

Once the image is prepared, the analysis can be run by entering the program function into the Matlab command line. Matlab will ask for information about your imaging setup and then run the detection, tracking and analysis. The results are then saved in an excel sheet.

The analyses here;

- Automatically detect spots which are significant compared to their background *
- Links the detections together to create tracks of the detections in the movie *
- Determines the lifetimes of tracks
- Determines the density of tracks
- Determines the fluorescent intensity profile of tracks with the mean lifetime
- Calculate the number of persistence tracks

* These steps make use of the cmeAnalysis package in Aguet et al., 2013, which has been slightly modified.

Requirements

You need Matlab and the following toolboxes installed;

- Curve Fitting
- Image Processing
- Optimization
- Statistics and Machine Learning
- Parallel Computing (optional – but significantly increases the speed of the analysis)

You can check if you have these by entering;

```
>> ver
```

Into the command window.

It has been tested on Windows, Mac (Mojave) and Ubuntu with Matlab 2013b, 2016a and 2019a. Also for Mac (Catalina) with Matlab 2019b.

Image Preparation Images are normally cropped to remove excess background and the edges of the cell should be excluded to further remove the possibility of false detections and ‘noisy’ events.

The different channel images need to be saved as separate files and naming the images is a key step. They have to follow a certain format, or the programs will not work. You should use a three-digit number, followed by an underscore, followed by either red or green. And they should be saved in the tiff format. For example; 001_green.tif, 001_red.tif...

The cellSurfaceAnalysis folder containing the scripts should be copied to the Matlab path directory. For further detailed information about this, see this link;

https://uk.mathworks.com/help/matlab/matlab_env/add-remove-or-reorder-folders-on-the-search-path.html

Data analysis, viewing and combining

Single Chanel data:

Enter the program name into the Matlab command line;

```
>> singChan_cellSurfaceAnalysis
```

Enter the requested information and select the data folders. The lifetimes, density and profiles will be saved in the 'condition' folder as a mat file.

To view the raw cmeAnalysis package data use;

```
>> cmeDataViewer(data(i));
```

 (as detailed in the cmeAnalysis package manual. This version of the data viewer does not work on matlab versions newer than 2013b). It is important to note that the tracks in this data viewer are not the same as ones included in the lifetime time analysis, as this does not use the same filtering of tracks as the cellSurfaceAnalysis.

However, to view a movie which has all the tracks used for analysis, enter;

```
>> trackingMovie
```

This produces frames of the movie with the detections and tracks of tracks used in the analysis. Which has been tested on newer versions of Matlab.

To combine experiments, enter;

```
>> combineSingChanData
```

This combines all the output results into one 'mat' file. It will also display a lifetime histogram of the combined tracks and produce an overall mean profile.

Dual Channel Data:

Enter the program name into the Matlab command line;

```
>> dualChan_cellSurfaceAnalysis
```

Enter the requested information and select the data folders. The lifetimes, density and profiles will be saved in the 'condition' folder as a mat file.

To view the raw cmeAnalysis package data use;

```
>> cmeDataViewer(data(i));
```

 (as detailed in the cmeAnalysis package manual). This version of the data viewer does not work on matlab versions newer than 2013b). It is important to note that the tracks in this data viewer are not the same as ones included in the lifetime time analysis, as this does not use the same filtering of tracks as the cellSurfaceAnalysis.

To view a movie which has all the tracks used for analysis, enter;

```
>> trackingMovie
```

This produces frames of the movie with the detections and tracks of tracks used in the analysis.

To combine experiments, enter;

```
>> combineDualChanData
```

This combines all the output results into one mat file. It will also display a lifetime histogram of the combined tracks and produce an overall mean profile.

Persistence assay

The `singChan_cellSurfaceAnalysis` program must be run before using this analysis.

Enter the program name into the Matlab command line;

```
>> persistTest
```

This uses the raw tracks to determine how many persist for the 1st 100 frames of the movie. Short tracks and tracks too close to the edge of the movie are removed for analysis.

To view the data, enter;

```
>> persisPlot
```

Here a movie of the frames will be made highlighting the tracks used for analysis. Green tracks are ones which persist. Red tracks are ones present and end within this 100 frame window.

Example Data

Single and dual channel cell surface analysis

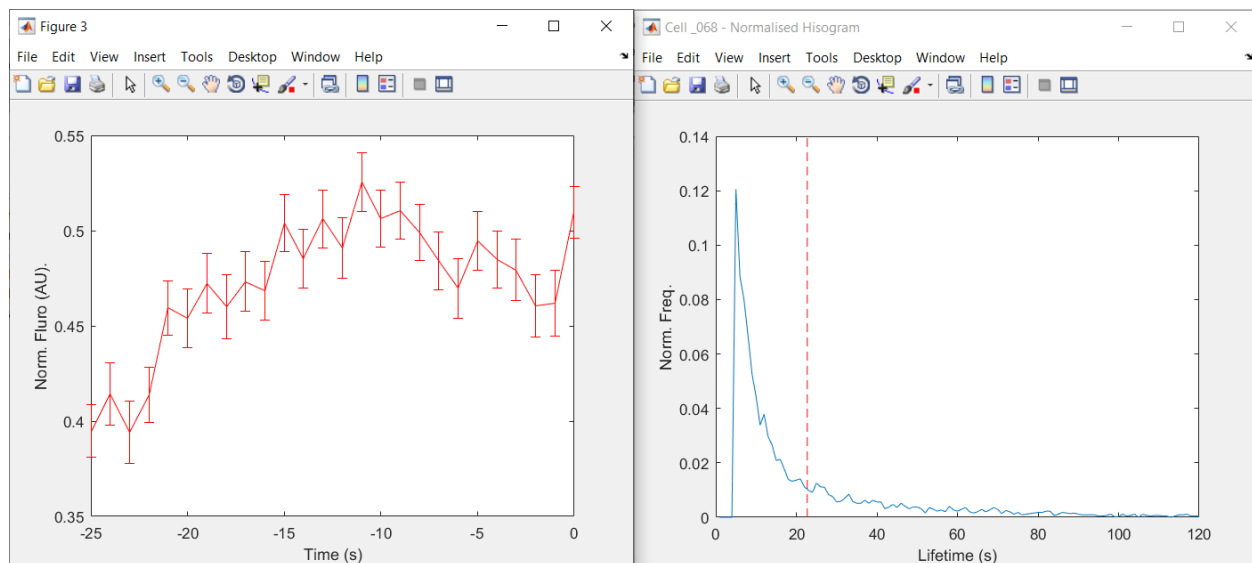
Both the single channel and dual channel protocols follow the same steps, with the exception of entering the number of channels and using the program names as detailed above. Below is a walk through with single channel data (but a dual channel example data set is also provided).

Run the program and enter the following values when prompted (underlined in red). You will be asked to confirm some folders by pressing enter.

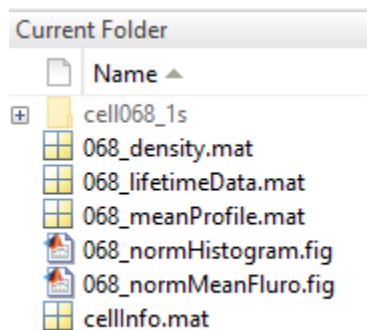
```
Command Window
>> singChan cellSurfaceAnalysis
Enter the time between frames (e.g., 500ms, 1s): 1s
Converting: cell1068_1s.tif
Enter the N.A. of the objective: 1.49
Enter the magnification of the objective: 100
Enter the camera pixel size, in [um]: 16
Root directory: C:\Users\ajohnson\Desktop\Test\068\Experiment\Condition\
Enter the number of channels: 1
Enter the fluorescent marker for channel 1: EGFP
```

For the dual channel data, the RFP movie is picked as the master and the number of channels entered is 2, and the fluorescent markers for `chan1 = RFP`, `chan2 = EGFP`.

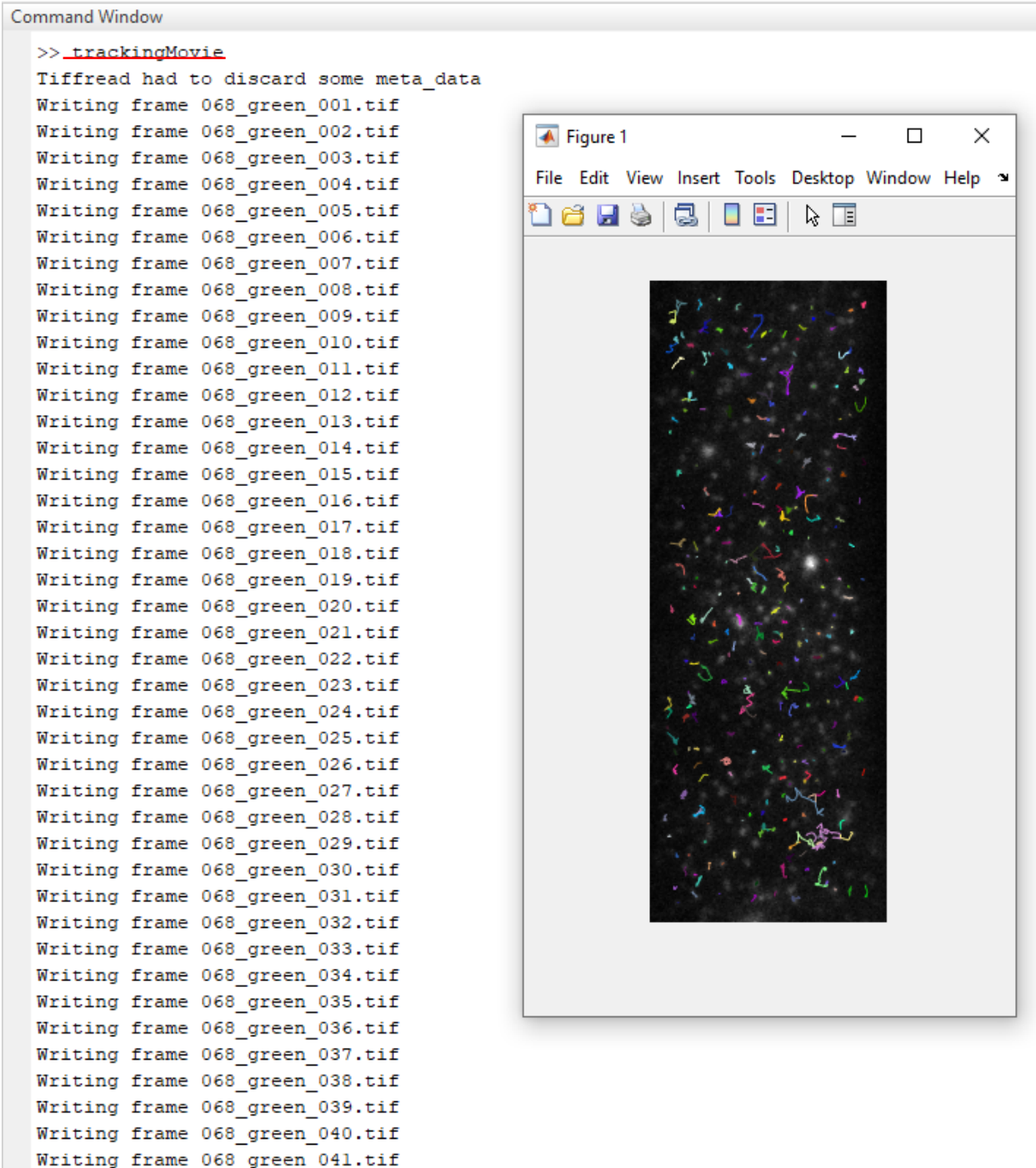
The output is;



And the key data is saved in these mat files;



To make a movie with the tracking enter trackingMovie into the command line (underlined in red). A figure window will open to show you the current frame being saved.



To use the cmeAnalysis package data viewer (this version only works on matlab > 2014) enter the following inputs (underlined in red);

Command Window

```
>> cmeDataViewer(data)
Loading frames ... done.
Loading detection masks ... done.
Loading tracks ... Tracking results found for this data set:
[1] ProcessedTracks.mat
[2] ProcessedTracks_Clean.mat
Please enter the number of the set to load: 1
done.
```

To combine the data from multiple experiments;

- Enter the program name
- Provide a name for combined data set
- Select which folder the data is in (in this case – example)
- Enter the number of cells you want to combine
- Select the parent folder (eg, 068) for each cell you want to combine, and press enter.

Command Window

```
>> combineSingChanData
Enter the protien or condition you are combing; CLC_Example
Enter the frame rate (eg 1s, 500ms): 1s
Enter the number of cells you want to combine: 2
```

```
combinedlifetimeMean =
```

```
22.5108
```

```
combinedlifetimeSEM =
```

```
0.2294
```

```
noOfCellsCombined =
```

```
2
```

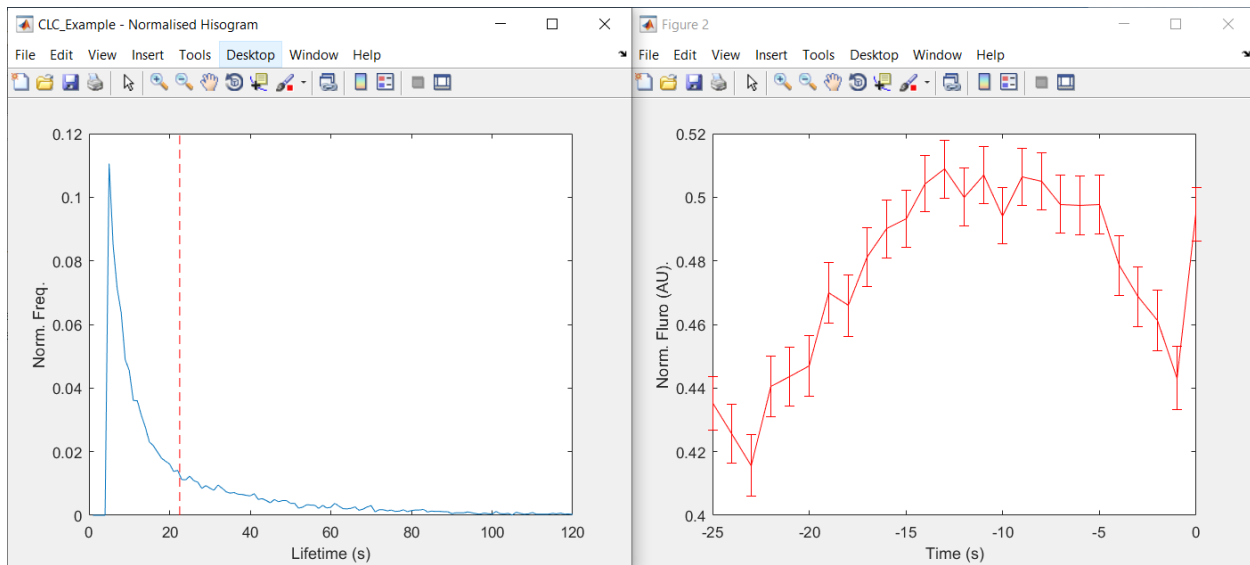
```
noOfCombinedEvents =
```

```
11588
```

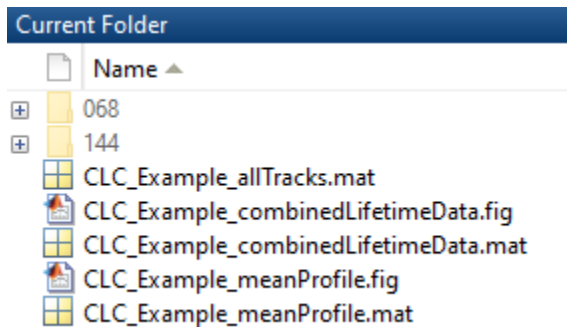
```
Current plot held
```

```
fx >> |
```

The output is;



And the key data is saved in the folder selected when asked ‘Select the folder with the data’;



Dual Channel – TPLATE x CLC TIRF Example

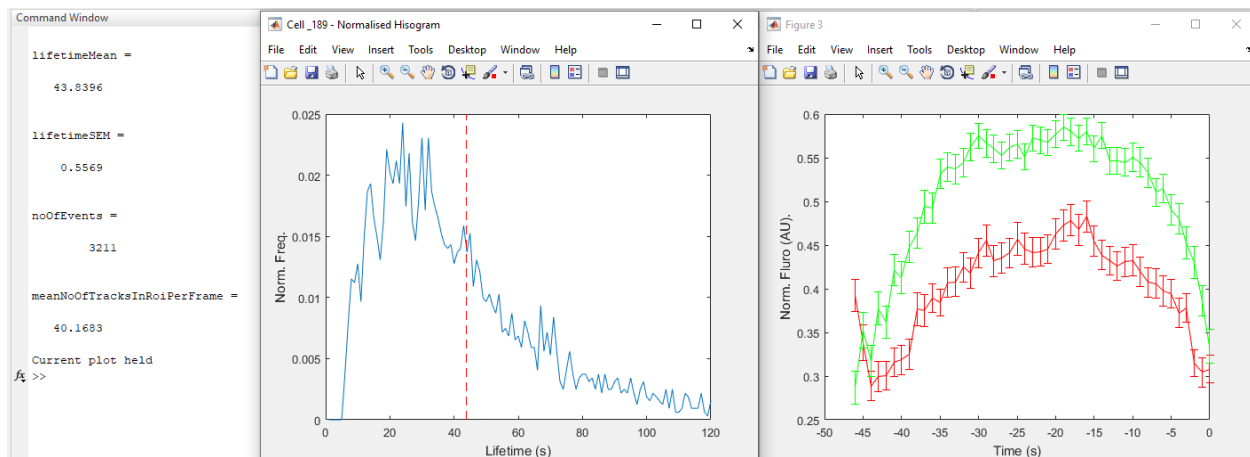
Red is CLC2 and green is TPLATE, and red should be used as the primary (master) channel.

The analysis is started by entering;

```
>> dualChan_cellSurfaceAnalysis
```

Follow the prompts, enter the data and select the appropriate folders as detailed above. The precise details of the experimental system are; NA – 1.49, Mag – 100, Pixel size – 16, no of channels – 2, Red master, Green slave, channel 1 marker – RFP, channel 2 marker – EGFP

The output is;



Then the trackingMovie program was run using the red movie.

Persistence test example

The example movies have already been analyzed using singChan_cellSurfaceAnalysis.

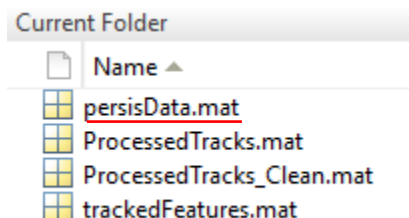
Run;

```
>> persistTest
```

Select the raw tracking file (.../Experiment/Condition/cell132_1s/tracking/ProcessedTracks.mat)

Select the cellInfo file (.../Experiment/Condition/cellInfo.mat)

The command window will display the result, but it is also saved here (underlined in red)



Then run;

```
>> persisPlot
```

Select the movie

Select the persisData.mat file