Micro-Manager Manual

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Introduction: Why Micro-Manager?

Micro-Manager (MM) is a microscope control software developed in Ron Vale's Lab at UCSF with support form NIH. The idea was to provide a free, open-source platform based on Java using ImageJ (which is also funded by NIH). Basically, MM tries to imitate the function of packages such as MetaMorph, ImagePro and SlideBook, but for free whereas these other packages go for up to $10,000.

In Bi1X we will be using mostly the GUI user interface. However, MM has a good scripting language. Its libraries for microscope control can also be accessed from Matlab, giving the chance to integrate data acquisition and data analysis.

Because it's a program in constant development it will always have some bugs. Please report any of them to the TAs so we can pass that information along to the programmers and, also, be patient!

The next few paragraphs give guidelines to be followed every time you sit in front of the microscope.

Some safety words

Three of our scopes (Kratos, Nemesis and Wheels) use external filter wheels. This means that when using fluorescence if you look through the eyepiece you might get some of the UV excitation light. **DO NOT LOOK THROUGH THE EYEPIECE WHEN USING FLUORESCENCE**. This is not a concern in the Zeiss.

After turning on the fluorescence lamp it should be kept on for at least 30 minutes. When turned off it should be kept off for at least 30 minutes. Changes to the bulb before it's equilibrated can break it.

Turning everything on

There's no particular order you should turn things on. However, make sure to turn on the fluorescence only if you need it and to turn everything off once you're done with your experiment.
Special warning about the scopes with a Lumen (Nemesis and Wheels): The Lumen combines a fluorescence light source with fluorescence filter wheel and an ND filter wheel. Both wheels are controlled from the Prior controller. The only role of the switch on the Lumen is to turn the fluorescence lamp on and off. If, for any reason, you need to restart your system and turn all controllers off and back on make sure you don't do this with the Lumen! You'd only be hurting the fluorescence light bulb.
Special warning about Wheels: When turning on the Prior controller you might hear a weird "buzzing" sound coming from the brightfield shutter. If you hear that just turn the Prior controller off and back on.

Logging in

Use the Bi1 account. If you find the computer already logged on, make sure to log off and log on using the Bi1 account. Do not install any software or change anything in the computer configuration.

Make sure to log yourself on the microscope logbook and to write down the number on the fluorescence illumination source (if you're using it).

Data storage

You should take data and save it in a folder inside the "Bi1" folder located on the desktop. Always save data directly to the local machine! After that you can move it to a USB drive or to our server (snowdome). In order to mount snowdome on a Windows machine you need to connect to \\snowdome.caltech.edu\aph162. The username is "aph162" and the password is "162sharing".

Loading Micro-Manager

On the desktop there should be a link to "MicroManager1.3.XX", with "XX" being some version. The software keeps getting updated constantly so load the latest one if there happen to be multiple links.

Scope	Dichroic position
Kratos	4
Nemesis	3
Wheels	4

Table 1: Dichroic to be used for each particular microscope in the class.

MicroManager will ask you for a configuration file. Make sure that it's loading the ".CFG" file located in the folder "C:\MMConfig". The ".CFG" file should have the name of the scope you're using (Kratos, Nemesis, Wheels or Zeiss). The previous user might have used a different configuration file, that's why it's key you make sure you choose and load the right one.
Special warning about Zeiss: Unfortunately, the camera on Zeiss sometimes has issues when starting MicroManager. If you have any problems just turn of the scope and camera, turn them back on and restart the computer.

Setting the dichroic mirror

Each scope has different dichroics. In Bi1X we will use only one of them unless otherwise noted by your TA. Make sure that the dichroic is set to the right position. Table 1 shows the positions you should use for each one of the available microscopes in the class.

Making sure everything's ready

Make sure that all diaphragms are open and that you know what phase ring (if any) is set on the condenser before starting.

Live mode

Each scope has a channel defined as "BrightField" and one defined "BrightField-Live". The difference between the two is that the latter bins the image. This decreases the resolution of the image, but allows for a much faster update of the screen with exploring your sample in "Live" mode. When you actually take data you can use "BrightField", but you should use "BrightField-Live" when moving through your sample.

Note that the "Auto shutter" option needs to be enabled. If you wanted to open the shutter manually in order to look at a sample in brightfield

through the eyepiece, for example, you can unselect it and click on "Open" or "Close".

Multi-D Acquisition

This is one of the great strengths of Micro-Manager. It allows you to go to multiple XY-positions, take Z-slices, take pictures using different channels (i.e. different wavelengths of excitation), perform image-based autofocus, and do time lapse microscopy! Make sure you familiarize yourself with this window. Also, before clicking on "Acquire!" check which of these options are set. For example, you might be interested in taking a snapshot of the particular position you're on, but the "Use XY list" option might be turned on.

Time-lapse microscopy

During the course we'll be taking movies of bacteria over many hours. Before taking a movie it is very important to make sure that the results are saved to disk choosing "Save files to acquisition directory". Also, you should select "Display in Live window". This will only show on the screen the very last frame that was taken. It ensures that there are no memory problems.

Autofocus

This option is only available together with the multiple "Use XY list" option. If you want to do this for a single position just choose a single position on the position list! You'll find a couple of parameters regarding the autofocus that can be adjusted.

- Channel: This is the channel that will be used to do the image-based autofocus. We usually use "BrightField".
- 1st number of steps and 1st step size: If the parameters are 5 and 1, respectively, it will take a total of 11 snapshots for the first search range. Each one will be spaced by 1 micron and will be centered around the starting position.
- 2nd number of steps and 2nd step size: After doing the first search it will perform a finer focus. One set of parameters that works is 6 and 0.3.

- Threshold: Set this to 1.
- Crop ratio: What fraction of the image should be used for the finding the focus. Often, you'll start with one cell in the middle of the field. You want most of the information to come from that area. In that case a crop ratio of 0.25 is good. If you're focusing on samples that span the whole image you can set that to 1.