

Upright Nikon A1R Multiphoton Microscope

**A1R - STEP BY STEP INSTRUCTIONS CONTENTS PAGE**

Page 6	-----	STEP 1	System On
Page 11	-----	STEP 2	Focus On Your Sample
Page 13	-----	STEP 3	Change From Eyes To Camera View
Page 17	-----	STEP 4	Choose A Laser Scanning Mode
Page 21	-----	STEP 5	Setting Up Initial Live View
Page 31	-----	STEP 6	Optimising Your Camera Settings ( When You Have Too Much Signal )
Page 35	-----	STEP 6	Optimising Your Camera Settings ( When You Don't Have Enough Signal )
Page 40	-----	STEP 6	Optimising Your Camera Settings ( When There Is Too Much Background Signal – Use Offset )
Page 43	-----	STEP 6	Optimising Your Camera Settings ( When There Is Too Much Background Noise – Use Averaging )
Page 45	-----	STEP 6	Optimising Your Camera Settings ( Now Do Step 6 For Every Channel You Want To Use )
Page 48	-----	STEP 7	How To Avoid Bleed-through
Page 50	-----	STEP 8	Optimise Your Image Resolution
Page 70	-----	STEP 9	Acquisition Settings ( Save To File )
Page 73	-----	STEP 9	Acquisition Settings ( Order Of Acquisition Tabs )
Page 75	-----	STEP 9	Acquisition Settings ( Lambda $\Lambda$ Tab – Laser Channels)
Page 79	-----	STEP 9	Acquisition Settings ( Z-stack )
Page 84	-----	STEP 9	Acquisition Settings ( Large Image )
Page 87	-----	STEP 9	Acquisition Settings ( XY Positions )
Page 89	-----	STEP 9	Acquisition Settings ( Time )
Page 92	-----	STEP 10	At The End Of Your Session ( Save And Shut Down Procedures )
Page 100	-----	MORE	More Advanced Instructions ( Reuse Pervious Camera Settings )
Page 102	-----	Contact	How To Contact George And Chen

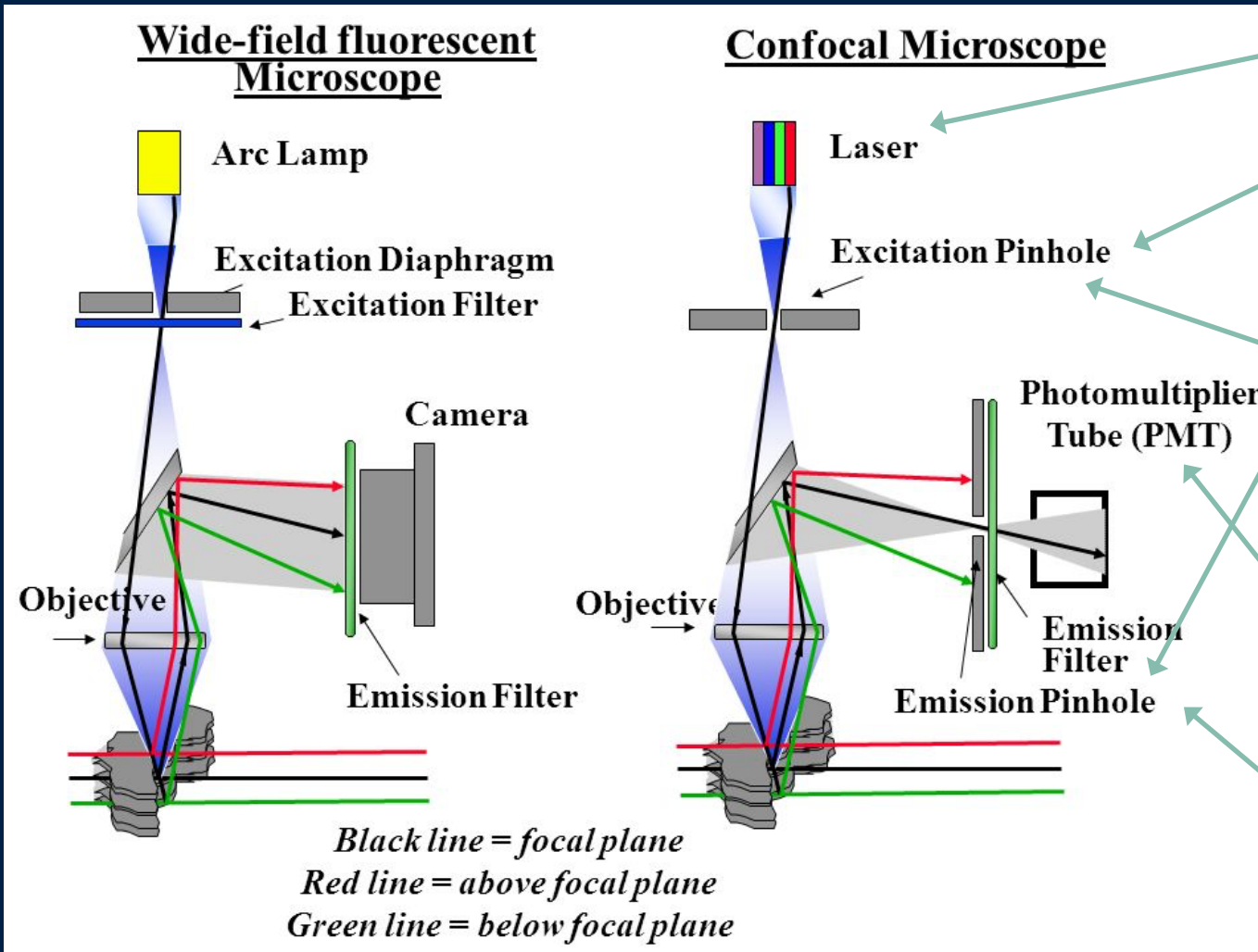
# Before Using The Facility...

To use the facility the individual must undergo training...

- Tour of the facilities
- T & C agreements
- First training session
- Second training session with user's samples
- Additional training sessions may be required
- Additional lens installation training (optional)
- Access to booking system: <http://ppms.eu/kcl-wohl>

# Basic Principles Of A Confocal Microscope

Confocal microscopy improves image resolution by using a more focused excitation light source and allow users to observe more selective emitted light. Confocal microscopy bridges the gap between widefield and electron microscopy.



Lasers produce intense monochromatic light which excites a small target zone instead of flooding a large area like a Arc lamp.

The excitation pinhole force lasers into a spot shaped light source.

The excitation and emission pinhole focuses on the same exact spot on the specimen, thus making them confocal.

If you enlarge the pinhole to much, the microscope will behave like a wild-field system.

The photomultiplier tube (PMT) is the detector and amplifier for emitted signals. The charge of the PMT affects the amplification (gain). PMT is more sensitive for blue light (15%) than red (4%).

The emission pinhole act as a spatial filter, restricting any light not originated from the focal point.



# STEP BY STEP INSTRUCTIONS

The rest of this document will take you from focusing on your sample to optimising your image to what to do at the end of your session.



Additional  
Information  
Slides

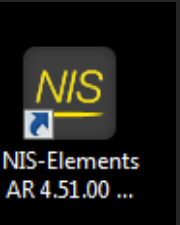
The blue slides contains additional information you might find helpful.

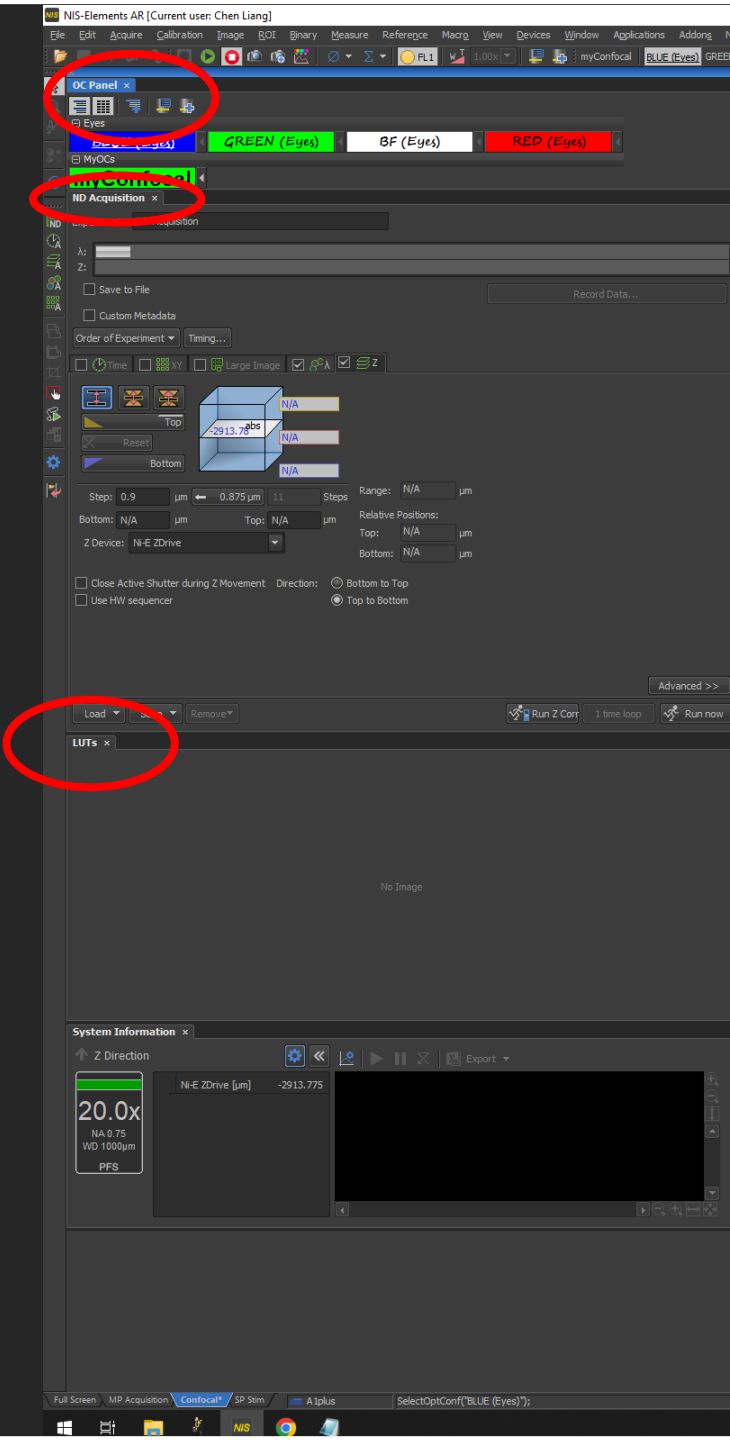
# STEP BY STEP INSTRUCTIONS

## STEP 1

System ON

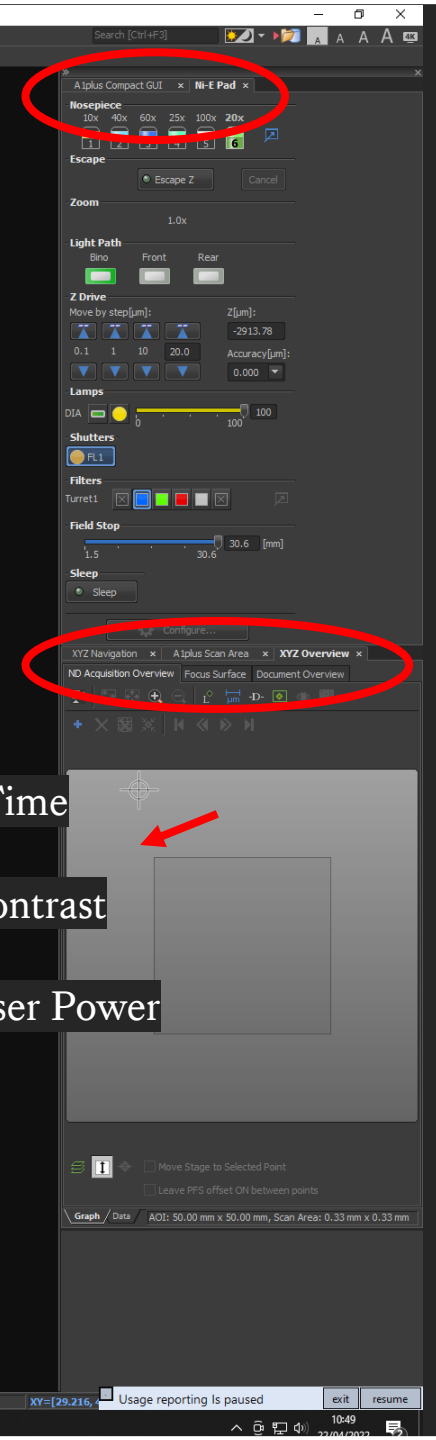
# System On

1. Switch on the A1R confocal system by following the numbered switches.
2. Do not switch on number 1 unless you are trained to do so.
3. Do not switch on number 5 unless you are trained to do so.
4. ALWAYS login to NIS-Elements Software  before loading any sample, this checks if all systems are connected.



After loading NIS Elements check you have the basic pads loaded...

- Optical Configuration (OC) Pad – Channel settings
- Acquisition Pad – Save to file,  $\lambda$ , Z-stack, Large image, XY, Time
- LUTs – Intensity histogram (X=intensity, Y=log intensity), Contrast
- Camera Settings – Scanning Mode, Speed, Pinhole, Gain, Laser Power
- Ti Pad – lens changes, light path, perfect focus
- Scan Area – Scan Direction, Zoom, Pixel Size





# Add to...and save software set up

Right click on blank space to add missing tabs and dock it in the docking panes at either side ( but check they are not just hidden behind another tab first ).

Right click to save configuration (top) and layout (bottom)

The screenshot shows the Nikon NIS-Elements AR software interface. A red arrow points from the top-left corner of the software window to the 'Add to...' button in the 'myAcquisition' tab. Another red arrow points from the bottom-right corner of the software window to the 'Save' button in the 'myAcquisition' tab. The interface includes a menu bar (File, Edit, Acquire, Calibration, Image, ROI, Binary, Measure, Reference, Macro, View, Devices, Window, Applications, Help), a toolbar, and several docking panes. The 'OC Panel' on the left shows various acquisition settings. The 'myAcquisition' tab is active, showing a 3D cube visualization and acquisition parameters. The 'A1plus Compact GUI' and 'TI Pad' panes are docked on the right, showing detailed acquisition settings. The status bar at the bottom displays the current acquisition parameters and the user's login information.

File Edit Acquire Calibration Image ROI Binary Measure Reference Macro View Devices Window Applications Help

OC Panel

Eyes

DAPI (eyes) CFP (eyes)

Green (eyes) YFP (eyes)

Red (eyes) Brightfield eyes

General

Confocal (DAPI\_GFP\_RFP)

CFP YFP Imaging

Confocal (CFP\_YFP)

myOCs

myConfocal (CFP\_YFP) myConfocal

ND Acquisition Z Intensity Correction

Experiment: ND Acquisition

Z: [Z-axis scale]

Save to File

Path: C:\Users\Nikon\Pictures\Chen

Filename: 19112018\_003.nd2

Custom Metadata

Order of Experiment Timing

Time XY Large Image Z

Top Bottom

Step: 0.06  $\mu\text{m}$  0.15  $\mu\text{m}$  87 Steps Range: 5.13

Bottom: 2788.28  $\mu\text{m}$  Top: 2793.40  $\mu\text{m}$  Relative Positions: Top: +2892.40 Bottom: +2887.28

Z Device: Nikon A1 Piezo Z Drive Piezo

Close Active Shutter during Z Movement Direction: Bottom to Top Top to Bottom

Use HW sequencer

Advanced

Load Save Remove Run Z Corr 1 time loop Run

LUTs

No Image

myAcquisition

A1plus

Apo 60x Oil XS DIC N2 (0.41  $\mu\text{m}/\text{px}$  512 x 512)

XY=[0.001, -0.001]mm, Z=-99.00 $\mu\text{m}$ , Piezo

Logged in as chenlang (2m) logout

9:32 AM 12/12/2018

A1plus Compact GUI TI Pad

Nosepiece

10x 20x 40x 60x 40x 100x

Escape

Escape Z

Light Path

E100

PFS

On Memory Recall

L100 L80 R100

Focus Offset: 5041

Dichroic Mirror: LM

Z Drive

Move by step[ $\mu\text{m}$ ]: Z[ $\mu\text{m}$ ]:

0.1 1 10 20.0 Accuracy[ $\mu\text{m}$ ]: 0.000

Shutters

DIA

Filters

Turret1

Condenser

5 DICN2

Zoom

1.00x

Configure...

A1plus Scan Area XYZ Navigation

Zoom: 1

Pixel size: 0.21 Nyquist XY

Scan size: 1024 Rotation: 0

Width: 1024 Height: 1024

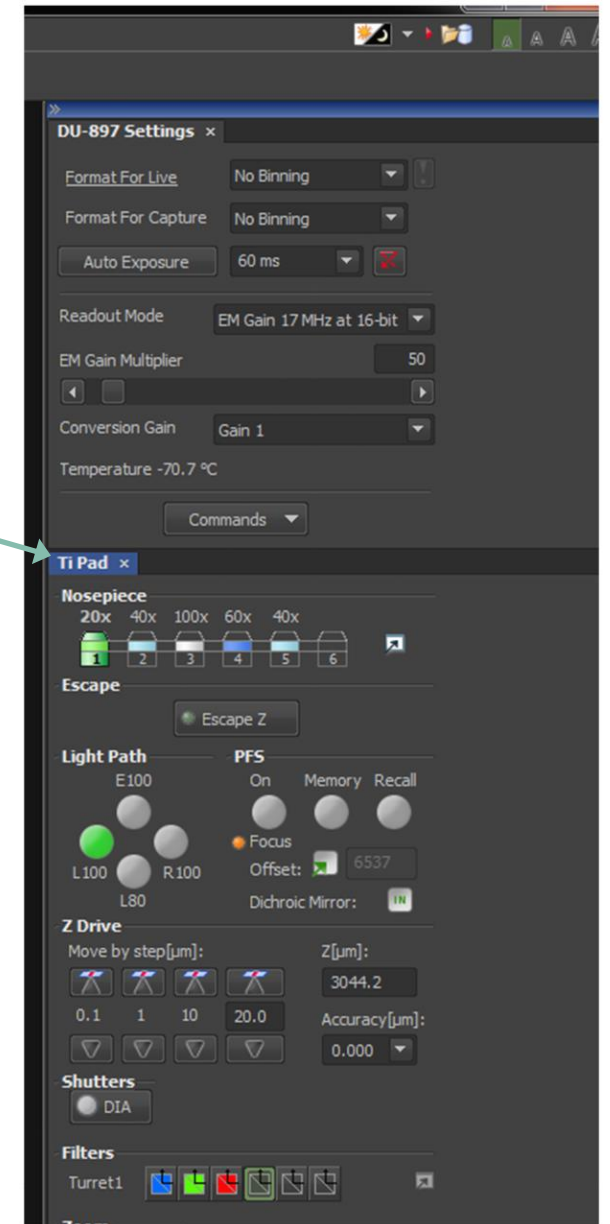
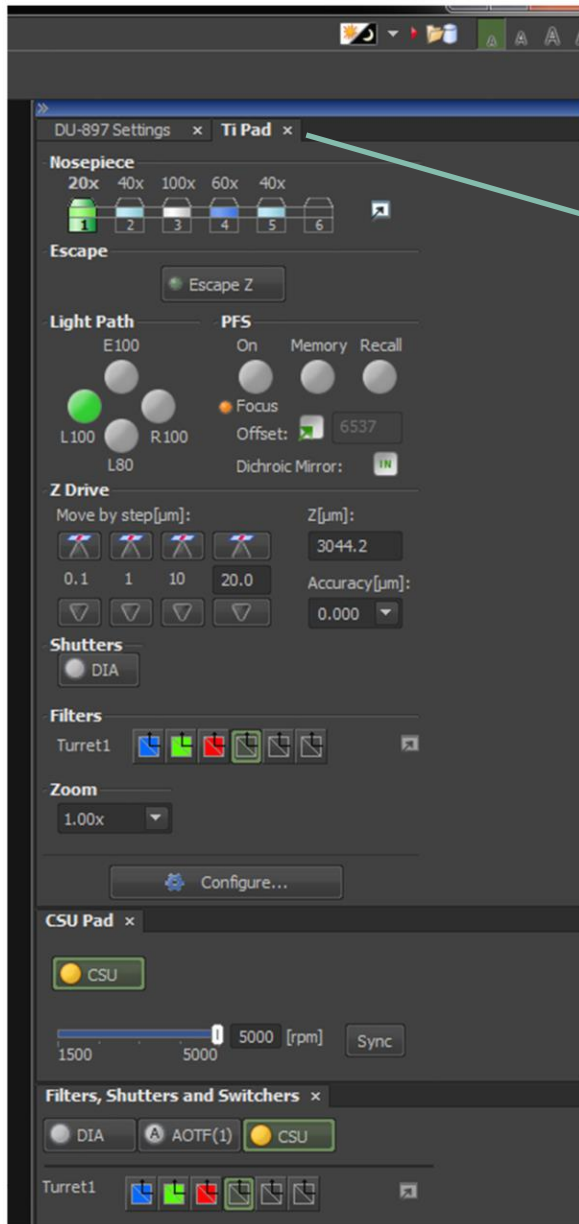
Dwell time: 0.05  $\mu\text{s}$

Pixel size: 0.21  $\mu\text{m}$  Optical resolution: 0.23  $\mu\text{m}$

Z step size: 0.15  $\mu\text{m}$  Optical sectioning: 0.47  $\mu\text{m}$

# Software Hidden Panels

Mouse left click hold, drag and drop  
to rearrange docking pane.



# STEP BY STEP INSTRUCTIONS

## STEP 2

Focus on your sample.

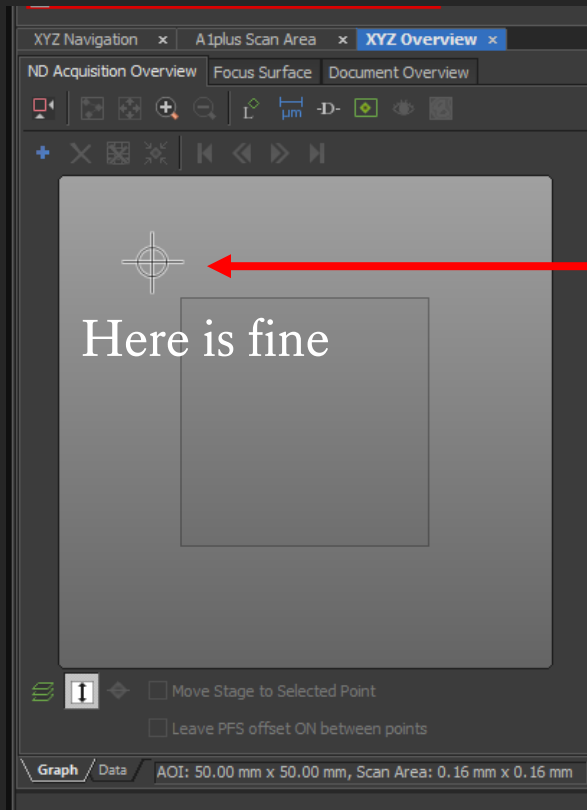
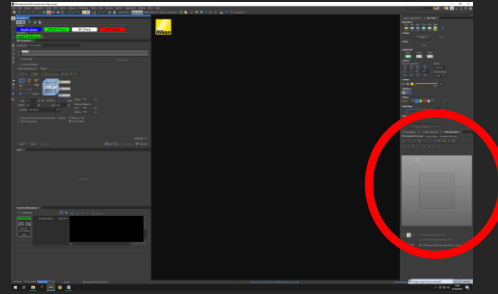
This will be included during your training session.


If you need a reminder, please contact us or watch the instruction video:

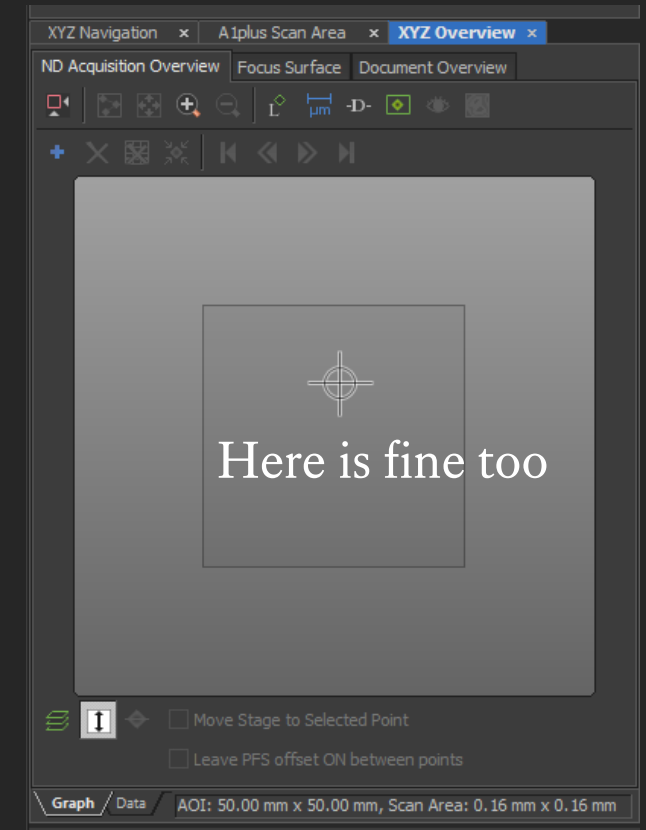
[https://www.youtube.com/watch?v=KY98YZ8M0h0&ab\\_channel=WohlCellularImagingCentre](https://www.youtube.com/watch?v=KY98YZ8M0h0&ab_channel=WohlCellularImagingCentre)



# Make sure the stage location is within range on the 'map'.



After you've loaded your sample, you should see this  on the grey 'map', if you don't see this, please double click anywhere on the 'map' to bring your stage location within range before the next step.



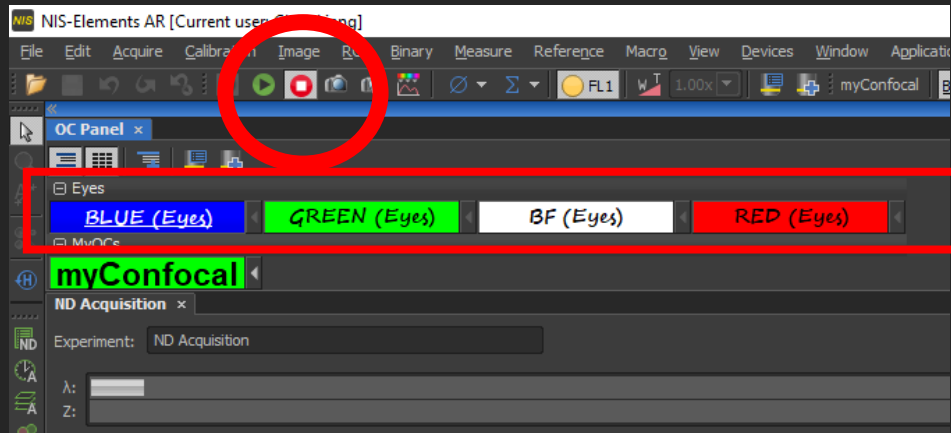
# STEP BY STEP INSTRUCTIONS

## STEP 3

Change from Eyes to Camera View

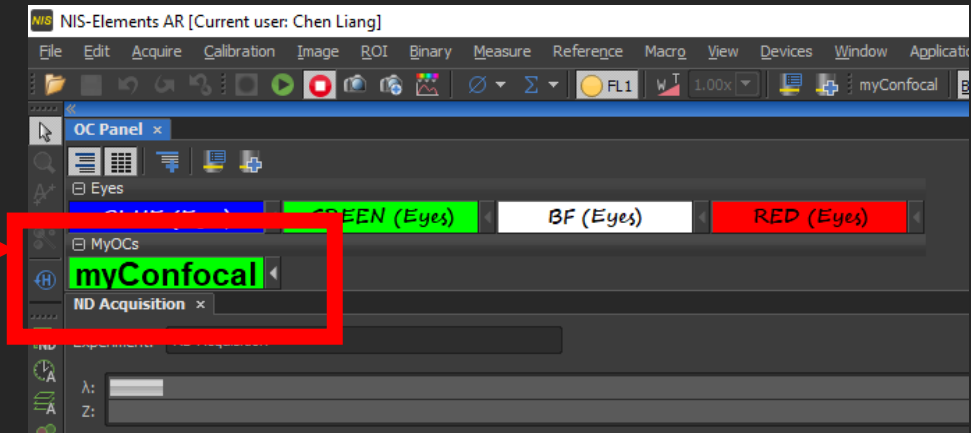
From this point forward we will be adjusting things on the computer screen so we need to work in camera mode instead of looking down the eye piece.

Remain on STOP setting.

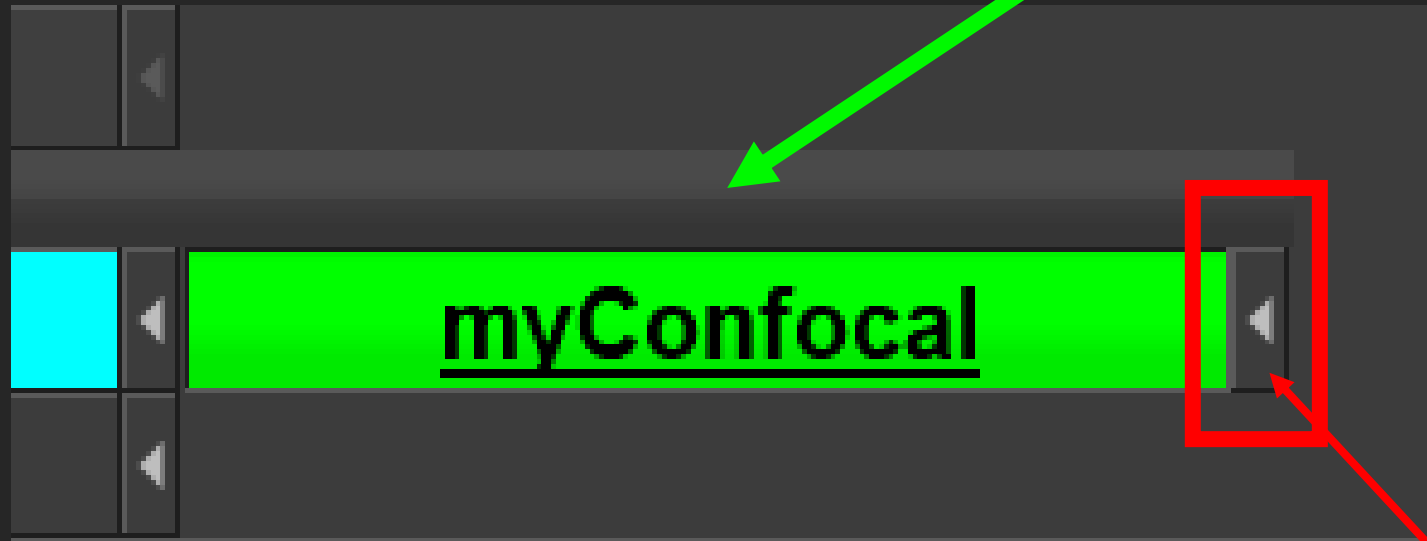


Click on  
'myConfocal'

This moves from  
eyes to camera.

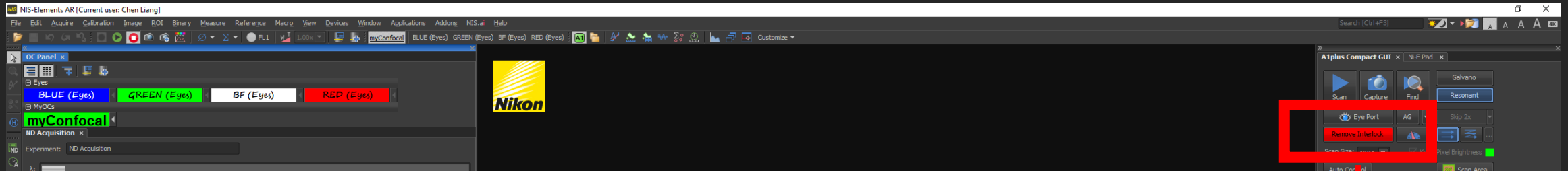


Click on the green area “myConfocal”

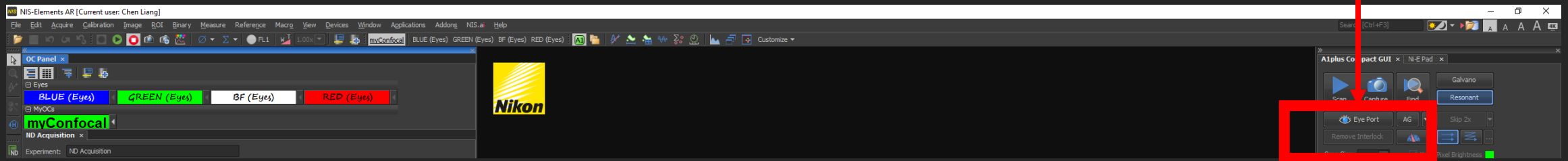


DO NOT CLICK ON  
THIS AT THE MOMENT

# Click on “Remove Interlock”



After clicking on myConfocal, WAIT for a few seconds for the system to change from eyes to camera, then click on “Remove Interlock”. Now your lasers are ready to be used, they are not on yet, but they will turn on when you are ready to scan your sample.

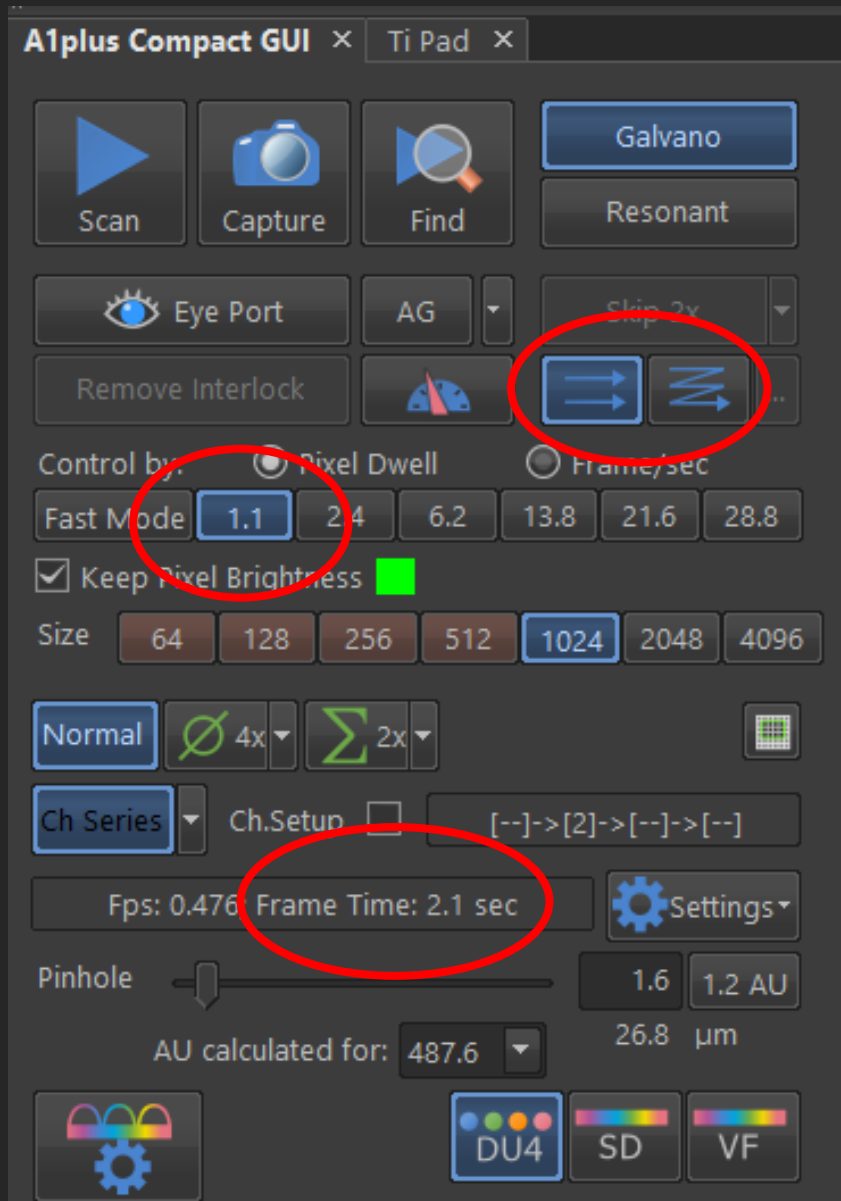


# STEP BY STEP INSTRUCTIONS

## STEP 4

Choose A Laser Scanning Mode

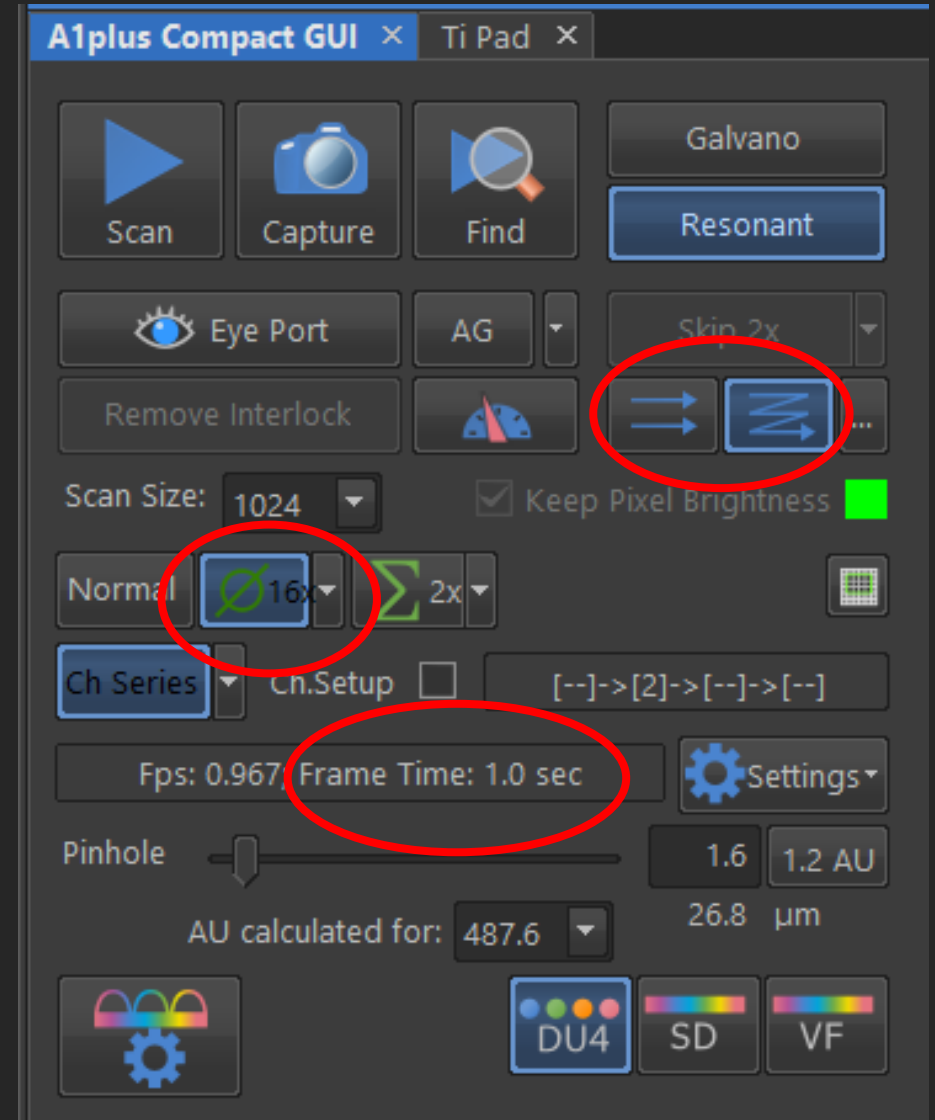
## Galvano: Precision point laser scanning



Even when Galvano option scans your sample once and Resonant has 16 times averaging (scans your sample 16 times) Resonant mode still only take half the time to capture.

Lets compare background noise and image quality...

## Resonant: faster laser scanning (recommended for most imaging, less bleaching)





# Galvano vs Resonant (with 16X averaging)

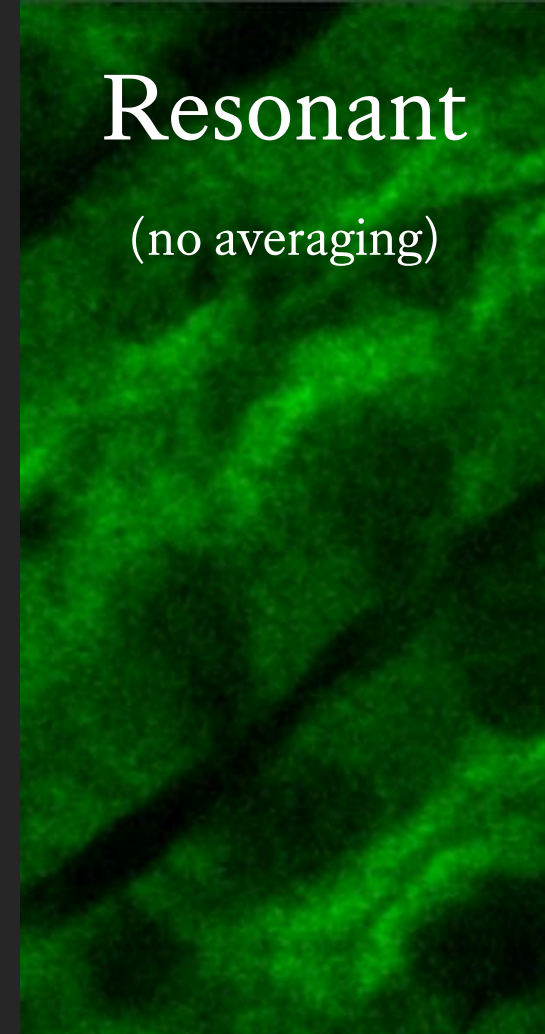
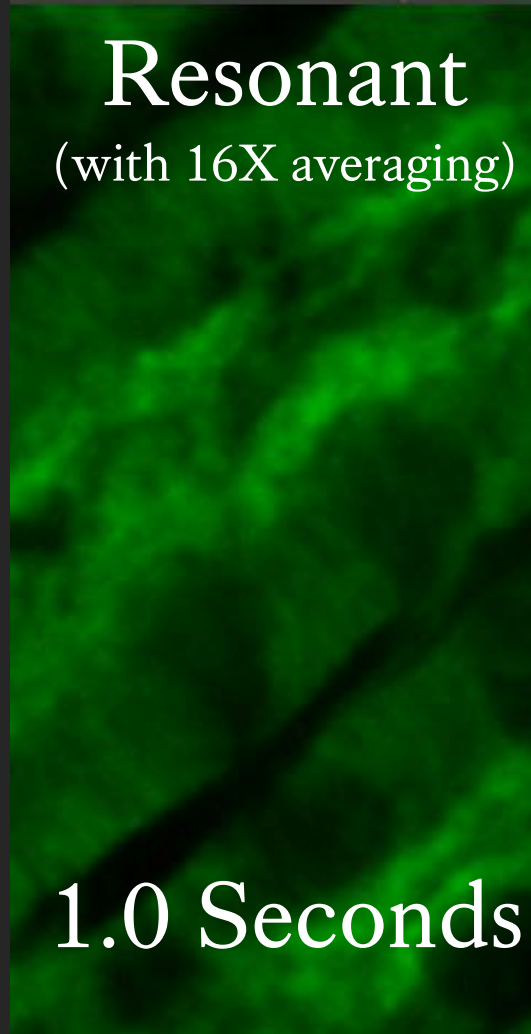
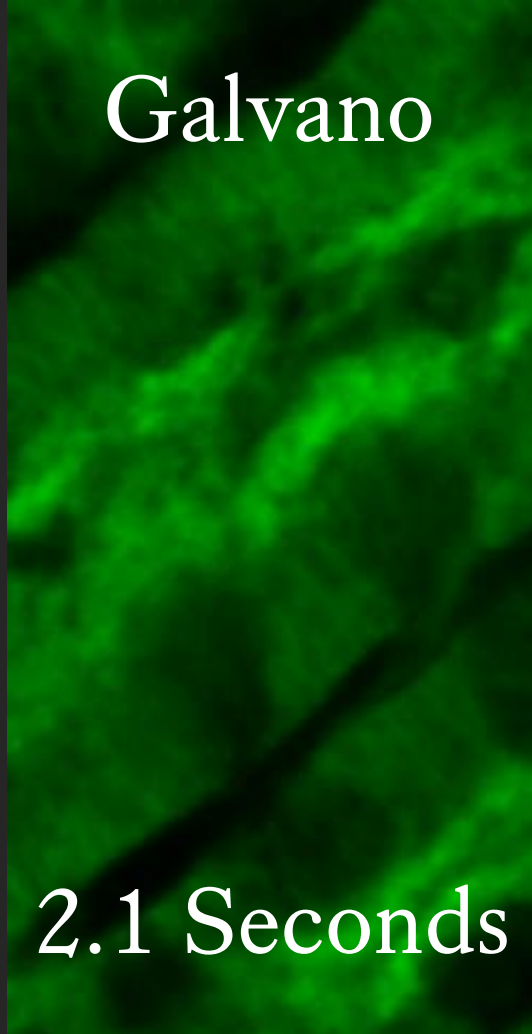
Galvano

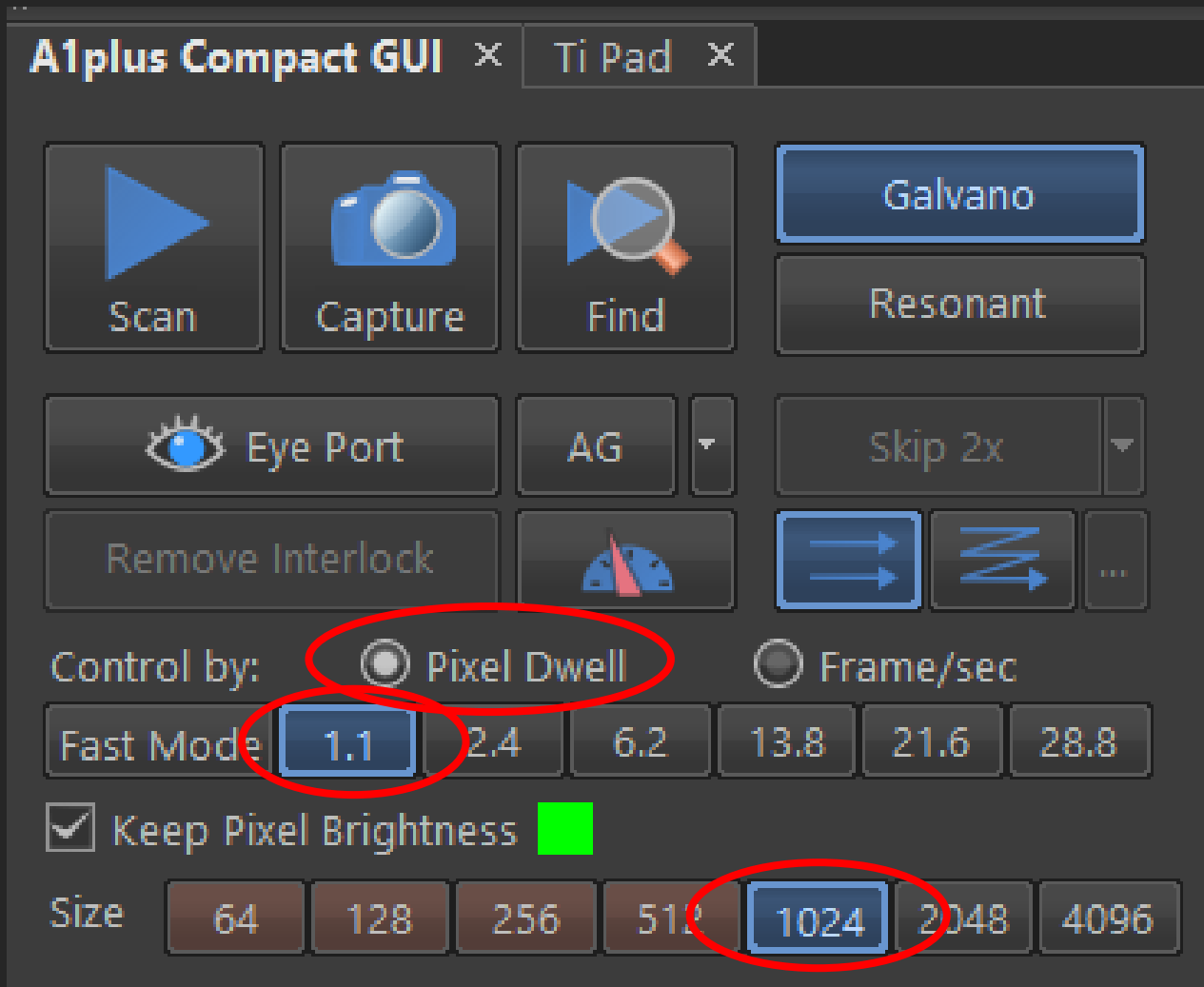
2.1 Seconds

Resonant  
(with 16X averaging)

1.0 Seconds

Resonant  
(no averaging)





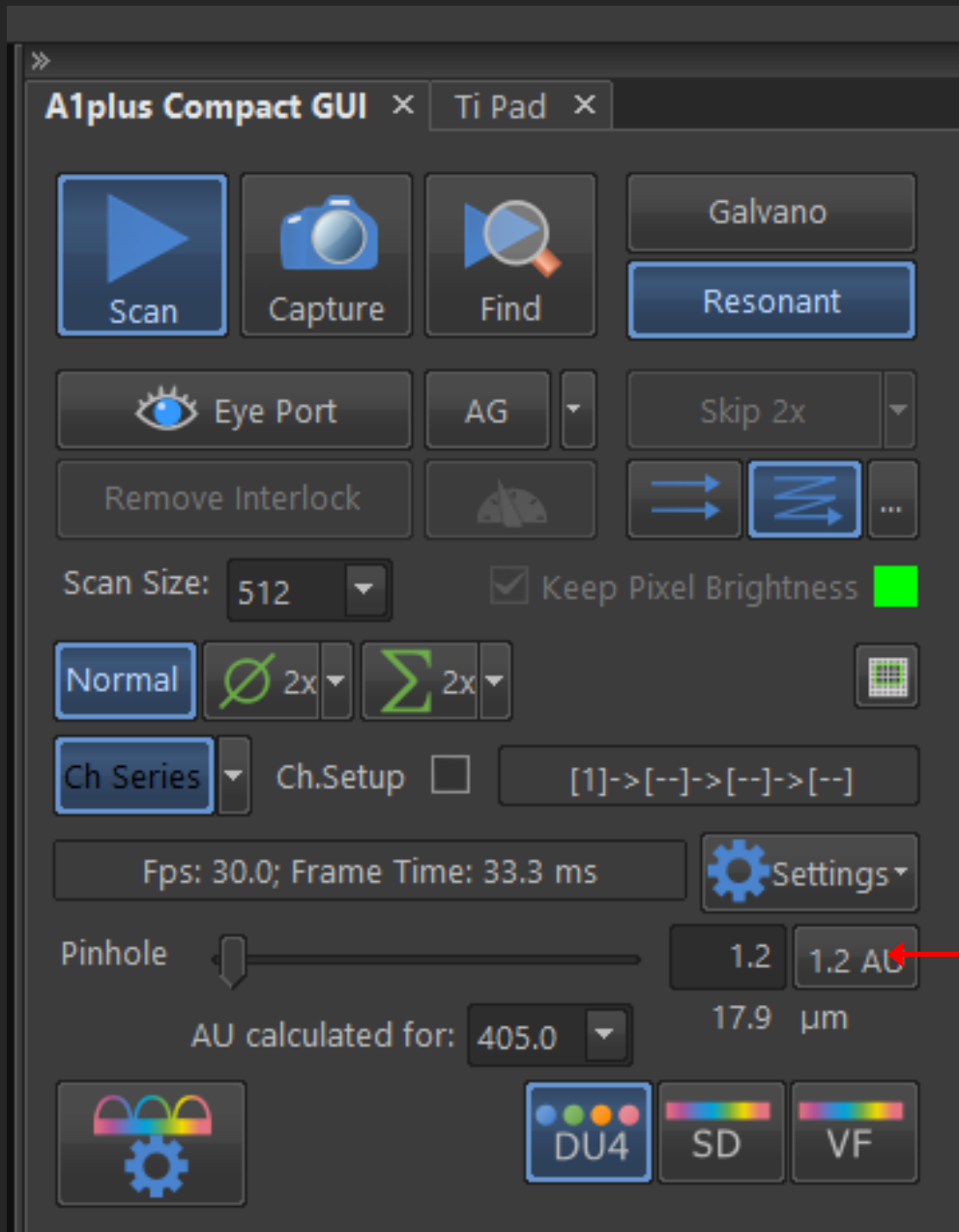
When switching from Resonant to Galvano, the software resets these settings every time, so please do the following:

1. Select Pixel Dwell
2. 1024
3. 1.1

# STEP BY STEP INSTRUCTIONS

## STEP 5

### Setting Up Initial Live View



Set your Pinhole size to 1.2AU

Click

To set pinhole to  
recommended starting point.

For New Samples Use these initial numbers!!!

DAPI Laser 405.4 nm 0.0

HV 70

Offset 0

405 5.00

✓ Alexa 488 water → Laser 487.6 nm 0.0

HV(G) 1

Offset 0

487 1.00

✓ Alx568 Laser 561.7 nm 0.0

HV(G) 1

Offset 0

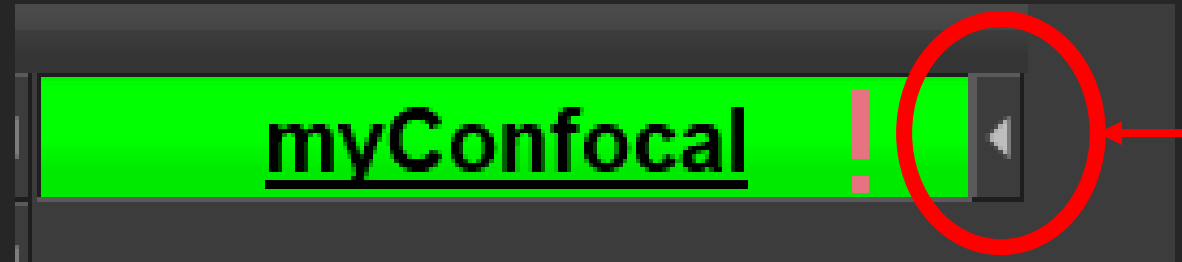
561 1.00

✓ Alx647 Laser 640.0 nm 0.0

HV 70

Offset 0

640 5.00



- 1) First input these initial setting numbers into your channel set up and update the myConfocal button. (This gives you a safe starting point)
- 2) Choose a single channel you want to see first.
- 3) Uncheck all the other channels but that one.

e.g. 488 channel

DAPI Laser 405.4 nm 0.0

✓ Alexa 488 water → Laser 487.6 nm 0.0

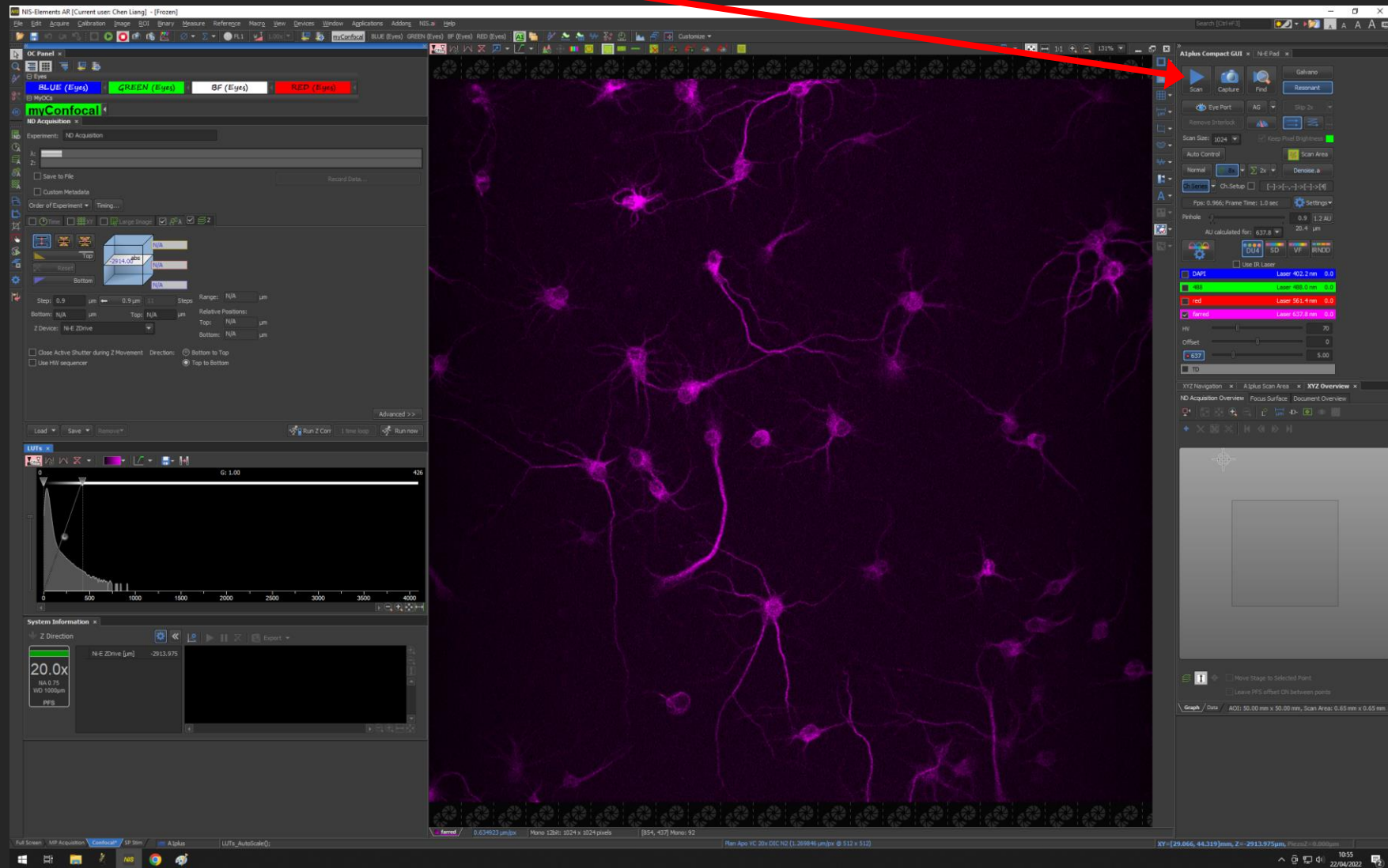

HV(G) 1

Offset 0

487 1.00

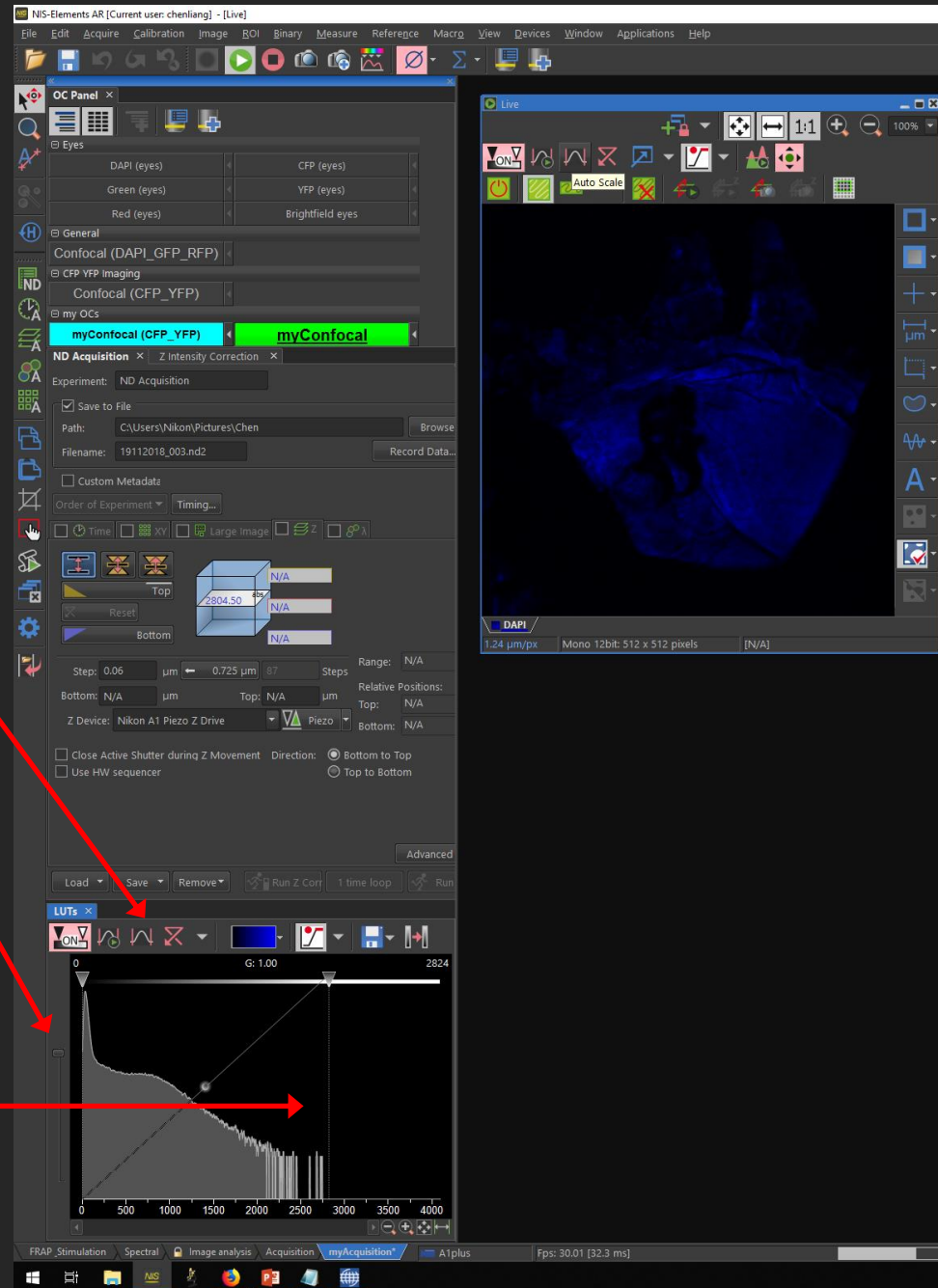
Alx568 Laser 561.7 nm 0.0

Alx647 Laser 640.0 nm 0.0



# To visualise your image...

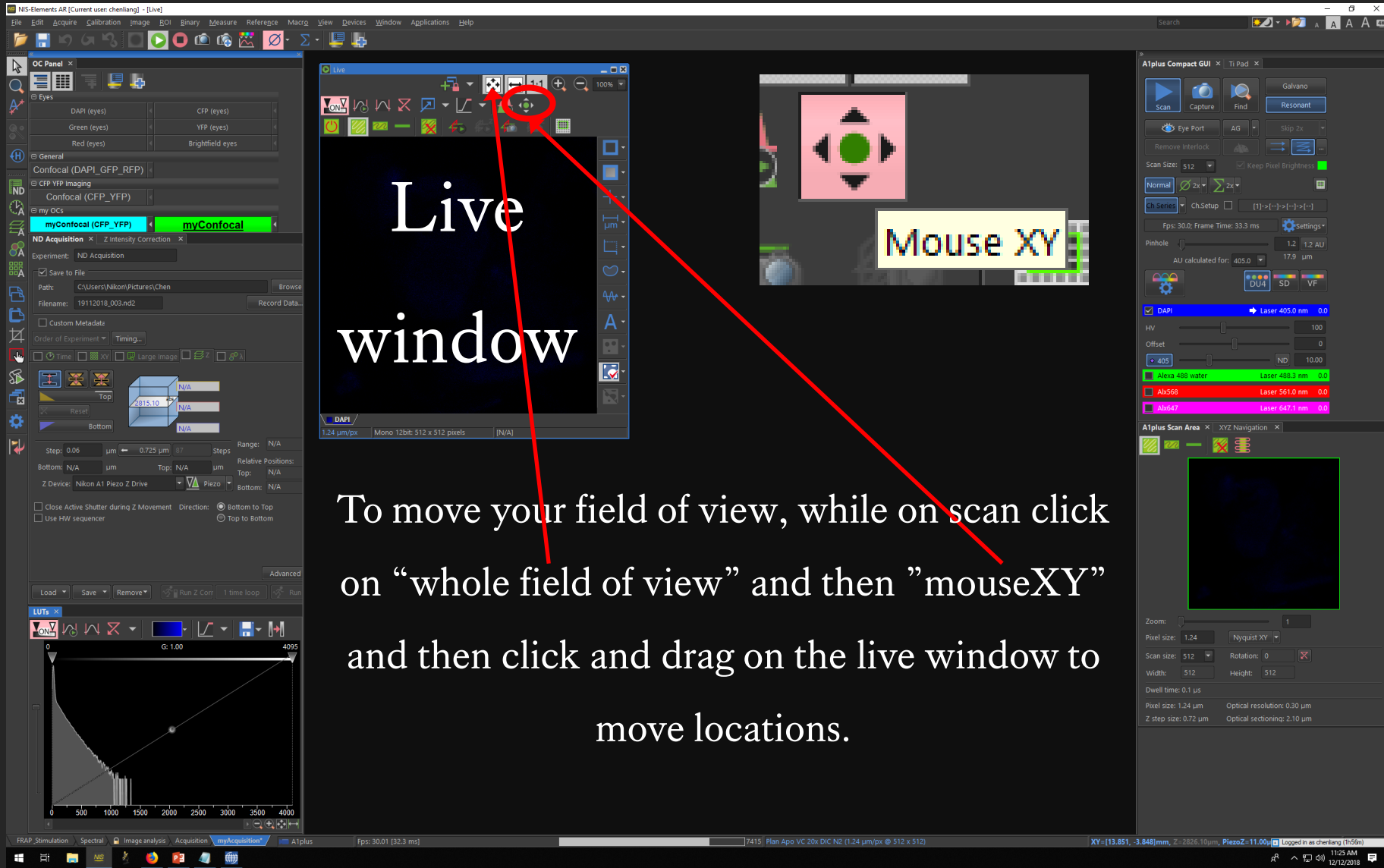
1. Click on auto contrast
2. Slide this bar to the top
3. Drag the contrast line towards the left.



4. Starting to see something on the screen...
5. Now bring it into focus (hover mouse over image and use mouse wheel to change focus.)
6. Once in focus STOP scanning to preserve your sample.



You can move your field of view  
But only do this when your sample is visible and in focus in your live window

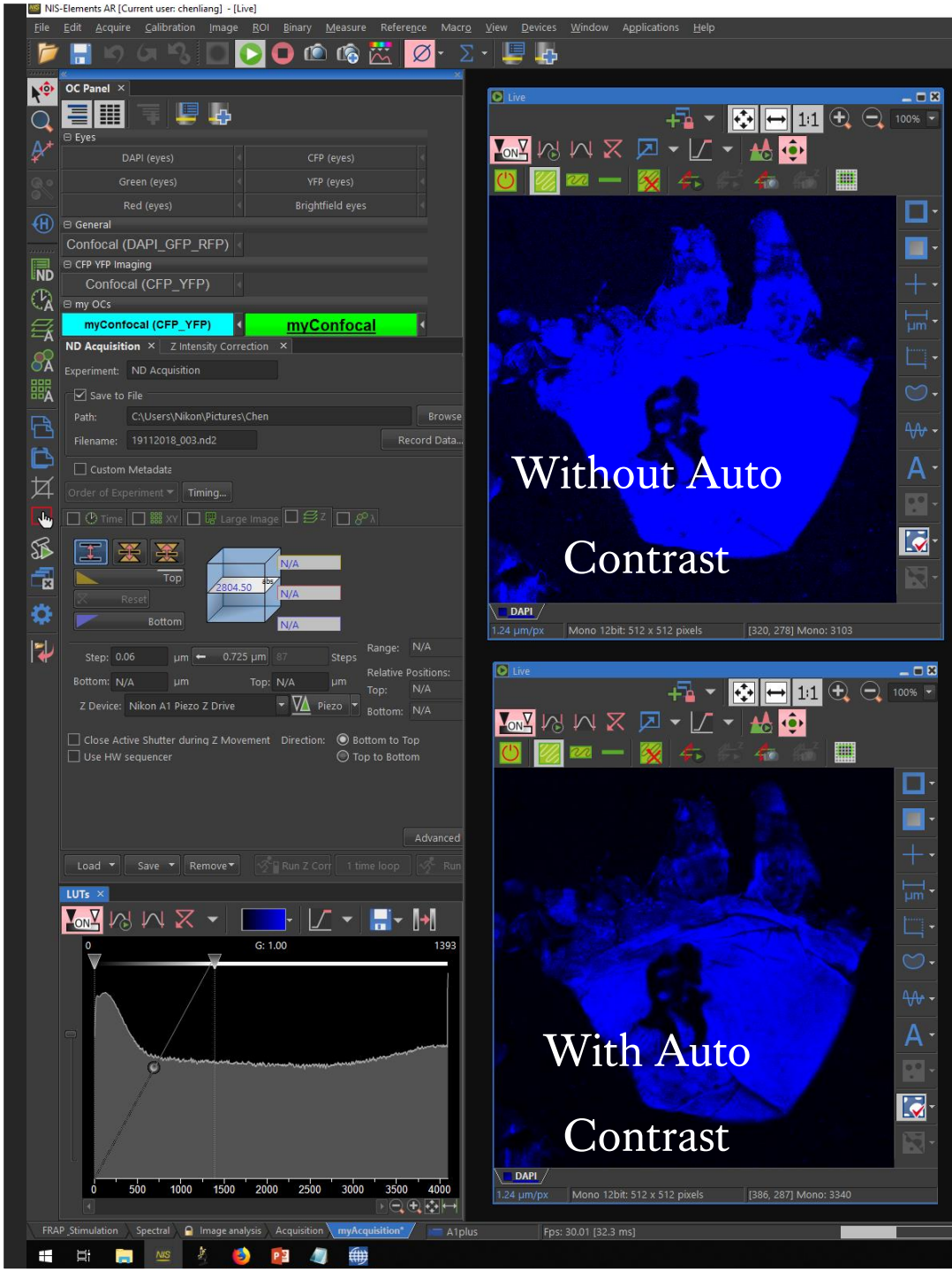


The screenshot displays the NIS-Elements AR software interface. The central 'Live' window shows a dark field with a bright, circular spot. A red circle highlights a button in the top toolbar of the Live window, with a red arrow pointing to it. A yellow box labeled 'Mouse XY' is positioned over the Live window. The left sidebar contains the 'OC Panel' with various acquisition settings. The right sidebar shows the 'A1plus Compact GUI' with scan parameters and a 'Scan Area' window. The bottom status bar displays acquisition details.

Live window

Mouse XY

To move your field of view, while on scan click on “whole field of view” and then ”mouseXY” and then click and drag on the live window to move locations.



While you are changing focus or field of view, your LUTs will change accordingly...

You may find you have too much or too little signal...

So now we move on to optimising your camera settings to get your signal just right...

LUTs

Additional Information Slides...

# LUTs And Contrast

## LUTs explained.

- Changing LUTs to visualise your sample better does not change your raw data (signal intensity).
- This means when you open your image again in e.g. ImageJ, LUTs will not be applied.
- If you change LUTs before quantitative analysis, it is recommended to save the LUTs and apply it to all comparable images.

## Why do we need to change the contrast?

The camera in this microscope captures shades from 0 to 4000, initially the LIVE window shows you all these different shades, but the signal from this sample only reach roughly 2500 therefore we only need to work within the 0 to 2500 range.

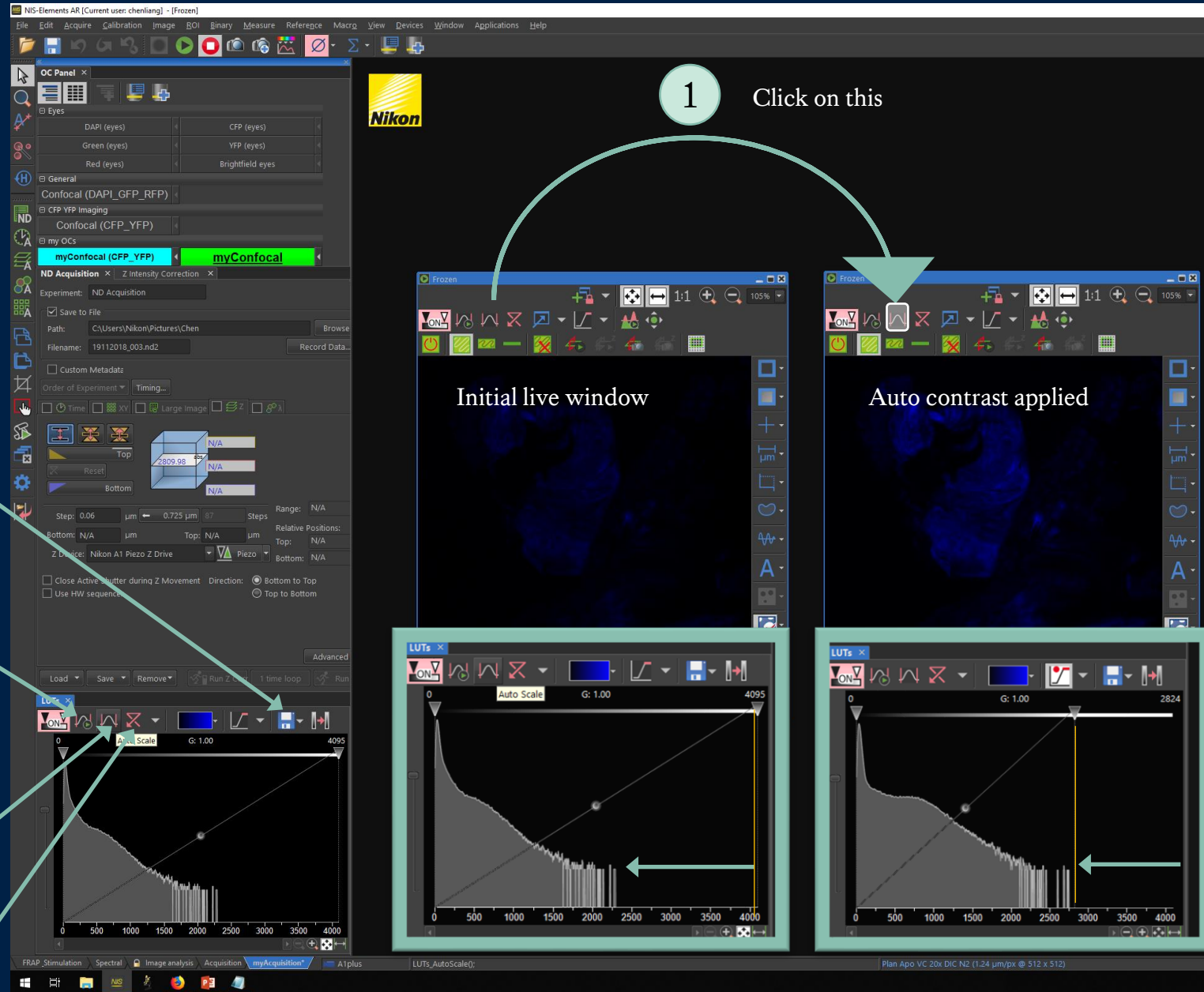
Auto contrast brings the contrast into a range for you to better visualise your sample.

LUTs can be saved in a separate file and applied to other images.

Continuous auto contrast (there may be flickering while in live view)

Auto Contrast

Reset LUTs



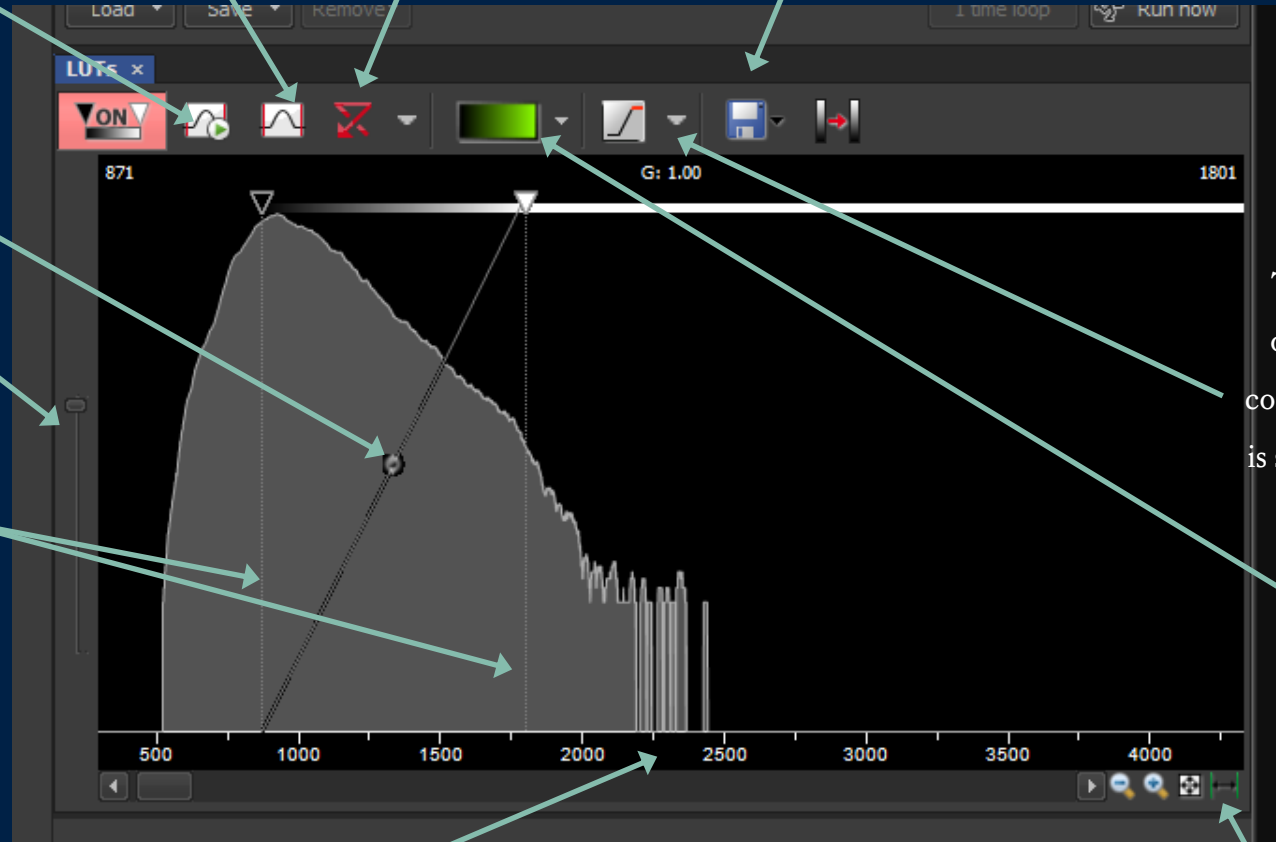
# LUTs in more detail...

Continuous auto-contrast while in live view – can cause a flickering effect.

Auto-contrast

Delete all contrast adjustments

To keep analysis consistent, you can copy and paste LUTs across different captured images.



Makes dim targets brighter while keeping bright target the same.

Controls the Y axis log graph

Controls the X axis contrast, this doesn't change the captured raw data.

The spinning disk does not over saturate, but on other microscopes, this is for viewing saturation, complementary colour recommended. Once an area is saturated, it loses any intensity value information.

The camera is a black and white camera. You can assign any colour combination to your captured image.

Drag and change the threshold of this histogram for better visualisation.

X axis - relative to #-bit camera (e.g. 16-bit CCD camera gives 65,536 different intensity values, of which you should not go over 50,000). Y axis – log intensity scale

Fit the histogram to this space

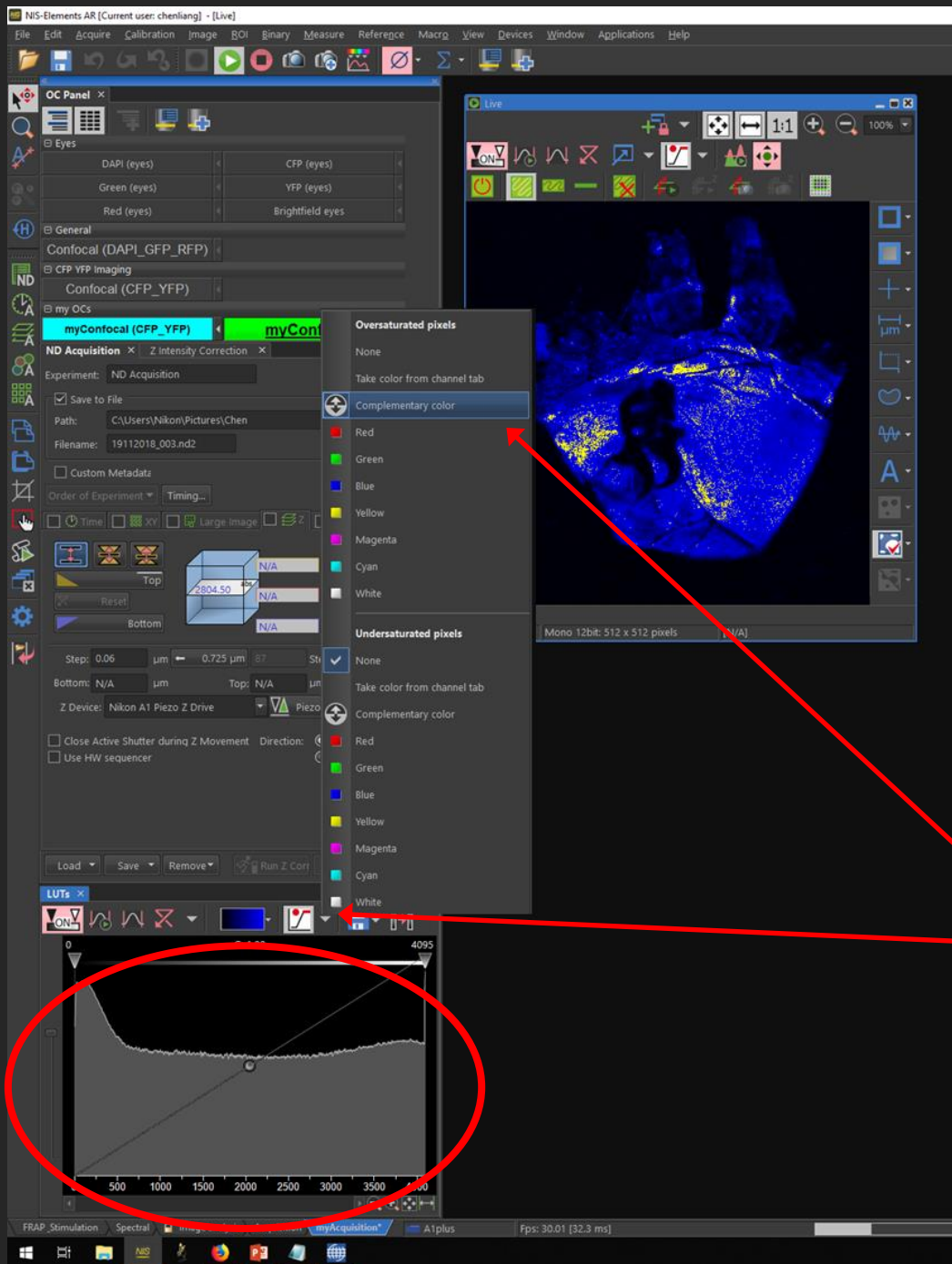
# STEP BY STEP INSTRUCTIONS

## STEP 6

Optimising your camera settings

...when you have too much signal





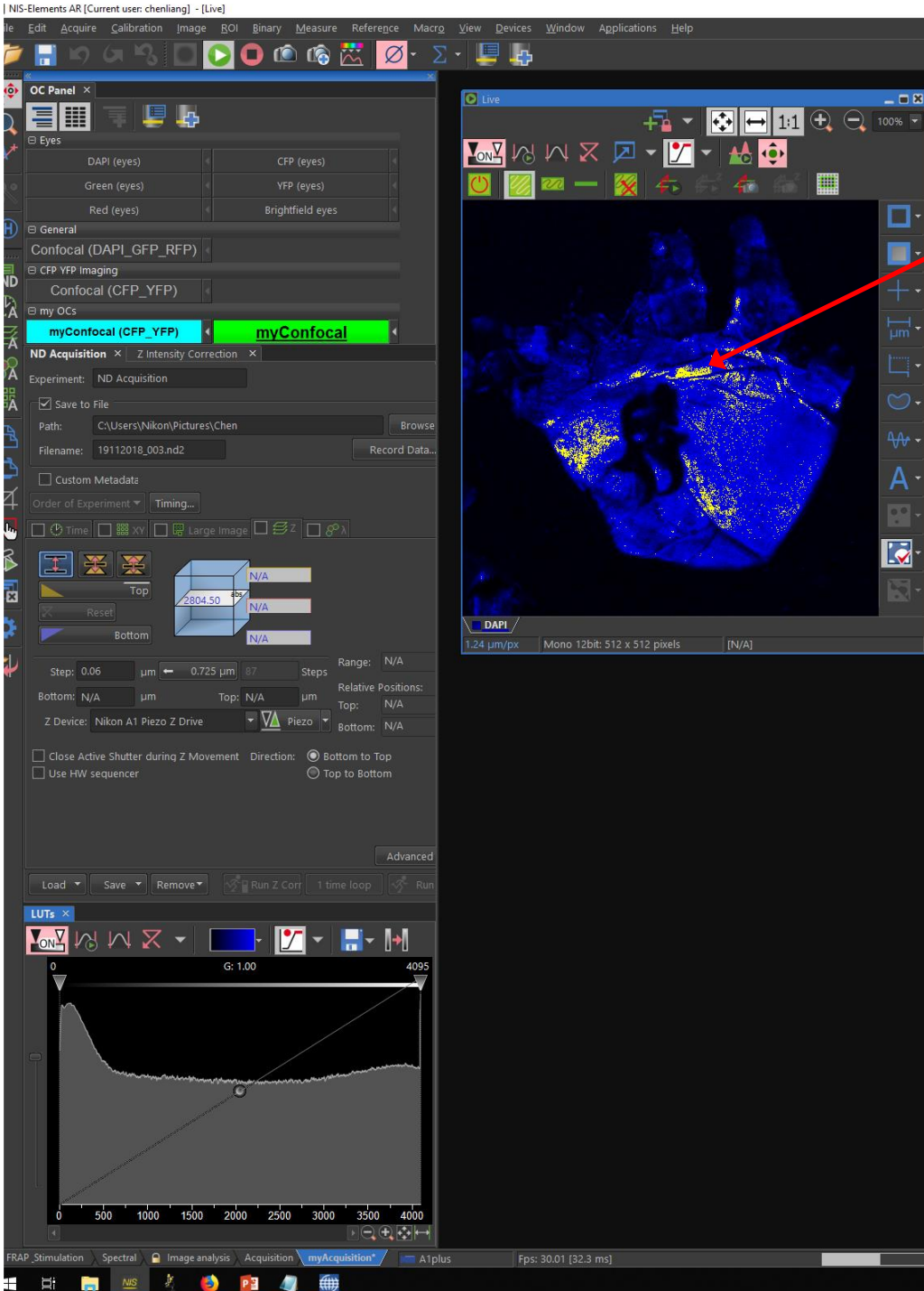
Do I need to optimise my settings?

How to tell if you have too much or not enough signal?

When your LUTs graph is filled up like this, you may be oversaturating your sample.

Turn on your oversaturating indicator by selecting complementary colour in the drop down.

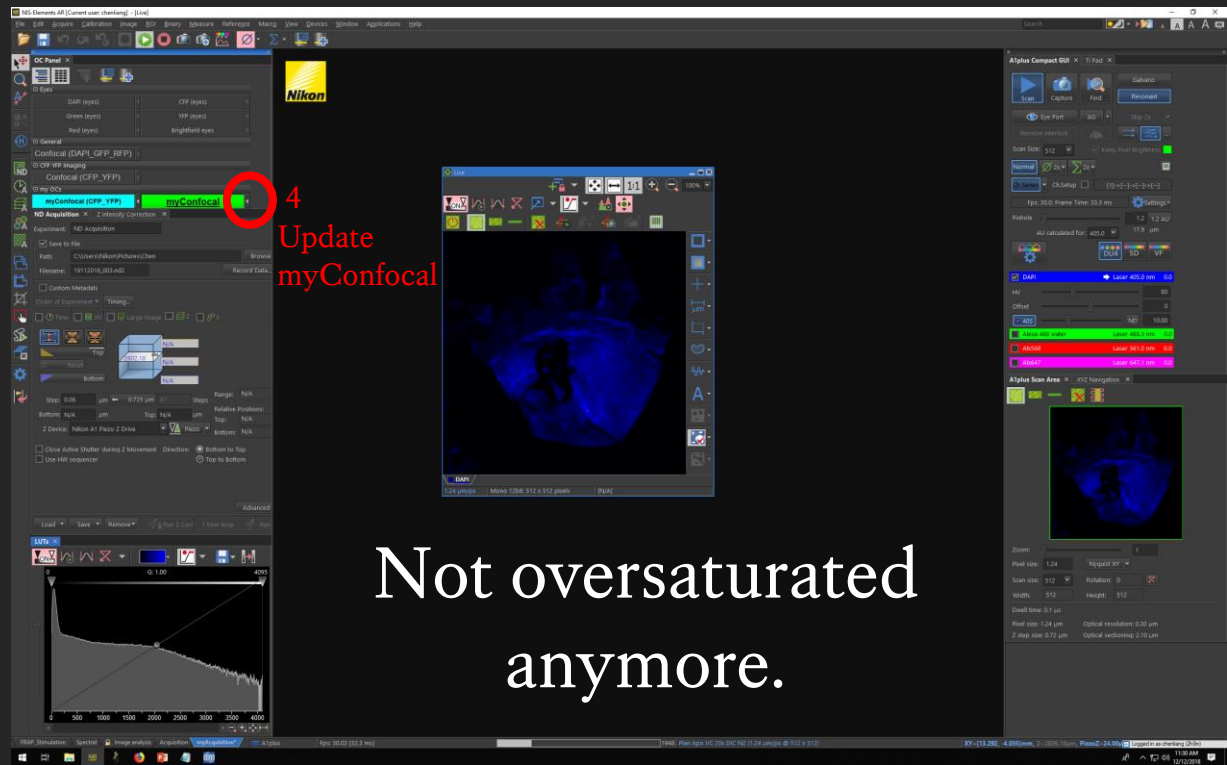




# Oversaturation!

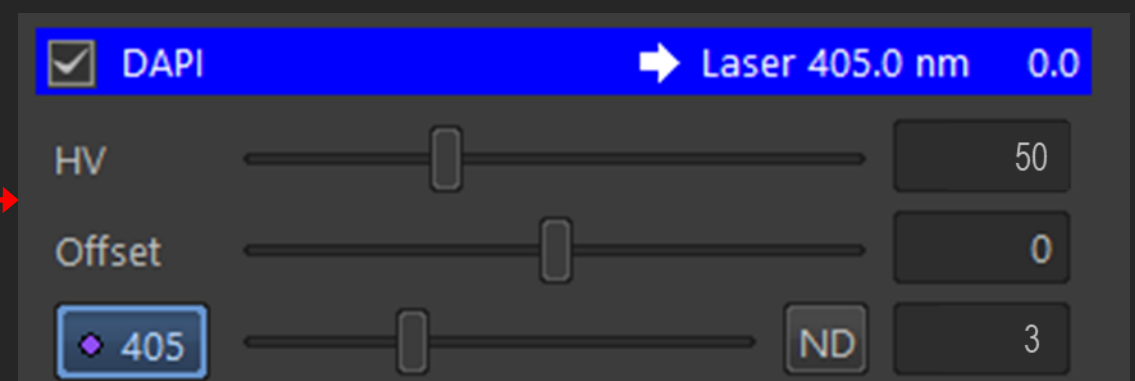
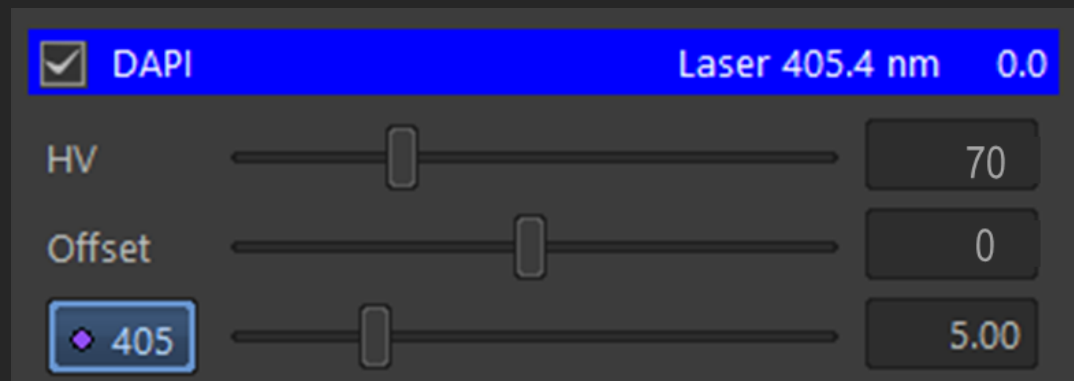
Oversaturation means the camera is picking up too much signal, and the camera can no longer determine the actual intensity of your signal, it just knows that sample is 'bright'.

This can cause you problems during analysis, because you won't have intensity information.



# To fix oversaturation...

1. Reduce the Gain (HV) and laser power to reduce oversaturation.
2. Press ENTER to confirm change.
3. Go to live view again to check.
4. Update changes in myConfocal



# STEP BY STEP INSTRUCTIONS

## STEP 6

Optimising your camera settings

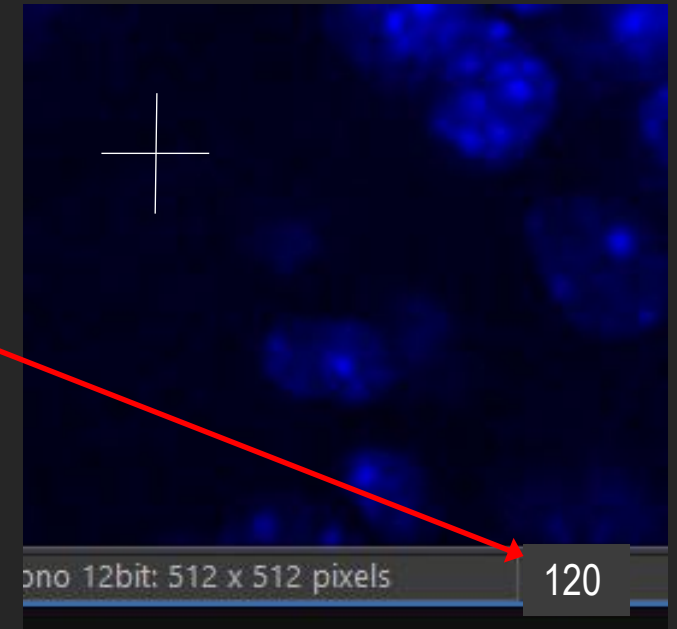
...when you don't have enough signal

# How much signal is enough signal?

This is not a straightforward answer because this really depends on what you want to measure and what analysis you want to carry out, we should be able to advise you on this during your second training session. For this slide, I'm going to use a common analysis request: "I want to use thresholding to count my cells."

To check your signal intensity:

- 1) Make sure you're in focus, stop scanning to minimise bleaching, then click on your image.
- 2) Hover your mouse over the background in several places to measure background intensity, read and remember the intensity shown at the bottom of your image.
- 3) Do the same for your target, make sure you only measure what is in focus and what you want to analyse.
- 4) If you want to count your cells by eye, as long as you can see your cells clearly, then there's enough signal.
- 5) If you want to set up thresholding to carry out automated analysis, then the difference in intensity between your target intensity and background intensity need to be above 1000.
- 6) E.g. Average DAPI intensity 1800, average background intensity 200, then  $1800 - 200 = 1600$  intensity difference, enough to threshold with.



If you don't have enough signal, you can  
increase gain (HV) and laser power to  
increase signal intensity, but there are a few  
rules you need to follow.

# RULES and maximum numbers

The screenshot shows a control panel for a microscope with four channels. Each channel has a checked checkbox, a label, a laser wavelength, and a gain value. Below each channel are three sliders: HV, Offset, and a gain/laser value.

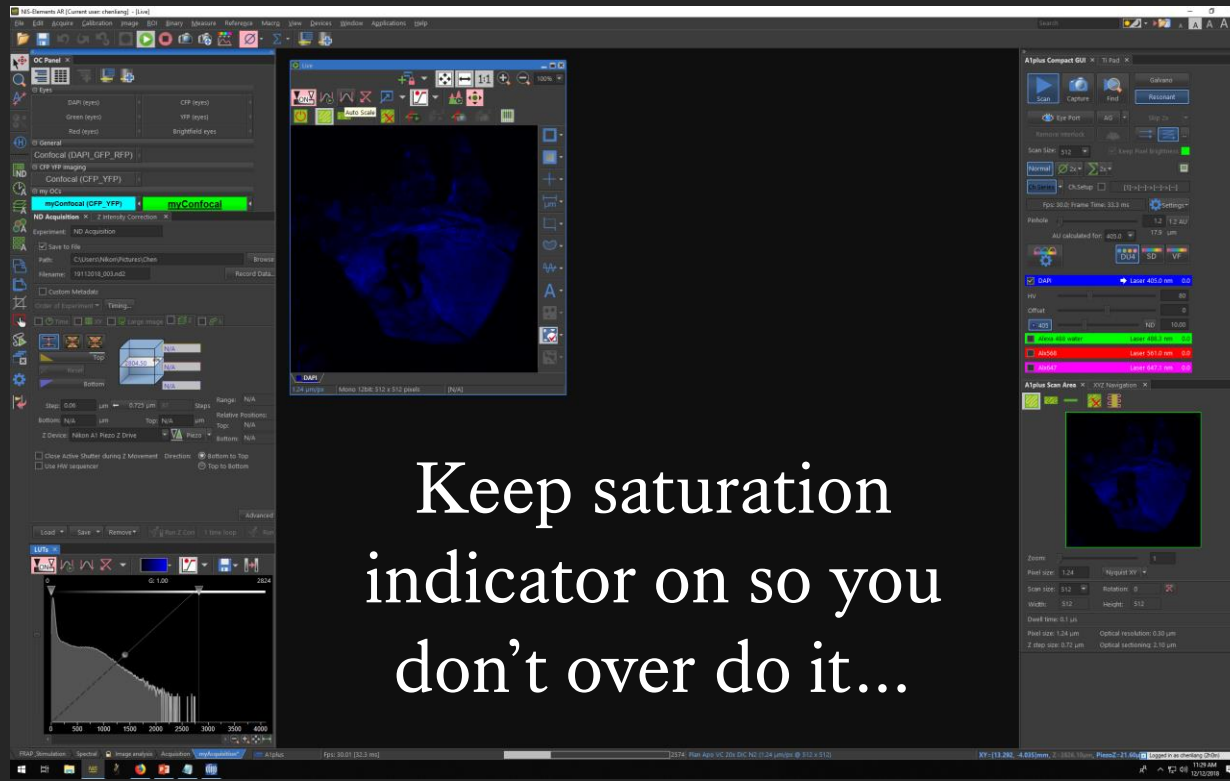
Channel	Label	Laser Wavelength	Gain Value	HV	Offset	Gain/Laser Value
DAPI	DAPI	405.4 nm	0.0	70	0	5.00
Alexa 488 water	Alexa 488 water	487.6 nm	0.0	1	0	1.00
Alx568	Alx568	561.7 nm	0.0	1	0	1.00
Alx647	Alx647	640.0 nm	0.0	70	0	5.00

Make small incremental increases for green and red channels, increase gain / laser by no more than 5 each time and DO

NOT Increase HV (G) above 50 !!!

For DAPI and far-red try changing by no more than 10 at a time and do not increase HV above 100.

Keep checking that you're not oversaturating!



# To increase signal...

1. Increase Gain (HV) and laser power.  
(In SMALL increments for green and red channels)
2. Press ENTER to confirm change.
3. Go to live view again to check.
4. Update changes in myConfocal



# STEP BY STEP INSTRUCTIONS

## STEP 6

Optimising your camera settings



background  
SIGNAL



background  
NOISE

...when there is too much background SIGNAL

use OFFSET.



# OFFSET...

helps to adjust the background voltage level to appear black on the computer screen, offset does this by shifting the entire amplitude of the signal without altering actual amplitude.

e.g. when offset -5 is applied to amplitude voltage 5 – 15 (figure 5a) it will shift to voltage 0 – 10 (figure 5b) but the actual difference between the voltage remains at 10 volts difference. Gain can then be applied to amplify the amplitude of the voltage (figure 5c).

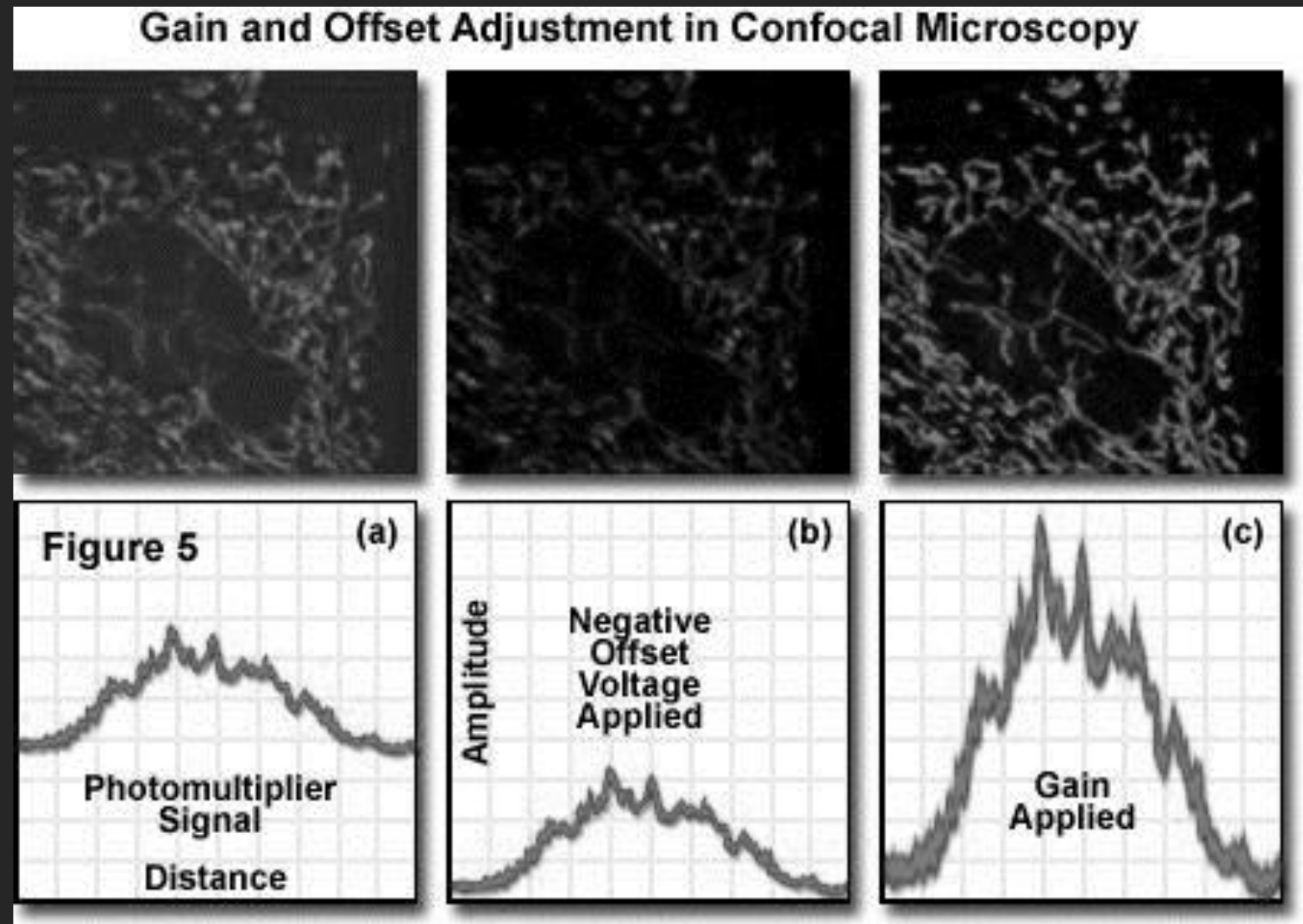


Image modified from: <https://www.olympus-lifescience.com/en/microscope-resource/primer/techniques/confocal/pmtintro/>



Please speak to us before you use offset, if used  
incorrectly, it could affect your research integrity.

# STEP BY STEP INSTRUCTIONS

## STEP 6

Optimising your camera settings



background  
SIGNAL



background  
NOISE

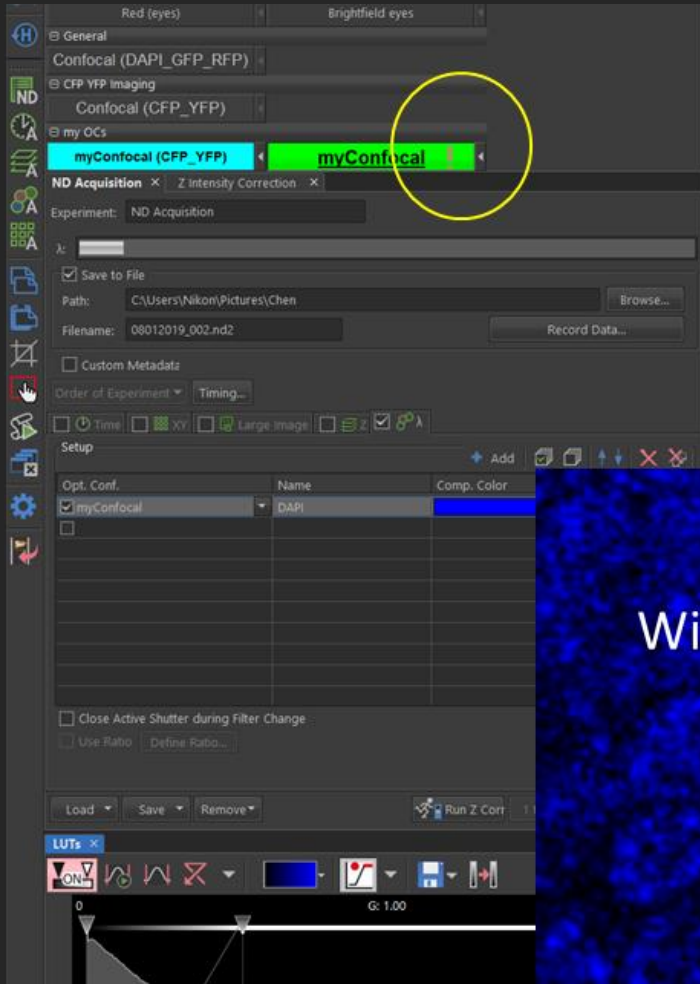
...when there is too much background NOISE

use AVERAGING.

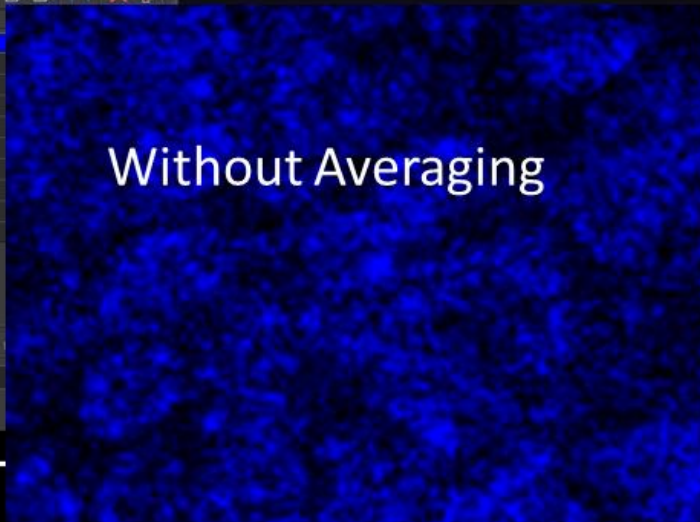
# Averaging



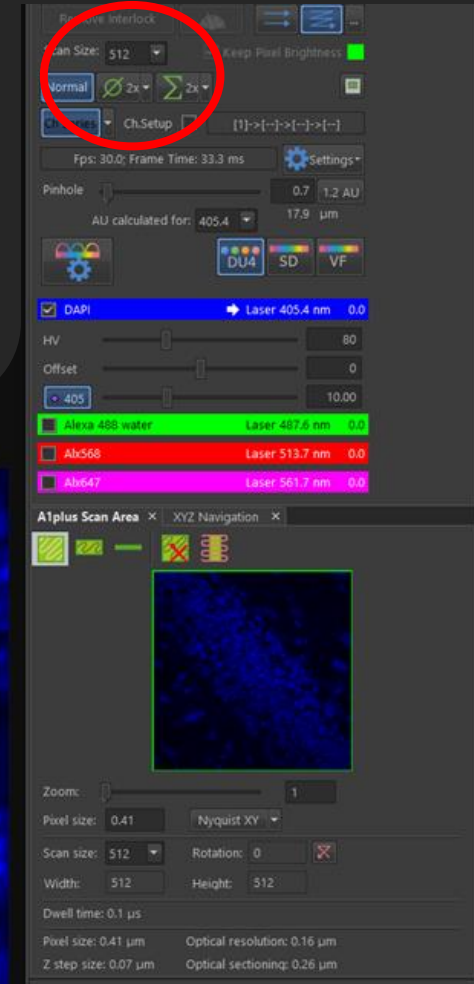
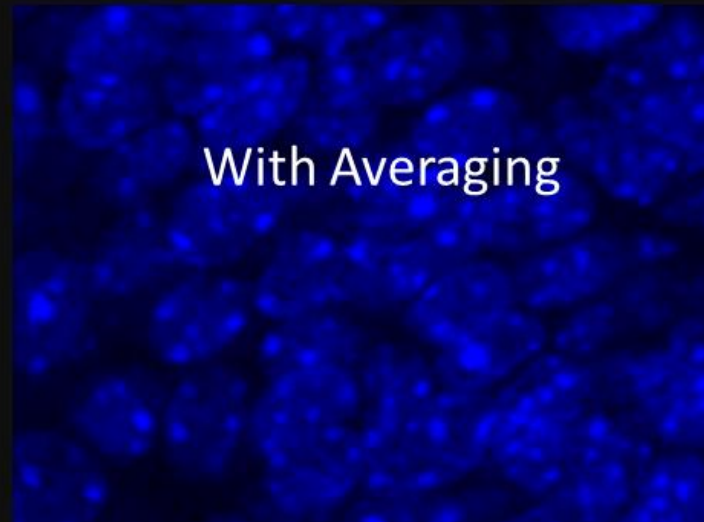
- Takes multiple images and averages them out.
- Beware 8X averaging increase your imaging time by 8 folds.
- Remember to update myConfocal



Without Averaging



With Averaging



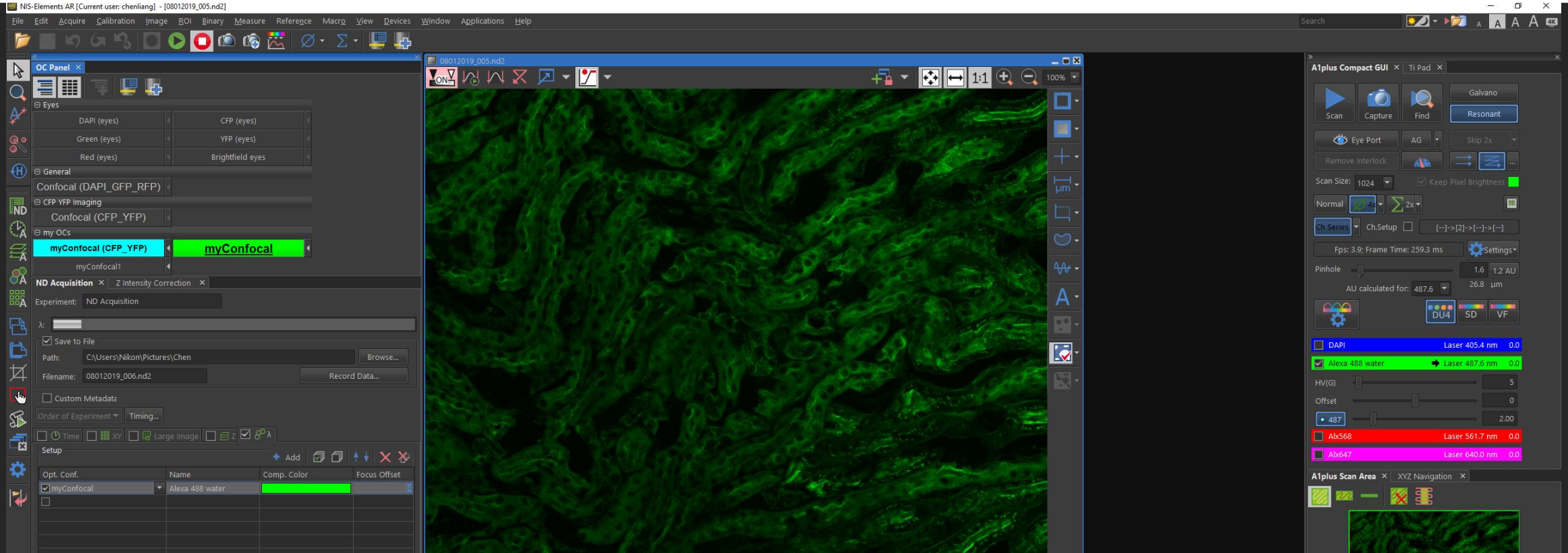
# STEP BY STEP INSTRUCTIONS

## STEP 6

### Optimising your camera settings

...now do STEP 6 for every channel you want to use.





Optimise your camera settings for each channel you want to use during imaging.

- 1) While you are on STOP.
- 2) Check the box of another channel you want to use.
- 3) Uncheck any other selected channels
- 4) i.e. Check GFP channel then unselect DAPI in this case
- 5) Input the initial gain and laser power!!! If you haven't done so at the beginning of your session.
- 6) **Be very GENTLE with your GREEN and RED channels.**
- 7) Click on LIVE and continue to optimise by repeating STEP 4 for each channel you want to use.

## A Helpful Table: Camera Settings – What Does It Do?

Camera Settings	Concept	Pro	Con	When To Change This...
Averaging	The camera takes multiple images and form an averaged image.	<ul style="list-style-type: none"> <li>• Reduce noise</li> </ul>	<ul style="list-style-type: none"> <li>• Increase acquisition time drastically</li> </ul>	<ul style="list-style-type: none"> <li>• Increase when your image have lots of noise.</li> </ul>
Binning	Combines the charges (signal) from adjacent pixels to form one “super” pixel.	<ul style="list-style-type: none"> <li>• Faster read out</li> <li>• Increase signal to noise ratio</li> </ul>	<ul style="list-style-type: none"> <li>• Trades resolution for sensitivity</li> </ul>	<ul style="list-style-type: none"> <li>• Increase when there is very little signal from your sample, causing low intensity in your image.</li> </ul>
Exposure	Longer exposure means the camera has longer to collect the emitted light. Ideally exposure time should be just below the saturation threshold.	<ul style="list-style-type: none"> <li>• Detector receive more signal from your sample</li> </ul>	<ul style="list-style-type: none"> <li>• Phototoxicity</li> <li>• Bleaching</li> <li>• Fade</li> <li>• Acquisition time</li> </ul>	<ul style="list-style-type: none"> <li>• Increase when signal captured is not enough to give you the intensity level you need. <ul style="list-style-type: none"> <li>• Decrease to preserve your sample.</li> </ul> </li> </ul>
Gain	Controls how much the signal is amplified before reaching the detection system. Increased gain makes photomultiplier tube plates more negatively charged = more amplification.	<ul style="list-style-type: none"> <li>• Amplifies signal without causing bleaching or phototoxicity</li> </ul>	<ul style="list-style-type: none"> <li>• Gain increases noise</li> </ul>	<ul style="list-style-type: none"> <li>• Increase when signal is low and you have sensitive samples.</li> <li>• Decrease if you have too much noise.</li> </ul>
Laser Power	The % power of a very photon-dense light source, focused in a very tight beam.	<ul style="list-style-type: none"> <li>• Penetrates deeper into sample</li> <li>• Increase signal</li> </ul>	<ul style="list-style-type: none"> <li>• Bleaching</li> <li>• Heating</li> <li>• Harmful to sample</li> </ul>	<ul style="list-style-type: none"> <li>• Increase when you have thick samples or need more signal.</li> <li>• Decrease if you have sensitive sample, especially live samples.</li> </ul>

# STEP BY STEP INSTRUCTIONS

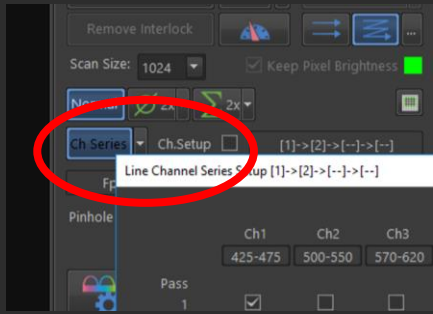
## STEP 7

How to avoid bleed-through

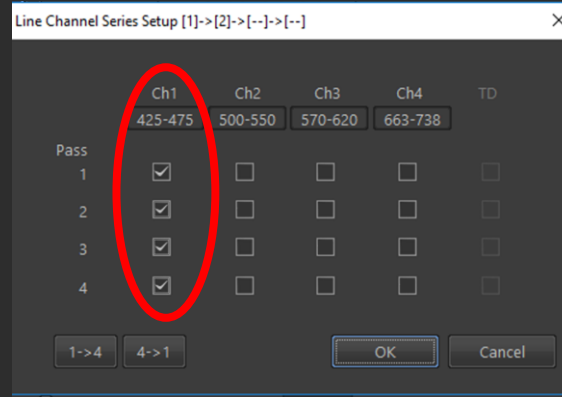
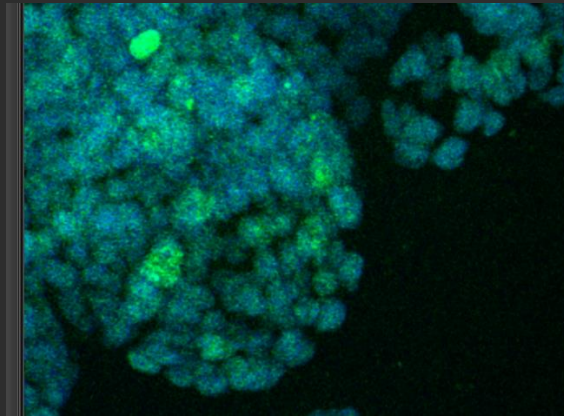


# Channel Series

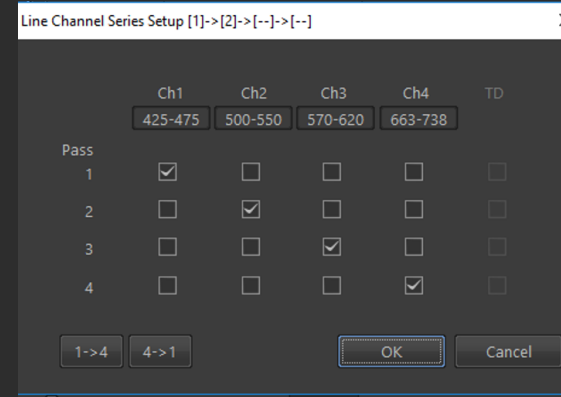
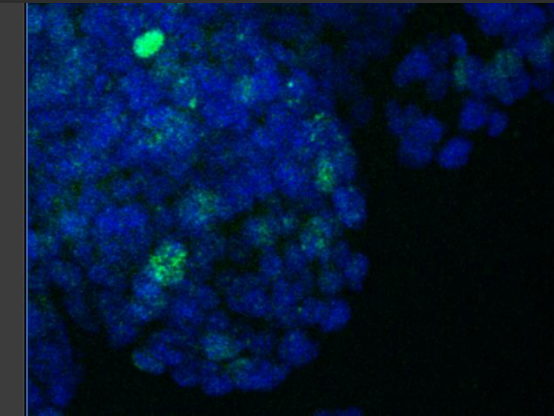
In your  
camera  
settings



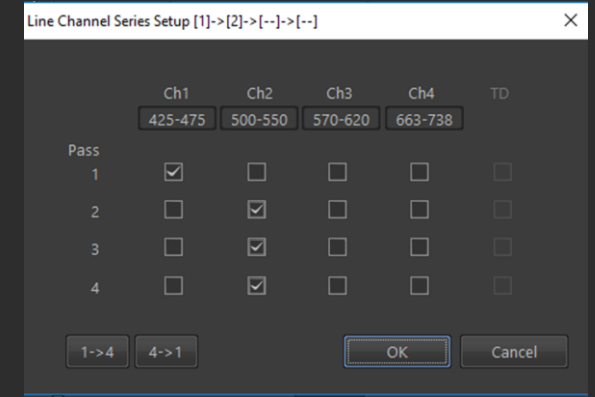
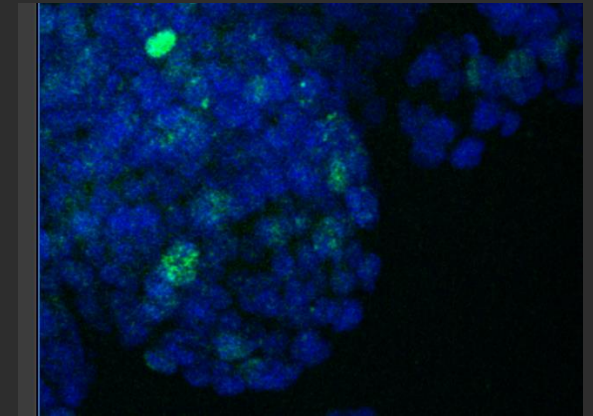
In this example DAPI  
signal is bleeding into  
the GFP channel.



This indicated you can  
scanning all channels at the  
same time, this is quick but  
can give false signal if bleed  
through occurs.



This indicated you are  
scanning each channel  
individually, this avoids bleed  
though but is a slower  
scanning method.



If DAPI is the only channel  
bleeding through, then you  
can scan DAPI alone and  
scan all other channels  
together to save time.

# STEP BY STEP INSTRUCTIONS

## STEP 8

Optimising your image resolution.

Up until now I've gone over how to change your camera settings to change the amount of signal intensity you can get. But the amount of signal and how much detail you can see within that signal are two different things.

Confocal microscopes can provide high resolution images, so here are some concepts to help you understand what actually changes image resolution and also what you need to change on the microscope and in this software to get the best possible image resolution.

But keep in mind, this is just a demonstration and when you come to do your own imaging, you'll have to consider what analysis you want to do and decide on how much image detail or resolution you actually need. Because the higher the resolution generally the more bleaching and the longer it takes to image.

To optimise your resolution you have 3 decisions to make...

1. Choosing a suitable lens and immersion medium
2. Set pinhole size (recommended: equal to or smaller than 1.2 AU)
3. Set sampling frequency (Zoom and Z stack step size)

# Resolution

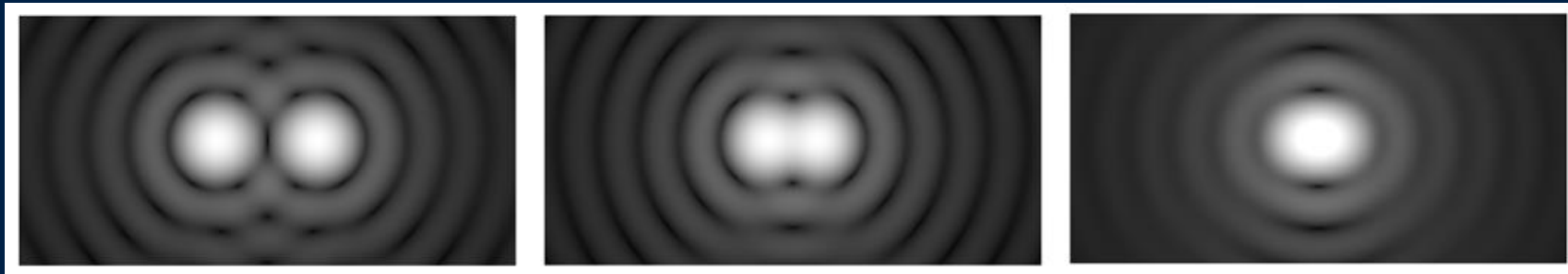
## Additional Information Slides

Please read the following additional information slides to familiarise yourself with these microscope concepts.

# Resolution is...

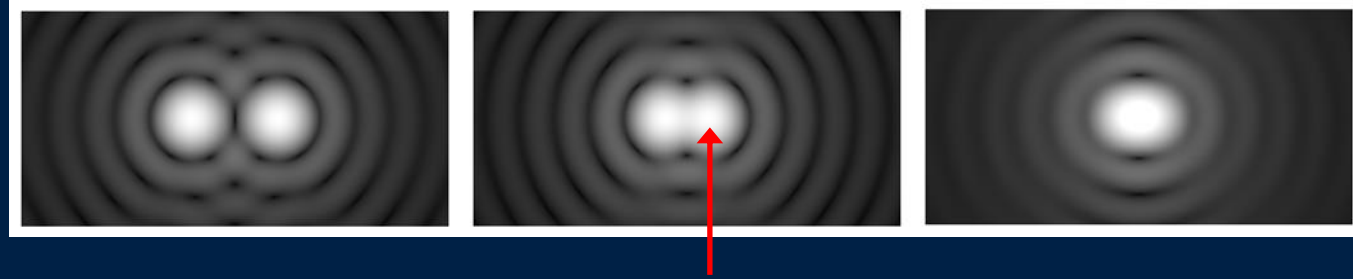
The ability to distinguish separate objects.

The highest resolution you can achieve is called the resolution limit, basically it's as close as 2 objects can get and still be visualised as 2 separate objects.



This limit exists because...

# Light coming from a very small point spreads out in Airy Disks



When another object overlaps the first peak intensity ring, the drop in intensity between the two objects is enough for us to categorise them as 2 different objects.

$$\text{Lateral (XY) Resolution} = 0.61 \lambda / \text{NA}$$

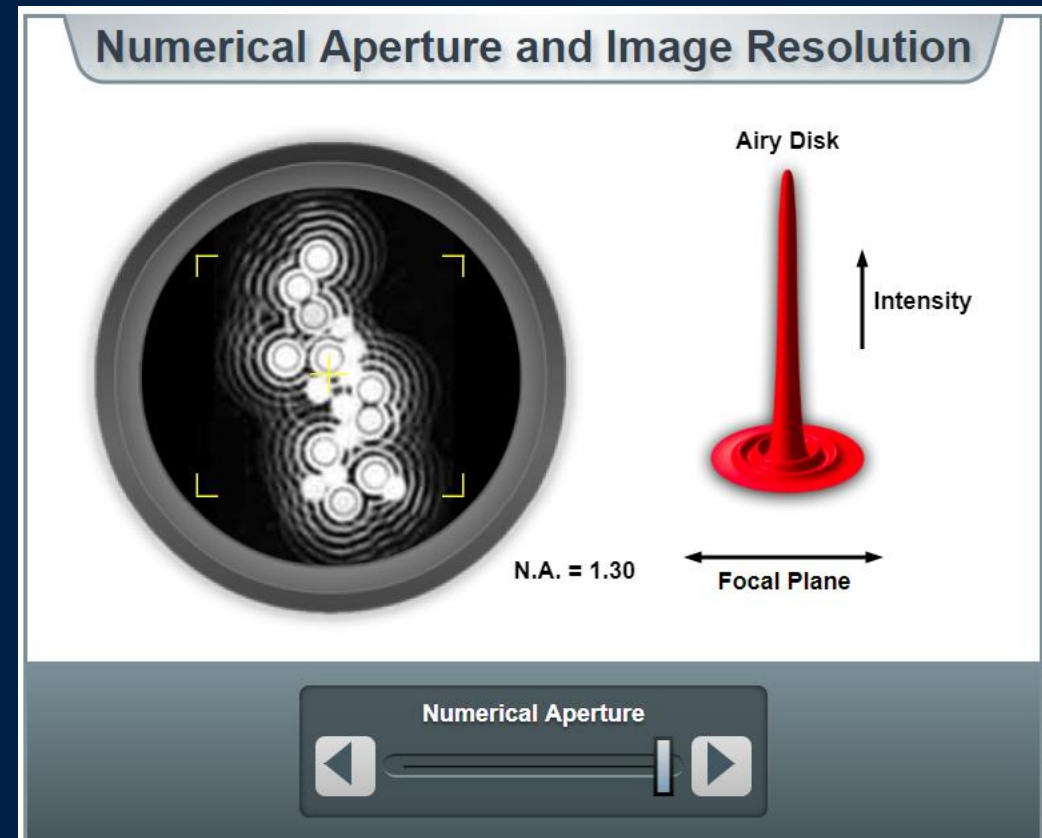
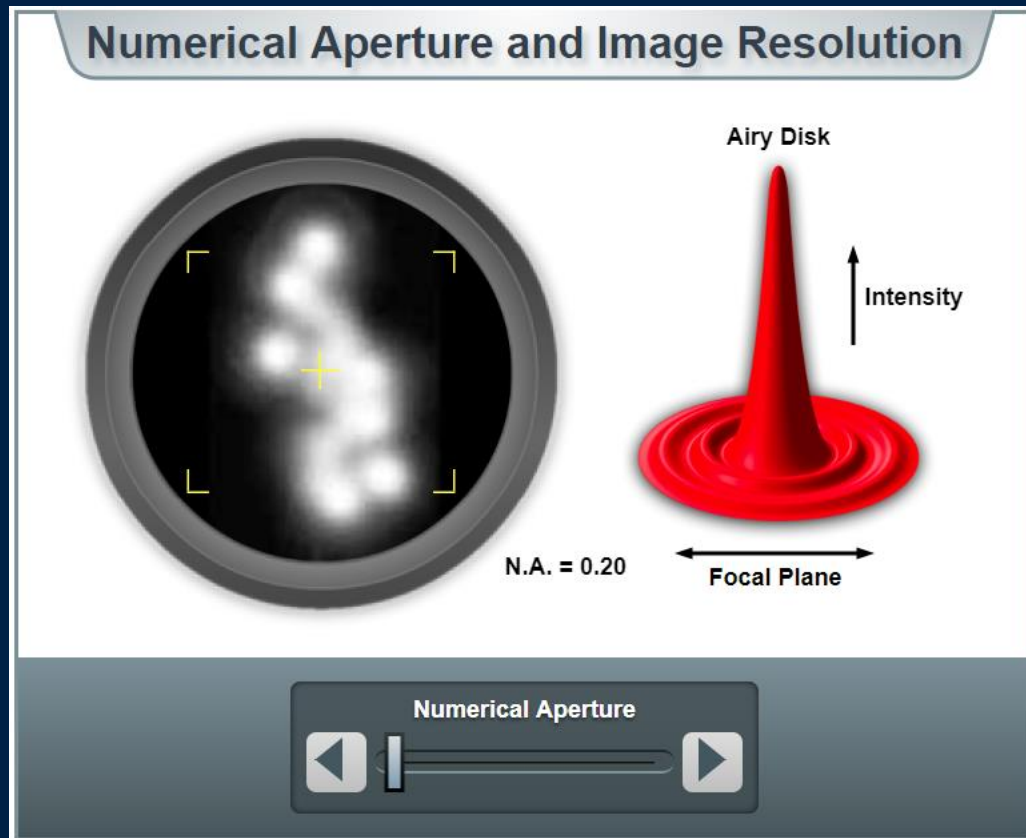
$$\text{Axial (XZ) Resolution} = 2 n \lambda / \text{NA}^2$$

## Rayleigh Criterion

$$\text{Lateral (XY) Resolution} = 0.61 \lambda / \text{NA}$$

E.g.  
 $0.61 \times 480\text{nm} / 0.8 = 366\text{nm}$   
 $0.61 \times 480\text{nm} / 1.4 = 209\text{nm}$

$$\text{Axial (XZ) Resolution} = 2 n \lambda / \text{NA}^2$$



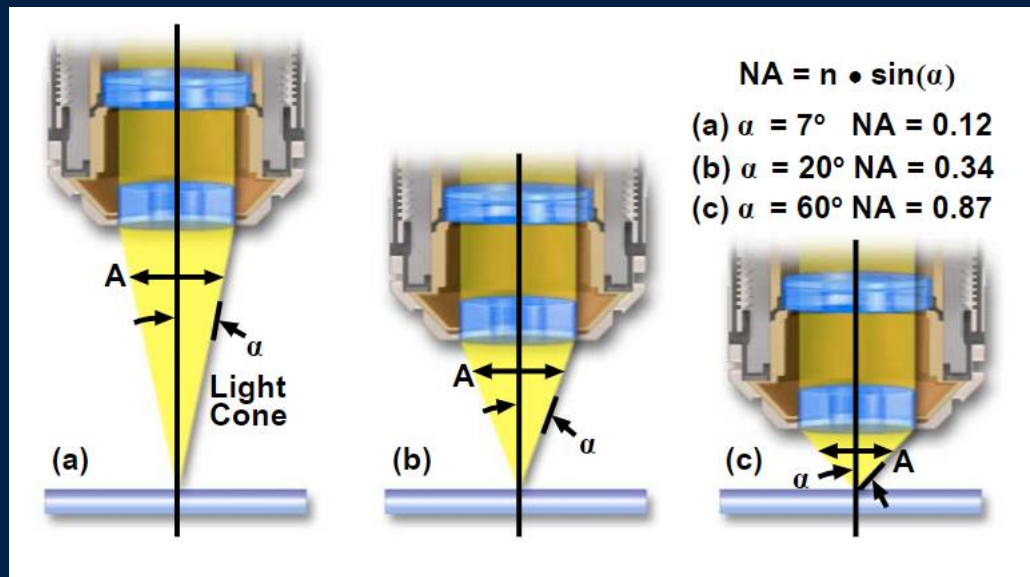
Changing the Numerical Aperture changes resolution!



# Numerical aperture (lens dependent)

NA is a measure of a lens' ability to gather light and detail.

Different lenses have different Numerical apertures



The closer the lens is to your sample and the better matched your immersion medium means you'll have a higher NA and can gather more detail from your sample.

Higher NA = better resolution

$$\text{Numerical Aperture (NA)} = n \times \sin(\alpha)$$

The immersion medium's refractive index:

Air = 1

Water = 1.33

Glycerine = 1.47

Immersion Oil = 1.51 = Glass (coverslip)

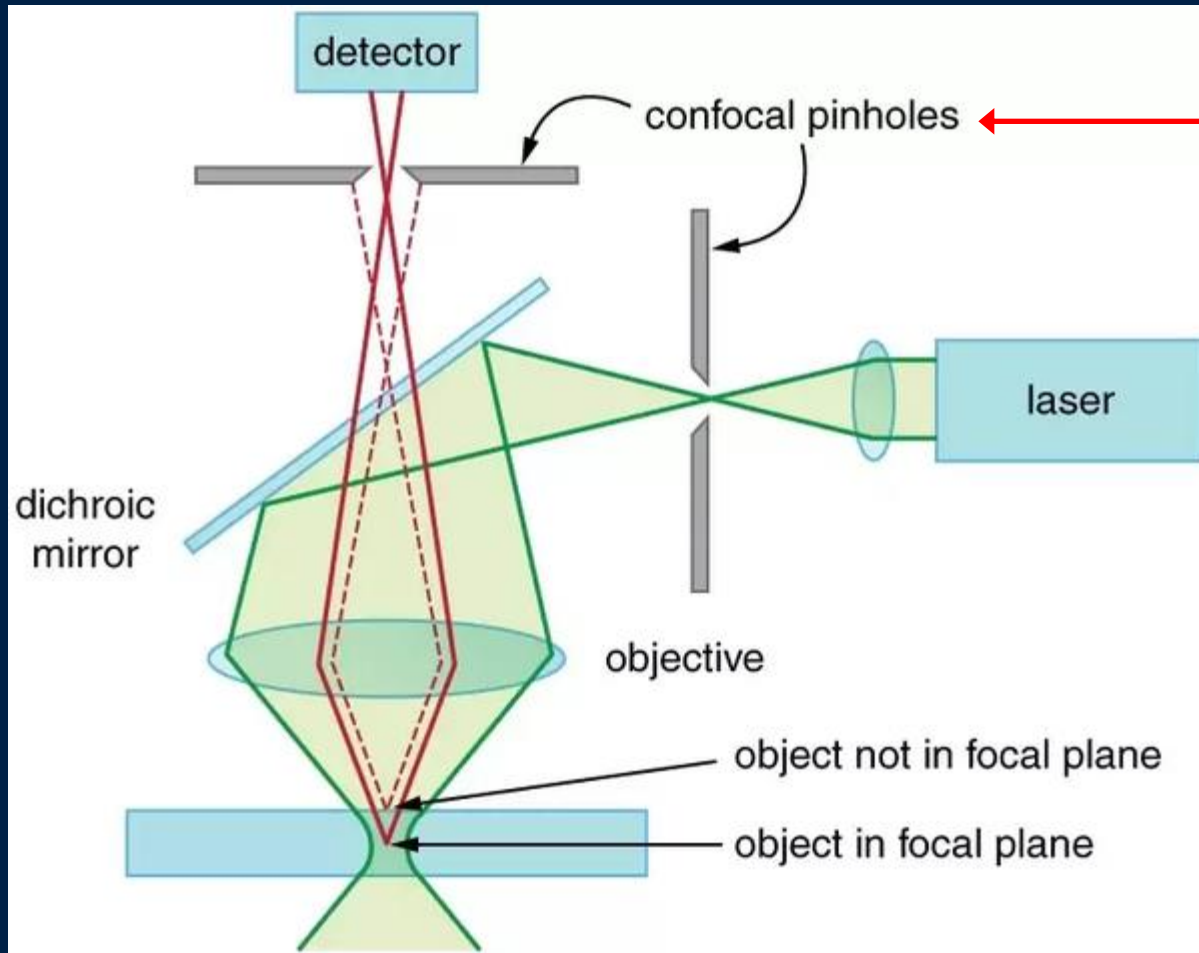
Z Resolution =  $2 n \lambda / NA^2$  is affected further by refractive index.

Angle between  
lens and sample.

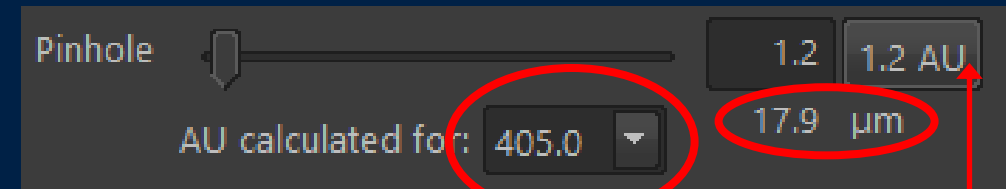
# Optimise your image resolution by...

1. Choosing a suitable lens and imaging medium
2. Set pinhole size (recommended: equal to or smaller than 1.2 AU)
3. Set sampling frequency (Zoom and Z stack step size)

Airy disk and numerical aperture affect all microscope machines  
so what makes confocal systems higher resolution than widefield?



Pinholes : minimises the detection of out of focus signal.



Recommended Pinhole size is 1.2AU

Be aware 1.2AU for different wavelength  
results in different pinhole sizes.

Chose a size and keep it that size when  
adjusting all channels

# Optimise your image resolution by...

1. Choosing a suitable lens and imaging medium
2. Set pinhole size (recommended: equal to or smaller than  $1.2 \text{ AU}$ )
3. Set sampling frequency (Zoom and Z stack step size)

After optimizing the NA and pinhole size, the next thing to consider is how to actually take the image...

You can have the most powerful lens and smallest pinhole but if you don't set up sampling frequency correctly, then you won't capture enough detail to end up with a high resolution image.

## Sampling frequency

(pixel and step size dependent)

- When your specimen emit fluorescent light, it is picked up by the camera and gets translated into digital pixels.
- When we decide on sampling frequency we are telling the microscope how many pixels we want to record within a fixed distance.
  - For optimal resolution, that fixed distance would be the smallest resolution limit you can achieve (lens and pinhole).
- If you only sample once, that is unreliable, like if you ran an experiment without any repeats.
- So you need to sample more than once, within your resolution limit.

A standard sampling frequency can be worked out using the Nyquist Limit (N) equation.

$$N = 0.3 \lambda / NA$$

Most researchers uses the Nyquist limit equation to works out a sampling frequency of 2.2, which means you need to sample 2.2 times within your resolution limit ( $0.61 \lambda / NA$ ) to be sure the signal you are sampling is real.

So how do you apply this 2.2 times sampling frequency...

So how do you apply this 2.2 times sampling frequency...

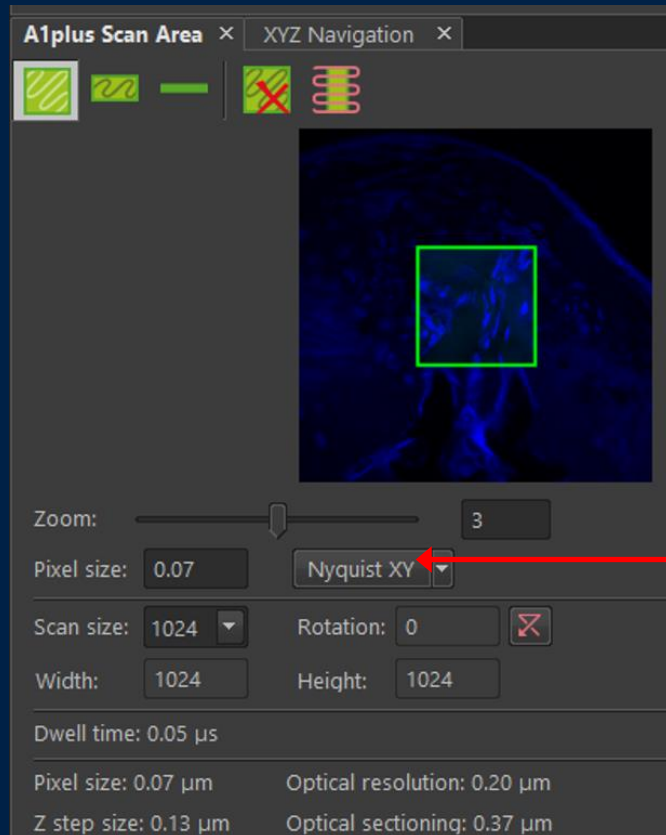
$$\begin{aligned} \text{Lateral (XY) Resolution} \\ = 0.61 \lambda / \text{NA} \end{aligned}$$

Scan area / Zoom

$$\begin{aligned} \text{Axial (XZ) Resolution} \\ = 2 n \lambda / \text{NA}^2 \end{aligned}$$

Z stack step size

# Lateral (XY) sampling frequency can be changed using the ZOOM option.



The software knows the lens and pinhole size you are using, so it calculates your resolution limit and Nyquist limit for you.

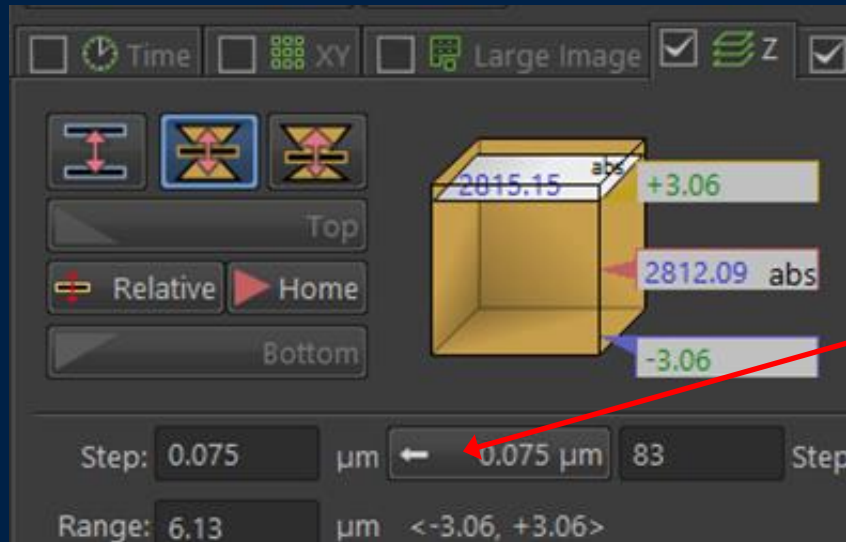
All you need to do is click on Nyquist XY for recommended pixel size. The correct Zoom will be automatically applied.

You are at your resolution limit, so even if you zoom in even further, you won't be able to distinguish any more detail from your specimen.



Axial (Z) sampling frequency can be changed using the  
Z stack step size option.

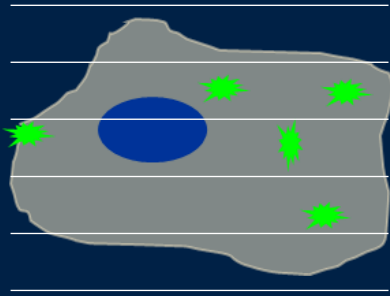
You can apply this during your acquisitions set up later...



The software again calculated your resolution limit for you. All you need to do is click here to apply the recommended step size during Z stacking.

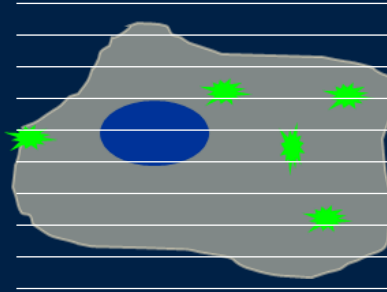
# You don't have to use the recommended step size...

Under-sample



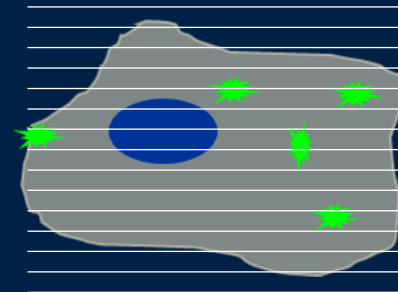
Make step size bigger  
Step size e.g.  $0.5\mu\text{m}$

Recommended  
Step Size



Step size e.g.  $0.3\mu\text{m}$

Oversample



Decrease by  $1/3$   
Step size e.g.  $0.2\mu\text{m}$

Faster acquisition  
Less bleaching

Slower acquisition  
More bleaching

# What happens when you under-sample

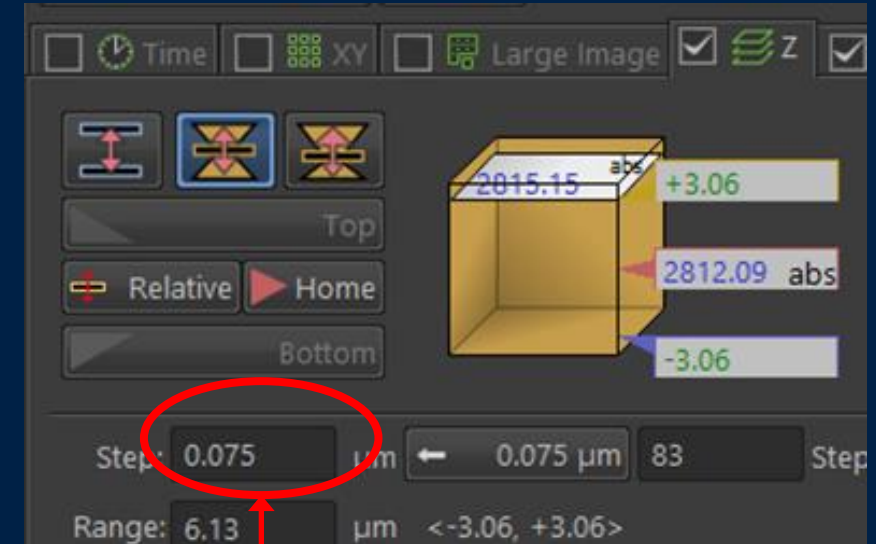
Actual Image



Under-sampled Image

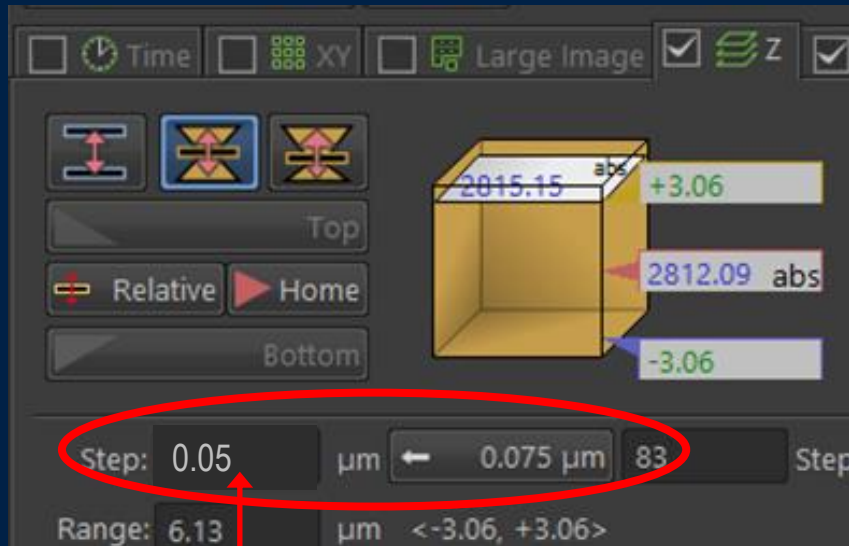


Aliasing occurs when signal becomes indistinguishable and create distortions.

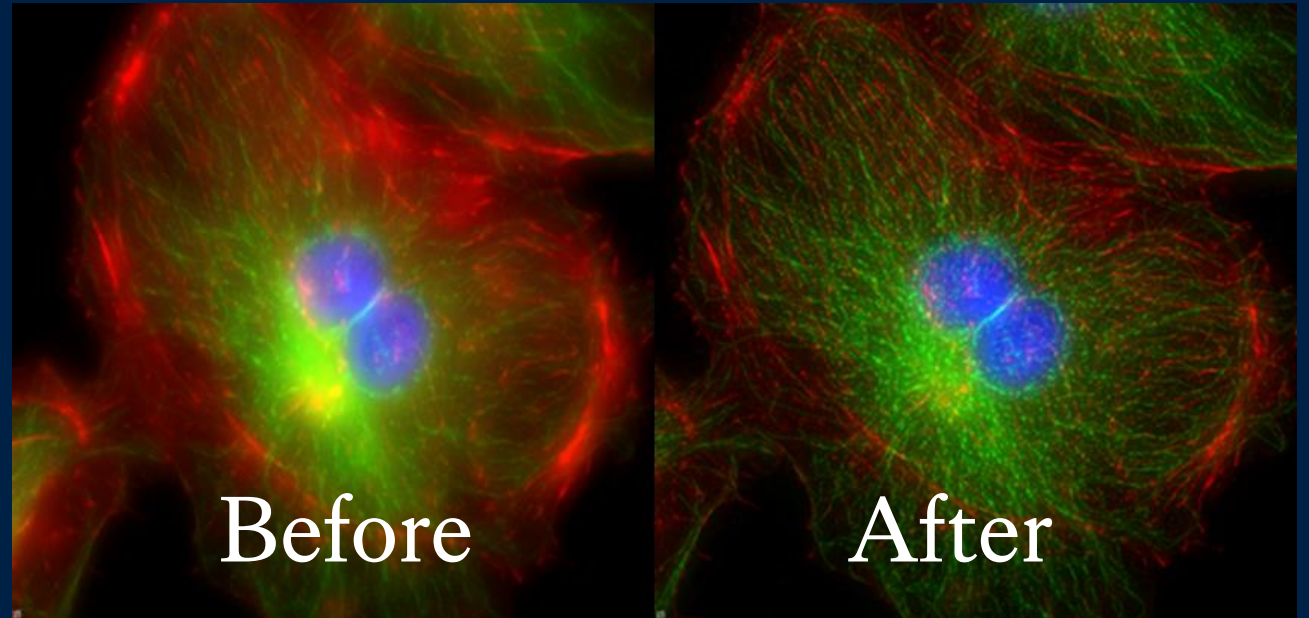


- Under-sampling can save time and minimise bleaching but you do lose information.
- To under-sample, type in a step size bigger than the recommended, E.g. in this case  $0.1\mu\text{m}$

# What happens when you oversample



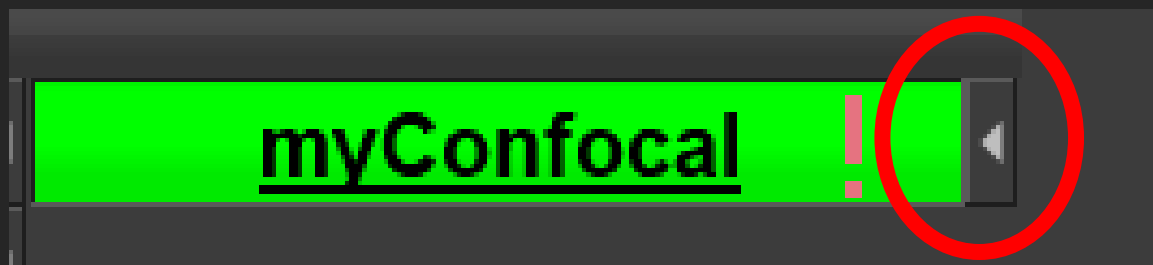
- You are capturing more information than you need when you oversample.
- But computer algorithms can use this information to 3D Deconvolve, digitally relocate signals for higher resolution images.
- To oversample, type in a step size smaller than the recommended, E.g. in this case 0.05μm



## 3D Deconvolution

Image copied from <http://www.biology.wustl.edu/imaging-facility/specs-deltavision.php>

Once everything is optimised, remember to save the camera settings in myConfocal



Moving on to Acquisition Settings...

# STEP BY STEP INSTRUCTIONS

## STEP 9

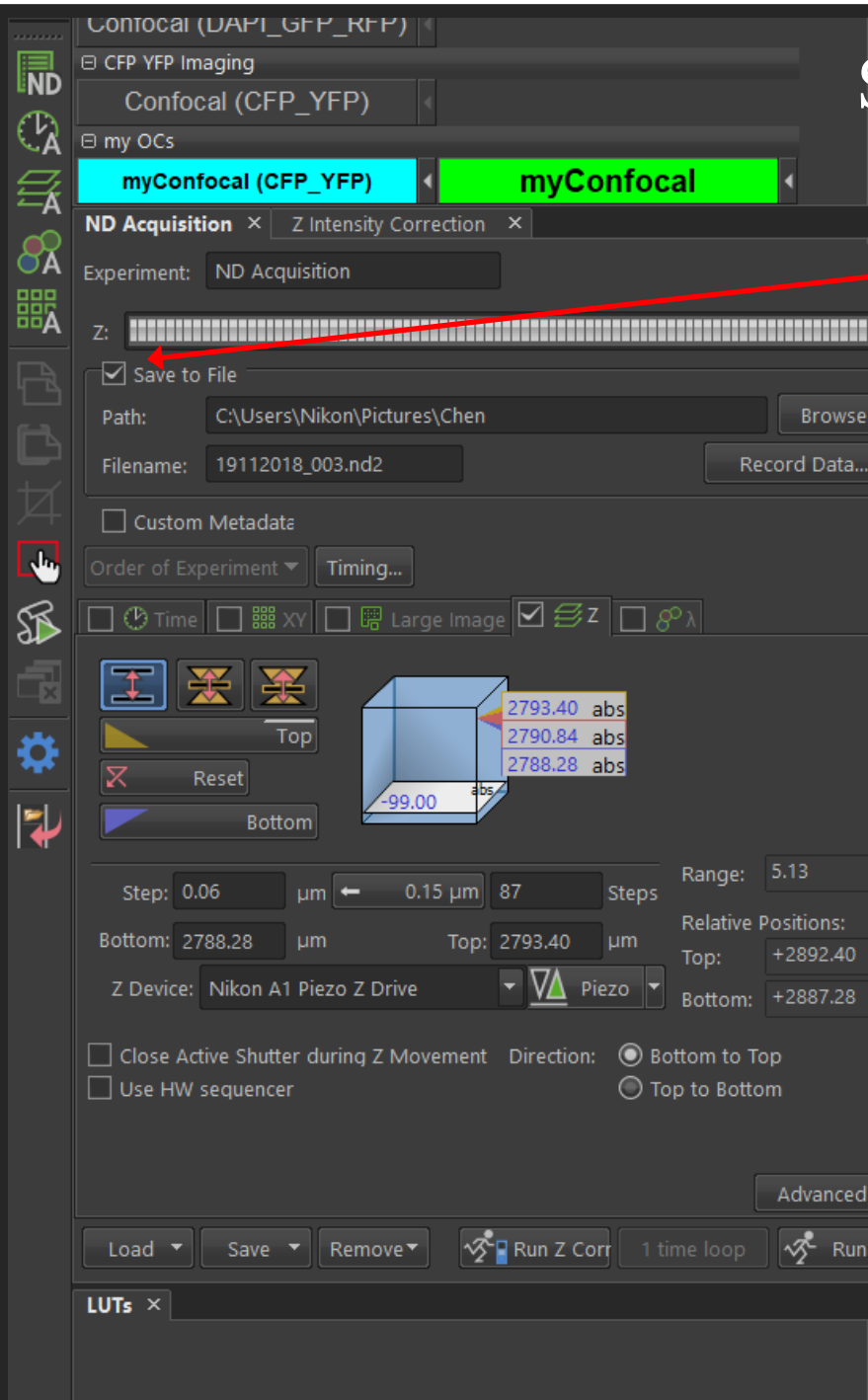
### Acquisition Settings

... Save to File

# Set Up File Path (SAVE)

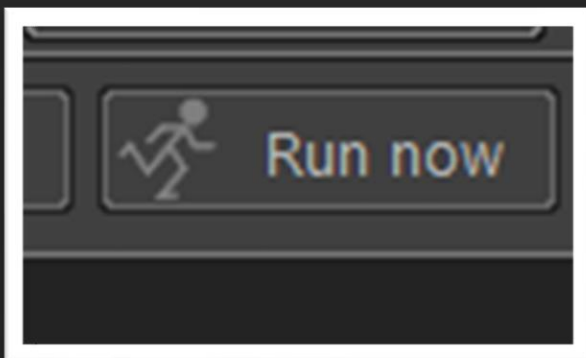
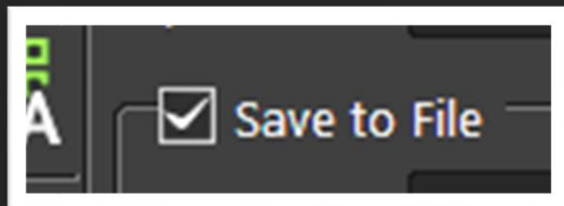
- 1) Check Save to file option
- 2) Go to Browse and select C:\Users\Nikon\Pictures
- 3) Create/find your folder, set up new folder for this session if needed.
- 4) Recommended file name: Experiment\_Name\_Date\_001
- 5) Finished, anytime you press "Run now" a new file will be automatically saved in the nd2 format.

If you don't put "\_001" at the end of the first file name, the software will automatically name your second image .....\_001, then \_002, \_003 for subsequent images.

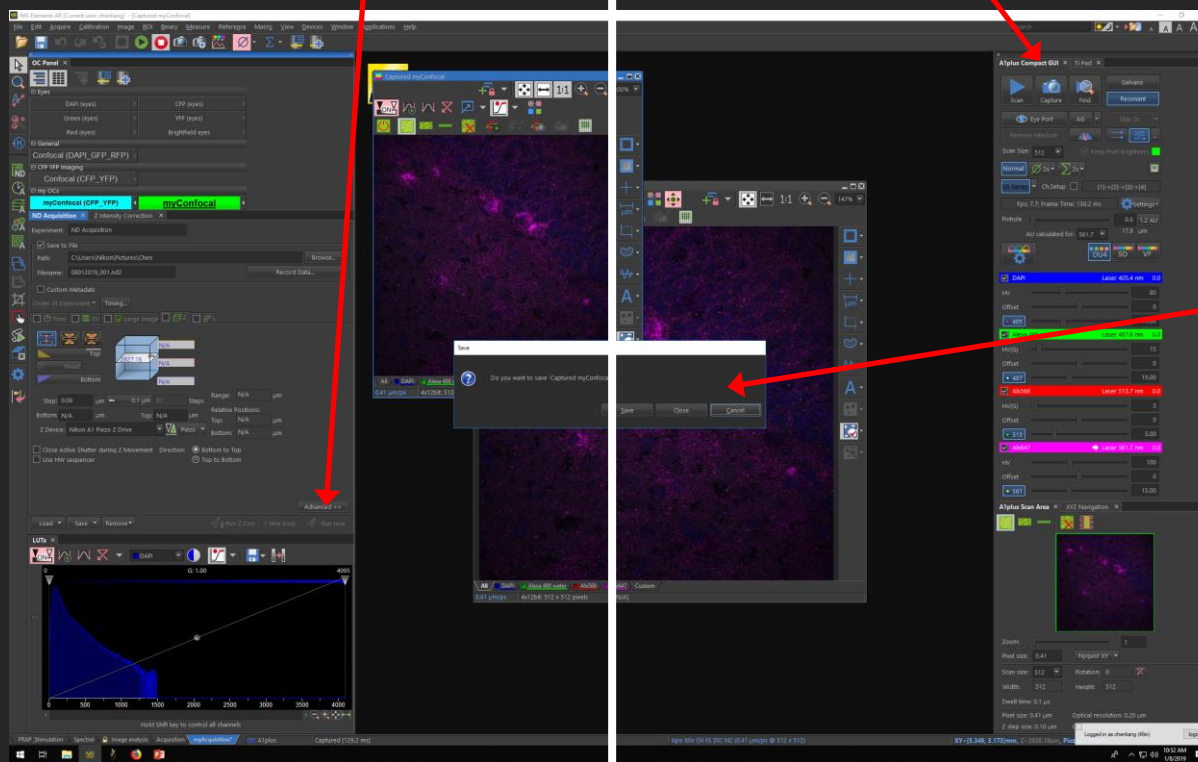




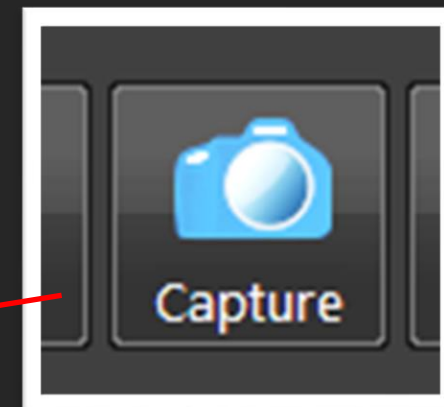
Automatically saved if  
'Save to File' is ticked



Run VS Capture



Not automatically saved



Box will appear  
and you can save  
or discard image.

After 'Run now' if you make changes to your image (such as adding ROIs) then save this  
'new' image by going to file and 'SAVE AS' so you don't overwrite your raw data.



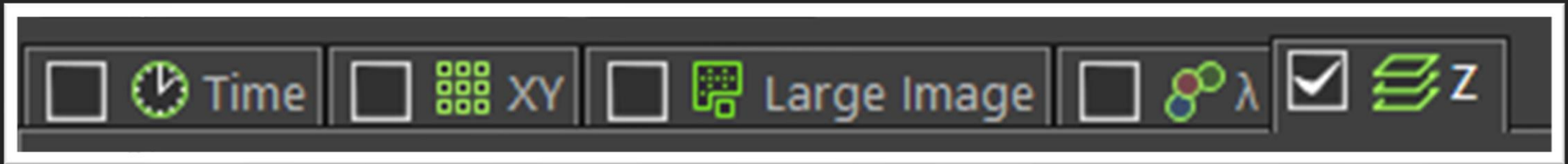
# STEP BY STEP INSTRUCTIONS

## STEP 9

### Acquisition Settings

... Order or acquisition tabs

## Fastest acquisition tab sequence



The Software will prioritise the tab on the RIGHT.

Do not put 'Large Image' tab on the right.

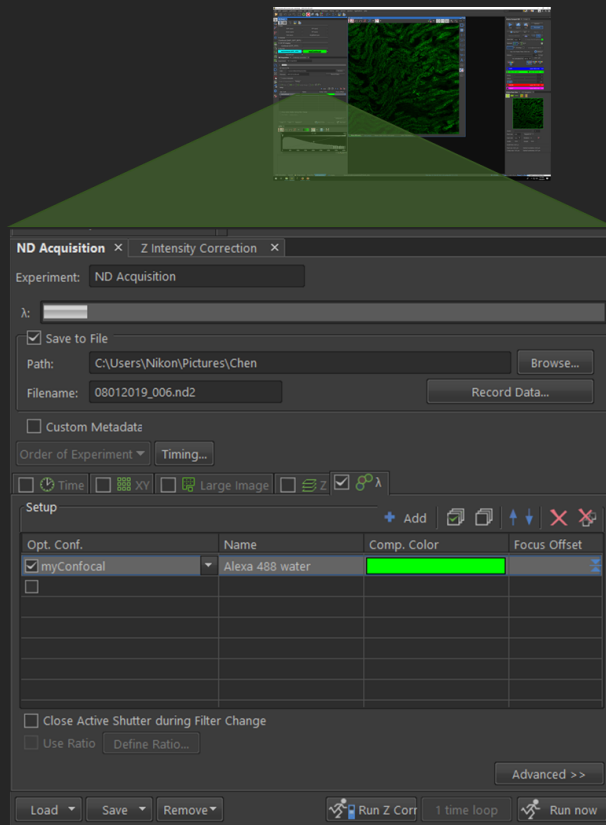
Tick the box for all acquisition functions you want to use.

# STEP BY STEP INSTRUCTIONS

## STEP 9

### Acquisition Settings

... Lambda (laser channels)



NIS-Elements AR [Current user: chenliang] - [Frozen]

File Edit Acquire Calibration Image ROI Binary Measure Reference Macro View Devices Window Applications Help

OC Panel

Eyes

DAPI (eyes)	CFP (eyes)
Green (eyes)	YFP (eyes)
Red (eyes)	Brightfield eyes

General

Confocal (DAPI\_GFP\_RFP)

CFP YFP Imaging

Confocal (CFP\_YFP)

my OCs

myConfocal (CFP\_YFP) myConfocal

ND Acquisition Z Intensity Correction Assign Current Settings

Experiment: ND Acquisition

λ:

Save to File

Path: C:\Users\Nikon\Pictures\Chen

Filename: 08012019\_002.nd2 Record Data...

Custom Metadata

Order of Experiment Timing

Time XY Large Image λ

Setup

Opt. Conf.	Name	Comp. Color	Focus Offset
<input checked="" type="checkbox"/>	myConfocal	DAPI	
<input type="checkbox"/>		Alx647	

Close Active Shutter during Filter Change

Use Ratio Define Ratio...

Advanced >>

Load Save Remove Run Z Corr 1 time loop Run now

LUTs

ON DAPI

G: 1.00

643

Hold Shift key to control all channels

FRAP Stimulation Spectral Image analysis Acquisition myAcquisition A1plus FreezeQ

Apo 60x Oil AS DIC N2 (0.41 μm/px 512 x 512)

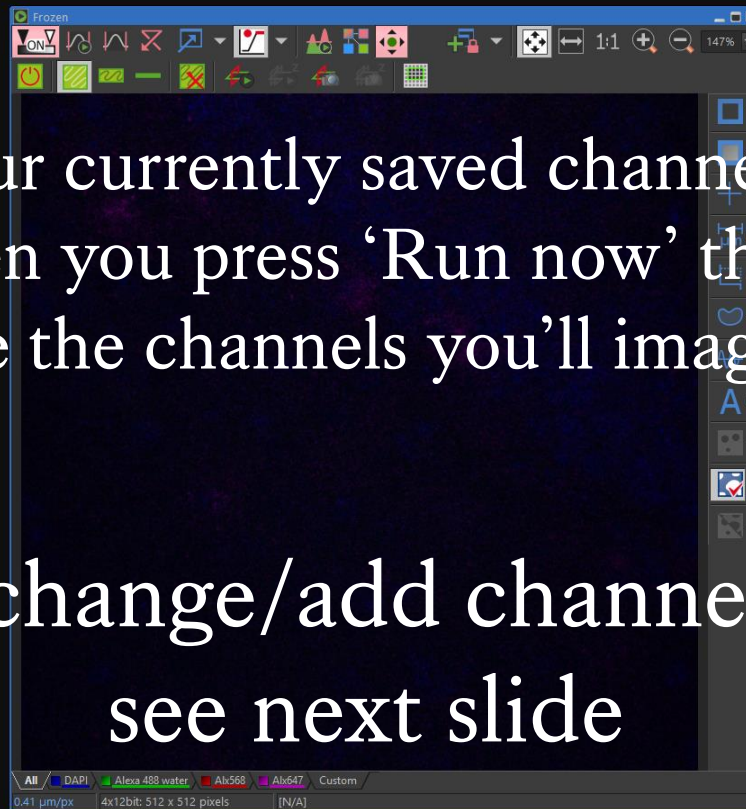
XY=[5.349, 3.173]mm, Z=2828.18μm, Pie

10:59 AM 1/8/2019



Your currently saved channels,  
when you press 'Run now' these  
are the channels you'll image.

To change/add channels...  
see next slide



A1plus Compact GUI Ti Pad

Scan Capture Find Galvano Resonant

Eye Port AG Skip 2x

Remove Interlock

Scan Size: 512 Keep Pixel Brightness

Normal 2x 2x

Ch Series Ch.Setup [1]->[2]->[3]->[4]

Fps: 7.7; Frame Time: 130.2 ms Settings

Pinhole 0.5 1.2 AU

AU calculated for: 561.7 17.9 μm

DU4 SD VF

DAPI Laser 405.4 nm 0.0

HV 80

Offset 0

405 10.00

Alexa 488 water Laser 487.6 nm 0.0

HV(G) 15

Offset 0

487 15.00

Alx568 Laser 513.7 nm 0.0

HV(G) 3

Offset 0

513 5.00

Alx647 Laser 561.7 nm 0.0

HV 100

Offset 0

561 15.00

A1plus Scan Area XYZ Navigation

Zoom: 1

Pixel size: 0.41 Nyquist XY

Scan size: 512 Rotation: 0

Width: 512 Height: 512

Dwell time: 0.1 μs

Pixel size: 0.41 μm Optical resolution: 0.20 μm

Z step size: 0.10 μm

Logged in as chenliang (53m) logout

NIS-Elements AR [Current user: chenliang] - [Frozen]

File Edit Acquire Calibration Image ROI Binary Measure Reference Macro View Devices Window Applications Help

OC Panel

Eyes

DAPI (eyes) CFP (eyes)

Green (eyes) YFP (eyes)

Red (eyes) Brightfield eyes

General

Confocal (DAPI\_GFP\_RFP)

CFP YFP Imaging

Confocal (CFP\_YFP)

my Ocs

myConfocal (CFP\_YFP) myConfocal

ND Acquisition Z Intensity Correction

Experiment: ND Acquisition

Save to File

Path: C:\Users\Nikon\Pictures\Chen

Filename: 08012019\_002.nd2

Custom Metadata

Order of Experiment Timing

Time XY Large Image z

Setup

Opt. Conf. Name Comp. Color Focus Offset

myConfocal DAPI

Alx647

Close Active Shutter during Filter Change

Use Ratio Define Ratio

LUTs

ON

DAPI

Hold Shift key to control all channels

FRAP Stimulation Spectral Image analysis Acquisition myAcquisition A1plus Freeze

Apo 60x Oil AS DIC N2 (0.41 µm/px @ 512 x 512)

XY=[5.349, 3.173]mm, Z=2828.18µm, Pie

Zoom: 1

Pixel size: 0.41 Nyquist XY

Scan size: 512 Rotation: 0

Width: 512 Height: 512

Dwell time: 0.1 µs

Pixel size: 0.41 µm Optical resolution: 0.20 µm

Z step size: 0.10 µm

Logged in as chenliang (53m) logout

10:59 AM 1/8/2019

To change/add channels...

2) Update myConfocal

3) New settings will be subsequently updated...see next slide

1) Select the channels you want to use

A1plus Compact GUI

Scan Capture Find Galvano Resonant

Eye Port AG Skip 2x

Remove Interlock

Scan Size: 512 Keep Pixel Brightness

Normal 2x 2x

Ch Series Ch.Setup [1]->[2]->[3]->[4]

Fps: 7.7; Frame Time: 130.2 ms Settings

Pinhole 0.5 1.2 AU

AU calculated for: 561.7 17.9 µm

DU4 SD VF

DAPI Laser 405.4 nm 0.0

HV 80

Offset 0

405 10.00

Alx488 water Laser 487.6 nm 0.0

HV(G) 15

Offset 0

487 15.00

Alx680 Laser 513.7 nm 0.0

HV(G) 3

Offset 0

513 5.00

Confocal (DAPI\_GFP\_RFP)

CFP YFP Imaging

Confocal (CFP\_YFP)

my OCs

**myConfocal (CFP\_YFP)** **myConfocal**

**ND Acquisition** × Z Intensity Correction ×

Experiment: ND Acquisition

λ:

☒ Save to File

Path: C:\Users\Nikon\Pictures\Chen

Filename: 08012019\_002.nd2





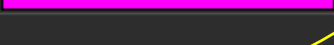
☐ Custom Metadata

Order of Experiment Timing

☐ Time ☐ XY ☐ Large Image ☐ Z ☒ λ

Setup

+ Add ☐ ☐

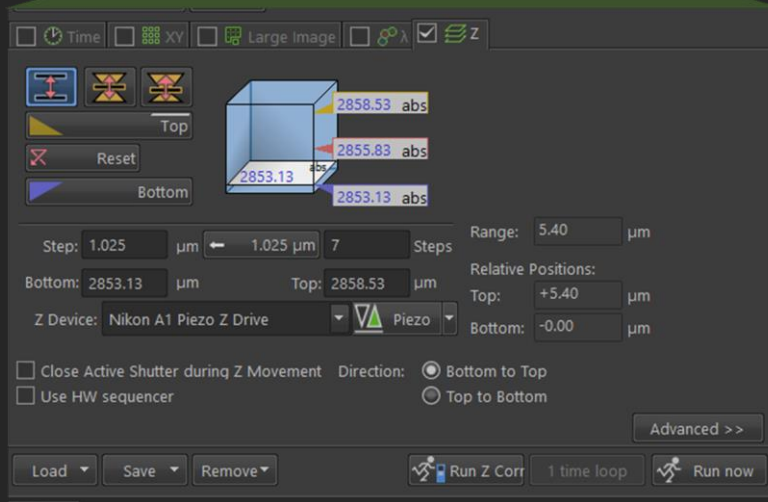
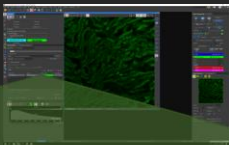
Opt. Conf.	Name	Comp. Color	Focus Offset
<input checked="" type="checkbox"/> myConfocal	DAPI		
	Alexa 488 water		
	Alx568		
	Alx647		
<input type="checkbox"/>			

New settings updated.

# STEP BY STEP INSTRUCTIONS

## STEP 9

### Acquisition Settings




... Z stack



# Z stack Basic Options

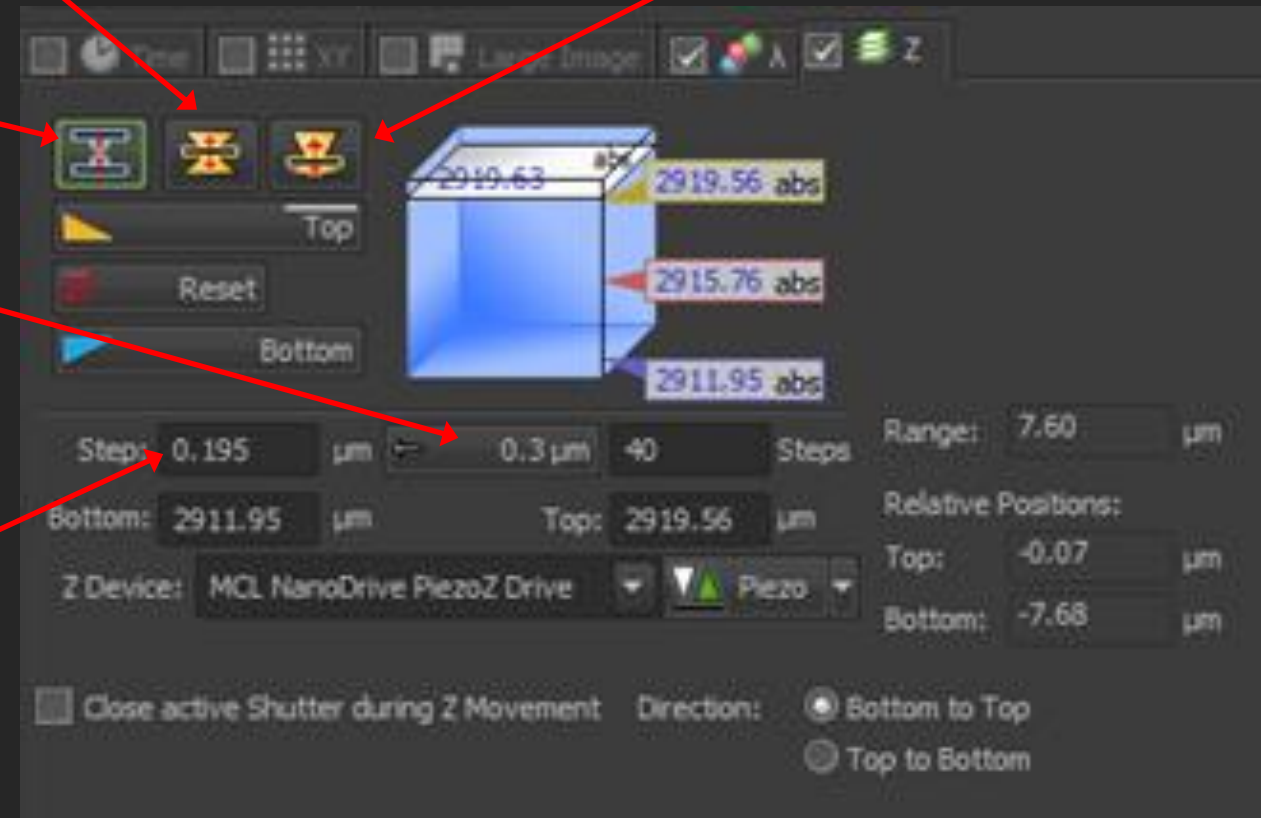
**Set top and bottom:** use mouse wheel to focus and define the exact range of your Z stack.

**Set Middle:** use mouse wheel to find the mid point of your Z focus and set equal distance above and below the focal plane. (Useful if your sample is symmetrical along the Z axis)

**Asymmetrical:** find focal plane and then set different distances above and below. (Useful for  like cells)

## Step Size

- You can set step size or number of steps.
- Use recommended step size to capture all the information.
- Fewer steps than the recommended is called under-sampling and you may lose information.
- More steps than the recommended is called over-sampling (typed in here) which is required for 3D deconvolution.





Choose an option to set your Z stack.

1 Tick to select

2

Reset

3

Reset

4

In Live mode use mouse wheel to define top/bottom/focus of your sample.

Watch the numbers to orientate if you're going up or down.

5

Set step size...Click (for recommended step size)

Step: 1.025  $\mu\text{m}$  1.025  $\mu\text{m}$  7 Steps

Bottom: 2853.13  $\mu\text{m}$  Or type in here to Over/Under-sample

Z Device: Nikon AT Piezo Z Drive

Piezo

Range: 5.40  $\mu\text{m}$

Relative Positions:

Top: +5.40  $\mu\text{m}$

Bottom: -0.00  $\mu\text{m}$

☐ Close Active Shutter during Z Movement Direction: ☒ Bottom to Top  
☐ Use HW sequencer ☐ Top to Bottom

Run now to image your Z stack

6

Load

Save

Remove



Run Z Corr

1 time loop



Run now

# Z stack View Modes

The screenshot displays the NIS-Elements AR software interface, showing various Z-stack view modes and acquisition settings.

**View Modes:**

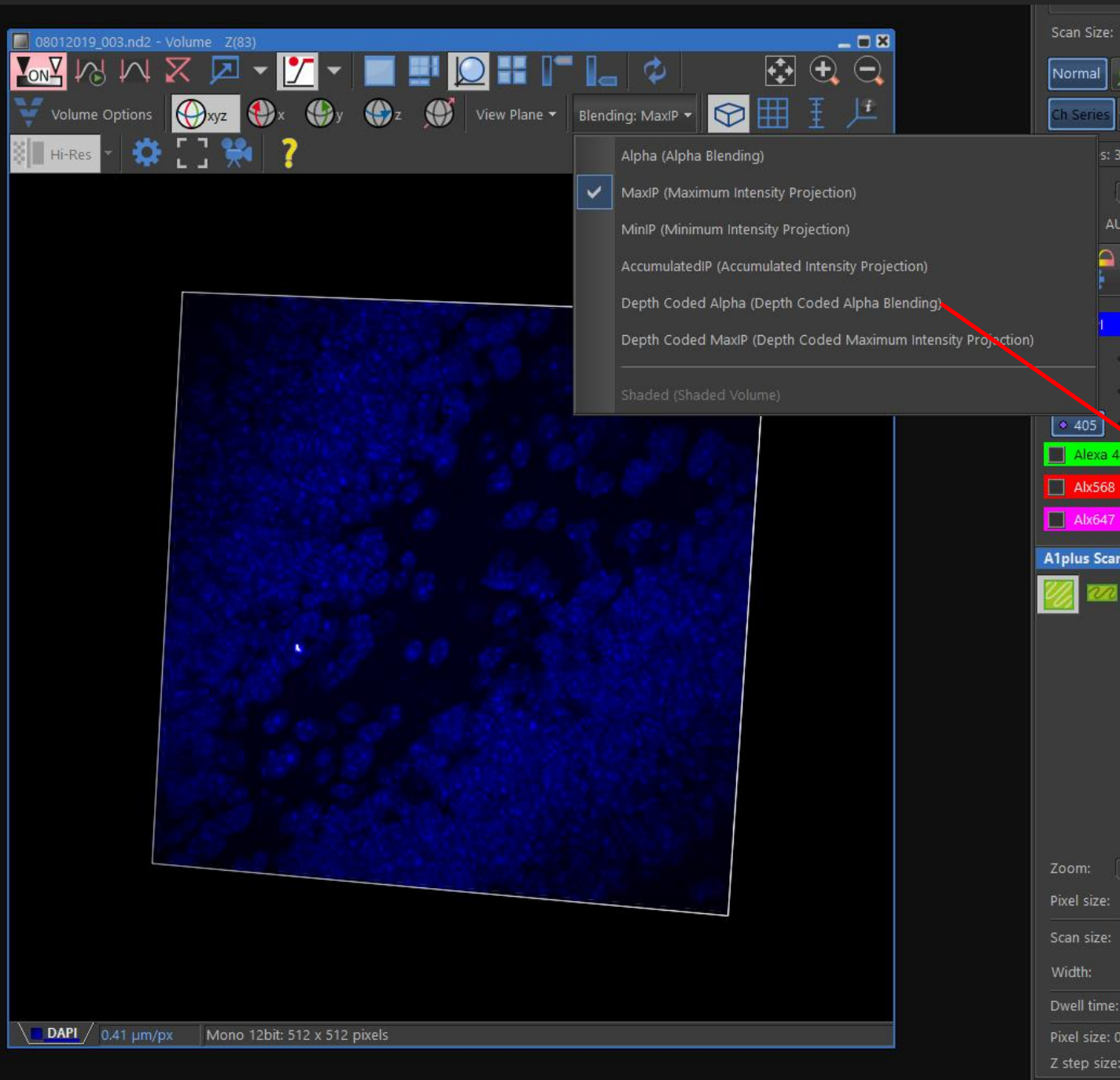
- Scroll through:** A single Z-stack slice view.
- Cross section:** A view showing the Z-stack slices in a cross-section.
- 3D Render:** A 3D visualization of the Z-stack data.
- Tile:** A view showing the Z-stack slices tiled together.
- Maximum Intensity Projection:** A view showing the maximum intensity projection of the Z-stack.
- Minimum Intensity Projection:** A view showing the minimum intensity projection of the Z-stack.

**Right Panel (A1plus Compact GUI):**

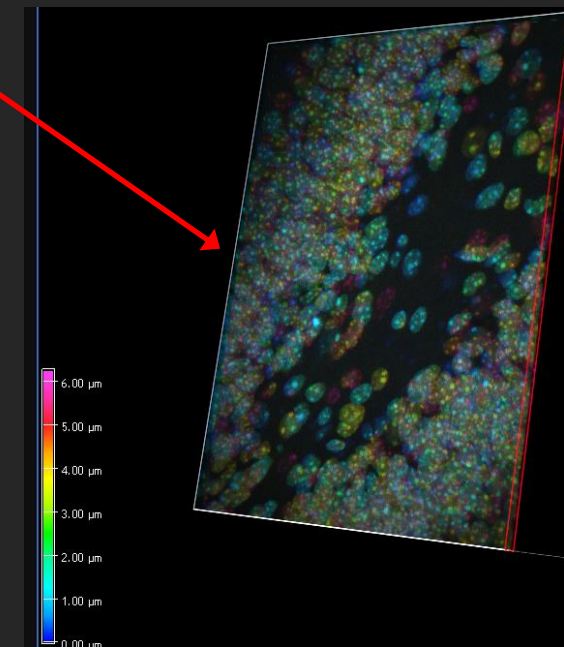
- Scan:** Play button, Capture button, Find button, Resonant button.
- Eye Port:** AG button, Skip 2x button.
- Remove Interlock:** Remove Interlock button.
- Scan Size:** 512.
- Keep Pixel Brightness:** Checked.
- Normal:** 2x, 2x.
- Ch Series:** Ch.Setup, (1)->[-]>[-]>[-]>[-].
- Fps:** 30.0, Frame Time: 33.3 ms.
- Settings:** Settings button.
- Pinhole:** 0.6, 1.2 AU.
- AU calculated for:** 405.4, 15.3  $\mu\text{m}$ .
- Laser Settings:**
  - DAPI: Laser 405.4 nm, 0.0.
  - Alexa 488 water: Laser 487.6 nm, 0.0.
  - Abi568: Laser 513.7 nm, 0.0.
  - Abi647: Laser 561.7 nm, 0.0.
- A1plus Scan Area:** XYZ Navigation button.
- Zoom:** 1.
- Pixel size:** 0.41, Nyquist XY.
- Scan size:** 512, Rotation: 0.
- Width:** 512, Height: 512.
- Dwell time:** 0.1  $\mu\text{s}$ .
- Pixel size:** 0.41  $\mu\text{m}$ , Optical resolution: 0.15  $\mu\text{m}$ .
- Z step size:** 0.07  $\mu\text{m}$ , Optical sectioning: 0.25  $\mu\text{m}$ .

**Bottom Panel:**

- FRAP Stimulation:** Stimulation button.
- Spectral:** Spectral button.
- Image analysis:** Image analysis button.
- Acquisition:** Acquisition button.
- A1plus:** A1plus button.
- ND\_ShowMinIntProjectionImage(0):** ND\_ShowMinIntProjectionImage(0) button.
- XY=[5.593, 3.007]mm, Z=-2828.03 $\mu\text{m}$ , Piezo:** XY=[5.593, 3.007]mm, Z=-2828.03 $\mu\text{m}$ , Piezo button.
- Logged in as chenliang (1h49m):** Logged in as chenliang (1h49m) button.
- Logout:** Logout button.



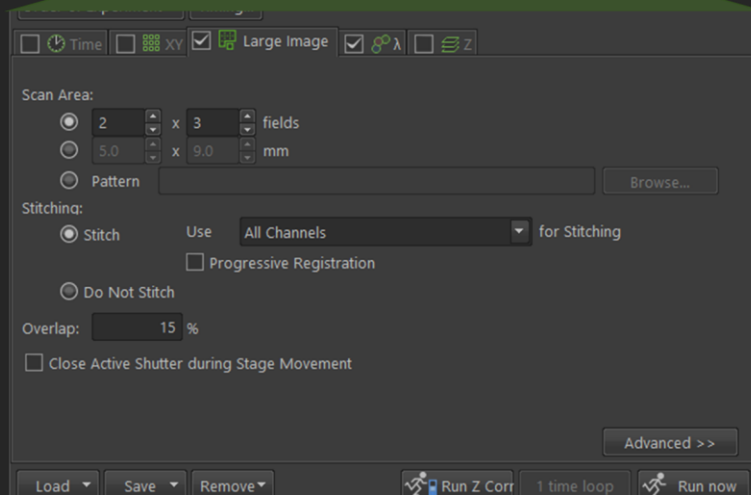
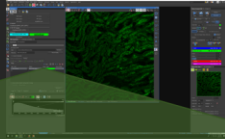
In 3D rendering you  
have different  
rendering modes.



# STEP BY STEP INSTRUCTIONS

## STEP 9

### Acquisition Settings



... Large Image

☐ Time ☐ XY ☒ Large Image ☒  $\lambda$  ☐ Z

1

Tick to select, Lambda must be ticked too if not using Z stack.

Set scan area

3

Scan Area:

☒ 2 X axis x 3 fields  
☐ 5.0 x 9.0 mm  
☐ Pattern

2

In Live mode, move to the centre of your field of view.

4

Stitching:

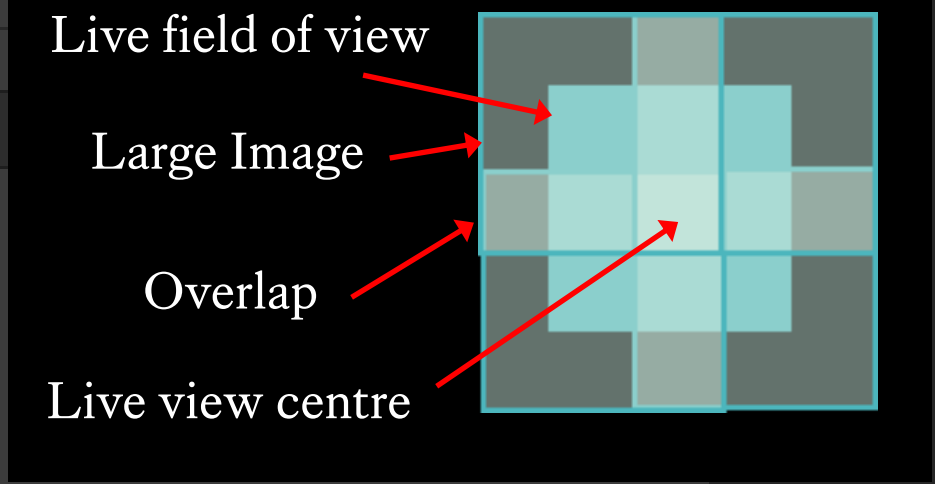
☒ Stitch ☐ Progressive Registration  
☐ Do Not Stitch

Use overlapping image edge to stitch

5

Overlap: 15 % 15% overlap minimum!

☐ Close Active Shutter during Stage Movement



Run now to image your Large Image

6

Load Save Remove

Run Z Corr 1 time loop Run now



NIS-Elements AR [Current user: chenliang] - [08012019\_010.nd2]

File Edit Acquire Calibration Image ROI Binary Measure Reference Macro View Devices Window Applications Help

OC Panel

Eyes

DAPI (eyes) CFP (eyes)

Green (eyes) YFP (eyes)

Red (eyes) Brightfield eyes

General

Confocal (DAPI\_GFP\_RFP)

CFP YFP Imaging

Confocal (CFP\_YFP)

my OCs

myConfocal (CFP\_YFP) myConfocal

myConfocal1

ND Acquisition Z Intensity Correction

Experiment: ND Acquisition

L:

λ:

Save to File

Path: C:\Users\Nikon\Pictures\Chen

Filename: 08012019\_011.nd2

Record Data...

Custom Metadata

Order of Experiment Timing

Scan Area:

2 x 3 field

5.0 x 9.0 mm

Pattern

Stitching:

Stitch Use All Channels for Stitching

Progressive Registration

Do Not Stitch

Overlap: 15 %

Close Active Shutter during Stage Movement

Advanced >>

Load Save Remove

Run Z Corr 1 time loop Run now

LUTs

ON

G: 1.00

4095

0 500 1000 1500 2000 2500 3000 3500 4000

08012019\_010.nd2

Click on this to fit your whole large image into the window

Y=3

X=2

A1plus Compact GUI

Scan Capture Find Galvano Resonant

Eye Port AG Skip 2x

Remove Interlock

Scan Size: 1024

Keep Pixel Brightness

Normal

Ch Series Ch.Setup [-]>[2]>[-]>[-]

Fps: 3.9; Frame Time: 259.3 ms

Pinhole 1.6 1.2 AU

AU calculated for: 487.6 26.8 μm

DU4 SD VF

DAPI Laser 405.4 nm 0.0

Alexa 488 water Laser 487.6 nm 0.0

HV(G) 5

Offset 0

487 2.00

Ab568 Laser 561.7 nm 0.0

Ab647 Laser 640.0 nm 0.0

A1plus Scan Area XYZ Navigation

Zoom: 1

Pixel size: 0.62 Nyquist XY

Scan size: 1024 Rotation: 0

Width: 1024 Height: 1024

Dwell time: 0.05 μs

Pixel size: 0.62 μm Optical resolution: 0.36 μm

Z step size: 1.02 μm Optical sectioning: 3.07 μm

FRAP Stimulation Spectral Image analysis Acquisition myAcquisition A1plus

ZoomFitToScreen0

Plan Apo VC 20x DIC N2 (1.24 μm/px @ 512 x 512)

XY=[12.631, -1.321]mm, Z=2861.88μm, PiezoZ=8.72μm Logged in as chenliang (48m)

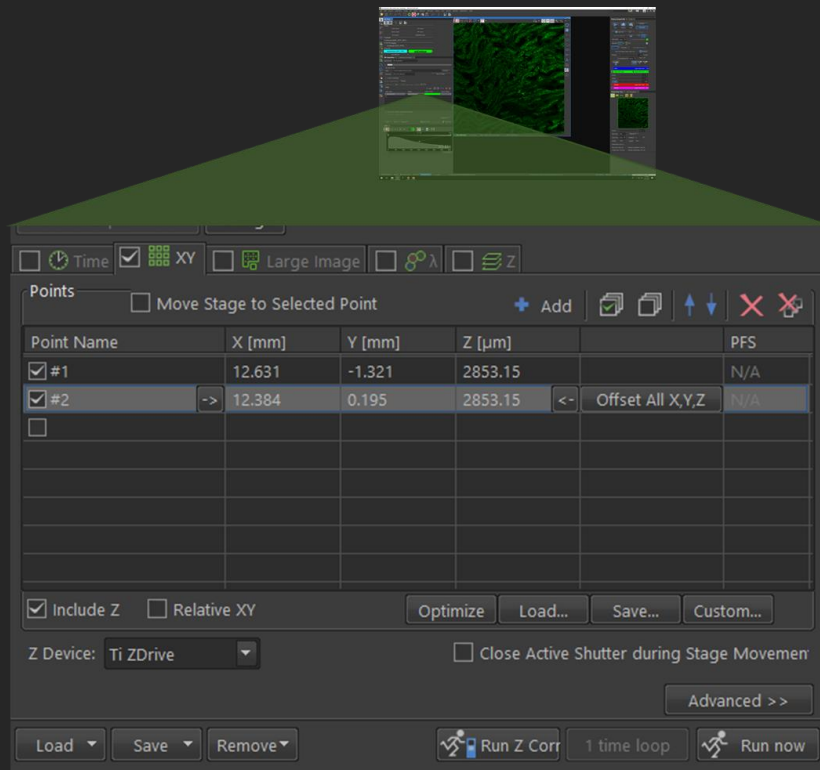
1:59 PM 1/28/2019

# STEP BY STEP INSTRUCTIONS

## STEP 9

### Acquisition Settings

... XY Positions



**1 ALWAYS DELETE ALL PREVIOUS POSITIONS BEFORE YOU START!**

3 Add that field of view to your positions.

2 In Live, focus on your field of view.

4 In Live, drag and move to another field of view.  
(repeat 2-4 until you have all your positions)

5 Run now : the microscope will scan and move to the next position as fast as it can.

Each position will be a separate scanned image.

6

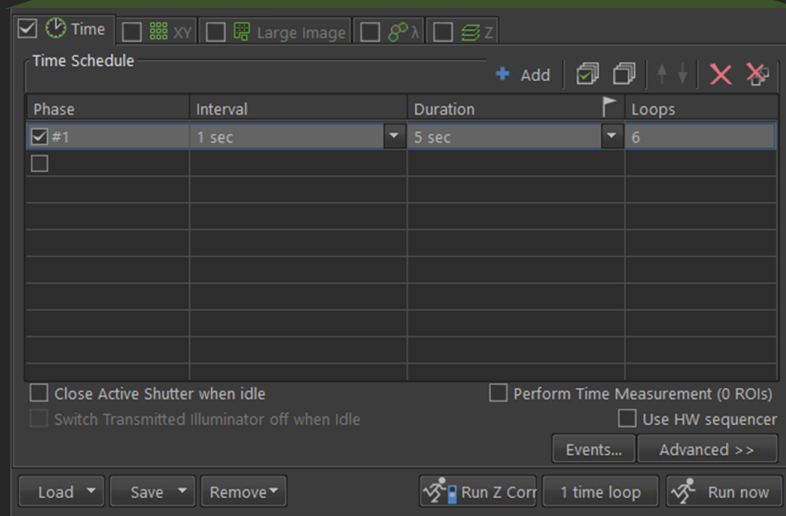
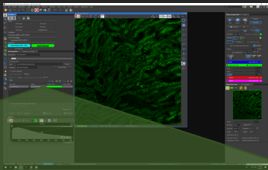
XY(2/2)  
Pos. X: 12384.00  $\mu\text{m}$   
Pos. Y: 193.10  $\mu\text{m}$   
Pos. Z: 2853.15  $\mu\text{m}$   
Req.Pos. X: 12384.20  $\mu\text{m}$   
Req.Pos Y: 195.30  $\mu\text{m}$   
Req.Pos Z: 2853.15  $\mu\text{m}$   
Alt key: starts Drag and Drop



# STEP BY STEP INSTRUCTIONS

## STEP 9

### Acquisition Settings



... Time



Continue with your imaging...

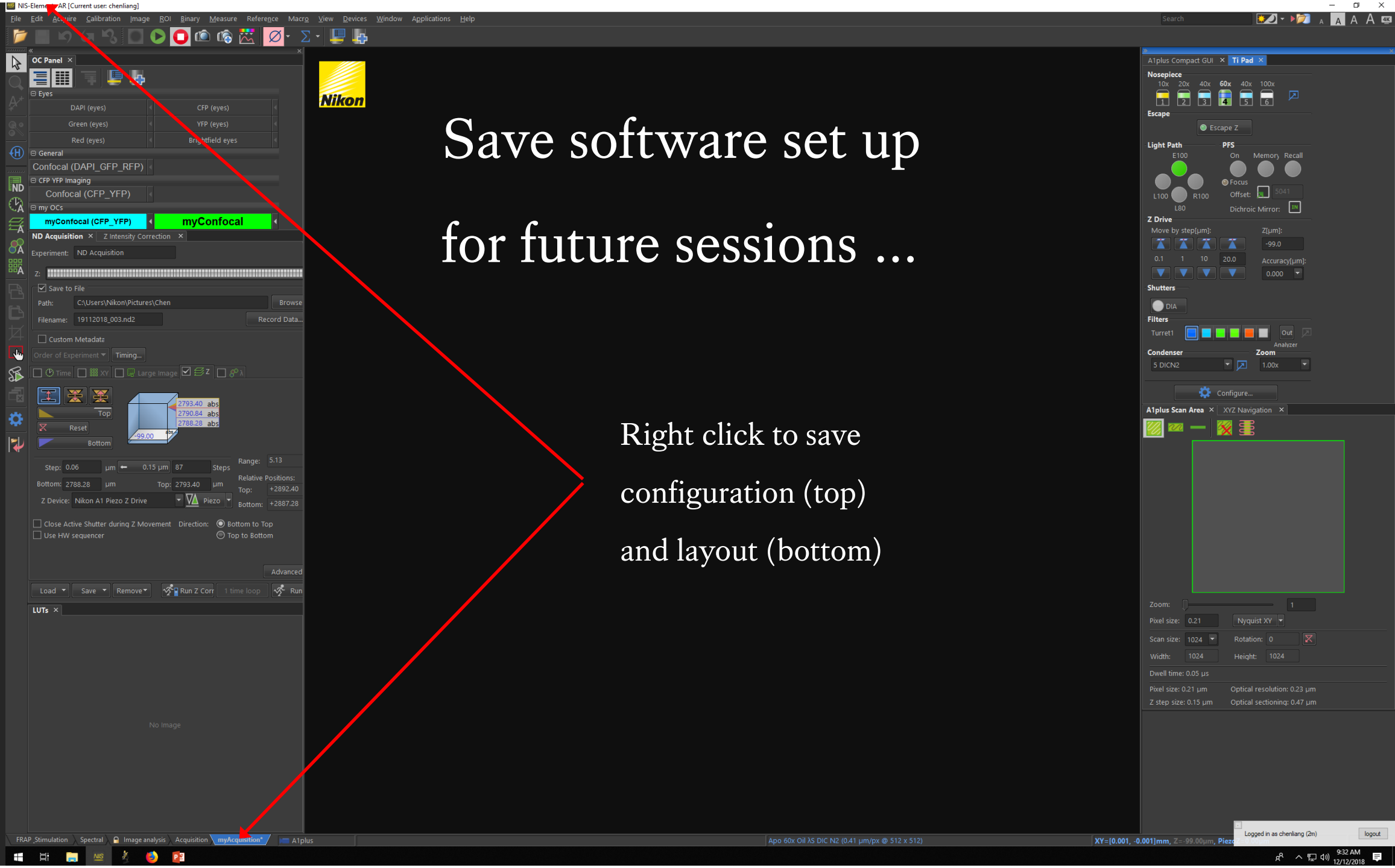
# STEP BY STEP INSTRUCTIONS

## STEP 10

At the end of your session

... Save your software settings

... Shut down procedure



Save software set up  
for future sessions ...

Right click to save  
configuration (top)  
and layout (bottom)

# Shut down procedure

The screenshot shows the Nikon Elements AR software interface. The interface is divided into several panels. On the left, the 'OC Panel' is visible, showing various acquisition parameters. In the center, the 'ND Acquisition' panel is active, displaying a 3D model of the sample and acquisition settings. On the right, the 'Light Path' and 'Z Drive' panels are visible, showing the current light path and Z-axis position. A red circle with the number '1' is placed over the 'STOP SCAN' button in the top right corner. A red circle with the number '2' is placed over the 'Brightfield eyes' button in the 'OC Panel'. A red circle with the number '3' is placed over a yellow box containing the text 'LOWER the objectives REMOVE your sample CLEAN the lens'. A red circle with the number '5' is placed over the 'Close' button in the 'Light Path' panel.

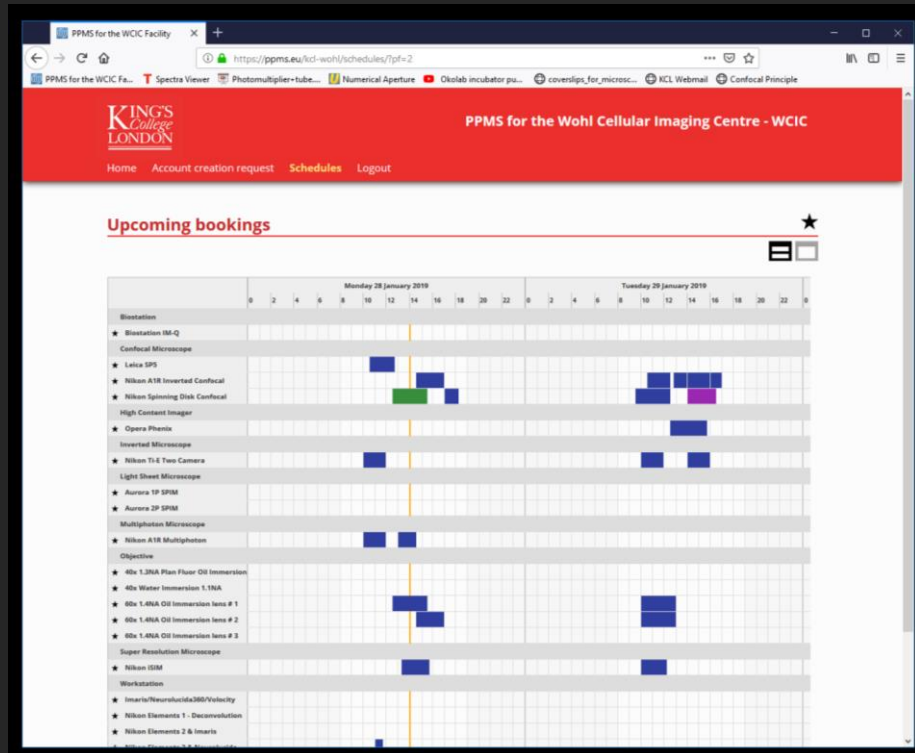
1 STOP SCAN

2 Put it in Brightfield Eyes

3 LOWER the objectives  
REMOVE your sample  
CLEAN the lens

5 Close

# Check the booking schedule!



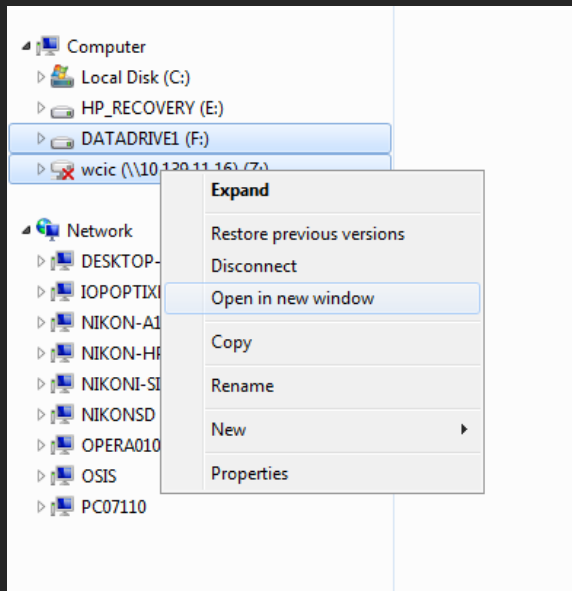
If no one is using the system within  
2 hours, shut down the system.

If someone is booked on within 2  
hours, leave the system on.

# Transfer Data To Shared Drive (1 of 3)

1

Open file  
Find your saved data in  
Pictures

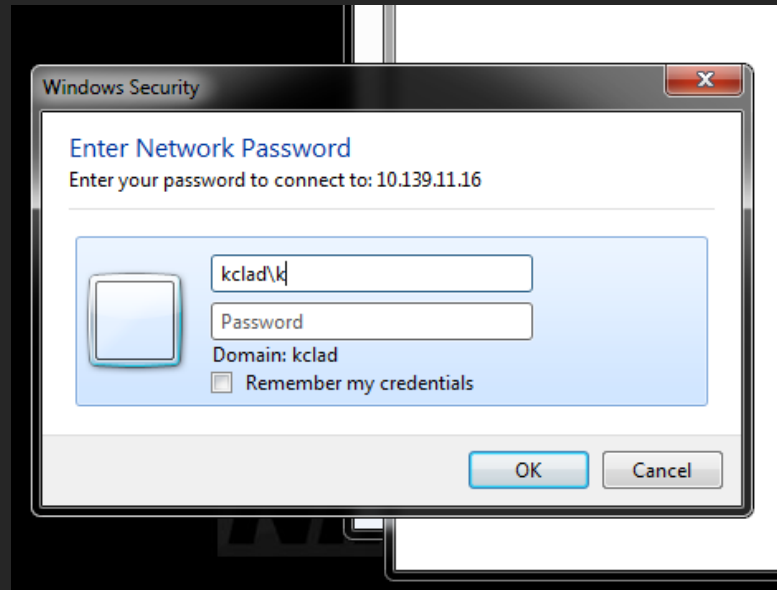


2

Right click on Shared Drive  
(WCIC) and select Open in  
new window.

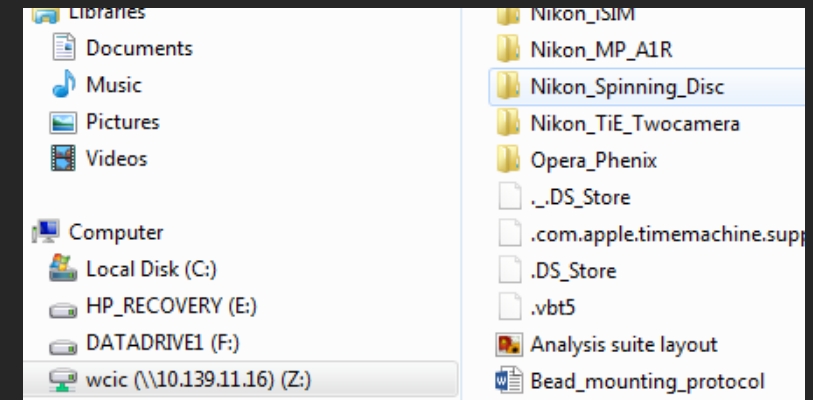
3

You need to login to this pop-up window, user name is  
normally: kclad\k number  
DO NOT click on remember my credentials



4

In the Network  
drive, open the  
Nikon\_A1R  
folder



5

In the Nikon\_A1R folder  
open your personal folder

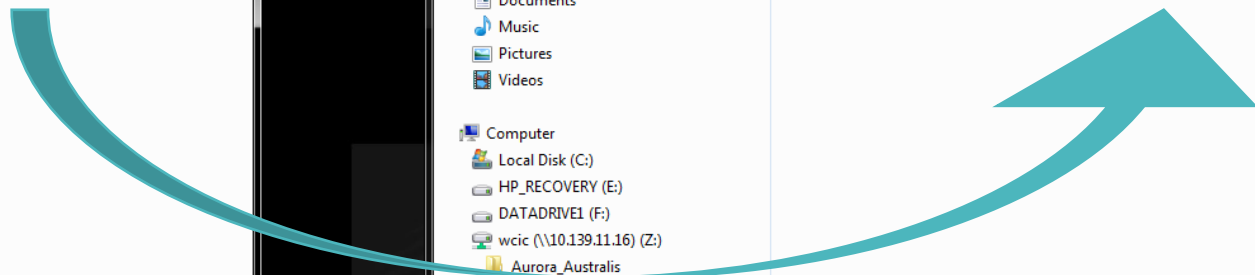
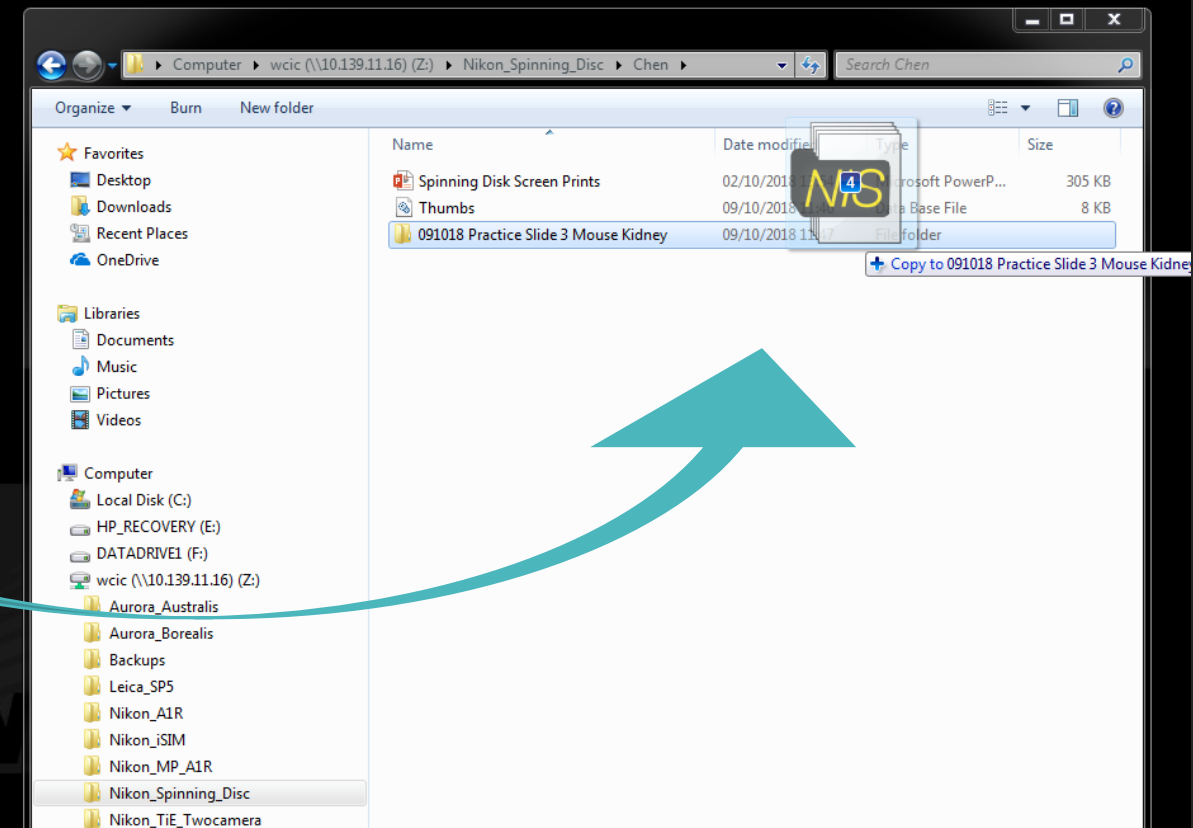
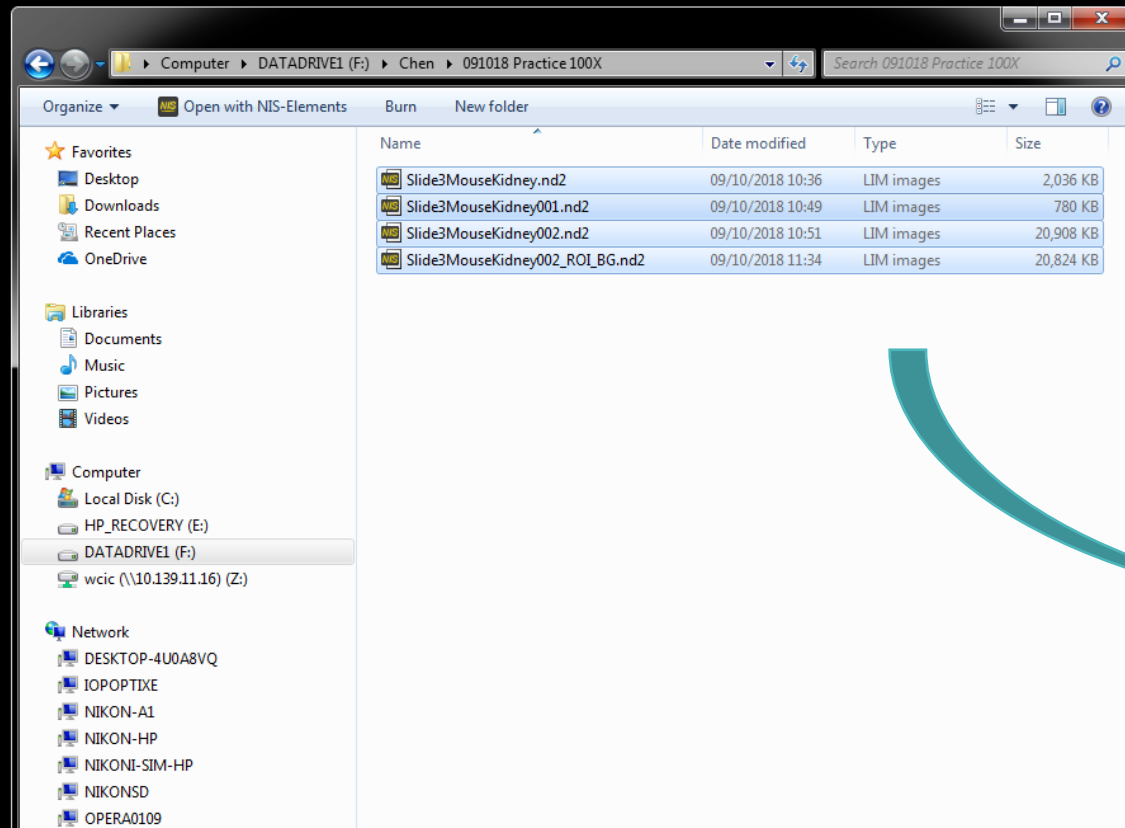


## Transfer Data To Shared Drive (2 of 3)

- **DO NOT USE USBs ON ANY COMPUTERS IN THE MICROSCOPE ROOMS!**

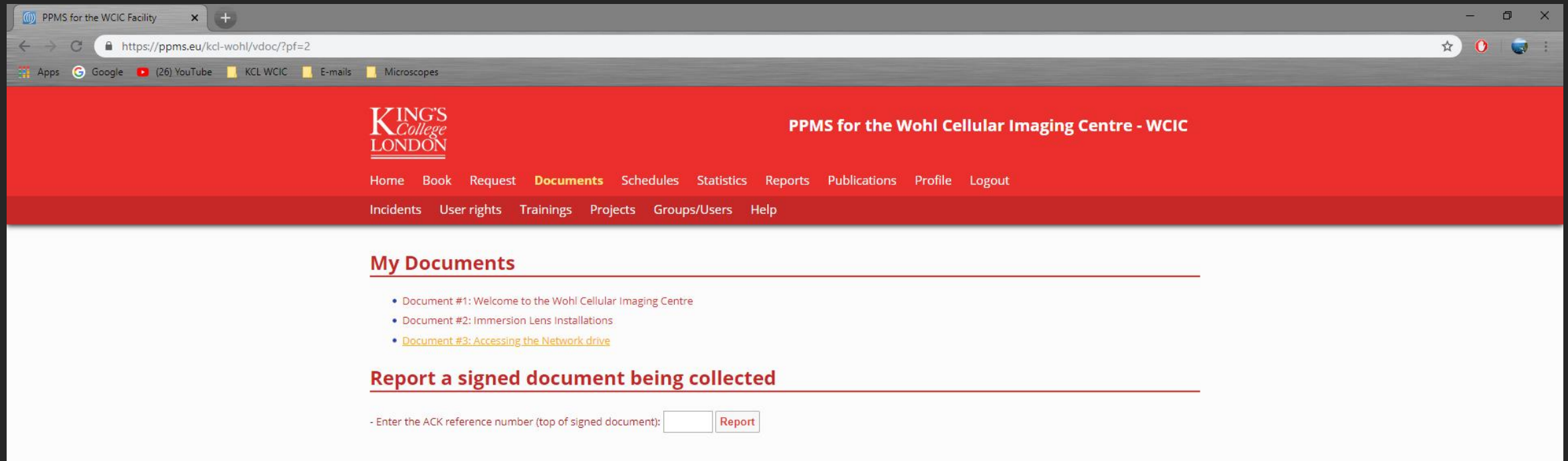
- Drag and drop the files you saved in this session.

- The Shared Drive can be accessed from the workstations (where you can use USBs), or your personal computers, from there please BACK-UP your data.



# Transfer Data To Shared Drive (3 of 3)

If you have trouble connecting to the shared drive, or need to re-map the network drive, please login to the booking system, go to documents, the 3<sup>rd</sup> document contains instructions.



The screenshot shows a web browser window with the address bar displaying <https://ppms.eu/kcl-wohl/vdoc/?pf=2>. The browser's address bar also shows several bookmarks: Apps, Google, (26) YouTube, KCL WCIC, E-mails, and Microscopes. The website has a red header with the King's College London logo on the left and the title "PPMS for the Wohl Cellular Imaging Centre - WCIC" on the right. Below the header is a navigation menu with links: Home, Book, Request, Documents (highlighted), Schedules, Statistics, Reports, Publications, Profile, and Logout. A secondary navigation bar contains links: Incidents, User rights, Trainings, Projects, Groups/Users, and Help. The main content area is titled "My Documents" and lists three documents: "Document #1: Welcome to the Wohl Cellular Imaging Centre", "Document #2: Immersion Lens Installations", and "Document #3: Accessing the Network drive" (highlighted in yellow). Below this list is a section titled "Report a signed document being collected" with a form that includes a label "- Enter the ACK reference number (top of signed document):", an input field, and a "Report" button.

PPMS for the Wohl Cellular Imaging Centre - WCIC

Home Book Request **Documents** Schedules Statistics Reports Publications Profile Logout

Incidents User rights Trainings Projects Groups/Users Help

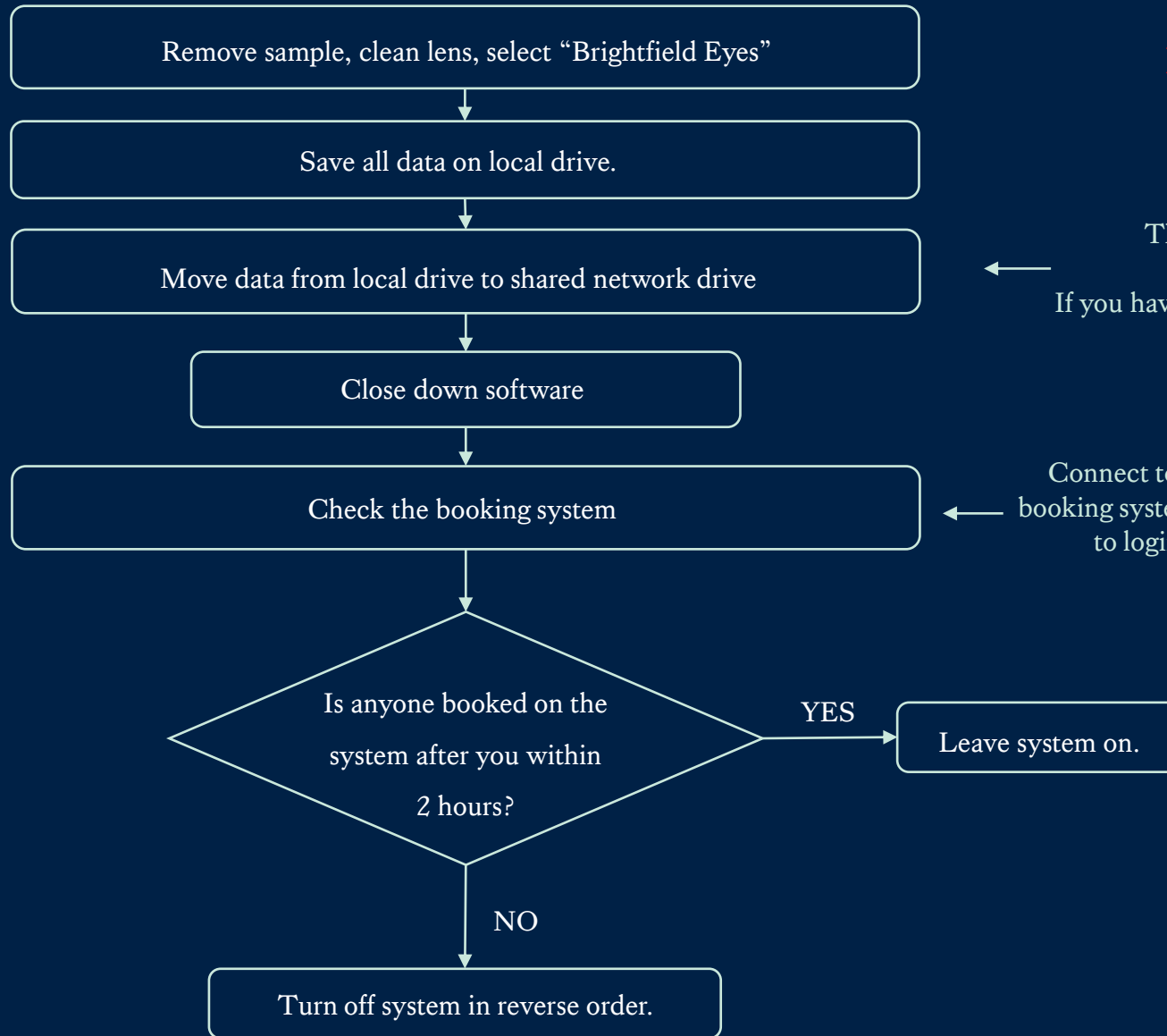
### My Documents

- Document #1: Welcome to the Wohl Cellular Imaging Centre
- Document #2: Immersion Lens Installations
- Document #3: Accessing the Network drive**

### Report a signed document being collected

- Enter the ACK reference number (top of signed document):

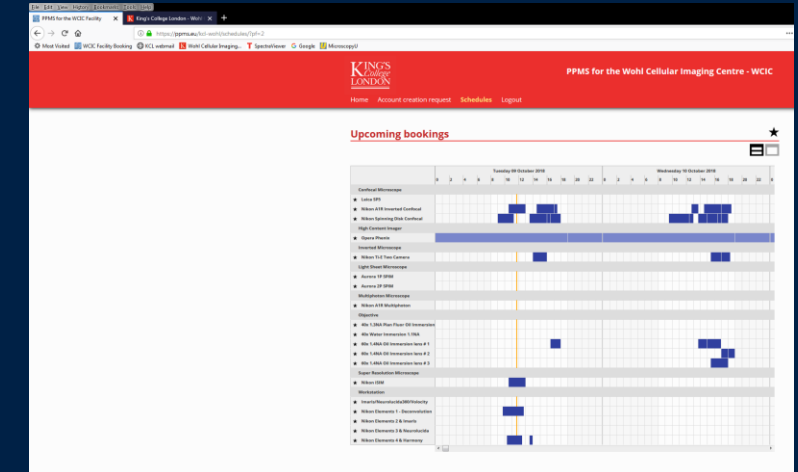
## At The End Of The Session...



This can then be accessed from the workstations and personal computers.

If you have trouble connecting to the shared drive, please login to the booking system, go to documents and the 3<sup>rd</sup> document contains instructions.

- Connect to internet and click on booking system in favourites, no need to login, go to schedules.



# STEP BY STEP INSTRUCTIONS

More advanced instructions

...reuse previous camera settings

# What if you are imaging similar samples and want to re-use camera settings you've optimised before...

- 1) In NIS Elements software, open a previous image with camera settings you want to mimic.
- 2) Right click on the image once it's open
- 3) Select reuse camera settings
- 4) Be aware, this uploads camera settings only, acquisition setting (Z-stack, large image etc.) will not be reloaded

If you need any help, please contact:

George Chennell (07771926760)

or

Chen Liang (07883166321) via WhatsApp

Happy Imaging!