Zeiss Axiovert Inverted Microscope

Epifluorescence, Brightfield, Phase Contrast
Automated Multi-D image acquisition

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: Turn on the Microscope / Computer

- Switch on Lamp
- Switch on microscope
- 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 on desktop“ZeissAxiovert”

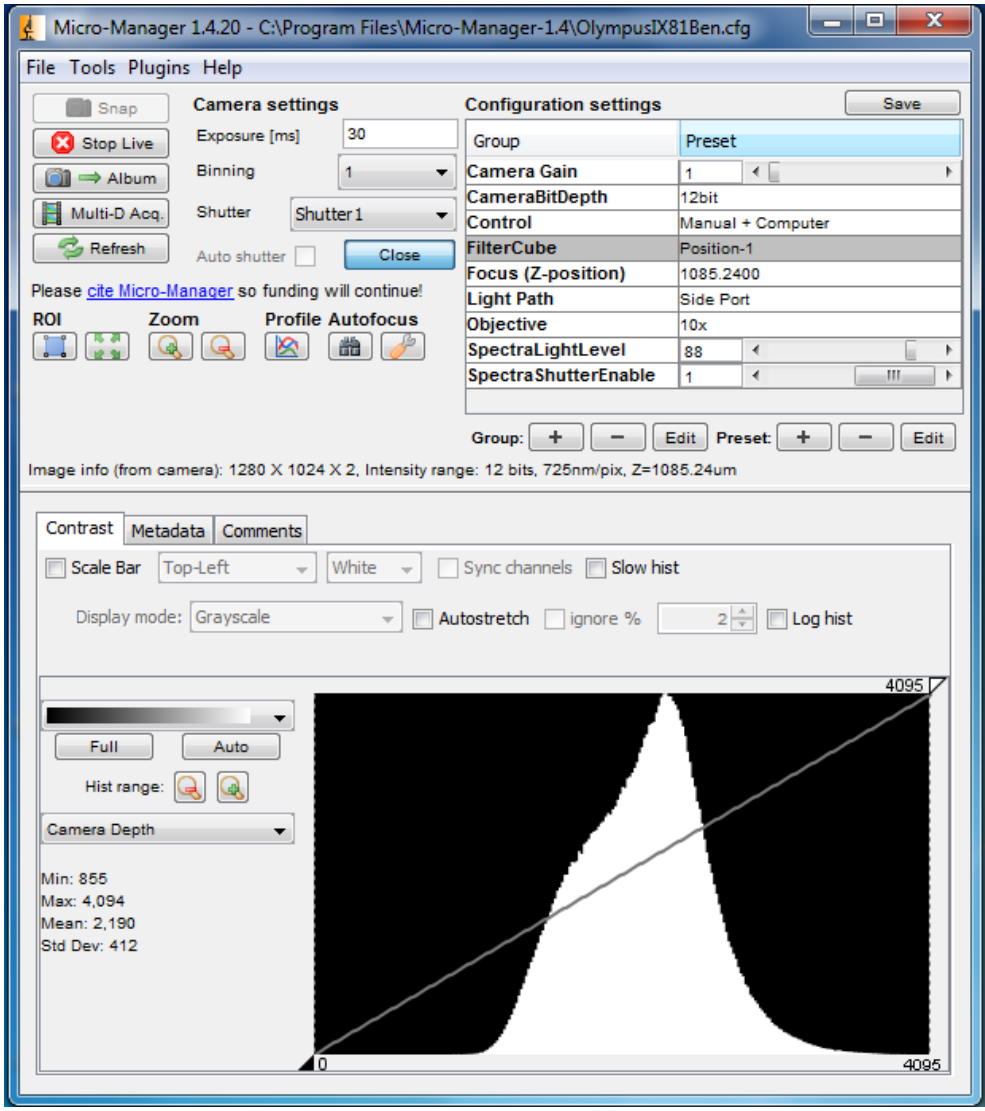
Preparing for Viewing and Imaging

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

General Prep – Initial Settings

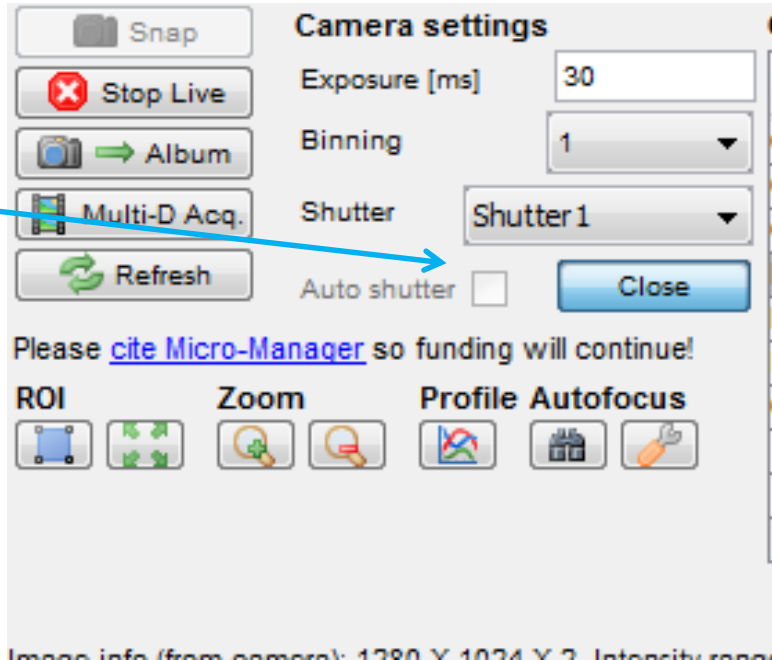
- The camera, filters, objectives, shutters, light path, and z-position can all be controlled through Micro-Manager.

- Light source changes automatically with FilterCube/Shutter selection.



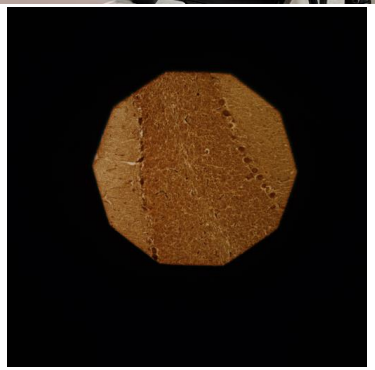
Part 2: Preparing the microscope for transmitted imaging

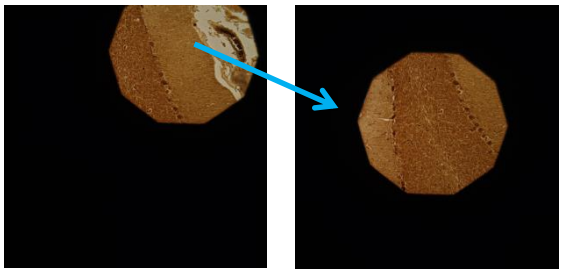
- Direct light to the transmitted path.
  - Set FilterCube to “Brightfield”
  - Set LightPath to “side port” for camera or “eyepiece”
- Adjust the light intensity.
- If “Auto shutter” is enabled the shutter will open and close depending on the selection of “live” camera
- Disable “Auto shutter” to allow 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

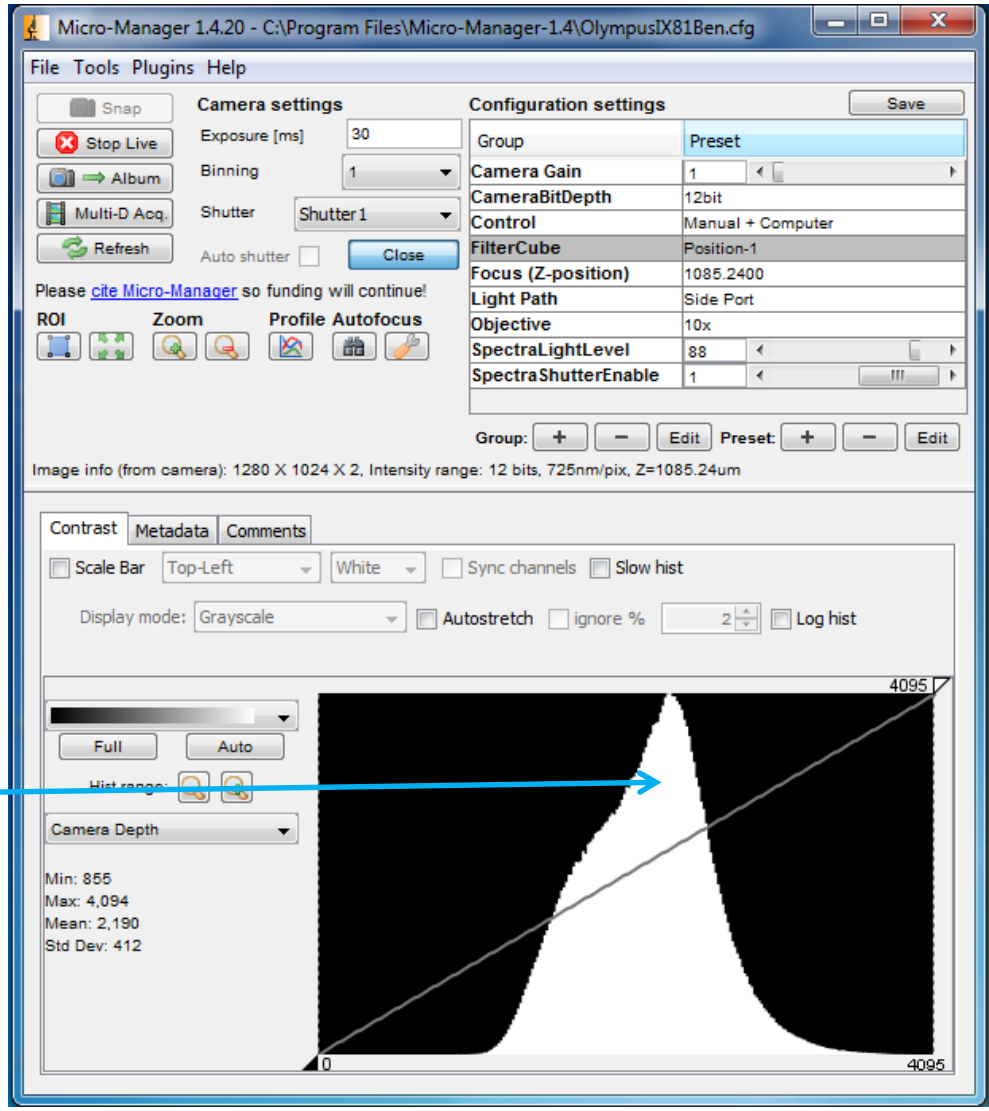
- Place a slide on the stage
- Rotate the 10x objective into position.
- Use the microscope focus knob to bring the sample into crisp focus.
- Rotate the field diaphragm to the closed position.
- Use the condenser focus knob to adjust the condenser height so that the field diaphragm appears crisply focused 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





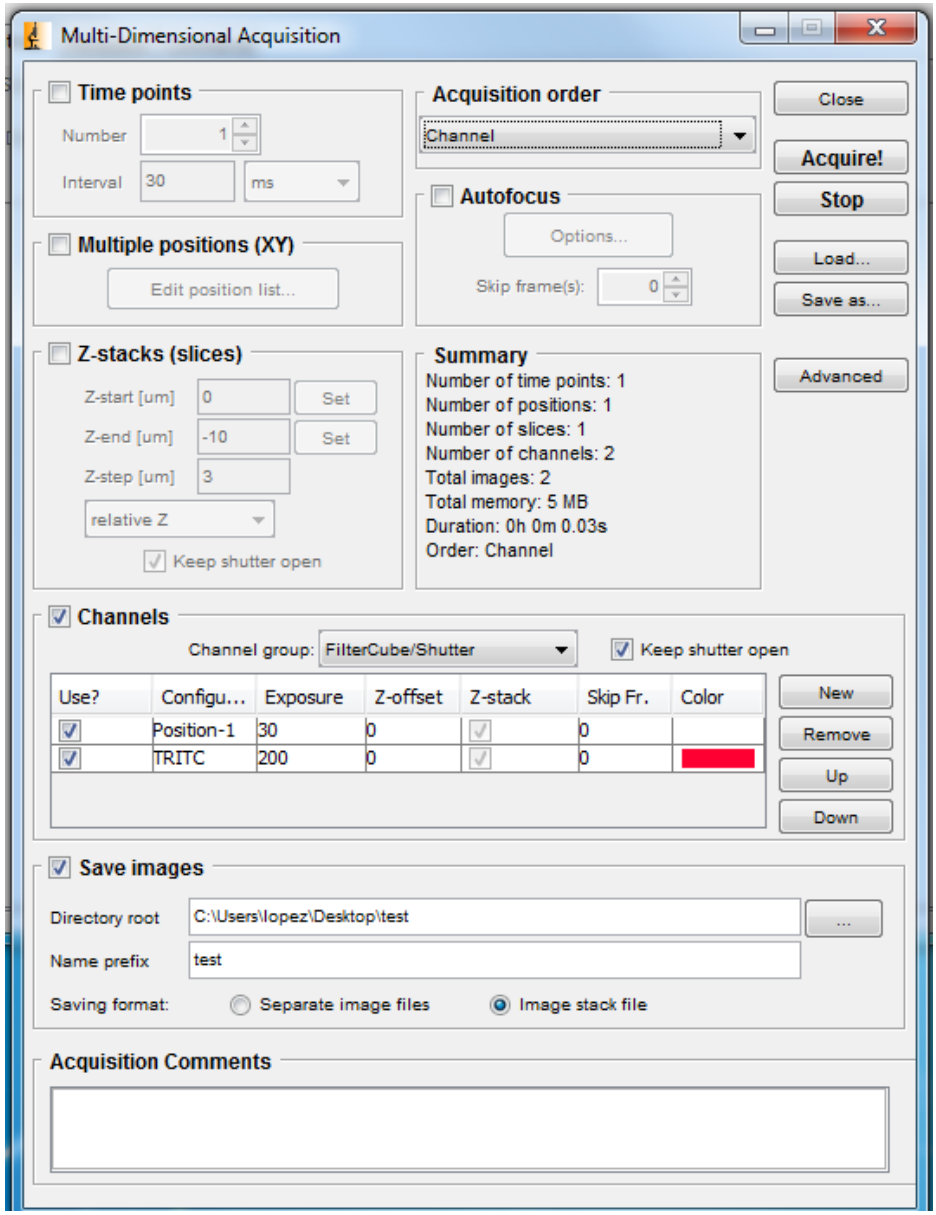
Part 3: fluorescence imaging

- Choose desired FilterCube (DAPI, GFP, RFP, Cy5)
- Shutter automatically opens and closes with live viewing
- Adjust exposure time to achieve appropriate brightness
- Pay attention to histogram. Very low brightness values will result in grainy images



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
- Adjust your online reservation end-time if you finished early or late
- Shut off the computer
- Turn off microscope and lamp

Specifications

- 4 objectives

	Camera Calibration
– 10x/0.5	0.625 µm/pixel
– 10x/0.25 Phase (not installed, swap with 10x)	
– 20x/0.8	0.313 µm/pixel
– 20x/0.4 Long Working Distance	0.313 µm/pixel
– 40x/1.3 Oil	0.157 µm/pixel
– 40x/0.8 Long Working Distance	0.157 µm/pixel
– 100x/1.4 oil	0.0633 µm/pixel

- 4 fluorescence filters
  - DAPI
  - GFP
  - RFP
  - Cy5
- Zeiss AxioCam