter Sets in the turret:
Empty position to perform confocal microscopy
DAPI. Excitation: 340-380 nm Emission: 435-485nm
FITC. Excitation: 465-495 nm Emission: 515-555nm
Cy3. Excitation: 512-552 nm Emission: 565-615nm
TxRed. Excitation: 540-580 nm Emission: 600-660 nm
Camera (Quad Tirf filter)

Confocal Microscopy





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Switch on the system

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1- Turn on Switch #1

If you want to have temperature and CO2 follow step #2 if you don't need the incubation system go to step #10

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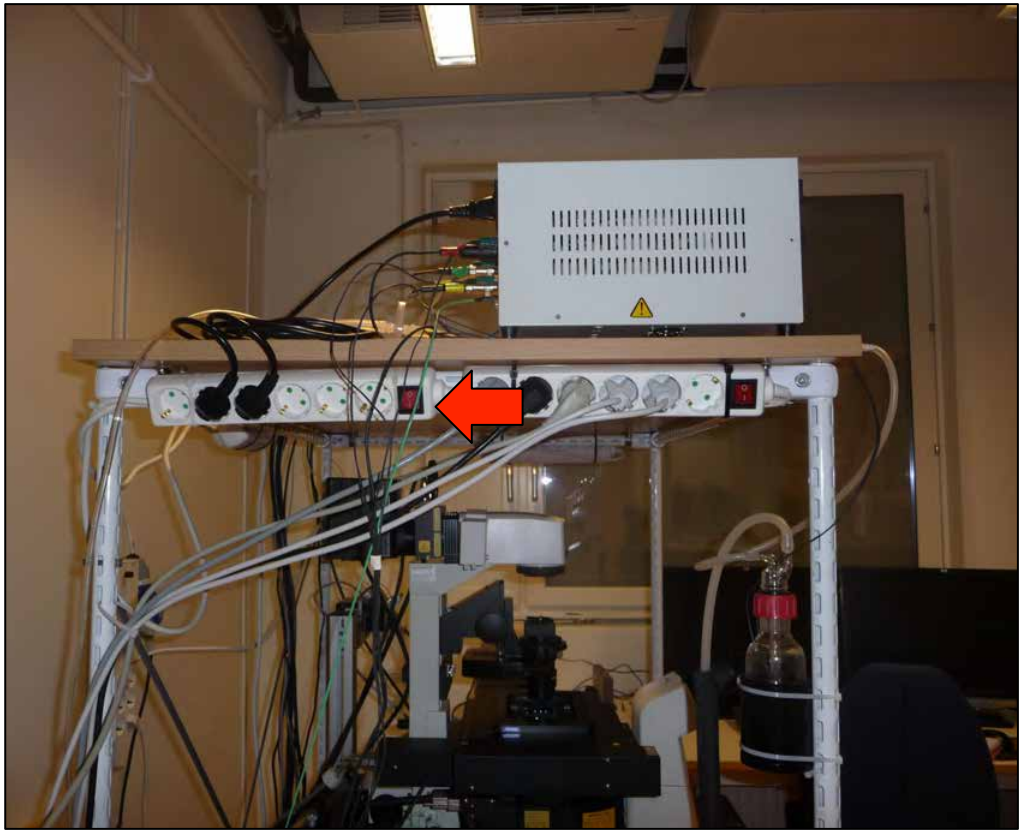
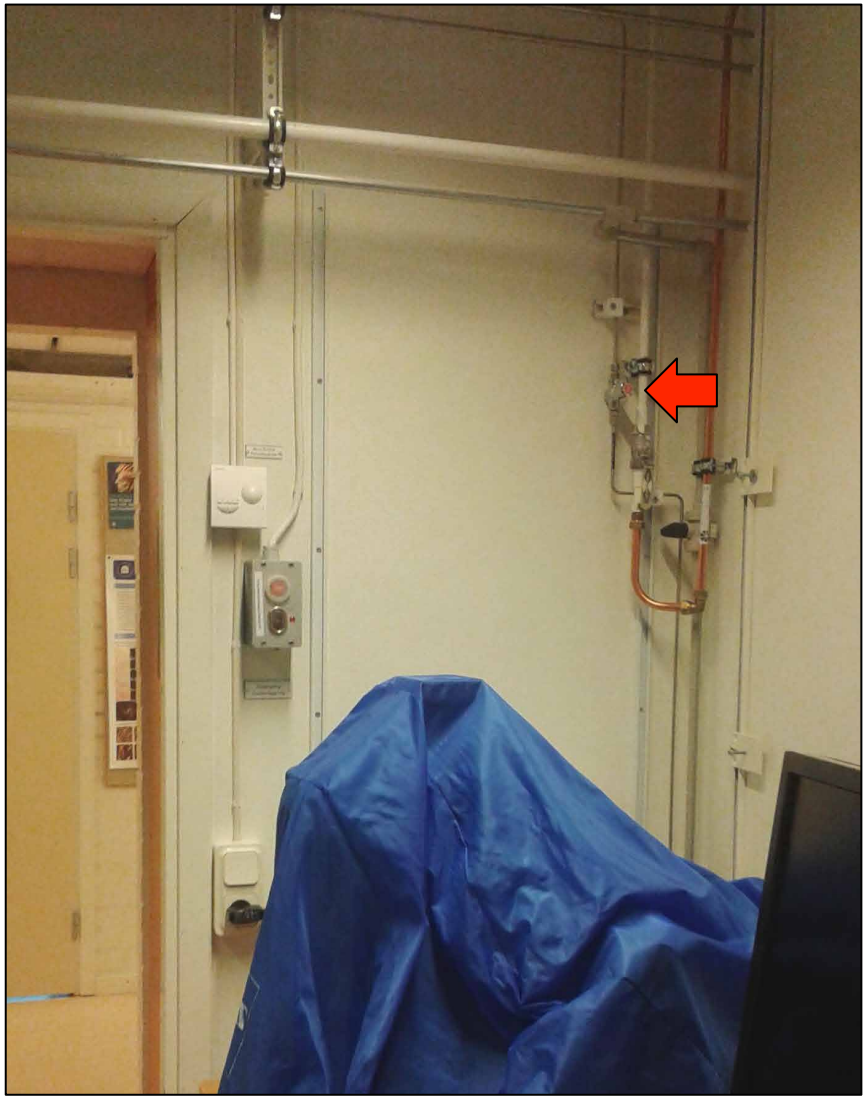
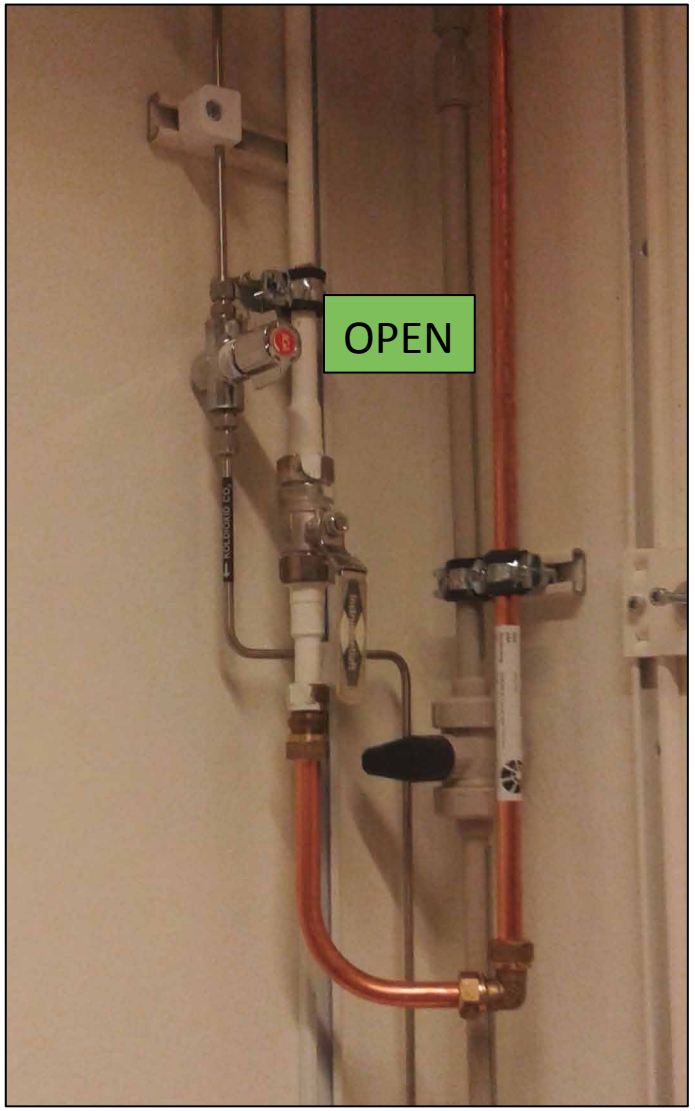
Switch on the temperature and CO2 at least 1h before starting acquisition:

2- Switch on the switch #3



3- Turn on CO2 key located in the wall close to the room door.





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4-. Make sure the objectives are placed all the way down. ALWAYS lower the objective to avoid any lense damage. Place the stage incubator carefully

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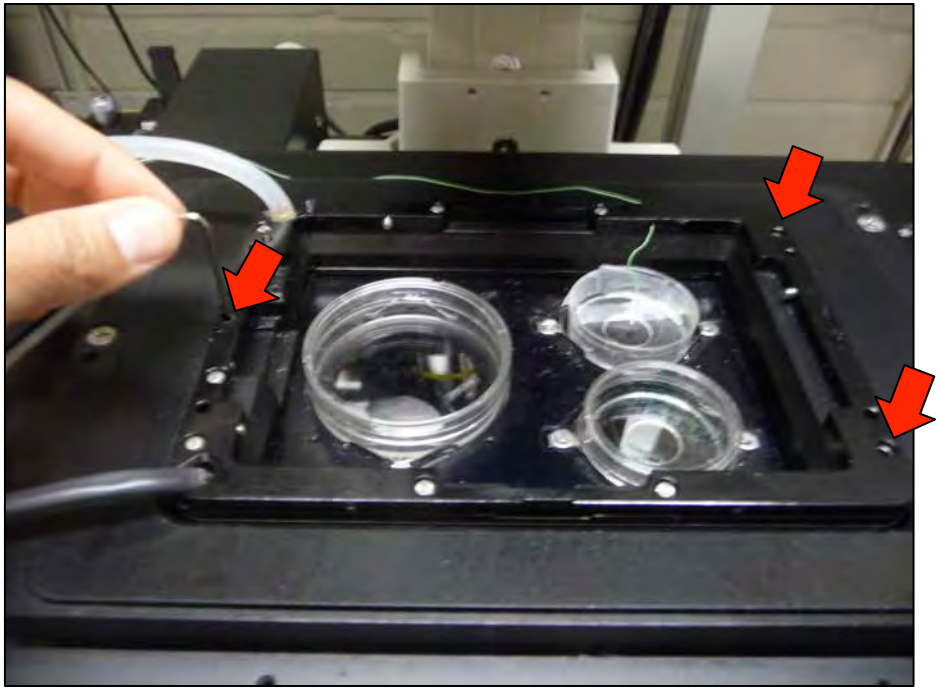
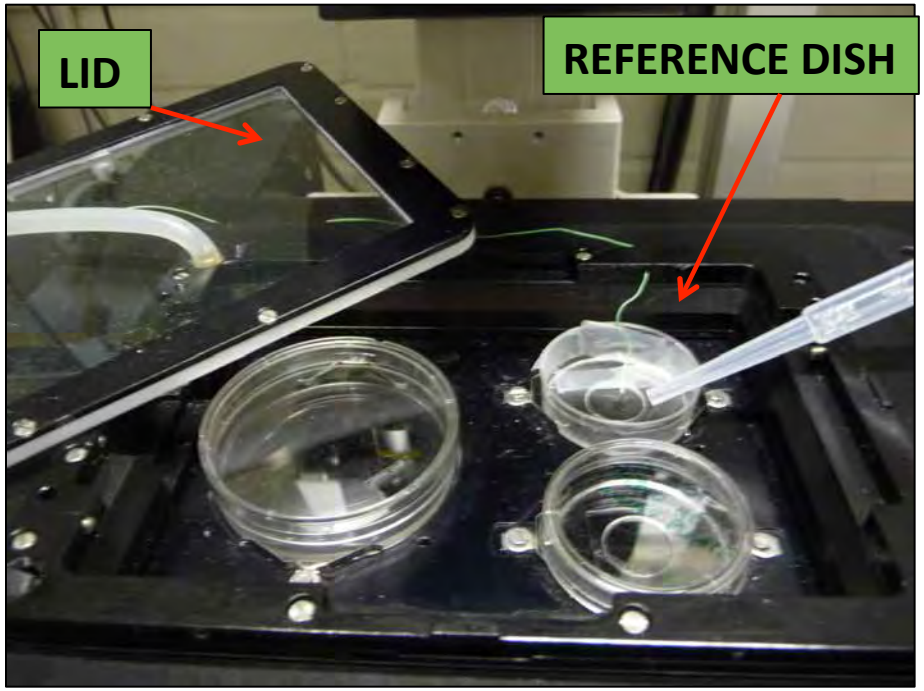
5- Screw the chamber. Make sure you are in the right positions



6- Add 3ml MiliQ water to the reference dish.

7- Put the chamber lid



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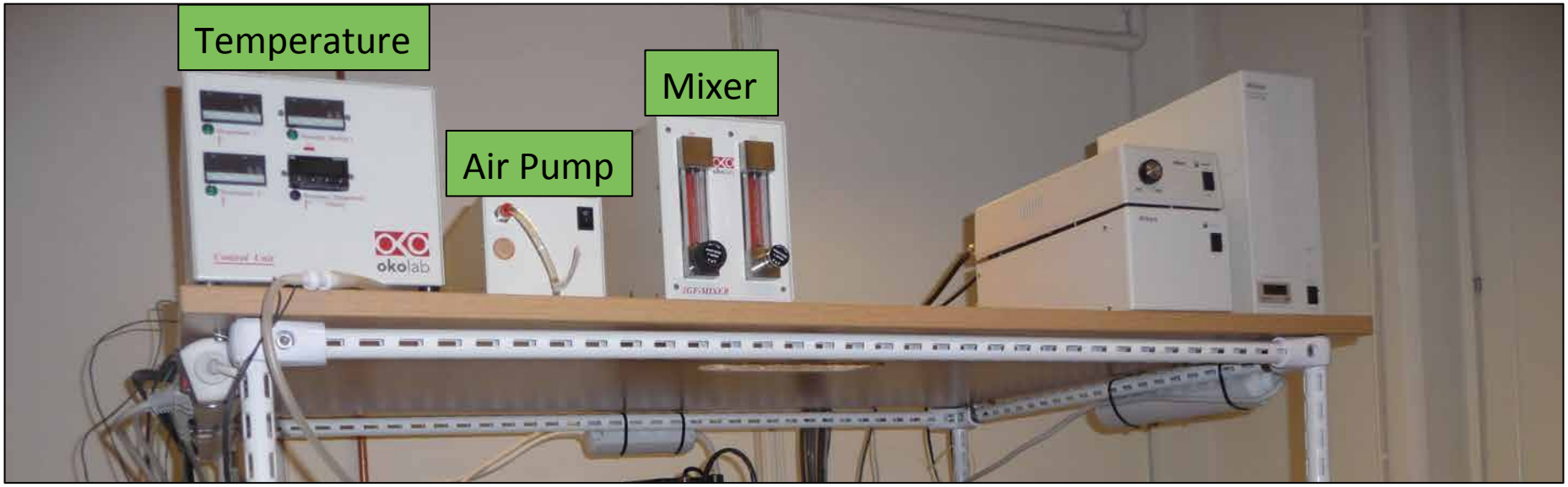
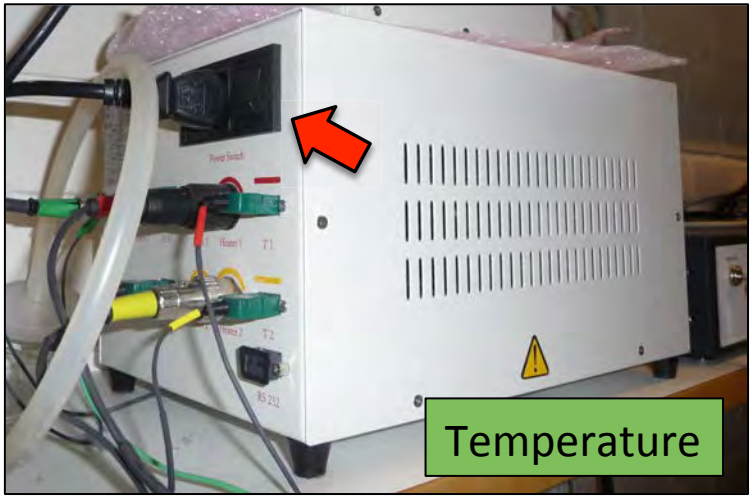
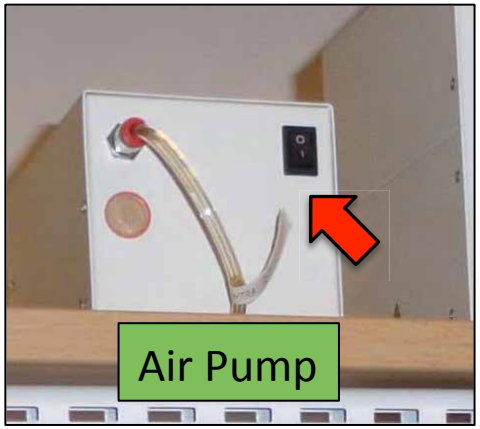


7- Switch on the temperature from the back



8- Switch on the Air Pump



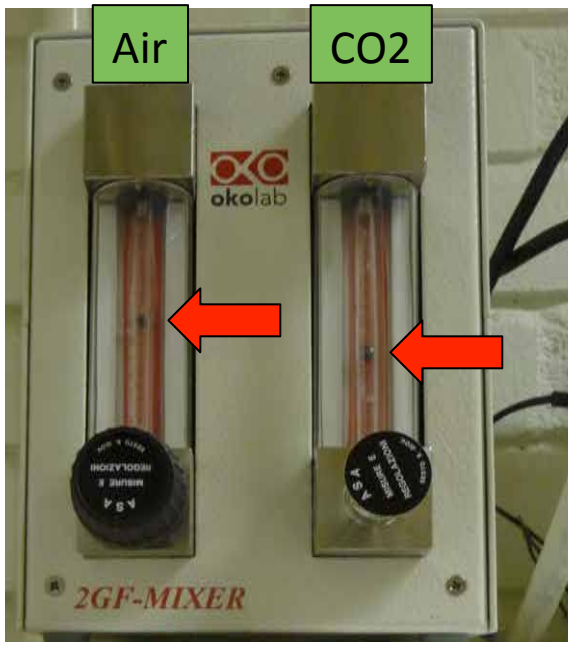
9- Make sure the mix is correct and stable:

For 5%CO2:

0.8 l/min Air
0.04 l/min of CO2

Or

0.6 l/min Air
0.03 l/min of CO2



If not, please contact BICU personnel: irene.martinez@umu.se

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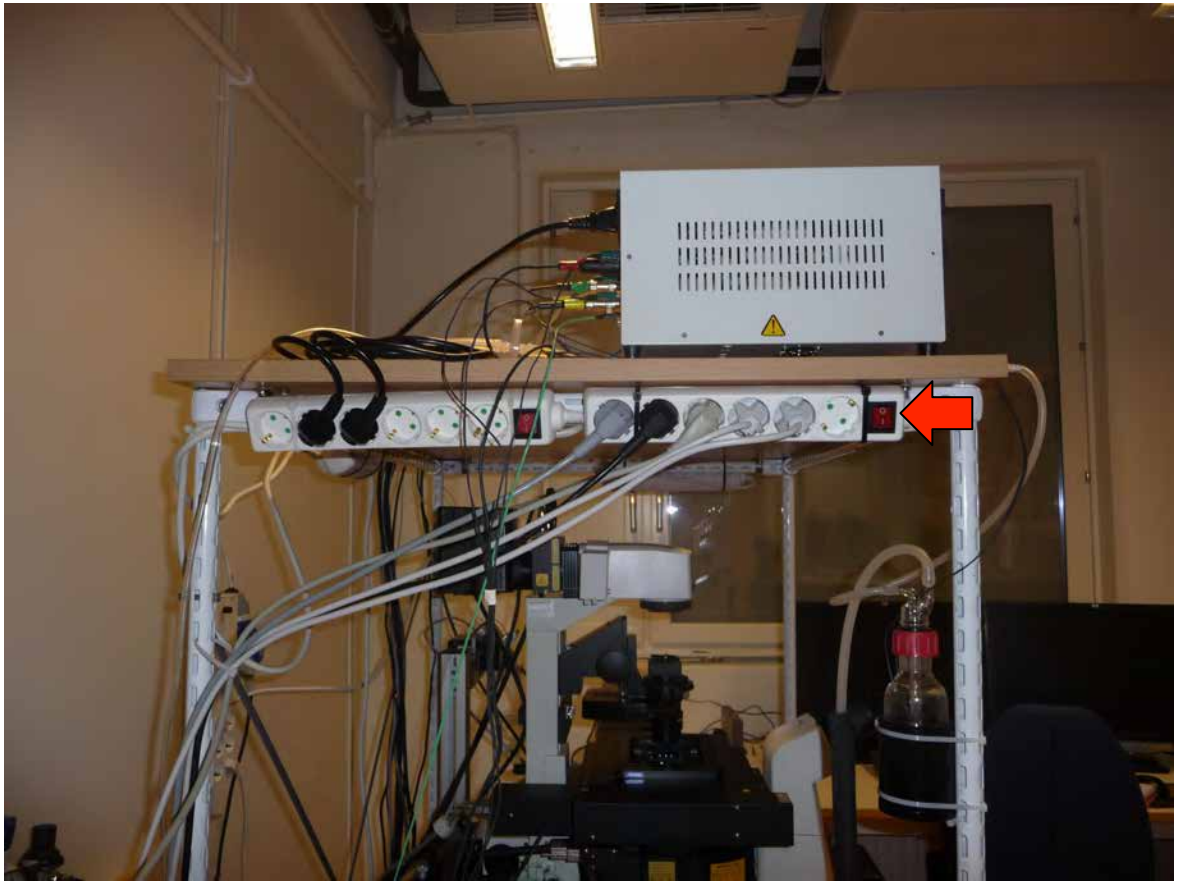
10- Turn on Switch #2



11- Switch on the Epi-Fluorescence Lamp

12- Wait few minutes and then: Switch on the Computer

13- Double click Nis-Elements AR 4.20.01

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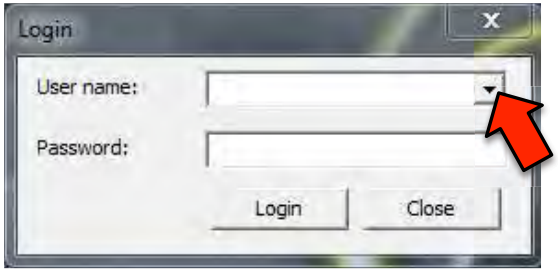
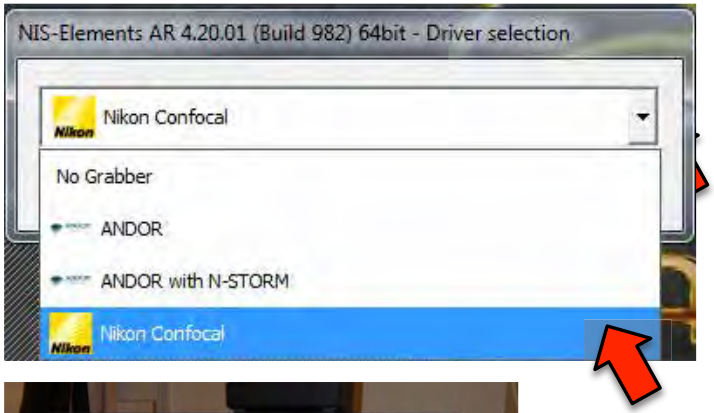
14- Login with your Name from the list and type your Password



15- Select Nikon Confocal



16- Once everything is on and the software has started normally turn on the laser box with the key (to perform confocal, TIRF or STORM microscopy).

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In case you need the 647 Laser for far red exitation, please follow this instructions:

17- Switch on the 647 laser 1 . Wait until the SHG led turns green and turn the key 2

18- Open the 647 Laser software

19- Press On and activate

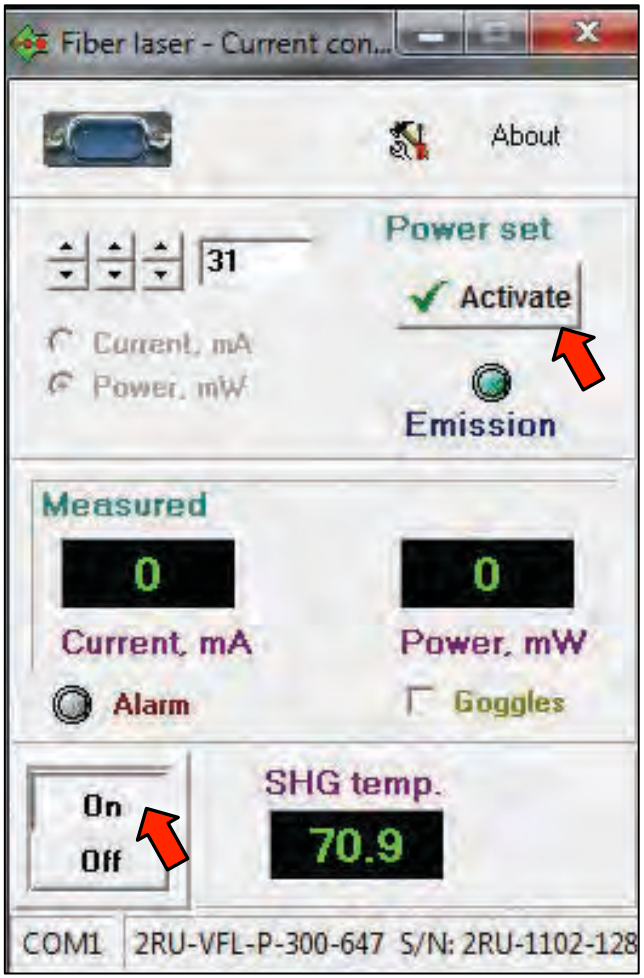

Image Acquisition

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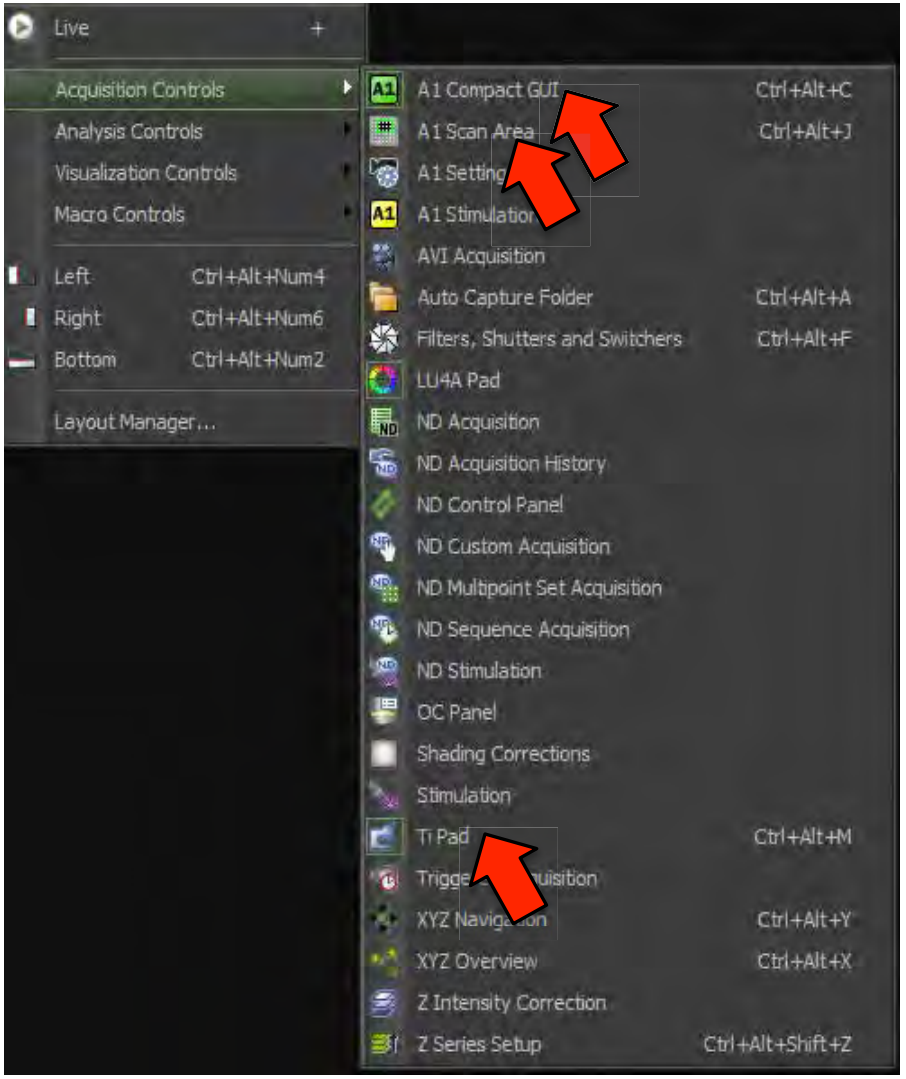
1- Right click and select: A1 Compact GUI, A1 Scan Area and Ti Pad.

You can also save this layout as “confocal” and every time you open the confocal driver all the windows will be loaded.



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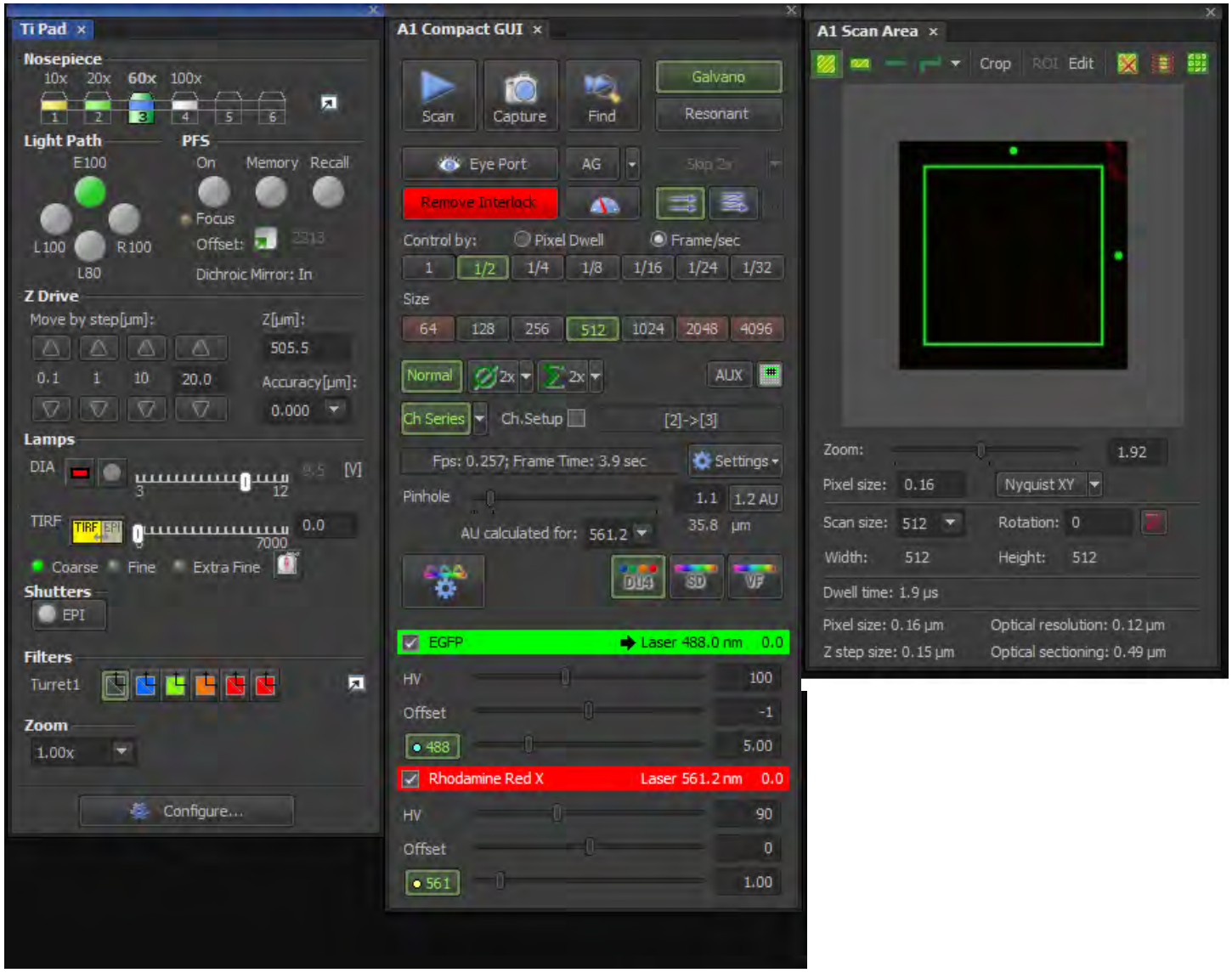

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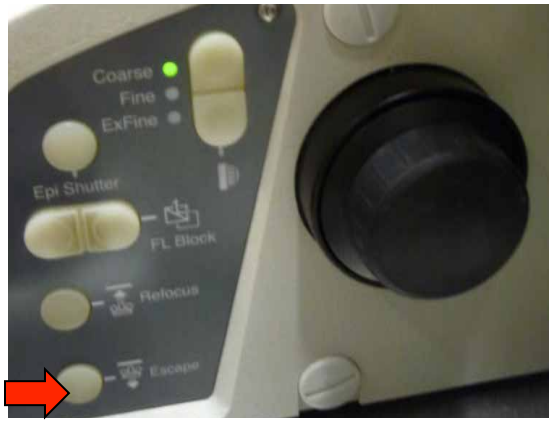
2- Switch off the DIA lamp (if it is on)

3- Lower the objectives



4- Choose the objective with the TiPad (click on the objective you want to use) or with the microscope

5- Add oil if needed just a drop (60x, 100x)

6- Place your sample propertly. Remember that with an inverted microscope you must turn upside down your sample if it is fixed, the working distance is 0.17 (cover slid thickness). You must take that into account before preparing your slides/dishes.

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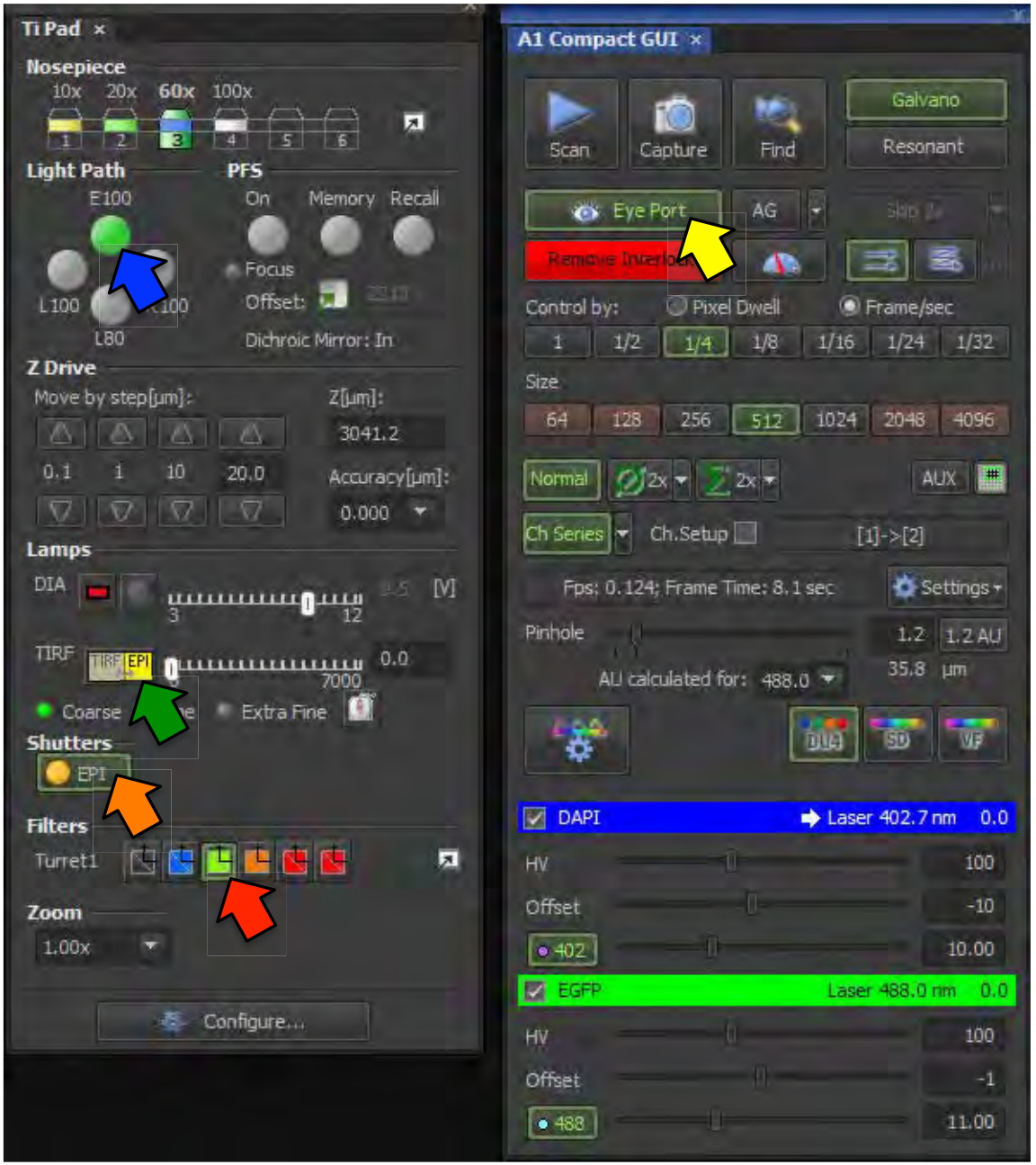
7- Press Eye port ➡ to check the specimen under the microscope. Open the shutter ➡

8- To change filters use the TiPad menu or the ➡ microscope



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9- Focus. You can select the movement accuracy

10- Move the stage with the joystick

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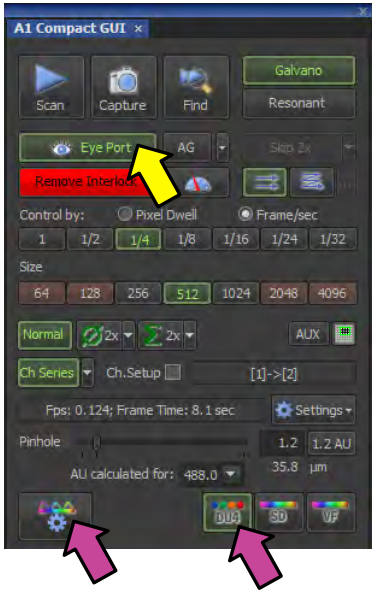
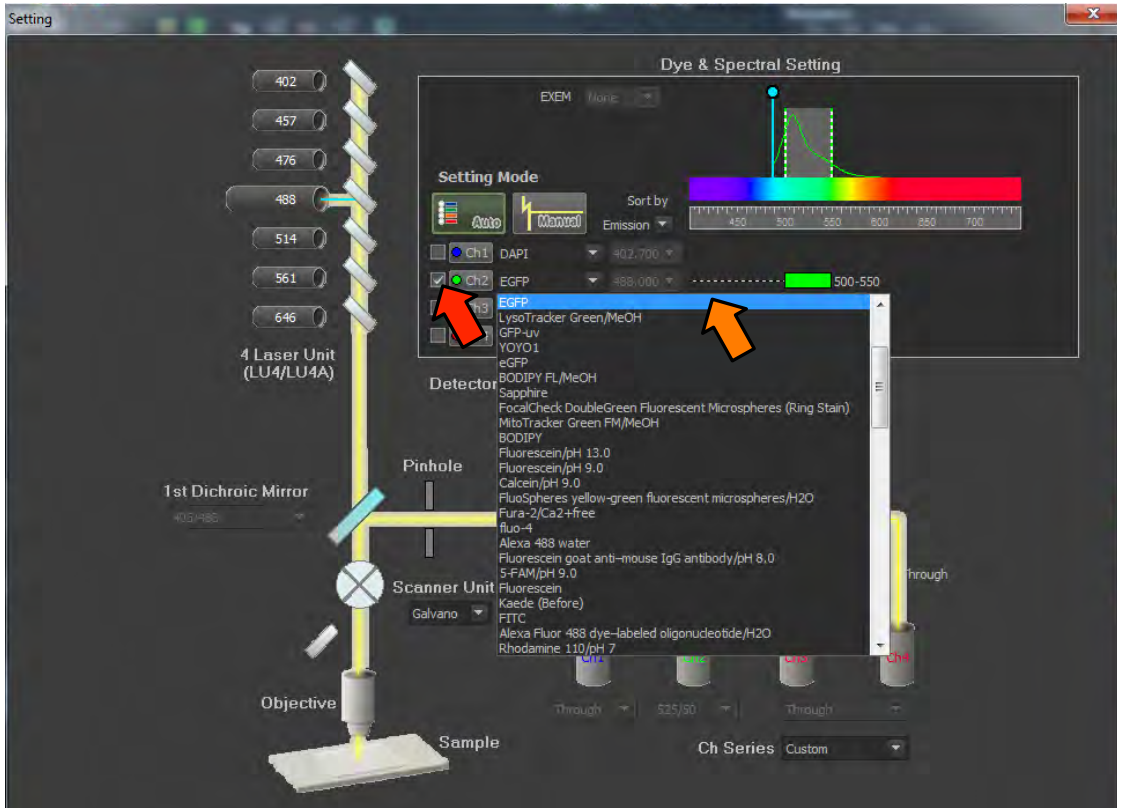
11- Press eye port ➡



12- Select the acquisition method ➡ and activate ➡ the channels to visualize and choose the dyes from the list ➡ to get the best signal. Then press ok



You can also reuse conditions from other images. Open your image/ right click and reuse camera settings.

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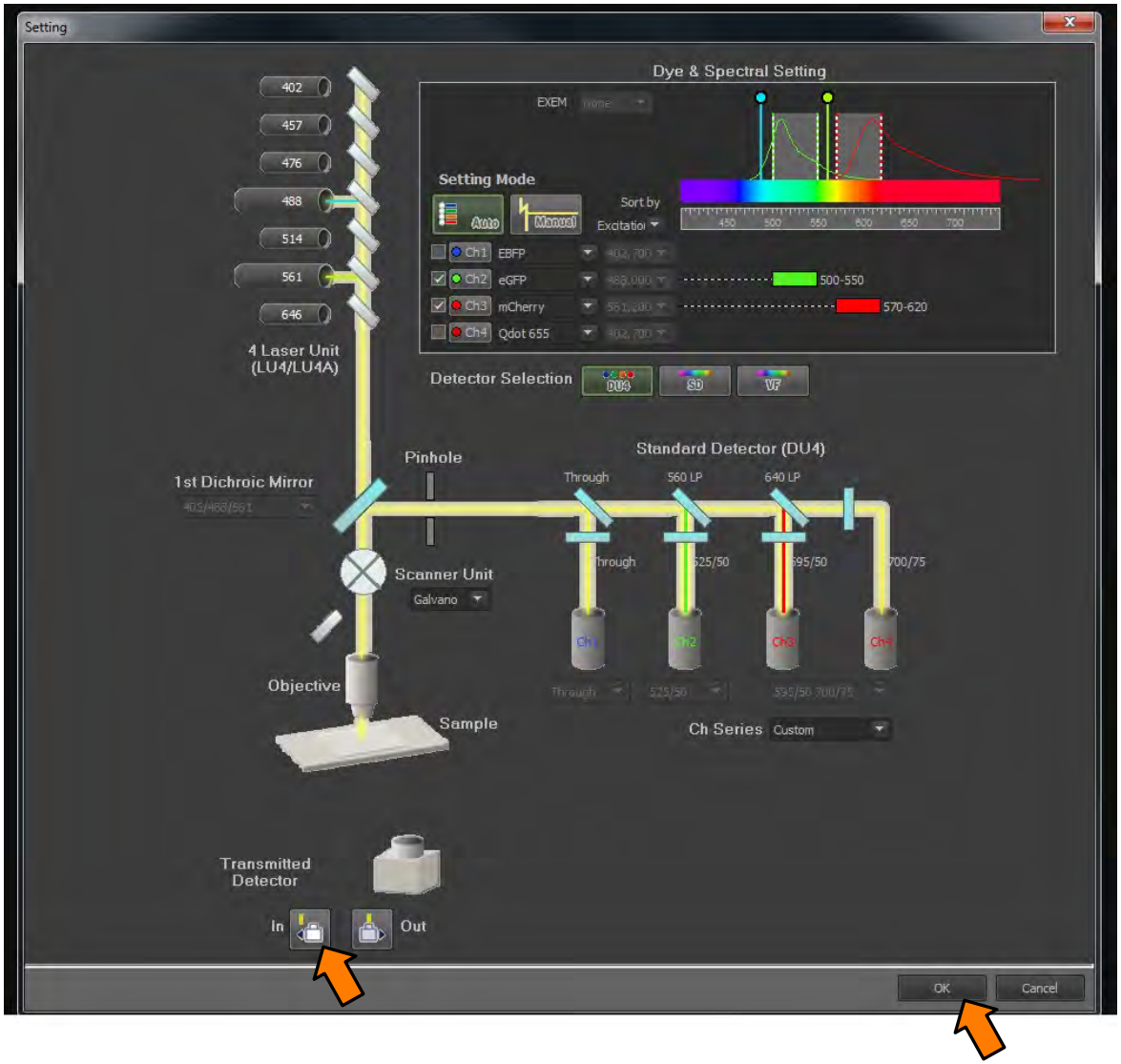
Transmitted Light:

To perform Transmitted light: Insert the detector and press OK.

The condensor must be in place in order to get the signal



13- Select first one channel ➪ ☑ . The most important one to find the focal plane.

14- Remove Interlock ➪ and Press Scan➪ to scan the image and adjust conditions for that chanel.

To find the focus use fast speed and small image size. This will give you less signal but you will find faster the focal plane.

Then, adjust the conditions. Activate the next one ☑ and Desactivate the first one Adjust conditions for this one. ☐

If you want to meassure intensity, do not saturate your image. Use the Pixel Saturation indication tool to check it.

A1 Compact GUI

Scanners

Scan mode: unidirectional / bidirectional. Use unidirectinal

Scan Speed (Pixel Dwell or fps) Use 1/2 or 1/4

Normal/Line average/ integrate. Use normal or line average of 2 if the image is noisy. The acquisition time will be double!!

Image size (pixels) 512x512 is recommended

Secuential/Simultaneus Acquisition

Acquisitin Time

Pinhole size 1.2 AU recommended

Channel

HV Gain around 100 is recommended

Offset

Laser power

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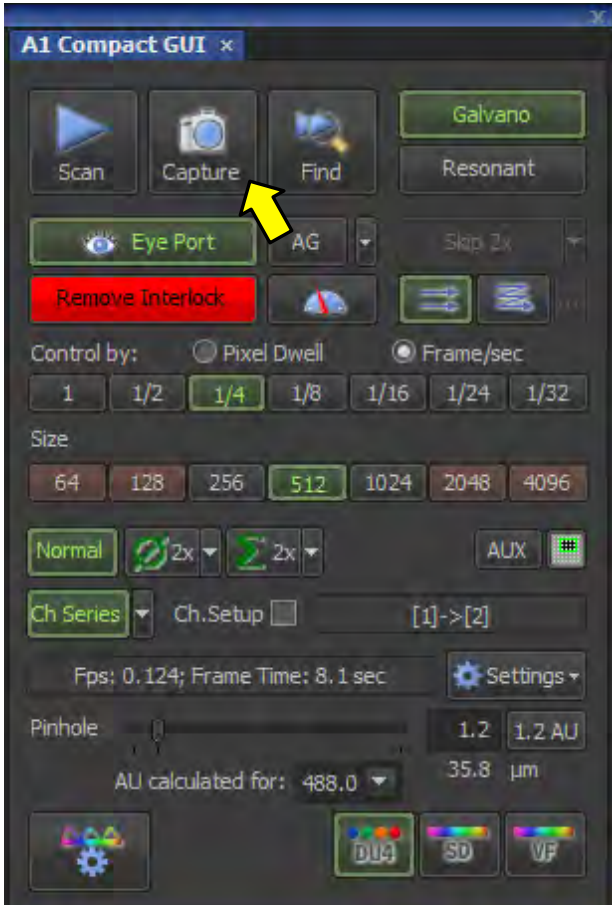
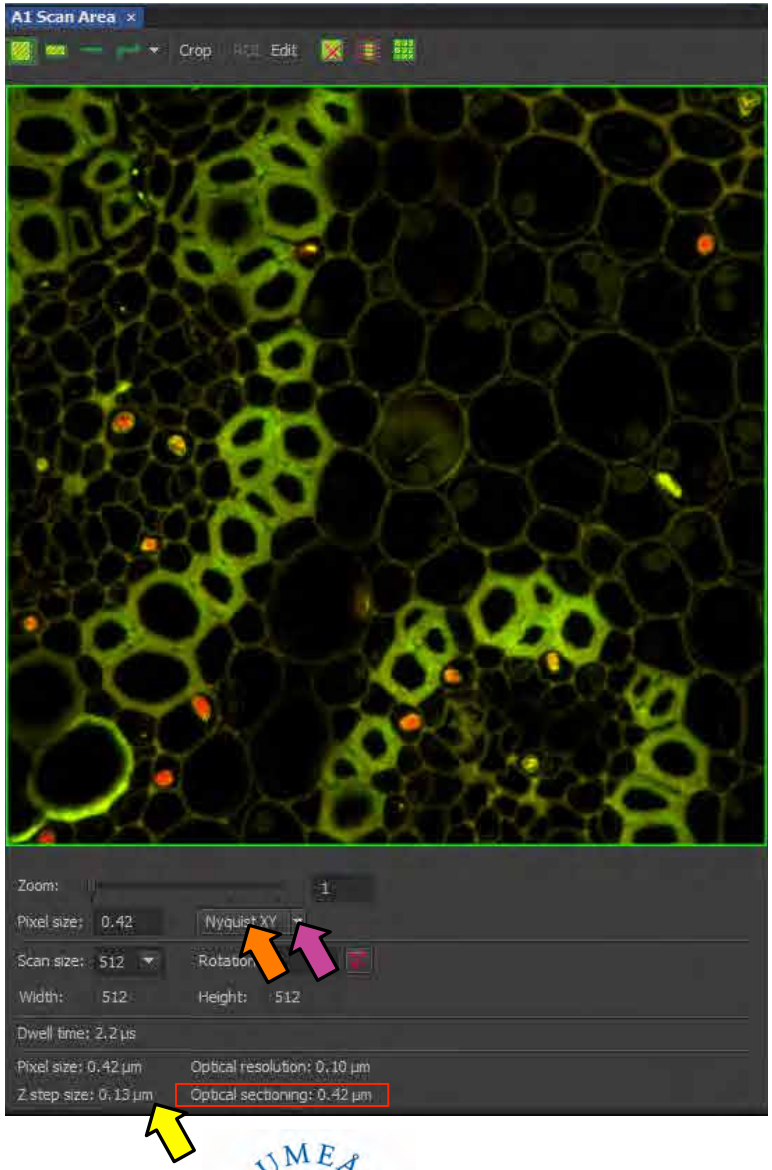
15- Press capture to get the final image. Save as nd2 file.

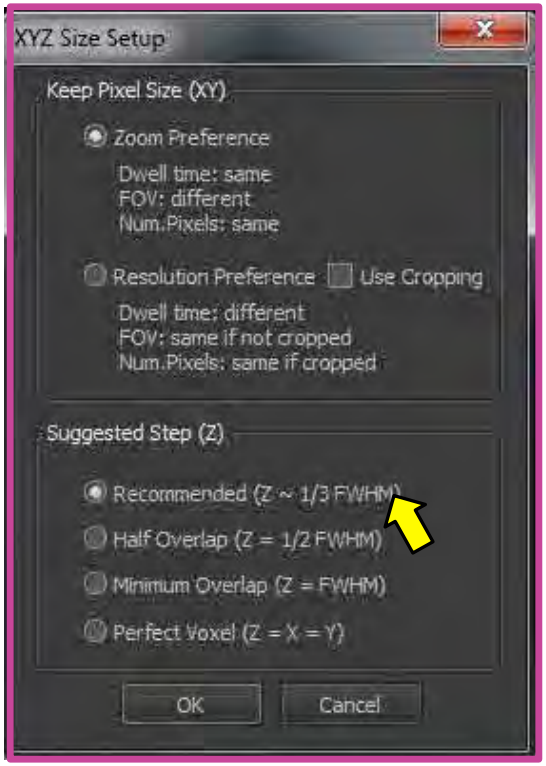
16- Save your images and transfer. You can also use the server to transfer your files.



A1 Scan Area



➪ Adjust the zoom to get pixel size according to the Nyquist criteria



Important information from the picture

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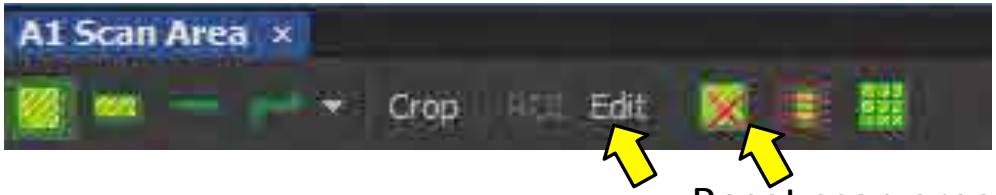
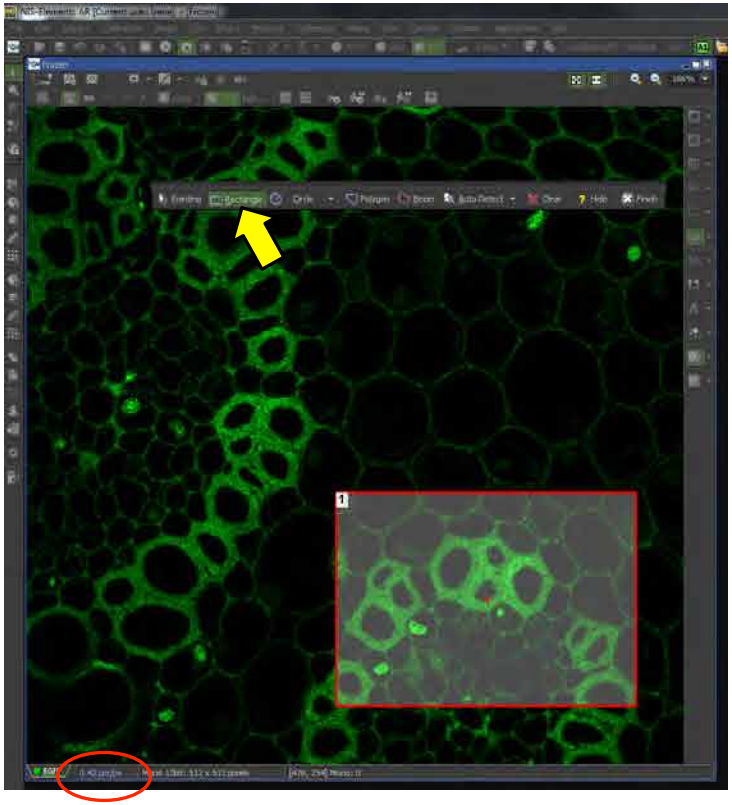
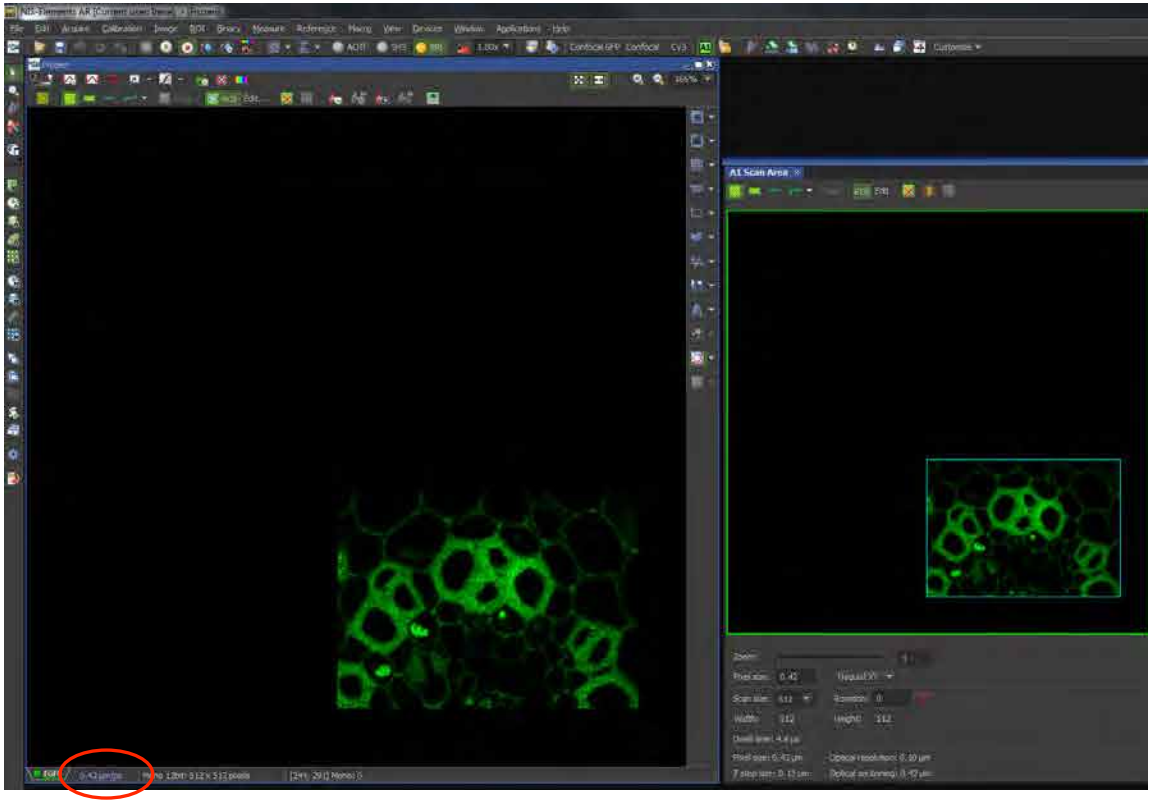
Use Edit to scan a smaller region without changing pixel size with the ROI manager tool. Finish ROI manager and scan







Same pixel size

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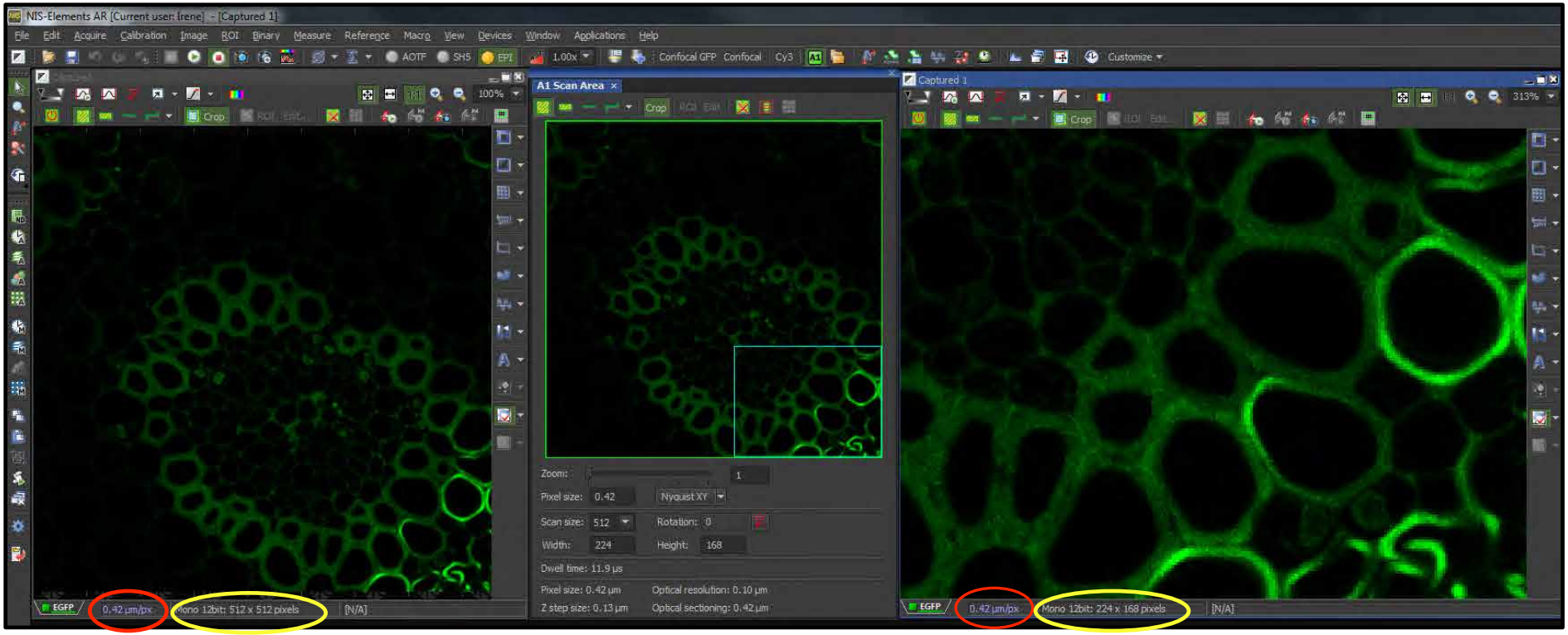
With the ROI tool you will just scan the ROI without any zoom Pixel size will be the same. Image size, smaller



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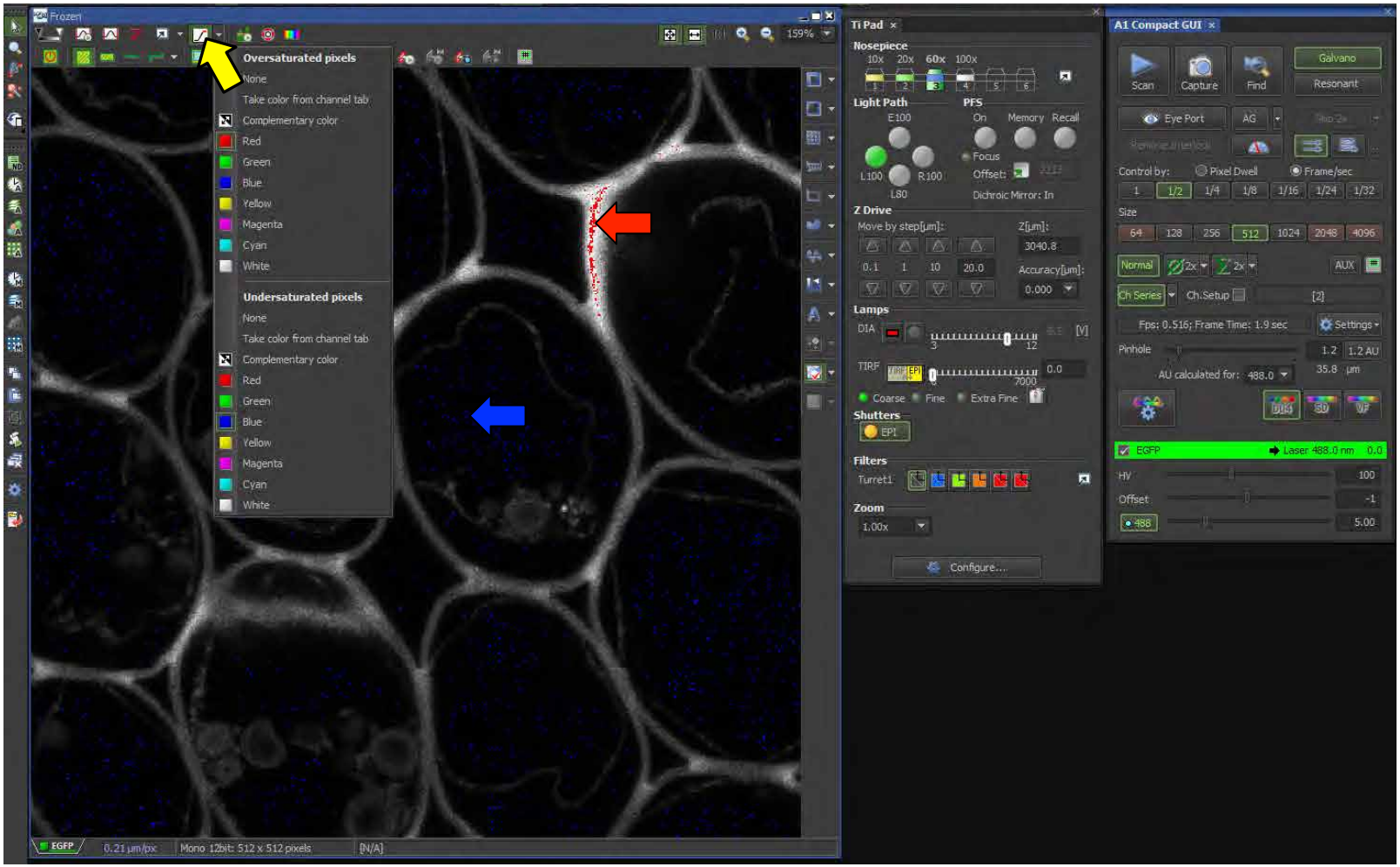
Pixel saturation Indication: Red pixels (Saturated 4095 value) Blue pixels (0 value)



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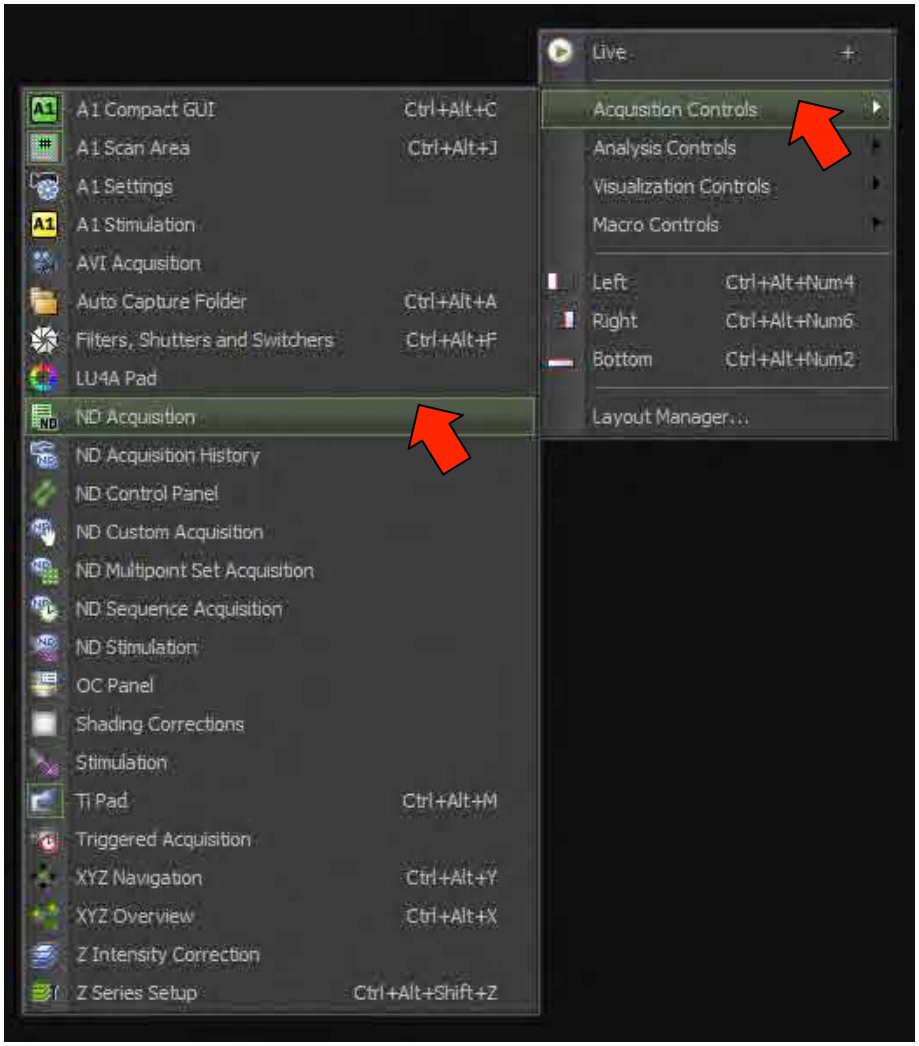
ND Acquisition



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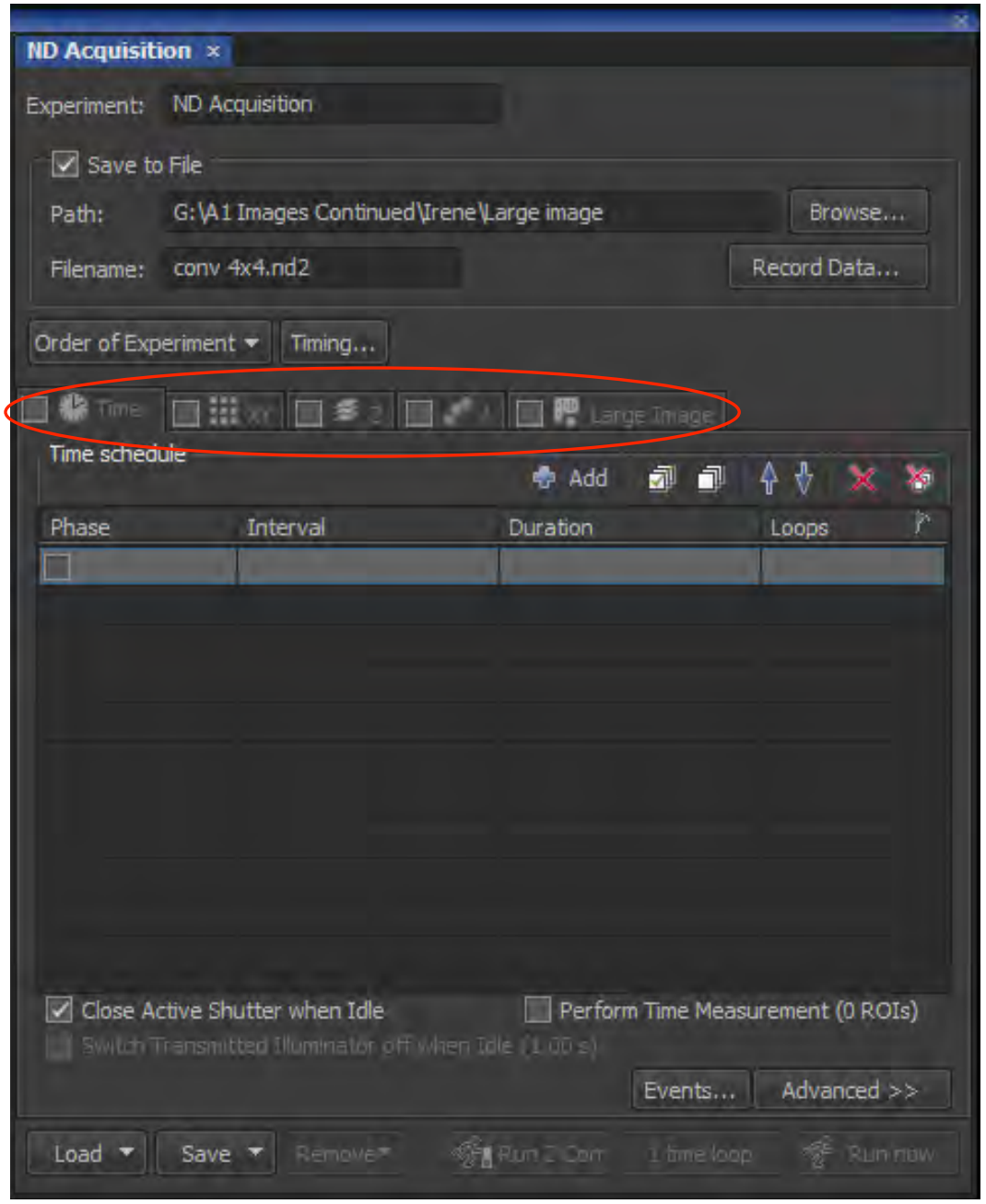
ND Acquisition: With this menu you will automatically acquire images and the Options listed bellow

Pathway to Save your files →

Options →

Time: Time lapse experiments.
XY: Image different positions.
Z: Z Stack
λ: Optical Configurations to perform an automatic acquisition with different wavelenghts.
Large Image: Acquisition and stiching of a large image.



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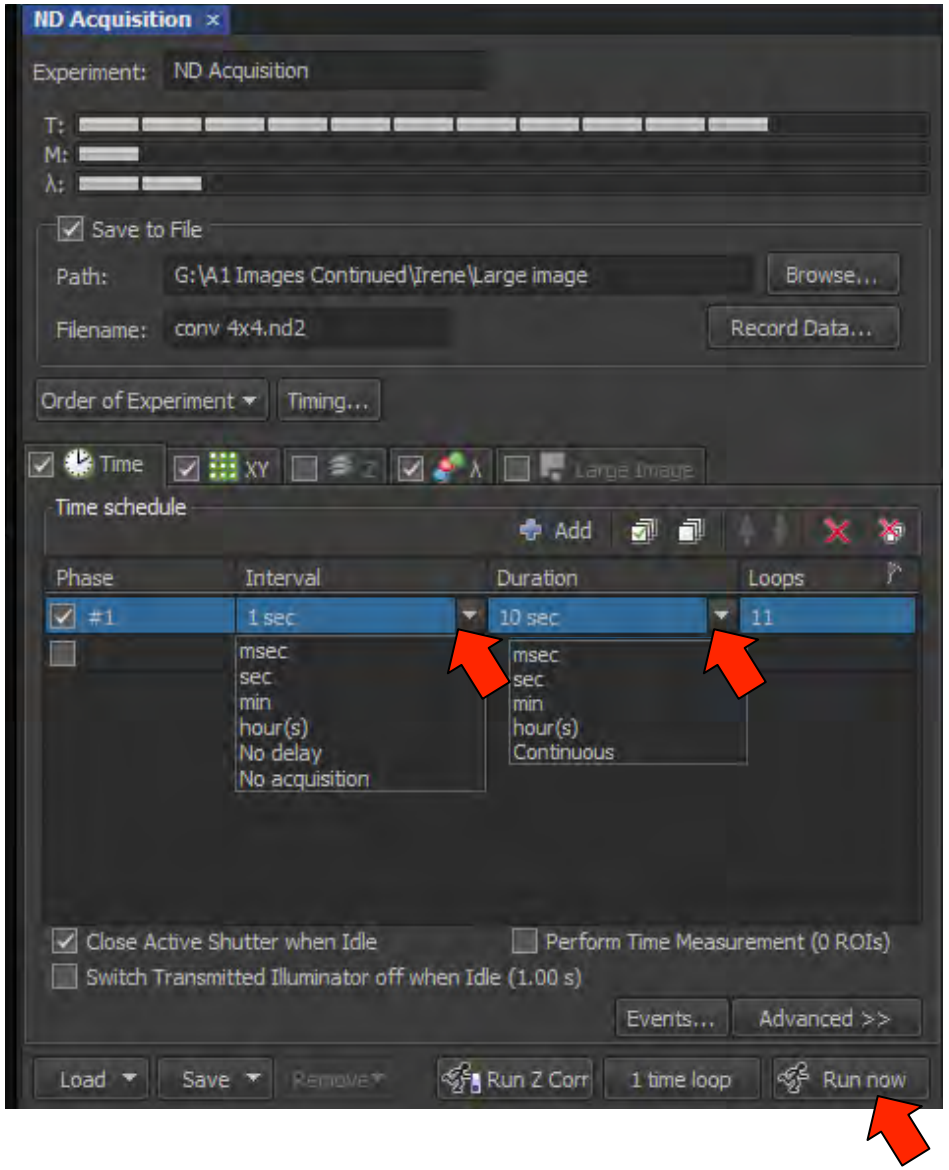
Time Lapse:

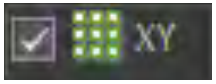
Go to ND Acquisition menu and select Time

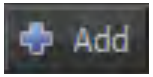
You can choose the Interval between acquisitions and the duration. The software will calculate loop numbers under those conditions.

Press Run Now to start the experiment. You can run Time Lapse with Positions, zstack and large images.



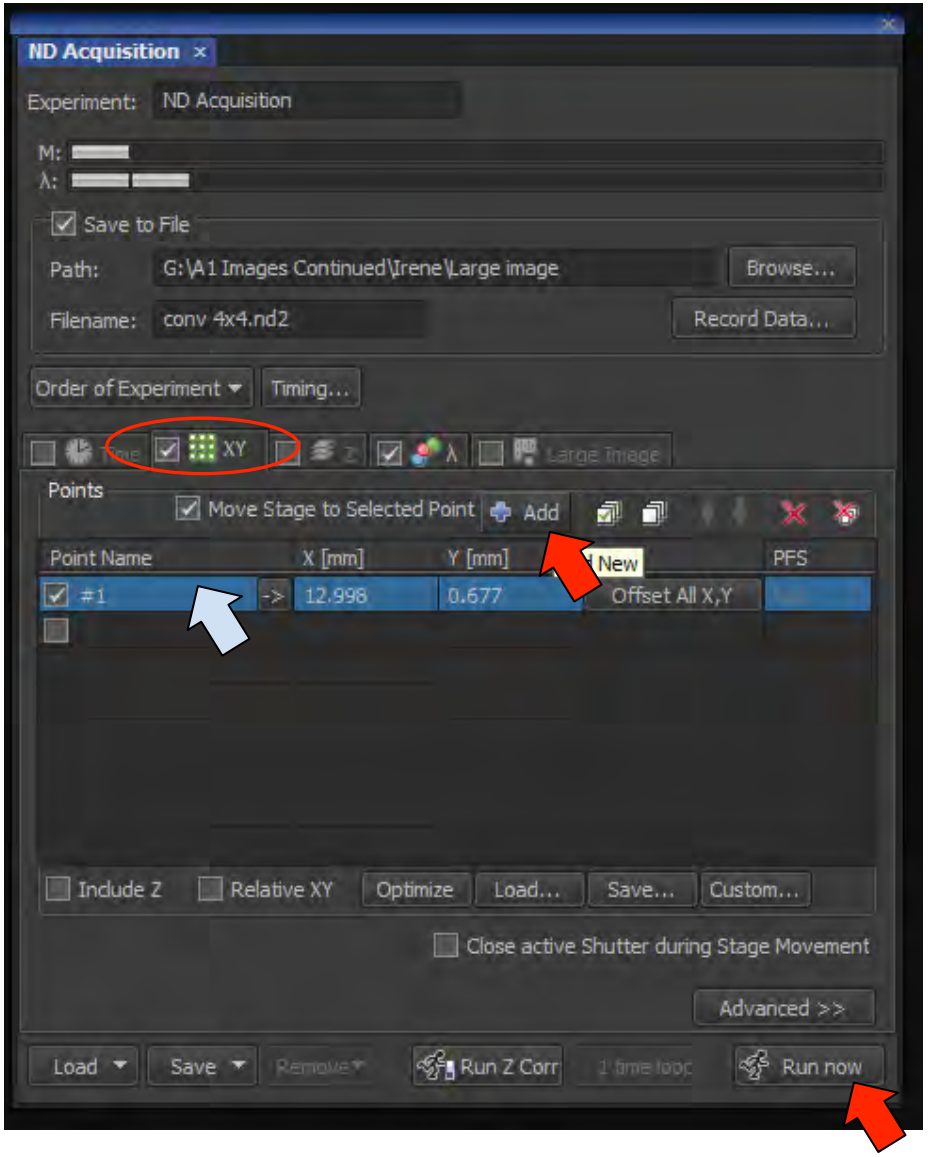
Positions:

Go to ND Acquisition menu and select ☑ **Positions** 

Press Add 

to add a new position. The coordinates will be kept, then you can move to another area an select a new position. You can travel within the positions selected by clicking with the mouse on each position (blue arrows).

If you are using oil objectives do not travel much along the xy to avoid loosing oil.



Z Stack: Go to ND Acquisition menu and select ☑ Z.

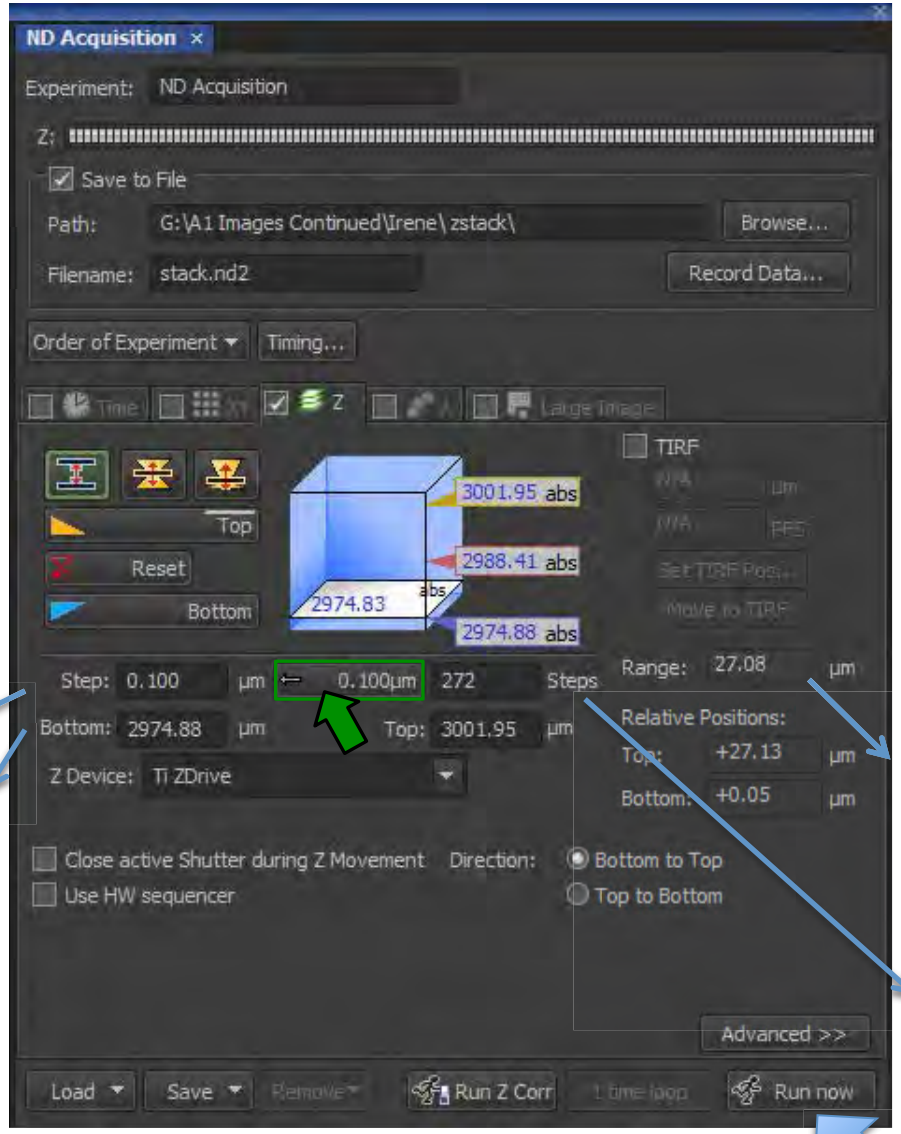
Pathway to save and name the file

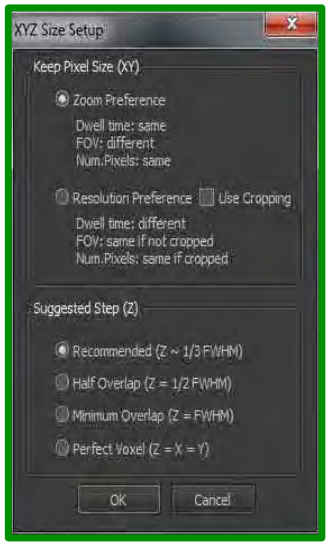
Zstack Top position. Objective is close to the slide

Zstack Botton position. Objective is far from the slide

Step thickness

Bottom and Top position





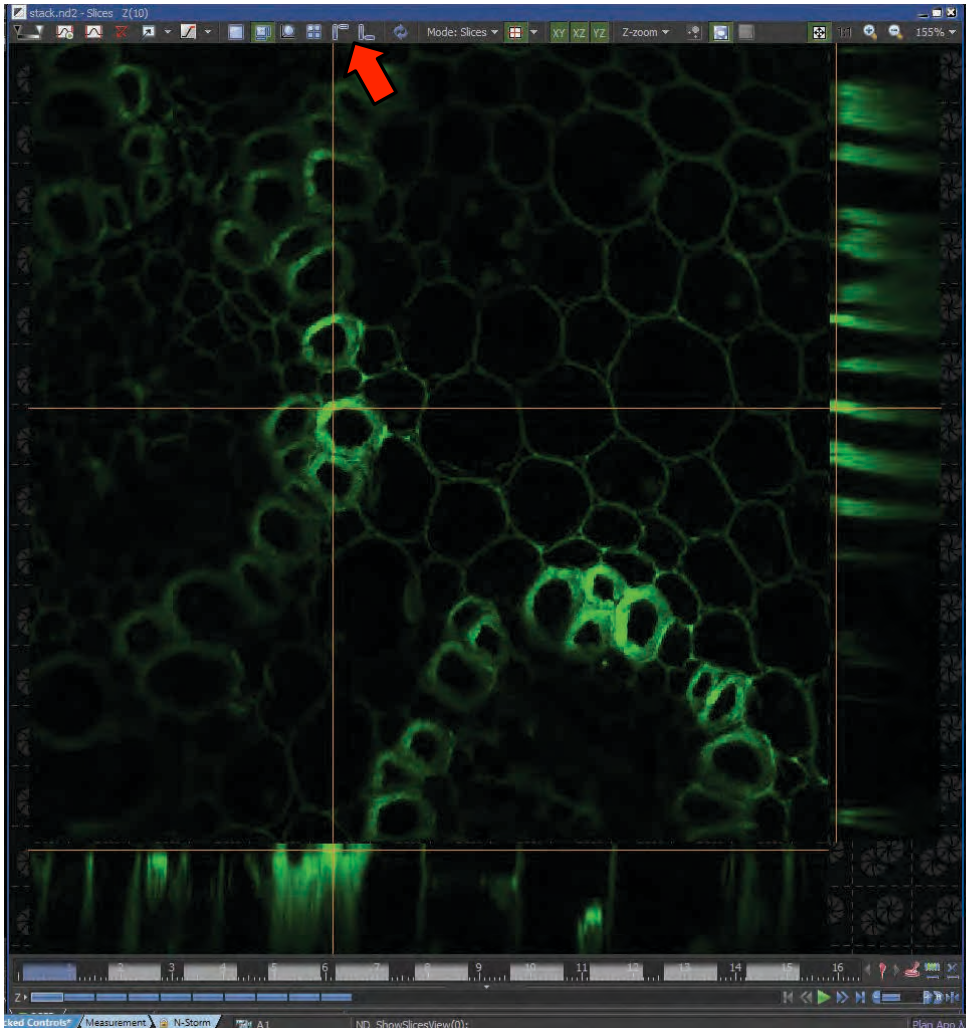
Suggested step size based on XYZ Size set up

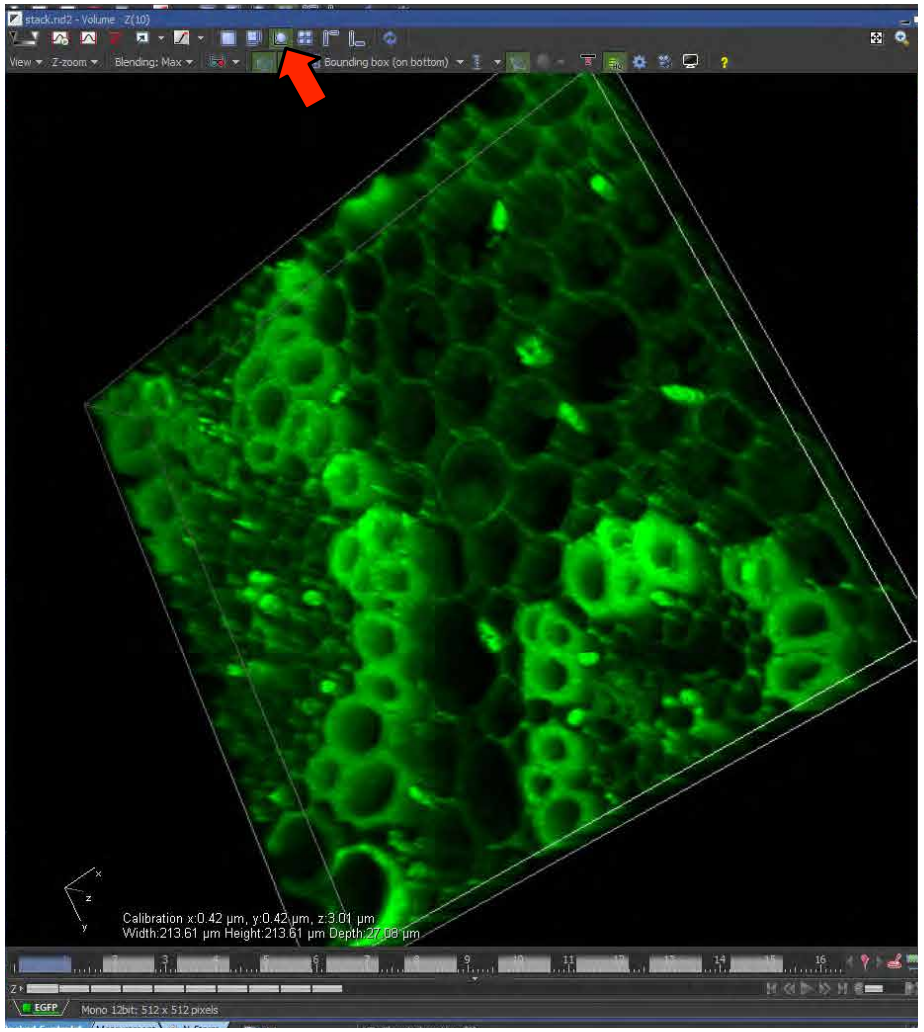
Range between Top and Bottom

Step numbers

Press Run Now to start acquisition

Z Stack Visualization. 27.08um range; 10 steps:





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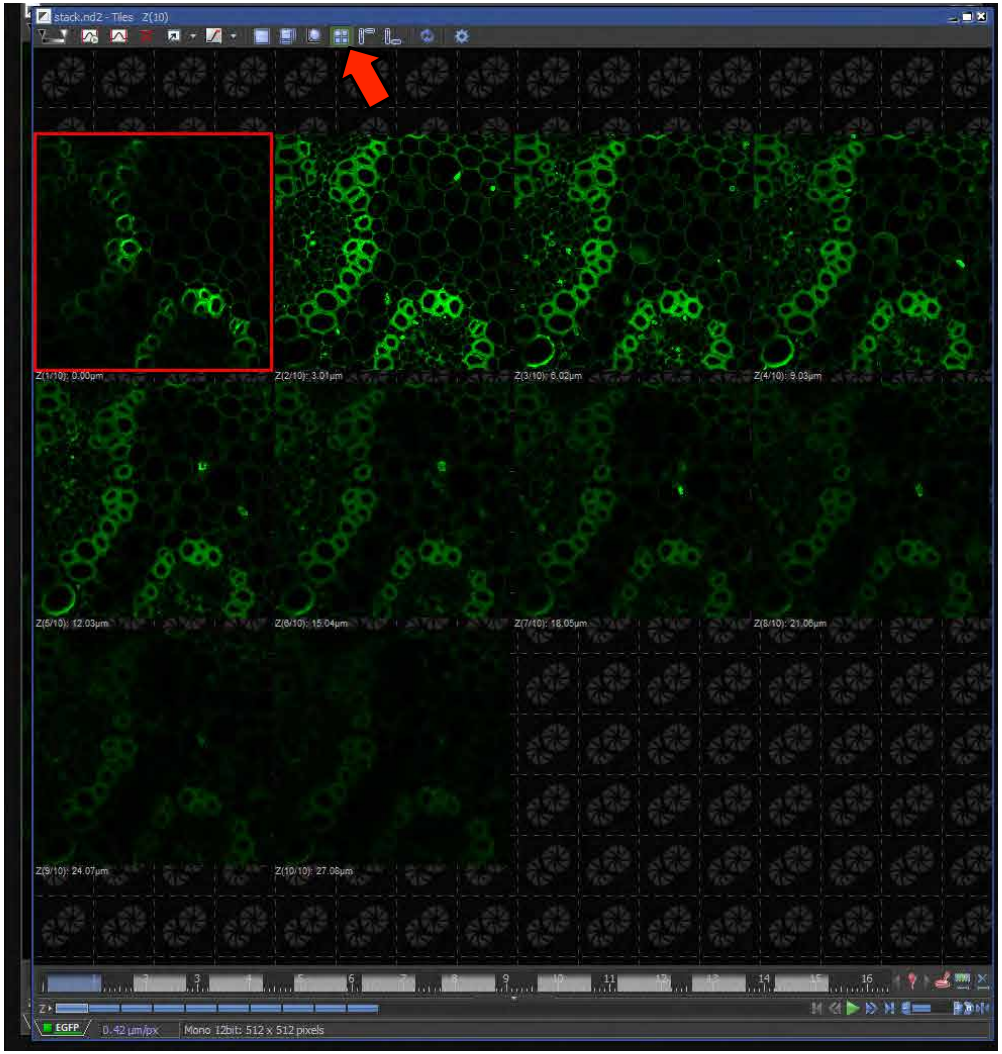
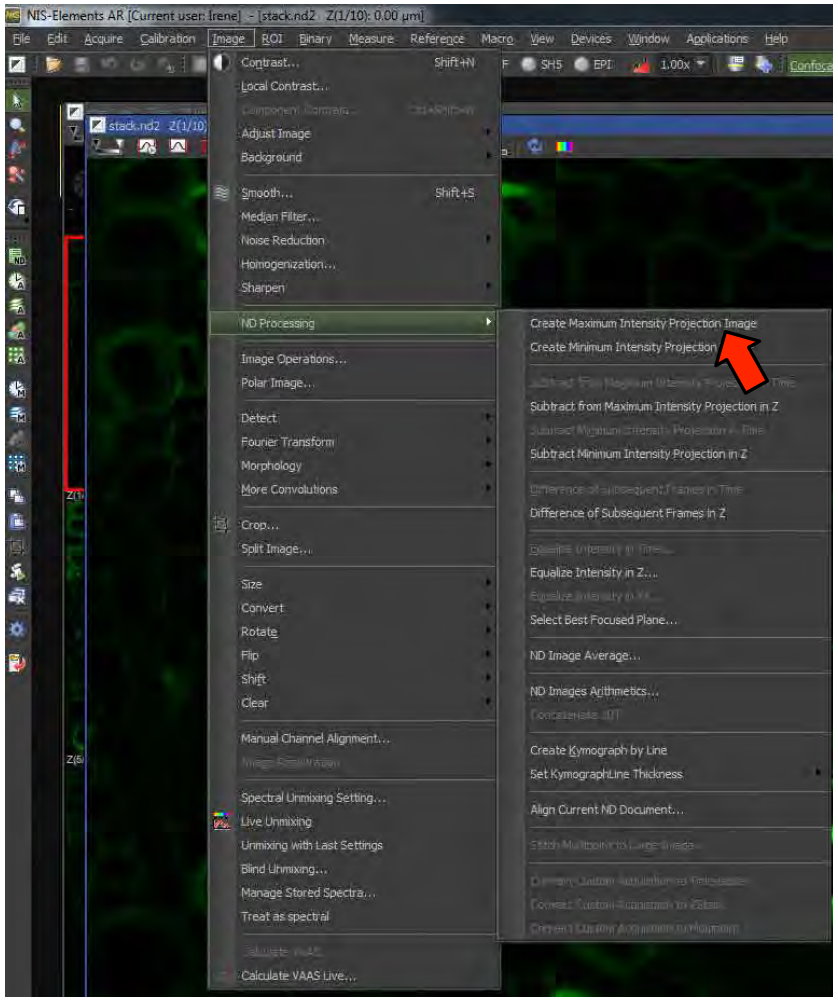
Z Stack Visualization. 27.08um range; 10 steps and Maximun Intensity Projection





Image/ND Processing /Create maximum intensity projection Image. The file will be saved as: “stackfilename”-MaxIP.nd2

Large Image:

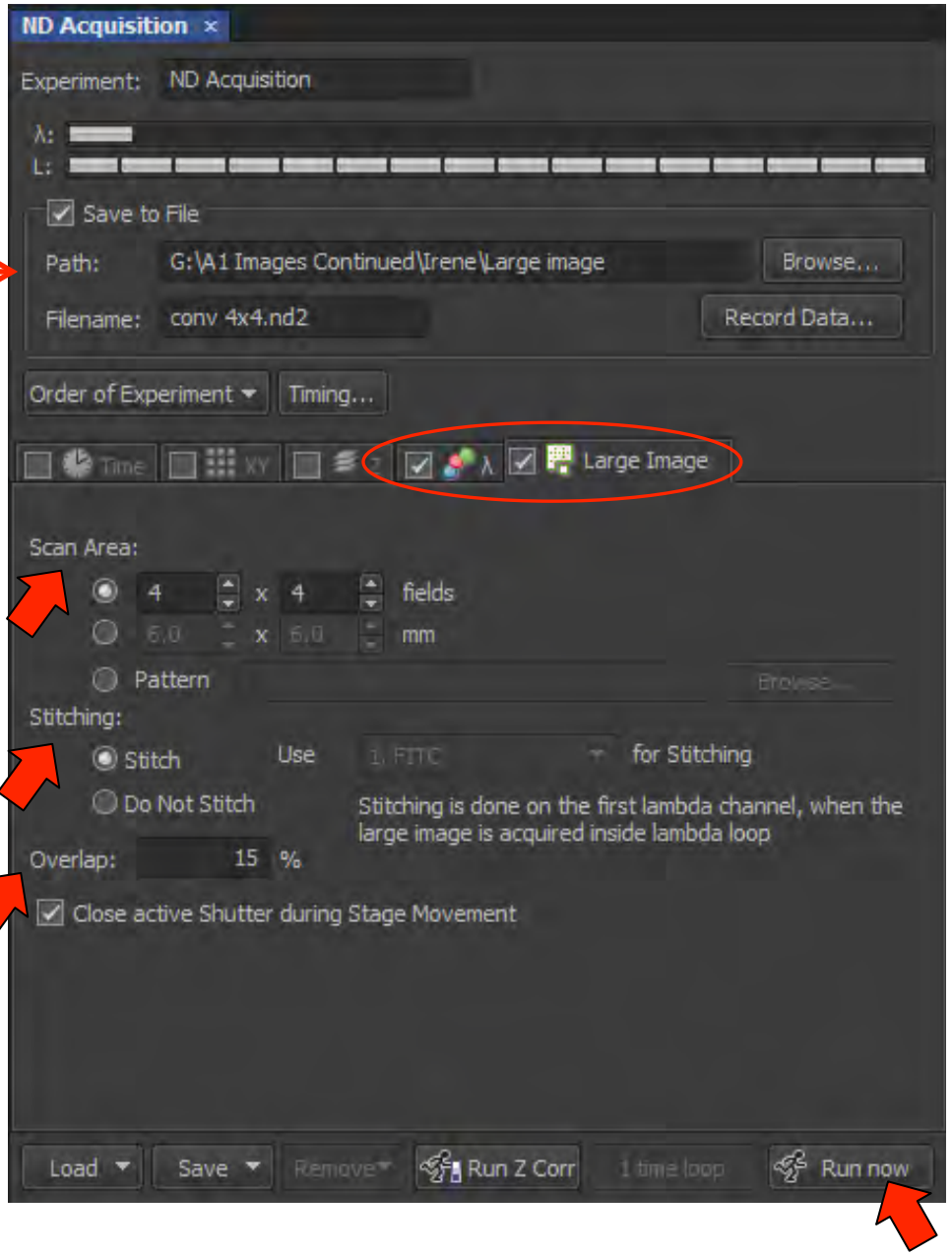
Go to ND Acquisition menu and select ☑
Large Image.

Pathway to save and name the file

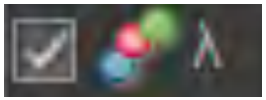
To use this option alone (without TimeLapse) you have to select an optical configuration within the λ menu.

Select the scan area , if you want to stich the large image and the overlap %.

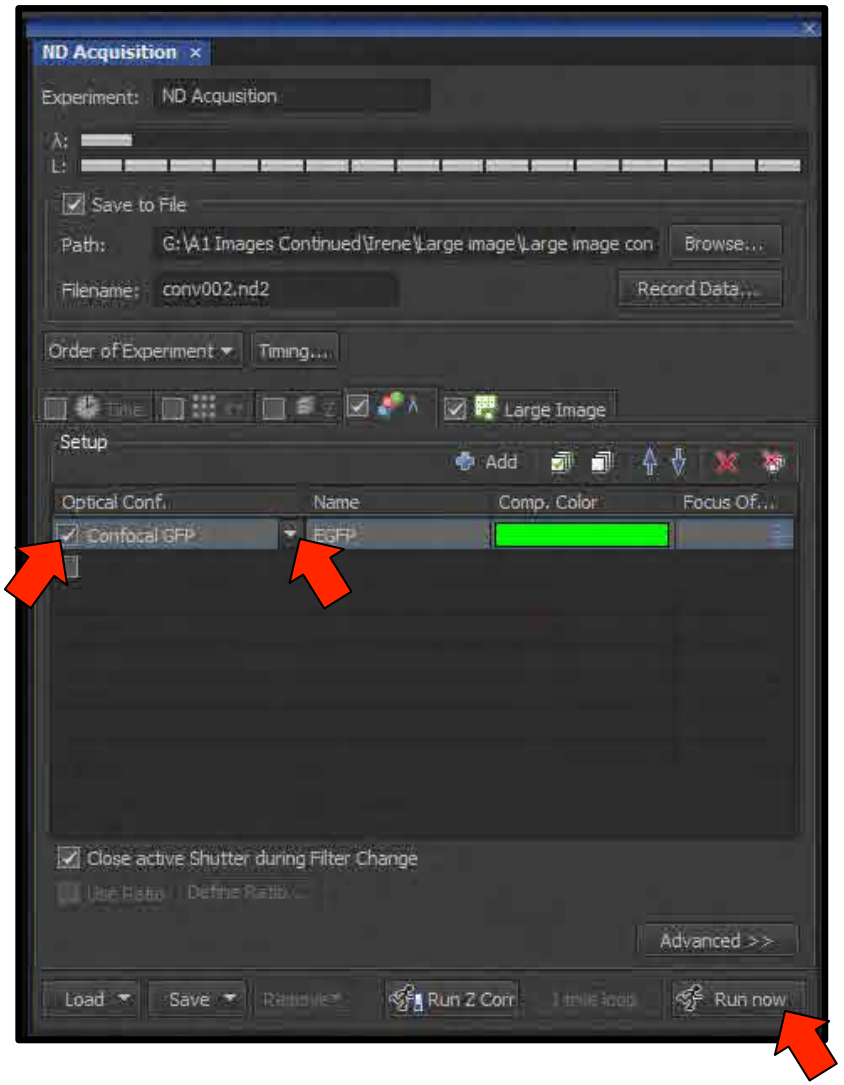
Press Run Now to start acquisition



Go to ND Acquisition menu and select ☑ λ

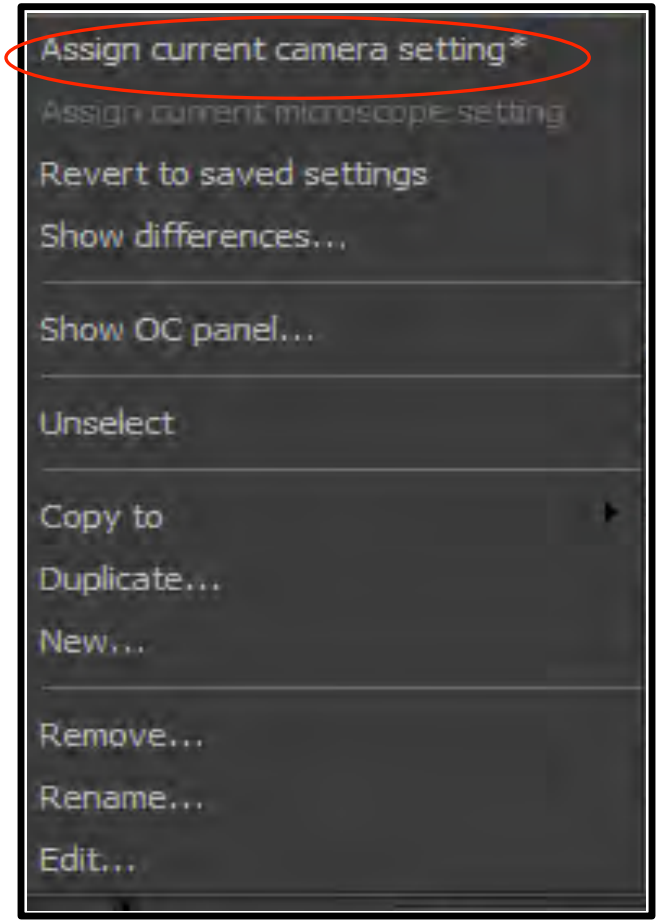


Choose the Optical Configuration you want to use from the list and load them within the SetUp.



In some cases you will need to use the optical configurations (recorded optical pathways) to run an ND Acquisition (for a large image for example). Select the OC from the main menu:

Adjust settings for that OC with the A1 compact GUI . An exclamation tag will show up. To keep those settings: right click and select: “Assign current camera settings”. Those settings will be used to acquire the images when you are running an experiment within ND Acquisition. In order to create new OC please ask BICU personnel to help you.



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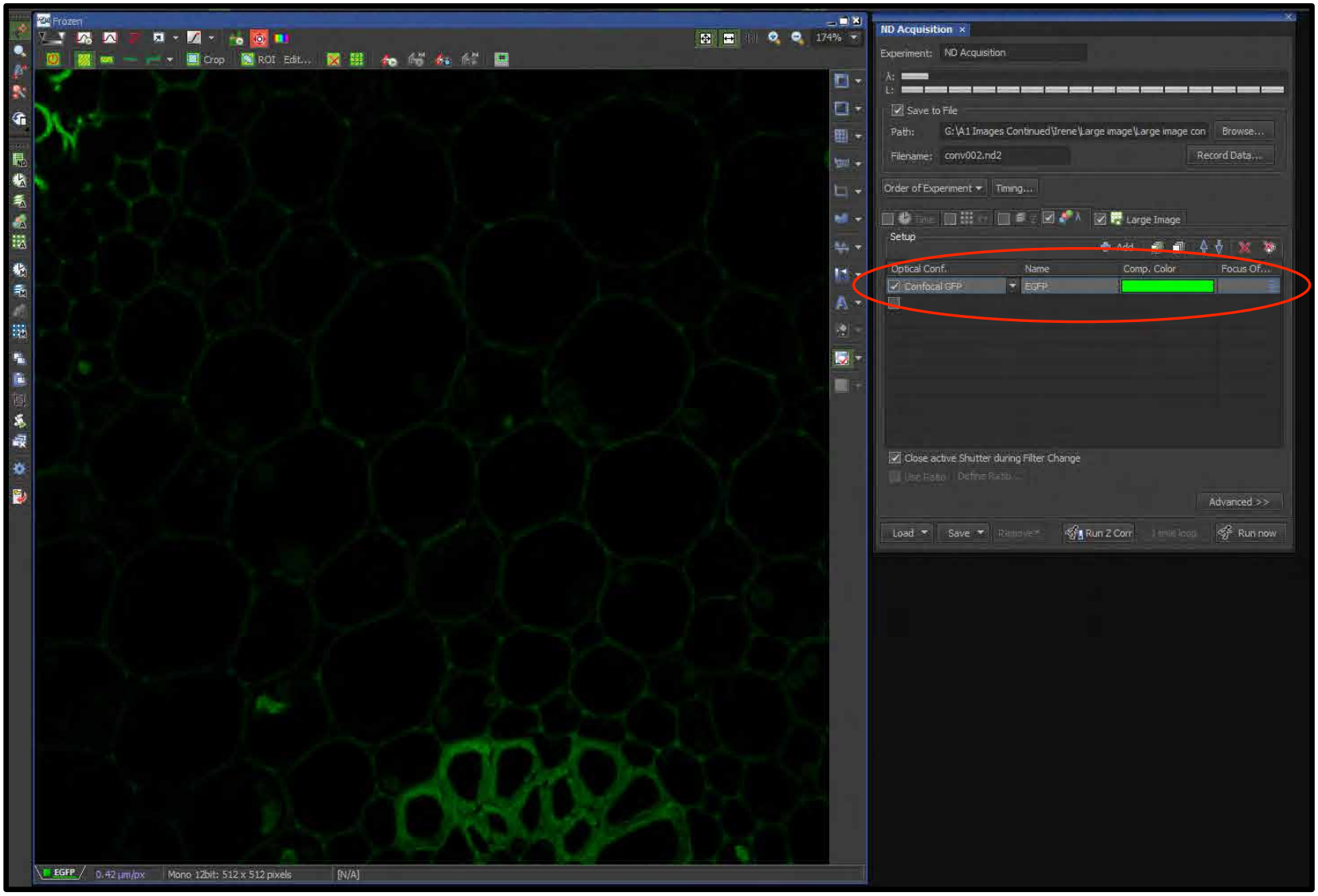
Large Image:



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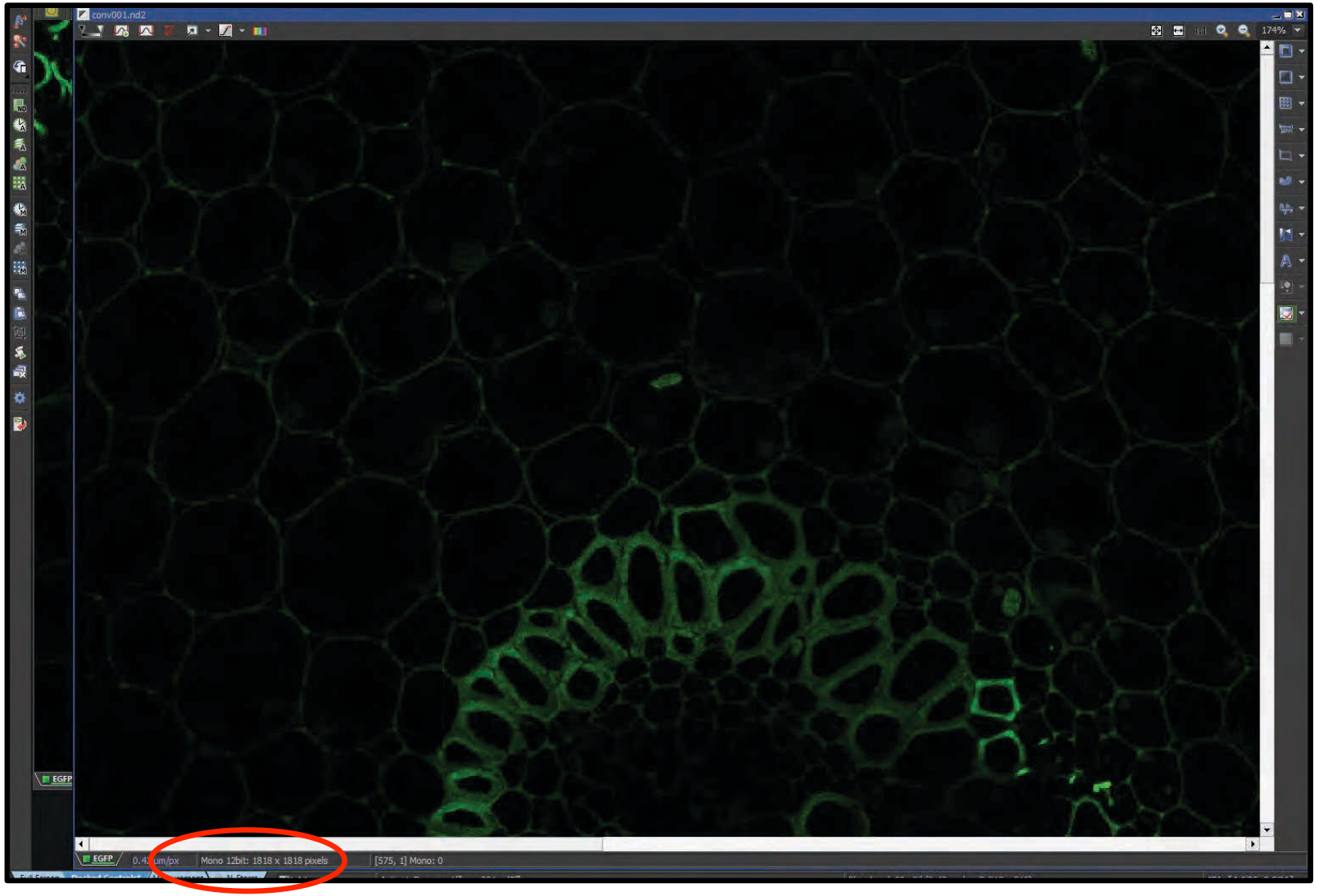
Large Image:



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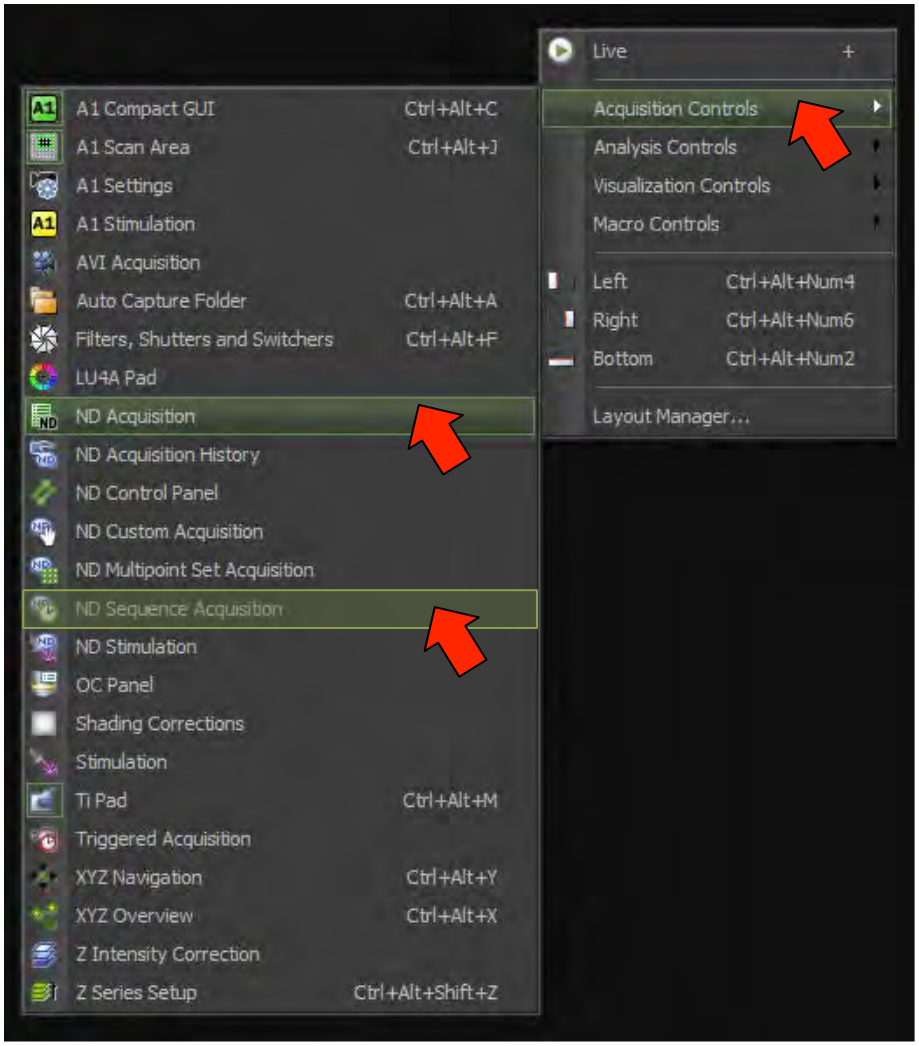
This is part of the large image. Size: 1818x1818 (15% overlap)

ND sequence acquisition: You can run a sequence on ND acquisitions to perform complex experiments



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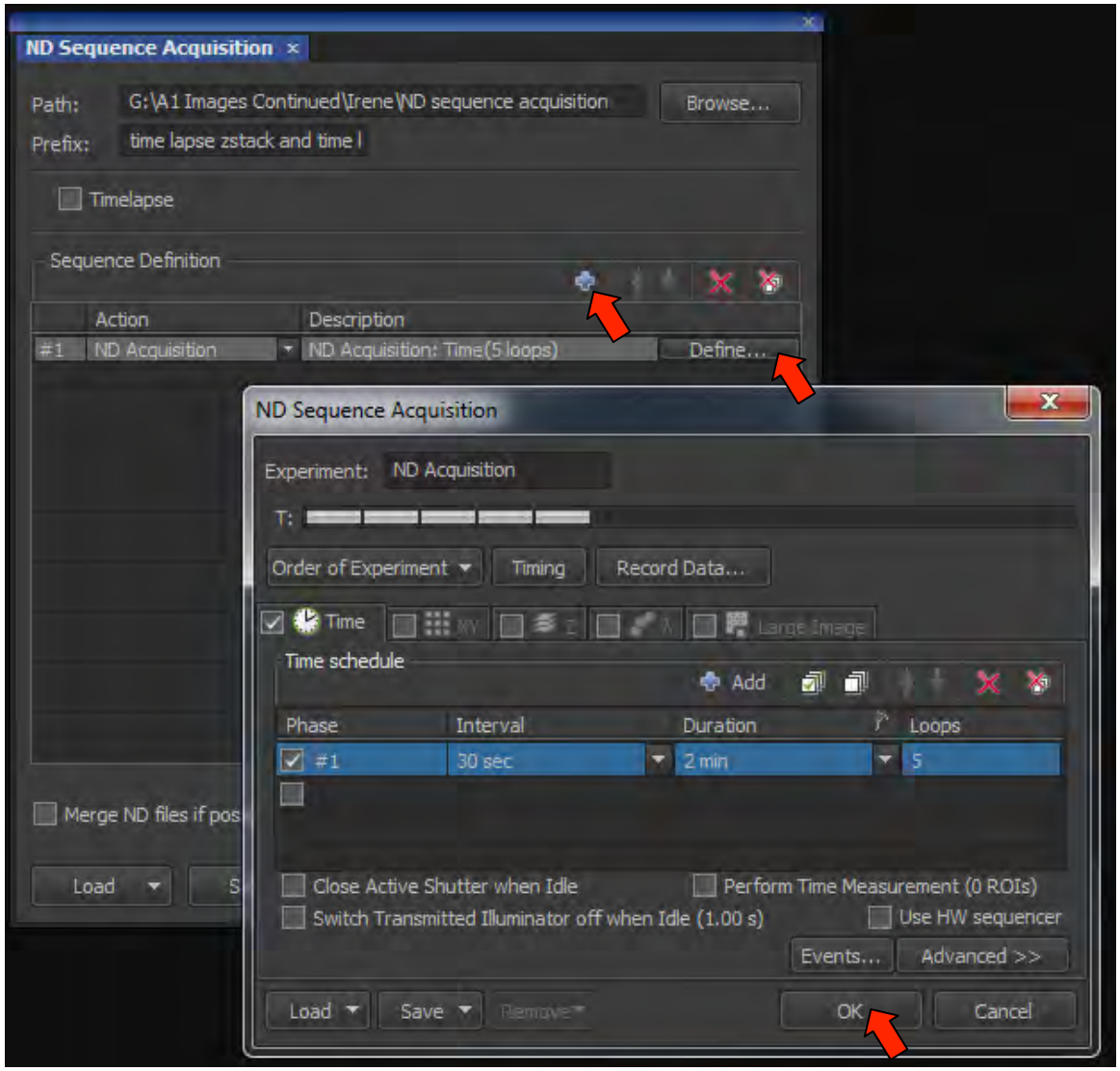
ND Sequence Acquisition: Time Lapse, Zstack and Time Lapse

Pathway to save the file

Define the ND Sequence. In this case a Time Lapse. Press ok and move to the next Action



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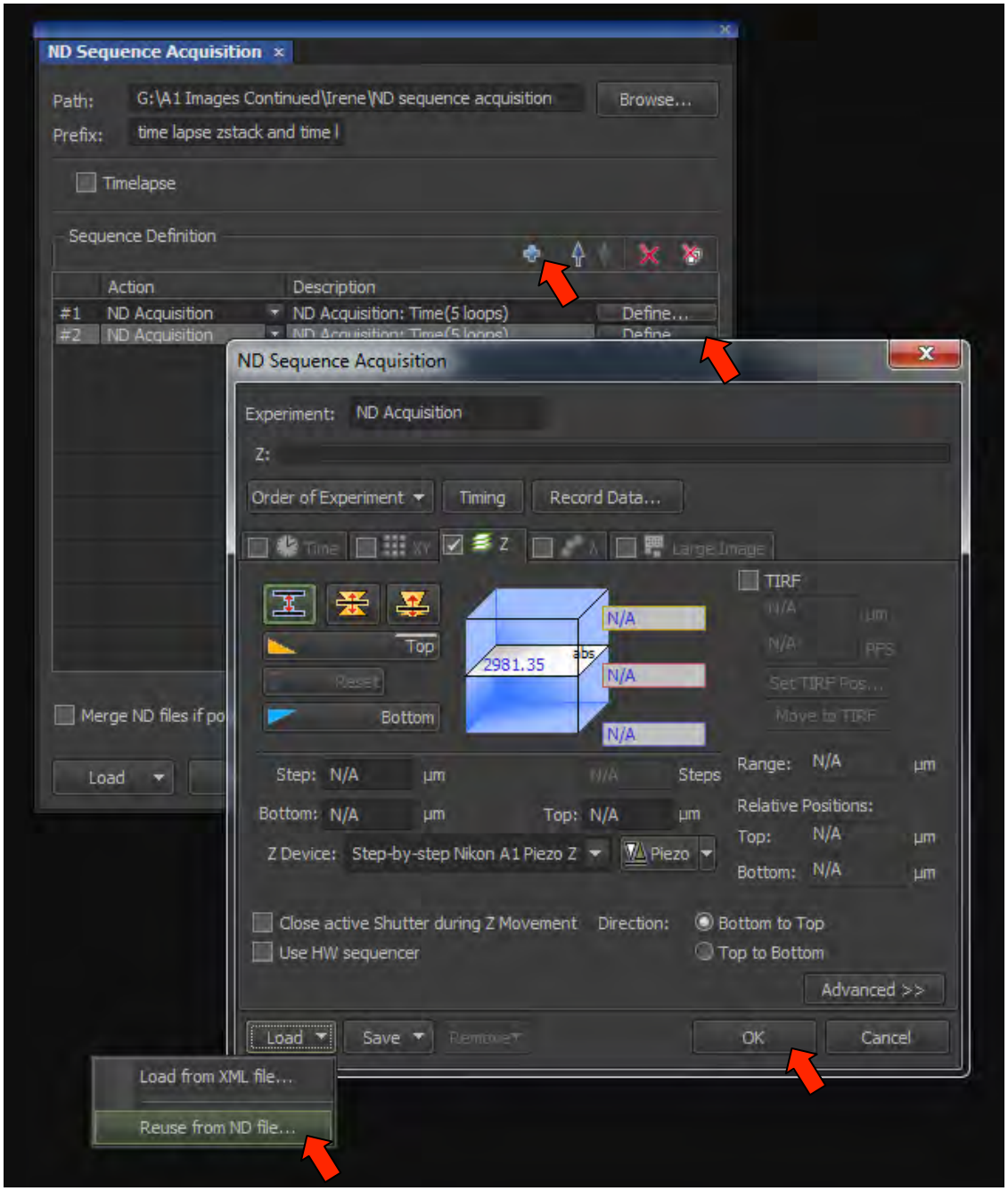
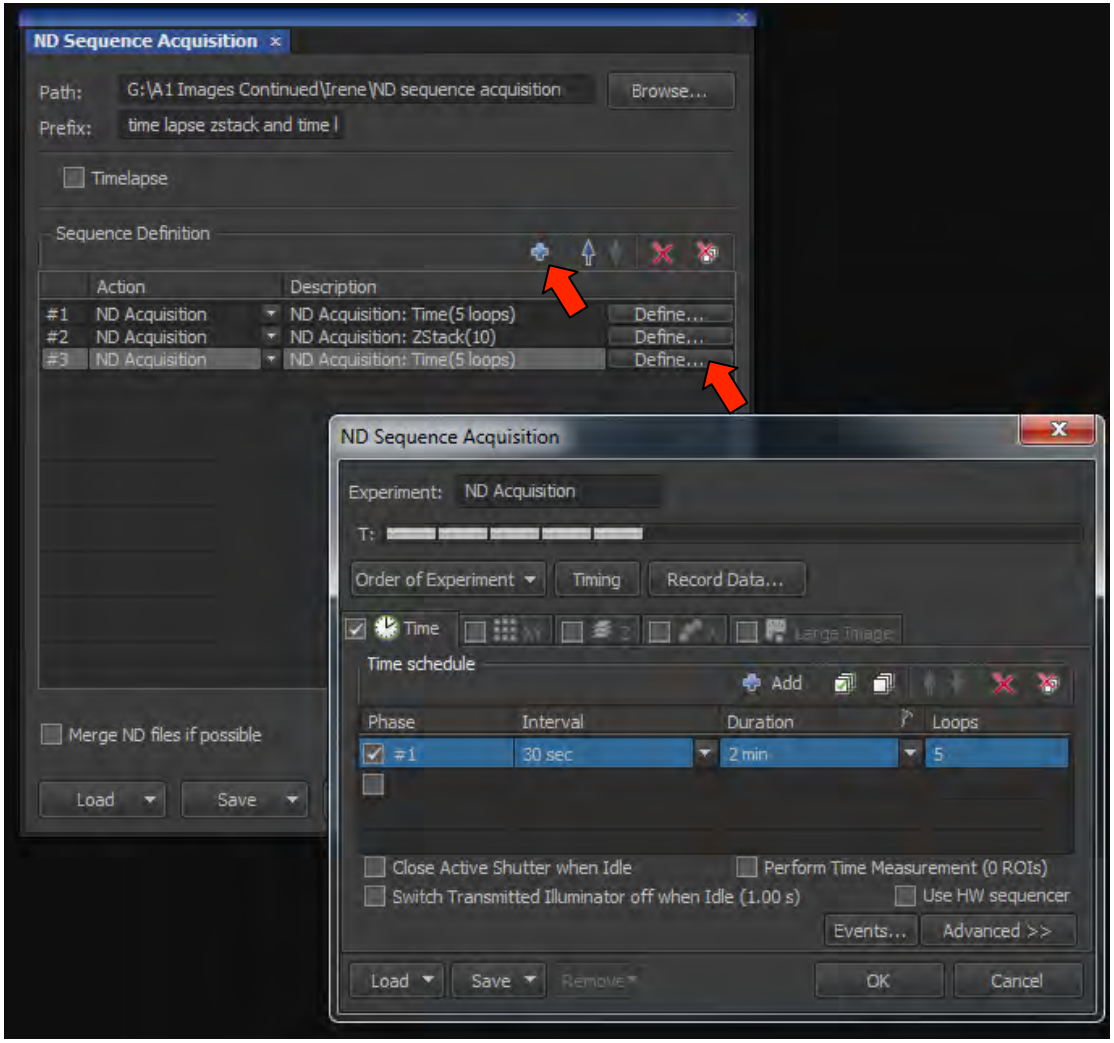
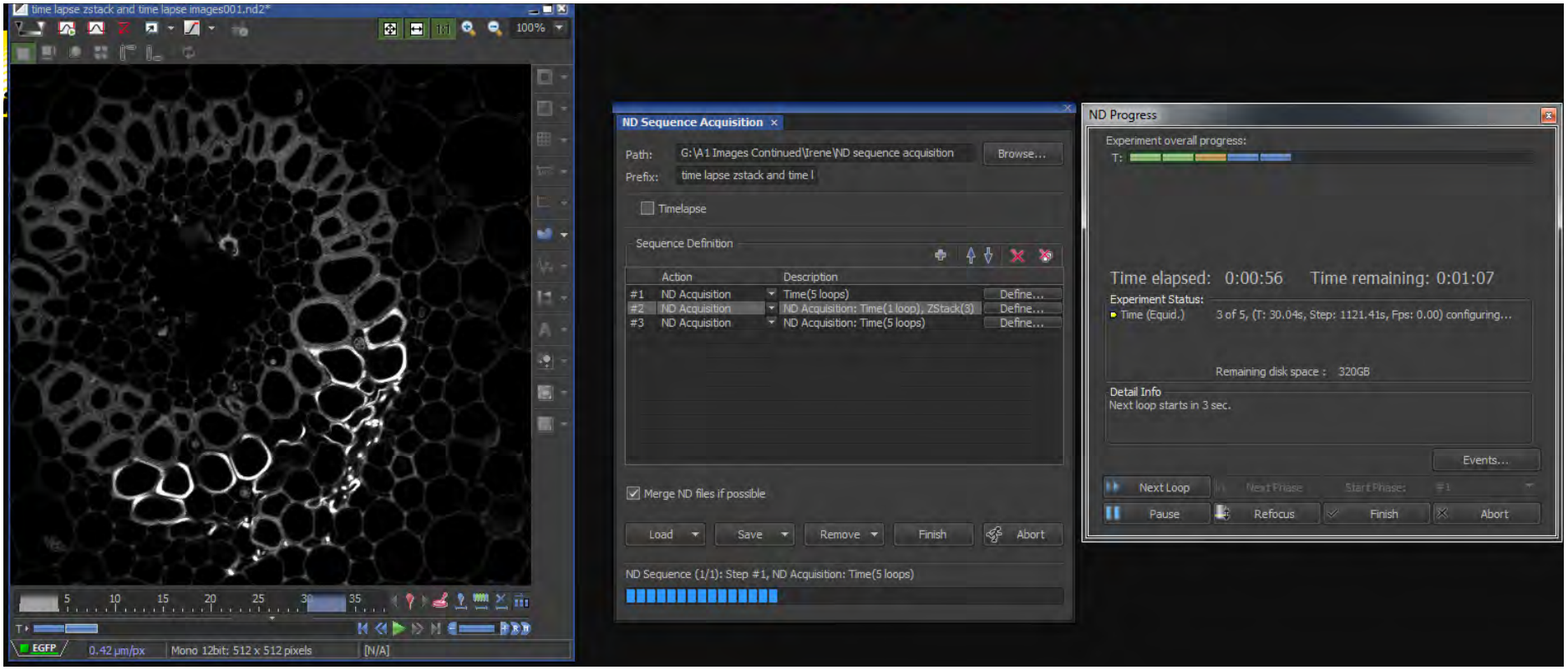
In this casethe second Action is a z-stack. We have to load settings from a previous ND fil, press ok and then move to the next Action



The las Action is Time Lapse.
Once you finish mark “Merge ND Files if possible” and Press Run





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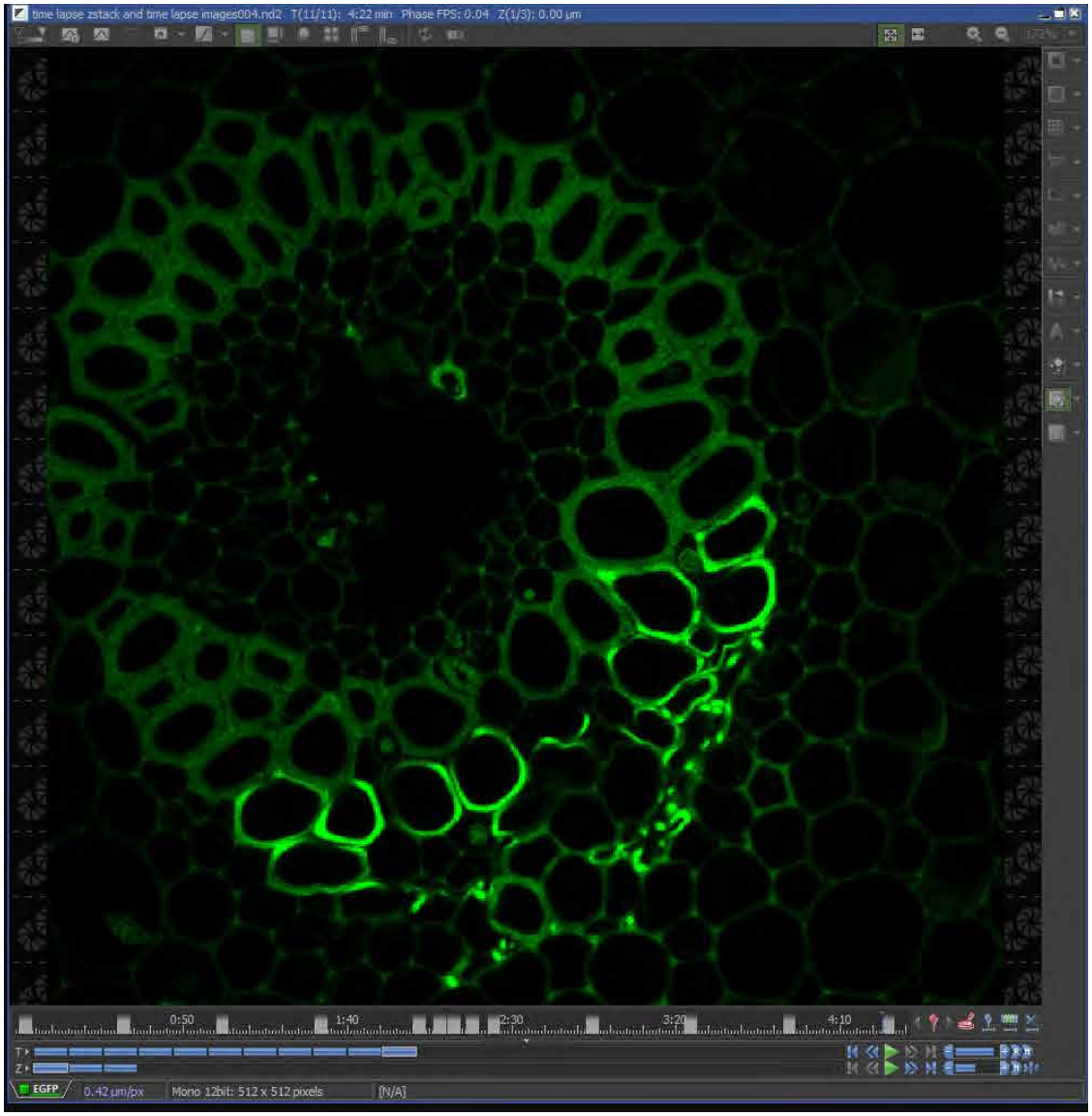
This is the final image with the nd files merged



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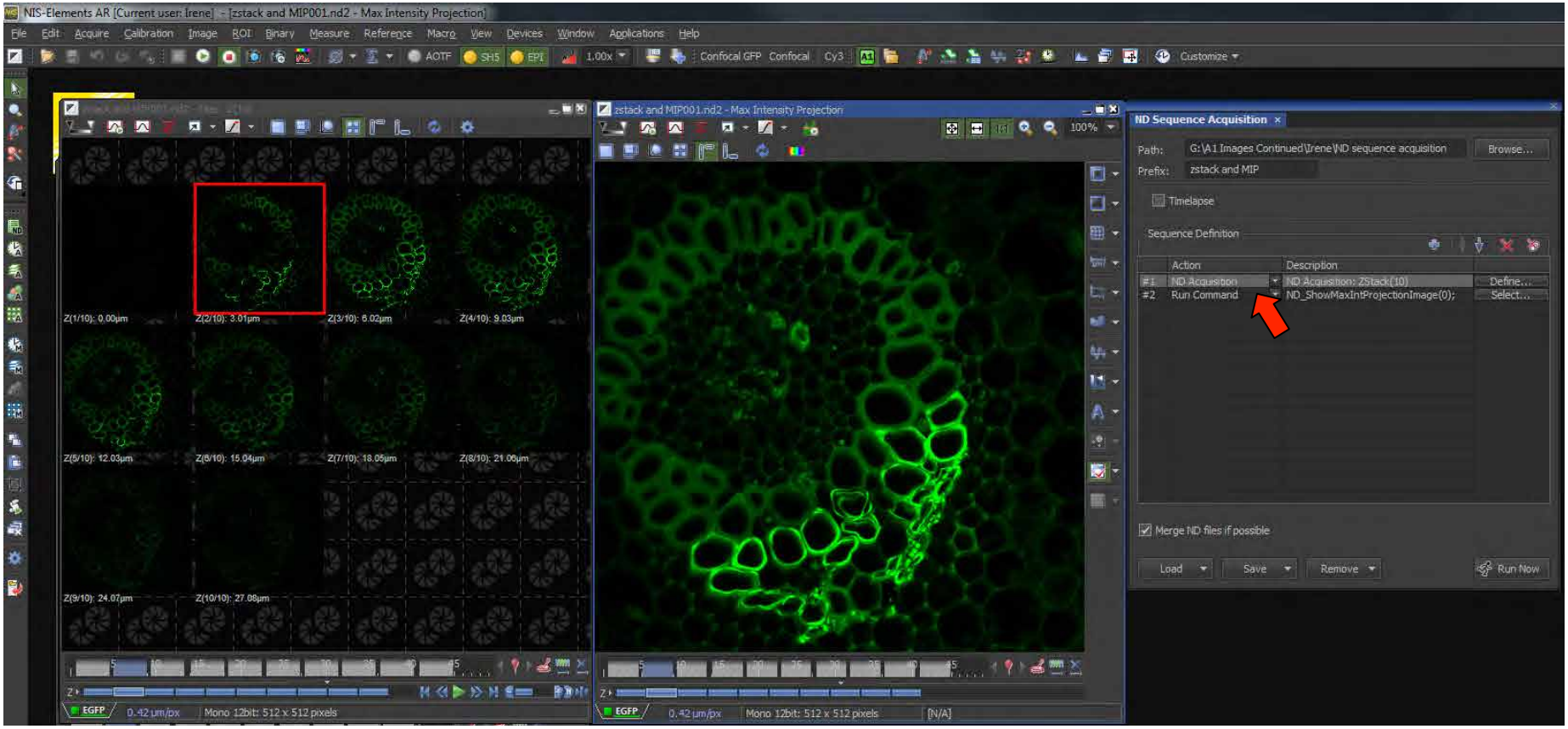
ND Sequence Acquisition: Zstack and Maximun Intensity Projection. You can also select to Run a Command within the nd sequence acquisition



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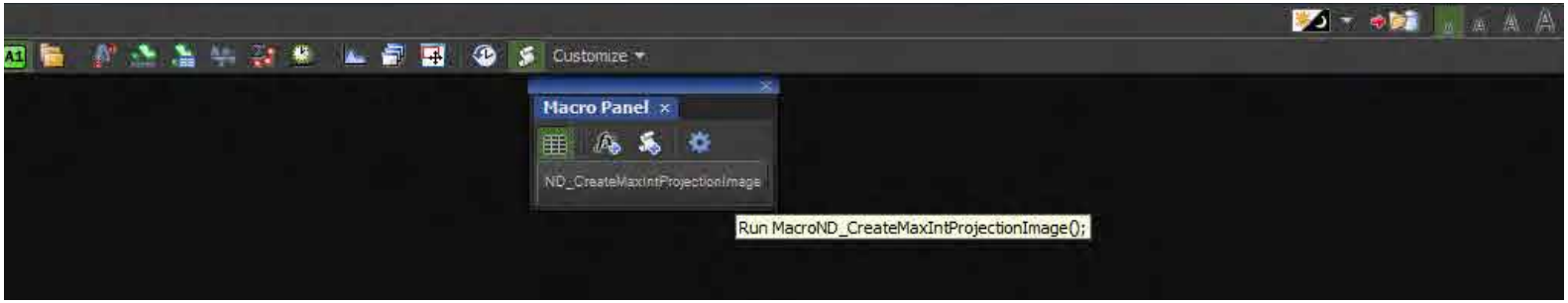
Macros: Maximun Intensity Projection button



You can also record a sequence of actions and save your macro as .mac (See Nis Elements Image Analysis PP)

Switch off the system

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SWITCH OFF THE SYSTEM

1- Lower the objectives and remove the excess oil from the objective with lens paper

Don't switch off the microscope if someone has booked within 1 hour after you.

2- ALWAYS Check if that person is going to use the microscope and let him/her know the microscope will stay on.

If you are the last user or next is comming in more than 1 hour:

3- Close Nis Elements

4- Transfer your files: connect the computer to internet to transfer your folder from: Computer / Iomega HDD 3TB (G:) A1 Images Continued to the server. There is a link to the server in the desktop. Once the transfer is done, please unplug the computer from the network for security reasons.

It is forbidden the use of usb or hard disks to transfer your files.
You cannot use the computer, hard disk or the server to keep your data as a storage place
The server is only to transfer your files.

5- Shut down the computer

6- Turn off the lasers: Turn off the key

7- Turn off the Lamp and check the running time.
Write down that info in the logbook.

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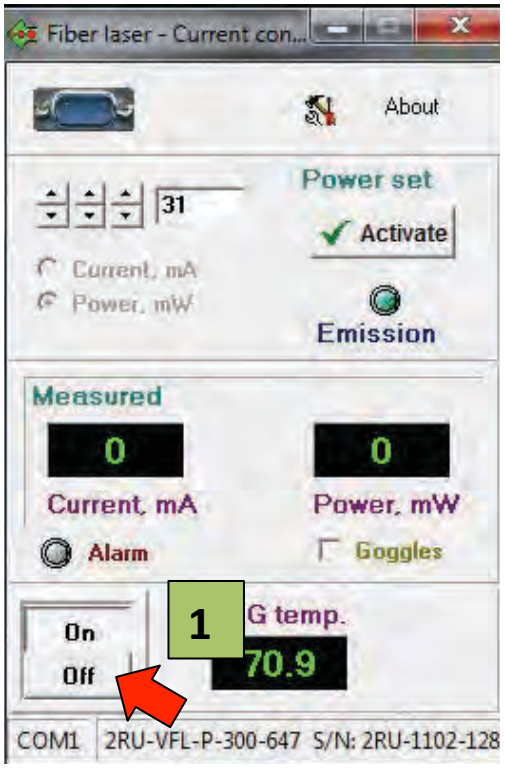
If you have used the 647 laser, follow the instructions bellow to switch it off, if not go to step #16

8- Turn off 647 laser from the software **1**



9- Turn off the key **2**

10- Turn off the switch **3**

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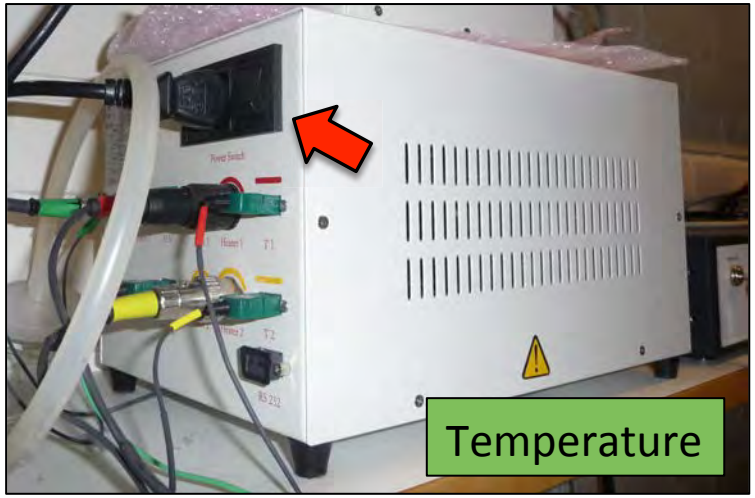
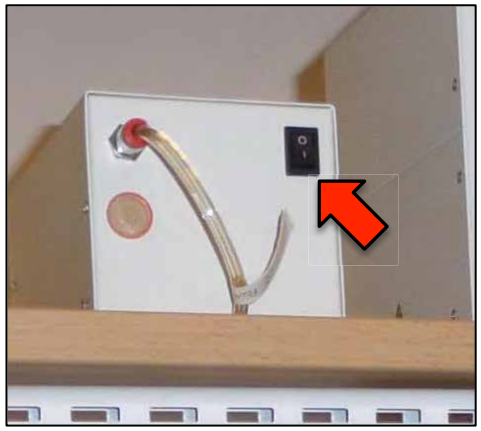
If you have used the incubation follow the steps 11 to 13, if not, go to step #16

11- Turn off the Temperature

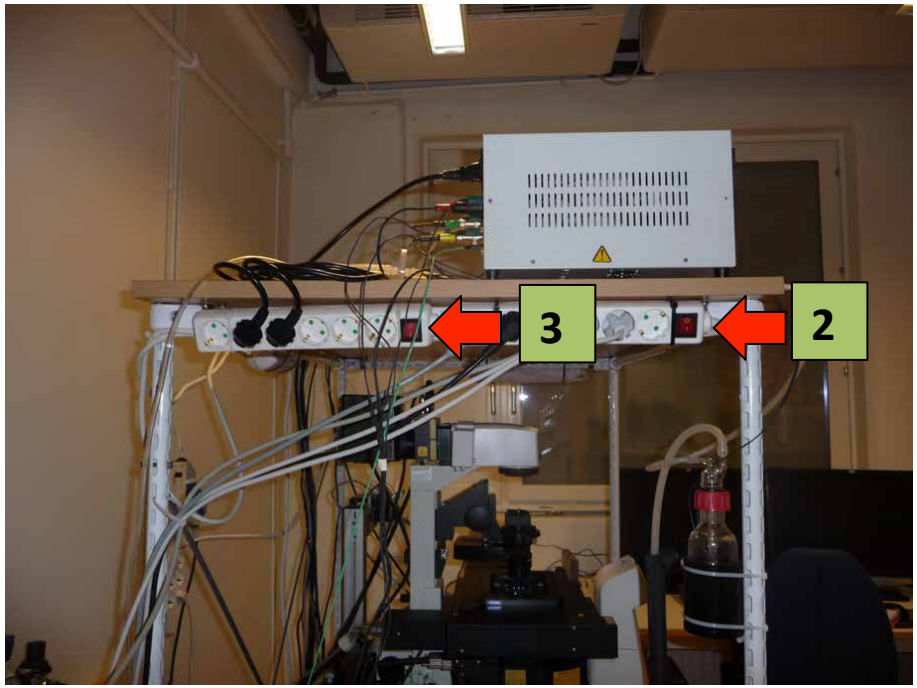


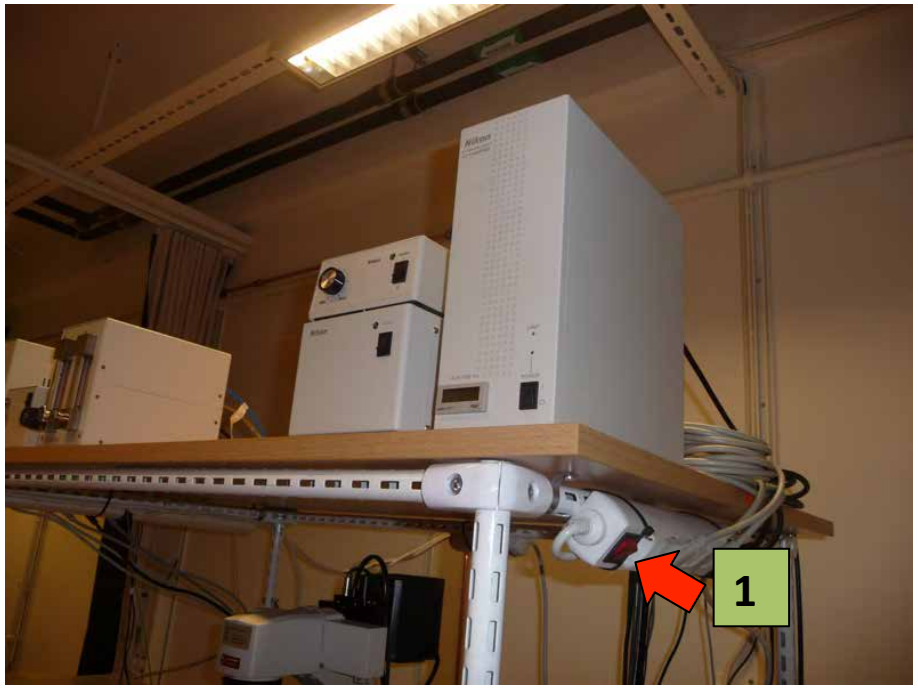
12- Turn off the Air pump



13- Close the CO2 main key in the wall

14- Turn off Switch #3, #2 and #1





15- Put the plastic cover to protect the microscope

16- Sign the logbook with all the information

If you have noticed any anomaly during the acquisition please contact Irene Martinez:

irene.martinez@umu.se

Cell phone: 072 220 6774 ONLY FOR URGENT MATTERS