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);
```

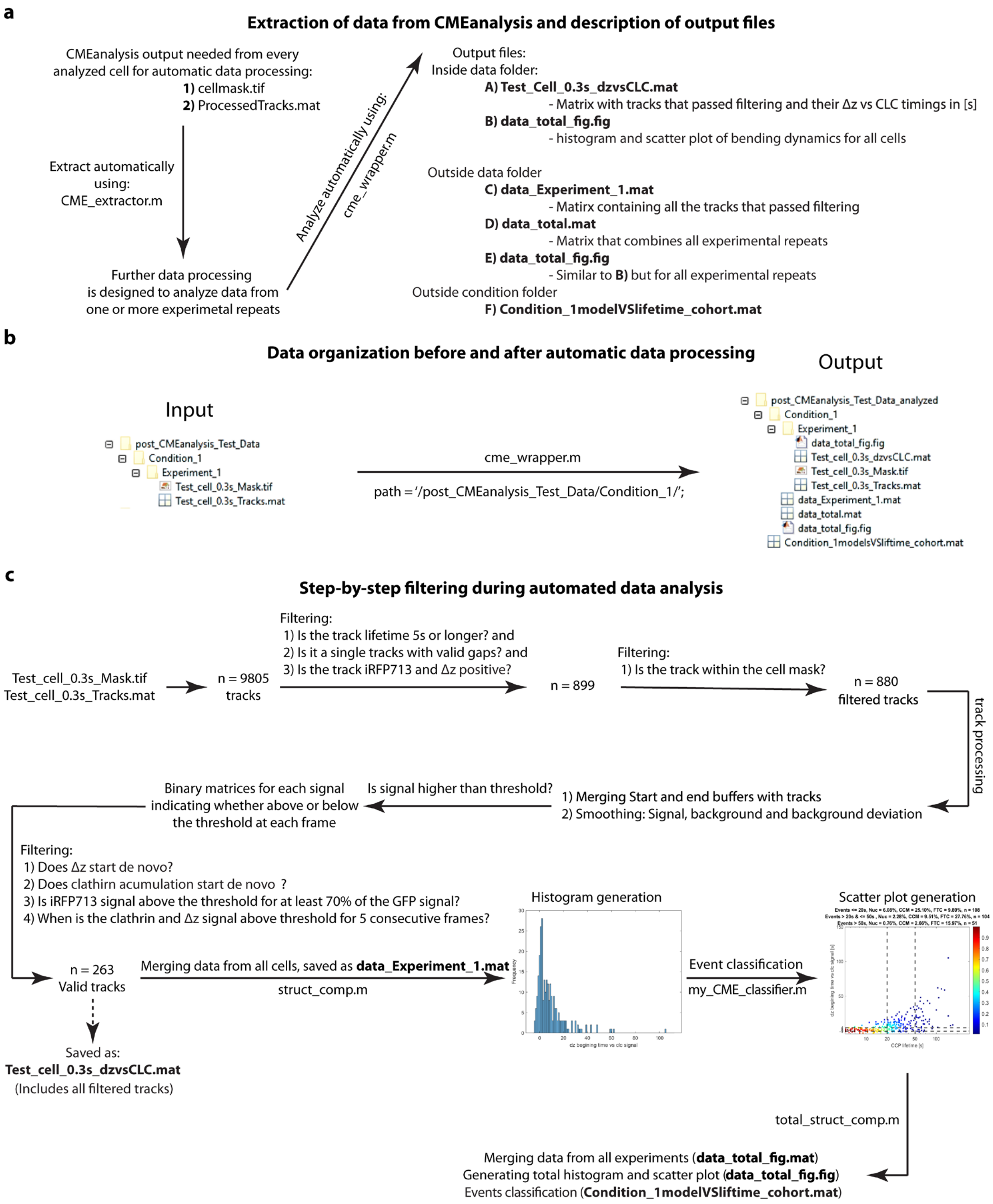
Example code run for experimental group:

```
>> 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

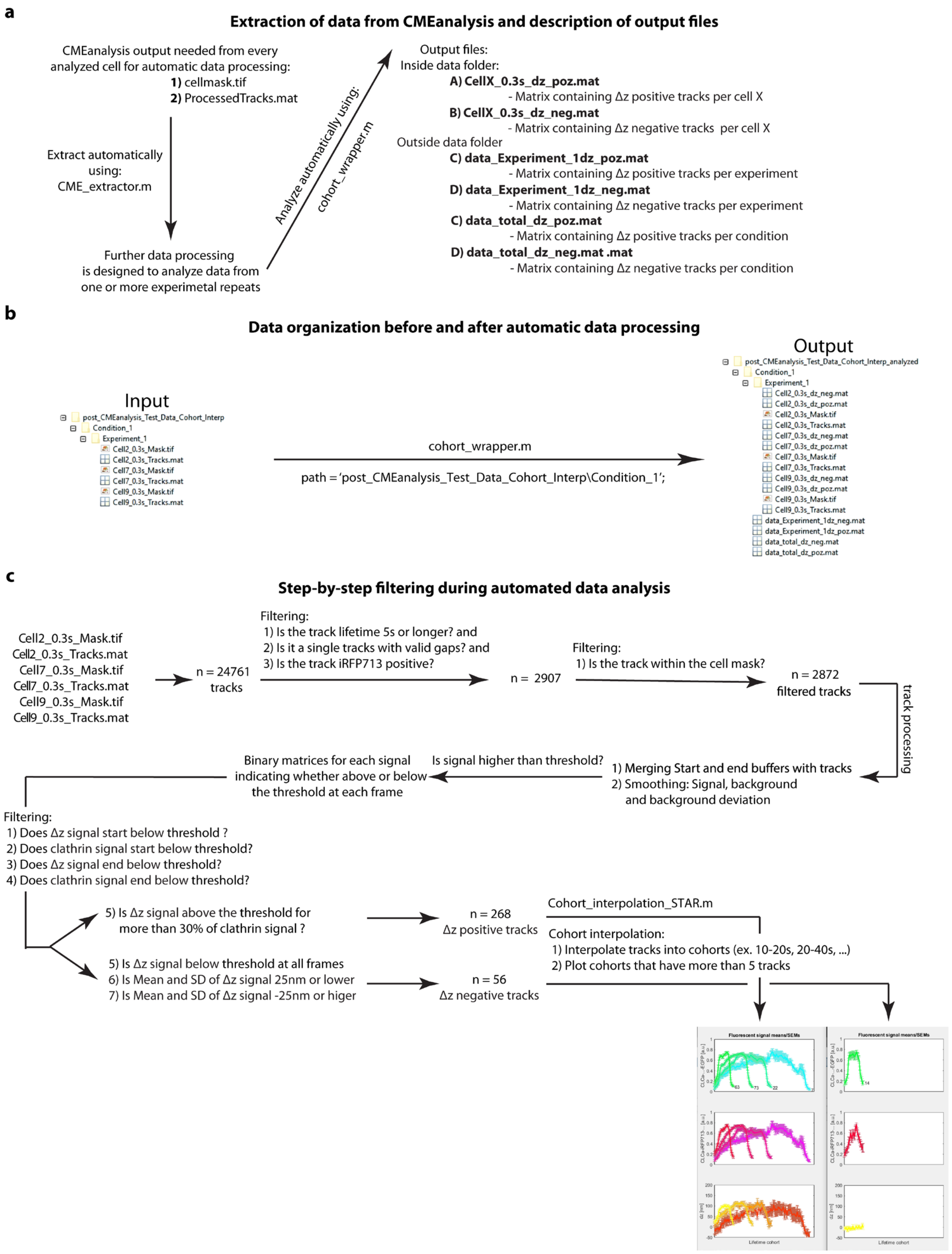
B. **post_CMEanalysis** (MATLAB R2020b) – processing of tracks detected by CMEanalysis

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


a
Extraction of data from CMEanalysis and description of output files
CMEanalysis output needed from every analyzed cell for automatic data processing:
1) cellmask.tif
2) ProcessedTracks.mat
Extract automatically using: CME_extractor.m
Further data processing is designed to analyze data from one or more experimetal repeats
Analyze automatically using: cme_wrapper.m
Output files:
Inside data folder:
A) Test_Cell_0.3s_dzvsCLC.mat
- Matrix with tracks that passed filtering and their Δz vs CLC timings in [s]
B) data_total_fig.fig
- histogram and scatter plot of bending dynamics for all cells
Outside data folder
C) data_Experiment_1.mat
- Matirx containing all the tracks that passed filtering
D) data_total.mat
- Matrix that combines all experimental repeats
E) data_total_fig.fig
- Similar to B) but for all experimental repeats
Outside condition folder
F) Condition_1modelVSlifetime_cohort.mat
b
Data organization before and after automatic data processing
Input
post_CMEanalysis_Test_Data
Condition_1
Experiment_1
Test_cell_0.3s_Mask.tif
Test_cell_0.3s_Tracks.mat
cme_wrapper.m
path = '/post_CMEanalysis_Test_Data/Condition_1/';
Output
post_CMEanalysis_Test_Data_analyzed
Condition_1
Experiment_1
data_total_fig.fig
Test_cell_0.3s_dzvsCLC.mat
Test_cell_0.3s_Mask.tif
Test_cell_0.3s_Tracks.mat
data_Experiment_1.mat
data_total.mat
data_total_fig.fig
Condition_1modelsVSliftime_cohort.mat
c
Step-by-step filtering during automated data analysis
Test_cell_0.3s_Mask.tif
Test_cell_0.3s_Tracks.mat
n = 9805 tracks
Filtering:
1) Is the track lifetime 5s or longer? and
2) Is it a single tracks with valid gaps? and
3) Is the track iRFP713 and Δz positive?
n = 899
Filtering:
1) Is the track within the cell mask?
n = 880 filtered tracks
track processing
1) Merging Start and end buffers with tracks
2) Smoothing: Signal, background and background deviation
Is signal higher than threshold?
Binary matrices for each signal indicating whether above or below the threshold at each frame
Filtering:
1) Does Δz start de novo?
2) Does clathirn acumulation start de novo ?
3) Is iRFP713 signal above the threshold for at least 70% of the GFP signal?
4) When is the clathrin and Δz signal above threshold for 5 consecutive frames?
n = 263 Valid tracks
Saved as:
Test_cell_0.3s_dzvsCLC.mat
(Includes all filtered tracks)
Merging data from all cells, saved as data_Experiment_1.mat
struct_comp.m
Histogram generation
Frequency
0
5
10
15
20
25
30
0
20
40
60
80
100
dz begining time vs clc signal
Event classification
my_CME_classifier.m
Scatter plot generation
Events <= 20s, Nuc = 6.08%, CCM = 25.10%, FTC = 9.89%, n = 108
Events > 20s & <= 50s , Nuc = 2.28%, CCM = 9.51%, FTC = 27.76%, n = 104
Events > 50s, Nuc = 0.76%, CCM = 2.66%, FTC = 15.97%, n = 51
dz begining time vs clc signal [s]
50
100
150
10
20
50
100
CCP lifetime [s]
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
total_struct_comp.m
Merging data from all experiments (data_total_fig.mat)
Generating total histogram and scatter plot (data_total_fig.fig)
Events classification (Condition_1modelVSliftime_cohort.mat)

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)



a
Extraction of data from CMEanalysis and description of output files
CMEanalysis output needed from every analyzed cell for automatic data processing:
1) cellmask.tif
2) ProcessedTracks.mat
Extract automatically using:
CME_extractor.m
Further data processing is designed to analyze data from one or more experimetal repeats
Analyze automatically using:
cohort_wrapper.m
Output files:
Inside data folder:
A) CellX_0.3s_dz_poz.mat
- Matrix containing Δz positive tracks per cell X
B) CellX_0.3s_dz_neg.mat
- Matrix containing Δz negative tracks per cell X
Outside data folder
C) data_Experiment_1dz_poz.mat
- Matrix containing Δz positive tracks per experiment
D) data_Experiment_1dz_neg.mat
- Matrix containing Δz negative tracks per experiment
C) data_total_dz_poz.mat
- Matrix containing Δz positive tracks per condition
D) data_total_dz_neg.mat .mat
- Matrix containing Δz negative tracks per condition
b
Data organization before and after automatic data processing
Input
post_CMEanalysis_Test_Data_Cohort_Interp
Condition_1
Experiment_1
Cell2_0.3s_Mask.tif
Cell2_0.3s_Tracks.mat
Cell7_0.3s_Mask.tif
Cell7_0.3s_Tracks.mat
Cell9_0.3s_Mask.tif
Cell9_0.3s_Tracks.mat
cohort_wrapper.m
path = 'post_CMEanalysis_Test_Data_Cohort_Interp\Condition_1';
Output
post_CMEanalysis_Test_Data_Cohort_Interp_analyzed
Condition_1
Experiment_1
Cell2_0.3s_dz_neg.mat
Cell2_0.3s_dz_poz.mat
Cell2_0.3s_Mask.tif
Cell2_0.3s_Tracks.mat
Cell7_0.3s_dz_neg.mat
Cell7_0.3s_dz_poz.mat
Cell7_0.3s_Mask.tif
Cell7_0.3s_Tracks.mat
Cell9_0.3s_dz_neg.mat
Cell9_0.3s_dz_poz.mat
Cell9_0.3s_Mask.tif
Cell9_0.3s_Tracks.mat
data_Experiment_1dz_neg.mat
data_Experiment_1dz_poz.mat
data_total_dz_neg.mat
data_total_dz_poz.mat
c
Step-by-step filtering during automated data analysis
Cell2_0.3s_Mask.tif
Cell2_0.3s_Tracks.mat
Cell7_0.3s_Mask.tif
Cell7_0.3s_Tracks.mat
Cell9_0.3s_Mask.tif
Cell9_0.3s_Tracks.mat
n = 24761 tracks
Filtering:
1) Is the track lifetime 5s or longer? and
2) Is it a single tracks with valid gaps? and
3) Is the track iRFP713 positive?
n = 2907
Filtering:
1) Is the track within the cell mask?
n = 2872 filtered tracks
track processing
1) Merging Start and end buffers with tracks
2) Smoothing: Signal, background and background deviation
Is signal higher than threshold?
Binary matrices for each signal indicating whether above or below the threshold at each frame
Filtering:
1) Does Δz signal start below threshold ?
2) Does clathrin signal start below threshold?
3) Does Δz signal end below threshold?
4) Does clathrin signal end below threshold?
5) Is Δz signal above the threshold for more than 30% of clathrin signal ?
n = 268
Δz positive tracks
5) Is Δz signal below threshold at all frames
6) Is Mean and SD of Δz signal 25nm or lower
7) Is Mean and SD of Δz signal -25nm or higer
n = 56
Δz negative tracks
Cohort_interpolation_STAR.m
Cohort interpolation:
1) Interpolate tracks into cohorts (ex. 10-20s, 20-40s, ...)
2) Plot cohorts that have more than 5 tracks
Fluorescent signal means/SEMs
CLCa-...-EGFP [a.u.]
0
0.2
0.4
0.6
0.8
1
63
73
22
7
CLCa-iRFP713-... [a.u.]
dz [nm]
-50
0
50
100
150
200
Lifetime cohort
Fluorescent signal means/SEMs
14
Lifetime cohort

        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

   - Test data set: https://drive.google.com/drive/folders/1oWKjh2ApkxeQiIzW3caPmq0vaUJhT9T0?usp=sharing

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.

   - Test data set: https://drive.google.com/drive/folders/1Or-NHN4S1h4X0DzA50RWLMEbKmBLsKZA?usp=sharing

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