BGSUBFIT Quick Start Guide

Installation

1. Download and unzip the BGSUBFIT directory.
2. Move the @TIFFStack directory to your MATLAB home directory
   a. Move it as is (leave it as a folder with that name). The TIFFStack README has more details if you have problems.
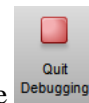
Running BGSUBFIT

The quick start guide shows how to fit the test data in the *Test data and simulations* directory. The steps outlined here will familiarize you with the most basic operations of BGSUBFIT; please see the User Guide for more details and discussion of various options and parameters.

1. Make sure that the Matlab working directory is the BGSUBFIT directory.
2. First let's check the guessing, using the default threshold. Call *BGSUBFit* with the following inputs:
   a. The first input is the directory containing the data, *Test data and simulations.*
   b. The second input is the nominal size (in pixels) of a diffraction limited spot, in this test data it is ~7. This input needs to be an integer.
   c. The third input is the width of the temporal window (in frames) to be used for the average subtraction; for this movie, let's use 99 frames.
   d. The fourth input is the width of the temporal window (in frames) to make the off-frame list; for this movie, let's use 50 frames.
   e. Let's check the guessing for this practice run, this means using the optional name-value pair *'check_guesses',1*
   f. Putting this all together, enter: *BGSUBFit('Test data and simulations',7,99,50,'check_guesses',1)*
3. Choose the movie to analyze
   a. A window will open allowing you to choose a .tif stack. Choose *SimData_wGNRs.tif*
4. The program will perform the average subtraction and then when the guessing starts you will see the following figure appear (where the red circles indicate guessed molecular positions):
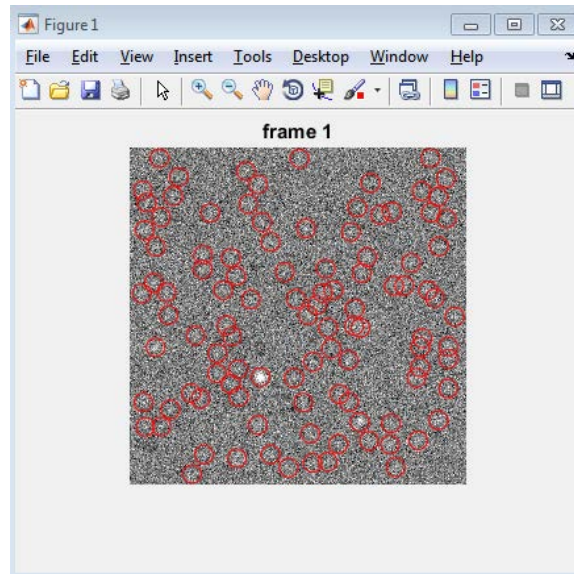
frame 1

5. You can click through the frames either using [Continue] button (in the editor tab) or by entering *dbcont* in the workspace. By doing this you will see that the threshold is set too low, *i.e.,* that the program has a lot of false positive guesses.
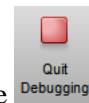
6. To remedy this, let's change the threshold. Stop the program, either by pressing the [Quit Debugging] button (in the editor tab) or by entering *dbquit* in the workspace. Then rerun the program using the same function call as previously, but adding in another name-value pair to change the threshold. For this movie, a value of 98 works well. Add *'bpthrsh',98* to the argument of BGSUBFit.
    a. Note: when the movie selection window opens you should choose the original movie, choosing the *SimData_wGNRs_avgsub.tif* movie would cause that movie to also have its average subtracted creating a *SimData_wGNRs_avgsub_avgsub.tif* movie.

7. If you're now satisfied with the threshold and the guessing, go ahead and stop the program again. Then remove the *'check_guesses',1* argument, or change it to *'check_guesses',0.* Running the program again will execute it fully.

8. You will see a movie called *SimData_wGNRs_ViewFits.avi* in the folder containing the data (*Test data and simulations*) when it is done. Note that in this movie, there are several stationary GNR point sources which look identical to single molecules, however they are not fit as they are part of a low frequency background.

9. The fits data are stored in a .mat file in the same directory called *SimData_wGNRs_AccBGSUB_fits.mat.* See the User Guide for more details.