Get started with ZEN 2009

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1- Start computer

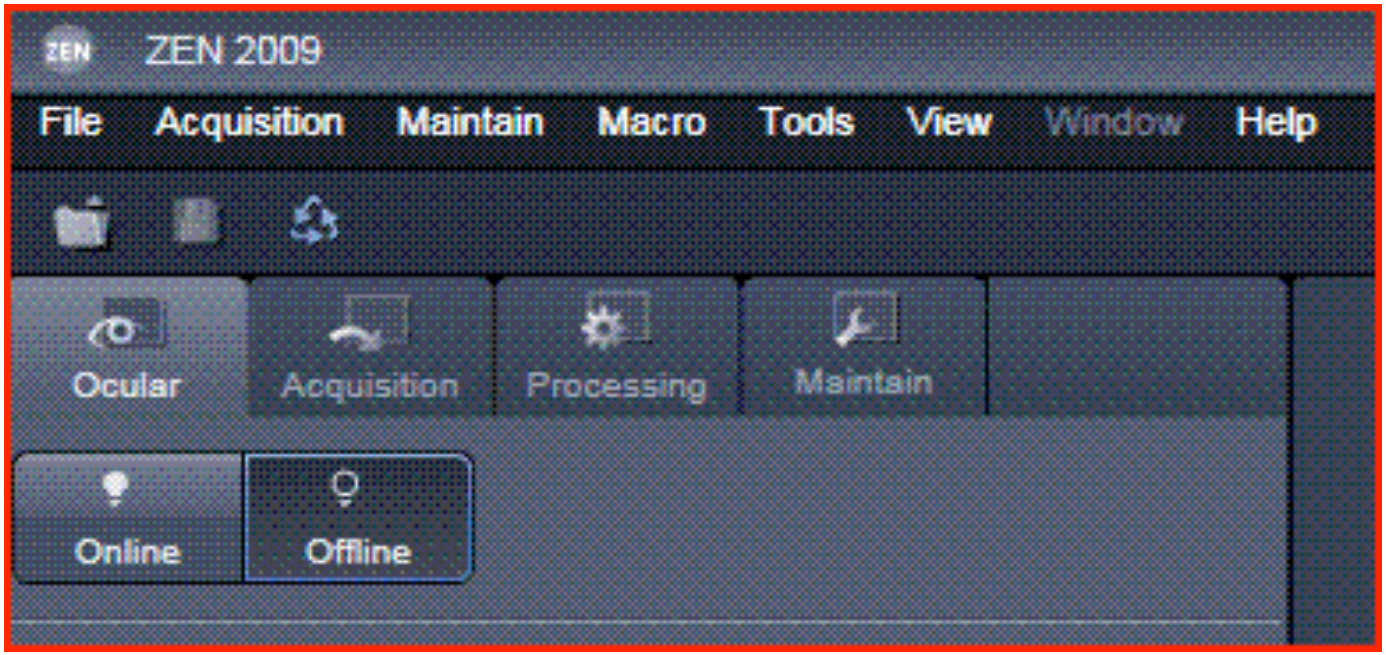
2- Log in “LSM User”

3- Start software ZEN 2009

4- Start System

3

4

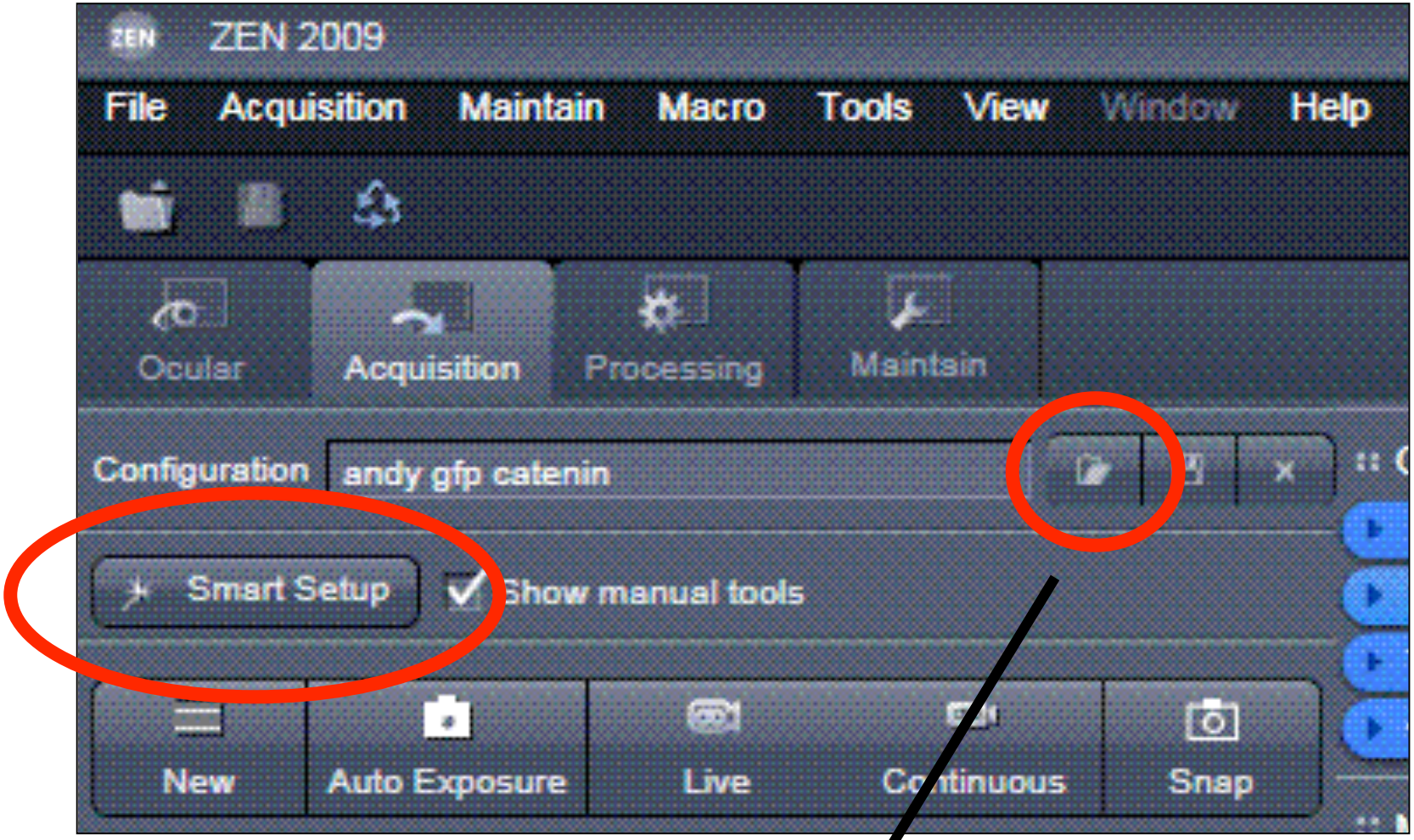


Ocular Tab : widefield microscope controls for direct observation via the eyepieces

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

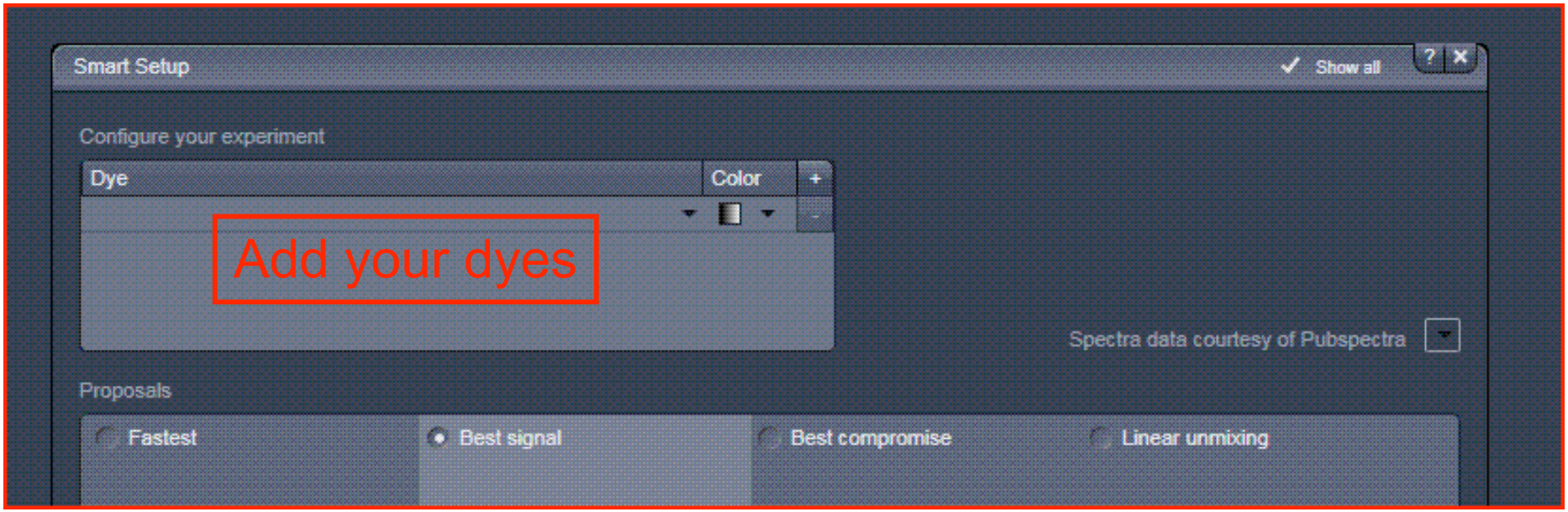
Acquisition Tab : confocal microscope mode

Track(s) selection : Smart Setup



Or load a saved configuration

Smart Setup window



Smart Setup : selecting dye(s)

+ Add Dye

- Remove Dye

Dye selector

Color displayed

Color selector

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

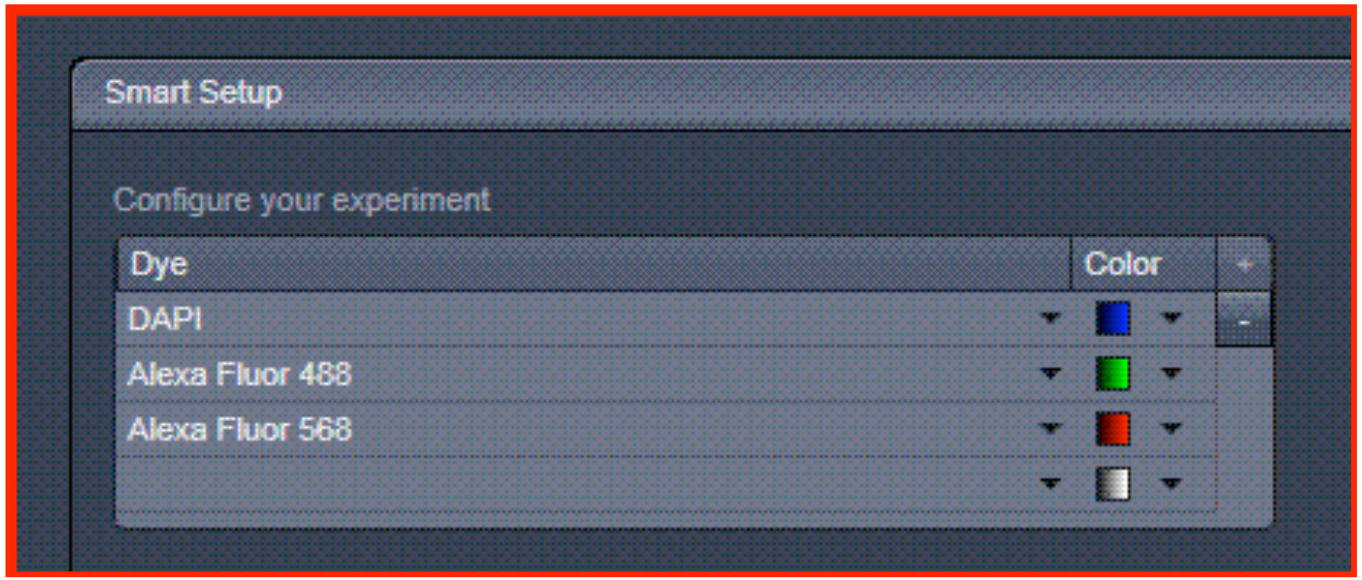
1) DAPI
2) FITC
3) Cy5

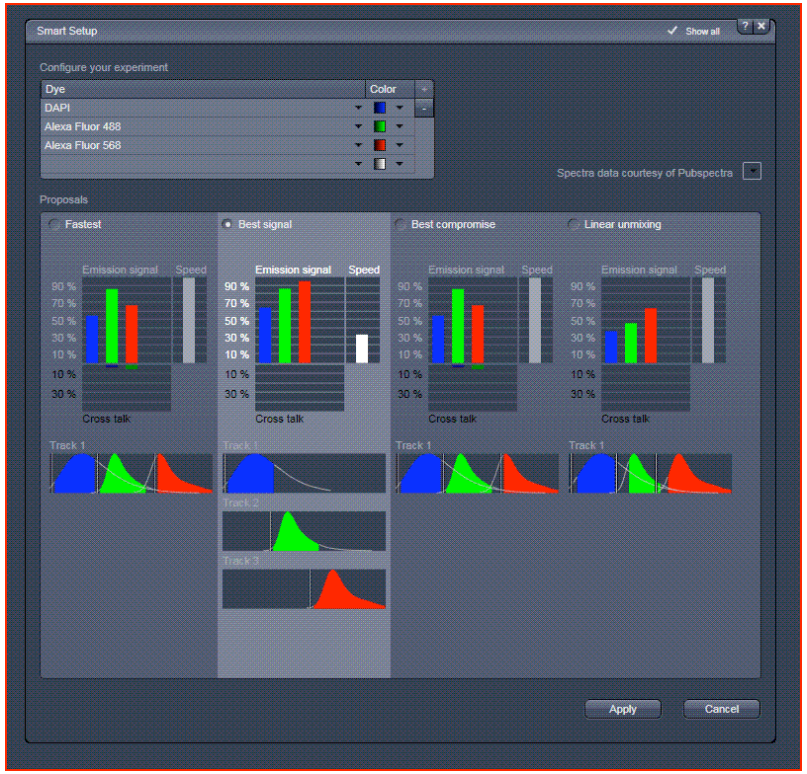
Example : multi-labelling

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

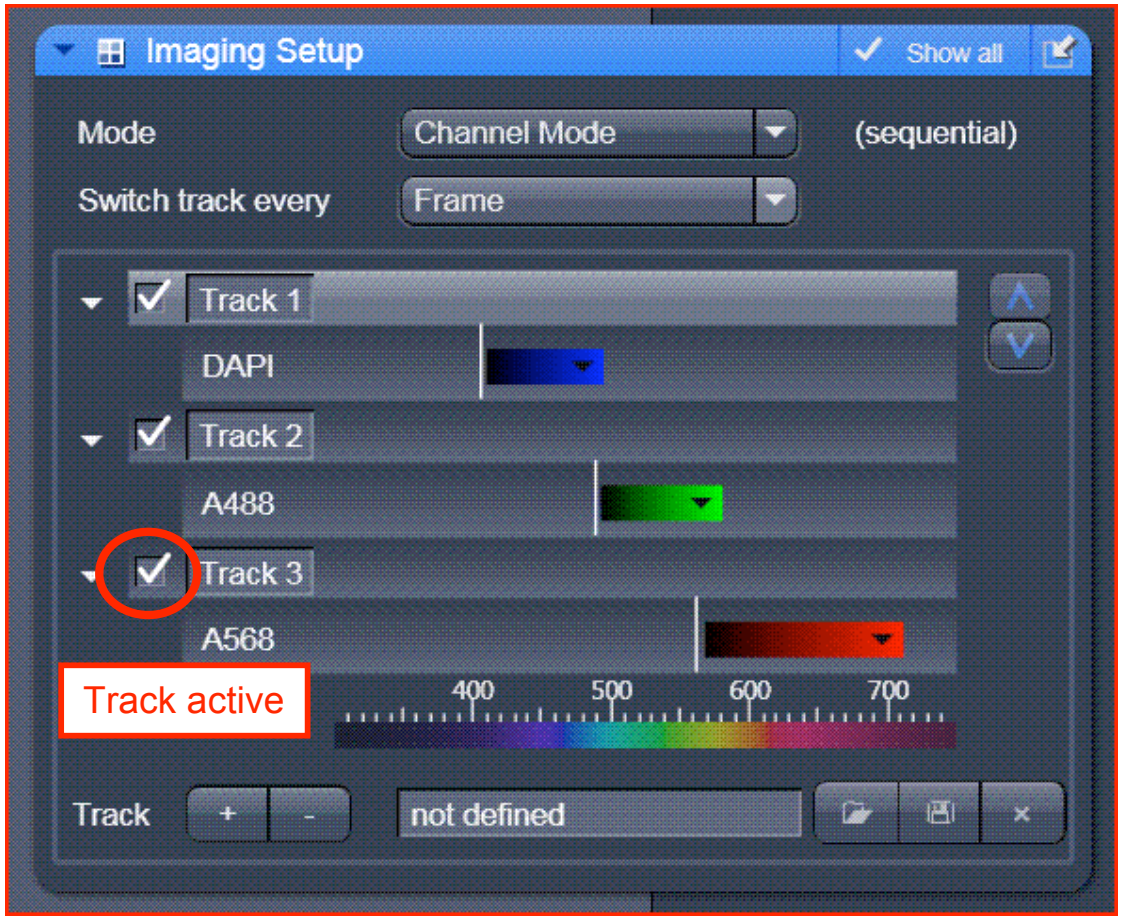
for multiple labelling if there no bleed-through between the dyes

↪**CAUTION : CHECK FOR BLEED THROUGH WITH APPROPRIATE CONTROLS**

BEST SIGNAL mode

for multiple labelling

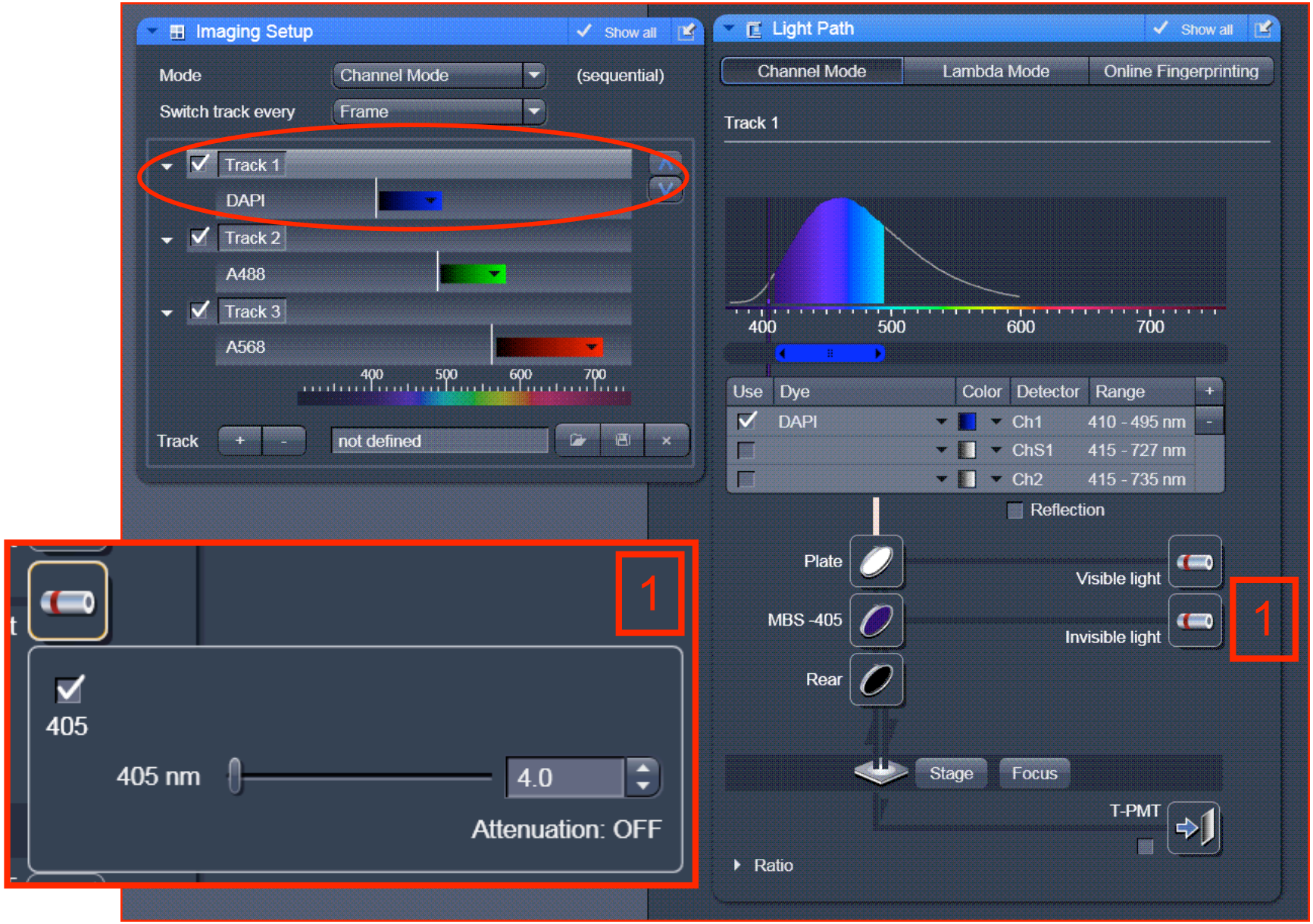
Imaging Setup



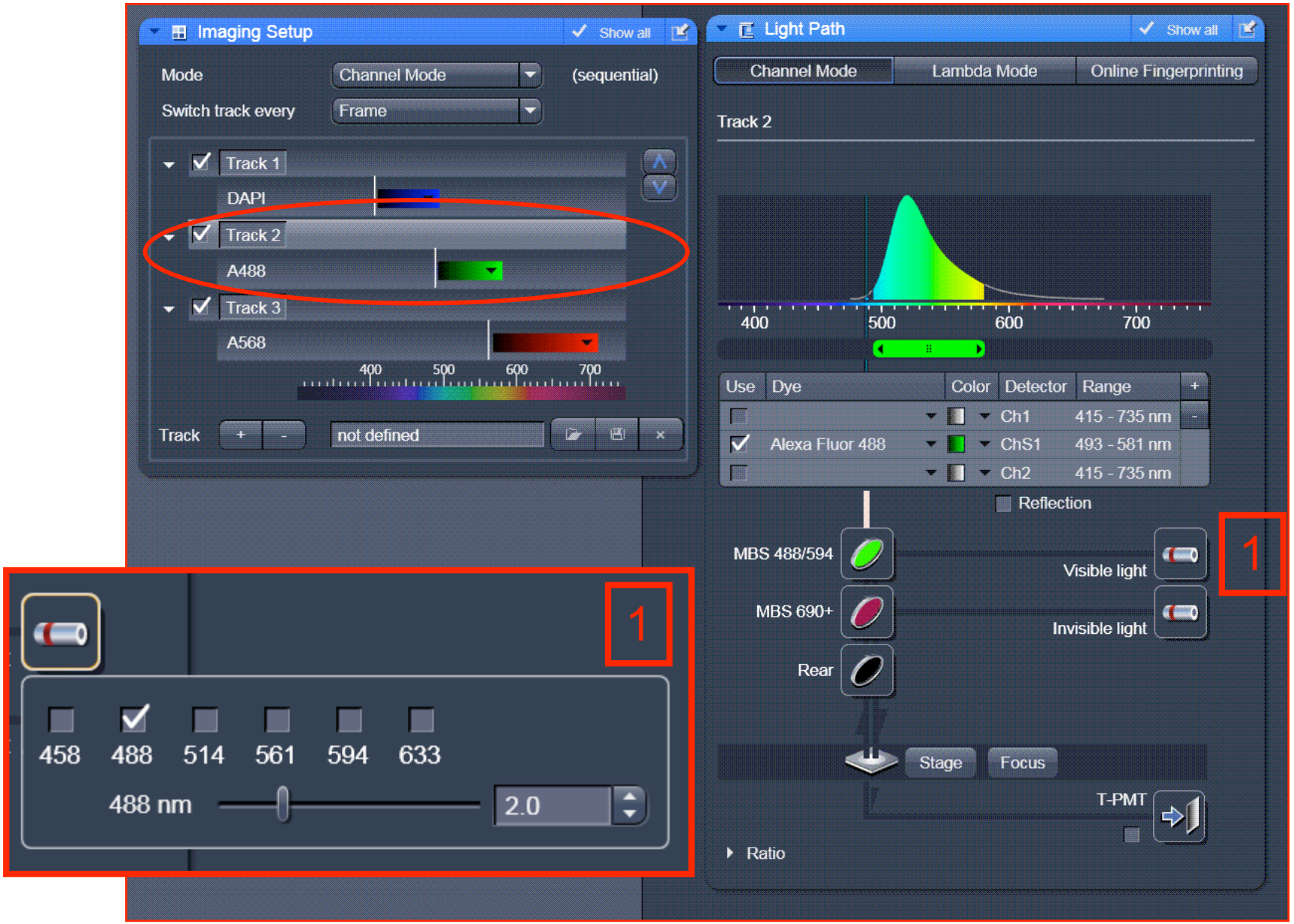
For each track

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

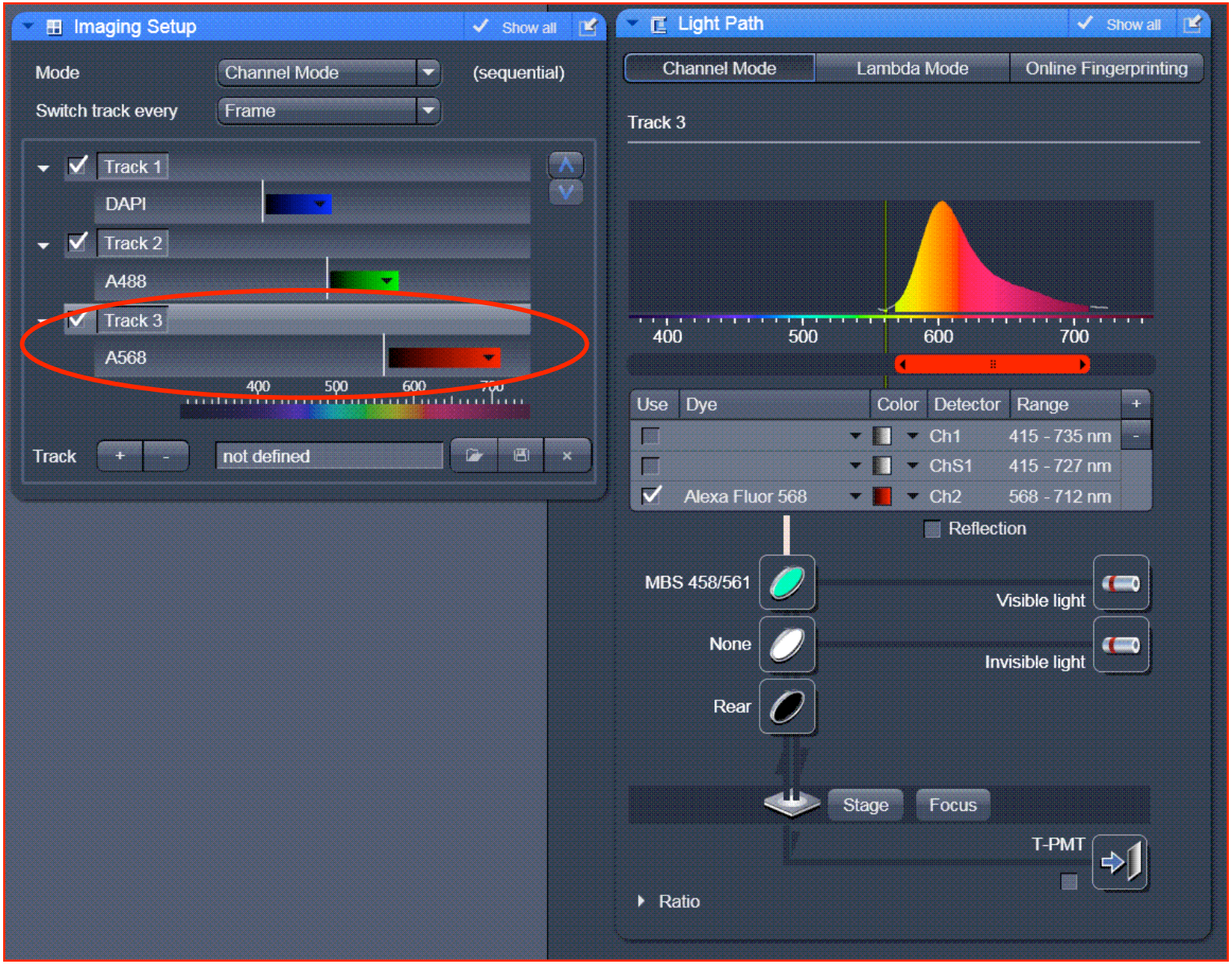
Sequential Scan :Beam light Path



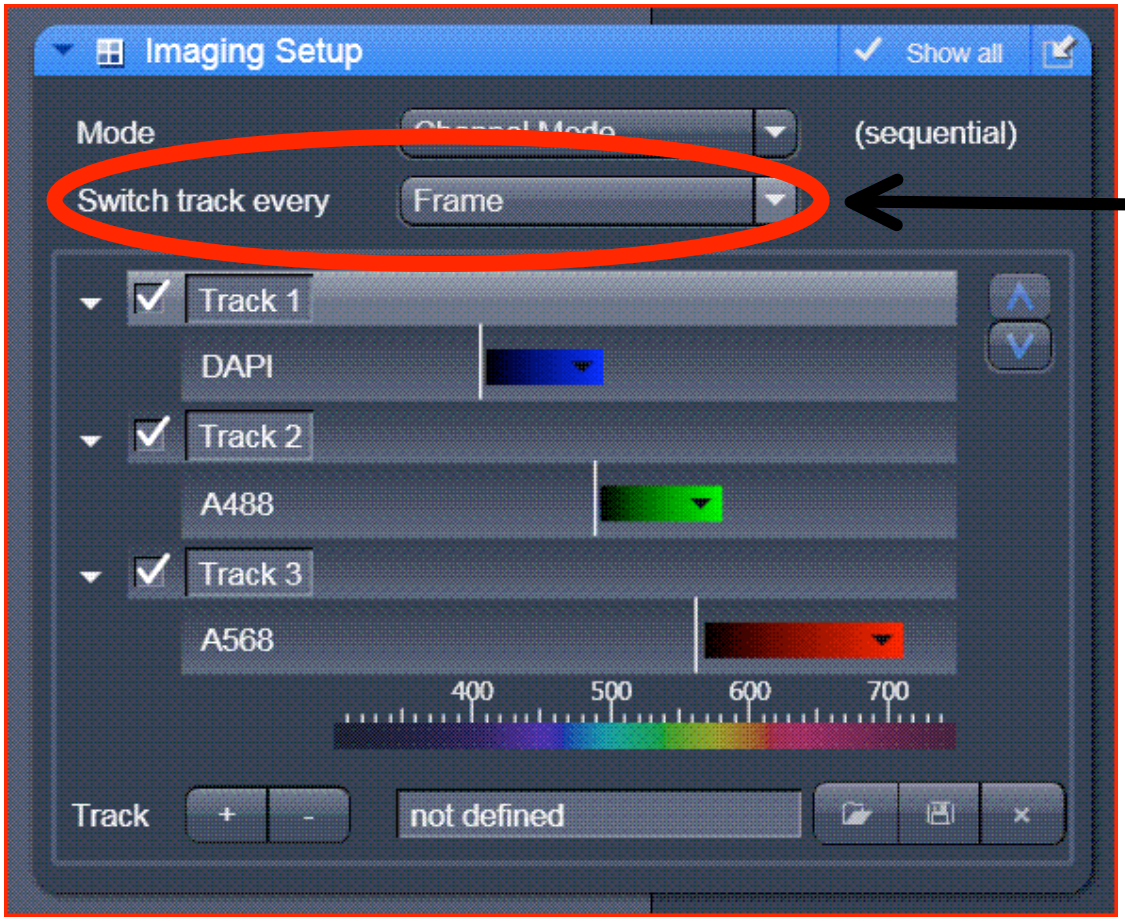
Sequential Scan :Beam light Path



Sequential Scan :Beam light Path



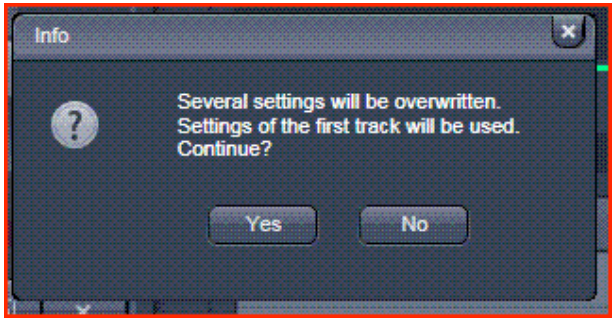
Switch track every Line



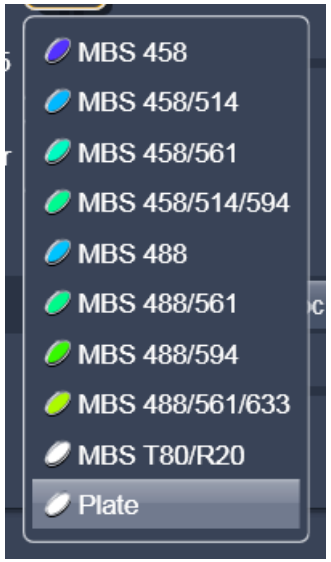
Select LINE

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 !

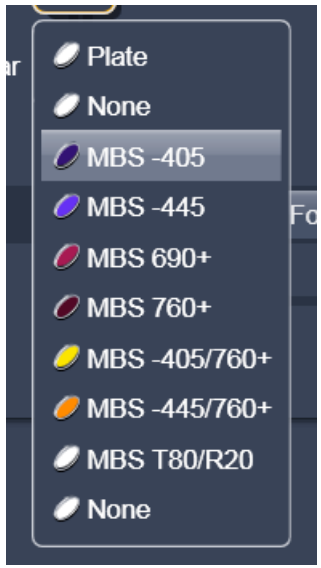


All tracks will use the same MBS settings. The MBS reflect the excitation light onto the sample. To select the appropriate MBS match the excitation wavelength in use with the MBS number(s)



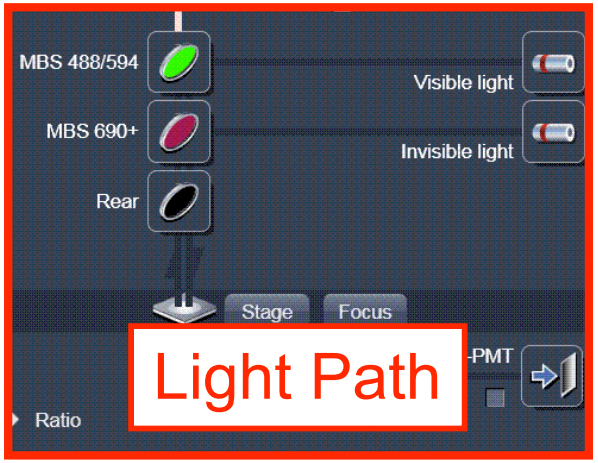
VISIBLE LIGHT

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



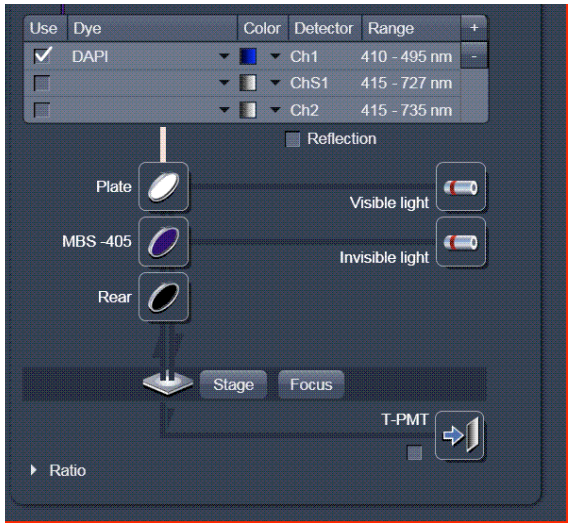
INVISIBLE LIGHT

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

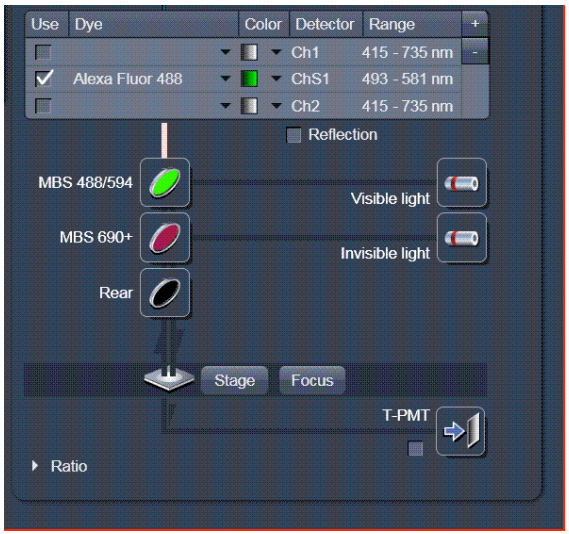


All tracks will use the same MBS settings

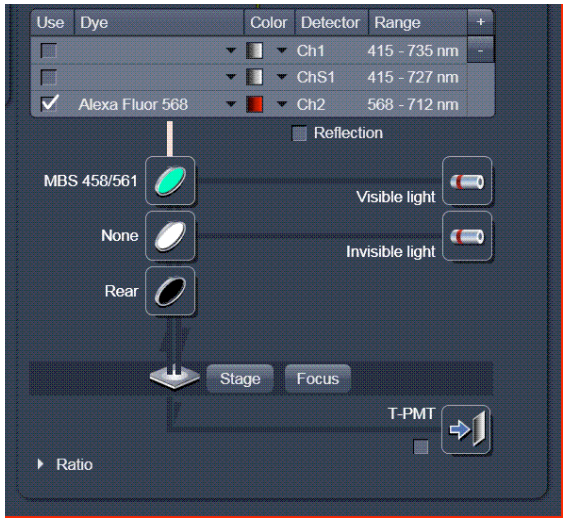
DAPI (405 nm)



Alexa 488 (488 nm)

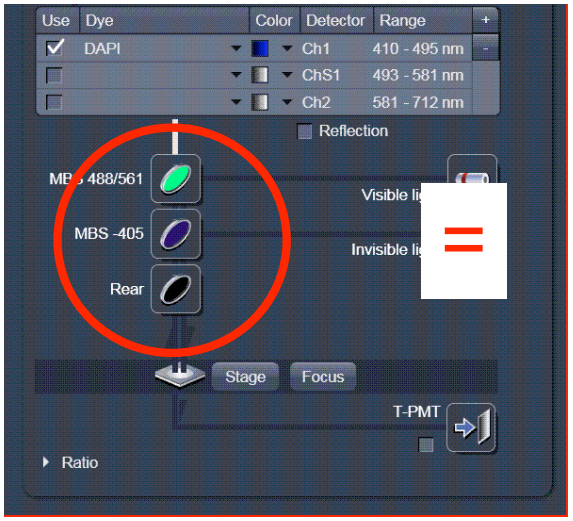


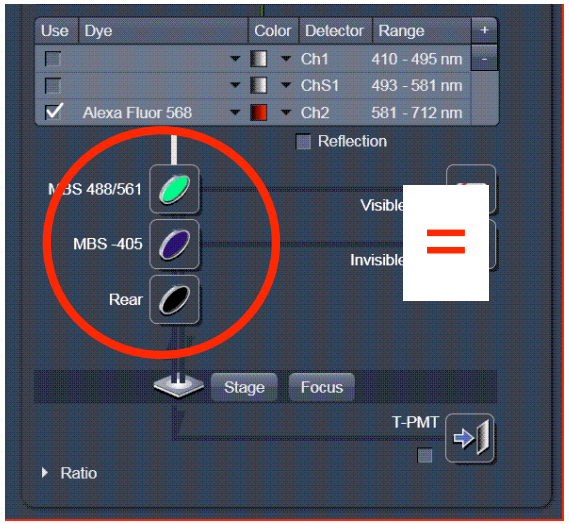
Alexa 568 (561 nm)

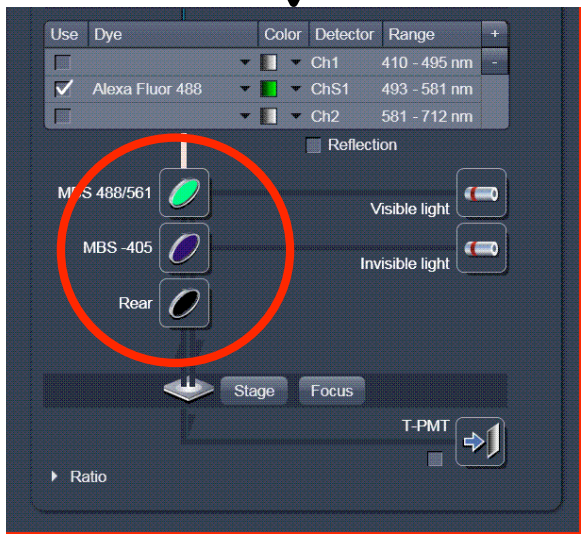


FRAME

LINE

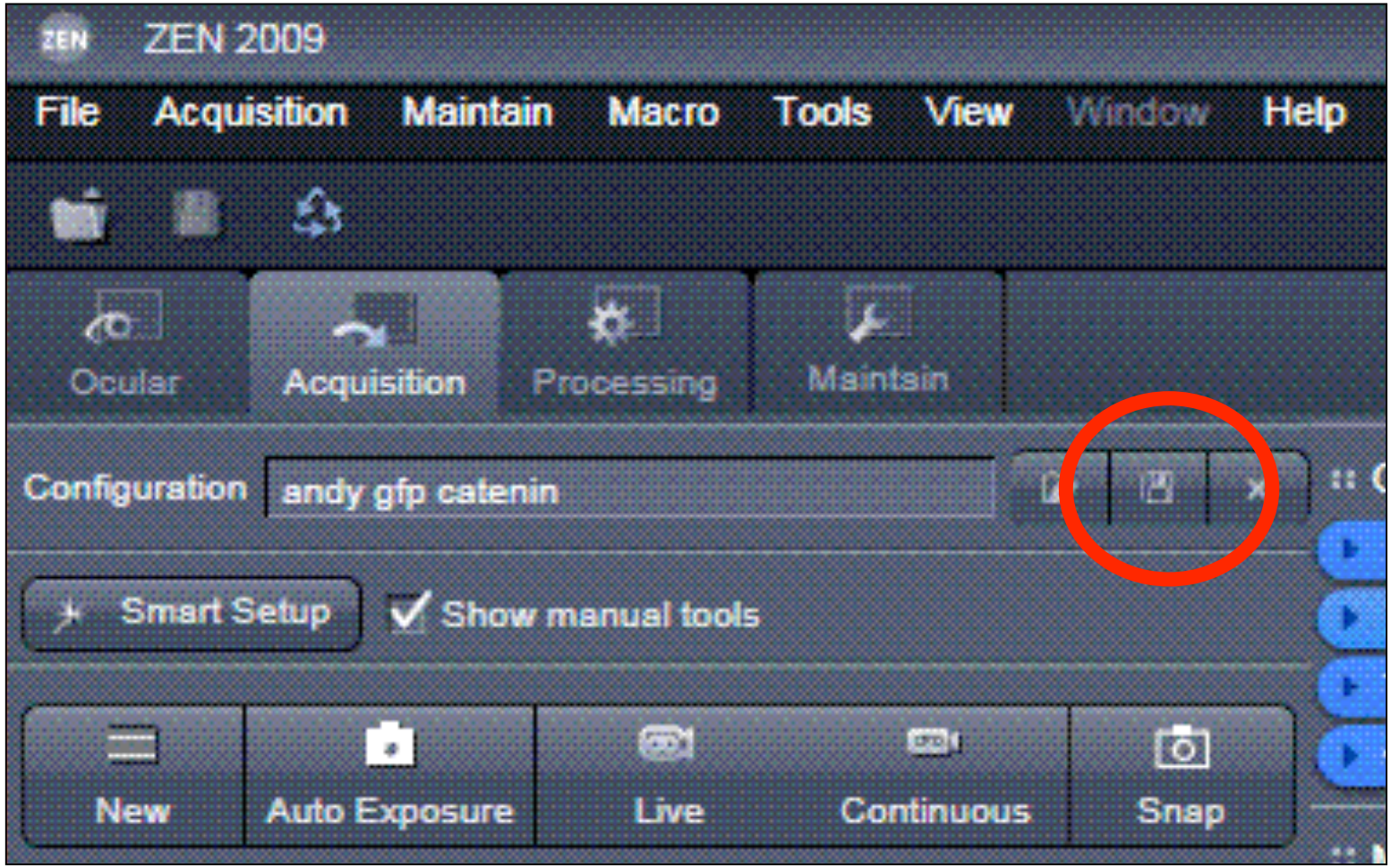






MBS selection are automatically applie to all tracks

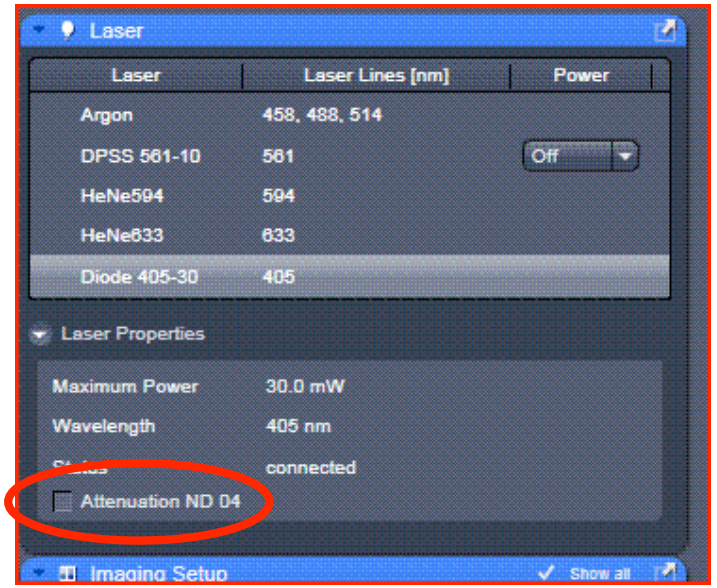
Save your configuration now



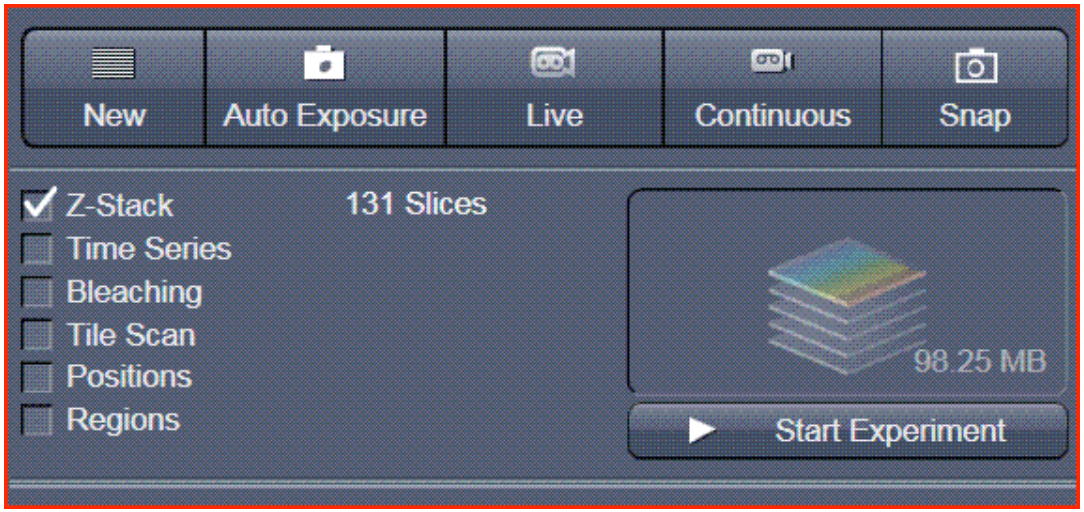
Laser setup

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

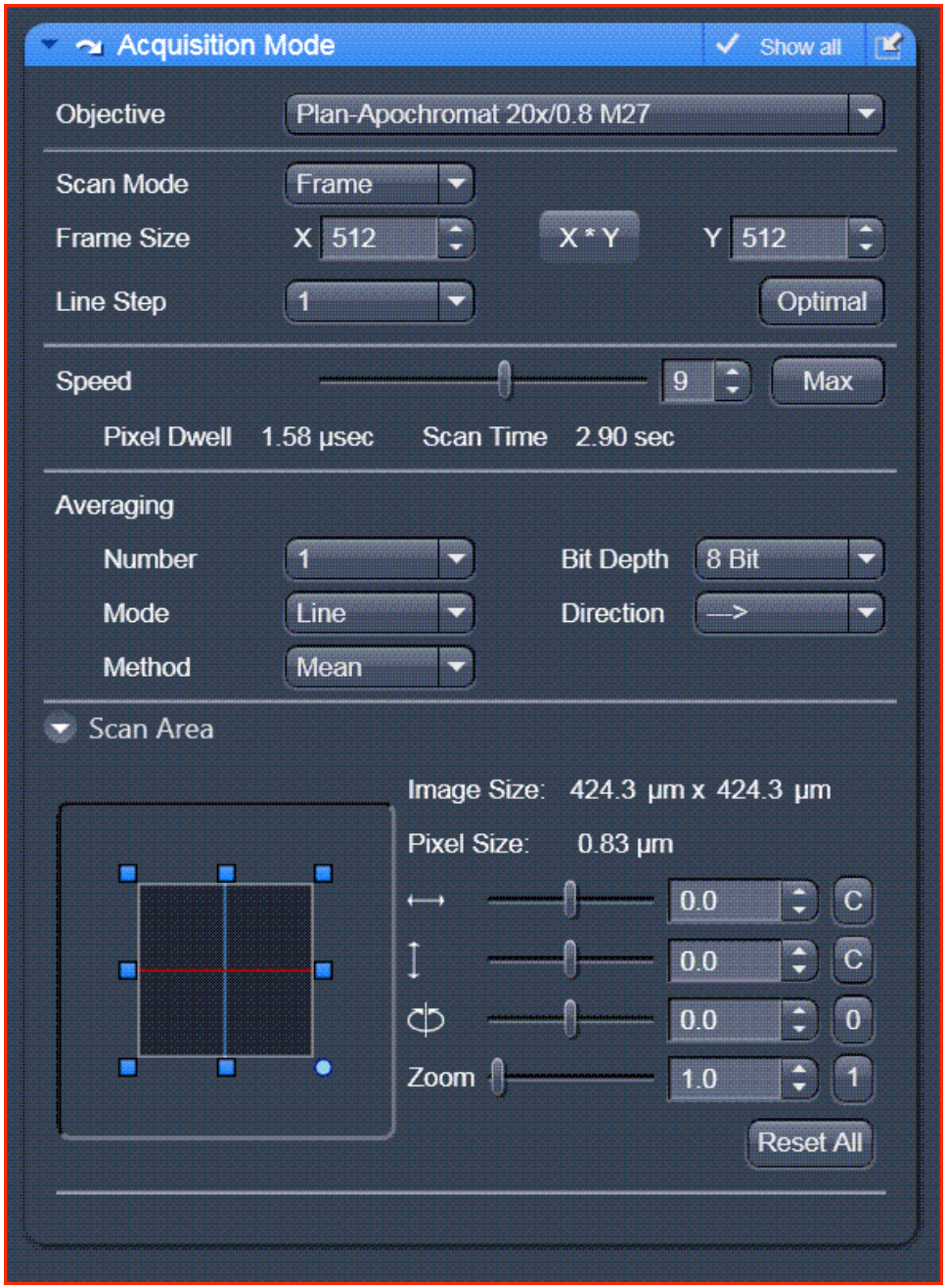
A neutral density filter can be use to reduce the 405 nm diode intensity : Attenuation ND 04



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

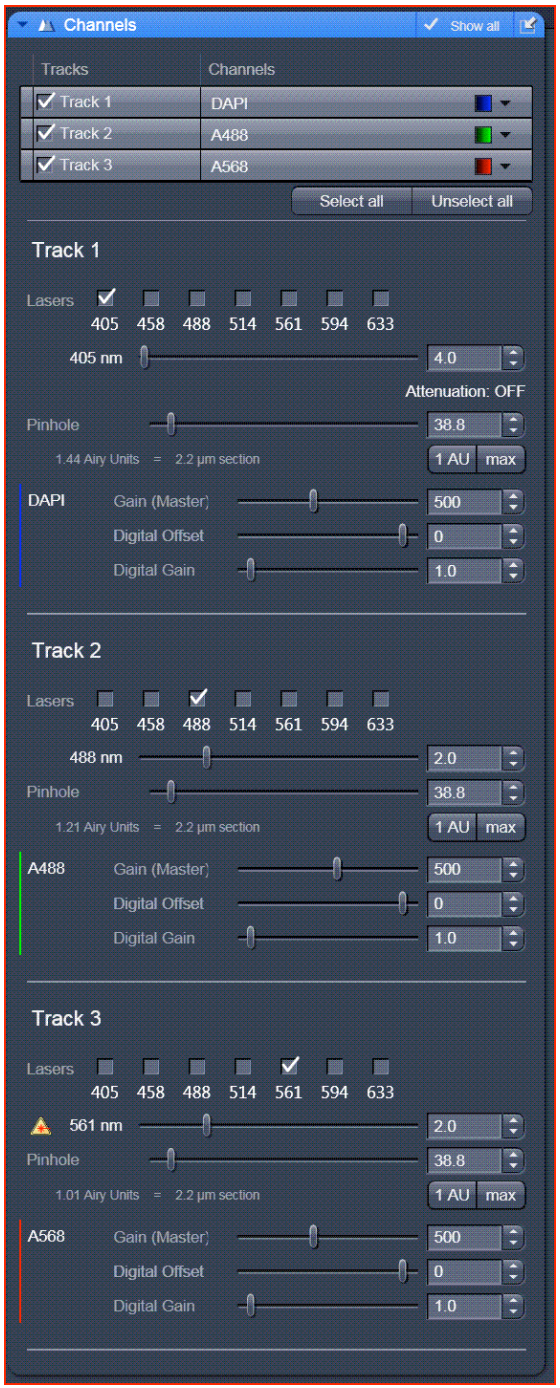
Averaging : number 1-16

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)