# Microscopy Shared Resource Facility Icahn School of Medicine at Mount Sinai

## **Olympus IX-70 Imaging Protocol**

#### 1) System Startup

- Please note our sign-up policy. You must inform the facility at least 24 hours beforehand if you can't come; otherwise, you will receive a charge for unused time. The facility will allow for extenuating circumstances (cells dying, sick day, etc.) if you inform us in a timely fashion.
- **Follow each step** of the startup poster, log into your account, and open Metamorph software.

#### 2) Lens Cleaning

T If necessary, please lift the condenser by its centering screws (highlighted in red) as shown below:



Please clean all of the lenses (used and unused) before your session and afterwards if you use the 60x oil immersion lens. Refer to the lens cleaning poster if you need any help recalling the rules and steps.

### 3) Microscope control and loading your sample

- **To load your slide, plate, or dish** and take care for the following issues:
  - Make sure that the stage adapter that correctly matches your sample fits securely and flush with the stage.
  - For slides:
    - Select and inspect each slide. If it is dirty, gently clean with a KimWipe and/or cotton swab. You should do this with all of your slides before you come.
- *<sup>conservent of the stage movement joystick to position your specimen over the lens.*</sup>
  - For faster movement, hold down button Joystick for X & Y stage movement

- ${}^{\mbox{\tiny \ensuremath{\mathcal{C}}}}$  Set the microscope into viewing mode:
  - Turn Camera dial: turn to





- Filter wheel: set to A
- *<sup>ce</sup>* Use the illumination control menu in the software to select your channel:
  - Click the magnification drop down menu: Select your channel from this menu.

ire Devices		Display	Process L
Mag:	[None]		4
1	[None]		M 1
	20x		
	40x 60x oil		

• Click the channel dropdown menu:



• Use this button to open and close the fluorescence light shutter:



*Focus on your sample and center your region of interest using the following controls:* 



Take care not to use the focus knobs with X's.

- Check all of your fluorophores in your specimen. You need to have an idea of what to expect before you start your scan.
- *Close the reflected light shutter to prevent photobleaching of your sample.*



#### 4) Your first image

- *S*et the microscope into camera mode:
  - Camera dial: turn to



• Set the filter wheel to the position that best matches your experiment:

B. DAPI, GFP, TxRed, TransC. CFP, YFPD. GFP, TxRed, Cy5

- Camera
- The Walti Dimensional Acquisition Menu:

Multi Dimensional Ac	quisition	
Main Saving	Timelapse	Summary
Wavelengths W1: DAPI	Multiple Stage Positions      Multiple Wavelengths      Z Series	Save State
W2: FITC	☐ Stream	Load State
Summary	🔽 Run Journals	
	Previous Next	
🛍 Bin: 2 🛨 📫	Bin: 2 🛨 🔳 🔳 1:DAPI 💌 📰 🛙	Acquire Close

1. Click the **Saving** tab within the window.

Multi Dimensional Ac	quisition	- • 💌
Main Saving Stage Wavelengths	Description: *Images automatically saved with base file* Multi Dimensions Experiment	
W1: DAPI W2: FITC Display	Select Directory Select Directory C:\User Data\huqr01 Increment base name if file exists Base Name: Experiment	
Summary		
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- a. Select you data folder in the Select Directory
- b. Create a base name
- 2. Click **Wavelengths** to set the number of wavelengths; set each wavelength illumination

Multi Dimensional Acc	juisition
Main Saving Stage	Number of Wavelengths: 3 ÷ ☐ Allow separate binning for each wavelength
W1: DAPI W2: FITC W3: TXRED Display Summary	Main       Illumination:       DAPI         Saving       Gain:       3         Stage       Gain:       3         Wavelengths       Digitizer:       20 MHz         W1: DAPI       Exposure:       590       •         W2: FITC       Target Intensity:         W3: TXRED       Auto Expose:       No Auto Expose       3000         Display       Auto Focus:       No Auto Focus       2       Configure         Alignment Cropping       X: 0       Y: 0       Set Alignment
1000 Bin: 2 🛨 🏧 E	Previous Next Bin: 2 : Bin: 2 : Bin: 2 : Acquire Close Page 6 of

*<sup>cr</sup>* Use the Acquire window to acquire your images:

Acquire	
Acquire Imag	e: 📑 Acquired
Save Image Save	e to: C:\MM\\Acquired001.tif Set Save
Save w/Sequence Disp	olay Acquire Correct Annotate Special Live Replay
Exposure Time:	Digitizer: 20 MHz
AutoExpose	Info
Binning: 2	Offset: 0 🕂 📢
Camera Area:	,
Center Quad.	Trigger Mode: Normal (Internal)
Use Active Region	Show Focus Indicator
Show Live	Frames To Avg: 1 🛨 Reset
Live Bin: 2	
Temp:n/a	
Setting [Modified]:	
Close Less << Setti	ng: Load Save Save As

- Make sure the gain is above 1; you can use a recommended starting point of 3
  - 1. Select a channel from **Wavelengths**
  - 2. Click Show Live to view the image.
  - 3. Click AutoExpose to calculate an exposure time or calculate your own exposure time; note

Display Saturation Markers

in the **Display tab** to see the full range of intensity.

- a. Red pixels = saturation; Blue green pixels= black
- b. Use the MAcquire you find Acquire

remember to check

Acquire	Image: Acquired
Save 'Live'	Save to: C:\MM\\Acquired001.tif
C Save w/Sequence	Display Acquire Correct Annotate
Exposure Time: 272.13 🔆 ms 💌 AutoExpose	Image Scaling:
Binning: 2 🕂	Autoscale
Camera Area: -> Full Chip	Scale within the active region
Center Quad.	Inage Gamma. ▲ ▶ 1.0
Use Active Region	
F2: Stop Live Live Bin: 2 ÷	
Setting [Modified]:	Jienlay Saturation Madeer
	V Display Saturation Markets
Close Less <<	Setting: Load Save

histogram to help the best exposure:

4. Click **F2:** Stop Live after calculating the exposure.

• Repeat the above steps for each channel.

5) Returning to the microscope

To return to the microscope to find another field, and set the camera dial and filter wheel for viewing. Focus and center another region of interest.

You also can perform the following operations after returning to the microscope: Using the oil immersion lens

If you need to switch to the 60x oil immersion lens, first use the course focus to drop the lenses.

Apply one drop of oil to the center of the lens.

Carefully raise the lenses and stop when you see oil contact between the lens and slide.

Focus & center your specimen. Please remember to use the fine focus control!

6) Multichannel Imaging

7) System Shutdown

Back up all your data.

If you used the 60x oil lens, please clean all the lenses.

Check the microscope calendar to see when the next user has an appointment.

If the user comes within 2 hours, log off your account.

Otherwise, follow the shutdown poster steps.