BASIC Z-STACK AND TIME SERIES SCAN ON THE ZEISS LIGHTSHEET Z. 1

The front door of the main body of the instrument may be open when you arrive. Take the sample chamber and slide it into position with the hole in the back to receive the objective if using the 20x dipping lens. Push the holder all the way to the back of the chamber and then hand tighten into position with the set screw on the front. **NOTE:** There is also a 5x air objective that can be used. Please see Imaging Center Manager if you need this option.

Attach the two cables with the silver ends to the corresponding position on the right side of the



opening with the red dots facing up. These simply push straight in. Then attach the two hoses containing blue fluid to the middle two positions by pushing them into place. The order of these two hoses is not important. The clear tubing coming from the syringe with the preferred liquid for your sample attaches to the bottom right port of the chamber by pushing it on and rotating ¼ turn to the right.



Turn on the system by toggling the three switches on the white control panel to the left of the monitor labeled system, PC, and incubation from the top down. <u>NOTE:</u> Please allow 3-5 seconds between turning on each switch or the system will not initiate properly. After the system has initiated and the computer has booted click on the ZEN software icon on



the left side of the monitor. NOTE: If you need to adjust the temperature of your media in the sample chamber before starting now is the best time to add your media to it. Even if you only require room temperature for your session, you should use the

temperature controller because the laser will heat up the solution in the chamber. Fill the chamber by depressing the syringe and observing the liquid level through the front window of the chamber. The chamber will hold 20 ml. Start the heating or cooling cycle by expanding the



Incubation window at the bottom left of the screen. Check the box labeled Peltier Unit to turn on the mechanism and then type in the desired value or use the arrows to set your temperature. You may do the same to adjust for CO2 percentage if needed.

Move to	Load Position	
	Set Home Position	Home Position

Make sure that the sample holder is in the LOAD position by clicking the icon at the bottom left of the software.



Load the sample holder into position making sure that it sits flat and the white lines on the holder and the ring surrounding it are aligned.



Then click **LOCATE** and **LOCATE CAPILLARY** at the top left. This turns on the webcam at the front of the chamber and allows you to see a front view of the chamber. This is the best time to fill the chamber if

you have not already, because you can see the liquid level increase on screen. Fill the chamber all the way to the top but do not overfill. **NOTE:** The front door of the system must be closed in order for the webcam to work and other parts of the software to function.

There is a holder attached to the right side of the microscope box that is designed to hold your



syringe in place. Make sure to feed the tubing underneath the bottom of the door so that it isn't crimped when the door is closed.

The sample chamber and Lightsheet Z. 1 system are designed for aqueous media with a refractive index of 1.33 such as water, PBS, cell culture media, etc. Media based on organic solvents can evaporate within the system and cause damage to the optical surfaces as well as the motors of the system. Please **do not** use anything other than aqueous media in the system without consulting either Dr. Doug Harrison or Jim Begley.



Move the capillary into position by sliding the blue outlined Z plane forward and the green outlined Y plane down in the specimen navigator window until the bottom of the capillary lines up with the horizontal line on the image of the objective. Open the specimen door at the top of the system and gently push down on the plunger inside the capillary. Check for air bubbles at the bottom of the capillary and remove them by tapping the

specimen holder if necessary. While looking at the screen push a small amount of agarose from the end of the capillary using the plunger until your sample is centered in the "crosshairs" that appear at the center of the objective.





Turn off the Locate Capillary function and then click on LOCATE SAMPLE. This will turn off the

webcam and now you will see your sample with transmitted light through the objective. You can center your sample with the joystick and if needed rotate the sample by clicking the

top button on either side of the joystick and then using the topmost control knob to rotate. Click the bottom button with the arrows designation to return to normal left-right and up-down joystick functions.



The wheel at the back of the joystick will move through your sample in the Z plane.

When you are satisfied with the position of your sample in the X, Y & Z planes you can turn off the Locate Sample function.

Page | 5

Next click on the ACQUISTION tab to open up the Experiment Manager window. Click on the



file folder icon and select one of the saved Acquisition Configurations. Please **do not** save any

changes you make to these configurations as they are templates needed by all users. You may set up a new Acquisition Configuration if needed.





Click CONTINUOUS to

view your image and confirm that it is in the right position. You may

now turn off the image by clicking the **STOP** button, which replaces the continuous button during an active scan. In the Channels window, uncheck the TL channel and all but the lowest

🔺 🛆 Channels		🗸 Show All 🛃
Tracks	Channels	
488	Cam1	•
	Cam2	

wavelength laser. The lowest wavelength laser should be used to adjust the lightsheets if you are using

more than one laser line.

488 - Light Sheet							
Lasers 405 445 488 514 561 638							
🔺 488 nm —	5.0 ‡						
Light Sheet Auto-Adjust Left	0.92						
Right	-1.01						

You may adjust the lightsheets by clicking the **AUTO-ADJUST** button and following the onscreen prompts. Alternatively, you may adjust them manually by pushing the

🔽 🛥 Acquisition Mode		✓ Show All	
Bit Depth Zoom	16 Bit 🔻	Advanced	
Light Sheet	Single Side		•
Direction	🖲 Left 🔵 Right		
Thickness	5.34 μm		
	Online Dual Side Fusion		

page up and page down keys on the keyboard during a continuous scan. Change directions of

the lightsheets by clicking left or right in the Acquisition window. Make sure the corresponding left or right light sheet is active in the adjustment window as well. You should try to get the image bright and focused well with minimal difference when switching between left and right. **NOTE:**

Some samples may do better with single side illumination from either the left or right if there is a dense area that the laser has to travel through.

and using Experiment Manager the wheel H on the m 488 405 TL Frame Zeiss joystick turn 90 6 through Z until you Set Exposure Continuous Snap New reach the desired ✓ Z-Stack 508 Slices start point for your ✓ Time Series 144 Images scan. Click Multiview Set First in the Z-Stack Start Experiment window. Turn the

Under the Experiment Manager section, check the box for **Z-STACK**. Start a continuous scan

wheel in the opposite direction until you reach the desired endpoint for your scan and click **Set Last** on the screen. It doesn't matter which order you set the first and last scans in as your scan will be the same either way.



You will see a range, number of slices, and interval between slices if you select optimal. You

In order to do a time series scan make sure that the **TIME SERIES** box is checked in the Experiment Manager window. You should then see a time series box opened beneath the z-



specific values. You must determine how many cycles you need based on the interval chosen to equal the total amount of time desired. In the example seen here with an interval of 5 min there would be 12 cycles in an hour and 144 cycles would be a 12-hour total scan time.

Once you have adjusted all of the parameters for your scan click on **START EXPERIMENT** in the Experiment Manager window to start your scan. You should name the experiment and choose where to save the images when prompted on screen. Choose the SWAP (X:) drive which is networked to the system to save your experiment as it has enough memory to handle the large files and keeps the Lightsheet computer free to operate the software. Each lab should create their own folder in which to save their data as well as individual user folders if desired.

Note: All data files should be removed from the SWAP (X:) drive and placed onto your own storage device within 14 days of the experiment or you will risk having your data deleted.

Please perform the following functions after your scan is finished:

- 1. Using the attached syringe remove the liquid that is filling the chamber.
- 2. Remove your sample and push out any remaining agarose inside the capillary. Rinse the capillary with water and place it in the tray with the other used capillary tubes.
- 3. Disassemble the sample holder and put the individual pieces back into the case.
- 4. Replace the syringe and tubing with the one labelled MilliQ H₂O and rinse the chamber several times removing as much water as possible on the last rinse.
- 5. Disconnect all of the hoses and connectors, unscrew the set screw holding the sample chamber in place, and remove the chamber from the machine. Place the chamber on a paper towel and leave it to dry.
- 6. Clean up any spills inside the machine in the tray that sits underneath the sample chamber.
- 7. Using a piece of **lens paper** "blot" the residual water from the surface of the objective lens in the back of the instrument if you used a dipping lens.
- 8. Close the software, shut down the computer, and turn off the three switches in the reverse order from how you turned them on.

Contact Information: E-mail: jgbegl2@uky.edu Phone: (859) 323-9647