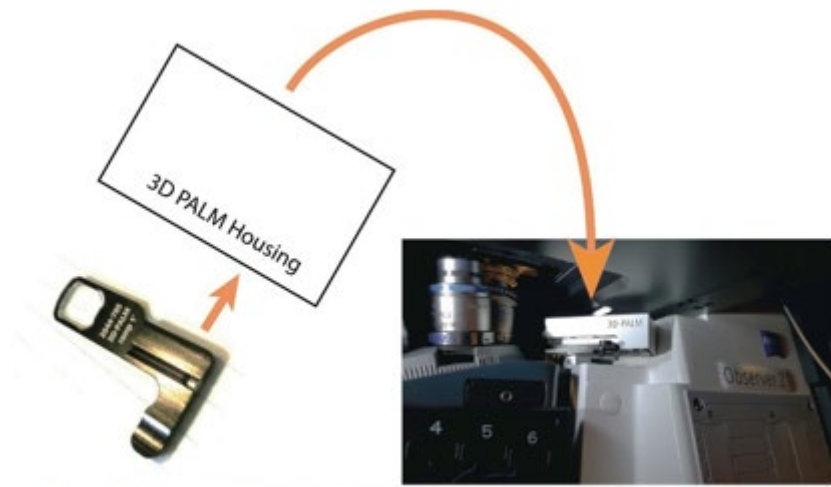


3D PALM/STORM (Elyra Super Resolution)

Acquisition

1. Make sure the 3D PALM slider is in place (writing facing up).
2. Find the focus and then switch into 3D mode. When you do this, the system will automatically kick into HiLo mode.
3. Proceed to imaging.



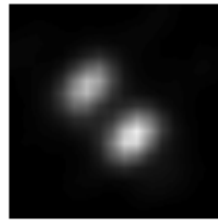
3D PALM slider and where to insert it (below objective turret)



Light Path setup shows the 3D button to set PALM slider in position (when ON).
The HILO button is selected when doing 3D PALM.

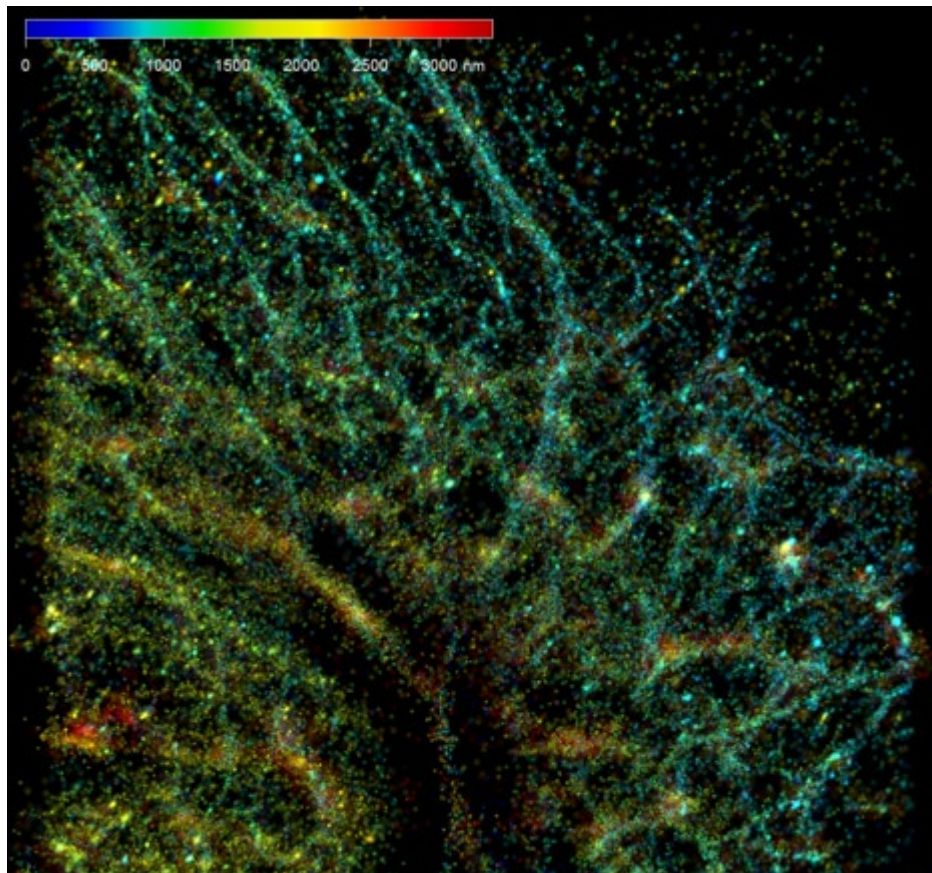
Reconstruction: Determining Experimental PSF

When you image, the corkscrew PSF should be apparent. Each spot looks the figure below and rotates with z.



*Image of
corkscrew PSF*

1. To reconstruct your 3D image, you will need an experimental PSF with localized precision. These can be found in D:\3DPALM_PSFs or you can make your own by following the steps below:
 - a. Use the TetraSpeck bead slide in the black box in the top drawer.
 - b. Take a Z-series with ~10 nm step sizes using the same conditions you used to collect your dataset. Decreasing the laser power is fine.
 - c. Process that data set with the *PALM > Experimental PSF* reconstruction algorithm that averages all the PSF's into one average dataset.
 - d. Process the experimental PSF data set using the *PALM > Localization Precision* algorithm. These files have a lookup table attached to them (even though it is not visible) that provides the z location and precision data when you process the data. (This takes a while.)
2. Process your image under the processing tab using PALM->PALM and your chosen Experimental PSF with localization precision. To start, just use default values.
3. You may want to do drift correction or filter according to come of the many variables. However again just use default everything to start with.
4. Make sure the *Show All* button is checked just to the right of PAL-Drift, PAL-Grouping, PAL-Stats, etc. tabs. Otherwise, random things will be missing and you will be confused.
5. Under the PAL-Rendering tab, set the Z pixel resolution to the same as the XY pixel resolution.
6. Under the Dimensions tab, go to a Z-position where you have the most spots. Adjust contrast levels (including gamma) to view optimally all the spots.
7. In order to colorize, go to Color-Coded Projection in the processing menu. Select the PALM processed image and your favorite LUT, and apply.



Color-coded projection of reconstructed PALM image (Abberior CAGE 552 Tubulin)