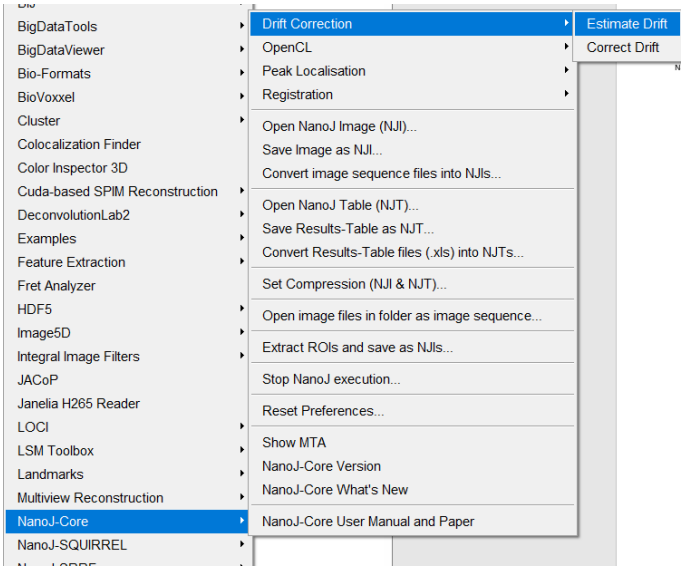
Image Reconstruction for STORM images.

In Fiji download the plugins

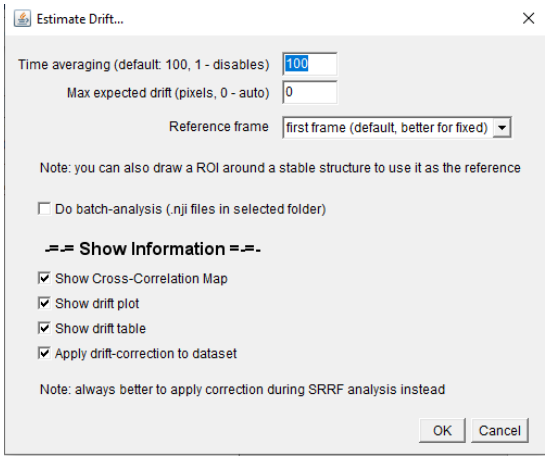
NanoJ-Core https://github.com/HenriquesLab/NanoJ-Core and ThunderSTORM https://zitmen.github.io/thunderstorm/

Nano-J Core is used for pre-processing drift correction of the data.

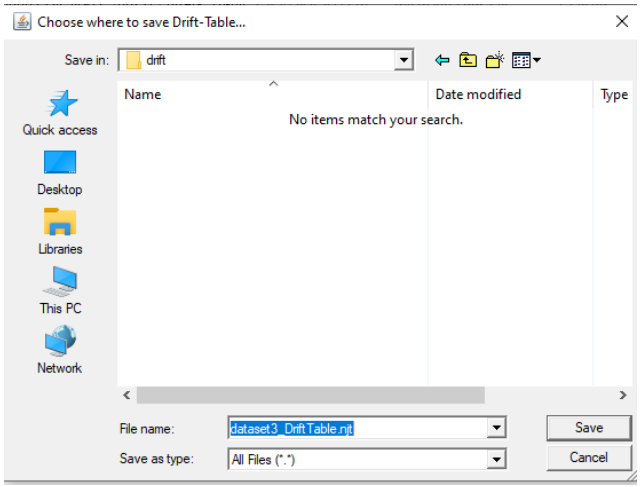
1. **Import the image sequence to Fiji**
2. **Go to Plugins -> NanoJ-Core -> Drift correction -> Estimate Drift**



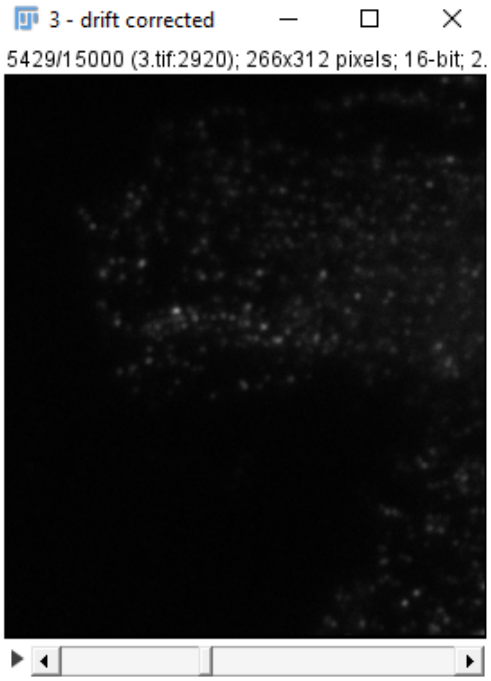
Use the default estimation of drift for the STORM images



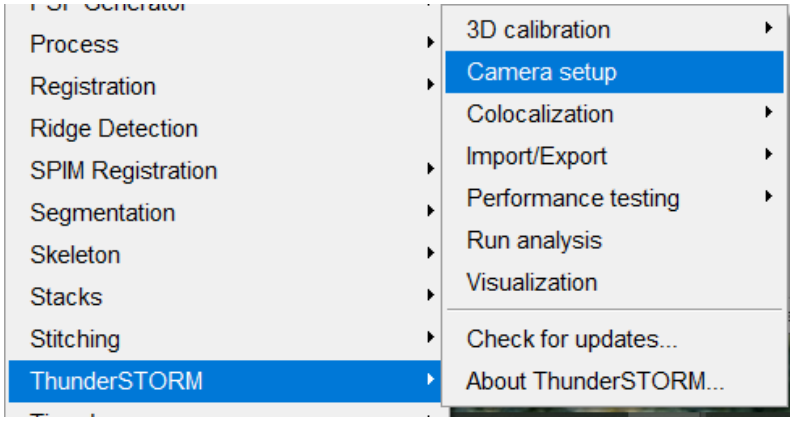
Choose to save the drift table named unique for the dataset:



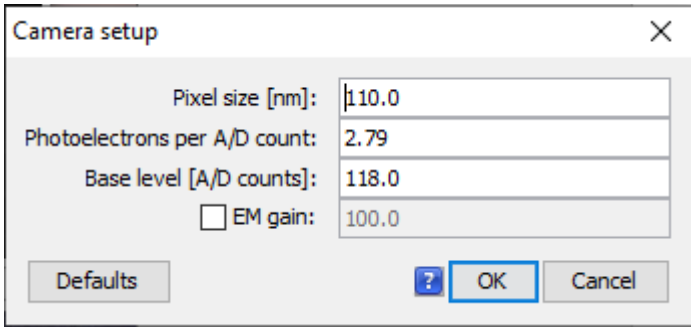
Save the drift-corrected image sequence for further processing.



3. **Now Open the ThunderSTORM plugin for image reconstruction of the drift-corrected file. Go to Plugins -> ThunderSTORM -> Camera Setup**

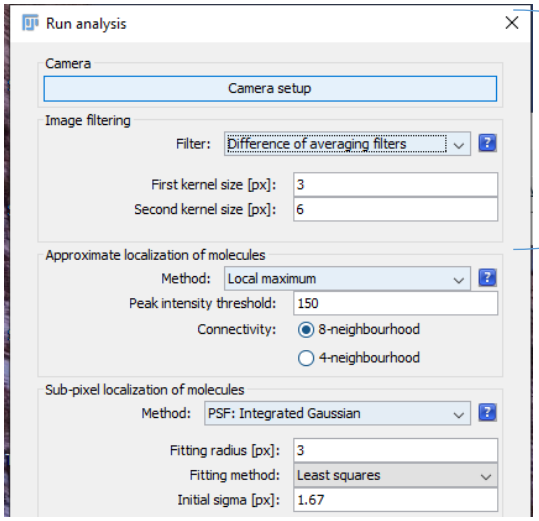


Load the following parameters for camera setup



After setting up the camera values, Go to Plugins -> ThunderSTORM -> Run Analysis

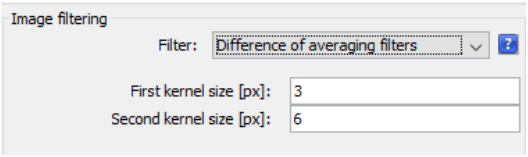
4. Run Analysis – (i) Image Filtering



The first part of the 'Run analysis' is image filtering, this is just a pre-processing of the images performed to visualise and detect the spots in the image better. Choose any filter for optimal filtering of the image

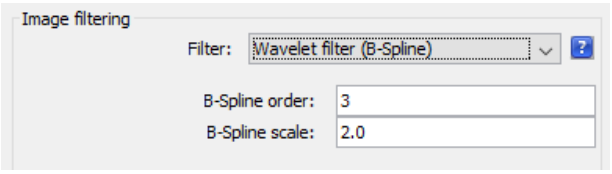
We can use 'difference of averaging filter' or 'wavelet' for better performance.

a. If using Difference of averaging filters:
Use the following features that is based on the PSF beads imaging on the system

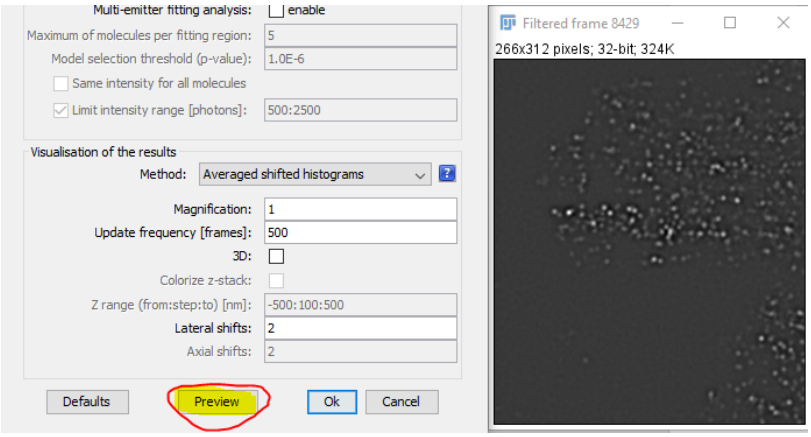


Parameter	Example in paper	beads
Width of PSF estimation	2 pix	3
FWHM of PSF (from profile)	2.5 pix	3.1
Sigma (std) of PSF	FWHM/2.35 = 1.1 pix	1.03
First kernel size (> FWHM of PSF)	3 pix	3 pix
Second kernel (> 2x FWHM of PSF)	5 pix	6 pix

b. If using the Wavelet filtering, you may use the default settings.



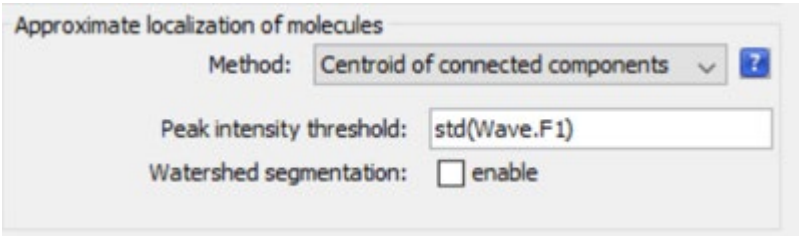
After setting up the image filtering options,
On the bottom of the 'Run Analysis' window click preview, to preview the filtered images, if distinct spots are easily visualized after the pre-processing image filtering step.



5. Run Analysis – (ii) Approximate localization of molecules

You can choose to use ‘local maximum’ or ‘Centroid of connected components’ for optimal results

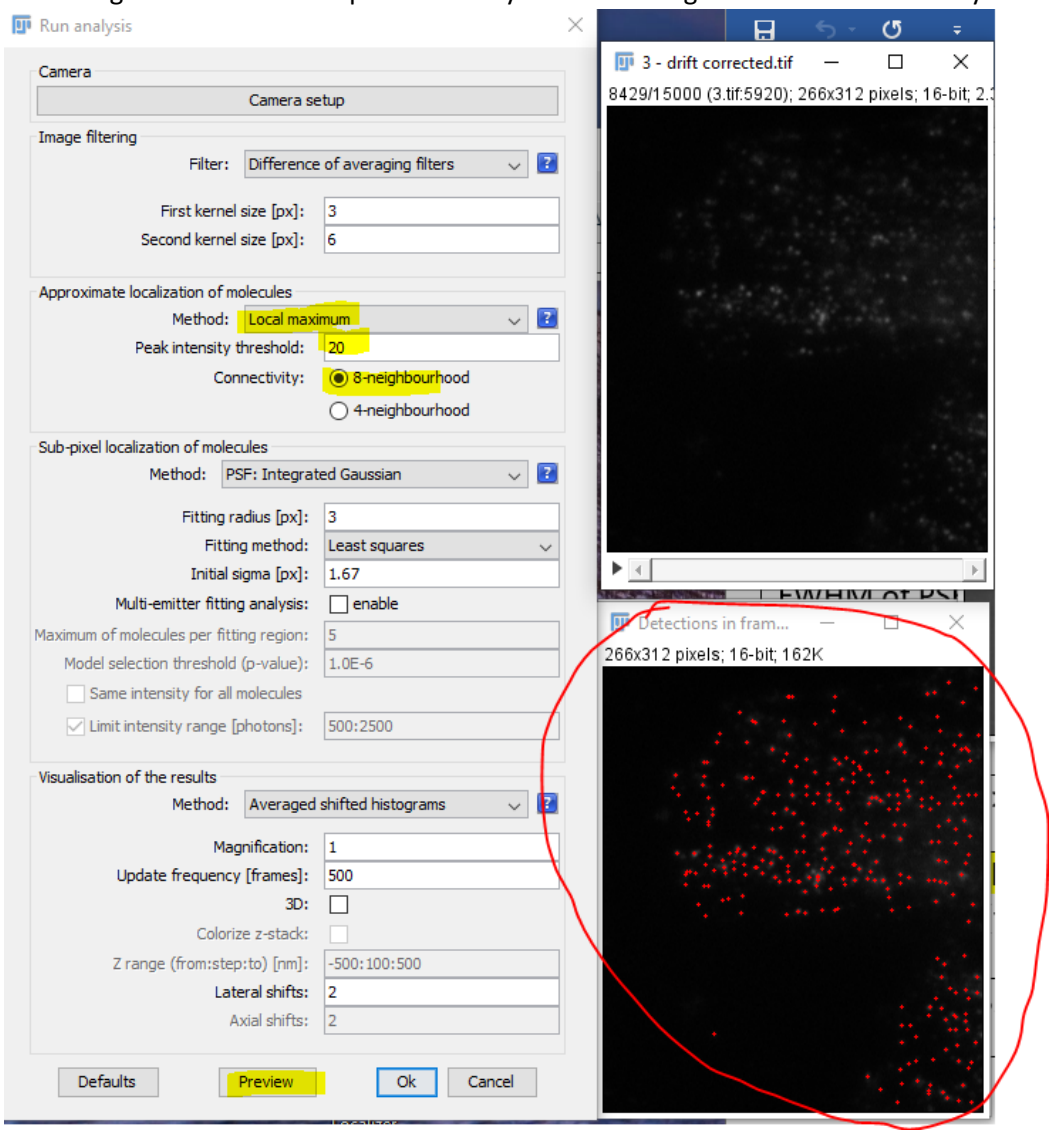
a. Using Centroid of connected components



b. Using Local Maximum

In this step, we will need to make sure that the spots are detected correctly.

Choose ‘Local Maximum’ and enter the value of the peak intensity threshold for the spot detection based on the histogram values of the spots. You may choose ‘8-neighbourhood connectivity’.

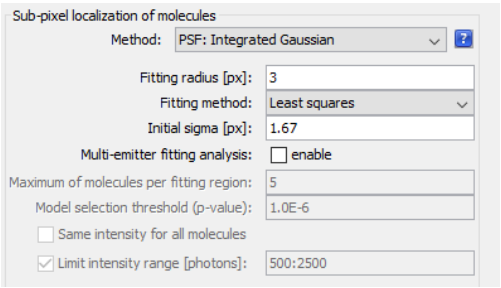


Once defined the values, select ‘Preview’ and make sure the necessary spots are detected in the ‘Detections in frame’ image output. If not, change the peak intensity threshold value accordingly.

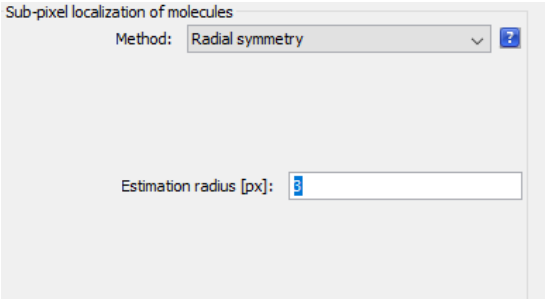
6. Run Analysis – (iii) Sub-pixel localization of molecules

Choose PSF: Integrated Gaussion or radial symmetry

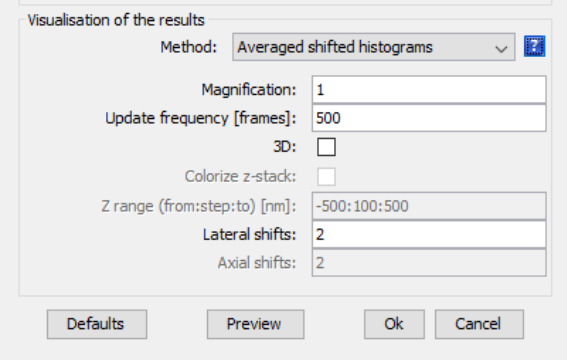
- For PSF: Integrated Gaussian



- For Radial symmetry:
  Radius estimation 2 or 3 based on the image structure size.



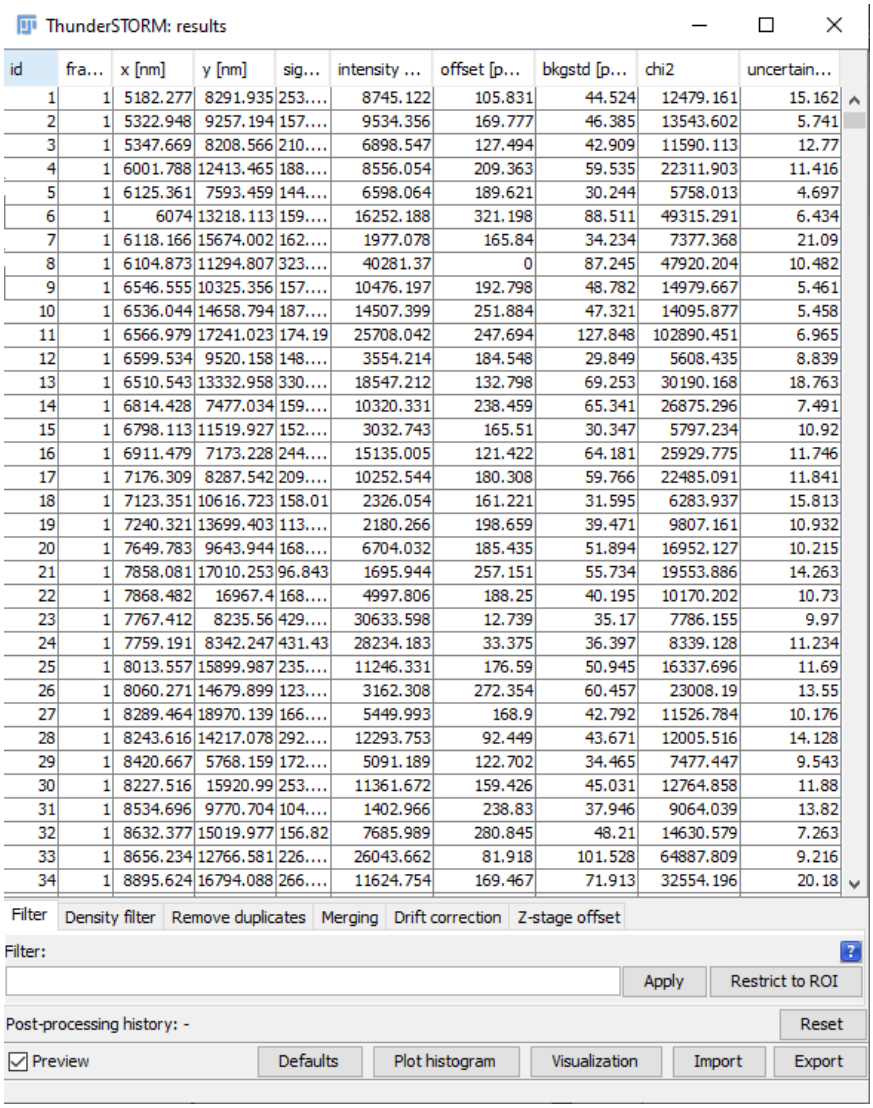
7. Run Analysis – (iv) Visualization of the results



For the first time you run the process, start with magnification 1 and view the results.
For a bigger image, once confirmed the image reconstruction parameters, you may increase the magnification, it will increase the image and processing time.
Click 'OK' when ready with all the steps.

Save the 'Averaged shifted histograms' image.

After the reconstruction is over, the ThunderSTORM results will show the number of detections.
You can choose to do post-processing (such as drift correction and flitering) if required.


ThunderSTORM: results
id fra... x [nm] y [nm] sig... intensity ... offset [p... bkgstd [p... chi2 uncertain...
1 1 5182.277 8291.935 253.... 8745.122 105.831 44.524 12479.161 15.162
2 1 5322.948 9257.194 157.... 9534.356 169.777 46.385 13543.602 5.741
3 1 5347.669 8208.566 210.... 6898.547 127.494 42.909 11590.113 12.77
4 1 6001.788 12413.465 188.... 8556.054 209.363 59.535 22311.903 11.416
5 1 6125.361 7593.459 144.... 6598.064 189.621 30.244 5758.013 4.697
6 1 6074 13218.113 159.... 16252.188 321.198 88.511 49315.291 6.434
7 1 6118.166 15674.002 162.... 1977.078 165.84 34.234 7377.368 21.09
8 1 6104.873 11294.807 323.... 40281.37 0 87.245 47920.204 10.482
9 1 6546.555 10325.356 157.... 10476.197 192.798 48.782 14979.667 5.461
10 1 6536.044 14658.794 187.... 14507.399 251.884 47.321 14095.877 5.458
11 1 6566.979 17241.023 174.19 25708.042 247.694 127.848 102890.451 6.965
12 1 6599.534 9520.158 148.... 3554.214 184.548 29.849 5608.435 8.839
13 1 6510.543 13332.958 330.... 18547.212 132.798 69.253 30190.168 18.763
14 1 6814.428 7477.034 159.... 10320.331 238.459 65.341 26875.296 7.491
15 1 6798.113 11519.927 152.... 3032.743 165.51 30.347 5797.234 10.92
16 1 6911.479 7173.228 244.... 15135.005 121.422 64.181 25929.775 11.746
17 1 7176.309 8287.542 209.... 10252.544 180.308 59.766 22485.091 11.841
18 1 7123.351 10616.723 158.01 2326.054 161.221 31.595 6283.937 15.813
19 1 7240.321 13699.403 113.... 2180.266 198.659 39.471 9807.161 10.932
20 1 7649.783 9643.944 168.... 6704.032 185.435 51.894 16952.127 10.215
21 1 7858.081 17010.253 96.843 1695.944 257.151 55.734 19553.886 14.263
22 1 7868.482 16967.4 168.... 4997.806 188.25 40.195 10170.202 10.73
23 1 7767.412 8235.56 429.... 30633.598 12.739 35.17 7786.155 9.97
24 1 7759.191 8342.247 431.43 28234.183 33.375 36.397 8339.128 11.234
25 1 8013.557 15899.987 235.... 11246.331 176.59 50.945 16337.696 11.69
26 1 8060.271 14679.899 123.... 3162.308 272.354 60.457 23008.19 13.55
27 1 8289.464 18970.139 166.... 5449.993 168.9 42.792 11526.784 10.176
28 1 8243.616 14217.078 292.... 12293.753 92.449 43.671 12005.516 14.128
29 1 8420.667 5768.159 172.... 5091.189 122.702 34.465 7477.447 9.543
30 1 8227.516 15920.99 253.... 11361.672 159.426 45.031 12764.858 11.88
31 1 8534.696 9770.704 104.... 1402.966 238.83 37.946 9064.039 13.82
32 1 8632.377 15019.977 156.82 7685.989 280.845 48.21 14630.579 7.263
33 1 8656.234 12766.581 226.... 26043.662 81.918 101.528 64887.809 9.216
34 1 8895.624 16794.088 266.... 11624.754 169.467 71.913 32554.196 20.18
Filter
Density filter
Remove duplicates
Merging
Drift correction
Z-stage offset
Filter:
Apply
Restrict to ROI
Post-processing history: -
Reset
Preview
Defaults
Plot histogram
Visualization
Import
Export