

# 4D rendering of Lattice Light Sheet data

**Slidebook and Imaris** 

V2 18th February 2019 / CAMDU @ WMS / Helena Coker

#### Slidebook; Deskewing Data



It is important to ensure that the z spacing is correct for each image.

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- Right click on image > Properties
- Spacing is what you input into the lattice software when you collected the images.
- Step size (Z) for **deskewed images** should be Spacing\*cos(57.2) and is shown on the acquisition programme in brackets next to the spacing you input



#### Slidebook; Deskewing Data

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| Home                  | Analyze Scripting Modules                                                           | Support Administration                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                         |                                                  |                                                                                        |                                            |                           |
| Focus Capture         | e Special Multi- Photo- Light<br>Capture - photon - manipulation - Sheet<br>Acquire | ►xport -     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓     ↓ | E Montage Windows • K                   | Tools Select Select Lookup<br>Regions            | ar Notes Photomanipulation<br>amp Regions Z Plane<br>s Table Object IDs<br>Annotations | Settings Set Default<br>Yettings Default - |                           |
| Cide1                 |                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                         |                                                  |                                                                                        |                                            | _                         |
| 🔓 🜒 20181             |                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Image Info                              |                                                  |                                                                                        |                                            |                           |
| Image                 |                                                                                     | Comments Capture Date                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Capture Type Edit Info                  |                                                  | X Edition                                                                              |                                            |                           |
| <b>1</b>              | Annulus055_050_20                                                                   | 3D sample scan (35 11/5/2018 15:47:36                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 3D Capture Capture Type:                | 3D Capture  Interval                             | l (ma): 0                                                                              |                                            |                           |
|                       | Annulus040_0325_20                                                                  | 3D sample scan (35 11/5/2018 15:59:01                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 3D Capture Objective:                   | Contents Optical Paramete<br>Microne/)           | pixel: 0.104 OK                                                                        |                                            |                           |
|                       | Annulus040_0325_20.Deskew                                                           | 3D sample scan (35 11/5/2018 15:59:01                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 3D Capture Camera:                      | Camera poe<br>Custom camera v<br>Camera magnitic | tice NA Cancel                                                                         |                                            |                           |
| <b>              </b> | Annulus040_0325_20.Deskew.Project Maximum Z                                         | Maximum Z projecti 11/5/2018 15:59:01                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 20 Capture                              | Numerical aper<br>Working dat                    | ture: 1.1<br>anoa 2000                                                                 |                                            |                           |
|                       | Annulus055_050_20.Deskew                                                            | 3D sample scan (35 11/5/2018 15:47:36                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 3D Capture Interplane s                 | g (um): 0.168 Index of verta                     | tion 1.333<br>(eta):                                                                   |                                            |                           |
| <b></b> ′             | Annulus055_050_20.Deskew.Project Maximum Z                                          | Maximum Z projecti 11/5/2018 15:47:36                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 2D Capture Channels Filter              | Configuration Lambda (um) Time (ms)              |                                                                                        |                                            |                           |
| <b>    </b>           | Annulus040_0325_20PSF                                                               | 3D Z objective sca 11/5/2018 16:15:47                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 3D Capture Channel 1: Oth<br>Channel 2: | er (488 MB) • 0.509 10                           |                                                                                        |                                            |                           |
|                       | Annuka040_0325_20PSF Sub                                                            | 3D Z objective sca 11/5/2018 16:15:47                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 30 Capture Channel 3:                   |                                                  |                                                                                        |                                            |                           |
|                       | Annulus040_0325_20.Deskew - 1                                                       | 3D sample scan (35 11/5/2018 15:59:01                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 30 Capture Channel 5:                   |                                                  |                                                                                        |                                            |                           |
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- If the spacing is incorrect, click
   Edit info
- Correct the spacing here and click okay

#### Slidebook; Maximum projections and compressed movies



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 For quick reference, smaller 2D timelapses can be created by maximum projection of your 4D volume.

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- Home > Create Projection
- These can be saved as massively compressed 'series movies' which can be emailed easily
- Home > Export > Create Series Movie

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#### Slidebook; 4D viewer



- For full volumetric view use the 3D or 4D Viewer depending on your data set
- Home > View > 3D/4D viewer
- Adjust look up table as necessary

Note Nov2020: SlideBook 6.0.21 and .22RC deskew by default when opening volumes so only open raw data



#### Slidebook; Saving 4D Volumes

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|                                          | Export all channels as 8-bit TIFFs | 736 S C M 4 S C Bue: None                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |            |
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| - Pad                                    | Movie Export                       | 5901                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |
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- Movies of the volume can be exported
- A limited number of rotations and movements are available.
- Home > Export > Movie
- Home > Export > OME Tif 16 bit can be used to export a tif to open in Fiji. Note: best opened with Bioformats Importer, do not open as virtual stack which compresses to 8 bit.



Slidebook; Getting ready for Imaris

- Open a new slide and save it.
- Copy the deskewed file of interest into the new slide.
- Resave
- Open Imaris File Converter (downloaded from <u>http://www.bitplane.com/download#fileconverter</u>)
- Drag and drop the Slide into IFC and click 'start all'



#### Imaris; Check image dimensions



- Open the .ims file saved by the converter in Imaris
- Check the z step size is correct Edit > Image Properties
- The value should be the deskewed step size as discussed earlier and NOT the spacing



Imaris; if the step size is wrong

- Imaris may crash if you change the step size and then try to save the file. To get around this...
- Reopen the file
- Click Save As and give the file a new name. Wait for it to save
- Go back into properties and edit the step size
- Click save
- Delete the older file



#### Imaris; Visualisation



- Open the .ims file saved by the converter in Imaris
- Check the z step size is correct Edit > Image Properties
- The value should be the deskewed step size as discussed earlier and NOT the spacing



#### Imaris; 4D movies



- Use the Animation tab to set up movies.
  - Add transition and rotations here and adjust them on the slide bar
  - When you have the rotations your want click the record button, and save the file.



#### Notes

- Please make use of the many resources on the internet available from other sources
- Although not for the exact release we have licensed, the Imaris 9 manual has some relevant information
- Slidebook is being continuously developed so if you have any suggestion for 3i let me (Helena) know and I'll pass them on





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

**Slidebook and Imaris** 

V2 18th February 2019 / CAMDU @ WMS / Helena Coker