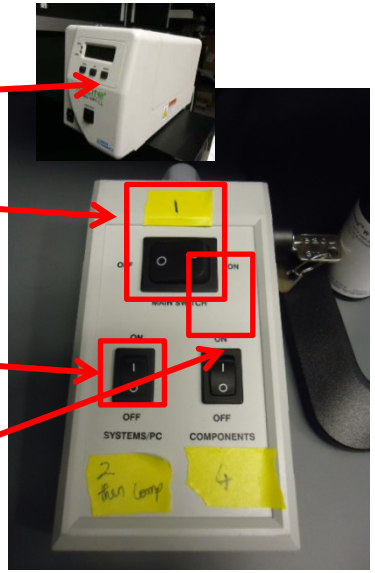
Zeiss LSM 780
Laser Scanning Microscope
Basic Operating Instructions

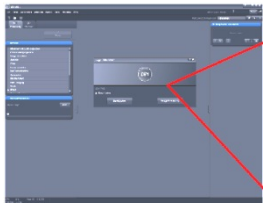
Start the system



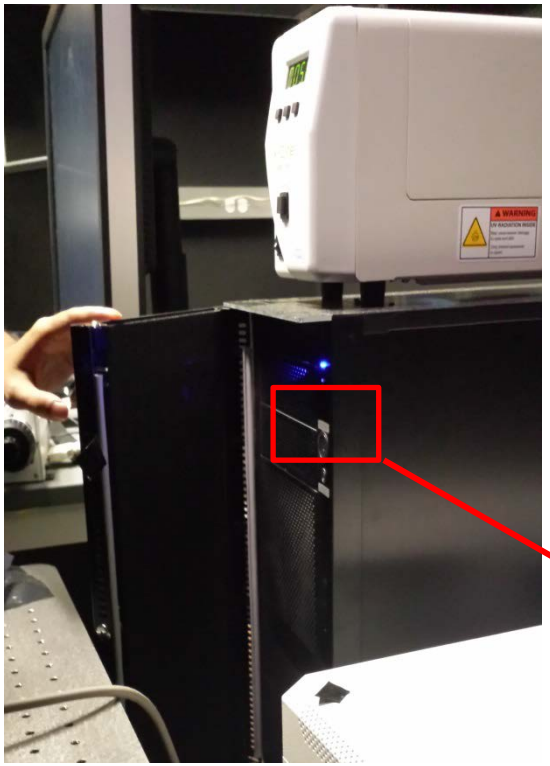
1. Turn on the "Arc lamp (6)" (if needed)
2. Turn on Main switch (1)
   Wait for 1-2 mins
3. Turn on System/PC (2)
   wait for 1-2 mins;
4. Press the computer power button (3)
   (under the table)
5. Wait for the login screen and
   log-on to the computer with your KCCI account.
6. Turn on Components (4) (Only for acquisition, NOT for exporting images)
   wait a few minutes until you hear sounds from the h are

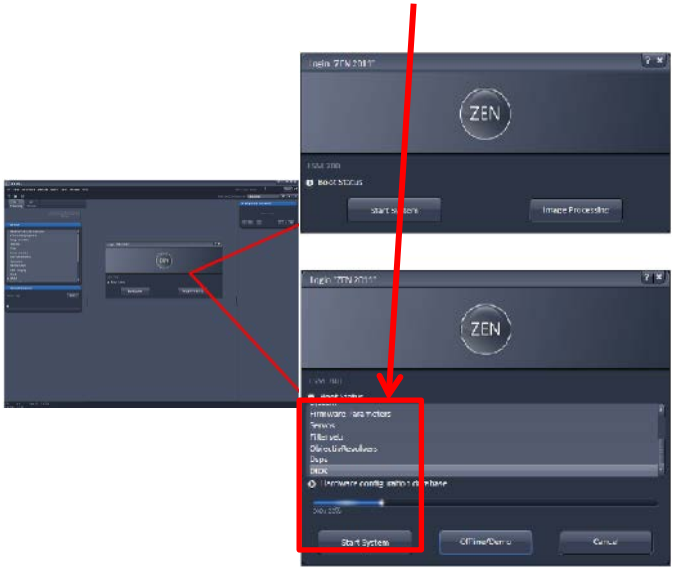
7. Turn on the "Laser (5)" switch, IF the 488nm wavelength is needed
8. Record the starting time at the KCCI website
9. Start the "ZEN" software on the desktop.

10. Click 'Start System'. Wait until the ZEN software Menu appears on the computer desktop. This may take A couple of minutes



If any error occurs during “Start system”





If you see any error message such as “Software stops working” then close the software and “log off”. After that reboot your machine. Press the big button, wait for 5 minutes and restart the Zen software.

Shut down the System

NOTE: Multiphoton Laser, Arc Lamp, and Hardware cannot be turned on again within 30 minutes after having been turned off. Check with KCCI sign up website or staff whether somebody is going to use the system after you or **ask the KCCI staff to shut down the system!!!**

1. Exit the "ZEN" software.
2. Export your data if needed (do not use a USB stick – use the server)
3. Record the ending time and submit the usage time.
   NOTE: Make sure to choose Zeiss 2p if you use the 2p laser.
4. Copy your data to the K drive for your data transfer.
5. Shut down the computer through software.
6. Turn off the "Arc lamp (6)"
7. Switch off the Laser (5).
8. Switch off the Components (4).
9. Switch off the System/PC (2).
10. Wait for 1-2 minutes.
11. Turn off the "Main Switch".
12. Clean the objective lens and stage.

Reuse settings if method already exists

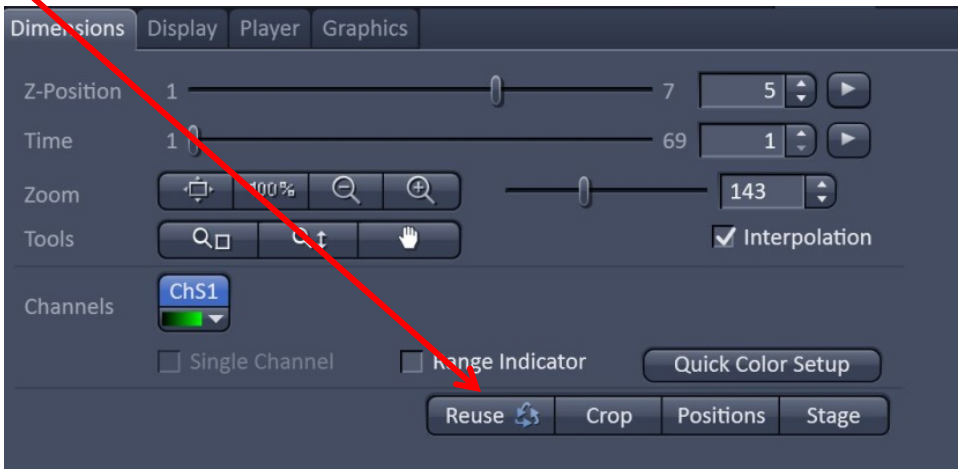
(For setting up new methods, proceed to next slides)

Load the method BEFORE putting the sample on the stage as objective will be moved by the software and may damage your specimen or objective.

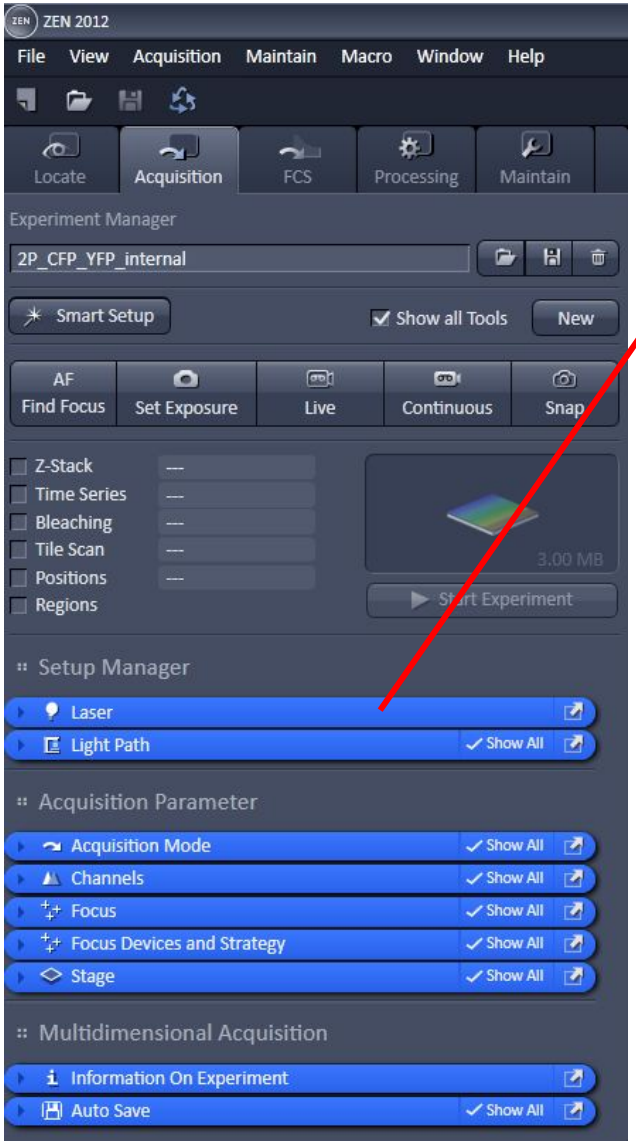
Make sure that the "laser(s)" is ON. (check next slide)

NOTE: The imaging method should be set up and stored with the help of KCCI staff. Talk to the KCCI staff when creating a new method or if the previous method does not work well.

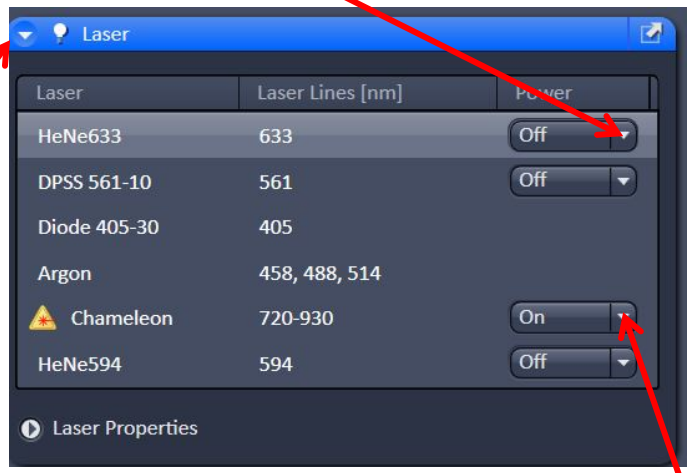
Choose the image from the 'File > Open' of the ZEN software.
Apply the method by clicking Reuse function from a previously saved image (Recommended).



How to turn ON the "Laser(s)"

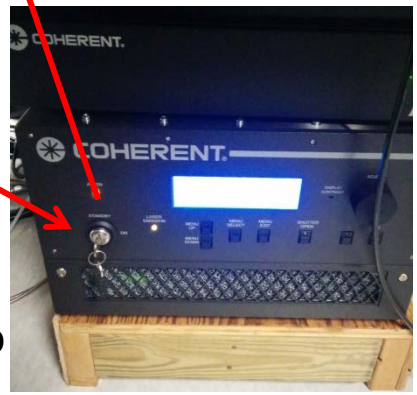


Turned on laser through the software by selecting "ON"



2p laser (if needed)

Remember to turn on the key of coherent BEFORE starting the Zen software. The laser controller is under the optical table. If the coherent laser is selected without turning on the key, it will corrupt the software and have to restart the software.



Select Objective Lens

BEFORE mounting the sample, check the objective lens by LCD display, Click on "Microscope" then choose "Objective lens".

Make sure to read and understand the labels on a objective lens and add the correct immersion fluid. 10X and 20X are dry lenses – no immersion fluid!

Clean the objective lens before and after use.

For dry lenses, although no immersion is required, cleaning is still needed at the beginning and end to remove any dirt or accidental fluid.

Follow instruction given by the center staff for cleaning an objective.

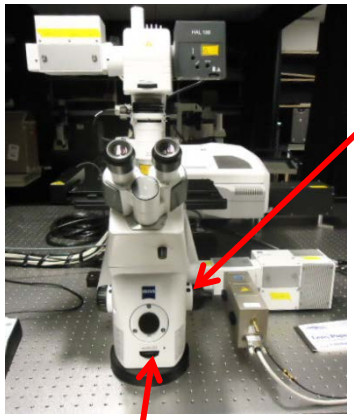
Step 1,2

Set up your specimens on the stage and select field of view

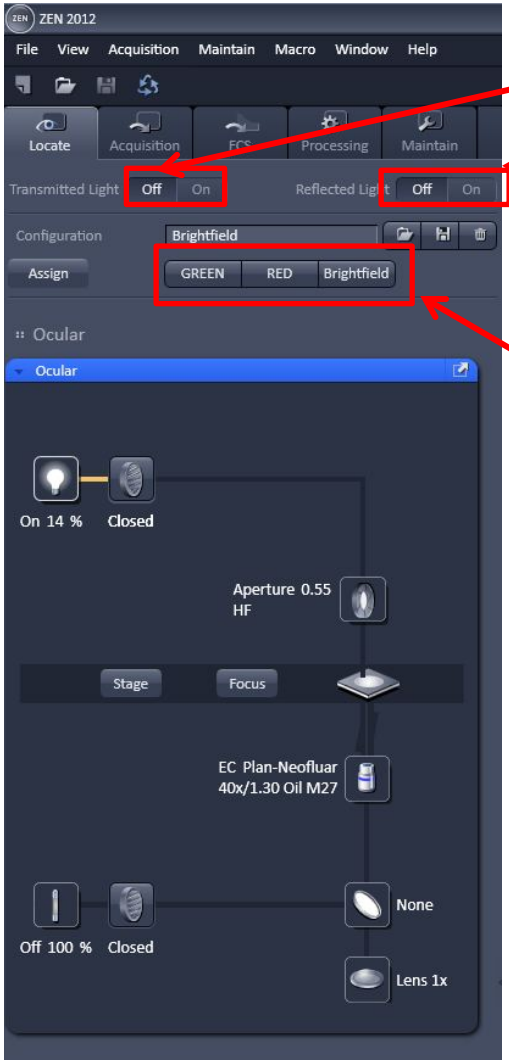
NOTE: Be careful to lift the pillar up and back when you put your specimens on the microscope stage.

Grab here to lift the pillar, if necessary.

NOTE: adjust focus by rotating knob.



Adjust the intensity of bright field illumination.



Turn off "Brightfield"

Turn off "Arc lamp"

Select "arc lamp" or "bright field" cube

Normally Green, Red cube will be there.

If you want other color, ask KCCI staff.

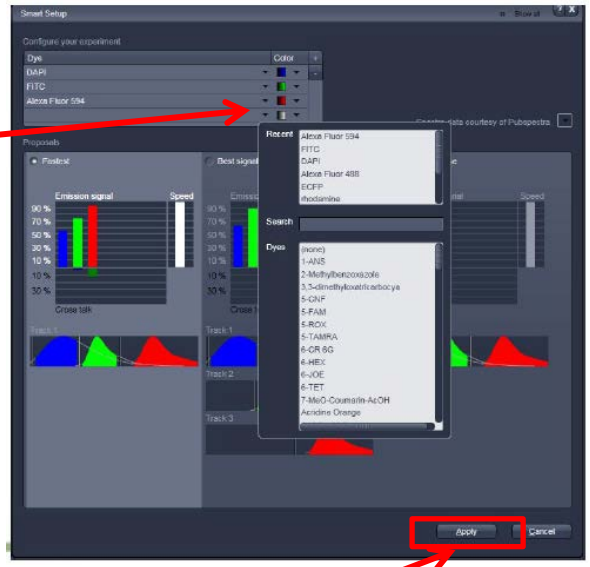
Step 3a

Smart setup

(In acquisition mode)

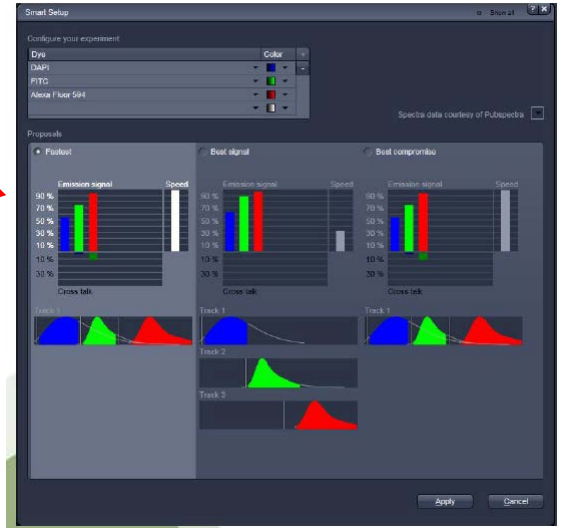
Automatic choice of dyes

Select your dye(s)



Click "Apply" to implement the conf.

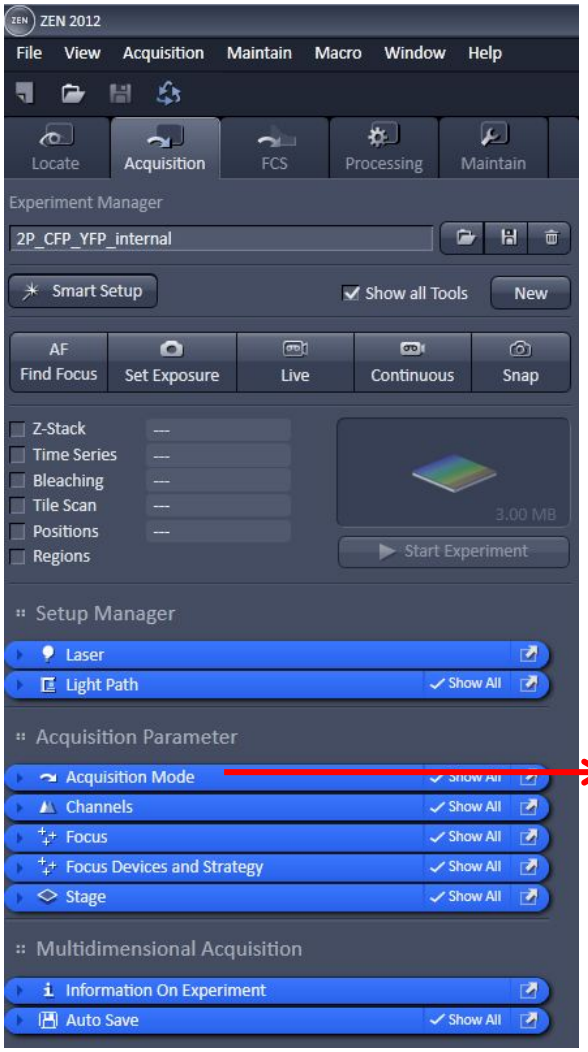
The Smartest configuration will also select lasers and emission filters automatically and configure the light path.



Depending on your imaging needs, the smart setup will select four different dye emissions.
Fastest, best signal and smartest.

Minimum adjustment of size of the image, laser power, PMT gain should made in next steps.

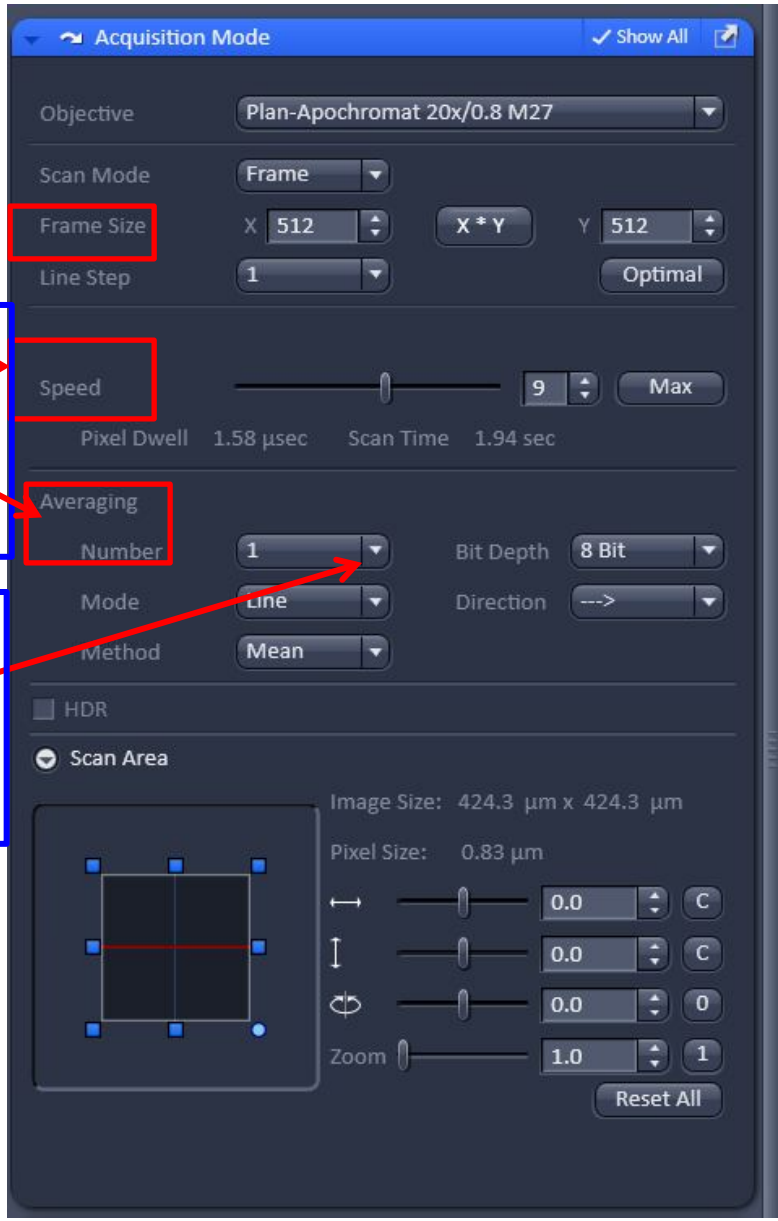
Step 3b Open Acquisition Mode



Frame Size
Adjust if necessary

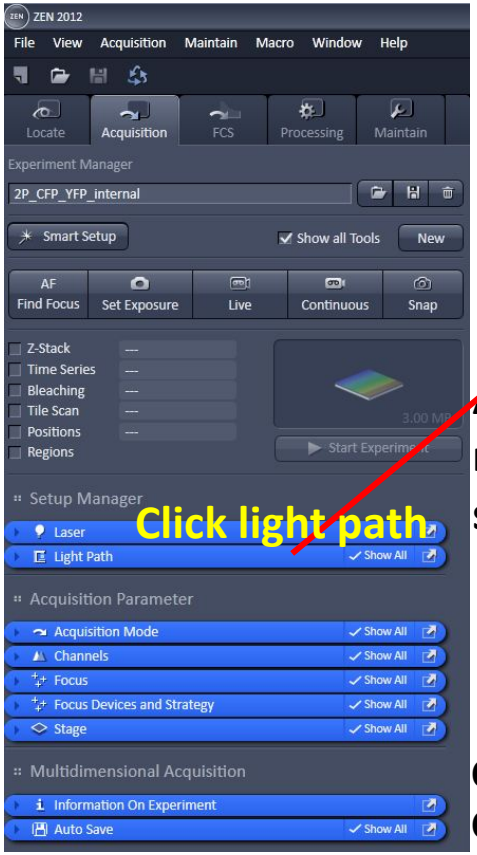
Scan Speed
(9 or 10) and averaging 1 for fine tuning the focus

Select higher averaging for better Signal to Noise ratio for the final image



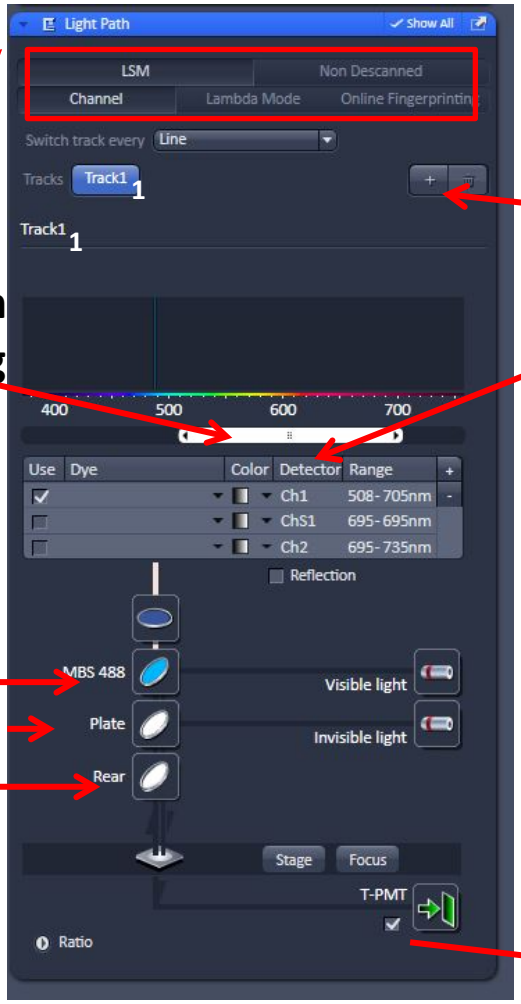
Light path-for 1p descan mode (not needed for smart setup)

Step 3c



Click light path

Adjust Emission range on sliding scale.



Create "Track" for additional laser and detector.

Detectors

Ch1 and Ch2 are normal PMTs. ChS1 is the spectral PMT, e.g. for establishing sample's fluorescence spectrum

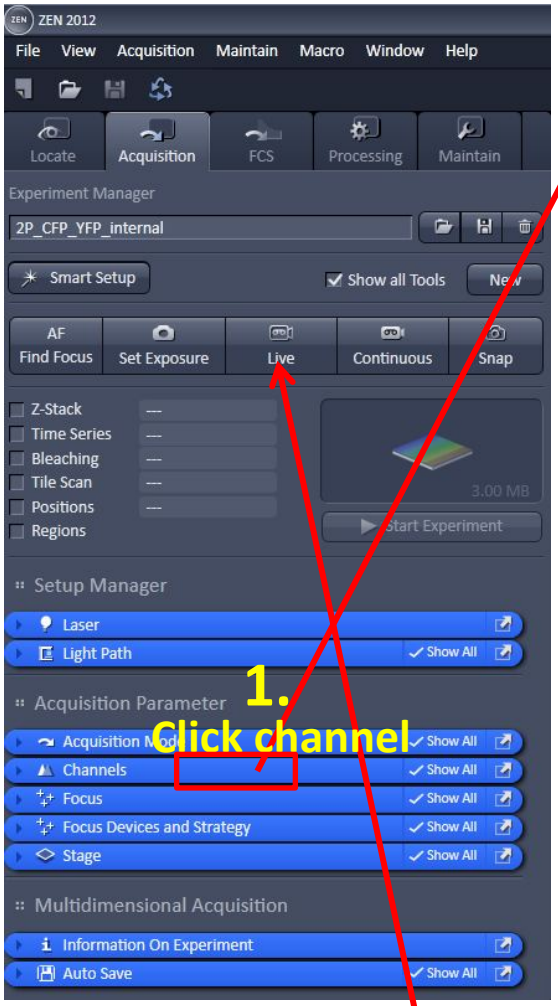
Choose "Filter"

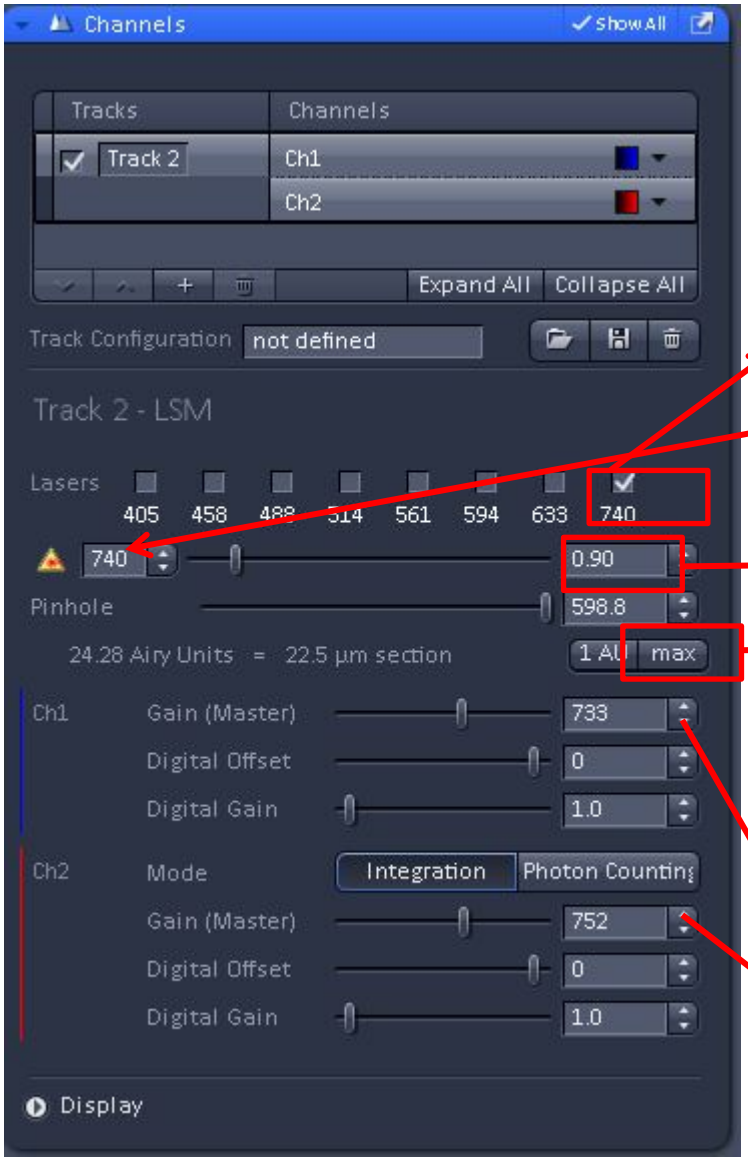
Choose "Plate"

Check Rear

Check Transmitted light Detector if needed.

Channel selection for 1p applications (descan & non-descan)





check laser
Type wavelength
and press "Enter"
wait for 30 sec.

Adjust laser power

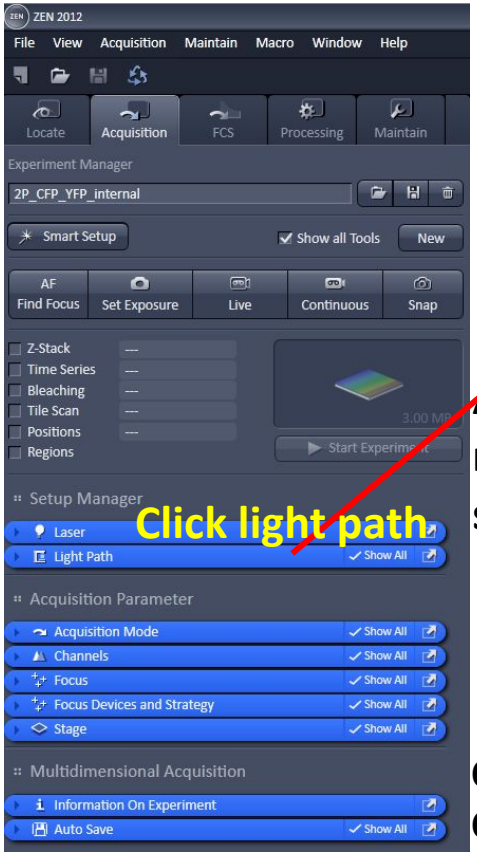
"Max"

PMT gains
(700 is sweet spot)

2. Click "Live" after selecting the laser, pinhole, channels.
In live mode, adjust power, gain etc.

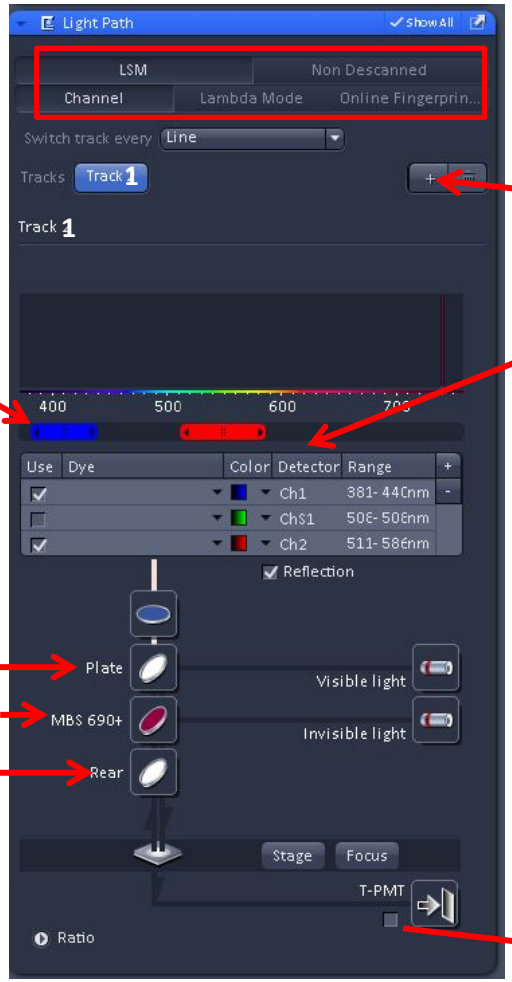
Light path-for 2p descan mode

(Descan configuration is default for 2p intensity using **internal PMTs**)



Click light path

Adjust Emission range on sliding scale.



Create "Track" for additional laser and detector.

Detectors

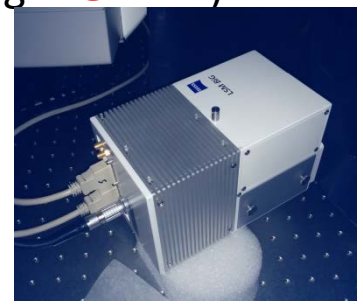
Ch1 and Ch2 are normal PMTs. ChS1 is the spectral PMT, e.g. for establishing sample's fluorescence spectrum

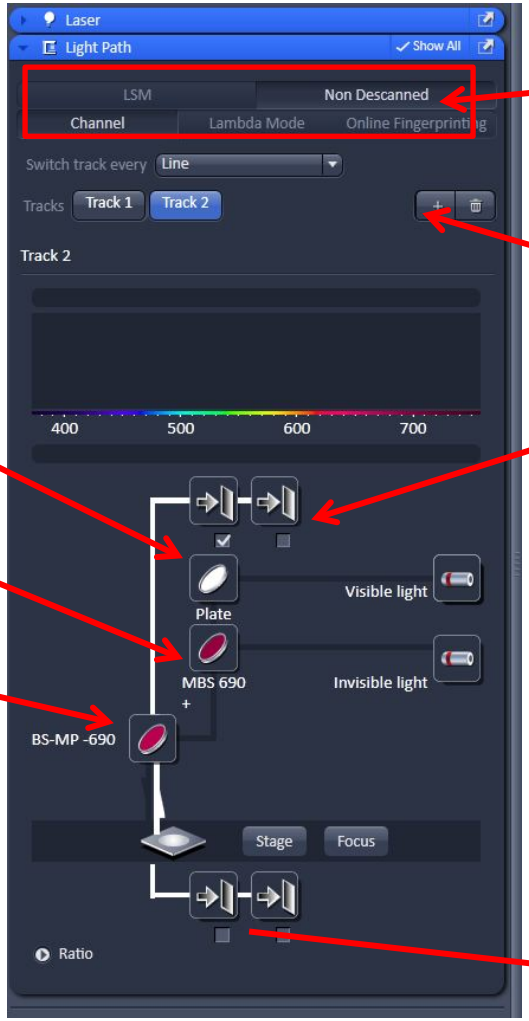
Choose "Plate"
Choose MBS 690+
Check Rear

Check Transmitted light Detector if needed.

Light path-for 2p non-descan mode

(Non-descan configuration is for high sensitivity/weak fluorescence signal using **BiG NDD**).





Select "**Non Descanned**" for 2P with BiG NDD

Create "**Track**" for additional channel.

Choose "**Plate**"

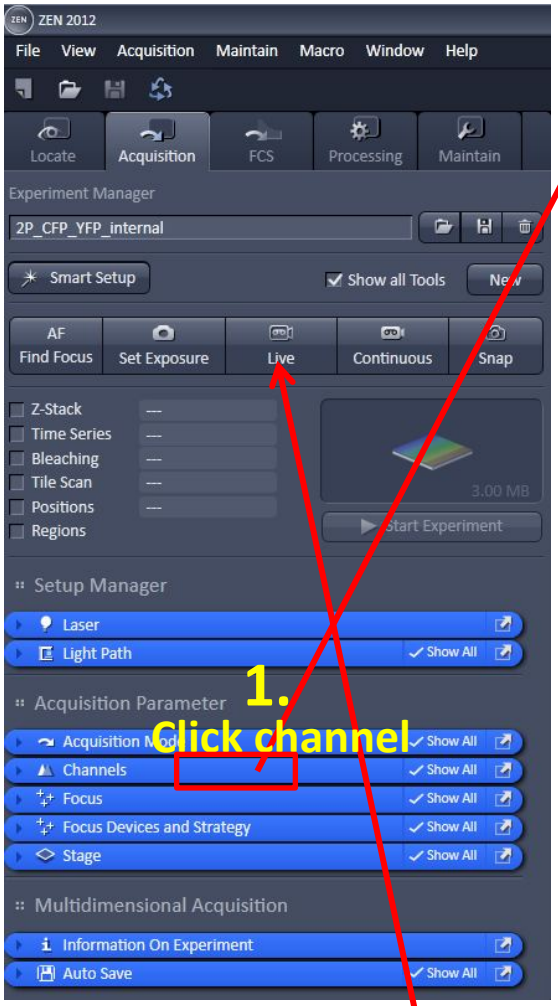
Choose "**MBS 690+**"

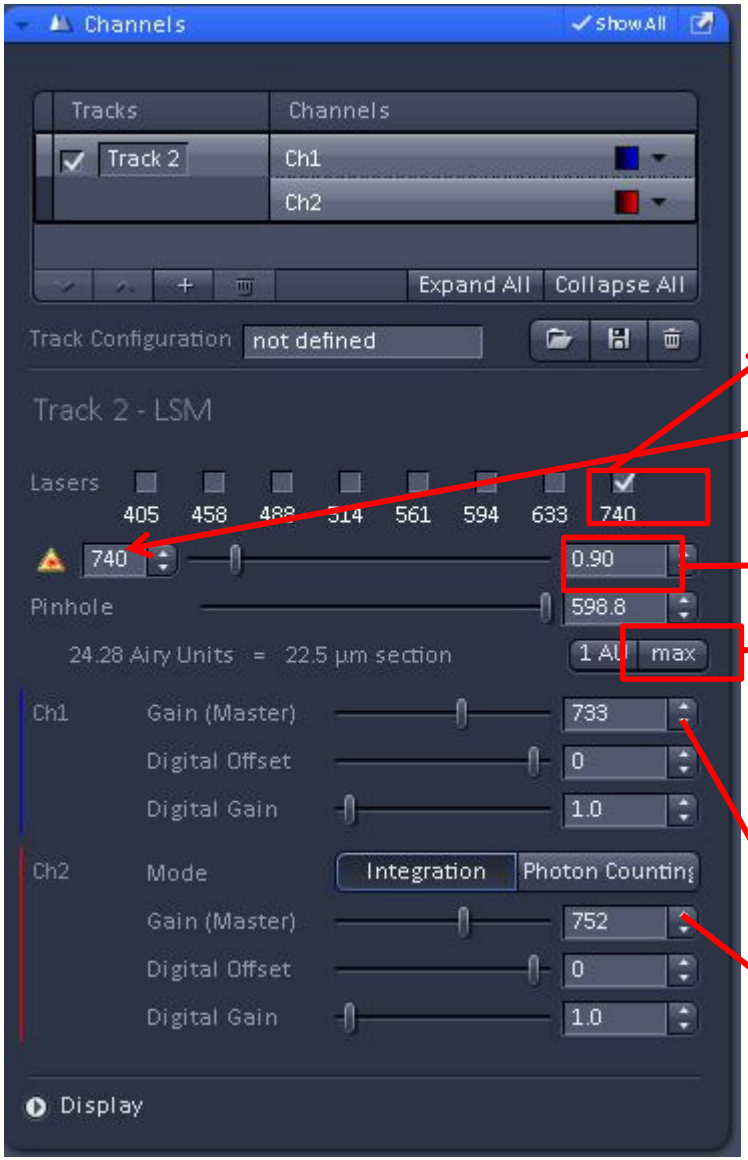
Select "**BS-MP 690**"

Check one or two "**BiG NDD**" PMTs.

Check for **Transmitted light detector** if needed.

Channel selection for 2p applications (descan & non-descan)





check laser
Type wavelength
and press "Enter"
wait for 30 sec.

Adjust laser power

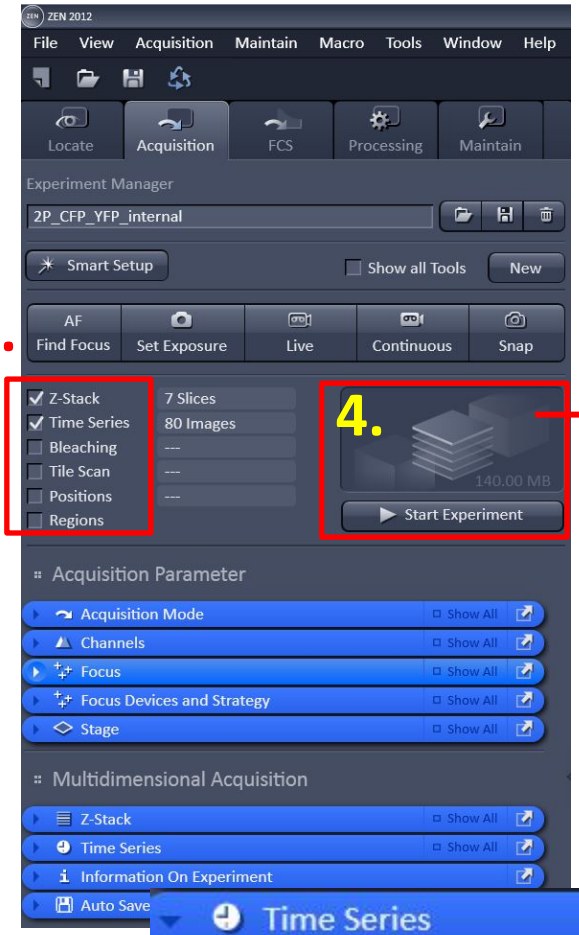
"Max"

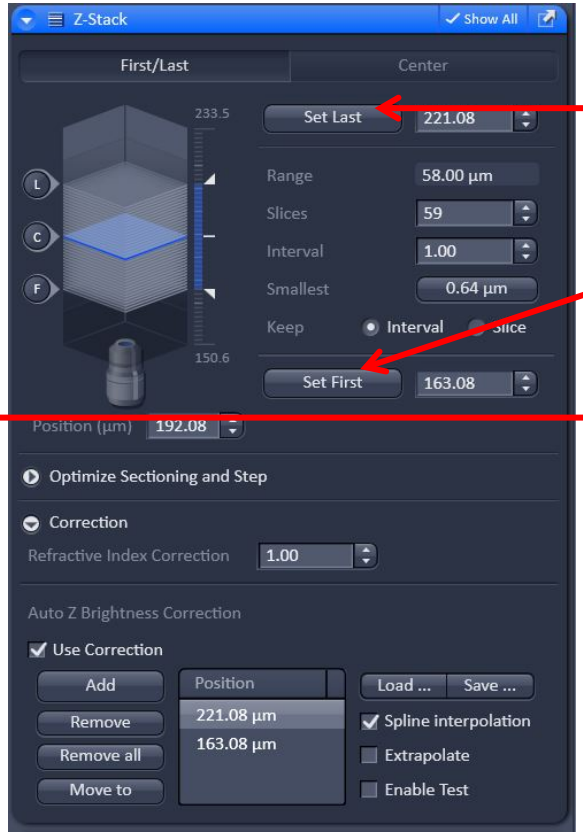
PMT gains
(700 is sweet spot)

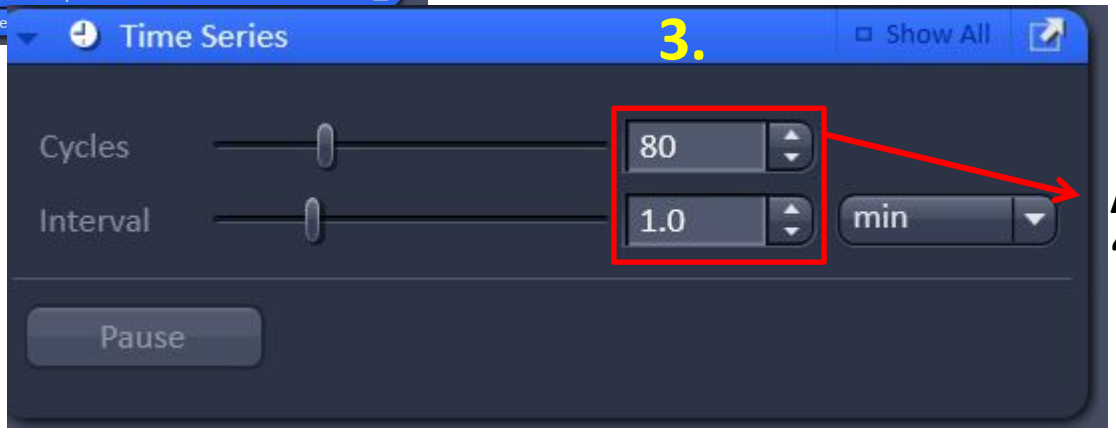
2. Click "Live" after selecting the laser, pinhole, channels.
In live mode, adjust power, gain etc.

Step 5

Z stack and Time series







2.

In "Live" view > move focus to the end > "set last"

> Move focus back to the other end > "set first"

Adjust the slice and interval.

➢ **"Start the experiment"**

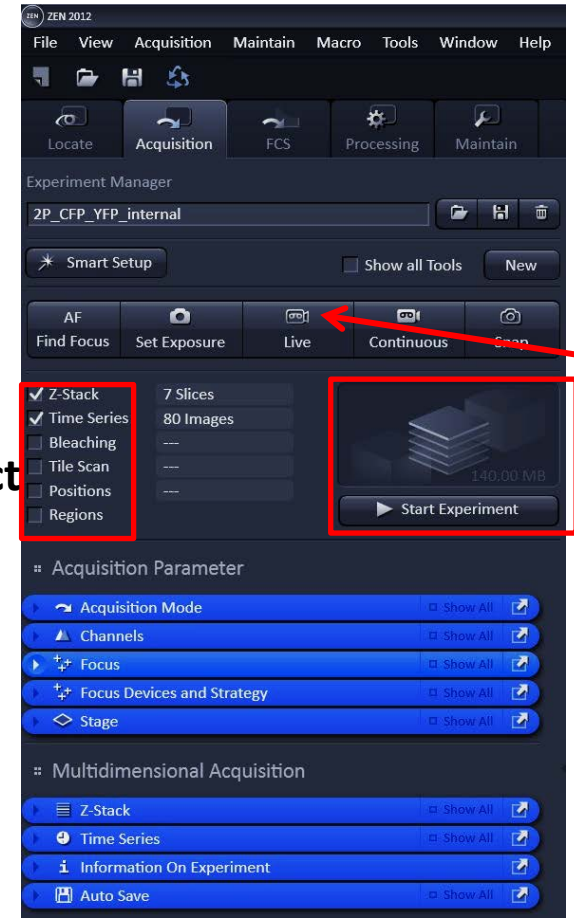
Adjust the slices, interval, Cycles depending upon the sample.

Adjust time interval

"Start the experiment"

Tile scanning (stitch) For a panorama view

- ✓ Apply stitch mode for specimen (e.g. tissue) where a 10X or 20X dry objective is sufficient.
- ✓ View a complete image for the stitch area to determine where to start the first frame.
- ✓ In "**Live**" mode acquire the first frame by clicking "**Add**", move the stage to the adjoining area for 2nd frame, click "**Add**" and so on. Finally click "**Start experiment**" for all frames.



1. Select Tile

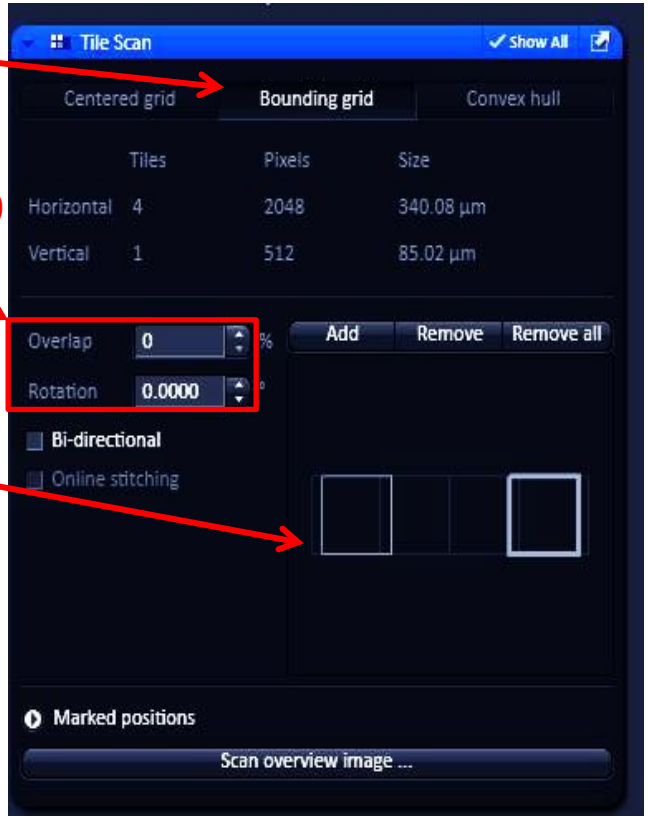
Select "Bounding grid"

2. Overlap 5 or more, Write rotation -0.4849

3. Click "Live"

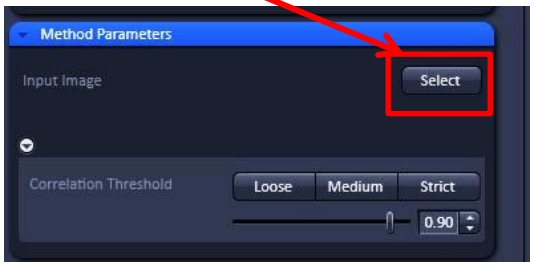
4. Click "Add"/ frame

5. Start experiment



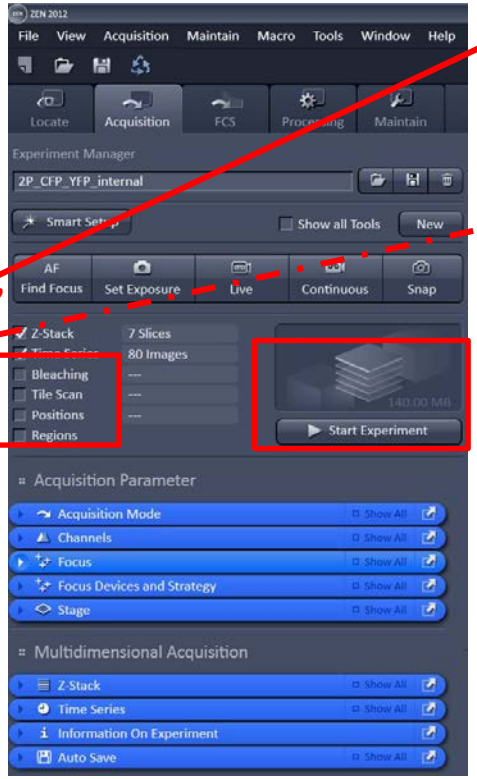
Processing the raw stitch image

Before “save”: Processing > Method > Stitch > Select > 0.90 > Apply (for stitched image)

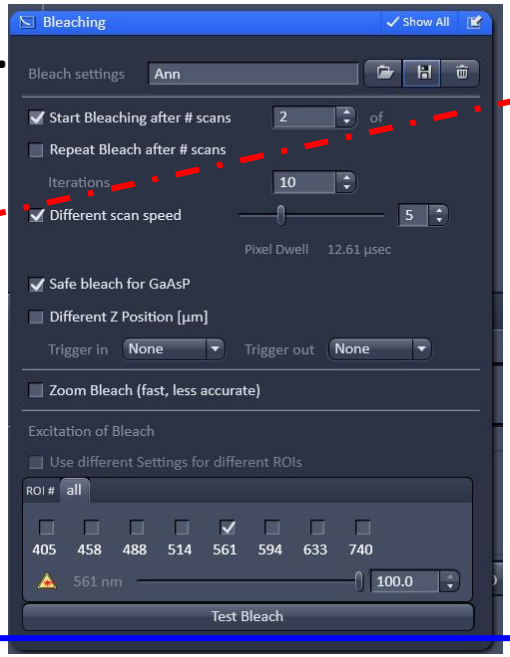


Bleaching setup (a more complete description is on the table)

1. Select **Bleaching, Positions, Regions.**

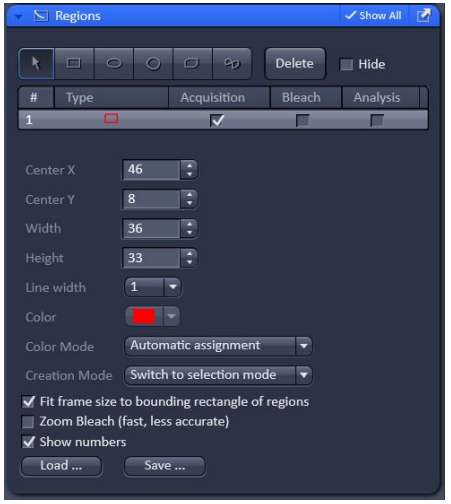


2.



3.

4.



2. Select laser, adjust iteration based on degree of bleaching of your sample.

4a. Acquisition: selection of a section of image containing ROIs to be bleached. Draw acquisition ROI.

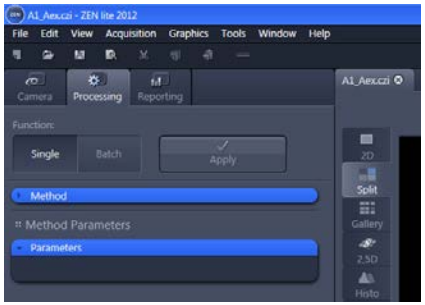
4b. Bleaching: Draw bleaching ROI shapes inside the acquisition ROI using shape selection. Selected ROI coordinates will be appeared in position window.

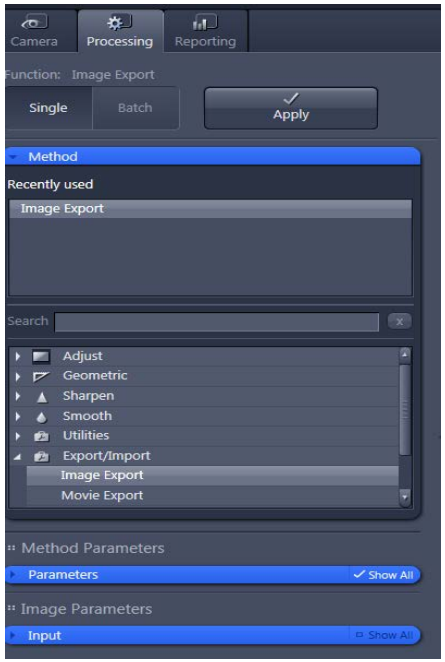
"Start Experiment"

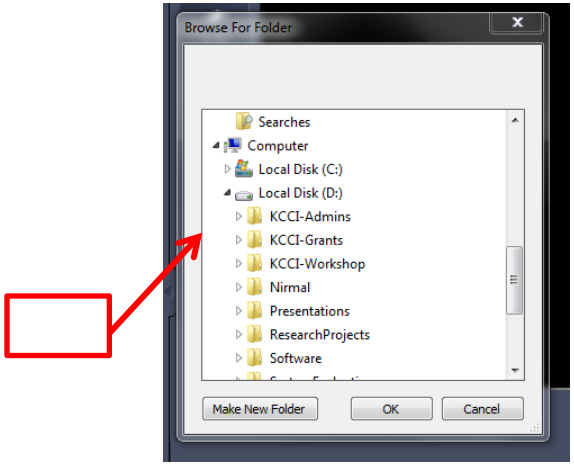
How to save and export images

Save images in **Zen black software** as .tiff, .jpg, .czi etc. In addition, we recommend **saving original data as .czi** format. To process .czi files download the free **Zen blue software** from Zeiss website.
http://www.zeiss.com/microscopy/en_de/products/microscope-software/zen-lite.html

Steps how to export images as .tiff, .jpg etc.







File > Open > .czi file > Processing > Method > image export > parameters > check format > individual channels image > create folder.

Shut down the System

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1. Exit the "ZEN" software.
2. Export your data if needed (do not use a USB stick – use the server)
3. Record the ending time and submit the usage time.
   NOTE: Make sure to choose Zeiss 2p if you use the 2p laser.
4. Copy your data to the K drive for your data transfer.
5. Shut down the computer through software.
6. Turn off the "Arc lamp (6)"
7. Switch off the Laser (5).
8. Switch off the Components (4).
9. Switch off the System/PC (2).
10. Wait for 1-2 minutes.
11. Turn off the "Main Switch".
12. Clean the objective lens and stage.

Auto Z brightness setup

Linear change of laser power from last/first.

In "Live" view > move focus to the end > "set last"
Stop Scan > check "use correction" > add position.

Move back to the other end > "set first" Stop Scan > add position.

Once you set up power, Z position
➢ **"Start the experiment"**