Spinning Disk Microscope

System	Spinning Disk Confocal (Zeiss)
Location	IMB: Department of Integrative Medical Biology. 4th floor
Room	C4:15:17
contact	irene.martinez@umu.se
Price	200kr/hr. Driver License 1000kr

For funding purposes, it is essential to acknowledge the Biochemical Imaging Centre Umeå in all publications that include data derived from the Facility. Include this statement: "Microscopy was performed at the Biochemical Imaging Centre Umeå"

Use these phrases to mention the microscope in your publications:

If you have used the **Spinning Disk**:

Zeiss Cell Observer Spinning Disk Confocal controlled by ZEN interface with an Axio Observer.Z1 inverted microscope, equipped with a CSU-X1A 5000 Spinning Disk Unit and a EMCCD camera iXon Ultra from ANDOR.

If you have used **Tirf**:

Zeiss Axio Observer.Z1 inverted microscope, equipped with a EMCCD camera iXon Ultra from ANDOR and an alpha Plan-Apochromat TIRF 100X/1.46 Oil objective controlled by ZEN software.

BICU

UMEÅ UNIVERSITET

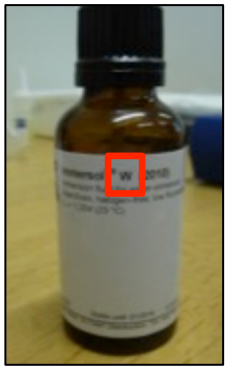
MIMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UCMR Umeå Centre for Microbial Research

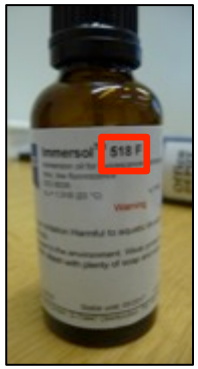
Spinning Disk Technical Information:

Objectives
20X: Plan-Apochromat 20X/0.8 M27
40X: C-Apochromat 40X/1.2 W Corr M27 (SPECIAL WATER)
63X: Plan-Apochromat 63X/1.40 Oil DIC M27
100X: alpha Plan-Apochromat TIRF 100X/1.46 Oil DIC M27

40X Oil W:



63X 100X Oil F:



Laser lines
405nm
488nm
561nm
647nm

BICU

UMEÅ UNIVERSITET

MiMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UCMR Umeå Centre for Microbial Research

Spinning Disk Emission Filter Changer
446 523 600 677
BP 525/50
BP 629/62
BP 690/50
DBP 480/22 + LP 530
DBP 527/54 + 645/60
BP 450/50 This one is not inside the Emission Filter Changer. If you need this one you have to tell BICU personnel in advance.

BICU

UMEÅ UNIVERSITET

MiMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UCMR Umeå Centre for Microbial Research

Motorized Reflector Turret (The microscope one) has 6 positions:
1- Empty for the Spinning Disk
2- #76: HE CFP/GFP/DsRed BP 390-422 BS 427 BP **488-472** BP 484-501 BS 503 BP 512-538 BP 549-573 BS 578 BP 585-631
3- #77: HE GFP/mRFP/Alexa 633 BP 469-497 BS 506 BP 510-542 BP 552-577 BS 582 BP 587-614 BP 629-650 BS 659 BP 665-711
4- #52 HE TIRF 488 BP 478-496 BS500 BP510-555
5- BS CFP/GFP/DsRed 80/20 to perform Frap in SD mode.
6- Analyzer/DIC/TL
In the Box #86 HE TIRF 561

BICU

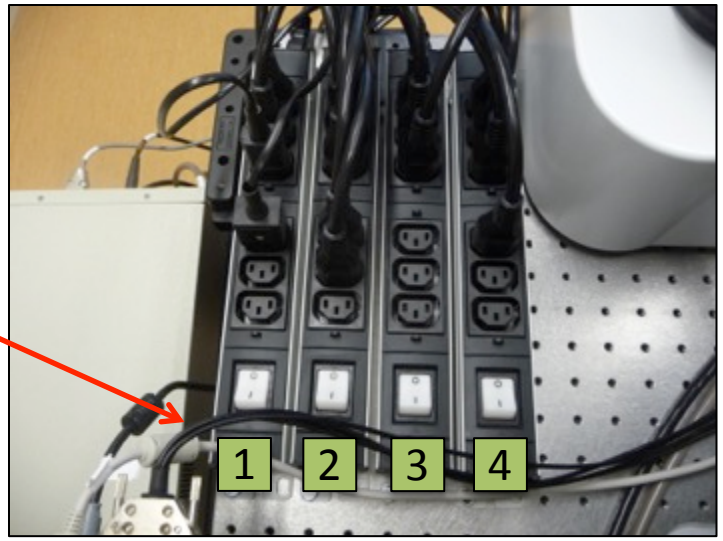
UMEÅ UNIVERSITET

MiMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

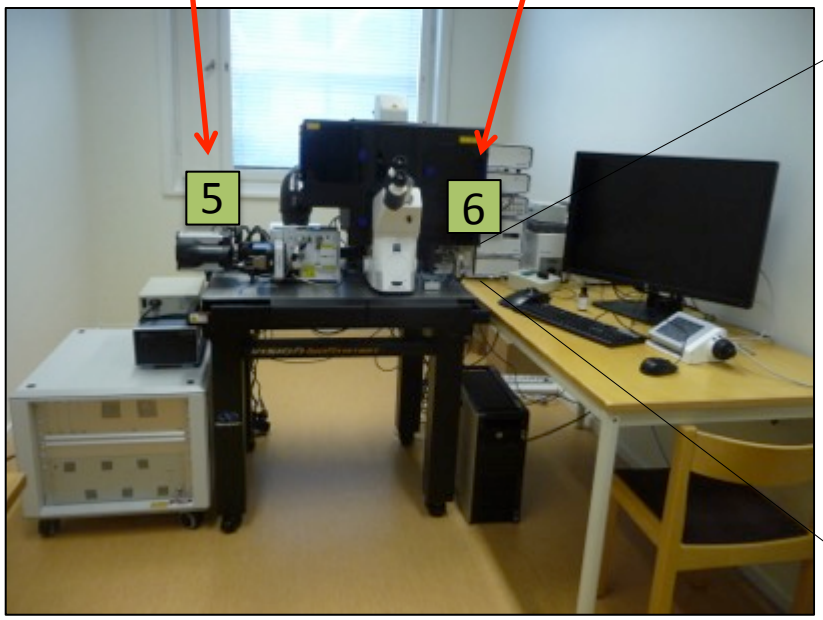
UCMR Umeå Centre for Microbial Research

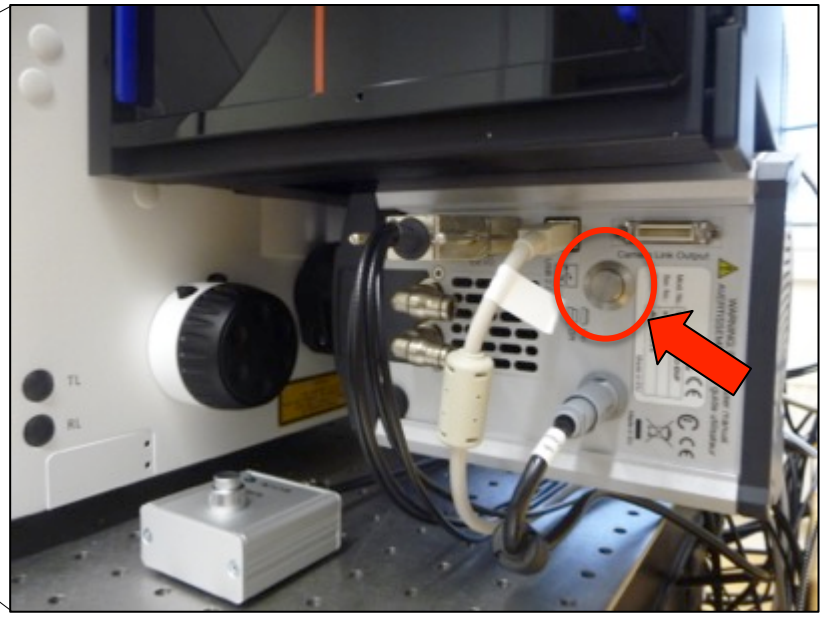
Switch on the system

1- Switch on: 1, 2, 3, 4 Please wait few seconds in between.



2- Switch on the camera: SpinningDisk Camera (5) or Tirf camera (6)

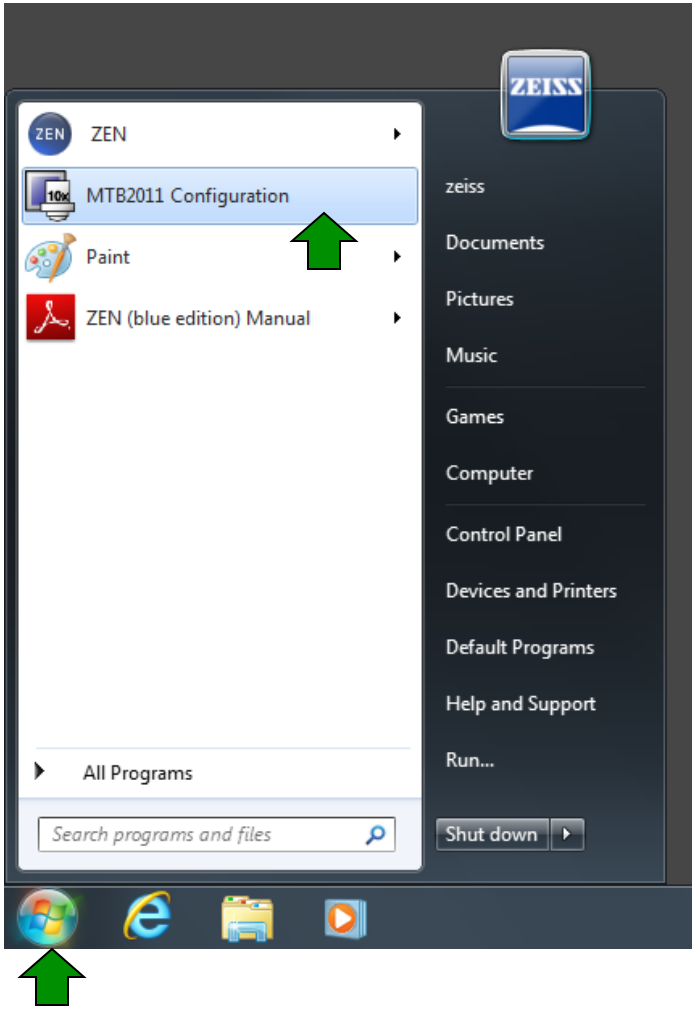




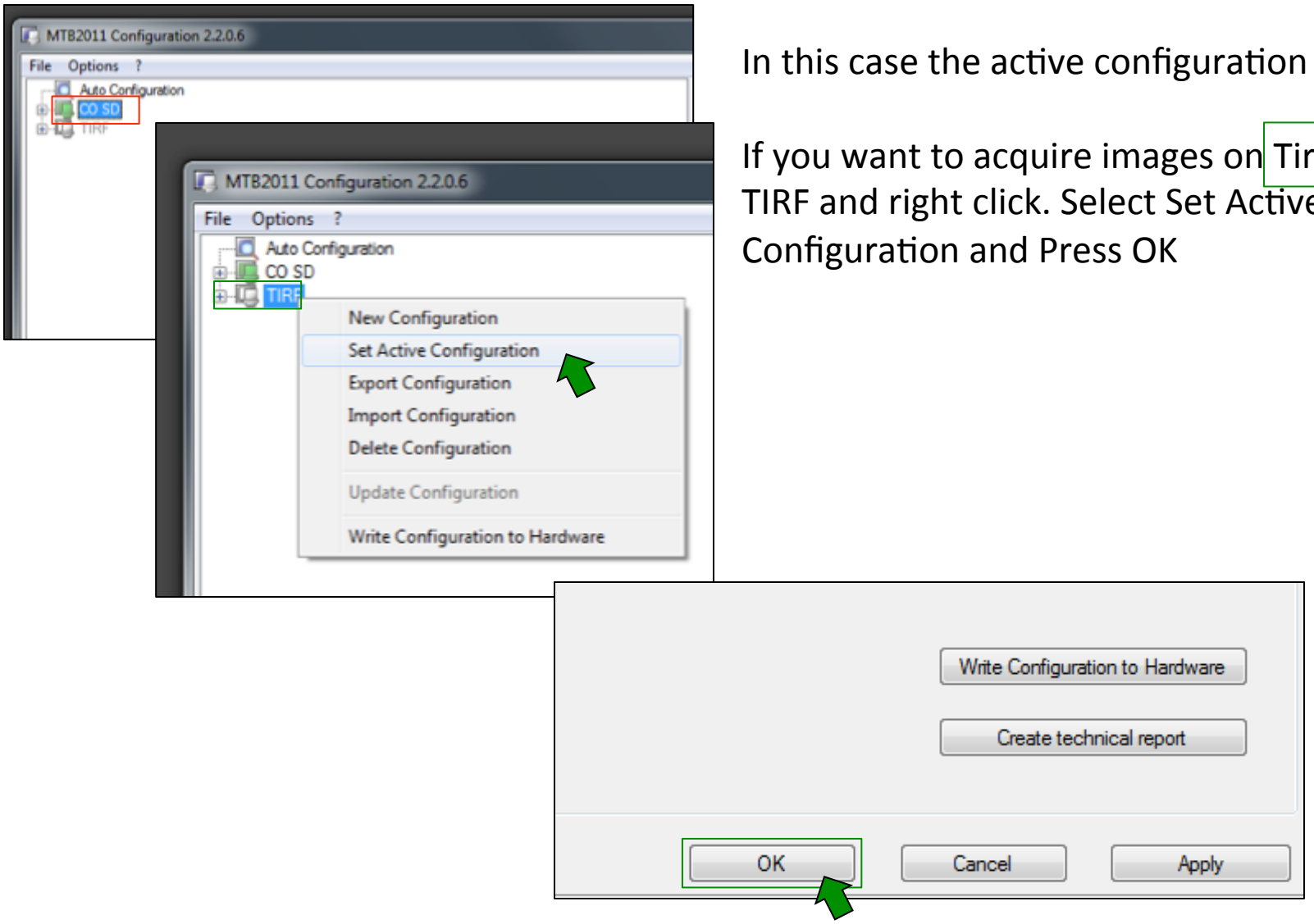
3- Turn on the computer

4- Click Zeiss icon

5- In order to select Spinning disk Acquisition or Tirf Acquision: Go to Start and click MTB2011 Configuration.



6- Select SD or Tirf/ right click and choose “Select Active Configuration”. Press OK



In this case the active configuration is SD.

If you want to acquire images on Tirf Go to TIRF and right click. Select Set Active Configuration and Press OK

7- Double click ZEN software

8- Click Zen System (It take few minutes to initialize)

9- Choose ZEN Imaging Procesing if you want to analyze your images

Switch on In Vivo system

BICU

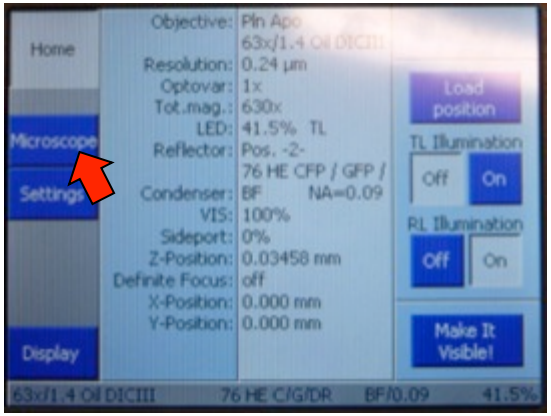
UMEÅ UNIVERSITET

MiMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

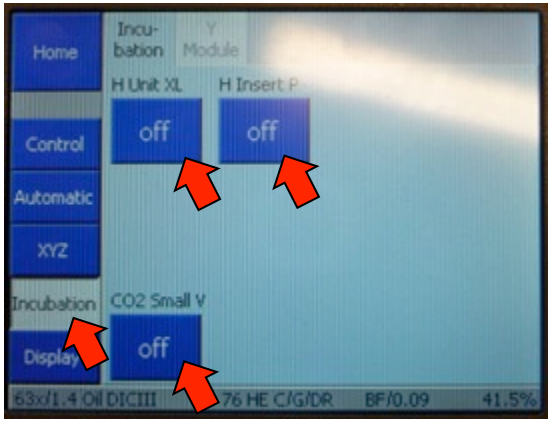
UCMR Umeå Centre for Microbial Research

Turn on the temperature for the Insert, the incubator and the humidifier 1hr before starting acquisition. With the screen:

1- Go to Microscope

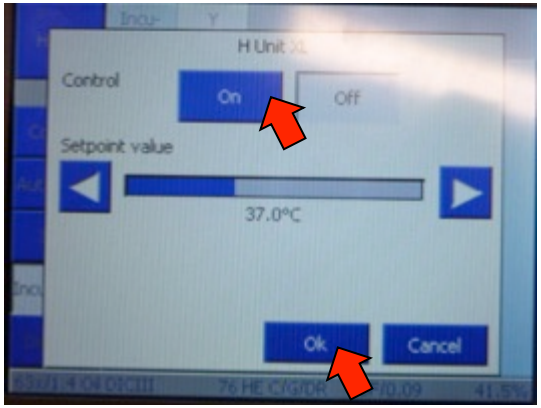


2- Press Incubation



3- Press:

H Unit XL, Press On and press ok
H Insert P, Press On and press ok
H Dev Humid, Press On and press ok



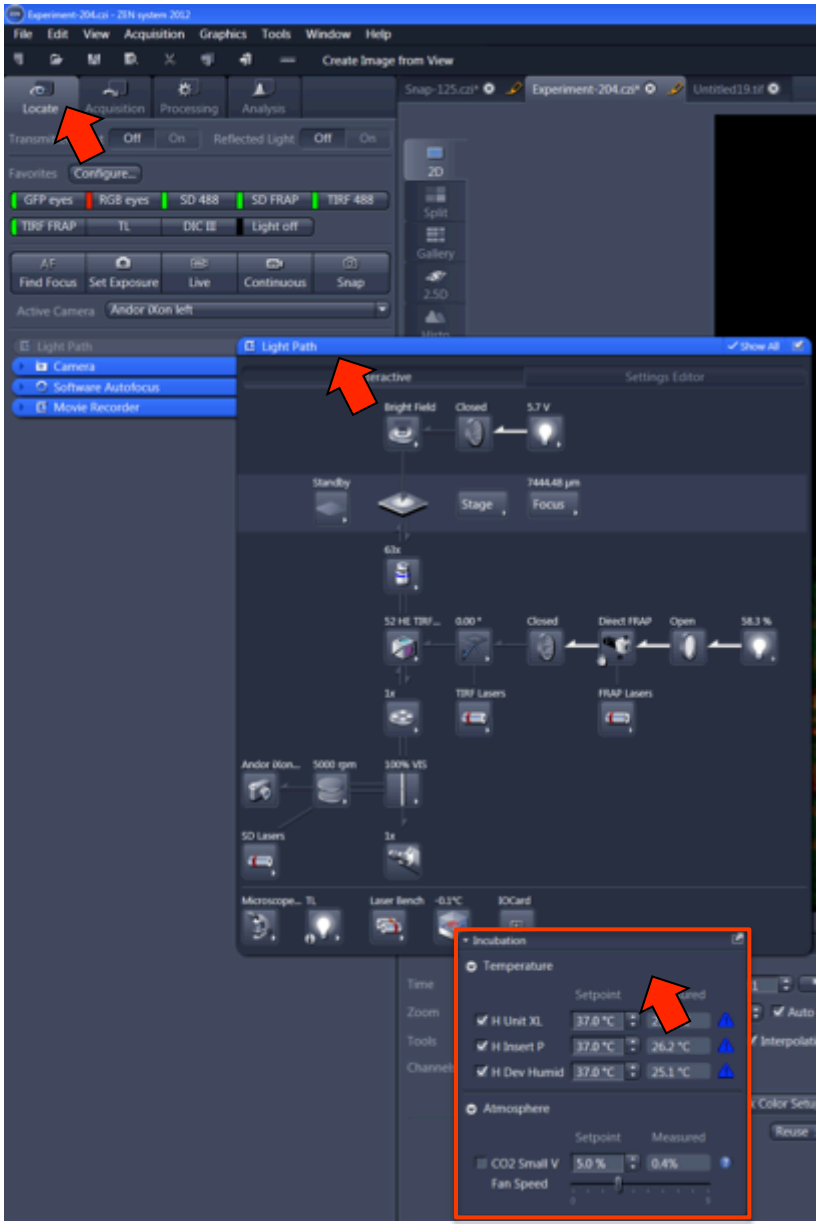
BICU

UMEÅ UNIVERSITET

MiMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UCMR Umeå Centre for Microbial Research

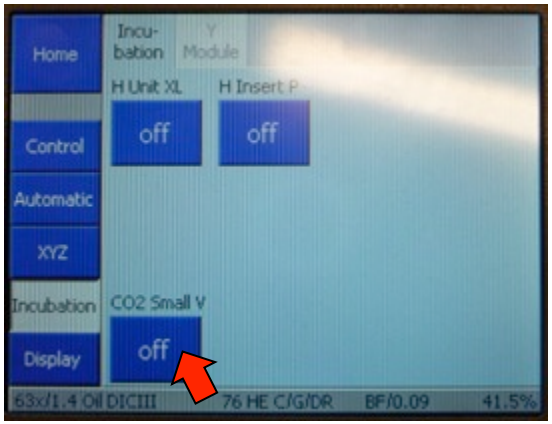
If you cannot find the Incubation in the TFT (It is disabled if you don't switch the incubation at the beginning)



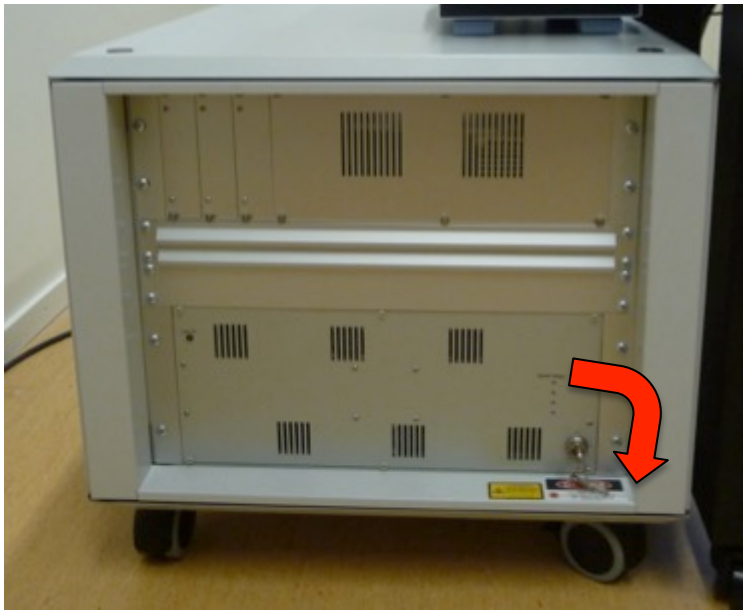
Go to Locate/ Light Path/ Expand the incubation menu and click the box to switch all the devices on.

15 min before starting acquisition: Prepare CO2 and Lasers

2- In the screen press CO2 Smal V (off symbol), Turn it on and press ok



3- Turn on the laser box key



Check your sample under the microscope

BICU

UMEÅ UNIVERSITET

MIMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UCMR Umeå Centre for Microbial Research

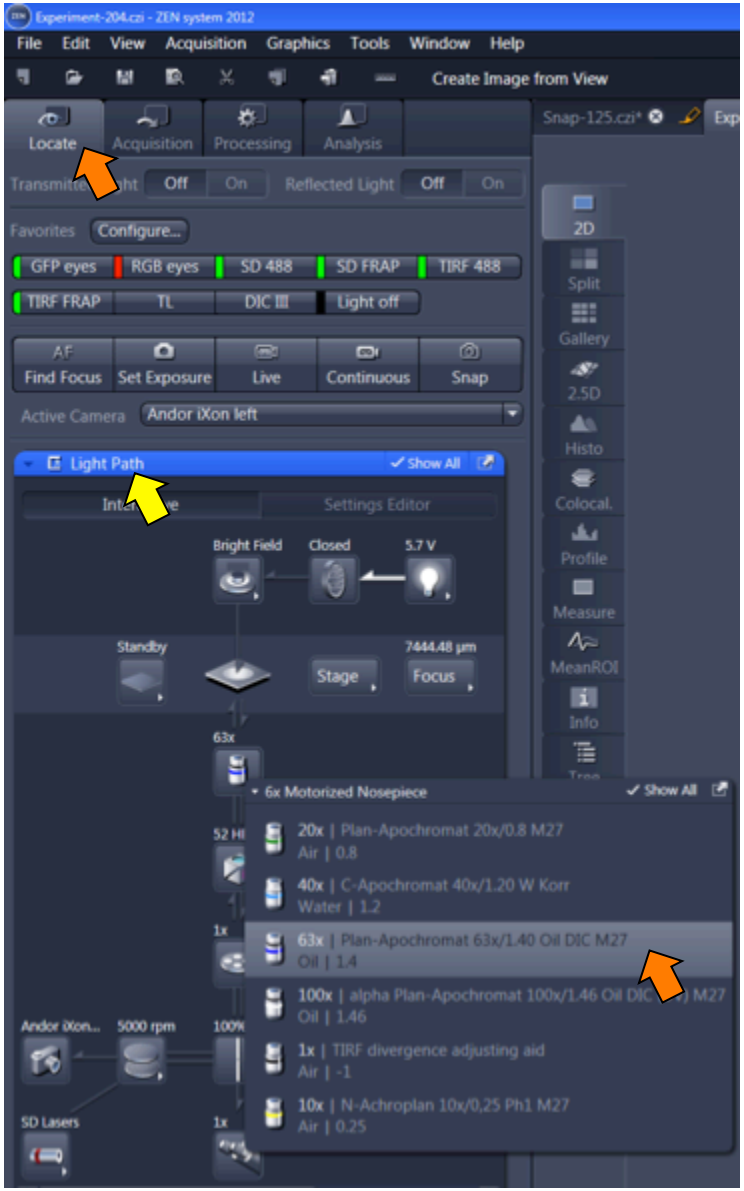
1- In the software go to Locate

2- Expand the Light Path Menu clicking the blue bar
Expand the objective Menu

Select the objective within the light path menu from the software

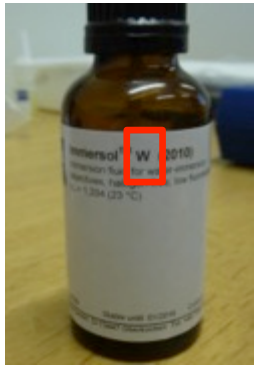
or with the screen (Microscope/Control/ Objectives)

Select the objective with the Light Path Menu:

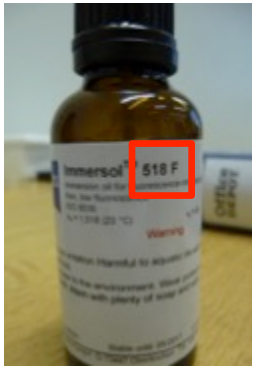


3- Add oil. Remember: W: 40X / F: 63X & 100X

40X Oil W:



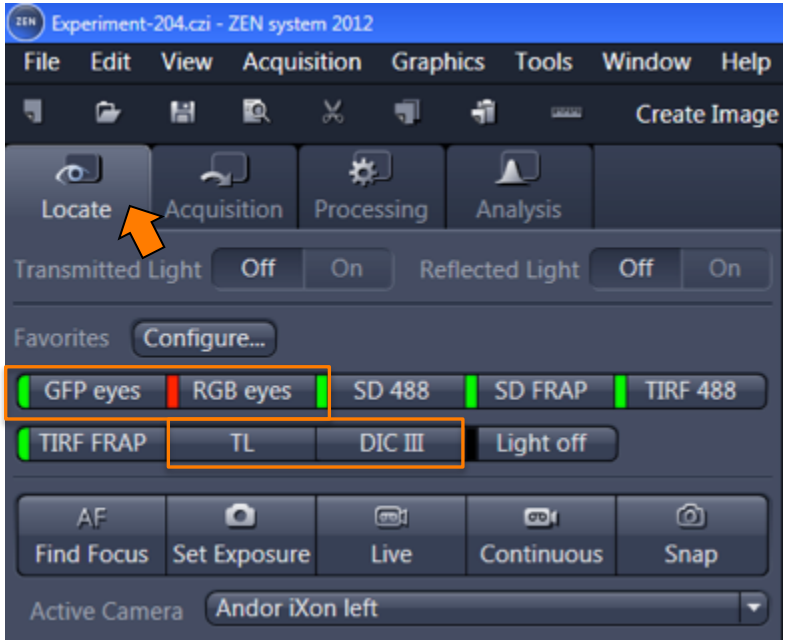
63X 100X Oil F:



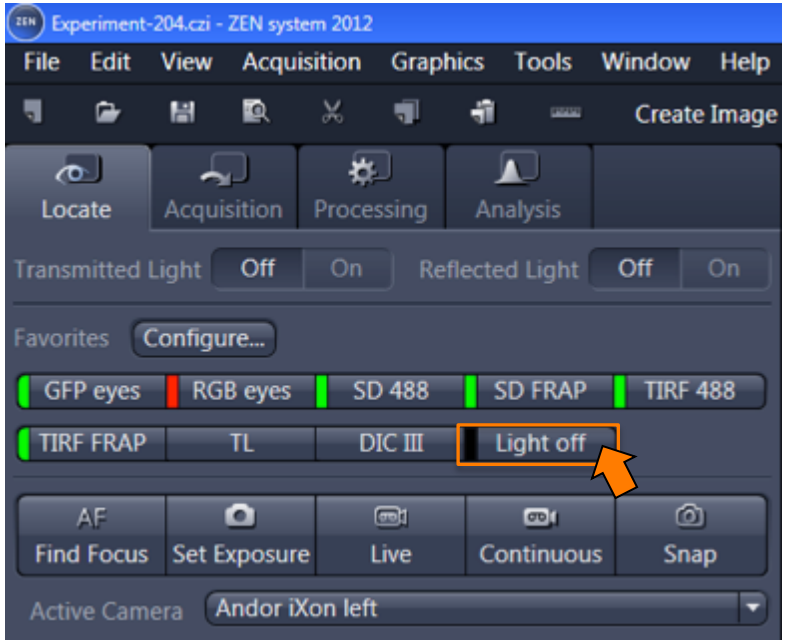
4- Close the incubator carefully.

5- Go to Locate/ Press Eye Green to see green fluoresence or TL: Transmitted Light to focus the sample. The laser safety box has to be off

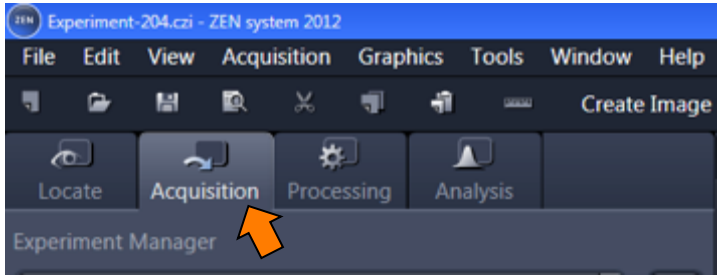
GFP eyes	To see green emission with the eyepieces
RGB eyes	To see blue/green/red emission with the eyepieces
TL	To see Transmited Light (BrightField)
DIC III	To see DIC (Nomarski)



6- Once you focus, press lights off to close the fluorescence shutter



7- Go to Acquisition

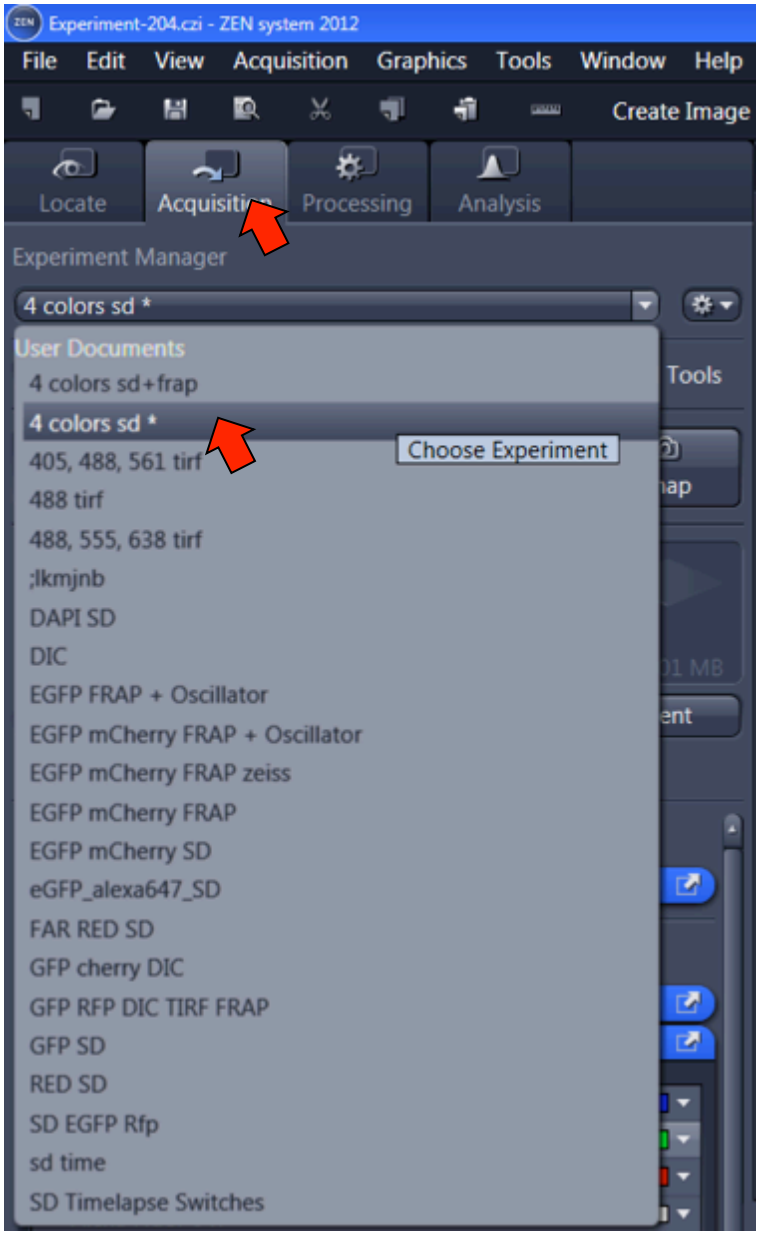


8- Active Safety.(Active: blue led and Safe green led should be on)

9- select within the exp manager your experiment set up (channels or colors you want to see)

The experiments for the Spinning Disk are named as “SD”. The ones for tirf as “tirf”

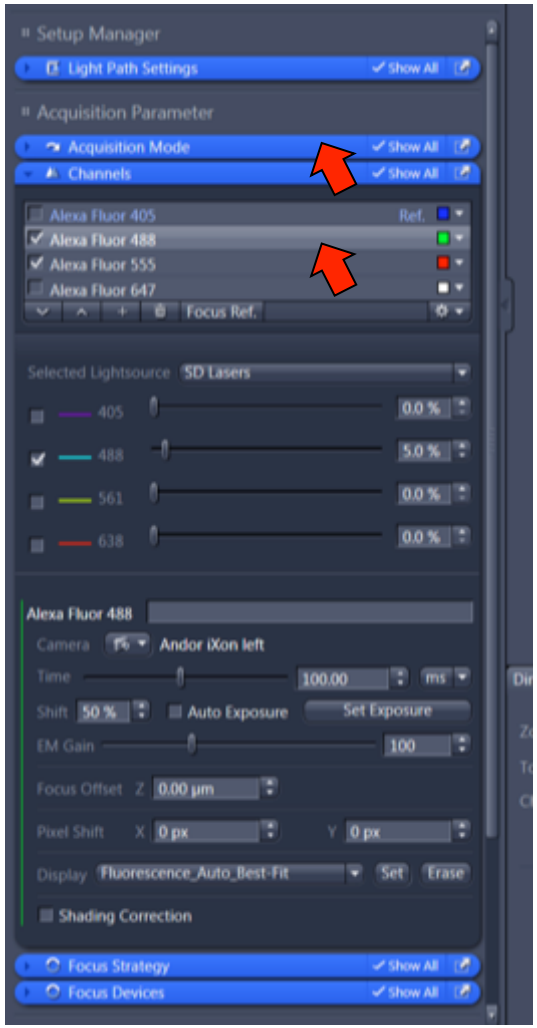
You can also open an old image and reuse the settings instead of loading the settings from the Experiment Manager.

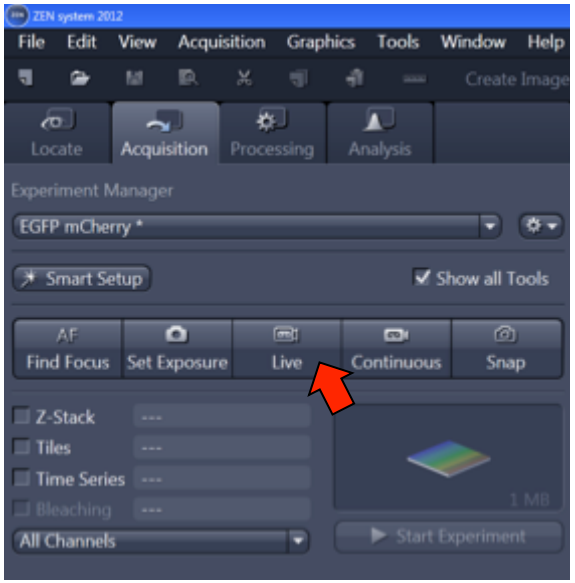


10- Go to channels

11- Select the channel you want to focus first → grey bar to see that channel in live mode

12- Press Live





13- Change conditions channel by channel. To speed up use the same exp time and EM gain and play with the laser power.

Live mode

4 channels experiment

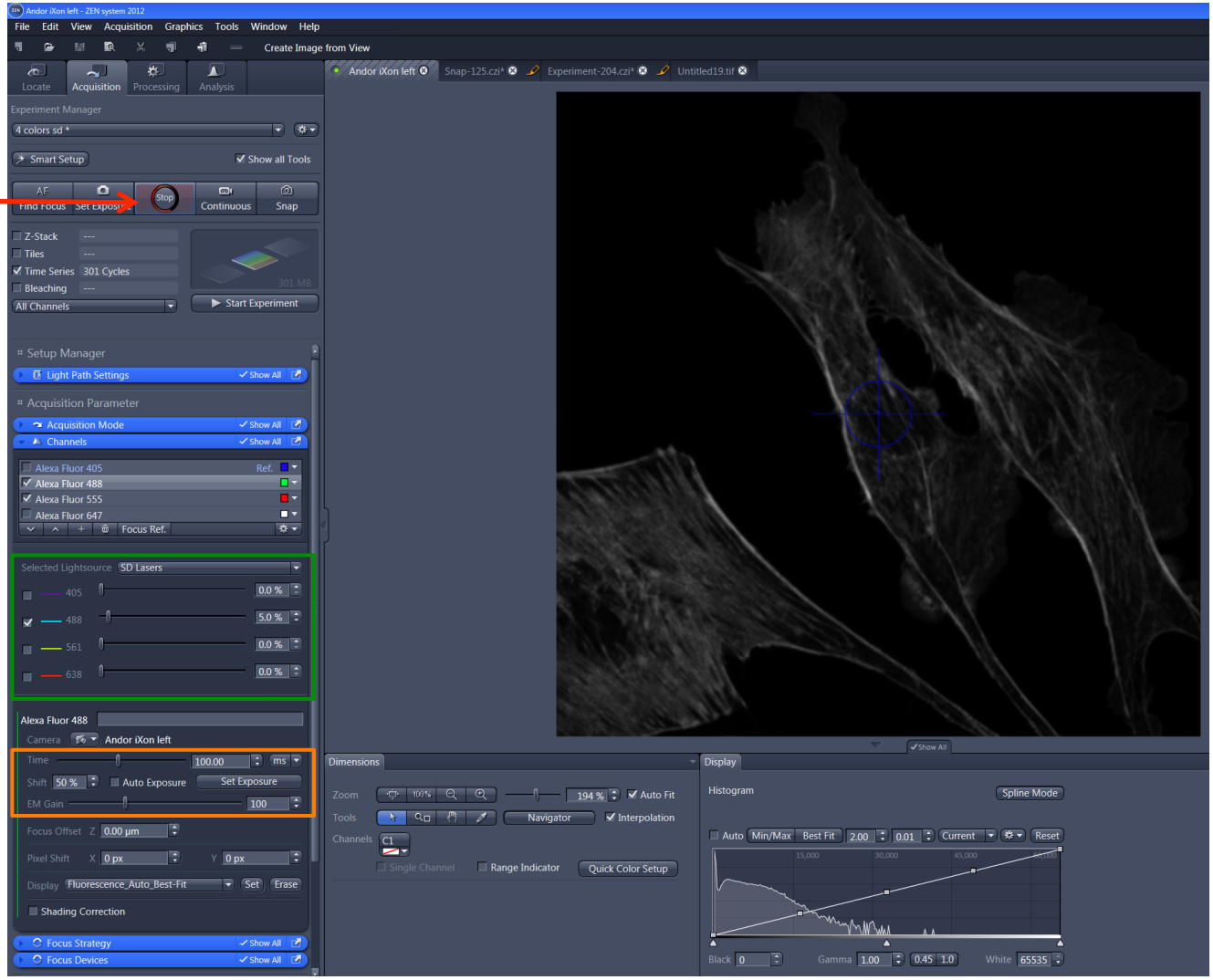
Green channel live

Acquisition conditions to change:

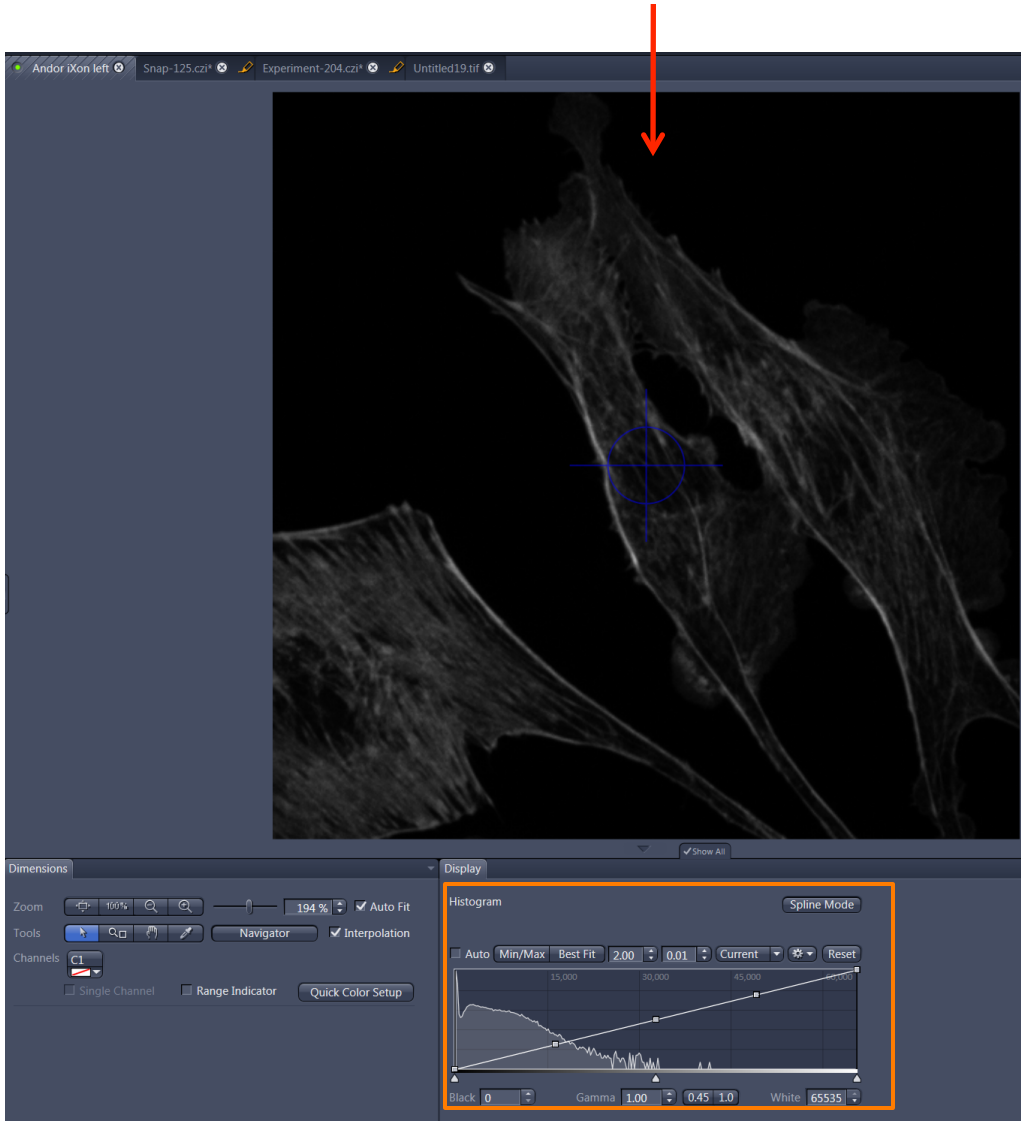
1-Laser power. Keep it low to avoid cell death

2-Exposure time in ms

3-EM Gain



14- To find the best location to image you can move x,y direction with the joystick. Within the image you can move up and down with the blue arrows.



Also you can centre with a mouse double click moving this icon where you want to have the center of the image.

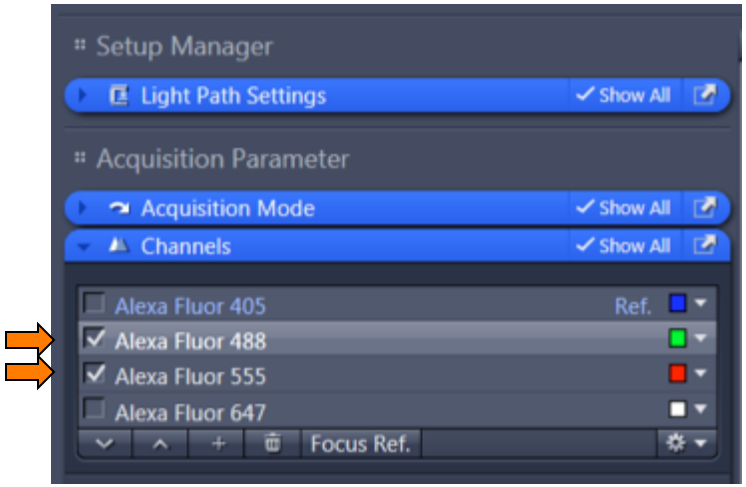
Image Histogram

Here you have the distribution of pixel intensities.

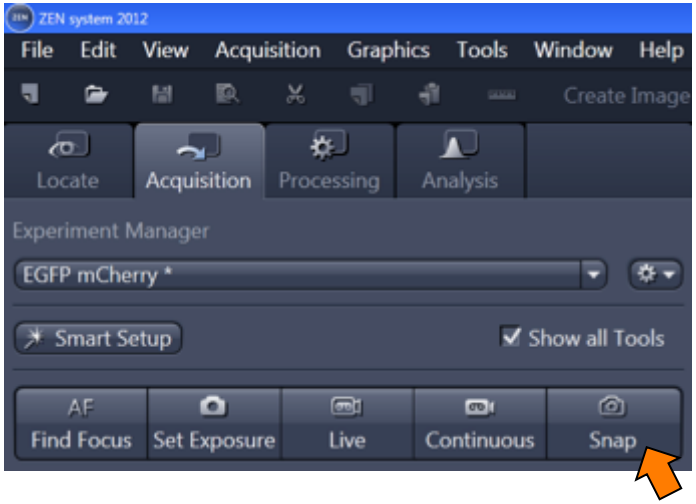
16 BITS images: 2^{16} grey intensity values

Gamma:1.00 as default

15- Select the channels that you want to image ☑. In this case we have loaded an experiment with 4 channels but we are going to image only 2 (green and red)



16- Press Snap in order to make the final picture



Time series

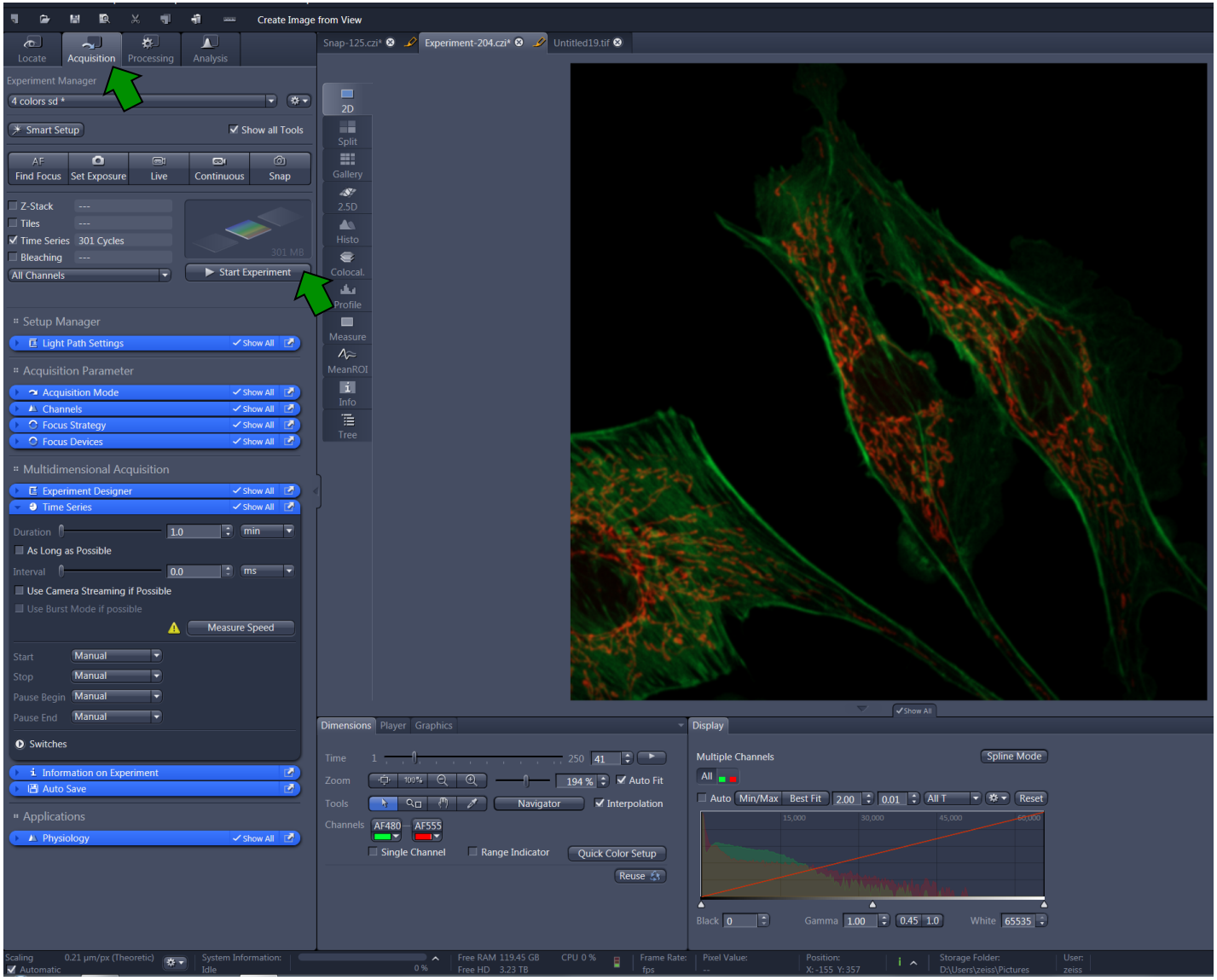
Time series: With this dialog you can image your cells along the time with an interval.

1- Click Time series from Acquisition.

2- Expand the Time Series Dialog and Select:

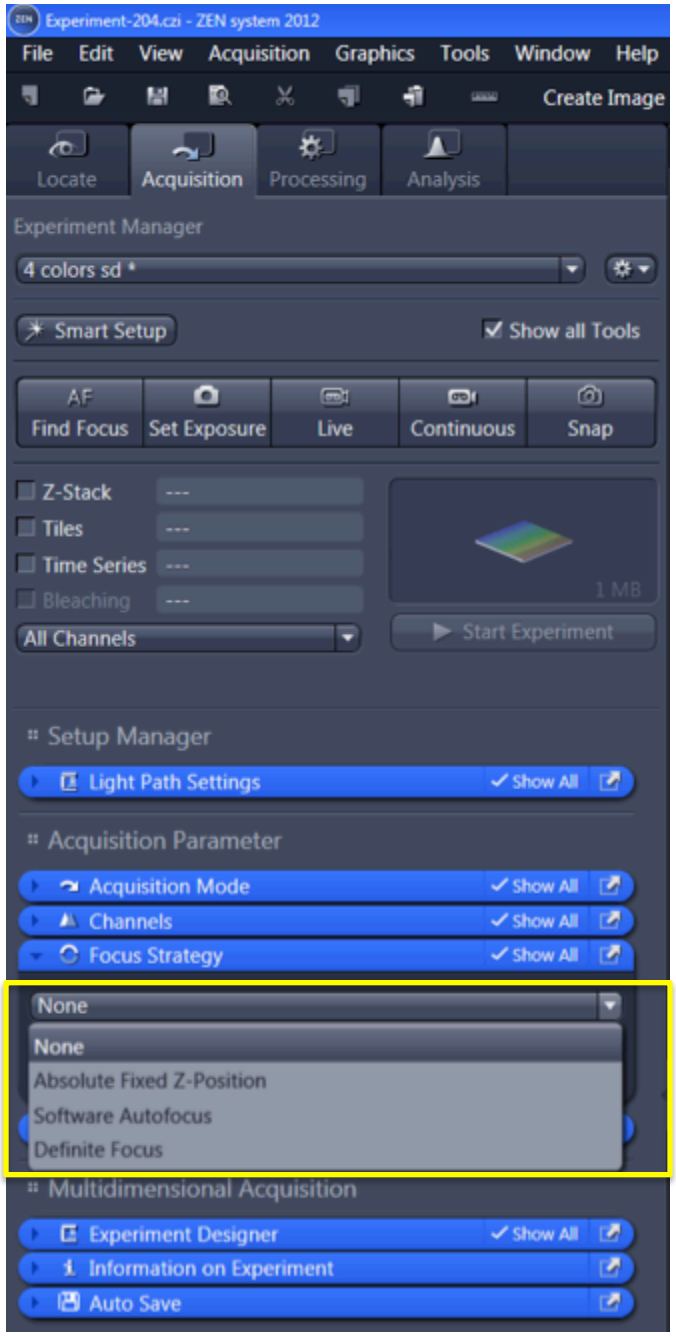
3- Duration of the experiment and the Interval between acquisitions.

4- Press Start Experiment to perform acquisition



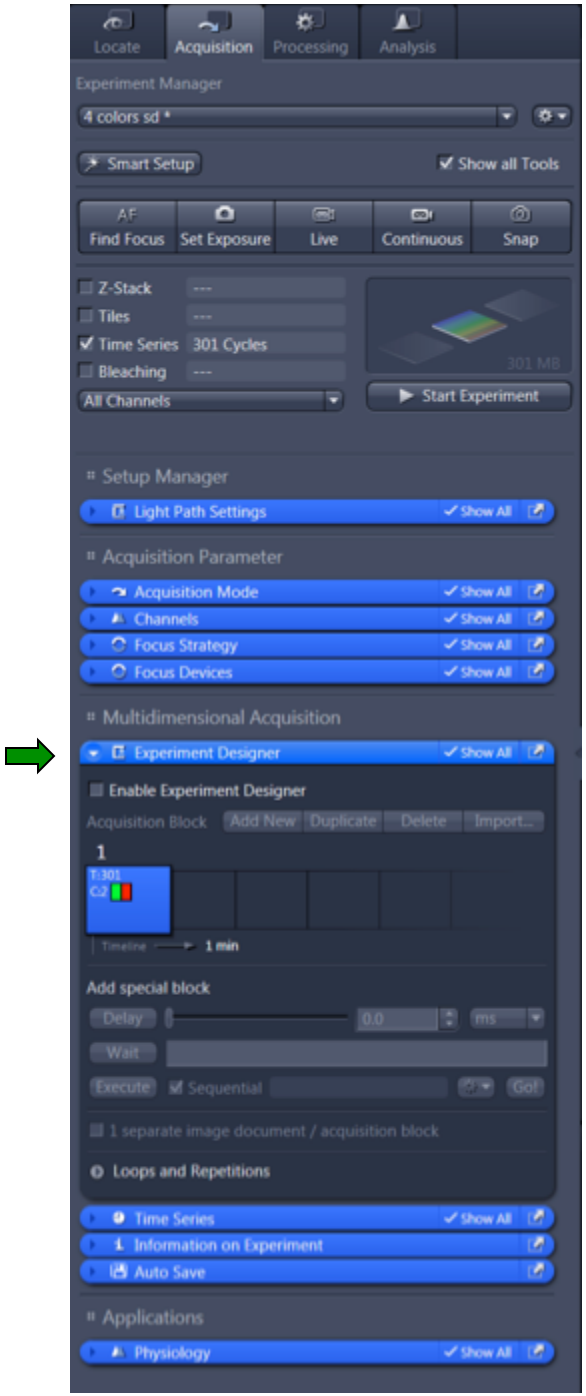
Time series:

Before Starting the experiment, you can select Focus Strategy to maintain the focal plane among the serie.



You can have a complex Time Series if you Enable Experiment Designer ☑.

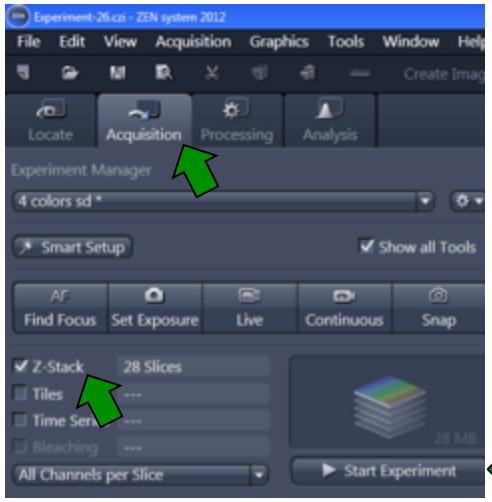
With this dialog you can combine different Time series among the time as blocks. You configure them independently and then you run a Time series.



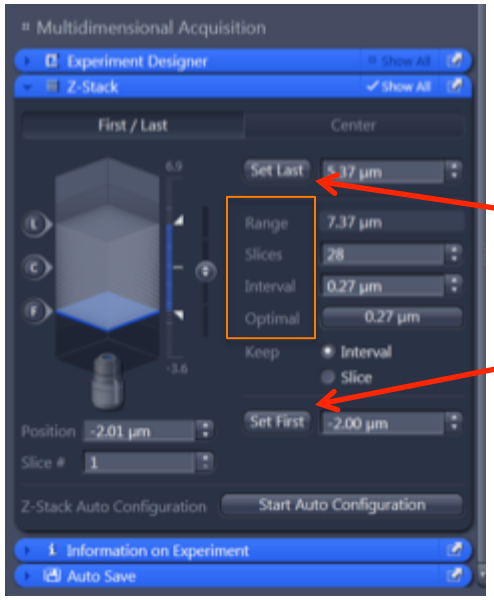
Z-Stack

Z-Stack:

1- Select Z-stack from Acquisition



Click Start Experiment when you are ready to start acquisition.



Range: thickness of the z-satck in um
Slices: number of planes in the z-stack
Interval:step (in um) in between planes
Optimal: Optimal interval in between planes (depending on the optical thickness)

Click show all and select First/Last option

Select z-stack limits: First and Last by pressing this buttons

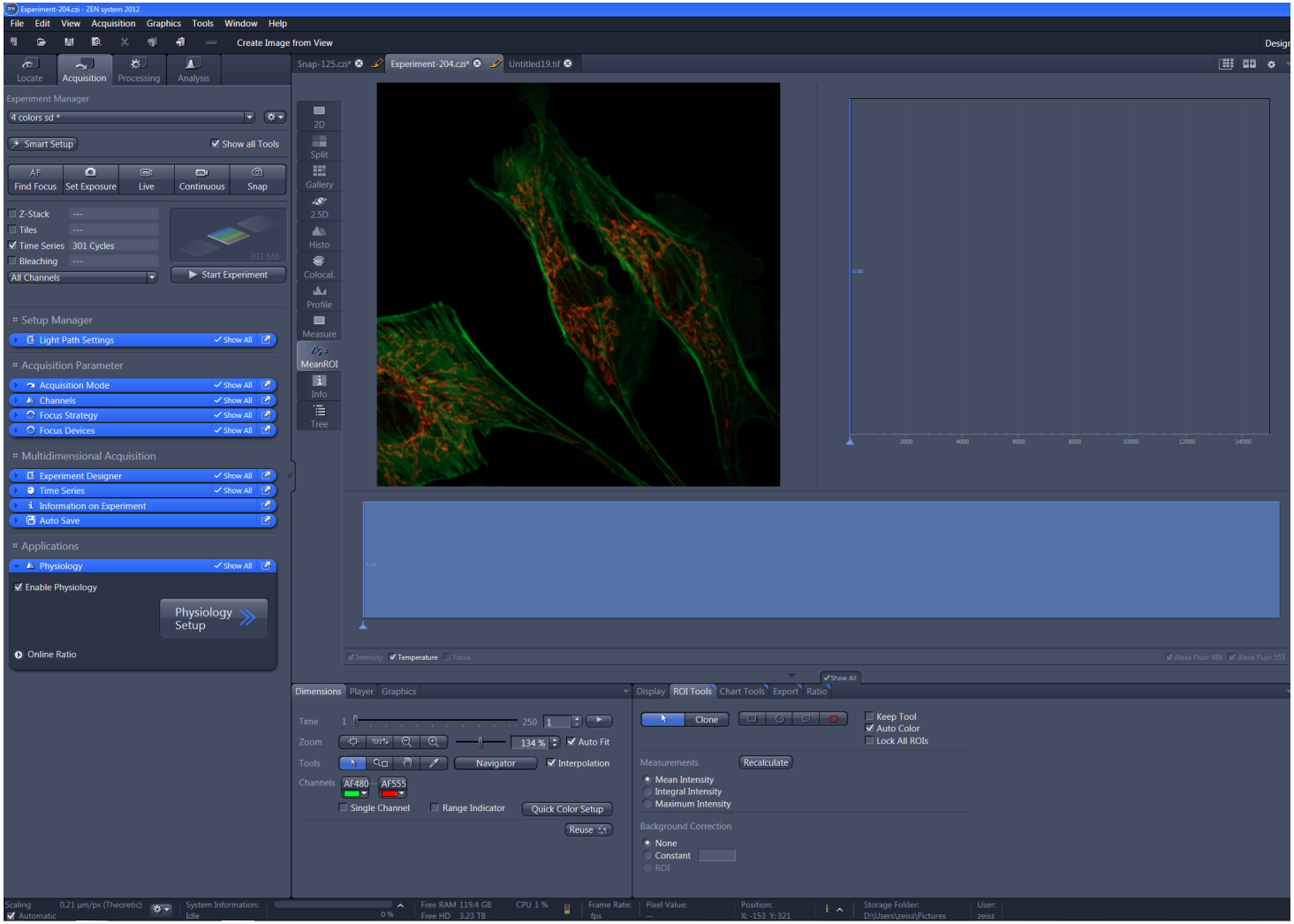
BICU

UMEÅ UNIVERSITET

MiMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UCMR Umeå Centre for Microbial Research

Physiology


Experiment-204.czi - ZEN system 2012
File Edit View Acquisition Graphics Tools Window Help
Create Image from View
Locate Acquisition Processing Analysis
Experiment Manager
4 colors sd *
Smart Setup
Show all Tools
AF Find Focus
Set Exposure
Live
Continuous
Snap
Z-Stack
Tiles
Time Series 301 Cycles
Bleaching
All Channels
301 MB
Start Experiment
Setup Manager
Light Path Settings
Acquisition Parameter
Acquisition Mode
Channels
Focus Strategy
Focus Devices
Multidimensional Acquisition
Experiment Designer
Time Series
Information on Experiment
Auto Save
Applications
Physiology
Enable Physiology
Physiology Setup
Online Ratio
Snap-125.czi*
Experiment-204.czi*
Untitled19.tif
2D
Split
Gallery
2.5D
Histo
Coloca.
Profile
Measure
MeanROI
Info
Tree
Dimensions Player Graphics
Time
Zoom
Tools
Channels
AF480
AF555
Single Channel
Range Indicator
Quick Color Setup
Reuse
Navigator
Interpolation
Auto Fit
Display ROI Tools Chart Tools Export Ratio
Clone
Keep Tool
Auto Color
Lock All ROIs
Measurements
Recalculate
Mean Intensity
Integral Intensity
Maximum Intensity
Background Correction
None
Constant
ROI
Scaling
Storage Folder:
User:

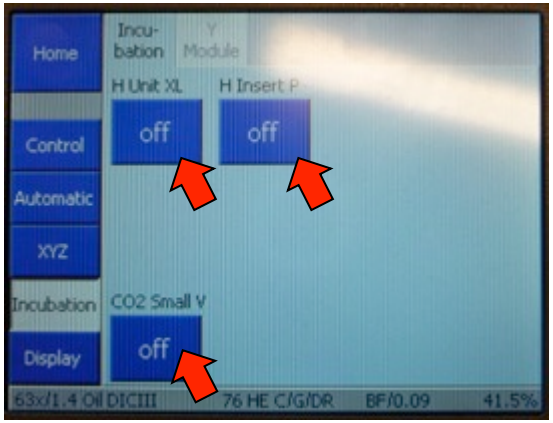
Switch off the system

Before turning off the system check the e-booking system. If someone has booked after you, leave it one and let that person known that the system is on.
If the next users has booked in 60 minutes or longer after you, continue with the instructions bellow to switch the system off:

1- Switch off Incubation:

A) With the screen:

Press CO2 Smal V, Turn it off and press ok
Press H Unit XL, Turn it off and press ok
Press H Insert P, Turn it off and press ok
Press H Dev Humid, Turn it off and press ok



BICU

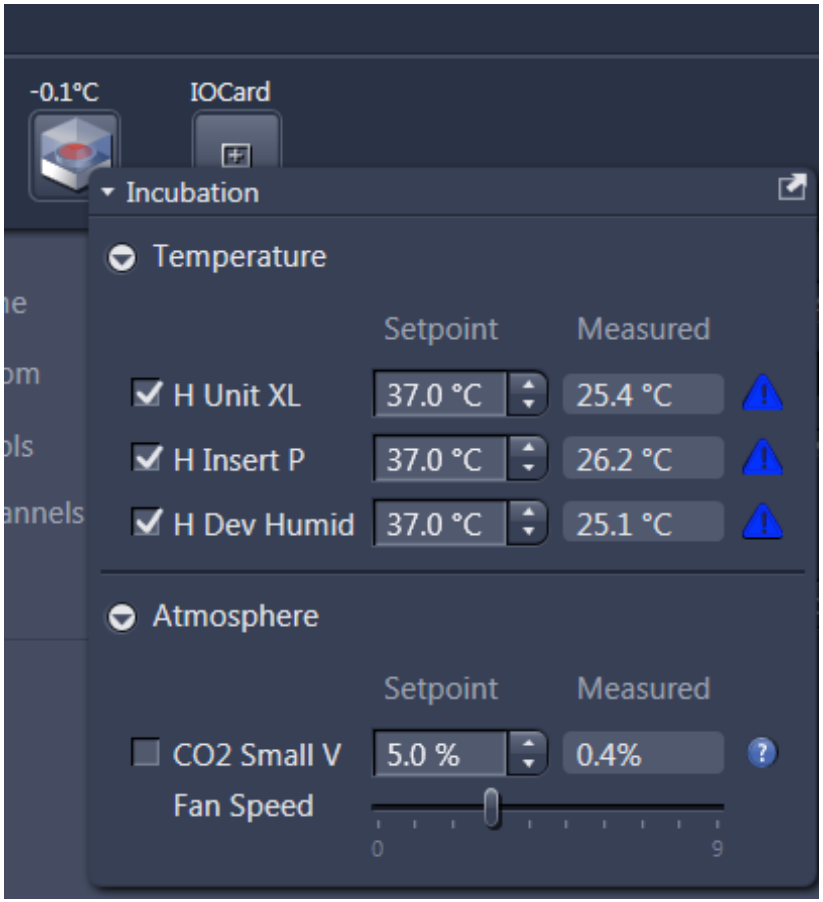
UMEÅ UNIVERSITET

MiMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UCMR Umeå Centre for Microbial Research

B) With the software:

Go to Locate/ Light Path/ Expand the incubation menu and un-click ☐ the boxes to switch all the devices off.



2- Save your pictures and close them.

3- Close Nis Elements.

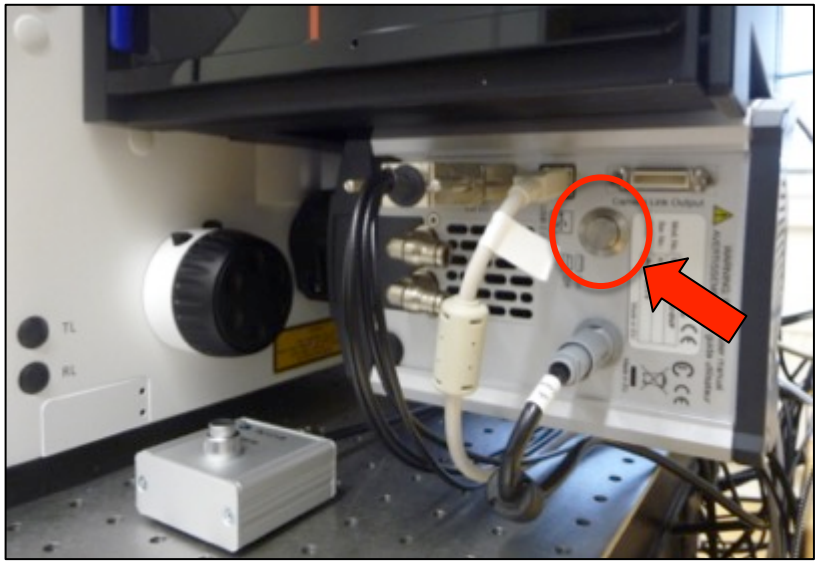
4- Turn All the laser power off from the software. Wait until all lasers are off, then press OK. While the lasers are turning off, clean the objective, the stage incubator and place the objectives down.

5- Transfer your files: connect the computer to internet and transfer your folder from: Computer / (C:) / Users to the server. There is server link on the desktop. Once the transfer is done, please unplug the computer from the network for security reasons.

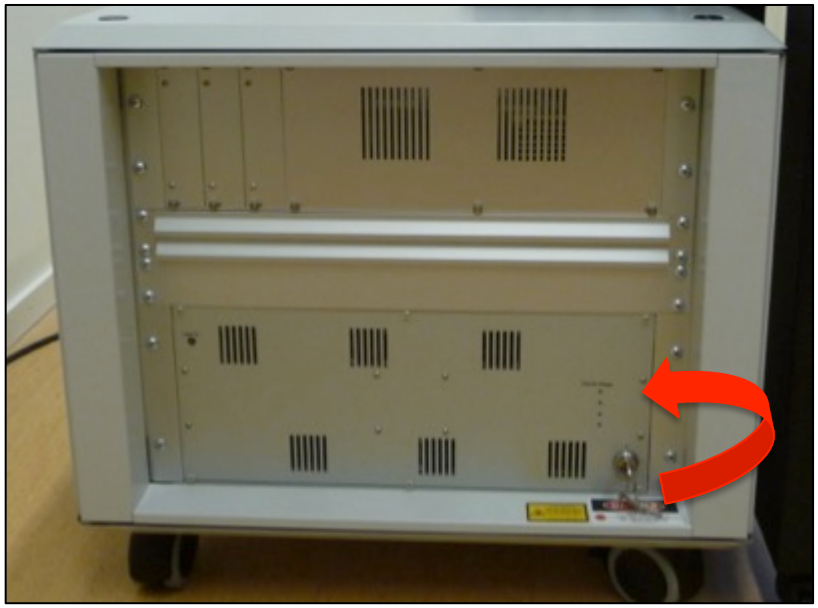
REMEMBER: It is forbidden the use of usbs or hard disks to transfer your files.

6- Shut Down the computer

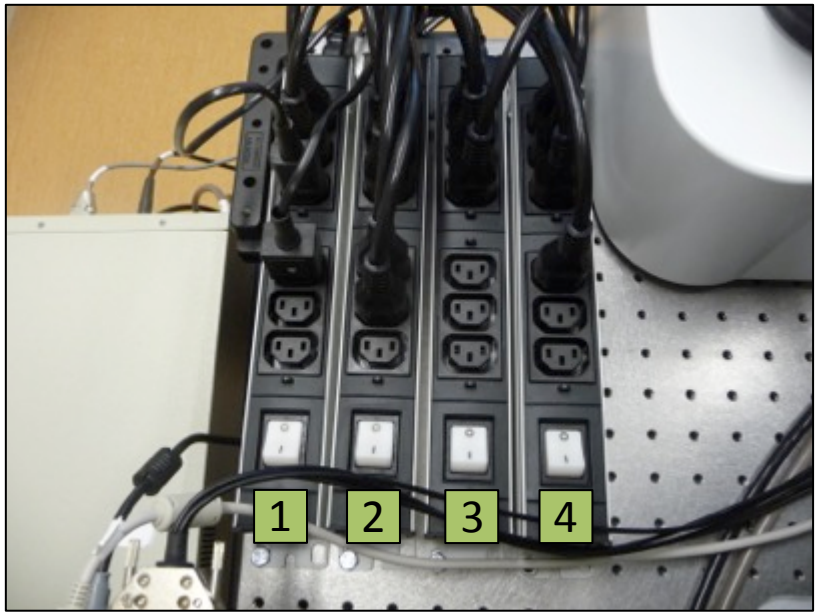
7- Turn the camera off



8- Turn the Lasers off with the key



9- Switch off 4, 3, 2, 1



10- Write the information in the logbook