STORM/DNA-PAINT image reconstruction guide

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### Necessary files for STORM image reconstruction:

* Raw experimental video files with 15000-30000 frames for each channel
* In case of multicolour imaging calibration video with fluorescent beads in each channel (100 frames per video)

### Necessary files for DNA\_PAINT image reconstruction:

* Raw experimental video files with 10000-20000 frames for each channel
* In case of multicolour imaging calibration video with fluorescent beads in each channel (100 frames per video)
* Fluorescent or brightfield video before washing and after washing for drift detection. Video files should be short (100 frames) and acquired in big field of view (512 x 512 px)

### Procedure of STORM/DNA\_PAINT image reconstruction:

* **ThunderStorm program to detect all blink localisations and their parameters**

Run ImageJ → Plugins → Run → search for location of “batch\_thunderSTORM\_reconstruction” macros → specify folder with ONLY raw videos for channels. It can be whatever number of videos. This step takes time, so I usually put all raw videofiles in the same folder and leave it work overnight. As result program will create localization csv files (excel) per each video.

* **In case of multicolour imaging prepare a matlab file (s) for transformation between channels using videos of fluorescent beads**

1. Run ImageJ → Plugins → Run → search for location of “batch\_beads\_reconstruction” macros → specify folder with ONLY short videos of fluorescent beads. Resulting files will be saved in the folder “bead\_reconstructions” inside a folder with bead video files
2. Go to software → “SR-registration-master” folder and open “getTransform.m” code
3. Specify:
   1. DefaultPath (path of folder “bead\_reconstructions”)
   2. Area\_token (the main name of the file, usually – beads)
   3. RefCh\_token (name of the reference channel)
   4. tformCh\_token (name(s) of the channel(s) to be transformed)
4. Run “getTransform.m”. As result, files with names “Trafo\_tform\_beads\_....” will be created for each channel that need to be transformed in the folder “Output\_registration\_polynomial”

* **ONLY IN CASE of multicolour DNA\_PAINT – take to account drift that was induced by washing**

1. Move videos with fiducial markers before and after washing in the same folder. Move to the same folder file with localizations corresponding to second acquired channel (localizations from channel imaged after washing will be corrected for the drift)
2. In folder “Drift correction” open “AccountDrift.m”
3. Specify:
   1. BeforeDriftName (end of the name of the video file taken before washing)
   2. AfterDriftName (end of the name of the video file taken after washing)
   3. LocFileName (end of the name of localization csv file that will be corrected)
   4. LocFileNewName (end of the desired name of output file with corrected position of localizations)
   5. Npoints (number of beads will be used to measure the drift)
   6. Type (‘w’ – for white spots; “b” – black spots)
4. Run “AccountDrift.m”
5. Chose the folder with the files from step 1)
6. In opened image chose with mouse beads that need to be taken to account. Press Enter after you picked each bead
7. In case of unsuccessful drift evaluation (nan instead of numbers) please play with thresholding levels (levelA and levelB). If you want to use Otsu’s thresholding instead, make sure you exclude levelA and levelB from input in function FindSpotPos (lines 71 and 94)
8. File with corrected position of localizations will be saved in the same folder from step 1)

* **Move all important files in the same folder**

Move localization csv files to one folder. Move to the same folder matlab file produced for position correction - “Trafo\_tform\_beads\_....”

* **Create registered file with localisations for the channels need to be transformed**

1. In folder “SR-registration-master” open “useTransform.m” code
2. Specify:
   1. DefaultPath (the path to the folder with localization files and “Trafo\_tform\_beads\_....”)
   2. area\_token (the main name of the file, usually – a1, a2, b1,..)
   3. RefCh\_token (channel that you assume being reference channel – localisations in this channel will be unchanged)
   4. tformCh\_token (channels, that will be corrected for dichroic aberrations)
3. Run “useTransform.m”
4. After program finished, transfer newly created localization files (their naming will finish with “\_reg”) from the folder “Registered\_data” to the folder with main localization files

* **Reconstruct images**

1. In folder “Synaptosome-analysis-master” open “synaptosomeAnalysis.m”
2. Specify:
   1. Directory (path for the folder with localizations files)
   2. Output\_dir (path for the folder where reconstructed images with other output files will be saved). Tip: use for the folder name parameters yopu specify below
   3. Channel\_token (ends of the localization filenames you want to analyze. Make sure you are using registered localization files in case of multicolour imaging – they ends with “\_reg”)
   4. Min\_sigma, max\_sigma (defines allowed range for localizations width. Usually is setted between 100 nm and 250 nm)
   5. Min\_uncertainty, max\_uncertainty (defines allowed range of uncertainties defined in thunderSTORM. Typical values are from 5 nm to 40 nm)
   6. Nloc (defines number of localizations in each channel per 10 frames. This number is used to compute a threshold for localization’s intensity density in each channel. Typical values are between 20-200, it varies strongly between different samples and field of views, so it need to be adjusted every time)

All other parameters can also be setted but they are not essential

1. Run “synaptosomeAnalysis.m”
2. The output files and reconstructed images will be situated in the folder specified in “output\_dir”