This note accompanies codes used for characterizing the microscopy image series either Image Correlation Spectroscopy (ICS) or Single Particle Tracking (SPT) and was used in the manuscript submitted to Nature Communications titled “Dynamics of T cell receptor clustering detected by intermolecular FRET”.

There are two main Matlab scripts included in this submission, *LoadTrackingDataAndGetTrajectoriesCharacteristics* and *DAfromImageSeries*. First one is used for importing the SPT data from Diatrack (http://www.diatrack.org/) and extracting the information from trajectories of clusters. The second is for computing ICS correlation function per frame and corresponding degree of aggregation (DA).

Ensure that all the included m files are included in Matlab directory that is added and saved to the Matlab path file.

These scripts prompt user to select a multi tif files for donor and acceptor channels but can be adapted to import other types of image files, such as lsm or czi raw data (see BioFormats for Matlab online).