SMALL-LABS Quick Start Guide

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This Quick Start Guide will show you how to analyze the test data that we have provided as an example. The steps outlined here will familiarize you with the basic operations of SMALL-LABS. Please see the User Guide for more details and discussion of various options and parameters.

Installation

Clone or download (and unzip if downloading) the SMALL-LABS directory: click the green button marked *Clone or download* at https://github.com/BiteenMatlab/SMALL-LABS

Running SMALL-LABS

1. Set the Matlab working directory to the SMALL-LABS directory
2. First let's run SMALL-LABS and check the guessing (detection) parameters. Run *SMALLLABS_main.m* with the following command:

```
>>SMALLLABS_main('Test data and simulations', 7,...
400, 100, 'checkGuesses', true)
```

   (a) The first input is the *Test data and simulations* directory, which contains the movie.

   (b) The second input is the nominal diameter (in pixels) of a diffraction-limited spot; in this test data, a value of $\sim$7 works well. This input needs to be an integer.

   (c) The third input is the length of the temporal window (in frames) to be used for the average subtraction; for this movie, let's use 400 frames.

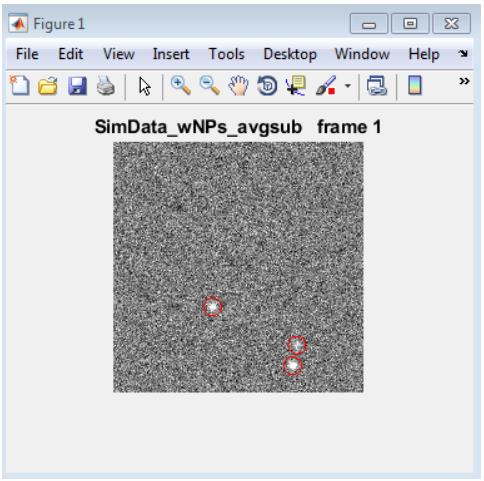
   (d) The fourth input is the length of the temporal window (in frames) to be used to make the off-frames list; for this movie, let's use 100 frames.

(e) Lastly, we want to check the guessing parameters, so we set the optional input `'checkGuesses'` to `true` (in *SMALLLABS_main.m* optional inputs are input as name-value pairs).

3. Choose a movie to analyze by clicking on *SimData_wNPs.mat* (which in Matlab appears as *SimData_wNPs*) then press enter.

   (a) This file contains a simulated single-molecule imaging movie with a background made of stationary (in time and space), bright punctate spots that look just single molecules.

4. SMALL-LABS will perform the average (approximate) subtraction, and then when guessing starts, the *Overall progress* indicator will pause and the following figure will appear. In this figure, red circles indicate guessed positions:



5. Look through the movie frames to check the guessing. This is accomplished either by pressing the *Continue* button  in the Matlab Editor tab, or entering `dbcont` in the command window.

   (a) You shouldn't have to look through the entire movie, but only enough frames to get a sense of false-positive and false-negative rates.

   (b) Note: The default guessing parameters are very accurate for this example (low false-positive and false-negative rates). However, to ensure the accuracy of the background subtraction in SMALL-LABS it is best to slightly over-guess (include more false-positives).

6. To include more false-positives, let's reduce the guessing threshold to 91% from its default value of 95%. Stop the program, either by pressing the *Quit Debugging* button  or entering `dbquit` in the command

window. Call *SMALLLABS_main.m* as before, but now with the additional optional parameter `'bpthrsh'` set to `91`:

```
>>SMALLLABS_main('Test data and simulations', 7,...
400, 100, 'checkGuesses', true, 'bpthrsh', 91)
```

(a) Note: when the movie selection window appears, be careful to still choose *SimData_wNPs.mat* (and NOT *SimData_wNPs_avgsub.mat*).

7. Go through the guesses again as before (you will notice increased false-positives, red circles where there are no molecules). After a few frames, if you're satisfied with the guessing results, stop the program as before (e.g., with `dbquit`).

8. Now let's fully run SMALL-LABS by changing `'checkGuesses'` to `false` or removing it entirely from the function call (as `false` is the default value):

```
>>SMALLLABS_main('Test data and simulations', 7,...
400, 100, 'bpthrsh', 91)
```

9. Once again choose the *SimData_wNPs.mat* movie. SMALL-LABS will now run fully, you will see progress indicated on the progress bar and in the command window. Note: depending on your computer this process could take some time.

   (a) If you have the Matlab Parallel Computing Toolbox, and don't already a parallel pool open, one will start up automatically. This can be a somewhat slow process, but only needs to happen once.

10. The final step in SMALL-LABS creates the *View-Fits* movie. You will see frames rapidly appear in Matlab, and when completed the file will be saved in the *Test data and simulations* directory as *SimData_wNPs_ViewFits.avi* which can be viewed later to qualitatively check the success of the fitting process.

    (a) In the *View-Fits* movie, green circles are fit locations that passed the default false-positive checks, red circles are guessed locations that did not pass those checks. The pink circles indicate molecules which were successfully tracked *and* passed the tracking filter described in the User Guide.

    (b) In this *View-Fits* movie, you will see that "real" molecules, which appear and disappear (blink/bleach) are successfully fit, whereas the stationary (nonblinking) fluorescent punctate spots that are part of the background are successfully removed by SMALL-LABS.

11. The measurement results (localizations, intensities, tracks, etc.) are stored in the same directory in a file called *SimData_wGNRs_AccBGSUB_fits.mat*; see the User Guide for more details.