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Light Sheet Microscope (LaVision BioTec) Condensed User Guide

This light sheet microscope is designed around an Olympus MVX-10 zoom macroscope. All samples need to be mounted so they are optically accessible on three sides and ideally refractive-index-matched to whatever solution is being used in the large quartz cuvette.

Turn On Sequence

1. If the computer is off, turn it on and let it boot.
2. Turn on the laser switch.
3. Turn on the motor switch.
4. Turn on the camera (back right-hand side).
5. Log in to the computer using your net ID and password.
6. Start ImSpector Pro.
7. Choose the objective collar setting according to chart. Default is 3.5.
8. Remove the aluminum imaging chamber cover with the "stage" Allen wrench.
9. Clean imaging chamber if smudgy. Fill with appropriate index-matched liquid and set it gently into the microscope using the sliding pedestal underneath the chamber.
10. Replace the imaging chamber cover.
11. Attach sample to imaging basket and submerge in imaging liquid.
12. Choose corrected 2x objective adapter (A), excitation lasers (B) and imaging solution (C) in software.

Turn Off Sequence

1. Remove sample and rinse out the imaging chamber; set upside down to dry.
2. Clean up any spills around and in the microscope using ethanol-saturated wipes.
3. Cover the imaging chamber to minimize dust.
4. Exit ImSpector Pro. Turn off computer.
5. Turn off camera
6. Turn off the camera, motor switch and laser switch.

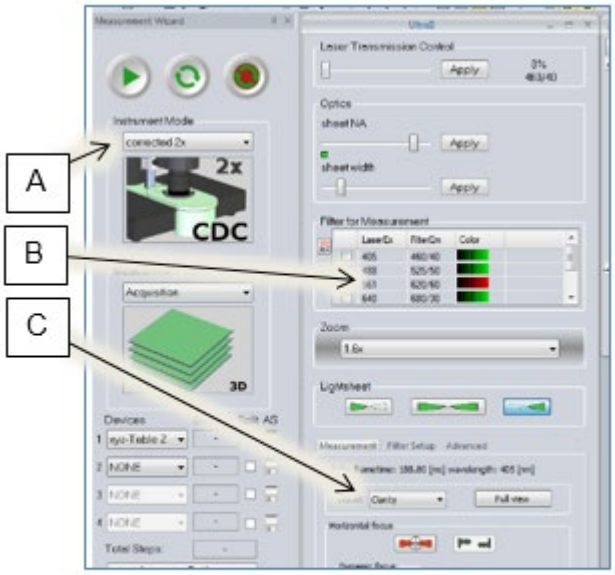


Image Acquisition Parameters: A: Objective adapter, B: lasers, and C: imaging solution

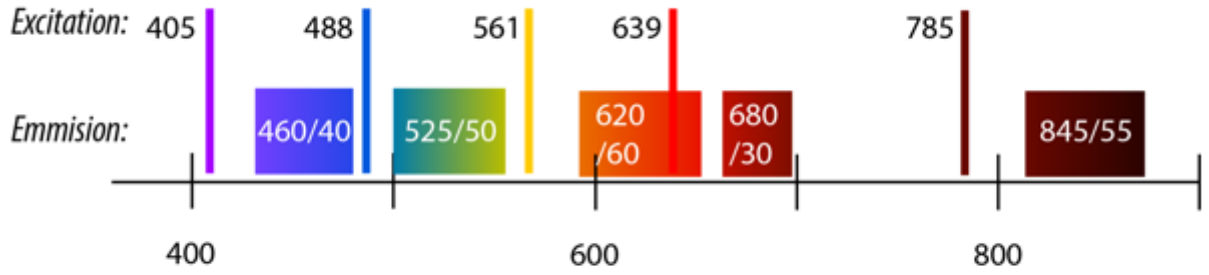
Objective Collar – Imaging Solution Settings

Imaging Solution	Refractive Index	Obj. Collar Setting
PBS (water)	1.33	0
60% TDE (CLARITY, DIX)	1.46	3.5
100% TDE (DBE)	1.52	5

Rough Imaging Sequence

1. Put stage in fast most by pressing blinking button at right bottom of the stage controller.
2. Orient the sample so this it is located in the center of the light sheet using the stage controller.
3. Choose a laser and scan. Put bowties from both left and right sheets in the center of the screen to correct software bug if they are not already there.
4. At the lowest possible zoom, focus objective to the sheet.
5. Choose appropriate zoom and re-focus. Set zoom in software. This ensures correct image scaling.
6. Choose a sheet NA for giving the thinnest sheet for the Field-of-view (FOV) required. See Table below for suggested sheet NA – FOV settings.
7. Choose a sheet width that ensures uniform illumination across the image vertically.
8. Adjust horizontal focus.
9. Adjust laser intensity such that levels are ~50,000 at the brightest part of the sample. “Apply” all settings.
10. If you are using multiple colors, focus to the sheet using the shortest wavelength. For all other colors, use the chromatic aberration adjustment to focus.

Excitation Laser Lines and Emission Filter Bandwidths



Available excitation laser lines covering the visible and near infrared regions of the spectrum and corresponding bandpass emission filters

Sheet NA – FOV Settings

Microscope Zoom	FOV Corrected 2x (mm)	Sheet NA for FWHM=FOV	Sheet NA for FWHM=0.5*FOV	Sheet NA for FWHM=0.25*FOV
0.63	10.36	0.03	0.042	0.261
0.8	8.16	0.034	0.047	0.283
1.0	6.52	0.037	0.053	0.283
1.25	5.22	0.042	0.059	0.283
1.6	4.08	0.047	0.068	0.283
2.0	3.26	0.053	0.078	0.283
2.5	2.61	0.059	0.089	0.283
3.2	2.04	0.068	0.107	0.283
4.0	1.63	0.078	0.132	0.283
5.0	1.31	0.089	0.183	0.283
6.3	1.04	0.108	0.261	0.283

New User Setup

1. Start ImSpector.
2. Navigate to edit -> set *Config* dir.
3. Ensure that path is set to: "C:\Imspectorconfig\config" and hit "ok".
4. Restart ImSpector.