Robust Quantitative Scratch Assay: Instructions and Descriptions of Scripts.

To run in MATLAB, the Image Processing and Statistics Toolboxes are required.

To run in Octave (GUI), the Image package is required.

1. All images should be organized in subfolders by well number and condition as follows:

- Parent Directory
  - Experiment Name
    - Condition1: Non-treated
      - Well 1
        - Frame 1 …
        - Frame n
      - Well N
    - Condition 2: Cisplatin
      - Well 1
        - Frame 1 …
      - Well n

2. In **Start_run_all_conditions.m** enter the Parent Directory and Experiment Name and Experiment name in lines 2 and 3, respectively.

The RQSA Start_run_all_conditions is divided into four modules:

1) Image processing: processing of individual images in all wells over all experimental conditions. Arrays of area values are created, saved to : ‘Parent Directory\Results_*Experiment_name*\Processed_*Condition name*\ Areaarrays_*Condition’*. Processed images are saved under: ‘Parent Directory\Results_*Experiment_name*\Processed_*Condition name*\procc_*well name’*
2) Polynomial fits of all area arrays. Saves information of fits, plots arrays with fit. Saved to: ‘Parent Directory\Results_*Experiment_name*\Areatimeplots _*Condition’*
3) Statistical analysis, estimate of average migration rates, standard deviations, uncertainties in parameters and identification of potential outliers. Text files of analysis per condition are saved to: ‘Parent Directory\Results_*Experiment_name*
4) Final plotting of average migration rates and standard deviations of each condition.
   Note: Name of conditions can be manually entered for the final plot

Description of scripts:

1. **Single_frame _area():** Calculates open wound area for an individual frame using structural elements and threshold parameters set by algorithm. Processed image is saved under: ‘Parent Directory\Results_*Experiment_name*\Processed_*Condition name*\procc_*well name*’.

2. **Well_avg_area():** Creates open wound area vs. time array for a well and saves it under: ‘Parent Directory\Results_*Experiment_name*\Areaarrays_*Condition name*’.

3. **Polynomial_fit():** Takes well area arrays and determines polynomial fits,outputs polynomial information (coefficients and confidence values, well labels) using Degree_polynomial().

4. **Degree_of_polynomial():** Fits polynomials of degree 1 through 4 on open wound area vs. frame arrays. Saves plot of raw data and information of the fit under: ‘ParentDirectory\Results_*Experiment_name*\Areatimeplots_*Condition name*’.

5. **Single_condition():** Organizes data of a single well for processing, creates folder to save processed images .

6. **Stat_analysis()**: Determines outliers, rates of change, and statistics using all wells of one condition, prints them to a text file, creates frequency of polynomial degree graph and creates an array with only migration rates and errors. All are saved under: ‘Parent Directory\Results_*Experiment_name*’.

7. **Check_islands_holes():** Checks for stray cells in the wound area for removal and checks for holes in the cell-covered area for filling.

8. **Create_directories()**: Creates all subfolders to save all processed data.