New LSM microscopes
–
Adaptation of Strip-FRAP

Protocol 3rd floor LSM510:

Feb-2005

1) **Switch on system (remote switch), general login Computer**

2) **Click LSM510 icon – Scan New Images**

   Start Expert Mode

3) **Acquire – Laser : Laser Control:**

   Switch on Argon Laser 488, (prewarming), adjust output (%) to tube current = 6.1 A

4) **Acquire – Micro : Microscope Control:**

   Microscope Settings: GFP

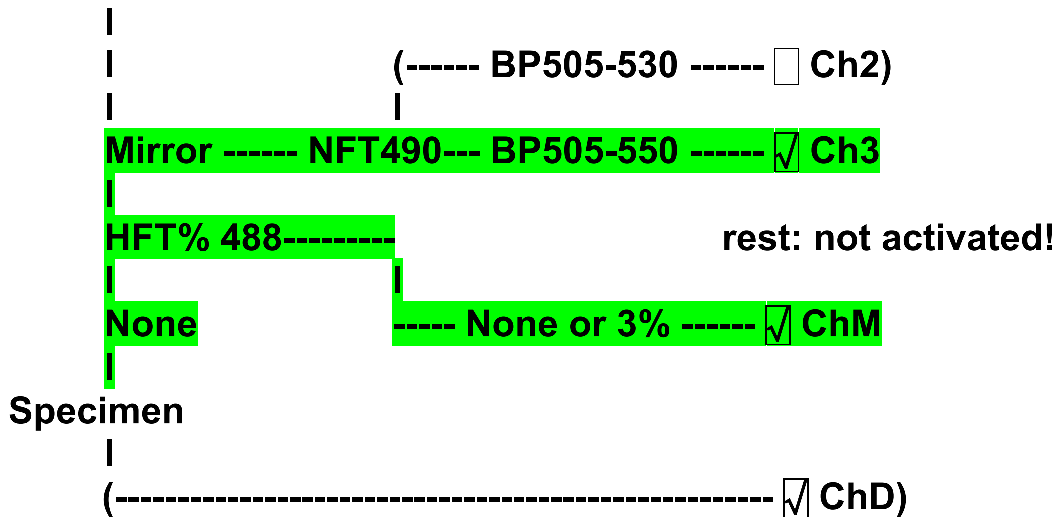
   Objective: 40x/Oil
   Reflector: none
   Tube Lens: Lens LSM

   - close window

5) **Acquire – Configuration : Configuration Control:**

   Channel Mode
   Single Track

Filter:



6) Acquire – Vis : find your cells in transmission light (microscope)

   - LSM

7) Configuration Control:

   (Possibility to switch on √ ChD = transmission light to easier find cells via Find ; (Careful: Find alters some of your settings, like Detector Gain!)

   Scan Control:

   during finding of cells, pinhole can be put to max.

   - switch off ☐ ChD), → Single

8) Acquire – Scan : Scan Control: Frame

Channels:

√ 488 nm to 0.XX% (~ 500-800 nW, measure with external laser meter! This laser power is around 21-22 in internal measure ChM)

Pinhole : to Optical Slice = 2 µm

Detector Gain = 904
Amplifier Offset = 0.1
Amplifier Gain = 1

Data Depth = 8 Bit
Scan Direction = →
Scan speed = 9!

If low fluorescence: you can use Sum 2-4 for finding cells at Zoom 1 or 0.7 (Careful: zoom 0.7 alters scan speed to 8!)

Center cells: 1st use Gert-Center in Macros, to center your desired cell, zoom in to 3 or 6.

→ Gert-Center off!

At Zoom 3-6: → Z-stack → Range

- set middle line to fluorescent cell middle (in fact a little closer to the glass slide line)

→ XY scan

→ Z-stack off!

Center cell carefully with Crop function in image window, to Zoom = 6. Be sure that the cell is straight upright (rotate it, if necessary!), and the Crop-Cross well in the center of the nucleus!

9) put Sum back to 1!

→ use ROI :

Acquire → Edit ROI :

Y = 10 pixels
X = adaptable to cell width

(center ROI to x=256, y=256)

→ save ROI to list or use ROI from list by clicking on it (strfr100 etc.)!

10) Acquire → Edit Bleach : Bleach Control:

Define Region: click on (proposed) region = same as for Acquire!

11) Bleach Control:

Settings: bleach after scan number 200 =~4 sec
488 nm = 100%
Iterations: 1! (= 20msec)

12) Acquire → Time Series : Start Series : Manual

Stop series: Manual Number 4000 (=~80 sec), 2100 (=~40 sec), or 1100 (=~20 sec)
Time Interval = 20 msec

13) → Mean ROI in Time series control!

14) Save image in MDB file

Trouble Shooting:

1) **1st time Mean ROI does not give any bleaching = "bug in the programme"! Solution: press 1st time StartB instead of Mean ROI in Time series Control, let run for a few seconds, then take image of your cell again, activate ROI and normally press Mean ROI !**
2) **Scan Speed switches from 9 to 8: can happen, if you decrease Zoom below 1, or go too far to the edge of your field of examination (at low zoom). Be sure to switch back to zoom=9, before you start your time series, otherwise the timing is incorrect!**
3) **You loose focus, or Mean ROI cannot be activated: switch off Z-stack ! Switch off center-macro!**

Feb 2005
Angelika Zotter