



Observing Life As It Happens

QUICKSTART GUIDE: CONFOCAL 5: ZEISS LSM 510 INVERTED (SAFB 408)



Start-up procedure	2
Switching on the lasers	2
Observing your specimen through the eyepiece	2
Adjusting brightfield Köhler illumination	3
Acquiring images with the Zeiss ZEN software	3
Scanning.....	4
Creating a new Setup (Select "Acquisition" tab	4
Saving/ Exporting data.....	6
Z-Scan	6
Time scan	6
Shutdown procedure	7
Additional information	7
Control buttons on the left side of the microscope:.....	7
File formats (selection):.....	7
Offline image viewer for Zeiss LSM files	8

Start-up procedure

Turn on in this order

1. Main power switch (high voltage switch on the left wall if you are standing in front of the microscope)
2. On the panel at the right side of the microscope:
 - Components
 - System/ PC
3. Start PC (under microscope)
4. Run ZEN 2009
5. Click "Start System"

Switching on the lasers

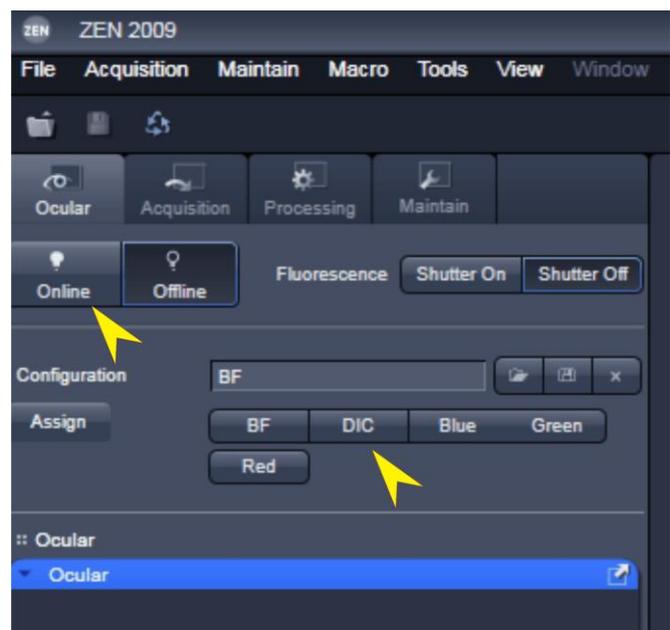
- In the software: select "Acquire Tab" and "Laser" and expand laser properties
- For the HeNe543 and HeNe633: click "ON"
- For the Diode 405-430: click on "Standby", after "Warming up" changes to "Ready" (usually very fast) click "ON"
- Argon laser: click on "Standby", after "Warming up" (Status) changes to "Ready" click "ON" and adjust the "Output %" so that you get a tube current of 6.1A



Observing your specimen through the eyepiece

Go to the **Ocular tab**, click **online** and choose one of the pre-sets for viewing:

- "BF" (Brightfield),
- "DIC",
- "Blue" (Fluorescence).
- "Green" (Fluorescence).
- "Red" (Fluorescence).



Guide to using the new LED Systems for viewing fluorescence down the eyepieces



In addition to the microscope controls

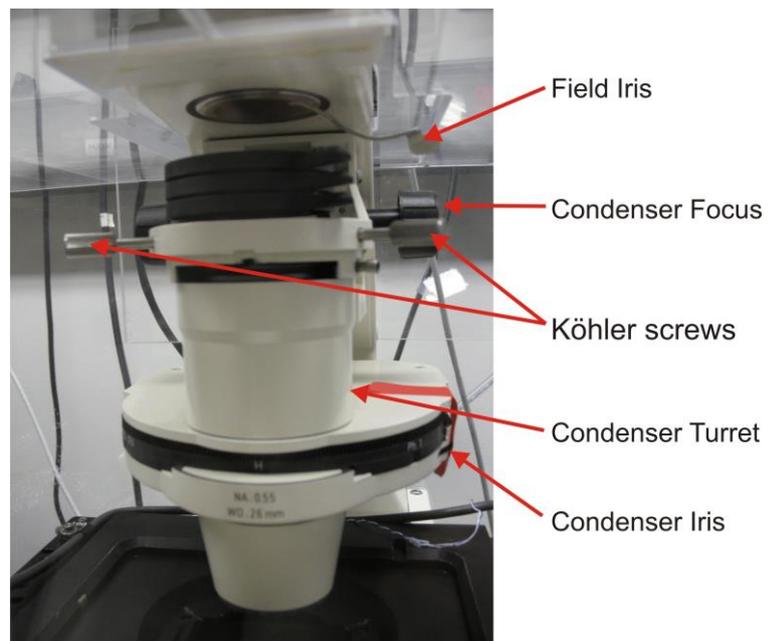
- Select all or individual channels using the select buttons
- Use the on/off button to switch on/off
- Use the +/- buttons to change intensity

!! Please remember to switch off after viewing and at the end of your session !!

Adjusting brightfield Köhler illumination

always recommended, essential for transmitted light confocal mode

- Select "Ocular" tab & select brightfield (BF)
- switch to 10x objective
- adjust brightness with large black button on front / bottom of microscope)
- make sure condenser iris is not completely closed (side of condenser wheel)
- focus on your sample
- completely close field iris (top silver lever) (if the image turns completely black, reopen until you see some light, proceed to next step and close again after you have focussed the iris)
- focus iris (black condenser focus wheel)
- centre iris (2 silver Köhler screws)
- reopen field iris until the edge is just not visible anymore
- readjust every time you switch to a different objective



Acquiring images with the Zeiss ZEN software

After starting the software you can either open an old image and click "Re-Use", open "Experimental Settings" or create a new setup

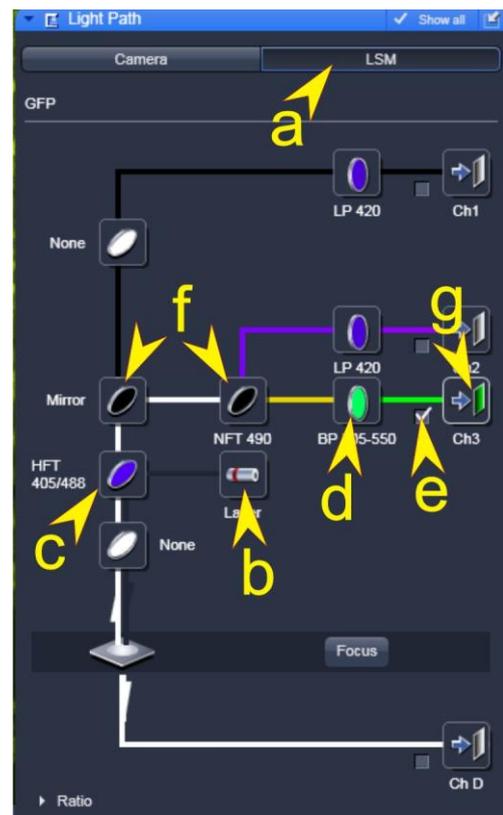
Due to some issues with the visible light shutter, **run one of the visible lasers (488, 543, 633) first** whenever you start ZEN, before running the 405 laser.

Scanning

There are 2 scanning modes:

<u>Single Track</u>	<u>Multi Track</u>
<ul style="list-style-type: none"> ○ use for single, double and triple labelling ○ simultaneous excitation/ emission of all fluorophores 	<ul style="list-style-type: none"> ○ use for multiple labelling with fluorophore crosstalk ○ sequential scanning (line by line or frame by frame)
<u>Advantages</u> faster image acquisition	<u>Advantages</u> selective laser/ detection channels for each fluorophore, one at a time, avoids crosstalk
<u>Disadvantages</u> possible crosstalk between channels	<u>Disadvantages</u> slower image acquisition

Creating a new Setup (Select "Acquisition" tab)



Under "Acquisition" tab select "Imaging Setup" and "Light Path"

In the Imaging setup

- select first Track

In the "Light Path"

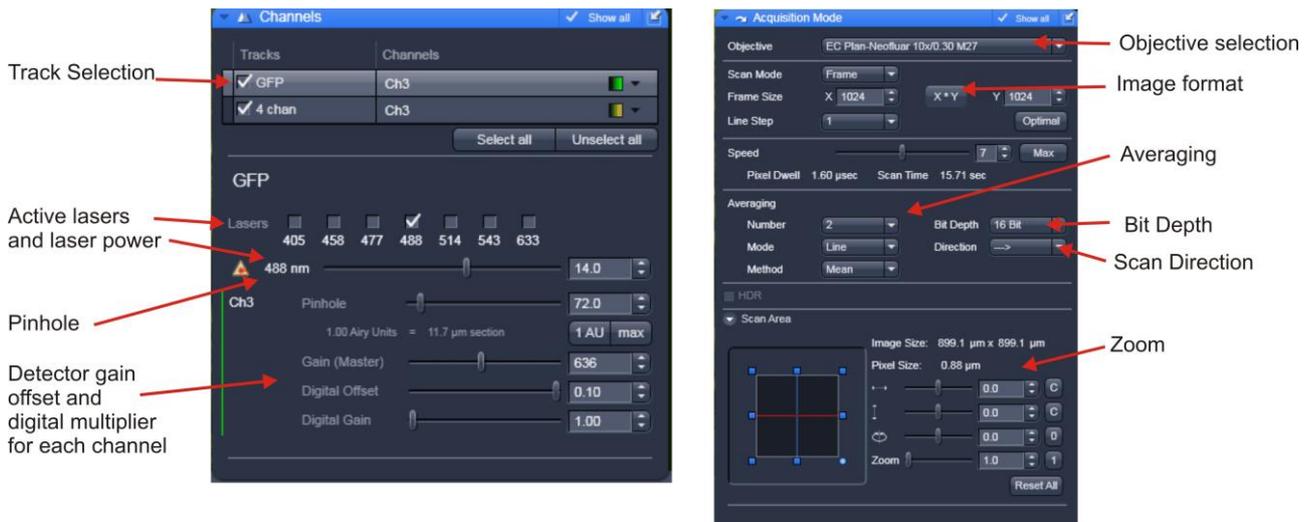
Select "LSM" (a)

Select required laser (b) and relevant dichroic (c)

Find a suitable emission filter (d) and detector (e)

- HFT: reflects the indicated wavelengths, all other wavelengths pass
- NFT: reflects the indicated and all shorter wavelengths, longer wavelengths pass
- BP: wavelengths of the indicated range pass, others are blocked
- LP: the indicated and longer wavelengths pass, shorter wavelengths are blocked

Change light path to detector (f) and the colour overlay for the detector(g)



For Multi Track mode select the “+” in the Imaging setup to add a new T rack. Select the new track and repeat the selection in the “Light Path” tab. Repeat for each new channel required. NB it is recommended (In Line/ Frame Fast mode: essential) to keep the settings for dichroics and filters constant (each change needs additional time during acquisition)

Each track can be stored using the save button in the imaging setup window. To store the experiment settings (all tracks) use the save button above the “Acquisition” tab

Experiment settings can also be restored by loading a previous image and using the “Re-Use” button

In “Acquisition Mode”

- Select the frame size, line step should be 1 and the objective is recognized automatically
- Select bit depth (8, 12 or 16 bit), scan direction (→ is recommended) and average mode (higher averaging gives you a better image quality but increases sample bleaching)

In the "Channel" tab

- Select each of the channels in turn (de-select tick of other channels and highlight bar) and adjust the following settings separately during “Live” scan.
- Pinhole - pressing "1" (for 1 airy) gives you a good starting point (A trade-off between z-axis resolution and brightness. (A pinhole of one Airy unit gives essentially gives you the best resolution, opening it slightly from there will allow you to collect more signal. With each new setup/channel pinhole needs to be set)
- Adjust Laser power (Higher laser powers will give less noise but increase photobleaching. The lowest value you can get away with is best to minimize fluorophore saturation and phototoxicity.)
- Adjust the "Gain (master)" so that just a few saturated pixels (shown in red) are visible
- Adjust the "Digital Offset" so that just a few black pixels (shown in blue) are visible.
- NB. (To help adjust gain and offset select the "Range Indicator" palette from the "Image Display" window)
- If you cannot obtain a bright enough image with adjusting the above parameters, turn up the "Digital Gain", otherwise leave it on 1



- Zoom - you can do this with the "Crop" button underneath the image - resize the box that appears - or using the scan area controls in the Acquisition Mode panel.
- Capture image – Select all channels (tick boxes) then press the “**Snap**” button to acquire an image with the settings you have specified above

Saving/ Exporting data

Note: Snap will overwrite the current image unless it is first saved. Images are not saved automatically.

Select image from view in right-hand window, select save icon, save to disk. Open images that are not yet saved have a warning icon next to them. Images are usually saved as ZEN format (.czi) files but can be saved as LSM (for those wishing to use the old LSM Browser) or exported as TIFF. Generally it is best to save to the local E:/User Data drive and after your session move the data to a server, USB drive etc. after you have closed the software.

Z-Scan

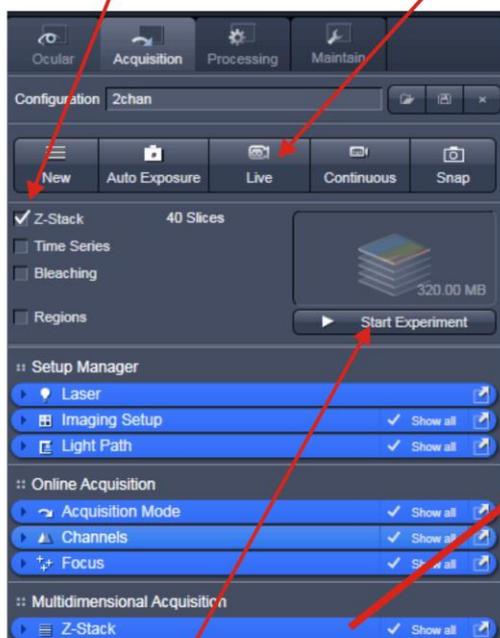
Follow steps 1-7 in the diagram below.

1) Check the **Z-stack** box and expand Z stack window

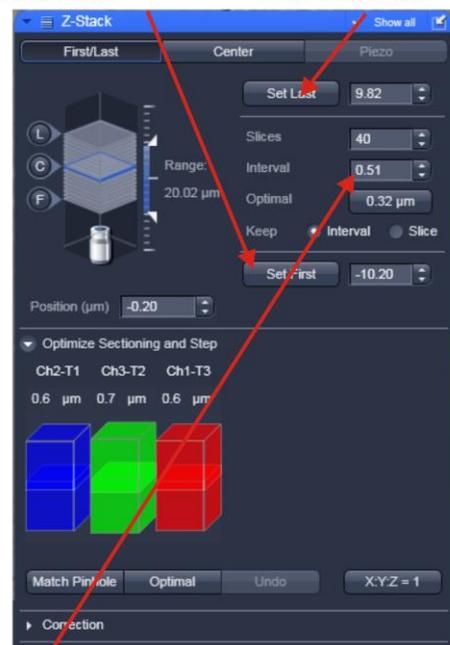
2) Click the **Live** button

3) Adjust the focus to the bottom of the specimen then **Set First**

4) Adjust the focus to the top of the specimen then **Set Last**



1) expand window



7) Click **Start Experiment** to collect Z stack

6) Select either optimal slice, interval or number of slices as desired

5) Click the **Live** (now Stop) button to stop scanning

Time scan

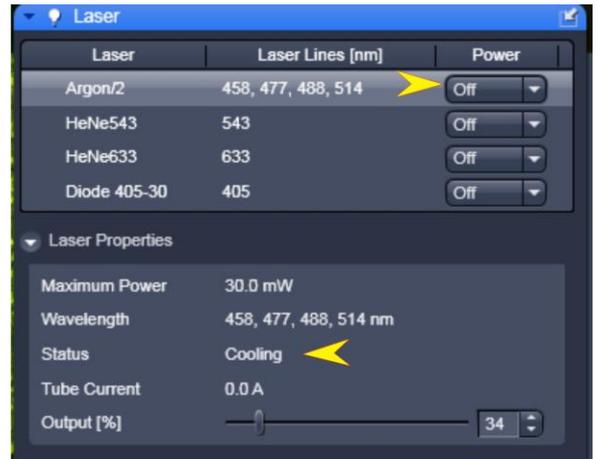
1. Select the **Time Series** box underneath the Z-series box of above
2. Choose the number of time points and the interval between them
3. Press start experiment

Shutdown procedure

- check if anyone is booked after you within 2 hours

If nobody is booked within two hours:

- go to "Laser"
- select lasers and switch them off
- **for the argon laser:**
- switch off
- wait until "connected" is displayed instead of "cooling"
- now you can proceed with shutting down the software
- go to "File" - "Exit"
- update the time in Sharepoint
- shut down computer
- turn off everything in the reverse order of the Startup Procedure
- remove specimen
- if oil was used, clean objective with fresh lens tissue



If someone's booked within two hours:

- update usage in Sharepoint
- remove your samples
- clean objective lenses with fresh lens tissue and close incubation chamber
- clear up the desk
- save files onto the server or on a mobile harddrive
- log off

Additional information

Control buttons on the left side of the microscope:

bottom button: cycles between frontport (empty) <> eyepiece <> baseport (camera)

middle button: switches between sideport 100% (empty) <> 50/50 <> eye/ front/ base 100%

top: Optovar in / out



File formats (selection):

LSM: original data with original bit depth and all enhancements and metadata

TIFF: raw (original) data with original bit depth, loses all enhancements and metadata

TIFF for publication: 8-bit screen version of your displayed dataset

- SAVE AS: all selected layers in 1 file as TIFF layers
- SAVE AS MULTIPLE: all selected layers in separate files

Movie format: AVI

Offline image viewer for Zeiss LSM files

ZEN Lite freely available, see link on FILM homepage

FIJI (Image J)