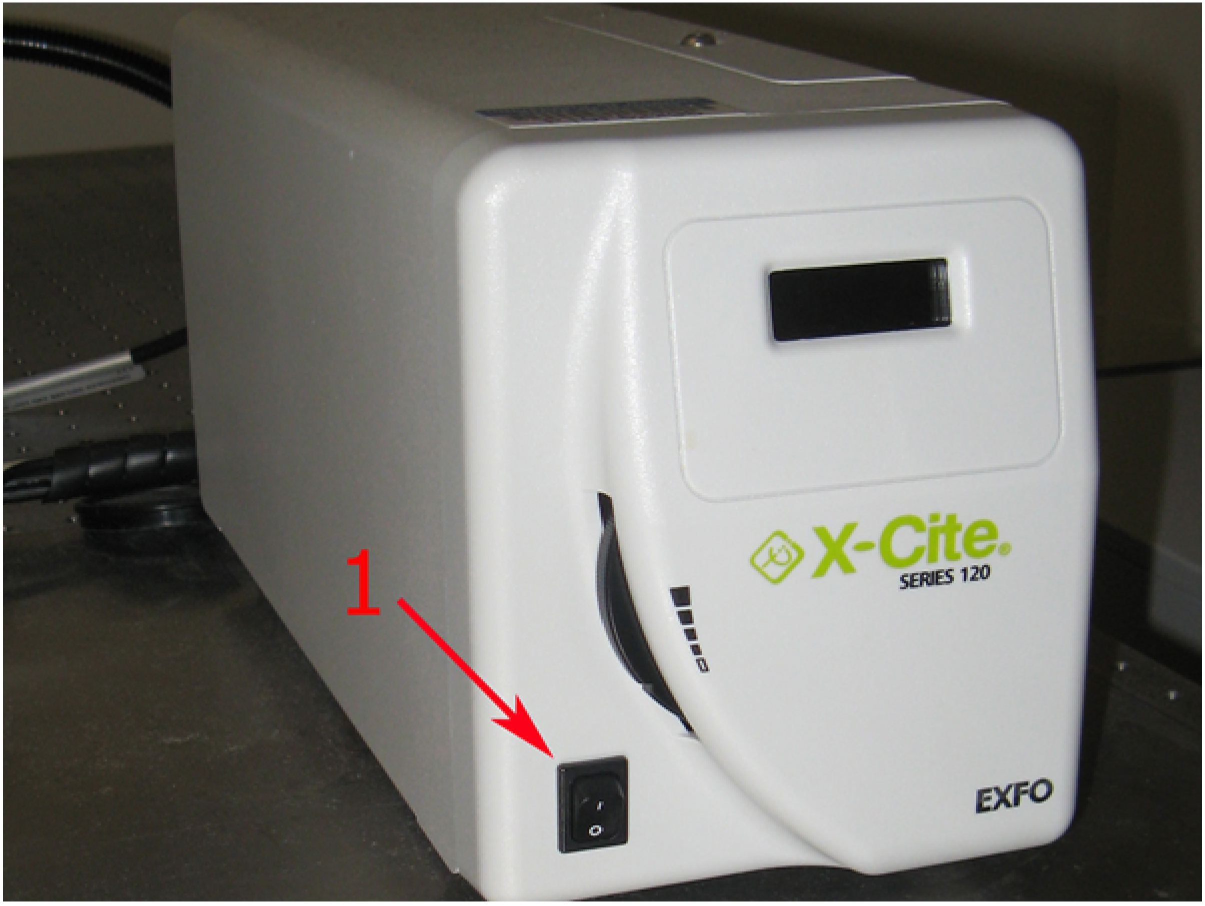
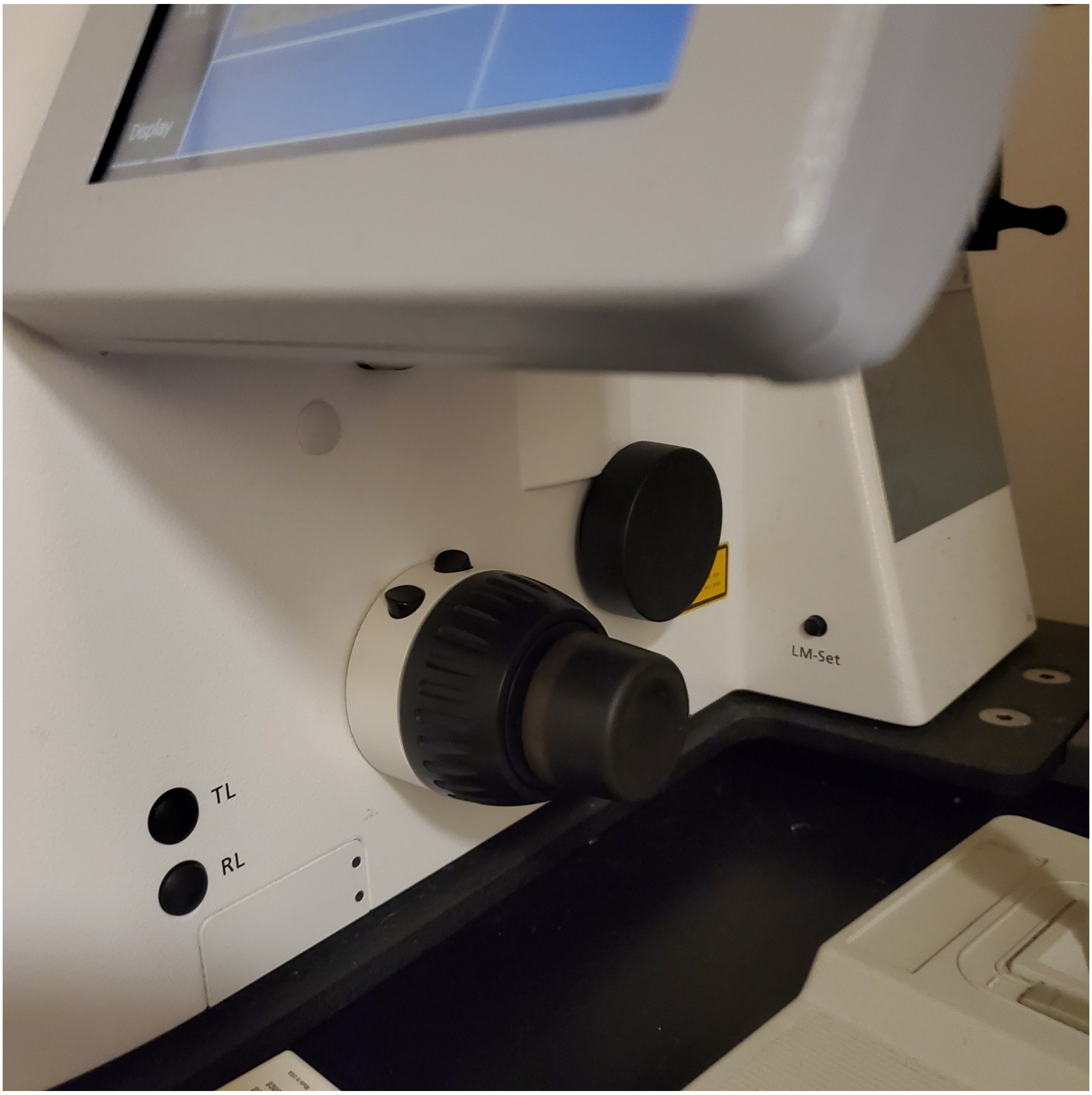
LSM 700 Basic Operation

Startup

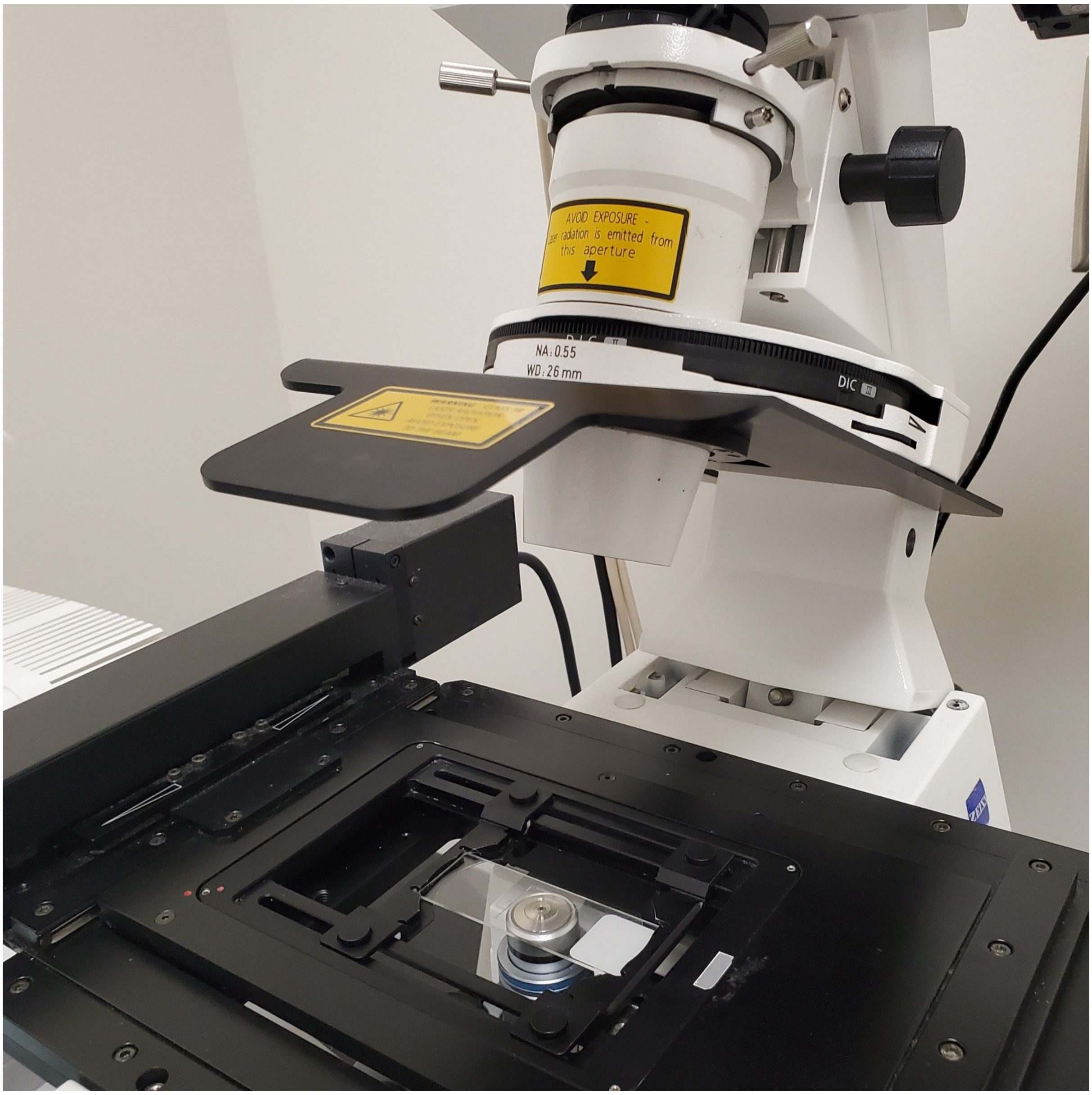
- Turn on the **laser lamp**
- 
1
X-Cite
SERIES 120
EXFO
- Login into the **computer**
- Start **[ZEN]** software - Start System

Visual Operation

Loading a slide (#1.5 cover slides are the best)

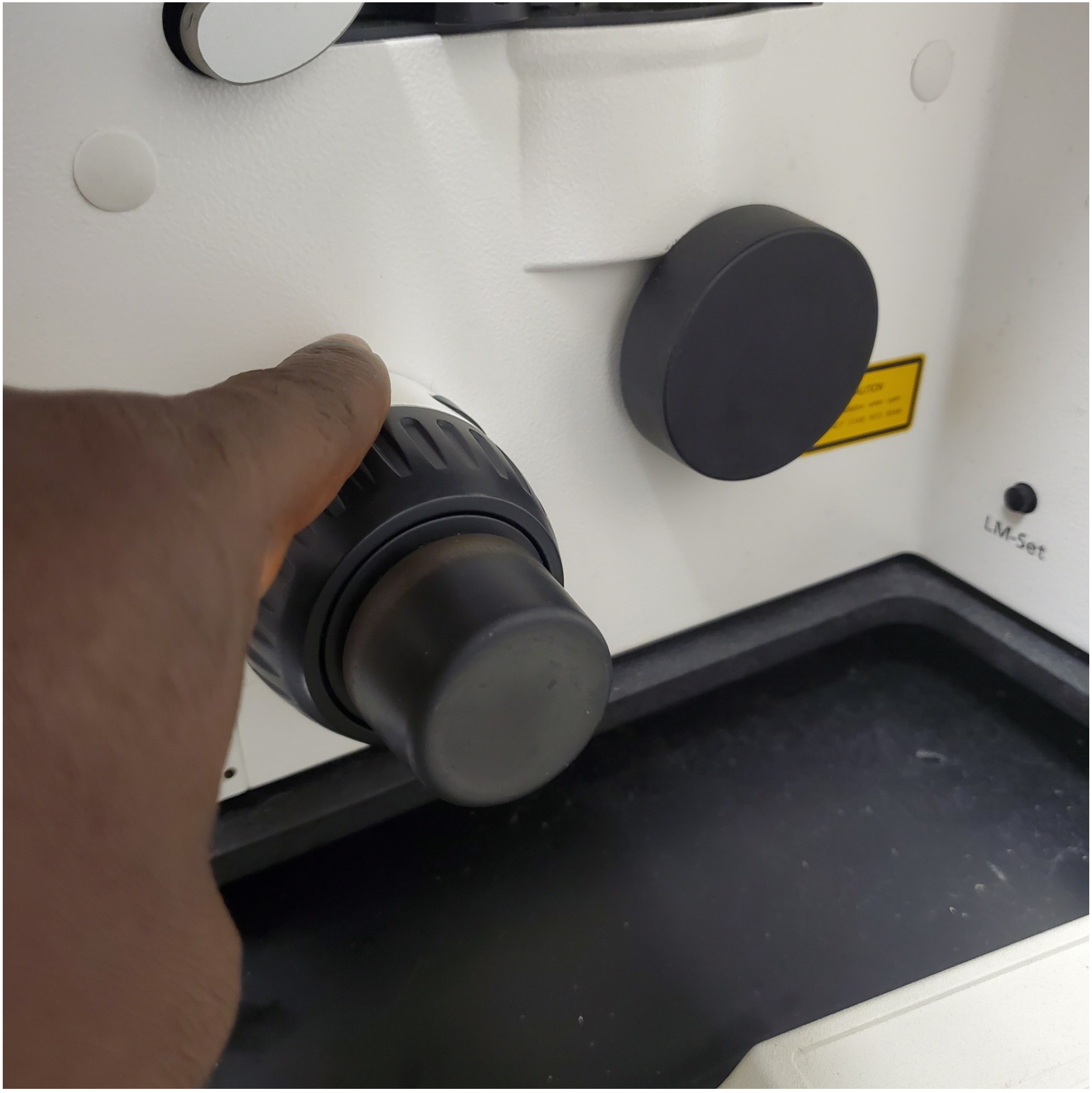
- Set the objective to **10x** using the touch pad on the microscope (recommended to always focus first on 10x – as the objectives are parfocal you can then go up in magnification as necessary while remaining in focus).
- Click on load button on knob attached to microscope **[Microsope] [Load position]**
- 

- Tilt transmitted light source back by placing one hand on the back side of the base of the eyepieces and one hand on the white part of the transmitted light source.


AVOID EXPOSURE - Laser radiation is emitted from this aperture
NA: 0.55
WD: 26 mm
DIC

- Load the slide into the slide holder cover slip down (to move the sliders apart press down on both buttons slide as necessary)

- Click **on working position knob [behind load position]** to return to working position – **Be careful to make sure the slide does not run into the objective** (this is why you should always focus on 10x first)



Note: Do not depend on the preset load and work settings. Samples and slides of different thicknesses may have been used by the previous investigator.

- Tilt the transmitted light source back into place
- Focus on your sample by using either transmitted light or fluorescence:
    - This can be done in a number of ways:
    - § Method of choice - In Zen Select **[Locate]**
        - Select **[Online]**
        - Select the desired fluorophore or transmitted light
        - Locate region of interest and focus the samples
        - Select **[Light Off]** to prevent bleaching § Using the touch pad on the microscope §§ Using the buttons on the microscope itself and touchpad. Choose TR (transmitted light) or RL (reflected light and the filter: DAPI/ 488 /Rhod).

        - Focus the specimen. With coaxial knob you can move the sample on the x-y level.
    - Change the objective as necessary for your imaging.
    - To add oil (only for the 40x and the 63x) – remove the slide

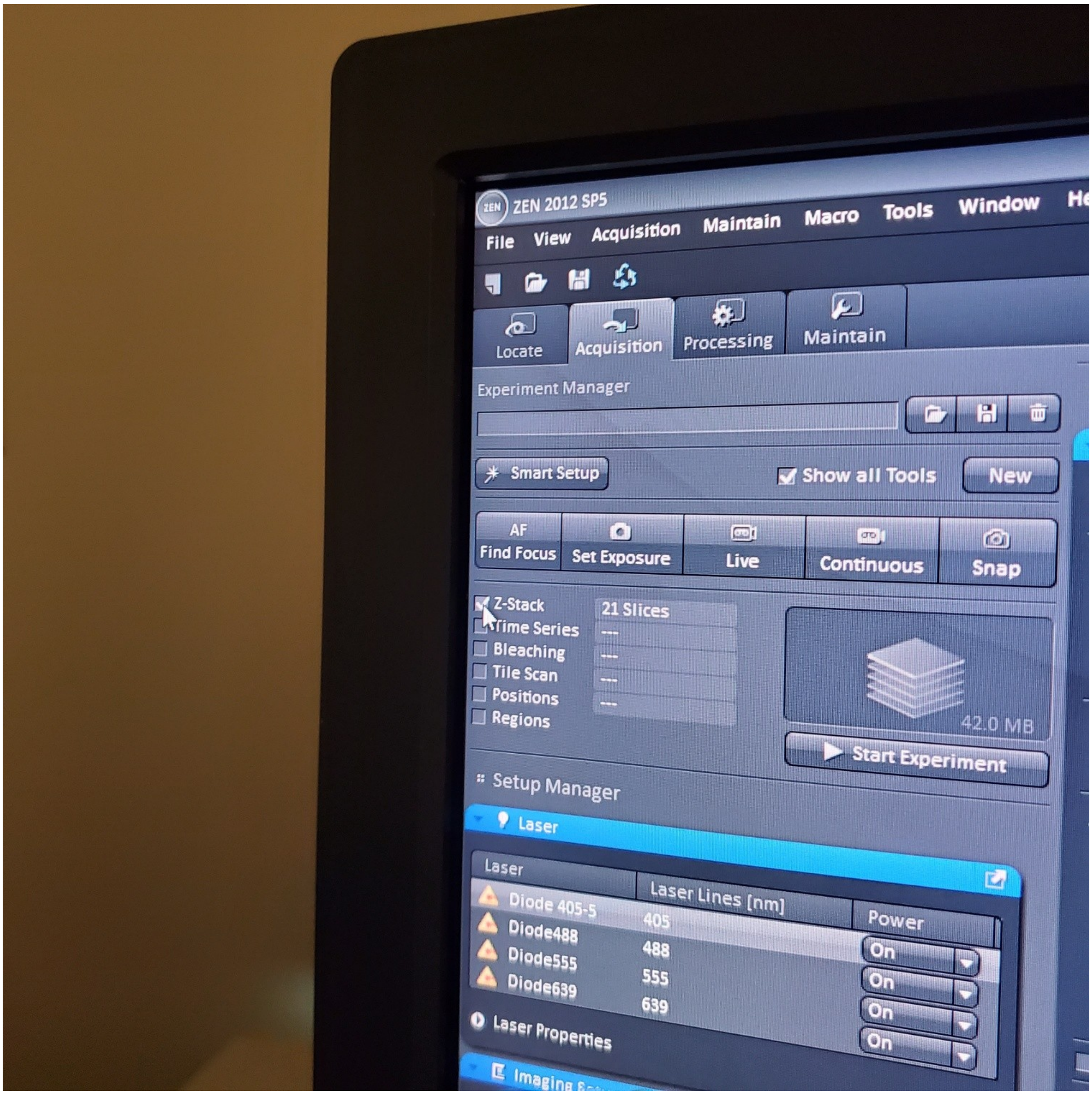
- Open the lid of the bottle and scoop some of the oil with the plastic oil probe.
- Turn it sideways (so that the longest edge is vertical) – there should not be oil dripping off of it

- Move it over the objective and turn it flat – one drop should fall onto the center of the objective
- **Never touch the oil probe to the objective**

- **If you need to return to a lower magnification from an oil objective you must use the 10x.** (The 20x has a short working distance and will get oil on it, which is bad for the objective and will hinder imaging with it. Additionally, the 20x objective is a high NA objective and is therefore expensive)

Imaging

- Select **[Acquisition]**


ZEN 2012 SP5
File
View
Acquisition
Maintain
Macro
Tools
Window
Locate
Acquisition
Processing
Maintain
Experiment Manager
Smart Setup
Show all Tools
New
AF Find Focus
Set Exposure
Live
Continuous
Snap
42.0 MB
Start Experiment
Setup Manager
Laser
Laser Properties

☑ Z-Stack 21 Slices
☐ Time Series ---
☐ Bleaching ---
☐ Tile Scan ---
☐ Positions ---
☐ Regions

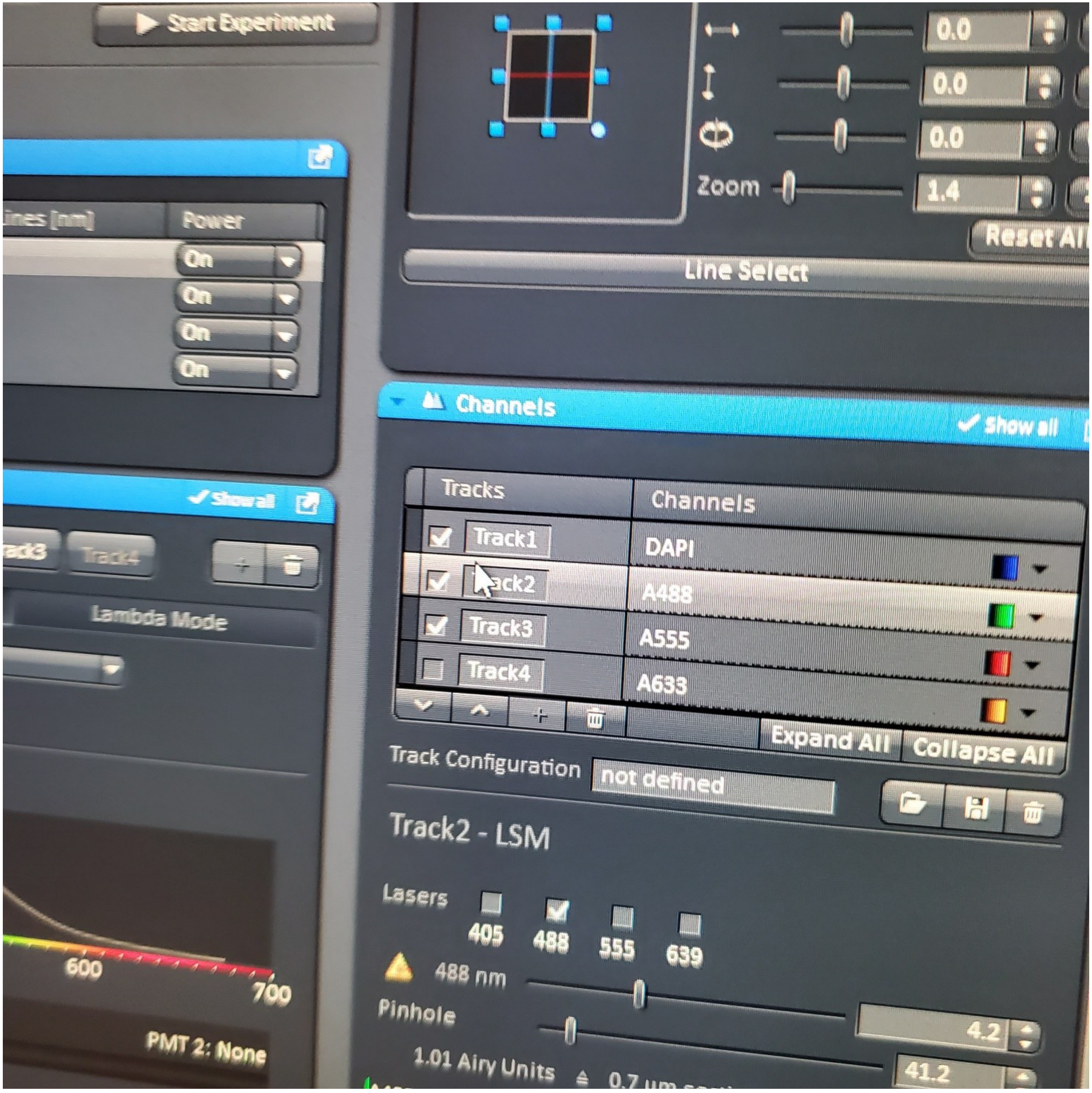
Laser	Laser Lines [nm]	Power
Diode 405-5	405	On
Diode488	488	On
Diode555	555	On
Diode639	639	On

- Load a user profile image file Select **[Reuse]** or use **[Smart Setup]**
- Select **[Dye]**, select show all, click on arrow and pick dyes (do this for each fluorophore your sample is labeled with). This is where you pick the pseudocolor for each channel.

o Pick imaging sequence:

- § Fastest: 2 channels per track (meaning 2 channels are imaged at the same time with 2 PMTs)
- § Best = slowest: each channel is in its own track and is imaged separately (this prevents bleed through)
- § Best-compromise = some channels are in a track together and others are in their own tracks (medium time to get image)
- Select **[light path]:** select track (if not showing click show all manual settings towards the top left of the software window)
  - If possible it is best move the VSD (variable secondary dichroic) to be at the same nm for all the tracks
  - § VSD is the white vertical line on the wave length graph and the nm is listed under Split

- You can use filters (show all and click on the arrow in the center of the colored bar below each emission spectra to see the filter options) to further narrow the emission collection

- For **acquisition** o Settings will depend on your sample. A good starting point is:
  - Scan mode: Frame o Frame size: 1024 x 1024 o Line step: 1 (always!)
  - Speed: 7-9 o Average: 1-4 o Mode: line
  - Method: mean o Bit depth: 8 -16 bit
  - Unidirectional scanning (arrow points in only one direction) or Bidirectional scanning makes imaging faster.
- For **scan** o Select one track by unclicking the other tracks o Live: center, orient and focus samples (use one track) o Turn on all the tracks
  - In the **[Channels]** menu adjust the **[laser power],** 2% default (1-5% is usually all you will need), and **[pinhole,** 1 airy unit – 1 AU**]**
  - 

Start Experiment
Lines [nm]
Power
On
On
On
On
Show all
Track3
Track4
Lambda Mode
600
700
PMT 2: None
Zoom
Reset All
Line Select
Channels
Show all
Expand All
Collapse All

Tracks	Channels
☑ Track1	DAPI
☑ Track2	A488
☑ Track3	A555
☐ Track4	A633

Track Configuration: not defined

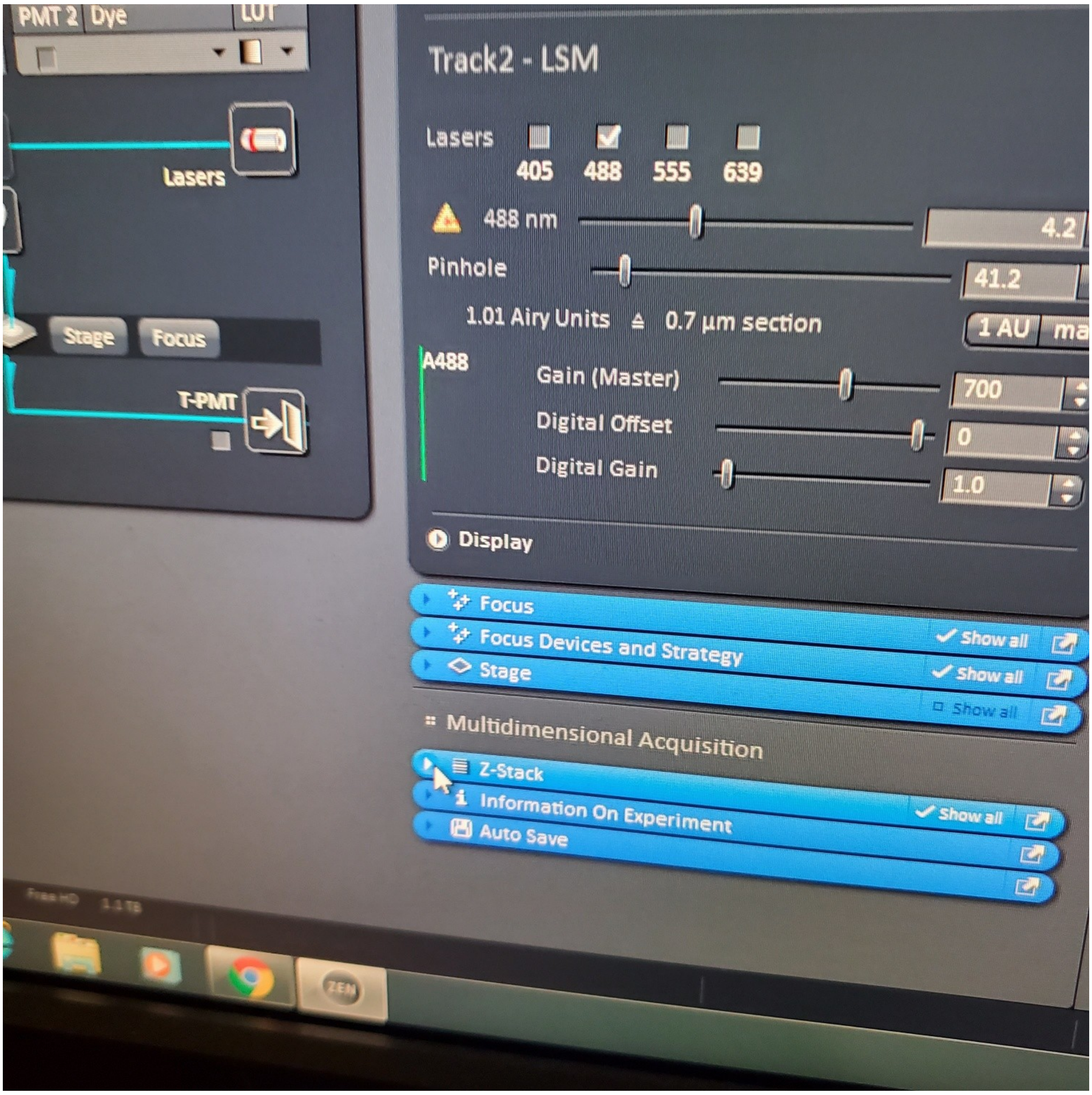
Track2 - LSM

Lasers ☐ 405 ☑ 488 ☐ 555 ☐ 639

488 nm 4.2

Pinhole 41.2

1.01 Airy Units

- 
PMT 2
Dye
LUT
Lasers
Stage
Focus
T-PMT
Display
Focus
Focus Devices and Strategy
Stage
Show all
Multidimensional Acquisition
Z-Stack
Information On Experiment
Auto Save
ZEN

Track2 - LSM

Lasers ☐ 405 ☑ 488 ☐ 555 ☐ 639

488 nm 4.2

Pinhole 41.2

1.01 Airy Units ≙ 0.7 µm section 1 AU

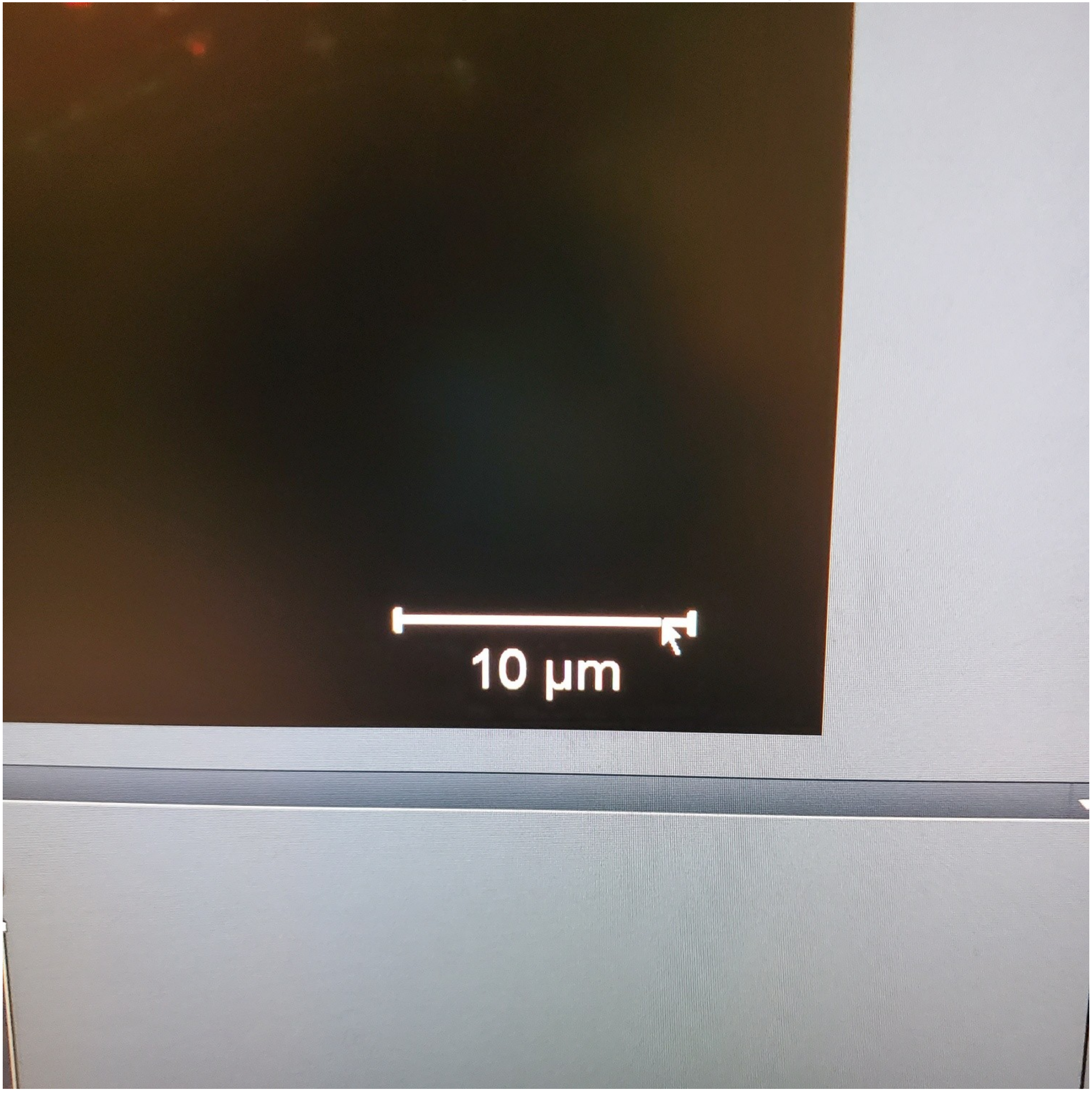
A488 Gain (Master) 700

Digital Offset 0

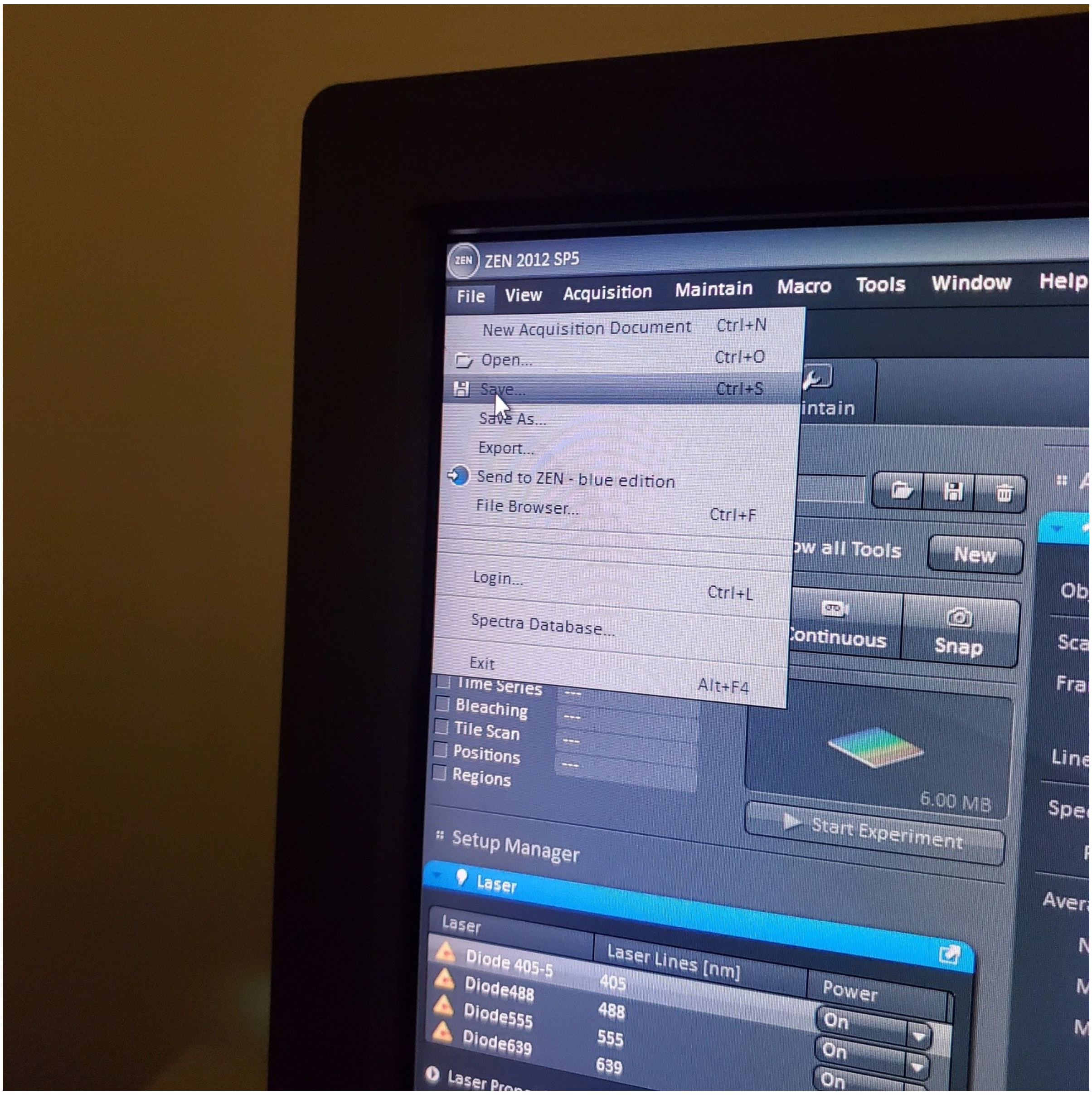
Digital Gain 1.0

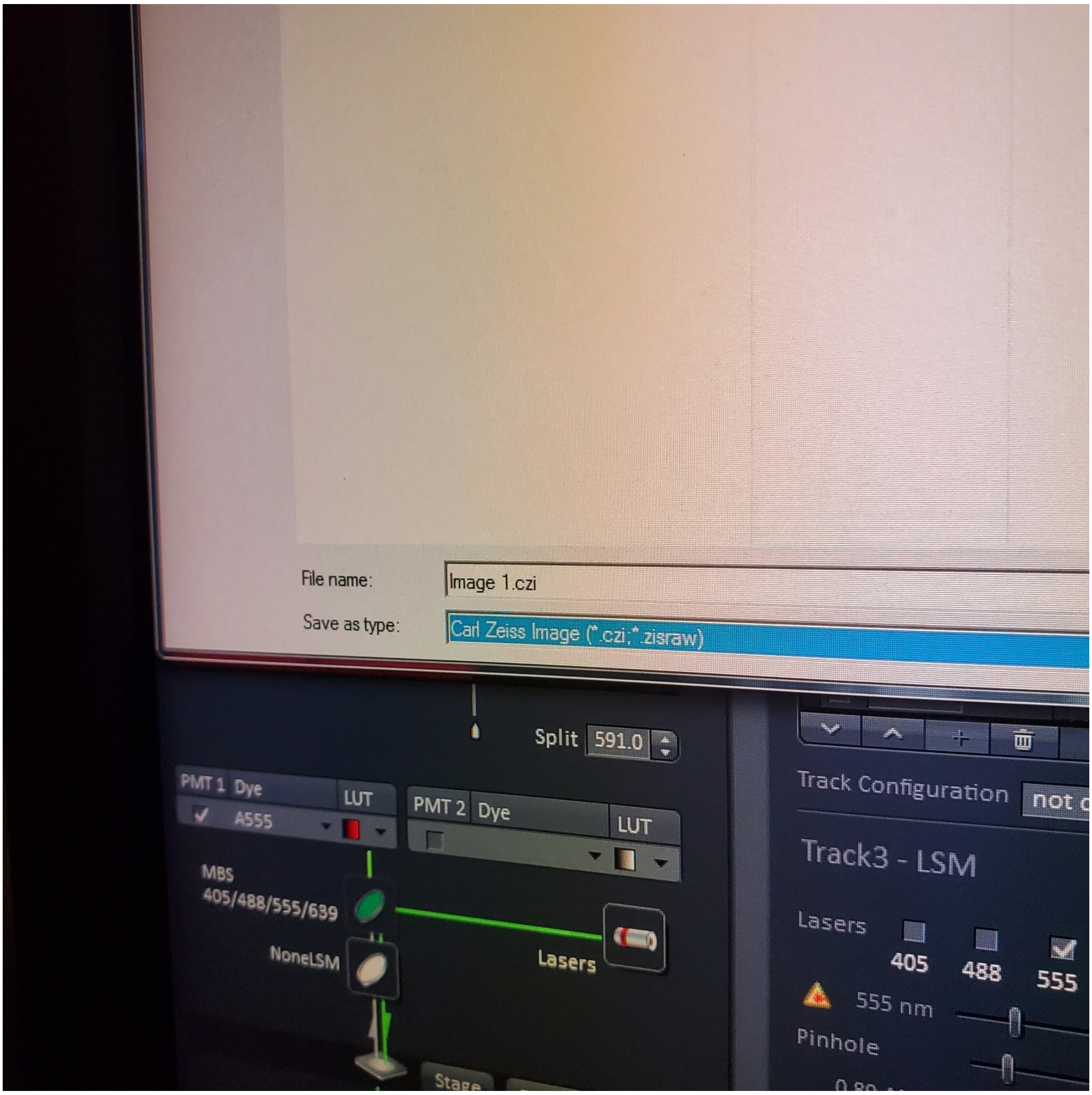
- Select **[Live]**
- Select **[Dimensions]:** (sometimes this is easier to do with one channel or track selected at a time)
- § use **range indicator mode** (click on color of channel and it will automatically switch to this or by clicking the color field in the View-Dimensions you get the scanned image in a false-color. Red color indicates saturated pixels (maximum) and Blue = zero (minimum). To avoid saturated pixels set the laser power and gain so that the red pixels just disappear
- § split channels
- § Select one channel within a track by clicking on it (light gray bar line is the selected one)
  - Focus on the brightest plane of this channel in your sample
  - adjust **[Gain]**, to just below when red (overexposed) pixels appear (you want this between 500-750; if it is too low then lower the laser power) o Gain = voltage on PMT. Higher number than you can detect weaker signals and also more noise.

o On histogram you want to shoot for 75% of the range

- Adjust **[offset]** – only move up or down by 1-2 to get background to blue speckles (starry night)
- **[Digital gain]** set to 1.0, do not change.
- Repeat for all the channels and all the tracks
- Click **STOP** –for stopping the current scan procedure
- To take a single image: Select **[Snap]** to collect the final image and add a scale bar
- 

- § Save image to groups-server or to drive D: user file deleted anytime folder

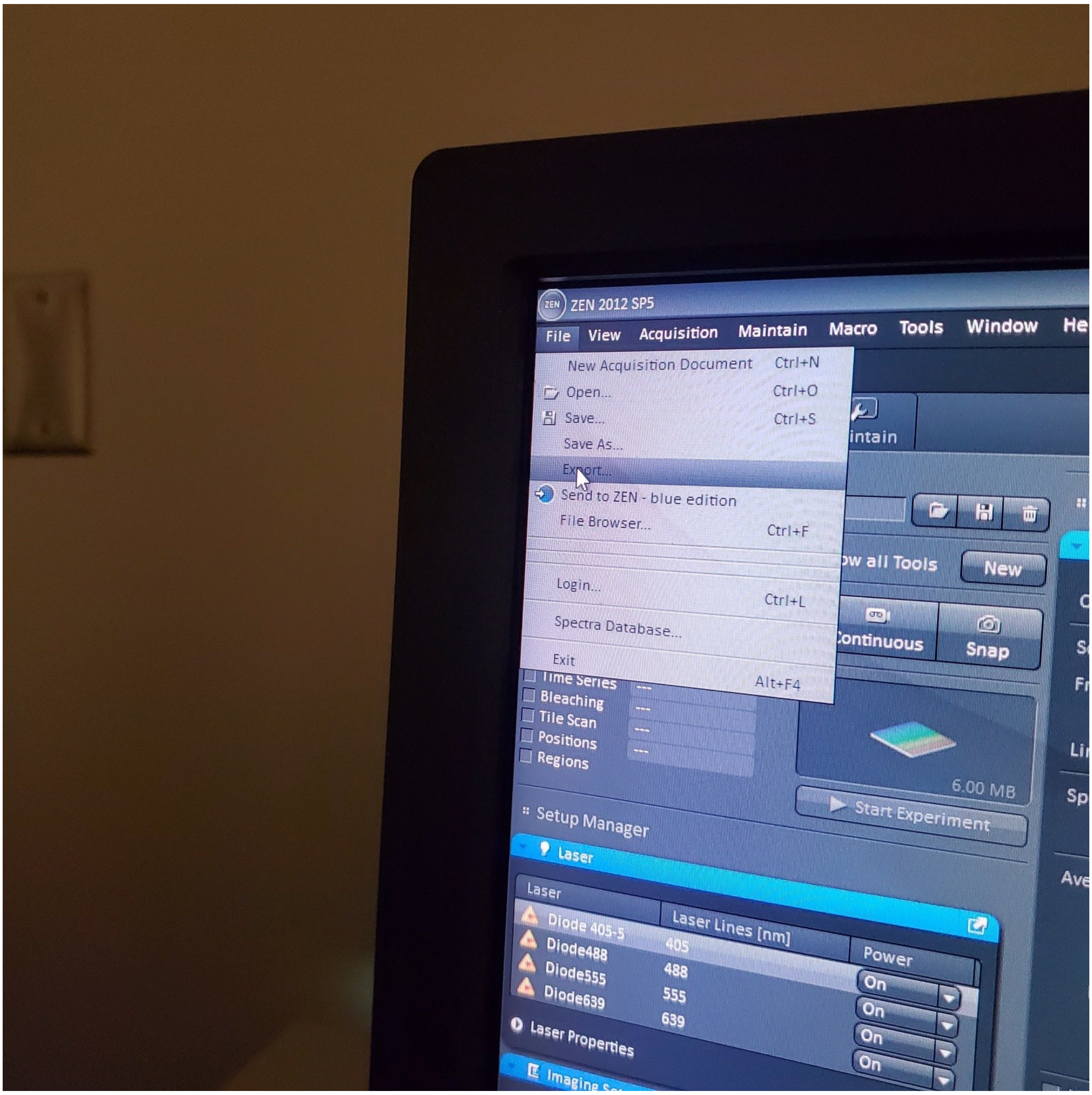
- 
ZEN 2012 SP5
File
View
Acquisition
Maintain
Macro
Tools
Window
Help
New Acquisition Document Ctrl+N
Open... Ctrl+O
Save... Ctrl+S
Save As...
Export...
Send to ZEN - blue edition
File Browser... Ctrl+F
Login... Ctrl+L
Spectra Database...
Exit Alt+F4
Time Series
Bleaching
Tile Scan
Positions
Regions
New
Continuous
Snap
6.00 MB
Start Experiment
Setup Manager
Laser
Laser Lines [nm]
Power
Diode 405-5 405 On
Diode488 488 On
Diode555 555 On
Diode639 639
- § Always save as a lsm/czi file first before exporting as tiff. In CZI-format all the information and hardware settings are saved together with the image. Export -function saves the contents of the image display window including analysis and overlays in selected format

- 

  File name: Image 1.czi

  Save as type: Carl Zeiss Image (*.czi;*.zisraw)

  Split 591.0
PMT 1 Dye
LUT
A555
PMT 2 Dye
LUT
MBS
405/488/555/639
NoneLSM
Lasers
Stage
Track Configuration
not c
Track3 - LSM
Lasers
405
488
555
555 nm
Pinhole
- You may also export a tiff file

- 

ZEN 2012 SP5
File
View
Acquisition
Maintain
Macro
Tools
Window
He
New Acquisition Document Ctrl+N
Open... Ctrl+O
Save... Ctrl+S
Save As...
Export...
Send to ZEN - blue edition
File Browser... Ctrl+F
Login... Ctrl+L
Spectra Database...
Exit Alt+F4
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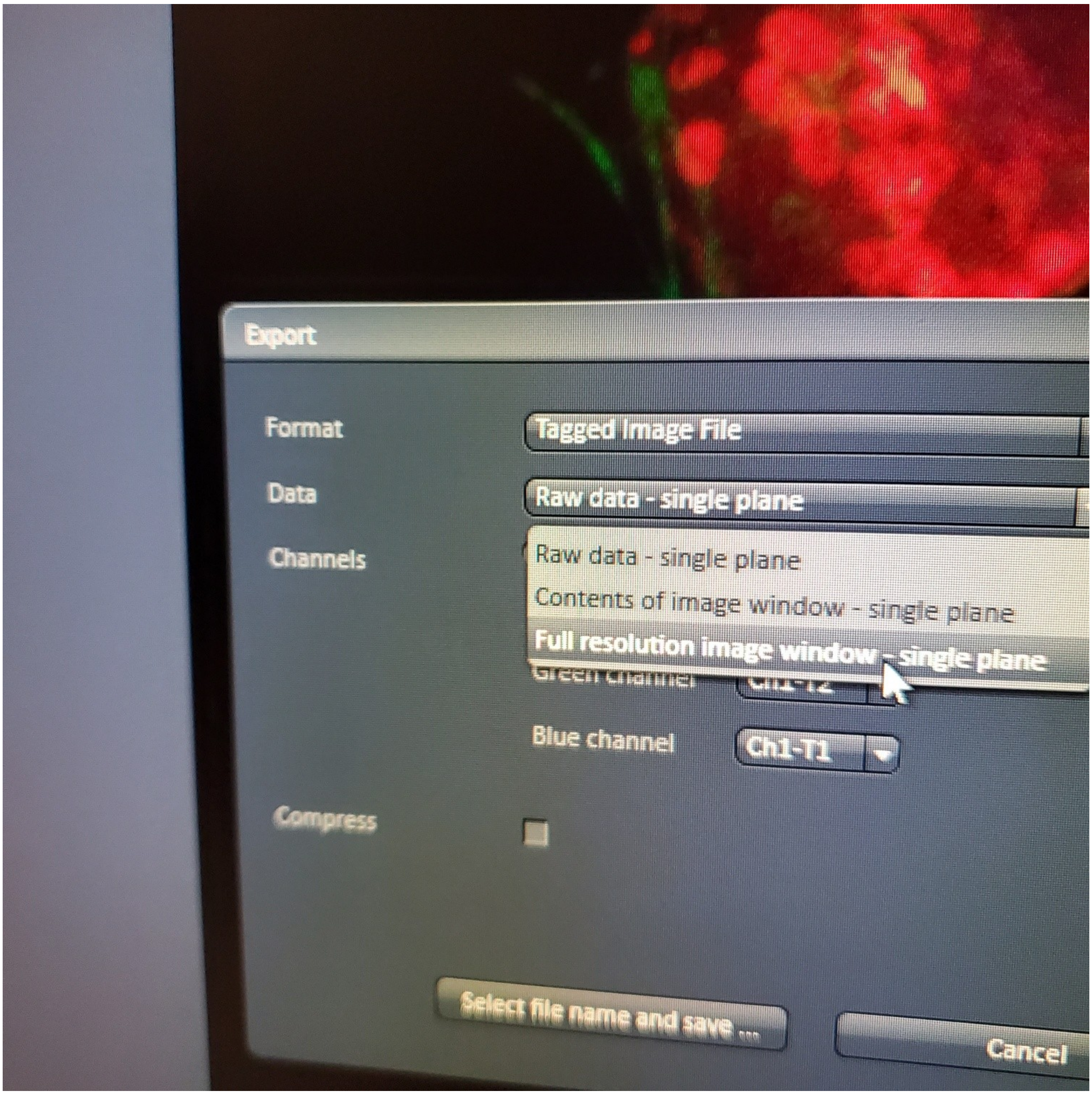
ow all Tools
New
Continuous
Snap
6.00 MB
Start Experiment
Setup Manager
Laser
Laser Properties
Imaging Se

☐ Time Series ---
☐ Bleaching ---
☐ Tile Scan ---
☐ Positions ---
☐ Regions ---

Laser	Laser Lines [nm]	Power
Diode 405-5	405	On
Diode488	488	On
Diode555	555	On
Diode639	639	On

- 

Export

Format: Tagged Image File

Data: Raw data - single plane

Channels

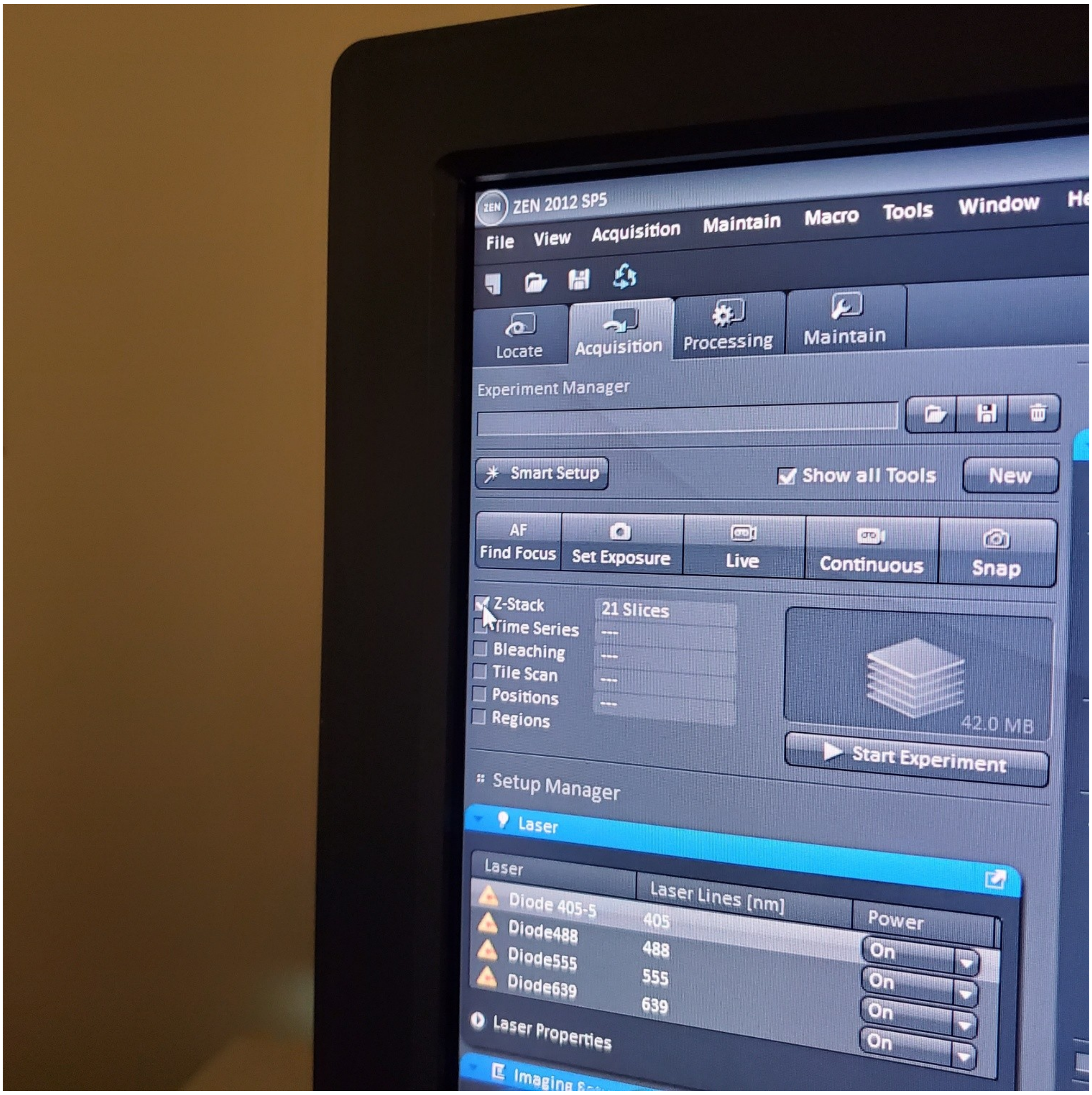
Green channel: Ch1-T2

Blue channel: Ch1-T1

Compress ☐

Raw data - single plane
Contents of image window - single plane
Full resolution image window - single plane
Select file name and save ...
Cancel

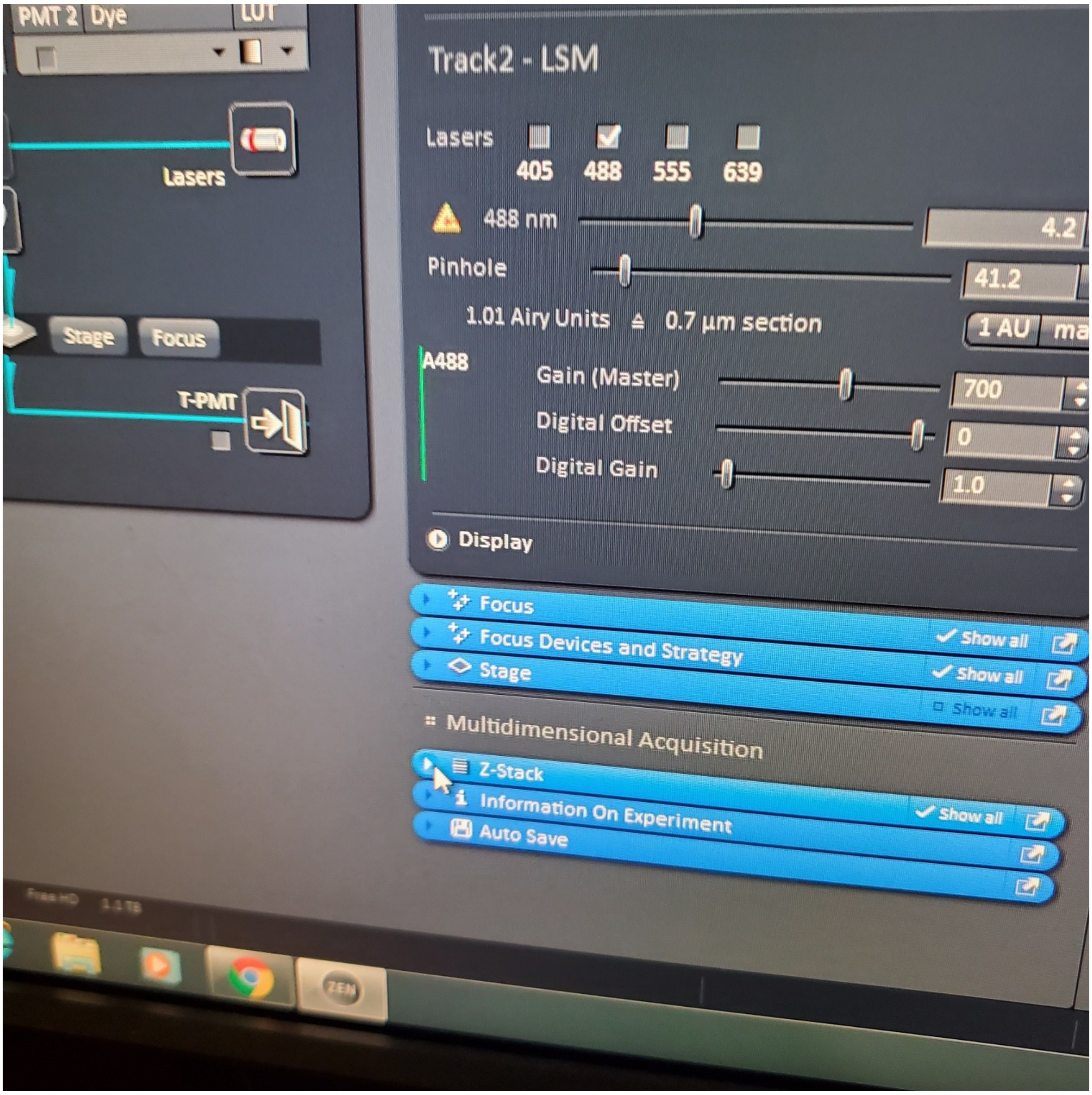
- § **Do not store on drive C:\**
- § Once you have moved your data to the core server please delete it on the confocal computer (any images over one month old will automatically be deleted)
- **Z-stack (select at upper left)**

- 

ZEN 2012 SP5
File
View
Acquisition
Maintain
Macro
Tools
Window
Locate
Acquisition
Processing
Maintain
Experiment Manager
Smart Setup
Show all Tools
New
AF Find Focus
Set Exposure
Live
Continuous
Snap
42.0 MB
Start Experiment
Setup Manager
Laser
Laser Properties
Imaging

☑ Z-Stack 21 Slices
☐ Time Series ---
☐ Bleaching ---
☐ Tile Scan ---
☐ Positions ---
☐ Regions ---

Laser	Laser Lines [nm]	Power
Diode 405-5	405	On
Diode488	488	On
Diode555	555	On
Diode639	639	On

- 

Track2 - LSM

Lasers ☐ 405 ☑ 488 ☐ 555 ☐ 639

488 nm 4.2

Pinhole 41.2

1.01 Airy Units ≙ 0.7 µm section

A488

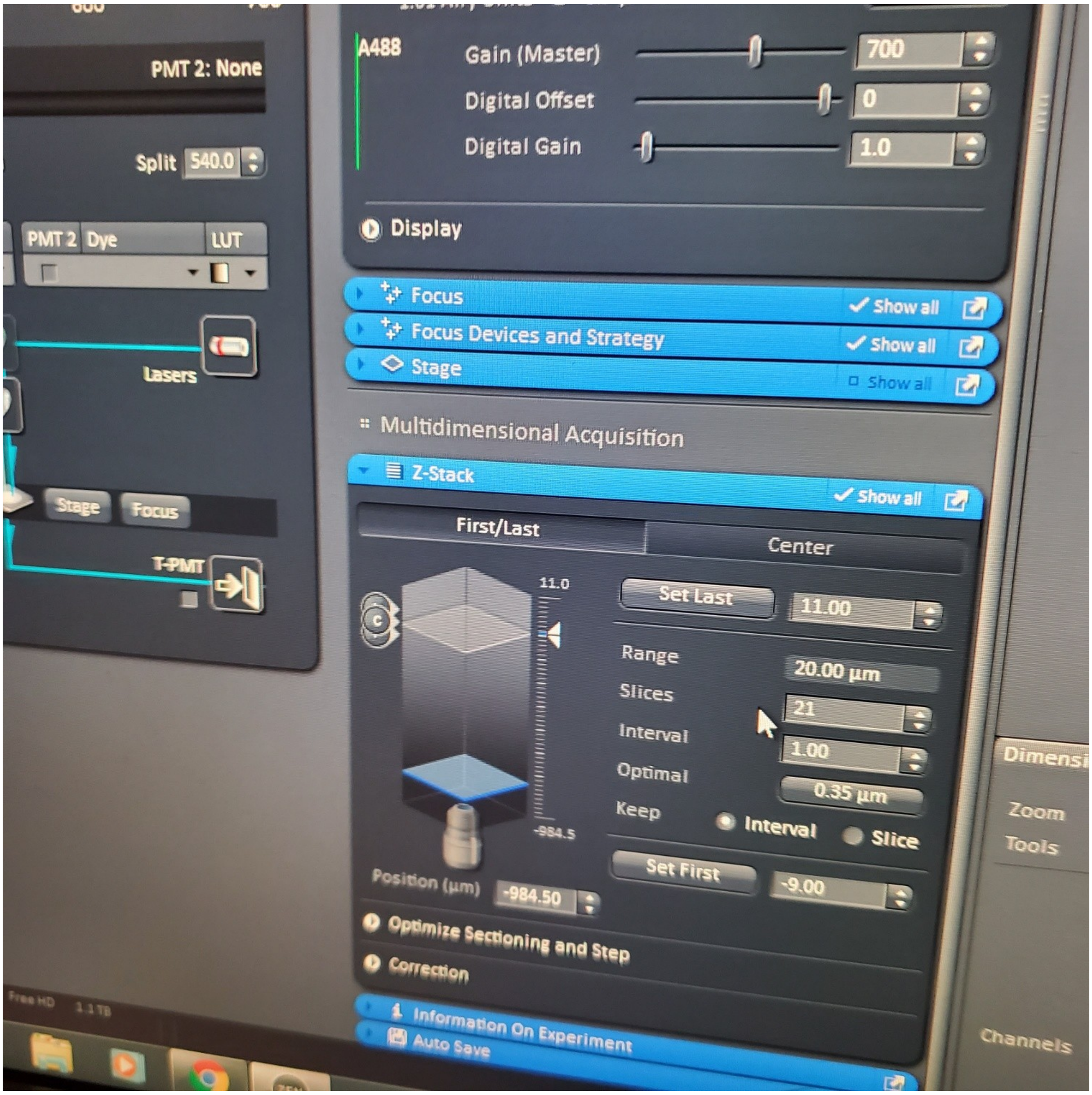
Gain (Master) 700

Digital Offset 0

Digital Gain 1.0

PMT 2
Dye
LUT
Lasers
Stage
Focus
T-PMT
1 AU
ma
Display
Focus
Focus Devices and Strategy
Stage
Show all
Multidimensional Acquisition
Z-Stack
Information On Experiment
Auto Save
ZEN

- § Choose one track to focus
- § To optimize interval click [optimal]
- § Set the first and last

- 

PMT 2: None

Split 540.0

A488

Gain (Master) 700

Digital Offset 0

Digital Gain 1.0

Multidimensional Acquisition

Z-Stack

First/Last | Center

Set Last 11.00

Range 20.00 µm

Slices 21

Interval 1.00

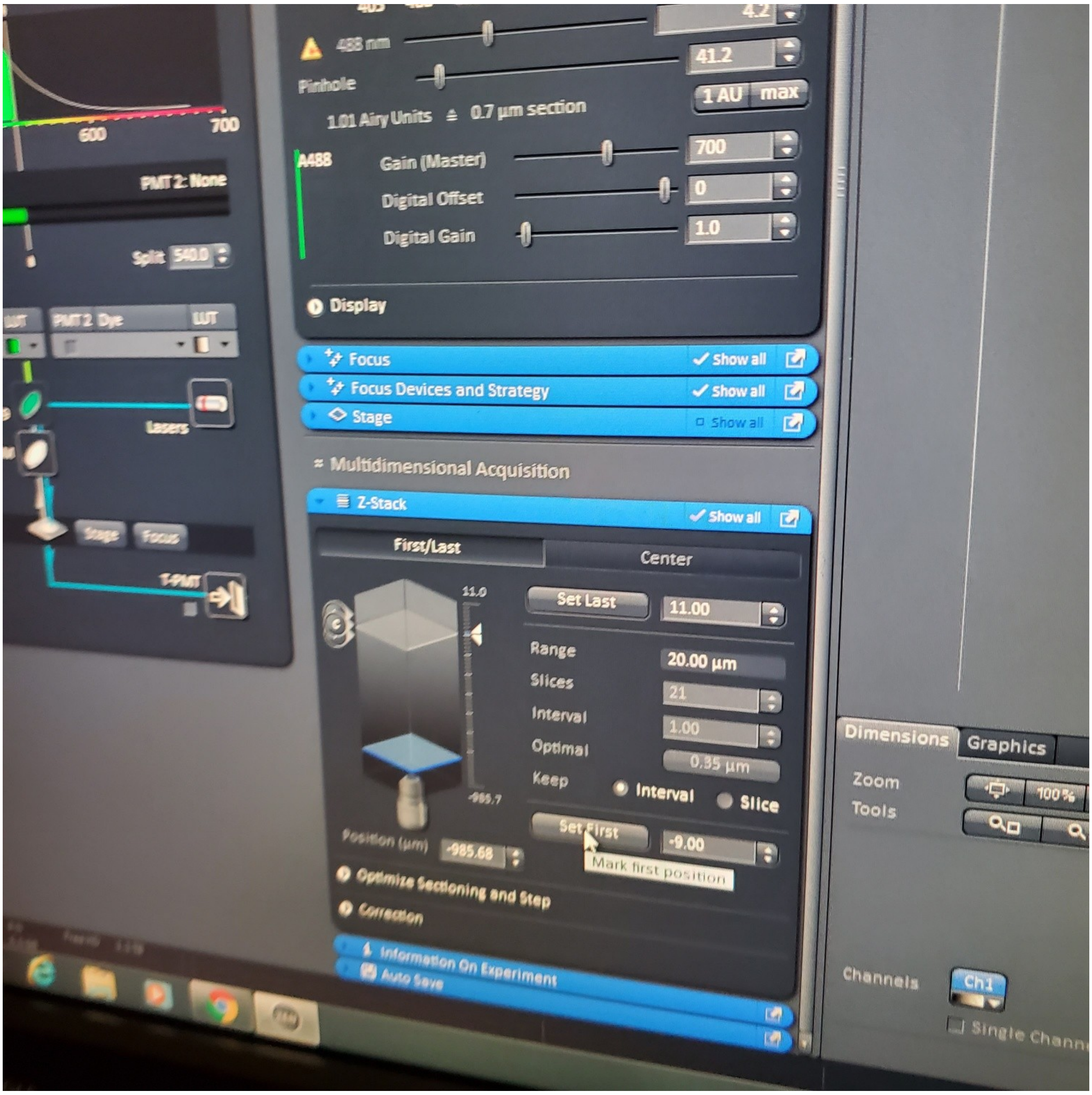
Optimal 0.35 µm

Keep ● Interval ○ Slice

Set First -9.00

Position (µm) -984.50

PMT 2
Dye
LUT
Lasers
Stage
Focus
T-PMT
Display
Focus
Show all
Focus Devices and Strategy
Show all
Stage
Show all
Show all
11.0
-984.5
Optimize Sectioning and Step
Correction
Information On Experiment
Auto Save
Dimensi
Zoom
Tools
Channels

- 

488 nm 41.2

Pinhole 1 AU max

1.01 Airy Units ≙ 0.7 µm section

A488 Gain (Master) 700

Digital Offset 0

Digital Gain 1.0

Split 540.0

Set Last 11.00

Range 20.00 µm

Slices 21

Interval 1.00

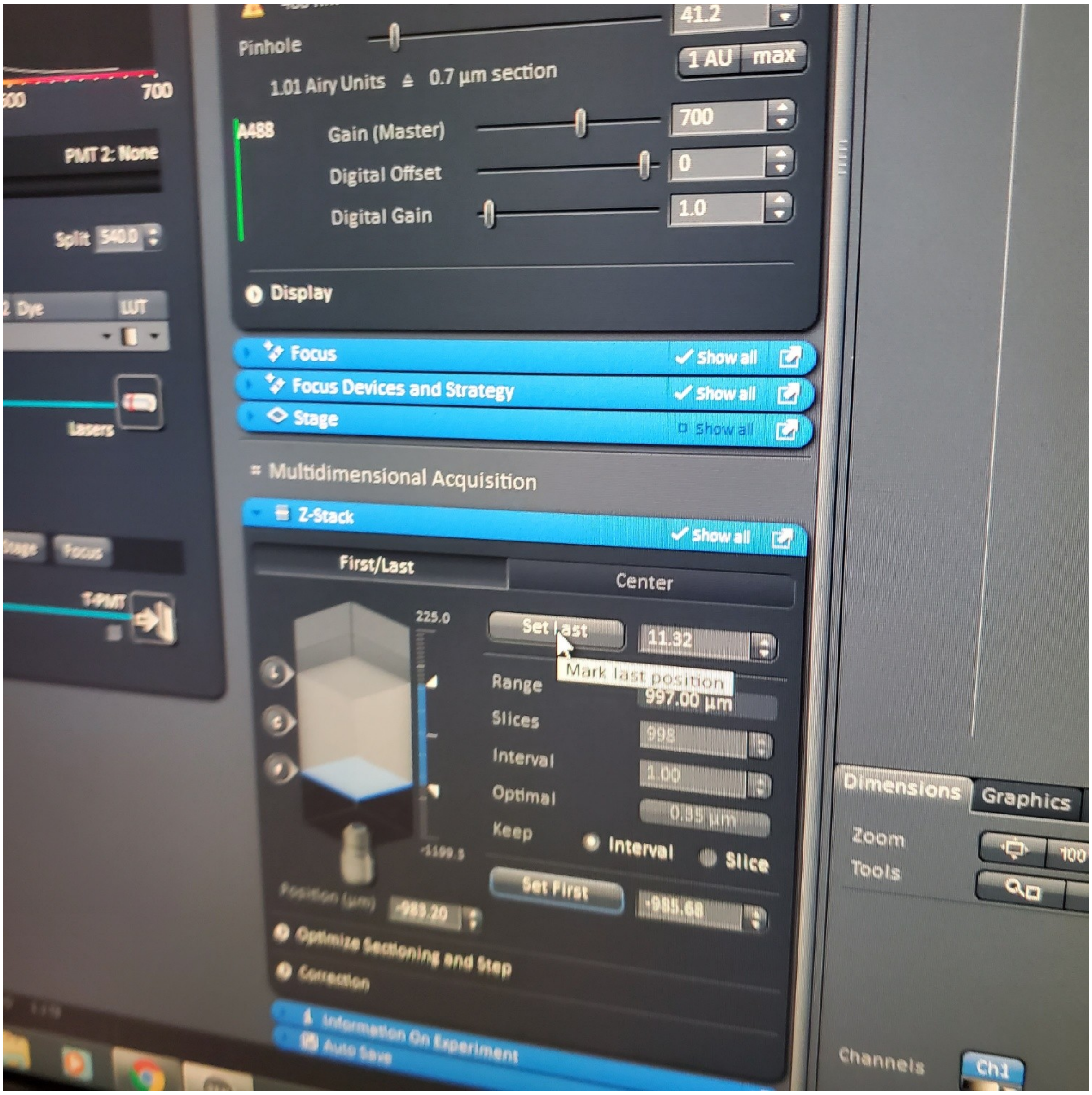
Optimal 0.35 µm

Keep ○ Interval ○ Slice

Set First -9.00

Position (µm) -985.68

600
700
PMT 2: None
PMT 2 Dye
LUT
Lasers
Stage
Focus
T-PMT
Display
Focus
Focus Devices and Strategy
Stage
Show all
Multidimensional Acquisition
Z-Stack
First/Last
Center
11.0
-985.7
Mark first position
Optimize Sectioning and Step
Correction
Information On Experiment
Auto Save
Dimensions
Graphics
Zoom
Tools
100 %
Channels
Ch1
Single Chann

- 

Pinhole 41.2

1.01 Airy Units ≙ 0.7 µm section

A488

Gain (Master) 700

Digital Offset 0

Digital Gain 1.0

Z-Stack

First/Last

Set Last 11.32

Mark last position

Range 997.00 µm

Slices 998

Interval 1.00

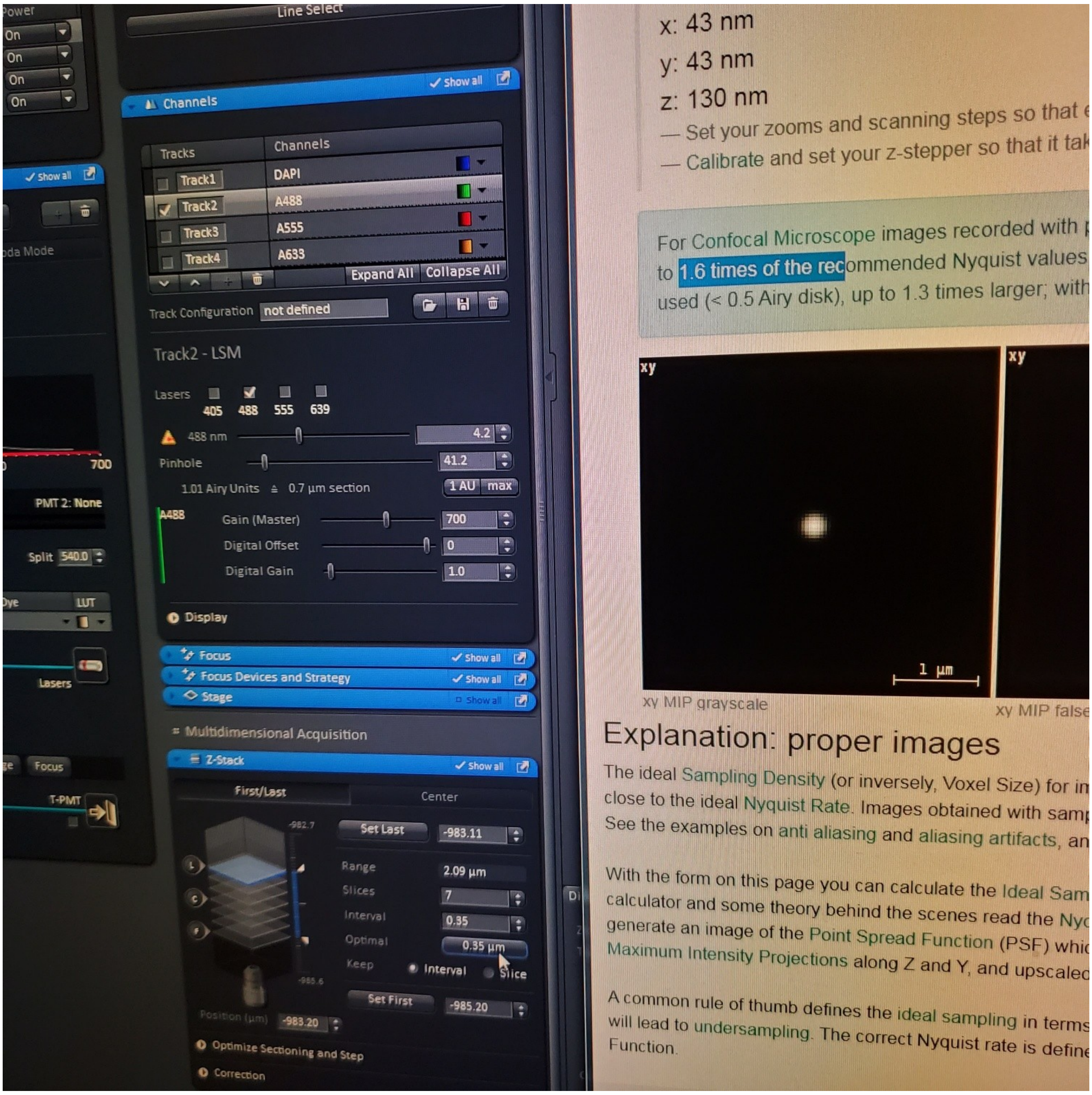
Optimal 0.35 µm

Keep ● Interval ○ Slice

Set First -985.68

Position (µm) -983.20

700
PMT 2: None
Split 540.0
Dye
LUT
Lasers
Stage
Focus
T-PMT
1 AU
max
Display
Focus
Show all
Focus Devices and Strategy
Show all
Stage
Show all
Multidimensional Acquisition
Show all
Center
225.0
Optimize Sectioning and Step
Correction
Information On Experiment
Auto Save
Dimensions
Graphics
Zoom
Tools
Channels
Ch1

- 
  Power
On
On
On
On
Line Select
Channels
Show all
Expand All
Collapse All
PMT 2: None
Split 540.0
Dye
LUT
Lasers
Focus
T-PMT
Display
Focus
Focus Devices and Strategy
Stage
Show all
Multidimensional Acquisition
Z-Stack
First/Last
Center
Optimize Sectioning and Step
Correction
xy
xy
1 µm
xy MIP grayscale
xy MIP false

Tracks	Channels
☐ Track1	DAPI
☑ Track2	A488
☐ Track3	A555
☐ Track4	A633

  Track Configuration not defined

  Track2 - LSM

  Lasers ☐ 405 ☑ 488 ☐ 555 ☐ 639

  488 nm 4.2

  Pinhole 41.2

  1.01 Airy Units ≙ 0.7 µm section

  A488 Gain (Master) 700

  Digital Offset 0

  Digital Gain 1.0

  Set Last -983.11

  Range 2.09 µm

  Slices 7

  Interval 0.35

  Optimal 0.35 µm

  Keep ● Interval ○ Slice

  Set First -985.20

  Position (µm) -983.20

  x: 43 nm
  y: 43 nm
  z: 130 nm
  — Set your zooms and scanning steps so that e
  — Calibrate and set your z-stepper so that it tak

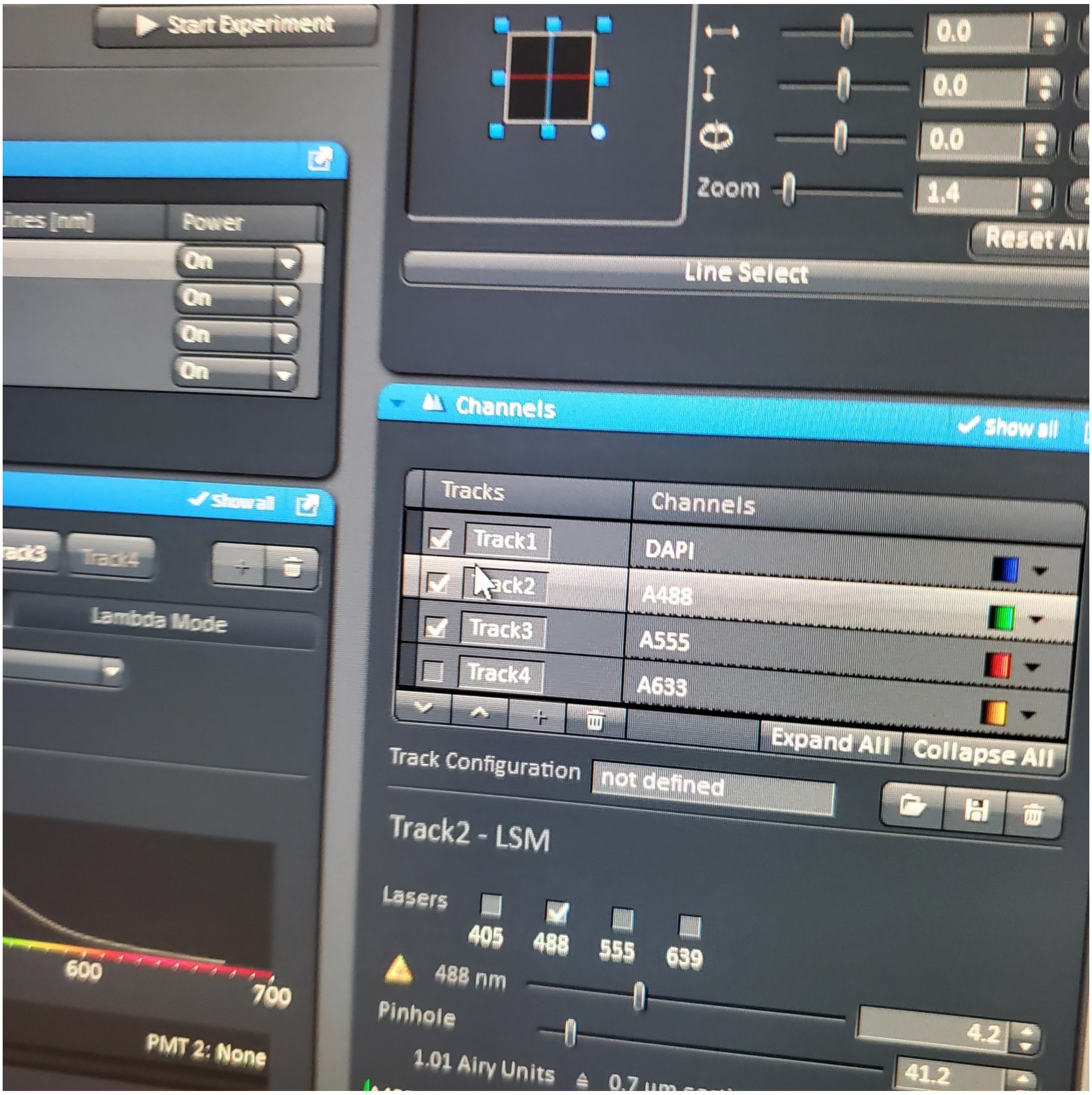
  For Confocal Microscope images recorded with p
  to 1.6 times of the recommended Nyquist values
  used (< 0.5 Airy disk), up to 1.3 times larger; with

  Explanation: proper images

  The ideal Sampling Density (or inversely, Voxel Size) for im
  close to the ideal Nyquist Rate. Images obtained with samp
  See the examples on anti aliasing and aliasing artifacts, an

  With the form on this page you can calculate the Ideal Sam
  calculator and some theory behind the scenes read the Nyq
  generate an image of the Point Spread Function (PSF) whic
  Maximum Intensity Projections along Z and Y, and upscaled

  A common rule of thumb defines the ideal sampling in terms
  will lead to undersampling. The correct Nyquist rate is define
  Function.
- § Re-select all the tracks

- 

Start Experiment
Lines [nm]
Power
On
On
On
On
Show all
Track3
Track4
Lambda Mode
600
700
PMT 2: None
Zoom
Reset All
Line Select
Channels
Show all
Expand All
Collapse All

Tracks	Channels
☑ Track1	DAPI
☑ Track2	A488
☑ Track3	A555
☐ Track4	A633

Track Configuration: not defined

Track2 - LSM

Lasers: ☐ 405 ☑ 488 ☐ 555 ☐ 639

488 nm

Pinhole: 4.2

1.01 Airy Units

0.0

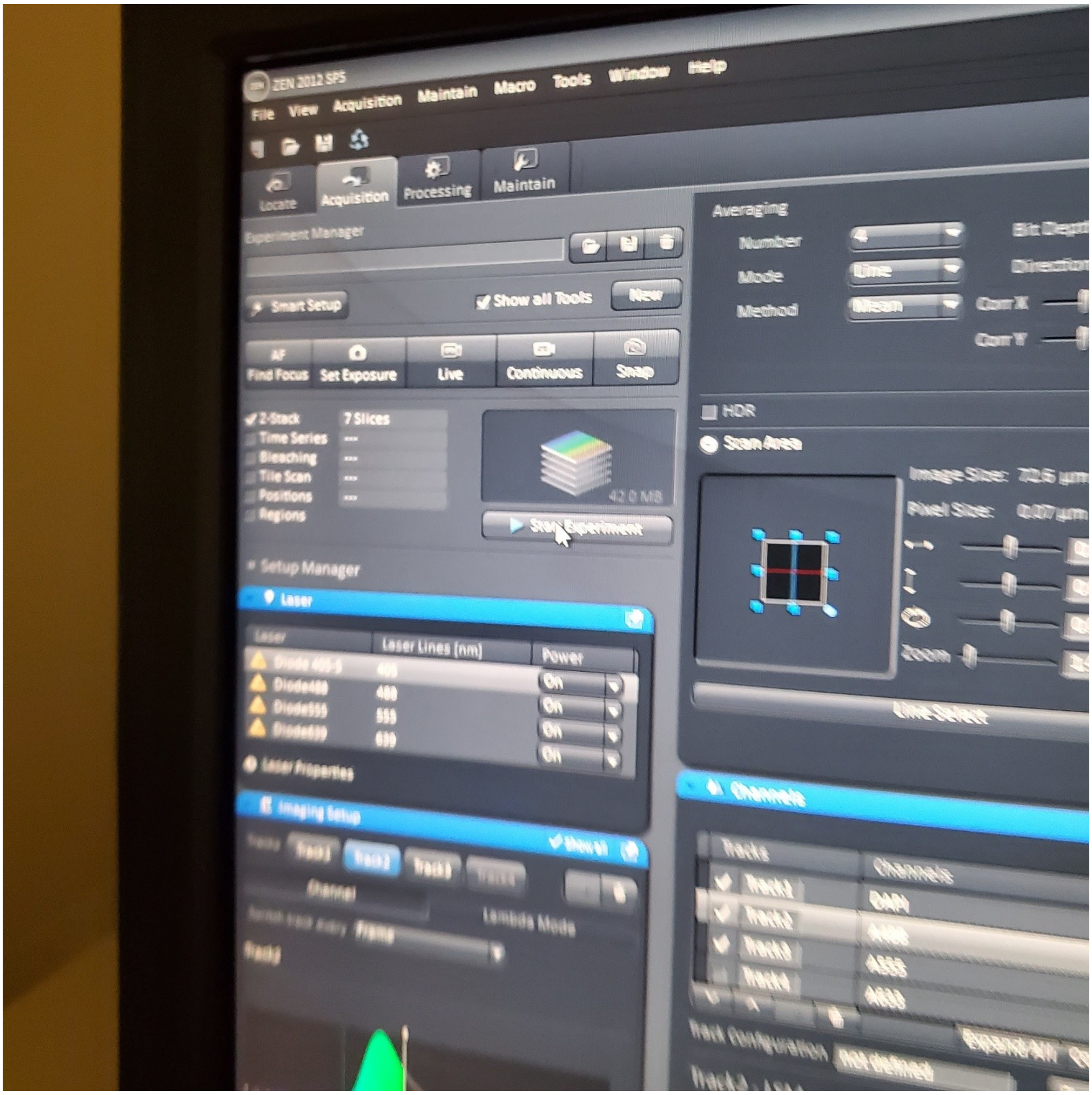
0.0

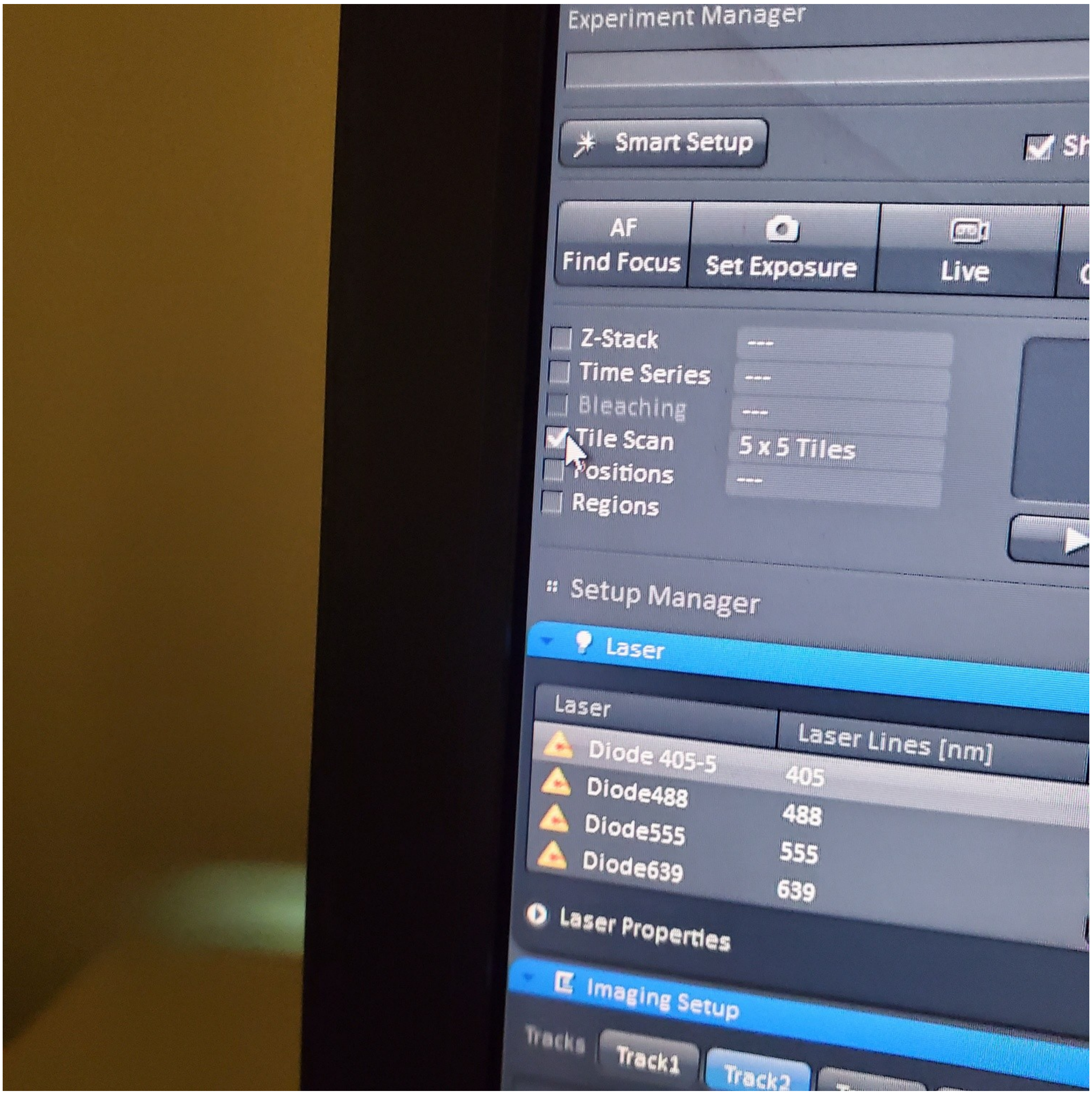
0.0

Zoom: 1.4

41.2

- § Start experiment (upper left window)

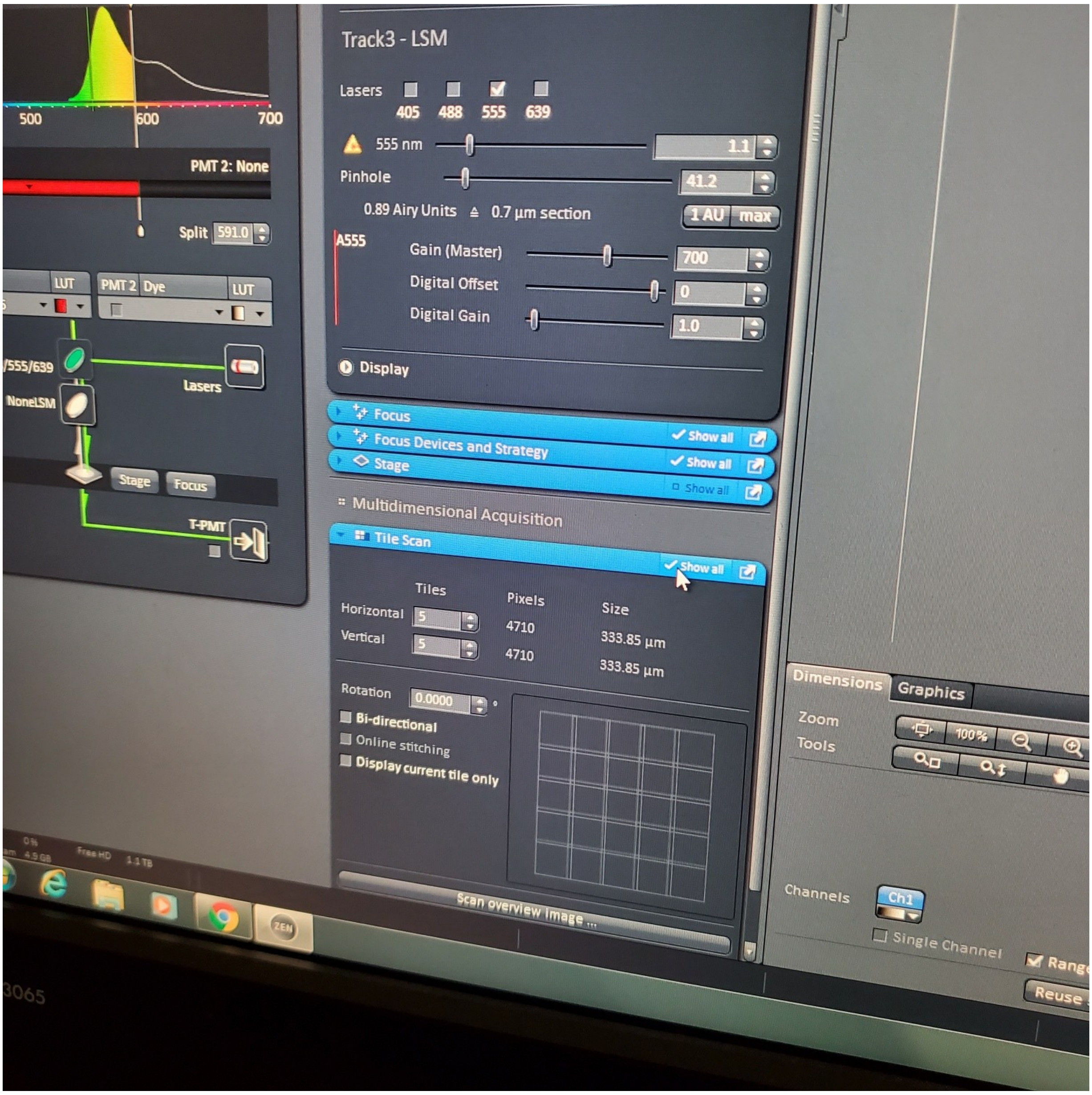
- 
ZEN 2012 SP5
File View Acquisition Maintain Macro Tools Window Help
Locate
Acquisition
Processing
Maintain
Experiment Manager
Smart Setup
Show all Tools
New
Find Focus
Set Exposure
Live
Continuous
Snap
Z-Stack 7 Slices
Time Series
Bleaching
Tile Scan
Positions
Regions
42.0 MB
Start Experiment
Setup Manager
Laser
Laser Lines [nm]
Power
Laser Properties
Imaging Setup
Show all
Track1 Track2 Track3 Track4
Channel
Lambda Mode
Averaging
Number
Mode
Method
Corr X
Corr Y
HDR
Scan Area
Image Size:
Pixel Size:
Zoom
Line Select
Channels
Tracks
Channels
Track1 DAPI
Track2 A488
Track3 A555
Track4 A633
Track Configuration
- § If you have a dramatic loss of brightness as you reach the end of your stack see core staff about Auto Z Brightness Correction
- **Tile Scan (select at upper left)**

- 
Experiment Manager
Smart Setup
AF Find Focus
Set Exposure
Live
Setup Manager
Laser
Laser Properties
Imaging Setup
Tracks
Track1
Track2

☐ Z-Stack ---
☐ Time Series ---
☐ Bleaching ---
☑ Tile Scan 5 x 5 Tiles
☐ Positions ---
☐ Regions

Laser	Laser Lines [nm]
Diode 405-5	405
Diode488	488
Diode555	555
Diode639	639

- Click on show all and select tile (example 5x5 for 25 images) or (3 x 3)

- 

500
600
700
PMT 2: None
Split 591.0
LUT
PMT 2 Dye
LUT
/555/639
Lasers
NoneLSM
Stage
Focus
T-PMT
Display
Focus
Focus Devices and Strategy
Show all
Stage
Show all
Show all
Multidimensional Acquisition
Tile Scan
Show all
Scan overview image ...
Dimensions
Graphics
Zoom
Tools
100%
Channels
Ch1
Single Channel
Range
Reuse

Track3 - LSM

Lasers ☐ 405 ☐ 488 ☑ 555 ☐ 639

555 nm 1.1

Pinhole 41.2

0.89 Airy Units ≙ 0.7 µm section

A555

Gain (Master) 700

Digital Offset 0

Digital Gain 1.0

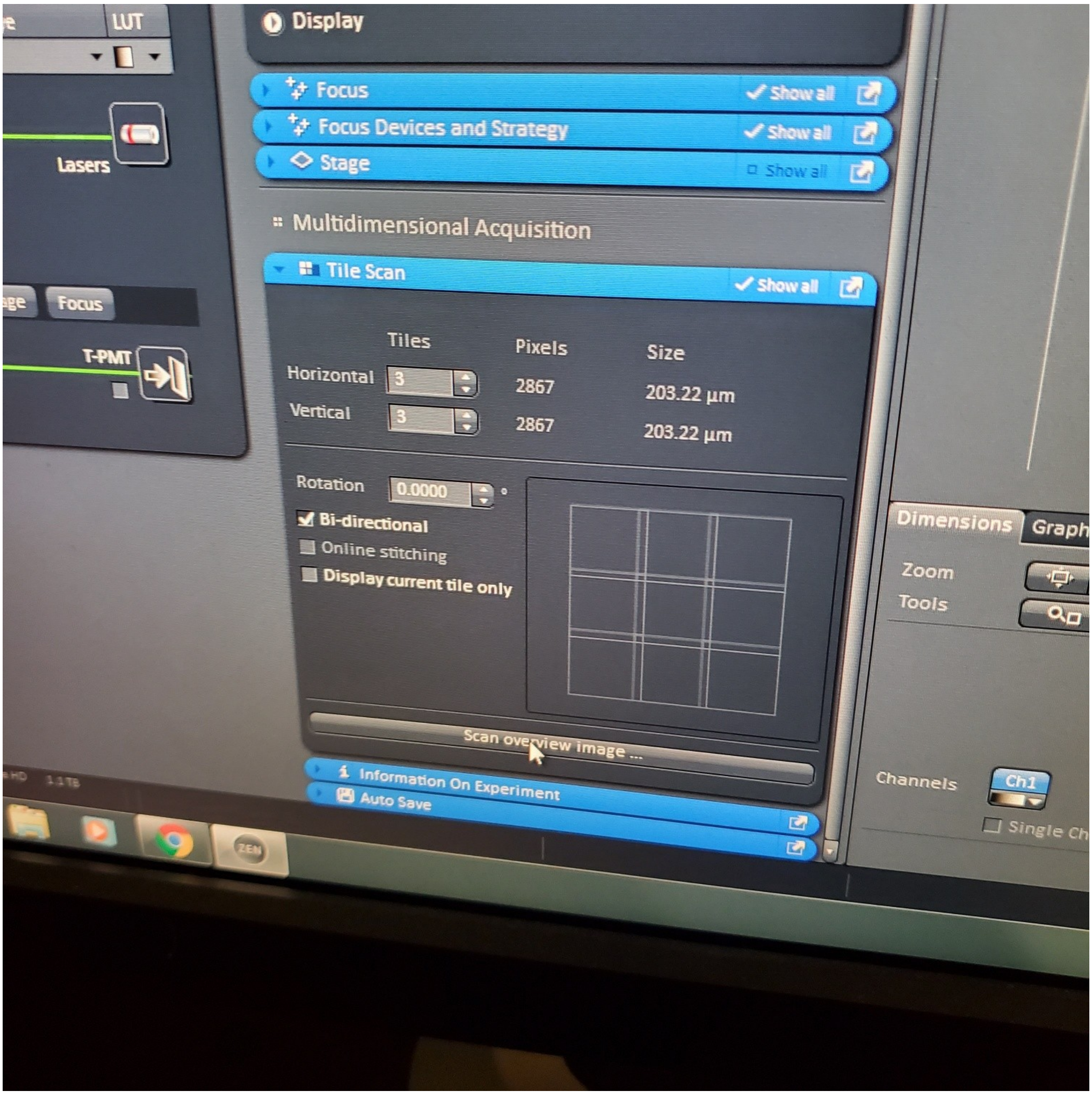
	Tiles	Pixels	Size
Horizontal	5	4710	333.85 µm
Vertical	5	4710	333.85 µm

Rotation 0.0000 °

☐ Bi-directional

☐ Online stitching

☐ Display current tile only

- 

LUT
Lasers
Focus
T-PMT
Display
Focus
Focus Devices and Strategy
Stage
Show all
Multidimensional Acquisition
Tile Scan
Scan overview image ...
Information On Experiment
Auto Save
Dimensions
Graph
Zoom
Tools
Channels
Ch1
Single Ch

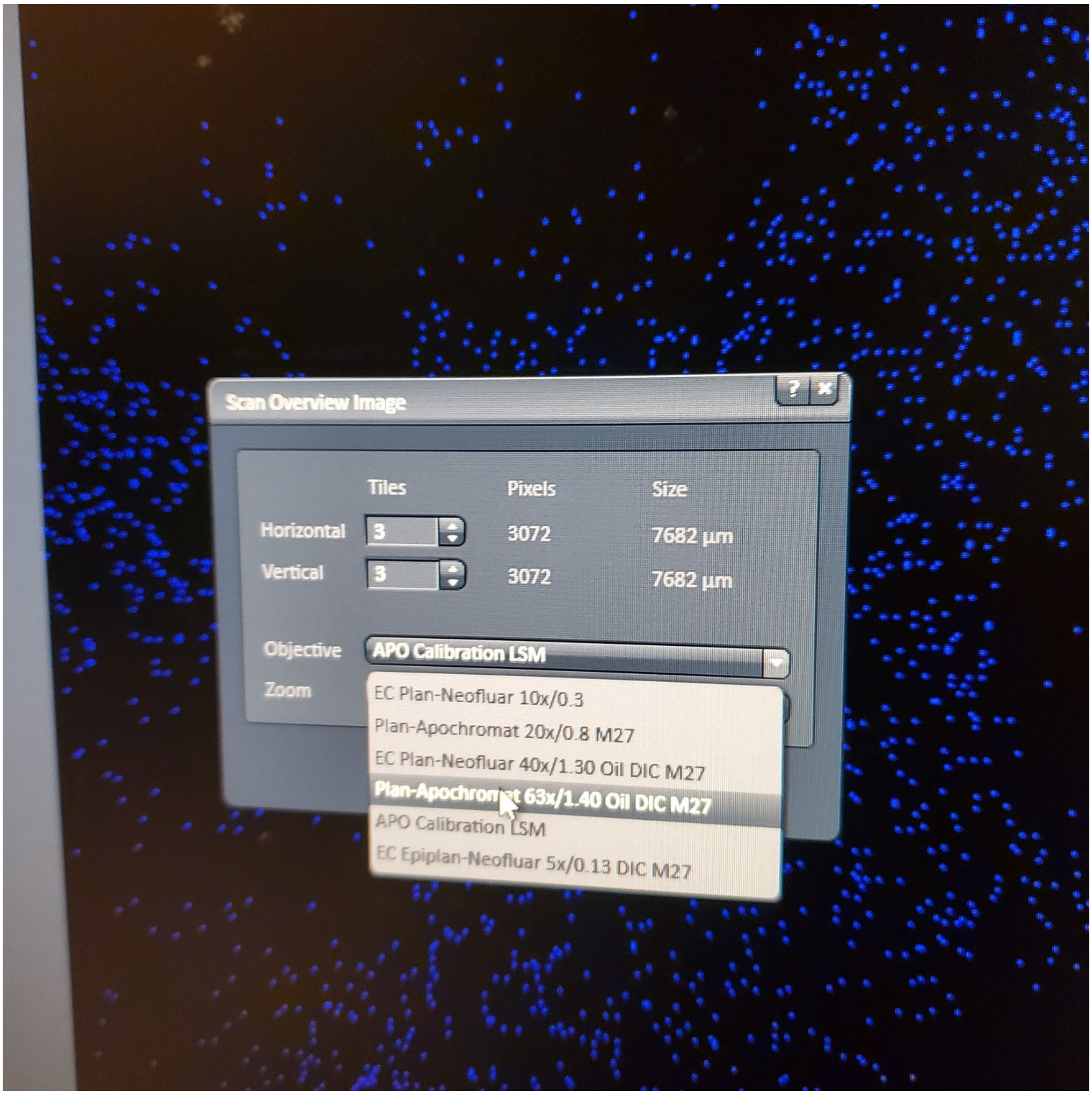
	Tiles	Pixels	Size
Horizontal	3	2867	203.22 µm
Vertical	3	2867	203.22 µm

Rotation 0.0000 °

☑ Bi-directional

☐ Online stitching

☐ Display current tile only

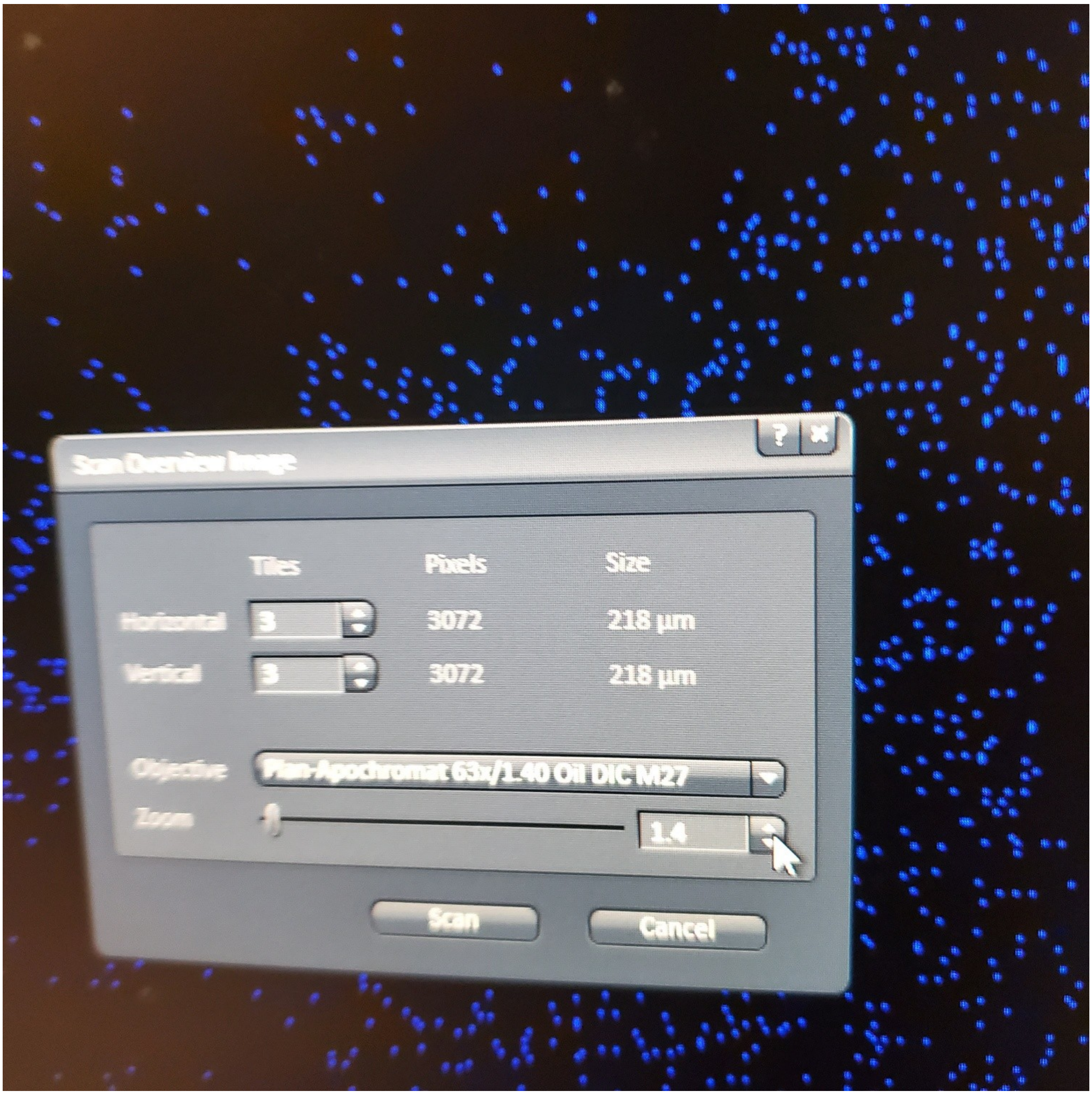
- 

Scan Overview Image

	Tiles	Pixels	Size
Horizontal	3	3072	7682 µm
Vertical	3	3072	7682 µm

Objective: APO Calibration LSM

Zoom
EC Plan-Neofluar 10x/0.3
Plan-Apochromat 20x/0.8 M27
EC Plan-Neofluar 40x/1.30 Oil DIC M27
Plan-Apochromat 63x/1.40 Oil DIC M27
APO Calibration LSM
EC Epiplan-Neofluar 5x/0.13 DIC M27

- 

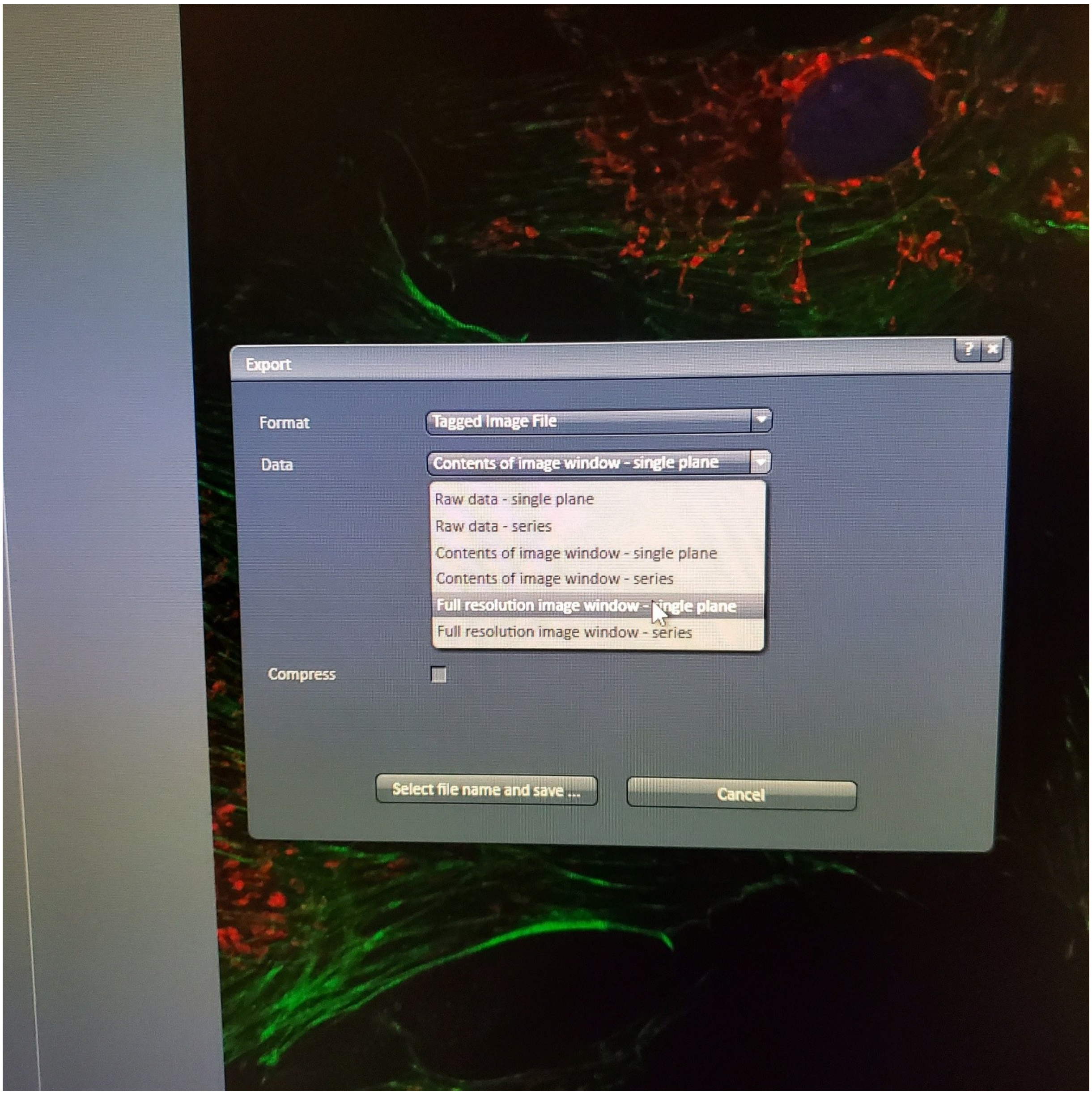
  Scan Overview Image

	Tiles	Pixels	Size
Horizontal	3	3072	218 µm
Vertical	3	3072	218 µm

  Objective: Plan-Apochromat 63x/1.40 Oil DIC M27

  Zoom: 1.4

- § Alternatively, for 10 or 20x just select the size of the image
  - Centered = current image is the very center of the size you select
  - Bounding grid lets you scan and move to positions an dmark
  - Convex hull generates an image the goes in a line from point a to point b.
  - Do not try to crop a tiled imaged by the crop tool – change the size using the image size (um) at the top of the right column.
  - If you see image issues, then set overlap to 10%.
  - § If you are using the 40 or 63x and doing tiling set the overlap to 10%
  - § Start experiment (upper left window)
  - You may export tiff file

  - 

Export

Format: Tagged Image File

Data: Contents of image window - single plane

Compress ☐

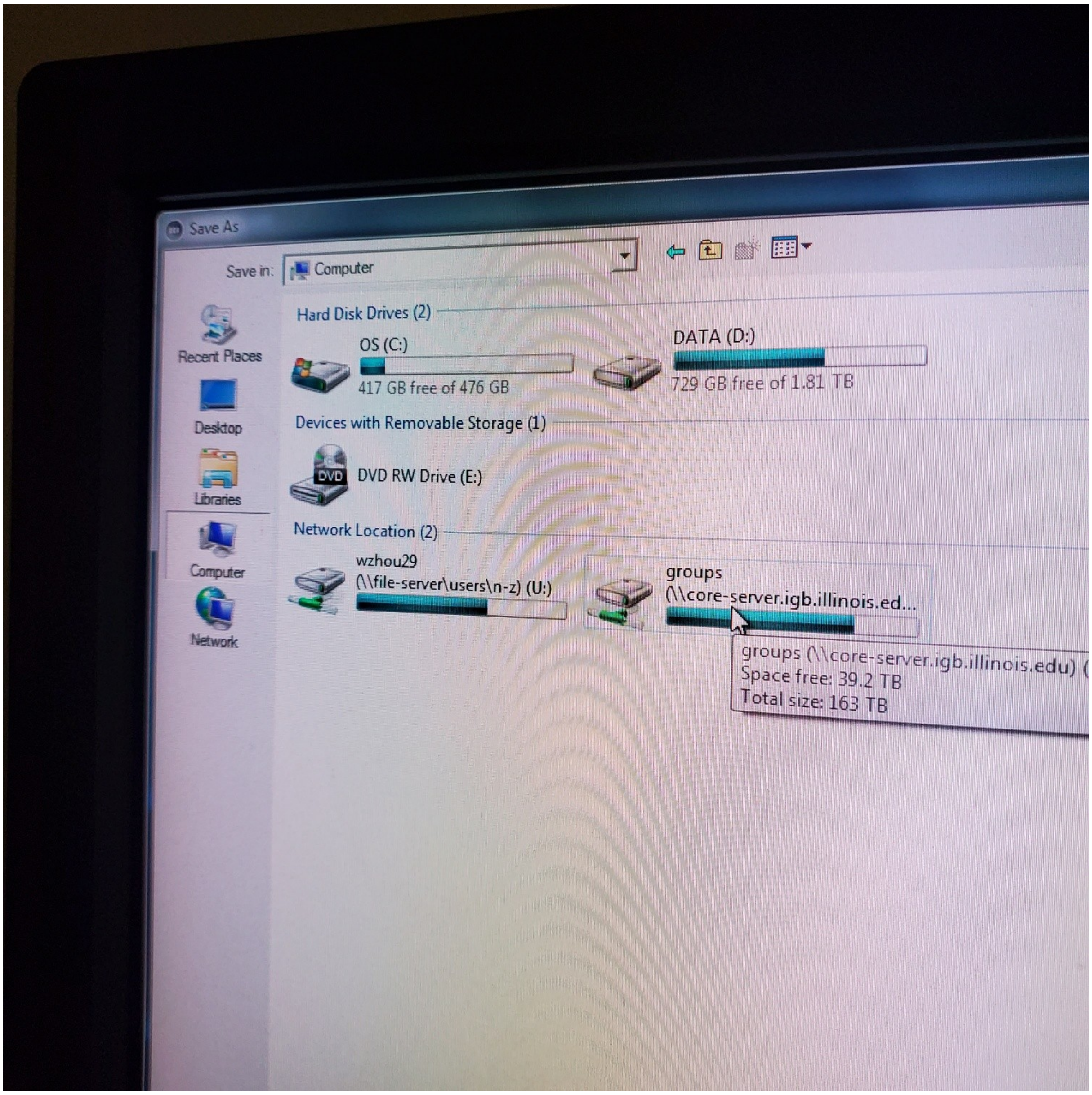
Raw data - single plane
Raw data - series
Contents of image window - single plane
Contents of image window - series
Full resolution image window - single plane
Full resolution image window - series
Select file name and save ...
Cancel

- o **Position (select at upper left)**
  - § Mark positions – marks X, Y, and Z.
  - If you want to do Z stacks at multiple positions, then each Z stack will be the same number of slices. Therefore, you need to make the Z position setting to be in the middle of your sample and then set the Z stack to go so many microns above and below your setting.
  - § Autofocus option
  - Fl = fluorescence is used to find sample. Takes a stack before it images and uses the brightest plane as the center. This can cause sample bleaching issues
  - Reflection = uses the transmitted light to detect the coverslip and you set the focus to be a certain number of microns above the converslip.
  - If you sample is sloshing around in liquid – none of these autofocus options will really help
- **Bleaching = photomanipulation (select at upper left)**
- § Time series and regions windows open by default
- § Scan image
- § Select the **[Regions]** and click show all:

  - Draw desired shape or shapes
  - Do not check acquisition box or you will only image the bleached region
  - The scope will scan the whole way across the X direction and only bleach the region boxed. This means your box should always be longest in the X direction (you can always rotate your sample).
  - Zoom Bleach – doesn’t scan all the way across in the X direction so it is faster, but less accurate.
  - § **[Bleaching]** and click show all:
  - Check start bleaching after a number of scans (2 is good)
  - Set - Repeat bleach after # of scans (pick a number)
  - Set - Stop when intensity drops to % starting intensity (after this it will no longer bleach, but it will continue to image)
  - **Iterations** – 20-100 is a good place to start (this and laser power are the points to adjust)
  - Select laser to use to bleach and laser power (**25%)**
  - § **[Time Series]** and click show all
  - Select number of cycles = number of time points.
  - Leave interval at 0 – usually you want to see this as fast as possible § Notes:
  - In the gallery view, the green boxes mark the upper left corner of the image that was taken before the bleach.
  - We currently do not have the analysis plugin for FRAP – you can analyze the data in Image J or metamorph.
  - Please note that when you select a region it will scan across the full X and only bleach in the region of interest. So if you have an oblong or rectangle region you want to bleach it is fastest to orient the longest part along the X plane.

When your session is done, make sure:

- You have transferred your files to IGB group server / lab folder

- 
Save As
Recent Places
Desktop
Libraries
Computer
Network

Save in: Computer

Hard Disk Drives (2)

OS (C:)
417 GB free of 476 GB

DATA (D:)
729 GB free of 1.81 TB

Devices with Removable Storage (1)

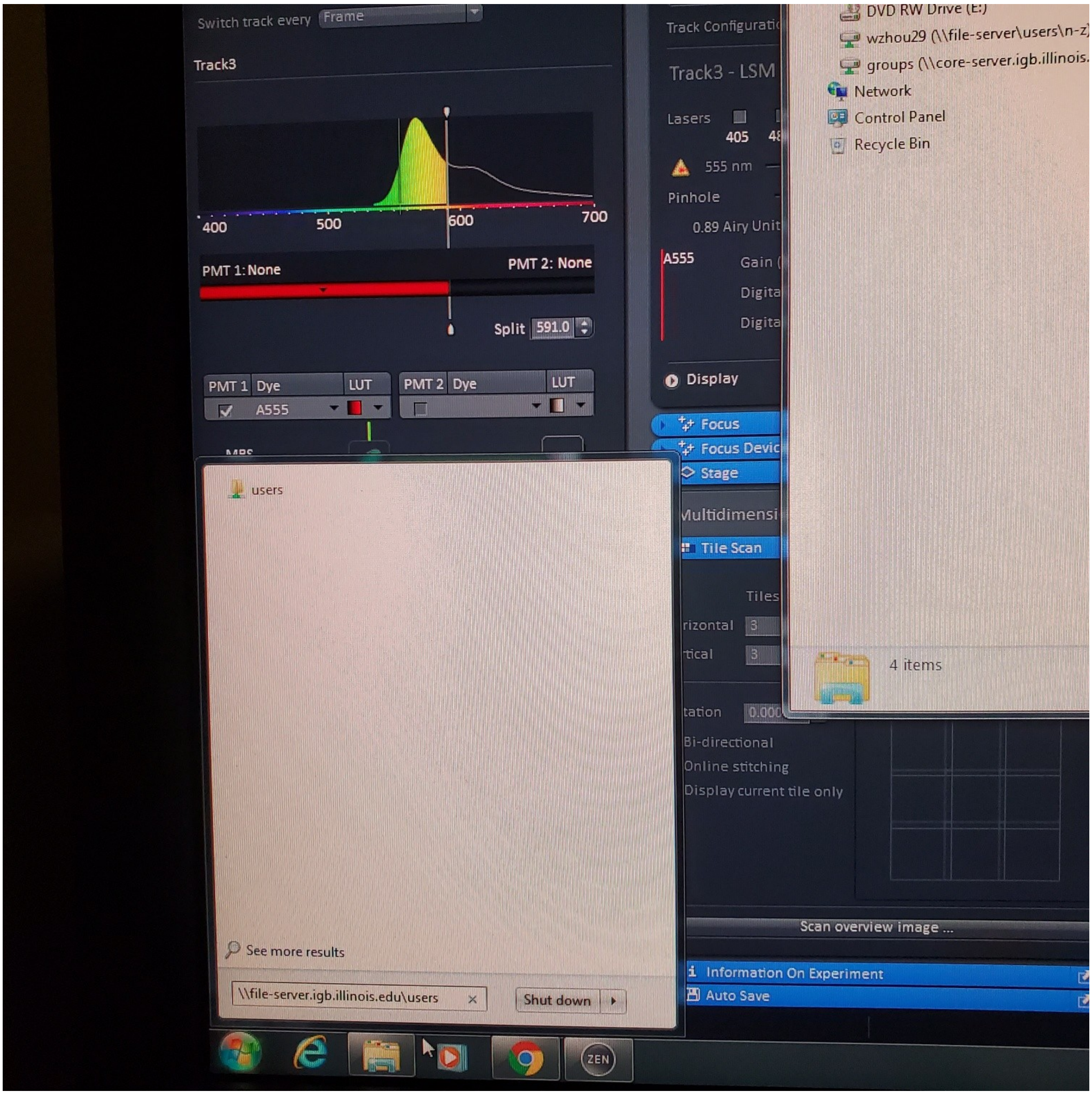
DVD RW Drive (E:)

Network Location (2)

wzhou29
(\\file-server\users\n-z) (U:)

groups
(\\core-server.igb.illinois.ed...

groups (\\core-server.igb.illinois.edu) (
Space free: 39.2 TB
Total size: 163 TB

- 
Switch track every
Frame
Track3
400
500
600
700
PMT 1: None
PMT 2: None
Split 591.0
PMT 1
Dye
LUT
A555
PMT 2
Dye
LUT
users
See more results
Shut down
Track Configuration
Track3 - LSM
Lasers
405
555 nm
Pinhole
0.89 Airy Unit
A555
Gain
Digital
Digital
Display
Focus
Focus Device
Stage
Multidimensional
Tile Scan
Tiles
rizontal 3
rtical 3
tation 0.000
Bi-directional
Online stitching
Display current tile only
Scan overview image ...
Information On Experiment
Auto Save
DVD RW Drive (E:)
wzhou29 (\\file-server\users\n-z)
groups (\\core-server.igb.illinois.
Network
Control Panel
Recycle Bin
4 items
ZEN

\\file-server.igb.illinois.edu\users

- Clean oil off 40x and 63x objectives with lens paper not kim wipes.
- Return to 10x objective
- Follow shutdown procedure

Shutdown

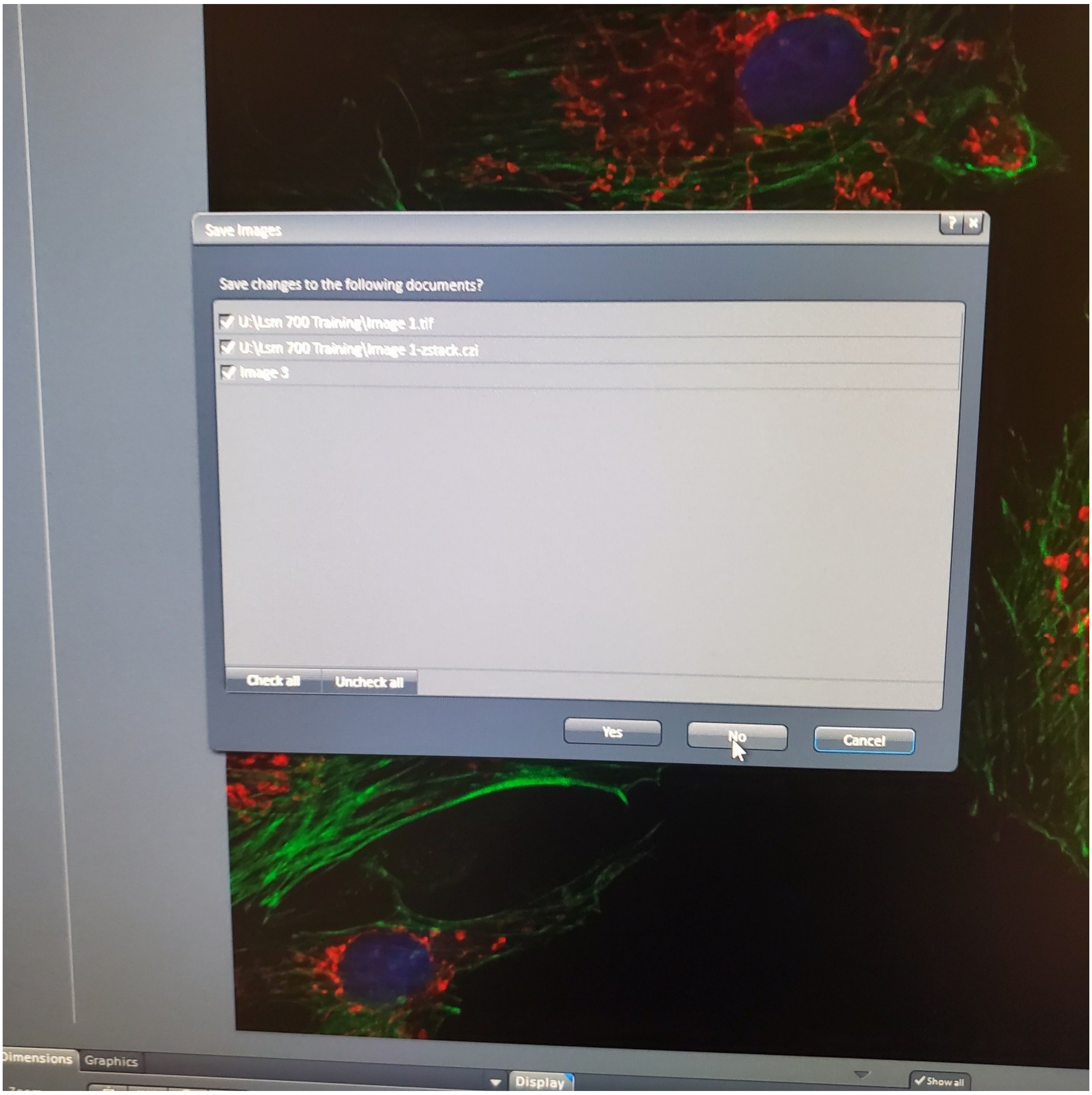
Check calendar to determine if someone is scheduled after you (within 1 hour).

IF Yes:

- Turn off the software and logout

IF No:

- Exit the Zen software and log off computer

- 

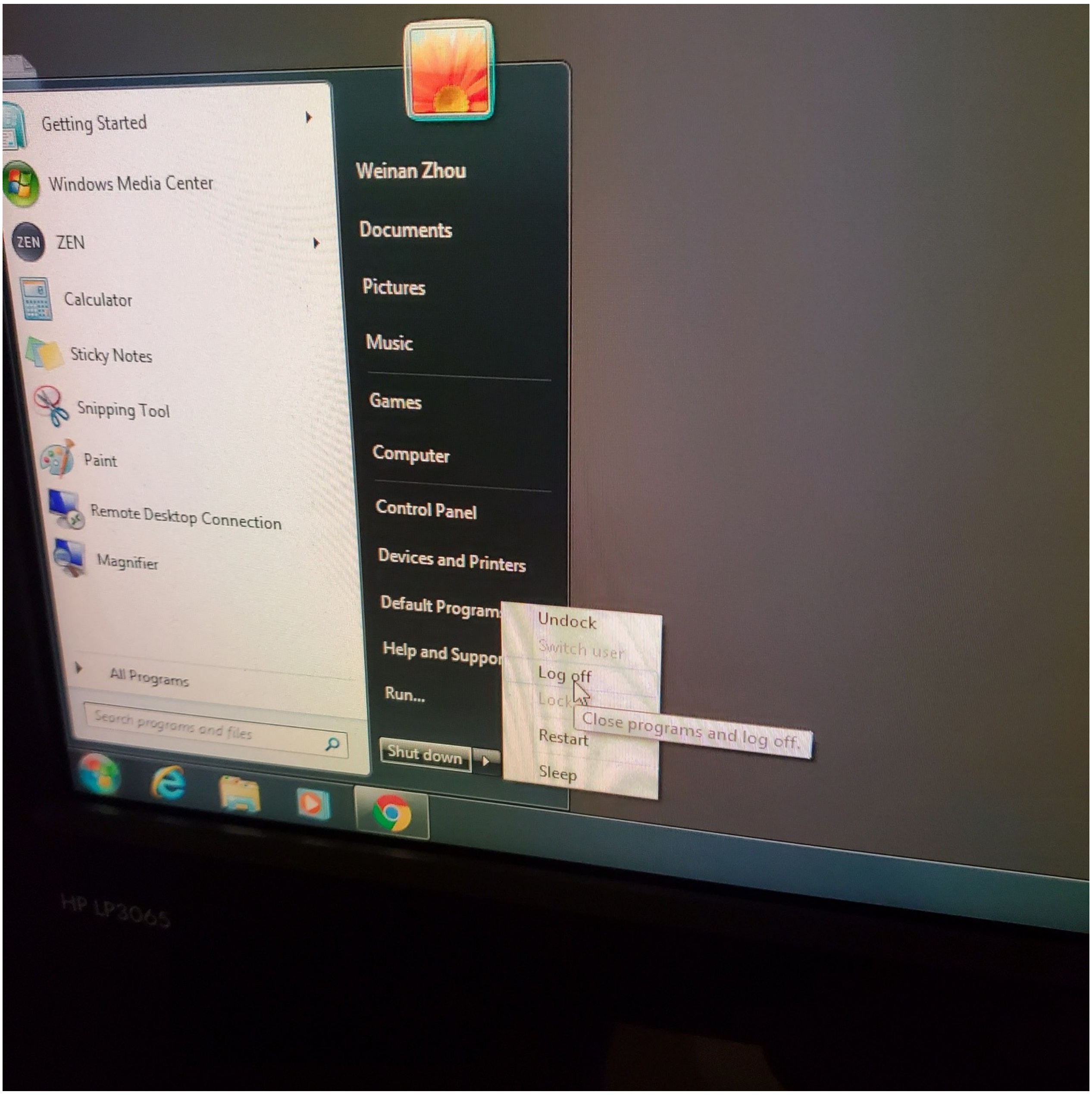
Save Images

Save changes to the following documents?

☑ U:\Lsm 700 Training\Image 1.tif
☑ U:\Lsm 700 Training\Image 1-zstack.czi
☑ Image 3

Check all
Uncheck all
Yes
No
Cancel
Dimensions
Graphics
Display
Show all

- Turn off laser lamp
- Don’t turn of the microscope or anything else.


Getting Started
Windows Media Center
ZEN
Calculator
Sticky Notes
Snipping Tool
Paint
Remote Desktop Connection
Magnifier
All Programs
Search programs and files
Weinan Zhou
Documents
Pictures
Music
Games
Computer
Control Panel
Devices and Printers
Default Programs
Help and Support
Run...
Shut down
Undock
Switch user
Log off
Lock
Close programs and log off.
Restart
Sleep
HP LP3065

If you have any problems:

- **Always report any problems to Core Staff**

Viewing and Processing Data

To view and process you images, ZEN 2012 lite freeware is available in www.zeiss.com/lsm. Fill the form and you will receive a link for installation via email

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Revision #3
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