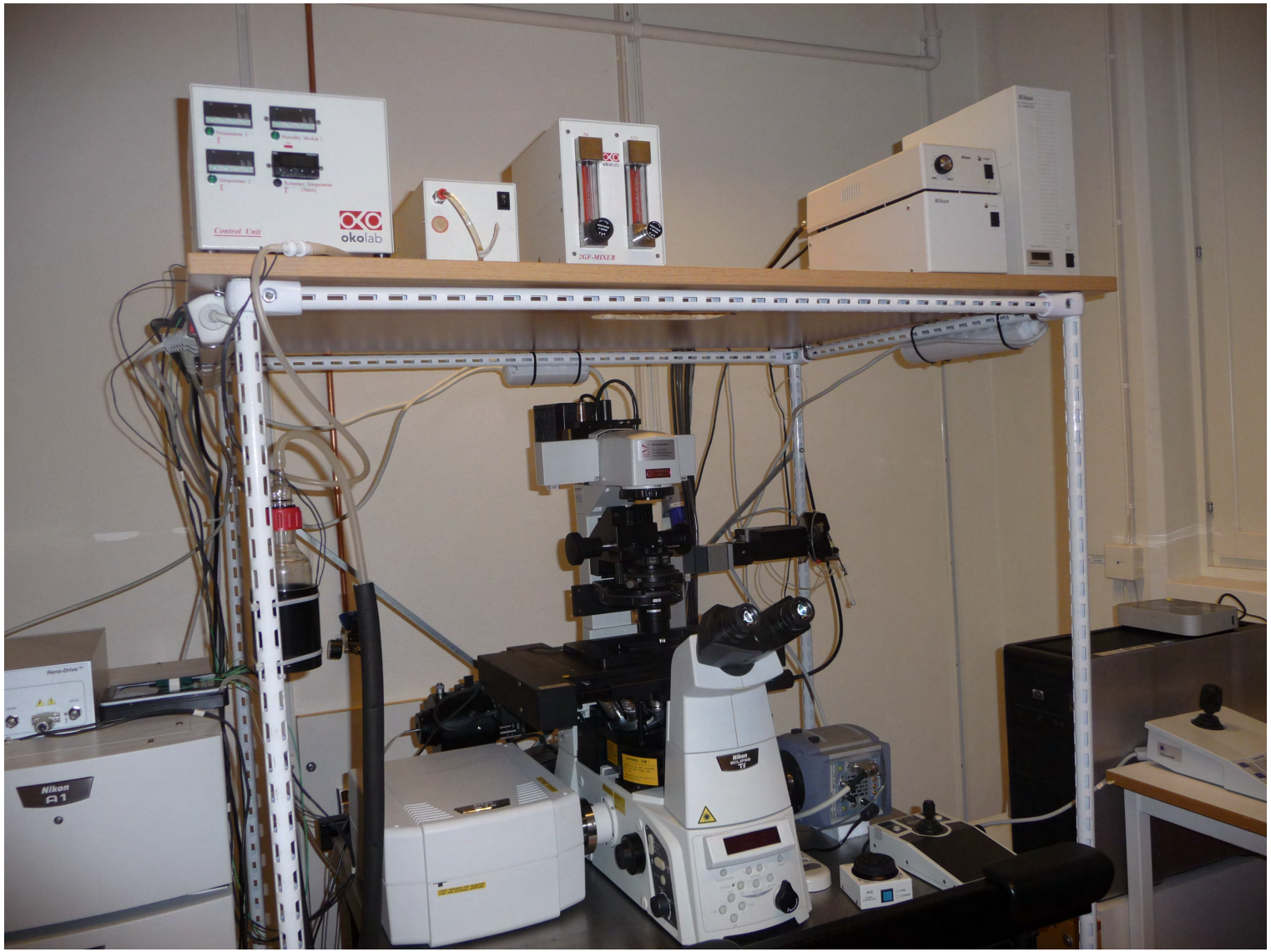
Nikon A1R. I Widefield Microscopy



Nikon A1R. I Widefield 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	50kr/hr. Driver License 1000kr

Use this phrase to mention the microscope in your publications: Nikon Eclipse Ti-E inverted microscope with an DU897 ANDOR EMCCD camera controlled by Nikon NIS Elements interface, 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

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)

Switch on the system

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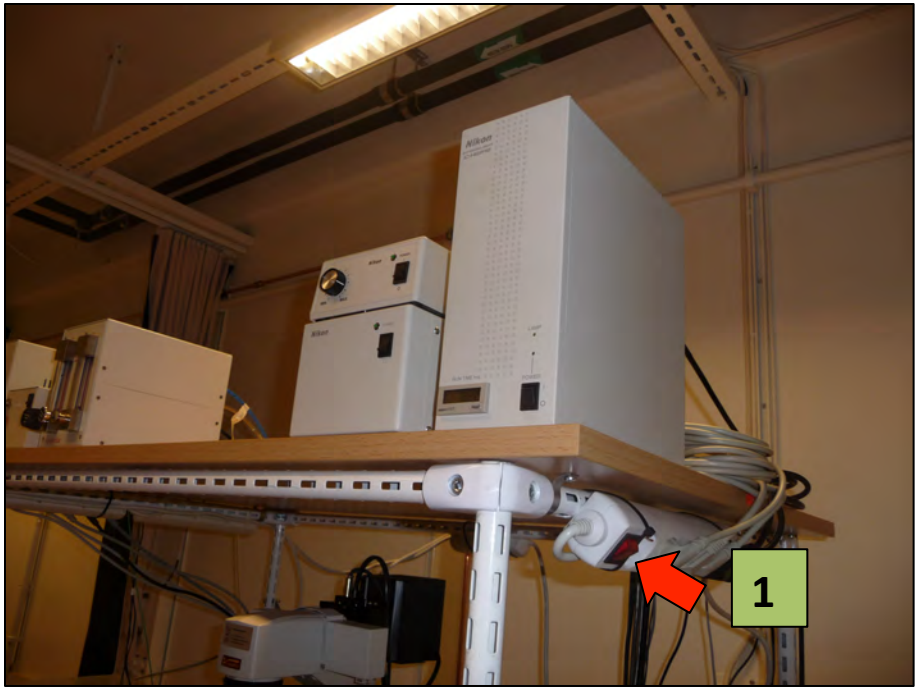
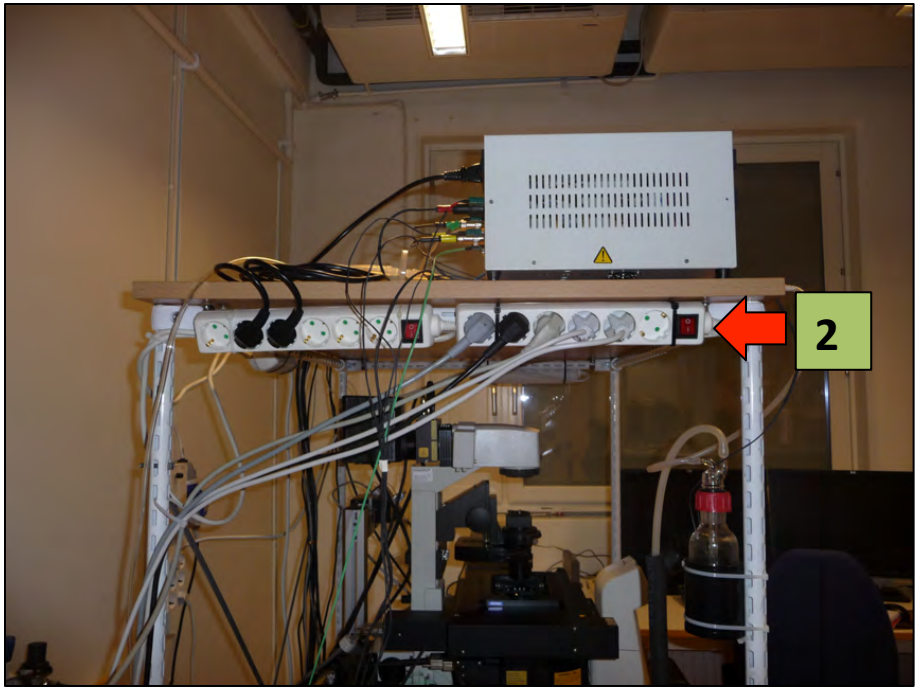
1- Turn on Switch #1



2- Turn on Switch #2



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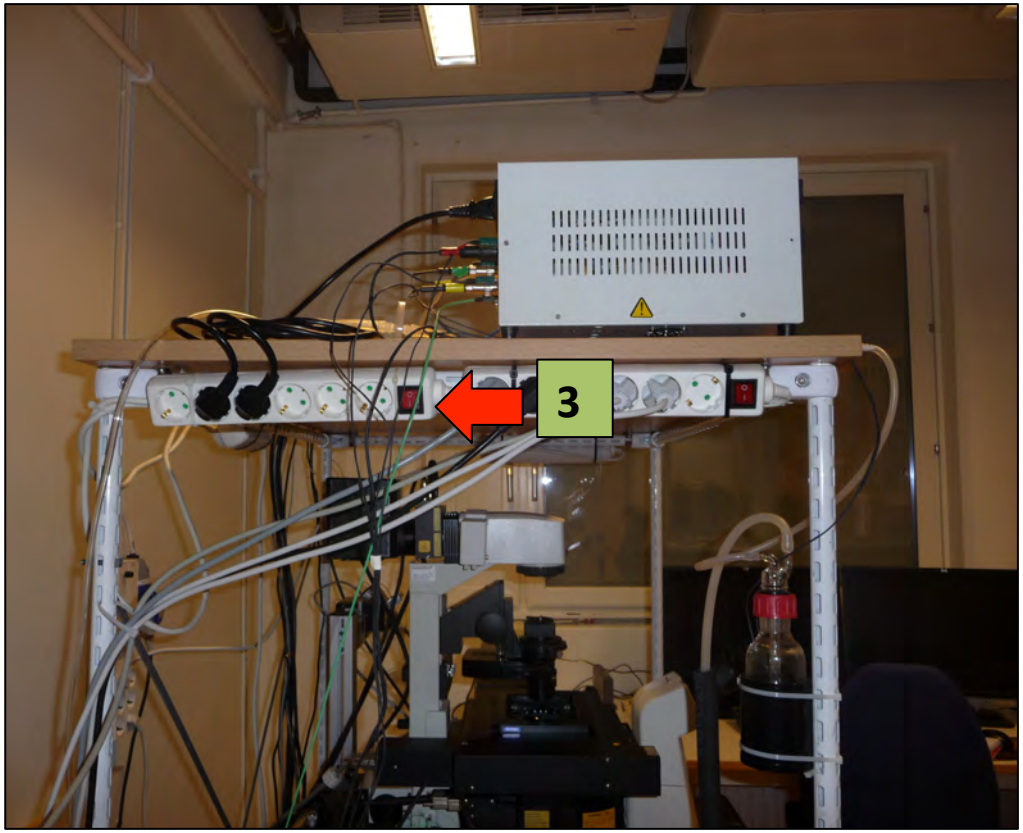
If you want to have temperature and CO2 follow step #2 if you don't need the incubation system go to step #12

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

3- Switch on switch #3



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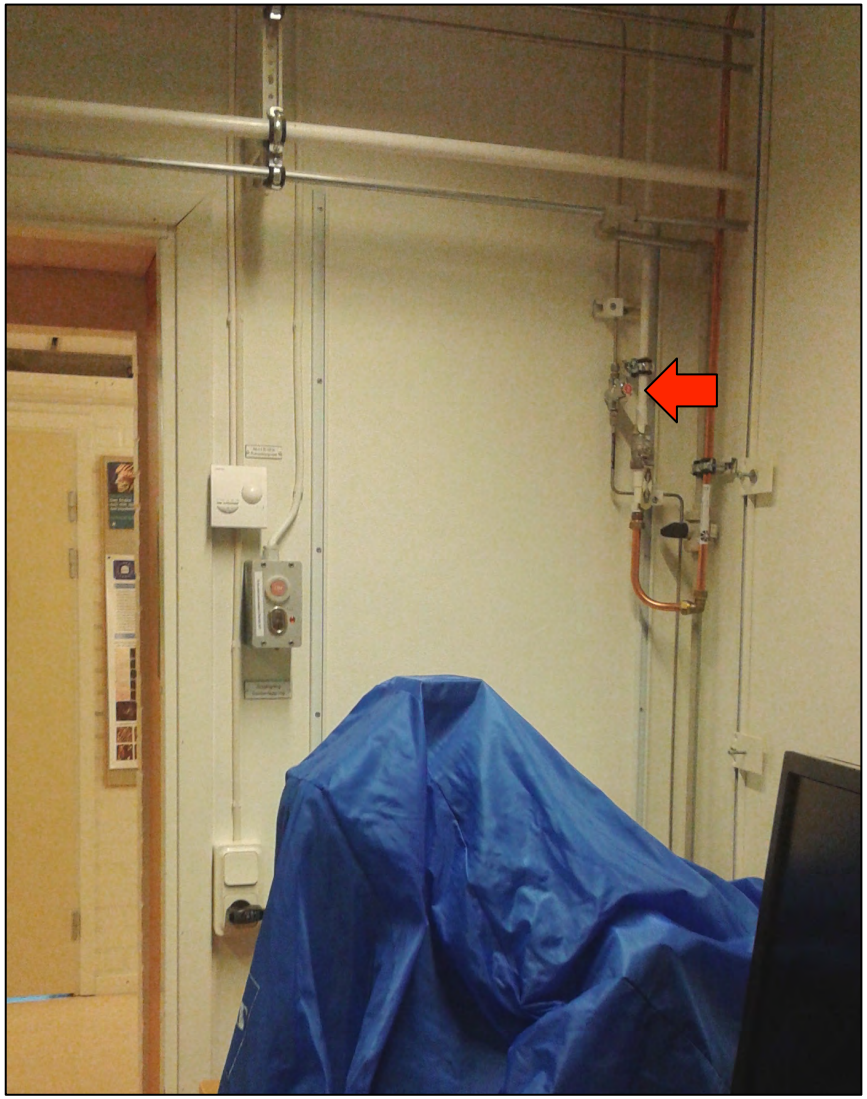
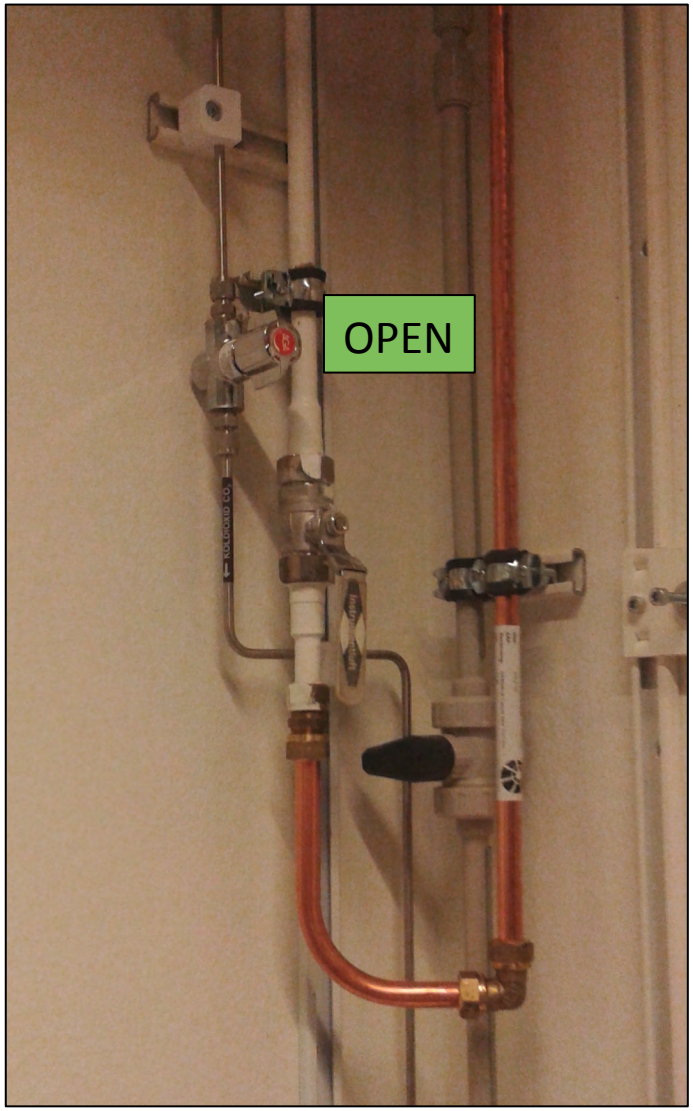
4- Turn on CO2 key located in the wall close to the room door.





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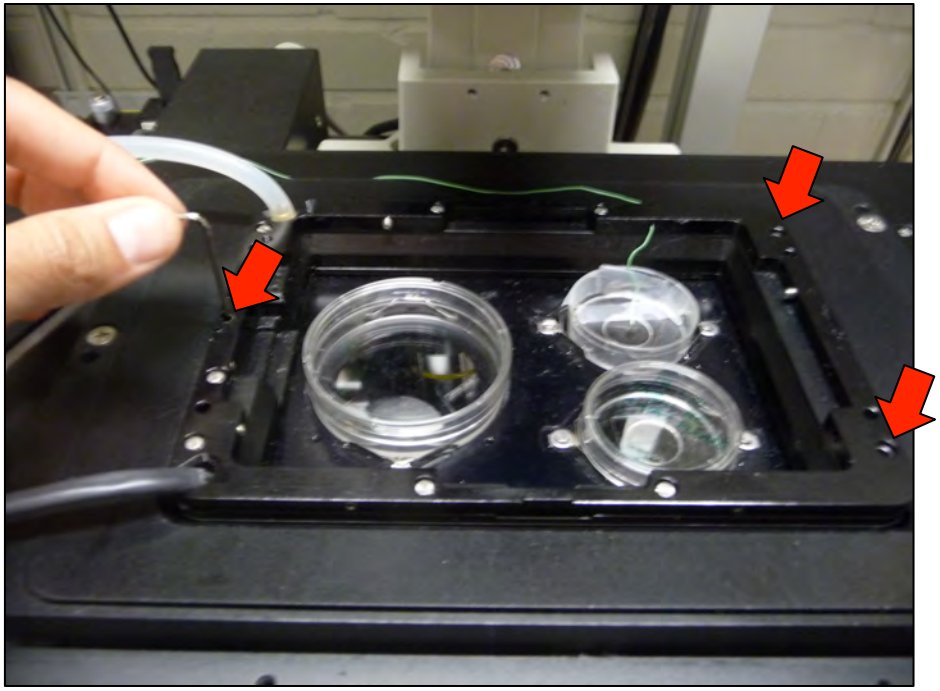
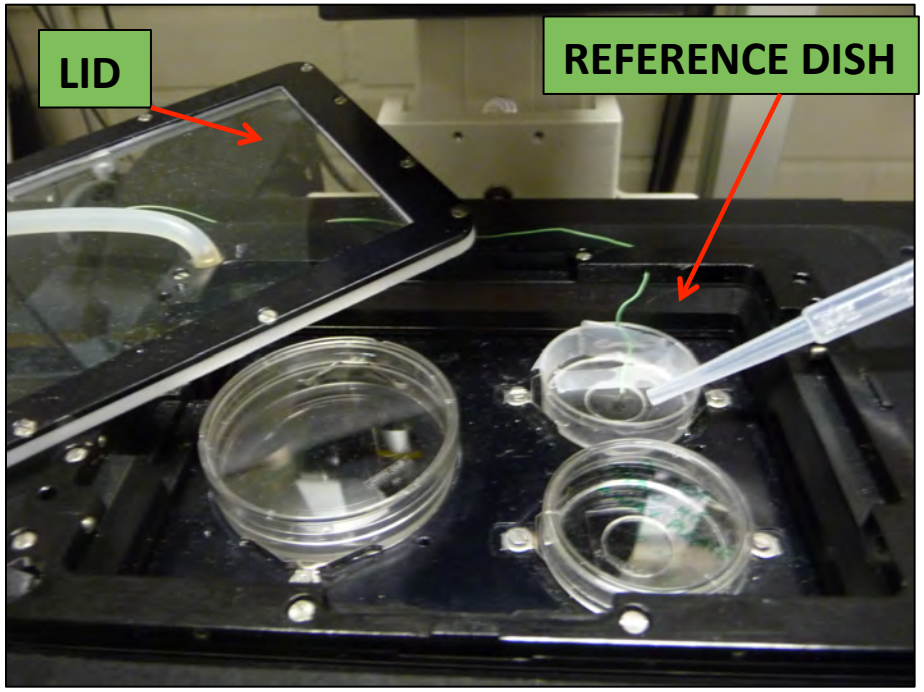
6- Screw the chamber. Make sure you are in the right positions



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

8- Put the chamber lid



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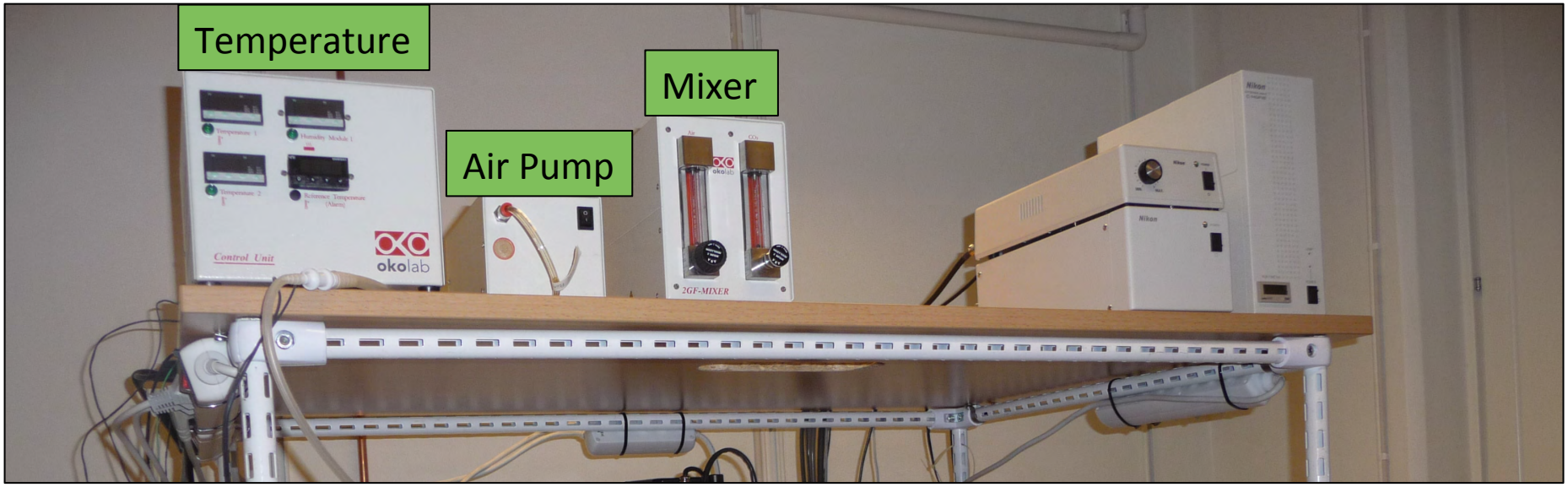
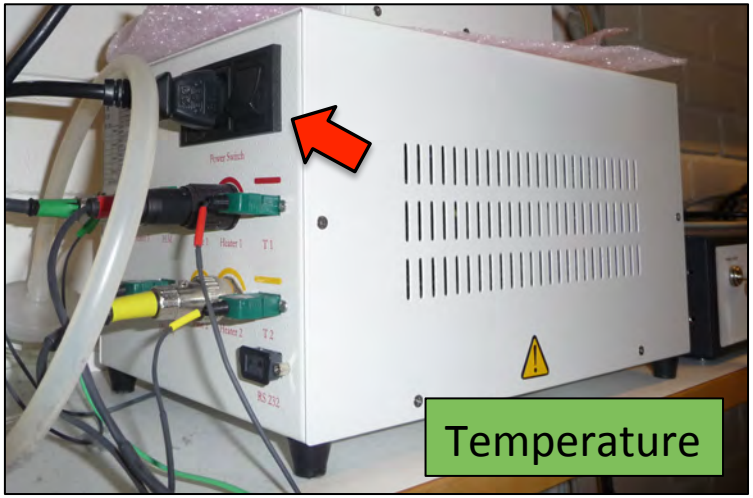
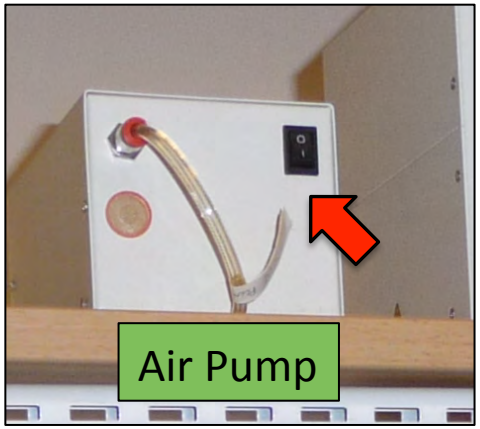


9- Switch on the temperature from the back



10- Switch on the Air Pump



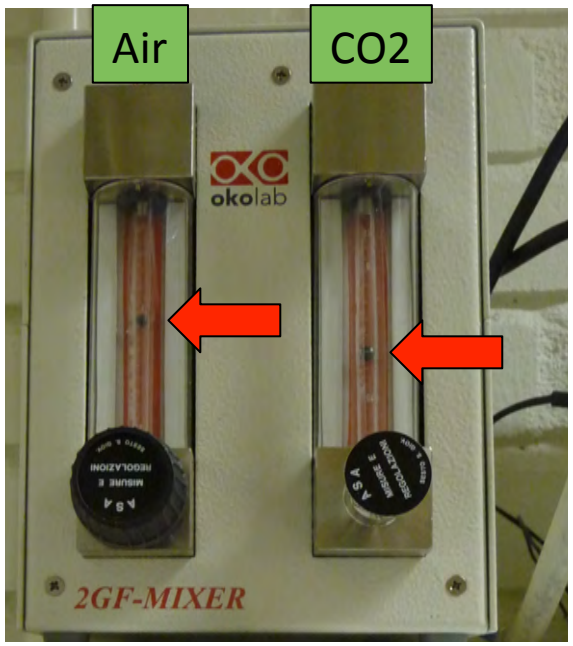
11- Make sure the mix is correct and stable:

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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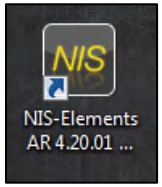
12- Switch on the Epi-Fluorescence Lamp

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

14- Double click Nis-Elements AR 4.20.01



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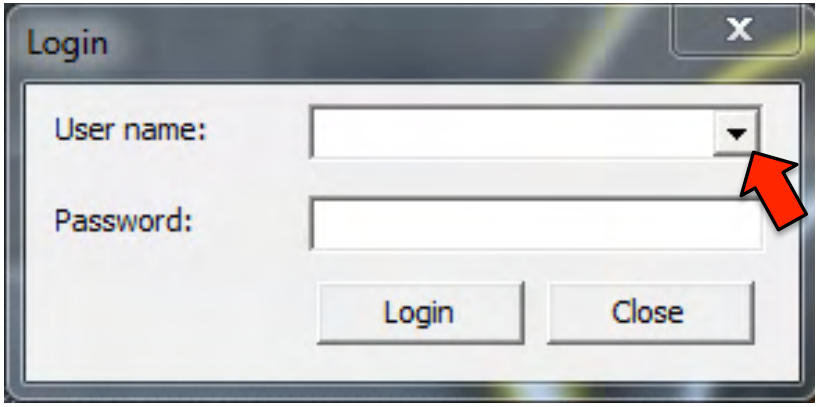
15- Login with your Name from the list and type your Password



16- Select ANDOR camera

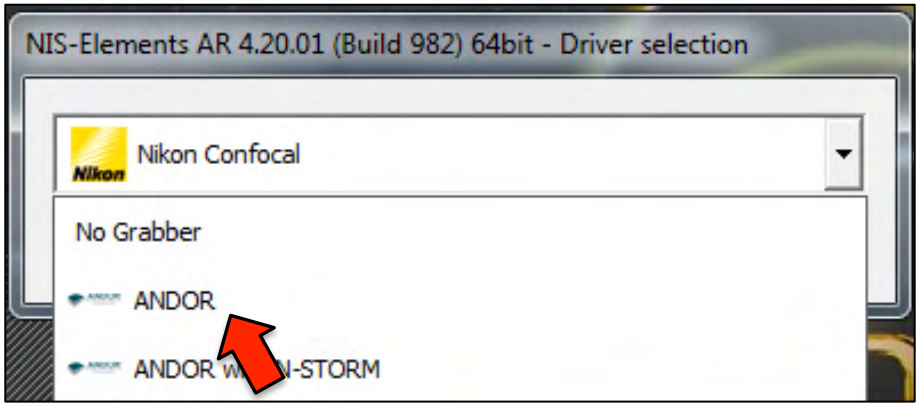


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Image Acquisition

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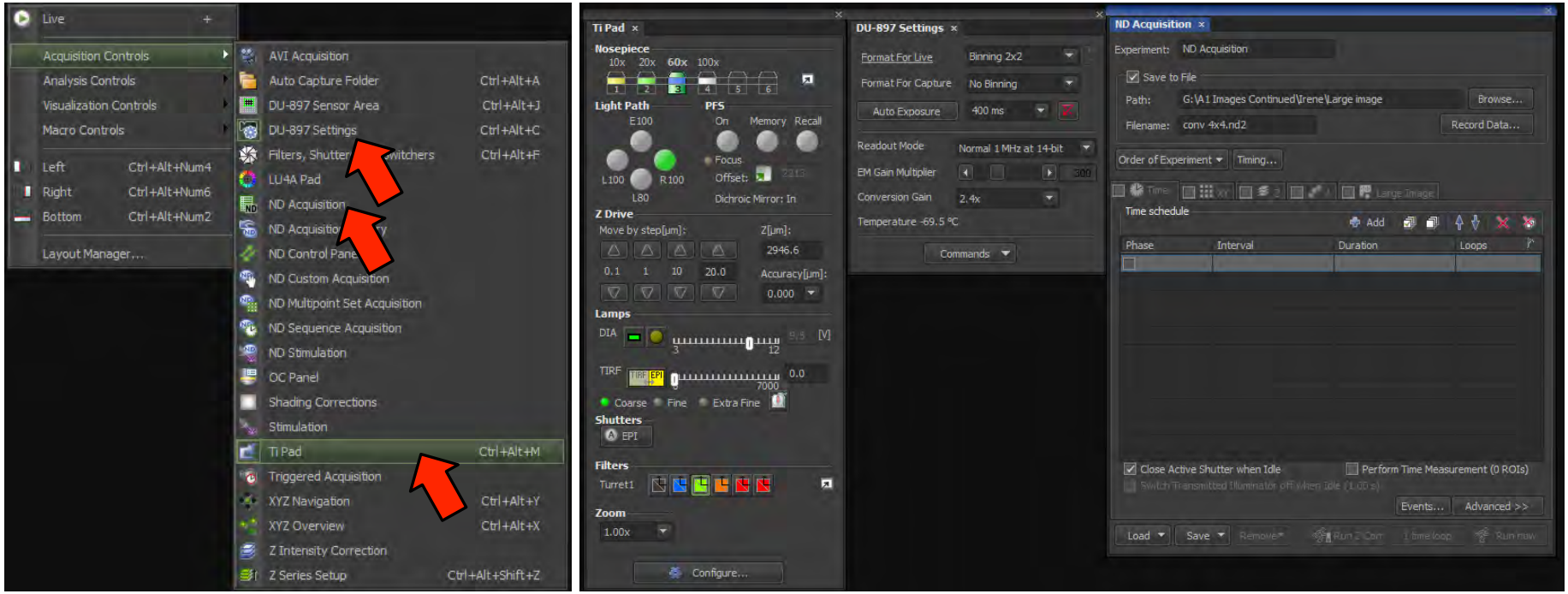
Main menu:

1- Right click and select the following panels:Ti Pad, DU897 Settings, ND acquisition



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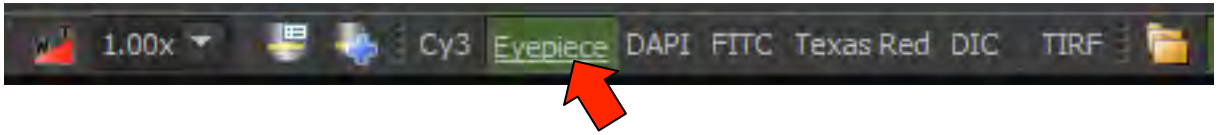
2- Select the objective with the TiPad menu or with the microscope botton on the left side

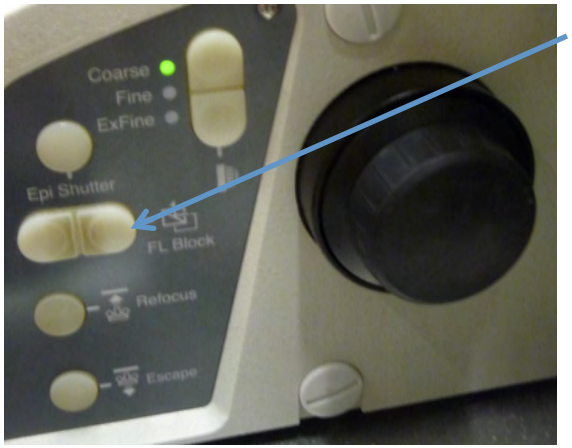
3- Add oil to the objective if needed (60X oil 100X oil)

4- Place the specimen:

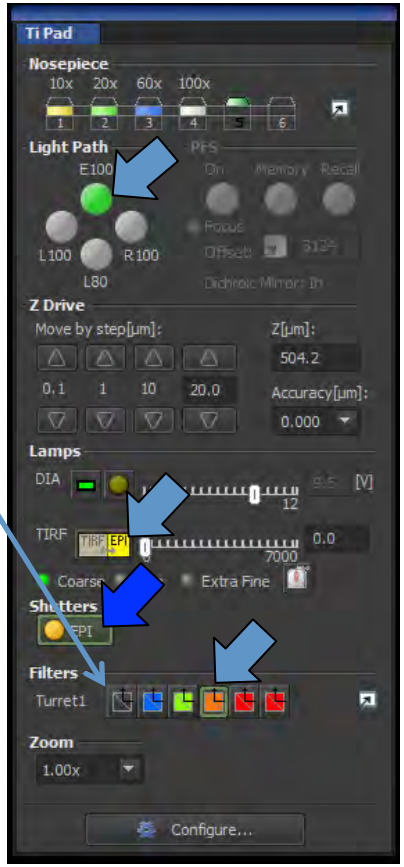
5- Press Eyepiece from the Optical Configuration menu



The last settings will be loaded automatically (blue arrows). In order to change filters (to visualize different wavelengths), use again the microscope or the TiPad menu. Press shutter if is not open



Filter change



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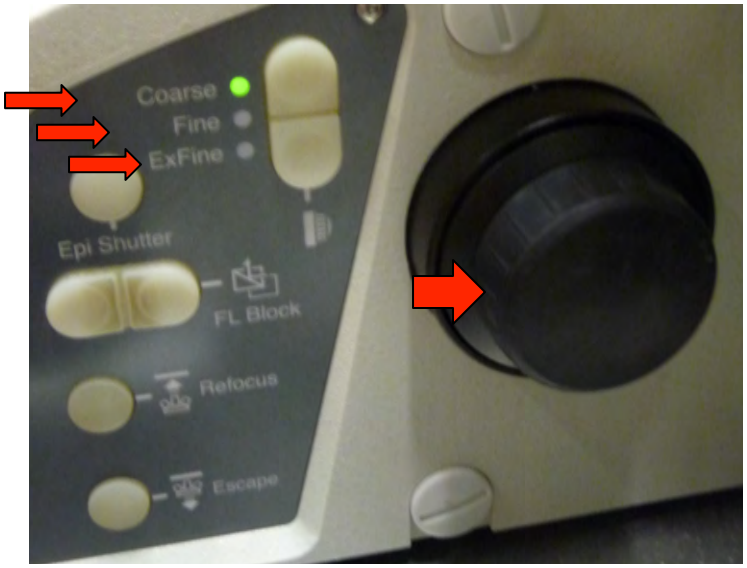
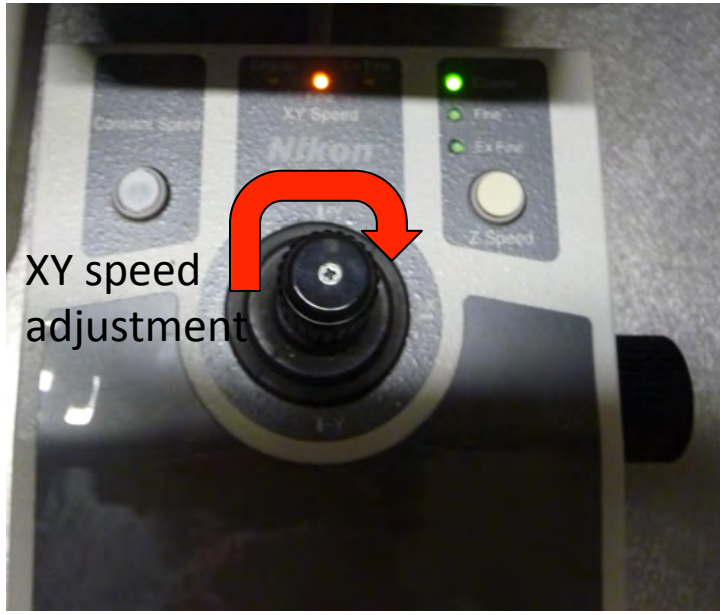
6- Focus the specimen.

movement accuracy adjustment



Move the stage with the joystick



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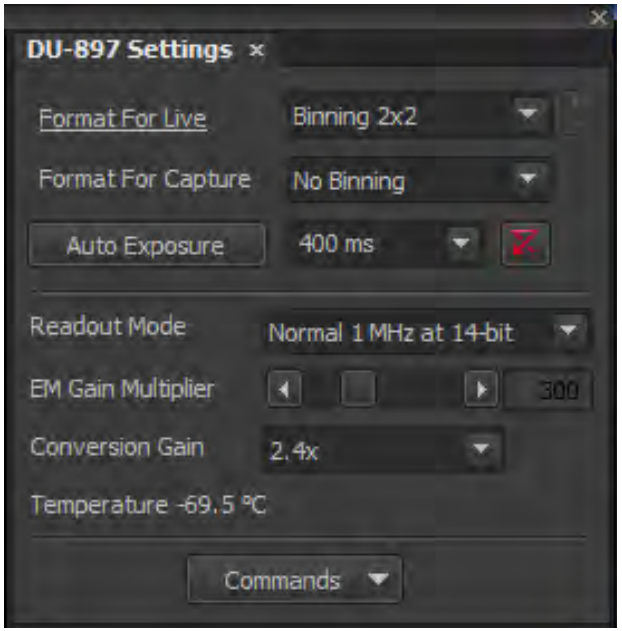
7- Press the OC

8- Press Live from the main menu 

9- Adjust Camera Settings:
Readout Mode: Readout Speed from 1MHz up to 10MHz
EM Gain Multiplier:
Conversion Gain:



Bining: Binning allows charges from adjacent pixels to be combined. You will get faster readout speeds and improved signal to noise ratios but reduces spatial resolution.

10- Make a picture: Press camera  from the main menu. Save the image as nd2 file.

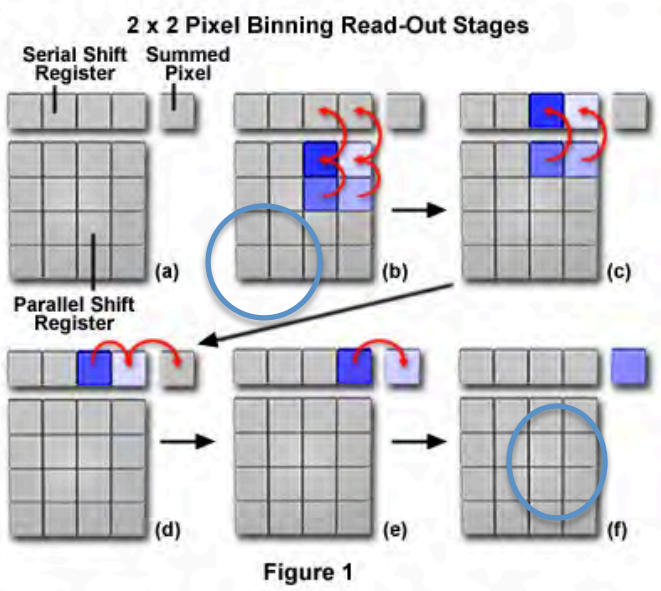


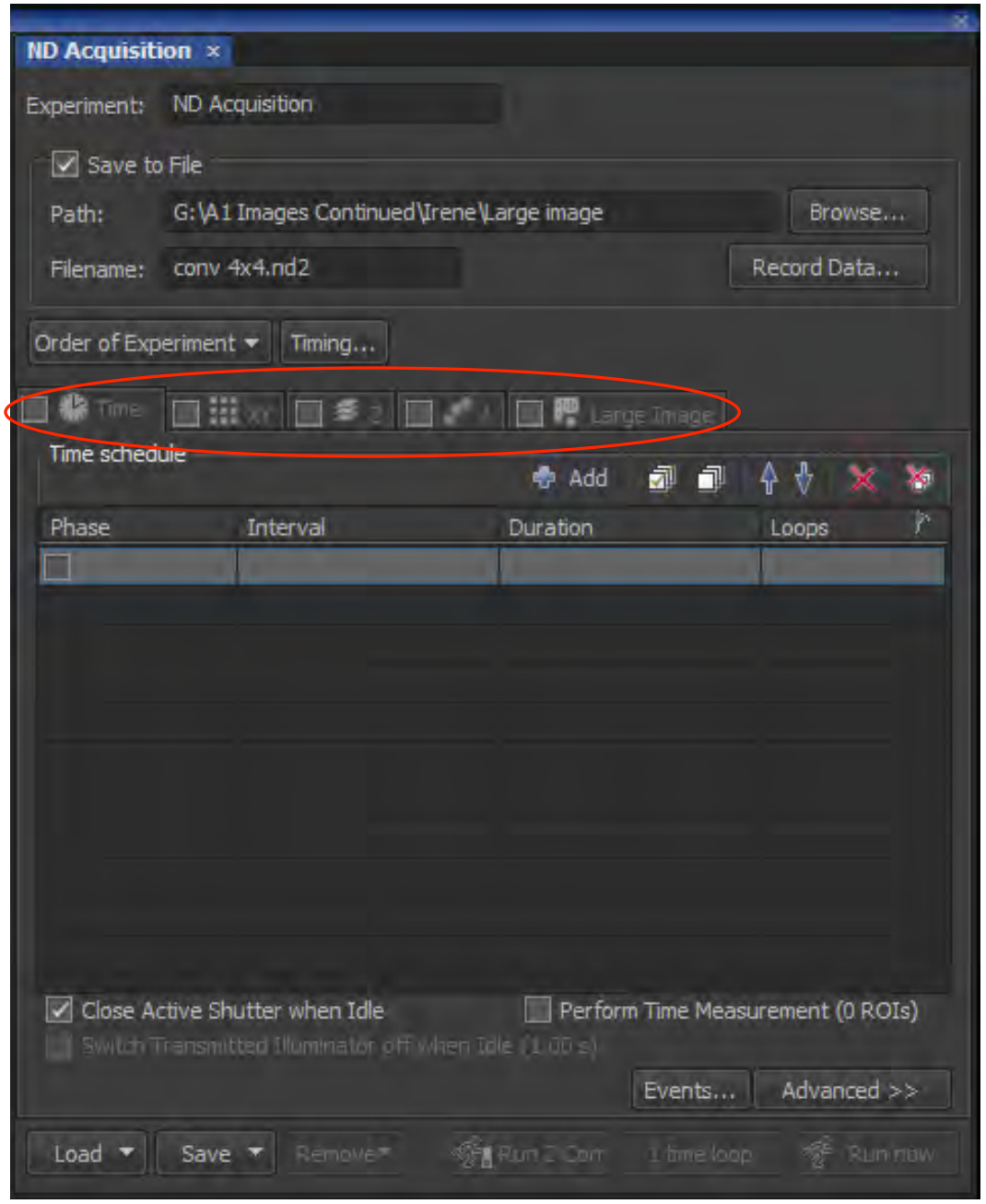
Figure 1

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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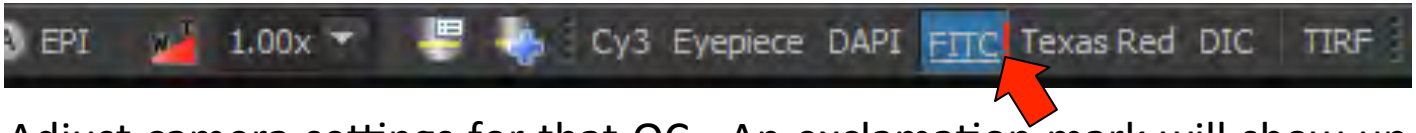
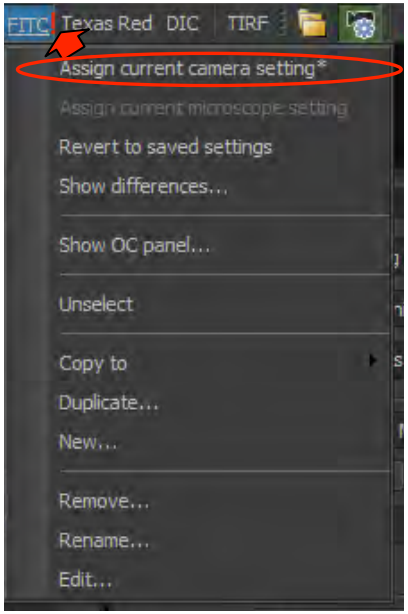
How to build an experiment with different OC. Select the OC from the main menu:



Adjust camera settings for that OC . An exclamation mark will show up. To keep those settings: right click and select: “Assign current camera settings”



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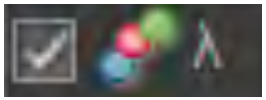
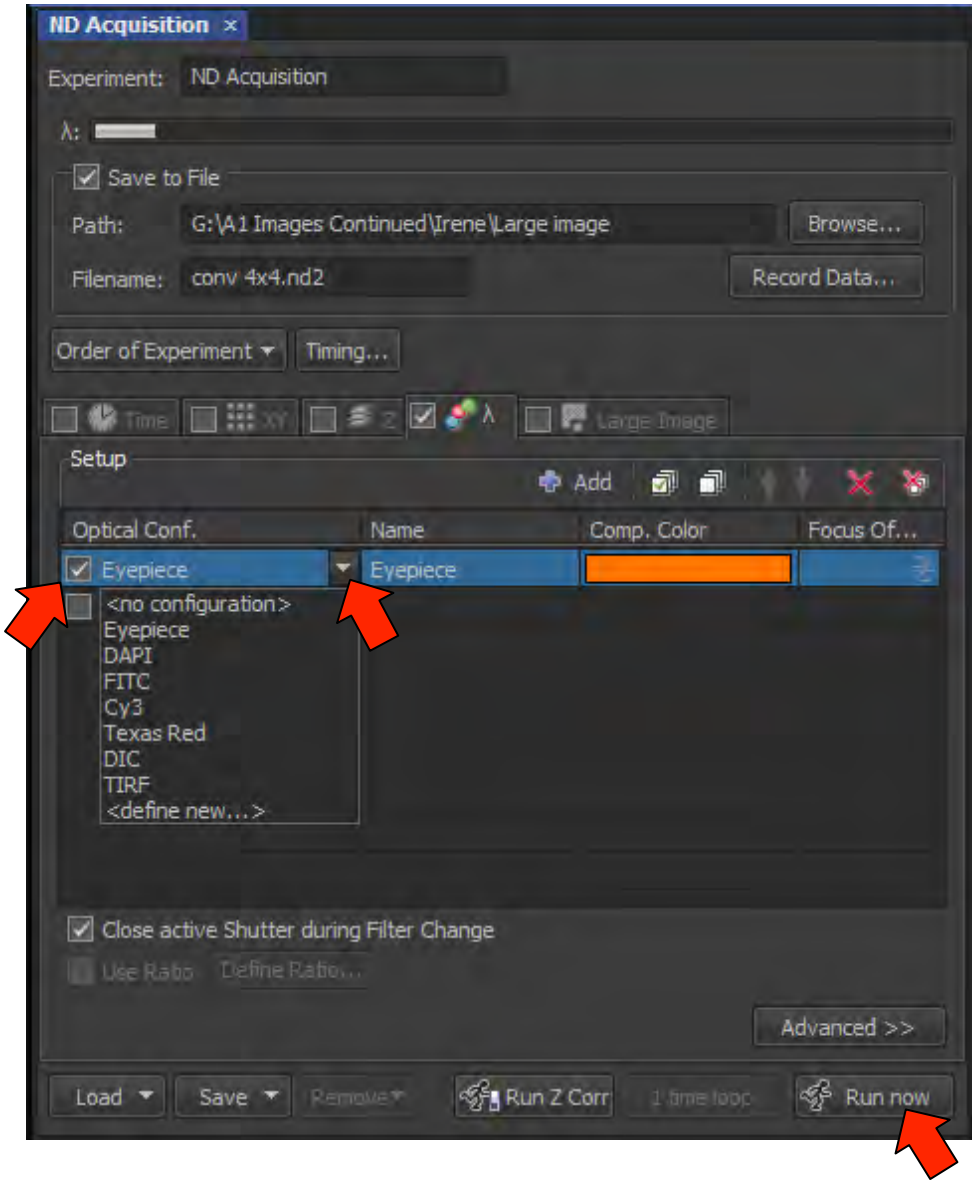
Go to ND Acquisition menu and select ☑ λ



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

To take an image you just have to press Run Now and the camera will acquire the OC selected and the files will be under the pathway you have previously selected.

In order to perform a complex experiment you can select the other parameters within the Set Up and add diferent positions to acquire .

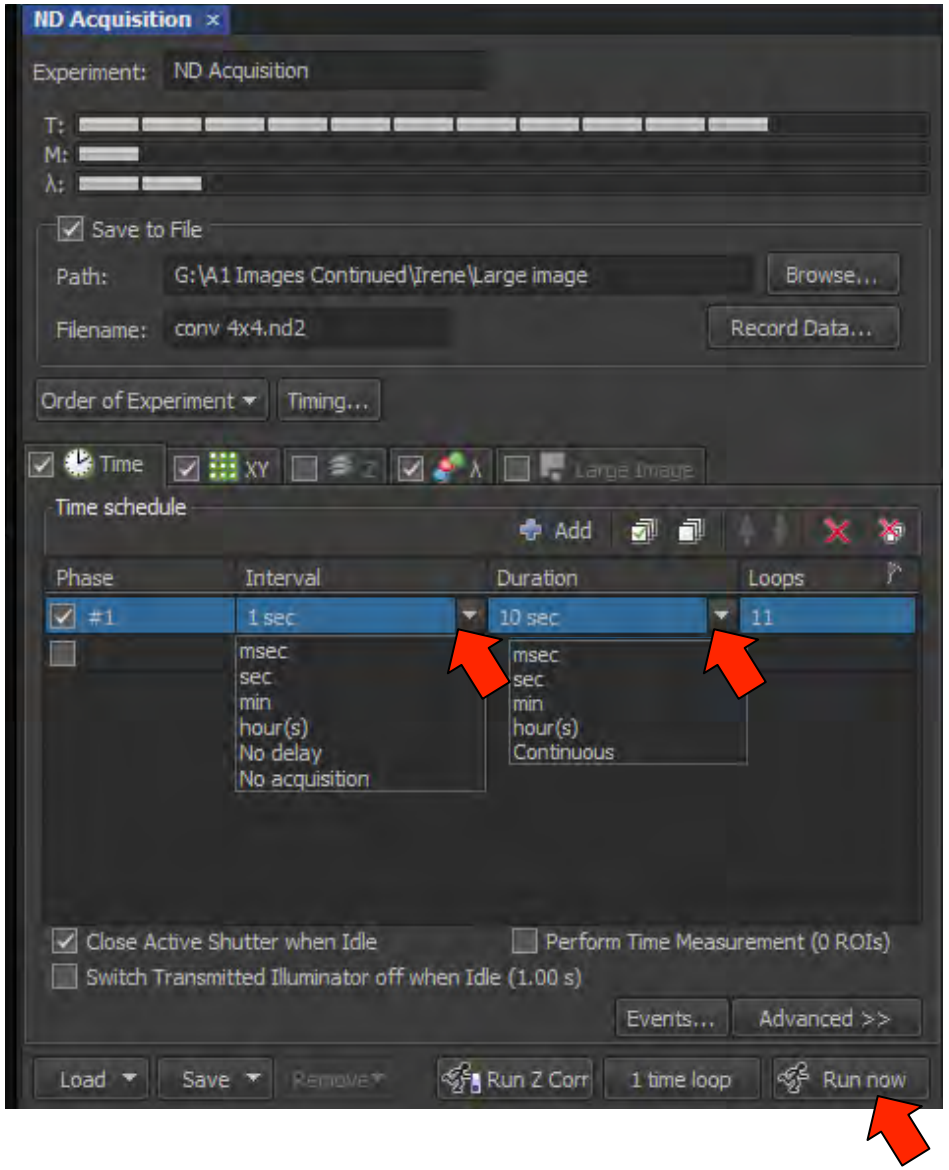


Time Lapse:

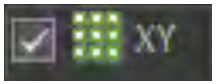
Go to ND Acquisition menu and select Time

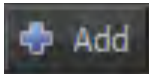
You can choose the Interval between acquisitions and the duration. The software will calculate the Loop number

Press Run Now to start the experiment



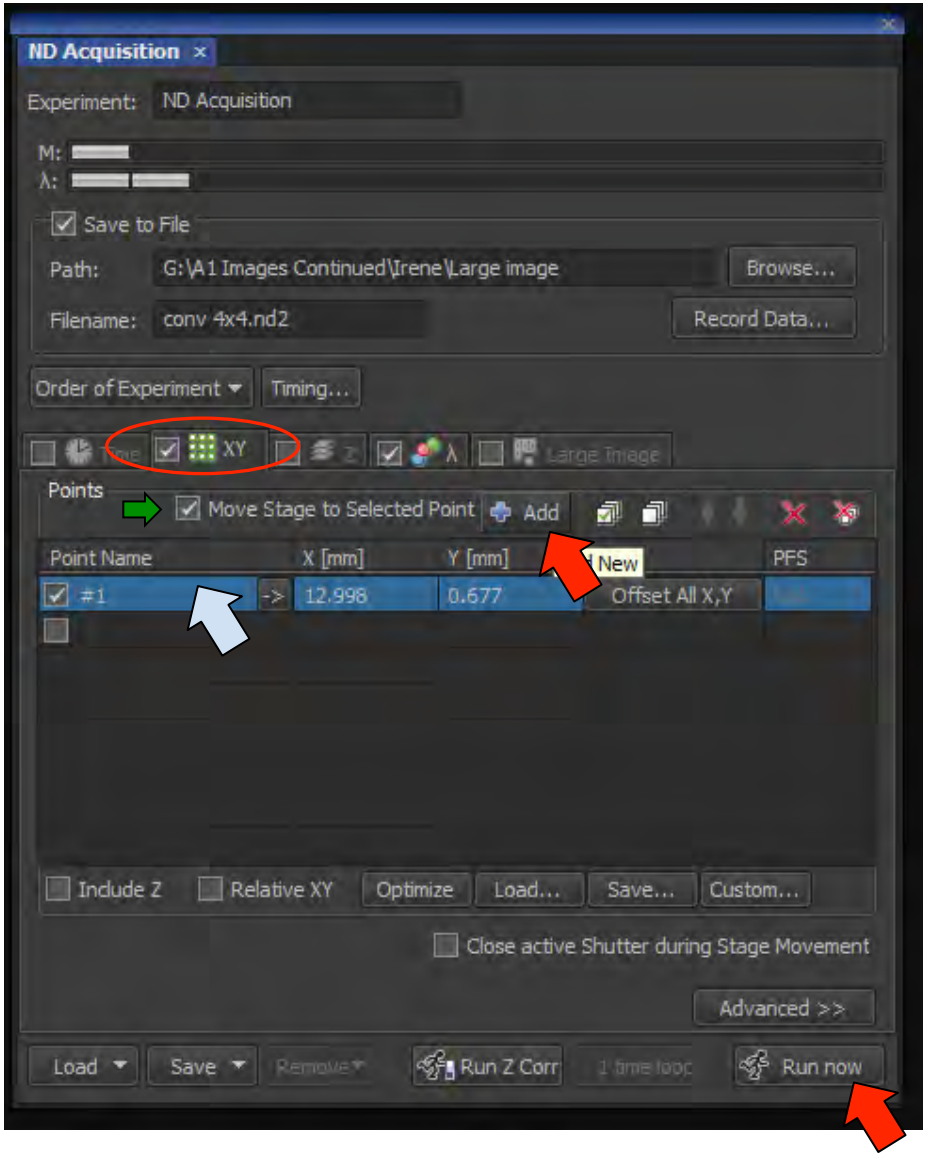
Positions:

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

Press Add 

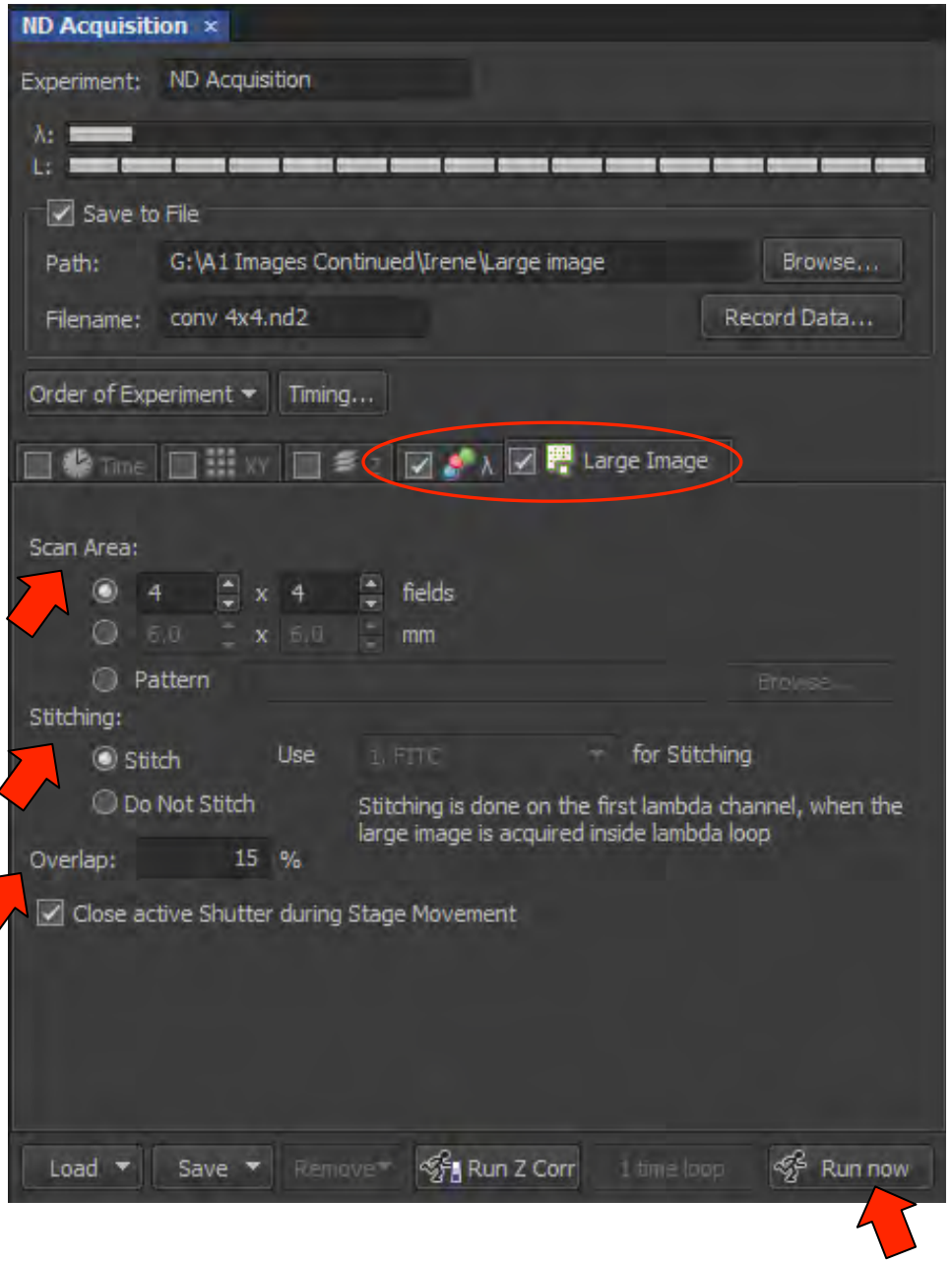
to add a new position. The coordinates will be kept, then you can move to another area an select a new position. You can travel within the positions selected by clicking with the mouse on each position (blue arrow). Make sure “Move Stage to selected Point” is selected ➡ ☑

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



Large Image:

Go to ND Acquisition menu and select ☑
Large Image. To use this option you have to select λ too.



Scan Area

Stiching

Overlap %

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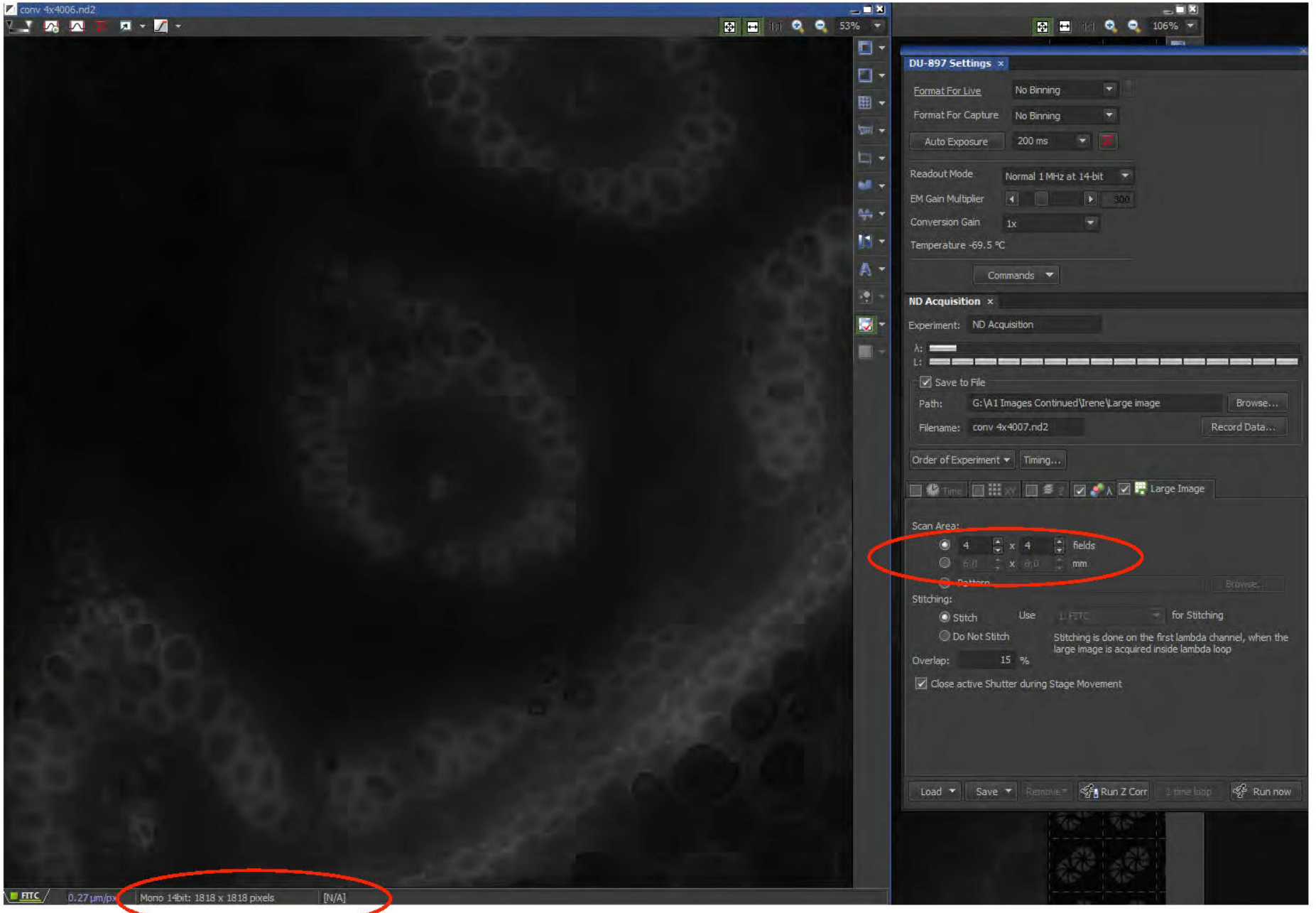

conv 4x4006.nd2
53%
106%
DU-897 Settings
Format For Live
No Binning
Format For Capture
No Binning
Auto Exposure
200 ms
Readout Mode
Normal 1 MHz at 14-bit
EM Gain Multiplier
300
Conversion Gain
1x
Temperature -69.5 °C
Commands
ND Acquisition
Experiment:
ND Acquisition
Save to File
Path:
G:\A1 Images Continued\Irene\Large image
Browse...
Filename:
conv 4x4007.nd2
Record Data...
Order of Experiment
Timing...
Large Image
Scan Area:
4
x
4
fields
mm
Stitching:
Stitch
Use
for Stitching
Do Not Stitch
Stitching is done on the first lambda channel, when the large image is acquired inside lambda loop
Overlap:
15
%
Close active Shutter during Stage Movement
Load
Save
Run Z Corr
Run now
FITC
0.27 µm/px
Mono 14bit: 1818 x 1818 pixels
[N/A]

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

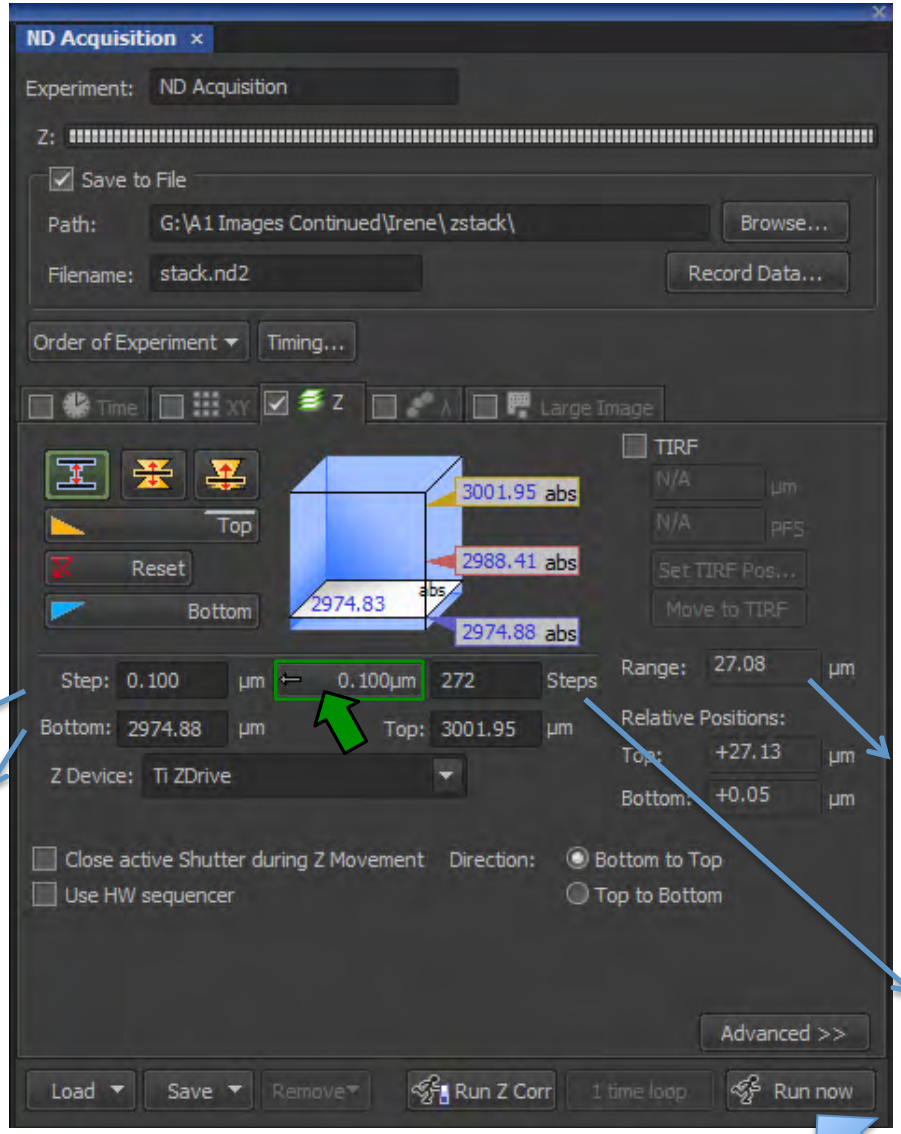
Pathway to save and name the file

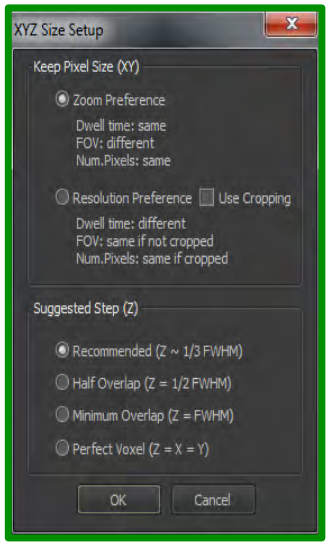
Zstack Top position. Objective is close to the slide

Zstack Botton position. Objective is close to the slide

Step thickness

Bottom and Top position





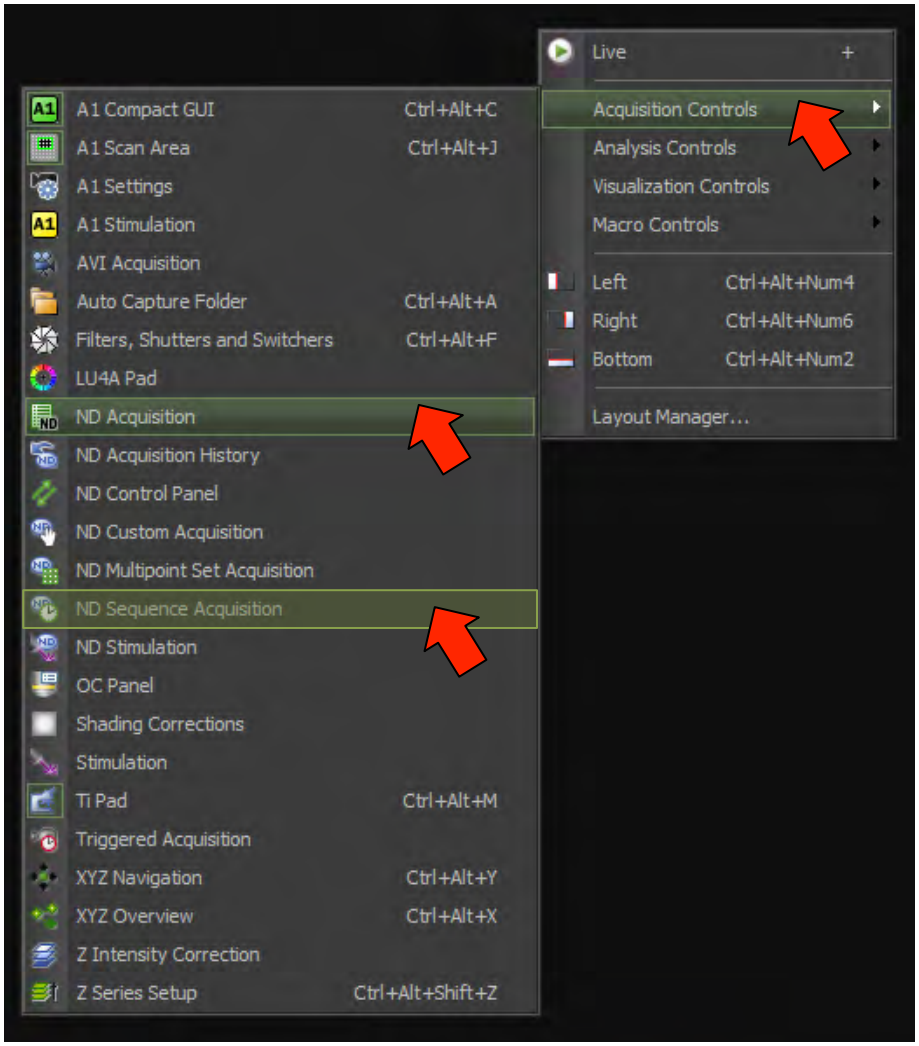
Suggested step size based on XYZ Size Set up

Range between Top and Bottom

Step numbers

Press Run Now to start acquisition

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



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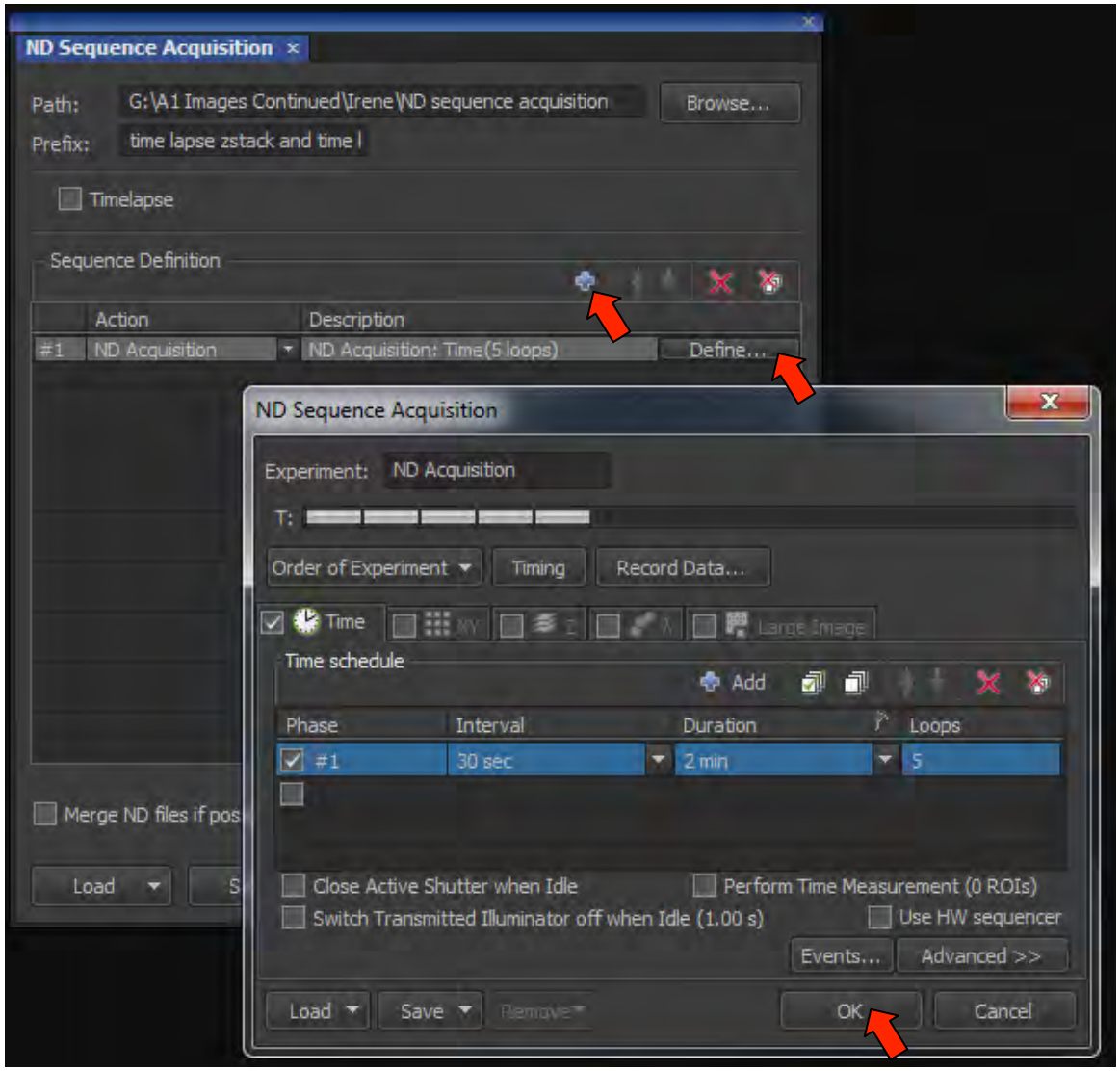
ND Sequence Acquisition: Time Lapse, Zstack and Time Lapse

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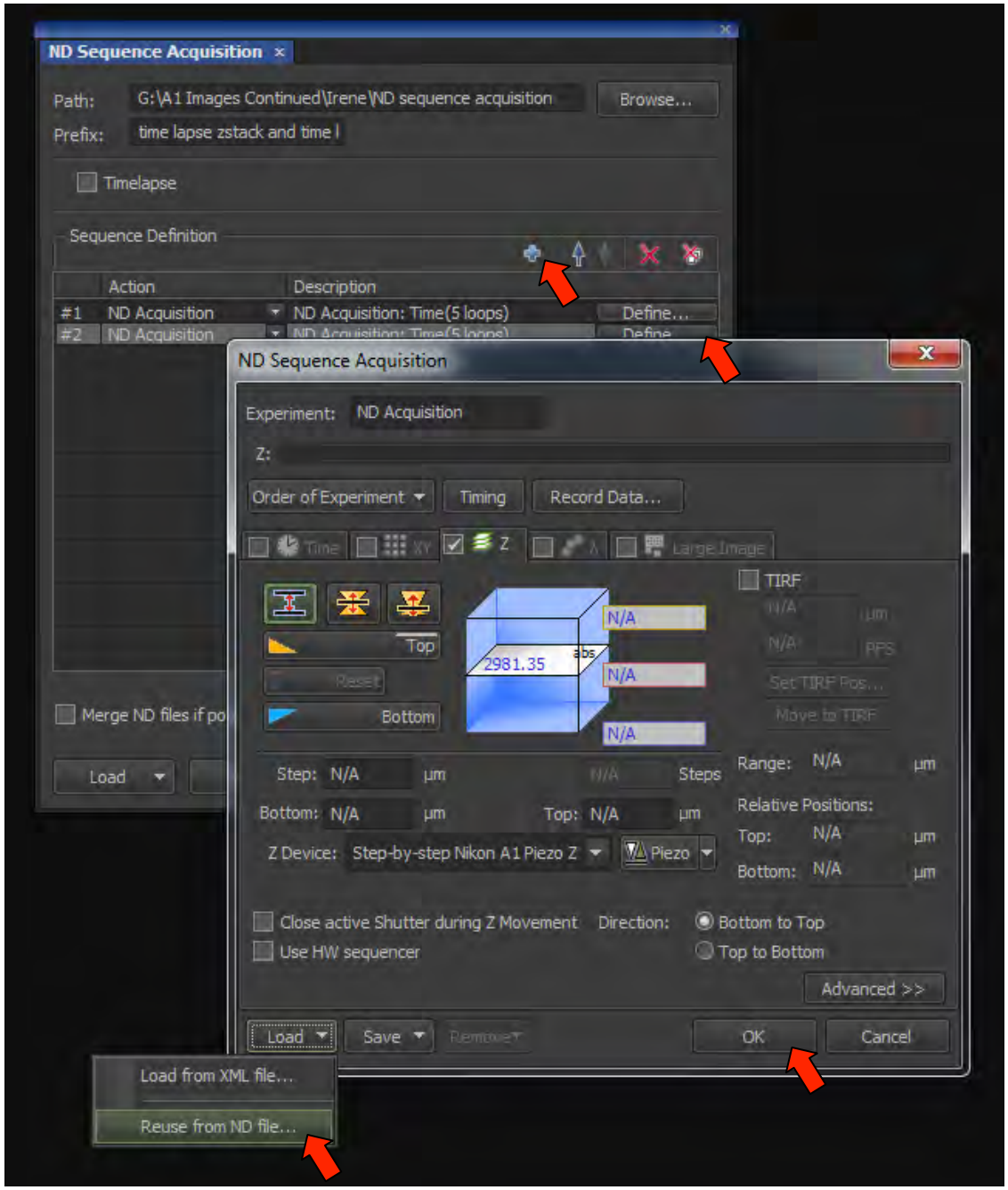
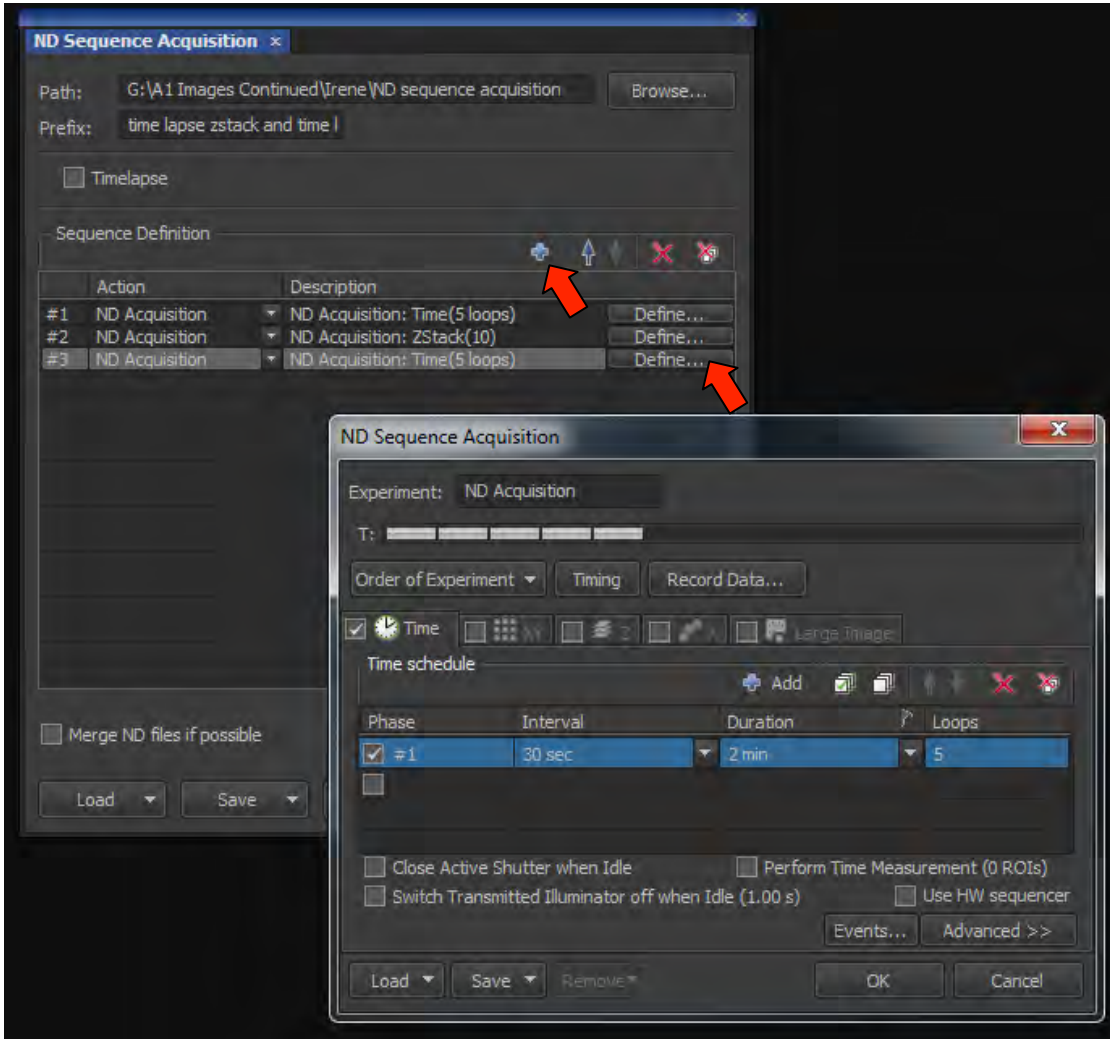
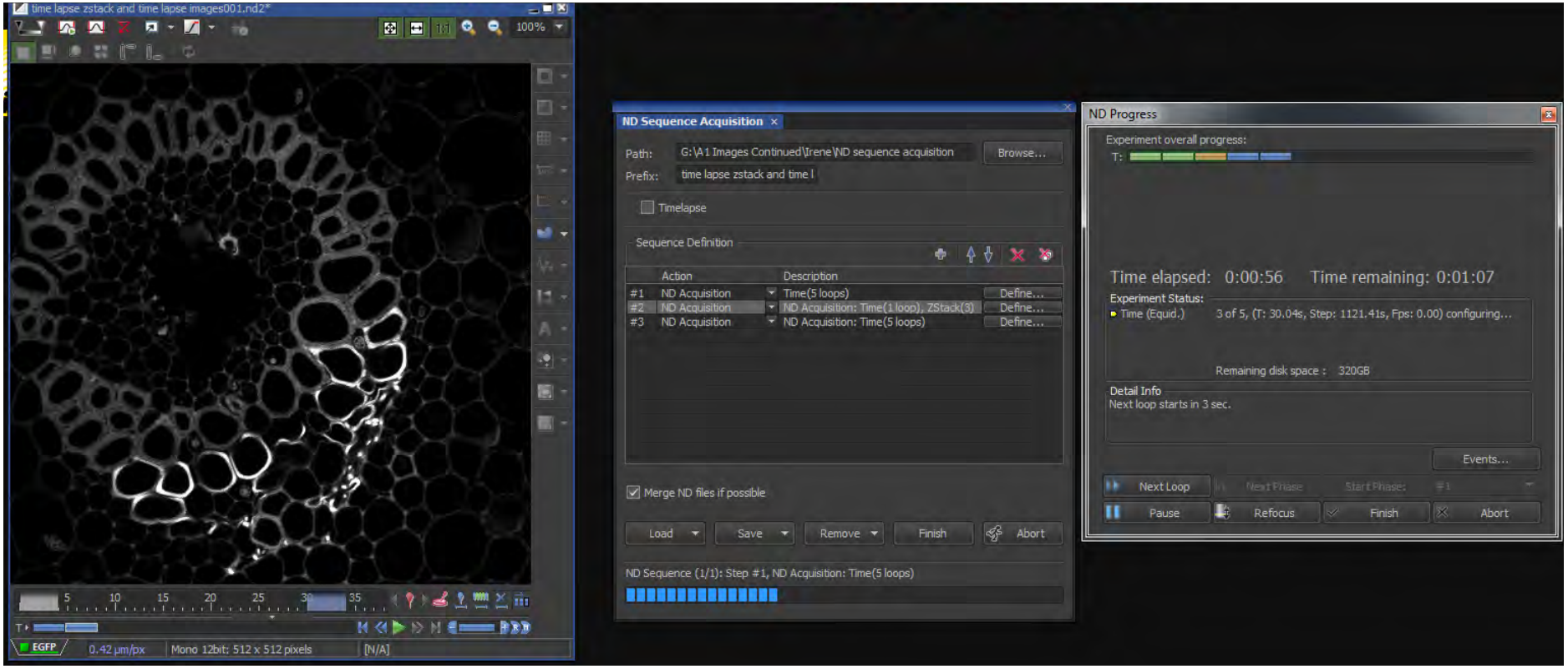
In this case the second Action is a z-stack. We have to load settings from a previous ND file, press ok and then move to the next Action



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



On going experiment…



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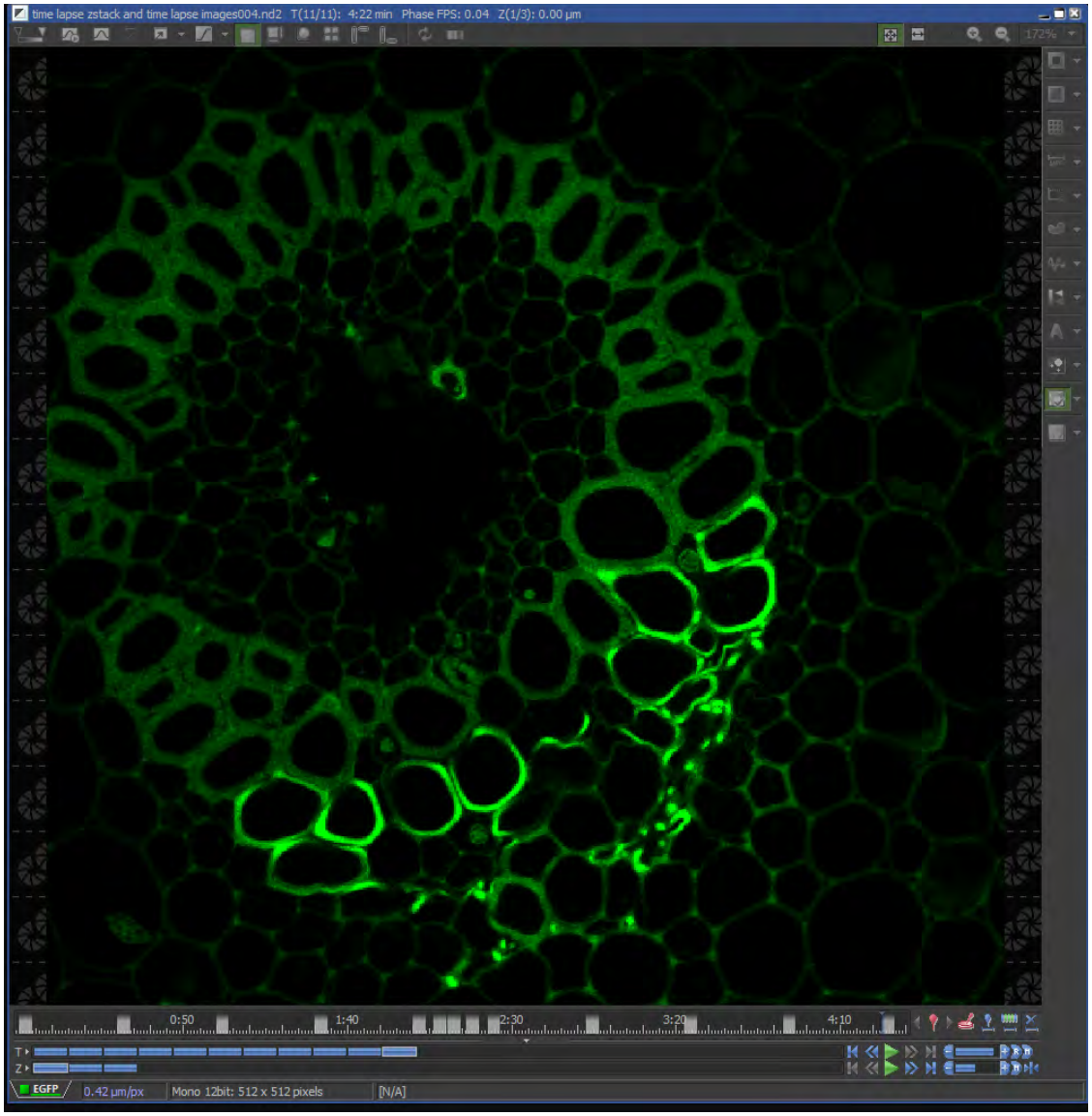
This is the final image with the nd files merged



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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:

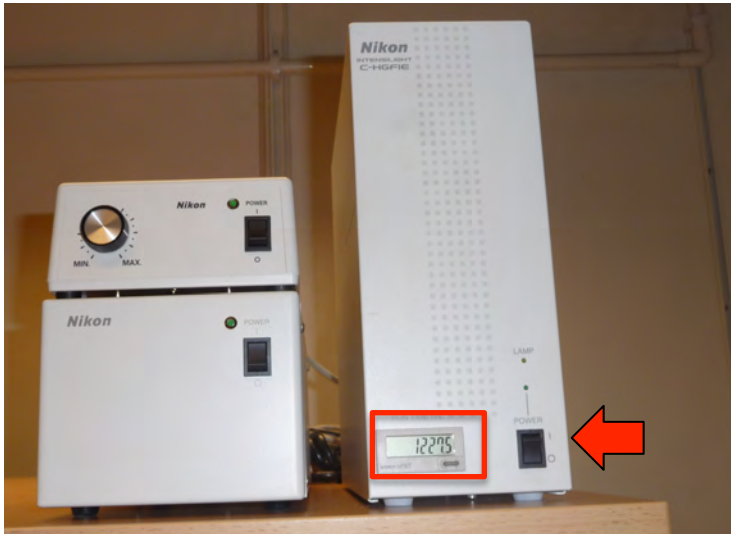
3- Close Nis Elements

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 Lamp and check the running time.
Write down that info in the logbook.



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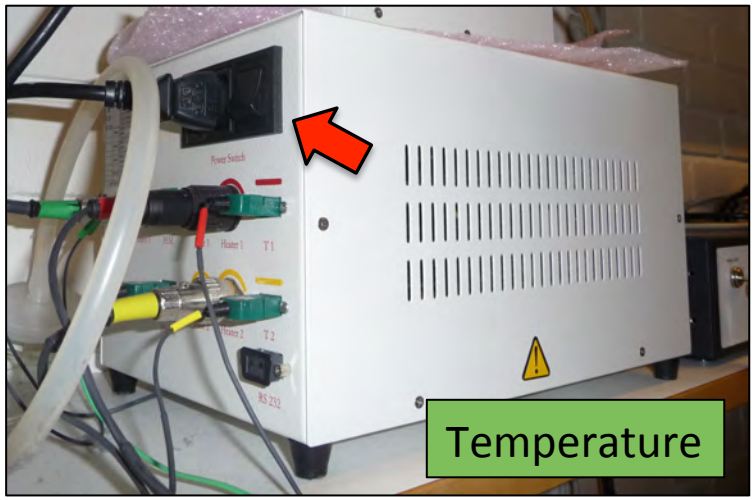
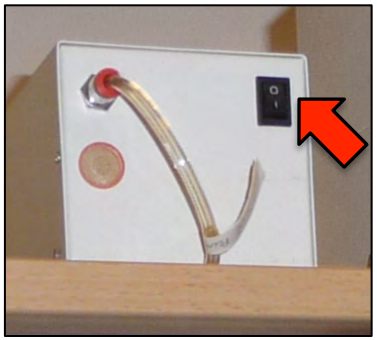
If you have used the incubation follow the steps bellow, if not, go to step #11

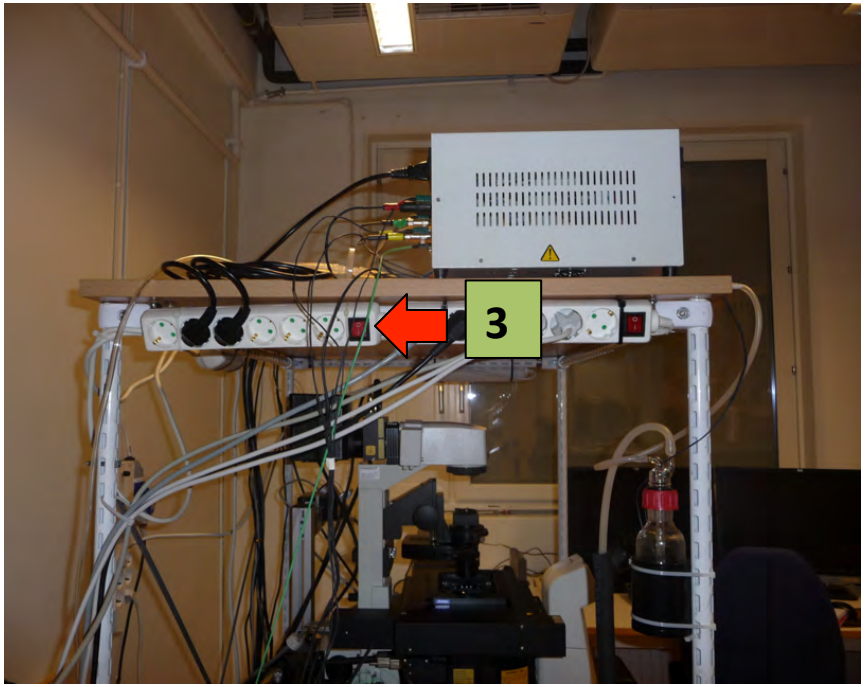
7- Turn off the Temperature



8- Turn off the Air pump

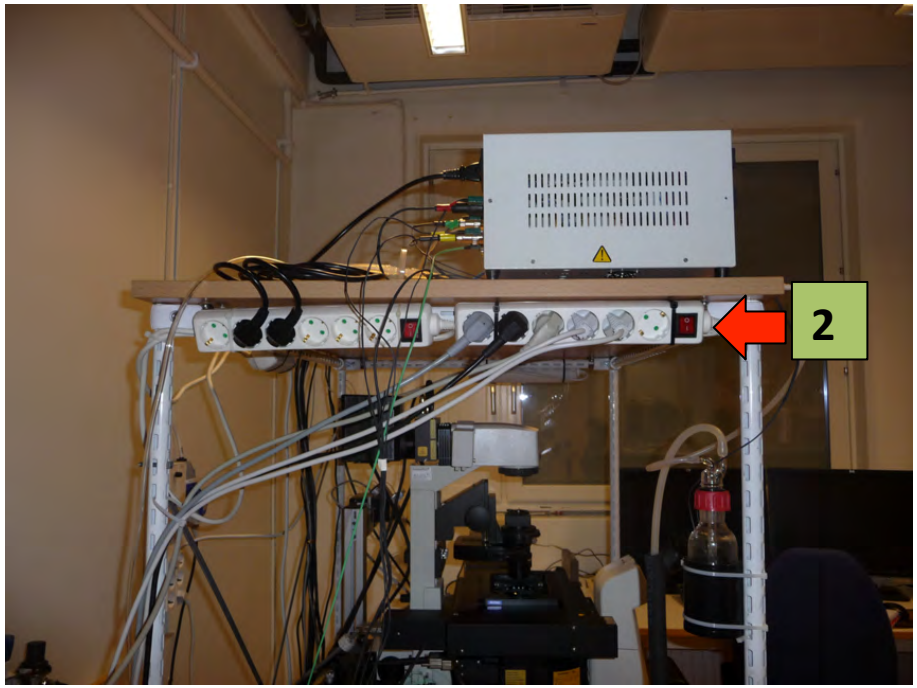


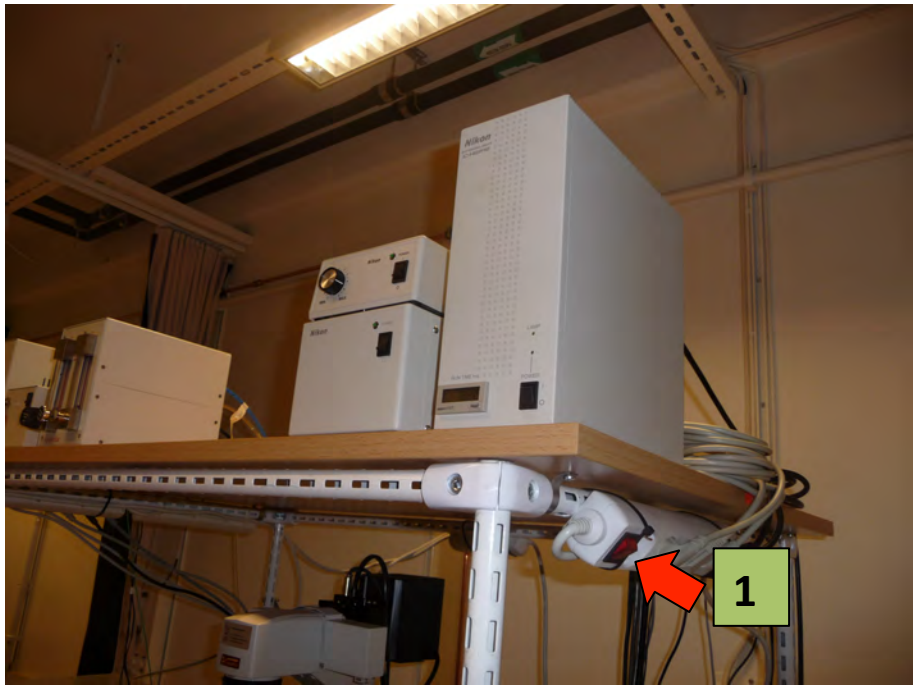
9- Turn off switch #3



10- Close the CO_2 main key in the wall

11- Turn off Switch #2 and #1





12- Put the plastic cover to protect the microscope

13- 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