SMALL-LABS Quick Start Guide

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This quick start guide shows how to analyze (provided) example data. The steps outlined here will familiarize you with the most basic operations of SMALL-LABS. Please see the User Guide for more details and discussion of various options and parameters.

> **Note**: Currently copying and pasting commands from this .pdf doesn't always work reliably, instead it's usually better to type the code directly.

## Installation

Download and unzip the SMALL-LABS directory

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 directory containing the movie, *Test data and simulations*

   (b) The second input is the nominal diameter (in pixels) of a diffraction limited spot; in this test data a value of ≈ 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.

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(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* (or *Sim-Data_wNPs.tif*) then press enter.

   (a) This is a simulated single-molecule imaging movie with a background made of stationary (in time and space) bright punctate spots (like fluorescent nanoparticles) that look just single molecules.

4. SMALL-LABS will perform the average subtraction and then when guessing starts you will see the following figure appear (where red circles indicate guessed molecular positions):
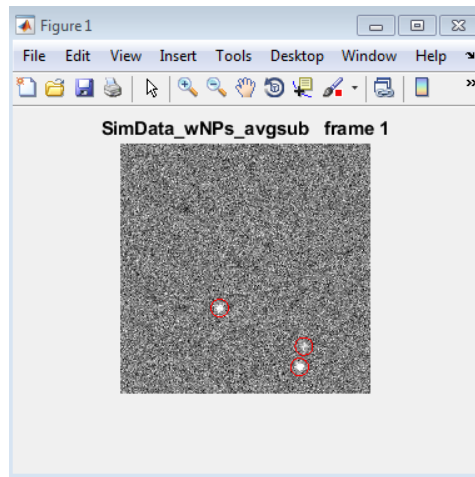
5. Look through the movie frames to check the guessing. This is accomplished either by pressing the *Continue* button in the editor tab, or entering `dbcont` in the command window.

   (a) You should see that the default guessing parameters appear to be quite accurate. However, in SMALL-LABS it's best to over-guess (include more false-positives) slightly.

6. To remedy this, let's reduce the guessing threshold to 91% from it's 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 you should still choose *SimData_wNPs.mat*; choosing *SimData_wNPs_avgsub.mat* would result in that movie underoging average subtraction, creating a *SimData_wNPs_avgsub_avgsub.mat* movie, which is not what we want.

7. Go through the guesses again as before, and if you're satisfied with the guessing results stop the program again. 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)
```

8. 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.

   (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.

9. The final step in SMALL-LABS is creating a *View-Fits* movie, you will see this 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 check the success of the fitting process.

   (a) In the *View-Fits* movie, green circles are fitted 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 track filter described in the User Guide.

   (b) In this *View-Fits* movie you will see molecules appearing and disappearing (blinking/bleaching) and several stationary fluorescent punctate spots which are part of the background that SMALL-LABS successfully removed.

10. The measurement results (localization, intensity, tracks, etc.) are stored in the same directory in a file called *SimData_wGNRs_AccBGSUB_fits.mat*, see the User Guide for more details.