BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

Wide Guy

Inverted Widefield Microscope

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Contents

1 Introduction

Colloquially known as "Wide Guy", the microscope is a conventional widefield microscope set upon the inverted Zeiss Axioscope 200M body.

The illumination source is a Sutter Lambda XL with appropriate excitation and emission filters for the common DAPI, FITC, TRITC, and Cy5 lines. Due to the configuration of the microscope, 7-color imaging (plus brightfield) can be achieved when taking advantage of the Brilliant Violet dye series.

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.

1. This can be done up to 3 weeks in advance for the owners' consortium.
2. For non-owners, reservations can be made beginning Friday for time during the following week.

2.1 Hardware Startup

Note: If you want to image cells in culture, turn ON the incubator **2 hours** *ahead of time and place the chamber on the microscope to allow the system to stabilize (Figure 1) .*

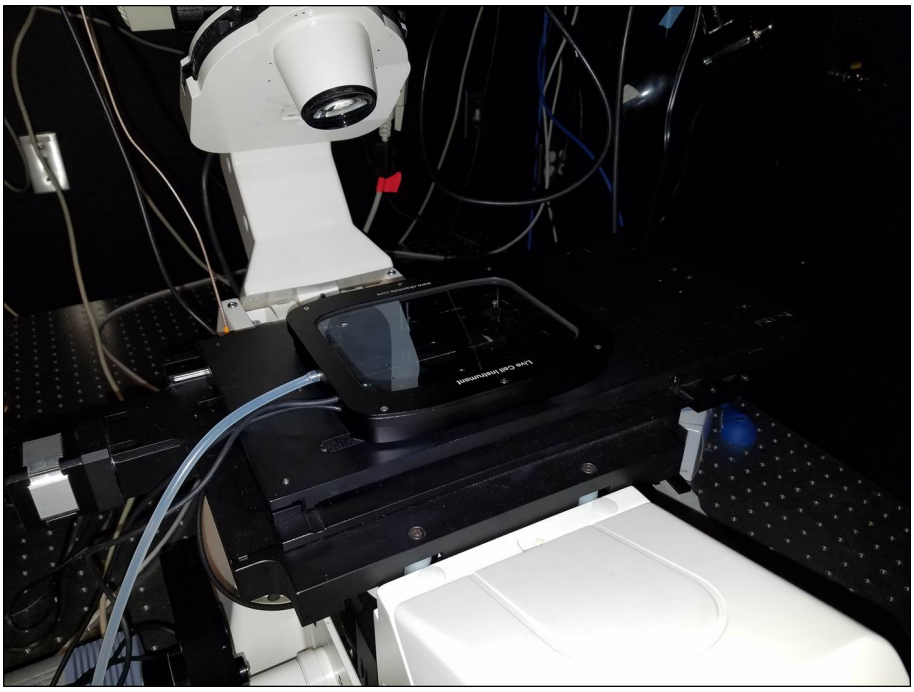

Figure 1: Cell Incubator on Stage

1. If the computer is not on, press the external power button taped to the outside of the case (Figure 2).
2. Turn ON the power strip integrated into the shelf behind the microscope (Figure 3).
3. Turn on the Lambda XL (Figure 4).
   (a) Flip the power switch on the back of the unit.
   (b) On the display press the "Local" button.
   (c) Press "1" to start lamp.

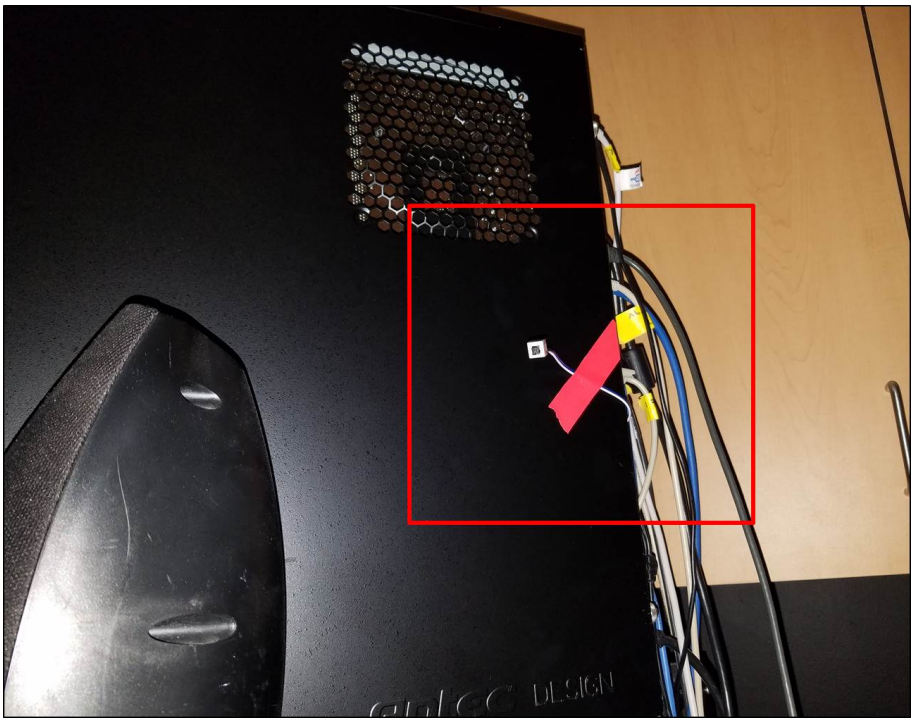

Figure 2: Computer Power Button

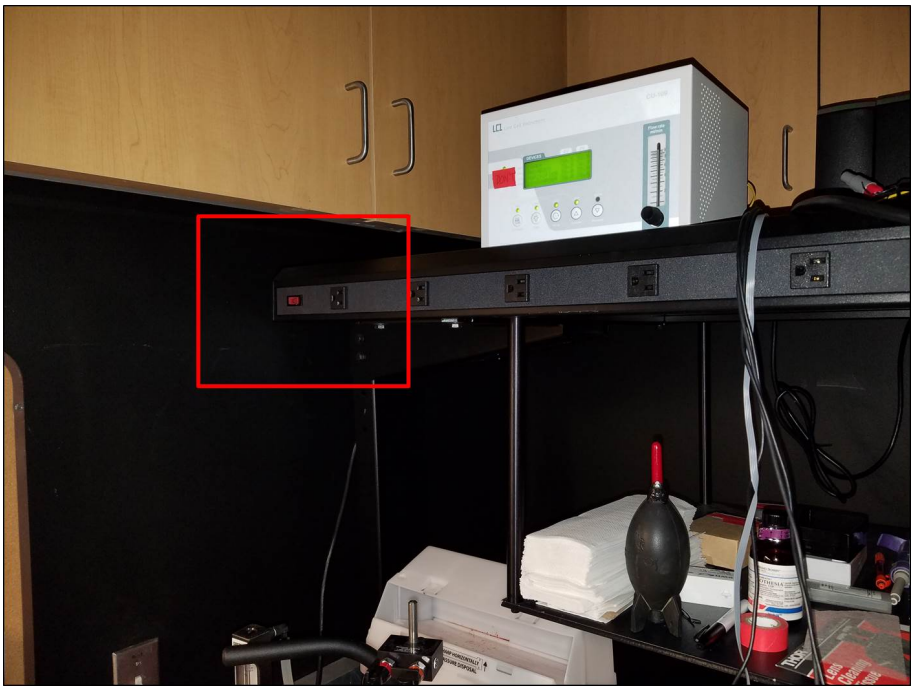

Figure 3: Power Strip for Components

Figure 4: Lamp for Microscope

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.
2. Upon start-up, select the configuration file.
   Note: This config file should already be loaded, and the user should only have to select OK.
   Bonus Note: If you become a heavy user, talk to a BIDC employee about making a custom config file to speed up your imaging time.
3. In the software choose the correct objective.
4. Align the sample focus by eye:
   (a) In the software under "Mode" choose "Eyes - color" where "color" is the emission range of your dye.
   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 using the focus mechanism.
   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 "Channel" choose the appropriate dye related excitation/emission combination.
   (b) 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.

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

(d) Use the scroll wheel of the mouse 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.
Note: 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.
Note: 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 μ Magellan. 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.
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!".

3.2 μMagellan

The Micro-Manager plugin μMagellan was written by the former BIDC employee Henry Pinkard. It has many built-in features designed to speed up sample exploration, region of interest defining, and sample acquisition time while limiting sample irradiance. Data is recorded in a special folder that includes image stacks of the regions of interest in TIFF format along with a .XML file that contains the metadata. To stitch the files together for data analysis in either ImageJ/FIJI or Imaris, the folder needs to run through a separate plugin known as "Imaricμ mpiler".

Below is a condensed user's guide. A more in depth article can be found at:

`https://www.micro-manager.org/wiki/MicroMagellan`

3.2.1 Explore Mode

Explore mode allows the user to quickly find regions of interest, define z-stack thickness, and create grids for image acquisition.

1. Initial Setup:
   (a) Assign the saving directory to your user folder.

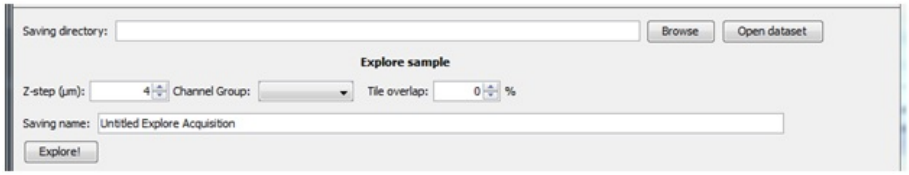

Figure 5: Explore Settings

(b) Assign a saving name (μ Magellan will save your exploration areas).

(c) Enter a z-step (this can be changed before data acquisition).

(d) Change "Channel Group" to channel.

(e) Enter a "Tile overlap" percentage (10% is a typical amount).

(f) Press "Explore!" (a new window will open).

2. Check the channels you wish to use in the upper right.

3. Click on the black space (a magenta box will appear).

4. Click on the magenta box to confirm image area (you should now see an imaged ROI in the colors you selected).

5. Use the look up tables (LUTs) on the right to adjust contrast for each color.

6. Use the scroll wheel to zoom in and out in the explore window and/or drag the screen to move around your sample.

7. You can click and drag to select multiple fields of view to be imaged.

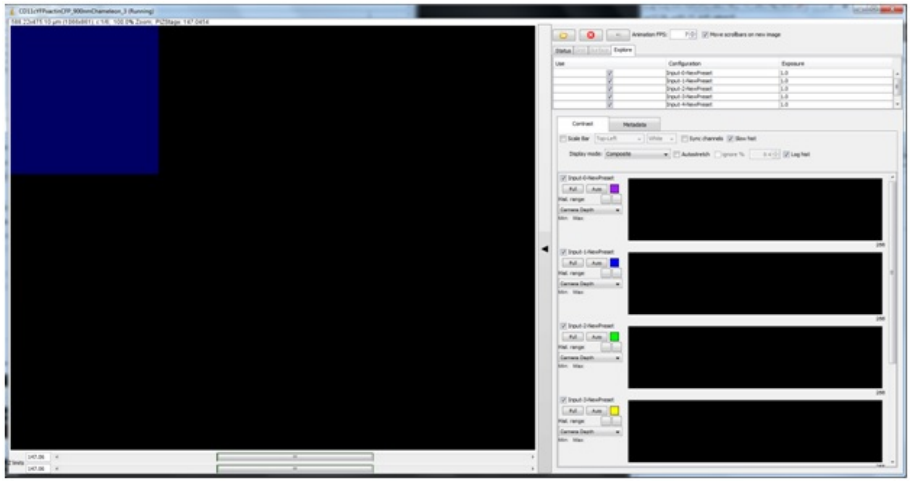

Figure 6: Explore Window

8. Adjust the z-limits by either pressing the arrow keys (which will step by the pre-defined step size) or dragging the scroll bar. Imaging a region will now include multiple image planes.

9. Define regions to be imaged:

(a) Click on the "Grid" tab in the upper right.

(b) Press "New Grid".

(c) Define the number of rows and column in the grid coresponding to fields of view.

(d) Drag the grid on the screen to the desired region on interest.

(e) Repeat for as many areas you would like imaged.

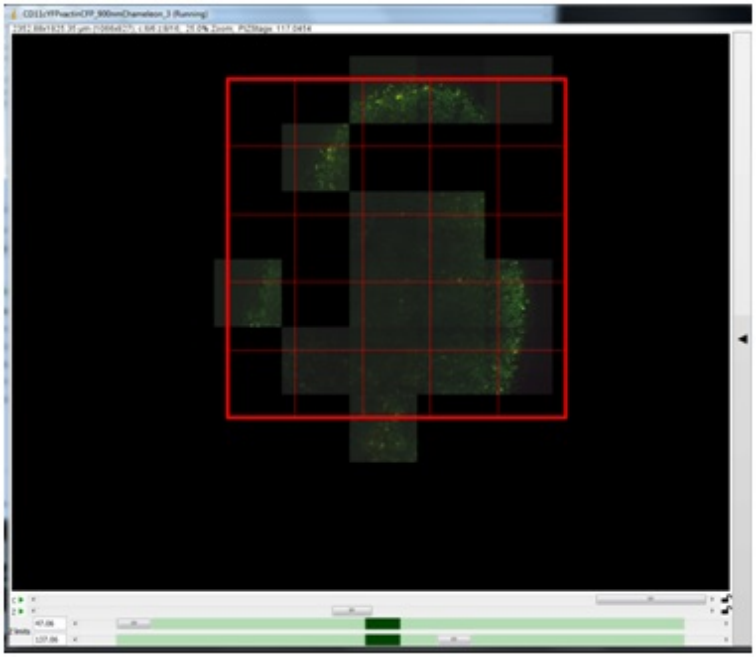

Figure 7: Explore Grids Image

3.2.2 Acquisition

There are many advanced features in the acquisition setting. Please follow the link above for more information.

1. Navigate back to the main μ Magellan window.
2. Remove any unwanted grids from the auto-populated list.
3. Press the "Saving" tab to direct data to the appropriate folders.
4. Press the "Time" tab to set up a time lapse.
5. Press the "Space" tab, select "Simple Z Stack" and input the appropriate z-limits for each grid.
6. To run a single grid, press the "Run Acquisition" button at the bottom of the window, otherwise press the "Run all" button under the "Setup multiple acquisitions".

More options for acquisition include:

1. Surfaces (3D grids)
2. Individual time lapse for each grid
3. Space (To reduce collection of "blank" data)
4. Covaried Setting (Changing hardware parameters based on imaging position)
5. Drift complensation

Please read the online guide or talk to a BIDC employee for more information.

4 Shut Down Procedure

One your data is saved, you can now shut down the instrument. Please note: Due to limited hard drive space on the computer, user's data will periodically be "purged" to make room available. The best practice is to immediately transfer data to a personal hard drive while you shut down and clean the area.

1. Exit the software.
2. Lower the objective and take off your sample.
3. Shut down the Lambda XL.
   (a) Press "Local" then "2" to shut down lamp followed by "1" to confirm.
   (b) Turn off the power at the back of the unit.
   Note: The lamp should be on for 30 minutes before turning off and off for 30 minutes before turning on. If someone is scheduled after you, please leave the lamp on.
4. Press the power switch on the shelf to turn off components.
5. Log off the computer.
6. Clean up the area.

5 Trouble Shooting

μ **Magellan 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.