https://github.com/Mattheyses-Lab/Nawara_et_al._NatCommun_2022.git contains all the automatic data processing and analysis software used in the manuscript.

1. **System requirements** must be compatible with:
    - MATLAB R2018a and R2020b (https://www.mathworks.com/support/requirements/previous-releases.html)
    - FiJi (ImageJ, https://imagej.net/downloads#:~:text=ImageJ%20will%20run%20on%20any,Java%20installed%20from%20java.com)
    - For CMEanalysis UAB Cheaha Supercomputer was used (https://www.uab.edu/it/home/research-computing/cheaha)

2. **Installation** – no installation is required. For MATLAB programs – double click on the code of choice, for FiJi drag the dz_channel_generator.ijm into to FiJi window to open it.

The three main programs that are used to analyze data are in the following folders:

A. **CMEanalysis_STAR** (MATLAB R2018a) – clathrin accumulation detection and tracing
    - Contains optimized for STAR microscopy CMEanalysis software (source: https://github.com/DanuserLab/cmeAnalysis). Data was organized as suggested by Aguet et al., Dev. Cell 26(3), pp. 279-291, 2013. and runed using the following executable codes:

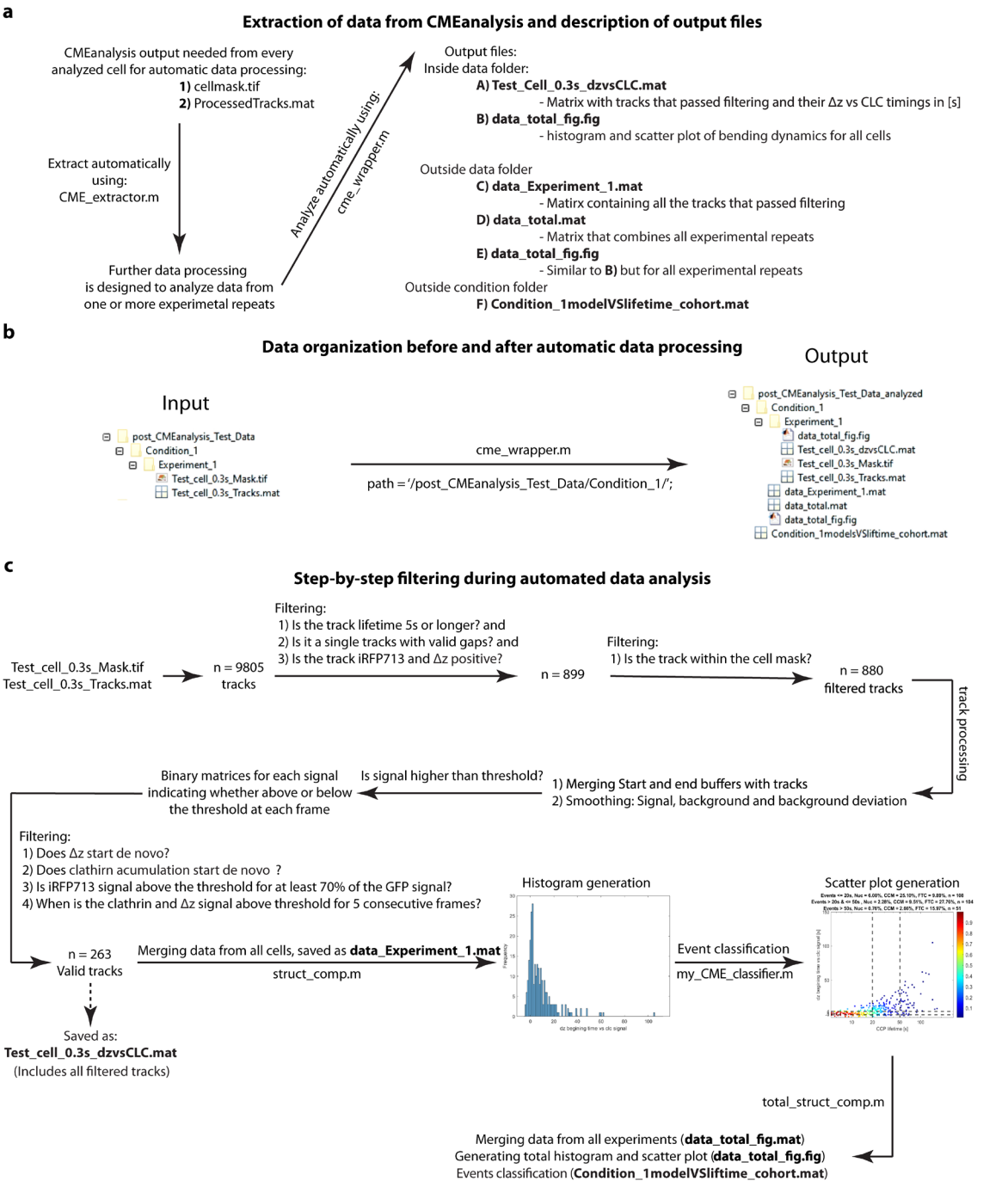
Example code run for control group:

>> data = loadConditionData('/data/user/tnawara/Data/Data analysis/AA_First paper/Revisions/siRNA/Exp3/CLCa_CTRL_exp3', {'Ch1', 'Ch2', 'Ch3'}, {'EGFP', 'iRFP713', 'dZ'}, 'Parameters', [1.49 60 6.45e-6]); >>[resCTRL, dataCTRL] = cmeAnalysis(data, 'ControlData', resCTRL, 'Overwrite', false, 'TrackingRadius', [1 3], 'TrackingGapLength', 13);
Example code run for experimental group:
>> data = loadConditionData('/data/user/tnawara/Data/Data analysis/AA_First paper/Revisions/siRNA/Exp3/CLCa_siRNA_exp3', {'Ch1', 'Ch2', 'Ch3'}, {'EGFP', 'iRFP713', 'dZ'}, 'Parameters', [1.49 60 6.45e-6]); >>[resKD, dataKD] = cmeAnalysis(data, 'ControlData', resCTRL, 'Overwrite', false, 'TrackingRadius', [1 3], 'TrackingGapLength', 13);

- Key parameters used for data processing are shown in Supplementary Table 1 (Nawara T. et al. 2022)
- Test data set: https://drive.google.com/drive/folders/1fd5narw6eAPgUsKROjOHWgRpWcxmboRb?usp=sharing

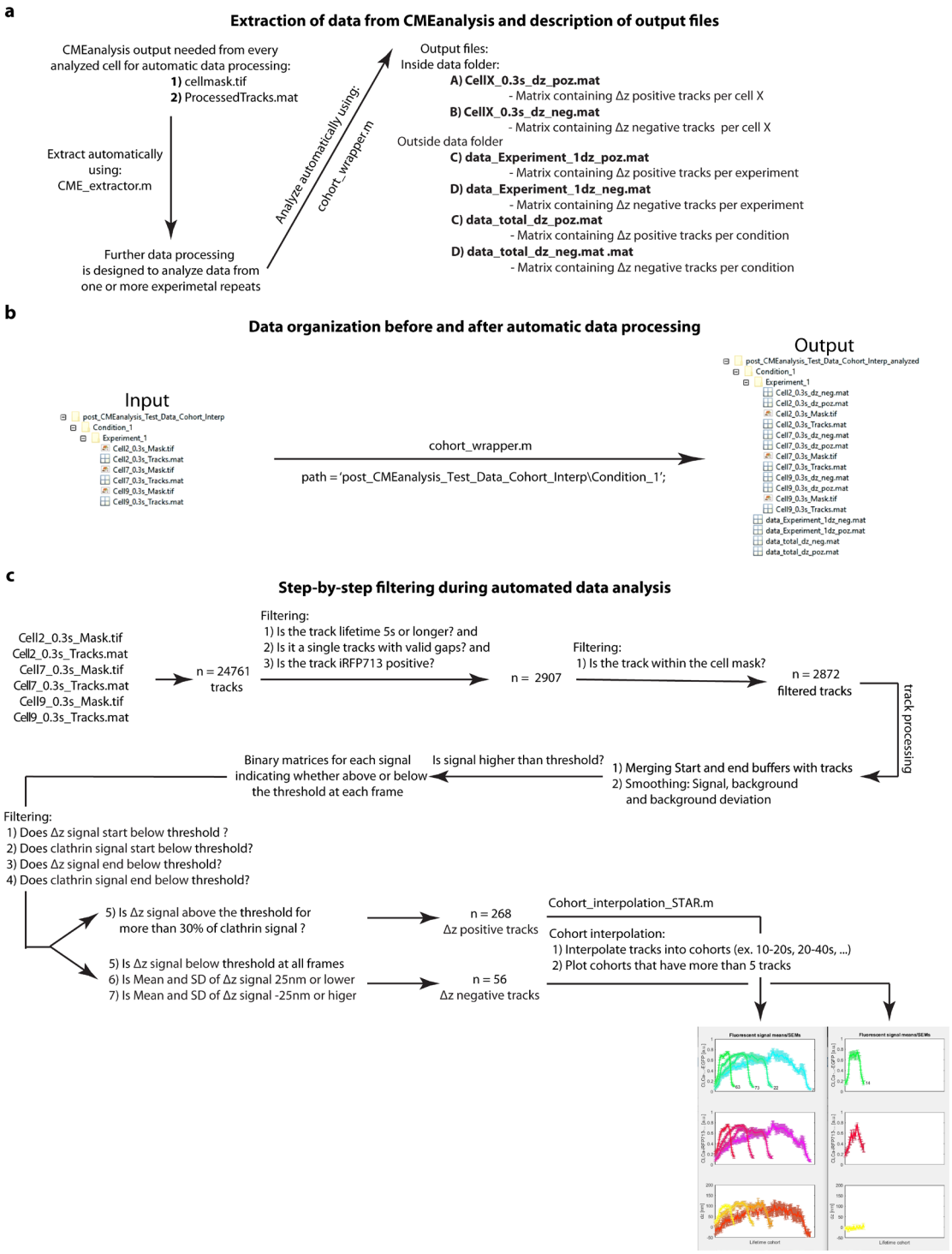
B. **post_CMEanalysis** (MATLAB R2020b) – processing of tracks detected by CMEanalysis

- **delta z Beginning** – measures when curvature develops in relation to clathrin accumulation. Data was organized and processed as follows (Sup Fig.9)



i. Test data set: https://drive.google.com/drive/folders/1v3B125oLSkMTZihWJZuGWquWwCAEv5XF?usp=sharing

- **flat curved sorting and interpolation** – separates and groups detected tracks on whether the curvature was induced (Flat vs. Curved CCSs - Sup Fig.10)



i. Test data set: https://drive.google.com/drive/folders/1GoBtimYqgoS-fsAevSEa2eaDGr1bDyoq?usp=sharing

Other programs used I the manuscript are listed below:

C. **FiJi** (ImageJ) – contains a FiJi plug-in that is used for generating the Δz/curvature channel

- Requires:
  i. Split, background subtracted, flat field and bleach corrected 488 channel time-lapse (end extension "_488_Cor.tif")
  ii. Split, background subtracted, flat field and bleach corrected and registered 647 channel time-lapse (end extension "_647_Cor_Reg.tif")
  iii. Sigma (interpolation correction adjust at line 21)
- Change path to folder @ line 10
- Change folder name @ line 9
- Change folder cell number in the experiment to go through all the cells one at the time @ line 7
- Change the 1/ γ value @ line 47
- Test data set: https://drive.google.com/drive/folders/1FKiySKlWkElbX4NqiqW8CdV_1CEqmel1?usp=sharing

D. **Beads** (MATLAB R2020b) – Measures theoretical and STAR measured Δz of microspheres

- Based on Stabley et al. 2015 (https://www.nature.com/articles/ncomms9307)
- Beads needs to be split, background subtracted and flat field corrected for TRIF modes pictures, required files and extensions:
  i. TIRF 488 – "_TIRF_488_Cor_Blur.tif"
  ii. TIRF 647 – "_TIRF_647_Cor_Reg.tif"
  iii. EPI 488 – "_EPI_488.tif"
- Define path to data @ line 15
- Define pixel size in nm @ line 16
- EPI and TIRF images will overlay, pick beads that TIRF signal is nicely in the middle of EPI ring. Right click when picking the last bead.
- Select beads with uniform fluorophores distribution by accepting it 'y' or rejecting 'n'
- It will scan through folders automatically

- Test data set: https://drive.google.com/drive/folders/11KzyFfA1jUVBUlwiGU2KhLohTEa7B5qo?usp=sharing

E. **EPI.STAR** (MATLAB R2020b) – similar to **delta z Beginning** but supports 4 channels and measures and displays the cumulative difference between the disappearance of clathrin-coated structures form TIRF and EPI, data organization for CMEanalysis:
   - Ch1 – TIRF 488
   - Ch2 – TIRF 647
   - Ch3 – Δz channel
   - Ch4 – EPI 488

F. **Video annotation** (MATLAB R2020b):
   - **image_annotation.m** - annotates time scale on live-cell videos
   - **image_overlay.m** - overlays a definable inset with the full size video

G. **Monte Carlo of stochastic variation of tagged and untagged molecules** (MATLAB R2020b):
   - **Delta_Z_Modeling_Figures.m** – prints the representative stages of vesicles formation
     i. Adjust the amount of snapshots @ line 4
     ii. Adjust percentage of vesicle coverage @ line 5
   - **Delta_Z_vs_CM_Spaghetti_Plot.m** – Performs Monte Carlo simulation of random distribution of tagged molecules at the modeled vesicle
     i. Adjust the amount of tagged molecules by decreasing the number @ line 14

H. IMGREG.m (**MATLAB R2020b**) – Program requires installation of bio-formats tool box details (https://docs.openmicroscopy.org/bio-formats/6.4.0/users/matlab/index.html) and Computer Vision Toolbox. Program registers 647 TIRF time-lapse to 488 TIRF Time-lapse, based on the NanoGrid. Requires path to data, and the split averaged pictures of the 488 NanoGrid and 647 NanoGrid post OptosplitIII calibration. Set manual detection to 0 if not using a predefined registration points. Will register all the images in the folder. For data organization please see test data set.
   - Test data set: https://drive.google.com/drive/folders/14xPjrXcs5lPZMedCzR5Vl069CdZaGwFf?usp=sharing

I. curv_topo.m (**MATLAB R2020b**) – Creates a mask of events detected by "**flat curved sorting and interpolation" package** (Flat vs. Curved CCSs - Sup Fig.10) – that can be then overlay with the live cell imaging data. Analyze one cell at the time and organize data as in Sup Fig.10

J. model_topo.m (**MATLAB R2020b**) - Creates a mask of events based on their model classification following the "**delta z Beginning" package**. Data was organized as in Sup Fig.9, analyze one cell at the time.

3. **Approximate Run time** (depends on computer specs):
    a. CMEanalysis – hours to days
    b. Post_CMEanalysis and EPI.STAR – several minutes
    c. Monte Carlo Simulation – few minutes
    d. Image registration –few hours per cell
    e. curv_topo.m – minutes
    f. model_topo.m – minutes