DNA PAINT/STORM Analysis

MONDAY, 10/4/2021

Analysis

1.Open ONI software to export

Load locb file

> (optional) Filter localization precision (x, y, 30 nm), also select sigma range (the PSF range to filter out of focus, e.g. for microtubules, we use 10-150 nm) (This can be done for each channel, usually we have 4 channels, 0, 3 are the real signals, 1 and 2 are the bleed through signals)

Export to CSV file

2. Extract each channel

Start Matlab

Go to the Matlab folder (software folder developed by **Peter Relich**)

> folder ONI

>Load ONI CSV_Chann_select_batch.m (this is to convert the CSV file to a bin file to be visulized by Insight3 program and the STORM analysis programe in Matlab)

Select folder to extract CSV to create bin file,

Type 0,1,2,3 (The numbers here correspond to the channel number during the acquisition. The acquisition channel number is designated as the acquisition sequence. The 4 channels correspond the laser program setting), hit'enter'

***!!! Note 1**, each time the code will extract one channel from all CSV files in the folder and write the bin files within the same folder. when extracting two different channels, this have to be repeated by selecting a different channel number, but make sure that the CSV files are duplicated into a different folder before selecting the 2nd channel because it doesn't give the bin files different names based on the channel and it will not overwrite the existing bin files in the same folder.*

Note 2, sometimes the code will show an erro message as follows, but it will still generate the bin files

Exception in thread "Java2D Disposer": java.lang.OutOfMemoryError: GC overhead limit exceeded

Exception in thread "Explorer NavigationContext request queue": java.lang.NullPointerException

3. Filter the signal

3.a. random imager binding filter-two peak analysis

(optional, this is for filtering out background blinking in DNA PAINT, due to imager randomly diffusing into the imaging plane)

Run 'Main' in the Matlab code 'two peak analysis' (to clean up signals that are noise from just the imager randomly binding to the sample.), the noise tend to be very low in density

The image and a distribution of voronoi area will show up

(single peak is a good sign, off target peak shows up on the **right** of the target peak as a second peak), select a threshold and filter out that peak, this should be done on a case by case basis as some images may only have a single peak

3.b. confidence interval filter

This is to get rid of outliers, particularly the highly dense area cluster, where repetitive blink occurs.

- Run 'data analysis software'
- load the bin file (this should follow the two peak analysis, if two peak analysis is to be used)
- plot
- Voronoi segmentation
- start clustering>> select a relative **stringent** threshold, because one wants to filter base on a percentage, and a stringent threshold here allows one to look at small clusters, and the outliers are usually tight and dense clusters if they are artifacts. **For example, here we use 0.025 for deY microtubule,** which is lower than what we ultimately used for clustering for statistical output
- send both clustered and non-clustered localizations to the workspace
- go to the clustered file, plot, go to 'clustering'> clusters remove outliers

- Select gaussian> sigma: 2 (this means that it is removing clusters beyond 2 standard deviation above average, one can certainly play with this number more but 2 seems to work fine), click 'filter data'
- send the plot to workspace
- now combine the filtered clusters with the non clusters open both files, and go to basic analysis tab, pile data. Now the filtered data and the non-clusters are recombined as a filtered set. This allows no loss of some of the low density data and re-clustering for future adjustment

4.Define ROIs for analysis
This is optional if analyzing the entire image is not optimal and sub regions of the image is better for analysis.

Go to ImageJ>plugin>AFIB>Insight3 ROI
change to 700, 4000
Browse>Select .bin file (one at a time)
>Set an ROI (to select only the relavent area that has the cell of interest)> Render (this is re-alalyze the ROI) > save the ROI
*Keep the different channel in different folders for analysis (This is optional but highly recommended)

5. Segmentation, clustering and staticsics
Go back to Matlab
Use the data analysis program from Arian
Load data> STORM microscopy
Select the .bin files with the ROI crop (this loads just the cropped region)
(you can also select the original .bin files, which loads the whole image)

Click on the image,
>Plot
>Image segmentation
>**voronoi segmentation** (can send to workspace, do this if not sure what threshold to use yet, otherwise don't because the file is huge)
>First use default number to test
If the result shows multiple colors (multiple clusters) for 1 object, then the area threshold is too stringent
increase the threshold

> **Clustering**
voronoi results> graph
Start clustering>cluster using voronoi area threshold

0.013 is generally too small,
play around with this number, if structures are broken up, increase the threshold, if it is clumping small structures, decrease threshold.

For example:
Beta tubulin threshold, 0.4
Detyrosinated tubulin threshold, 0.045

> combine two sessions (beta-tubulin and Detyrosination clusters)
>Open co-localization module: Use **colocalization module New**

Select all 29 beta tubulin clustering files (select from top to bottom as the order of selection determines the order of loading the reference), click 'set reference'
Select all 29 detyro clustering files (lower threshold), click set 'colocalization data'

>Start colocalization
>Threshold overlap Try 75 (default is 40) (%) for DeY and beta tub, 40%
pixel size 117 nm
Stastistics (post-processing): for washout time points for DeY clusters, loc # ≥ 20, area ≥ 500 nm^2.