First Steps - Training Procedure

Introduce rules/guidelines.

How to access the building, computer, and software; how to book the calendar; follow the SOPs; how to seek help; communication between users.

Introduce three portions of the whole imaging system.

Bulky hardware could be a power supply, control box, lasers, laser power supplies, xyz stage controller. The user should know the order of how to turn it on/off. Advanced: to recognize each box/part.

The second part is the mainframe of the microscope. Start from the Light sources, show the light path to tell upright or inverted configuration so they know how to put the sample on correctly; how many detection locations (usually four: three ports from microscope body and one is for eyepiece); main UNTOUCHED box, where the major optical components are located; how many objectives (air, water or oil immersion, advanced knowledge (features of the objective, working distance, NA, Correction…)); how many lasers, wavelength covered and how to switch channels (advanced knowledge: pulse or CW, solid-state or gas, single line or multiple lines, output energy, life span…); Epifluorescence light and how to switch channels; the right position to put samples on the stage; how to move the stage (xyz, coarse and fine); how to see the image and switch between the eye and detector; how to put a water/oil on the objective and clean the objective after you have done

The third part is the software on the computer to acquire data and transfer data.

Introduction of the software (the introduction is based on the Olympus Fluoview)

· Set up an individual account.

Log in as the Administrator, set up an individual account as “guest”?

The username format in each account is “User’s Firstname_PI last name”. The password is random. User/trainer should write this down.

· Log into a new account.

The first window (explorer):

Point out the “Jump to…” Icon

The second window (Data)

Do a quick live scan and show the data information. Introduce the memory issue if data was left in the memory

The Third Window (Setting)

· Scanning model

· Scanning shape

· Speed, pay attention to the unit. So, a user will understand the number they are using. Explain the pros and cons why use different values

· Time indication. L, F, S

· Resolution (512x512 for general, >=1024X1024 for publication, pros and cons)

· Aspect ratio

· Rotation

· Zoom (digital zoom vs real resolution change)

· Set laser power

· Lambda measurement (don’t teach)

· Z Stack drop down menu for objective selection; while running the live imaging, click the arrow to move the objective to the top, click “set”, then move to the opposite move direction, and “go” button; move back to the middle and make sure no saturation; high-low mode, ctrl+h; select Z step (z resolution)

· Time lapse (can mention, don’t teach the detail)

The Fourth window (acquire)

· Select three light sources to observe the sample.

· For fluorescence dyes, how to add/remove channels

· The next column can briefly introduce them.

· To acquire, explain FocusX2 and why; explain XY repeat; explain “Auto HV” back to “Speed” at the third window; use XY (ZTL) to take a final imagine;

· Channels (HV, Gain and Offset)

· Pinhole size

· Transmit light intensity

· Save file to Hard drive

Hands-on and introduction to the Fifth window (Live view)

· Left: how to select window and channel

· Single window vs multiple windows

· Merge channels

· 1:1 vs Full window

Save file

· Format of the raw data (*.oib for 2D ; *.oij for 3D)

· Export data

· “Save display” function and the trick

· “Add a view” function

Play move

Do/undo Z projection and save

To browse data back to office, Olympus Fluoview viewer (link) and ImageJ (link)

Transfer data by using USB portable device

Cleaning up the water or oil on objectives.

Login Book

Revision #2
Created 4 December 2024 23:05:05 by Elliot Francis
Updated 4 December 2024 23:11:30 by Elliot Francis