Installation Requirements:

- Matlab (v 2011 or higher)
- Java Runtime Environment (v 1.7 or higher)

1. Download the “Cropping and Visualization Tools” folder
    a. Open “CustomCrop_1.m”
        i. Edit input file name “fname1”
    b. Run “CustomCrop_1.m”
        i. It should generate an image of the input file
        ii. Draw a line around the area of interest
            - Choose a single cell to draw around
            - This generates the mask that is used in CustomCrop_2.m
    c. Open “CustomCrop_2.m”
        i. Edit output file name “fname2”
    d. Run “CustomCrop_2.m”
        i. This generates output images of the area of interest a blurred edge
            - The blurring can be made sharper or smoother by editing the “blurmask” variable
    e. Open “AntibodyShadowCrop.m” (to generate the same crop on separate antibody images)
        i. Edit input file name “fname3”
        ii. Edit output file name “fname4”
    f. Run “AntibodyShadowCrop.m”

2. Download the “Cell Image Analysis” folder (separate user guide included in the folder for more details)
    a. Create a folder inside the “data” folder with appropriate experiment name (eg. “test”)
    b. Specify the path of Matlab in the “config” file
    c. Can create multiple such folders and run together
    d. Create a folder named “actin” inside the data>“test” folder
    e. Put all the actin images in the “actin” folder
        i. Use files generated as output files from Step 1
        ii. Preferable format: .tif
    f. Open the Jar file “FilamentsAnalysis_v0.9_debug”
        i. Click on the “Add” button
            - This will open a separate GUI window
            - Write the name of the condition/experiment
            - Click the button next to the “Filaments Image”
            - Choose the proper “actin” folder
        ii. Multiple conditions can be added one after another

iii. Choose the parameters in the GUI (example screenshot provided"

g. Click "Run All"
    i. Progress of the analysis can be monitored
h. Output files will be stored on a folder named "res_filaments" inside the "actin" folder
i. The statistical information regarding single filaments is stored in the file named "dataM-[filename].tif.mat"

3. Download the "OOP Tools" folder

4. Preparing Angle List
    a. Open "get_angles_for_OOP.m"
        i. Edit input file name "fname1"
            - The input name should be same as the statistical output file from the Cell Image Analysis package ("dataM-[filename].tif.mat)
    b. Edit output file name "fname2"
    c. Run "get_angles_for_OOP.m"
    d. If a batch of output files need to be processed together, use the "get_angles_for_OOP_batch.m"
    e. Save all the output files in a specific folder (eg. "angles")

5. Calculate Orientational Order Parameter
    a. Open "OOP_maincode.m"
    b. Run "OOP_maincode.m"
    c. Choose the folder in which output files from Step 5 were saved ("angles")
    d. Enter the number of different conditions that will be analyzed
    e. Enter the name of the first condition
    f. Choose all the files (in the "angles" folder) that belong to the same condition
    g. Repeat for all the conditions
    h. Choose a name for the output/summary file
    i. It will create a file "[summaryname].mat"
    j. Open "[summaryname].mat"
    k. Average OOP values for individual conditions are stored in "OOP_cond" variable
    l. Standard deviation values for individual conditions are stored in "OOP_E" variable
    m. Each column in the OOP_VEC corresponds to the OOP values of individual cells in that condition
        i. The third column will contain OOP values of individual cells in condition #3
    n. Number of cells analyzed in each condition is stored in the variable "num_cs"