

LAB SHADOW – INSIGHTS

Shadower: Kat of Territory – UX research/design
Shadowing: Andres Miklosi of ONI – Imaging scientist

Oxford Oni Lab
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HEADLINES

Gained valuable insight of the imaging process and ONI software in use.
Felt I covered about 40% top level understanding of the software.

Next requirements:

More collaboration (face to face, skype, email contact):

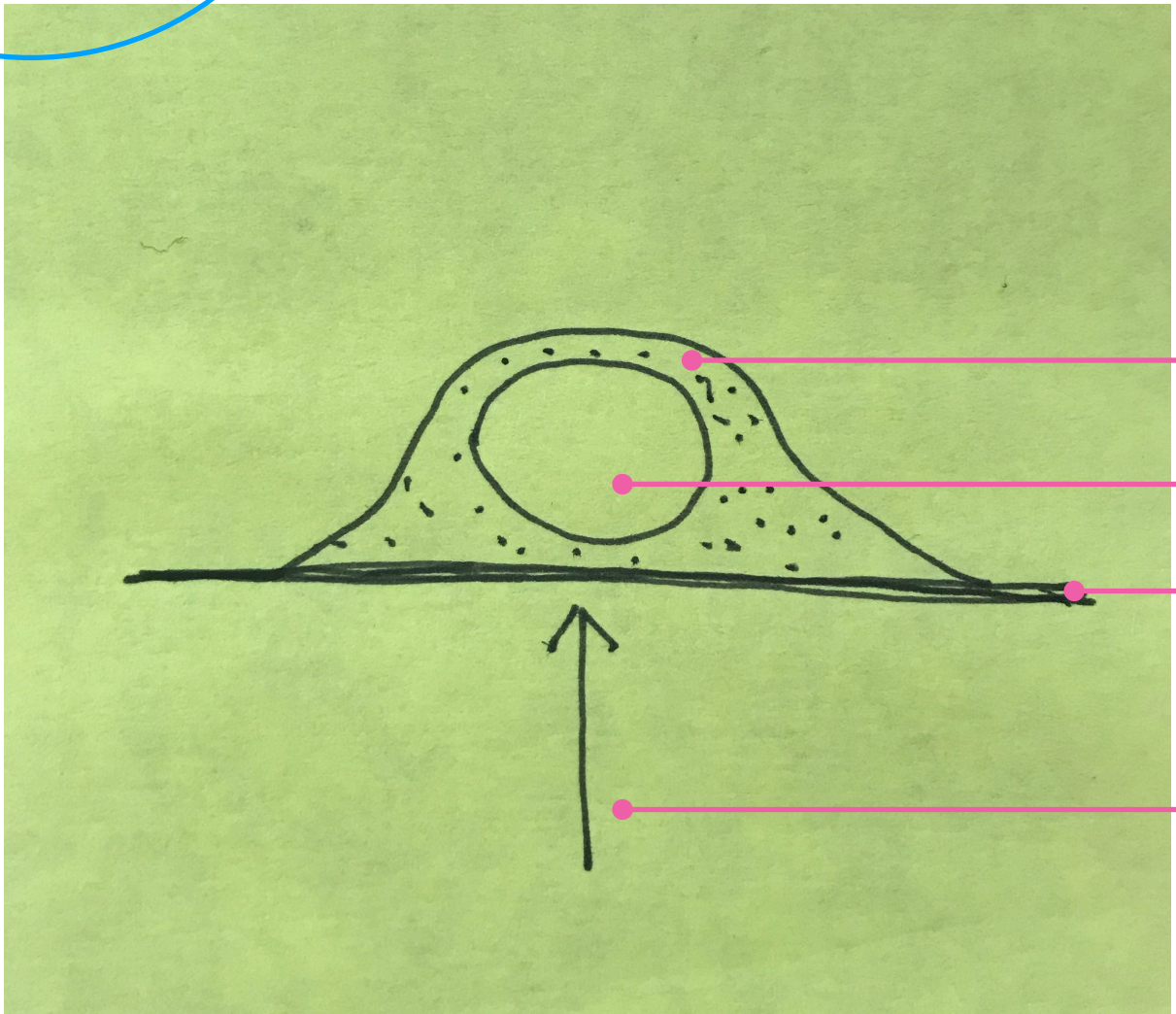
- 2 or 3 imaging scientists
- 1 application specialist
- The developer team – integration

Need another visit to:

- See imaging with 2 lasers in use
- See imaging with 4 lasers in use
- Take more screen grabs/photos
- Gather insights in menu options not yet covered
- Gather insights from different end users (preferably 3 in total)

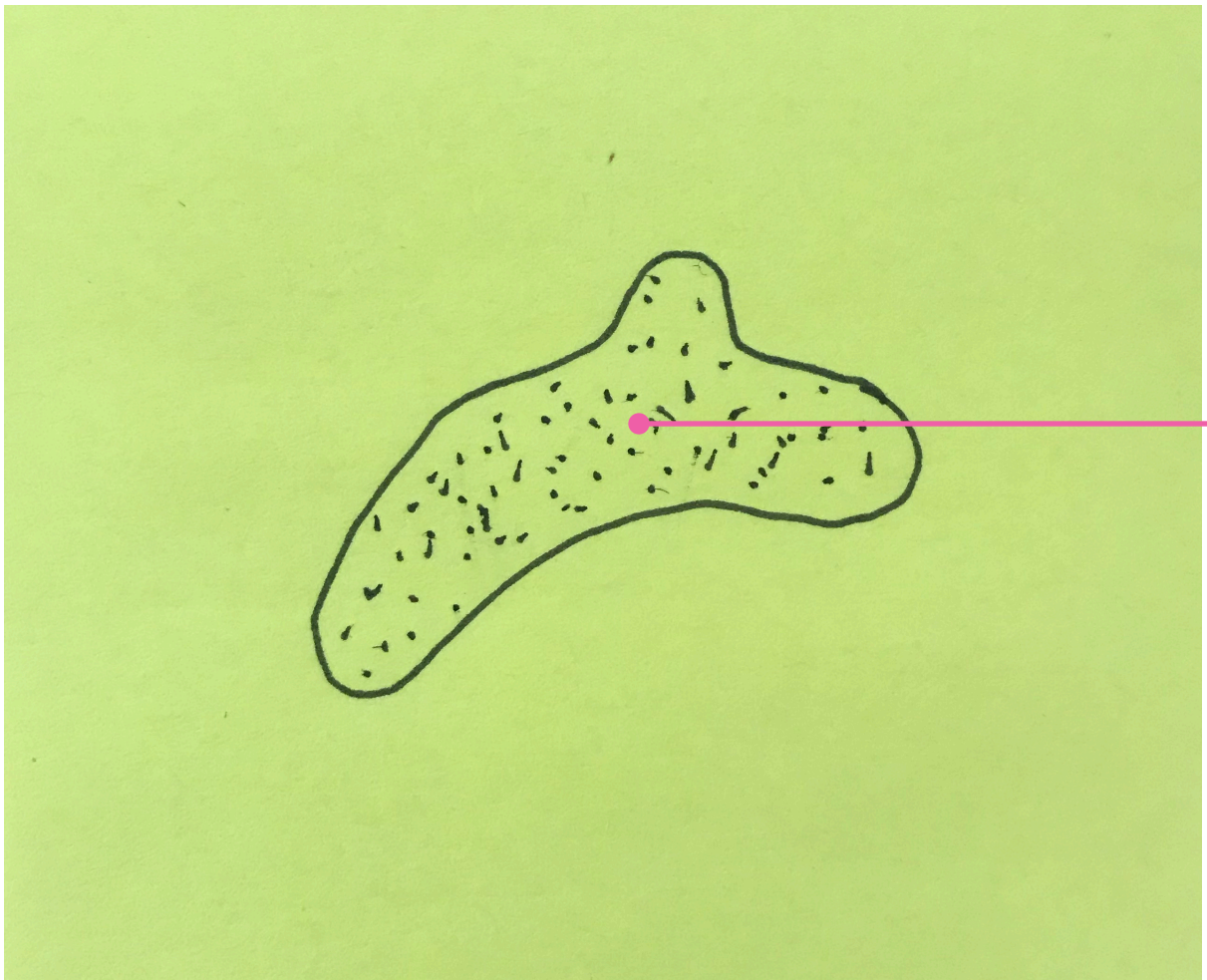
Imaging team

What are they imaging?



A single cell
The tiny dots = organelles

- Single cell
- Nucleus of cell
- Glass
- Laser



An organelle
The tiny dots = 'blinks' = single blinking molecules
The blinks build up to reveal the shape of the organelle

Aquire

In the lab

Captures raw data – and processes in real time.

Outputs = file.locb + 8 compressed image files

ALWAYS acquire a sequence!

Blinking molecules are captured over a series of frames. Each frame shows different blinks.

Usually capture up to 20,000 frames.
This can take 10 minutes or more.

Can see analyse view in real time using the options, but some variables are not editable. Useful, so can see if it's working / worth imaging.

Analyse

Out of the lab (?)

Filter the view

Change the colours

Why else use this area?

I think there is an analysis team that use more complex software to do this academically.

PREP



Optics

Interface

Laser

ONI lab
Oxford



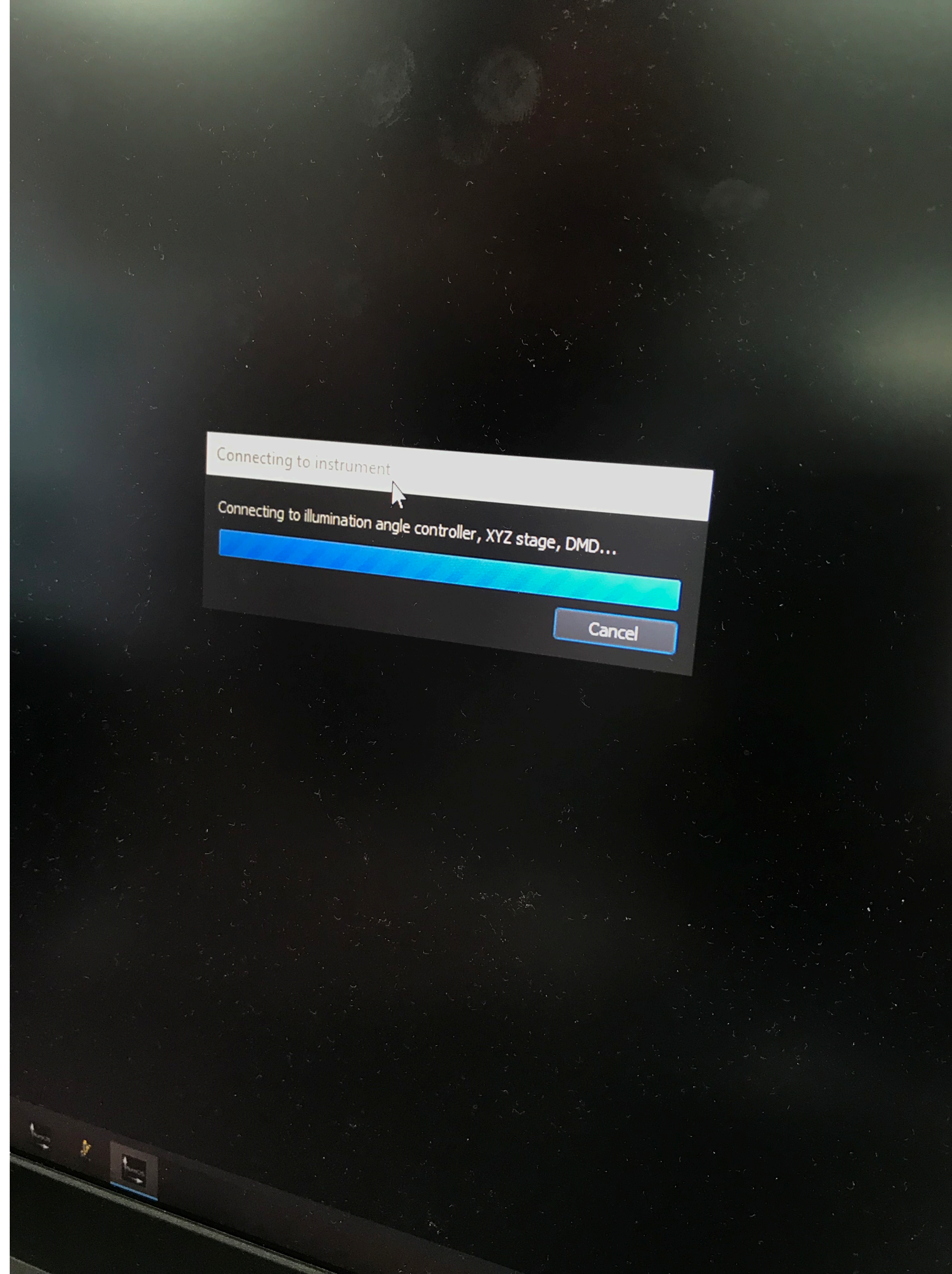
PREP

Setting up the sample

- Zera set up a sample on a slide with a florescent label (agent, reporter).
- Zera tells Andres (end user) which laser to use “640” – they refer to lasers using industry standard numbers.
- Florescent labels correspond with various lasers – they are matched to work together.
- The agent binds to something (eg proteins, DNA, lipids) to give it shape.



LASER	FILTER	FLUOROPHORE
405 nm	448/58	Pacific Blue, CFP, BV 421, DAPI
	526/52	AmCyan, BV 510
	585/42	BV 570
	622/22	BV 605
	722/44	BV 711
488 nm	795/70	BV 785
	513/26	FITC, GFP
	550/32	YFP
	620/29	PI
532 nm	710/45	PerCP Cy 5.5
	546/20	Alexa 532
	576/21	TRITC, Cy 3
561 nm	664/22	PI
	692/18	PE Cy 5.5
	579/16	PE
592 nm	614/20	RFP, tomato
	664/22	PE Cy 5, mFlum,
	795/70	PE Cy 7
633 nm	620/29	Texas Red, Alexa 594
	671/30	
640 nm	671/30	APC
	722/44	Alexa 700
	795/70	APC-Cy7



PREP

Connect to microscope.

takes a minute or 2.

Text descriptions goes through the progress.

PREP

Place sample on the optics

Drop of oil on the (lens?)

Place slide on top.

Close the lid.

Set the physical lock 'interlock'.



PREP

Pain point

The 'interlock' is a physical lock that needs to be pulled out.

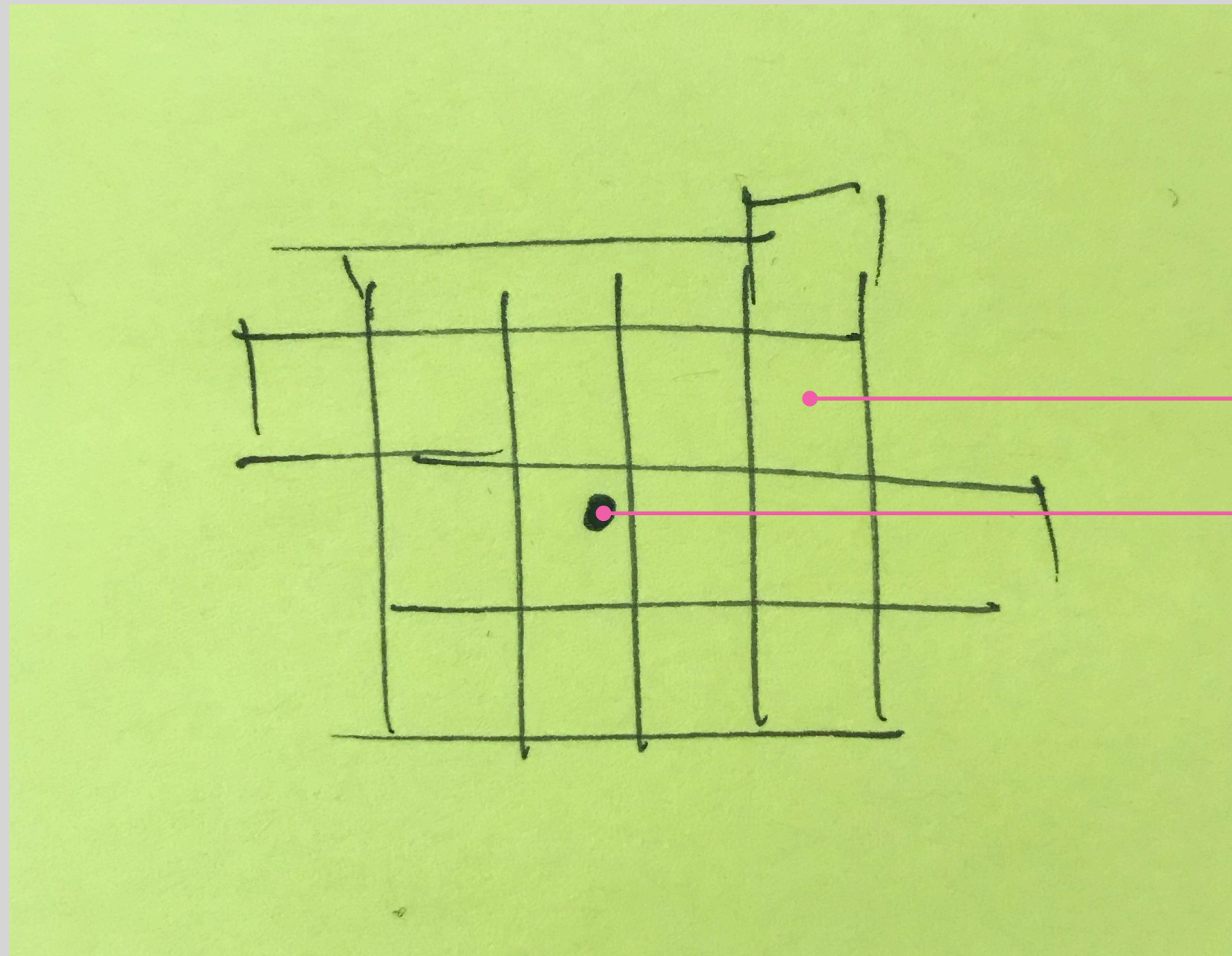
Sometimes people forget, will do an acquire and the screen is blank.

There is no signal in the interface that the lock is not on.



AQUIRE

AIM = looking for mitochondria shapes.



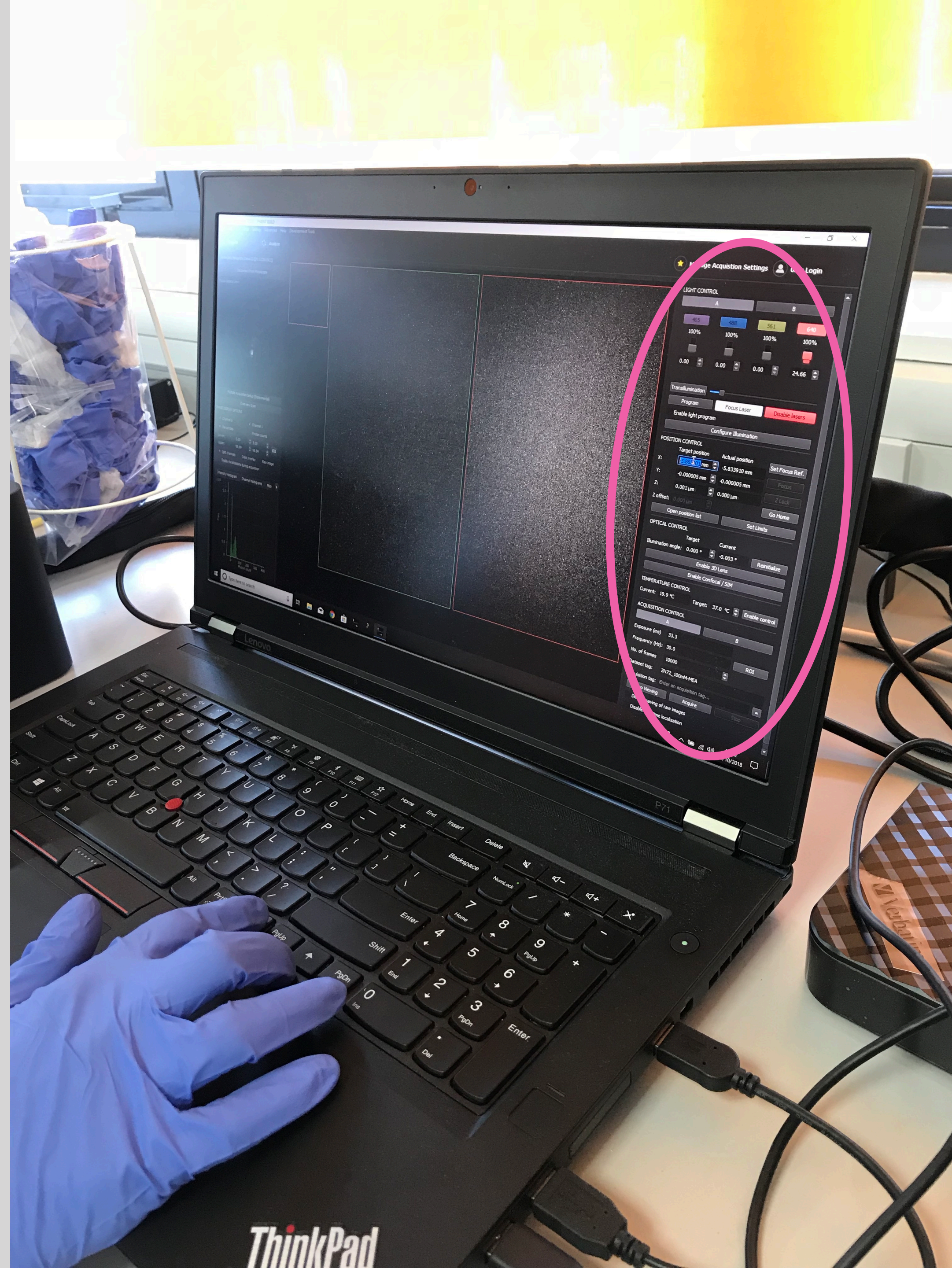
2 step process:
1 = detect blink
2 = find centre.

Blurry pixels

Central point

This processing is done on the fly in real time as acquiring.

During acquire – tick box ‘display’ – then you can see the squares blinking. A square means the centre has been processed. Andreas likes this view! Need screen grab.



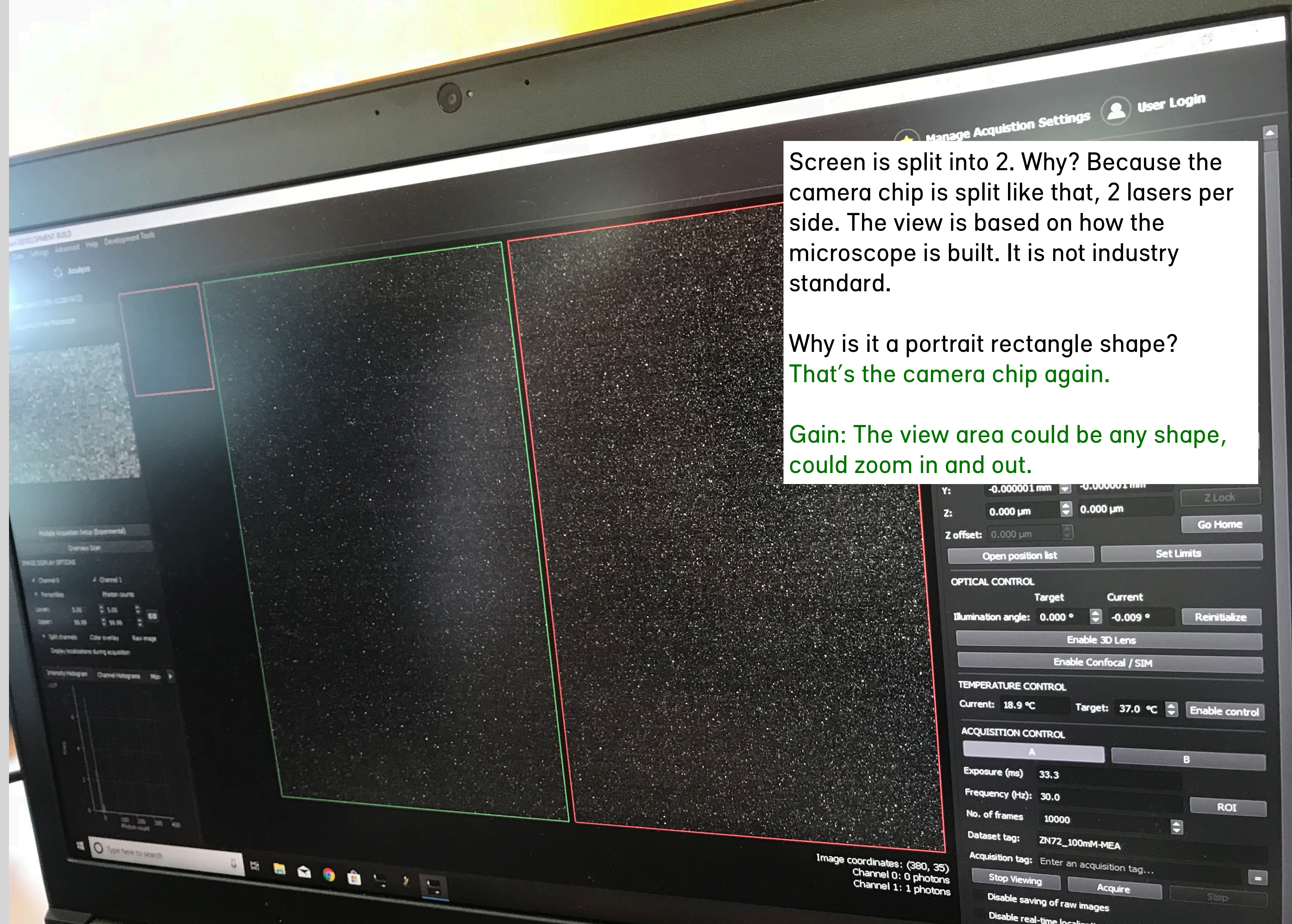
AQUIRE - LIGHT CONTROL

Once sample is placed in the optics unit, all action is at the laptop.

All of this panel information is useful and should be in view.

In analyse mode – some items are not editable so should be visually disabled or not in view.

In light control we do the bleaching and set the focus.



Screen is split into 2. Why? Because the camera chip is split like that, 2 lasers per side. The view is based on how the microscope is built. It is not industry standard.

Why is it a portrait rectangle shape?
That's the camera chip again.

Gain: The view area could be any shape, could zoom in and out.

Y: -0.000001 mm -0.000001 mm Z Lock
Z: 0.000 μm 0.000 μm Go Home
Z offset: 0.000 μm Set Limits
Open position list

OPTICAL CONTROL

	Target	Current	
Illumination angle:	0.000 °	-0.009 °	Reinitialize
Enable 3D Lens			
Enable Confocal / SIM			

TEMPERATURE CONTROL

Current:	18.9 °C	Target:	37.0 °C	Enable control
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ACQUISITION CONTROL

A		B	
Exposure (ms)	33.3		
Frequency (Hz)	30.0	ROI	
No. of frames	10000		
Dataset tag:	ZN72_100mM-MEA		
Acquisition tag:	Enter an acquisition tag...		
Stop Viewing	Acquire	Stop	
Disable saving of raw images			
Disable real-time localization			

Image coordinates: (380, 35)
Channel 0: 0 photons
Channel 1: 1 photons

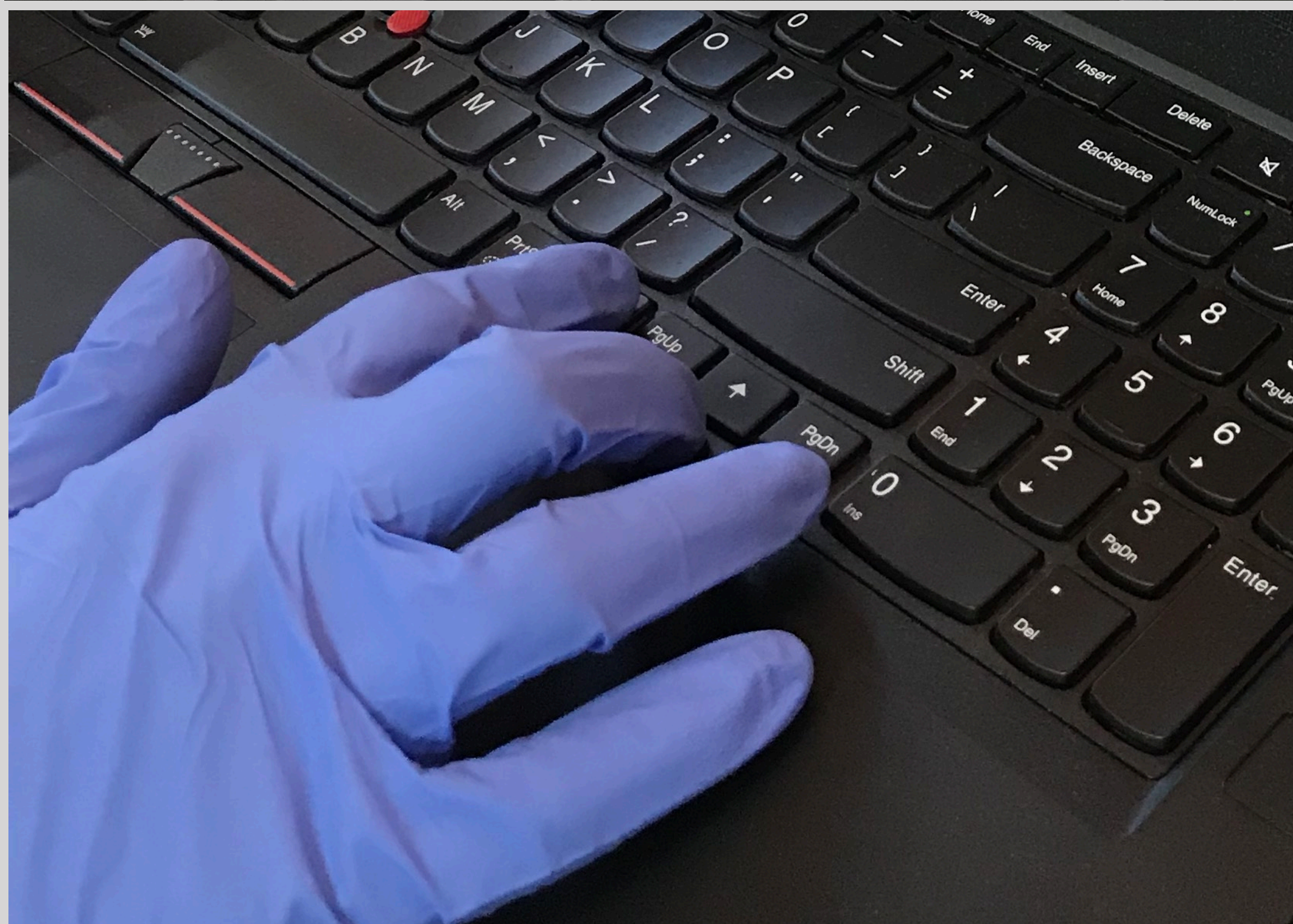


POSITION CONTROL

Moves the stage.

Mouse click in the view pane to then control X and Y with arrow keys.

Click in control to move Z dial.



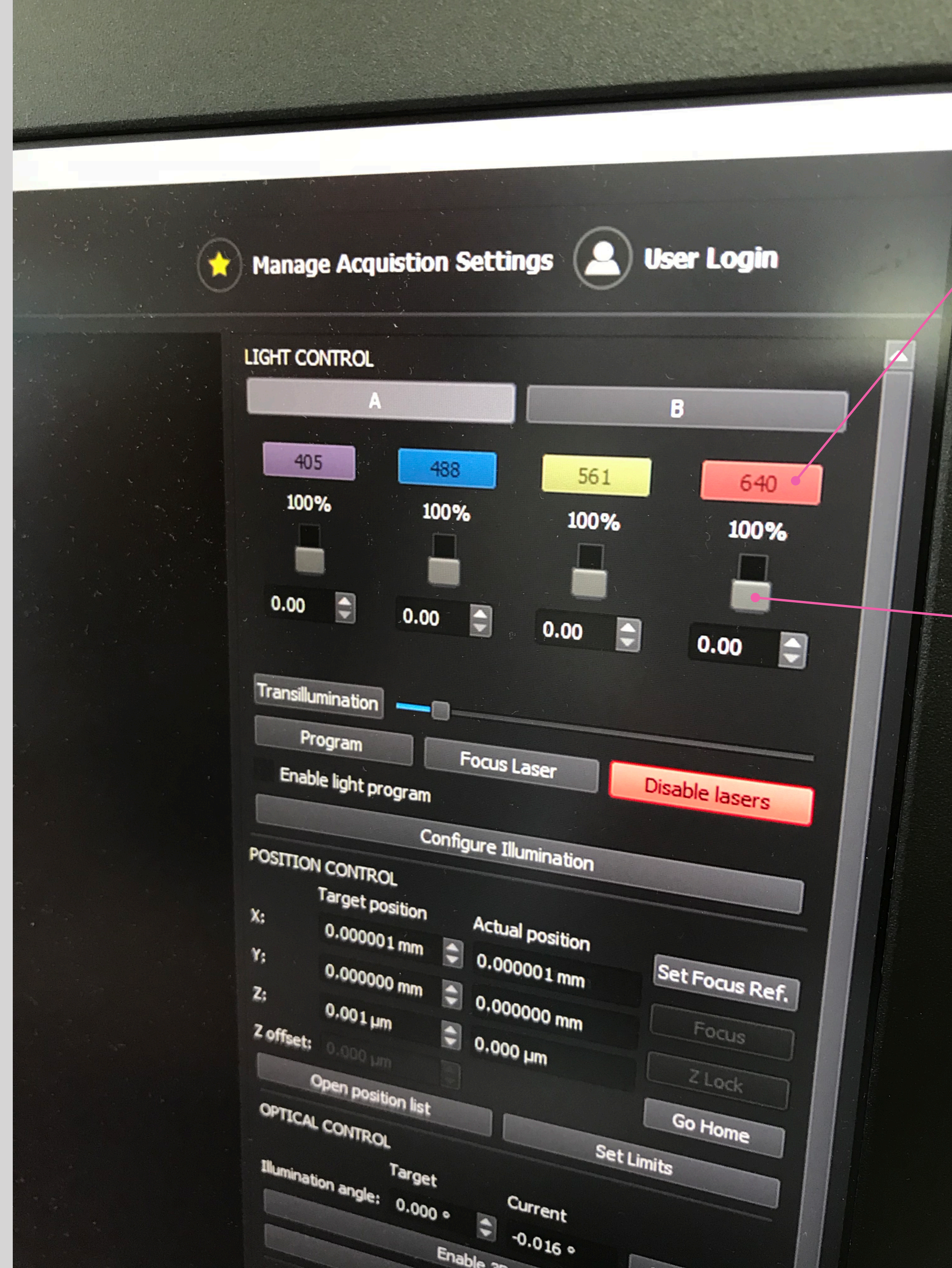
ACTIVATE LASER

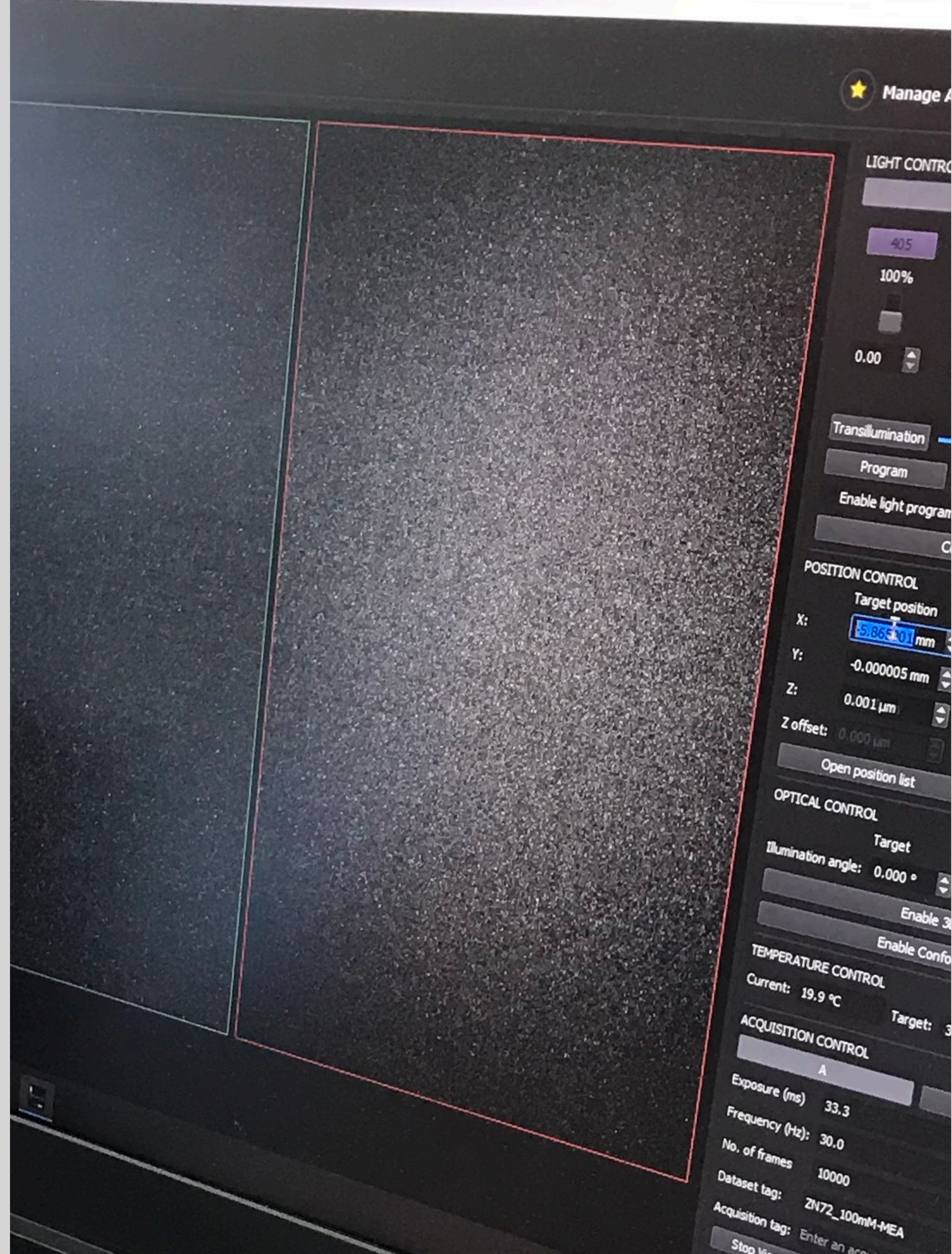
Andres sets the 640 laser power to 20%.

Laser names = industry standard numbers. Users know what these numbers mean and like to see them. They are in view for acquire mode, but not in analyse mode – **this is an issue.**

These sliders are too small, poor fidelity. Why have a vertical slider and a number slider?

Once set the laser – **hit the view button to turn the camera on. Why is it not on be default? No idea. No reason why it should be off.**





BLEACHING

He's looking for spacial and temporal defined 'blinks'. Blinks are molecules.

Reduce the blinking so can see things clearer – less noise.

The structure has never been luminated by a laser before. Light it up and it bleaches naturally over time. Photo-physics.

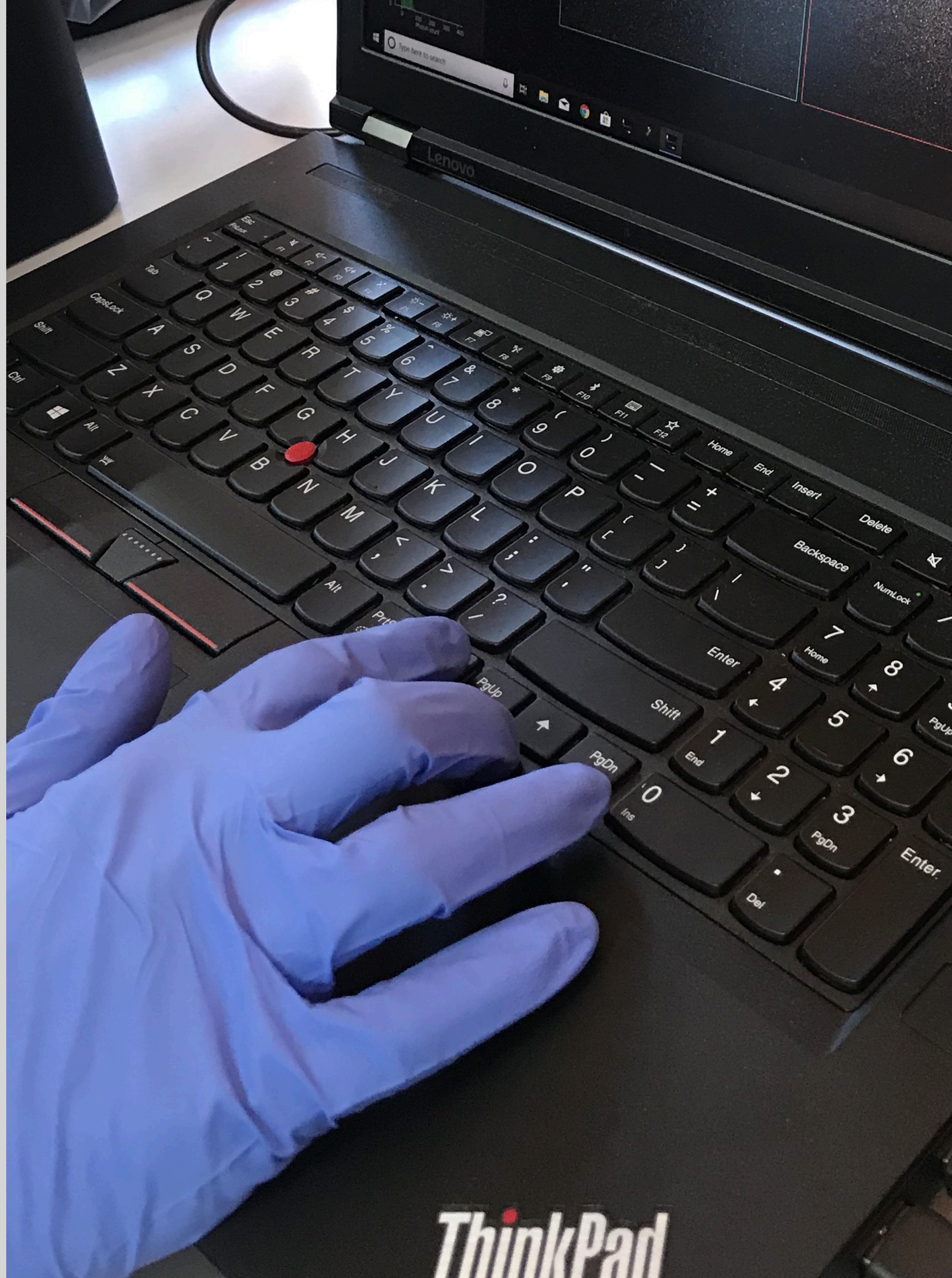
This is where the slider control fidelity is not great.

A/B



Andres said he never uses the A/B option and is not sure what the value is – “it overcomplicates things and is unnecessary”. I suggest they are for capturing different laser settings during acquire? He responds that you can't change a laser setting in the middle of acquiring – so is not sure why it's there.

Thoughts: Watch a user that uses it.



SETTING FOCUS

Gloves are on throughout (in the lab).

Uses arrow keys. **Andres does not use the xbox controller, says it's irrelevant.** Can use the controller for focus or stage direction. Maybe useful to others.

Gain: When moving the stage (arrow keys) the interface position numbers change – they should let you know when hitting the limit – instead the optics unit makes a noise – it's confusing and not clear what is going on.



Manage Acquisition Settings User Login

LIGHT CONTROL

A	B
405	561
100%	100%
0.00	17.50

Transillumination

Program Focus Laser Disable lasers

Enable light program

Configure Illumination

POSITION CONTROL

	Target position	Actual position	
X:	-5.863339 mm	-5.863339 mm	Set Focus Ref.
Y:	0.089992 mm	0.089992 mm	Focus
Z:	-258.295 μm	-258.295 μm	Z Lock
Z offset:	0.000 μm		Go Home

Open position list Set Limits

OPTICAL CONTROL

	Target	Current	
Illumination angle:	0.000 °	0.007 °	Reinitialize

Enable 3D Lens

Enable Confocal / SIM

TEMPERATURE CONTROL

Current: 22.1 °C Target: 37.0 °C Enable control

ACQUISITION CONTROL

A	B
Exposure (ms):	33.3
Frequency (Hz):	30.0
No. of frames:	10000
Dataset tag:	ZN72_100mM-MEA
Acquisition tag:	Enter an acquisition tag...

Stop Viewing Acquire Stop

Disable saving of raw images

Disable real-time localization

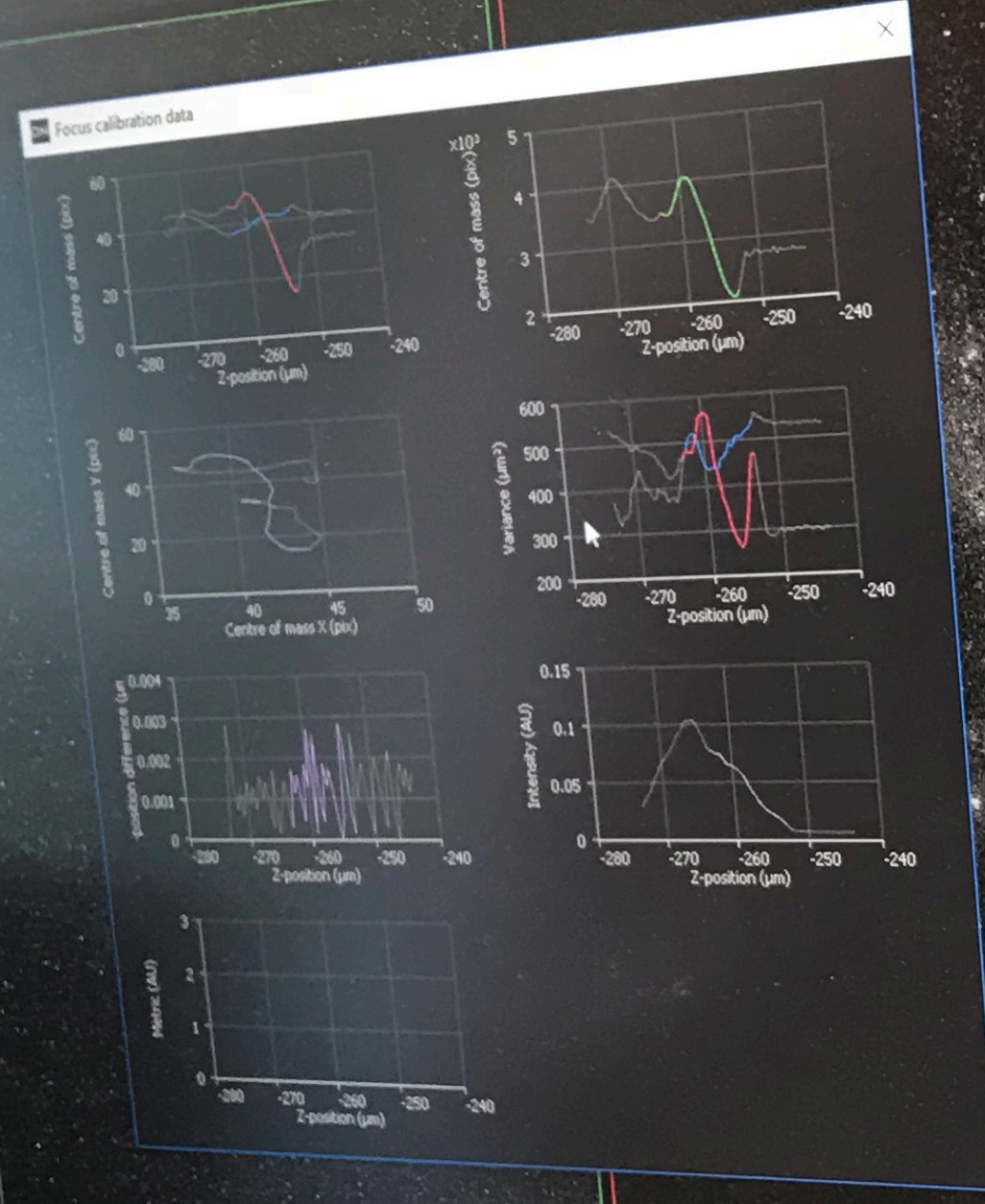


Image coordinates: (18, 278)
Channel 0: 1 photons
Channel 1: 4 photons

12:53
29/10/2018

SET FOCUS REFERENCE

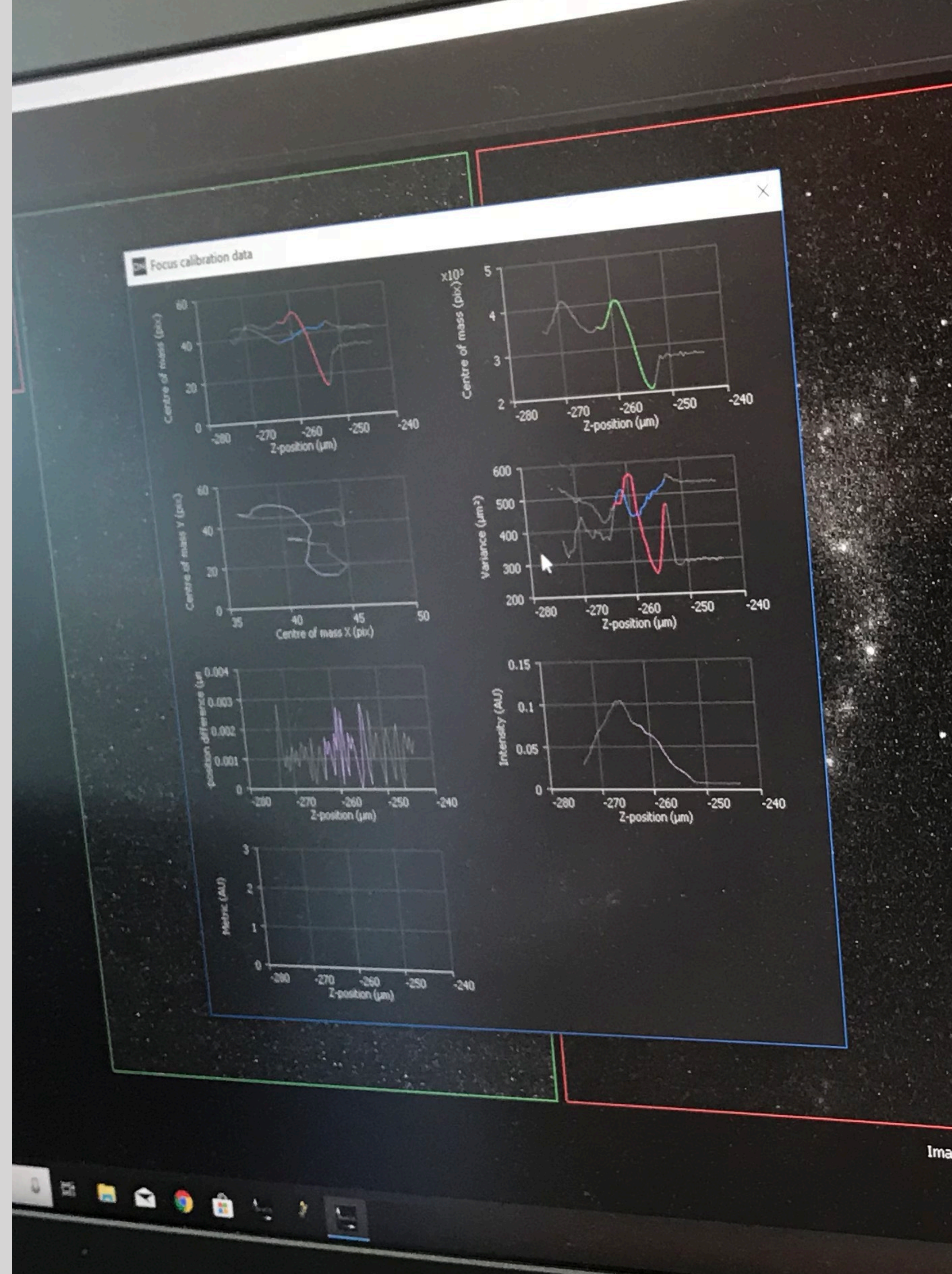
Shows how following the focus pattern.
"Focus calibration data" = Charts in an overlay window.

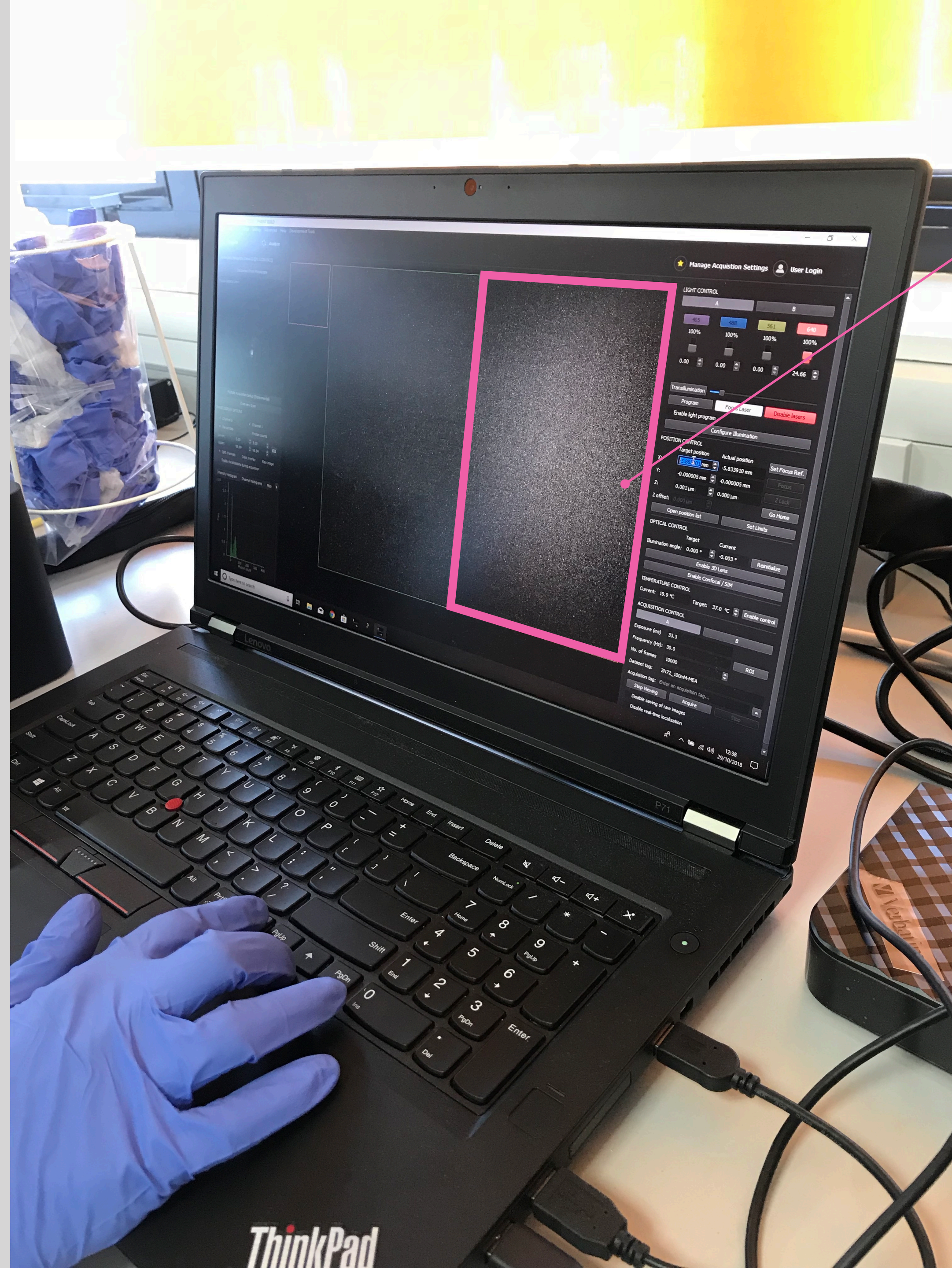
He looks at these graphs to assess if the focus is good. There are 6 graphs here. Andres looks at one graph and ignores the others. He's looking at the curve shape in 1 graph. He finds this data too mathematical and says it's too much data.

"I just want to see if this is in focus or not"

Very advanced info.

Once happy, click 'lock' – the focus is set.

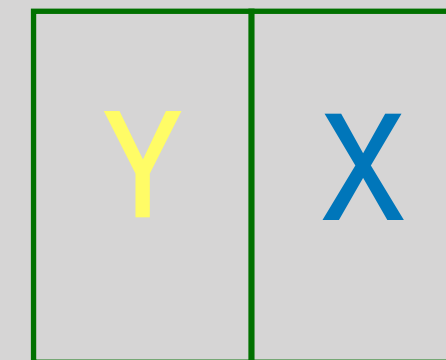




VIEWPORT

Painpoint:
During demo, the right panel was being used. Small space!

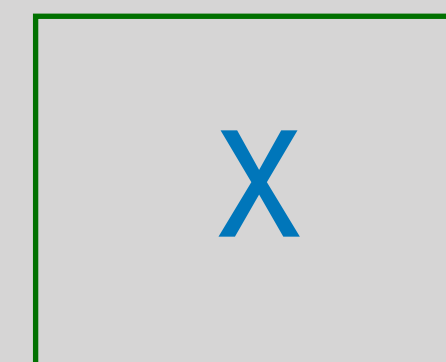
Gain:
Want's options for the view panel:



"I want to see shapes Y and X side by side."



"I want to see shapes Y and X layered."



"I want to see a single shape at a time"

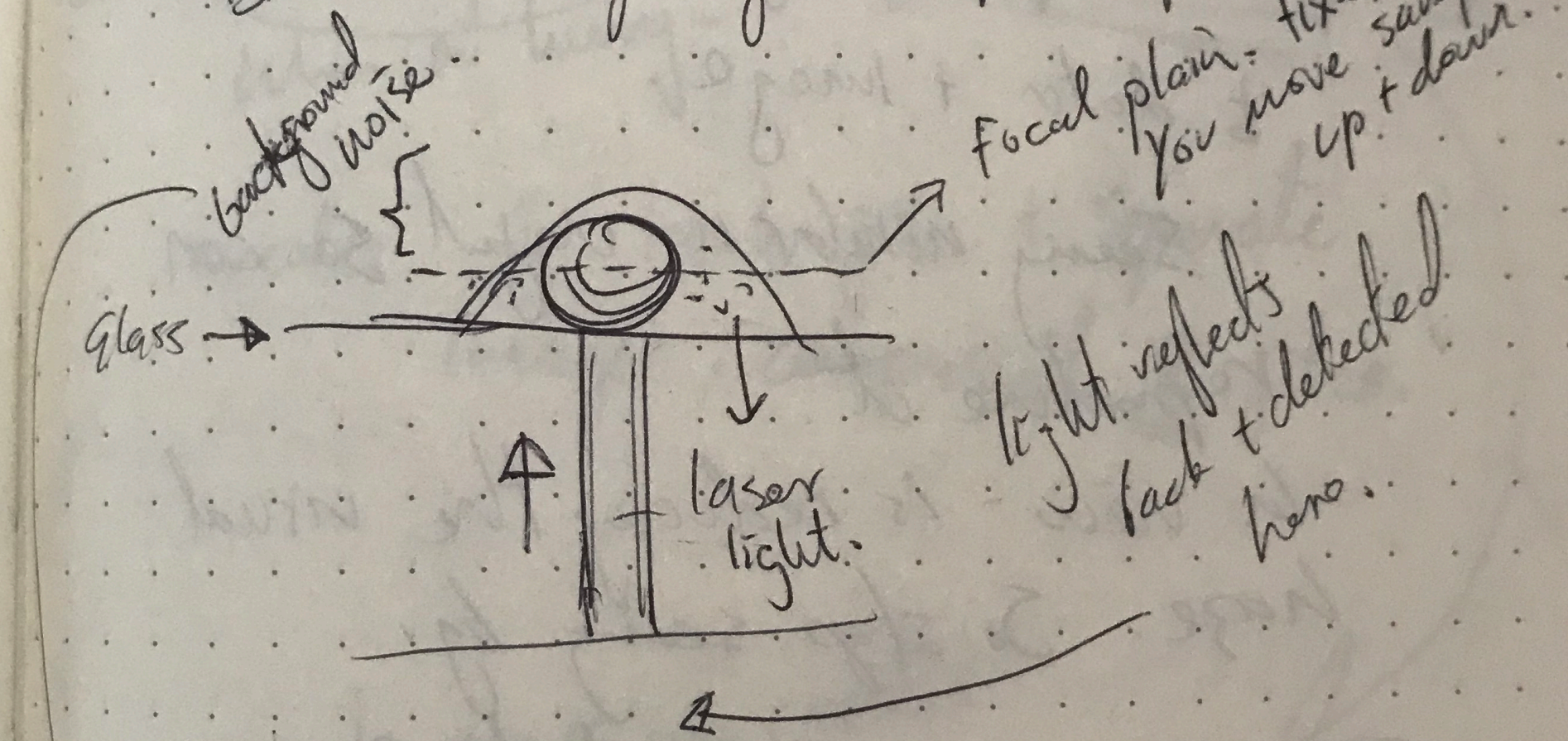
3. Z lock
Focus sorted!!

focus)

stem.

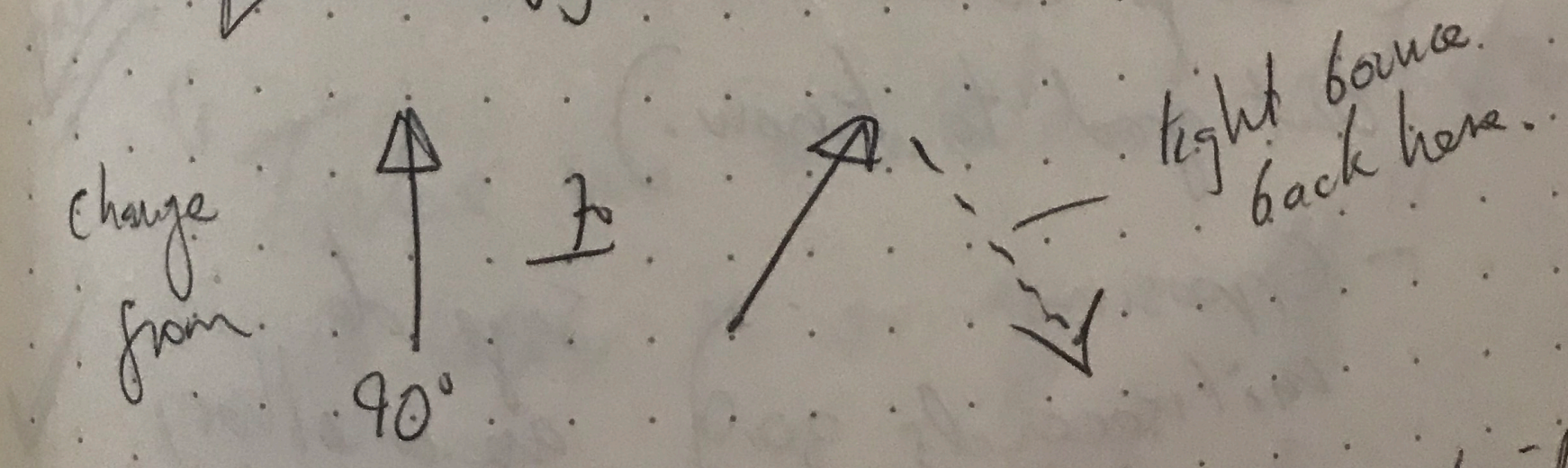
14. optical control

Based on physical principles



god.

Bg. noise = problem!
Modify angle in optical control



has narrow band of - optical

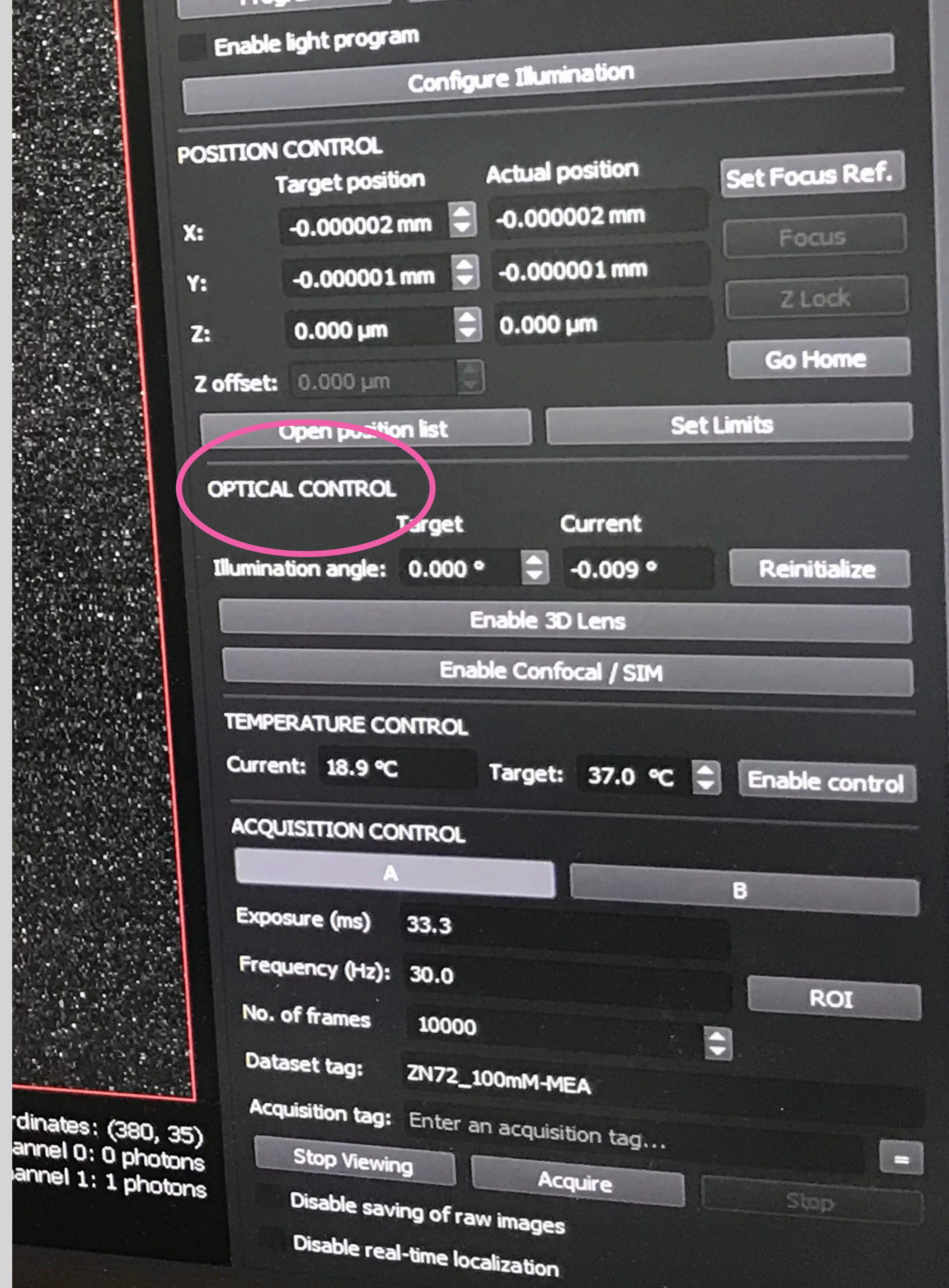
OPTICAL CONTROL

The problem... based on physical principles.

The focus plain is fixed - you move the sample up and down.

The laser light goes straight up, and reflects light back as detected.

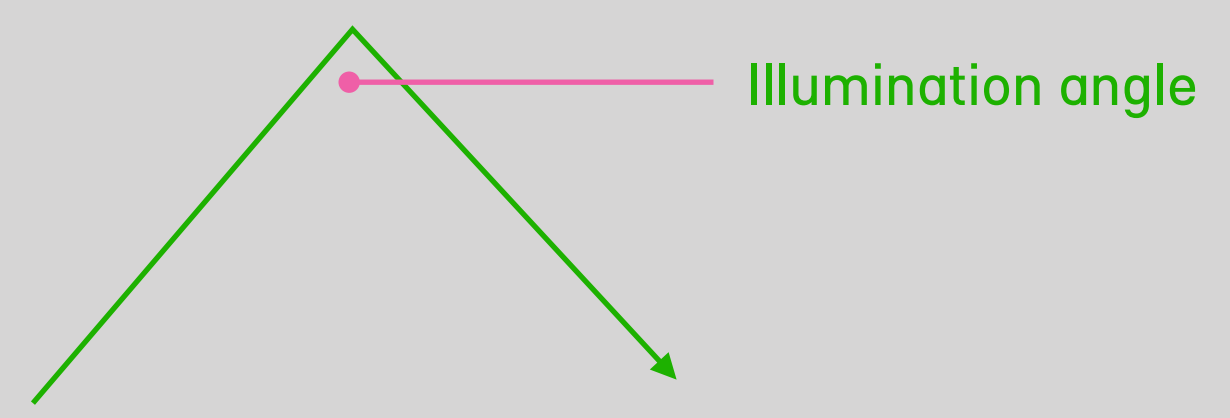
The problem is the laser light picks up background noise beyond the focus plain.



OPTICAL CONTROL

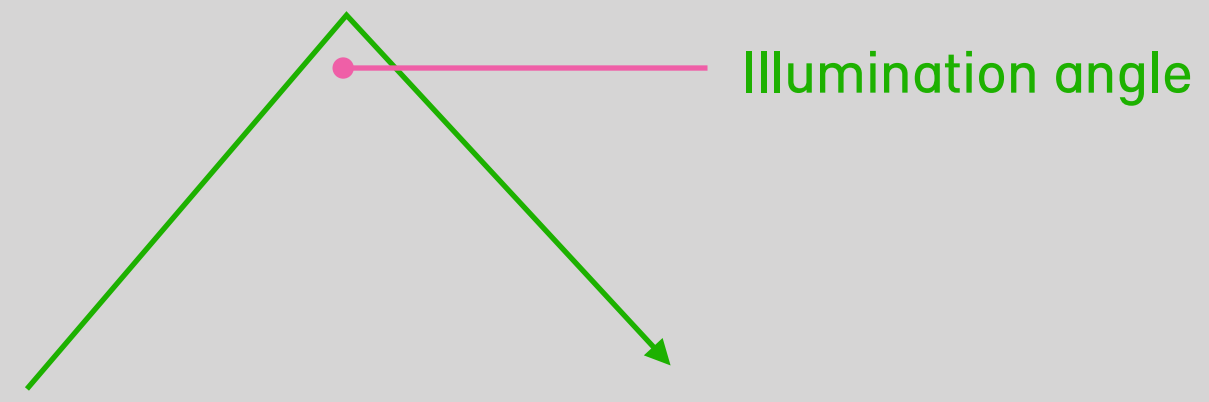
The solution... modify the 'illumination angle' (target) to change the laser beam from 90 degree position to bounce back at an angle – so it lights up 'excites' a narrower focal band – an optical slice. Reduces the bg noise.

Change laser beam from 'zero' (90 degree position) to...



In the view, this reduces the visual haze in the background (in real time).

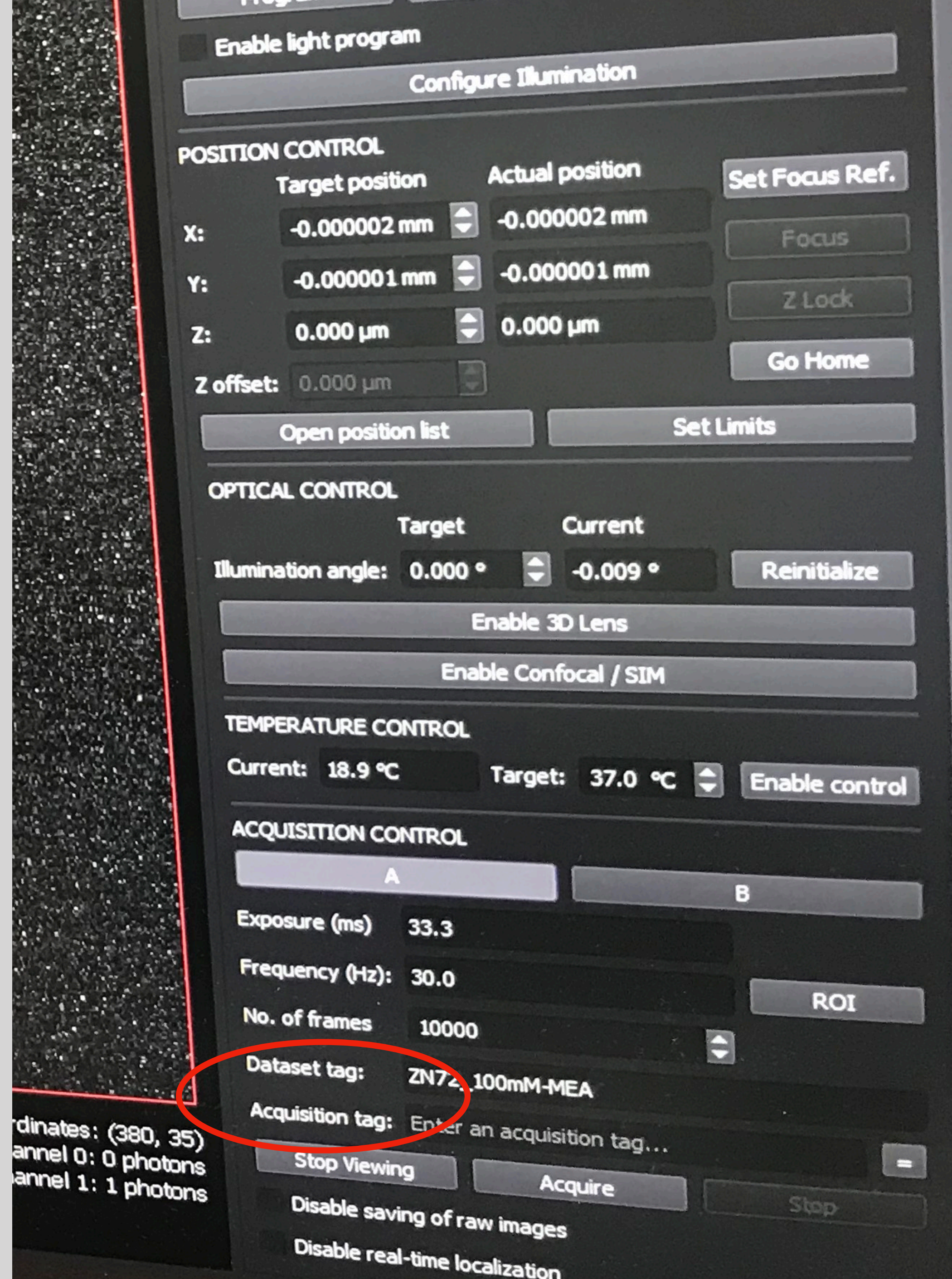
OPTICAL CONTROL



How do you know what angle is best?
Experience!!

Gain = Useful to know when this number is hitting a physical extreme (some numbers don't make sense, are impossible, but it lets you go to those numbers). When goes extreme, the screen is blank and user may not understand why. It is detrimental to the data and image.

Seeing the number is useful so can reproduce it if need to,



ACQUISITION CONTROL

Temperature control – Andres does not use this but it's good to know.

Exposure (miliseconds)
Frequency (Hz)

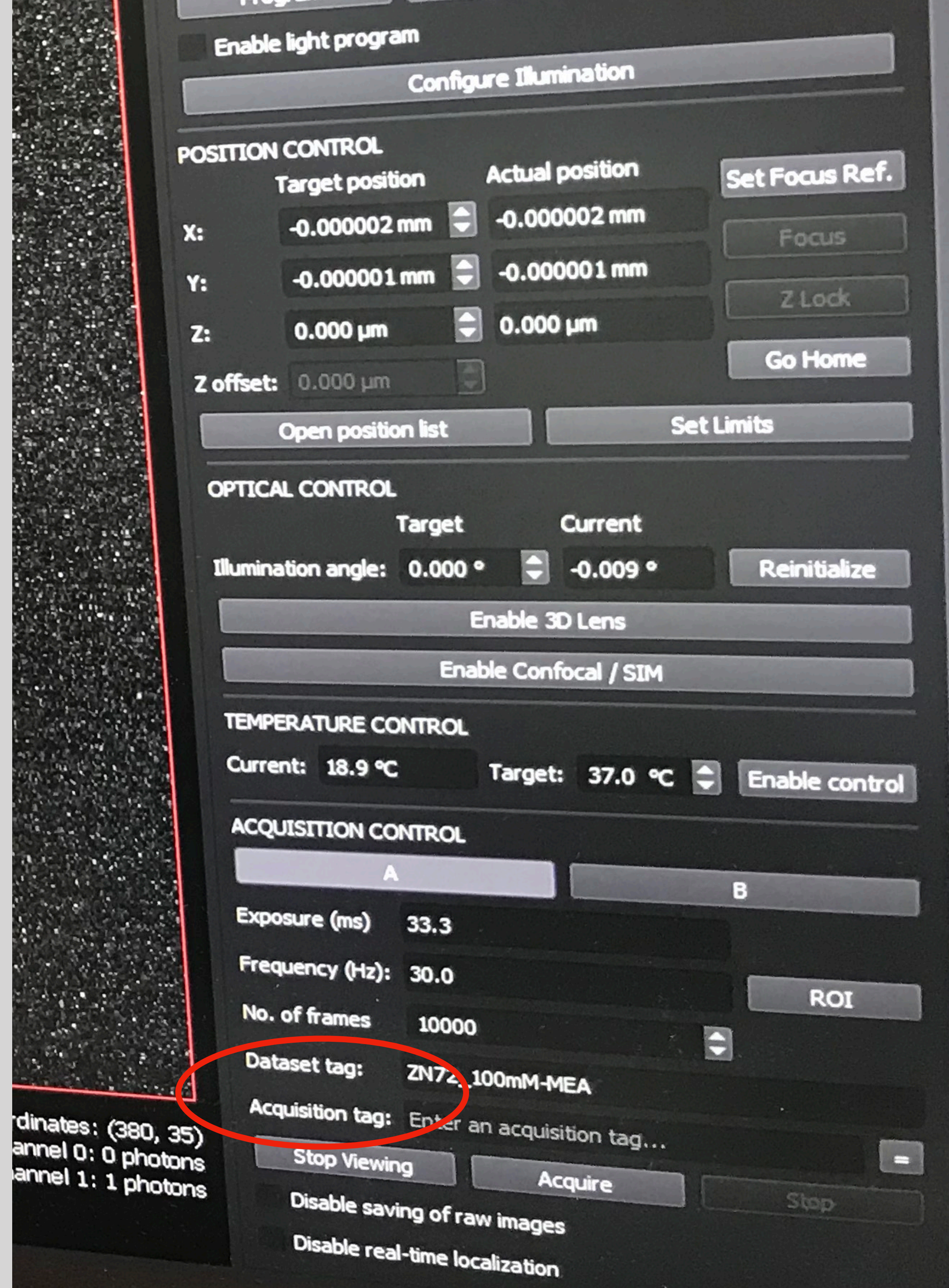
You edit one or the other – never both.
They coordinate and change automatically if one is changed.

He thinks this doesn't display clear well enough for that interaction.

Set number of frames – 10,000

Painpoint:

Dataset tag = file name; Acquisition tag = folder name. EVERYONE hates the labelling for this setting and can never remember what the labels mean and get it mixed up – which causes a mess with the output files. Output = 9 files, if it messed up they are all in separate folders.



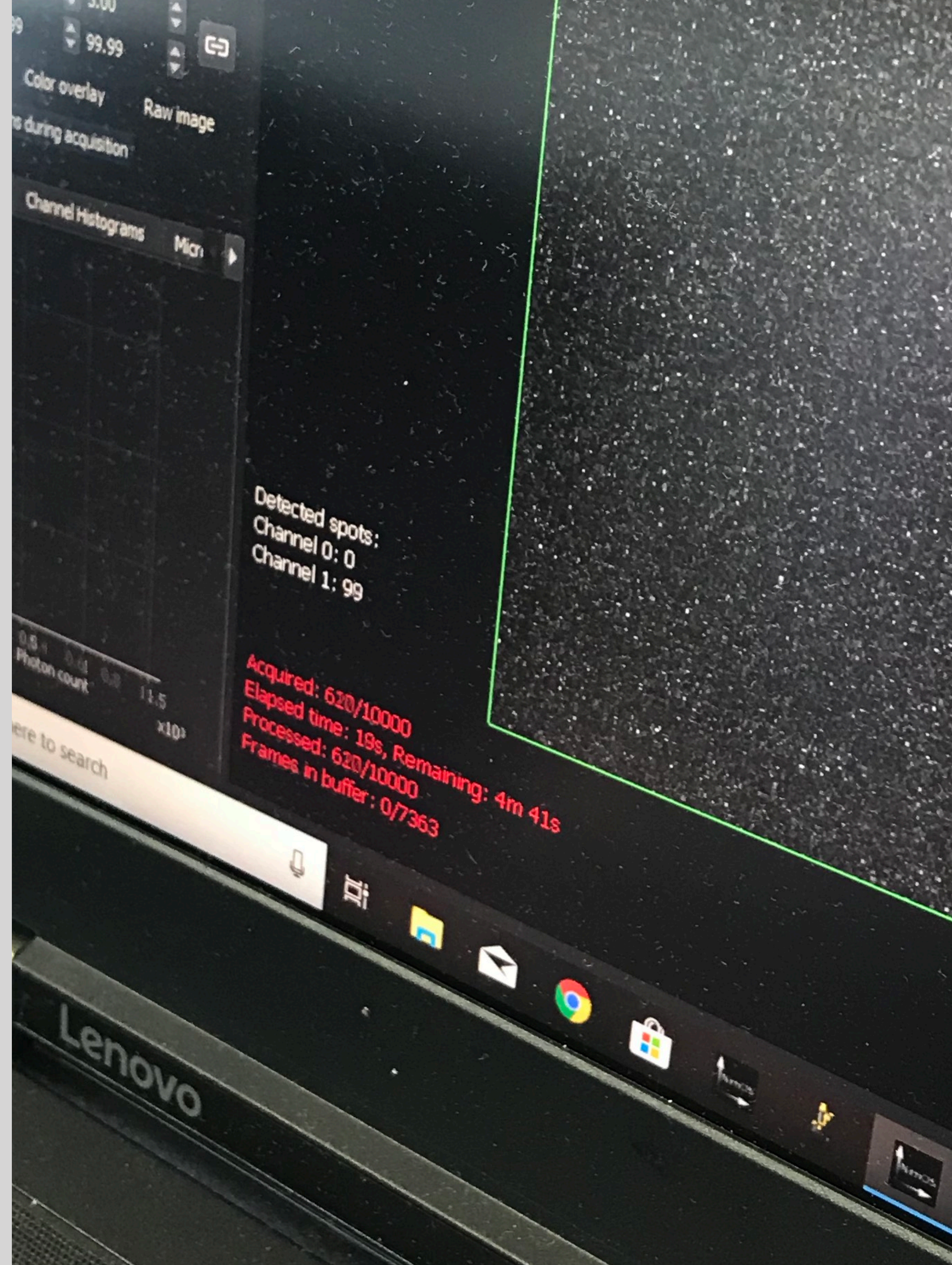
Painpoint:

“Takes me a while to understand if the laser is on or off”

Lets you acquire even if the laser is off. They suggest traffic light colour code of the UI for the laser area so can see clearly if a laser is on or off.

To see if a laser is on or off – look at the viewport. But if it’s greyscale mode, can’t distinguish the lasers.

If the laser is off, then the view options should be off too. Contextualised.



ACQUIRE

Hit the acquire button to start the process.

Pain: Does not notify you that the laser is off. It will create an empty file and you have to create a new file name and do it again.

Want to make sure the laser is on.

When running, the red text number data states the progress.

It's good to stay and monitor it.

Hit the analyse tab to see it being built up in real time. Very cool.

Issue: not being able to edit filter numbers here, can't modify, yet options are there – this is confusing – especially for a new user.

ANALYSE

An opened file

The screenshot displays a software application window titled "Nim Qt Application: DEVELOPMENT BUILD". The interface is divided into several sections:

- Top Bar:** Contains menu items (File, Instrument, Data, Settings, Advanced, Help, Development Tools) and a "User Login" button.
- Left Panel:**
 - Acquire/Analyze:** Two main tabs at the top.
 - Load Data...:** A section for loading image files, listing multiple "ImagingTeam_HACAT tom20.153311789686" files.
 - Warnings:** Three red warning messages: "Warning! Invalid camera parameters", "Warning! Invalid camera calibration", and "Warning! Invalid camera mapping".
 - Localization Parameters:** Includes "Override default localization parameters", "Sigma estimate" (0.90, 1.20), and "Iterations" (15, 15).
 - Run localization:** A button to execute the localization process.
 - Result files:** Lists "ImagingTeam_HACAT tom20.1533117896862.lc" and "ImagingTeam_HACAT tom20.1533117896862.n".
 - Export Localizations as .csv:** A button to export data.
 - TOOLS:** A list of analysis tools including Trace, Line Histogram, XYZ Plane View, Tracking, FRET Traces, and 3D Visualization (Experimental).
 - Calculate Drift Correction:** A button with a checked "Apply correction" checkbox.
 - STATUS:** A section at the bottom of the left panel showing "0%".
- Main View:** A large central area displaying a red-stained biological image. A dashed white box highlights a specific region. A scale bar in the bottom right corner indicates "10 µm".
- Right Panel:**
 - RESULTS FILTERING:** A section with two tabs (A, B) and a "Disable all filters" checkbox. It contains several filter settings with min/max values:
 - Photon count: Min: 300, Max: 100000000
 - Background: Min: 0, Max: 100000
 - Localization precision (x) (nm): Min: 8.00 nm, Max: 14.00 nm
 - Localization precision (y) (nm): Min: 8.00 nm, Max: 14.00 nm
 - Sigma X (nm): Min: 47.59 nm, Max: 142.77 nm
 - Sigma Y (nm): Min: 47.59 nm, Max: 142.77 nm
 - LLR: A checkbox at the bottom.
 - VIEWING OPTIONS:** A section for controlling the display:
 - Channel selection: "Channel 0" (green) and "Channel 1" (red, checked).
 - Advanced Viewing Options...: A button.
 - Display Image: A checkbox.
 - Display localized points: A checked checkbox.
 - Projection modes: "Max projection", "Single frame" (selected), and "Custom image".
 - Localization color: A dropdown menu set to "Channel".
 - Localisation rendering mode: A dropdown menu set to "Fixed".
 - Frame index: A slider and a numeric input field set to "4093".
 - Display filtered points in grey: A checkbox.

Menu: Data / view acquisition data

Results filtering

The screenshot displays the ONI software interface. The main window shows a large red fluorescence image of a cell. On the left, there are panels for 'Image files', 'Warning!', 'Sigma estimate', 'Iterations', 'Result files', 'TOOLS', and 'STATUS'. A central 'Acquisition Data Viewer' window is open, showing acquisition data for the current file and for frame 1. A pink line points from the 'Menu: Data / view acquisition data' label to the 'Acquisition Data Viewer' window. On the right, a 'RESULTS FILTERING' panel is open, showing various filter settings for photon count, background, localization precision, and sigma. A pink line points from the 'Results filtering' label to this panel. The 'RESULTS FILTERING' panel includes sections for 'RESULTS FILTERING' and 'VIEWING OPTIONS'. The 'VIEWING OPTIONS' section shows 'Channel 0' and 'Channel 1' (checked), 'Display Image' (unchecked), 'Display localized points' (checked), 'Localization color' set to 'Channel', and 'Localisation rendering mode' set to 'Fixed'. A '10 µm' scale bar is visible in the bottom right of the main image area. The system tray at the bottom shows the Windows taskbar with the time 11:20 and date 29/10/2018.

key	value
CameraFrameIn...	0
Exposure_ms	33.3354586466165
FormatVersion	2
Frames	20000
FramesPerSecond	29.9980869293213
Hash	58d22b2fbd3230e55a2b87480134bda90fb99752b...
IlluminationAn...	54.9996905987686
InstrumentSerial	376634539453
LaserActive	0 false 1 false 2 false 3 true
LaserPowerPerc...	
LaserProgramL...	0

key	value
AccelerationDet...	false
CameraFrameIn...	0
Hash	
IlluminationAn...	54.9996905987686
LaserActive	
LaserPowerPerc...	
StagePos_um	
TemperatureC	30.6159687042236
zFocusOffset_um	0

Menu: Data / view acquisition data

ONI Acquisition Data Viewer

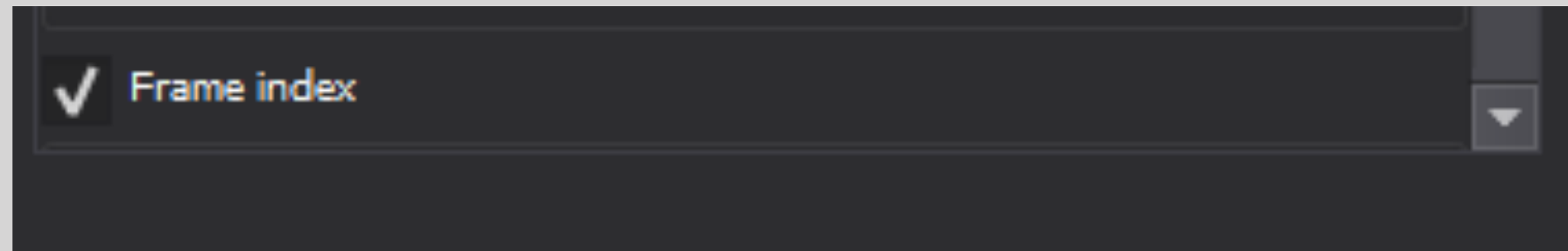
Acquisition data for current file:

key	value
CameraFrameIn...	0
Exposure_ms	33.3354586466165
FormatVersion	2
Frames	20000
FramesPerSecond	29.9980869293213
Hash	58d22b2fbdf3230e55a2b87480134bda907b99752b...
IlluminationAn...	54.9996905987686
InstrumentSerial	376634539453
▼ LaserActive	
0	false
1	false
2	false
3	true
▶ LaserPowerPerc...	
Laser_ProgramL...	0

Acquisition data for frame: 1

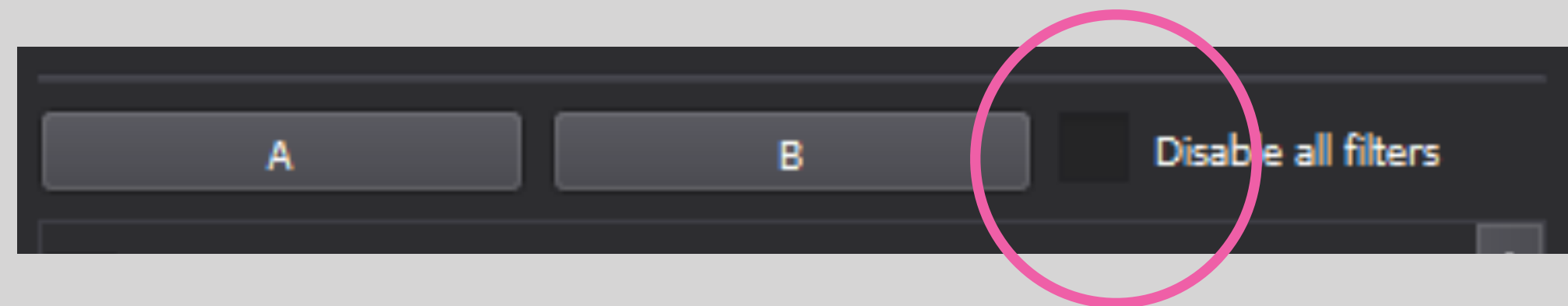
key	value
AccelerationDet...	false
CameraFrameIn...	0
Hash	
IlluminationAn...	54.9996905987686
LaserActive	
LaserPowerPerc...	
▶ StagePos_um	
TemperatureC	30.6159687042236
zFocusOffset_um	0

- Scrollable and nested data.
- True / false should read 'on/off'.
- Built by developers! End users want normal phrases – not 'true/false'.
- Laser numbers = 0 – 3. Confusing. Should be 1-4.



Frame index (parameters out of view here!)

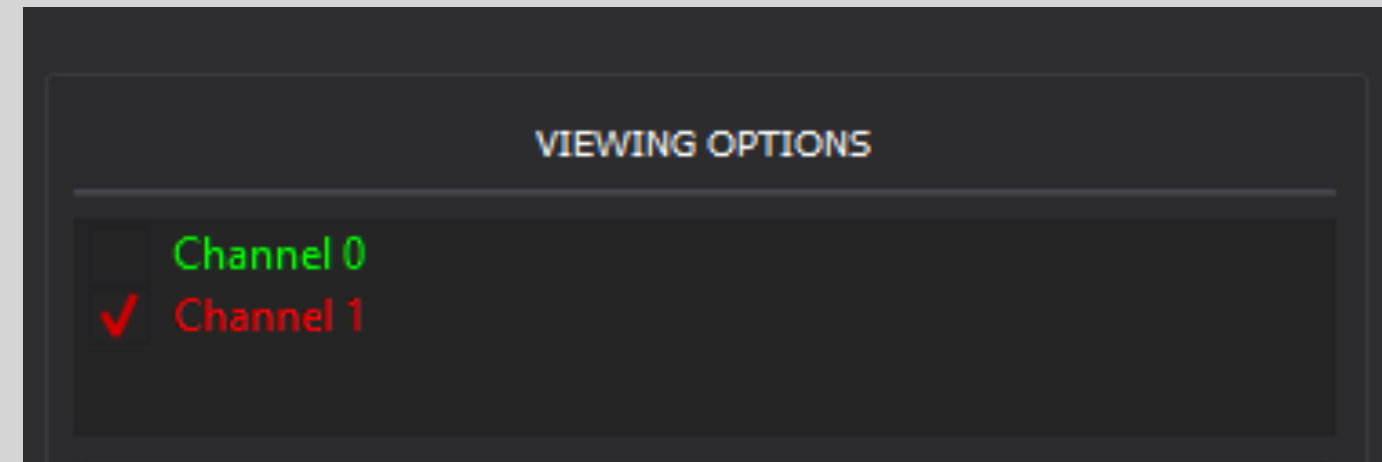
- min (say 10,000)
- max (say 20,000)
- Will show just those frames. Like Quicktime frame clipping!



Disable all filters – tick box

- If ticked you see everything!
- Untick this to set own filters.
- CRUCIAL step – to visualise relevant data and remove unspecific data. Useful step as labelling (agent) could be crap – need to know.
- Can do this during or after acquire. If during – acts as a real time filter of what you want.
- Filters are not fully functional during acquire due to processing power – but gives you a rough idea.
- Wants the unusable options hidden or greyed out, so clear can't use them. Contextualised view.

“Laser colours and labels here are super confusing”



Lasers of device:

0 = UV

1 = 488

2 = 561

3 = 647



‘Excites’
various dies
and agents.

Channel = laser

- Laser numbers = 0–3, confusing.
- Can use all 4, but generally use 1–3. Most frequent use 2.
- The laser corresponds to the marker/labelling
- Channel numbering here is just a sequence, doesn't reflect which laser was used.
- No reference of the laser number! User needs clarity of what the lasers are.
- The colour is arbitrary – set by the user. Does not reflect colour of the laser.
- Channel 0 shows by default, despite used only 1 laser. Known issue / industry issue due to how it's built. “A competitor has resolved this, ONI hasn't”. Confusing as may not know channel was active!
- There's no labelling of which channel was active.

Colour setting – screen 1/2

Nim Qt Application: DEVELOPMENT BUILD

File Instrument Data Settings Advanced Help Development Tools

Acquire Analyze User Login

Load Data...

Image files:

- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686

Warning! Invalid camera parameters
Warning! Invalid camera calibration
Warning! Invalid camera mapping

Override default localization parameters

Sigma estimate: 0.90 1.20

Iterations: 15 15

Run localization

Result files:

- ImagingTeam_HACAT tom20.1533117896862.lc
- ImagingTeam_HACAT tom20.1533117896862.n

Export Localizations as .csv

TOOLS

- Trace
- Line Histogram
- XYZ Plane View
- Tracking
- FRET Traces
- 3D Visualization (Experimental)

Calculate Drift Correction Apply correction

STATUS

0%

Advanced Viewing Options

Channel 0

- Raw Image: Color: ■ Contrast: 0.00 95.00
- Localizations: Color: ■ Contrast: 0.00 95.00 Gaussian Width (nm): 3.50
- Tracks: Color: ■ Override Default Range: ■ ■
- Charts: Color: ■

Channel 1

- Raw Image: Color: ■ Contrast: 0.00 95.00
- Localizations: Color: ■ Contrast: 0.00 95.00 Gaussian Width (nm): 3.50
- Tracks: Color: ■ Override Default Range: ■ ■
- Charts: Color: ■

Apply

RESULTS FILTERING

A B Disable all filters

- Localization precision (y) (nm)
Min: 0.00 nm 0.00 nm
Max: 14.00 nm 14.00 nm
- Sigma X (nm)
Min: 47.59 nm 60.98 nm
Max: 142.77 nm 182.95 nm
- Sigma Y (nm)
Min: 47.59 nm 60.98 nm
Max: 142.77 nm 182.95 nm
- LLR
Min: 0.00 0.00
Max: 100000000.00 100000000.00
- p-value
Min: 0.0000 0.0000
Max: 1.0000 1.0000
- Frame index

VIEWING OPTIONS

- Channel 0
- Channel 1

Advanced Viewing Options...

- Display Image Display localized points
- Max projection Single frame Custom image

Image display parameters:

Localization color: Channel

Localisation rendering mode: Fixed

Lower percentile: 39.00 Brightness: 74 %

Upper percentile: 100.00 Colour Greyscale

Frame index: 4093

Display filtered points in grey

0,50 μm

12:05
29/10/2018

Colour setting – screen 2/2

Nim Qt Application: DEVELOPMENT BUILD

File Instrument Data Settings Advanced Help Development Tools

Acquire Analyze User Login

Load Data...

Image files:

- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686
- ImagingTeam_HACAT tom20.153311789686

Warning! Invalid camera parameters
Warning! Invalid camera calibration
Warning! Invalid camera mapping

Override default localization parameters

Sigma estimate: 0.90 1.20

Iterations: 15 15

Run localization

Result files:

- ImagingTeam_HACAT tom20.1533117896862.lc
- ImagingTeam_HACAT tom20.1533117896862.n

Export Localizations as .csv

TOOLS

- Trace
- Line Histogram
- XYZ Plane View
- Tracking
- FRET Traces
- 3D Visualization (Experimental)

Calculate Drift Correction Apply correction

STATUS

0%

Advanced Viewing Options

Channel 0

Raw Image

Color: ■

Contrast: 0.00 95.00

Localizations

Color: ■

Tracks

Color: ■

Charts

Color: ■

Select Color

Basic colors

Click Screen Color

Custom colors

Add to Custom Colors

Hue: 0 Red: 255

Sat: 0 Green: 255

Val: 255 Blue: 255

HTML: #ffffff

OK Cancel

Apply

RESULTS FILTERING

A B Disable all filters

Localization precision (y) (nm)

Min: 0.00 nm 0.00 nm

Max: 14.00 nm 14.00 nm

Sigma X (nm)

Min: 47.59 nm 60.98 nm

Max: 142.77 nm 182.95 nm

Sigma Y (nm)

Min: 47.59 nm 60.98 nm

Max: 142.77 nm 182.95 nm

LLR

Min: 0.00 0.00

Max: 100000000.00 100000000.00

p-value

Min: 0.0000 0.0000

Max: 1.0000 1.0000

Frame index

VIEWING OPTIONS

Channel 0

Channel 1

Advanced Viewing Options...

Display Image Display localized points

Max projection Single frame Custom image

Image display parameters:

Localization color: Channel

Localisation rendering mode: Fixed

Lower percentile: 39.00 Brightness: 74 %

Upper percentile: 100.00 Colour Greyscale

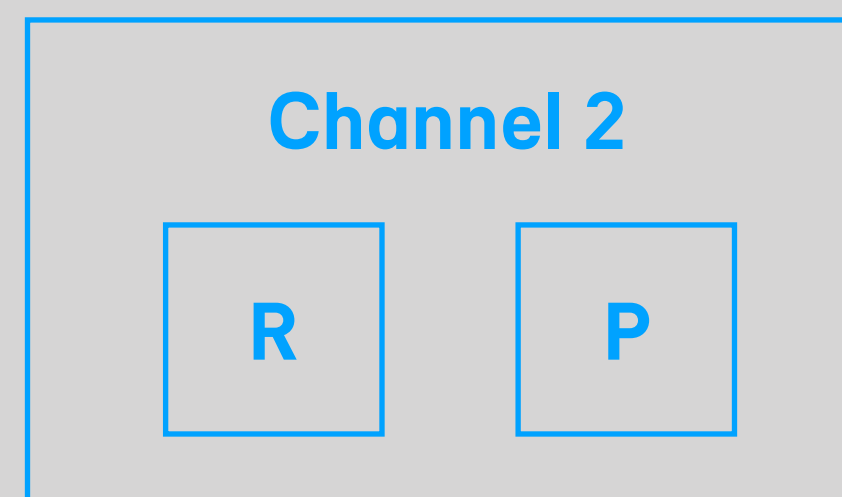
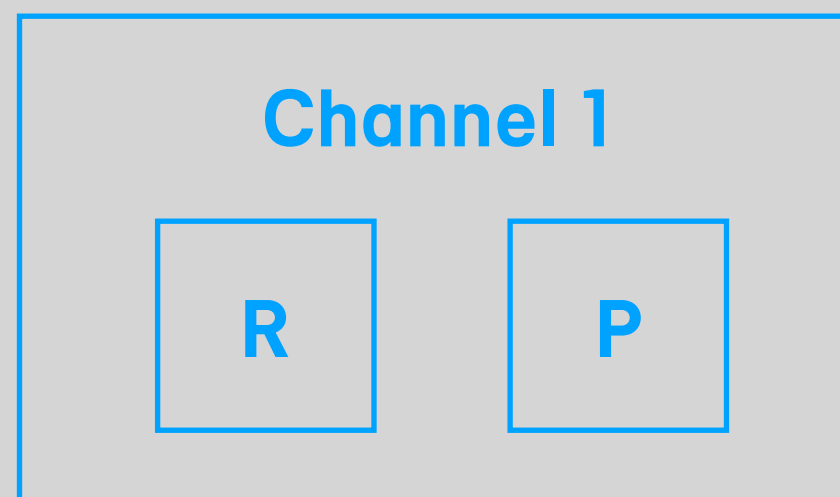
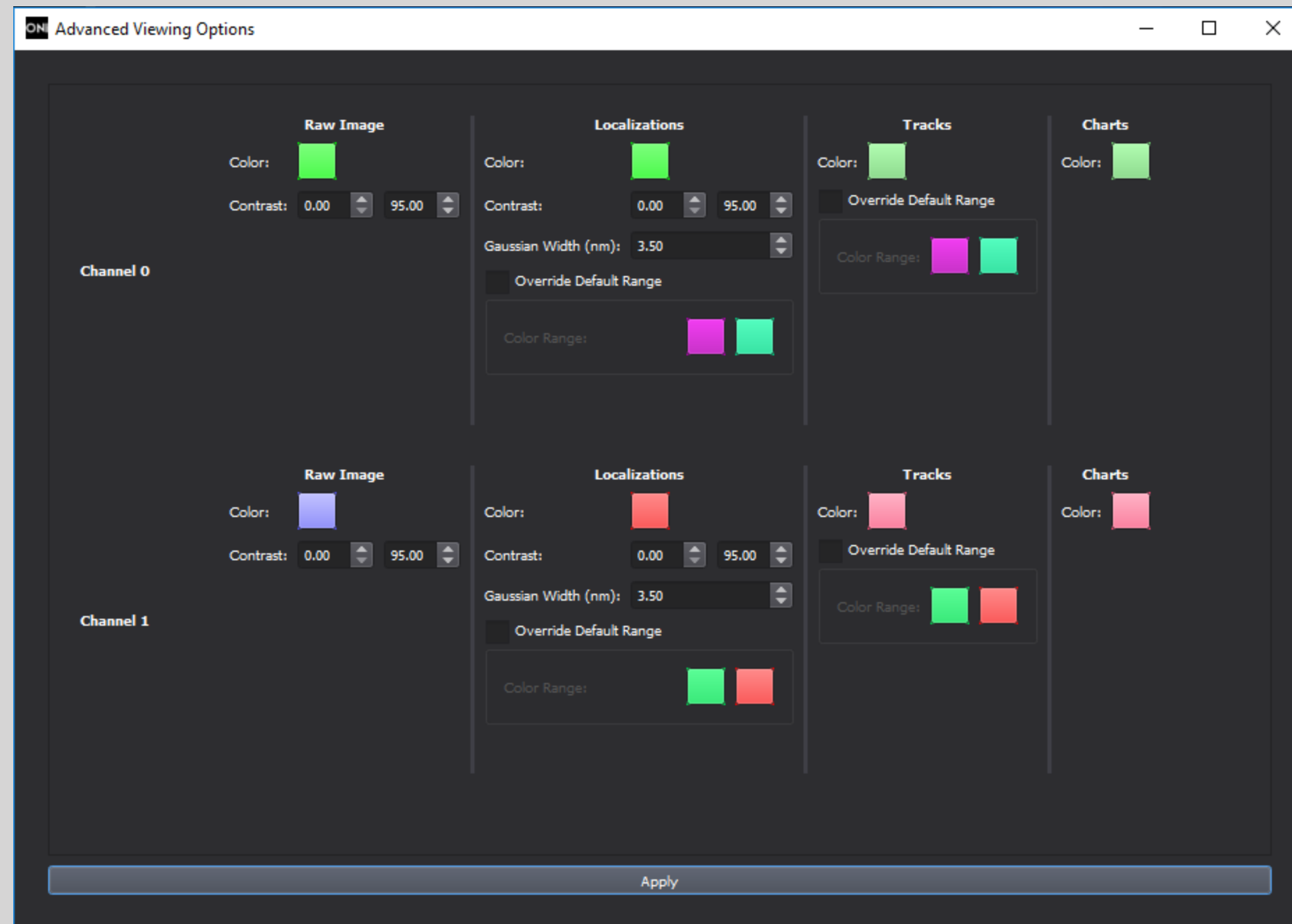
Frame index: 4093

Display filtered points in grey

0,50 μ m

12:06
29/10/2018

Colour setting



- Each channel laser has 2 colour settings: Raw / Process
- This is a useful tool.
- Andres talks of a competitor that has UI much better than here.
- If don't want to see/capture a channel R/P – have to set it to black – so still collecting data. Better to have option to turn it off!
- Want's tick boxes here for each colour.
- Colours are a personal preference of the user.
- Some publishers have their preferences of colour schemes.
- Colour blindness is a recognised issue!
- Can always change colours later.
- Wan't everything in single easy access rather than across two pop ups.
- Channel 0 is showing here even though user is not using it. Needs to be contextual.

