LAS AF Setup

1. All hardware has to be switched on
2. Start LAS AF
3. Choose configuration: MP_on or MP_off
4. Conventional Scanner (Default) or Resonant
5. Lasers – warm up
6. Objectives
7. Beam Path Settings
8. Control Panel
9. Image settings
10. Acquisition Mode
11. Others

Switch on Hardware

1. Green buttons and turn the key to On-1 (from left to right)

2. White Light Laser module –

- Turn key to laser on

- Reset interlock

- Press “Emission”

3. Log on Windows: TCS User

4. Wait for microscope to initialize

5. Click on LASAF icon

System configuration

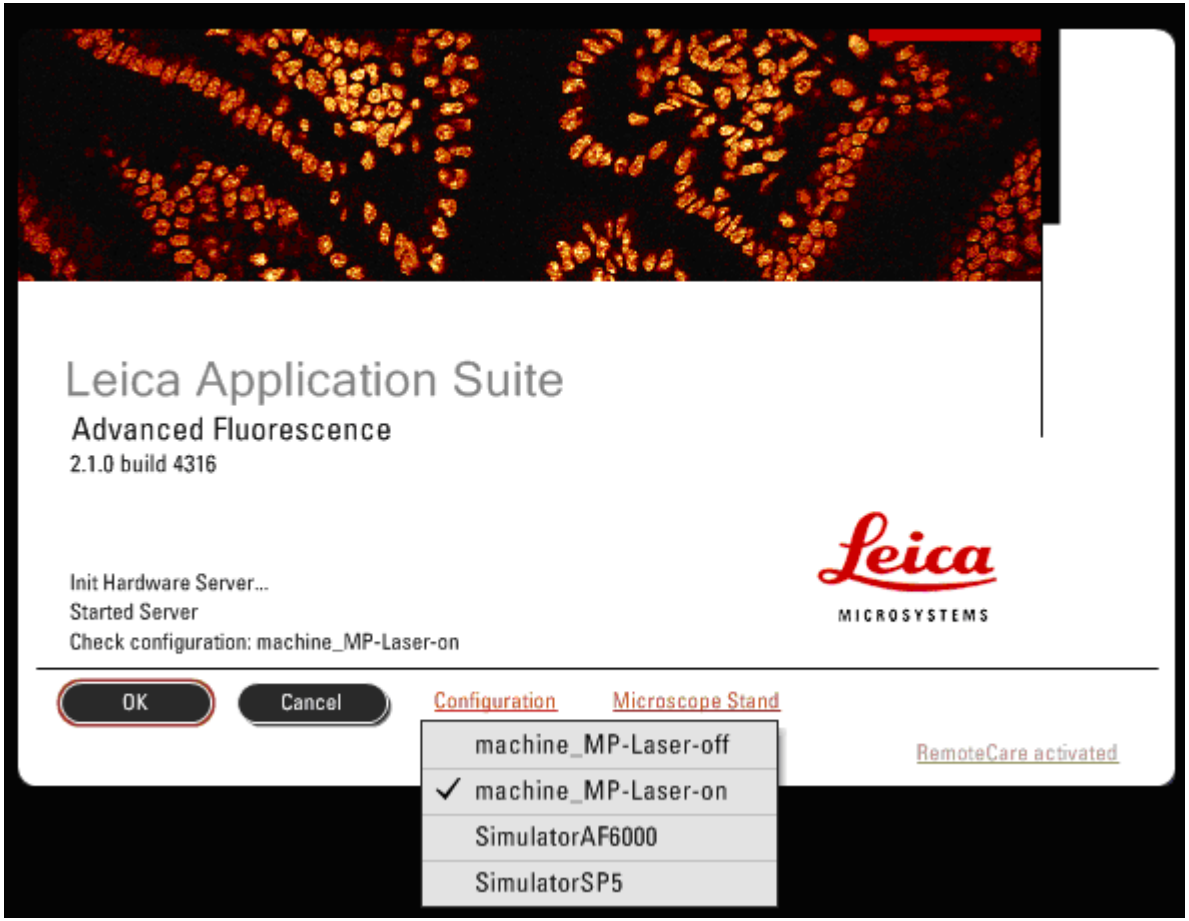
Choose from “Configuration”:

- machine_MP-laser-off
- machine_MP-laser-on

- Simulator

Microscope stand:

Always **DMI6000**

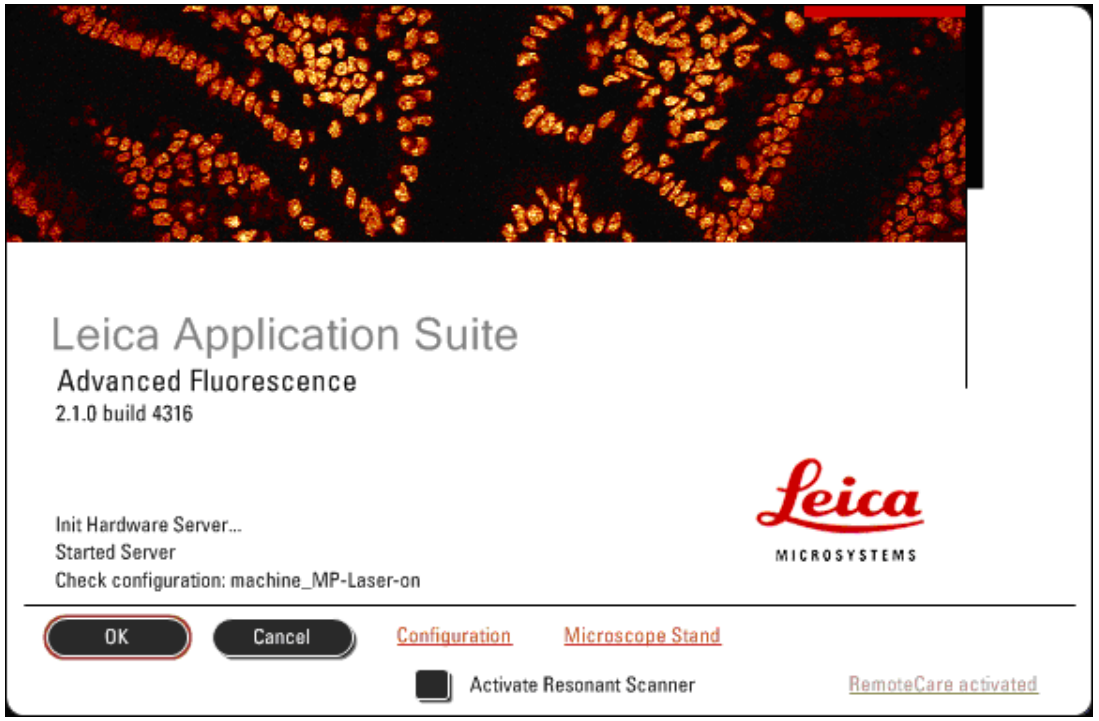


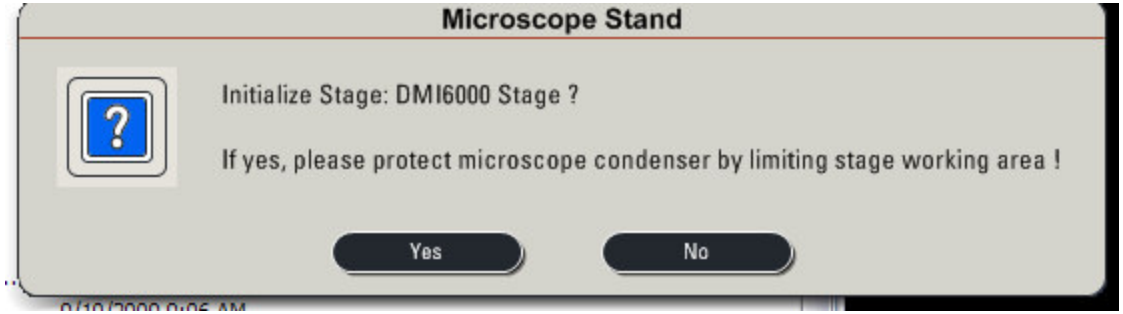
Choice of scanner

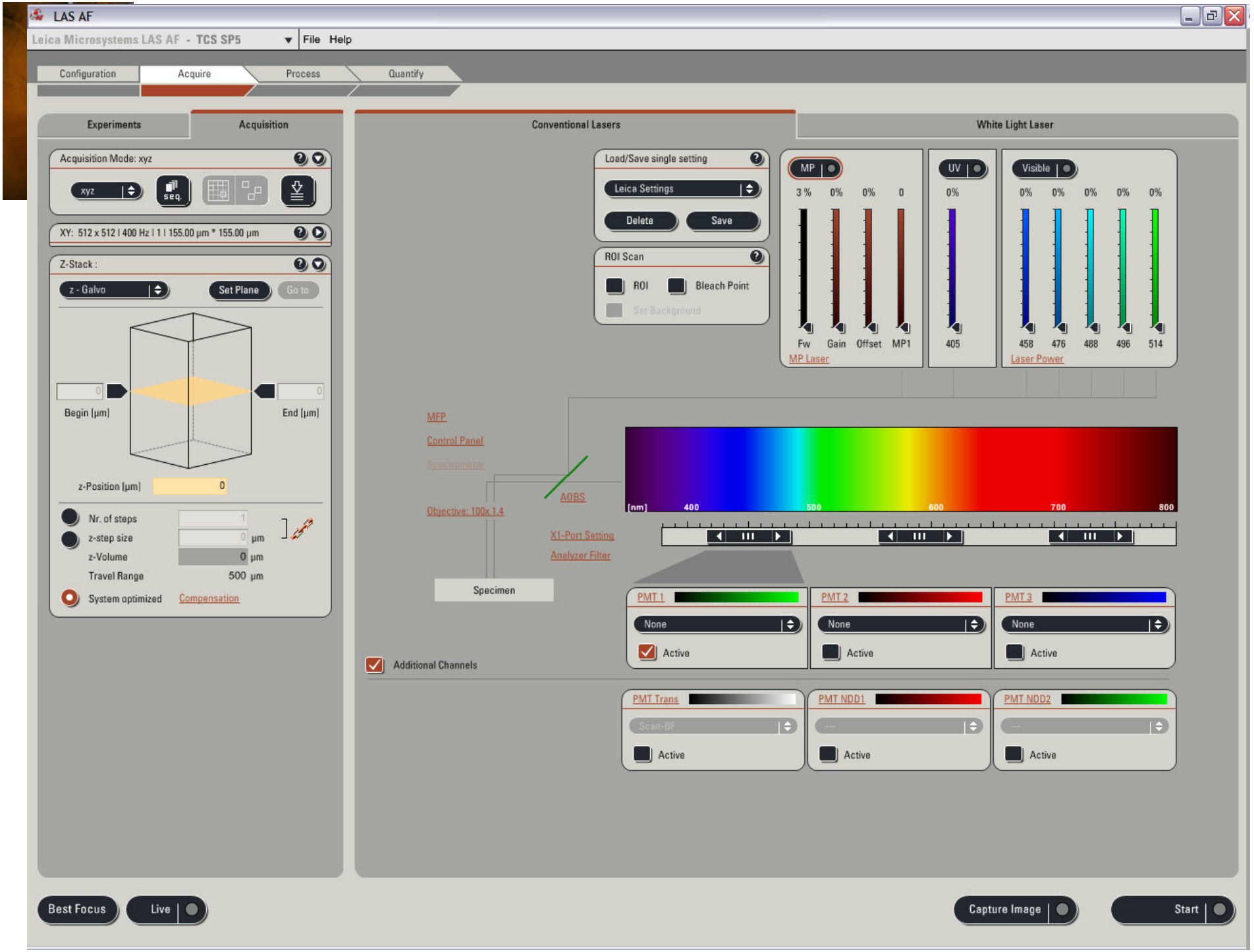
System is equipped with a Tandem scanner:

- Conventional
- Resonant (8KHz)

Initialization of scanning (xy) stage

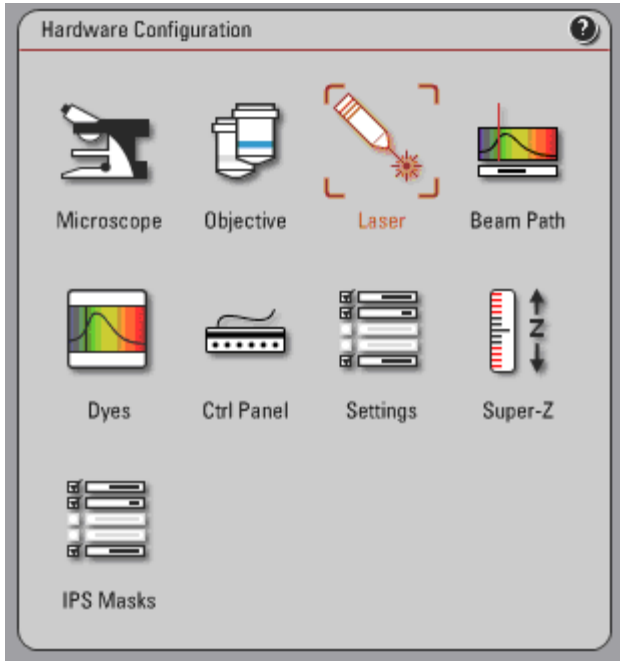




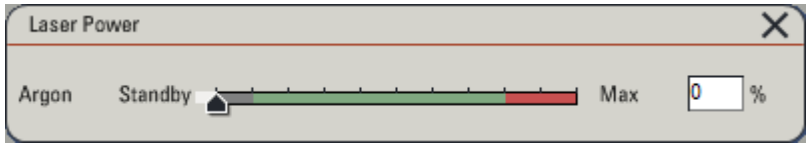

LAS AF
Leica Microsystems LAS AF - TCS SP5
File Help
Configuration
Acquire
Process
Quantify
Experiments
Acquisition
Acquisition Mode: xyz
xyz
seq.
XY: 512 x 512 | 400 Hz | 1 | 155.00 µm * 155.00 µm
Z-Stack :
z - Galvo
Set Plane
Go to
Begin [µm]
End [µm]
z-Position [µm]
Nr. of steps
z-step size
z-Volume
Travel Range
System optimized
Compensation
Conventional Lasers
White Light Laser
Load/Save single setting
Leica Settings
Delete
Save
ROI Scan
ROI
Bleach Point
Set Background
MP
Fw
Gain
Offset
MP1
MP Laser
UV
405
Visible
458
476
488
496
514
Laser Power
MFP
Control Panel
Spectrometer
AOBS
Objective: 100x 1.4
X1-Port Setting
Analyzer Filter
Specimen
[nm]
PMT 1
PMT 2
PMT 3
None
Active
Additional Channels
PMT Trans
PMT NDD1
PMT NDD2
Scan-BF
Best Focus
Live
Capture Image
Start

Lasers

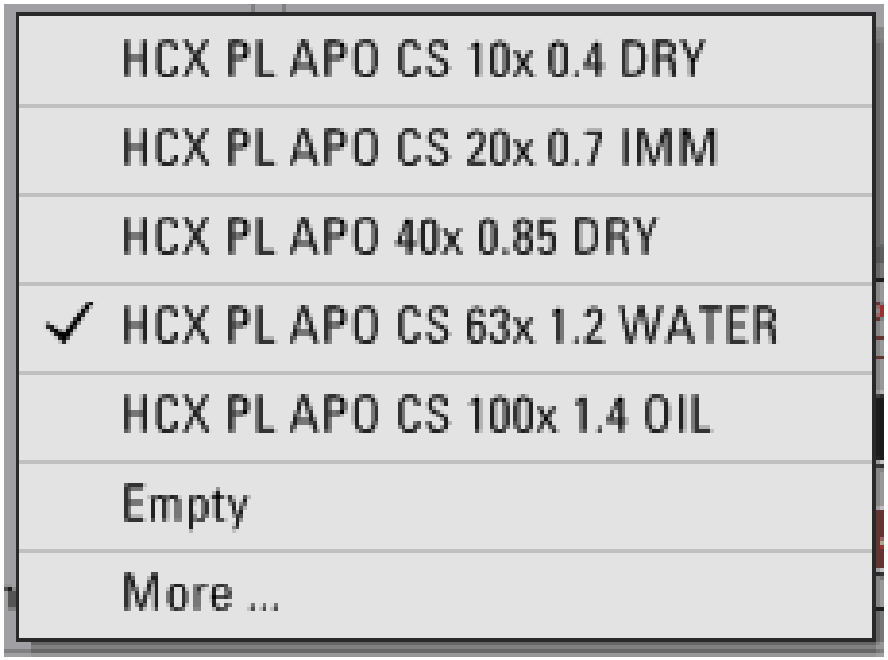
Work Flow Bar



Hardware Configuration



Objectives



DRY – air

IMM – multi immersion (water or oil)

Check correction ring

WATER – water

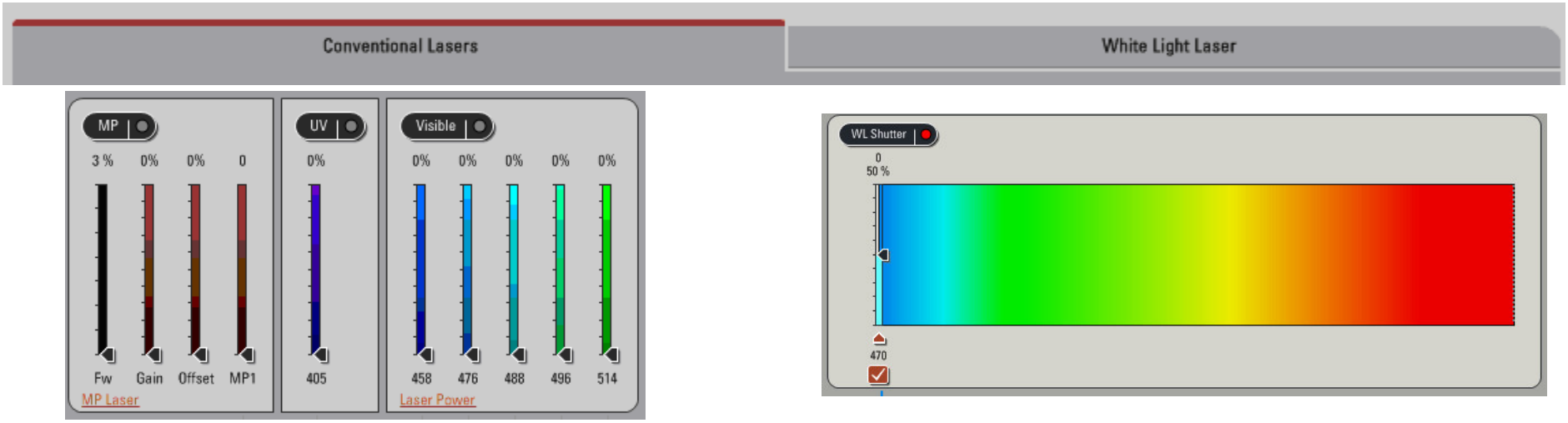
Oil – Oil (fluorescence free)

Important:

- Check Spring cap
- Clean after use or between

Beam Path Settings

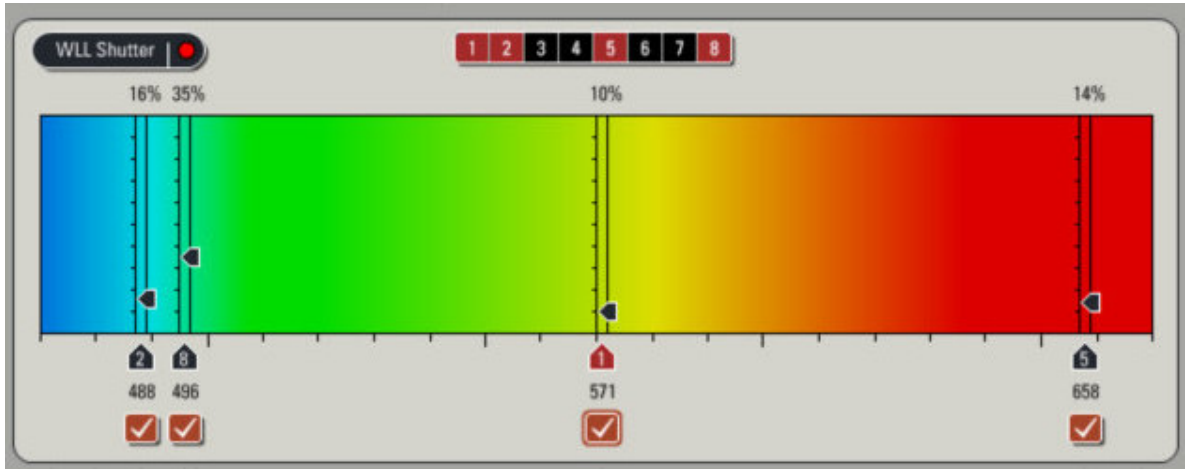
- Choose Excitation



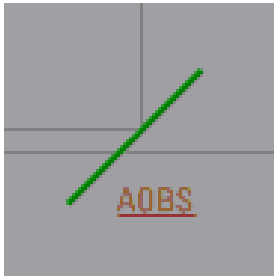
There are 2 tabs:

Conventional Lasers v/s

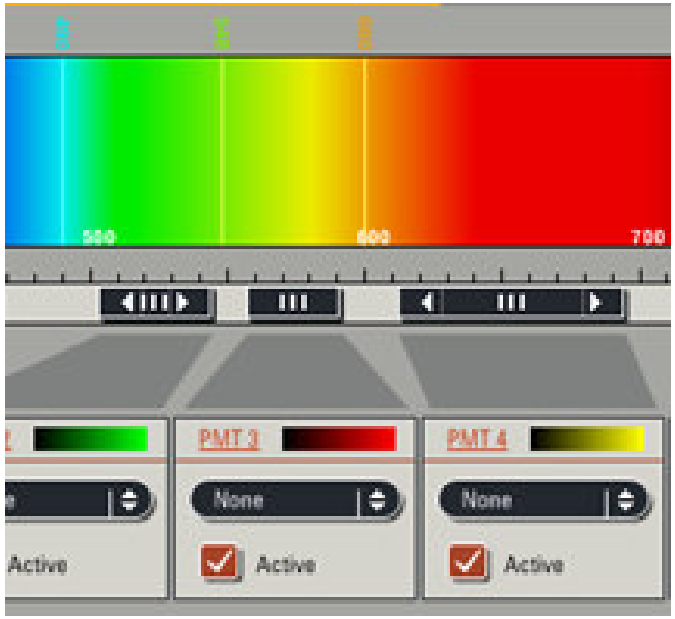
White Light laser



Beam Path Settings



- AOBS does the automatic selection
- Activate PMT(s)
- Adjust Spectral Sliders to emission bands
- Click Live

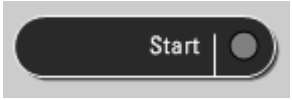


- Capture Image

  Will capture a single image or a single sequence

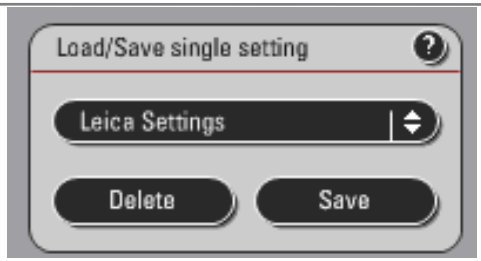
- Start

  Will start any sequence or stacks or time lapse

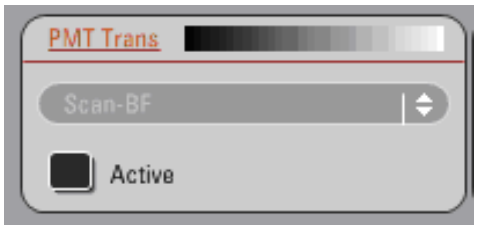


Beam Path Settings

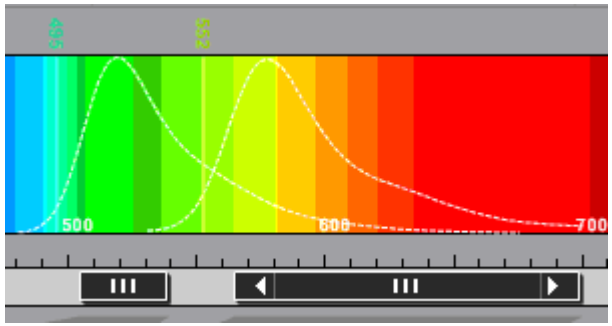
- Instrument Parameter Settings - IPS
  - Leica Settings
  - User Settings



- Transmitted Detector
  - Bright-field imaging



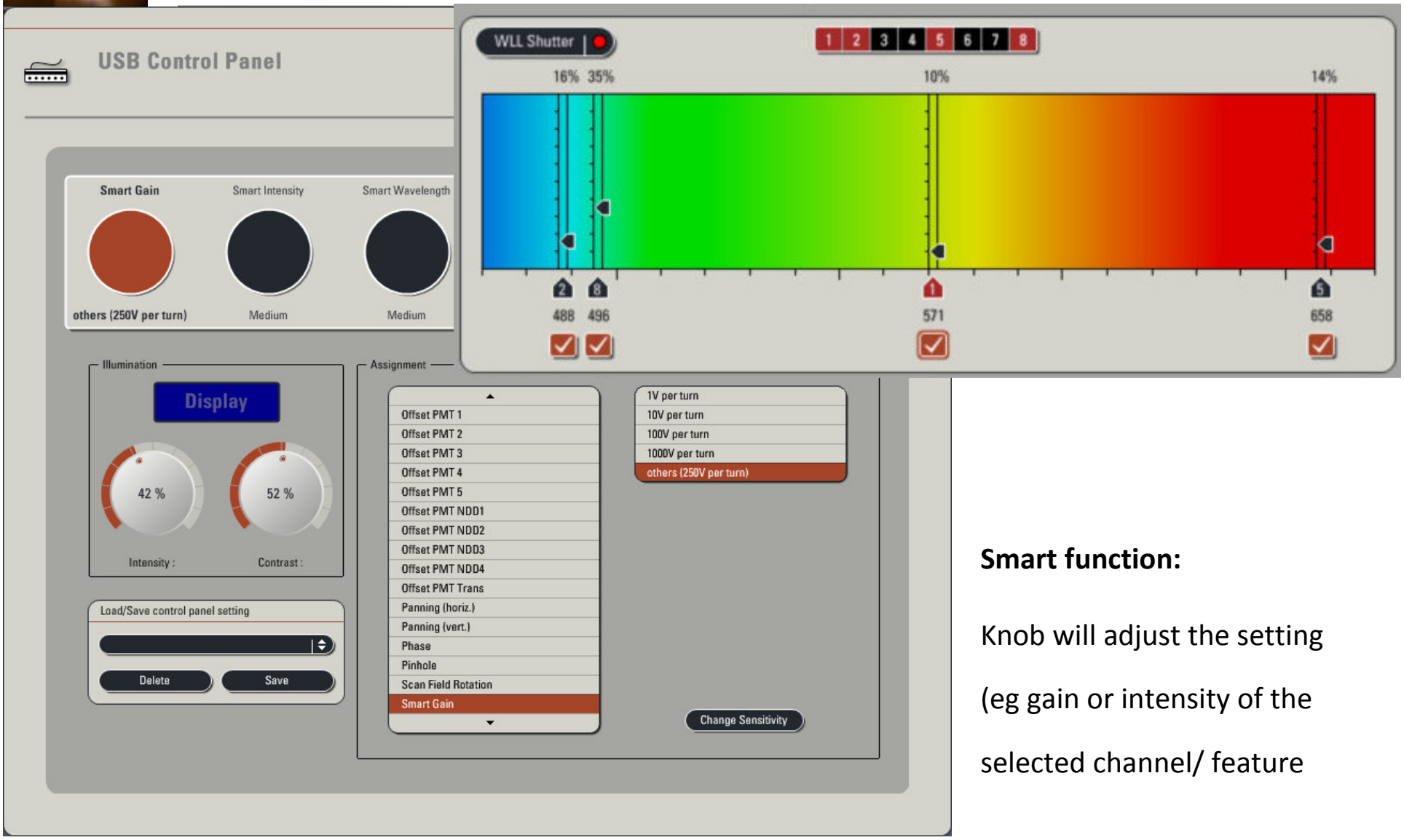
- Emission Peaks of common dyes



- Pseudo color


LAS AF
Leica Microsystems LAS AF - TCS SP5
File Help
Configuration
Acquire
Process
Quantify
Experiments
Acquisition
Acquisition Mode: xyz
xyz
seq.
XY: 512 x 512 | 400 Hz | 1 | 155.00 µm * 155.00 µm
Format :
512 x 512
Speed :
400 Hz
Pinhole
Bidirectional X
Zoom factor :
1
Zoom in
Image Size : 155.00 µm * 155.00 µm
Pixel Size : 303.33 nm * 303.33 nm
Line Average :
Accu:
Frame Average :
Auto Gain
Rotation:
0.0
Z-Stack :
Conventional Lasers
White Light Laser
Load/Save single setting
FITC-TRITC
Delete
Save
ROI Scan
ROI
Bleach Point
Set Background
WLL Shutter
1 2 3 4 5 6 7 8
20%
20%
495
552
631
MFP
Control Panel
Spectrometer
AOBS
Objective: 100x 1.4
X1-Port Setting
Analyzer Filter
Specimen
[nm]
400
500
600
700
800
PMT 1
Leica/FITC
Active
PMT 2
Leica/TRITC
Active
PMT 3
None
Active
Additional Channels
PMT Trans
Scan-BF
Active
PMT NDD1
Active
PMT NDD2
Active
Best Focus
Live
Capture Image
Start

Leica Control Panel



Smart function:

Knob will adjust the setting (eg gain or intensity of the selected channel/ feature

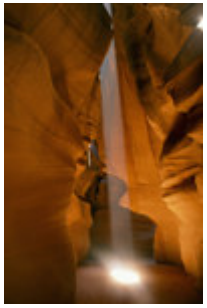
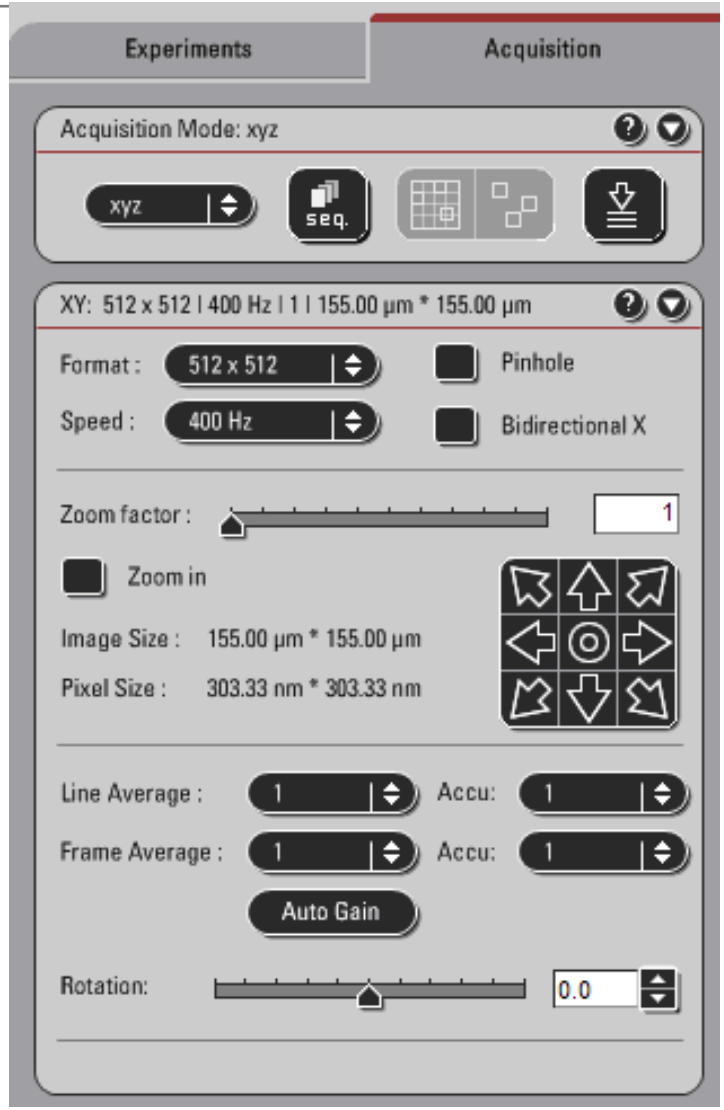


Image Settings

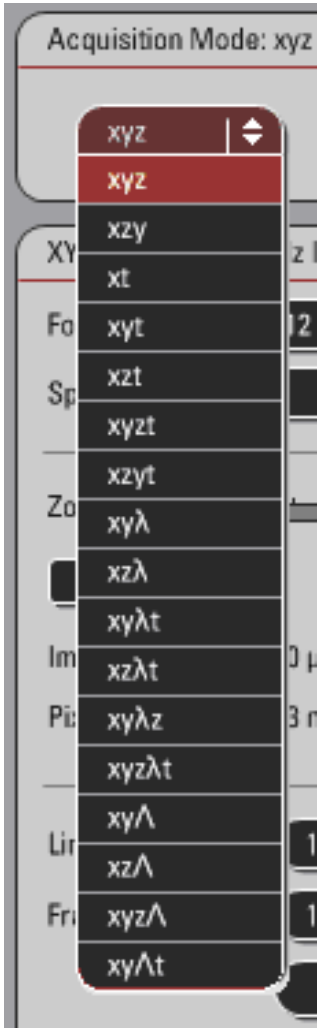
- 2 tabs:

Experiments: Data are classified into a tree.
Data is pre-saved into a Data-Container.
User need to SAVE data to the Hard Drive
Each experiment will create a single file with extension .LIF
User LASAF Lite to view data

Acquisition: Mode, XY… or Sequential
Select mode of imaging
Image format
Scan Speed
Zoom Factor
Image Size / Pixel Size (raster scan)
Averaging



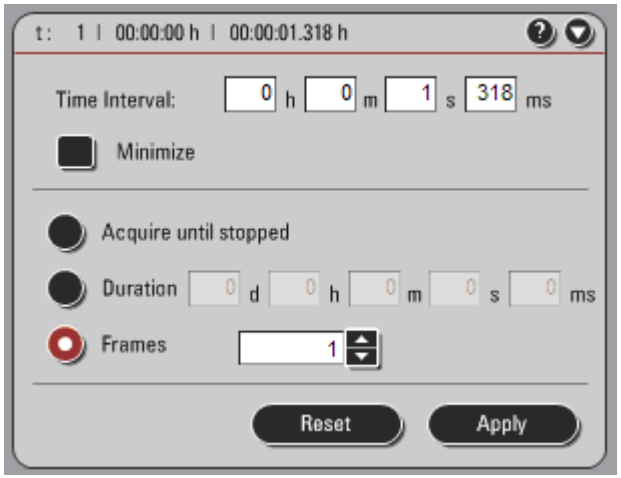
Acquisition Mode

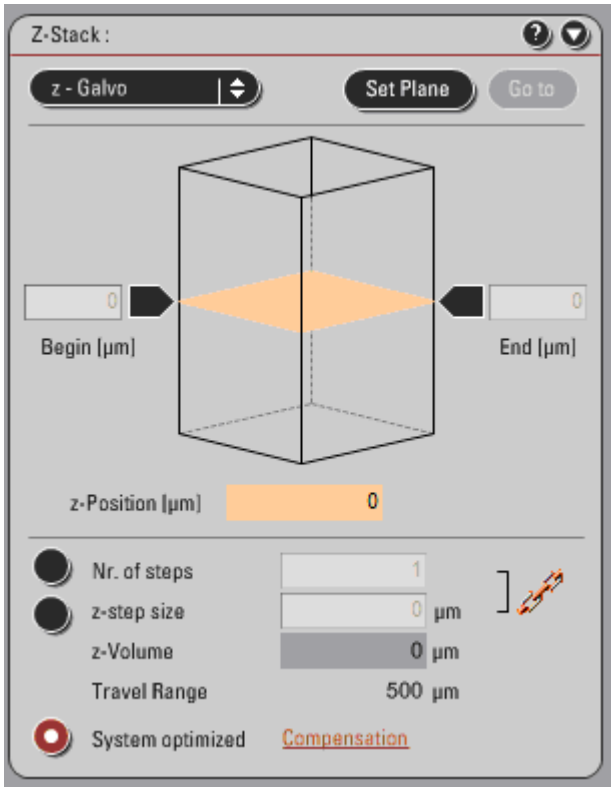


- Select mode of imaging

- xyt – time lapse

- Z-stack – Begin / End
  Steps





Others…

- 2 mode of focus:
  z-wide: Microscope focus.
  Objective turret moves up and down
  z-galvo: specimen moves

• Quick LUT - Saturation

Click once – image is displayed to overflow/underflow

Click twice – image is displayed RAW (greyscale)

Click thrice – image is displayed as pseudo color defined