Transcript: Navigating around your sample on the 3i Lattice LightSheet

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Note, closed captions are available on the video
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This tutorial will guide you through the SlideBook window as at version .22, and how to navigate around your sample.

Please get the running checklist from the training materials section of the website for reference.

First we will use this checklist to ensure that the window is set up correctly for imaging.

Step One, ensure that the correct mask is set, this is usually 0.55 by 0.493.

Step Two, if using the pre-set modes select this checkbox. Select the correct lasers, exposure time and ROI.

Tick on guides if you'd like to use.

Step three, ensure that the dither is on. The box next to 'x galvo range (um)' should read a positive number usually two or five.

steps four to five, if you are working outside of the preset then this is where you select exposure time and the lasers. Make sure you are using the appropriate MB for miltibeam.

Step 6, select an appropriate laser power. For live cells 488 is usually no more than 2%.

Step 7. Z-offset is set by the superuser during alignment and is usually between minus 5 and 5.

Step 8-9, the 3D tick box controls whether a stack is being collected. Number of slices and step size should be appropriate for your imaging. They are usually 101 and 0.57 for single cells.
MIP XYZ and deskew should be ticked. Mode should be 'sample scan'.

Step 10, this is where you will move the stage to find cells of interest. Tick jog only when you have found your cells and are searching for rare events.
Z stage controls which plane of the sample is in the focal plane of the sheet.

Step 11, wait for piezo ('wait for PZ') should be ticked if running very fast imaging with no wait time. It stops doubling and shaking artefacts at the edge as the piezo returns for the next stack.

Step 12, this is where you select the ROI if you are outside of the presets.

Step 13, tick timelapse, input the number of timepoints and select an appropriate time interval. 0 corresponds to running as quick as possible.

Step 14 finally programme SLM to ensure all the settings are synced.

If the live button is green you can click to open the live preview. If it is red, you need to program the SLM first. If you are prompted to programme the SLM now or at any stage in the imaging, click no, press stop and then manually trigger program SLM, this works more reliably than clicking yes.

With the checks complete we can find the sample.
Click live
Move up in Z using the up arrow above 10. This can be clicked quickly until the number next to the arrows reaches about 14.7 or when you first see signal. Use the arrows above 1 to fine tune the signal into the middle. This should look like a thin slice through your sample. This is caused by the geometry and will it never look like a full XY overview you might expect on a normal microscope. I will call this signal the sheet from now on and it takes practice to get used to finding a particular feature in this limited field of view.

You can then move in X and Y to find cells you want to image. It is important to remember that all the time you are in live view you are exposing your sample to laser.

The right arrow moves the sample to the right so that something on the left side of the view comes into the middle. The down arrow will move the sample down bringing something from above the middle towards it. The left arrow will move the sample left and the up arrow will move the sample up. You must think about moving your sample through a static frame of reference, rather than moving the frame.

You can change how far each click moves you by changing the step size, do not go above 15.

Click on jog to reduce the number of times you have to click. Clicking an arrow once will set the piezo in motion in that direction and clicking again will stop the jog.

You can see that the sheet is no longer in the middle of the crosshairs. Because of the geometry, movement in x will need to be corrected by a movement in z to keep the sheet in the middle of the view and therefore the same plane of your sample.
Moving down in Z takes your sheet to the left and moving up in z takes it to the right.

When you have found a cell you are interested in, ticking 3D and hitting live will take a preview stack. The MIPs help you see if the sample is inside the volume and the XY projection, which is more similar to conventional microscopy, is as you expect.

If the sheet is in the centre of the crosshairs, the sample should be in the middle of the volume. Turn off 3D before returning to live view.

To view something which appears to the right of the sheet, do not move the sheet to the right by clicking up in Z as this will put your sample too hight in the volume and you may lose data. Instead, with the sheet in the middle, move your sample to the left to meet it. It is likely that you will see a different plane of the cell now, make the correction in z by moving with the down arrow above the 1.

The same is true if you want to see something that appears to the left of the middle. Move your sheet to the middle using the z control arrows and then move your sample to the right.

Remember pressing live does not record any data. So when you are ready to record, check your settings, including for timelapse, tick 3D, name it and press execute. The data is not automatically saved so you must remember to save the slide periodically.

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