

README for running the various analysis functions in the CondensateAnalysis repository, as employed in Ditlev, Vega, Koster et al., ELife 2019. A composition-dependent molecular clutch between T cell signaling condensates and actin. <https://pubmed.ncbi.nlm.nih.gov/31268421/>.

The CondensateAnalysis repository includes new functions that were developed specifically for this work, or functions employed in this work but not previously published. While most functions will most likely run on any OS, we have only run them on Linux.

Previously published analyses are not included here. Instead, they can be downloaded separately from their own repositories, as specified in the manuscript.

Below are the various functions supplied in CondensateAnalysis. They are numbered following the organization of the Data Analysis and Display subsection of the Materials and Methods section of the manuscript (skipped numbers belong to previously-published functions), where the algorithms are also described in detail.

#### **1. Drift Correction for In Vitro Movies**

- scriptCorrectChannelDrift

#### **2a. Detection of pLAT Condensates**

- detectThreshLocMax

#### **4a. Synapse and cSMAC Segmentation**

- calcCellBoundaryImage

#### **4d. LAT Condensate Composition Analysis**

##### **& 4e. Measuring Deviation from Straight Path for Condensate Tracks**

- mapIntensity
- multiChannelTrackAnalysis:
- multiChannelPlotTime

NOTE: To run the functions in 4d and 4e, please download the particle tracking package u-track (<https://github.com/DanuserLab/u-track>), run the detection and tracking steps of u-track first, and then run the functions listed in 4d and 4e.