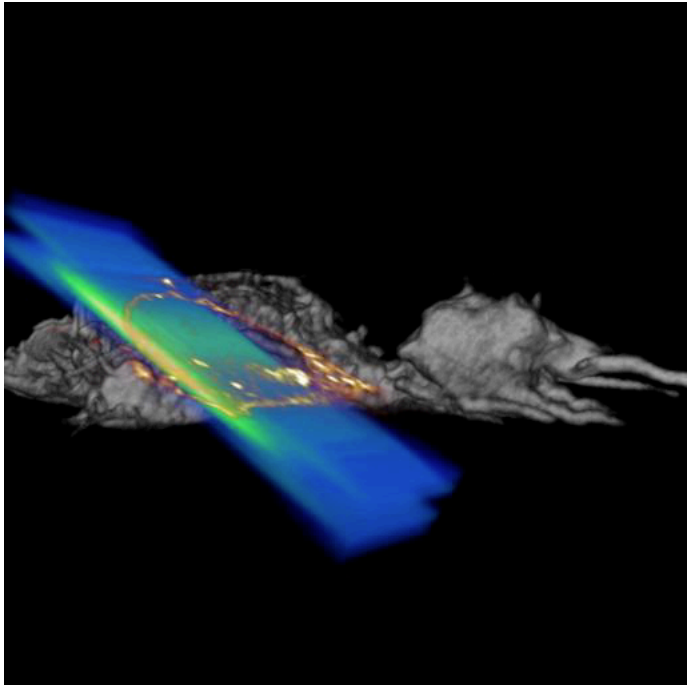
WARWICK
THE UNIVERSITY OF WARWICK

Geometry and Navigation in LLSM

How to move around your sample in Slidebook

24th January 2020 / CAMDU @ WMS / Helena Coker

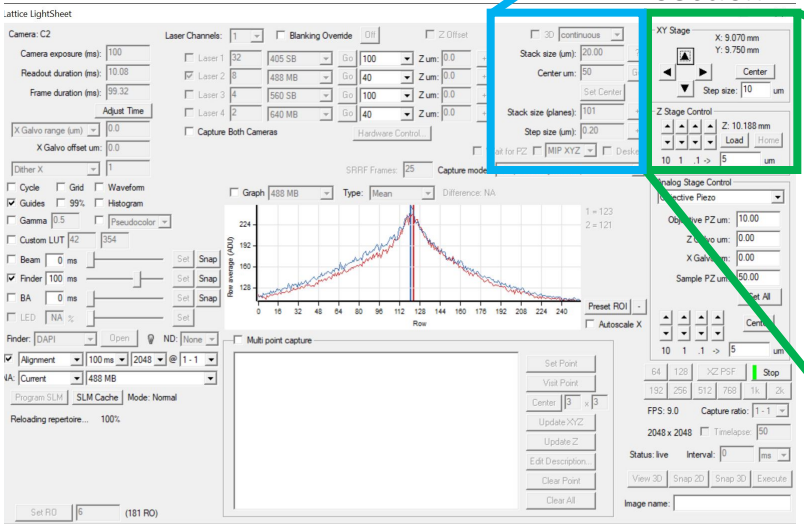
How the lattice collects images



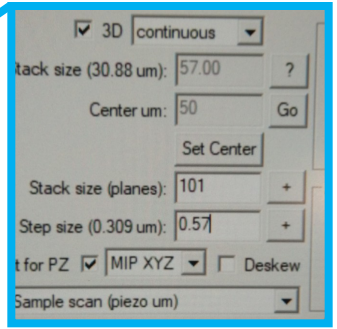
- When you are in live view you only look at one slice though the cell at a 32.8° angle to the coverslip.
- To record a volume, images are collected via a sample scan:
- The sample stage is moved by the set step size, through the fixed sheet .
- This is illustrated in the video. The sheet (green and blue stripes) are dithered to create a uniform illumination (@2.7s into the video). The sample is scanned through the sheet (example @ 9s).

Lattice Light Sheet Microscopy: Imaging Molecules to Embryos at High Spatiotemporal Resolution, Chen *et al*. Movie S2

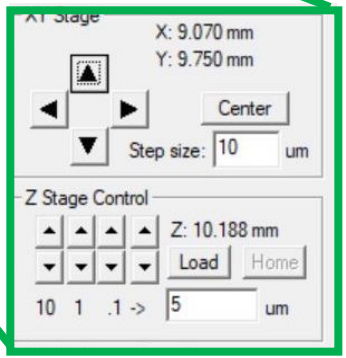
The SlideBook interface



Section 1

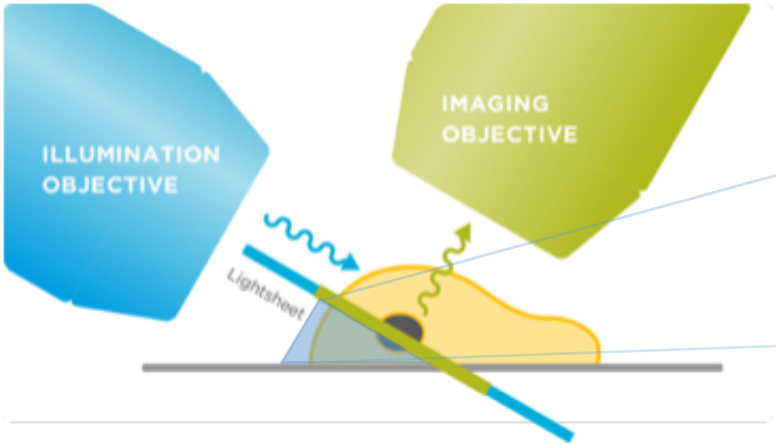


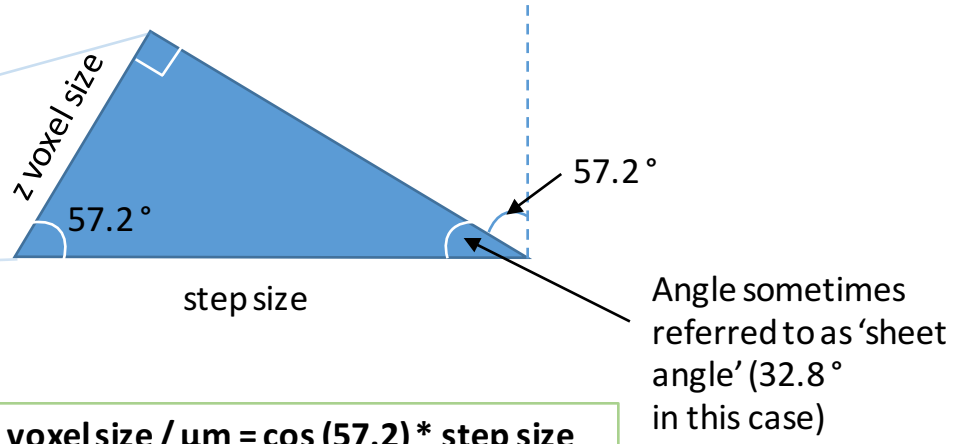
Section 2



Section 3:
Understanding deskewing

Section 1: Lattice Geometry



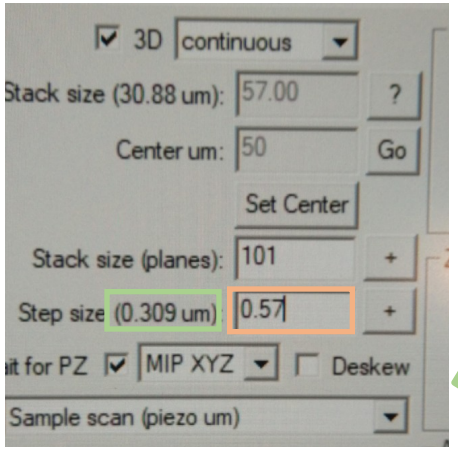


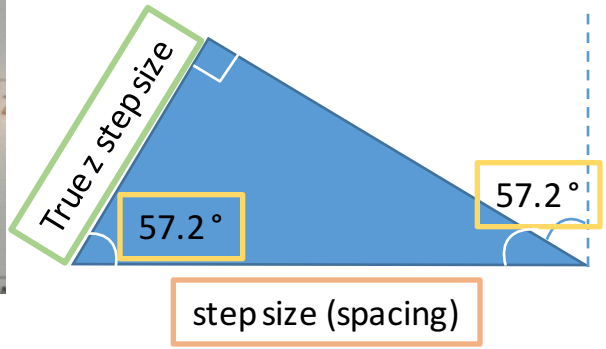
z voxel size / µm = cos (57.2) * step size
x, y voxel size / µm = 0.104

https://www.intelligent-imaging.com/lattice

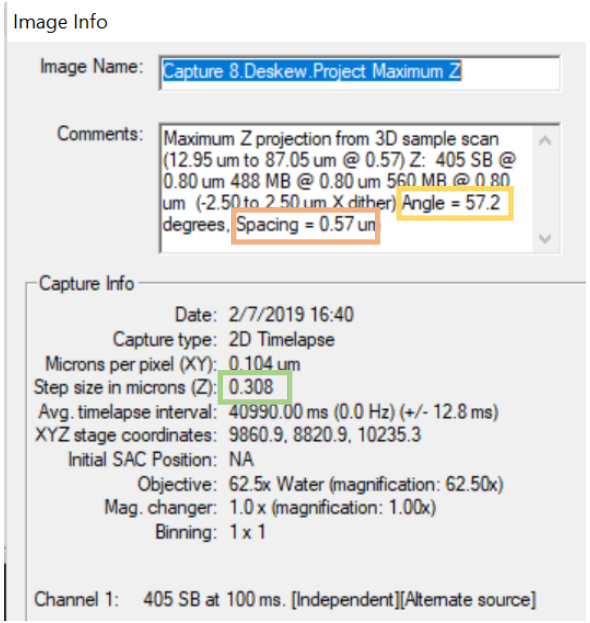
Finding the info

z voxel size / µm = cos (57.2) * step size

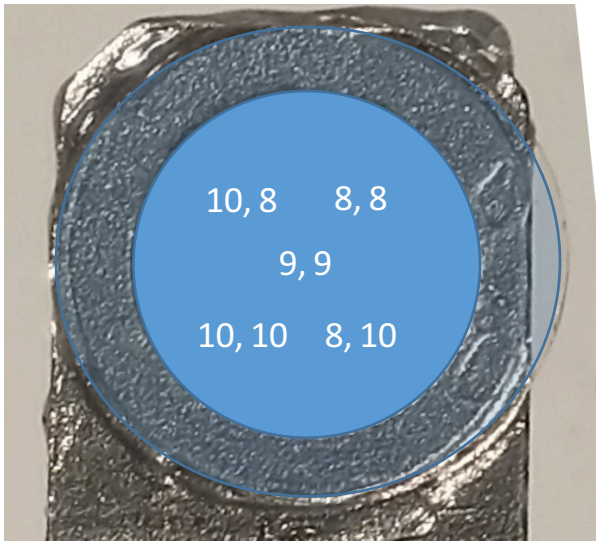




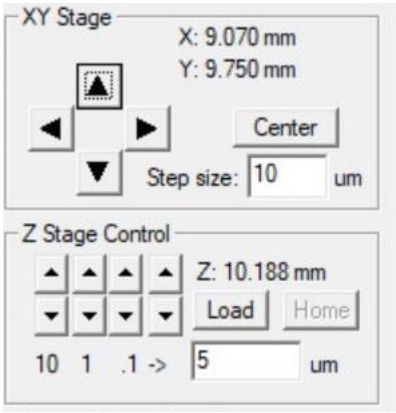
Right click on deskewed file in SlideBook, go to Properties



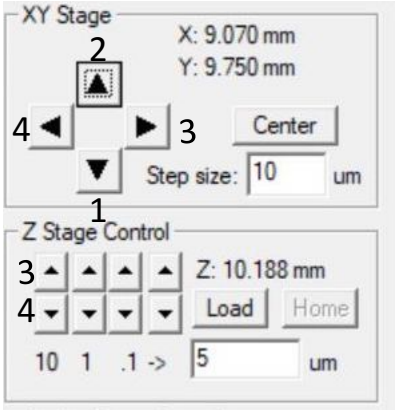
Section 2: Sample Holder



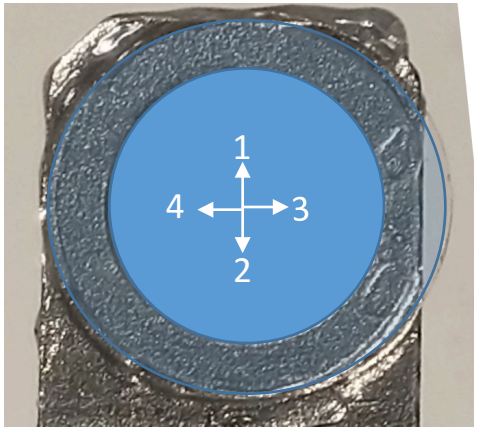
- X, Y coordinates
- 9, 9 is the middle of travel
- 7.5 – 10.5 mm is the range



Navigating

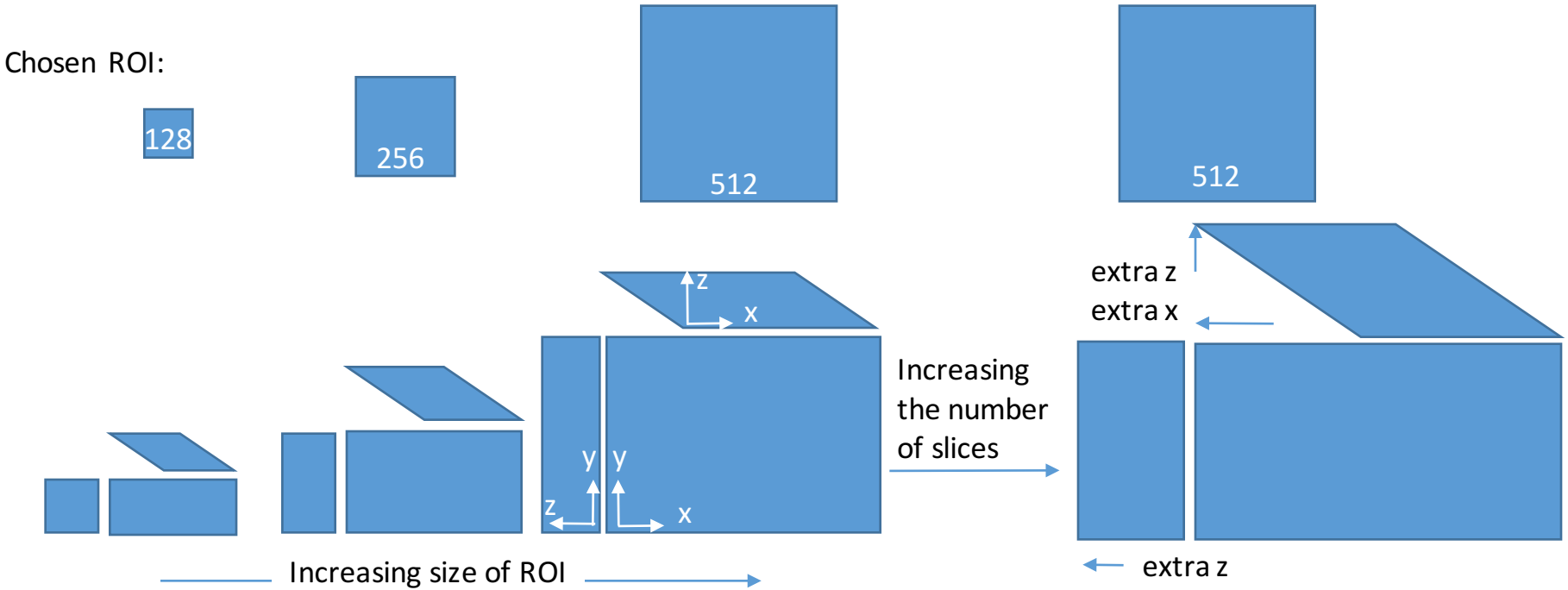


1. To look at something further towards the back of the scope (away from you, if sat in front of it), click the down arrow, the Y value will decrease.
2. To look at something further towards the front of the scope (closer to you, if sat in front of it), click the up arrow, the Y value will increase.
3. To look at something towards the right of the coverslip, click the right arrow, the X value will decrease. Click the up arrow in Z to compensate.
4. To look at something towards the left of the coverslip, click the left arrow, the X value will increase. Click the down arrow in Z to compensate.



Optimising the sample position in the volume

- It's really important to make sure your sample is correctly positioned in the volume so you don't lose any data. We do this by using the 3D deskewed MIP windows in live view.

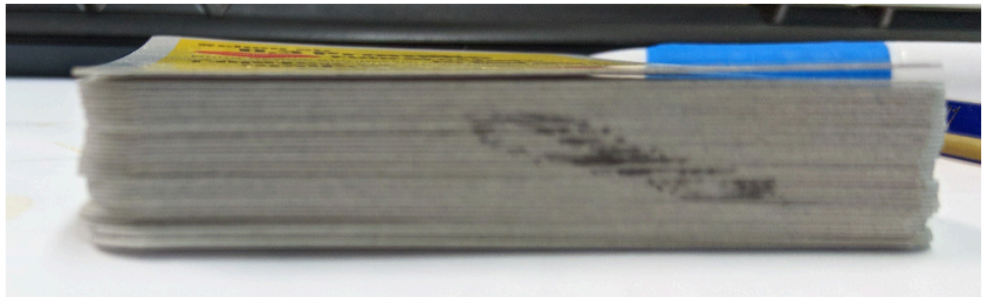


Section 3: What deskewing actually means

You should now be able to collect 3 or 4D image stacks of the region of your sample you are interested in.
Volker Hilsenstein (Monash University) has a really nice analogy to help visualise what lattice stacks look like and why they need to be deskewed…

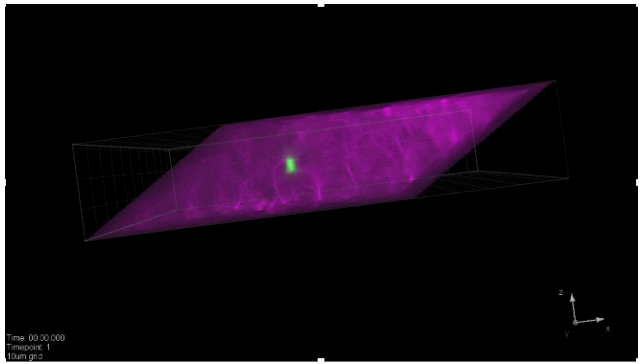
I found it helpful to use a deck of cards as a tactile model for the captured image stack. During acquisition, the deck of cards is oriented like this (the sample projection is represented by a circle drawn with pencil):

However the representation as a `.tif` stack is like this:



https://github.com/VolkerH/Lattice_Lightsheet_Deskew_Deconv/blob/master/Python/00_Lattice_Light_Sheet_Deskew.ipynb

What deskewing actually means



That's why in SlideBook, your deskewed volume is a parallelepiped.
The voxels should have size (x,y,z / µm): 0.104, 0.104, cos (57.4) * step size

https://github.com/VolkerH/Lattice_Lightsheet_Deskew_Deconv/blob/master/Python/00_Lattice_Light_Sheet_Deskew.ipynb

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Any questions? Find me in *in silico or drop me a line at* helena.coker@warwick.ac.uk

Navigating the LLSM

24th January 2020 / CAMDU @ WMS / Helena Coker

View Finder Camera

Orientation consistent with the sample as placed into the microscope. some background fluorescence needed to see large objects 488 nm if fluorescin, 647 nm if Alexa.

THE MAIN CAMERA IS A MIRROR IMAGE

what's in the top left portion of the viewfinder will be in the middle of the main camera view (but mirrored). Focus should be approximately right at the moment.