How to use LAS AF

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§ Workflow

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§ Acquire (Image Viewer, z-stack, time lapse, stage)

§ Data Management (+ licenses)

§ Process (+ deconvolution)

§ Quantify

§ Live Data Mode, FRET-wizard, FURA etc.

Installation:

§ Installation CD

§ 2 updates / year: FTP-server Leica Mannheim

§ Installs / updates Leica LAS (Basis software)

§ Firmware upgrade of microscope may be necessary

§ Hardware configuration and data are safe

§ Full software is installed: dongle protection

§ LAS AF lite: freeware software (Viewer, Export)

§ Review software with modules is available

Starting the software:

§ Select the correct configuration

§ Microscope, camera etc. must be switched on

§ System starts with the settings of the last session

§ Stage initialization not necessary,
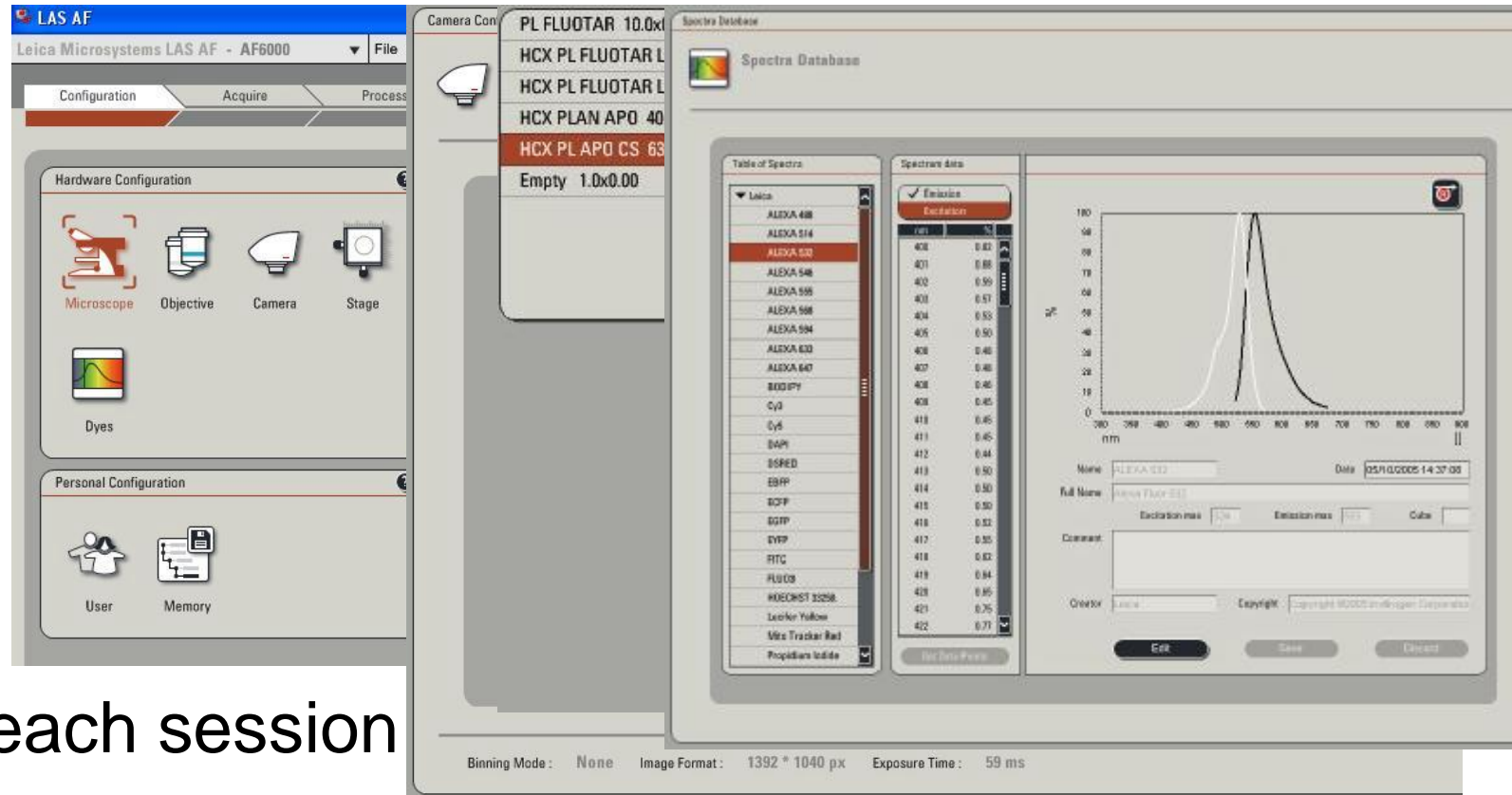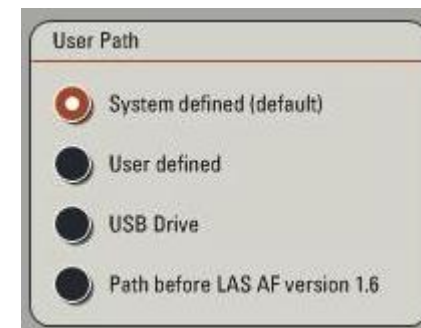if no motor stage functions
for this session
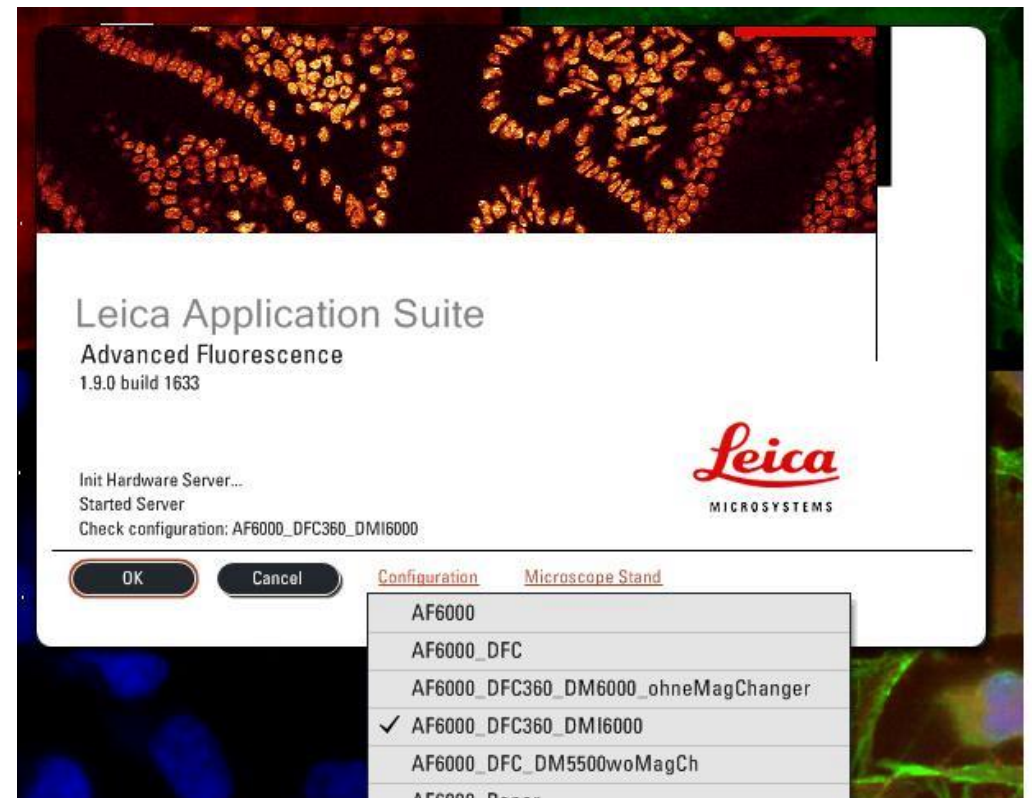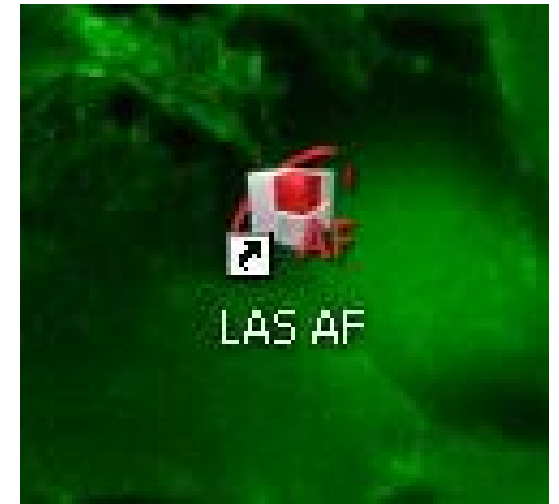
Workflow:
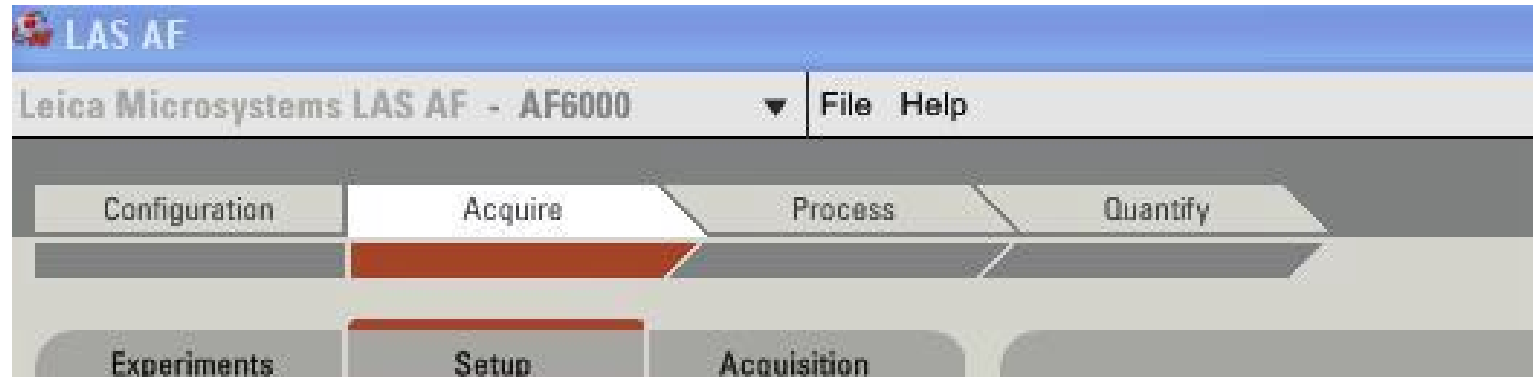
§ Directs the user

§ System starts in "Acquire"

Configure:

§ No need for each session

§ Change camera settings if necessary
(c-Mount, 8bit – 16 bit)

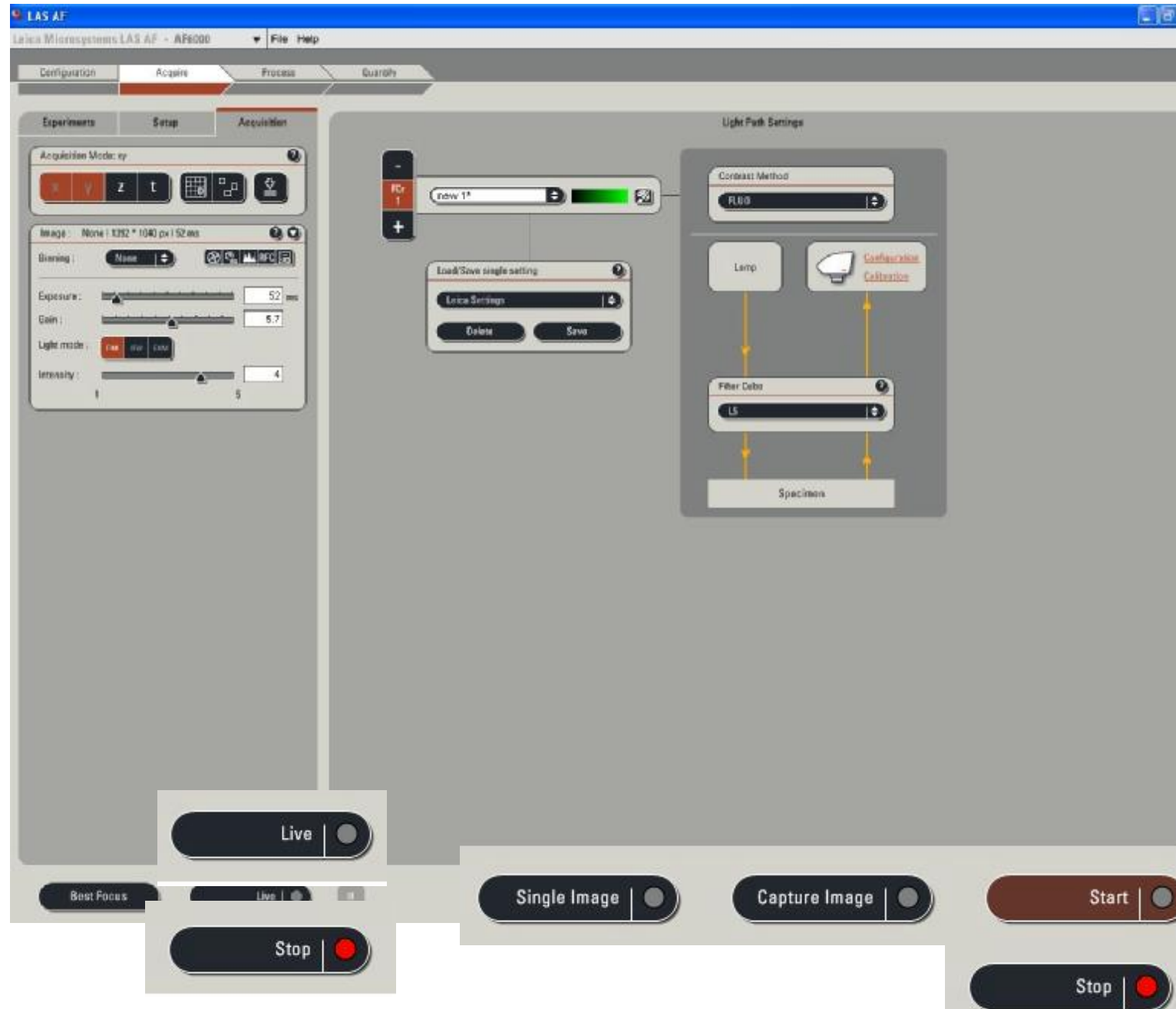§ Display of objective parameters

§ List of fluorochromes

§ User settings

Acquire:

§ Go to "Acquire" sub-menue

§ Define **Light Path**

§ Select contrast method

§ Select filter cube

§ Select look-up-table (LUT)

§ Go to "LIVE"

§ Adjust camera settings

§ Save channel (right mouse click)

§ Add more channels

Acquire:

§ Camera Settings

§ LIVE on/off:
Preview (frozen
image)

§ SINGLE IMAGE:
1 channel only

§ CAPTURE:
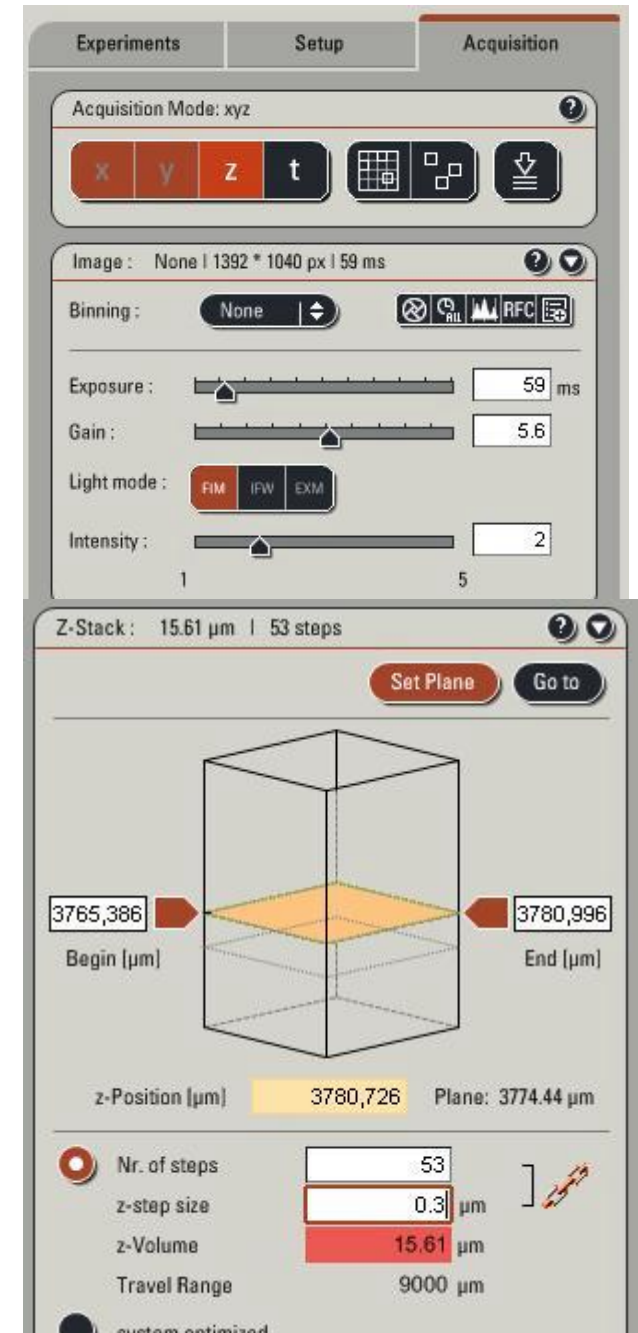1 set of channels

§ START:
1 experiment
(z-stack, time
lapse,..)

Image Viewer:
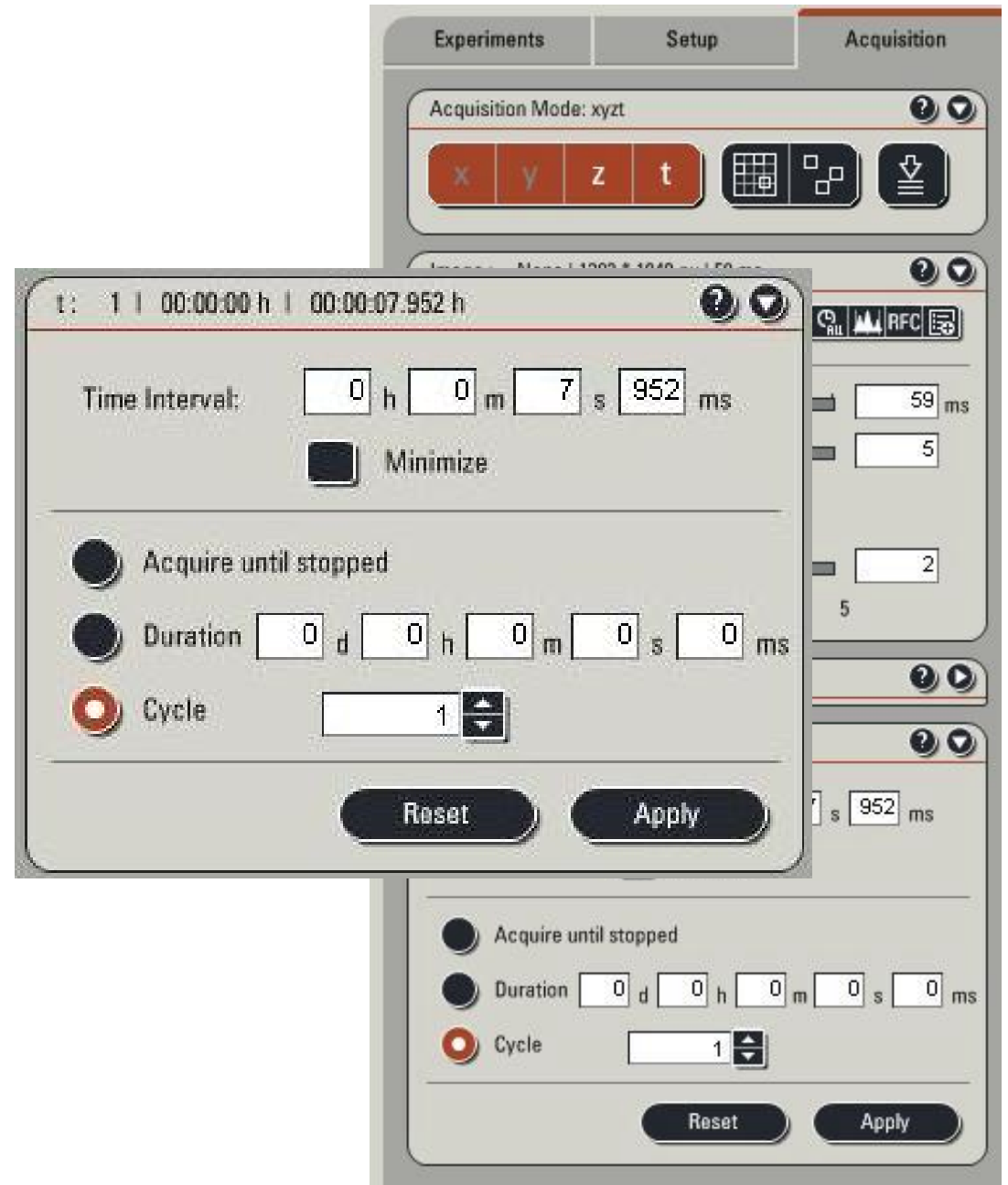
Acquire z-stacks:

§ Select Z

§ Define "PLANE" (in focus)

§ Focus to upper and lower border and select

§ Select "System optimized" or define step size individually
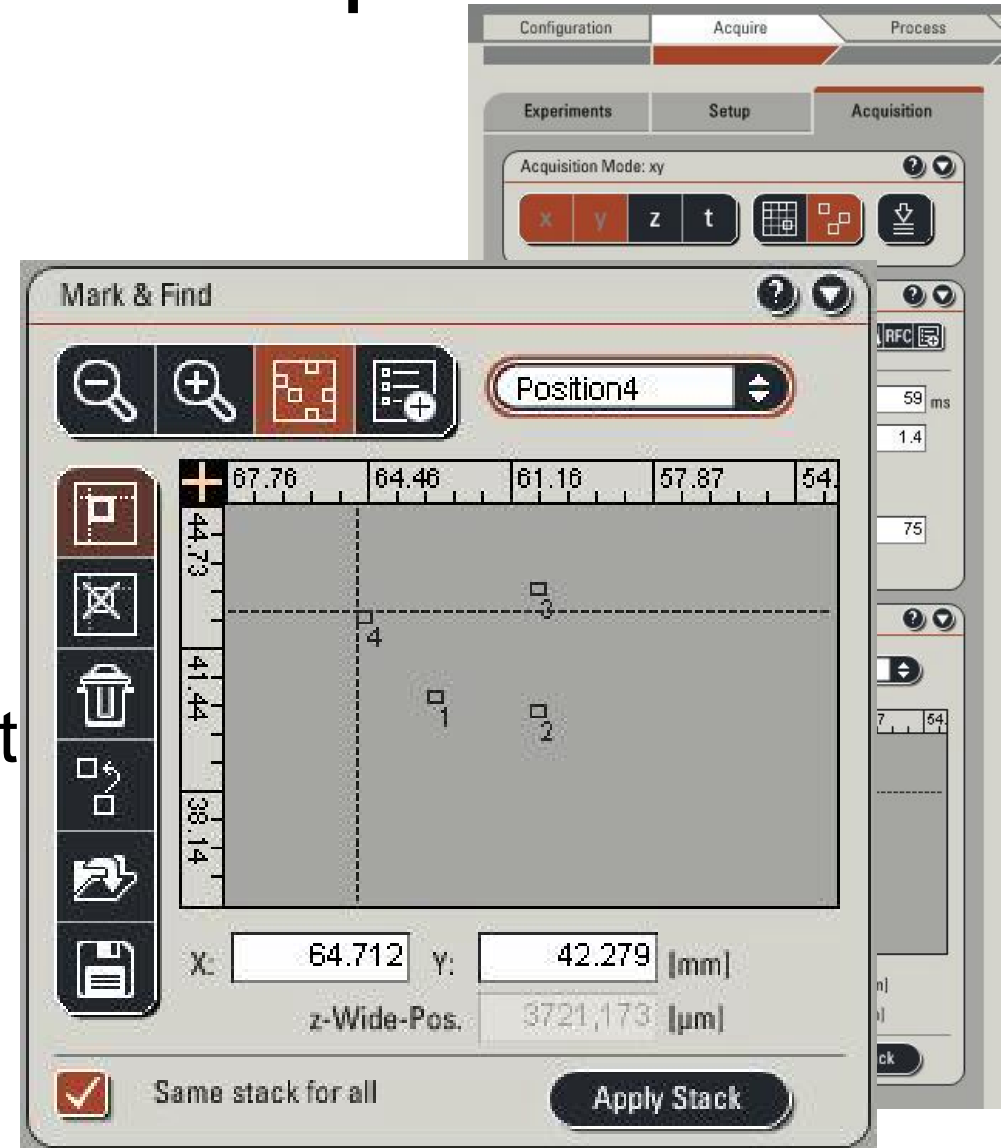
§ START

§ Setup: first channel then z or vice versa

Acquire time lapse:

§ Select T

§ Select DURATION or CYCLES or ACQUIRE UNTIL STOP
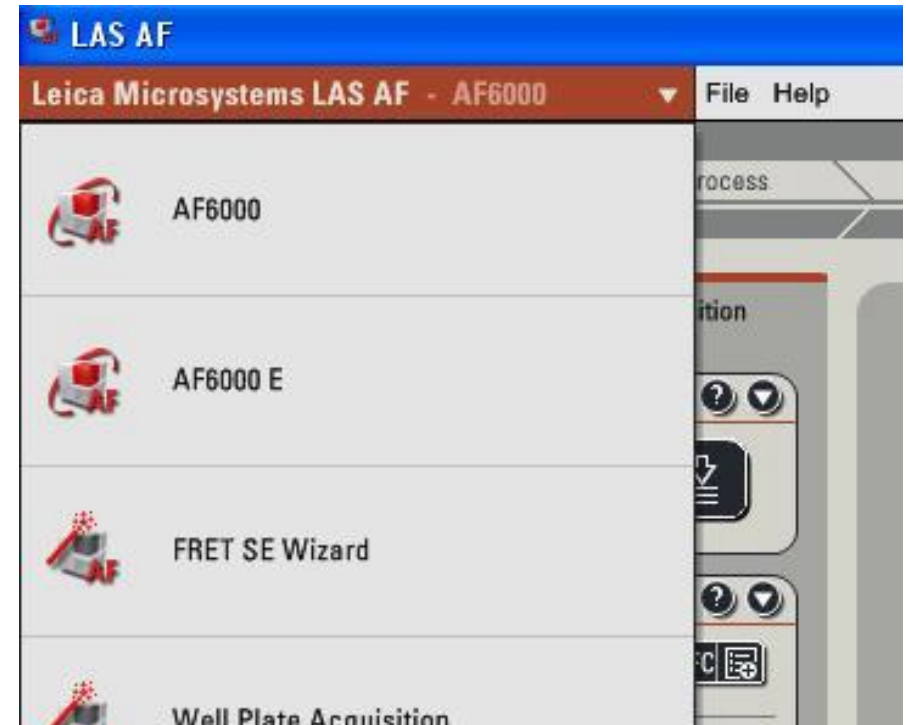
§ Select Interval

§ START

§ May be combined with Z

Acquire multi position time lapse:

§ Select Stage settings

§ Select positions and check in stage control box

§ Positions are available in XYZ

§ Start Time Lapse Experiment
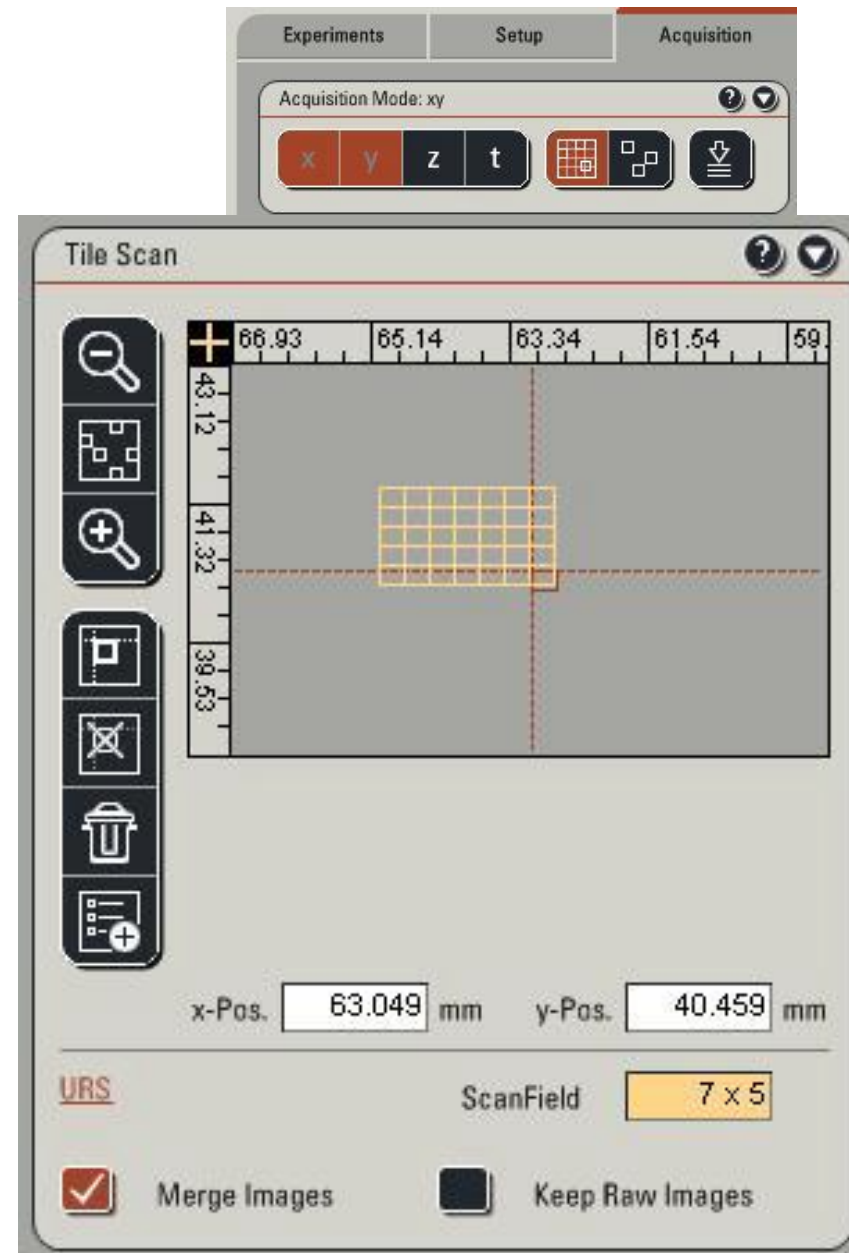
Live Data Mode:

§ Optional Module

§ Definition of Jobs and Macros

§ Definition of Loops, Pause, Trigger,..

§ Tool for complex experiments

Acquire Tile Scan:

§ Select Stage settings: Tile Scan

§ Select upper left and
lower right position

§ Save individual images ?

§ Special stage settings ?

§ Start Acquisition

§ No Stitching: precision
of the motor stage

Image Viewer: Gallery, Maximum Projection, sections

§ For complex experiments

§ Maximum Intensity Projection
for z-Stacks and
time lapse experiments

§ XZ- and YZ-Sections
for visualization of z-stacks

§ Gallery

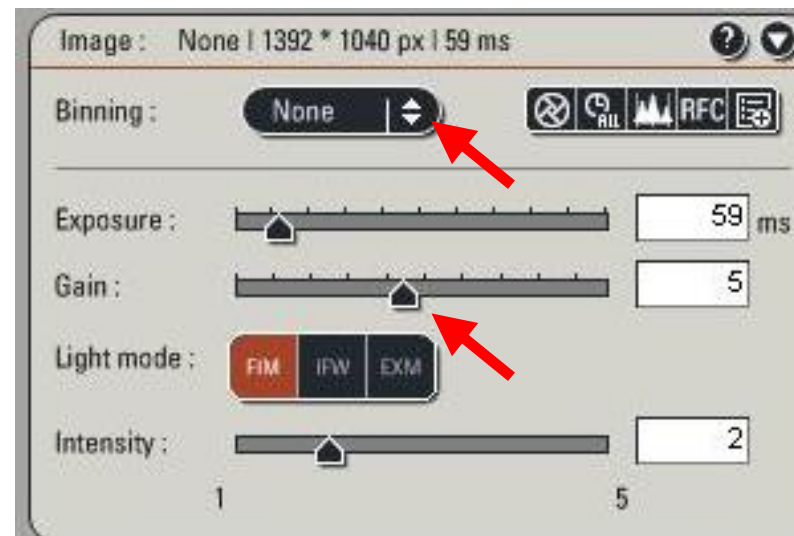§ Rescaling of 16 bit

§ Autoscaling

§ Gamma value

§ Colour (LUT)
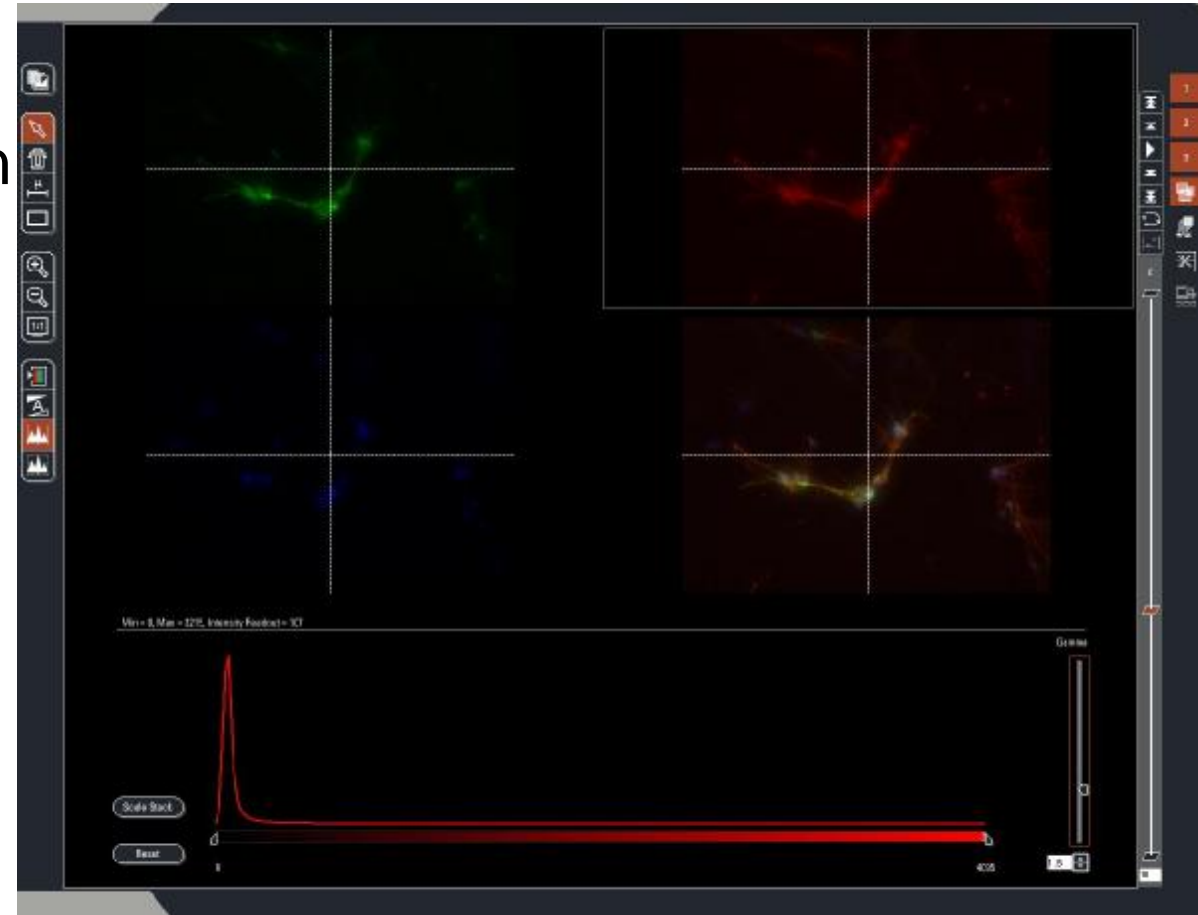
Acquisition:

§ **Save settings:**
each channel or
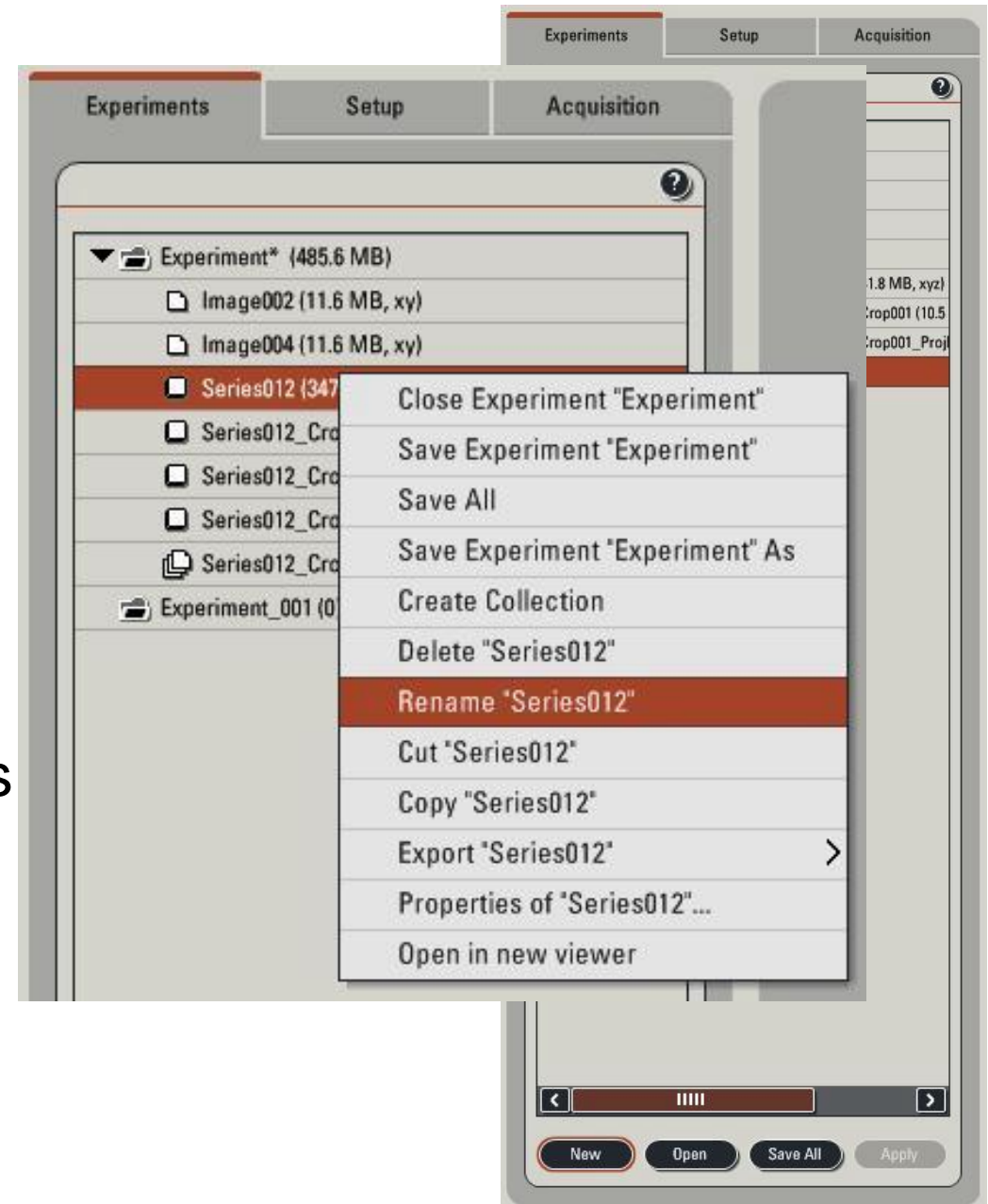each set of channels
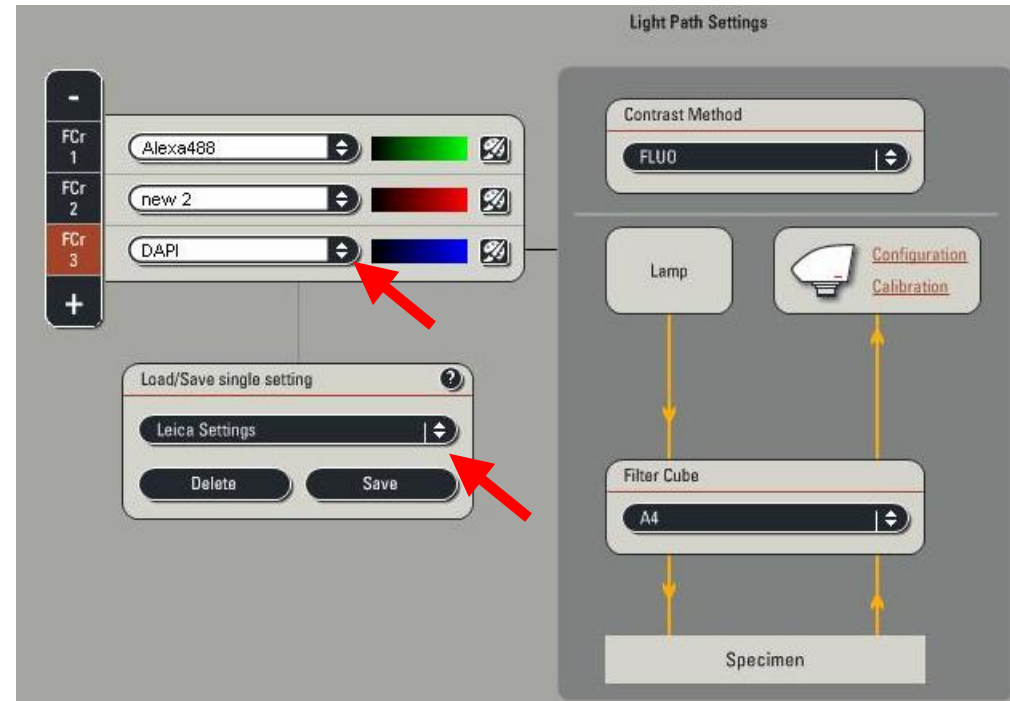
§ **Camera settings:**
Binning and
recommended gain
(half maximum)

Data Mangaement:

§ All image sets automatically in Data Container

§ Save "Experiments": LIF-format

§ LIF can be opened with LAS AF-lite (free of charge) on any PC-system

§ Moving data sets with Drag&Drop between experiments

§ Rename, Delete, Copy-Paste,.. With right mouse click

§ Export TIF, JPEG, AVI

Export:

- § Channel images or overlay
- § Export many data sets in 1 step
- § TIF: Raw images or
  as currently displayed
  in Image Viewer
- § Export annotations,
  Scale bar, Time Point,…
- § 1 file per image
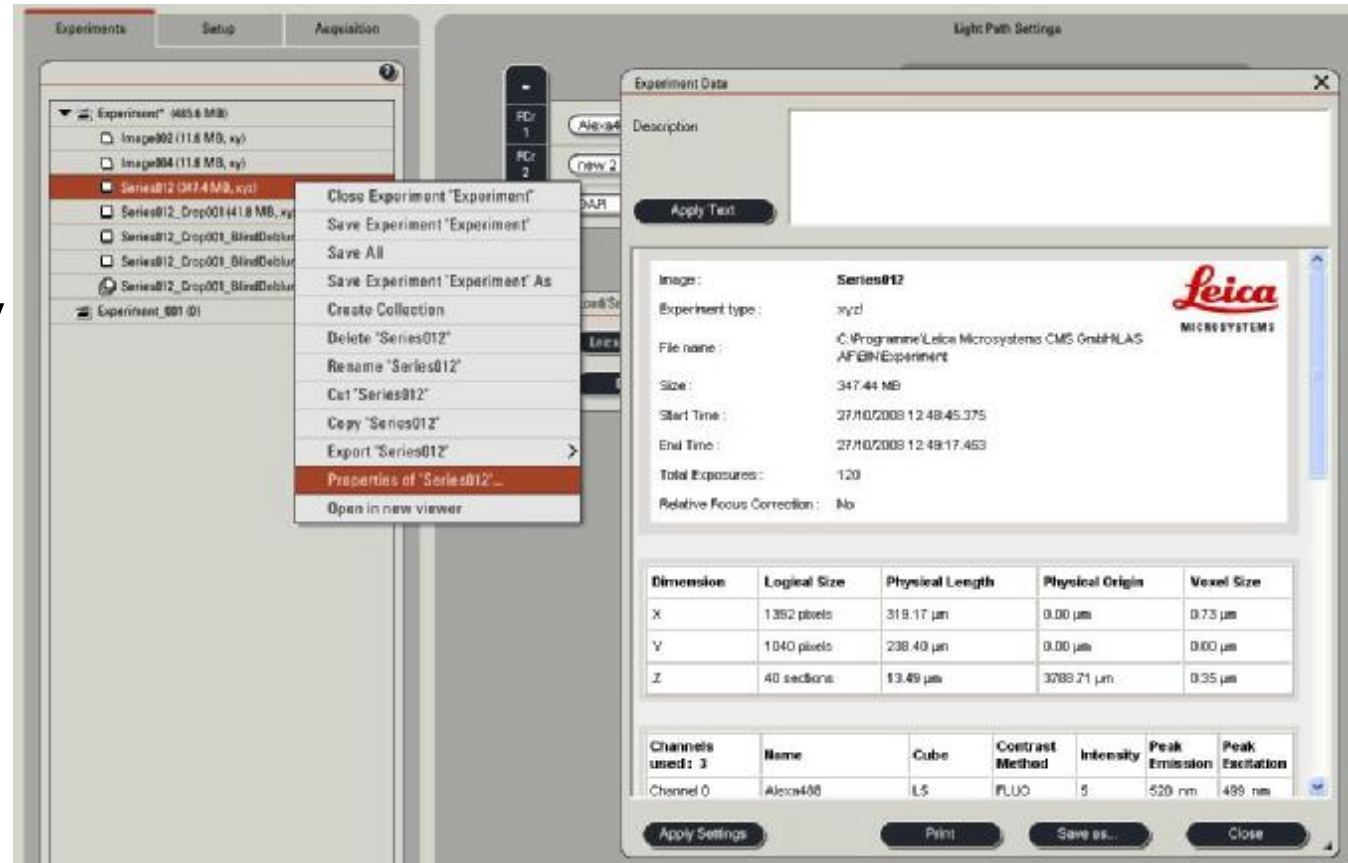- § AVI: select frame rate
  and compression
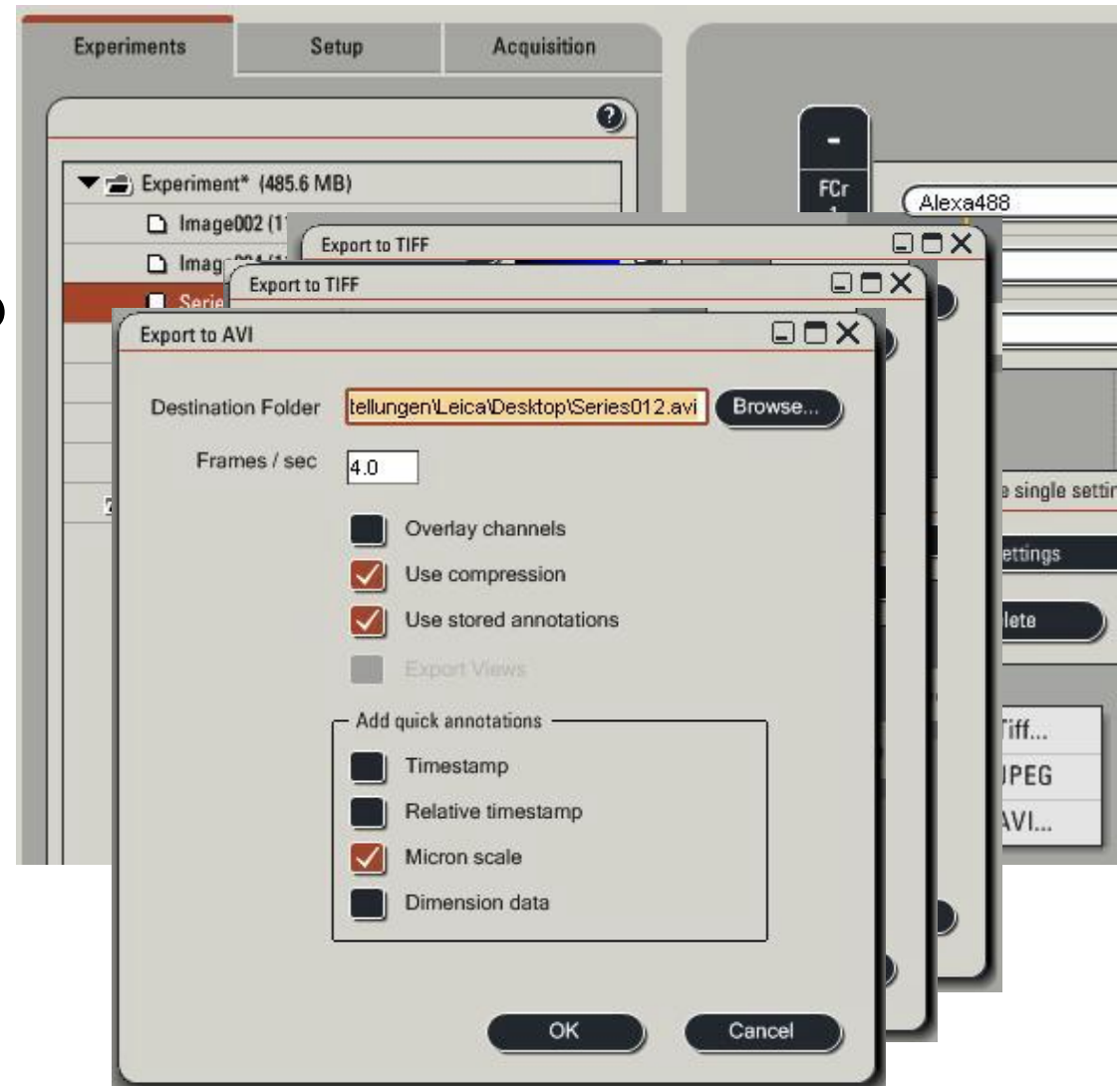
Metadata:

§ All relevant microscope and camera settings are saved automatically

§ Metadata visible as "Properties" in the experiment list

§ Properties can be exported as XML

§ Metadata are exported automatically with channel images

§ Complete experiment can be reproduced with "Apply settings" in the "Properties"-window

How to make Life easy:

§ Copy successful image set into an "Experiment" called "My templates"

§ Each user can open this experiment, select the appropriate data set and go to "Properties – Apply settings"

§ Appropriate settings are available, adjust exposure time, objective lens, z-stack definition and start imaging

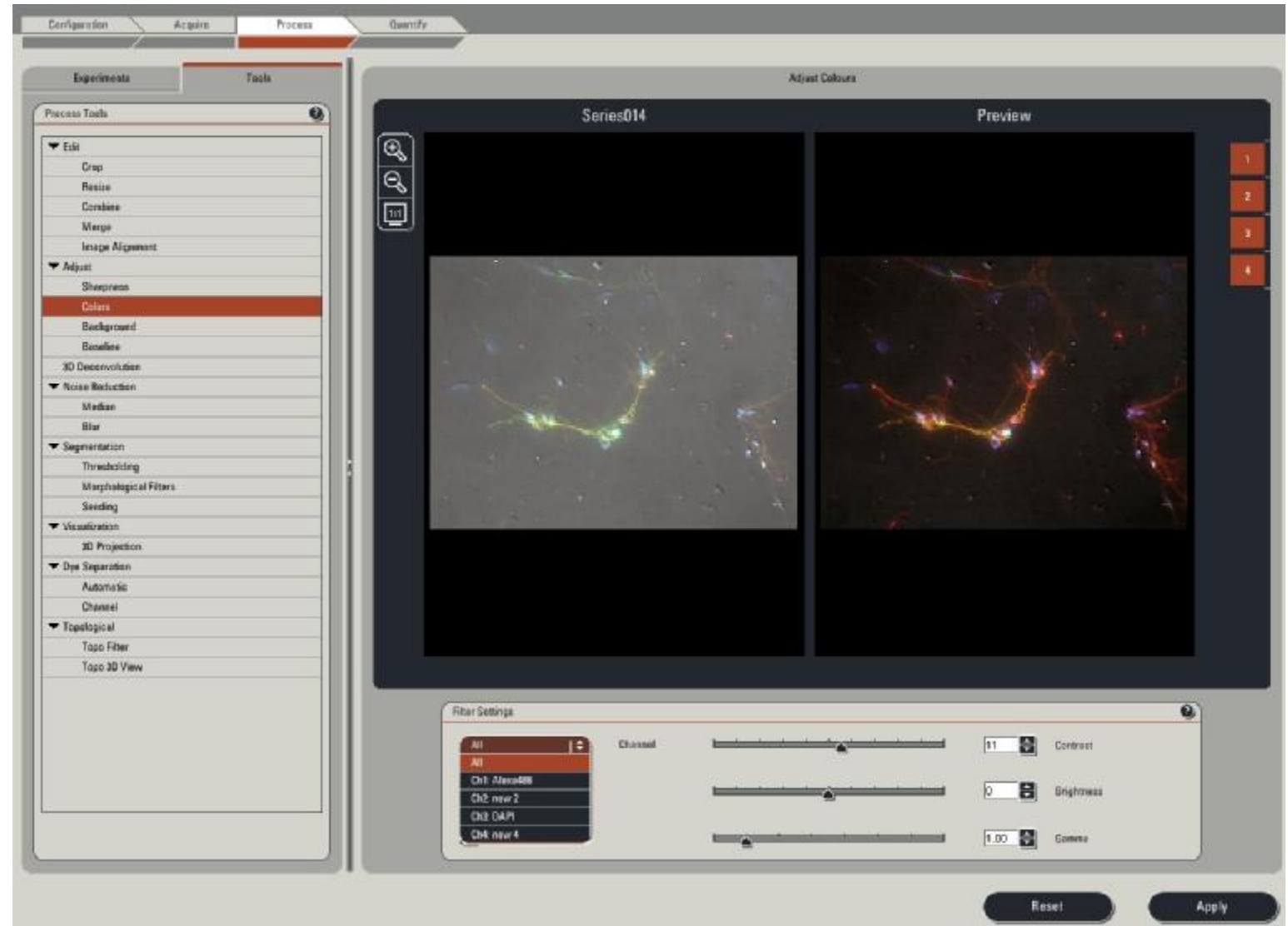§ Keep a reasonable list in definition of Channels and Settings

Process:

§ Crop: Select subsets
(ROI, z-levels,
time points, channels)

§ Merge: Glue together
(channels, time points,
z-positions, in X or in Y)

§ Combine: Arithmetics

§ Adjust Colours:
Mixing the channels
(also included in LASAF lite)
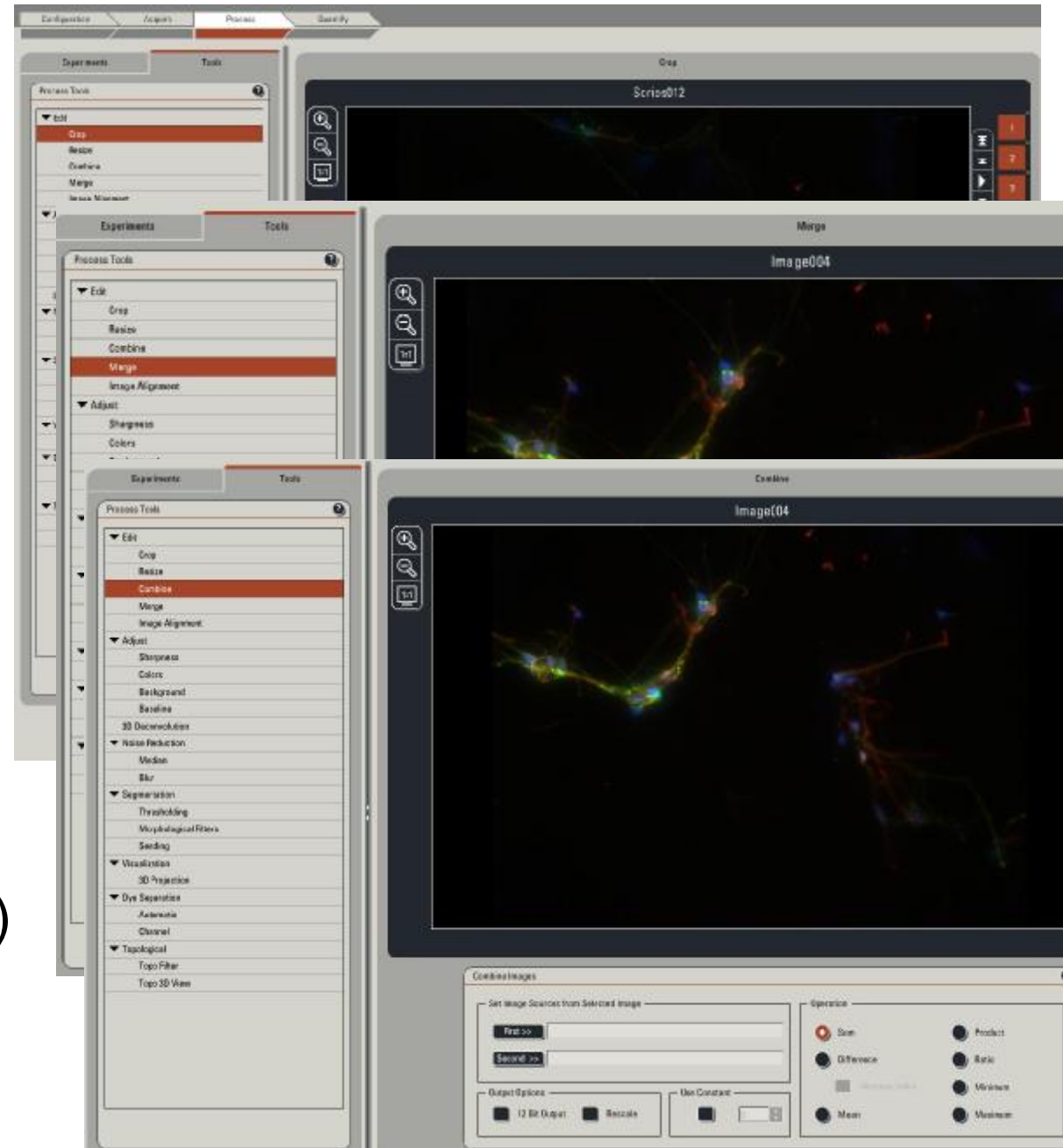
Process:

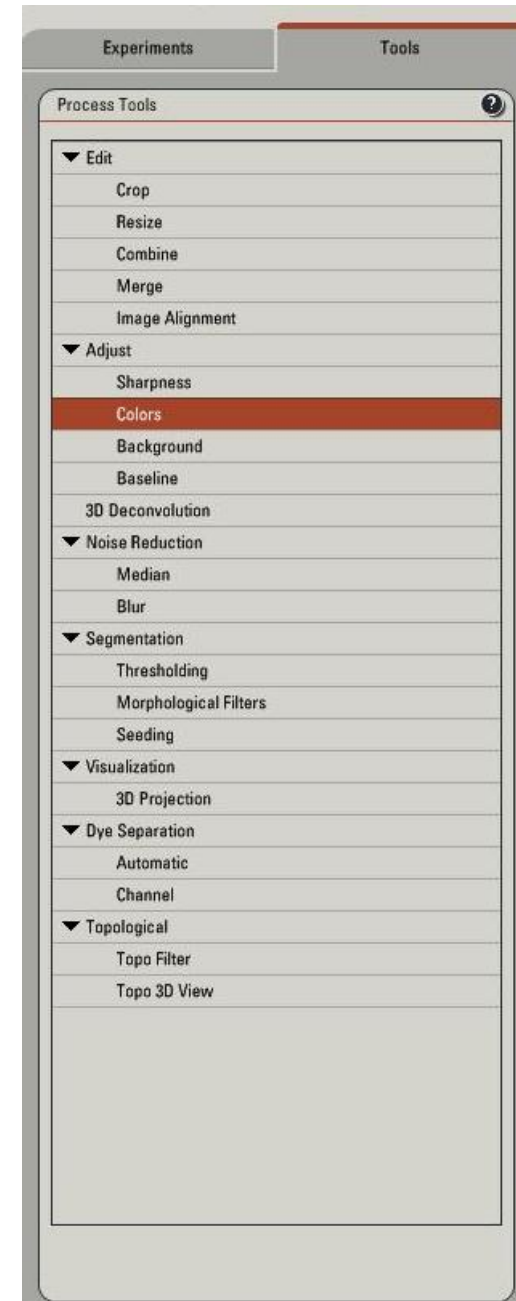§ Adjust Colours: Mixing the channels (also included in LAS AF lite)

Process:

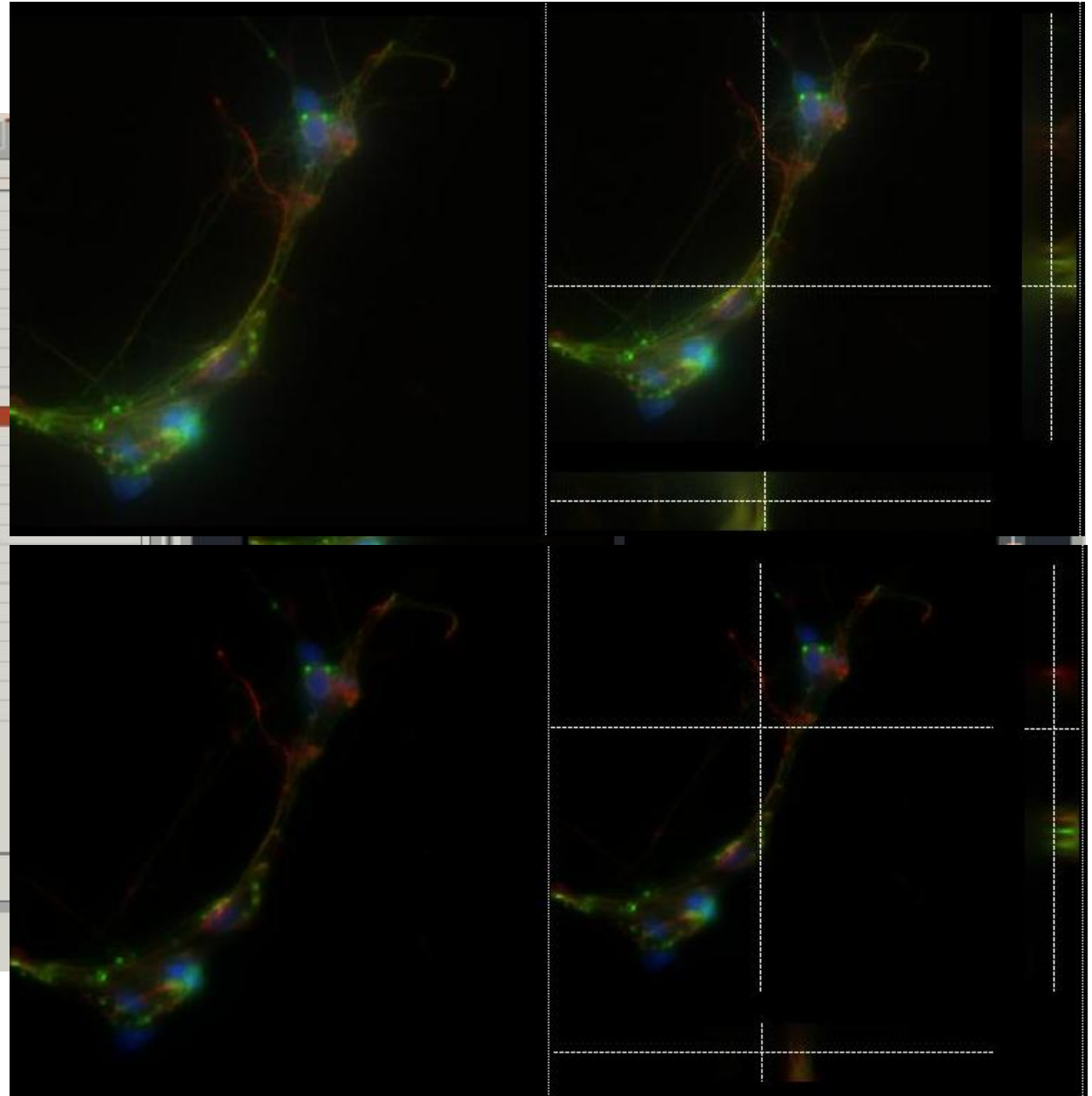§ Noise reduction

§ Sharpness

§ Background subtraction

§ …..

Optional:

§ Deconvolution

§ 3D Visualization

§ Dye Separation

Deconvolution:

§ All data auto-matically loaded
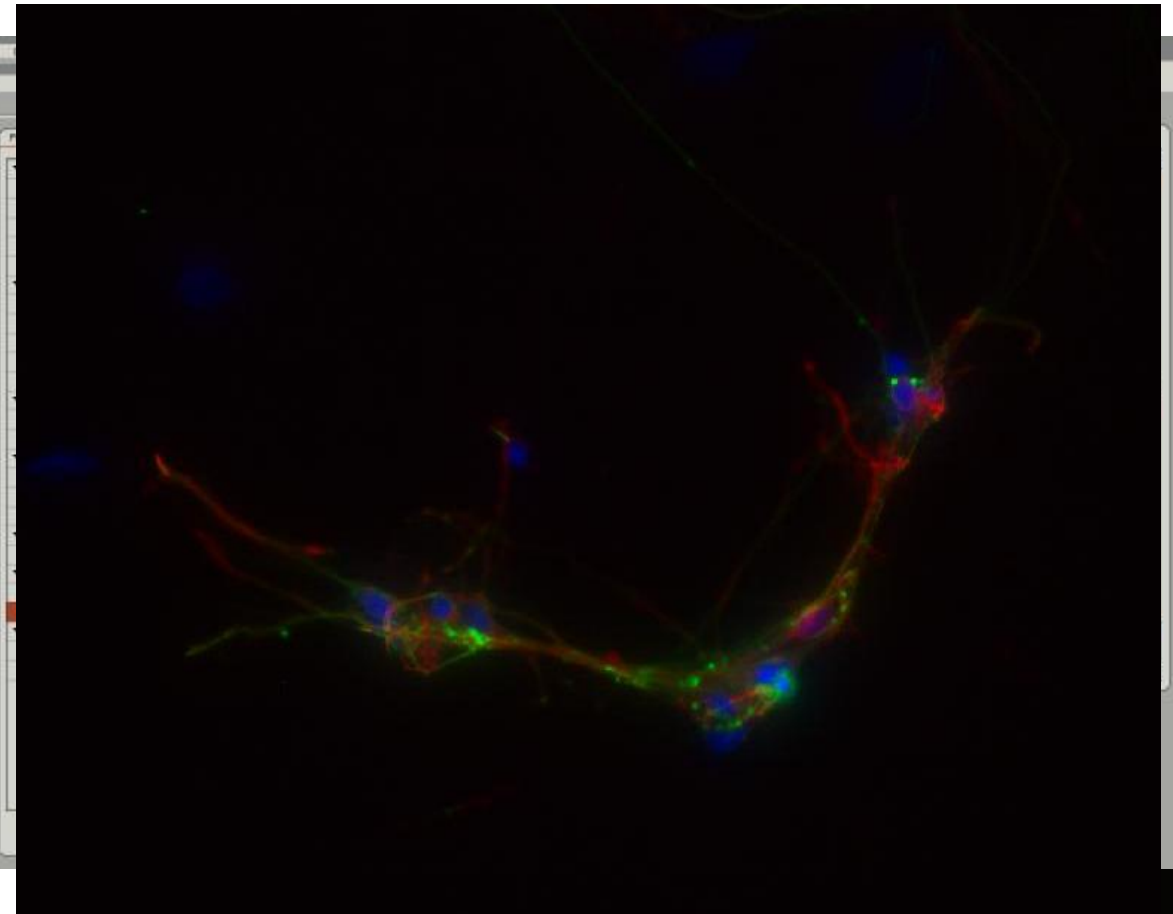
§ Recommendation: Blind, 5 iterations, deselect "fast"

3D Visualization:

§ Create movies (tilting)

§ "colours" tool also
for "projection series"

Dye Separation:

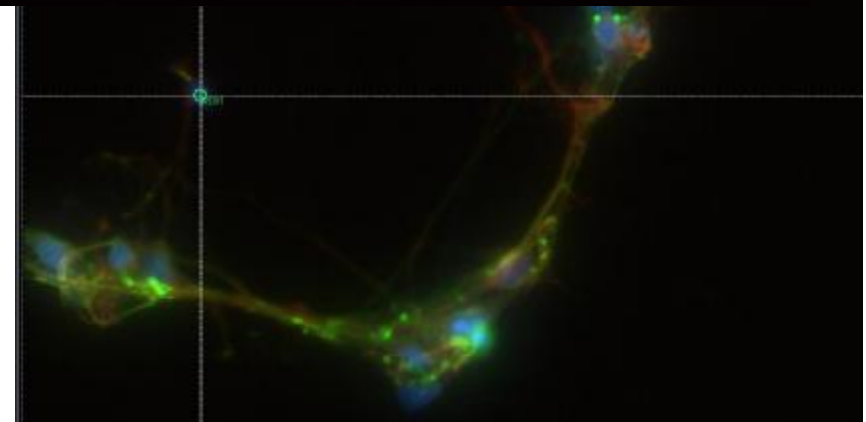§ Correction of Cross talk

§ Select areas of one fluorochrome only

Matrix	Ch1	Ch2	Ch3
Dye1	1	0	0
Dye2	0	1	0
Dye3	0	0	1

Reset Matrix   Save   Load   Apply

Quantification:

§ Profiles

§ Areas

§ Measurement "live"

§ Export as images
   or as Excel

§ No automatic detection !