# Features and methods introduction

* Image processing
  + Alignment
  + Deconvolution
  + Baseline adjustment
  + Denoise
  + Background adjustment
  + ΔF/F presentation
* Spatialtemporal analysis
  + Peak location identification
  + Peak extraction
  + Peak maximum response analysis
  + Peak exponential decay analysis

# Dependencies

The following JAR File has to be added to the java folder of Matlab

* [DeconvolutionLab\_2](http://bigwww.epfl.ch/deconvolution/deconvolutionlab2/)

Additional Matlab toolboxes are also required

* Curve Fitting Toolbox
* Statistics and Machine Learning Toolbox

# Input and output data

Input

* The default setting for input is in “.tif” format
* The images of one cell should be in the same folder number by a number (eg. 1), and each image should be named in sequence (eg. 1\_1, 1\_2,...)

Output

* Folders
  + \_edited\_1: aligned image from input
  + \_edited\_2: deconvolved from \_edited\_1
  + \_edited\_3: baseline adjusted from \_edited\_2
  + \_edited\_4: denoised from \_edited\_3
  + \_edited\_5: ΔF/F calculated from \_edited\_4
  + \_edited\_6; background adjusted ΔF/F calculated from \_edited\_5
* Images
  + Overlaid ΔF/F color image on cell image: currently saved in A6 folder as “xx\_xxxbmp\_transparant.png”
  + 3D image: manually save to PNG from matlab image after running jerky
  + Peak extraction: manually save to PNG from matlab image after running jerky
* Peak quantification
  + The data of response against distance to the maximum response is currently saved as **jacktotal,** which is background adjusted.

# Usage

* Run A0\_TestRun.m in the A0 folder
  + This code will perform simple calculation of ΔF/F and present results in color images
  + Proceed only if there are noticeable response without significant movements and noises
  + Manual options
    - The default setting for the start and end of the images are 401 and 1001, and they can be changed at the beginning of A0\_TestRun.m
* Run A6\_1main.m in the A6 folder
  + This code will perform alignment, deconvolution, baseline adjustment, denoise, and present ΔF/F in color images without background adjustment
  + Manual options
    - In STA5Calc.m line 40, change the noise level according to the setup and image quality to achieve cleaner images that show individual peaks
    - The default setting for the start and end of the images are 401 and 1001, and they can be changed at the beginning of A6\_1main.m
    - The default setting for the response starts at 501
* Run A6\_2F6.m
  + This code will present ΔF/F in color images after background adjustment
  + The background level is uniformly assumed to be the minimum value of the image before the response
  + Manual options
    - In line 18, the background value can be manually changed if the background is not uniform
* Run A6\_3Overlay.m
  + This code will overlay the one ΔF/F color image of choice on one denoised cell image for peak location identification
  + Manual options
    - Slice number can be changed at the beginning
    - In line 15, the range of bw can be changed to overlay large response
* Run the function jerky
  + This code will present the 3D image
* Run A6\_4PeakIdentification.m
  + This code will identify clusters of response using DBSCAN algorithm
  + The default epsilon and minpts were set to accommodate for individual peaks with a decay constant around 1
  + Manual option
    - In STAPeak.m, in line xx and xx, epsilon and minpts can be adjusted for clusters of different size
* Run A6\_5Pumpkintest.m
  + This code will plot the response against the distance to the maximum response point of a peak of selection
  + Input requires circling the region of interest
  + The output response will be adjusted by eliminating the background (similar in A6\_2F6)

# Practical suggestions