3i diSPIM data Reconstruction with Slidebook

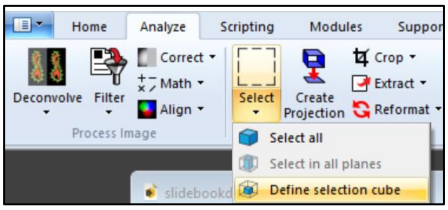
Multi-View Reconstruction in Slidebook:

Step 1: Crop data if necessary. (To reduce time processing)

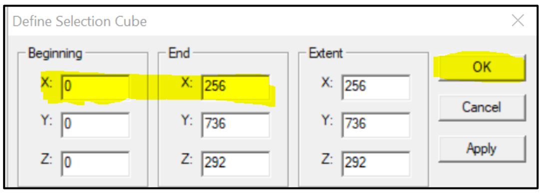
(i) Note cropping of scans:

(Optional) Crop the sample dimensions <u>to reduce unnecessary data size in processing</u>

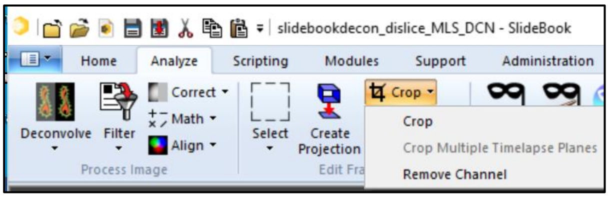
1) **Double-click** the image capture you would like to crop
2) Go to **Analyze -> Select -> define selection cube**



3) Make sure the number of pixels cropped in **Beginning** and **End** of the X-axis is same (Symmetric image along X-axis during post-processing). Click **OK** after selection



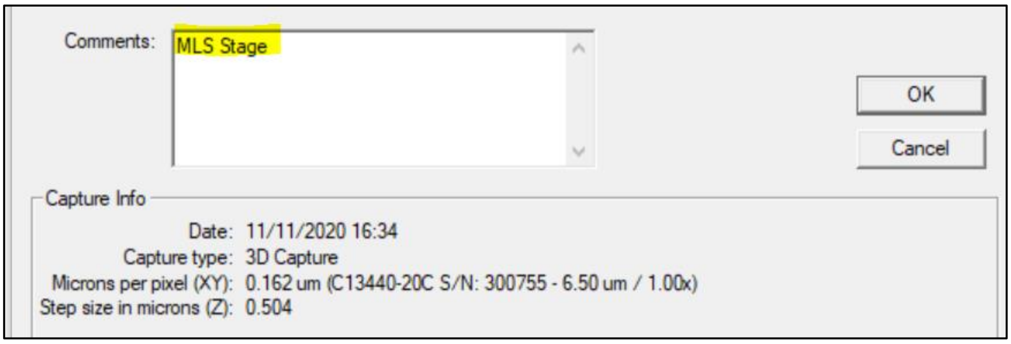
4) Select **Analyze->Crop**



Note: To remove any channel not required, **Analyze->Crop->Remove channel.**

(ii) Note: If distage scans:

1) Make sure when you right click on the properties of dataset, under comments, there is 'MLS stage', if not Enter into **Comments, 'MLS Stage'.**

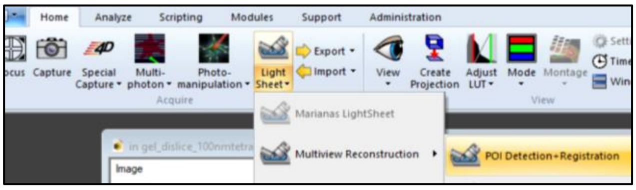


Step 2: Detect interest points for registration of the two sides of the scan

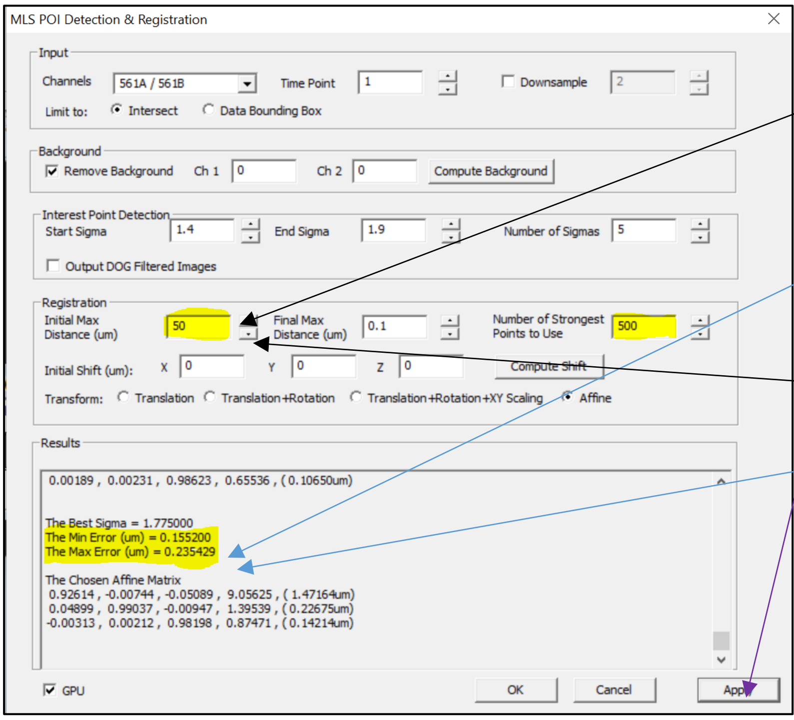
A. POI Detection for Registration (preferred if beads in sample)

Pre-processing step to detect interest points in the sample so as to register the two channels before fusion/ deconvolution.

1) Select the image capture you would like to perform registration
2) **Home -> Light Sheet-> Multi-view Reconstruction -> POI Detection + Registration**



Typical settings



1) Start with a bigger initial max distance value (typically 50/20 microns).

2) Click **Apply**

3) Observe the min and max error values generated.

4) With each consequent iteration of POI detection and registration, reduce the initial max distance value: 50-> 20-> 10-> 8-> 6->5>4->3->2->1, and with each iteration observe that the error should reduce.

5) If the registration performs poorly, increase the **number of strongest points** to use for better registration.

6) Once the best minimum error value has reached, click **OK**. Note the final affine matrix for the same.

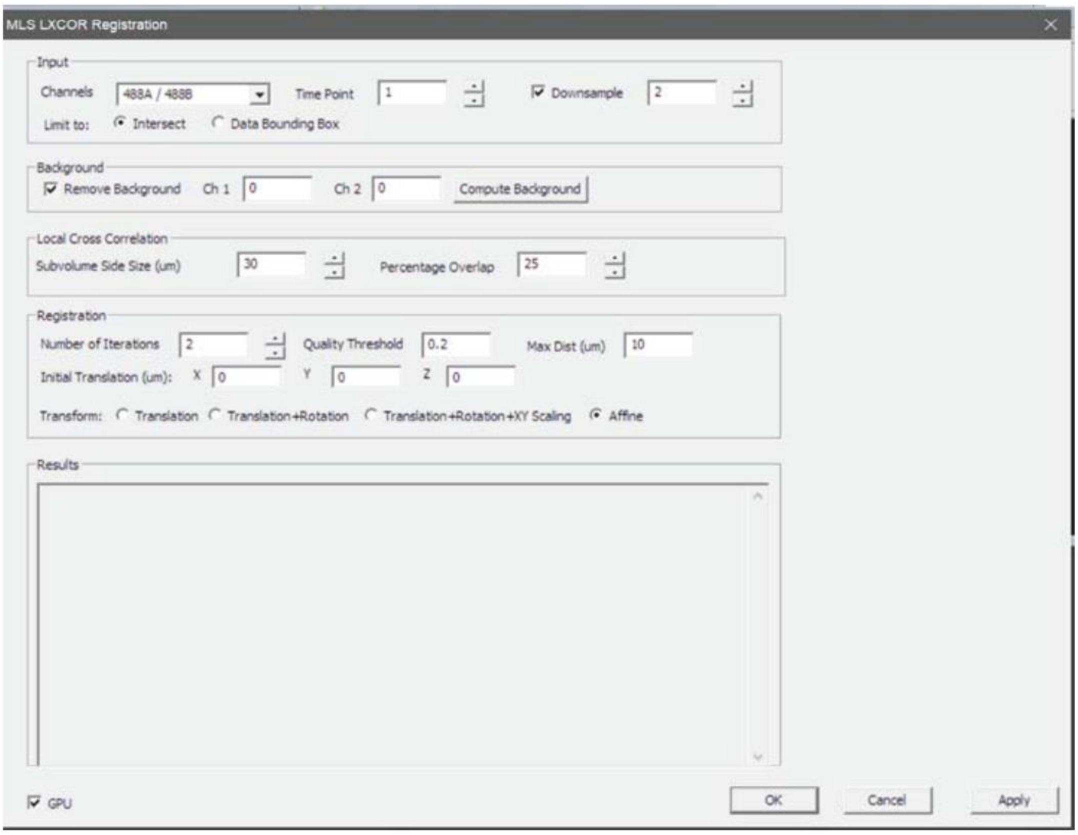
Note: Start sigma and end sigma are the size of the interest points in the sample. Start with the default start sigma (1.8) and default end sigma (1.8) – number of sigmas 1 first.
If the registration keeps failing, the sample interest points may be bigger or smaller than the default one. You may then vary the sigma values if the interest points can be smaller/bigger than typical values listed here.
Based on the start and end sigma value range, change the number of sigmas (for example 1.4 – 1.9; number of sigmas = 5)
Take note of the chosen affine matrix generated.

Observe the registration preview. If satisfactory, click 'OK'.

Use the following registration only if POI detection fails, in step 2:

B. Local XCOR Registration (if POI detection fails)

Alternatively to the POI Detection + Registration you can use 3i's Local Cross Correlation Registration: Select (highlight) the capture you would like to work with. In the Home tab of SlideBook, select ***LightSheet > Multiview Reconstruction > Local XCor Registration***. The 3i Local XCor Registration dialog and the ***3D Volume Viewer*** will open.



a. **Input:**

i. Choose the ***Channels*** for which to detect the transform

ii. Choose the ***Time Point*** for which to detected the transform.

iii. Select ***Downsample*** the data to gain speed. A downsampling value of 3 would keep only every third value. The volume size is 27 times smaller.

iv. Limit To:

i. ***Intersect***: This is the data volume common to path A and Path B that has been (deskewed) rotated.

ii. ***Data Bounding Box***: You can draw a Data Bounding Box in the Bounding Box 3D Volume Viewer beforehand.

b. **Background:**

i. ***Remove Background***: If the background is not 0, it should be removed, since a better contrast improves the results.

ii. ***Ch1***: The first channel (e.g. 488A) background value

iii. ***Ch2***: The second channel (e.g. 488B) background value

iv. ***Compute Background***: Choosing this option will compute the background, but it may be slow. Alternatively, you can choose and enter background values based on the channel histograms.

c. **Local Cross Correlation**

i. ***Subvolume Side Size (um)***:The process subdivides the volume in a number of smaller cube volumes. Specify the size in microns of the cube side.

NOTE: This volume needs to be small enough to offer a true localized cross-correlation, and yet large enough to include the maximum expected shifts.

ii. ***Percentage Overlap***: Set the percentage overlap between a subvolume and its adjacent one.

d. **Registration**

i. To start the algorithm with an ***Initial Shift*** click ***Compute Shift*** and the program will run a rough estimation.

ii. ***Number of Iterations***: Set the number of iterations to perform. At every iteration the current registration transform is applied to the data before the correlation process starts.

iii. Set the ***Quality Threshold***: First the maximum correlation in all subvolumes is detected. Then only subvolumes with correlation quality larger than the maximum correlation times the threshold are used.

iv. When looking for the correlation maxima, restrict the search for shifts less than the maximum distance in ***Max Dist (um)***.

v. ***Transform***: Check the box of the preferred transform. The default is ***Affine***, which applies a Translation, Rotation, Scaling and Shear.

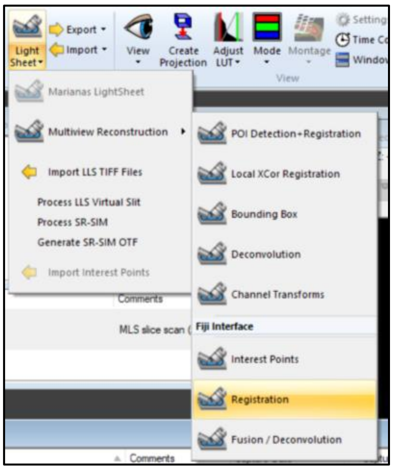
e. Check the box to ***GPU*** to use CUDA accelerated processing.

f. Click ***Apply*** to start the process. It might take a while depending on file size and how many different parameters it is checking.

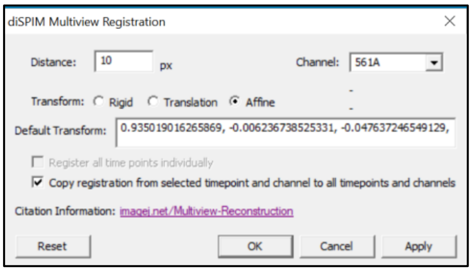
g. The ***Results*** panel will present the transform.

Step 3: Registration

A. Home-> Multiview Reconstruction-> Registration



Make sure the affine matrix values in the **default transform** matches the affine matrix generated in POI detection minimum error value in the previous step. Click **Apply, OK**

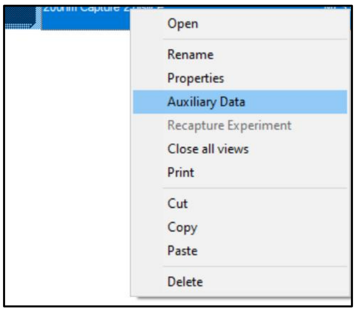


Note: Sometimes this module 'Registration' cannot be opened and shows error. In this case, ignore the error and proceed to the next step to B. check registration, as below.

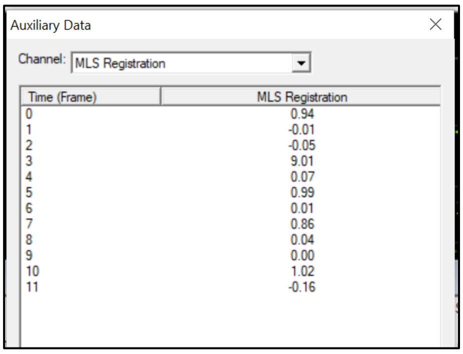
B. Check registration

Right click on the image capture, that you have just performed registration

Click **Auxiliary data**



Check if the **MLS registration** matches the best registration performed during POI detection + registration having the least error



Auxiliary Data

Channel: MLS Registration

Time (Frame)	MLS Registration
0	0.94
1	-0.01
2	-0.05
3	9.01
4	0.07
5	0.99
6	0.01
7	0.86
8	0.04
9	0.00
10	1.02
11	-0.16

Step 4: Create a new slide.

Copy the **defined data** and **PSF files** in it.

PSF File from:

(Z:\confocalcore\For diSPIM Users\Using Slidebook for Deconvolution - Post-acquisition\PSFlibrary.sld)

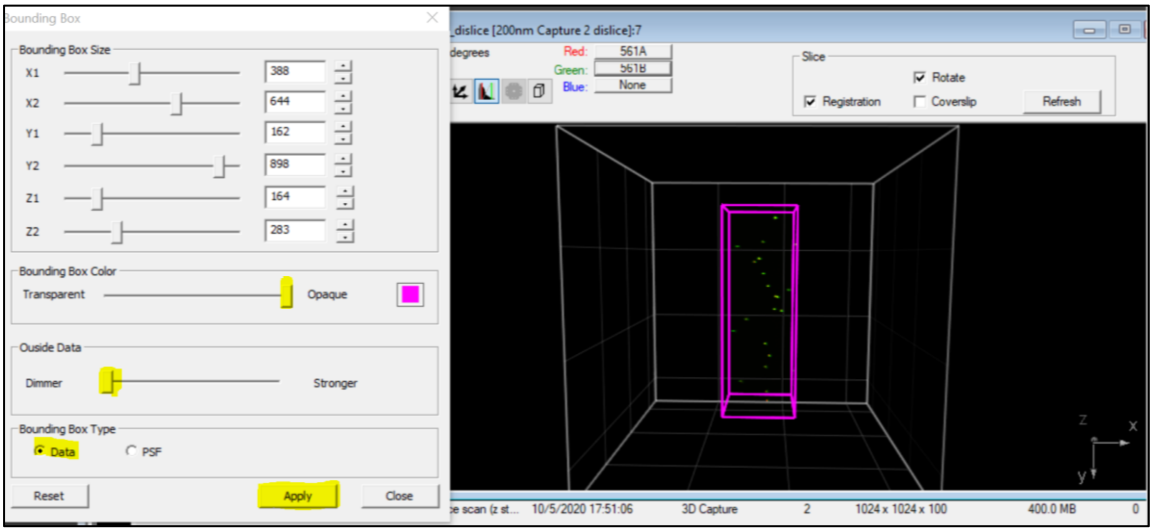
You can save it as 'ForDeconvolution_XYZ'.

Step 5: Now in the new slide,

Define bounding box for the Data

To perform the reconstruction limited to a specific volume in the sample (to eliminate any outliers or unnecessary data)

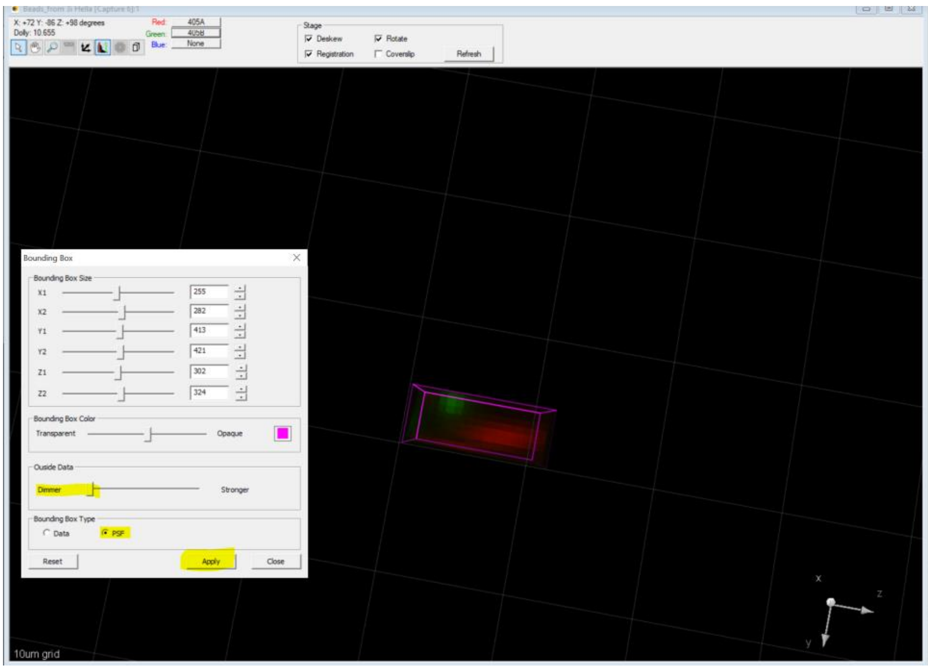
1) **Home -> Light Sheet -> Multi view Reconstruction -> Bounding Box**
2) Select Bounding Box Type: **Data**
3) Click **Apply** after defining bounding box size boundaries



Step 6:

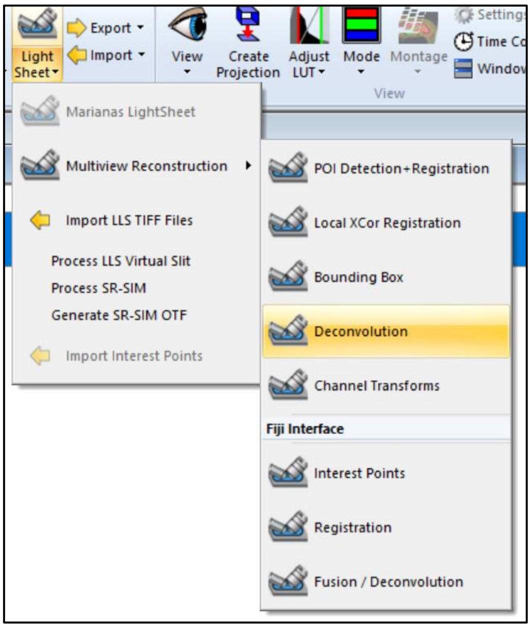
Define Bounding box for PSF

4) **Home -> Light Sheet -> Multi view Reconstruction -> Bounding Box**
5) Select Bounding Box Type: **PSF**
6) Click **Apply** after defining bounding box size boundaries. Make sure only a single bead is within the bounding box for every channel.

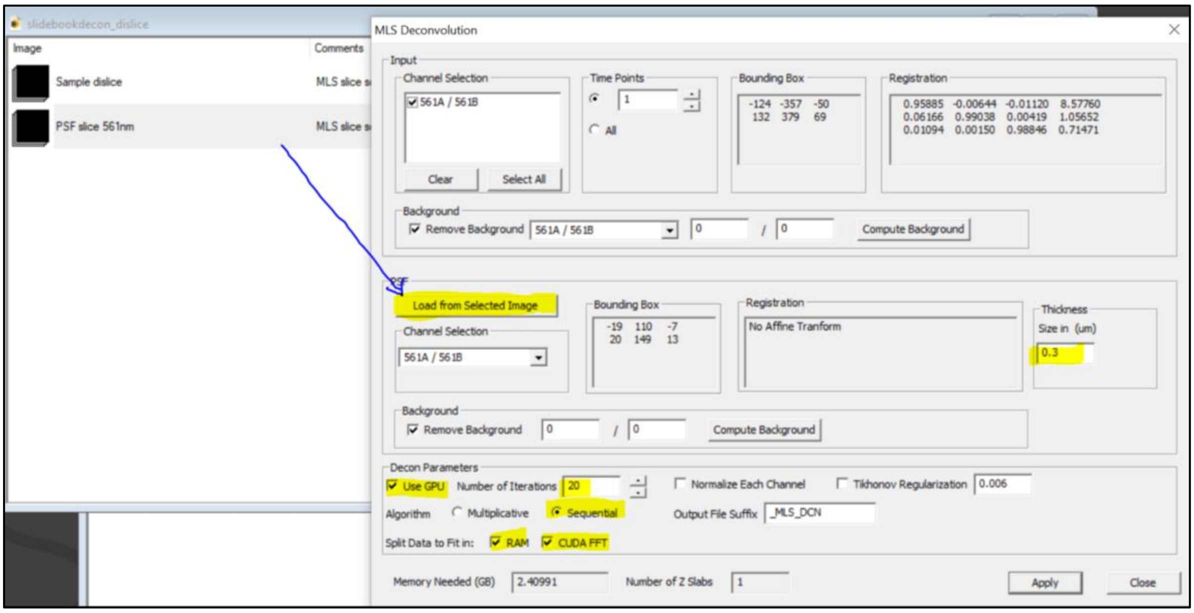


Step 7: Deconvolution

1) Select the image for deconvolution**.** Select **Home -> Light Sheet -> Deconvolution**



2) Select the PSF file and **click Load from Selected Image** under PSF
Choose the typical settings for deconvolution as follows:



Change the number of iterations as per the outcome for optimal deconvolution result.
Save the deconvolution result file.

Export-> 16-bit TIFF if you would like to save TIFF files.

If you want to export the files as TIFF of a stage scan at different orientation (coverslip view):

1. Go to Analyze -> Align -> 3 D transform, rotate 90 degrees or 45 degrees.
2. Export-> 16-bit TIFF