Basic Intructions for LSM 5 DUO

Starting the System

- turn on the System/PC switch and then the Components switch on the desk
- log in as Administrator (there is no password, just hit OK)
- launch LSM 5 DUO program (Scan New Images + Start Expert Mode)

Before Scanning

- in *Acquire/Config* choose Channel Mode and Single Track, then click Config, choose a desired configuration and load it by Apply. For example:

  GFP, FITC – Ex 488 Em (narrow band, FITC)
  green and red double labeling – Ex 488/561 (FITC, GFP / Rhodamin, ...)
  DAPI – Ex 405 (DAPI, eBFP)

- in *Acquire/Laser* switch on lasers required for this configuration (if On in not activ, click StandBy first)

Scanning

- in *Acquire/Scan* window Reset Zoom in Mode
- set the optimal Pinhole by clicking 1 in Channels (and after each objective change)
- set laser power in Channels (approx. 3-15%)
- set Images Size in Mode (512 at the beginning and for Z Stack, higher for final pictures), Optimal says the highest reasonable resolution (for objective and zoom used)
- set Scan Average in Mode (typicaly – Mode: Frame (or Line for Z Stack); Method: Mean; Number: 1 (fast scanning) or 2 and higher (for high quality pictures)

- click Find to gain the first picture, Fast XY (preview) when focusing or moving the specimen, Continuous (final quality) when focusing or moving too faint specimen, Single to make the final picture, Stop will stop Fast XY or Continuous scanning
- adjust Detector Gain and Amplifier Offset (contrast and brightness) in Channels using Palette of your image changed to Range Indicator (red and blue spots should disappear)
  in case of double labeling set it subsequentely for both channels
- if your sample is too faint open the Pinhole a little bit in Channels
- before scanning next image click New or save your previous picture

Z Stack:

activate Z Stack > in the fold Mark First/Last enter Number of slices of Interval between slices > hit Fast XY and mark First and Last section when focusing > focus back to the first > hit Start

deactivate Z Stack if you want to use scanning of single images

---

Button or Icon *Menu/Submenu* Fold (záložka)

Saving images

- in image window click Save choose your database, enter name of the image and save
- opened (or re-opened images from a database) can be exported to JPG or TIF via *File/Export*, choose Full resolution image and save
- this PC is offline, burn your data on DVD. Do not use USB flash drive

Switching off the System

switch off all lasers > close the software > shut down the computer > turn off the Components switch and then the System/PC switch - NOT LESS then **5 min after lasers** were switched off!

After Work

CLEAN immersion objectives (if used) > COVER the microscope > SIGN out in the book

General Comments

- if you don't intend to use UV lamp in your further work, switch it off (you can turn it on again after 20 min)
- if the software freezes, right down the actual time in the book
- in case of any problems contact Jan Petrášek or Katka Malínská (l. 435)
- for any special application (FRAP, FRET, Multitrack, Lambda Scan) ask for our help
- person who doesn't follow CLEAN - COVER - SIGN rules will be prosecuted (executed)

Reservations

http://lhr.ueb.cas.cz/res