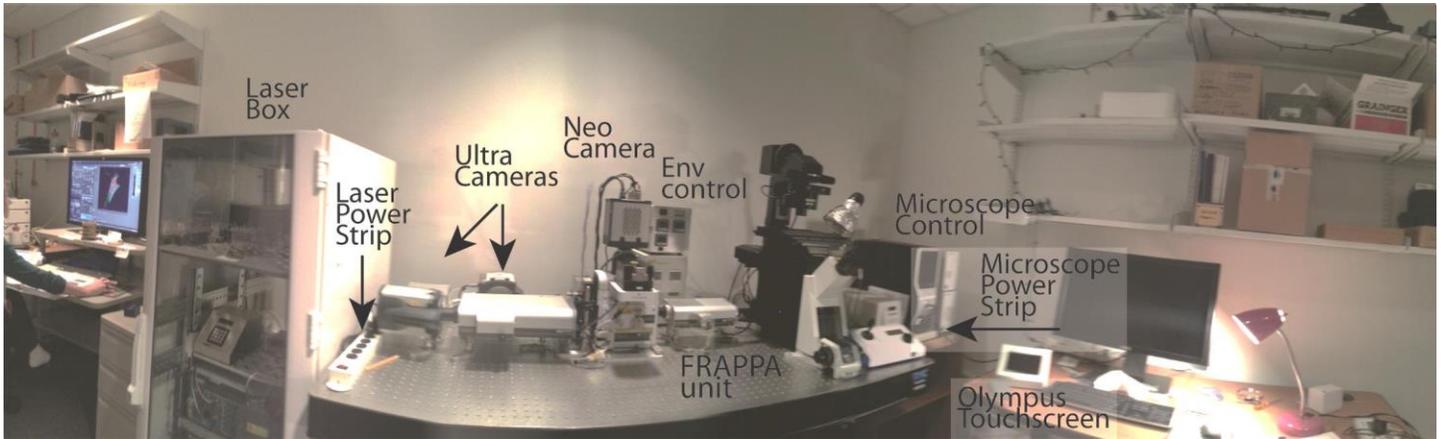

ANDOR/OLYMPUS SPINNING DISK USER GUIDE

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OVERVIEW



TURN ON SEQUENCE:

- Turn on laser power strip.
- Turn on microscope power strip.
- Turn on Olympus Touch Screen. (Do not use while Metamorph software is initializing.)
(Turn on X-cite illuminator if you require widefield fluorescence to focus.)
- Turn on computer if off.
- Log in using NetID and NetID password.
- Open Metamorph Premier.

TURN OFF SEQUENCE:

- Remove sample and clean off objective.
- Turn off heating and/or gas.
- Split any necessary files in SplitStack directory.
- Move files to shared drive.
- Close Metamorph Premier.
- Logoff Windows and shut down.
- Turn off Olympus Touch Screen.
- Turn off X-cite illuminator (if on).
- Turn off microscope power strip.
- Turn off laser power strip.

GETTING STARTED

- 1) Select the camera for single camera or dual camera mode (taskbar). Ensure that the TuCam slider is adjusted correctly (see Appendix 3).
- 2) Select the illumination setting in the main menu bar. If you are using e=eyepiece settings, use the shutter button to get light.



- 3) Select the objective magnification. **This will physically move the objective!**
- 4) Use the *Acquire* menu (taskbar) for collecting single images and adjusting your settings. The Show Live button starts continuous imaging. The camera settings are buried in the "Special" tab. For beginners, use only exposure time and gain.
- 5) Laser power is set under Configure Illumination (taskbar). **In this menu, do not change anything other than the laser power.**
- 6) If you want to see images from the TuCam merged, select Display->Splitview->Align.

MULTI DIMENSIONAL ACQUISITION (MDA)

Fast acquisition of Z series, Time series or 4D series are accomplished using the Multi Dimensional Acquisition menu (taskbar). The menu is relatively straightforward. The stream function enables the cameras to run uninterrupted.

1) The illumination configuration must be set under wavelength. **It does not use the illumination setting on the main toolbar.**

2) The camera settings are set under wavelength or stream. **It does not use the exposure and gain camera settings in the acquire menu.** (Ensure that the camera state is overlapped for fastest acquisition under configure camera mode.)

3) Laser powers are set in the Configure Illumination dialog box (taskbar). You must save the value or it will default back to the original value during acquisition.

4) For streaming in Z, the focus must be set to the ASI piezo unit (taskbar).

5) Test to ensure all settings are correct using the live button at the bottom left of the MDA menu. 

6) Press Acquire to start acquisition.

7) To cancel acquisition when cameras are streaming, use esc rather than the cancel button.

FRAP

1) Use Show Live in Acquire menu to optimize imaging settings.

2) Open *FRAPPA setup* (taskbar) and select the correct pulse and imaging illumination settings and the correct magnification only.

3) Acquire a single image.

4) Delete all previous ROI's

5) Draw an ROI using the drawing tools in the main menu bar.

6) Run the experiment by using the Show Live button in the acquire menu followed by the *Stream+FRAP in Live Replay* button (taskbar).

Parameters can be optimized by using the FRAP ROIs button in live mode. *Change Frappa settings* can be used to change the pulse length and repetition number. The laser power is set under *Configure Illumination*. The number of images before and after is set in the Live Replay tab of the Acquire menu.

FOCUS MOTORS AND LONG-TERM FOCUS STABILIZATION

The spinning disk system is equipped with two focus motors. The piezo unit integrated into the ASI microscope stage is used for acquiring fast Z-series. The Olympus motor is used for standard focusing and automated focus stabilization (ZDC=Zero Drift Compensation). You must select the correct motor when using these various functions. To set up the ZDC, see Appendix 2.



If the taskbar is missing, select Journal->Taskbars->Load Taskbar->Standard.JTB

APPENDIX 1: INSTRUMENT SPECIFICATIONS

Objectives on Olympus IX3 microscope							
Color Band	Mag	NA	WD	Imm	c. slip	Type	Field
Yellow	10x	0.4	3.1	dry	0.17	UPlanSApo	26.5
Green	20x	0.7	0.7	dry	0-.16	UCP PlanFL N	22
blue	40X	0.95	0.18	dry	.11-.23	UPlanSApo	26.5
Red+Green	30x	1.05	0.8	silicon oil	.13-.19	UPlanSApo	22
Red+Blue	60x	1.35	0.3	silicon oil	.15-.19	UPlanSApo	22
White	100x	1.3	0.2	oil	0.17	UPlanFL	26.5

Filters in Olympus IX3 microscope				
Name	Ex	Dichroic	EM	Semrock Code
DAPI	387/11 (381-392)	409	447/60 (417-477)	DAPI 1160B
GFP	482/18 (3473-491)	495	520/28 (506-534)	GFP 1828A
TX Red	562/40 (542-582)	593	624/40 (604-644)	TXRed 4040C

Filters in Tucam		
Dichroic	Red Emission	Green Emission
FF 580	617/73	525/40
	581-653	505-565

Laser Specs	
Wavelength	Power
405	100 mW
488	50 mW
561	50 mW
647	100 mW

APPENDIX 2: SETUP FOR ZERO DRIFT COMPENSATION (ZDC)

(1 beep = good. Three beeps = bad.)

SINGLE PLANE IMAGING (EASY)

- 1) SetZtoOlympus (taskbar).
- 2) Devices -> Focus -> Set Continuous Focus

Be patient. This step takes a while.

- 3) Start Continuous Focusing. Now you cannot move the focus. Set up the MDA however you want, but no Zseries!

IMAGING OVER TIME WITH MULTIPLE FOCAL PLANES (NOT AS EASY)

- 1) Open the MDA and set up the experiment, filling out saving, timelapse and wavelength information.
- 2) On the main menu, choose how often you want to autofocus.
- 3) If you are NOT doing multiple positions, SetZtoOlympus (taskbar). Find the focus that you want. Set Focus under the wavelength menu. GoToFocus (taskbar) to ensure you have the correct position. (If you are having problems, set AF offset to 0 and repeat.)
- 4) If you want to use the Olympus focus motor to do the Zseries (slower, but not limited to a 300um range), simply fill out the Zseries sub-menu.
- 5) If you want to use the ASI piezo focus (faster but limited to -150-150um), click Use Dual Z Motors in the main menu. Ensure the configuration is set right.

Z1:	Olympus Z
Z2:	ASI Piezo Z
Zseries:	ASI Piezo Z
AutoFocus:	Olympus Z

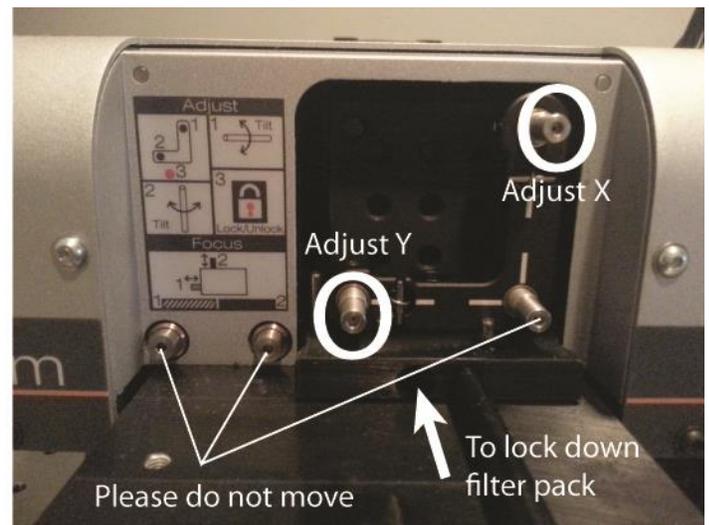
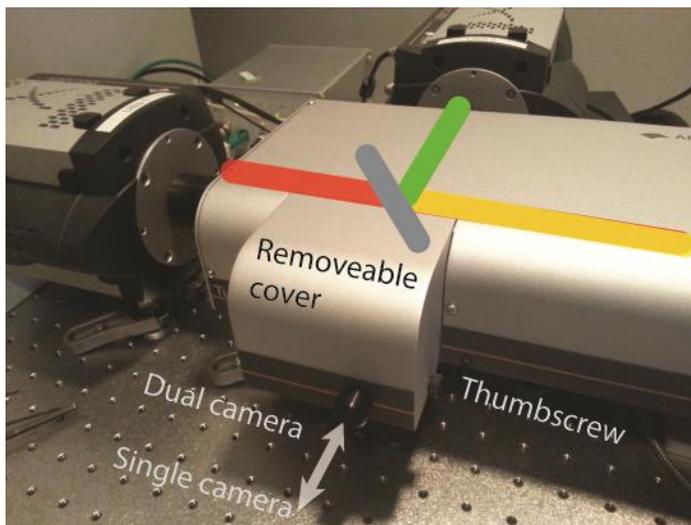
SetZtoASI (taskbar) and fill out the Zseries sub-menu. Choose correct Zseries focal positions using the wheel on the ASI stage controller.

- 6) For multiple positions, set multiple focus positions using the Olympus focus motor, using SetZtoOlympus (taskbar) and Set Focus in the stage sub-menu. Add positions using + and check positions using the Move To Position button.

APPENDIX 3: ALIGNING RED/GREEN FILTER PACK FOR TUCAM OPERATION

For single camera operation, where colors are selected by a filter wheel, the red/green filter pack is slid out of the way. Each time the filter pack is moved back into place, the second camera must be re-aligned manually to the first.

- 1) Loosen the cover thumb screw and remove the cover.
- 2) Slide the filter pack in and tighten the allen-keyed screw that locks it into place.
- 3) Put a slide on the microscope stage and adjust settings (c488-561) until signal is visible in both channels.
- 4) Align the filter pack for optimum co-localization using the indicated X- and Y- allen-keyed alignment knobs.



Red is collected in the through channel (Ultra 1, right image) and Green is collected in the reflected channel (Ultra 2, left image).