**Automated Physiological Phenotyping of Individual Neuronal Types (APPOINT) analysis script**

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**Overview**

*In vitro* calcium imaging analysis generally utilizes manual segmentation of individual cells and manual annotation of positive and negative responses, or utilizes a single empiric threshold for quantification of responsive cells. This analysis platform utilizes machine learning approaches to remove bias from analysis of *in vitro* calcium imaging data. This analysis takes as input data from a custom MetaXpress analysis module which provides unbiased cell segmentation and measures calcium dye intensity over time in individual cells.

**System Requirements**

*Hardware Requirements*

The APPOINT analysis script requires a standard computer with sufficient RAM. For minimal performance, this will be a computer with about 8 GB of RAM. For optimal performance, we recommend a computer with the following specs:

RAM: 12+ GB  
CPU: 2+ cores, 2.5+ GHz/core

The runtimes below are generated using a computer with the 12 GB RAM and 2 cores@2.5 GHz.

*Software Requirements*

The software has only been tested on Windows 10 x64 computers.

All required packages should be compatible with Windows, Mac, and Linux operating systems.

Before running APPOINT analysis, users should have R version 3.4.0 or higher, RStudio v1.0 or higher, and several packages set up from CRAN.

**Installation Guide**

*Installing R and RStudio on Windows*

The latest version of R can be downloaded and installed from <https://cran.r-project.org/>

RStudio provides a convenient graphic user interface for R and can be installed from <https://rstudio.com/products/rstudio/download/>

*Installing required R packages*

Instructions for installing all required packages are included in the APPOINT analysis script. The command (line 7) must be un-commented prior to running and should be re-commented upon successful installation.

Install times will vary depending on previously installed packages.

Specific versions of each package that have been tested are noted within the script.

**Instructions for use**

Raw data must be generated from MetaXpress custom analysis pipeline or other compatible image analysis software. Raw data should be in long format with individual columns denoting imaging time or image number within timelapse stack, individual cell identifier, and object intensity within specified stack image. Each unique image stack should generate an individual .csv file and all .csv files for an experiment should be placed into a folder titled “Data”. An additional folder title “Metadata” containing a single .csv file should be placed in the same folder containing the “Data” folder. The metadata .csv file should contain two columns containing a unique identifier for each image stack and the experimental condition for that sample.

Prior to running the analysis in R, the file path (line 50) must be updated to point to the folder containing the “Data” and “Metadata” folders. The final two sections of the script “Organize final results for plotting” and “Plot final results” must be modified for the specific conditions tested in the experiment to reflect the desired comparisons.