
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!

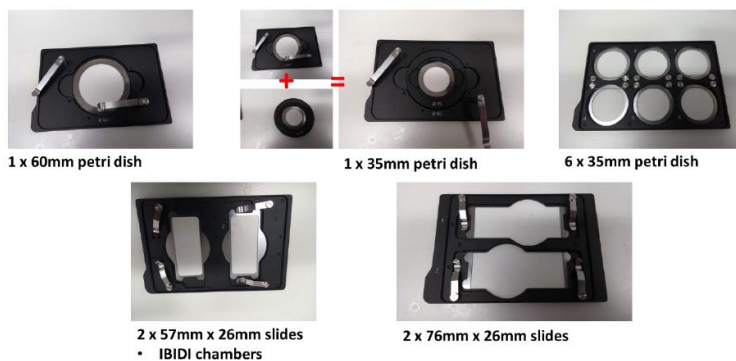


Figure 1 Inserts for Celldiscoverer (S1)



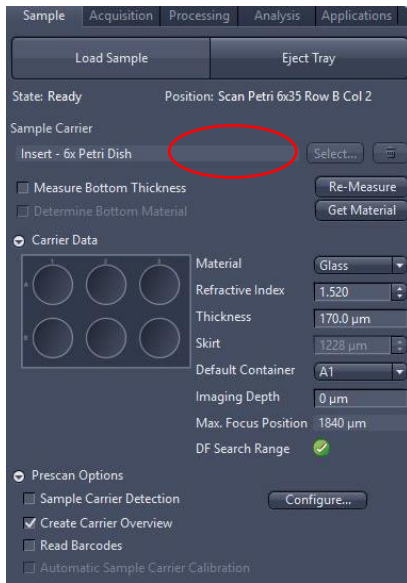
Figure 2 Trays for Celldiscoverer (S1)

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, CO2 and humidity)
- If you use INCUBATION wait at least 15 min to equilibrate.

Load Sample

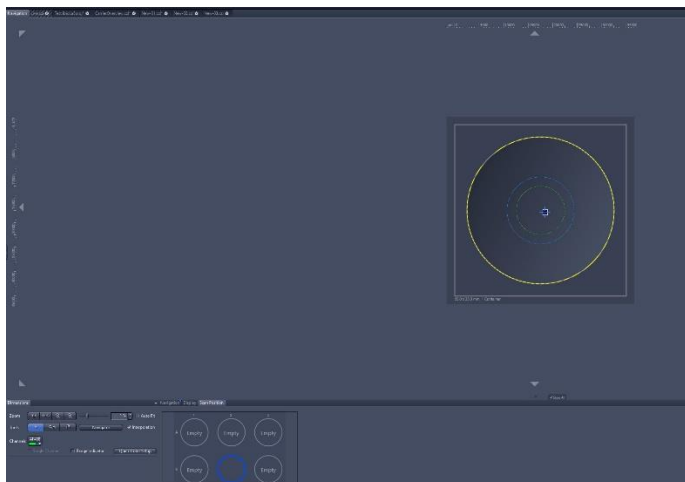
- If the tray is not outside, click '**eject tray**'
- Place you tray with your insert and sample on the stage
- Select the Sample carrier type, press **select** button
- You can use automation functions such as 'measurement of bottom material' and 'determine bottom material' or you choose bottom material 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



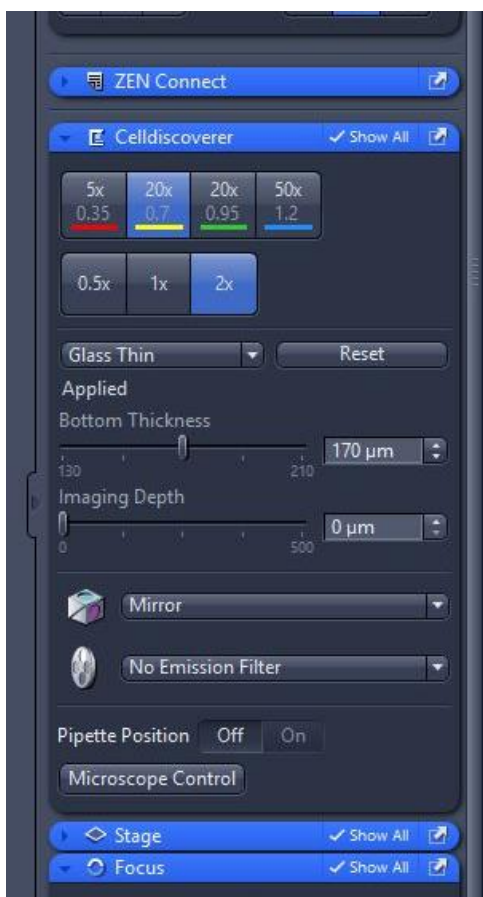
Carrier Overview



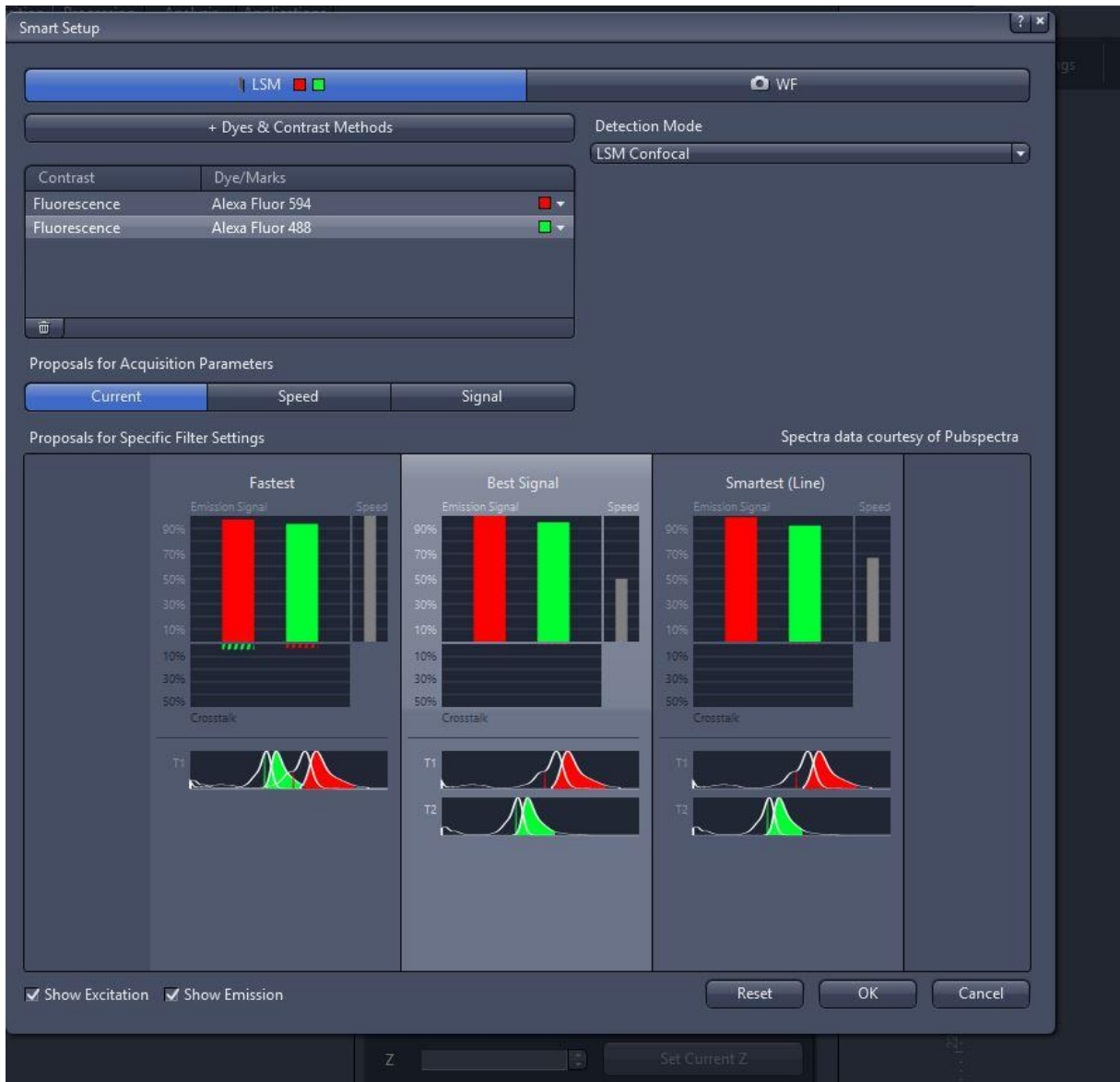
Navigation tab



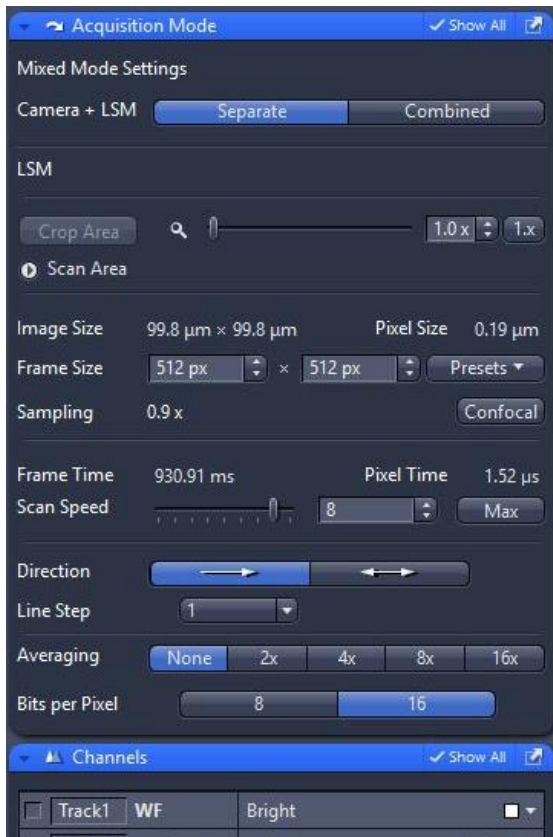
Objective



Smart Setup confocal and WF



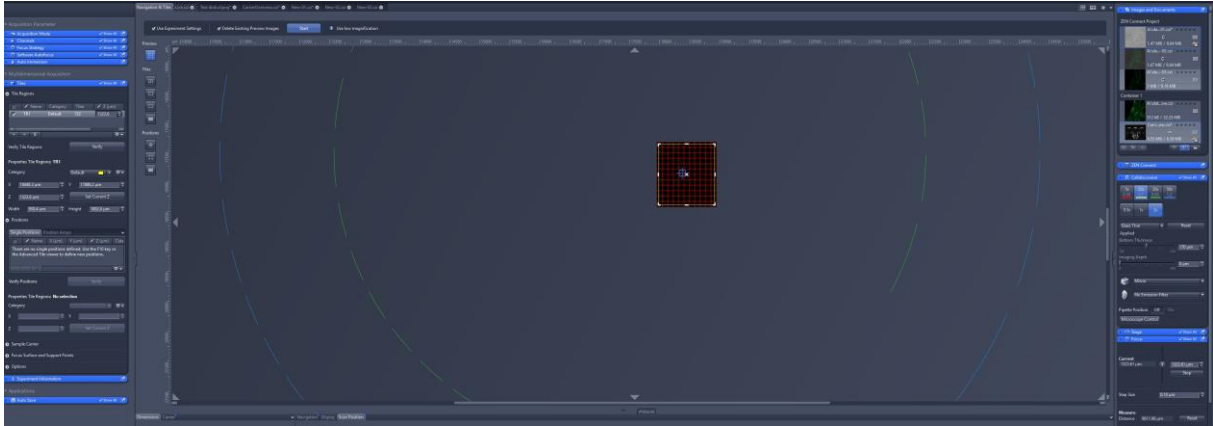
Acquisition separate confocal WF



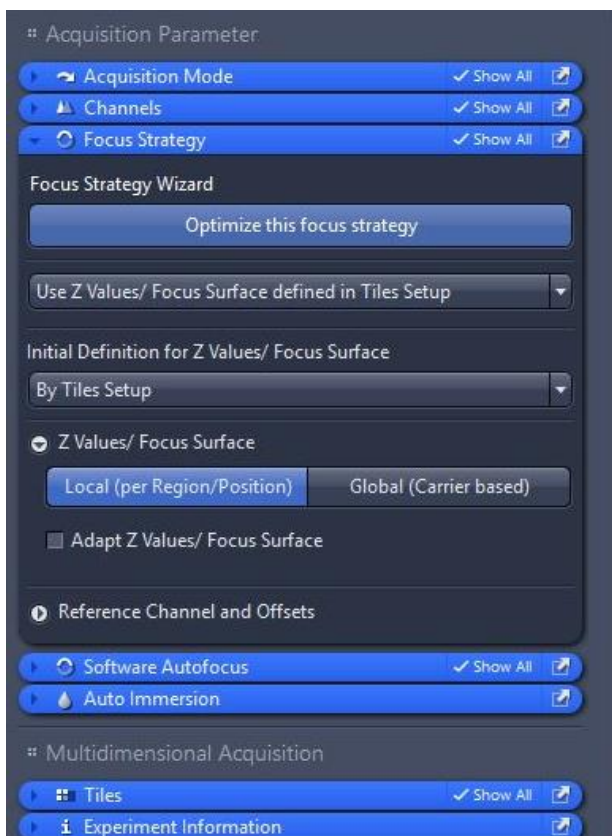
Acquisition combined confocal WF



Setup Tiles Scan



Setup Tiles Scan – Focus Strategy



Setup Z-stack

Acquisition Mode Show All

Channels Show All

<input type="checkbox"/>	Track1	WF	Bright	<input type="checkbox"/>
<input type="checkbox"/>	Track2	Confocal	AF594	Ref. <input type="checkbox"/>
<input checked="" type="checkbox"/>	Track3	Confocal	AF488	<input checked="" type="checkbox"/>

High Intensity Laser Range

Track3

Lasers 405 488 561 640

488 nm

Relative Laser Power: 0.12 %

Pinhole

1.00 Airy Units \pm 1.5 μm section

Alexa Fluor 488

Master Gain

Digital Offset

Digital Gain

Display Setting

Z-Stack Mode

Slice # Use center

Focus Strategy Show All

Software Autofocus Show All

Auto Immersion Show All

Multidimensional Acquisition

Z-Stack Show All

First / Last

Center

Range

Slices

Interval

Optimal

Keep Interval Slice

Position

Slice #

LSM Specific Settings

Optimize Sectioning and Step

Auto Z Brightness Correction

Setup Z-stack

Acquisition Mode ✓ Show All

Channels ✓ Show All

<input type="checkbox"/>	Track1	WF	Bright	<input type="checkbox"/>
<input type="checkbox"/>	Track2	Confocal	AF594	Ref. <input type="checkbox"/>
<input checked="" type="checkbox"/>	Track3	Confocal	AF488	<input checked="" type="checkbox"/>

Focus Ref. ⚙

High Intensity Laser Range

Track3

Lasers 405 488 561 640

488 nm 1.0 %

Relative Laser Power: 0.12 %

Pinhole 91 μm

i 1.00 Airy Units \pm 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

L *C* *F*

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

The screenshot displays the software interface for setting up a Z-stack acquisition. The main window is titled "Channels" and shows three tracks:

- Track1: WF, Bright
- Track2: Confocal, AF594, Ref. (Red)
- Track3: Confocal, AF488 (Green)

Below the channels, the "Track3" settings are detailed:

- Lasers:** 405, 488 (checked), 561, 640
- 488 nm:** Slider set to 1.0 %
- Relative Laser Power:** 0.12 %
- Pinhole:** Slider set to 91 μm
- Resolution:** 1.00 Airy Units \pm 1.5 μm section
- 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 (checked)

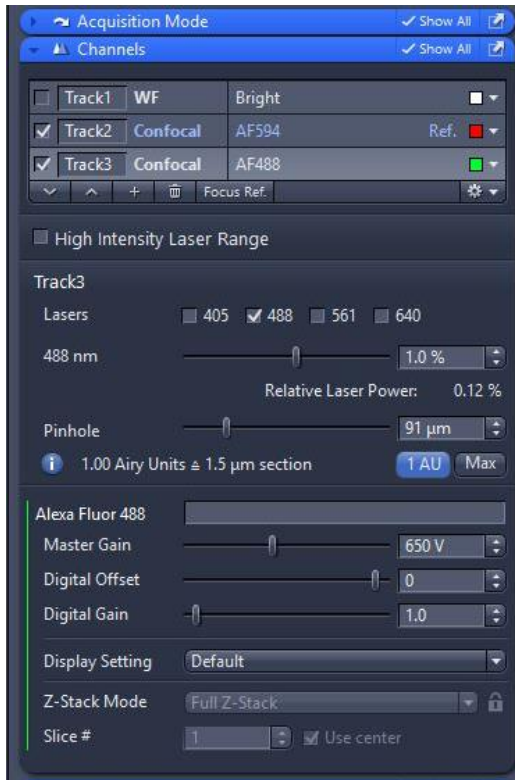
Below the Track3 settings, there are three expandable sections:

- Focus Strategy (Show All)
- Software Autofocus (Show All)
- Auto Immersion

The "Multidimensional Acquisition" section is expanded to show "Z-Stack" settings:

- First / Last:** Center
- Set Last:** 1333.11 μm
- Range:** 11.50 μm
- Slices:** 13
- Interval:** 0.89 μm
- Optimal:** 0.89 μm
- Keep:** Interval (selected), Slice
- Set First:** 1321.61 μm
- Position:** 1333.11 μm
- Slice #:** 12
- LSM Specific Settings:**
 - Optimize Sectioning and Step (unchecked)
 - Auto Z Brightness Correction (unchecked)

Pinhole 1 AU – compare setup different channel





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

Acquisition Mode Show All

Channels Show All

<input type="checkbox"/>	Track1	WF	Bright	<input type="checkbox"/>
<input checked="" type="checkbox"/>	Track2	Confocal	AF594	Ref. <input type="checkbox"/>
<input checked="" type="checkbox"/>	Track3	Confocal	AF488	<input checked="" type="checkbox"/>

Focus Ref.

High Intensity Laser Range

Track3

Lasers 405 488 561 640

488 nm
Relative Laser Power: 0.12 %

Pinhole

1.25 Airy Units ± 1.8 μm section

Alexa Fluor 488
Master Gain
Digital Offset
Digital Gain

Display Setting

Z-Stack Mode

Slice # Use center

Focus Strategy Show All

Software Autofocus Show All

Auto Immersion

* Multidimensional Acquisition

Z-Stack Show All

First / Last	Center
<input type="text" value="1335.6"/>	<input type="text" value="1333.11 μm"/> <input type="button" value="v"/>
	Range <input type="text" value="11.50 μm"/>
	Slices <input type="text" value="14"/> <input type="button" value="v"/>
	Interval <input type="text" value="0.88 μm"/> <input type="button" value="v"/>
	Optimal <input type="text" value="0.88 μm"/>
	Keep <input checked="" type="radio"/> Interval <input type="radio"/> Slice
	<input type="text" value="1321.61 μm"/> <input type="button" value="v"/>

Position

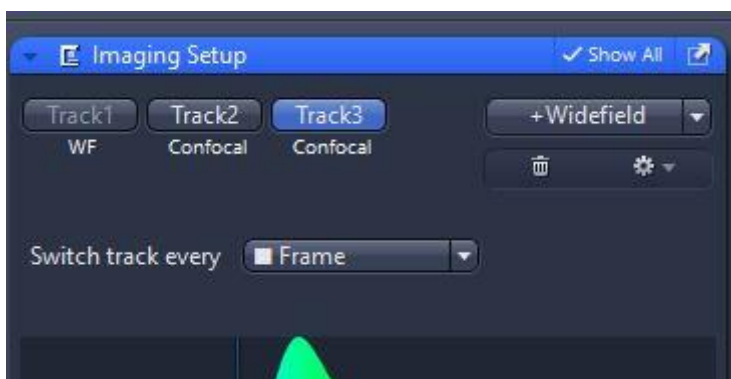
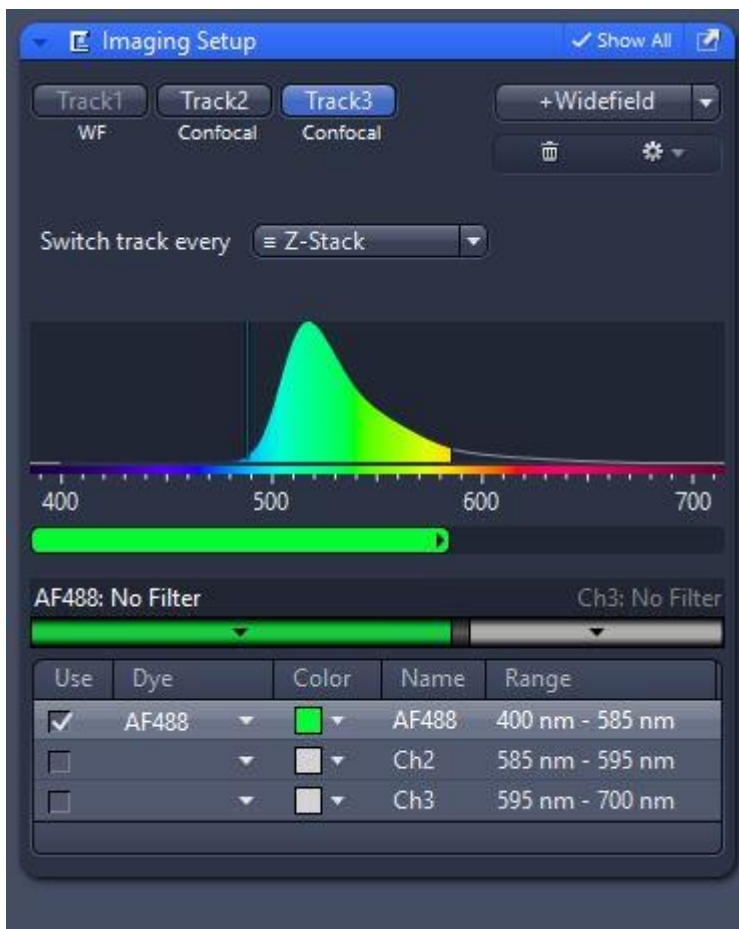
Slice #

LSM Specific Settings

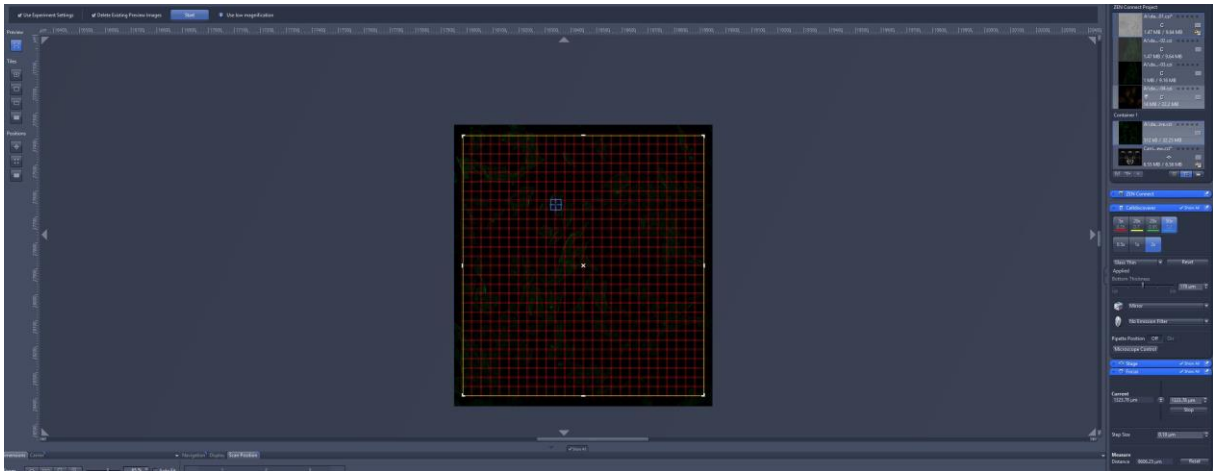
Optimize Sectioning and Step

Auto Z Brightness Correction

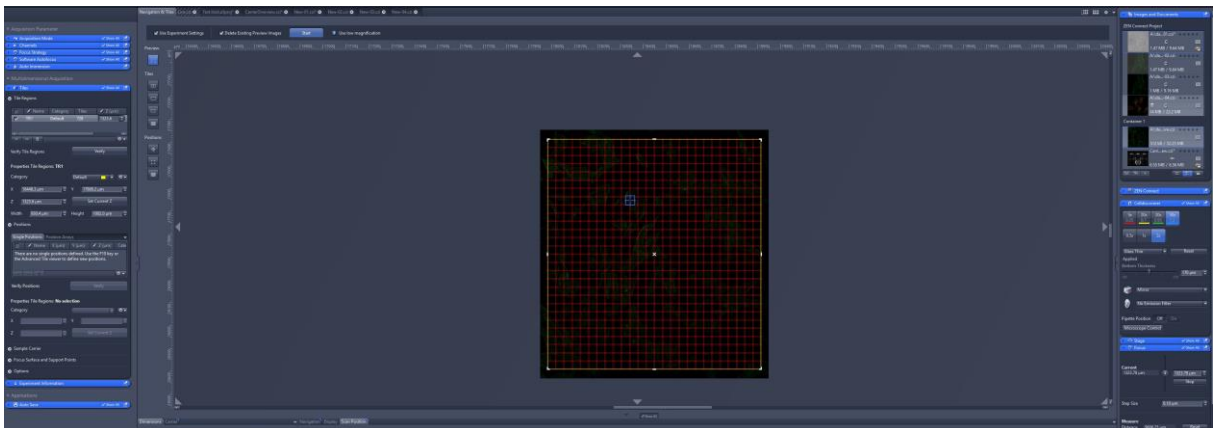
Setup Z-stack – Mode for switch



Tile scan now with 50x rectangle are changed automatically

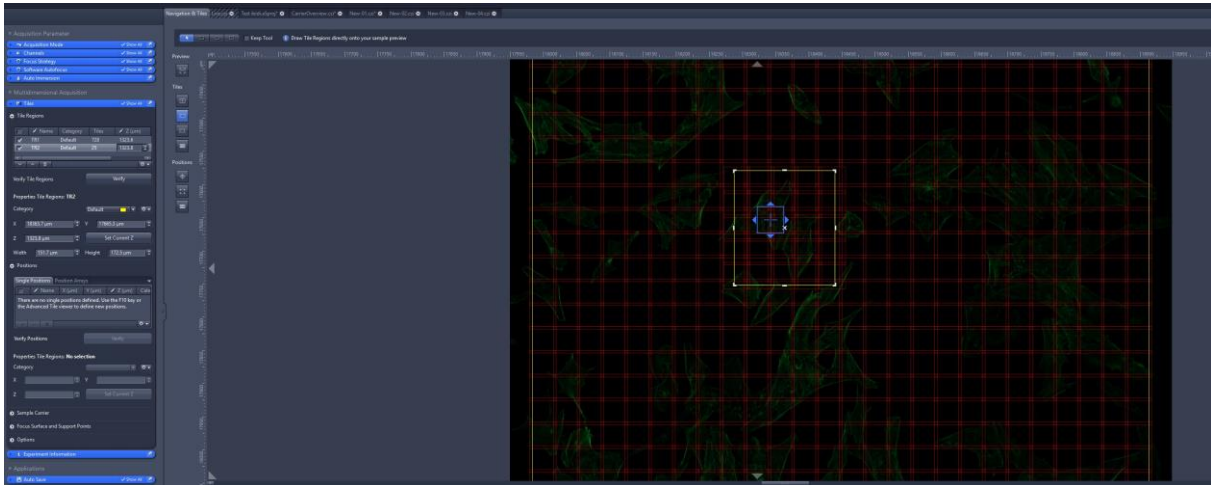


Also, if I have changed the Focus in live in the setup the old Focus will be used – update to new before start



Or define new tile region and delete old tiles

GUIDES / LIC





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 1.0 x

Scan Area

Image Size 39.9 μm \times 39.9 μm Pixel Size 0.10 μm

Frame Size 402 px \times 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

Track	Mode	Filter	Color
<input type="checkbox"/> Track1	WF	Bright	
<input checked="" type="checkbox"/> Track2	Confocal	AF594	Ref. ■
<input checked="" type="checkbox"/> 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

i 0.86 Airy Units \pm 0.5 μm section 1 AU Max

Alexa Fluor 488

Master Gain 650 V

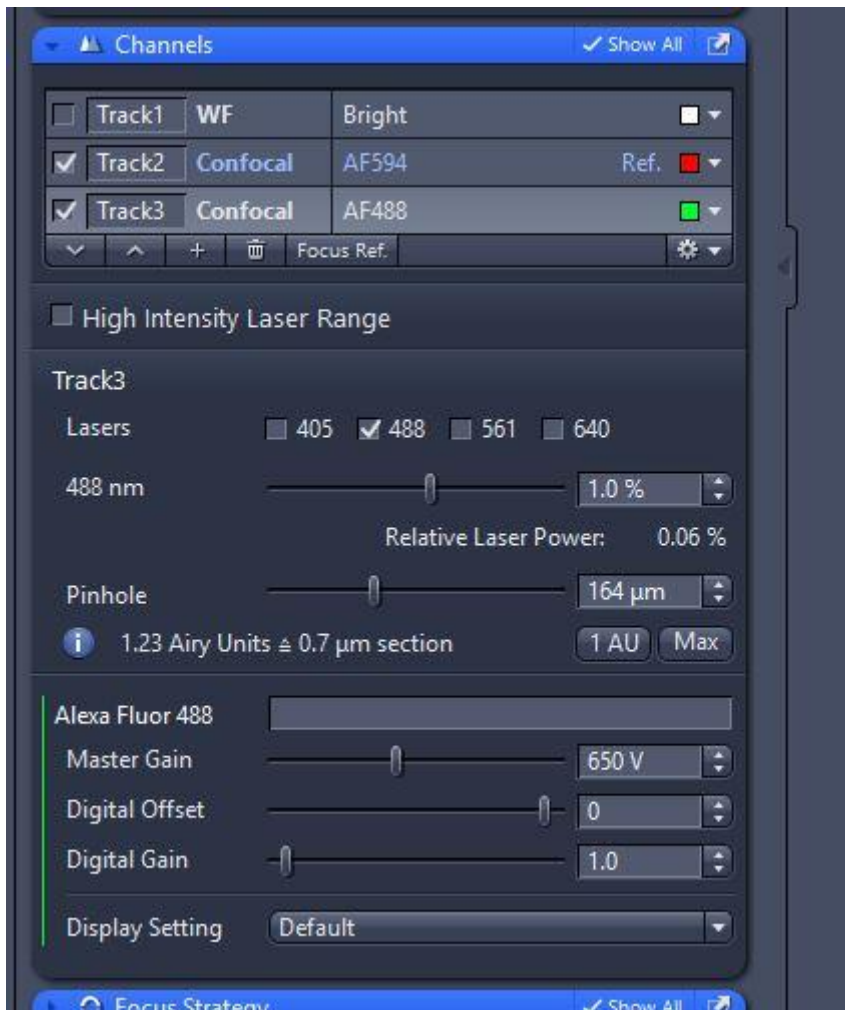
Digital Offset 0

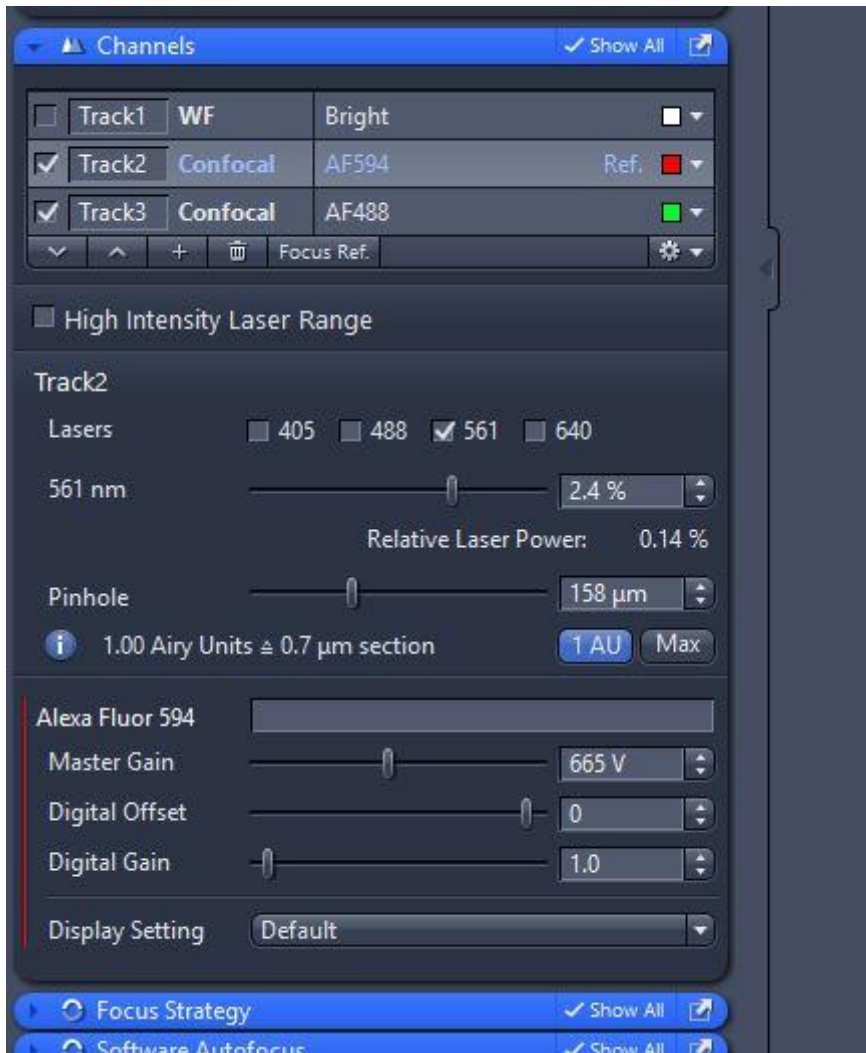
Digital Gain 1.0

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

Scan Area

Image Size 39.9 μm \times 39.9 μm Pixel Size 0.10 μm

Frame Size \times Presets

Sampling 1.0 x Confocal

Frame Time 1.47 s Pixel Time 1.93 μs

Scan Speed

Direction

Correction

Correction X

Correction Y

Line Step

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

<input type="checkbox"/>	Track1	WF	Bright	<input type="button" value="v"/>
<input type="checkbox"/>	Track2	Confocal	AF594	Ref. <input type="button" value="v"/>
<input checked="" type="checkbox"/>	Track3	Confocal	AF488	<input type="button" value="v"/>
<input type="button" value="v"/>	<input type="button" value="^"/>	<input type="button" value="+"/>	<input type="button" value="x"/>	Focus Ref. <input type="button" value="v"/>

High Intensity Laser Range

Track3

Lasers 405 488 561 640

488 nm

Relative Laser Power: 0.06 %

Pinhole

1.23 Airy Units \pm 0.7 μm section

Alexa Fluor 488

Master Gain

Digital Offset

Digital Gain

Display Setting

Focus Strategy Show All

Software Autofocus Show All

2D

Gallery

2.5D

Profile

Histo

Measure

Info

Dimensions Gr

Acquisition Parameter

Acquisition Mode Show All

Mixed Mode Settings

Camera + LSM

LSM

Crop Area

Scan Area

Image Size 39.9 μm \times 39.9 μm Pixel Size 0.10 μm

Frame Size 402 px \times 402 px

Sampling 1.0 x

Frame Time 1.47 s Pixel Time 1.93 μs

Scan Speed

Direction

Correction

Correction X

Correction Y

Line Step

Averaging

Mode

Method

Bits per Pixel

Channels Show All

Track	Mode	Filter	Color
<input type="checkbox"/> Track1	WF	Bright	
<input type="checkbox"/> Track2	Confocal	AF594	Ref. ■
<input checked="" type="checkbox"/> Track3	Confocal	AF488	■

High Intensity Laser Range

Track3

Lasers 405 488 561 640

488 nm

Pinhole

1.23 Airy Units \pm 0.7 μm section

Alexa Fluor 488

Master Gain

Digital Offset

Digital Gain

Display Setting

Focus Strategy Show All

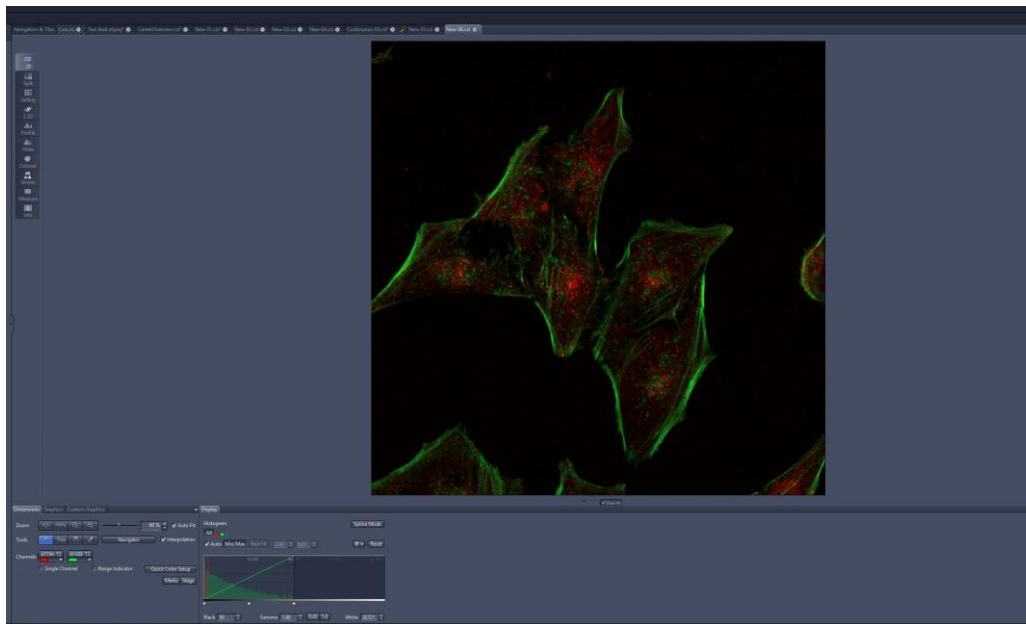
Dimensions Graphic

Tile-scan Overlap

The screenshot displays the 'Tiles' software interface with the following sections and settings:

- Tile Regions:** A table with columns for Name, Category, Tiles, and Z (μm). The entry 'TR2' is selected, with Category 'Default', 25 Tiles, and Z value '1324.3'. Below the table is a 'Verify Tile Regions' button.
- Properties Tile Regions: TR2:**
 - Category: Default (with a yellow color swatch)
 - X: 18363.7 μm, Y: 17665.3 μm
 - Z: 1324.3 μm (with a 'Set Current Z' button)
 - Width: 151.7 μm, Height: 172.5 μm
- Positions:**
 - Single Positions / Position Arrays tabs are visible.
 - A message states: "There are no single positions defined. Use the F10 key or the Advanced Tile viewer to define new positions."
 - 'Verify Positions' button is present.
- Properties Tile Regions: No selection:**
 - Category: (empty)
 - X, Y, Z input fields are empty.
 - 'Set Current Z' button is present.
- Sample Carrier**
- Focus Surface and Support Points**
- Options:**
 - Tile Overlap: 10 %
 - Stage Travel Optimization:
 - Travel in Tile Regions: Meander
 - Tile Regions/Positions: Sort by Y, then X
 - Carrier Wells/Container: Meander
 - Use Stage Speed from Stage Control:
 - Use Stage Acceleration from Stage Control:
 - Split Scenes into Separate Files:
 - Image Pyramid During Acquisition:

Start Tile Scan



Stitching

Function: Stitching

Single Batch

Method

Method Parameters

Parameters

Settings

Fuse Tiles

Correct Shading

Select dimension references for stitching

Get all dimensions from 2d view:

Channels

Parameters

Edge Detector

Minimal Overlap

Maximal Shift

Comparer

Global Optimizer

Image Parameters

Input

New-06.czi

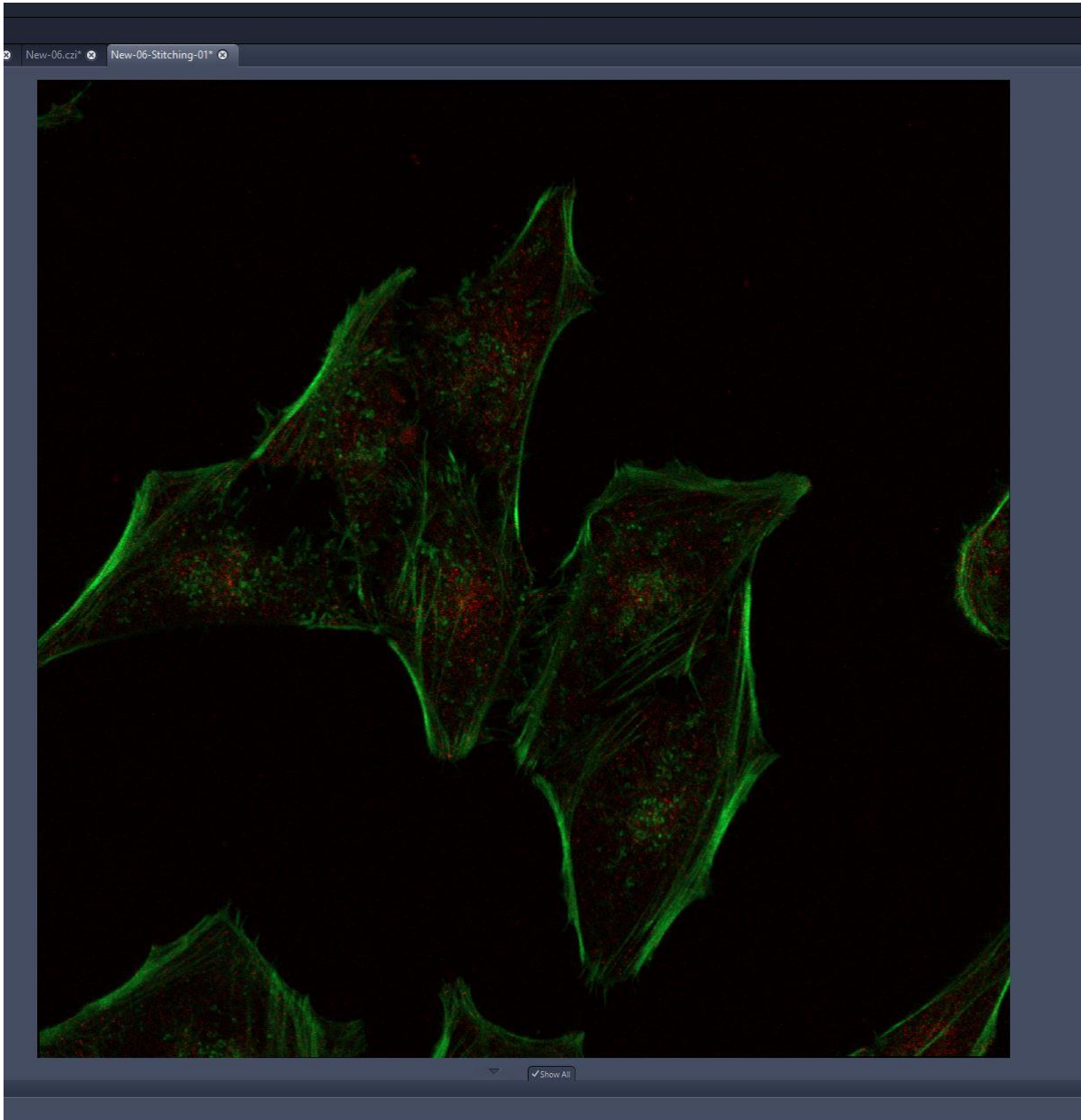
Input



Input Definition Set Input Automatically

After processing Switch to Output Remain at current view

Output



Smart Setup Airyscan HS

Smart Setup

LSM WF

+ Dyes & Contrast Methods

Contrast	Dye/Marks
Fluorescence	Alexa Fluor 594
Fluorescence	Alexa Fluor 488

Detection Mode: Airyscan HS

Proposals for Acquisition Parameters: Current, Speed, Signal

Proposals for Specific Filter Settings

Spectra data courtesy of Pubspectra

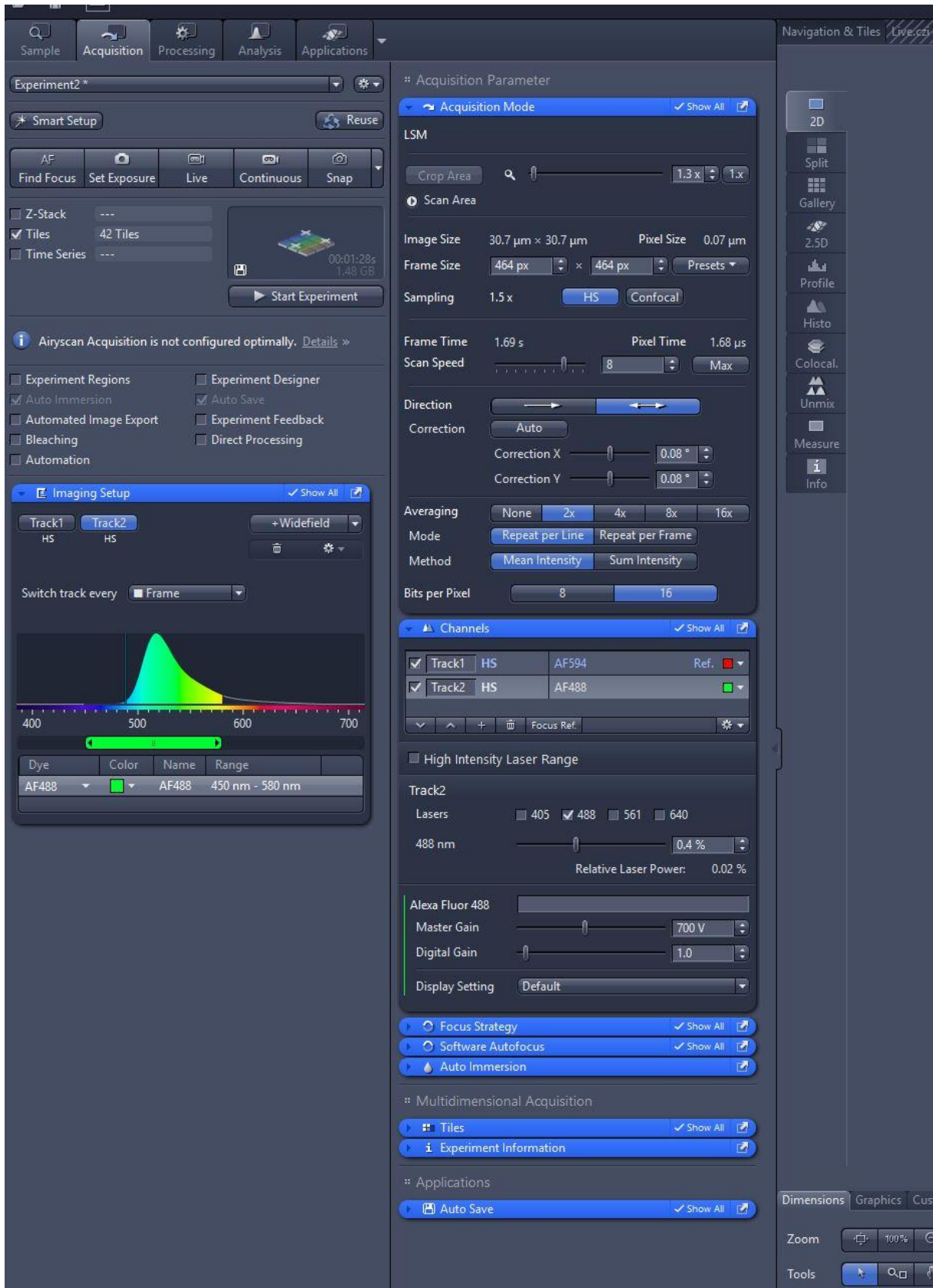
Best Signal vs Smartest (Line) comparison charts showing Emission Signal, Speed, and Crosstalk for channels T1 and T2.

Show Excitation Show Emission

Reset OK Cancel

Track3

Smart Setup Airyscan HS channel 1



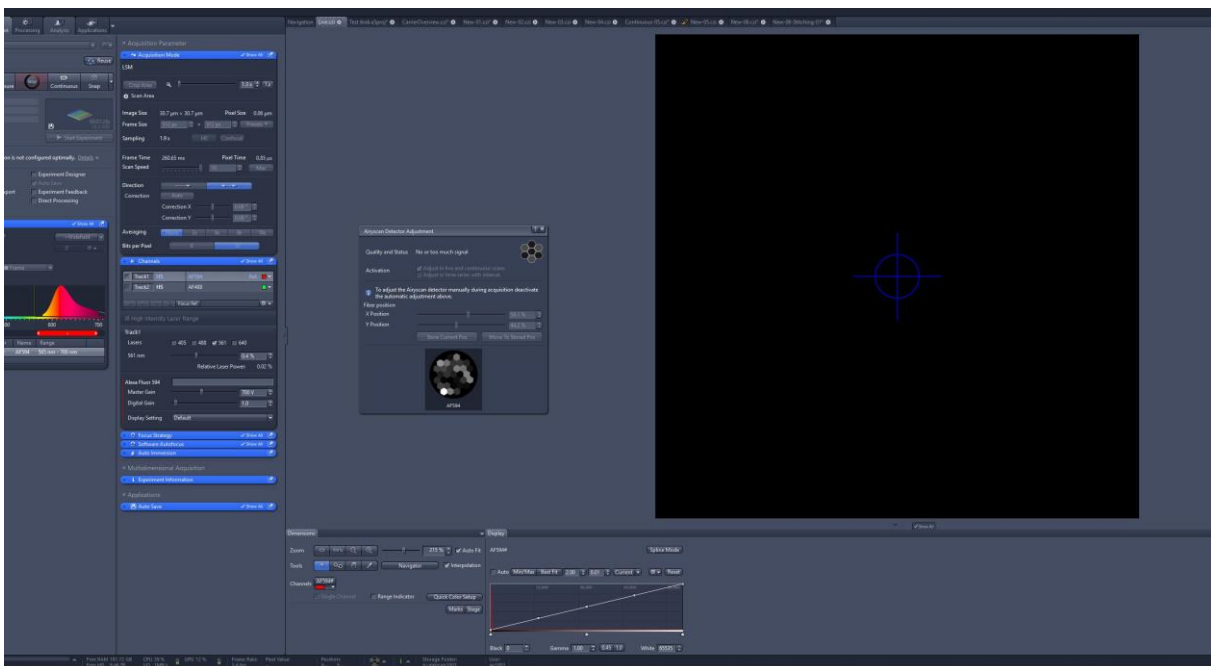
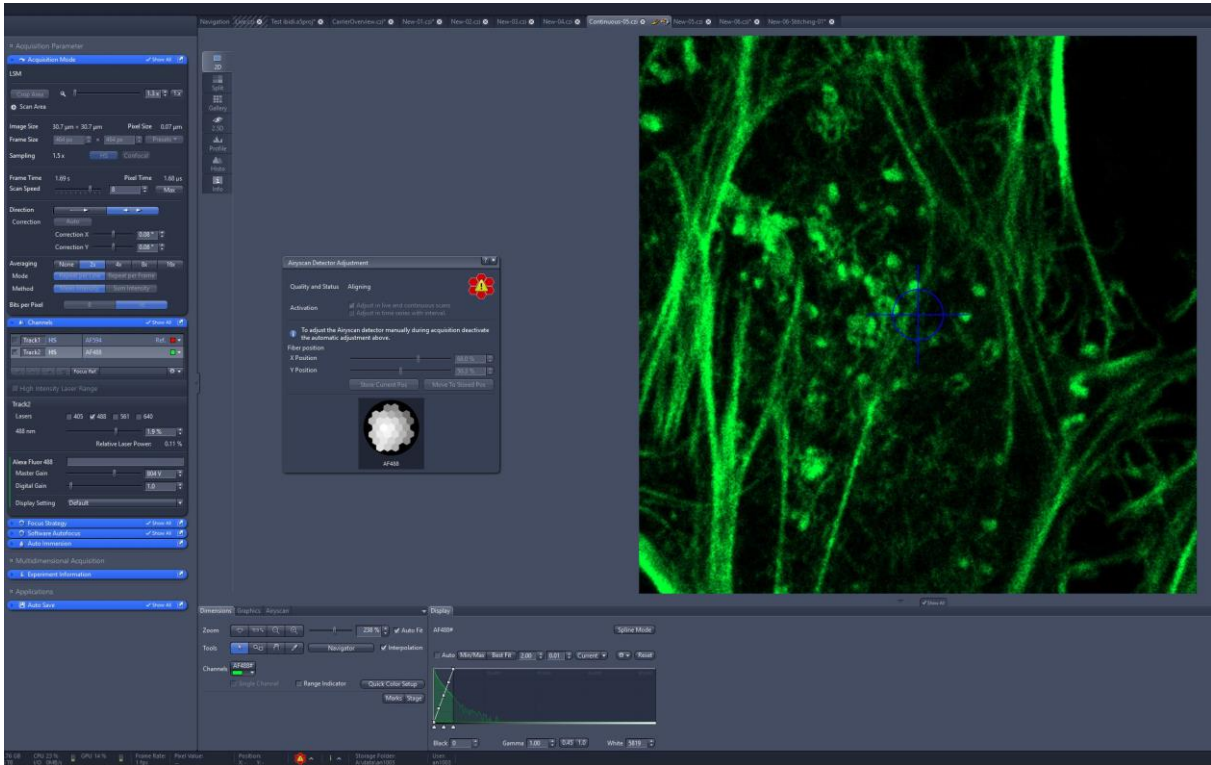
Smart Setup Airyscan HS channel 2

The screenshot displays the software interface for microscope acquisition, organized into several panels:

- Top Panel:** Navigation tabs for Sample, Acquisition, Processing, Analysis, and Applications.
- Left Panel (Experiment2*):**
 - Smart Setup:** Buttons for AF (Find Focus), Set Exposure, Live, Continuous, and Snap.
 - Acquisition Options:** Z-Stack, Tiles (42 Tiles), and Time Series.
 - Airyscan Acquisition:** A warning that it is not configured optimally.
 - Experiment Settings:** Checkboxes for Experiment Regions, Auto Immersion, Automated Image Export, Bleaching, Automation, Experiment Designer, Auto Save, Experiment Feedback, and Direct Processing.
 - Imaging Setup:**
 - Track1 and Track2 (both HS).
 - Widefield selection.
 - Switch track every: Frame.
 - Intensity histogram showing a peak around 600 nm.
 - Dye table:

Dye	Color	Name	Range
AF594	Red	AF594	565 nm - 700 nm
- Center Panel (Acquisition Parameter):**
 - Acquisition Mode:** LSM.
 - Scan Area:** Crop Area (1.3x), Image Size (30.7 μm x 30.7 μm), Pixel Size (0.07 μm), Frame Size (464 px x 464 px).
 - Sampling:** 1.5x, HS, Confocal.
 - Frame Time:** 1.69 s, Pixel Time: 1.68 μs.
 - Scan Speed:** 8, Max.
 - Direction:** Auto.
 - Correction:** 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:**
 - Track1: HS, AF594, Ref. (Red)
 - Track2: HS, AF488, (Green)
 - High Intensity Laser Range:** 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:** Software Autofocus, Auto Immersion.
 - Multidimensional Acquisition:** Tiles, Experiment Information.
 - Applications:** Auto Save.
- Right Panel (Navigation & Tiles):**
 - 2D, Split, Gallery, 2.5D, Profile, Histo, Colocal., Unmix, Measure, Info.
 - Dimensions: Graphics, Custom Grap.
 - Zoom: 100%.
 - Tools: Selection, Crop, Pan, Zoom.
 - Channels: AF594-T2 (Red), AF488-T3 (Green).

Smart Setup Airyscan Alignment 1



Smart Setup Airyscan Muliplex

The screenshot displays the 'Smart Setup' window for Airyscan Muliplex. At the top, it indicates 'LSM' and 'WF' settings. Below this is a section for '+ Dyes & Contrast Methods' with a table:

Contrast	Dye/Marks
Fluorescence	Alexa Fluor 594
Fluorescence	Alexa Fluor 488

The 'Detection Mode' is set to 'Airyscan MPLX HS'. Under 'Proposals for Acquisition Parameters', the 'Signal' tab is selected. The 'Proposals for Specific Filter Settings' section compares two options: 'Best Signal' and 'Smartest (Line)'. Each proposal includes a bar chart showing 'Emission Signal' (red and green bars) and 'Speed' (grey bar), and a 'Crosstalk' graph with two traces labeled 'T1' and 'T2'. The 'Smartest (Line)' option shows a red line on the T1 trace. At the bottom, there are checkboxes for 'Show Excitation' and 'Show Emission', and buttons for 'Reset', 'OK', and 'Cancel'.

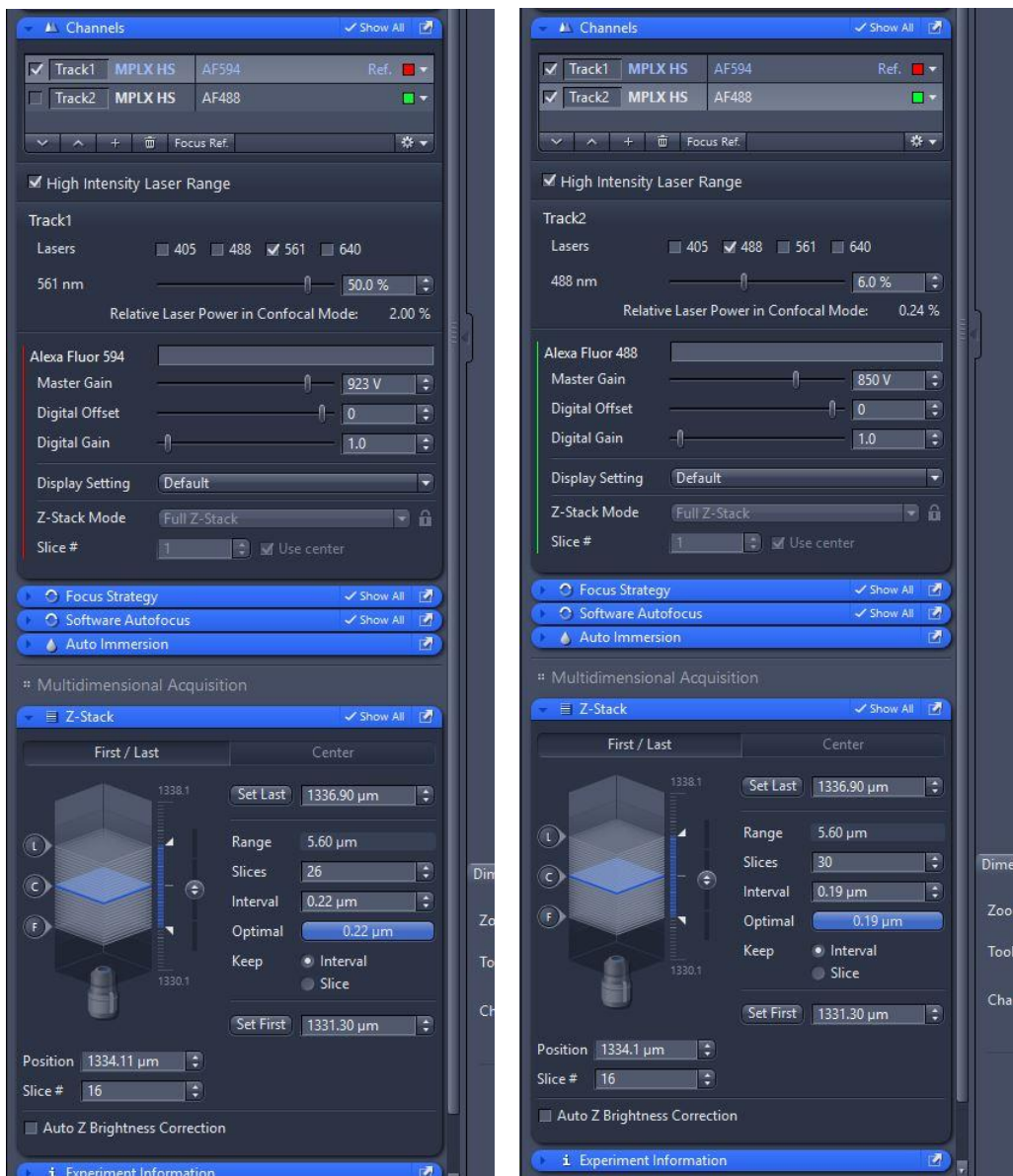
Smart Setup Airyscan Multiplex

The screenshot displays the microscope software interface with the following sections:

- Top Navigation:** Sample, Acquisition, Processing, Analysis, Applications.
- Experiment2* Panel:**
 - Buttons: Smart Setup, Reuse.
 - Buttons: AF Find Focus, Set Exposure, Live, Continuous, Snap.
 - Options: Z-Stack, Tiles, Time Series.
 - Start Experiment button.
 - Info: Airyscan Acquisition is not configured optimally. Details >>
 - Options: Experiment Regions, Experiment Designer, Auto Immersion, Auto Save, Automated Image Export, Experiment Feedback, Bleaching, Direct Processing, Automation.
- Imaging Setup Panel:**
 - Tracks: Track1 (MPLX HS), Track2 (MPLX HS), +Widefield.
 - Switch track every: Frame.
 - Graph: Intensity vs Wavelength (400-700 nm).
 - Table:

Dye	Color	Name	Range
AF594	Red	AF594	565 nm - 700 nm
- Acquisition Parameter Panel:**
 - Acquisition Mode: Show All.
 - LSM: Crop Area (1.3x), Scan Area.
 - Image Size: 30.7 μm × 30.7 μm, Pixel Size: 0.07 μm.
 - Frame Size: 464 px × 464 px.
 - Sampling: 1.5 x, HS-2Y, CO-2Y.
 - Frame Time: 237.09 ms, Pixel Time: 0.94 μs.
 - Speed: 4.22 fps, Max.
 - Scan Speed: 10.
 - Direction: Forward/Reverse.
 - Averaging: None, 2x, 4x, 8x, 16x.
 - Channels: Track1 (MPLX HS, AF594, Ref.), Track2 (MPLX HS, AF488).
 - High Intensity Laser Range: Track1 Lasers (405, 488, 561, 640), 561 nm, 0.8%, Relative Laser Power in Confocal Mode: 0.03%.
 - Alexa Fluor 594: Master Gain (850 V), Digital Offset (0), Digital Gain (1.0), Display Setting (Default).
 - Focus Strategy, Software Autofocus, Auto Immersion.
 - Multidimensional Acquisition: Experiment Information.
 - Applications: Auto Save.

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=2ahUKEwiir6KggN_5AhWFQPEDHWuTBtIQFnoECAcQAQ&url=https%3A%2F%2Fwww.imperial.ac.uk%2Fmedia%2Fimperial-college%2Fmedicine%2Ffacilities%2Ffilm%2FCell-Discoverer_Handbook.pdf&usg=AOvVaw00-1RpOoPzZGWTtDxAYRgv