



Zeiss 880 Airyscan

Microscope Users Guide

Zeiss 880 'Airyscan' User Guide - Contents

This user guide is not intended to replace training, but as an additional aid to help you get the best out of the microscope

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Zeiss 880 Airyscan Turn ON

- A**
WAIT
turn on 'Main Switch'
WAIT
After approximately 1 mins
the hardware will make a 'beeping' noise.



- B**
WAIT
turn on 'system/PC'
WAIT
After approximately 1 mins
the microscope touchpad will load up its
start-up page



- C**
turn on 'Components'



- D**
start the program
double click the icon 'ZEN Black'



- E**
click 'Start System'





10X
0.45NA

NO Oil



20X
0.8 NA

NO Oil



40X
1.30

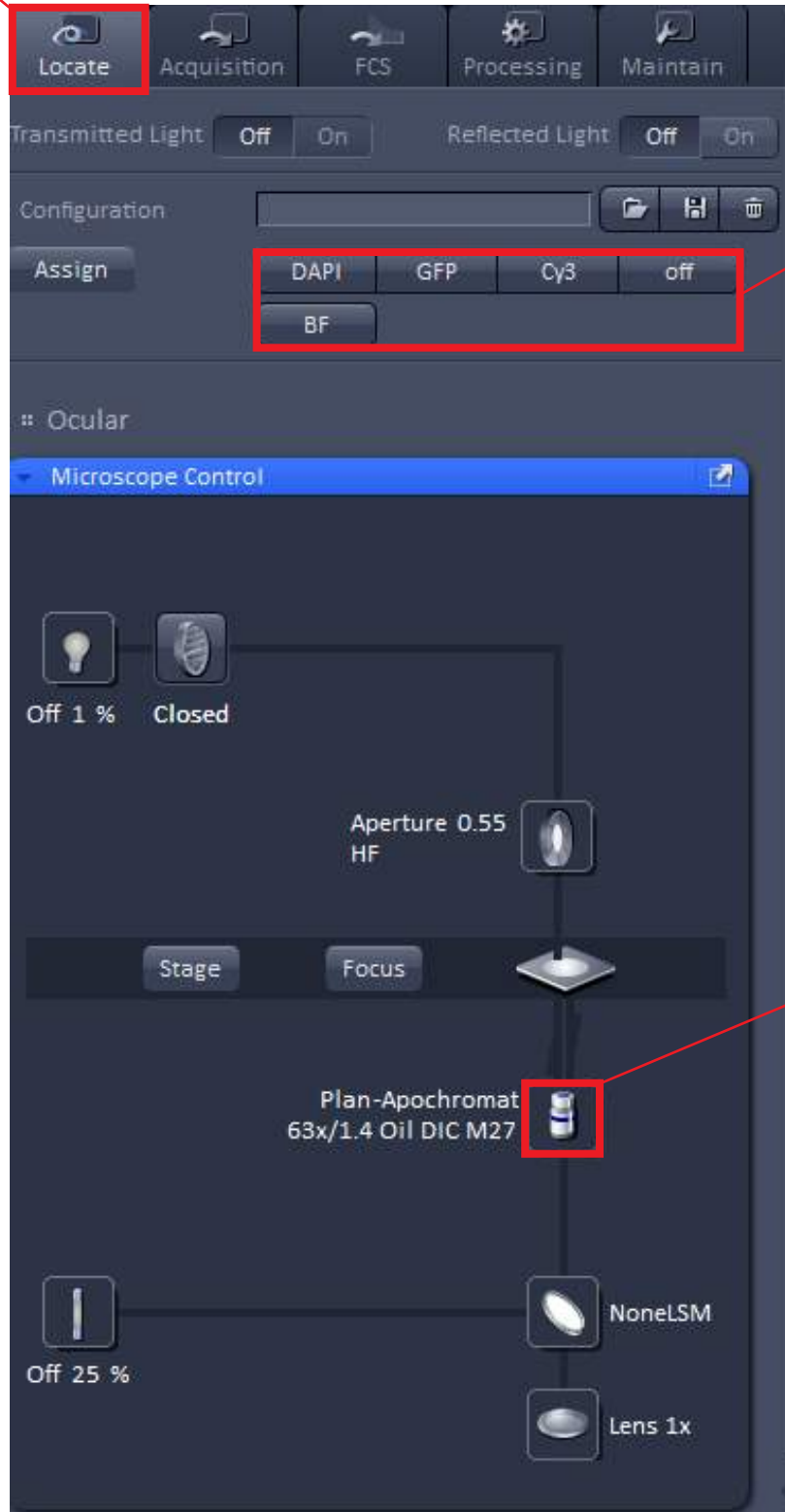
Oil



63X
1.4

Oil

microscope control



select filter

select objective lens

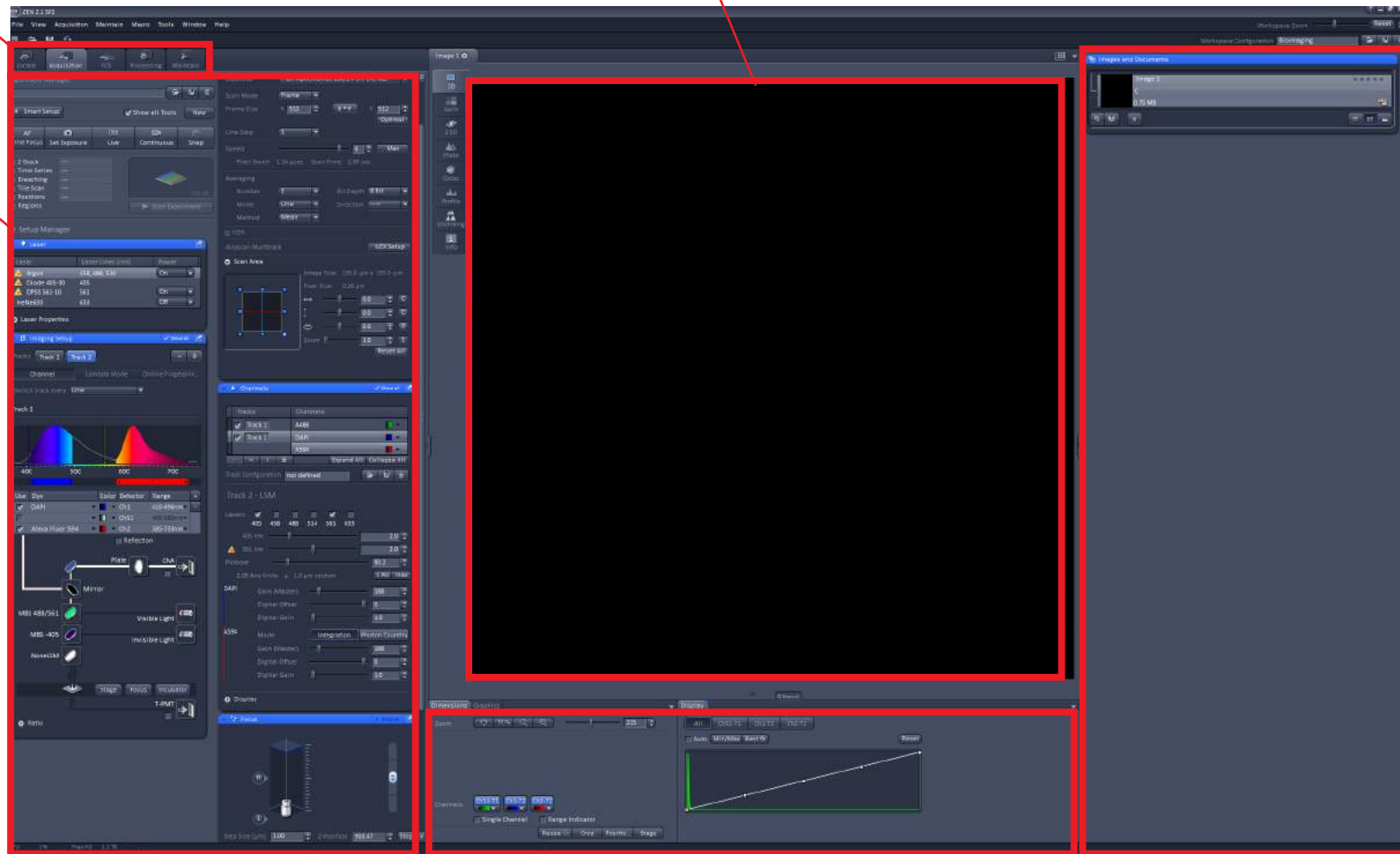
Function Tabs

Preview Window

Control Panel

Image Gallery

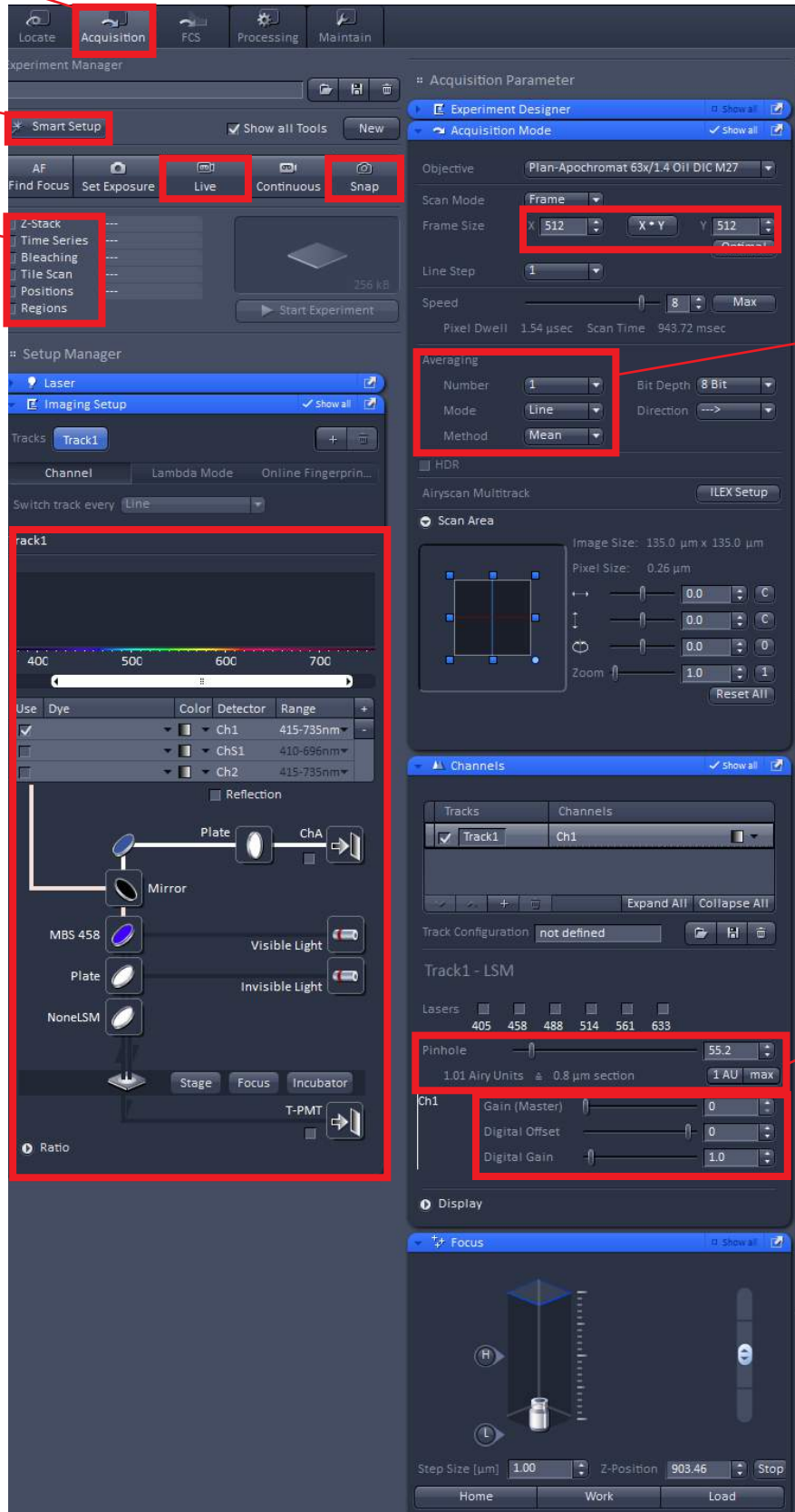
Display Control



'confocal' control

dye list setting

imaging mode selection



The screenshot shows the software interface for a microscope system. Several key settings are highlighted with red boxes:

- Acquisition Mode:** The 'Acquisition' tab is selected at the top.
- Smart Setup:** The 'Smart Setup' button is highlighted in the top left.
- Imaging Mode Selection:** The 'Live' button is highlighted in the top left toolbar.
- Frame Averaging:** The 'Averaging' section on the right is highlighted, showing 'Number' set to 1, 'Mode' set to 'Line', and 'Method' set to 'Mean'.
- Pinhole Aperture Control:** The 'Pinhole' control on the right is highlighted, showing a value of 55.2 and '1 AU max'.
- Dye List Setting:** The 'Dye' list in the 'Track1' section is highlighted, showing channels Ch1, ChS1, and Ch2 with their respective ranges.
- Confocal Control:** The 'MBS 458' and 'NoneLSM' options in the 'Track1' section are highlighted.

frame averaging

pinhole aperture control

Viewing with Transmitted or Epifluorescence Light

Before scanning the sample with laser light, look at the sample and find the region of interest first with either transmitted or fluorescent light.

Select the Locate tab to ensure that light emitted light is sent to the eyepieces



Click on brightfield light button for transmitted light viewing



or

Click on appropriate epifluorescence filter cube button for fluorescence viewing



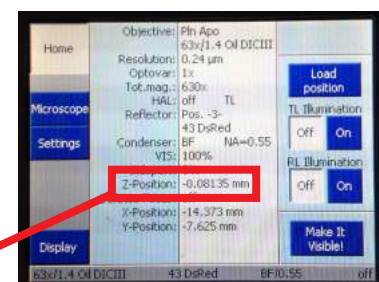
Make sure the slide and coverslip are clean and sealed.

Place the slide on the microscope stage.

Focus on the sample.

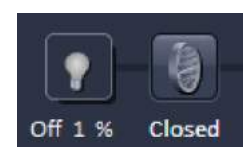
Course and fine focus control are located on both sides of the microscope.

If you are focusing on the left side of the microscope, the fine focus is located INSIDE the coarse focus.



Follow the direction of travel using the touchpad display

Adjust the transmitted light level with the lamp controller (or on the front of the microscope).



Adjust the fluorescence light level with the lamp controller.

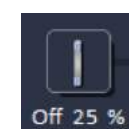
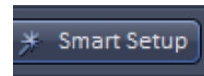


Image Acquisition - Confocal Mode

Select the Acquisition tab to use the lasers and detectors



Click on the 'Smart Setup' button

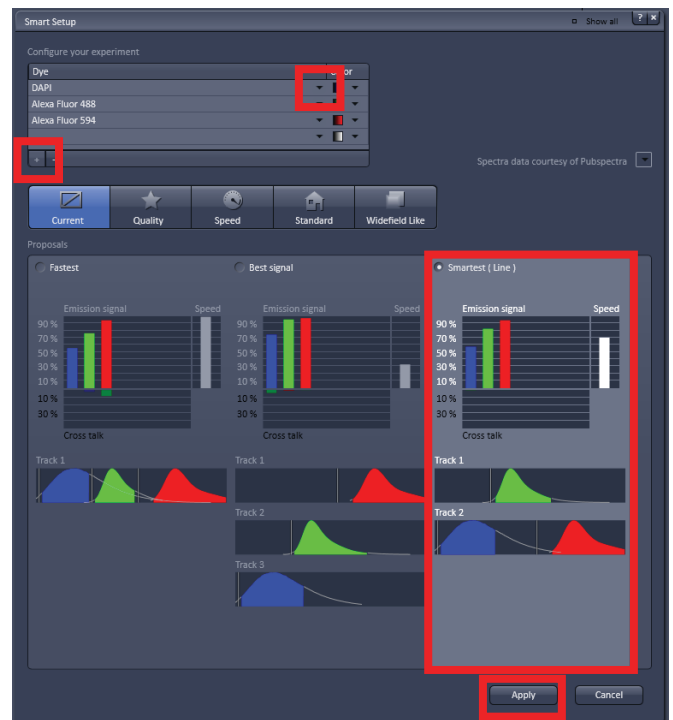


In the 'Smart Setup' window
click on the first inverted triangle and select dye from list.

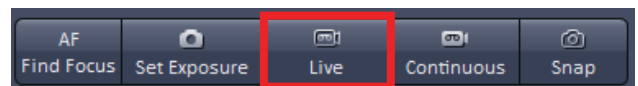
Add additional dyes by clicking on '+' button.

For most experimental conditions choose
"smartest (line)" option

click Apply. This will configure the microscope to
image the selected dyes using the 'standard' confocal
detectors in the fastest way to minimise, but not totally
rule out bleed-through.



Click 'Live' button to 'live' scan the sample.



Click Stop button to stop the 'live' scanning.



Image Acquisition

Open Channels zone

Click 'Expand All' to give control of all channels

Click 'Live' button to scan

Set the Laser output level

Click the 1AU button to set the pinhole size to one AiryUnit

Detector (CHS) Channel Setting:
Adjust the brightness of Gain Master and background black level (Digital offset) of individual channels.

Keep the Master Gain value below 800 (beyond 800 the background noise will show up).

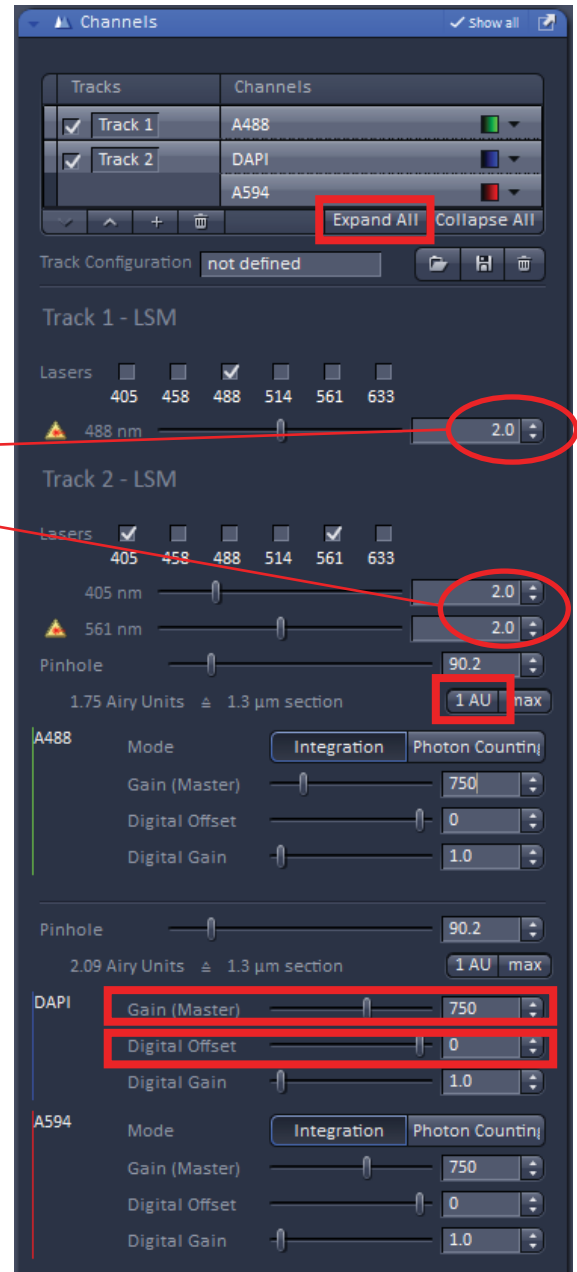
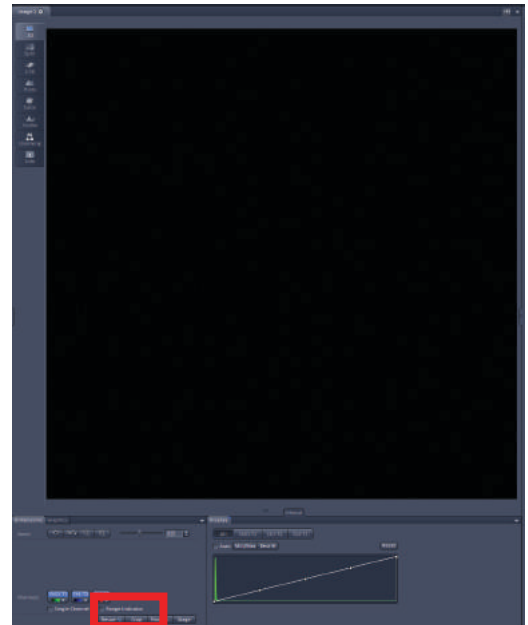


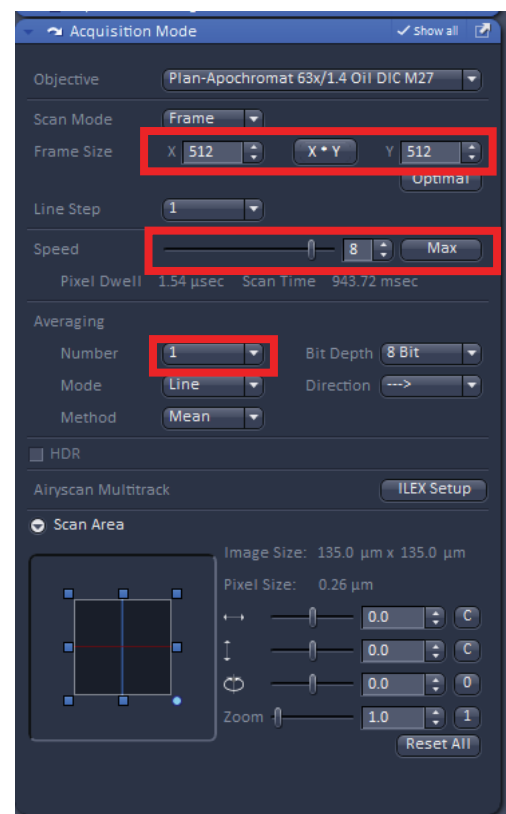
Image Acquisition

You can optimise the image quality with the Range Indicator.
(This is the display with red dots and blue dots)
Click range indicator tick box. Now the Image is displayed
using this specific 'look-up-table' where red pixels represent
maximum intensity (saturated)
and blue pixels represent zero pixel value.

To maximise the signal to noise ratio of the image, adjust
the acquisition settings (laser power level, Master Gain
and Digital Offset) such that the image shows a few red pixels
and a few blue pixels while you are scanning.

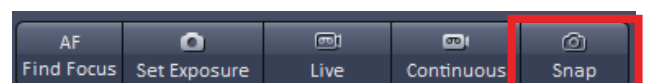


Change frame size, scan speed and line averaging depending
on the quality of image desired.



Once you are satisfied with the settings....

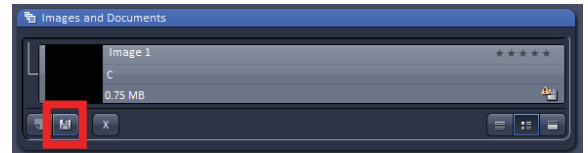
Click the 'Snap' button to acquire an image.
When acquisition is done, a 2D view window will appear.



Saving Images and Re-using Image Parameters

The 'snapped' image will appear in the Image Gallery on the Right hand side of the window

click on the save icon



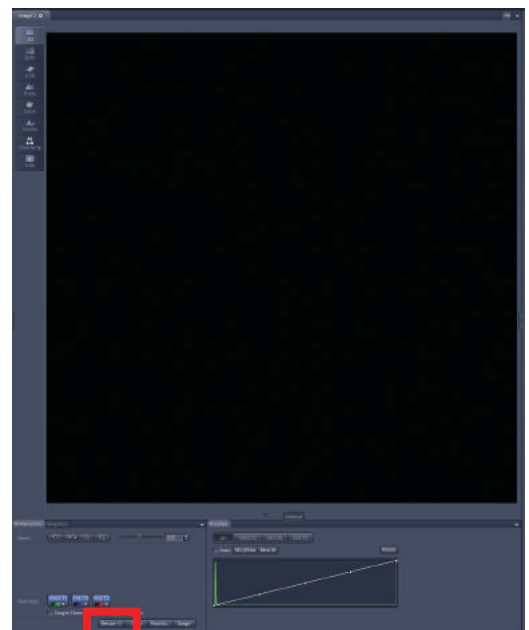
A **Save As** window will appear. Save the image in your own folder on the D: Data drive.

Select **Carl Zeiss Image*.czi** file type, type file name, and click on **Save** button. The .czi file type contains all the metadata including all acquisition parameters as well as the image.

The .czi files can be opened using
fiji / image J free software (<http://fiji.sc/Fiji>).

Open a saved image which was acquired with the settings you wish to reuse

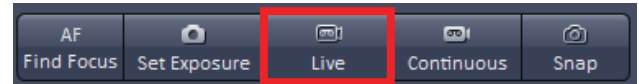
click the re-use button



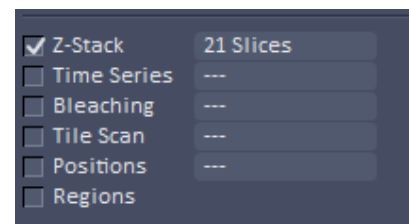
z-Series Image Acquisition

Use this mode to obtain an optical section through the depth (z dimension) of your sample. This 'z-stack' can then be used to visualise your sample in 3D:

Click 'Live' button to start 'live' scan.

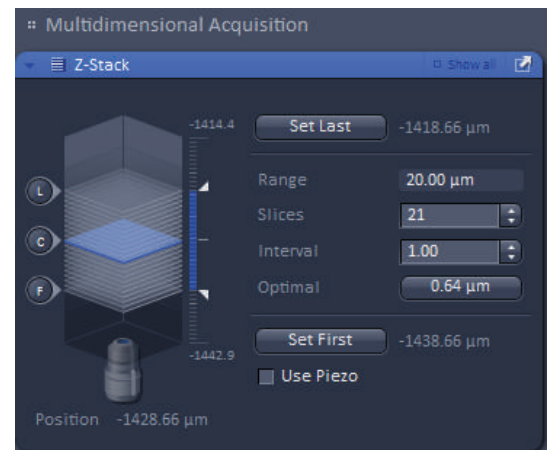


Click the 'Z-stack' tick-box

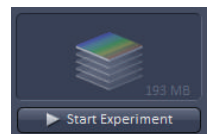


The Z-stack 'blue zone' should appear

When you find the lower limit of your sample (closest to the coverslip) click 'set first', then find the upper limit of your sample and click 'set last'. Define the step interval. If you need Nyquist sampling of the z-stack - click 'optimal' button.



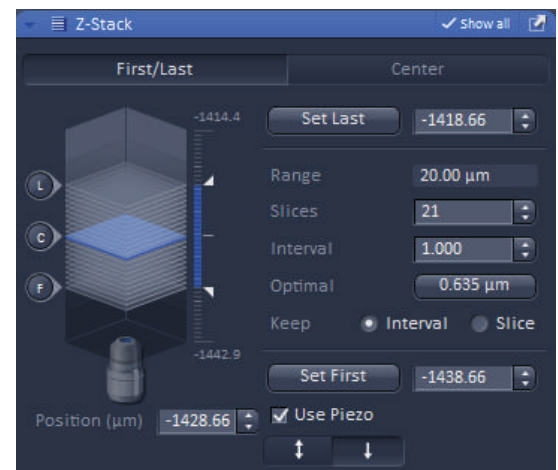
Click the stop button to stop the 'live scan'.
Click 'Start Experiment' to start the scan.



Save the image.

If you wish to define a centre point click 'show all' and then the center tab.

If you wish to use the piezo z-stage, this option is also given after clicking 'show all'

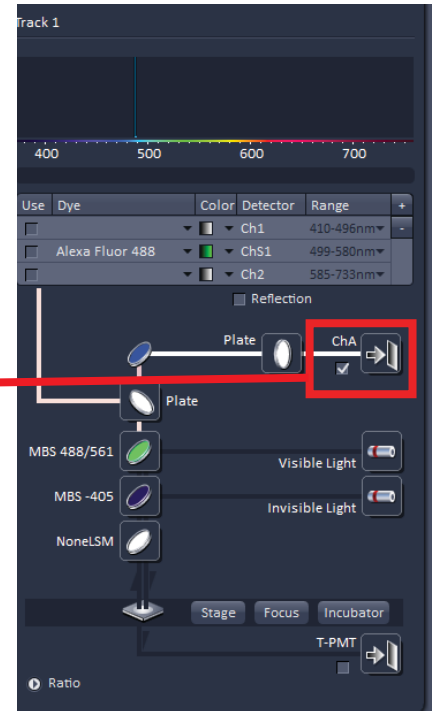


Airyscan Image Acquisition

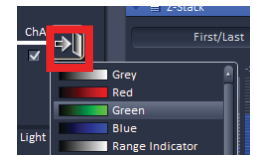
Use the smart set-up tab to create a multi-track configuration where each track has just one colour, and that there's no physical movement of components within the microscope (as described earlier)

If multiple colours are set to image in the same track add a new track so that there's only one colour per track

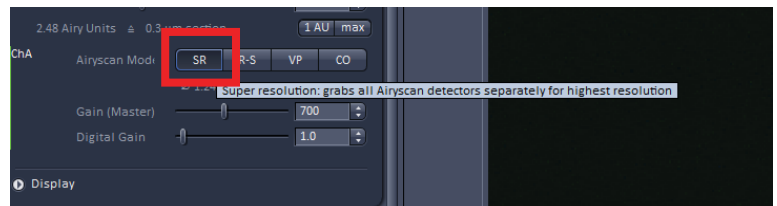
Activate the Airyscan detector



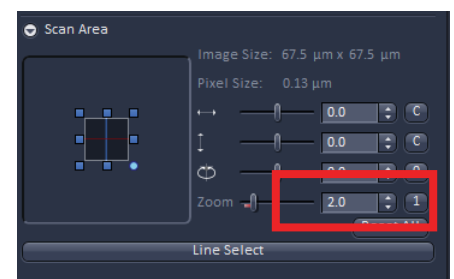
Set the 'colour' of the track



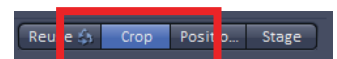
Ensure that 'SR' button is pressed in the channels 'blue zone' (this ensures the pinhole is correctly sized). Set the laser power and Gain (Master) according to the Range Indicator as described earlier.



The zoom must be set to higher than 1.8x

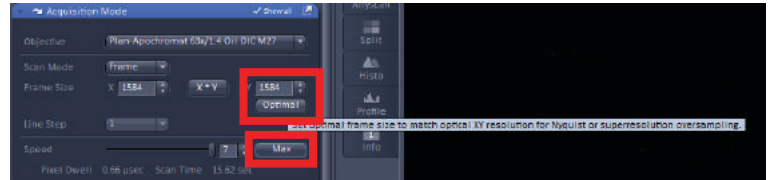


It is easy to zoom on a specific part of your field of view using the 'crop' button



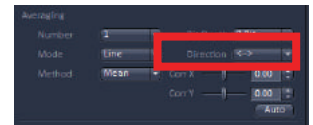
Airyscan Image Acquisition

'Click' optimal button to ensure Nyquist sampling in XY.

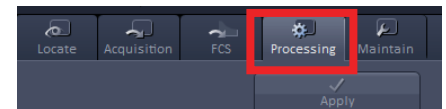


'Click' 'Max' button to ensure fastest possible scan speed.

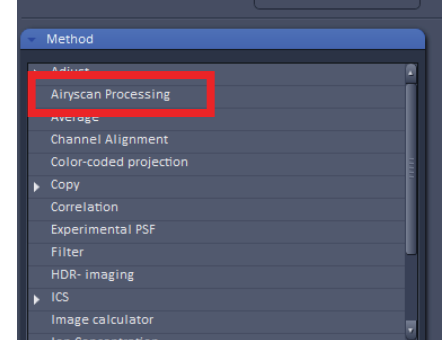
Select 'bi-directional' scanning for quicker scanning



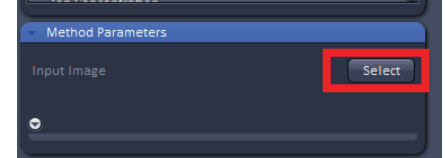
Click 'Snap' or 'Start Experiment' to carry out scan.



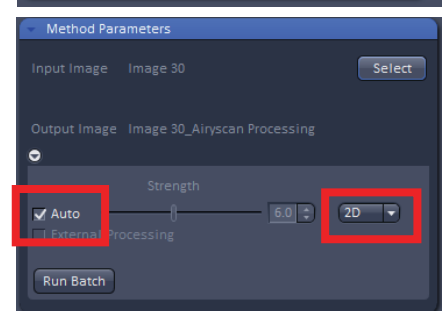
Once your image has been captured it must be processed. Select the Processing tab and choose 'Airyscan Processing' method.



Click select on image and click 'select' button

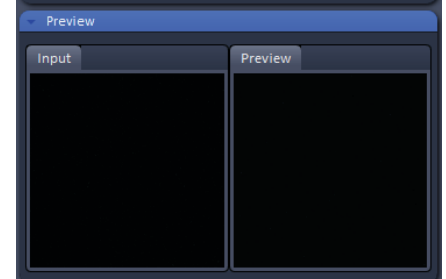


Typically leave processing 'strength' on Auto

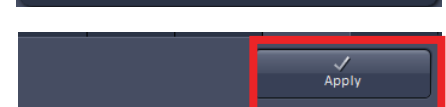


For a z-stack scanned with 'optimal' Nyquist sampling in z use 3D processing rather than 2D processing.

Click 'Apply' to process the image.



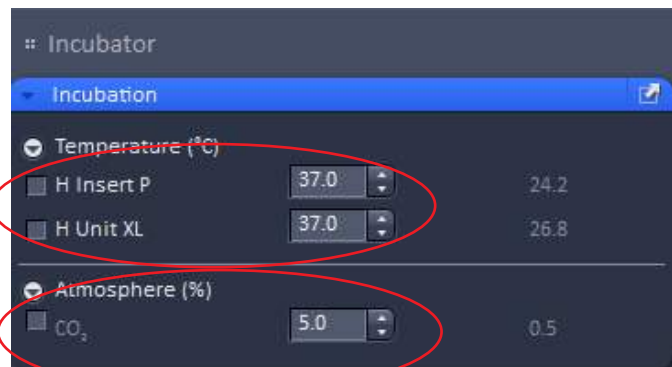
Save processed image



in vivo Experiments

tick check-box to turn on
warming of heated stage
insert ('H Insert P') and
incubator chamber ('H
Unit XL')

tick check-box to turn on
5% CO₂



Check the booking calendar. Are you the last user?

α

Close Metamorph software
Log off computer



β

1. remove your sample from the microscope
2. clean the objective lens using lens cleaning tissue
3. exit the 'ZEN Black' software



γ

turn off 'Components'



δ

turn off 'System/PC'



ε

turn off 'Main Switch'

