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):

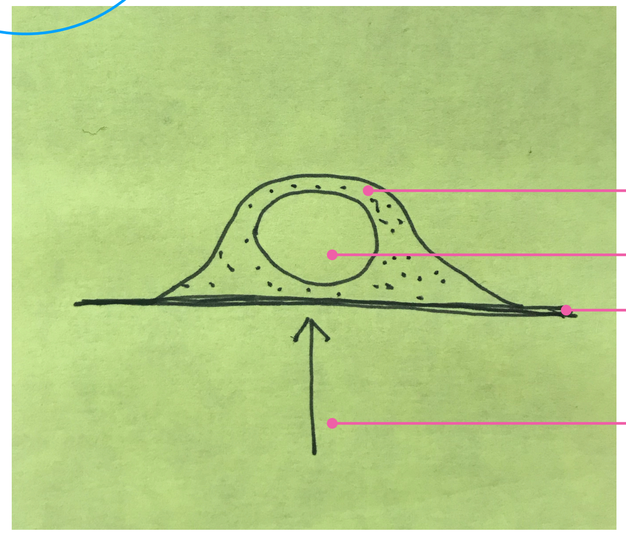
- 2 or 3 imaging scientists
- 1 application specialist
- The developer team – integration

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

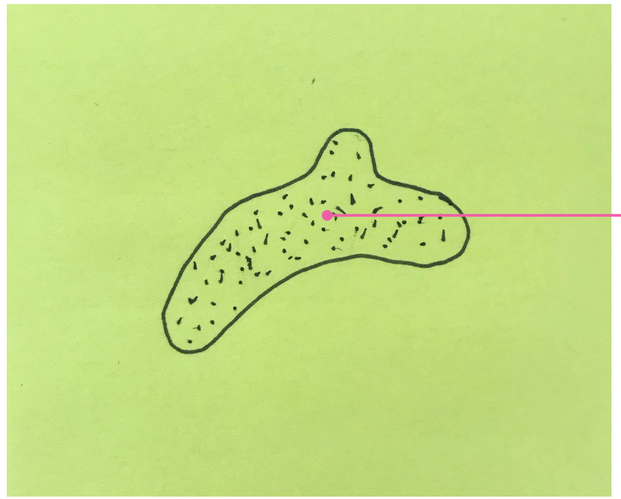
The tiny dots = organels

Single cell

Nucleus of cell

Glass

Laser



An organel

The tiny dots = ‘blinks’ = single blinking molecules

The blinks build up to reveal the shape of the organel

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

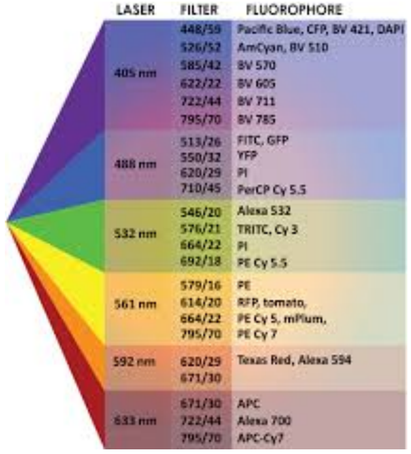
Optics **Interface** **Laser**

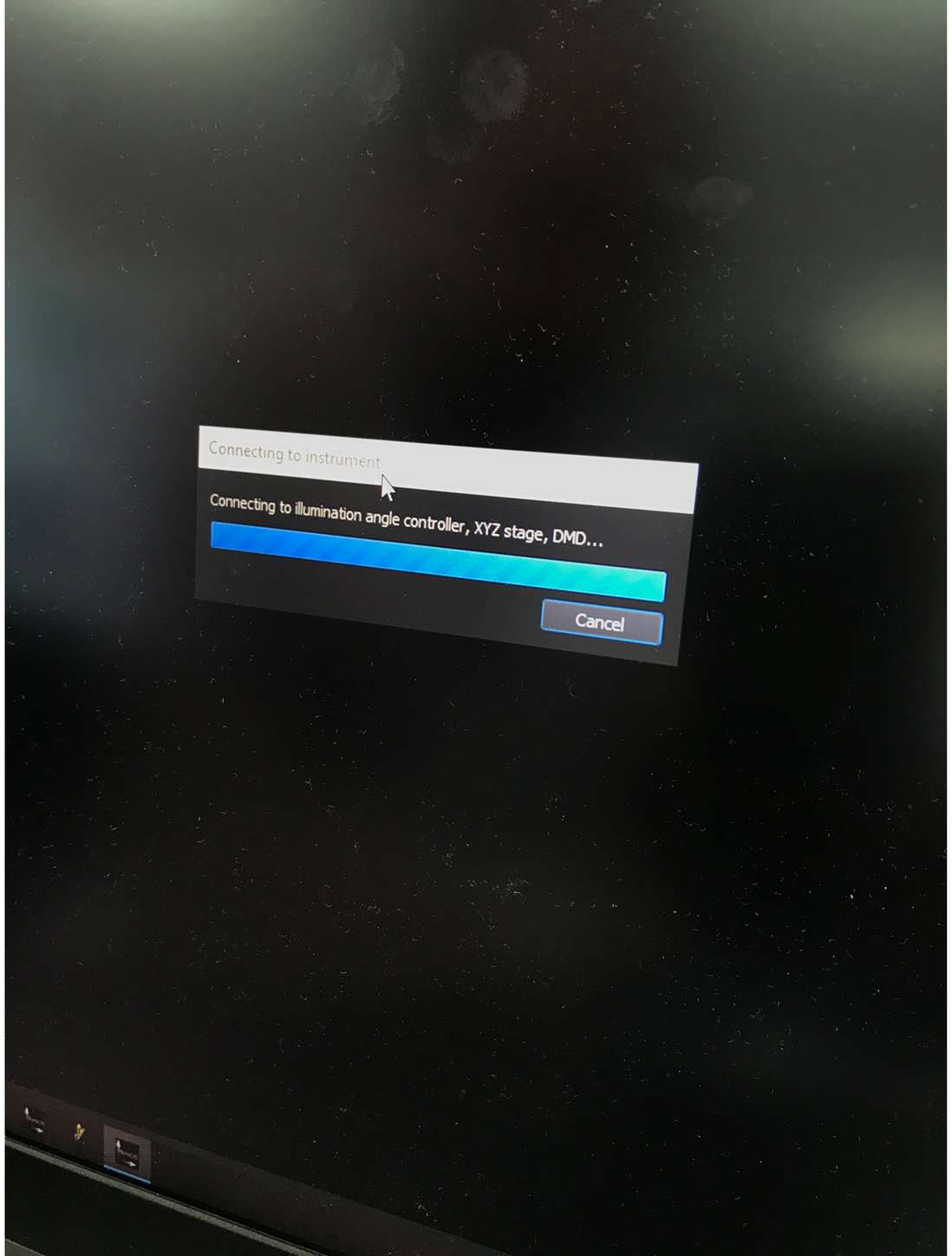
ONI lab
Oxford

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.





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’.

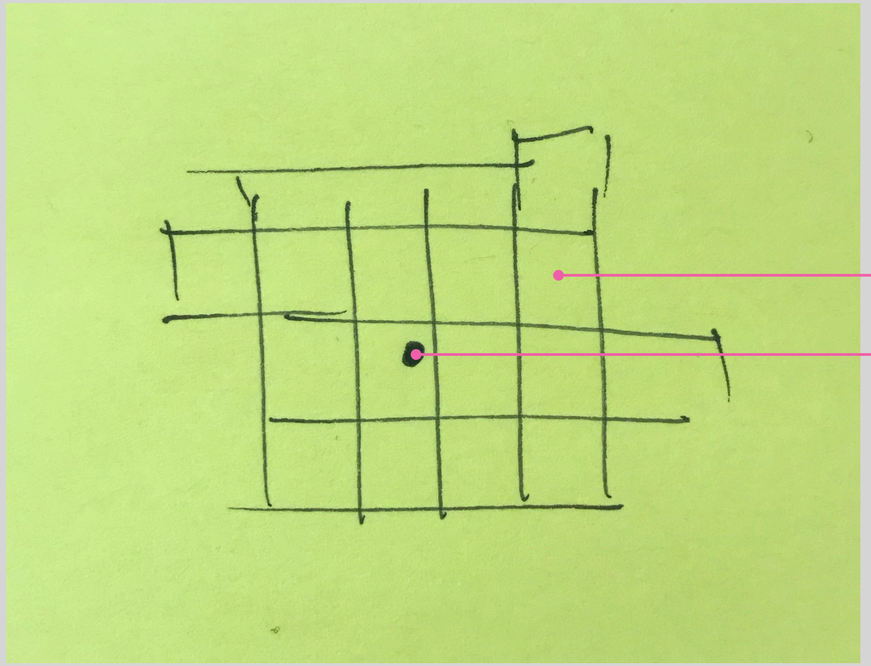
PREP

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.

Blury 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.

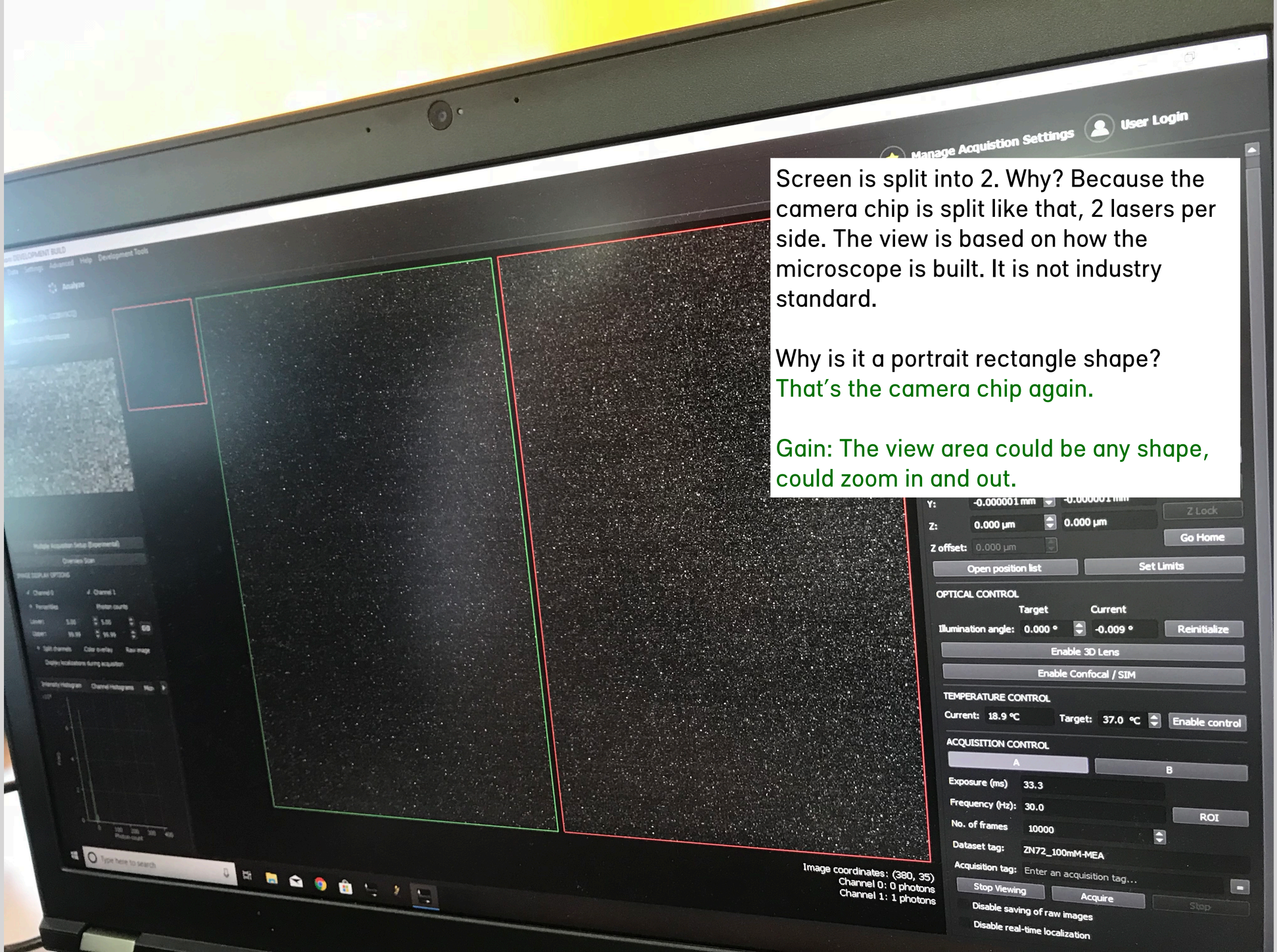
AQUIRE – LIGHT CONTROL

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.

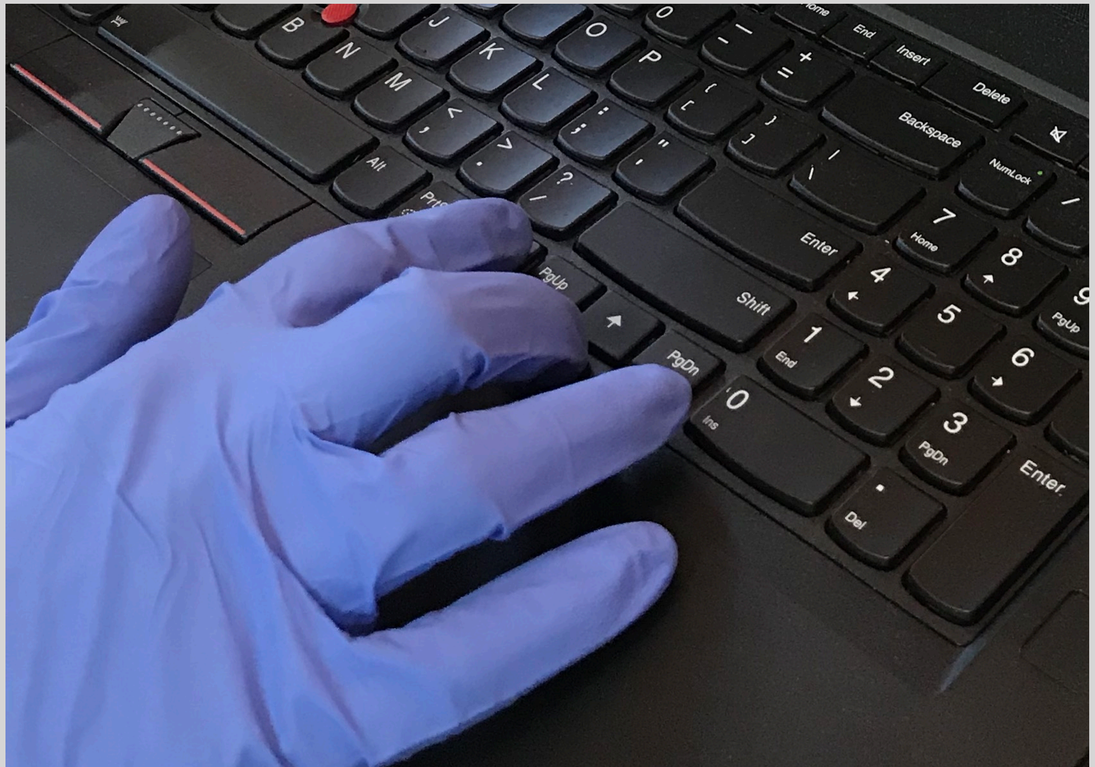
In light control we do the bleaching and set the focus.



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.

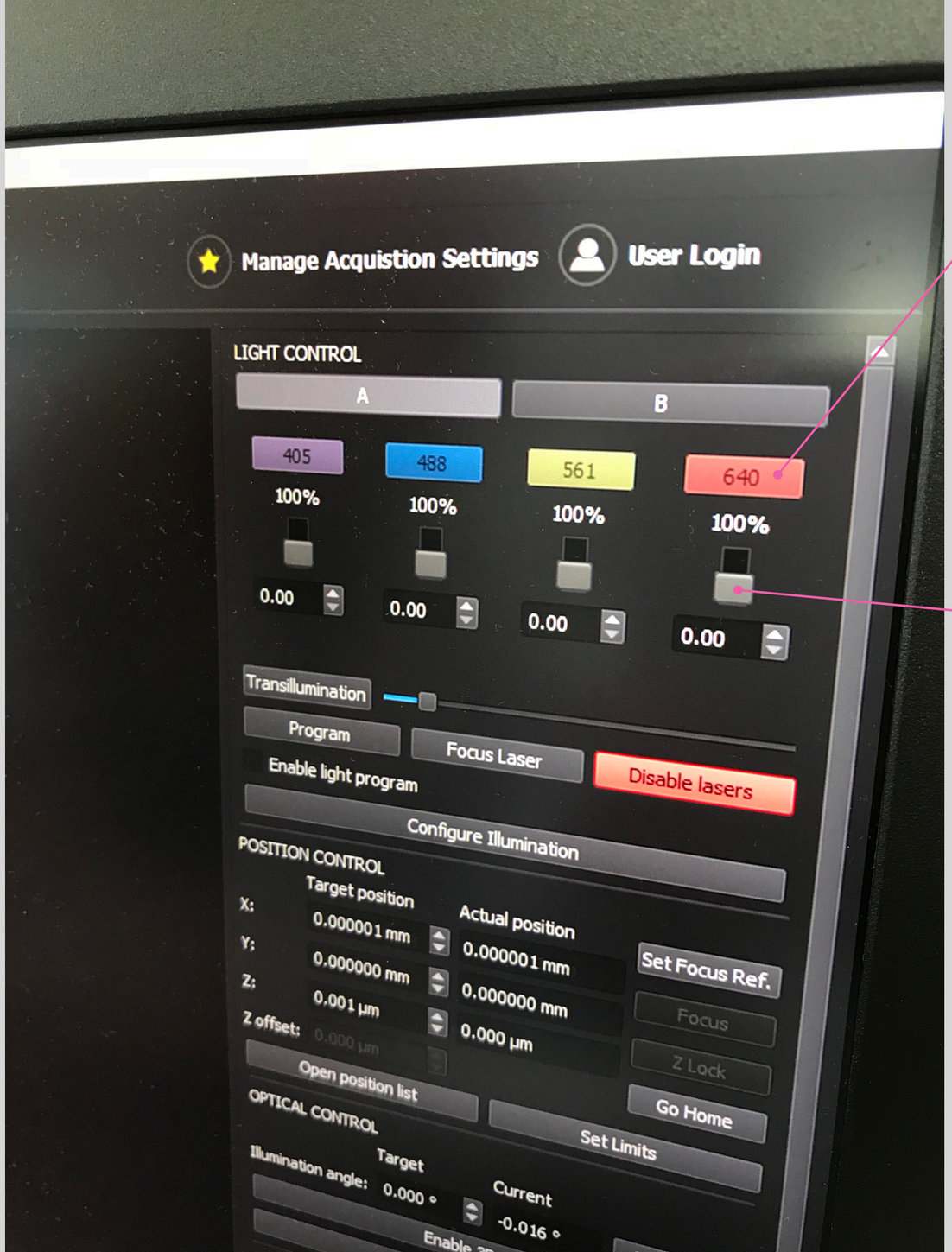
Why is it a portrait rectangle shape?
That's the camera chip again.

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



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.



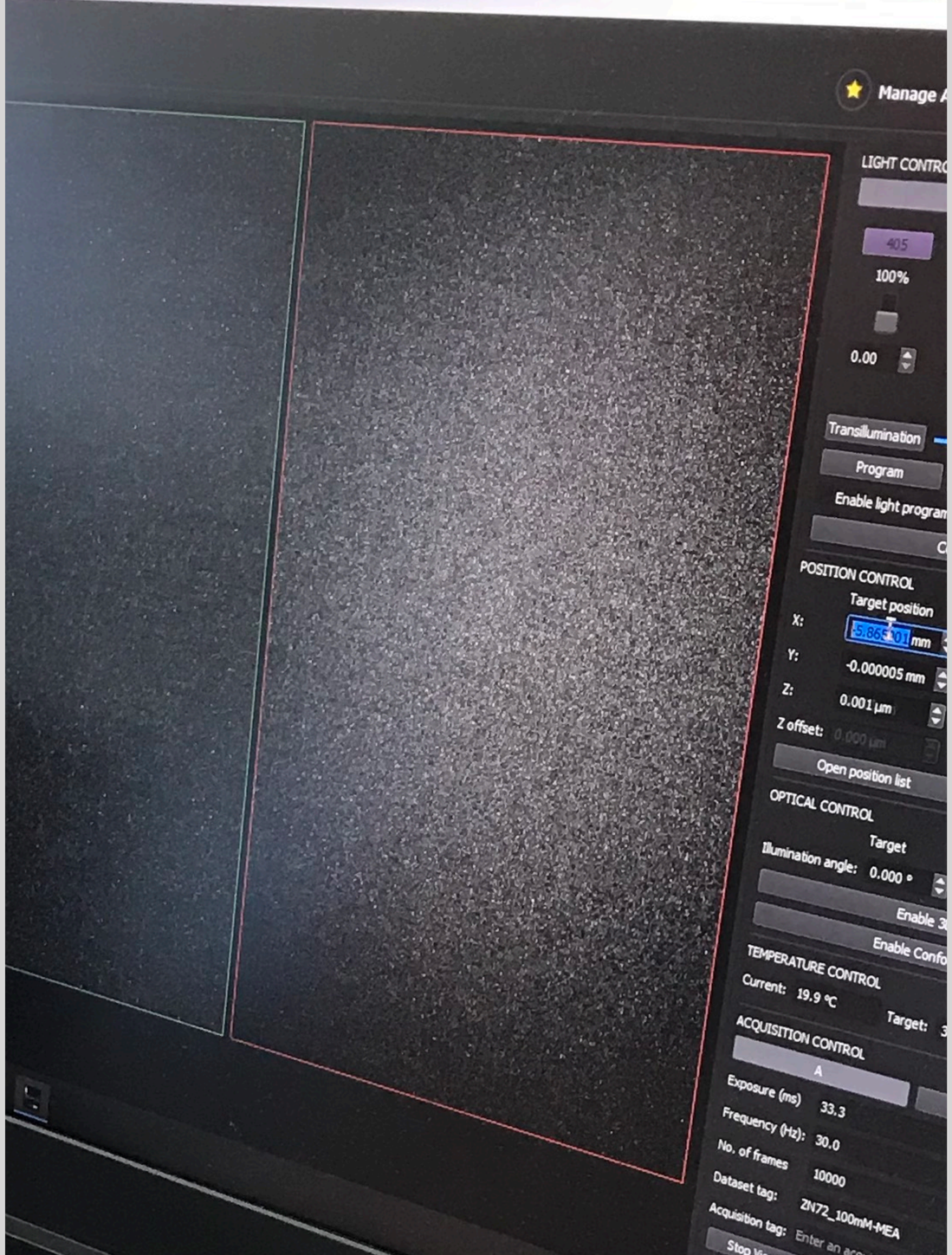
ACTIVATE LASER

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.



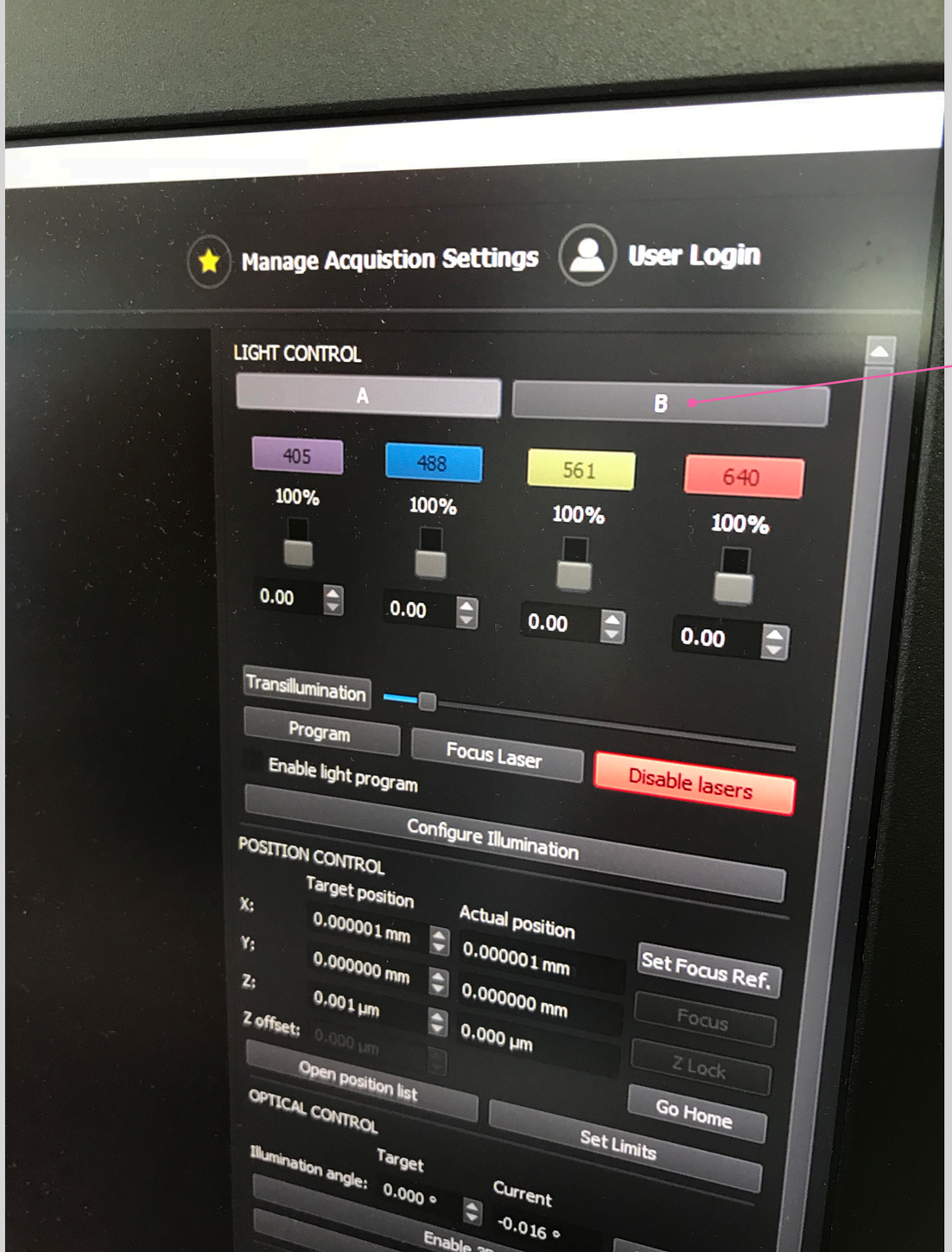
BLEACHING

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.

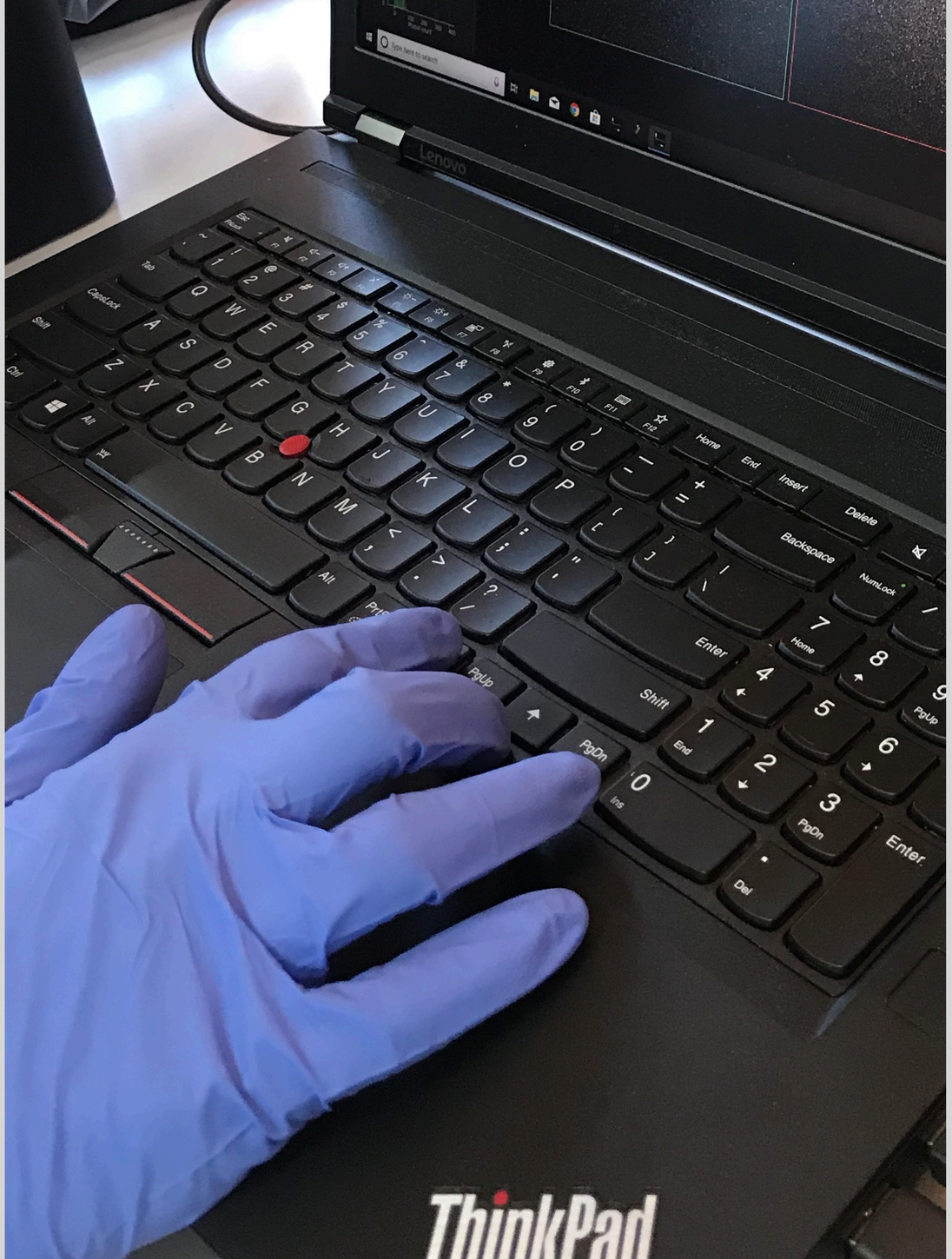
This is where the slider control fidelity is not great.



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.

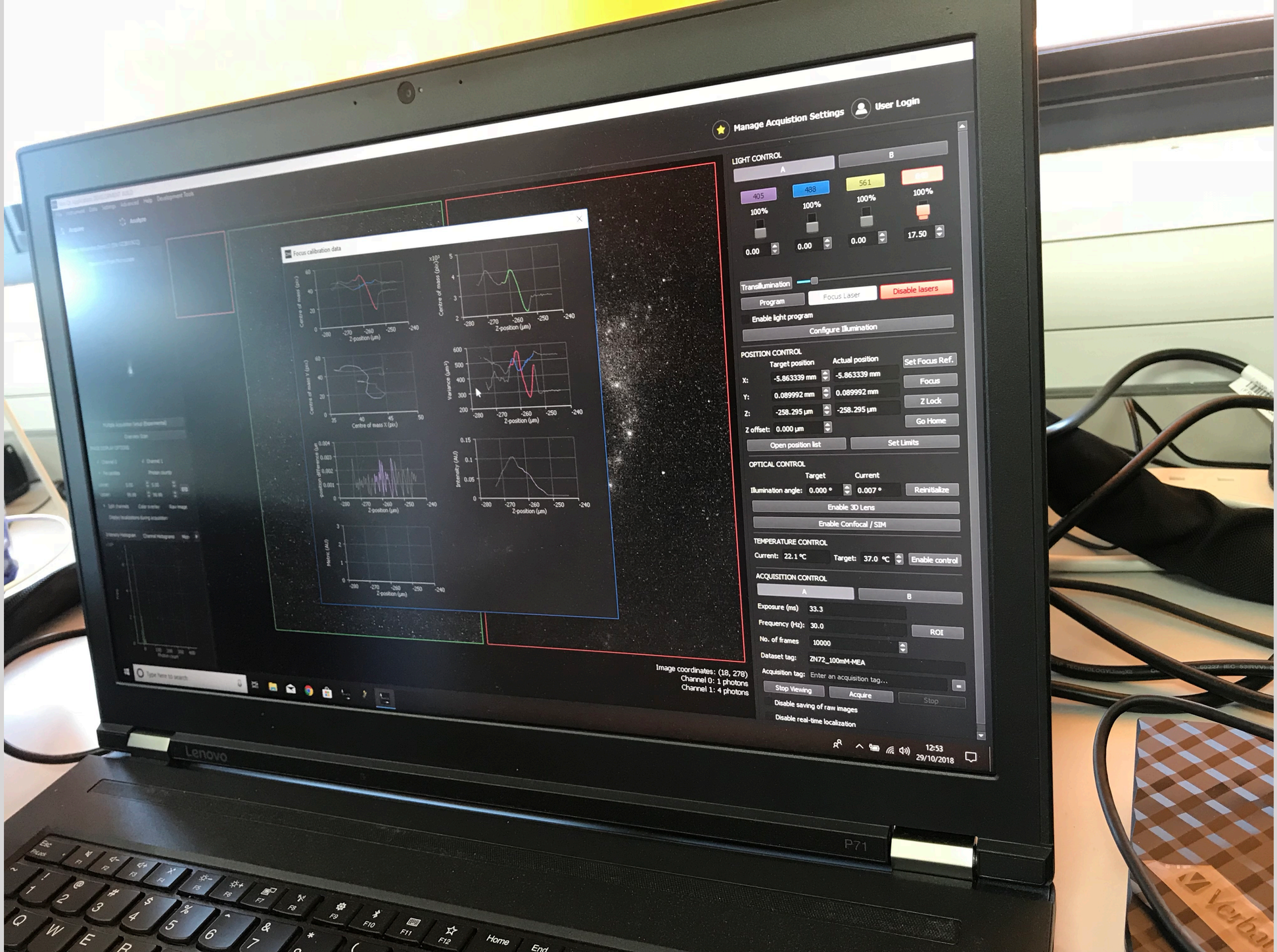


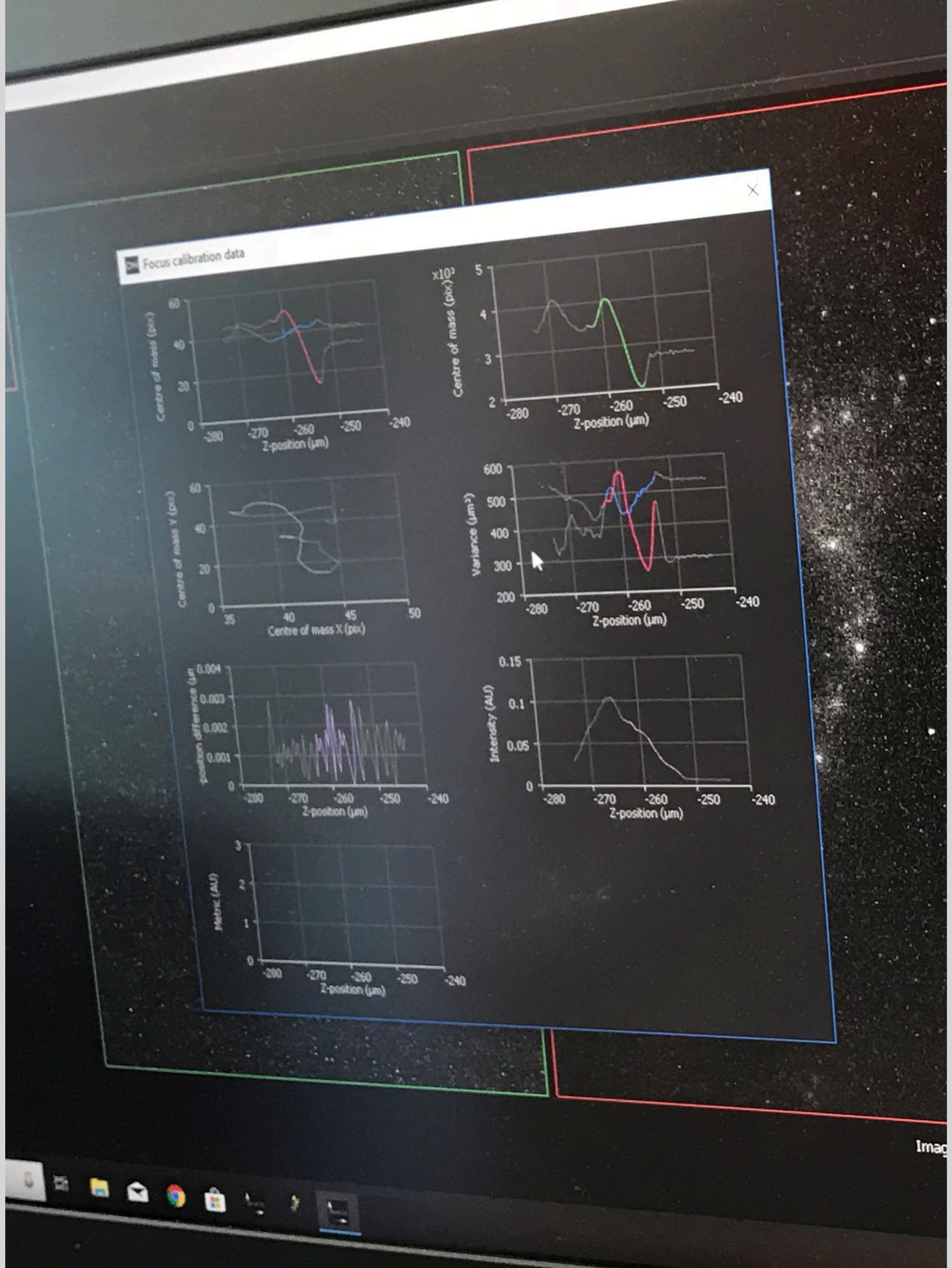
SETTING FOCUS

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 Acquistion Settings
User Login
LIGHT CONTROL
A
B
405
488
561
100%
100%
100%
100%
0.00
0.00
0.00
17.50
Transillumination
Program
Focus Laser
Disable lasers
Enable light program
Configure Illumination
POSITION CONTROL
Target position
Actual position
Set Focus Ref.
X: -5.863339 mm -5.863339 mm
Y: 0.089992 mm 0.089992 mm
Z: -258.295 µm -258.295 µm
Focus
Z Lock
Go Home
Z offset: 0.000 µm
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
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
Focus calibration data
Centre of mass Y (pix)
Centre of mass (pix²)
Centre of mass X (pix)
Variance (µm²)
Intensity (AU)
Astig. (AU)
Z-position (µm)
Image coordinates: (18, 278)
Channel 0: 1 photons
Channel 1: 4 photons
12:53
29/10/2018
Lenovo
P71



SET FOCUS REFERENCE

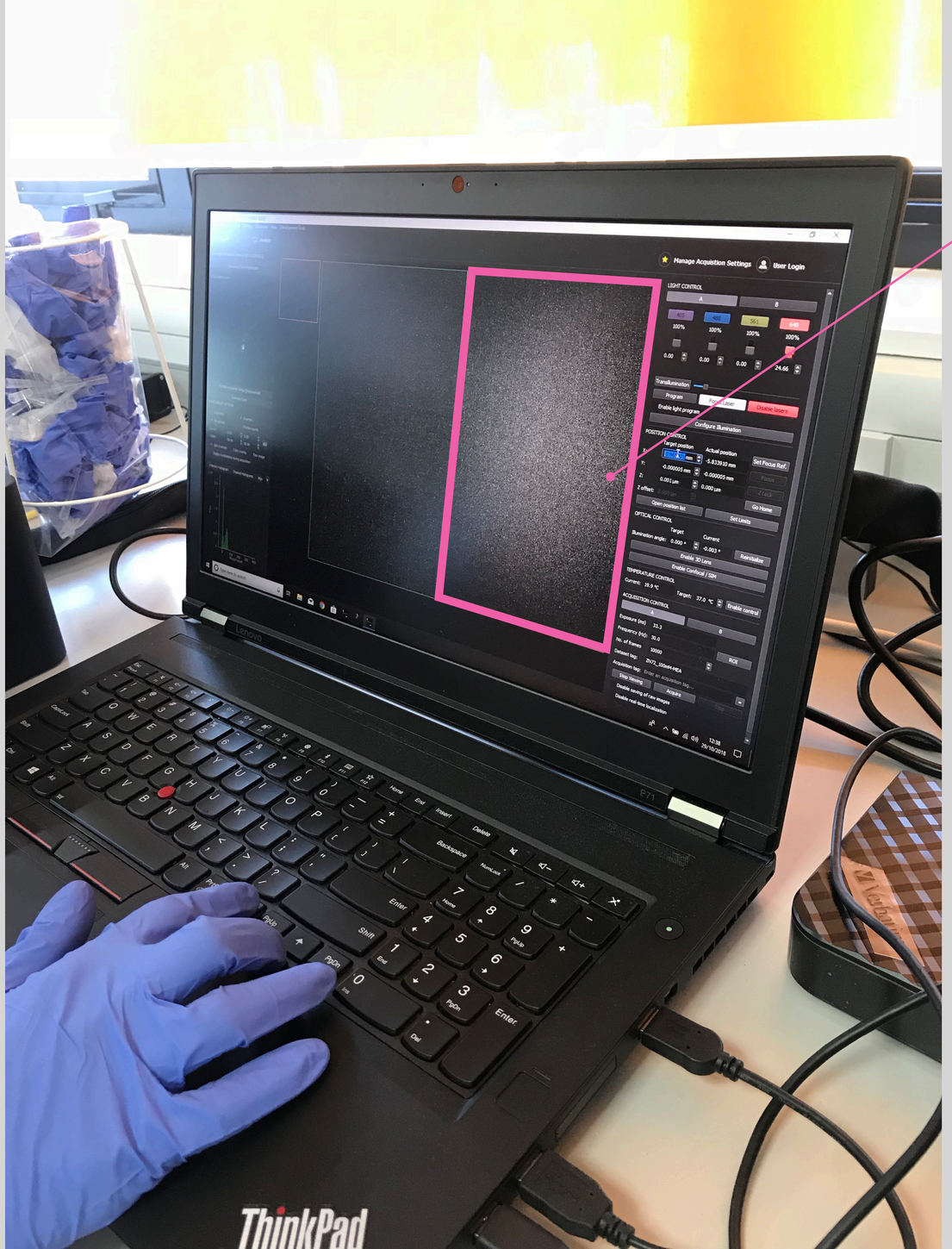
Shows how following the focus pattern. “Focus calibration data” = Charts in an overlay window.

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!

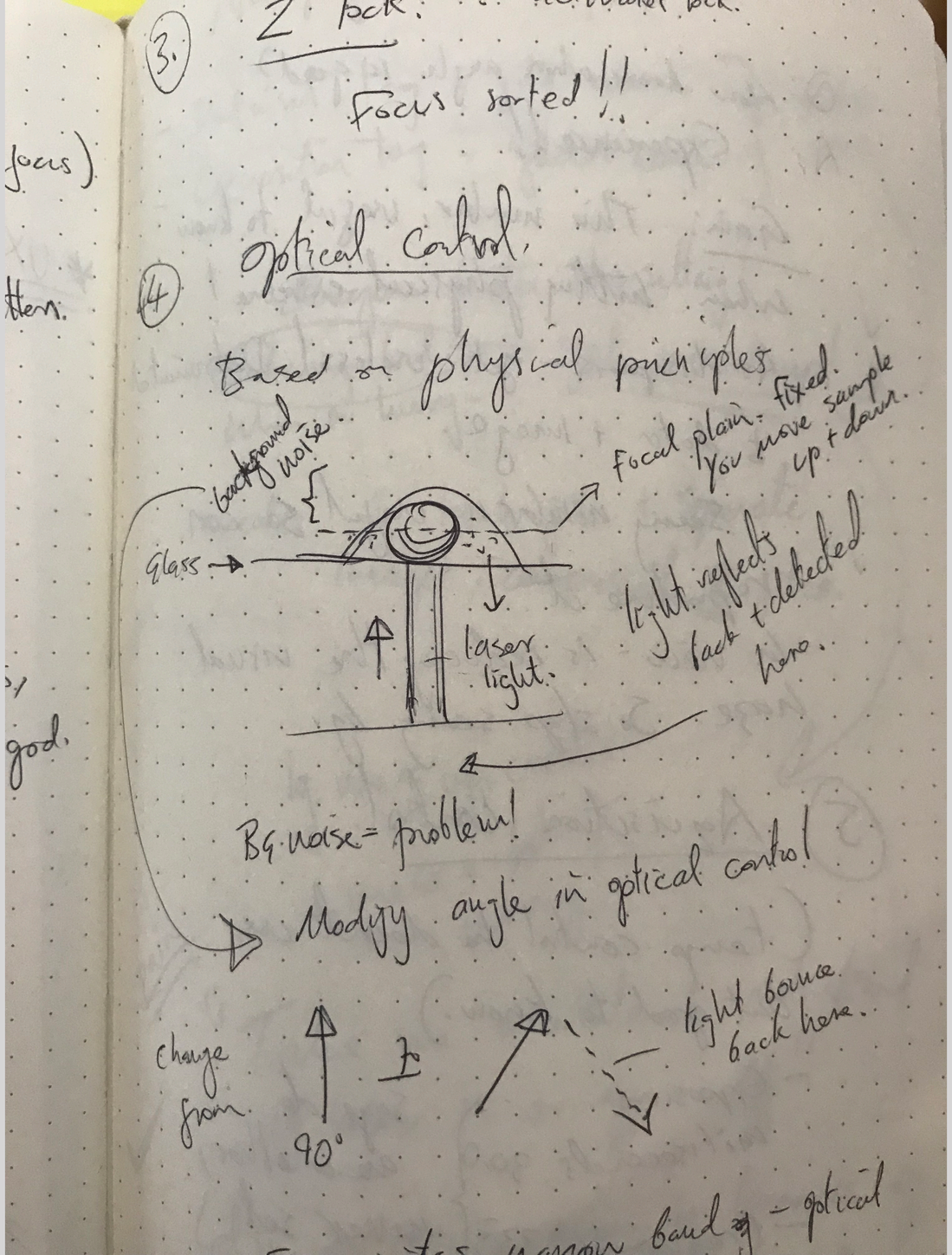
Gain:
Want's options for the view panel:

Y	X

"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"



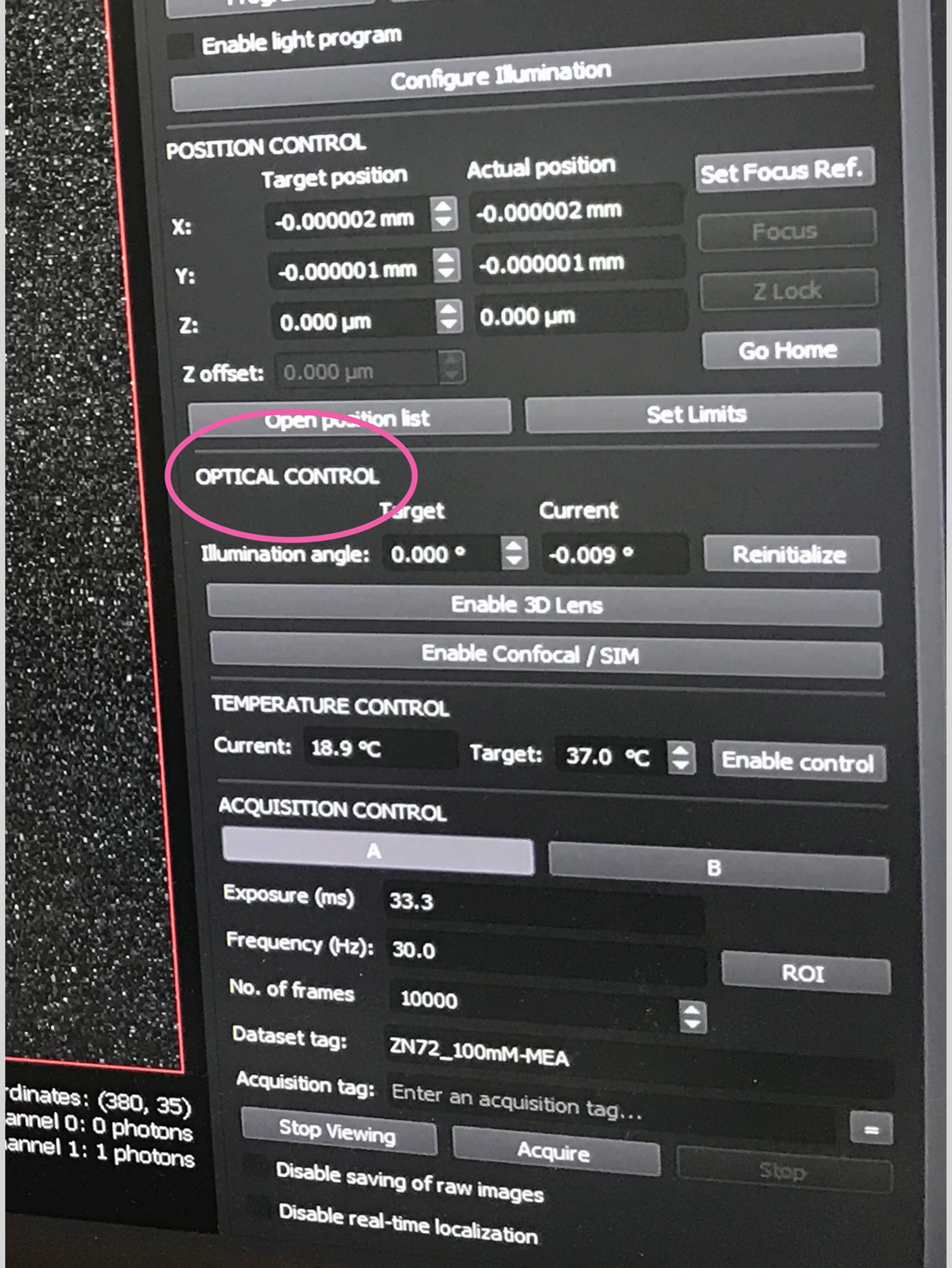
OPTICAL CONTROL

The problem... based on physical principles.

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

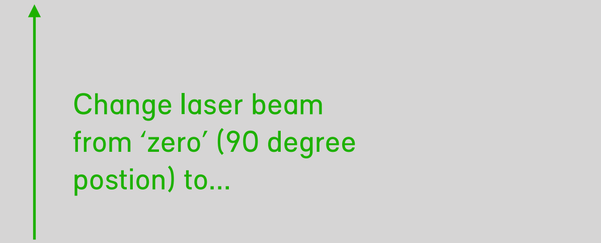
The laser light goes straight up, and reflects light back as detected.

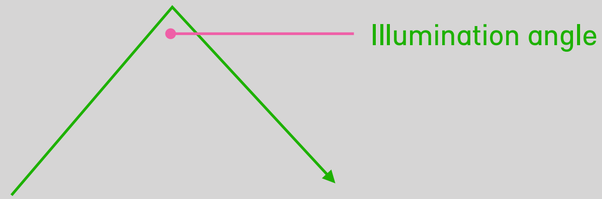
The problem is the laser light picks up background noise beyond the focus plain.



OPTICAL CONTROL

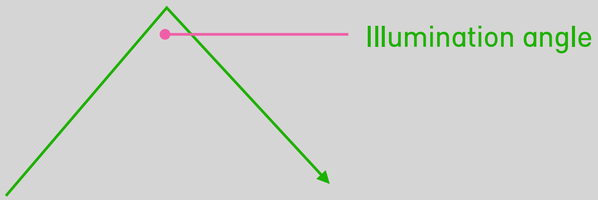
The solution... modify the 'illumination angle' (target) to change the laser beam from 90 degree position to bounce back at an angle – so it lights up 'excites' a narrower focal band – an optical slice. Reduces the bg noise.





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

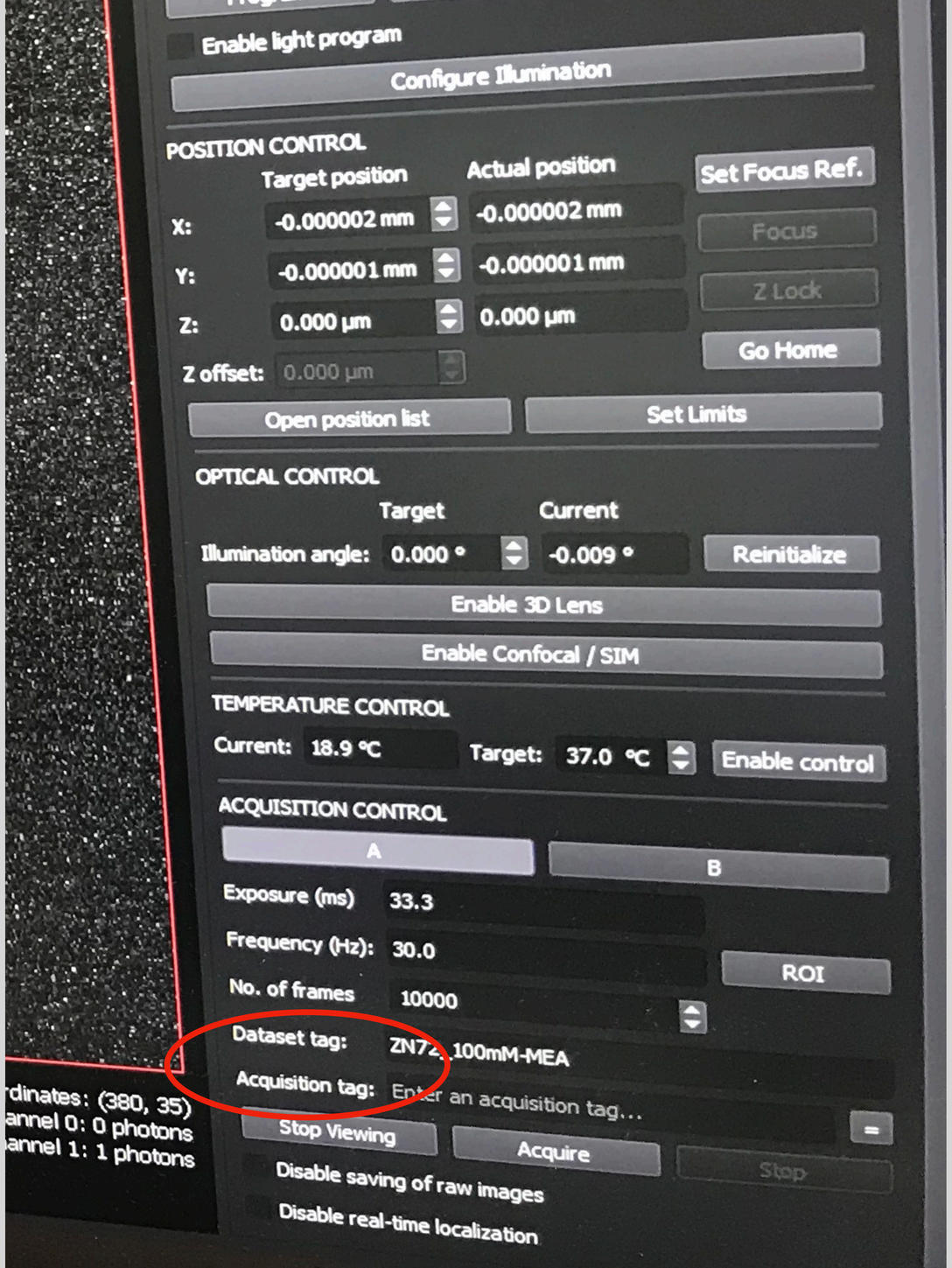
OPTICAL CONTROL



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.

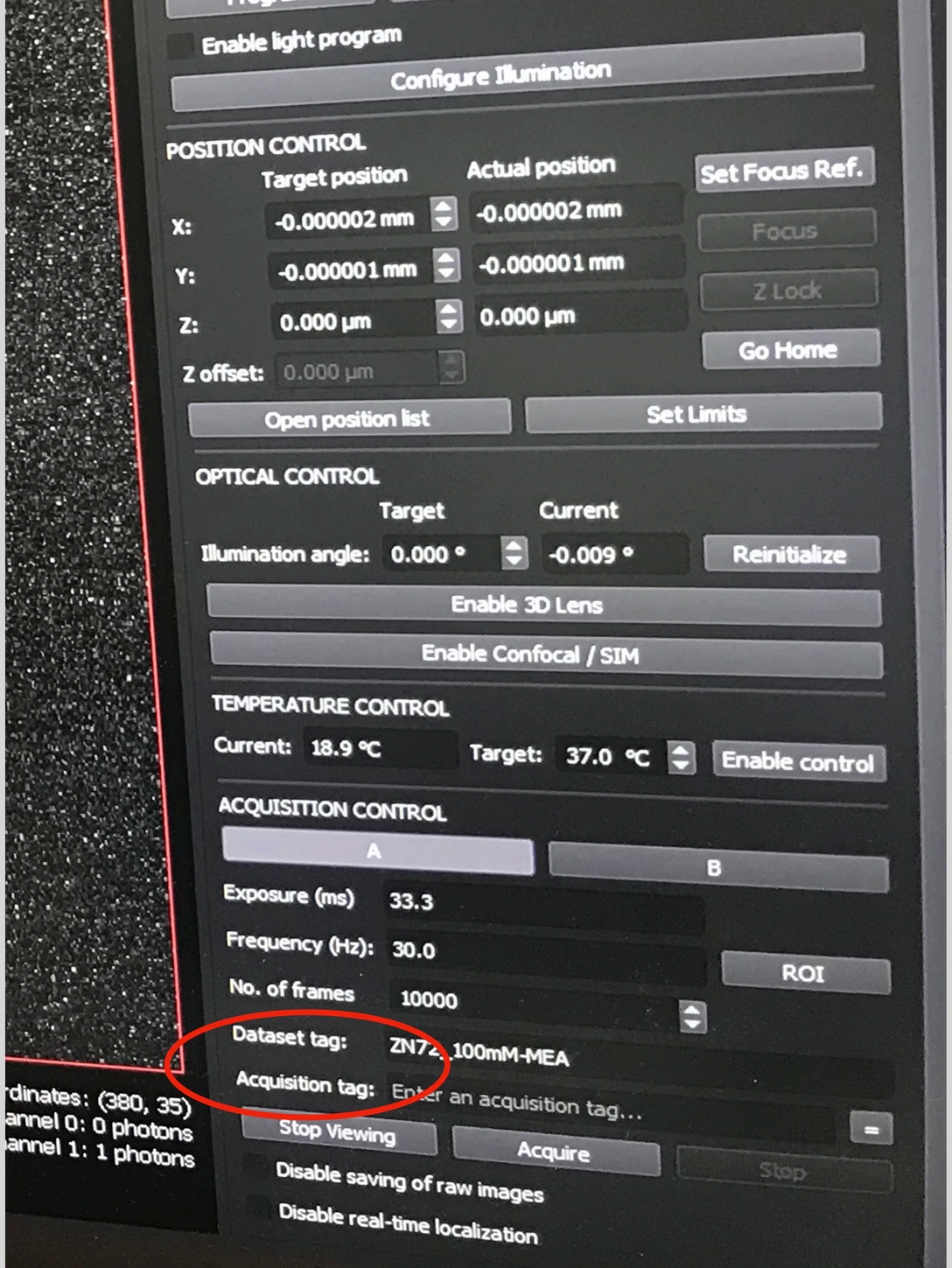
Exposure (milisecionds)
Frequency (Hz)

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.



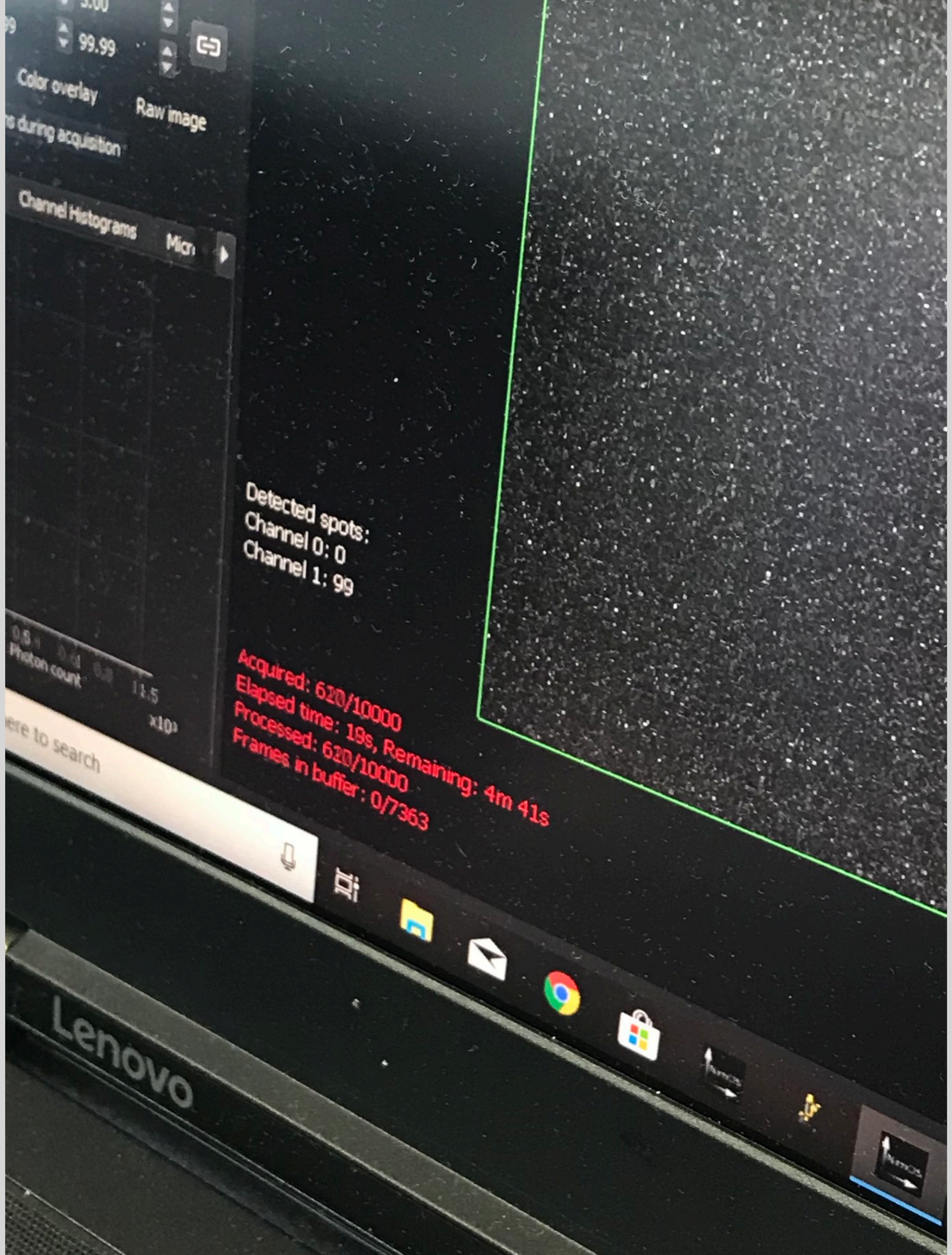
Painpoint:

“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.

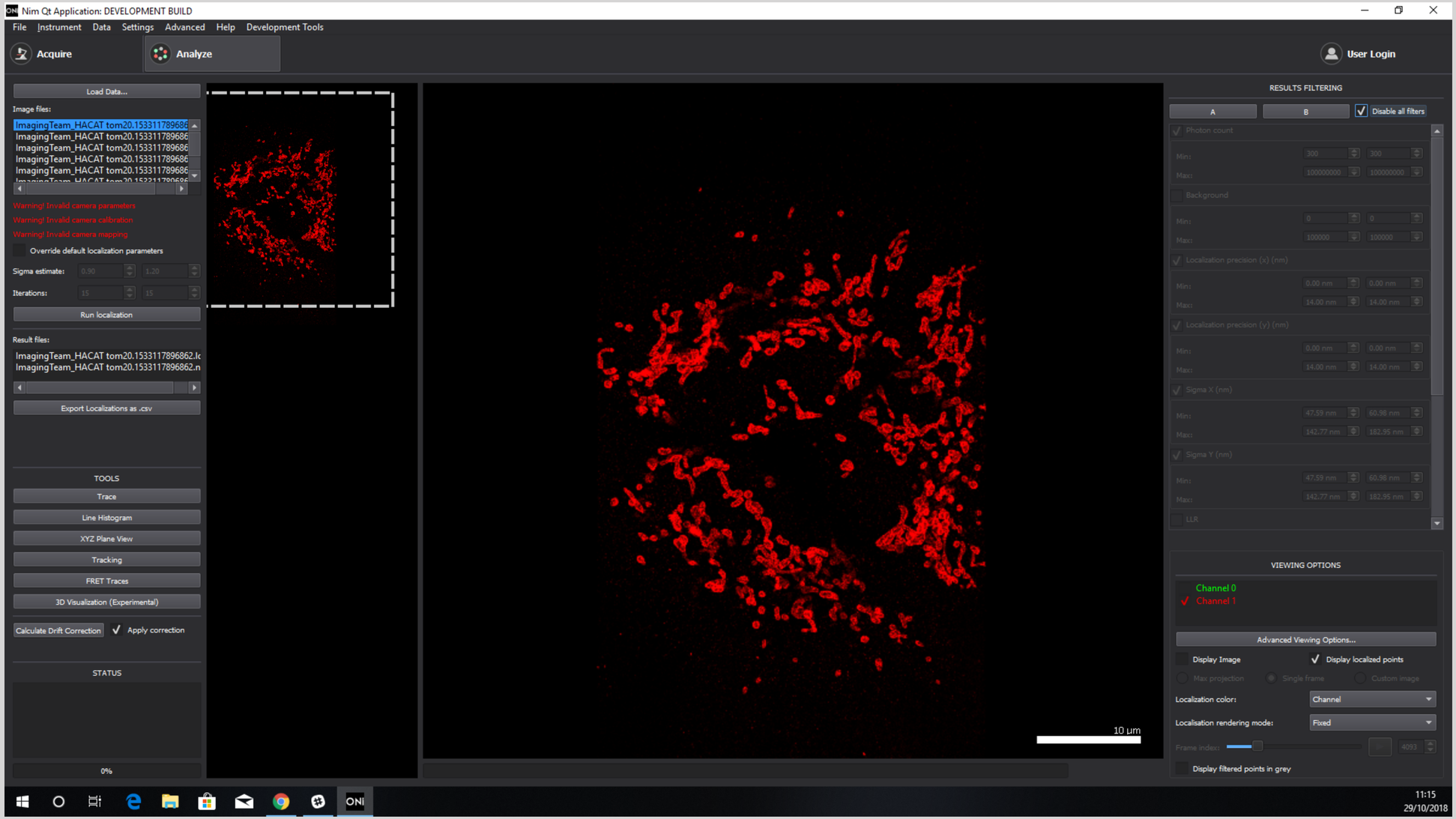
It’s good to stay and monitor it.

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.

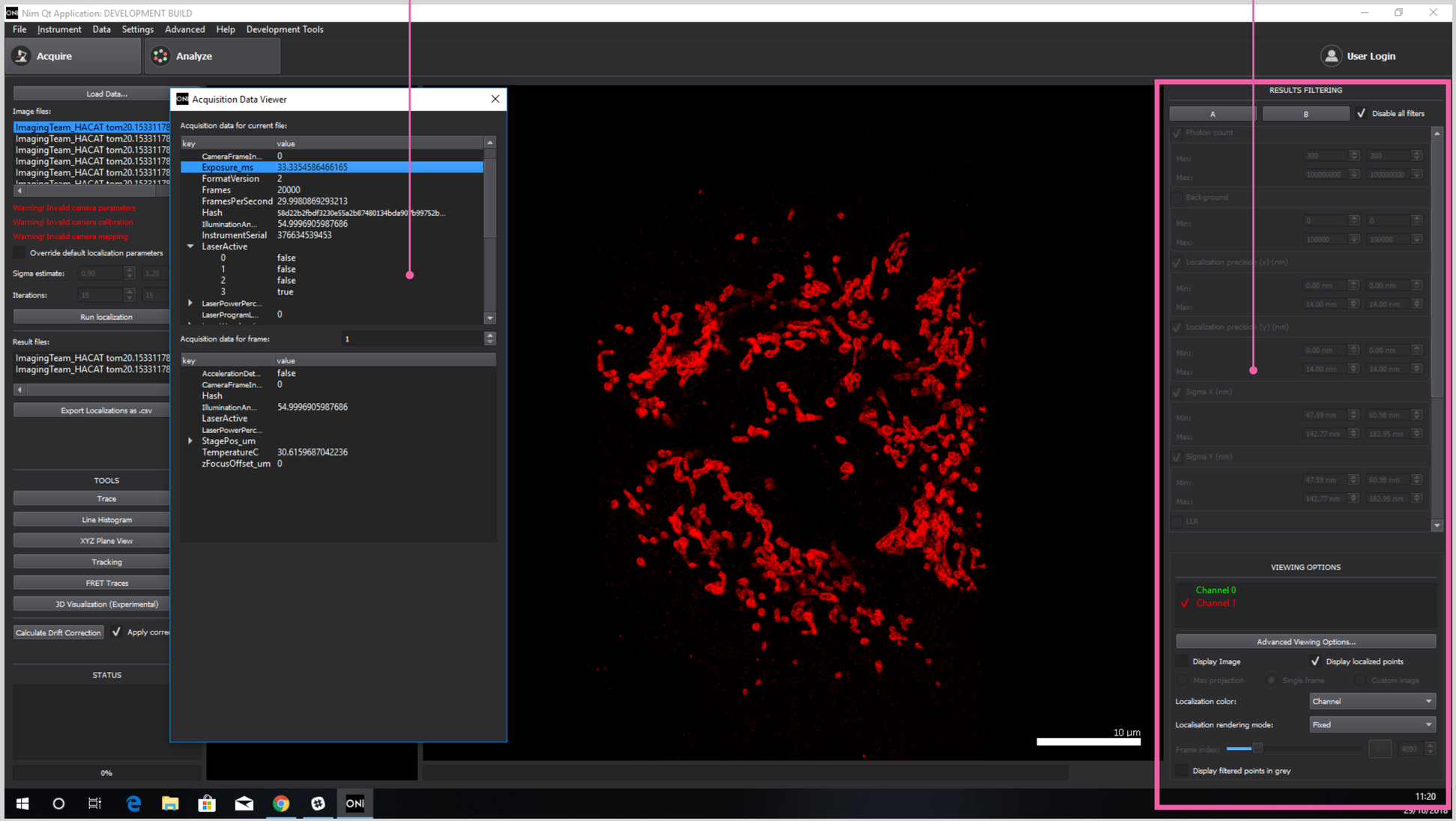
ANALYSE

An opened file

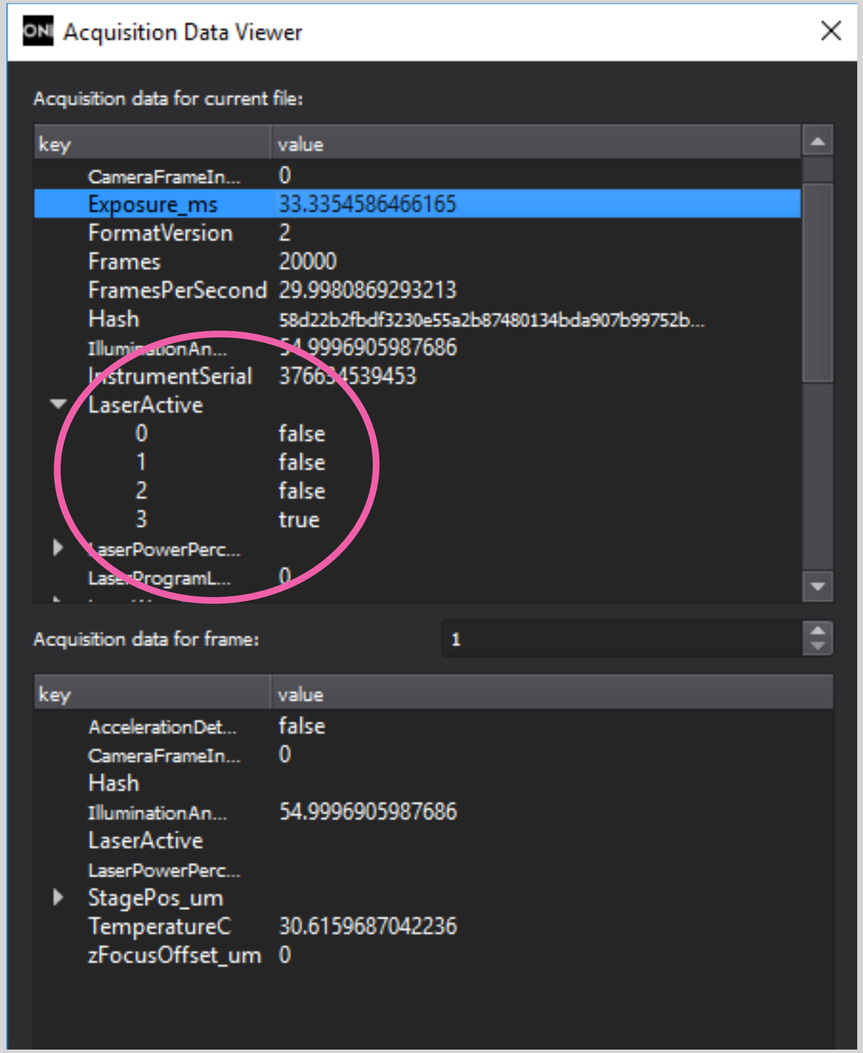


Menu: Data / view acquisition data

Results filtering

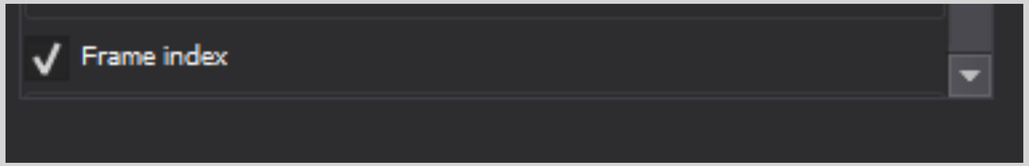


Menu: Data / view acquisition data



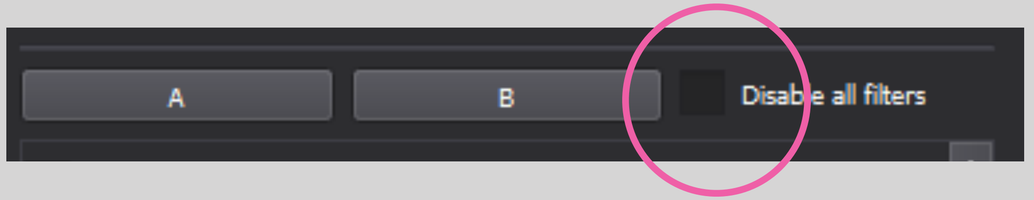
- Scrollable and nested data.
- True / false should read ‘on/off’.
- Built by developers! End users want normal phrases – not ‘true/ false’.

- Laser numbers = 0 – 3. Confusing. Should be 1–4.



Frame index (parameters out of view here!)

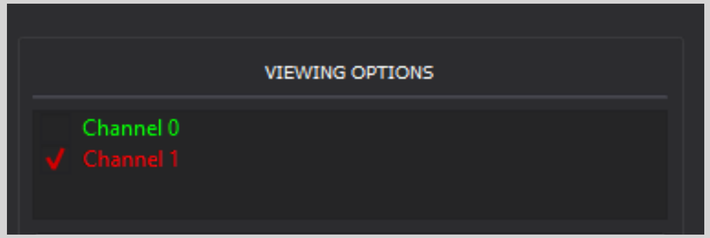
- min (say 10,000)
- max (say 20,000)
- Will show just those frames. Like Quicktime frame clipping!



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 aquire. 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:

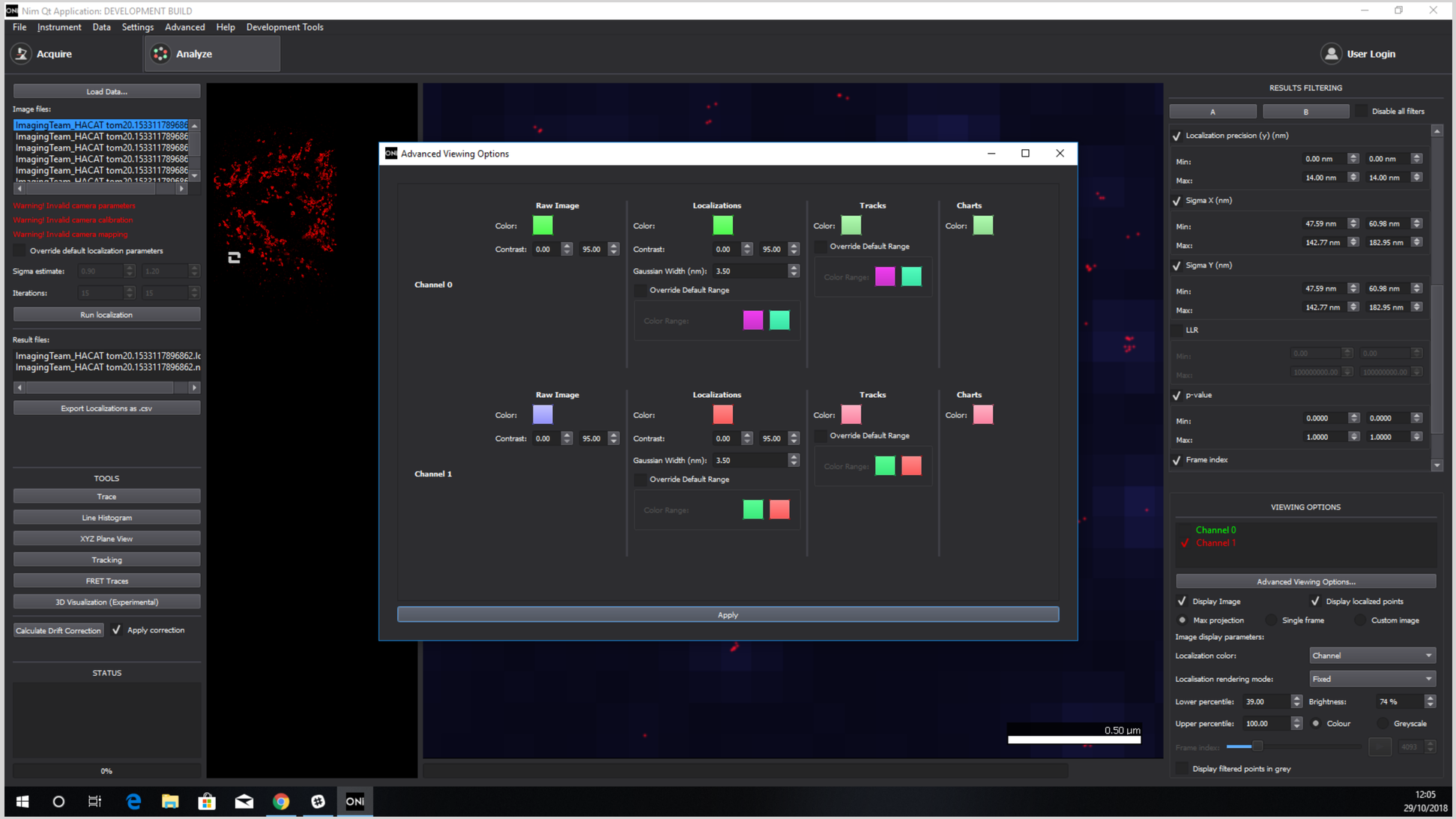
0 = UV
1 = 488
2 = 561
3 = 647

} ‘Excites’ various dies and agents.

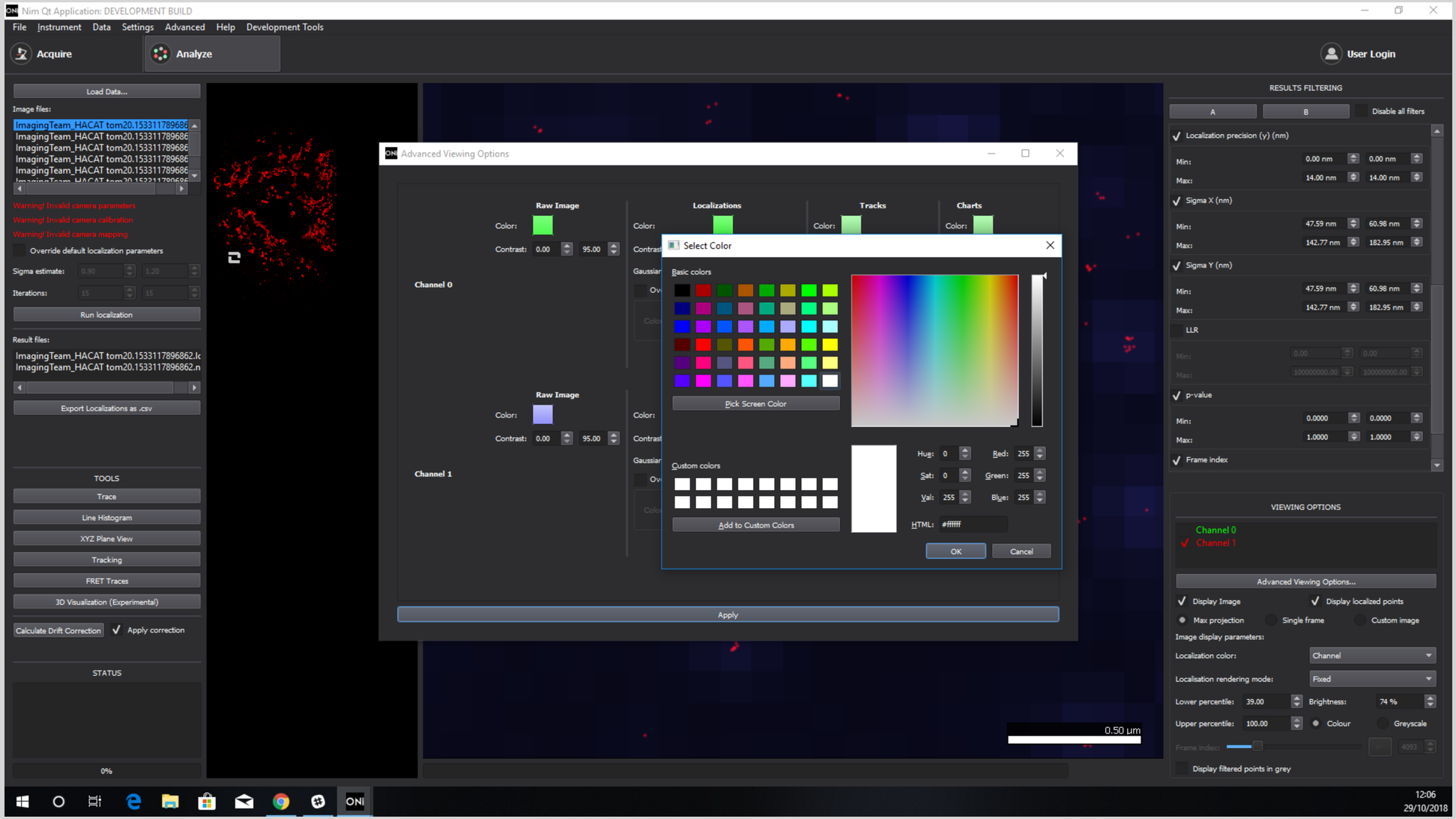
Channel = laser

- 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.

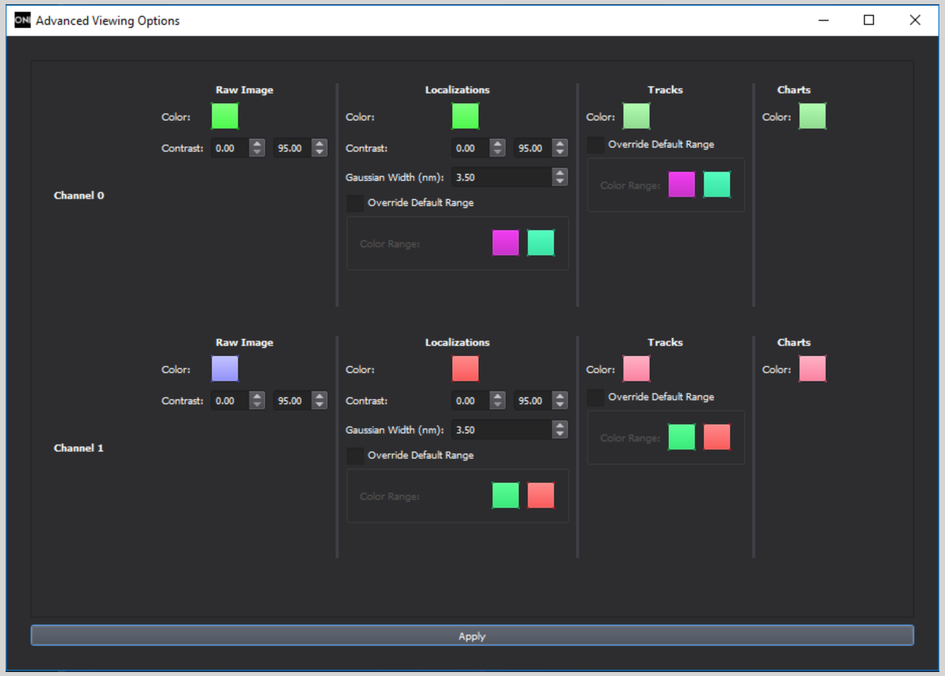
Colour setting – screen 1/2

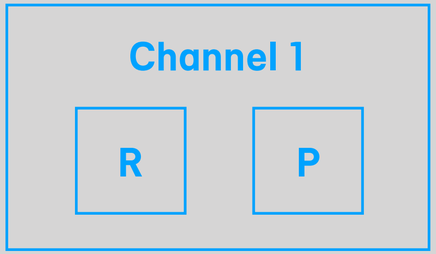


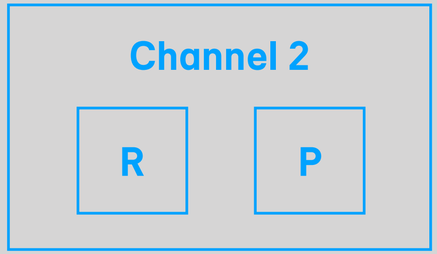
Colour setting – screen 2/2



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.

FIN