BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

Werb Scope

Spinning Disk Confocal Microscope

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

April 2019

Contents

1 Introduction

1.1 Background

"Werb Scope" is a Yokogawa style spinning disk confocal microscope. Compared to a traditional scanning confocal microscope that uses a single pinhole to direct light onto the sample and then to a PMT, a spinning disk microscope uses a rotating array of microlenses and pinholes to excite many sample locations which are then projected upon a camera. Advantages of this design include increased light throughput (attributed to the microlenses) and decreased image acquisition time (attributed to the camera). See Figure: 1

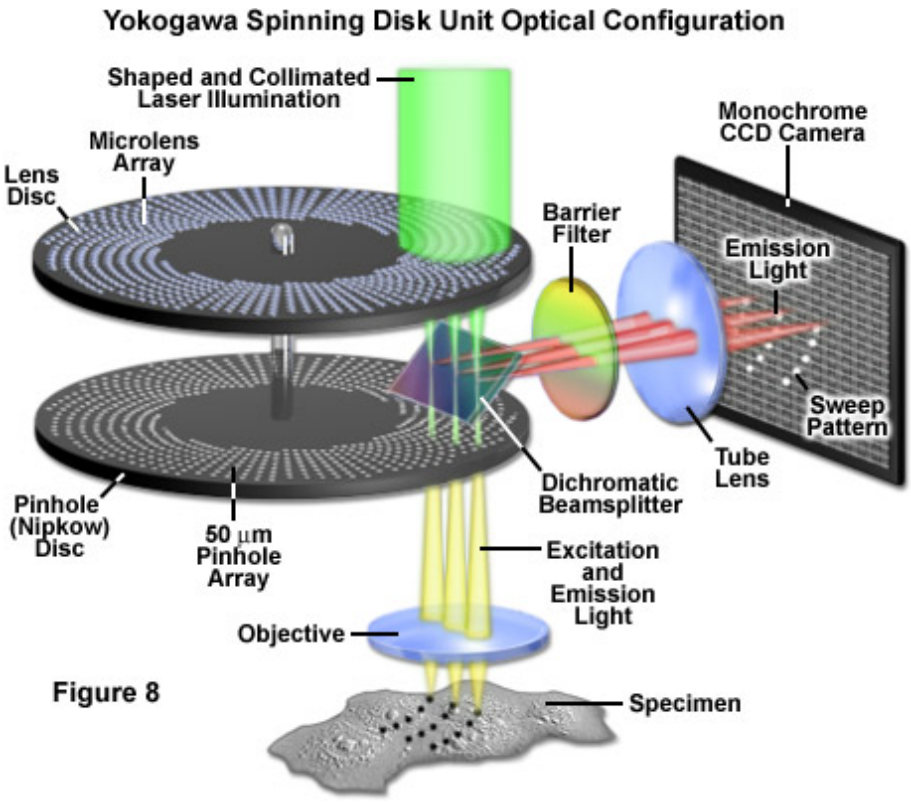

Figure 1: http://zeiss-campus.magnet.fsu.edu/articles/spinningdisk/introduction.html

1.2 Hardware

The current microscope configuration allows for a variety of objectives, laser excitation lines, and dichroic filters:

Objectives	Laser Lines	Dichroic Filters
10x	405 nm	440/40 nm
20x	488 nm	525/30 nm
20x high NA	561 nm	607/36 nm
40x (oil)	640 nm	630/90 nm
100x (oil)		684/24 nm

The microscope also includes components and features such as:

Andor Clara CCD Fast acquisition rates with large array resolution

Motorized microscope stage Controlled by either joystick or software

z-axis piezo Software controlled for 3D image stacks

Sample incubator Available for cells in culture imaging

μ**Magellan** Dynamic device control and acquisition

2 Initial Setup

Sign up for microscope time using the MyCores microscope scheduler.

2.1 Hardware Startup

1. If the computer is off, turn on the computer.
2. Turn on the power strips. One is to the back left of the microscope and one is to the back right.
3. Power on the lamp. The lamp will need to warm up for a couple of minutes before you can set the power and open the shutter. The power can not be set while the display screen is blinking.
4. Power on the Laser Launch and turn the emission key.
5. Turn on the Beam Conditioner.
6. If it is needed for your experiment, turn on the incubation unit.

For a photo guide of the set up components see 6

2.2 Software Initialization and Sample Alignment

All hardware components must be powered on before software can be loaded.

1. Double-click on the "Micro-Manager" icon, which is located on the Desktop, to start the data acquisition software. See Figure:2

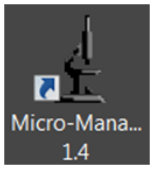

Figure 2: The Micro-Manager software can be found on the Werb scope desktop.

2. Upon start-up, select the configuration file. See Figure:3

Figure 3: Choose the proper configuration file from the drop down menu.

Note: This config file should already be loaded, and the user should only have to select OK.

3. In the software choose the correct objective. If the objective in the software does not match the objective currently being used on the microscope, the scaling of the image will be incorrect.

4. Align the sample focus by eye:

   (a) In the software under "Visualization" choose "Color Eyes" where "color" is the emission range of your dye (ex: Dapi,GFP,RFP). "Analyzer Eyes" can also be used in order to see all three colors.
   ProTip: Use the brightest, most robust dye in your sample for initial alignment. Additionally, Red has lots of background due to the autofluorescence of most sample substrates.

   (b) Click the "live" button in the software to send the "open shutter" command to the instrument.

   (c) Look through the eye-piece and bring your sample into focus.
   Note: Rotate the focus towards you, as this will move the objective away from your sample and align the focal plane.

   (d) Once you are satisfied with the focus, hit the "stop" button to close the shutter.

5. Parameter setup within the software:
   Note: The path length is slightly different between your eyes and the camera, so the focus will need to be adjusted by a few microns.

   (a) Under "Visualization" choose "CONFOCAL".

   (b) Under "Confocal Channel" choose the appropriate dye related excitation/emission combination.

   (c) Choose an exposure time (typically between 100 - 500 ms).
   Note: The appropriate exposure time is sample and dye dependent. Take note of final parameters for future reference.

   (d) Click the "live" button to get a continuous stream of your sample.

   (e) Use the scroll wheel of the mouse or the focus knob on the microscope base to finely adjust the focus of the objective until your sample is in view.
   Note: The focal change response time is dependent on exposure time. It is possible to scroll through your sample plane without it appearing on the screen. Typically, when the sample is brightest the focus is at the middle plane of your sample.

6. Repeat the adjusting of exposure time for each dye you wish to image. The Brightfield lamp intensity can be adjusted as well as the exposure time.

3 Data Acquisition

There are two effective ways to collect data on the Wide Guy; through the Micro-Manager interface or the plugin extension known as μMagellan. Both have their pros and cons and the choice ultimately comes down to the user.

3.1 Micro-Manager

Data collected from the Micro-Manager software itself produces image stacks at each location with the color channels incorporated into the file. The files are easily loaded and analyzed in ImageJ/FIJI.

1. When you are ready to acquire data, click on the "Multi-D Acq" button. See Figure:4

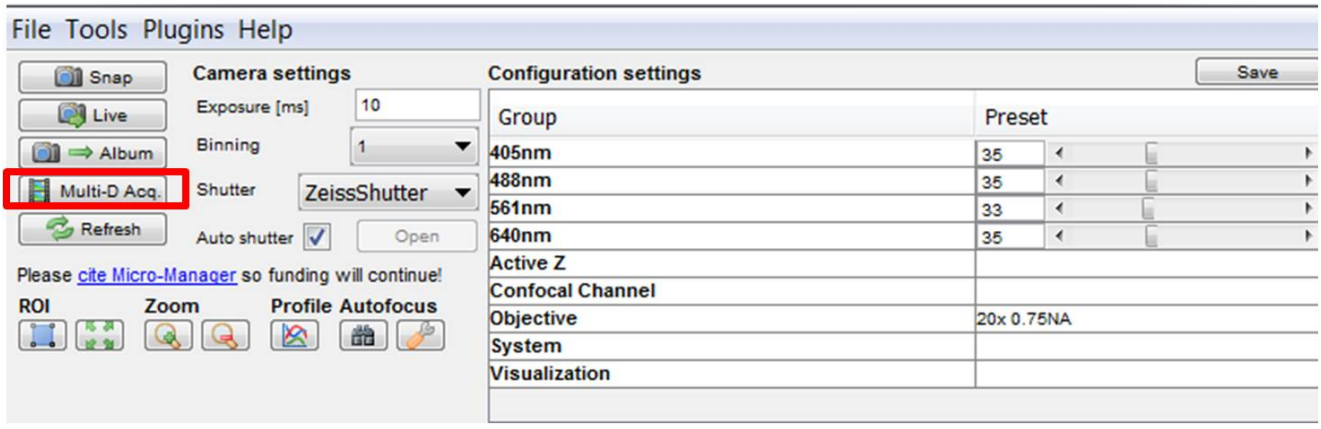

Figure 4: Click the Multi-D acq. button in order to set up imaging parameters.

2. To perform a z-stack:
   (a) Check the "z-stacks (slices)" field and switch to "absolute z".
   (b) Set the interval for your z-stack:
      - Manually enter the start and end positions, or
      - While in "live" mode, scroll the focus to the top and bottom plane of your sample. Set each position appropriately.
3. To use multiple channels:
   (a) Check the "Channels" field and select "Channel" for Channel group.
   (b) Select "Create Multiple Channels" and click "New" to add additional channels.
4. To image multiple x-y positions:
   (a) Check the "Multiple positions (XY)" field, and click the "edit positions list" button.
   (b) Select "Create Grid" near the bottom right.
   (c) "Set" positions while in "live" mode and choose "10% overlap".
5. Select the acquisition order for your Multi-D acquisition.
   Note: "Channel/slice" is much faster than "slice/channel".
6. Click "Acquire!". See Figure:5

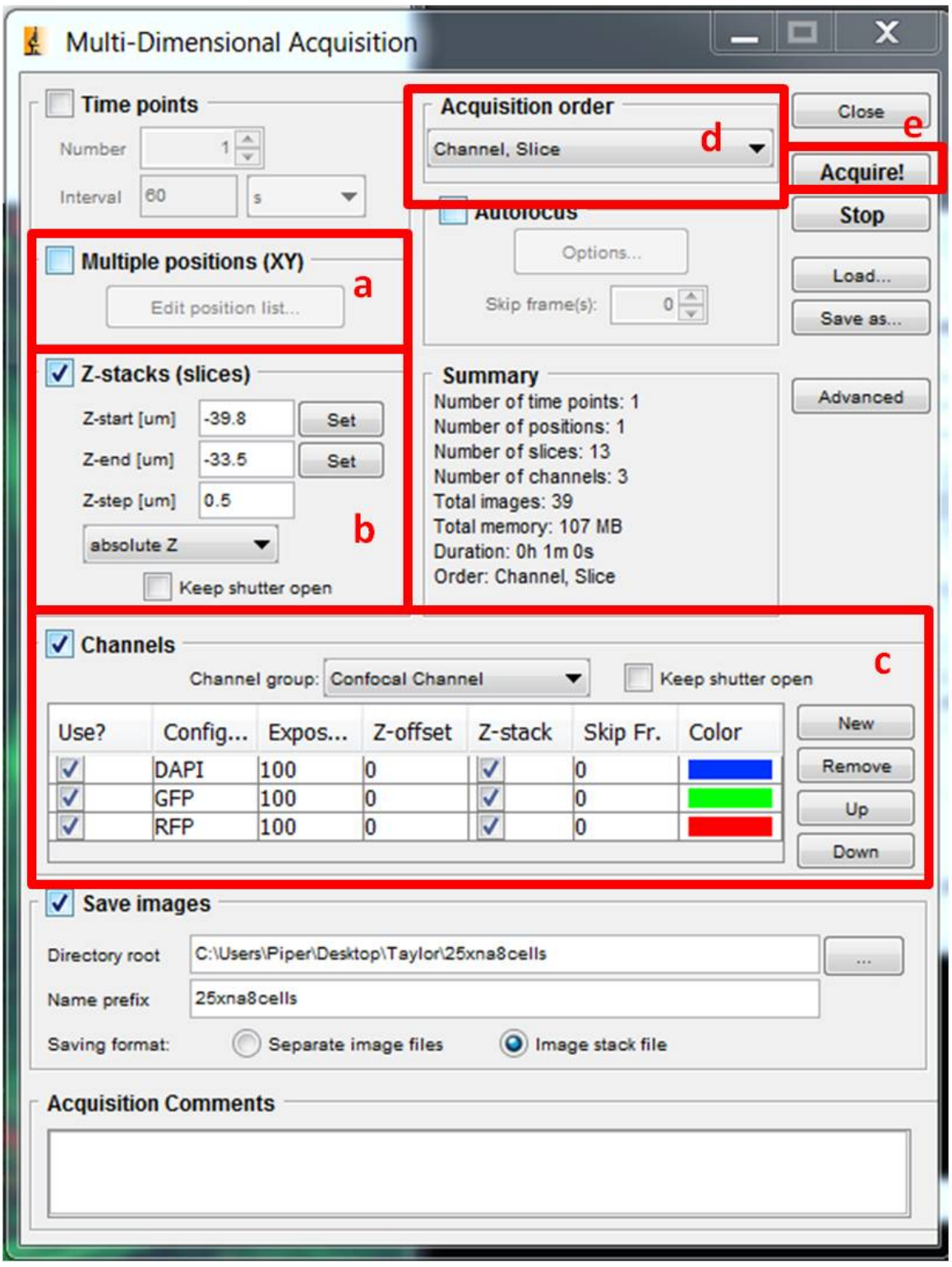

Figure 5: Witih this window acquisition settings such as Multiple Positions (a), Zstacks (b), Multiple Channels (c), Acquisition Order (d), and the Acquire! button (e) can be found.

4 Saving

1. To save images, click the "Save images" box in the Multi-Dimensional Acquisition window.
2. Choose the proper directory root to save the images to.
3. Choose a naming prefix.

5 Trouble Shooting

μMagellan Explore! mode launches with error Check that the "Channels" option is set to Channel.

Scroll wheel does not change focus in Micro-Manager 1) Select the "hand" from the ImageJ window and try again. 2) The z-piezo might be at its limits. Go into the Multi-D Acq window and set the z-positions to zero. Manually bring you sample back into focus.

Micro-Manager launches with an error Check that all the components are turned on.

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.

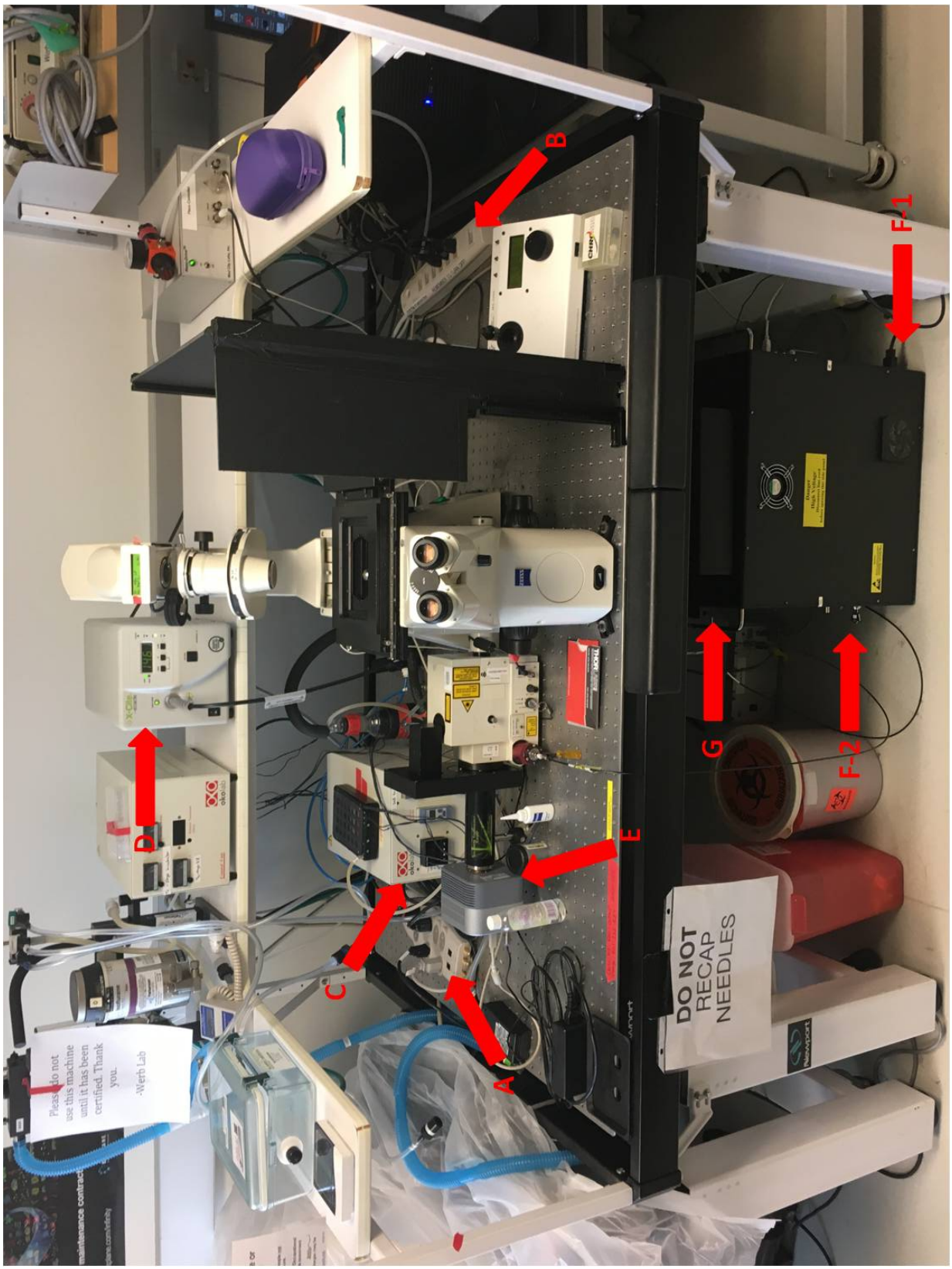

Figure 6: Powerstrip1 (A), Powerstrip2 (B), Inucbator (C), Xcite Box (D), Camera (E), Laser Lauch power switch (F-1), Laser Launch emission key (F-2), Beam Conditioner (G)