Open files for an image

Image -> Stacks -> Concatenate

Image -> Stacks -> Hyperstack -> Stack to hyperstack (set # of z planes based on metadata), SAVE

Image - > Stacks -> Max projection, SAVE

Run “linear stack alignment with SIFT” registration plugin on ImageJ and chose the translation method with default settings.

Bleach correction if needed? This can make detection weird if bleaching is significant

Analyze -> set scale - > 1080 px = **341.77248 um (3.16 px/um**) (from metadata)

TURN THIS SECTION INTO A MACRO

Freeform line selection

Measure now (to get length info for line drawn)

Edit -> selection -> line to area

Edit -> selection -> enlarge -> by 4 um

Add to ROI Manager

Save ROIset

ROI Manager -> More -> OR (this selects all ROIs as one)

RUN TRACKMATE

* No cropping
* DoG detector -> estimated obj diameter = **15 px**, quality threshold = **100**, pre-process **off**, sub pixel localization **on**
  + Idea is to capture more objects initially but filter them in a slightly more sophisticated way than the linear threshold would
* No initial thresholding
* Spot filtering
  + Min intensity Ch1 = **159.19**
  + Sum intensity Ch1 = **174219.03**
    - **New = 374676.00**
* LAP tracker -> Max frame to frame linking = **25 px/8um** (for 10s interval), gap closing **25 px/8um/10 frames**, splitting max distance **20 px/6.3um** (based on Marissa data showing little movement after splitting, will need to ensure this is always the case), max merge distance **20 px/6.3um**
* Click next 2x
* Number of spots per track >= **2 spots**
* ZOOM OUT, then capture overlay and SAVE resulting image
* Hit SAVE in bottom left to save the full XML (do NOT use Export to XML feature in dropdown menu – this creates a simple file that cannot handle splits and merges)

Proceed to Matlab analysis (for detailed info see supplement 8 in the paper: [https://www.sciencedirect.com/science/article/pii/S1046202316303346#s0140](https://www.sciencedirect.com/science/article/pii/S1046202316303346%23s0140))

3-230 sigNoise 0.8

4-256 sigNoise 0.9

5-171 sigNoise 0.75

6-325 sigNoise 1.04

7-170 sigNoise 0.8

8-170 sigNoise 0.8

6 – 81.8, 0.25

7 – same as 6

8 – same as 6

10 – same as 6

4/7/23 imagtes

4 – 100

5 - 82.7

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Image # | Initial threshold | Min intensity filter | SigNoise filter |
| 3/11/23 | 3 | 65.2 | 152.07 | .19 |
| 3/11/23 | 4 | 79.7 | 154.3 | .27 |
| 3/11/23 | 5 | 53.4 | 154.3 | .28 |
| 3/11/23 | 6 | 81.3 | 155.2 | .22 |
| 3/11/23 | 7 | 55.4 | 155.8 | .22 |
| 3/11/23 | 8 | 34.0 | 153.6 | .21 |
| 4/7/23 | 4 | 100 |  |  |
| 4/7/23 | 5 | 82.7 | 154.63 | .28 |
| 4/7/23 | 6 | 100.6 | 156.15 | .34 |
| 4/7/23 | 7 | 77.9 | 157.26 | .35 |
| 4/7/23 | 8 | 78.2 | 157.26 | .29 |
| 4/21/23 | 1 | 90.3 | 150.7 | .39 |
| 4/21/23 | 2 | 78.9 | 154.91 | .39 |
| 4/21/23 | 3 | 84.5 | 155.4 | .31 |
| 4/21/23 | 4 | 97.7 | 156.0 | .32 |
| 4/28/23 | 1 | 67.8 | 155.9 | .28 |
| 4/28/23 | 2 | 77.3 | 158.5 | .36 |
| 4/28/23 | 4 | 116.4 | 155.3 | .32 |
| 4/28/23 | 5 | 78.3 | 156.6 | .35 |
| 4/28/23 | 6 | 70.5 | 155.6 | .29 |
| 5/24/23 | 4 | 42.0 | 149.9 | .16 |
| 5/24/23 | 5 | 55.0 | 151.6 | .32 |
| 5/24/23 | 7 | 53.2 | 150.75 | .19 |
| 5/24/23 | 8 | 53.2 | 149.45 | .23 |

Graphical user interface, text, application

Description automatically generated Graphical user interface, text

Description automatically generated

These were actually pixel values for GAD65