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 zposition can all be controlled through Micro-Manager.
- Light source changes automatically with FilterCube/Shutter selection.
- There's a footswitch that controls the fluorescence shutter

Stop Live Exposure [ms] 30 Image info (from camera): 1 Image Image info (from camera): 1280 × 1024 × 2, Intensity range	·	
Stop Live Exposure [ms] 30 Image info (from camera): 1280 × 1024 × 2, Intensity range Contrast Metadata Comments Image info (from camera): 1280 × 1024 × 2, Intensity range	Group Camera Gain CameraBitDepth Control FilterCube Focus (Z-position) Light Path Dbjective SpectraLightLevel SpectraShutterEnable Group: + - E e: 12 bits, 725nm/pix, Z=108	Preset 1 Preset 12bit Manual + Computer Position-1 1085.2400 Side Port 10x 88 Preset: Edit dit Preset: Edit t Italian
Stop Live Binning 1 Binning 1 Multi-D Acq. Shutter Shutter Shutter Shutter Image info (from camera): 1280 × 1024 × 2, Intensity range Contrast Metadata Contrast	Camera Gain CameraBitDepth Control FilterCube Focus (Z-position) Light Path Dbjective SpectraLightLevel SpectraShutterEnable Group: + - E e: 12 bits, 725nm/pix, Z=108	1 • 12bit • Manual + Computer • Position-1 1085.2400 Side Port • 10x • 88 • 1 • 1 • •
Multi-D Acq. Shutter Multi-D Acq. Shutter Auto shutter Close Please cite Micro-Manager so funding will continue! ROI Zoom Profile Autofocus Image info (from camera): 1280 X 1024 X 2, Intensity range Contrast Metadata Contrast Metadata Comments Scale Bar Top-Left White Siplay mode: Grayscale	CameraBitDepth Control FilterCube Focus (Z-position) Light Path Dbjective SpectraLightLevel SpectraShutterEnable Group: + - E e: 12 bits, 725nm/pix, Z=108	12bit Manual + Computer Position-1 1085.2400 Side Port 10x 88<
Multi-D Acq. Shutter Shutter 1 Refresh Auto shutter Close Please cite Micro-Manager so funding will continue! ROI Zoom Profile Autofocus Image info (from camera): 1280 X 1024 X 2, Intensity range Contrast Metadata Comments Scale Bar Top-Left White S Display mode: Grayscale Auto	Control FilterCube Focus (Z-position) Light Path Dbjective SpectraLightLevel SpectraShutterEnable Group: + - E a: 12 bits, 725nm/pix, Z=108 Sync channels Slow hist	Manual + Computer Position-1 1085.2400 Side Port 10x 88 4
Image info (from camera): 1280 X 1024 X 2, Intensity range Contrast Metadata Metadata Contrast Metadata Contrast Metadata Contrast Metadata Contrast Metadata Contrast Metadata Contrast Metadata Metadata <t< th=""><td>FilterCube Focus (Z-position) Light Path Dbjective SpectraLightLevel SpectraShutterEnable Group: + - E e: 12 bits, 725nm/pix, Z=108</td><td>Position-1 1085.2400 Side Port 10x 88 4 1 1 4 11 b 1 1 b 1 b 1 b 1 b 1 b 1 b 1</td></t<>	FilterCube Focus (Z-position) Light Path Dbjective SpectraLightLevel SpectraShutterEnable Group: + - E e: 12 bits, 725nm/pix, Z=108	Position-1 1085.2400 Side Port 10x 88 4 1 1 4 11 b 1 1 b 1 b 1 b 1 b 1 b 1 b 1
Please cite Micro-Manager so funding will continue! ROI Zoom Profile Autofocus Image info (from camera): 1280 × 1024 × 2, Intensity range Contrast Metadata Comments Scale Bar Top-Left Display mode: Grayscale Full Auto	Focus (Z-position) Light Path Dbjective SpectraLightLevel SpectraShutterEnable Group: + - E a: 12 bits, 725nm/pix, Z=108 Sync channels Slow hist	1085.2400 Side Port 10x 88
ROI Zoom Profile Autofocus Image info (from camera): Image info (from camera): 1280 X 1024 X 2, Intensity range Image info (from camera): 1280 X 1024 X 2, Intensity range Contrast Metadata Comments Scale Bar Top-Left White S Display mode: Grayscale Image Full Auto	Dbjective SpectraLightLevel SpectraShutterEnable Group: + - E E: 12 bits, 725nm/pix, Z=108 Slow hist	10x 88
Image info (from camera): 1280 X 1024 X 2, Intensity range Contrast Metadata Comments Scale Bar Top-Left White S Display mode: Grayscale Full Auto	SpectraLightLevel SpectraShutterEnable Group: + - E a: 12 bits, 725nm/pix, Z=108 Sync channels Slow hist	88
Image info (from camera): 1280 X 1024 X 2, Intensity range Contrast Metadata Comments Scale Bar Top-Left White S Display mode: Grayscale Full Auto	SpectraShutterEnable Group: + - E a: 12 bits, 725nm/pix, Z=108 Slow hist	idit Preset: + - Edit 35.24um
Image info (from camera): 1280 X 1024 X 2, Intensity range Contrast Metadata Comments Scale Bar Top-Left White S Display mode: Grayscale Full Auto	Group: + - E e: 12 bits, 725nm/pix, Z=108 iync channels Slow hist	idit Preset: + - Edit 35.24um
Image info (from camera): 1280 X 1024 X 2, Intensity range Contrast Metadata Comments Scale Bar Top-Left White Auto Full Auto	e: 12 bits, 725nm/pix, Z=108	35.24um
Scale Bar Top-Left v White s S Display mode: Grayscale v Auto		
Camera Depth Min: 855 Max: 4,094 Mean: 2,190 Std Dev: 412		4095

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

Kicro-Manager 1.4.20 - C:\Program Files\Micro	-Manager-1.4\OlympusIX	81Ben.cfg						
File Tools Plugins Help								
Snap Camera settings	Configuration settings	Save						
Stop Live Exposure [ms] 30	Group	Preset						
Binning 1 ▼	Camera Gain	1 4						
Multi-D Acq. Shutter Shutter1 -	CameraBitDepth	12bit						
	Control	Manual + Computer						
Refresh Auto shutter Close	Focus (Z-position)	Position-1 1085.2400						
Please cite Micro-Manager so funding will continue!	Light Path	Side Port						
ROI Zoom Profile Autofocus	Objective	10x						
🔲 🛄 🔍 🔍 🖄 🛗 🥖	SpectraLightLevel	88 4						
	SpectraShutterEnable	1 ◀						
	Group: + -	Edit Preset: + - Edit						
Image info (from camera): 1280 X 1024 X 2, Intensity ran	ige: 12 bits, 725nm/pix, Z=10	185.24um						
Contrast Metadata Comments								
Scale Bar Top-Left Vhite V	Sync channels 🔲 Slow his	-+						
White V		si i						
Display mode: Grayscale 🗸 🔲 Autostretch 📄 ignore % 2 😓 🕅 Log hist								
		4095 🗸						
		4053						
Full Auto								
Hist range: 🔍 🔬								
Camera Depth 🗸								
Camera Deptri								
Min: 855								
Max: 4,094								
Mean: 2,190								
Std Dev: 412								
		4095						

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

🛃 Multi-Dimensio	nal Acquisition					
Number Interval 30	1 <u>*</u> ms •	Cha	quisition o			Close Acquire! Stop
	Edit position list			Dptions e(s): 0) 	Load Save as
Z-end [um]	ces) 0 Set -10 Set 3 	Num Num Tota Dura	mmary — ber of time ber of posit ber of slice ber of chan l images: 2 l memory: 5 ation: 0h 0m er: Channel	ions: 1 s: 1 nels: 2 MB		Advanced
		rCube/Shutt Z-offset 0 0	z-stack	 Ket Skip Fr. 0 0 	ep shutter o Color	New Remove
	:\Users\lopez\Deskto	op\test				Down
Name prefix ta Saving format:	Separate im	age files	Imag	e stack file		

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
 - 10x/0.4 UPlanSApo
 - 20x/0.7 UPlanApo
 - 40x/0.6 LUCPlanFLN
 - long working distance, coverslip thickness adjustment collar
 - 100x/1.3 oil UPlanFL
 - 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

Camera Calibration

- $0.725 \,\mu m/pixel$
- 0.361 µm/pixel
- 0.178 µm/pixel
- 0.0722 μm/pixel

