BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

FIRF

FRAP-enabled Total Internal Reflection Fluorescence

Jordan Briscoe Adam Fries Kyle Marchuk Taylor Shagam John Eichorst Austin Edwards

February 2019

Contents

1 Introduction

The FIRF is an inverted Zeiss microscope that can perform experiments involving FRAP, epi-fluorescence imaging and total internal reflection microscopy (TIRF). The laser lines include a 405 nm for experiments requiring FRAP as well as a 488 nm laser and a 561 nm laser for TIRF-based imaging. The epifluorescent illumination is controlled by a DG4 light source, with the filters for imaging GFP, Cy3, RFP and Cy5. The instrument is built around an inverted Zeiss Axiovert 200M microscope with a motorized ASI stage. The system is fully automated and can be controlled by the Metamorph software. Using the optical splitter installed on the system, the fluorescence emission from two distinct fluorescent probes can be collected simultaneously for experiments such as imaging intracellular calcium sensors.

Please sign up for time using the MyCores microscope scheduler. Reservations can be made up to 4 weeks in advance for owner's consortium and beginning on Friday for the following week for non-owners.

1.1 Features

1. 2 Color TIRF.
2. Image two channels simultaneously by splitting the camera vewiing area.
3. Image at 37 C.
4. 4-color Epi-fluorescent imaging.
5. FRAP.
6. Three objectives: 40x, 60x, and 100x.

1.2 Laser Safety

1. Complete the UCSF laser safety training before the start of using the FIRF microscope.
2. Do not uncover lasers for any reason.
3. Wear provided laser goggles at all times if any laser is in use on the FIRF microscope.

2 Turn on the microscope

1. Turn on the PC.
2. Turn on DG4 light source (Lamp Button then Main Button).
3. Turn on Evolve EMCCD camera.
4. Turn on microscope.
5. Turn on 488nm laser and/ or 561nm laser controller (If using TIRF).
6. Turn on the ASI stage controller.
7. Turn on MCL stage controller.
8. Turn on AOTF.

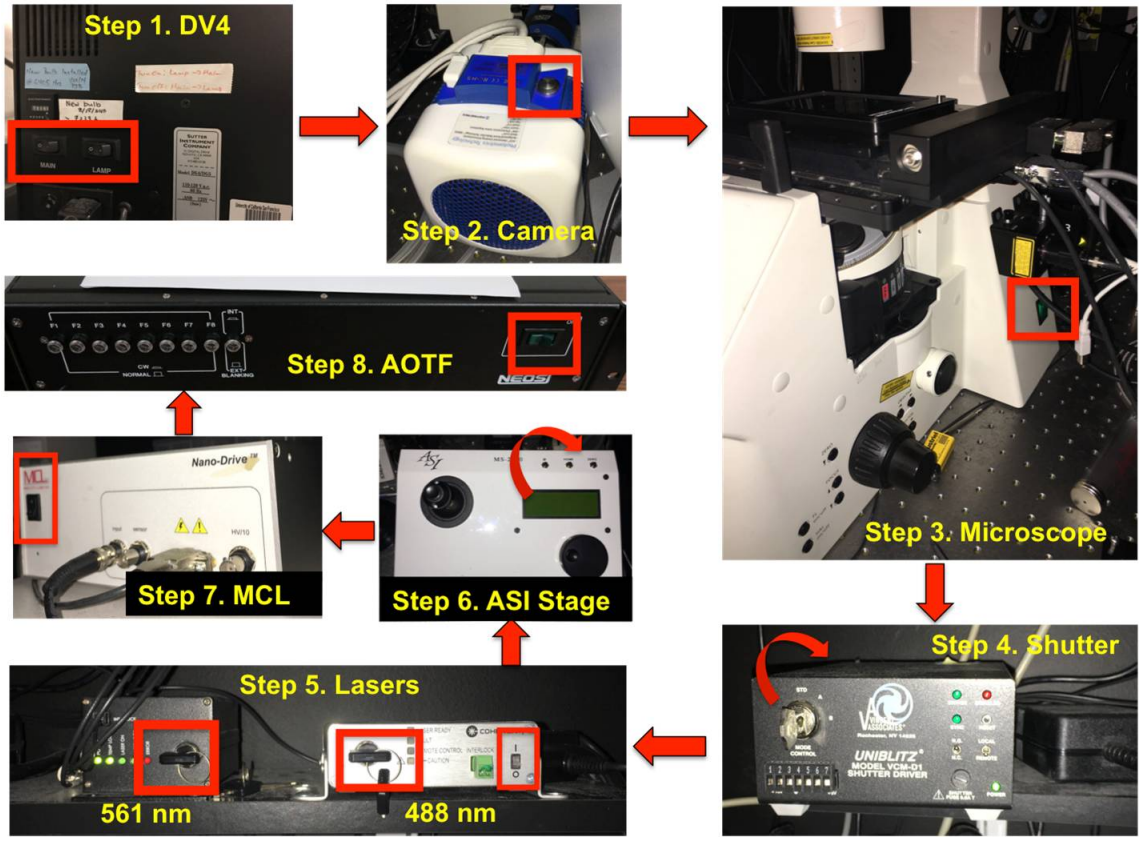

Figure 1: Start up procedures.

3 Load Samples

1. Prior to loading a sample and stage insert, lower the objective by hitting the "FOCUS ⇓" button (Figure:2).

Figure 2: Automatic Focus buttons on Zeiss Microscope.

2. After loading sample, move the stage so that the objective is in the center of your sample. .
3. Raise the objective manually using large black knob on right of microscope. If using oil objective, raise objective until oil just touches sample. If using air objective, raise objective until objective sits just below sample.

4. If you need to change samples between experiments from this point on in the experiment, lower the objective by hitting the "FOCUS ⇓" button (Figure:2, red box), and take off your old sample, and put on your new sample, then hit the "FOCUS ⇑" button. This will bring the sample into coarse focus automatically.

4 Metamorph software for microscope control and data collection

1. Double-click the MetaMorph icon on the desktop.Login to your account (Figure:3).

Figure 3: Location of Metamorph icon on desktop.

4.1 Locating Sample for Imaging-Camera

1. In the top left corner of the Metamorph software window, select your illumination setting (Figure:4A) and objective (Figure:4B).

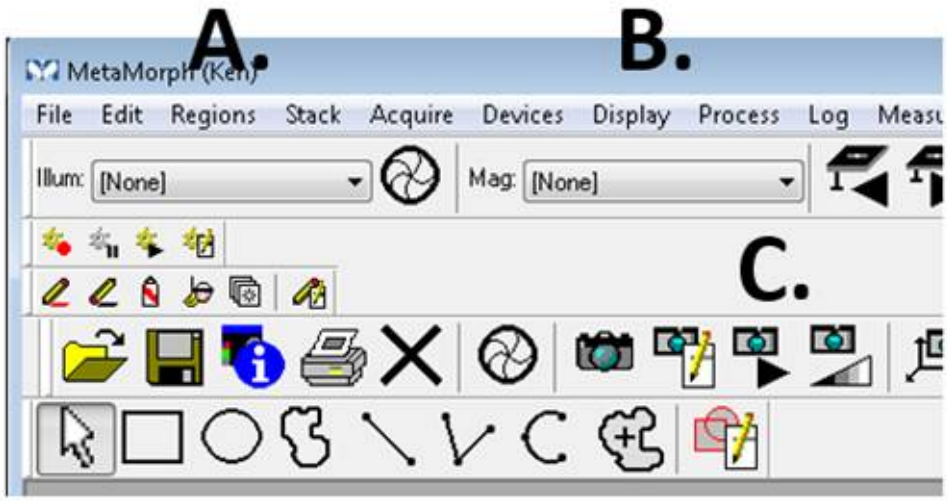

Figure 4: Visualizing Sample (A) Drop-down menu to select excitation light and mode. (B) Drop-down menu to select objective. (C) Play button to activate camera and visualize sample.

2. Press the play button to get a live view (Figure:4C).
3. If you do not see any excitation or brightfield light at the objective, press the FL on/off and/or the Hal on/off buttons on the right side of microscope.
4. Adjust focus know to obtain an image of your sample.

4.2 Creating method to acquire data(time series, z stack, multi-color imaging)

All image acquisition can be accessed through MetaMorph's multi-dimensional acquisition menu (Figure:9 – located at the end of document).

1. In the "main" tab, select the type of measurement that you will use.
2. In the "saving" tab, select the folder in which to save images.
3. The "timelapse" and "wavelengths" tabs will have options to select a number of time points to acquire, which channels to use and exposure time to use.
4. When setup, click acquire in the lower right corner of the multi-dimensional acquisition window.

5 TIRF Imaging

Prior to starting an experiment that requires TIRF-based imaging, the system must be inspected to insure that the optics are aligned properly to achieve total internal reflection.

5.1 Inspect alignment

1. Please load either your sample or a bead sample provided by the BIDC on the microscope.
2. Configure Metamorph for TIRF imaging at the wavelength(s) needed and send the emitted fluorescence to the eyepiece.
3. Using the black knob on the right side of the microscope, move the objective both above and below the sample and observe the image in the eyepiece.
4. In you have achieved TIRF, you will see an image of a single slice of sample become blurry as you move above and below it (Figure:5B).

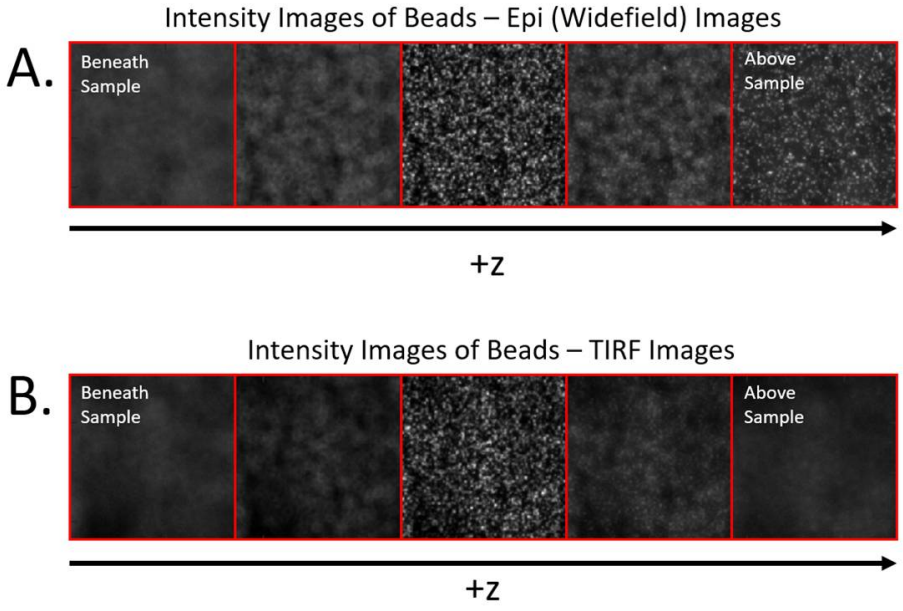

Figure 5: Bead sample for TIRF alignment. (A) In Epi, the image of the bead changes specifically as you image areas above the coverglass. (B) With TIRF, you only see a single image of the beads that defocuses as you image areas above and below the coverglass.

5. If you have not achieved TIRF, you will see a different image as you move above and below your sample (Figure:5A).

5.2 Adjusting alignment

1. With the sample still on the microscope's stage, rotate the small gray knob (Figure:6) to the right and if necessary, left of its current position and repeat inspection procedure.

Figure 6: Knob to adjust the position of TIRF excitation light a rear focus of objective.

2. Do not turn gray knob more than half a revolution.
3. If you are unable to achieve TIRF, please seek assistance from the people at the BIDC.

6 Shut-down

To keep the computer's hard drive available for recording data, the PC automatically clean up all files that are older than 30 days. Remember to transfer your at the end of your experiments.

1. When finishing your data collection, close Metamorph.
2. Use the 'subtract objective' to lower your objective to its lowest position.
3. Move the stage to the center.
4. Turn of the lasers.
5. Turn off AOTF.
6. Turn off Mad city labs controller.
7. Turn off ASI stage control.
8. Turn off microscope.
9. Turn off camera.
10. Turn off DG4 (Turn of Lamp Button, wait 30 minutes and then turn off main button)

7 Trouble shooting

1. No light is coming out of the objective when you select and activate an epi-fluorescent channel.
   (a) Press the "FL" button on the right side of the microscope.
   (b) Insure that the correct filter is installed in the DG4.
2. Samples measured with TIRF's system excitation are very dim even after alignment.
   (a) There is a wheel of neutral density filters inside the TIRF slide (Figure:7). Please try changing the position of filters in this wheel and see if your sample's fluorescence becomes more intense. Be gentle!

Figure 7: Location of wheel of neutral density filters.

3. You are unable to get a TIRF image of your sample regardless of alignment.
   (a) If you sample is mounted in a media that has an index of refraction higher than 1.4 (i.e. Prolong Gold), you will not be able to use objective-based TIRF.

8 Setting up the DG4 Lamp Source

Before experiments, make sure you have chosen the correct filters for the DG4. The filters we have are 405/10, 488/10, 560/10, 640/10, 340/10 and 380/10. The filters are located in sliders that can be inserted into the DG4 (8C). Each slider is labeled with the wavelength of the filter within it. There are only 4 slots on the DG4 (Figure:8A (red arrow) and Figure:8B), therefore each time you can only use 4 of the filters.

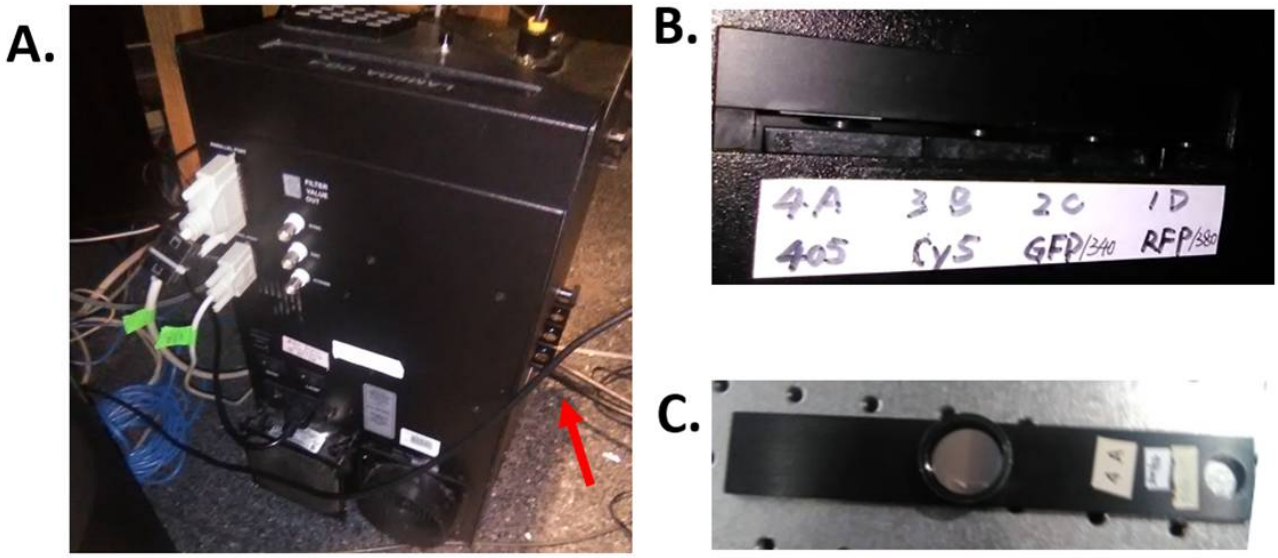

Figure 8: DG4 Excitation Source. (A) A picture of the DG4 is presented with a red arrow showing the location where the four filters can be inserted and removed. (B) A close-up image of the area highlighted by the red arrow in (A) is shown. Please not the letters in the labels. (C) This is a picture of the slide that can be inserted into the DG4. The label indicated the wavelengths that it passes is located near the handle (round hole at end).

If you need to switch filters, please put them in the correct position. As shown in Figure:8B, each slot on the DG4 is labeled 'A', 'B', 'C' and 'D'. In order to have the software select the correct color to excite your sample with, please follow the below table when inserting the filters.

405/10: A
488/10: B
560/10: C
640/10: D
340/10: A
380/10: B

Contact the BIDC

The BIDC office is located in Medical Sciences Building Room S1109.
The BIDC office phone number is 415-476-4550.
If you need immediate assistance and no one is available in the office, or it is after business hours, please call the **BIDC Hotline** at 415-745-2432.

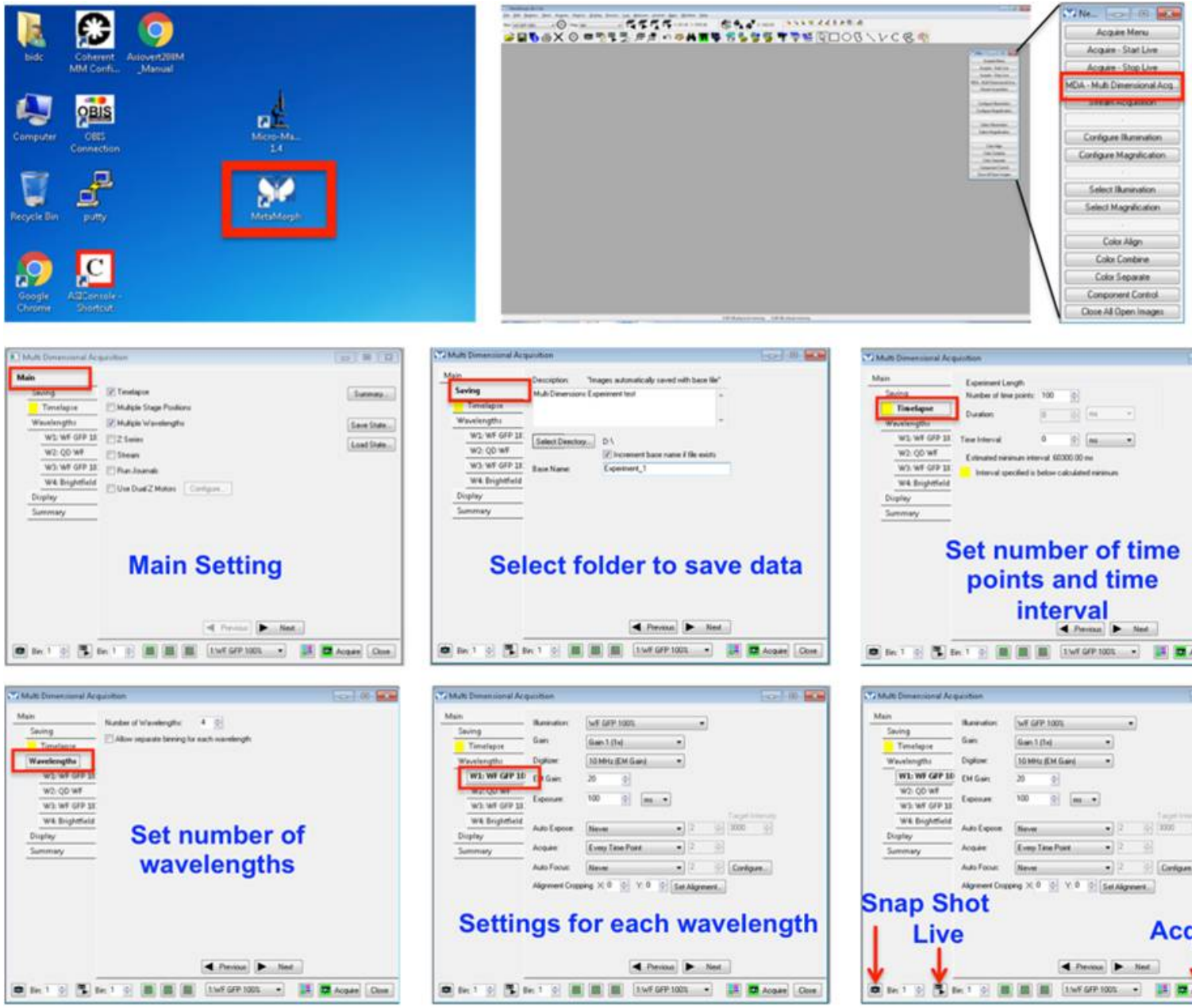

Figure 9: Metamorph's Multi-Dimensional Acquisition Interface