This protocol describes how to do state array analysis on PAPA and DR trajectories from an automated experiment.

# Required code

* Copy into each data folder:
  + segment\_and\_measure.py (if segmentation and measurement of cell ROIs have not already occurred while the macro was running)
  + cellpicker.m (version 6, or equivalent)
  + callCellPicker.m
  + sort\_selected\_trajectories.py
* Copy into an analysis folder:
  + autosmt\_utils.py
  + analysis\_settings.toml
  + autosmt\_papa\_saspt.ipynb

# Analysis steps

1. In each data folder:
   1. If segmentation and measurement have not already been done by realtime\_analysis.py script while the macro was running, then run segment\_and\_measure.py. Alternatively, run segment\_measure\_time.py to also retrieve timestamps from nd2 files.
      1. **Important: Rename any existing folder called “masks”**
      2. This will generate two output folders called “masks” and “roi\_measurements”
   2. Do cell picking with cellpicker, which gives the output file out.mat.
   3. Run sort\_selected\_trajectories.py from the command line. This takes as input the out.mat file from the previous step and exports csv files for each selected cell. CSV files for cells classified by the user in different categories are exported in different numbered folders.
2. In an analysis folder:
   1. In analysis\_settings.toml,
      1. enter source data directories for different conditions and different experiments within each condition.
         1. csv\_folder: Location of trajectory csvs sorted by cell from the previous step
         2. measfile: Location of csv file containing measurements for all cells. This is also in the cell sorting folder from the previous step
      2. Specify parameters for PAPA illumination sequence
      3. Specify sample\_size (maximum number of trajectories) and microscope focal depth.
   2. Run the notebook autosmt\_papa\_saspt.ipynb to do state array analysis on PAPA and DR trajectories in each condition.