

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

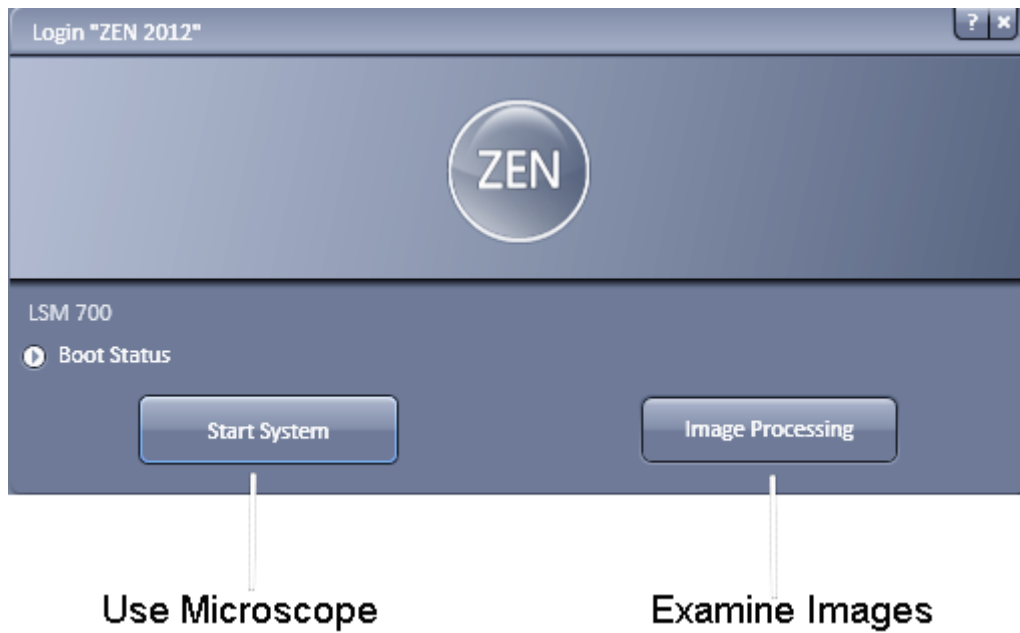
Objectives on LSM700* and Nyquist**

objectives	NA	Wrk dist	Widefield	Nyquist	Confocal Nyquist		Camera Pixels	xy Nyquist ratio
		mm	x,y nm	Z nm	x,y nm	Z nm	x,y nm	
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

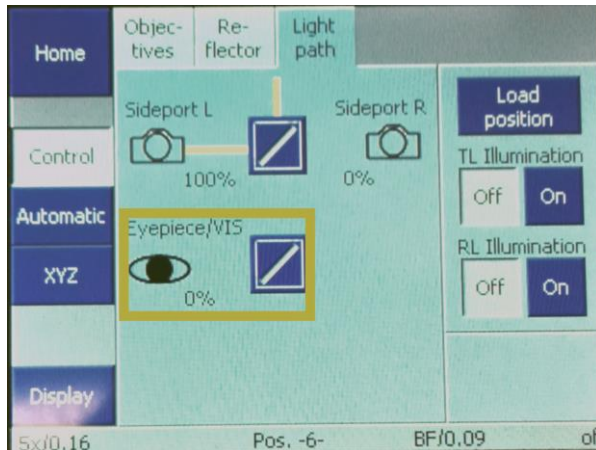
It is difficult to find and focus on a specimen with a confocal – the pinhole removes the out of focus image.

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.

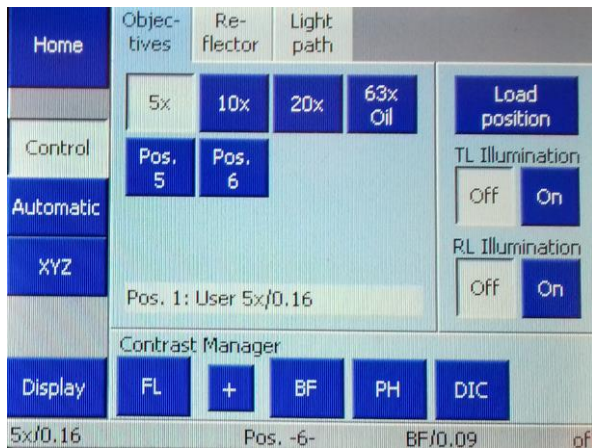
Use **widefield fluorescence** controlled from the stand alone TFT touch screen.

Select “Microscope” from the “Home” menu.

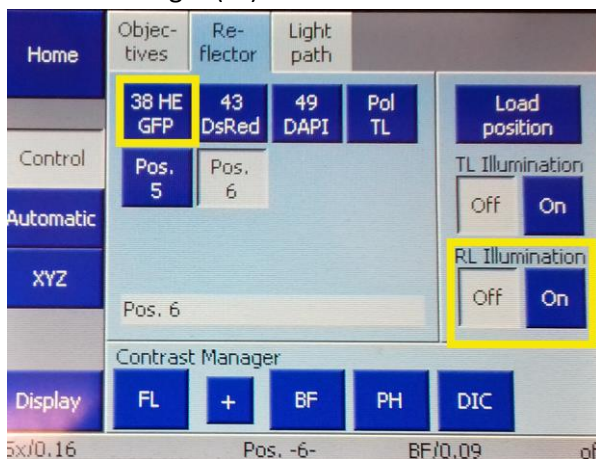
Light path (where the image goes) –select to your eyes.



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

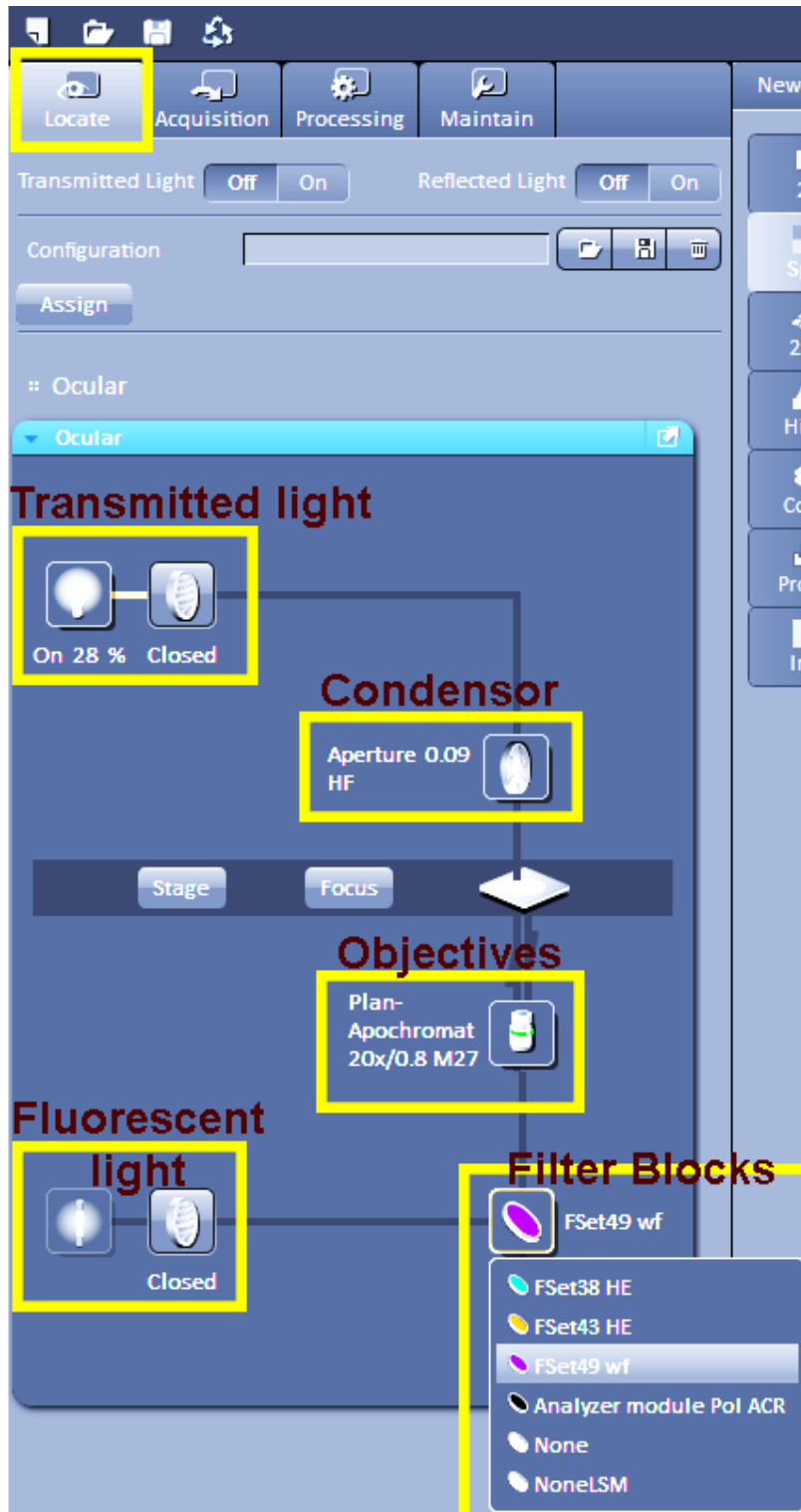


b) Select a **filter block** (GFP shown as selected) and turn on RL (fluorescent light source) or transmitted light (TL). Zeiss calls the filter blocks “Reflectors”.



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

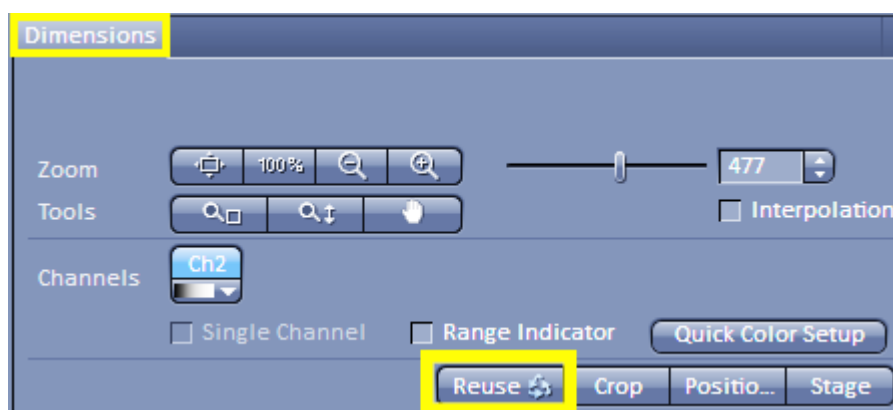
- a) Fluorescent images – one or two at the same time (track)
- b) A transmitted light image – can acquired at the same time as a fluorescence images
- c) 4 excitation laser lines - Note the widefield fluorescence lamp is not used
- d) Detection uses photomultipliers (PMT: two for fluorescence & one 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'

The image shows a screenshot of the ZEN software interface. On the left, the 'Experiment Manager' window is open, with the 'Acquisition' tab selected. A red arrow points from the 'Acquisition' tab to the 'Smart Setup' window. Below the 'Experiment Manager', a 'Smart Setup' button is highlighted with a yellow box. A yellow arrow points from this button to the 'Smart Setup' window. In the 'Smart Setup' window, the 'Dye' list is open, showing 'Alexa Fluor 488' and 'Alexa Fluor 405'. A yellow box highlights the 'Dye' list, with a yellow arrow pointing to it from the text 'choose fluorophores'. Another yellow arrow points from the 'Dye' list to the 'Add a fluorophore' text. Below the 'Dye' list, a 'Recent' list is shown, with a yellow box around it and a yellow arrow pointing to it from the text 'Choose from a list of fluorophores'. The 'Smart Setup' window also shows three acquisition proposals: 'Fastest', 'Best signal', and 'Smartest (Line)'. Each proposal has a 'Speed' bar and a 'Cross talk' bar. The 'Smartest (Line)' proposal is selected. A yellow arrow points from the 'Smartest (Line)' proposal to the 'Display colour for the fluorophore' text. At the bottom of the 'Smart Setup' window, there are 'Apply' and 'Cancel' buttons.

Locate Acquisition Processing Maintain

Experiment Manager
Mliana_2016

Smart Setup Show all To

Configure your experiment

Dye Alexa Fluor 488 Color
Alexa Fluor 405

choose fluorophores

Display colour for the fluorophore

Add a fluorophore

Choose from a list of fluorophores

Recent Alexa Fluor 488
Alexa Fluor 430
Alexa Fluor 405

Search

Dyes (none)
1-ANS
2-Methylbenzoxazole
3,3-dimethyloxatricarbocya
5-CNF
5-FAM
5-ROX
5-TAMRA
6-CR 6G
6-HEX
6-JOE
6-TET
7-MeO-Coumarin-ACDH
Acridine Orange
Acridine Yellow
Adirondack Green 520
AG

Proposals

Fastest Best signal Smartest (Line)

single track two tracks two tracks

2 fluorophores

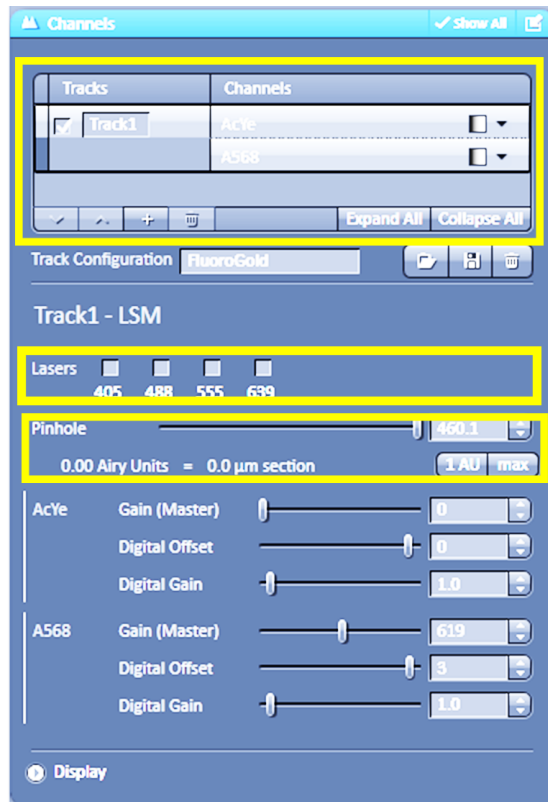
Speed Emission signal Speed Emission signal Speed Emission signal

Cross talk Cross talk Cross talk

Track 1 Track 1 Track 1
Track 2 Track 2

Apply Cancel

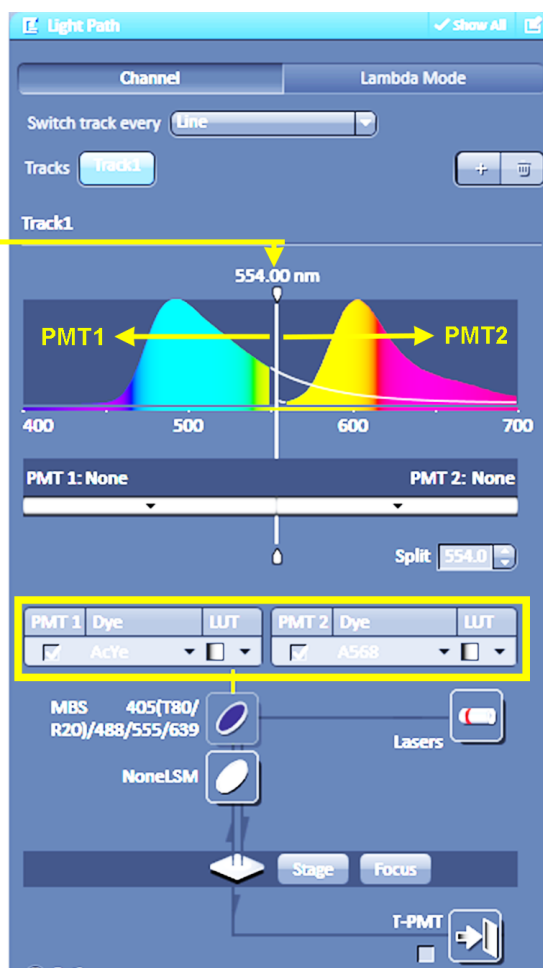
c) **Create your own tracks** – choose the settings yourself



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



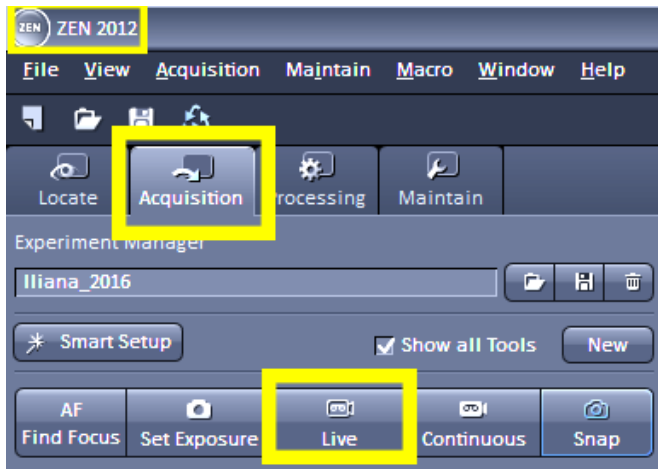
An adjustable
dichroic splits the
photons between
the 2 PMTs
<554 to PMT 1
>554 to PMT 2

3) Set the
emission range
for fluorescence
PMTs

2) Images - select
PMTs (tick boxes)
Both fluorescence
images ae active
the transmission
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, adjustments 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



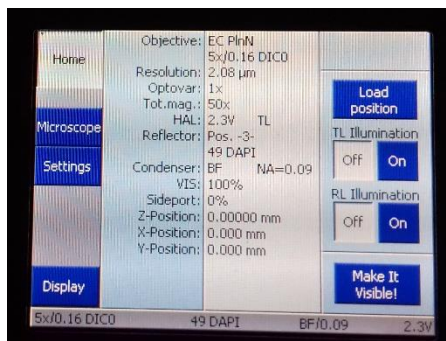
- iii) Microscope power supplies - on the shelf



- iv) No lasers – check is the laser box ON

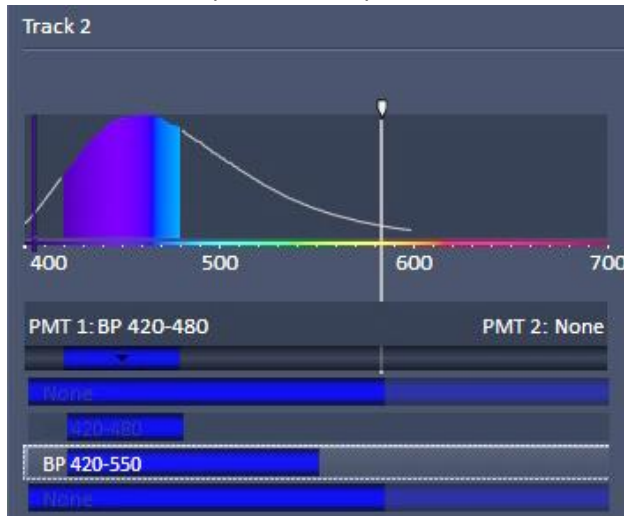
2) No fluorescence seen by eye

- 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

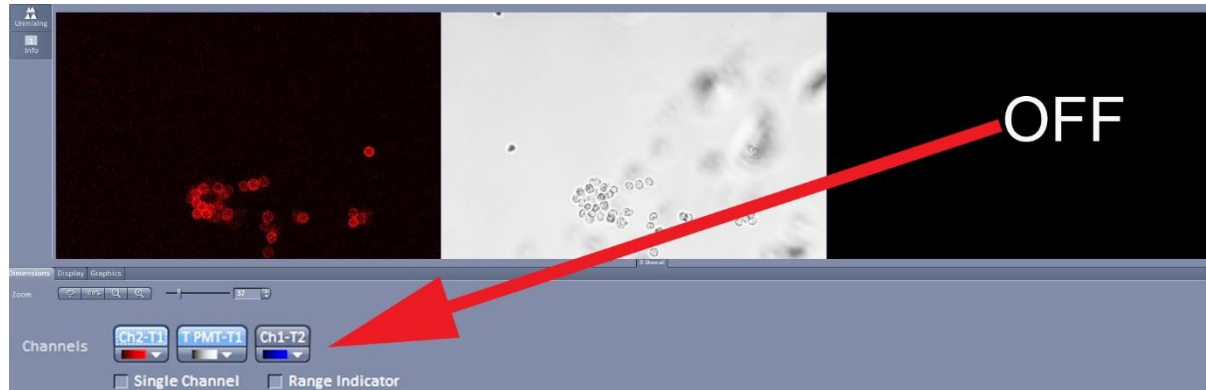
Slide/petri dish holder may not be flat – check stage



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

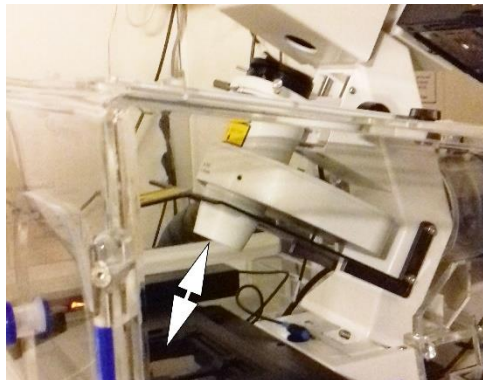
5) Blank fluorescence image

- 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.