Short Notes- Use of Celldiscoverer 7 with Airyscan (Typ2)

Starting up the System

- Turn on the PC power button
- Login with your user LIC account
- Double click the “ZenBlue Software button”
- Start “Zen system”
- After the first logon please change the **default storage path**
    - Tools – Options – Saving – AutoSavePath

Insert and Tray Options

- You can choose between different inserts
- ! Note that with the **6 x petri dish insert - NO multi-dish position experiment can be performed** as these 6 individual dishes as carrier cannot be calibrated together!

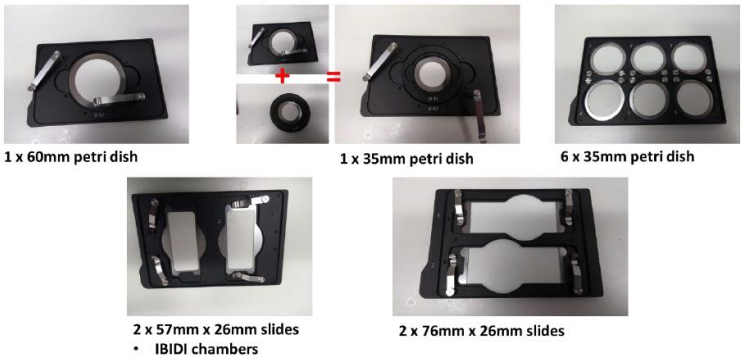


Figure 1 Inserts for Celldiscoverer (S1)

Figure 2 Trays for Celldiscoverer (S1)

If you use INCUBATION (temperature, Co2 and Humidity)

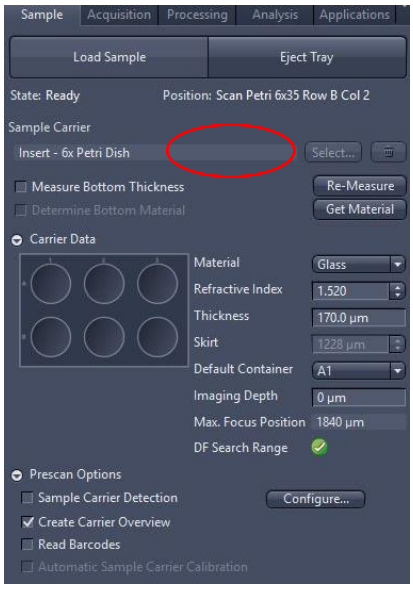
- Check the demineralized water level in the humidity bottle
    - if low, fill midway (no tap water)

- Active **in Zen software** (incubation window right sides tools) the required controls (heating, C02 and humidity)
- If you use INCUBATION wait at least 15 min to equilibrate.

Load Sample

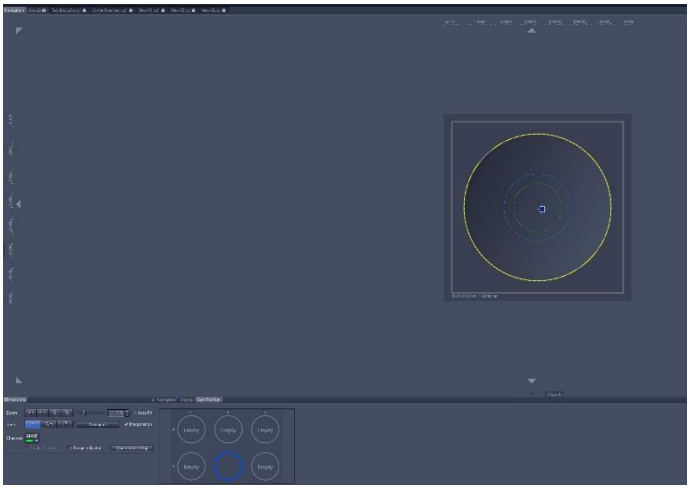
- If the tray is not outside, click '**eject tray'**
- Place you tray with your insert and sample on the stage
- Select the Sample carrier type, press **select** button
- You can use automation functions such as 'measurement of bottom material' and 'determine bottom material' or you choose bottom material from the menu.
- As well, there are prescan options as 'sample carrier detection', 'create carrier overview' and 'automatic sample carrier calibration' (works only for default templates **not IBIDI**
    - Ibidi must be calibrated with the 'Sample Carrier calibration Wizard')
- We suggest **select bottom material** from **top down menu** and select **'create carrier overview'**

- According to the bottom thickness the system will adjust correction rings of the objectives automatically



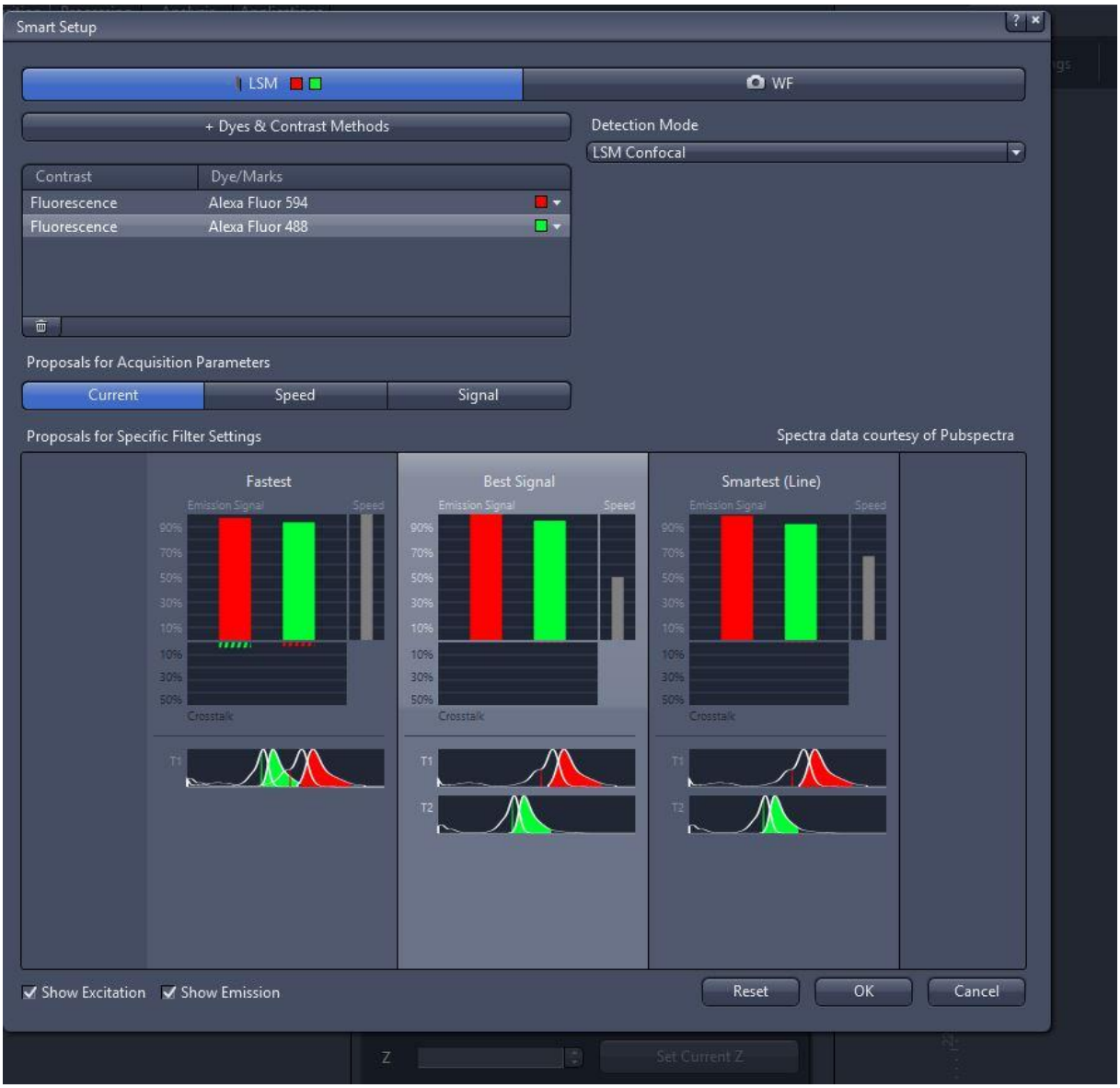
Carrier Overview

Navigation tab

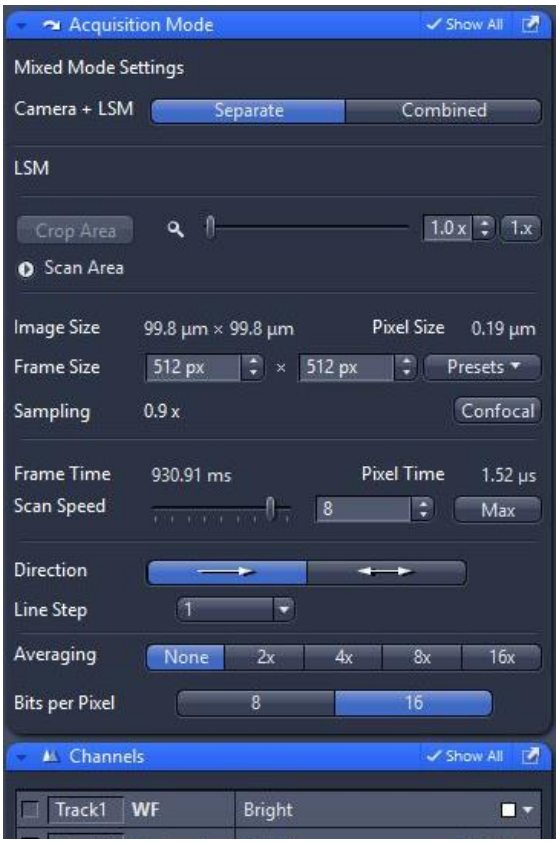


Objective

Smart Setup confocal and WF

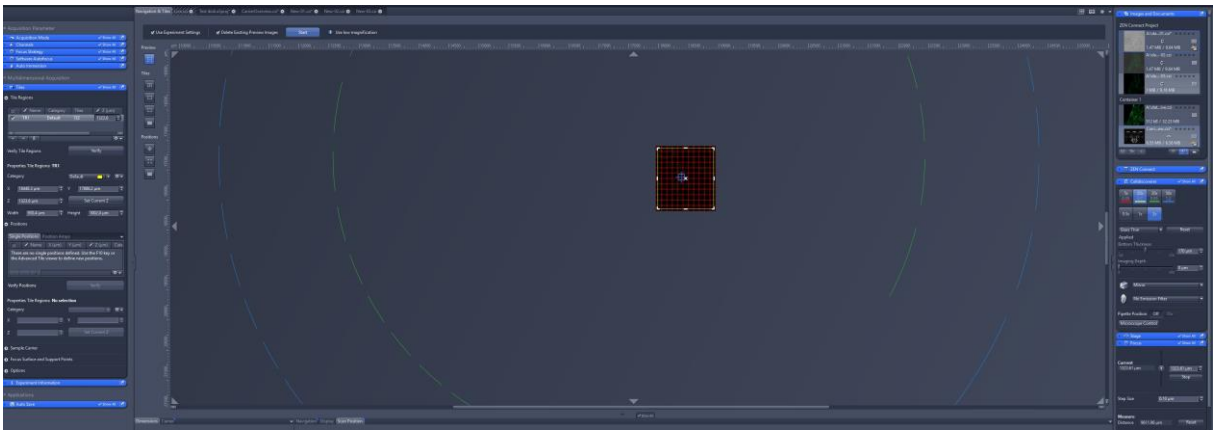


Acquisition separate confocal WF

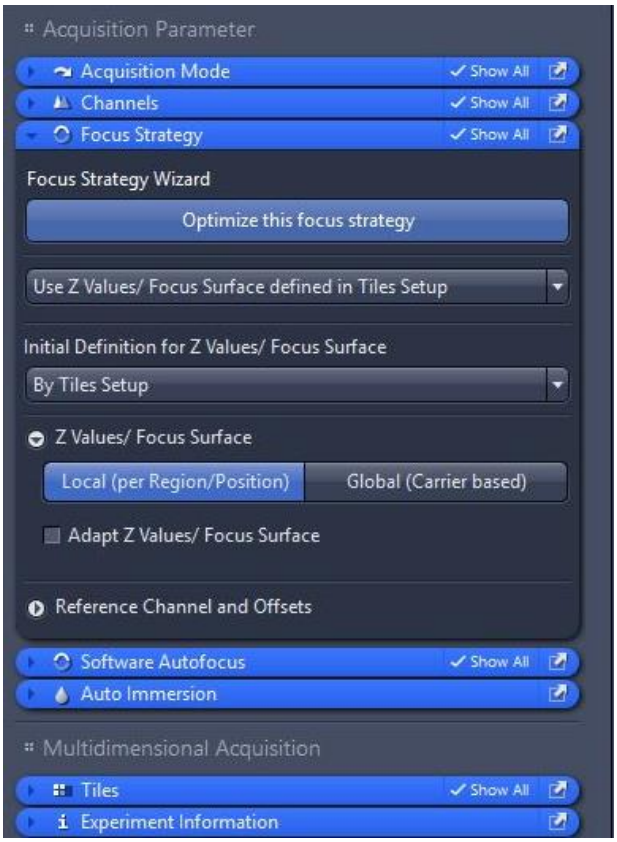


Acquisition combined confocal WF

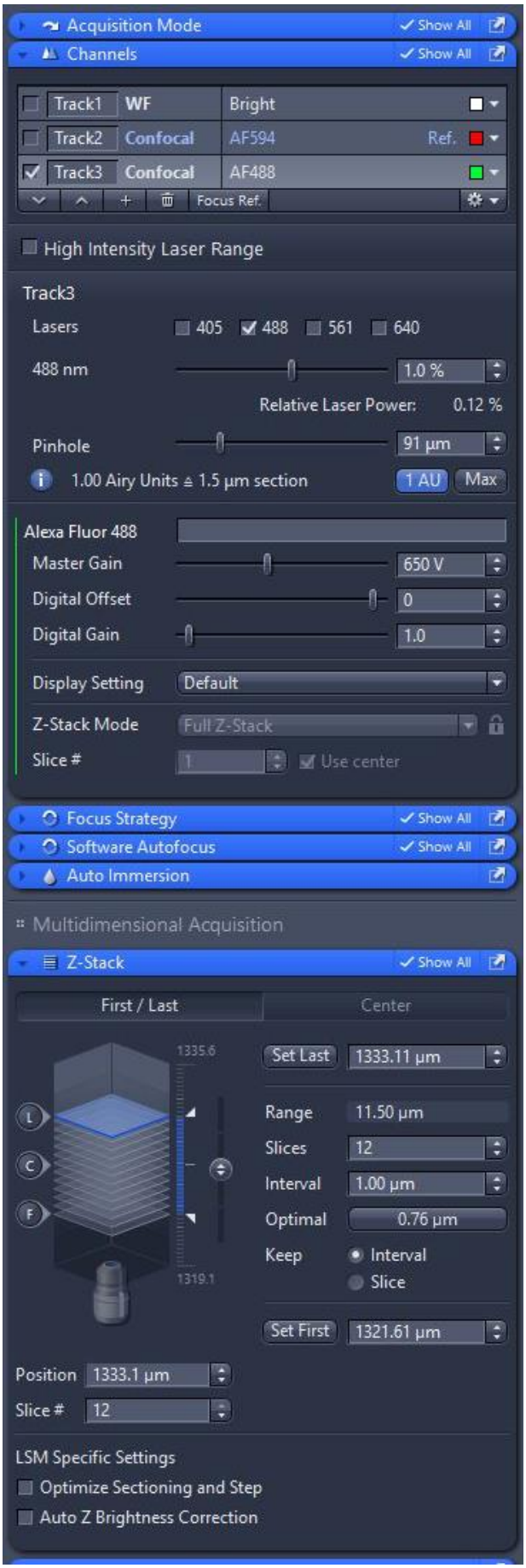
Setup Tiles Scan



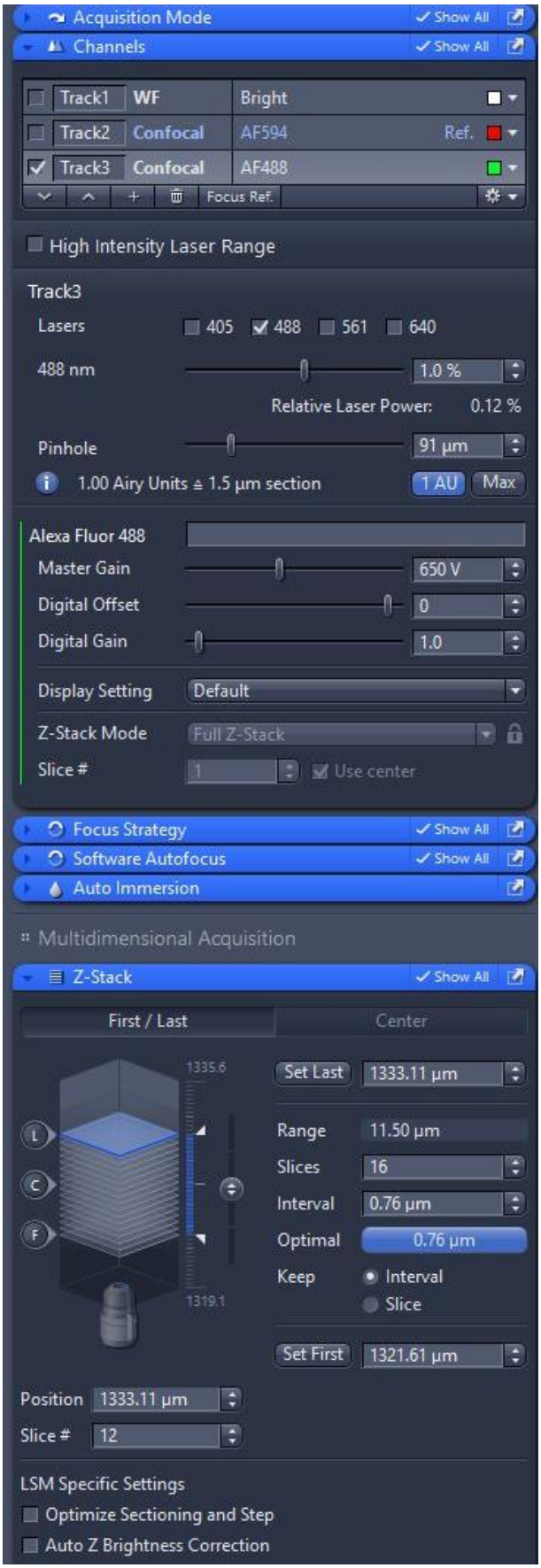
Setup Tiles Scan – Focus Strategy



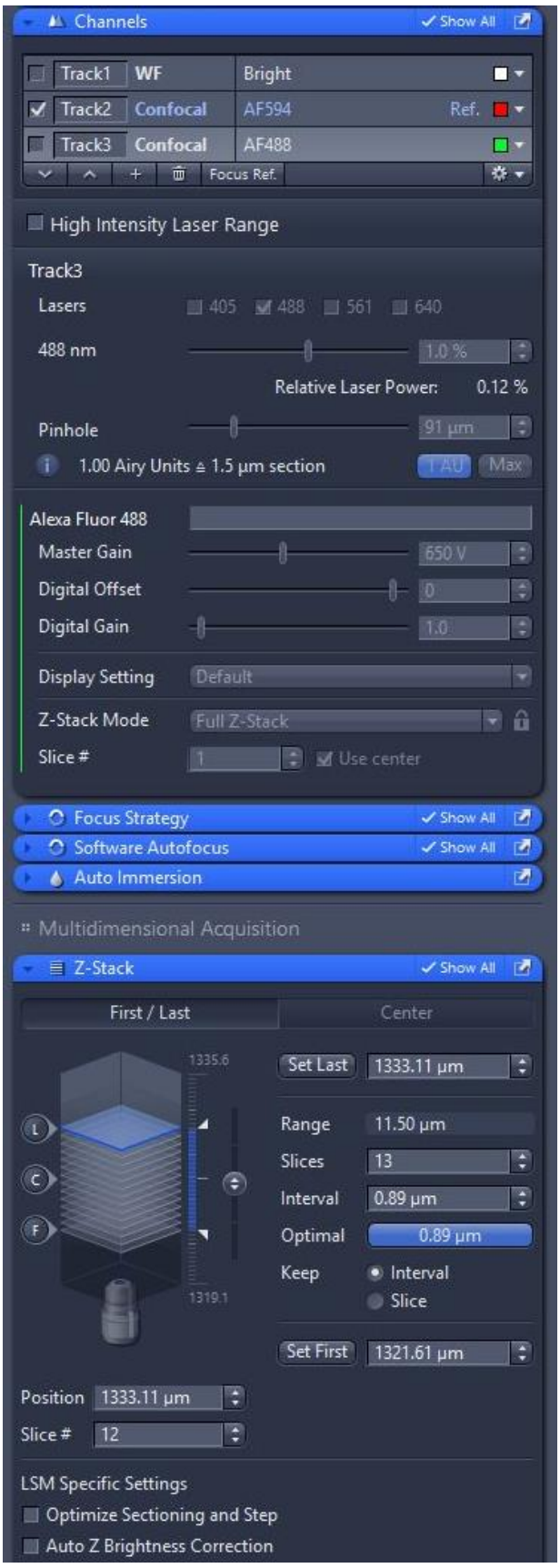
Setup Z-stack


Acquisition Mode
Show All
Channels
Show All
Track1 WF Bright
Track2 Confocal AF594 Ref.
Track3 Confocal AF488
Focus Ref.
High Intensity Laser Range
Track3
Lasers 405 488 561 640
488 nm 1.0 %
Relative Laser Power: 0.12 %
Pinhole 91 µm
1.00 Airy Units ≙ 1.5 µm section
1 AU
Max
Alexa Fluor 488
Master Gain 650 V
Digital Offset 0
Digital Gain 1.0
Display Setting Default
Z-Stack Mode Full Z-Stack
Slice # 1
Use center
Focus Strategy
Show All
Software Autofocus
Show All
Auto Immersion
Multidimensional Acquisition
Z-Stack
Show All
First / Last
Center
1335.6
1319.1
Set Last 1333.11 µm
Range 11.50 µm
Slices 12
Interval 1.00 µm
Optimal 0.76 µm
Keep Interval
Slice
Set First 1321.61 µm
Position 1333.1 µm
Slice # 12
LSM Specific Settings
Optimize Sectioning and Step
Auto Z Brightness Correction

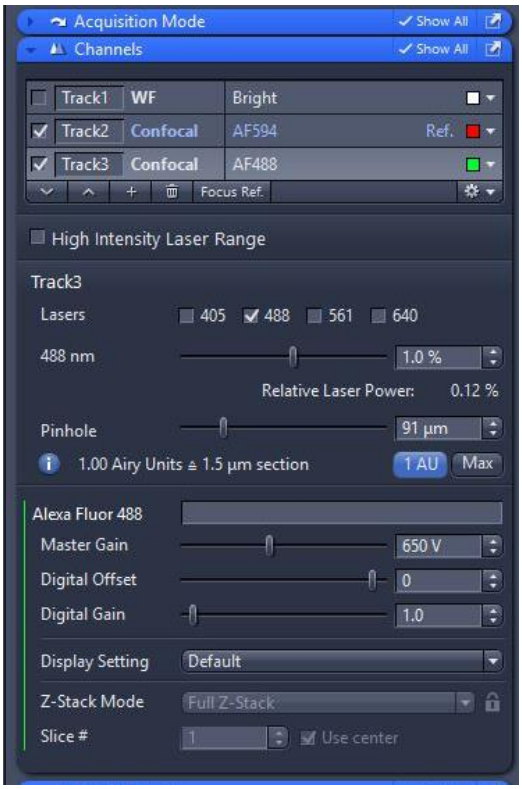
Setup Z-stack

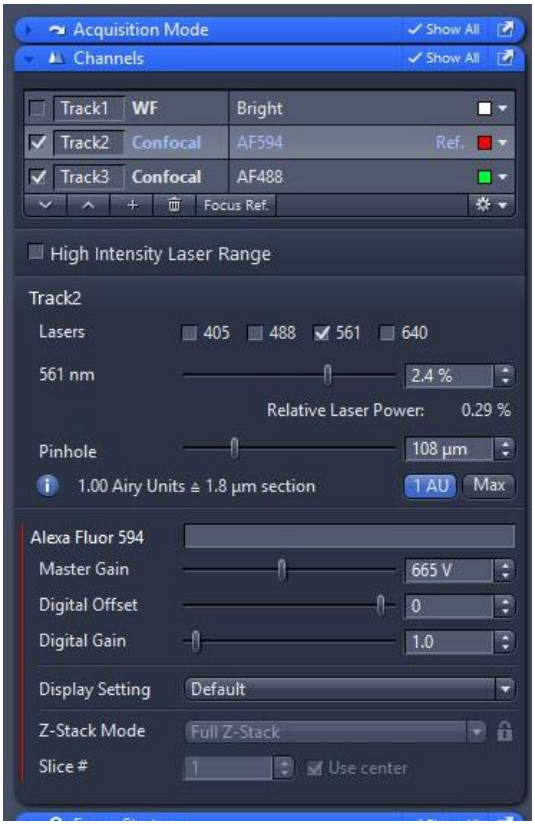

Acquisition Mode
Show All
Channels
Show All
Track1 WF Bright
Track2 Confocal AF594 Ref.
Track3 Confocal AF488
Focus Ref.
High Intensity Laser Range
Track3
Lasers 405 488 561 640
488 nm 1.0 %
Relative Laser Power: 0.12 %
Pinhole 91 µm
1.00 Airy Units ≙ 1.5 µm section
1 AU
Max
Alexa Fluor 488
Master Gain 650 V
Digital Offset 0
Digital Gain 1.0
Display Setting Default
Z-Stack Mode Full Z-Stack
Slice # 1
Use center
Focus Strategy
Show All
Software Autofocus
Show All
Auto Immersion
Multidimensional Acquisition
Z-Stack
Show All
First / Last
Center
1335.6
1319.1
Set Last 1333.11 µm
Range 11.50 µm
Slices 16
Interval 0.76 µm
Optimal 0.76 µm
Keep Interval
Slice
Set First 1321.61 µm
Position 1333.11 µm
Slice # 12
LSM Specific Settings
Optimize Sectioning and Step
Auto Z Brightness Correction

Setup Z-stack – Interval- automatically change

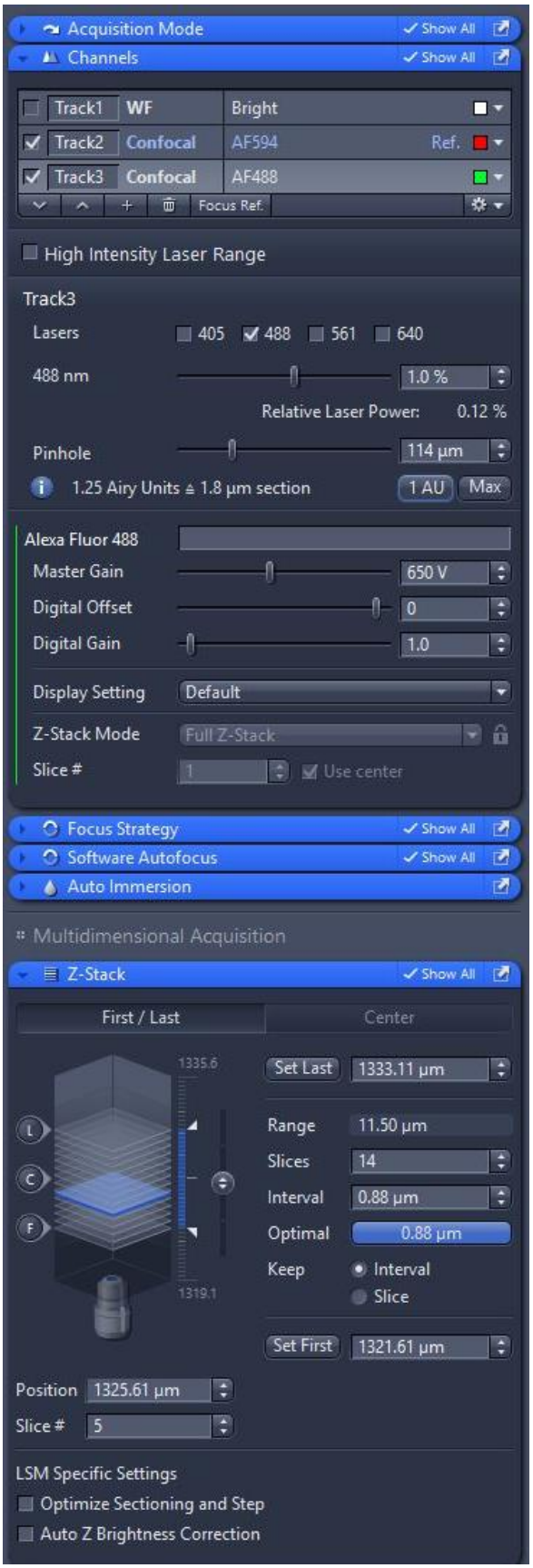


Pinhole 1 AU – compare setup different channel

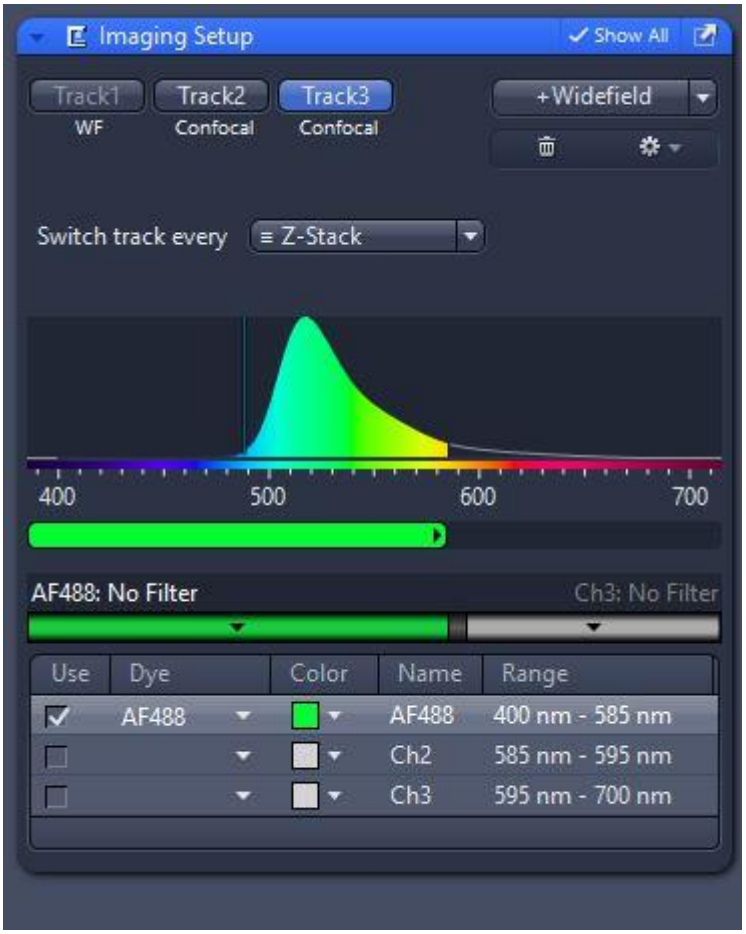


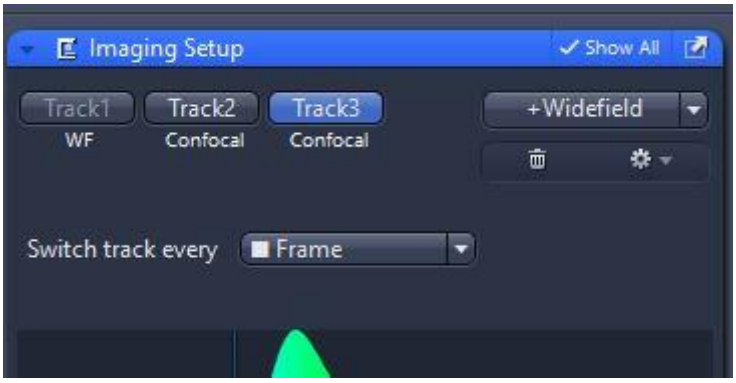


Setup Z-stack – Interval- is now same for both channel

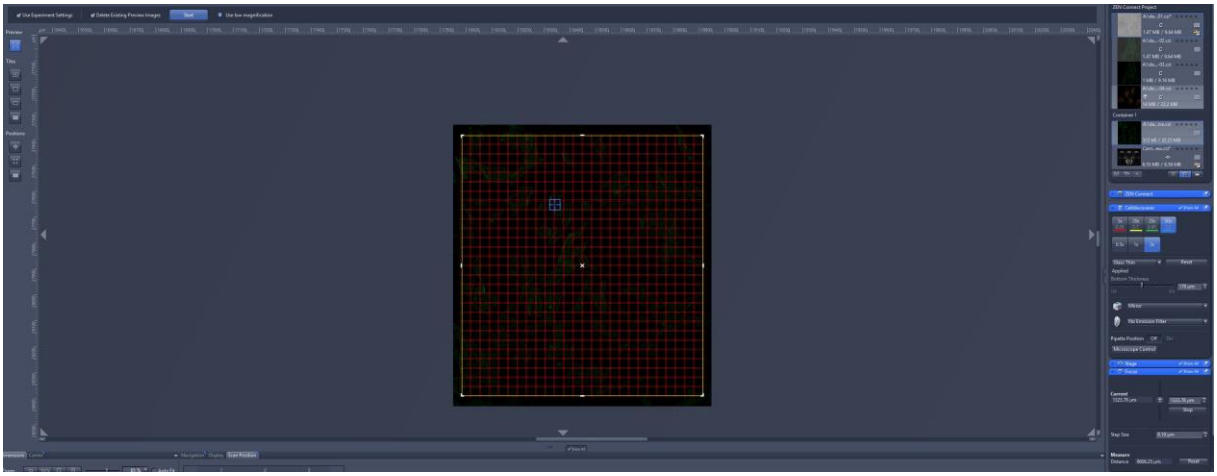

Acquisition Mode
Show All
Channels
Show All
Track1 WF Bright
Track2 Confocal AF594 Ref.
Track3 Confocal AF488
Focus Ref.
High Intensity Laser Range
Track3
Lasers 405 488 561 640
488 nm 1.0 %
Relative Laser Power: 0.12 %
Pinhole 114 µm
1.25 Airy Units ≙ 1.8 µm section
1 AU
Max
Alexa Fluor 488
Master Gain 650 V
Digital Offset 0
Digital Gain 1.0
Display Setting Default
Z-Stack Mode Full Z-Stack
Slice # 1
Use center
Focus Strategy
Show All
Software Autofocus
Show All
Auto Immersion
Multidimensional Acquisition
Z-Stack
Show All
First / Last
Center
1335.6
1319.1
Set Last 1333.11 µm
Range 11.50 µm
Slices 14
Interval 0.88 µm
Optimal 0.88 µm
Keep Interval
Slice
Set First 1321.61 µm
Position 1325.61 µm
Slice # 5
LSM Specific Settings
Optimize Sectioning and Step
Auto Z Brightness Correction

Setup Z-stack – Mode for switch

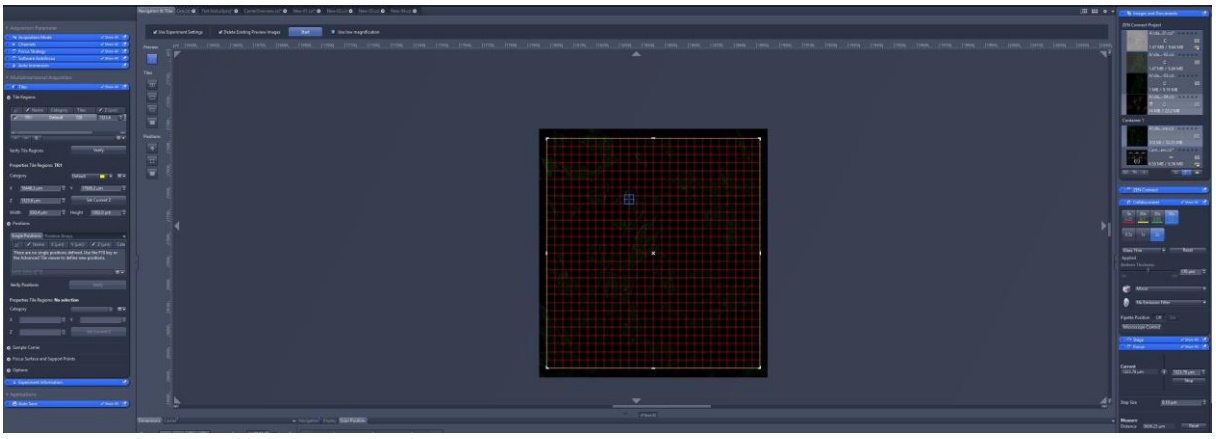




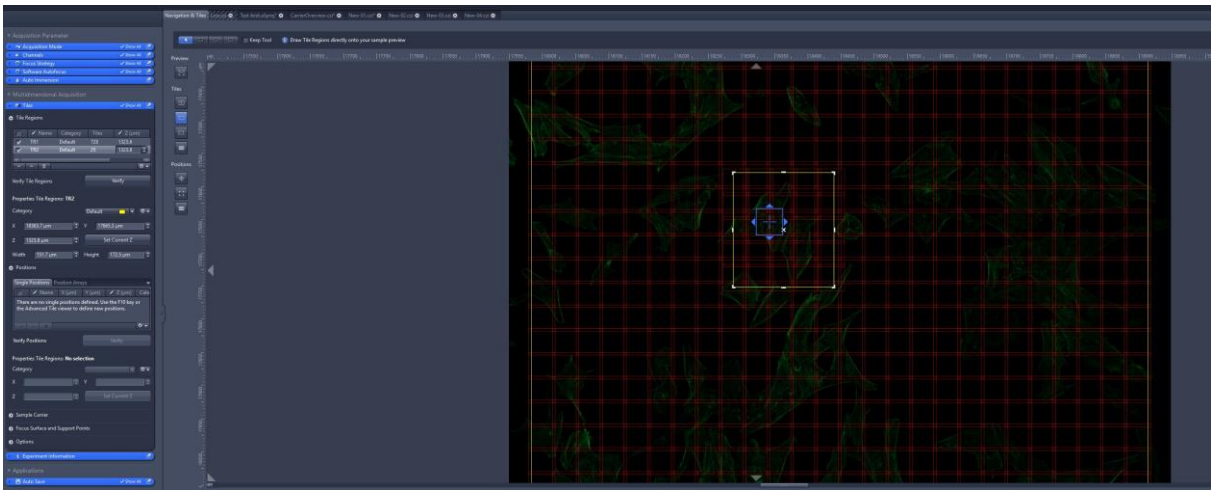
Tile scan now with 50x rectangle are changed automatically

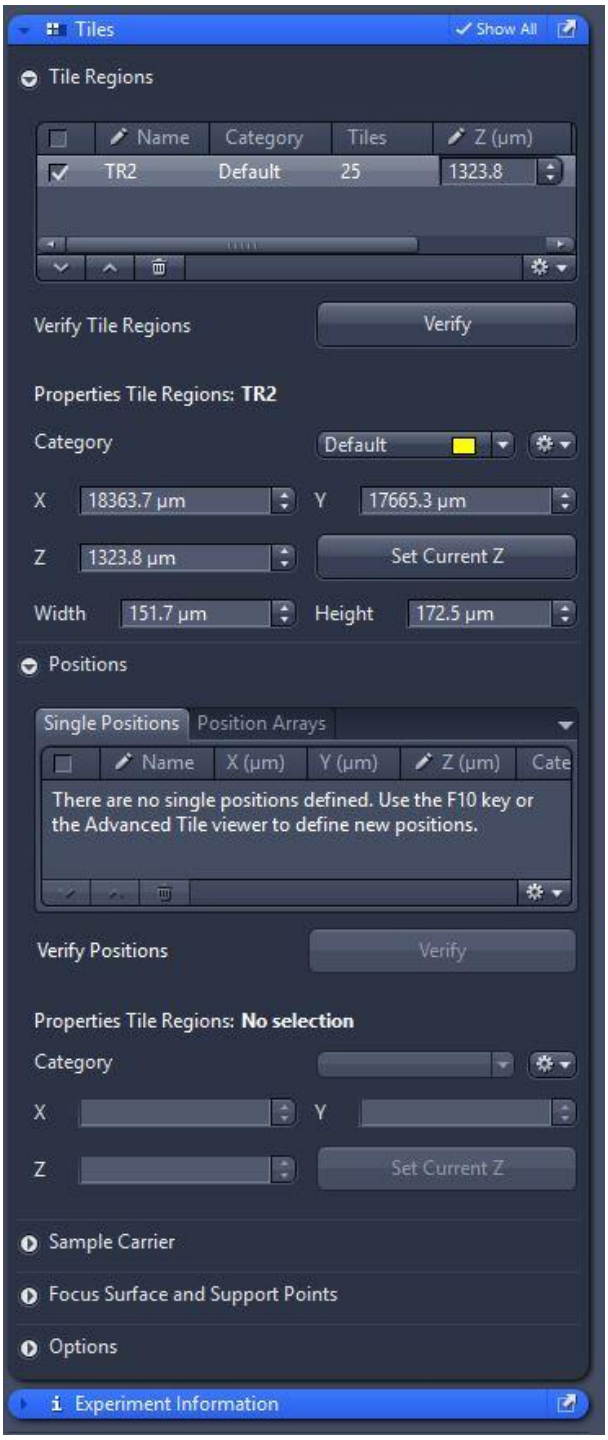


Also, if I have changed the Focus in live in the setup the old Focus will be used – update to new before start

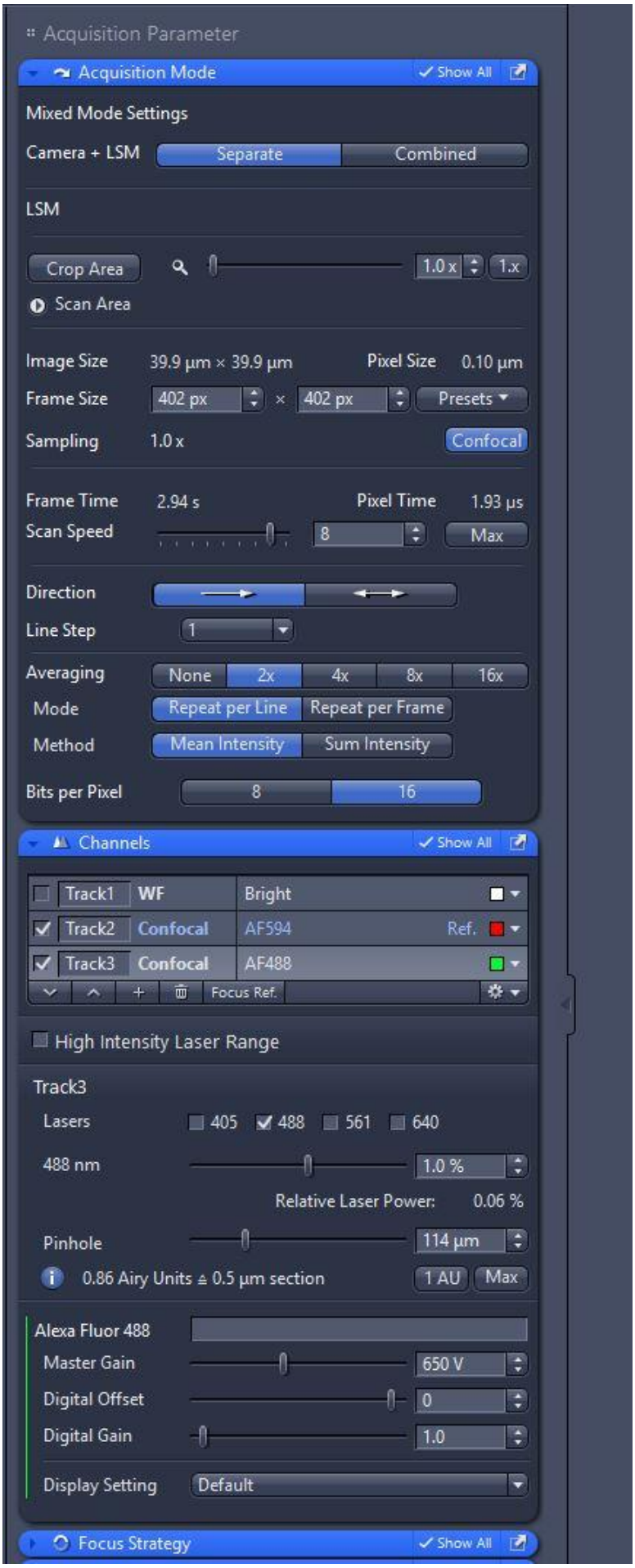


Or define new tile region and delete old tiles

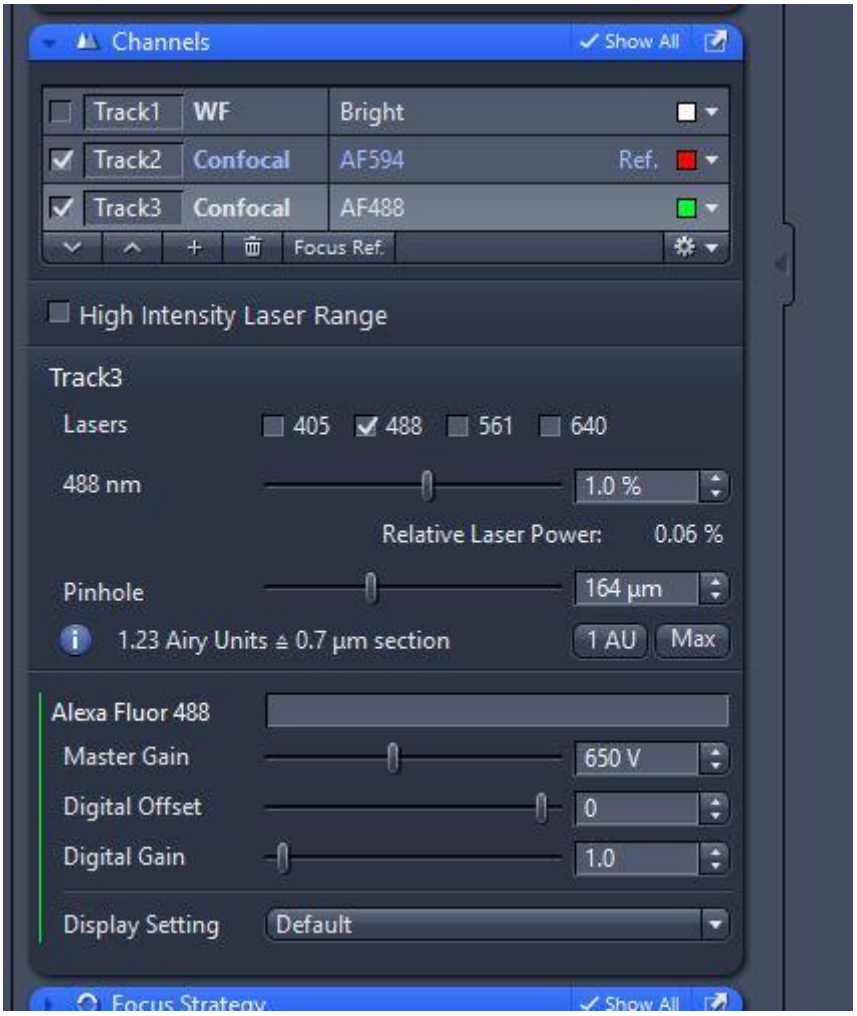


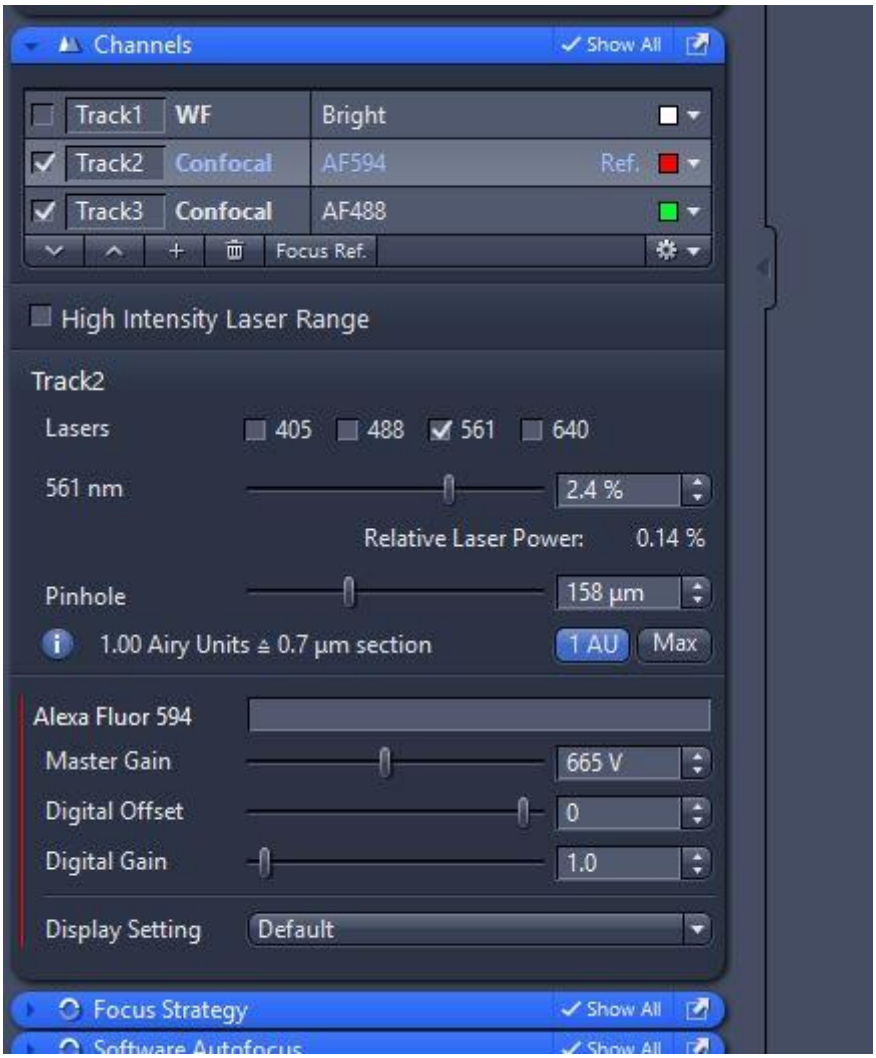


Last set now the best values for speed, sampling and averaging

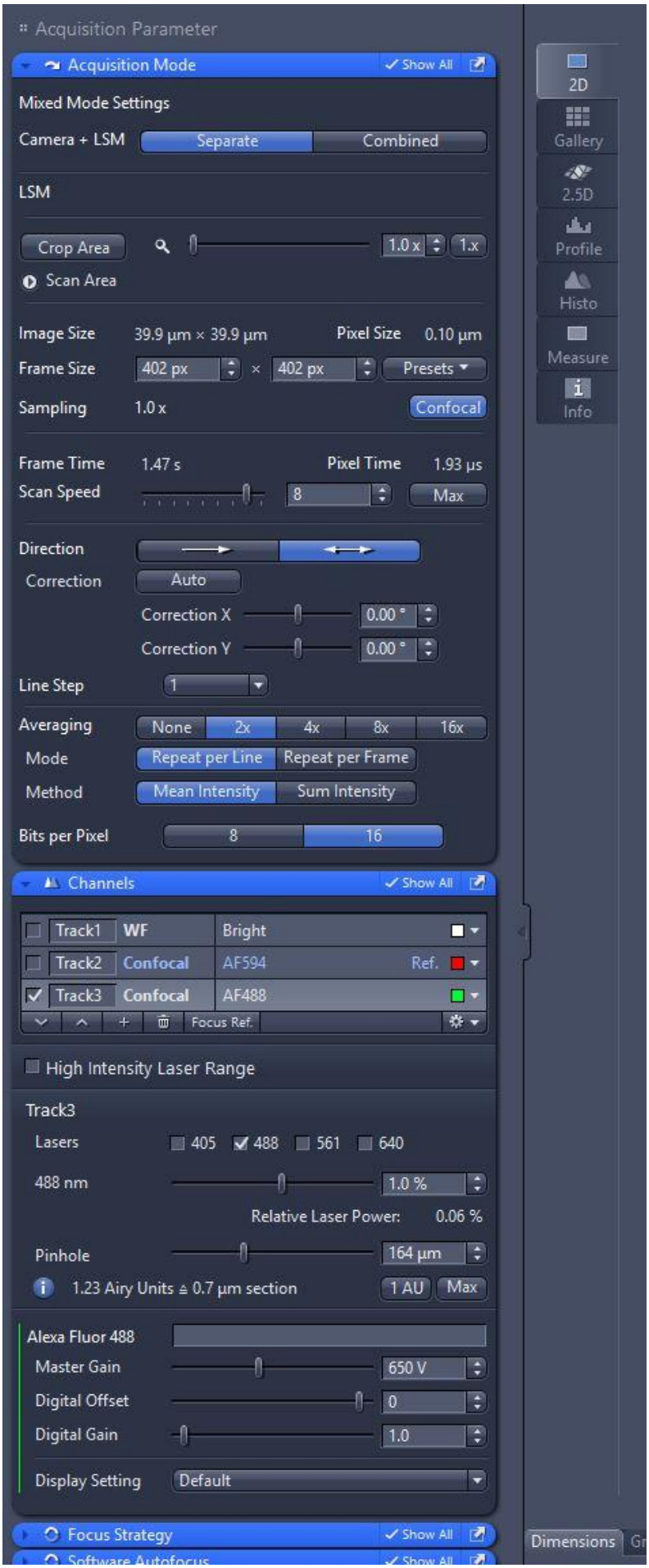

Acquisition Parameter
Acquisition Mode
Show All
Mixed Mode Settings
Camera + LSM
Separate
Combined
LSM
Crop Area
1.0 x
1.x
Scan Area
Image Size
39.9 µm × 39.9 µm
Pixel Size
0.10 µm
Frame Size
402 px
402 px
Presets
Sampling
1.0 x
Confocal
Frame Time
2.94 s
Pixel Time
1.93 µs
Scan Speed
8
Max
Direction
Line Step
1
Averaging
None
2x
4x
8x
16x
Mode
Repeat per Line
Repeat per Frame
Method
Mean Intensity
Sum Intensity
Bits per Pixel
8
16
Channels
Show All
Track1
WF
Bright
Track2
Confocal
AF594
Ref.
Track3
Confocal
AF488
Focus Ref.
High Intensity Laser Range
Track3
Lasers
405
488
561
640
488 nm
1.0 %
Relative Laser Power:
0.06 %
Pinhole
114 µm
0.86 Airy Units ≙ 0.5 µm section
1 AU
Max
Alexa Fluor 488
Master Gain
650 V
Digital Offset
0
Digital Gain
1.0
Display Setting
Default
Focus Strategy
Show All

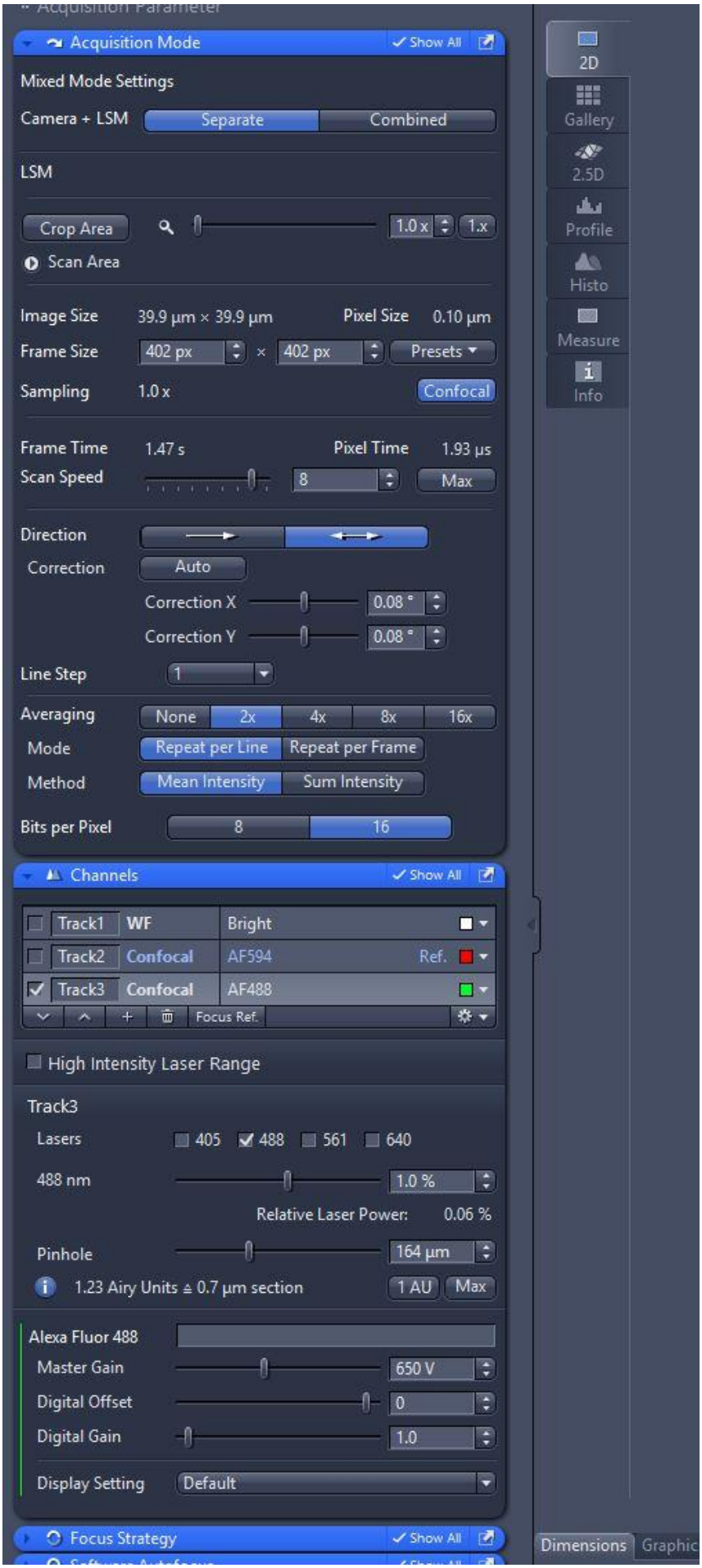
Check again for pinhole and section thickness





If you want Bidirectional scanning autoalign


Acquisition Parameter
Acquisition Mode
Show All
Mixed Mode Settings
Camera + LSM
Separate
Combined
LSM
Crop Area
1.0 x
1.x
Scan Area
Image Size
39.9 µm × 39.9 µm
Pixel Size
0.10 µm
Frame Size
402 px
402 px
Presets
Sampling
1.0 x
Confocal
Frame Time
1.47 s
Pixel Time
1.93 µs
Scan Speed
8
Max
Direction
Correction
Auto
Correction X
0.00 °
Correction Y
0.00 °
Line Step
1
Averaging
None
2x
4x
8x
16x
Mode
Repeat per Line
Repeat per Frame
Method
Mean Intensity
Sum Intensity
Bits per Pixel
8
16
Channels
Show All
Track1
WF
Bright
Track2
Confocal
AF594
Ref.
Track3
Confocal
AF488
Focus Ref.
High Intensity Laser Range
Track3
Lasers
405
488
561
640
488 nm
1.0 %
Relative Laser Power:
0.06 %
Pinhole
164 µm
1.23 Airy Units ≙ 0.7 µm section
1 AU
Max
Alexa Fluor 488
Master Gain
650 V
Digital Offset
0
Digital Gain
1.0
Display Setting
Default
Focus Strategy
Show All
Software Autofocus
Show All
2D
Gallery
2.5D
Profile
Histo
Measure
Info
Dimensions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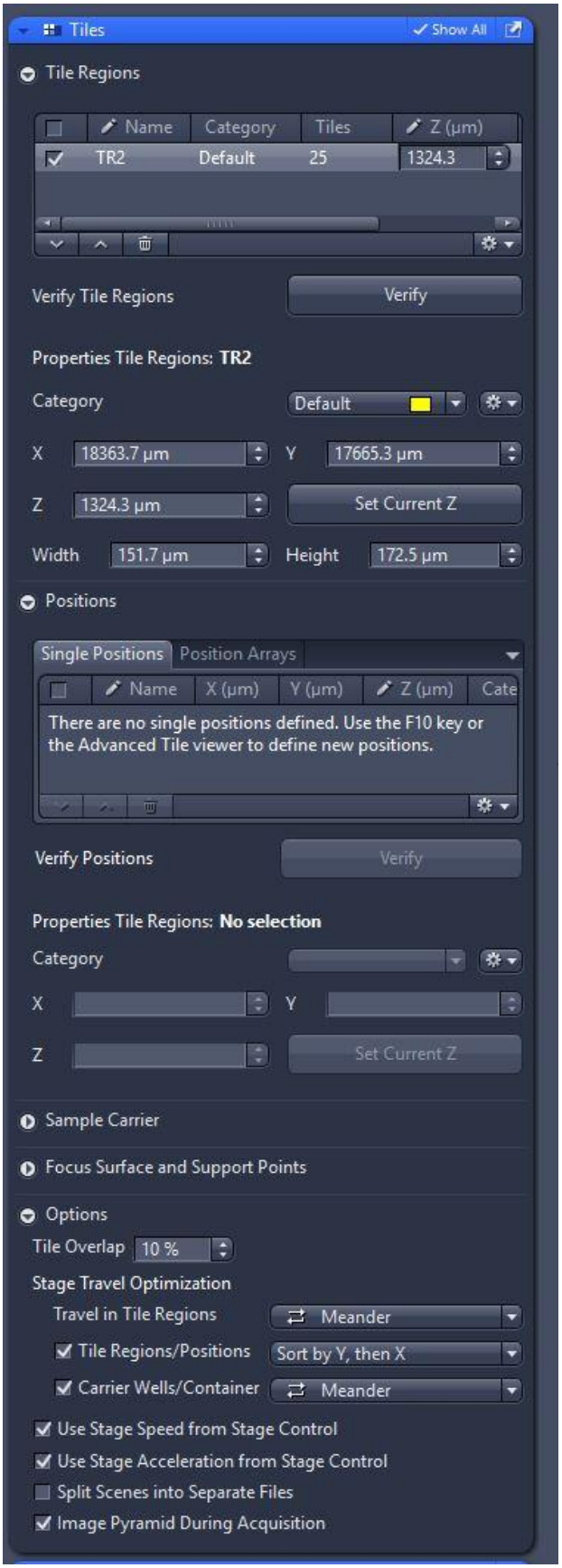
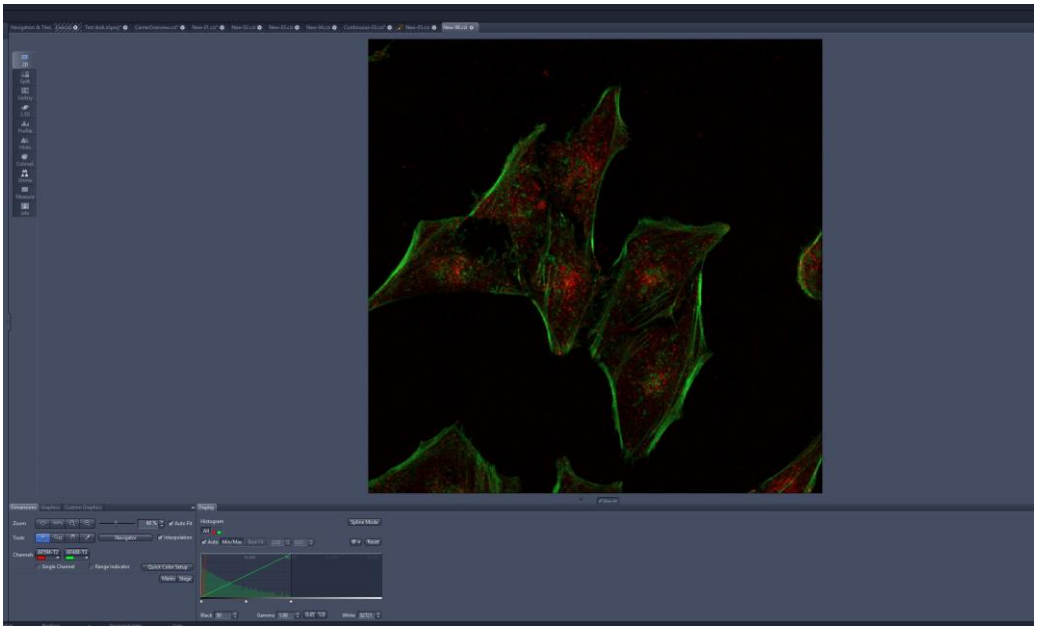
Mixed Mode Settings
Camera + LSM
Separate
Combined
LSM
Crop Area
1.0 x
1.x
Scan Area
Image Size
39.9 µm × 39.9 µm
Pixel Size
0.10 µm
Frame Size
402 px
402 px
Presets
Sampling
1.0 x
Confocal
Frame Time
1.47 s
Pixel Time
1.93 µs
Scan Speed
8
Max
Direction
Correction
Auto
Correction X
0.08 °
Correction Y
0.08 °
Line Step
1
Averaging
None
2x
4x
8x
16x
Mode
Repeat per Line
Repeat per Frame
Method
Mean Intensity
Sum Intensity
Bits per Pixel
8
16
Channels
Show All
Track1 WF
Bright
Track2 Confocal
AF594
Ref.
Track3 Confocal
AF488
Focus Ref.
High Intensity Laser Range
Track3
Lasers
405
488
561
640
488 nm
1.0 %
Relative Laser Power:
0.06 %
Pinhole
164 µm
1.23 Airy Units ≙ 0.7 µm section
1 AU
Max
Alexa Fluor 488
Master Gain
650 V
Digital Offset
0
Digital Gain
1.0
Display Setting
Default
Focus Strategy
Show All
2D
Gallery
2.5D
Profile
Histo
Measure
Info
Dimensions
Graphic

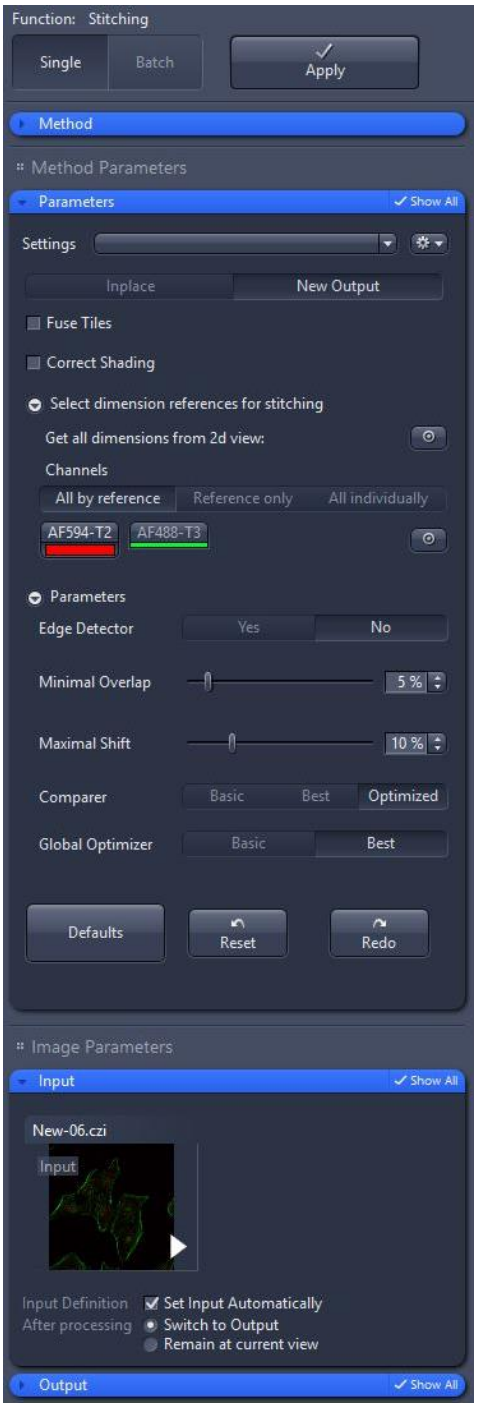
Tile-scan Overlap

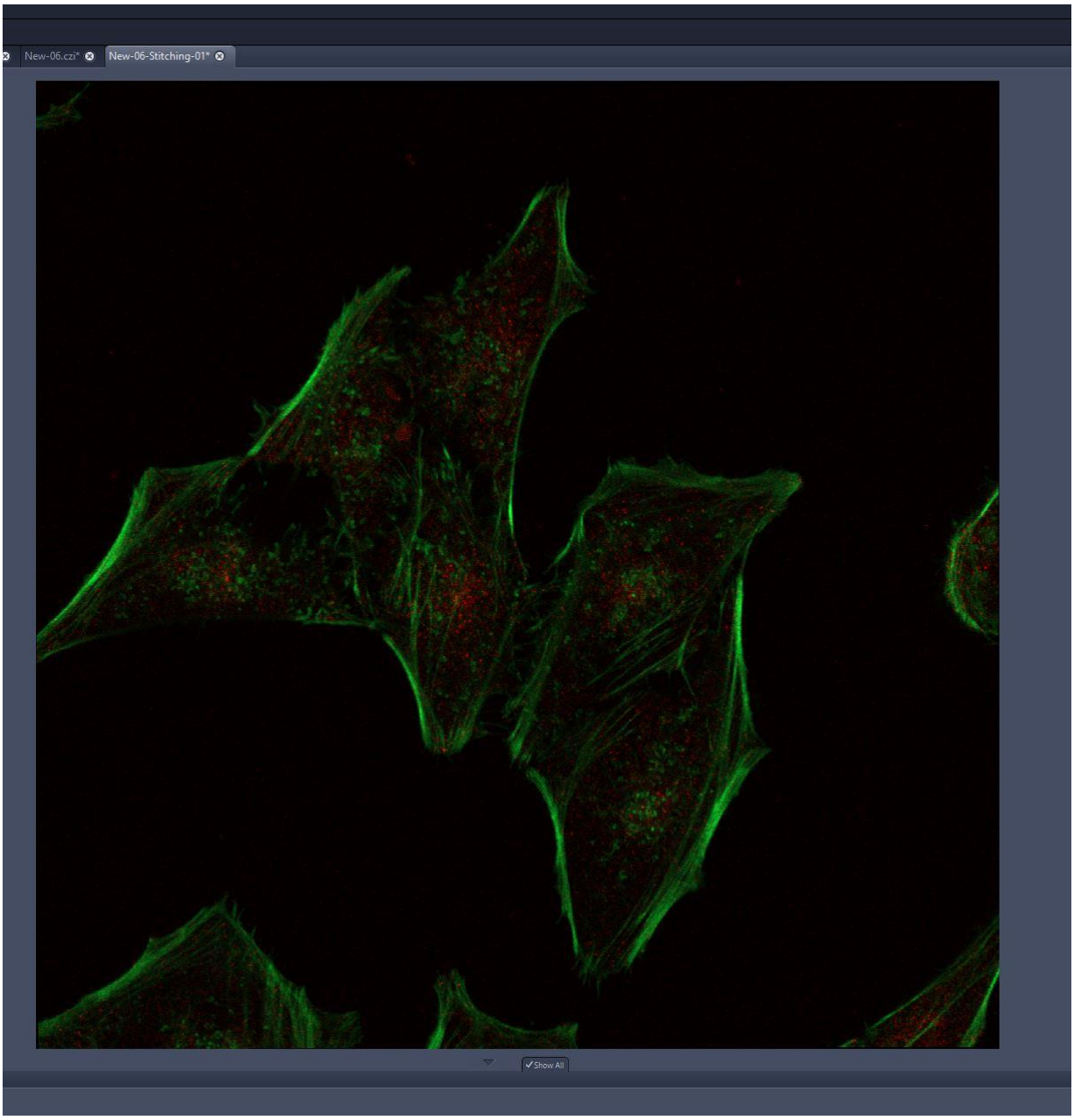


Start Tile Scan

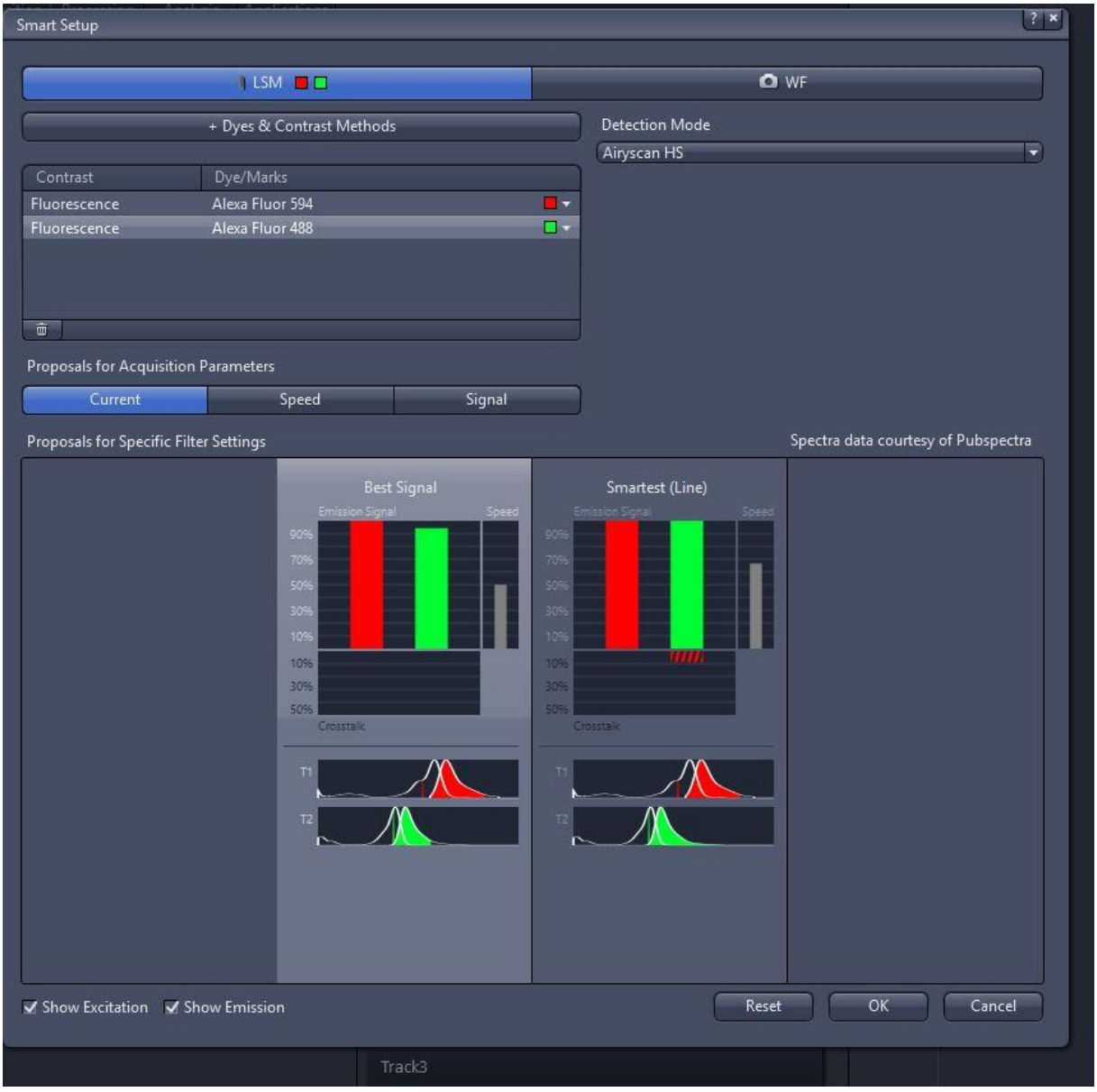


Stitching

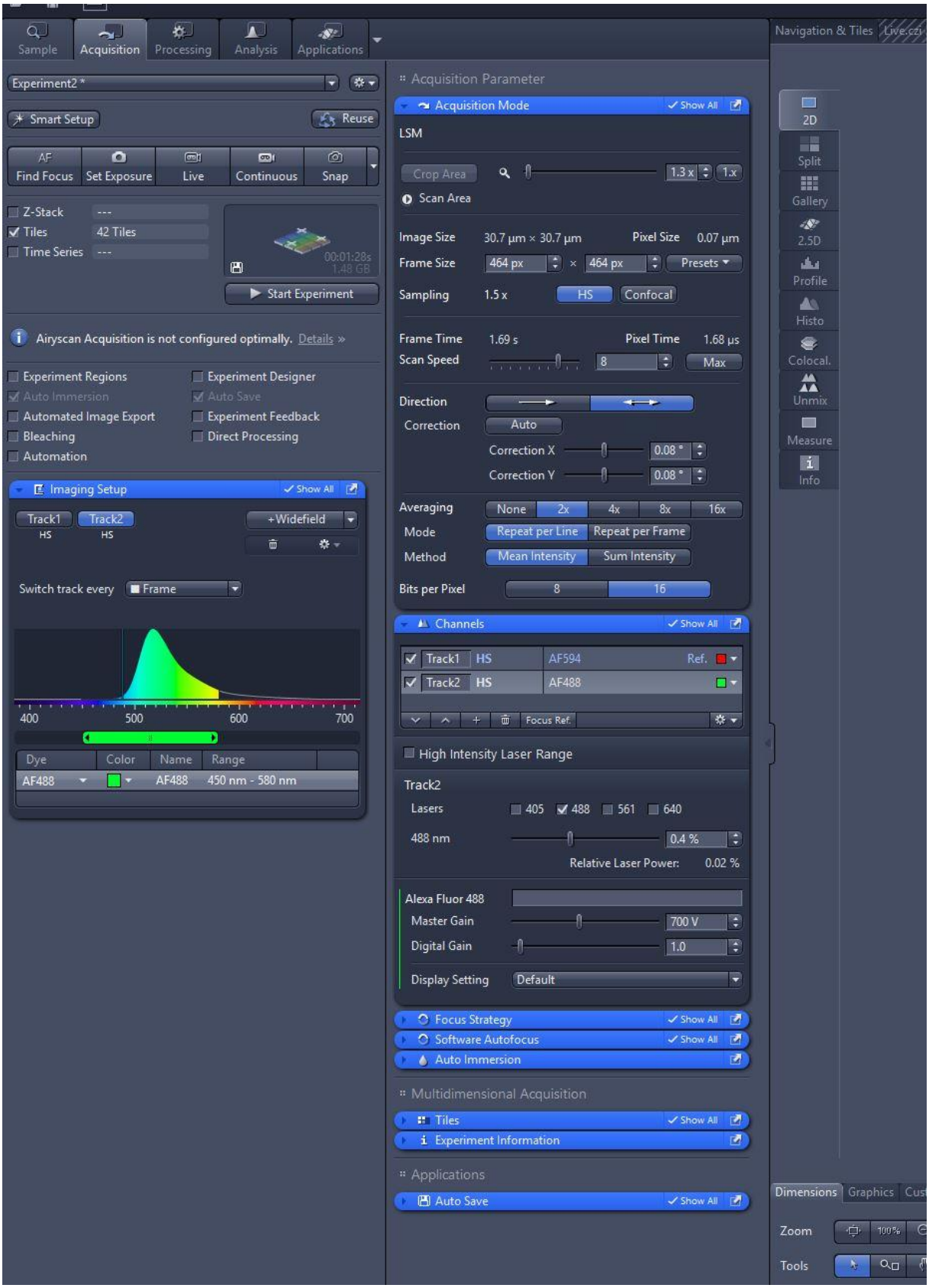

Function: Stitching
Single
Batch
Apply
Method
Method Parameters
Parameters
Show All
Settings
Inplace
New Output
Fuse Tiles
Correct Shading
Select dimension references for stitching
Get all dimensions from 2d view:
Channels
All by reference
Reference only
All individually
AF594-T2
AF488-T3
Parameters
Edge Detector
Yes
No
Minimal Overlap
5 %
Maximal Shift
10 %
Comparer
Basic
Best
Optimized
Global Optimizer
Basic
Best
Defaults
Reset
Redo
Image Parameters
Input
Show All
New-06.czi
Input
Input Definition
Set Input Automatically
After processing
Switch to Output
Remain at current view
Output
Show All


New-06.czi*
New-06-Stitching-01*
Show All

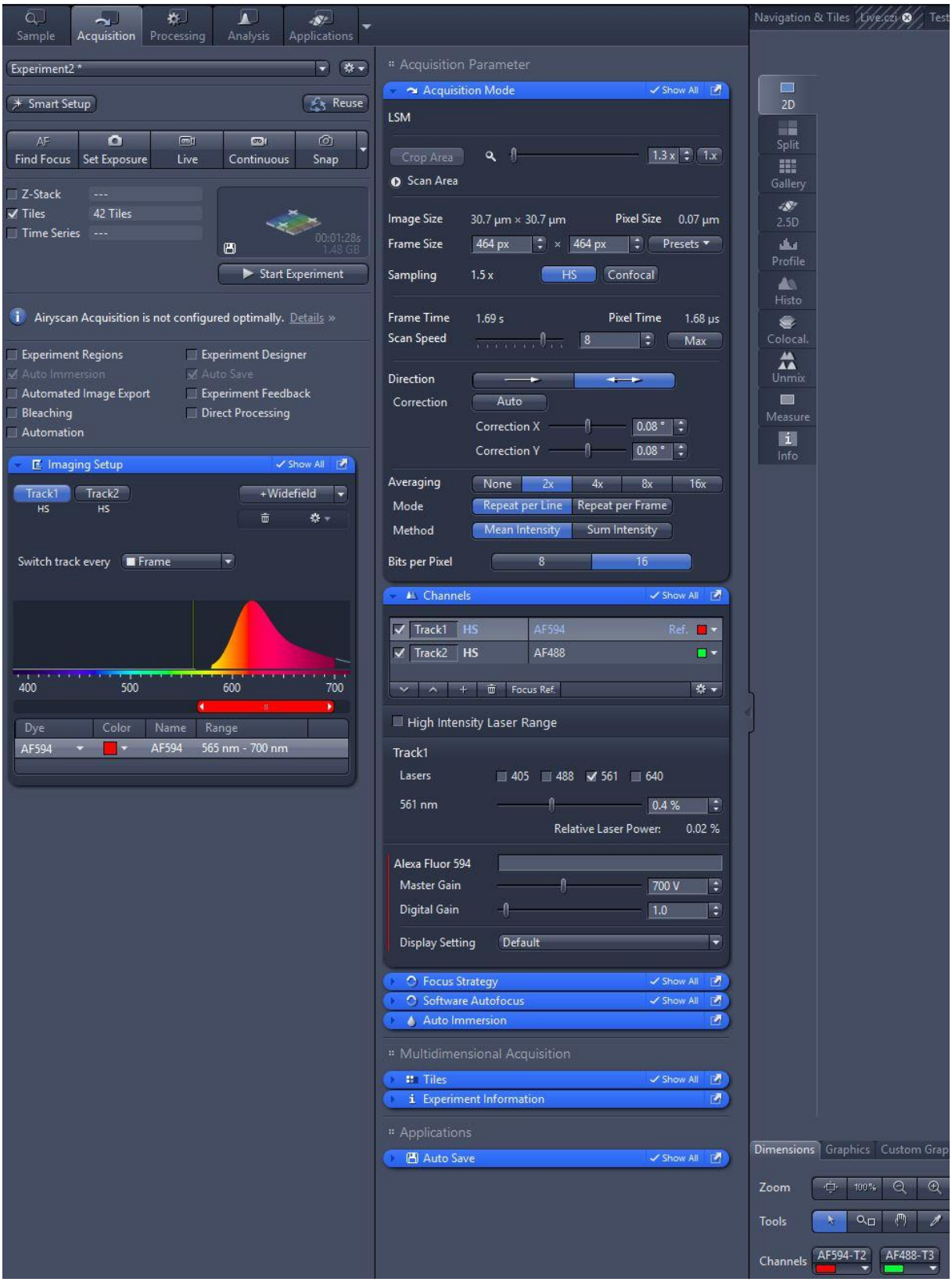
Smart Setup Airyscan HS



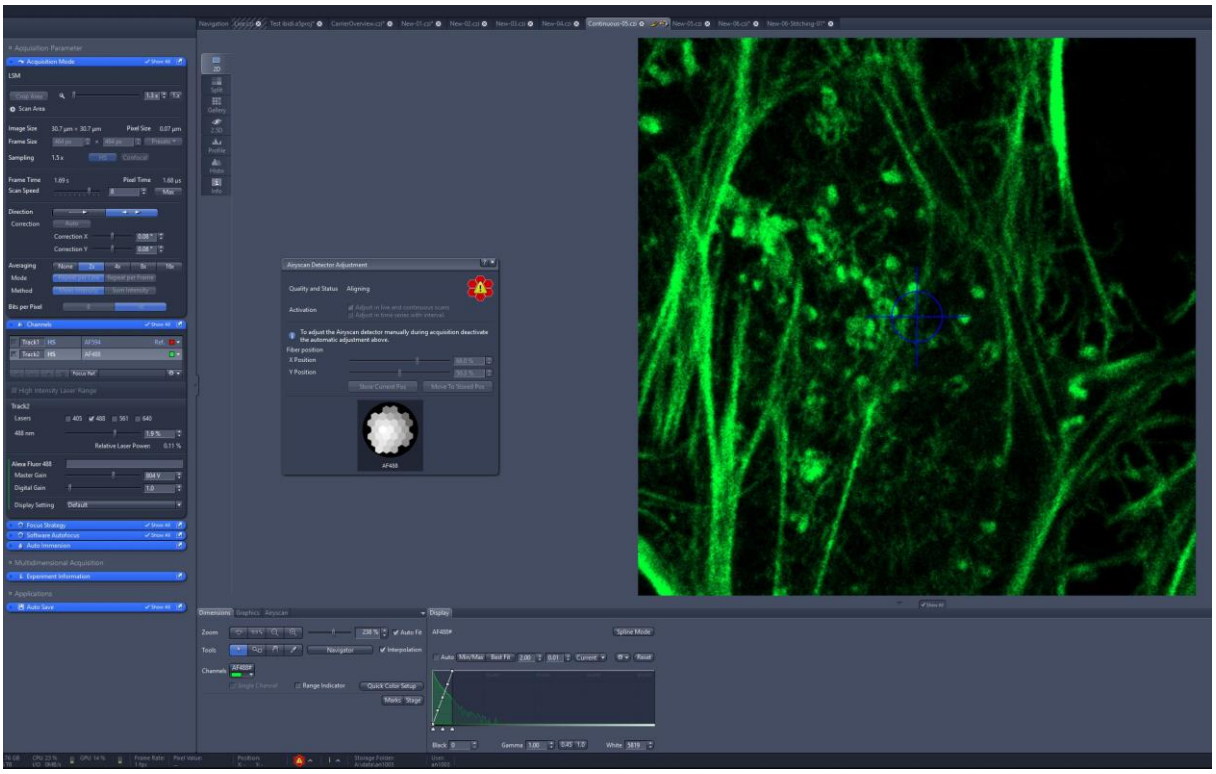
Smart Setup Airyscan HS channel 1

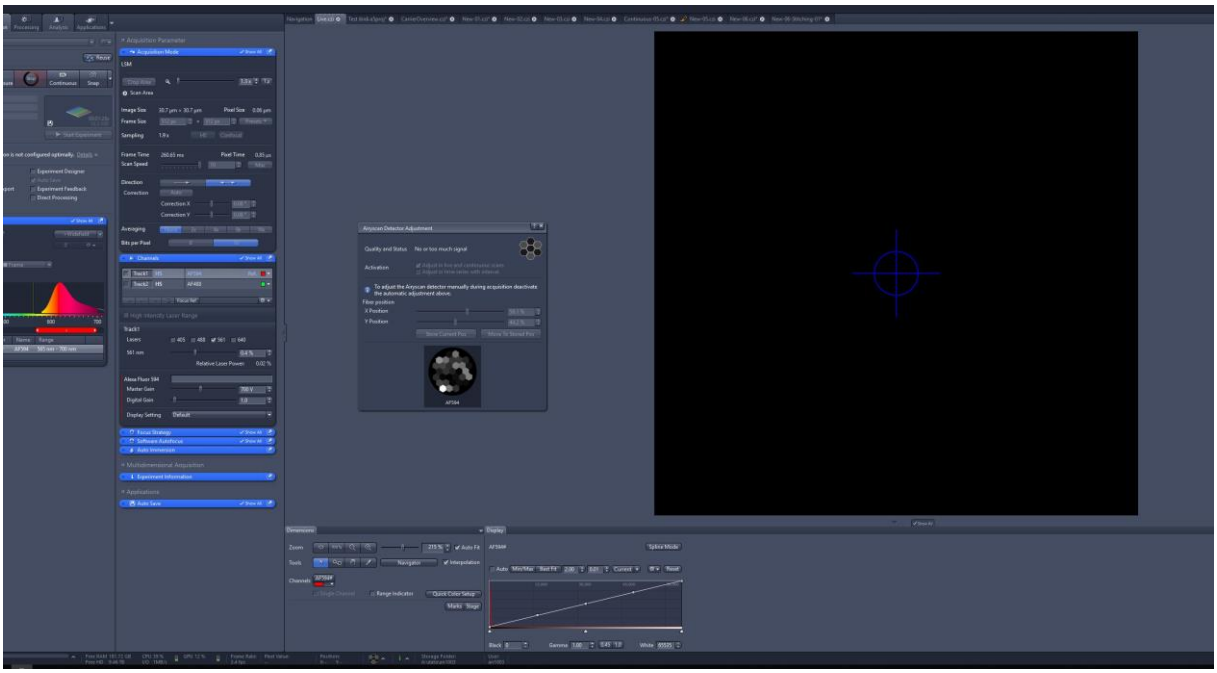


Smart Setup Airyscan HS channel 2

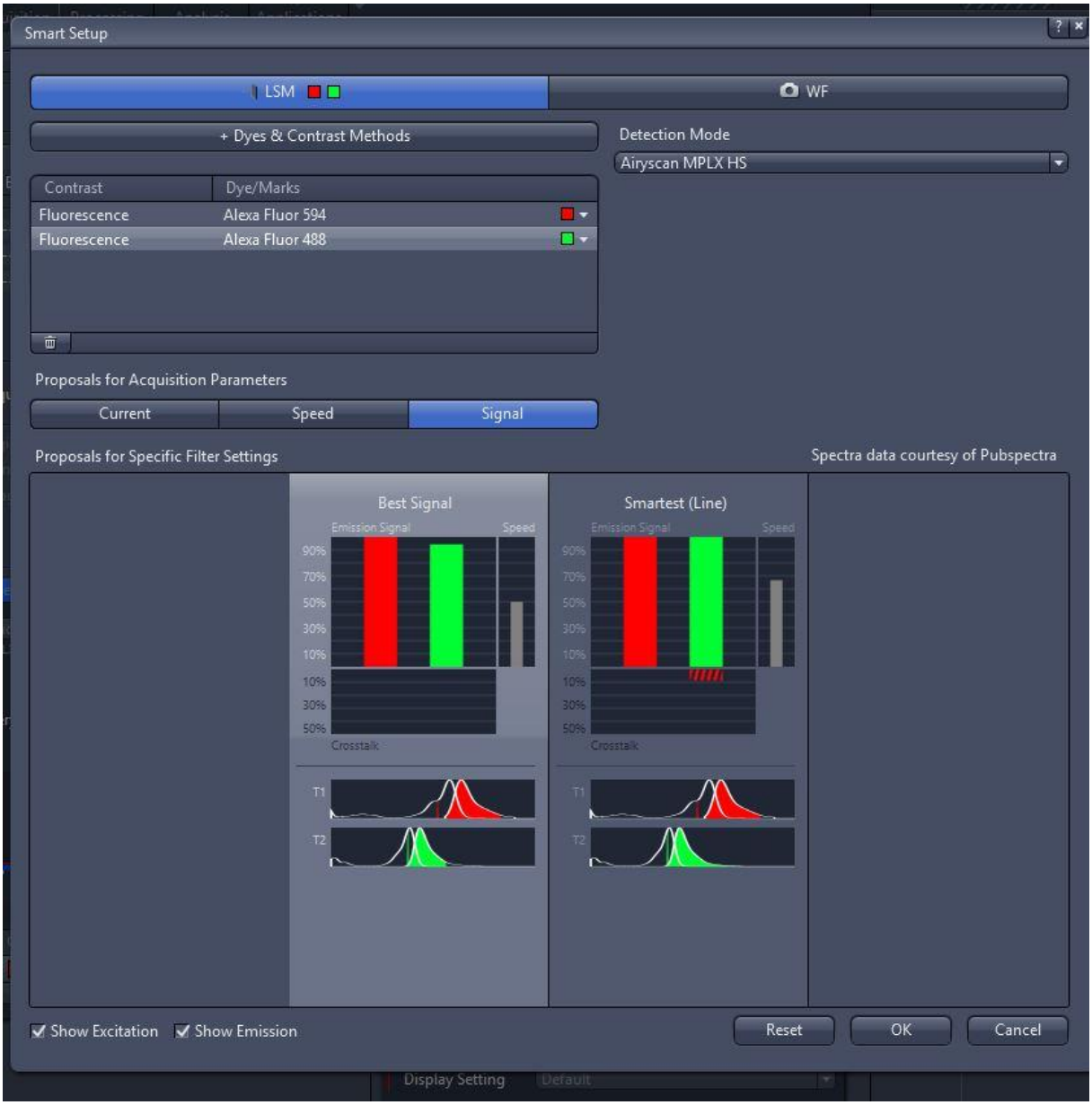

Sample
Acquisition
Processing
Analysis
Applications
Experiment2 *
Smart Setup
Reuse
AF
Find Focus
Set Exposure
Live
Continuous
Snap
Z-Stack
Tiles
42 Tiles
Time Series
00:01:28s
1.48 GB
Start Experiment
Airyscan Acquisition is not configured optimally. Details »
Experiment Regions
Auto Immersion
Automated Image Export
Bleaching
Automation
Experiment Designer
Auto Save
Experiment Feedback
Direct Processing
Imaging Setup
Show All
Track1
HS
Track2
HS
+Widefield
Switch track every
Frame
400
500
600
700
Dye
Color
Name
Range
AF594
AF594
565 nm - 700 nm
Acquisition Parameter
Acquisition Mode
Show All
LSM
Crop Area
1.3 x
1.x
Scan Area
Image Size
30.7 µm × 30.7 µm
Pixel Size
0.07 µm
Frame Size
464 px
464 px
Presets
Sampling
1.5 x
HS
Confocal
Frame Time
1.69 s
Pixel Time
1.68 µs
Scan Speed
8
Max
Direction
Correction
Auto
Correction X
0.08 °
Correction Y
0.08 °
Averaging
None
2x
4x
8x
16x
Mode
Repeat per Line
Repeat per Frame
Method
Mean Intensity
Sum Intensity
Bits per Pixel
8
16
Channels
Show All
Track1
HS
AF594
Ref.
Track2
HS
AF488
Focus Ref.
High Intensity Laser Range
Track1
Lasers
405
488
561
640
561 nm
0.4 %
Relative Laser Power:
0.02 %
Alexa Fluor 594
Master Gain
700 V
Digital Gain
1.0
Display Setting
Default
Focus Strategy
Show All
Software Autofocus
Show All
Auto Immersion
Multidimensional Acquisition
Tiles
Show All
Experiment Information
Applications
Auto Save
Show All
Navigation & Tiles
Live.czi
Test
2D
Split
Gallery
2.5D
Profile
Histo
Colocal.
Unmix
Measure
Info
Dimensions
Graphics
Custom Grap
Zoom
100%
Tools
Channels
AF594-T2
AF488-T3

Smart Setup Airyscan Alignment 1

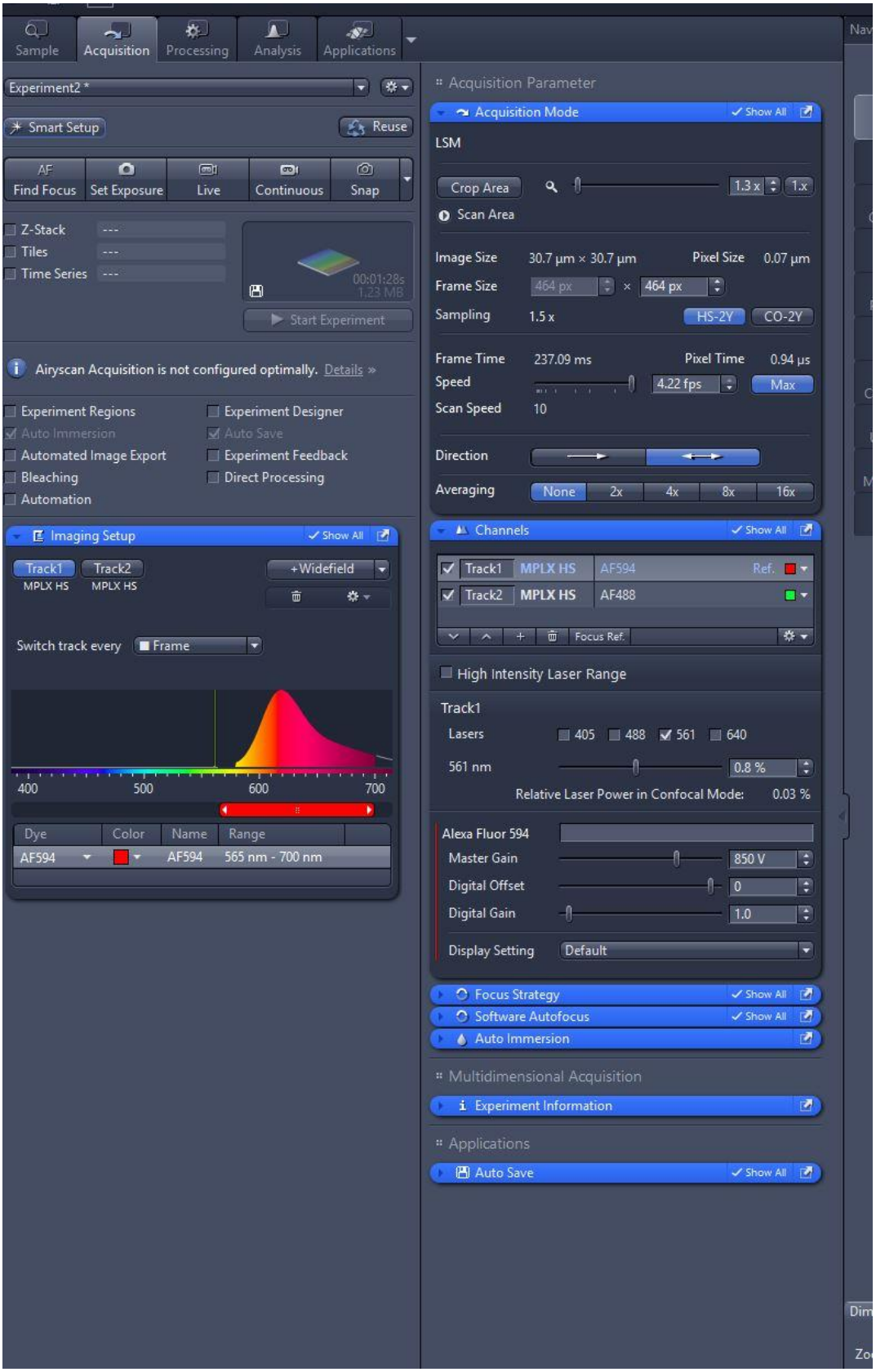




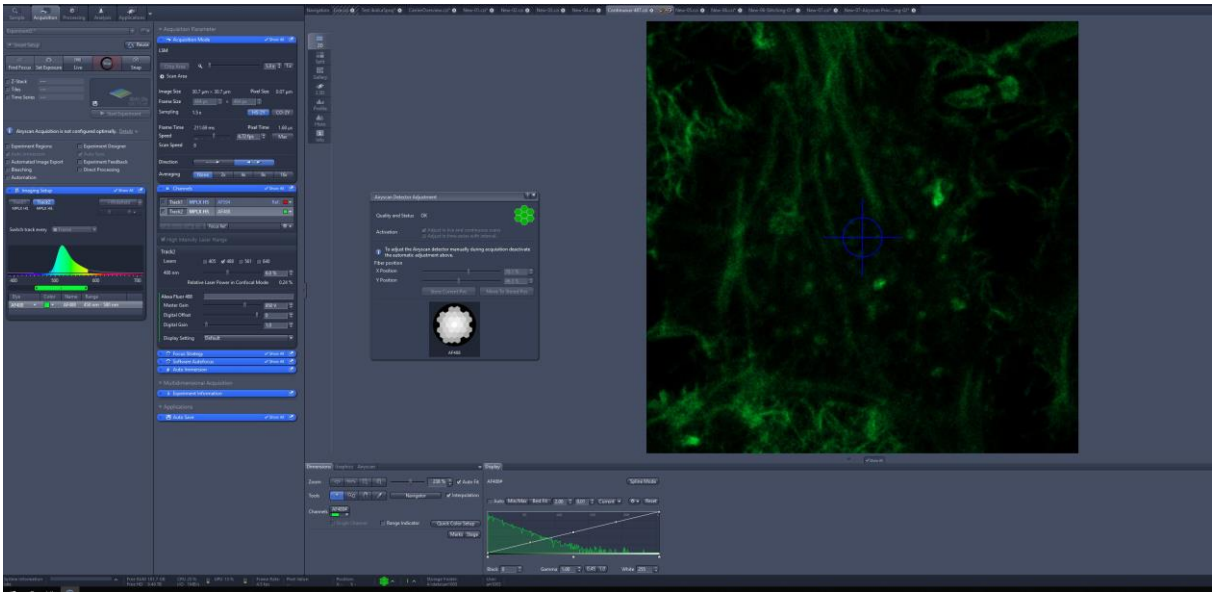
Smart Setup Airyscan Muliplex

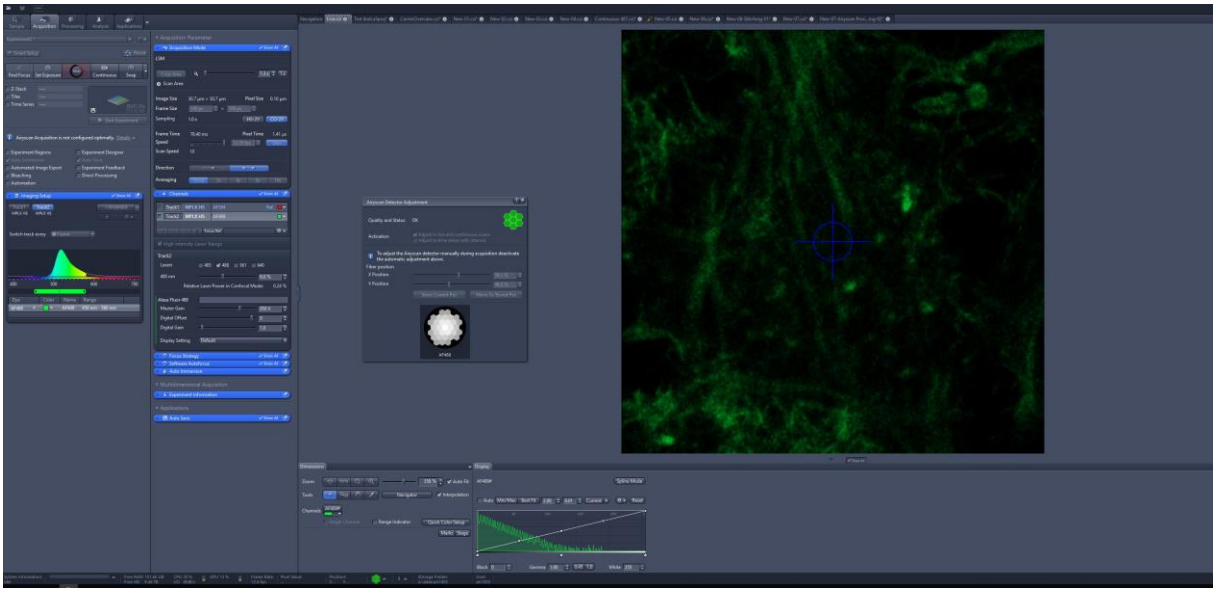


Smart Setup Airyscan Muliplex

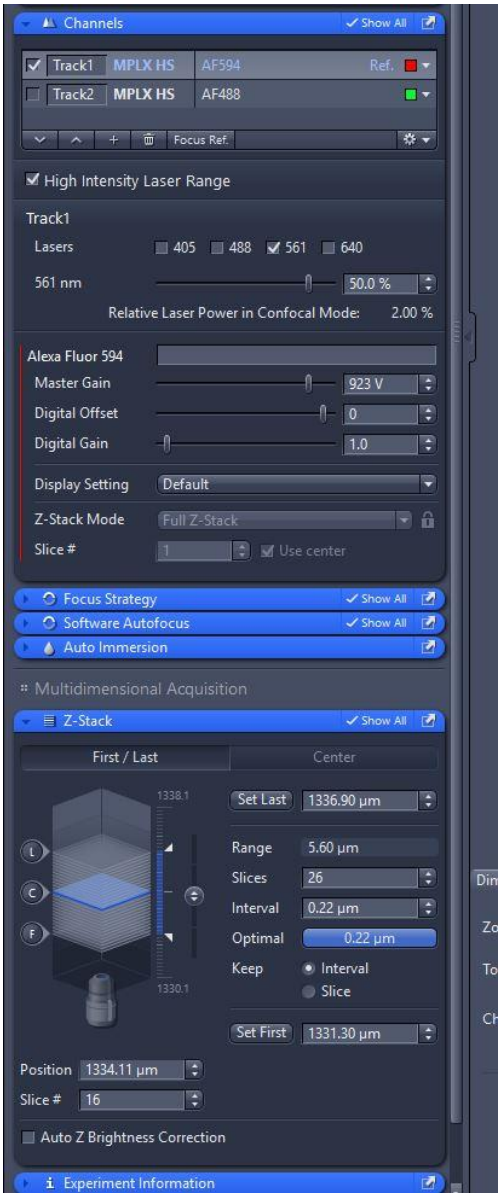


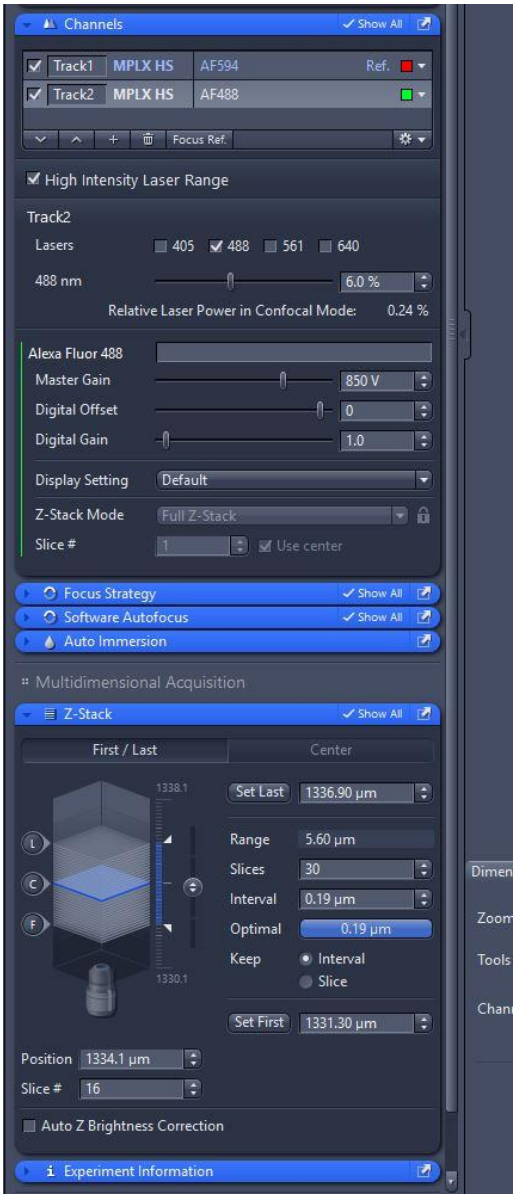
Smart Setup Airyscan Muliplex Alignment





Smart Setup Airyscan HS und Muliplex -higher laser power und unterschiedliches Interval





Source

1. Jessica Rowley:Cell Discoverer Handbook https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwiir6KgqN_5AhWFQPEDHWuTBtIQFnoECAcQAQ&url=https%3A%2F%2Fwww.imperial.ac.uk%2Fmedia%2Fimperial-college%2Fmedicine%2Ffacilities%2Ffilm%2FCell-Discoverer_Handbook.pdf&usg=AOvVaw0O-1RpOoPZzGWTtDxAYRgv