

LifeCanvas Technologies SmartSPIM User Guide

Turn-on sequence

- 1) Turn on main switch
- 2) Login to the acquisition computer.
- 3) (Ensure the Destripe and Stitch GUIs are open on the stitch computer)
- 4) Open SmartSPIM software

Imaging overview

1) Make sure you have the correct imaging solution in place (LCT Oil vs eCi).**Do not skip this step!!!**

- 2) Insert sample(s).
- 3) Remove gloves (if you are wearing them).
- 4) Insert objective lens (and select **Insert Objective** button).
- 5) Select **Illumination Focus** button and follow the indicated sequence. You will need to do this every time you insert a new objective. For both right and left lightsheets you will need to adjust both wheels on the hand unit to maximize kurtosis, followed by the y-adjustment knob to center the lightsheet on the camera.
- 6) Preview your image. You probably will need to adjust "Sample Z" such that your sample is in the lightsheet by eye (left wheel on hand unit). Once you have an image, focus it (right wheel on the hand unit).
- 7) You will need to set up an **Acquisition Sequence** (blue box) for each sample you want to image.
 - a) Check out your sample. Adjust laser powers and display parameters to best visualize your data at the different wavelengths. **Laser powers must remain the same between comparison samples**, but display parameters can change.
 - b) Find the thickest part of your sample, adjust the **Z stack parameters and the wavelength selection** for your data collection.
 - c) **Set Up Tiling**. The yellow and blue dotted lines determine the extent of your tiling. They can be dragged around or the white buttons below set them to cut to the current field-of-view. The white dashed line indicates which tiles will be illuminated by the left (green) vs the right (red) laser. If you have a small sample, use only the right laser by dragging the white line all the way to the left.
 - d) Do a **Left/Right alignment** if you are using both sheets. The arrow will bring you to the center of your sample. Grab a reference (right sheet), and then use the focus knob to adjust the left sheet height to illuminate the same objects.
 - e) **Set Up Focus** for at least 3 Z-planes throughout your stack for each wavelength you are using (adjusts for slight changes of focus with high index optical solutions).
- 7) Once you have green check marks after each item within each acquisition sequence, you are ready to **Start Sequence!**

Turn-off sequence

- 1) Push **Remove Objective** button in order to retract it to the upper position.
- 2) Physically remove the objective, clean it and cover it.
- 3) Remove sample and clean sample arm.
- 4) Clean up all the tools and any drips within the instrument with EtOH saturated wipes.
- 5) Exit the SmartSPIM software
- 6) Log out of BRC Login.
- 7) Turn off the main switch

Display parameters



Digital zoom (or un-zoom with shift).



Move magnified still image.



Fit in box.

Objectives

Mag	NA	Immersion media	Pixel Size (um)	Axial resolution (um A488/A790)	Working distance (mm)	Field-of-View (mm)
0.8x	0.06	Air	8.0	12.7/20.4	25.0	16.0 x 12.8
1.625x	0.1	Dipping	4.0	7.7/12.3	17.5	8.0 x 6.4
3.6x	0.2	Dipping	1.8	4.0/6.4	17.5	3.6 x 2.9
9x	0.3	Dipping	0.72	2.8/4.5	18.1	1.4 x 1.2

Lightsheet specifications

NA=0.125

Emission filters

Fluorophores	Excitation line (nm)	Emission band (nm)
FITC (Fluorescein, GFP, Alexa-488, Cy2, Green dyes)	488	500-550 (ET525/50m)
TRITC (Rhodamine, Cy3, Alexa-568, mCherry, tdTomato, PE)	561	575-625 (ET600/50m)
Cy5 (Alexa-647, APC, mRFP)	640	665-715 (ET690/50)
Alexa-790, ICG,	785	792 long pass (RET792LP)

Alexas and DyLights are named for their excitation wavelengths.

Camera

Hamamatsu ORCA Fusion sCMOS (ROI = 2000 x 1600 pixels)

Data progression:

- Original data in *D:\SmartSPIM_Data\date\datetime_filename_user*
- Destripe destripes and transfers to *S:\SmartSPIM_Data\date\datetime_filename_user* and when done appends *Destripe_DONE* to the folder and appends *RAW_Transferred* to the original folder.
- If you choose combine folders, the single channel standard stitched files will all be moved to the *All Channels* folder.
- If you choose to make an Imaris file, there will be a single *ims* file in the *Destripe_DONE* folder.