

MC_QuiescenceActivity_v1202 Readme
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Preview images tab

This tab allows you to scroll between images to visually inspect the images

1. Load image

Requires sequentially numbered images of the format: Image0001.bmp, Image0002.bmp, etc.

2. Scroll between images using the keyboard keys indicated in the program

Process images tab

This tab calculates activity in user selected ROIs and saves the data in a .mat file. Activity is calculated by subtracting sequential images, smoothing the difference image with a 2-D Gaussian filter, thresholding, and summing the number of pixels within a region of interest in which a change in intensity occurred. Therefore, the activity is a single number for each region of interest between each sequential image pair. With the frame skip option (described below) you can choose an image interval other than sequential to calculate the activity between. The code enables you to preview the smoothed and thresholded image for visual assessment of noise and image resolution prior to processing.

1. Click load image

a) Load the first image in the sequence to be processed

2. Select ROI

a) Choose Auto selection (default). This assumes a rectangular grid of evenly spaced wells

b) Designate the number of rows and columns in the grid

c) Follow the instructions in the program to choose ROI size and locations

i) Click the center of the wells in the upper left, upper right, lower left, and lower right

ii) Finally, choose the upper left and lower right boundaries of a single well (to calculate well dimensions)

d) Adjust ROI size or ROI centers using the push buttons

e) When finished, click Finish Adjustment

3. Set Parameters

a) Designate start and end frame numbers (if unsure of end frame, choose a large number (~100000))

b) Input the time/frame in seconds

c) Input image file prefix

d) Choose threshold for activity analysis (0.2 is good to start, this value can be adjusted later)

e) Input spatial filter size (1 is good to start, this can be adjusted later)

f) Input frame skip: This parameter enables choosing an interval to calculate image subtraction between. The program automatically records image subtraction between adjacent frames. The Frame Skip option enables you to calculate activity between another image interval (e.g. 10)

g) Indicate off-line or real-time analysis (Choose real-time)

h) Autosave per N frames (auto-save after N images are processed. A low value here is preferable in case MATLAB crashes. N=50 is a good choice)

4. Display thresholded image

a) The program will display the thresholded image. If noise is apparent, type 0 to return to the set parameters window and adjust the noise threshold or spatial averaging filter size accordingly. If satisfied with thresholded image, type 1 and continue.

5. Save file

a) After accepting the thresholded image, you will be prompted to name the save file and choose the directory to save in.

6. Start Analysis

a) Analysis begins and will auto-save every N frames as designated above

Output

The saved .mat file will contain a number of saved variables.

The sequential image activity is saved in the variable **ActVal**, where the first and second matrix dimensions represent image # and region of interest, respectively.

The frame skipped image activity is saved in the variable **ActValS**.

The time stamp for each image (the time it was recorded) is saved in the vectors **dayRecorded**, **hourRecorded**, **minuteRecorded**, and **secondRecorded**. With these vectors you can reconstruct the continuous time vector associated with the activity matrix. Ideally the interval between all consecutive images is the same and you can assume the data points are separated by a given delta t.