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.

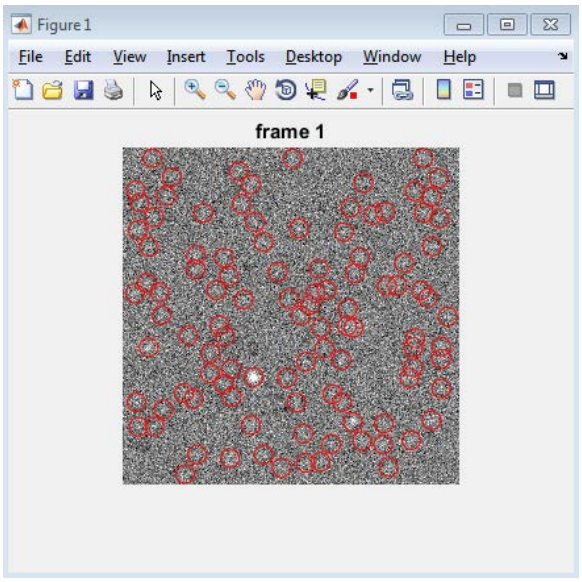
Running BGSUBFIT

In this quick start guide we'll be fitting test data in the *Test data and simulations* directory. The steps outlined here will familiarize you with the most basic operation 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 BGSUBFIT directory.
2. First let's check the guessing, using the default threshold. Call *BGSUBFit* with the following inputs:
   a. The first input being the directory containing the data (on my computer this is *C:\Users\isaacoff\Documents\BGSUBFit\Test data and simulations*)
   b. The second input is the nominal size of a diffraction limited spot, in this test data it is ~7 pixels
   c. The third input is the window size (in frames) to be used for the average subtraction, for this movie let's use 99 frames.
   d. The fourth input is the window size (in frames) to make the off-frame list, for this movie let's use 50 frames.
   e. Let's check the guessing, this means using the optional name-value pair *'check_guesses',1*
   f. Putting this all together, enter:

```
BGSUBFit('C:\Users\isaacoff\Documents\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



5. You can click through the frames either using 

button or by entering *dbcont* into the workspace. By doing this you should see that the threshold is too low, that the program has a lot of false positive guesses.

6. To remedy this lets change the threshold. Stop the program, either by pressing the 

button, or by entering *dbquit* into 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: 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 can see a viewfits movie called *SimData_wGNRs_ViewFits.avi* 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.
9. The fits data are stored in a .mat file called *SimData_wGNRs_AccBGSUB_fits.mat.* See the User Guide for more details.