Using the Zeiss LSM700 Confocal Microscope: ver ja13b Jeremy Adler 2021

Summary

Confocal or Widefield microscopy

Point scanning confocal microscope
Moves a spot of light over the specimen, building the image (slowly) pixel by pixel.

Heating

Objectives air 5x, 10x, 20x, 40x
water 63x
oil 63x

4 lasers 405, 488, 555, 637 nm

3 detectors (PMTs)-
2 fluorescence
1 transmitted light

Motorized stage (X, Y and Z movement)
Z stacks of images- 3D dataset
images from multiple points
tile scans - large image by combining many individual images

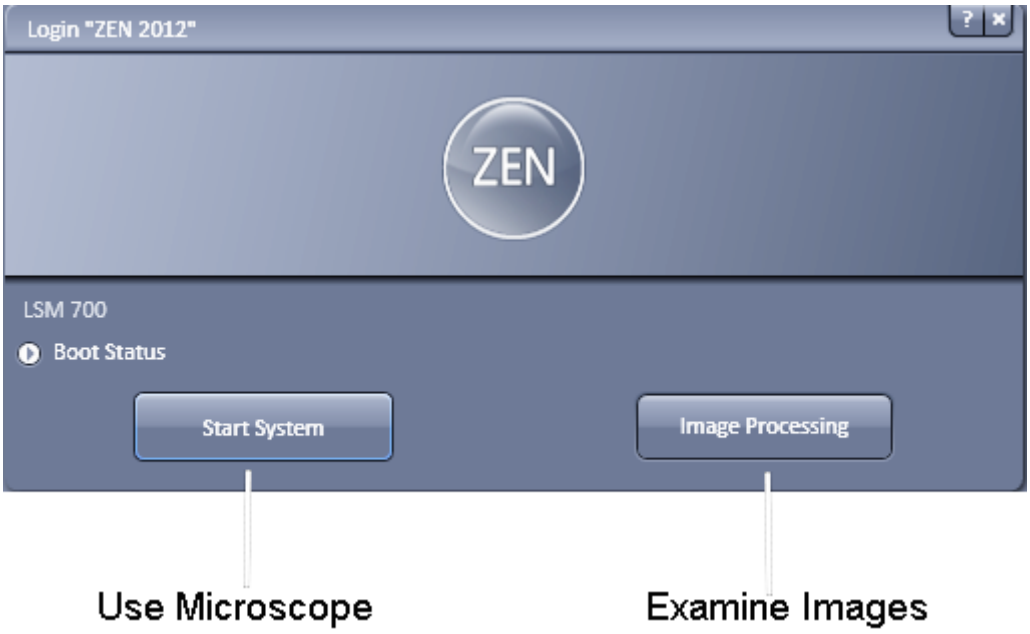
Objectives on LSM700* and Nyquist**

objectives		Wrk dist	Widefield	Nyquist	Confocal Nyquist		Camera Pixels	
	NA	mm	x,y nm	Z nm	x,y nm	Z nm	x,y nm	xy Nyquist ratio
5x	0.16	18.5	812	20181	381	9469	908	0.89
10x	0.5	5.2	260	1940	122	910	454	0.57
20x	0.8	0.55	162	650	76	305	227	0.71
40x	0.95	0.25	136	378	64	177	114	1.19
63x oil	1.4	0.19	92	277	43	130	72	1.28
63x water	1.2	0.28	108	348	50	163	72	1.50

* Other objectives maybe available – ask staff.

** Nyquist, the Voxel size that matches the resolution of each objective

First Zen Black menu – a choice



Note – a free "lite" version of Zen Blue & Zen Black can be downloaded from Zeiss – PCs only

STEP 1 – finding your specimen – the LSM700 as a widefield microscope

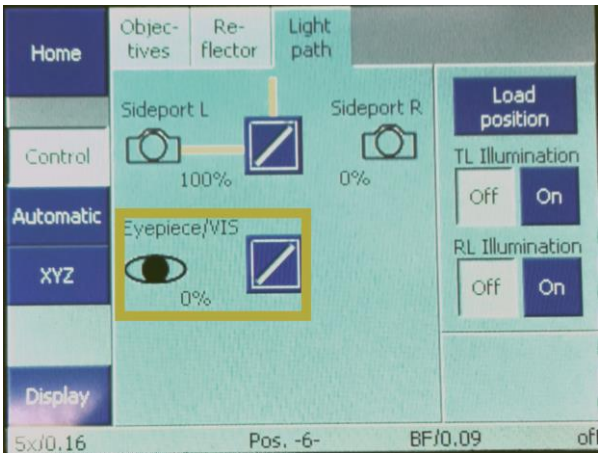
It is difficult to find and focus on a specimen with a confocal – the pinhole removes the out of focus image.

Worst case - a high magnification objective 63x and a low density of cells, so start with the 10x or 20x. The focus position for all objectives is similar.

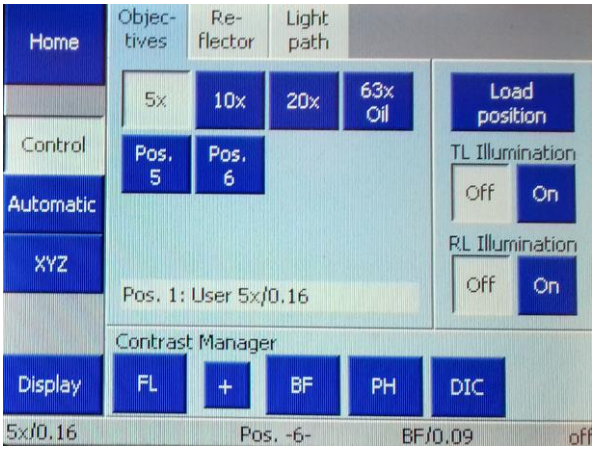
Use **widefield fluorescence** controlled from the stand alone TFT touch screen.

Select "Microscope" from the "Home" menu.

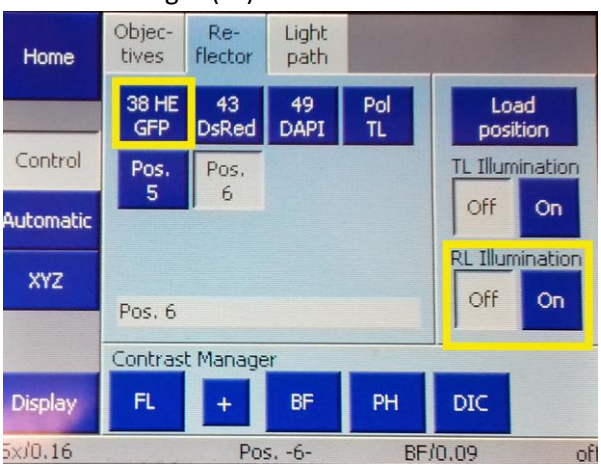
Light path (where the image goes) –select to your eyes.



a) Select an **objective** – the 5x has been selected

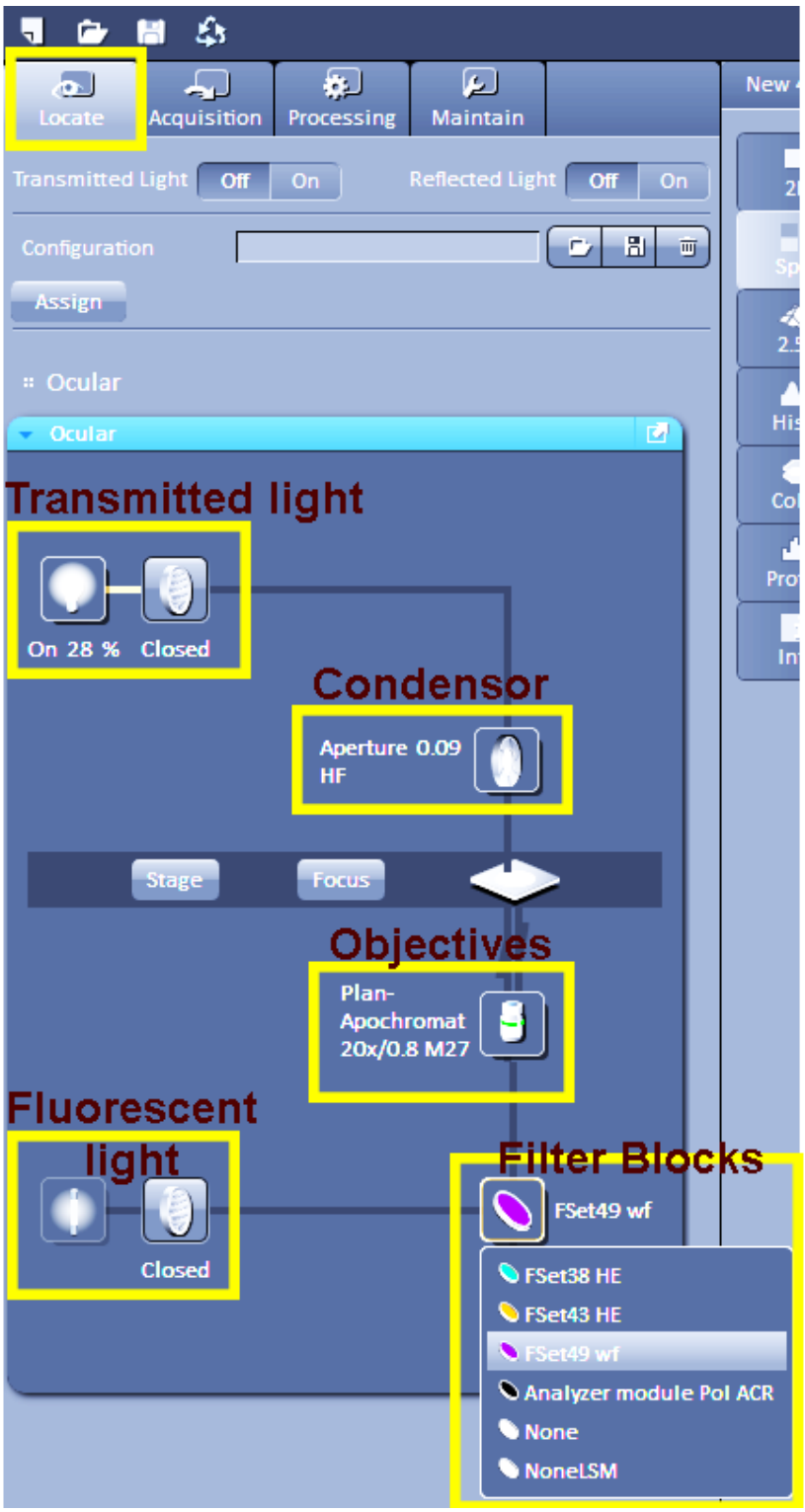


b) Select a **filter block** (GFP shown as selected) and turn on RL (fluorescent light source) or transmitted light (TL). Zeiss calls the filter blocks "Reflectors".



The LSM700 as a **widefield microscope** can also be controlled from the ZEN **Locate Menu**

The Filter blocks pull down menu is shown



STEP 2 – acquiring confocal images: setting up the microscope

The LSM700 as a confocal microscope

a) Fluorescent images – one or two at the same time (track)

b) A transmitted light image – can acquired at the same time as a fluorescence images

c) 4 excitation laser lines - Note the widefield fluorescence lamp is not used

d) Detection uses photomultipliers (PMT: two for fluorescence & one for transmission). The PMT's settings need to be adjusted

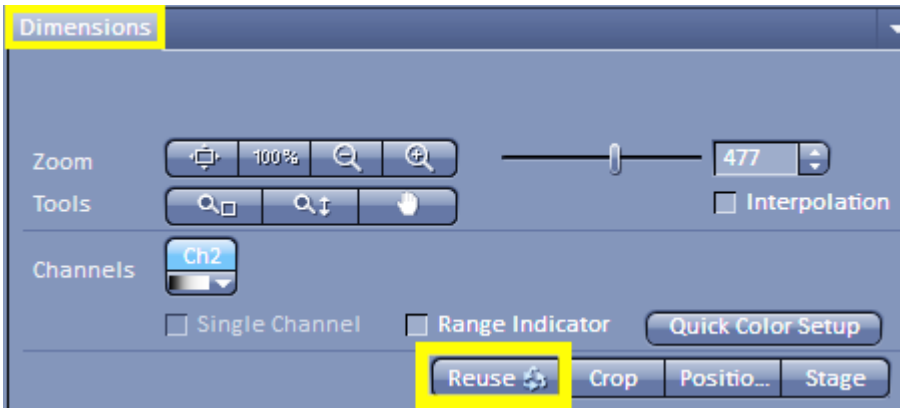
e) The confocal uses a pinhole to reject out of focus light – the pinhole is adjustable

f) The area, size of pixels, number of pixels, scan speed etc are all adjustable.

Words:
A Track – the complete settings for a single acquisition of one or two fluorescent images plus a transmission image. Up to 4 tracks can be created

Initial Setup of the Confocal: **3 options Reuse, Smart Setup, Manual**

a) Reuse previous settings – open your image then the 'Reuse' – in the Dimensions window

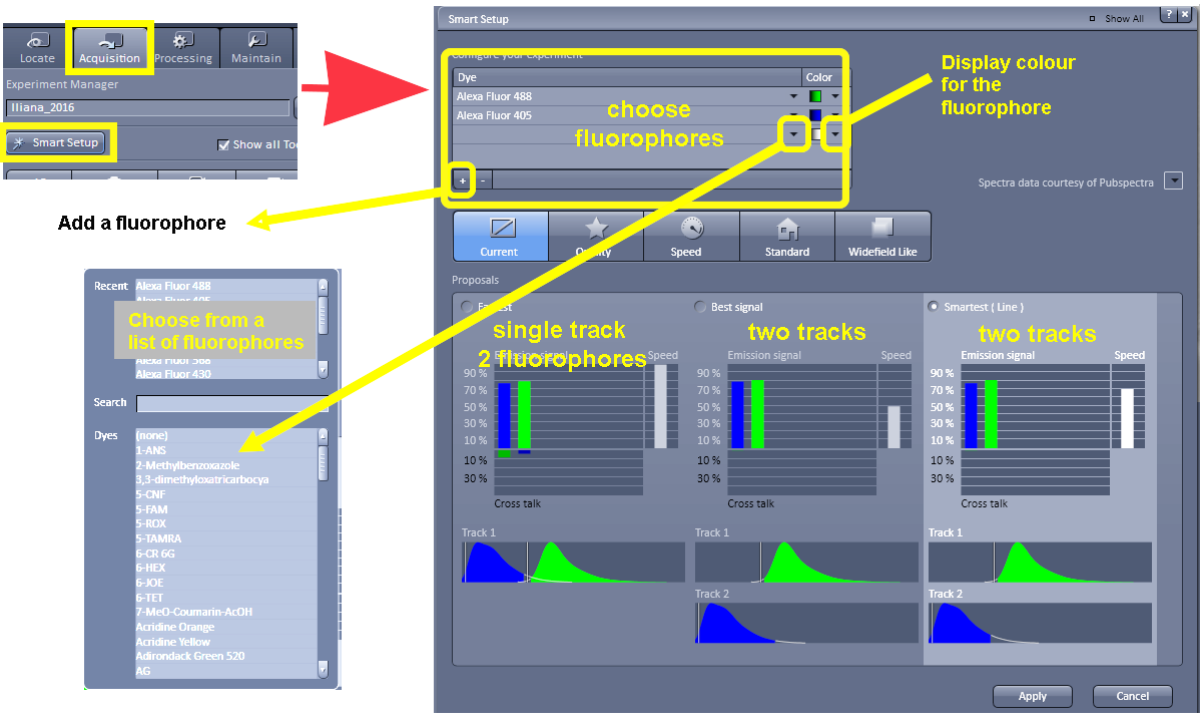


b) Smart Setup: enter your fluorophore(s), ZEN sets up the tracks and offers 3 options – choose.

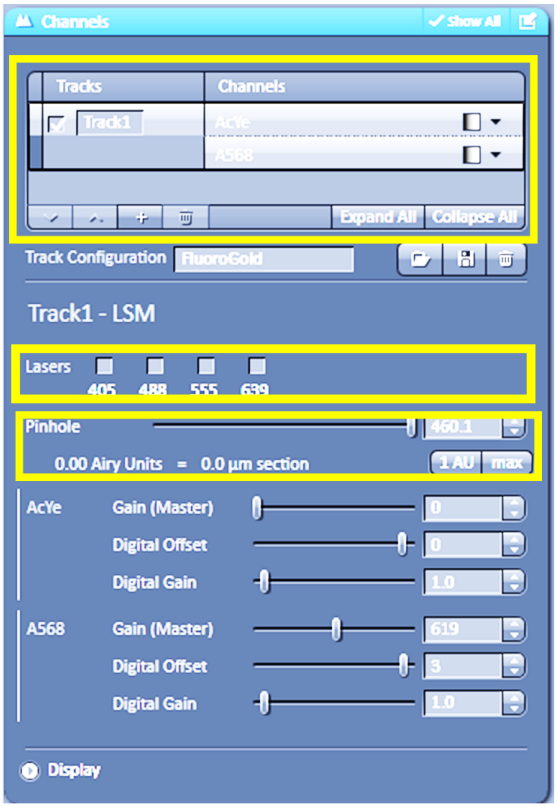
'Fastest' aims to acquire images rapidly by acquiring 2 fluorescent images at the same time, but the separation of photons from the 2 fluorophores may not be perfect.

'Best signal' separates the emitted photons from the two fluorophores. In this case by creating 2 Tracks – which is good, but the image acquisition takes longer.

'Smartest' is a compromise – in this case the same as 'best signal'



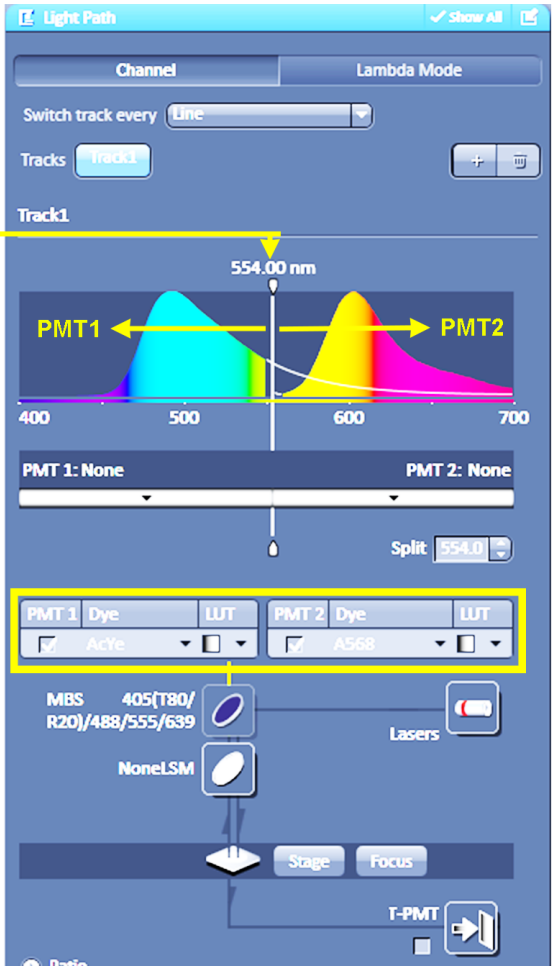
c) Create your own tracks – choose the settings yourself



1) Tracks
Create (+)
one track iwith 2 images is shown

4) Select laser lines - tick box none currently active

5) Set pinhole
1 AU is OK



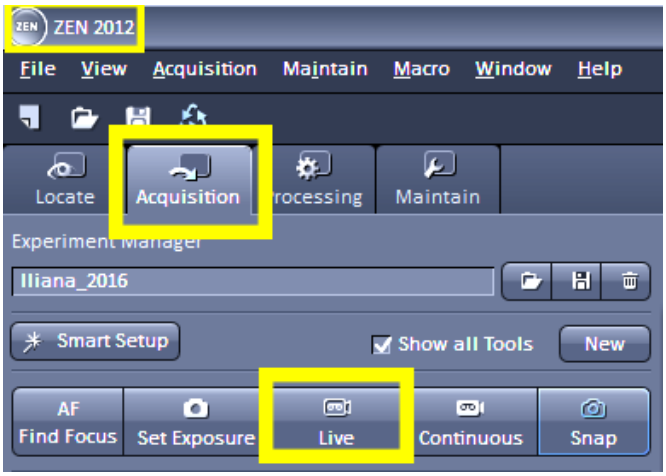
An adjustable dichroic splits the photons between the 2 PMTs
<554 to PMT 1
>554 to PMT 2

3) Set the emission range for fluorescence PMTs

2)
Images - select PMTs (tick boxes)
Both fluorescence images ae active
the transmiosion T-PMT is not selected

Having found your specimen and made the initial settings – START Imaging and make adjustments to the imaging setup – mainly the PMT gain and offset

Activate 'Live' in the 'Acquisition' menu



'Live' is scans quickly which allows for focusing, adjustment s to settings etc

1) Adjust the Z position (focus wheel on the microscope) to put your specimen in the imaging plane – there maybe a difference between the widefield in focus position and the confocal imaging plane.

2) Adjust the PMT settings – the range of intensities in the image should cover the full scale of the digital image (an 8 bit image covers a range of integers from 0-255).

STEP 3 – save images and copy them from the LSM700 computer.

NB – USBs and portable hard drives cannot be used with the LSM700 computer

Instead the LSM700 is locally networked to the a second computer 'Datashare' which is part of the IGP network –

Consider non fluorescent imaging

1) Transmitted light images – may differ for each laser line – images are not confocal and a rotatable polarization filter is available

2) Reflection imaging – best with 405nm laser on the BioVis LSM700, because there is no blocking filter for the 405 line in the light path –why an emission filter is needed for fluorescence

LSM700 Problems

Ask: Jeremy 070-1679349 **Matyas** 070-1679083 **Pacho** 070-1679338

1) Microscope does not work:

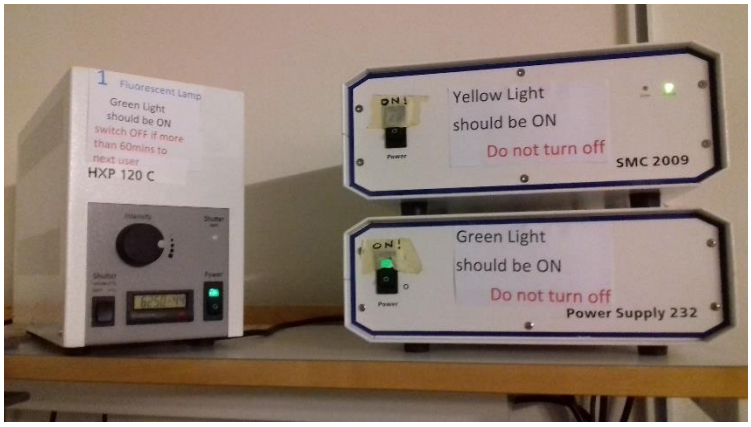
check the if all power switches are on

i) Two power blocks – on the desk

ii) Fluorescent light source – if you need it – on the shelf

Is the power ON

Is shutter (bottom left) in the out position



iii) Microscope power supplies - on the shelf

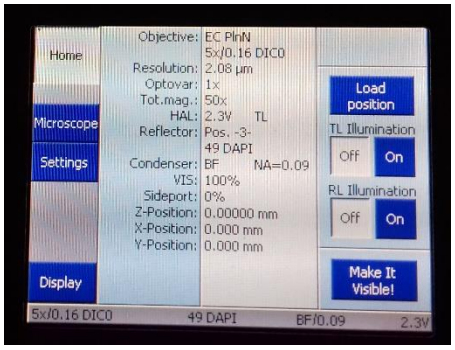
iv) No lasers – check is the laser box ON

2) No fluorescence seen by eye

i) is the Fluorescence light source OK – select GFP block, can you see blue light

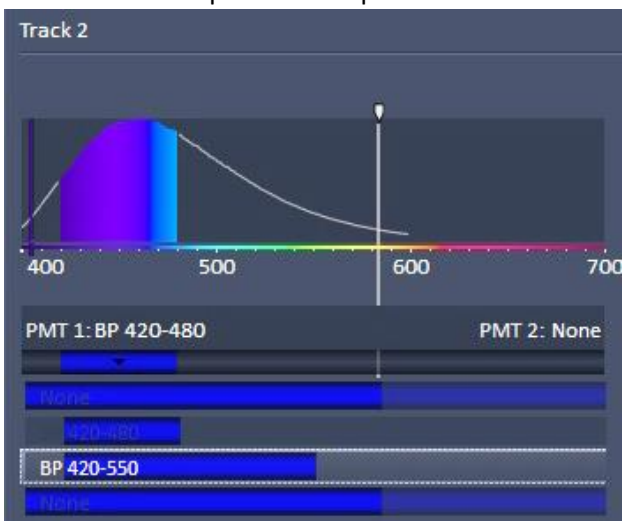
ii) check light path - on TFT screen, where is the image going, try Make It Visible

iii) Note Humans cannot see Far-red and Infra-red wavelengths



3) Dapi (405 laser) image is odd – a reflection image?

i) Is one of the band pass filter in place –to block reflection imaging



ii) A weak image, Turn up the laser power, the 405 excites Dapi inefficiently or open the pinhole

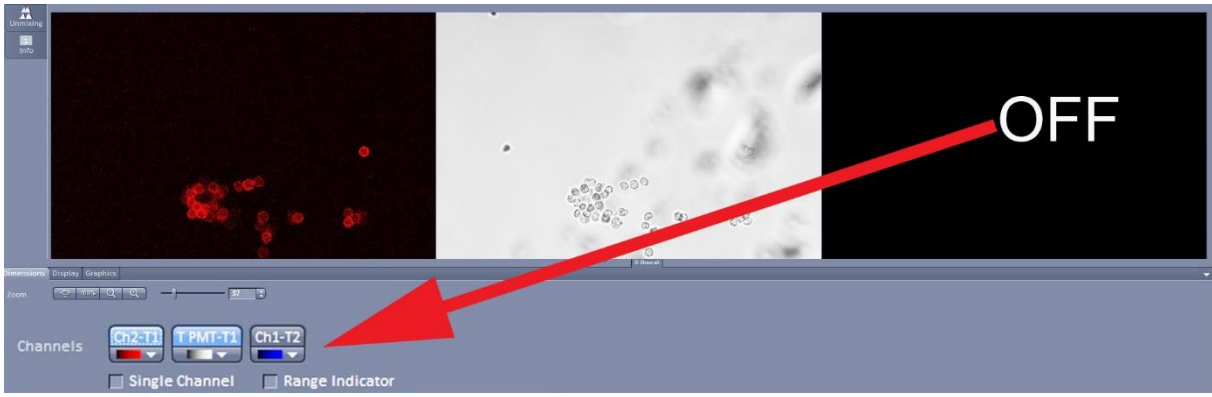
4) Sample goes out of focus when the stage is moved in XY

Slide/petri dish holder may not be flat – check stage

Your sample may not be flat – see……..doc

5) Blank fluorescence image

i) check that the display for the channel is ON



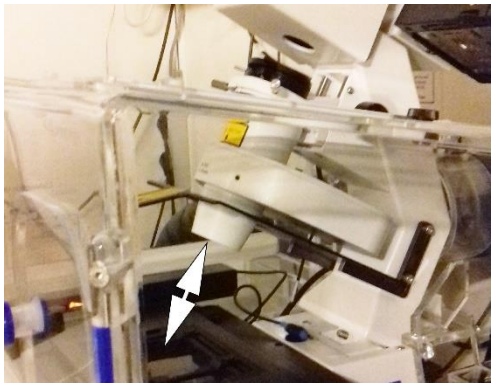
ii) can you see the laser scanning – No laser – check that the laser box switched ON see point 1) iv) and check laser power setting

iii) check the gain for the PMT

iv) The display of the fluorescence image may be switched OFF - check

6) No transmitted light image

Check Is the condenser in place



7) Connection to Datashare failed

i) Is the Datashare computer switched on

ii) Restart the LSM700 computer

Ask for help

If all else fails - restart ZEN

If this fails - restart everything.