# Formation of synthetic RNA protein granules using engineered phage-coat-protein -RNA complexes

## Full pipeline walkthrough

**Authors:** Naor Granik[1], Noa Katz[2], Or Willinger[2], Sarah Goldberg[2], and Roee Amit[2,3*]

**Affiliations:**

[1] Department of Applied Mathematics, Technion - Israel Institute of Technology, Haifa 32000, Israel.

[2] Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Haifa 32000, Israel.

[3] The Russell Berrie Nanotechnology Institute, Technion - Israel Institute of Technology, Haifa 32000, Israel.

***** To whom correspondence should be addressed: roeeamit@technion.ac.il.

# Prerequisites

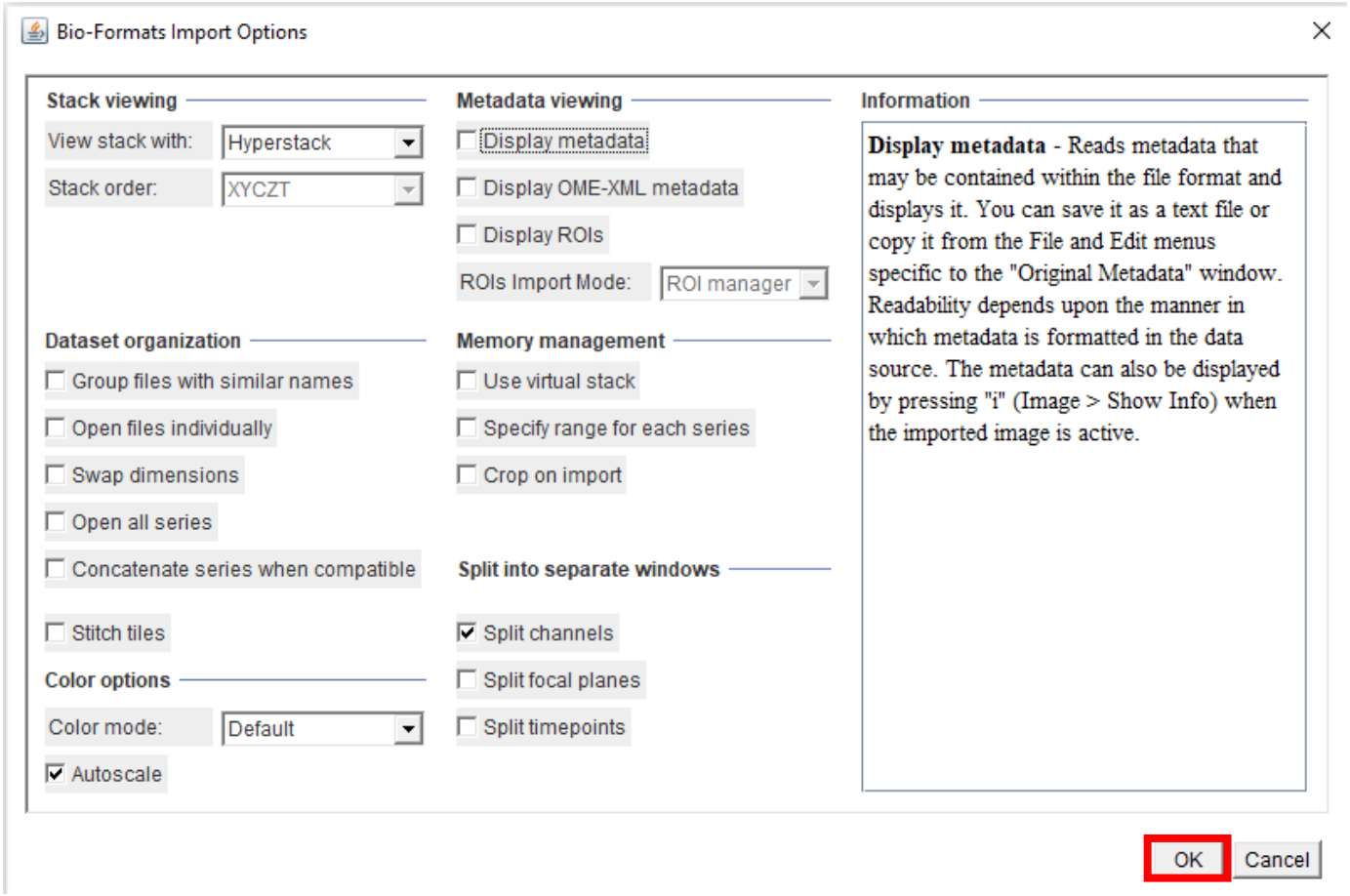
- The scripts and instructions provided here were tested on Matlab 2021a 64-bit with the following additions:
  - Curve Fitting Toolbox
  - Image Processing Toolbox
  - Statistics and Machine Learning Toolbox
- In addition, ImageJ FIJI (specifically, the Mosaic plugin) was used to read microscopy image files, detect bright spots, and convert to the formats required for the scripts.
- All scripts were built and tested in a Microsoft Windows 10 version 21H1 environment.

# Microscopy image files

- All microscopy files analyzed using the provided scripts were captured on a Nikon Eclipse Ti-E epifluorescent microscope with a 100x 1.45 NA oil immersion objective.
- Images were captured every 10 seconds for a duration of 1 hour. As such, the provided scripts are hard-coded for these settings.
- Alteration of the temporal definitions, and/or magnification requires changing of the scripts to accommodate for the new settings.

# ImageJ processing

- Open the microscopy file in imageJ-FIJI.
- Select OK in the Bio-Formats import window.

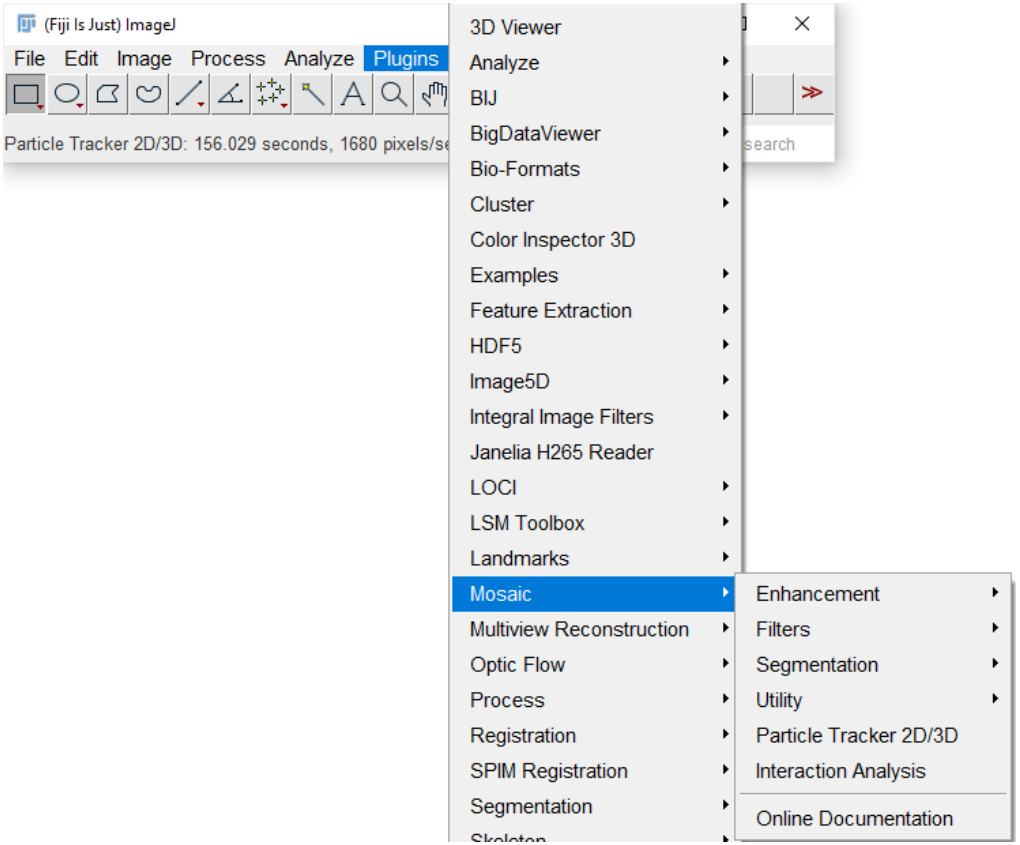


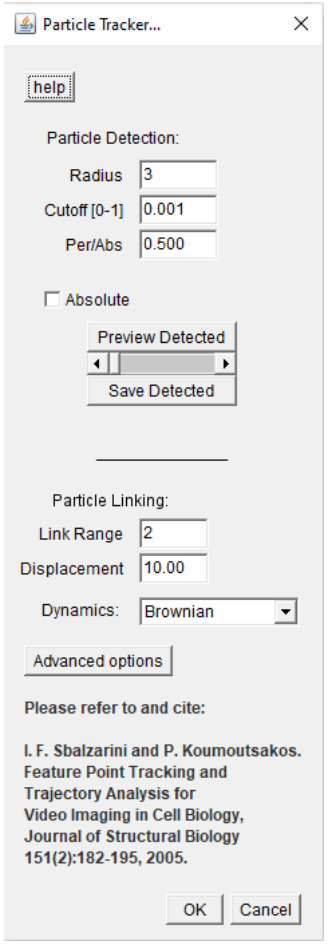
# ImageJ processing

- By default, each measured channel is opened in a separate window.
- Images might appear black or oversaturated due to viewing settings. You can change display setting by going to Image -> Adjust -> Brightness/contrast. You can select ‘Auto’ in the popup window or select the display limits yourself. This does no change the file in any way.
- **Save each channel separately as a .tiff file.**

# ImageJ processing

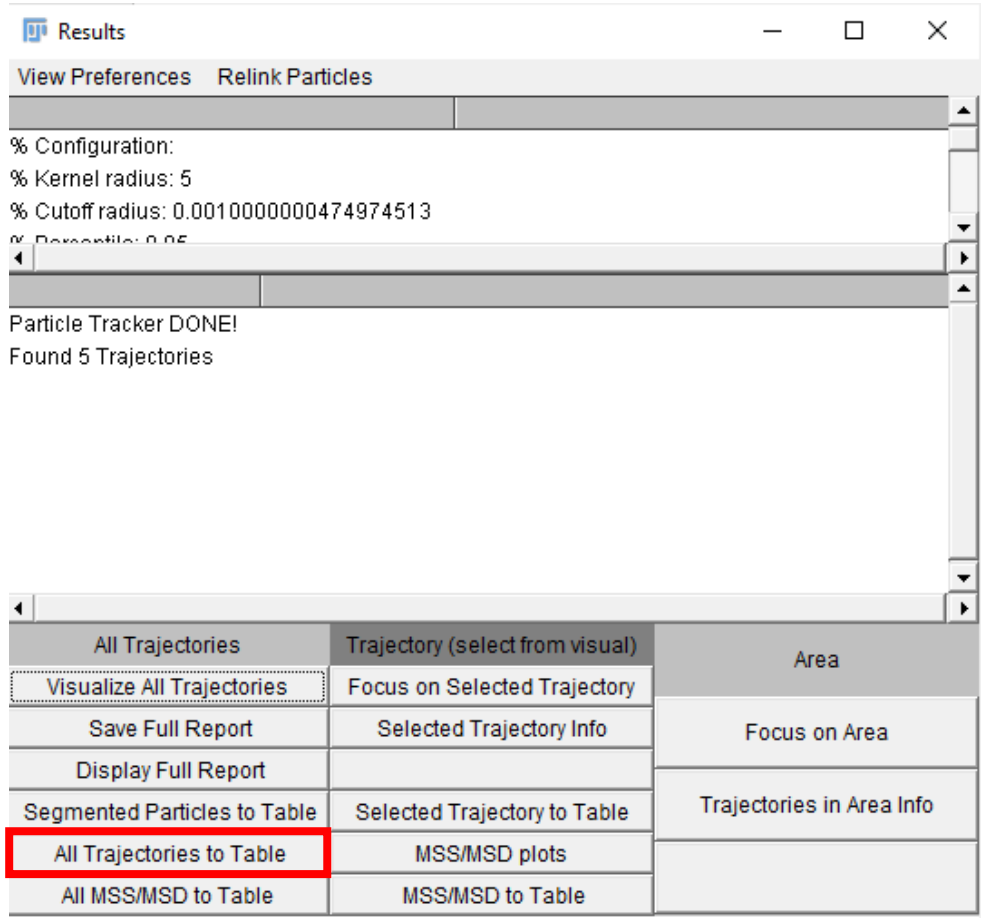
- For each channel separately, go to **Plugins -> Mosaic -> Particle Tracker 2D/3D**
- Particle Tracker identifies points of high fluorescence and tracks their movement over time (if there is any). The output is a matrix of spatiotemporal coordinates for each point.
- Fill in the parameters for particle detection - **our recommended settings:**
  - **Radius: 5**
  - **Cutoff 0.001**
  - **Per/Abs: 0.05**
- **Manual inspection is recommended** to verify that all visible high fluorescence points are captured in first frame (easily visible using the Preview Detected button).

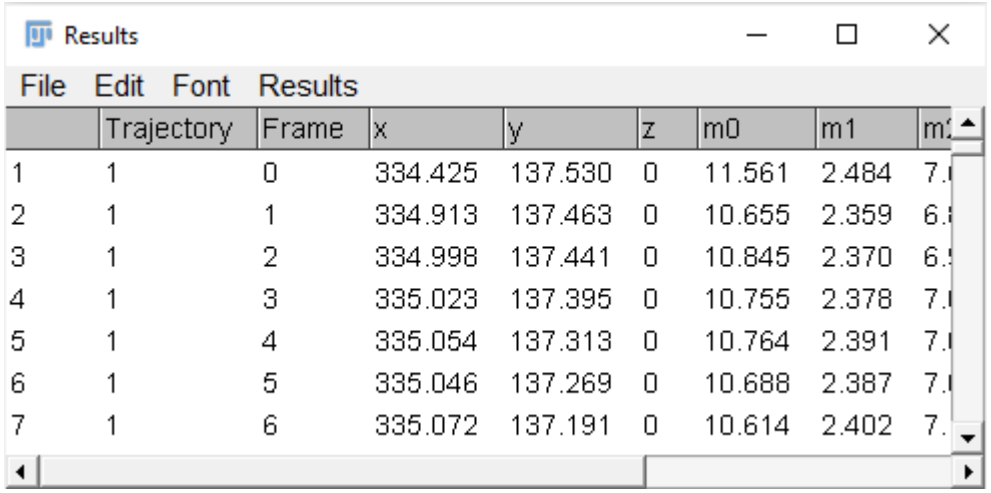


# ImageJ processing

- The results of Particle Tracker are presented in a new window, stating how many trajectories were found.
- Save the trajectory data by clicking on '**All Trajectories to Table**', and in the new window that appears clicking on File -> Save as.
- Files are automatically saved as .csv




| | Trajectory | Frame | x | y | z | m0 | m1 | m2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 334.425 | 137.530 | 0 | 11.561 | 2.484 | 7. |
| 2 | 1 | 1 | 334.913 | 137.463 | 0 | 10.655 | 2.359 | 6. |
| 3 | 1 | 2 | 334.998 | 137.441 | 0 | 10.845 | 2.370 | 6. |
| 4 | 1 | 3 | 335.023 | 137.395 | 0 | 10.755 | 2.378 | 7. |
| 5 | 1 | 4 | 335.054 | 137.313 | 0 | 10.764 | 2.391 | 7. |
| 6 | 1 | 5 | 335.046 | 137.269 | 0 | 10.688 | 2.387 | 7. |
| 7 | 1 | 6 | 335.072 | 137.191 | 0 | 10.614 | 2.402 | 7. |

# Matlab processing

- Step I: Run script named: read_tables.m
  - Extracts the (x,y,frame) data from the .csv file generated by Mosaic particle tracker and save as a .mat file.
  - **Script file should be in the same folder as the .csv files.**
  - Upon completion (matter of seconds), a .mat file will be generated for each .csv file in the folder.
  - **Note that all .csv files in the folder should be outputs from Mosaic particle tracker as the script assumes a certain file structure.**

# Matlab processing

- Step II: Run script named: Collect_intensity_data.m
  - The script loads all .tiff and .mat files containing (x,y,frame) data about detected fluorescent granules. The data from each movie is converted to an array of images, and the fluorescence data (intensity of the granule, and intensity of its local environment) from each granule is collected and saved for further analysis.
  - The script operates under the assumption that .tiff and .mat files are in the same alphabetical order. It is therefore recommended to use numbers when naming the files.
  - The script saves 2 files into the current folder:
    - Granule intensity values are saved in a *_spot.mat file
    - Background intensity values are saved in a *_background.mat file

# Matlab processing

- Step III: Run script named: Collect_statistical_data.m
  - This script takes as input granule intensity files, and background intensity files. For each signal (each granule), significant entry and exit events are found and their parameters measured. The output is a .mat file containing amplitude data and temporal data about burst events.
  - Upon running, the script will open two dialogue boxes in succession.
    - The first will ask for a signal file - this is the file with ‘spot’ in the name generated in the previous step.
    - The second will ask for a background file - this is the file with ‘background’ in the name generated in the previous step.

# Further parameter settings

- Plotting variables
  - plot_max – Maximum number of signals to plot (can be zero).
- Segment classification variables
  - match_probability – look for segments of nearly continues increment/decrement with a probability of occurrence lower than $\frac{1}{2^{match}_{probability}}$
  - Window_span – moving average filter span.

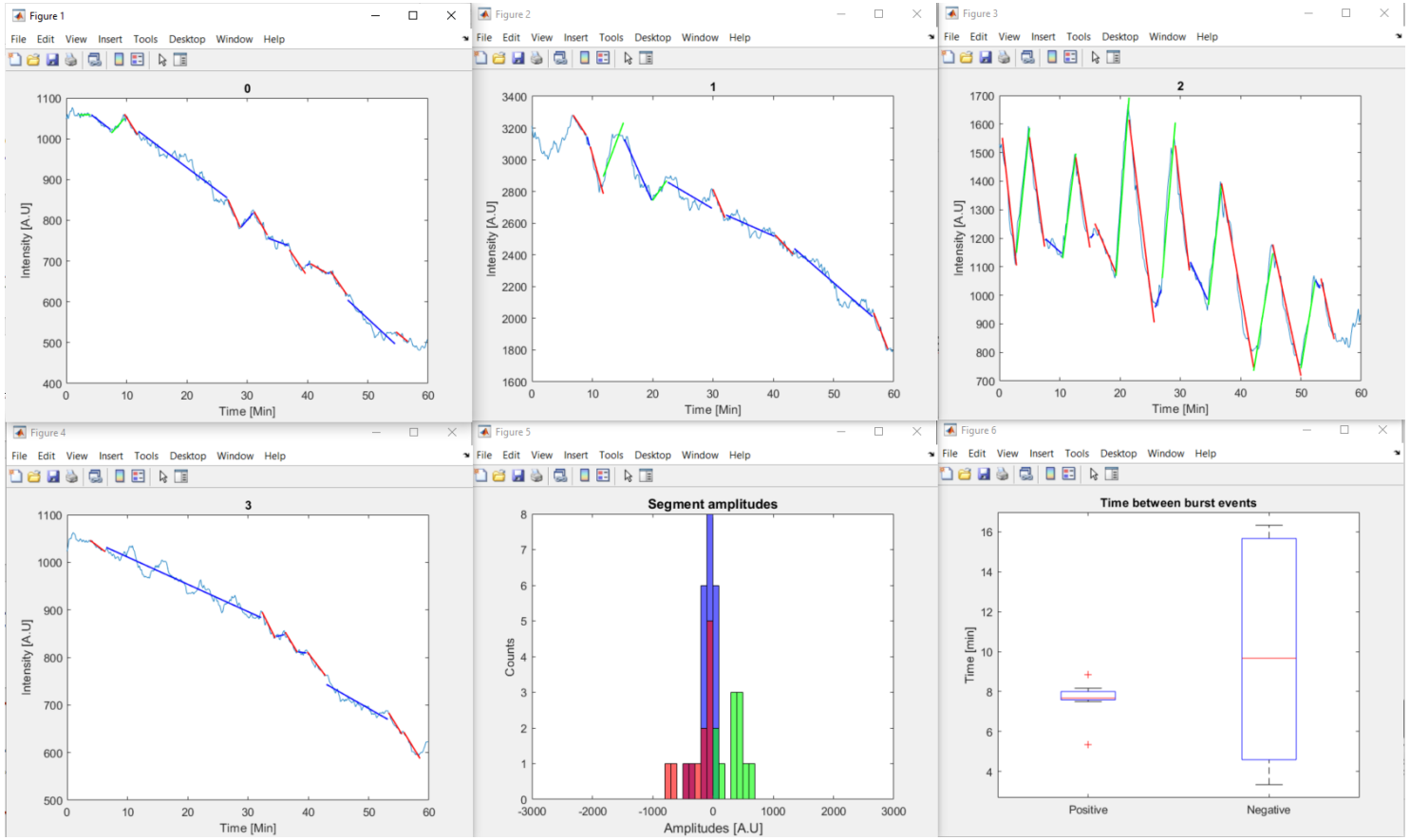
# Demo results

# Provided input

- The provided scripts are accompanied by microscopy output files– a 488 [nm] (green) measurement (corresponding to the Atto-488 labeled RNA), and a 585 [nm] (red) measurement (corresponding to the mCherry protein), of the same field of view
  The files can be found in the provided folder:
  - ./8x/green/ - RNA tracking files
  - ./8x/red/ - Protein tracking files

# Running the Demo

- To start from the image files, load the files into imageJ-FIJI and start from page 4 of this manual.
- To start directly from Matlab, start from page 9 of this manual as the .mat files needed are provided as well.
- The scripts can be run as is without any alterations.
- After running the colletct_statistical_data.m script, the following plots should appear when running on the red files:



# Running on your own files

- ImageJ processing should be handled manually on a case-by-case basis to ensure that fluorescent spots are identified and tracked correctly.
- Although the scripts are hard-coded to specific experimental settings, they can be made to fit by changing 2 variables.
  - Sub_frame_length variable which is defined in collect_intensity_data, determines the local environment of spot from which to calculate background intensity. This variable should be changed when changing the experimental system.
  - timeVectorTotal variable which is defined in collect_statistical_data, determines the temporal perspective of the experiment. It is presently defined as 60 minutes with a 10 second interval between time points and can be altered according to whatever duration and interval are used in the experimental system.