Olympus IX81 Inverted

Epifluorescence & Brightfield Automated Multi-D image acquisition

Introduction to the

NRI-MCDB Microscopy Facility

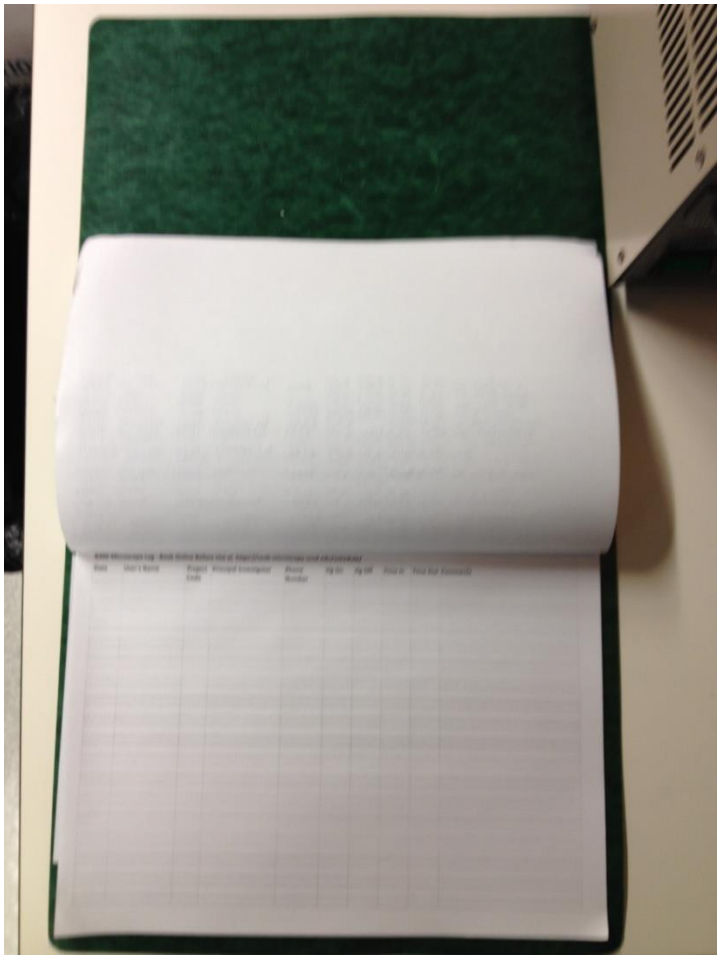
IX81 Inverted Microscope

Contents

- Start-up
- Preparing for Imaging
  - Part 1 – General
  - Part 2 – Transmitted – Brightfield
  - Part 3 – Fluorescence
  - Part 4 – Multi-D camera acquisition
- Shut-down

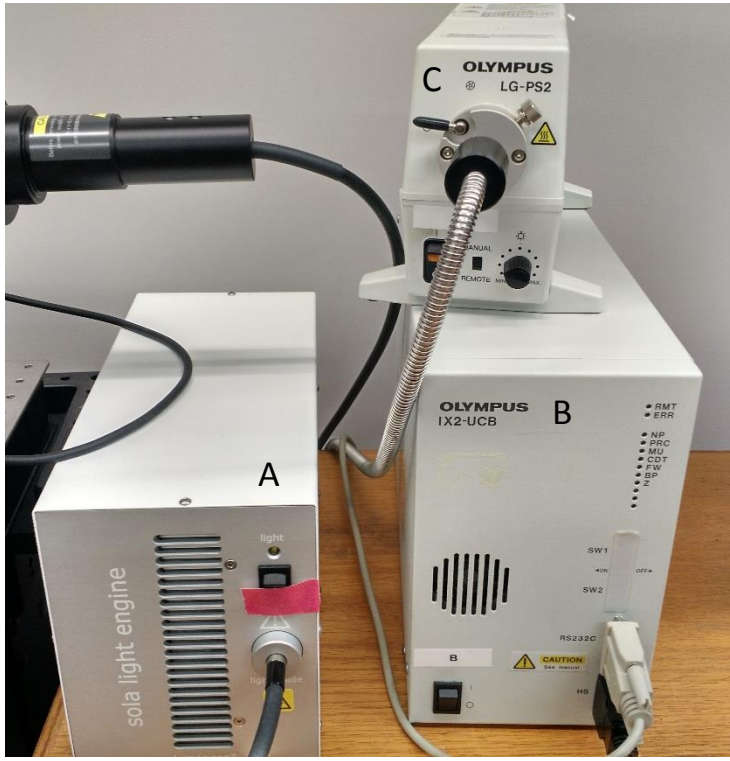
Step 1: Sign-in

- Record the following:
  - Date:
  - Your name:
  - Your Project Code (i.e. Index Code):
  - Your Principal Investigator (PI):
  - Extension (Optional):
  - Time-in (the time you arrived):
  - Time-off (the time you left):
  - Comments (any notes on system condition)



Step 2: Turn on the Microscope / Computer

- A: Sola Light Engine
  - Switch on back
  - Used for fluorescence
- B: Olympus IX2-UCB
  - Automated scope controls
- C: LG-PS2
  - Brightfield light source
- Turn-on the computer
- Log-in using your ADS account name and password.
- For access to the network drive, select Run and then type
- \\microscopy-nas1.nri.ucsb.edu
- Create a shortcut for future use.
- Open Micro-Manager 1.4
- Choose configuration file “OlympusIx81Ben.cfg” unless you’ve made your own.



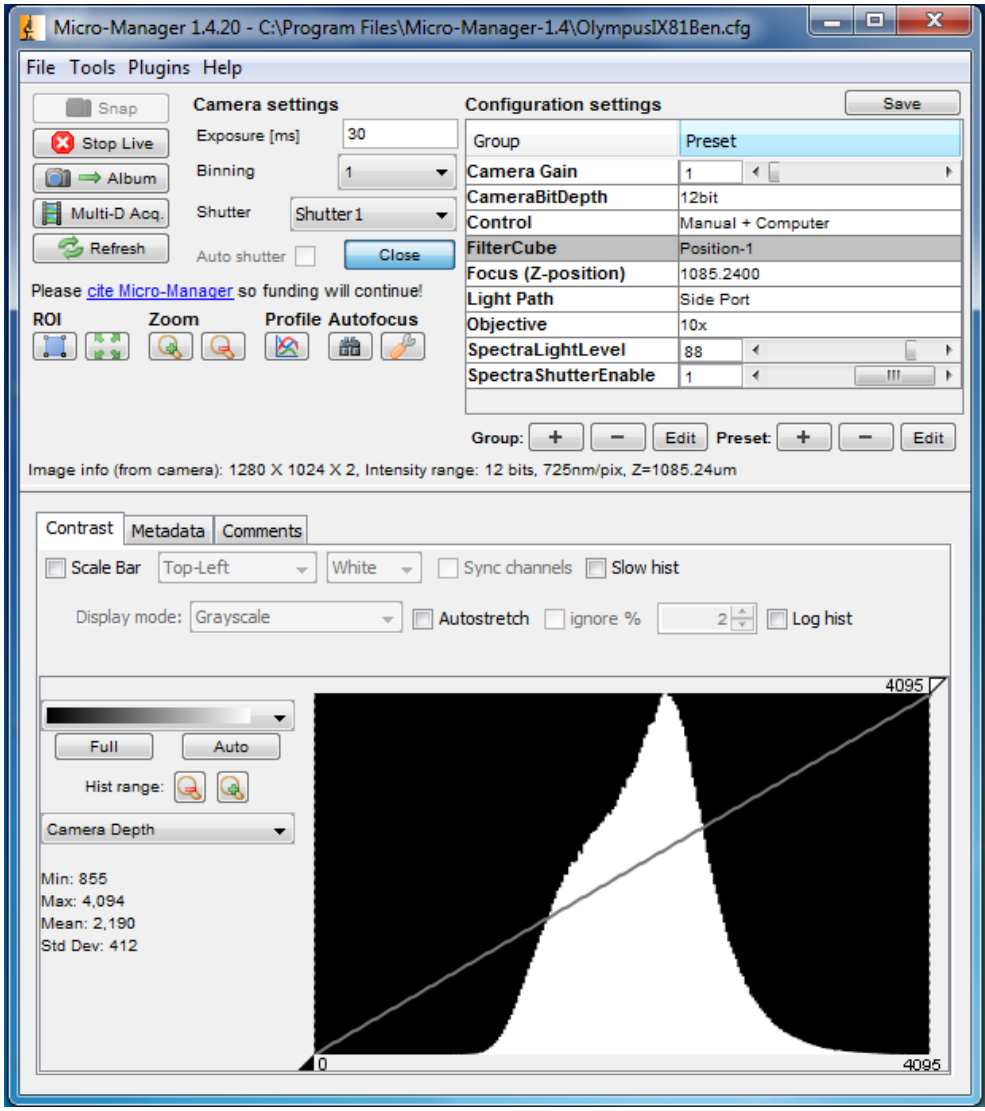
Preparing for Viewing and Imaging

- Part 1 – General Preparation
- Part 2 – Transmitted Light Applications
- Part 3 – Fluorescence Applications
- Part 4 – Multi-D Acquisitions

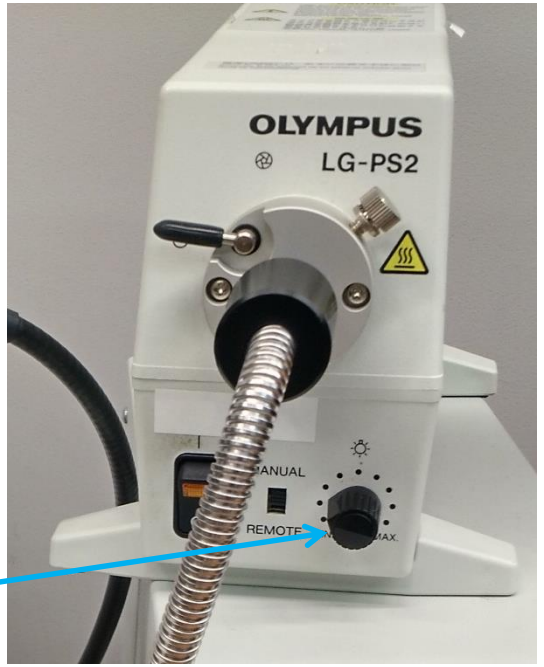
General Prep – Initial Settings

- The camera, filters, objectives, shutters, light path, and z-position can all be controlled through Micro-Manager.

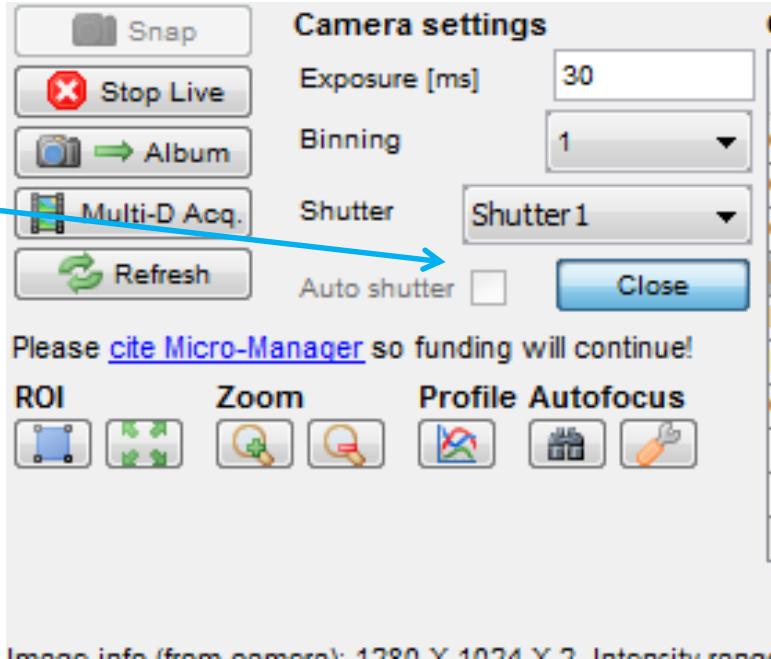
- Light source changes automatically with FilterCube/Shutter selection.
- There's a footswitch that controls the fluorescence shutter



Part 2: Preparing the microscope for transmitted imaging

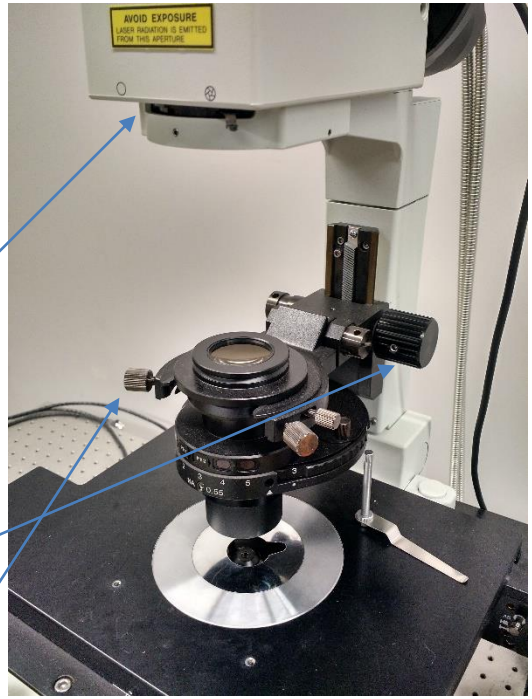


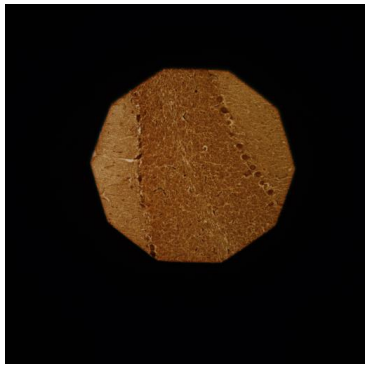
- Direct light to the transmitted path.
  - Set FilterCube to “Position-1”
  - Set LightPath to “side port” for camera or “eyepiece”
- Adjust the light intensity.
- If “Auto shutter” is enabled the shutter will open and close depending on the selection of “live” camera
- Disable “Auto shutter” to allow direct control of the shutter
- For single images, use “Snap” or “Album” for more complex operations use “Multi-D Acquisition”

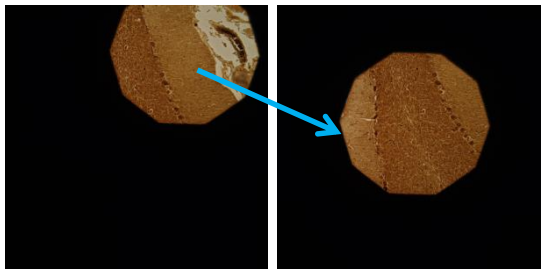


Part 2: Establish Kohler Illumination

- Place a slide on the stage
- Rotate the 10x objective into position.
- Use the microscope focus knob to bring the sample into crisp focus.
- Rotate the field diaphragm to the closed position.
- Use the condenser focus knob to adjust the condenser height so that the field diaphragm appears crisply focused when viewed through the microscope.
- Use the centering knobs located on the left and right sides of the condenser to center the view of the field diaphragm.
- open the field diaphragm just beyond the field of view
- Kohler illumination is objective specific

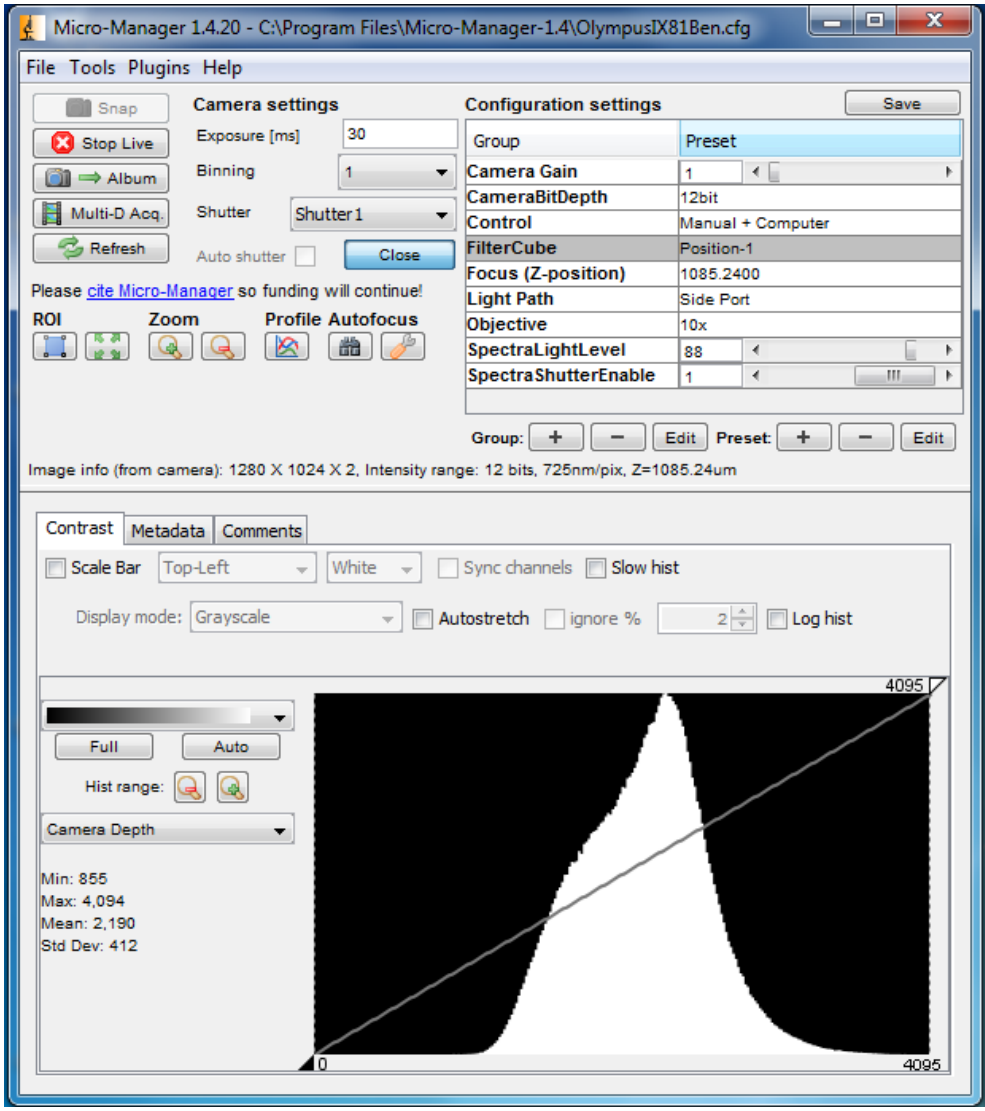






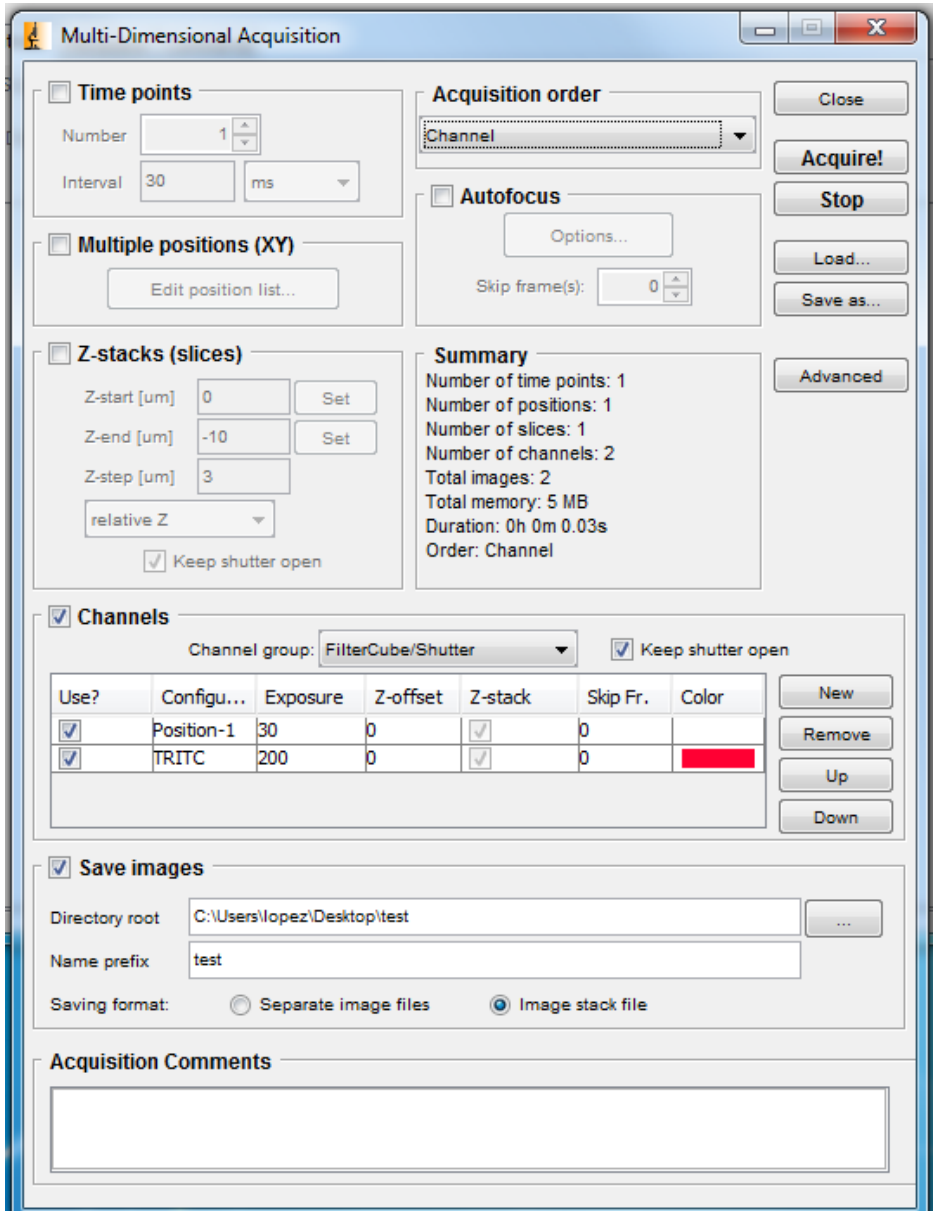
Part 3: fluorescence imaging

- Choose desired FilterCube (DAPI, CFP, FITC, TRITC)
- Shutter automatically opens and closes with live viewing
- Footswitch will also open and close Spectra shutter
- Adjust SpectraLightLevel to desired brightness to control photobleaching.
- Adjust exposure time to achieve appropriate brightness



Part 4: Multi-D image acquisition

- Time points: settings for a time series
- Z-stacks: if “relative Z” is chosen then positions are relative to current position
- Channels: lets you cycle between different scope configurations. “FilterCube/Shutter” lets you choose filter settings and light source
- “Acquisition order” is important



Shut-Down Procedure

- Check the online schedule
  - Shut-down if nobody is scheduled within the next hour
  - Leave the system on if somebody is using the system in the next hour but do the following.
    - Log-off the computer
    - Close the fluorescent shutter
    - Clean-up
    - Return to the 10x objective
    - Sign-off in the log.
- Adjust your online reservation end-time if you finished early
- Shut off the computer
- Turn off A, B, and C
- Complete the paper log by filling-in
  - Time you finished
  - Any comments
- Put dust cover over microscope

Specifications

- 4 objectives — Camera Calibration
  - 10x/0.4 UPlanSApo — 0.725 µm/pixel
  - 20x/0.7 UPlanApo — 0.361 µm/pixel
  - 40x/0.6 LUCPlanFLN — 0.178 µm/pixel
    - long working distance, coverslip thickness adjustment collar
  - 100x/1.3 oil UPlanFL — 0.0722 µm/pixel
    - oil immersion, NA adjustment collar
- 4 fluorescence filters
  - DAPI
  - CFP
  - FITC
  - TRITC
- Qimaging Scientific CMOS, 12-bit, 1280x1024, 30 fps full resolution
  - Model #01-ROL-BOLT-M-12