

# Get started with ZEN 2009

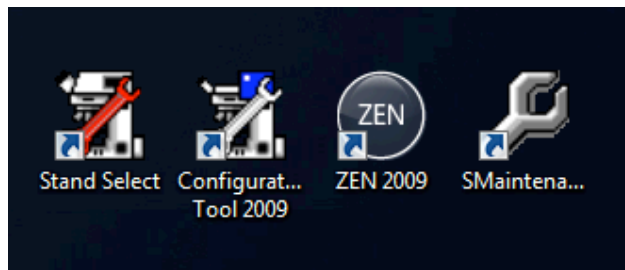
Dr Bertrand Vernay

Ext 2224

[b.vernay@ich.ucl.ac.uk](mailto:b.vernay@ich.ucl.ac.uk)

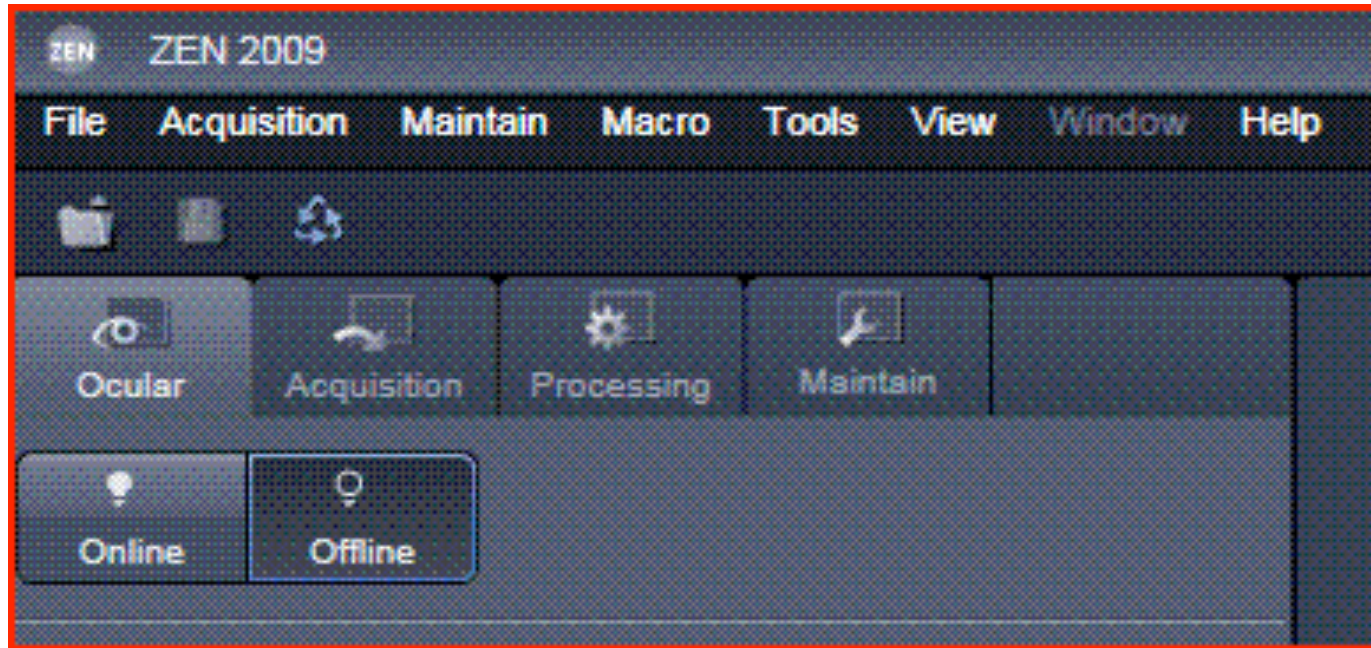
- 1- Start computer
- 2- Log in "LSM User"
- 3- Start software ZEN 2009
- 4- Start System

3



4



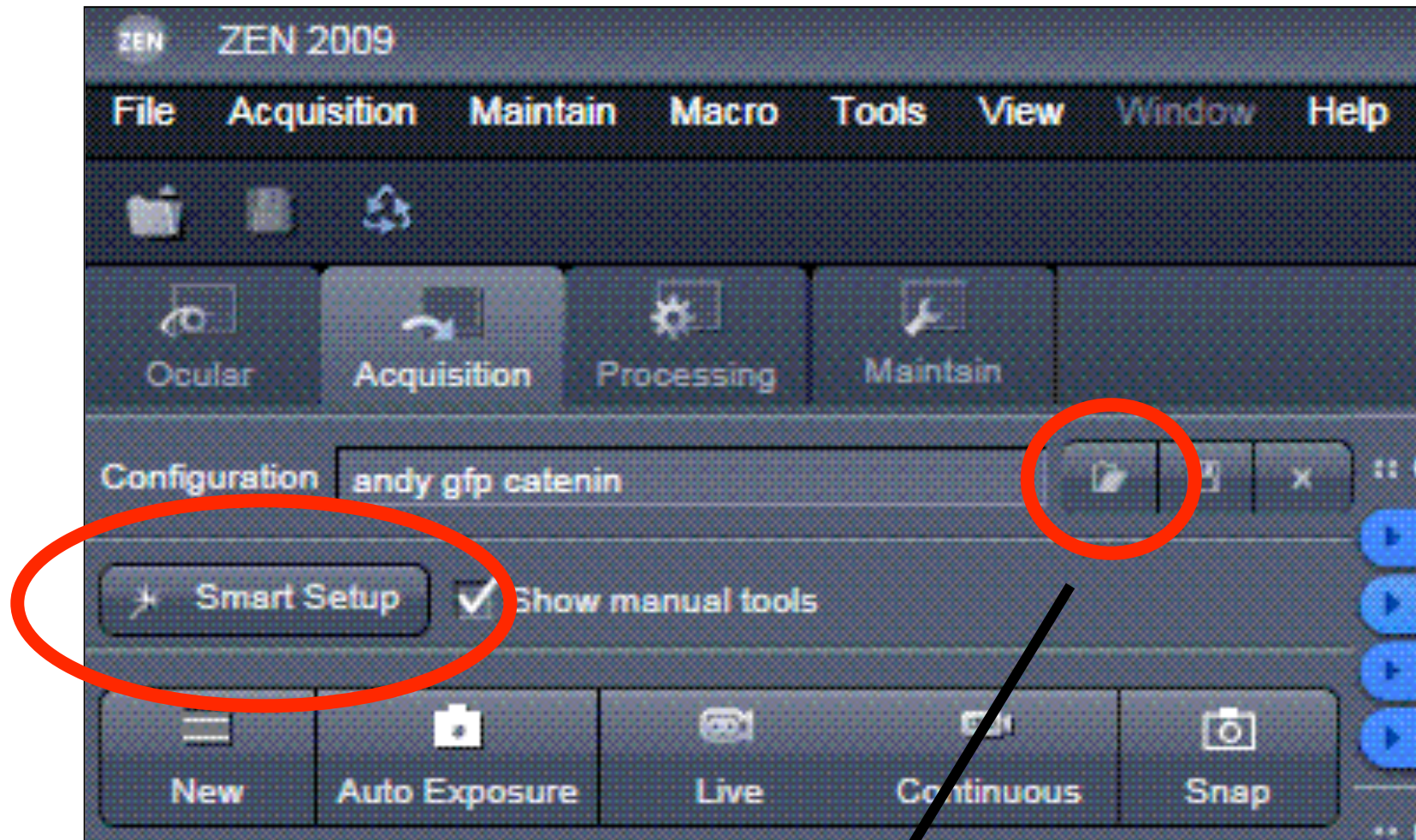


**Ocular** Tab : widefield microscope controls for direct observation via the eyepieces

- **Online** : to use the microscope
- **Offline** : to close the light shutters and use the confocal mode

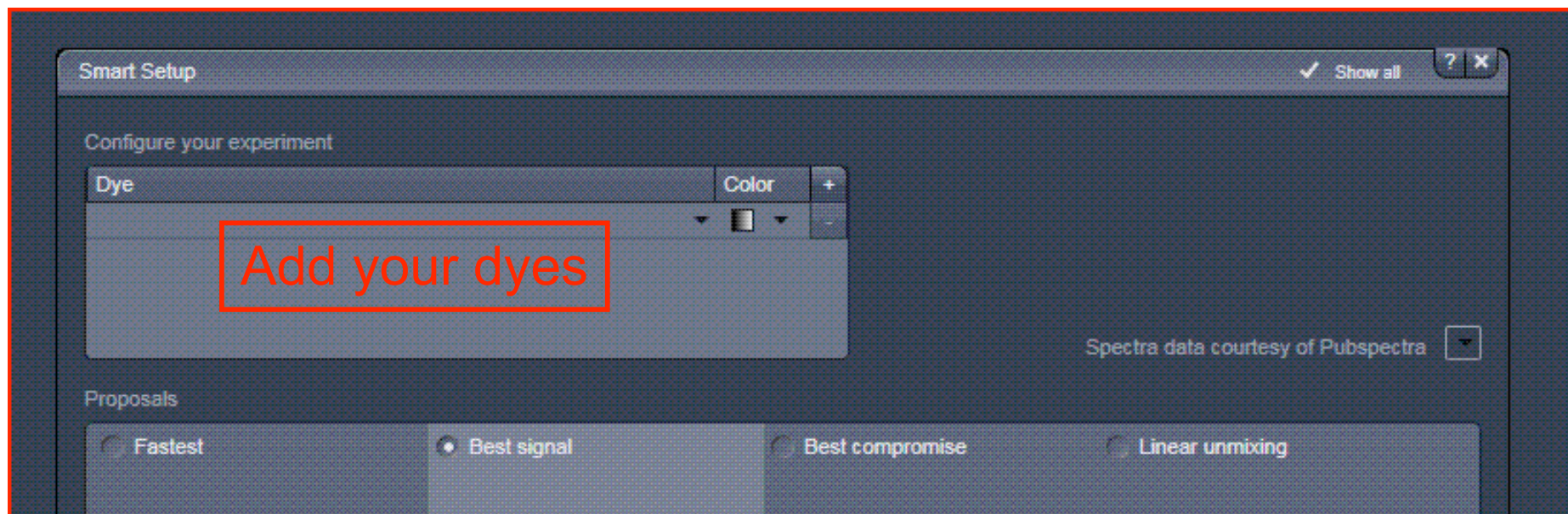
**Acquisition** Tab : confocal microscope mode

# Track(s) selection : Smart Setup

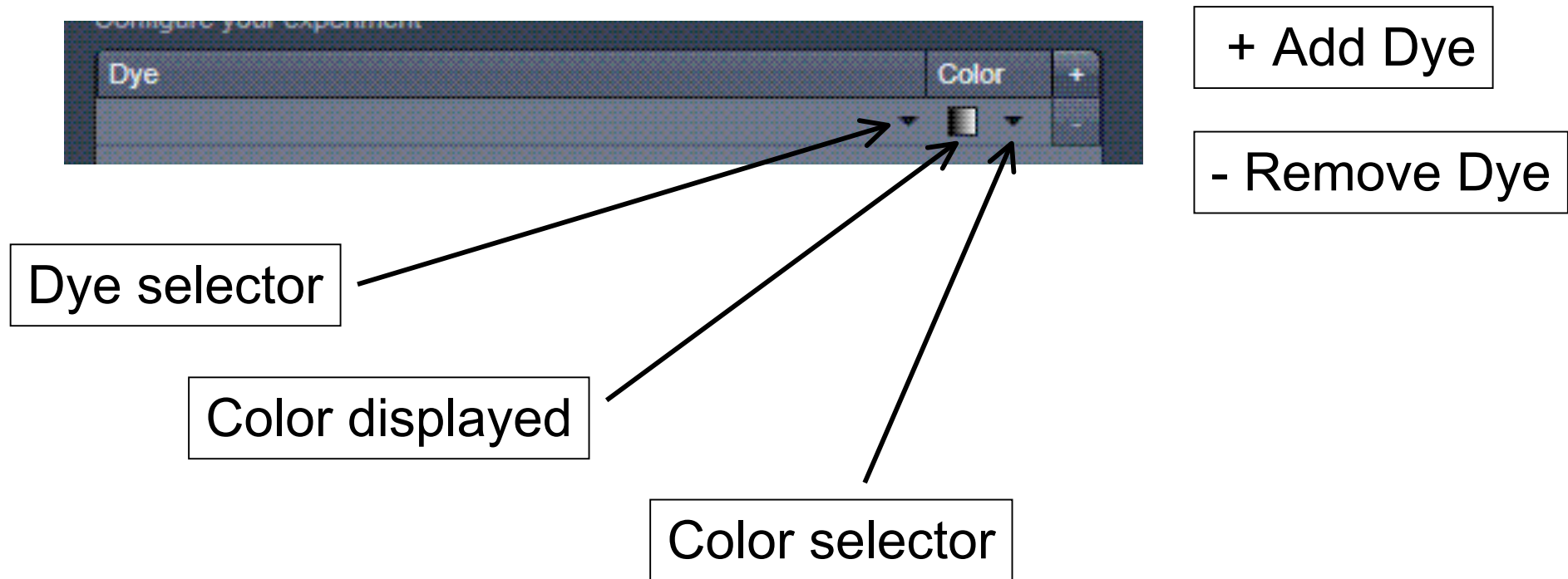


Or load a saved configuration

# Smart Setup window



# Smart Setup : selecting dye(s)



To add dyes, follow this order : from short to long emission wavelength

example :1) DAPI

2) Alexa Fluor 488

3) Alexa Fluor 568

4) Alexa Fluor 633

or

1) DAPI

2) FITC

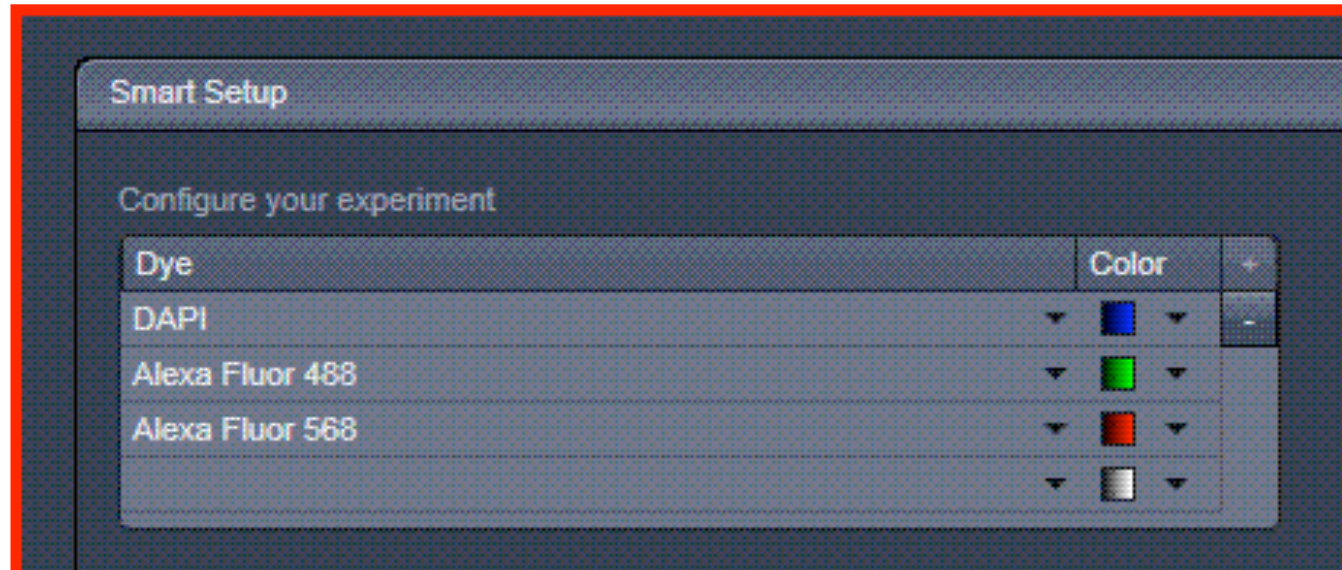
3) Cy5

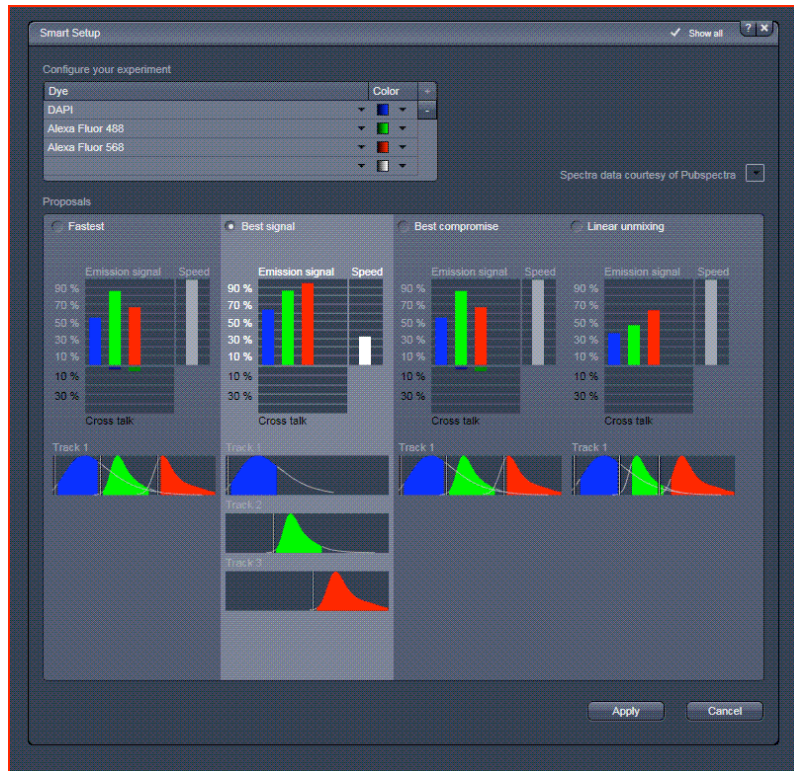
Example : multi-labelling

DAPI

Alexa Fluor 488

Alexa Fluor 568





### Fastest : simultaneous scanning

- :-) fastest mode
- :-( potential bleed-through between channel

### Best signal : sequential scanning

- :-) reduces bleed through by switching on only one laser and one detector at any one time
- :-( slower image acquisition

## **FASTEST mode**

for single labelling

for multiple labelling if there no bleed-through between the dyes

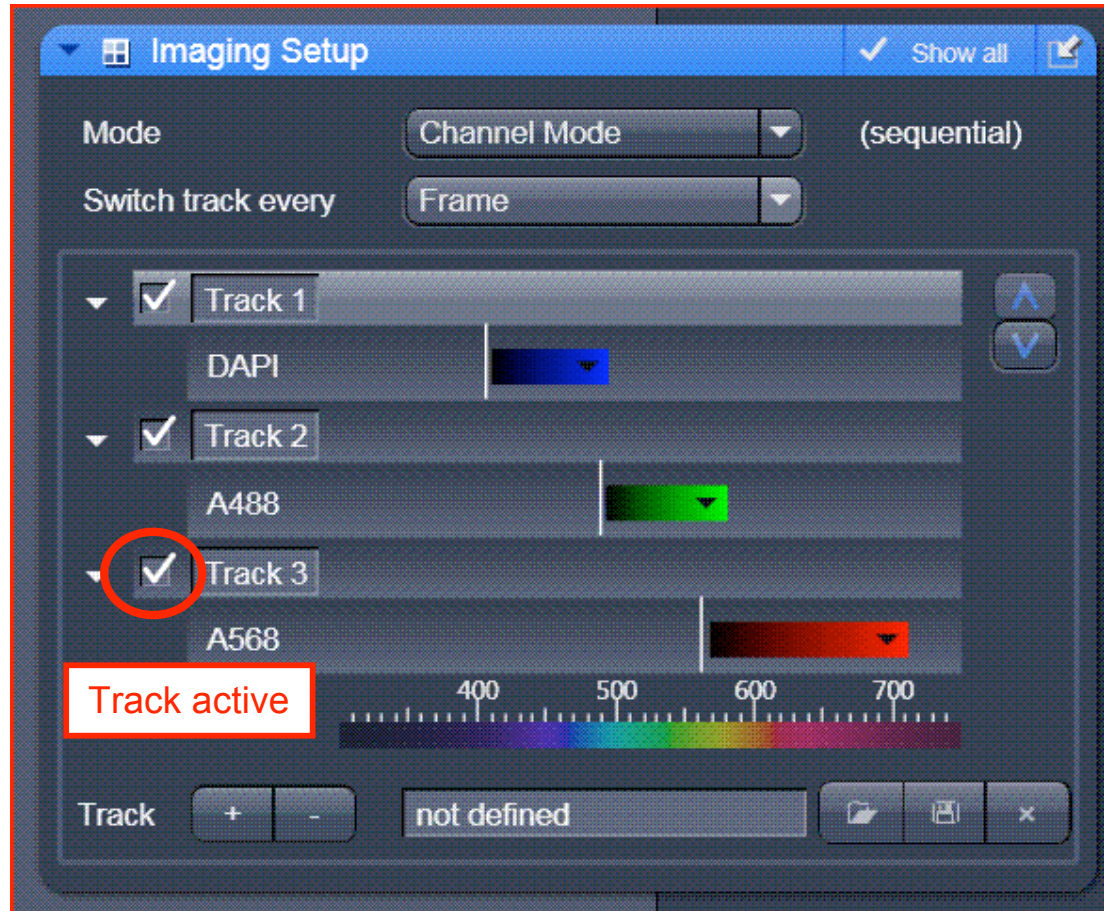
↳ CAUTION : CHECK FOR BLEED THROUGH WITH APPROPRIATE CONTROLS

## **BEST SIGNAL mode**

for multiple labelling



# Imaging Setup



## For each track

- Dye detected
- Laser (white line)
- Detection range (colored rectangle)

# Sequential Scan : Beam light Path

The image displays two panels from a microscope control software interface. The left panel, titled "Imaging Setup", shows three tracks: Track 1 (DAPI), Track 2 (A488), and Track 3 (A568). Track 1 is highlighted with a red oval. The right panel, titled "Light Path", shows a spectral graph and a table of dye configurations. A red box highlights a "405" laser source in the bottom left, and another red box highlights a "Visible light" source in the bottom right. The "Light Path" panel also shows a diagram of the optical path with components like Plate, MBS -405, Rear, Stage, Focus, and T-PMT.

**Imaging Setup Panel:**

- Mode: Channel Mode (sequential)
- Switch track every: Frame
- Track 1: DAPI (highlighted)
- Track 2: A488
- Track 3: A568

**Light Path Panel:**

Use	Dye	Color	Detector	Range	
<input checked="" type="checkbox"/>	DAPI	Blue	Ch1	410 - 495 nm	-
<input type="checkbox"/>			ChS1	415 - 727 nm	
<input type="checkbox"/>			Ch2	415 - 735 nm	

**405 Laser Source (Red Box 1):**

- 405 nm
- Attenuation: OFF

**Visible light Source (Red Box 2):**

- Visible light

# Sequential Scan : Beam light Path

The screenshot displays the software interface for a sequential scan, divided into three main sections:

- Imaging Setup:** Shows the configuration for three tracks. Track 1 is DAPI, Track 2 is A488 (highlighted with a red circle), and Track 3 is A568. The mode is set to Channel Mode (sequential) and the switch track every is set to Frame.
- Light Path:** Shows the excitation path for Track 2. A spectral plot shows the emission of Alexa Fluor 488. Below the plot is a table of channel configurations:

Use	Dye	Color	Detector	Range	
<input type="checkbox"/>			Ch1	415 - 735 nm	-
<input checked="" type="checkbox"/>	Alexa Fluor 488	Green	ChS1	493 - 581 nm	
<input type="checkbox"/>			Ch2	415 - 735 nm	

Below the table, the light path diagram shows the excitation path for the 488 nm laser line. The path includes the MBS 488/594, MBS 690+, and Rear mirrors, leading to the Stage and Focus. The T-PMT is also shown. A red box with the number '1' highlights the Visible light source in the diagram.

**488 nm Laser Line Detail:** A red box with the number '1' highlights a detailed view of the 488 nm laser line. It shows a list of available wavelengths: 458, 488 (checked), 514, 561, 594, and 633. Below the list is a slider for the selected wavelength, currently set to 488 nm, and a numerical input field set to 2.0.

# Sequential Scan : Beam light Path

The screenshot displays two panels from a microscope control software interface. The left panel, titled "Imaging Setup", shows the configuration for a sequential scan. It includes a "Mode" dropdown set to "Channel Mode" (sequential), a "Switch track every" dropdown set to "Frame", and three tracks: Track 1 (DAPI), Track 2 (A488), and Track 3 (A568). Track 3 is circled in red. The right panel, titled "Light Path", shows the configuration for Track 3. It includes a "Channel Mode" dropdown set to "Channel Mode", a "Lambda Mode" dropdown set to "Lambda Mode", and an "Online Fingerprinting" button. Below these are a spectral plot showing a peak at approximately 600 nm, a "Use" table, and a diagram of the light path.

**Imaging Setup**

Mode: Channel Mode (sequential)  
Switch track every: Frame

Track 1: DAPI  
Track 2: A488  
Track 3: A568

**Light Path**

Channel Mode | Lambda Mode | Online Fingerprinting

Track 3

Spectral plot: 400 - 700 nm

Use	Dye	Color	Detector	Range	+
<input type="checkbox"/>			Ch1	415 - 735 nm	-
<input type="checkbox"/>			ChS1	415 - 727 nm	-
<input checked="" type="checkbox"/>	Alexa Fluor 568		Ch2	568 - 712 nm	-

Reflection:

MBS 458/561 | None | Rear

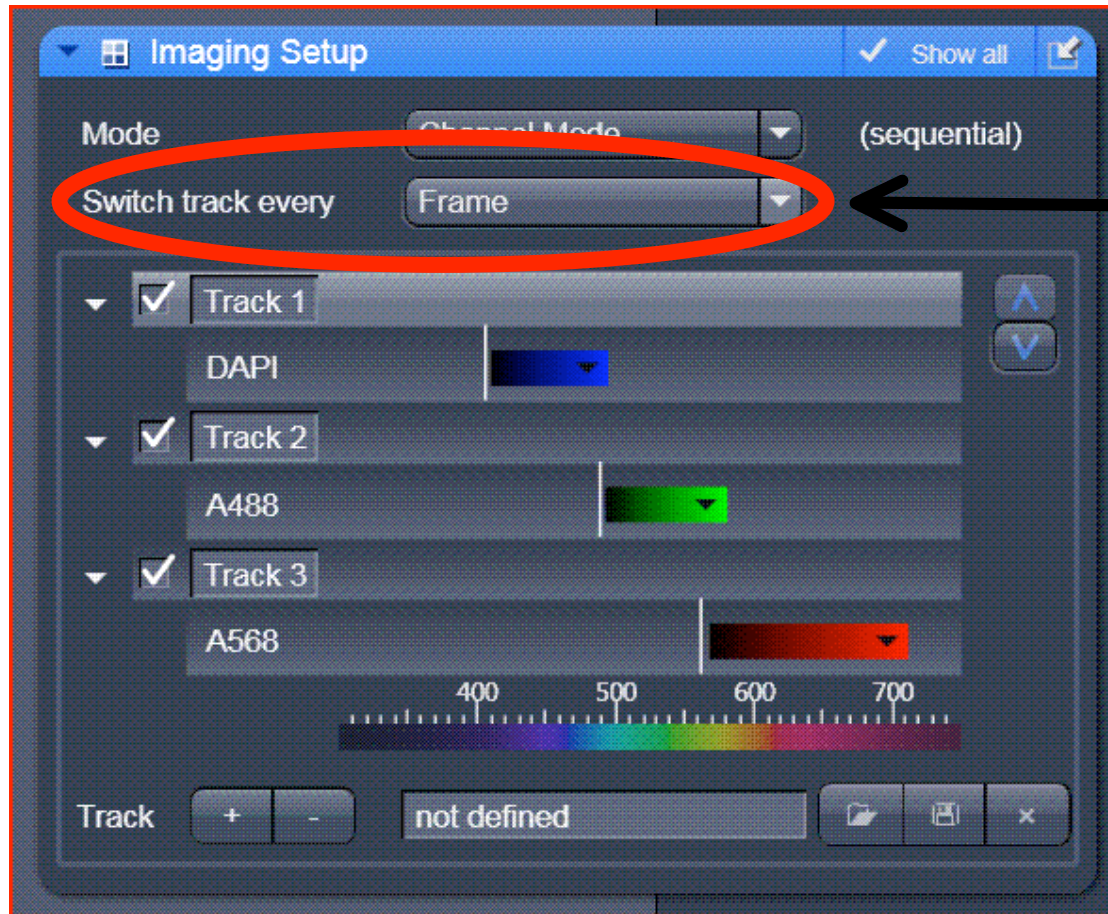
Visible light | Invisible light

Stage | Focus

T-PMT

Ratio

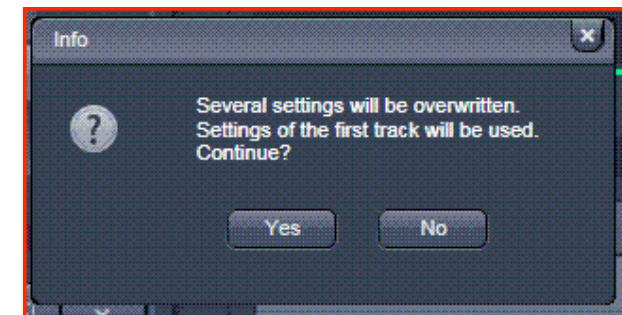
# Switch track every Line



Select LINE

Use **Switch track every Line** to limit artefact due to movement of the sample (live sample or vibrations). Setting up **Switch track every Line** requires some modification of the **Light Path**.

No worry, Press YES !



All tracks will use the same MBS settings. The MBS reflect the excitation light onto the sample. To select the appropriate MBS match the excitation wavelength in use with the MBS number(s)

- MBS 458
- MBS 458/514
- MBS 458/561
- MBS 458/514/594
- MBS 488
- MBS 488/561
- MBS 488/594
- MBS 488/561/633
- MBS T80/R20
- Plate

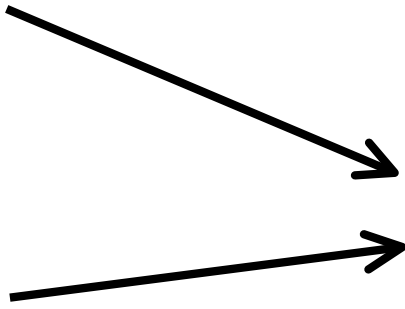
### VISIBLE LIGHT

**Plate** : no visible laser/diode in use  
**MBS -XXX**: XXX should match ALL the visible excitation wavelengths used (458, 488, 514, 561, 594, 633)  
**MBS T80/R20** : use when no specific MBS available.  
Example : 488, 594 and 633, no MBS -488/594/633

- Plate
- None
- MBS -405
- MBS -445
- MBS 690+
- MBS 760+
- MBS -405/760+
- MBS -445/760+
- MBS T80/R20
- None

### INVISIBLE LIGHT

**Plate** : UV diode 405 nm not in use  
**MBS -405** : UV diode 405 nm in use



The 'Light Path' control panel is shown with a red border. It features three rows of controls: 'MBS 488/594' with a green indicator, 'MBS 690+' with a red indicator, and 'Rear' with a black indicator. To the right, there are two sliders: 'Visible light' and 'Invisible light', both with active indicators. At the bottom, there are buttons for 'Stage' and 'Focus', and a 'Ratio' section with a 'PMT' indicator and a right-pointing arrow.

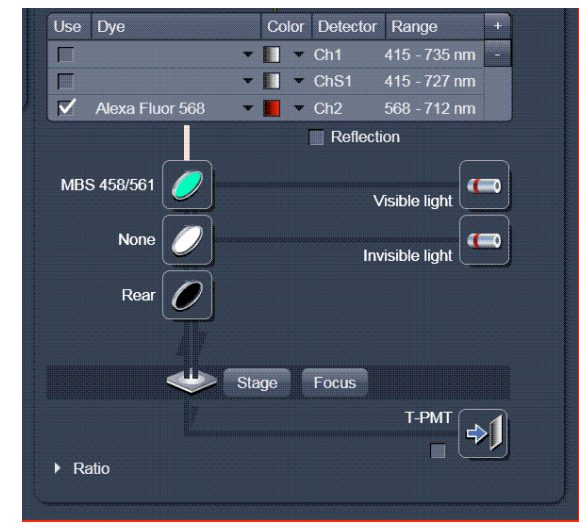
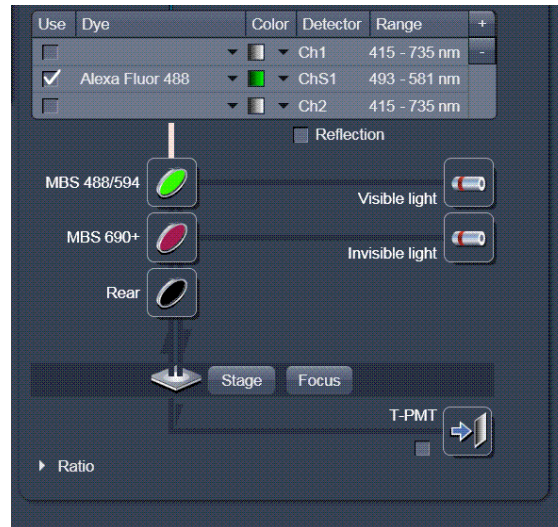
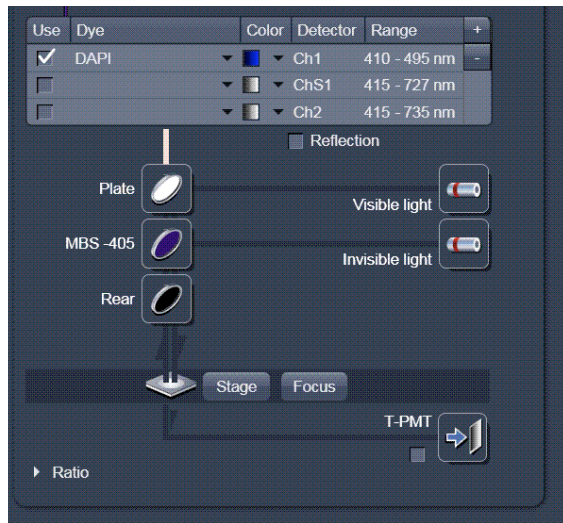
# All tracks will use the same MBS settings

## DAPI (405 nm)

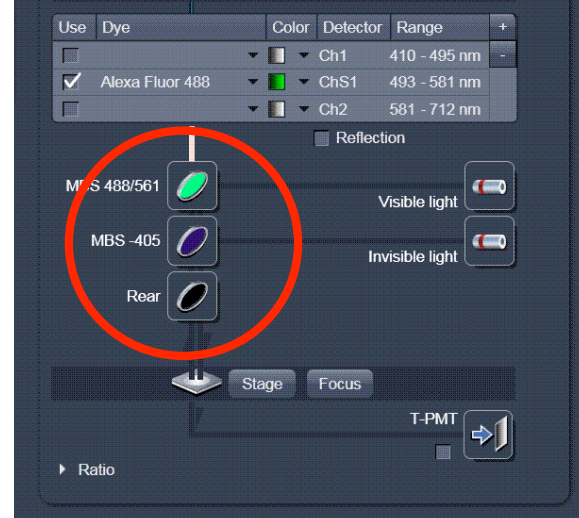
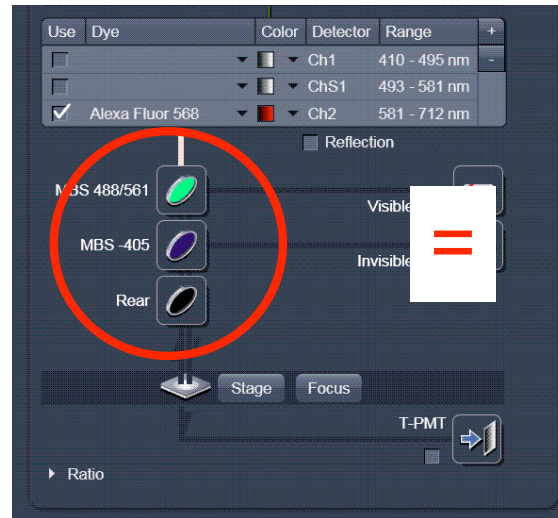
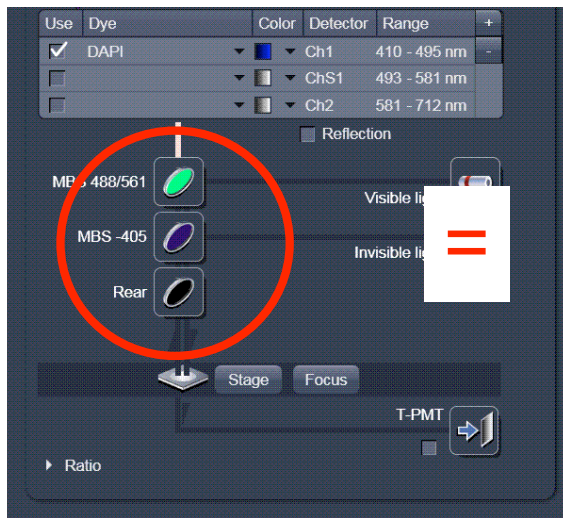
## Alexa 488 (488 nm)

## Alexa 568 (561 nm)

FRAME

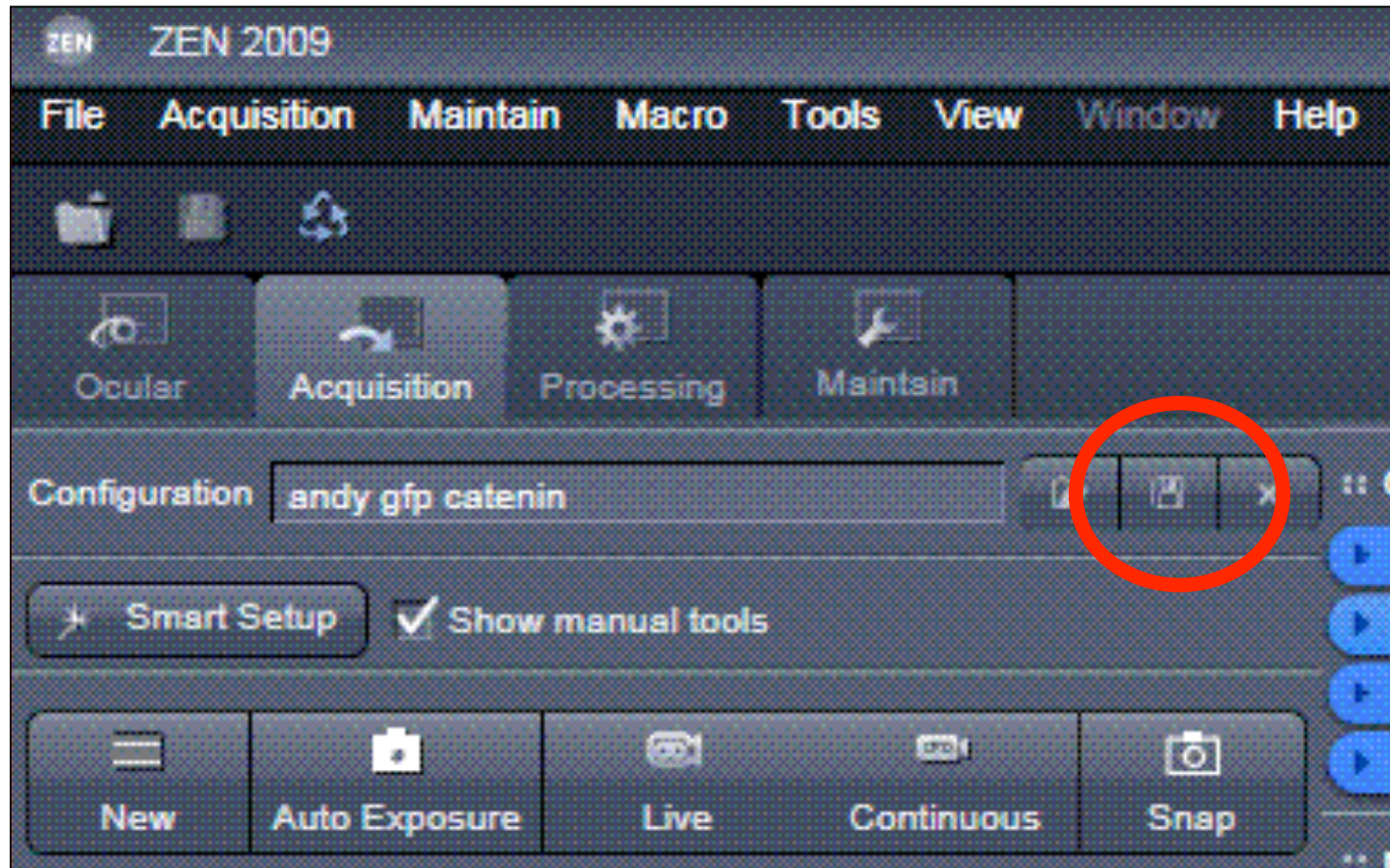


LINE



MBS selection are automatically applied to all tracks

# Save your configuration now



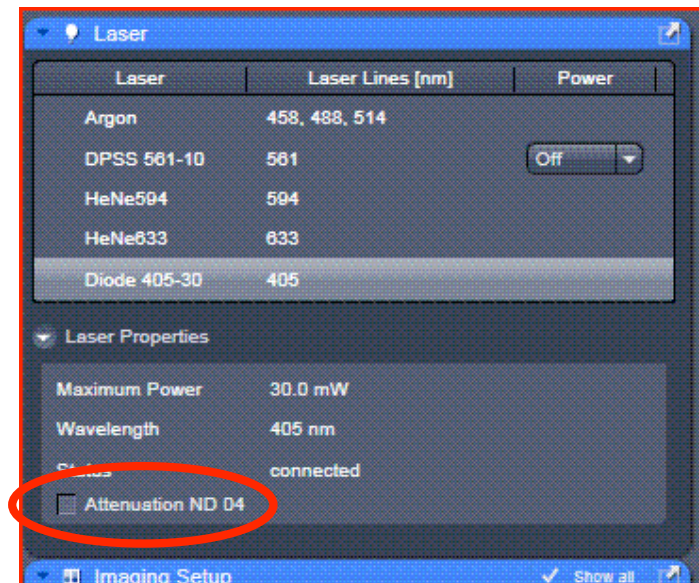


# Laser setup



The 561 nm Diode has to be turned on within ZEN 2009

A neutral density filter can be used to reduce the 405 nm diode intensity : Attenuation ND 04



# Acquisition



**New** : creates new image file.

**Auto Exposure** : automatic pre-adjustment of detector and gain by the ZEN 2009

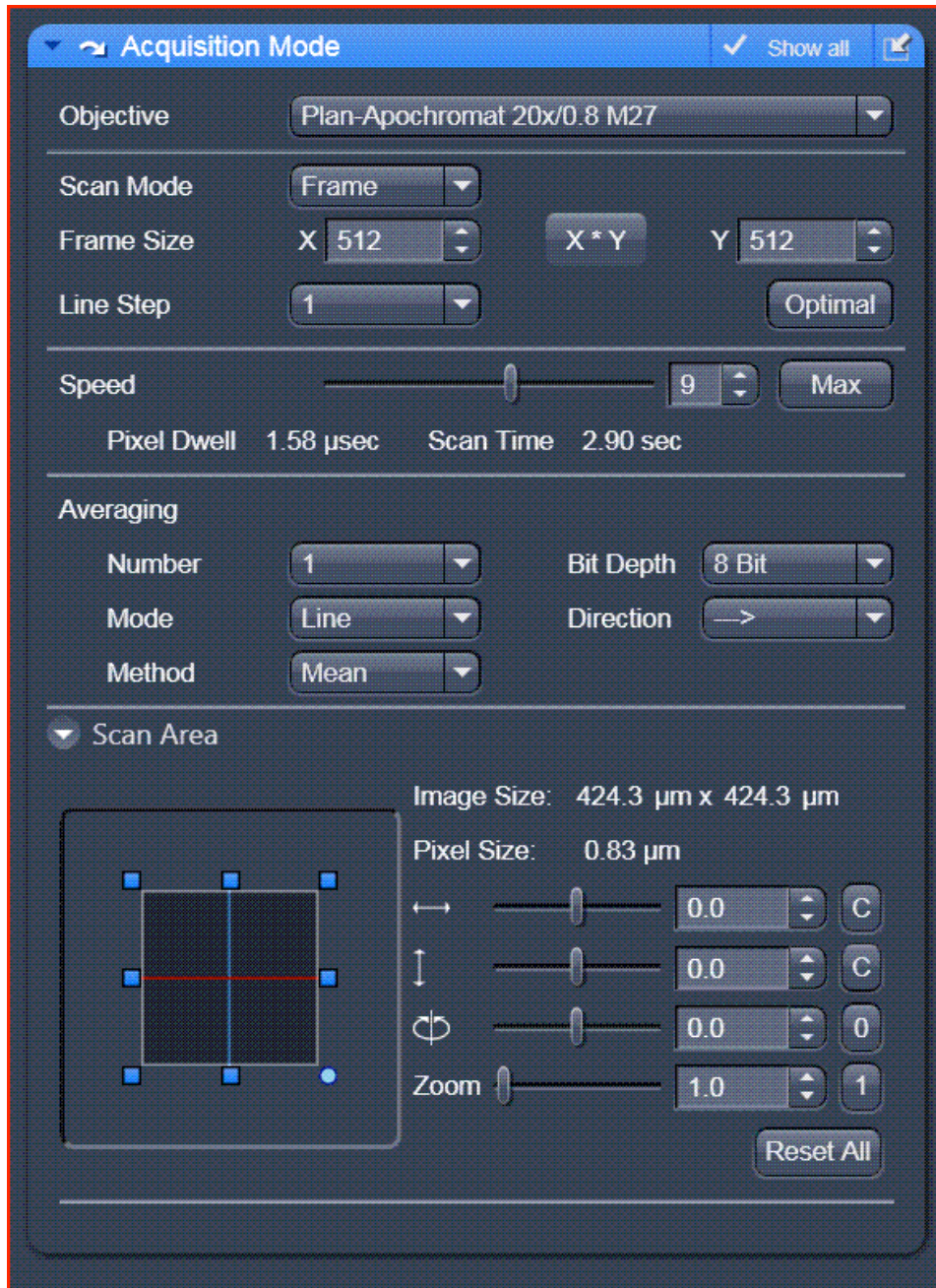
**Live** : continuous fast scanning (focusing, gain/offset adjustment)

**Continuous** : continuous scanning with the selected scan speed

**Snap** : records a single image

**Stop** : stops the current scan procedure

**Start Experiment** : records multiple images according to the options activated on the left



**Scan mode** : always on Frame

**Line Step** : always 1

**Frame size** : click **Optimal** for optimal image resolution according to the Nyquist theorem

**Speed** : 8 usually produces good results.  
Lower speed : improves signal-noise ratio  
Faster speed : reduces scanning time

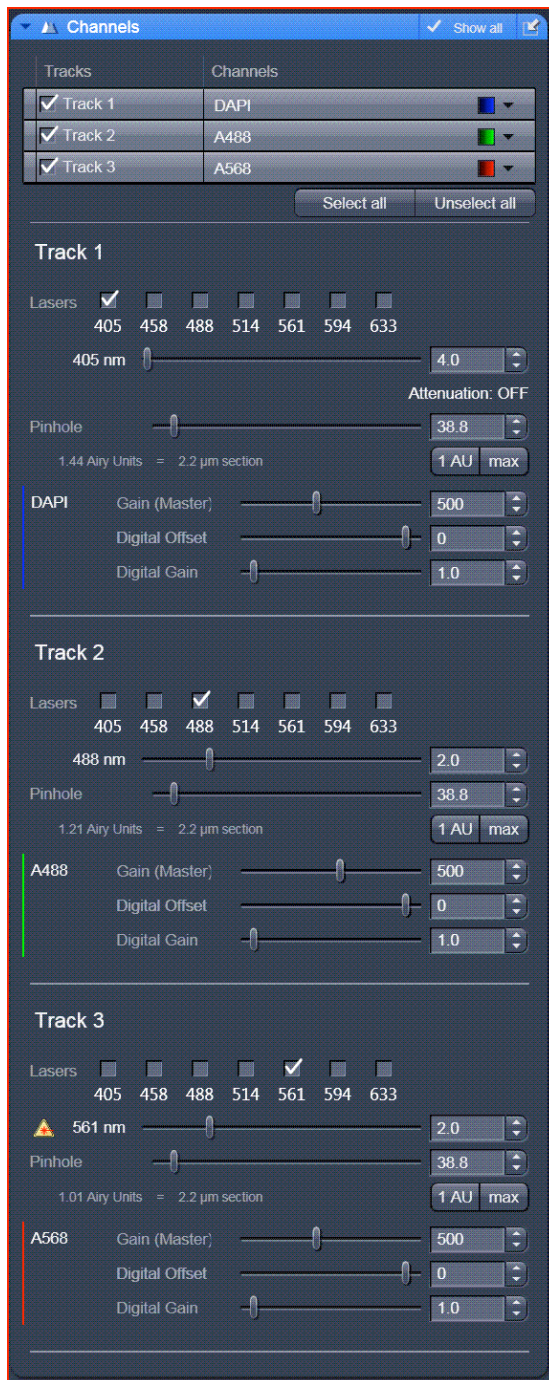
**Averaging** : number 1-16

**Mode** : Line

**Method** : Mean

**Bit depth** : 8-, 12- or 16-bit (mimimum 12-bits for intensity measurement)

**Scan area** : Position (X,Y), Rotation (360°), Optical Zoom (0.6x to 40x)



**Laser transmission** : modulate the intensity of the laser light to the specimen

**Pinhole** : set on 1 AU for best compromise between depth discrimination and detection efficiency. For multiple labeling experiments adjust the pinhole so that each channel as the same optical slice thickness. This is important for co-localisation study.

**Gain (master)** : sensitivity of the detector

**Digital Offset** : adjust the black level (background)

**Digital Gain** : signal amplification

**USE THE RANGE INDICATOR WHEN DEFINING THE DETECTION PARAMETERS**  
**Fill the dynamic range : some blue pixel (black) and some red pixels (saturation)**