SPtrack

Version 1.3

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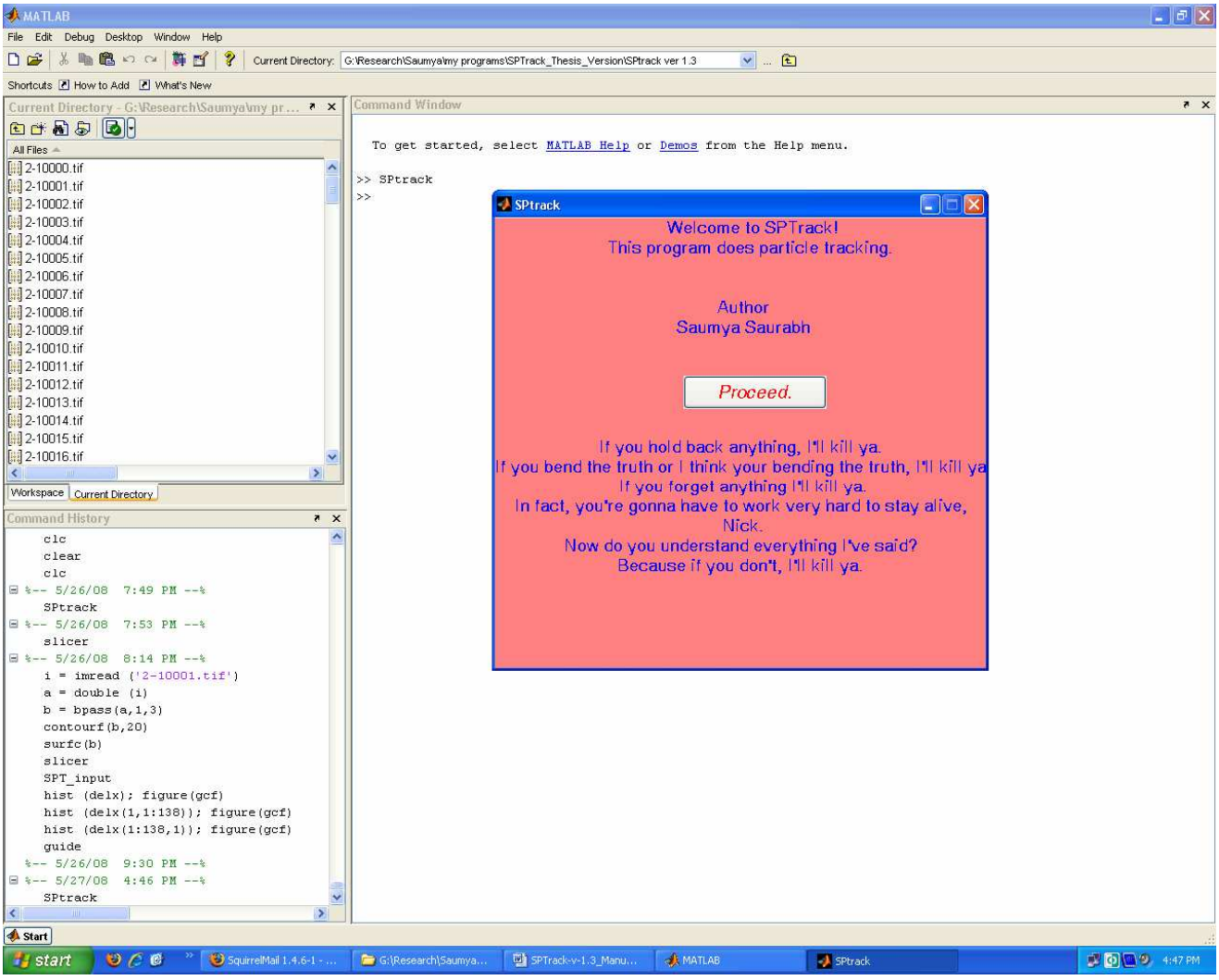
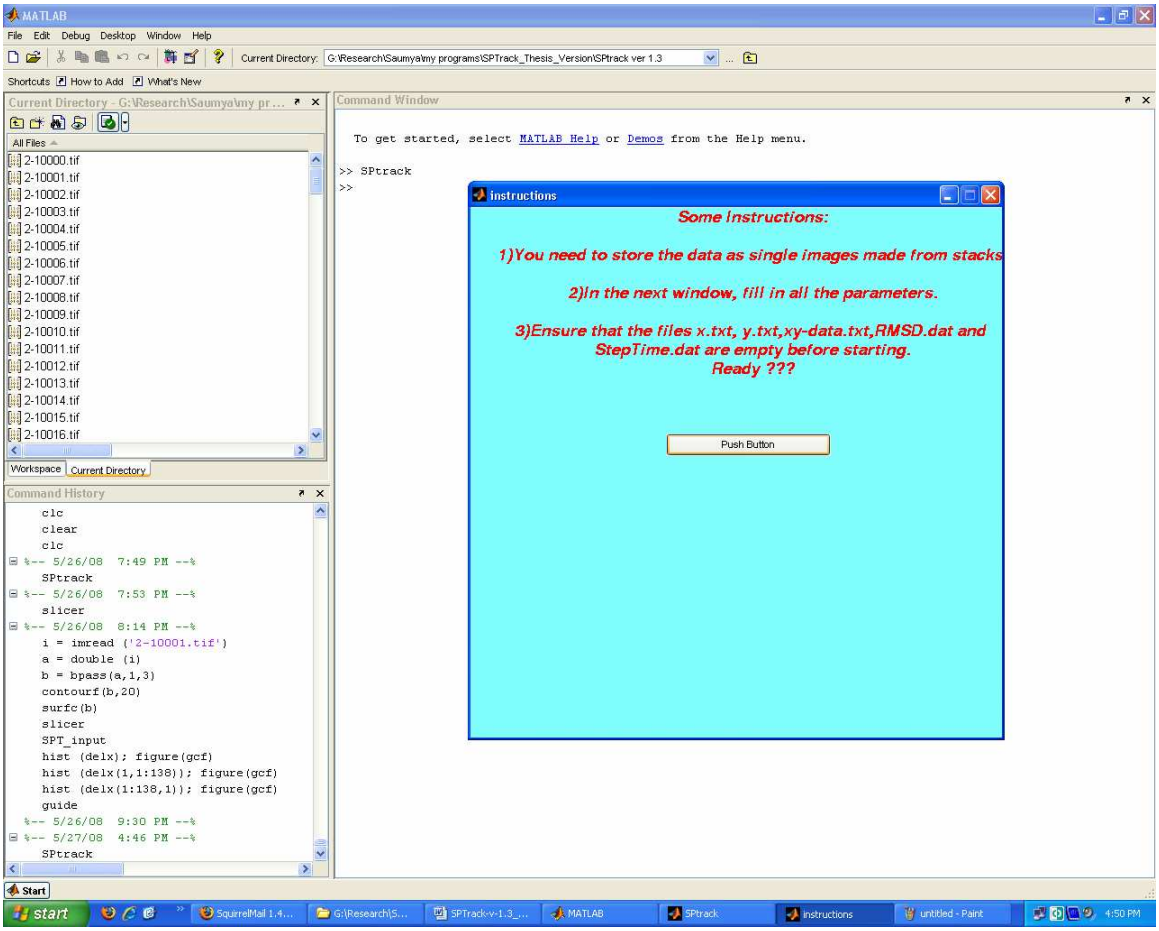
Introduction:
SPtrack is a GUI (Graphical User Interface) based program written in Matlab which is used for tracking single particles and finding Diffusion properties. It works on Matlab 6 and above platforms and requires the Image Processing Toolbox and the Curve fitting Toolbox (optional). The program analyzes each particle in an image sequence and then fits the centre of the particle to a 2 Dimensional Gaussian. The key feature of the program is its GUI based approach which is very user friendly and gives the user the freedom to analyze the data in more than one way.

Getting Started:
To start SPtrack just copy the image sequence that you want to track in the folder which contains SPtrack subroutines. Once you have done that just go to the command line and type:

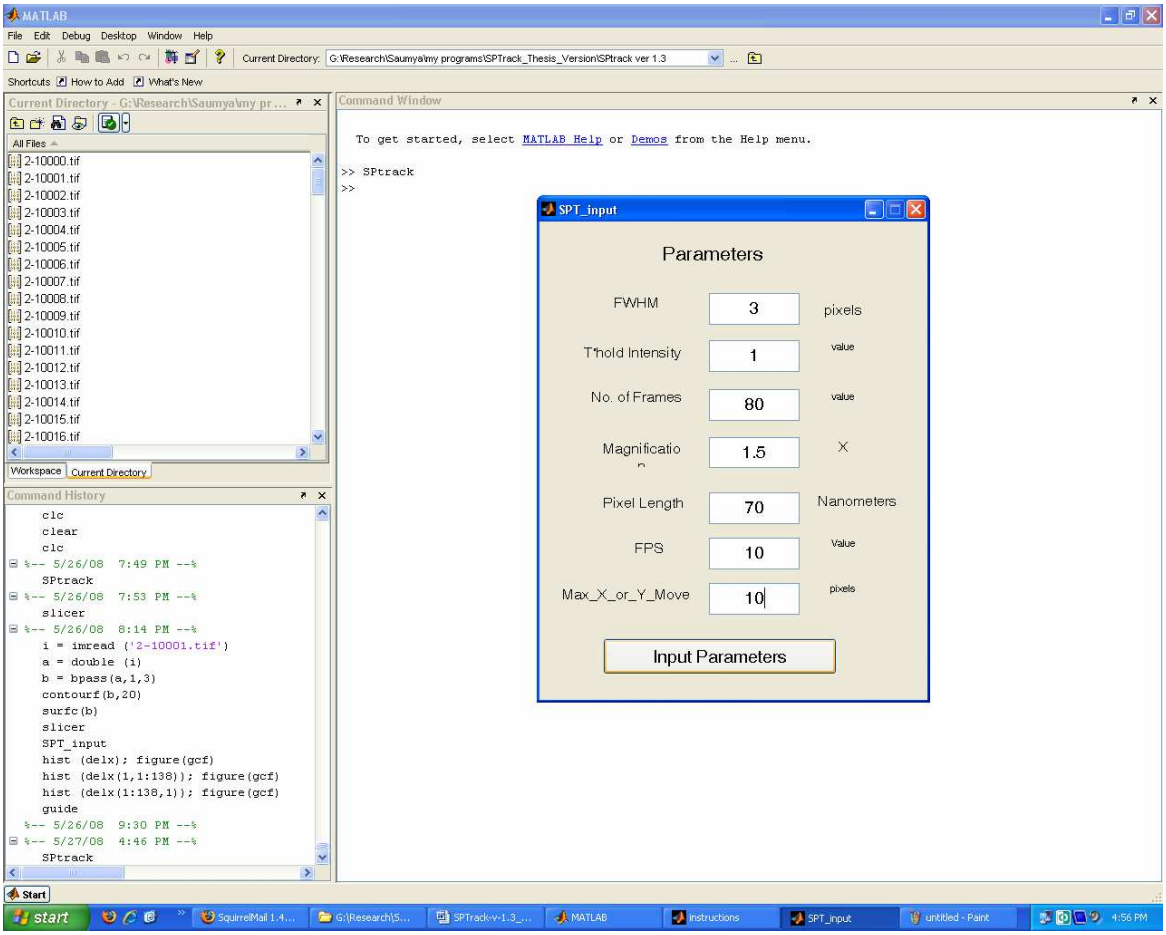
>> SPtrack



A GUI like the one shown above will open up and you can press the button **Proceed** to go further. Once you do that a window will appear which will look like:



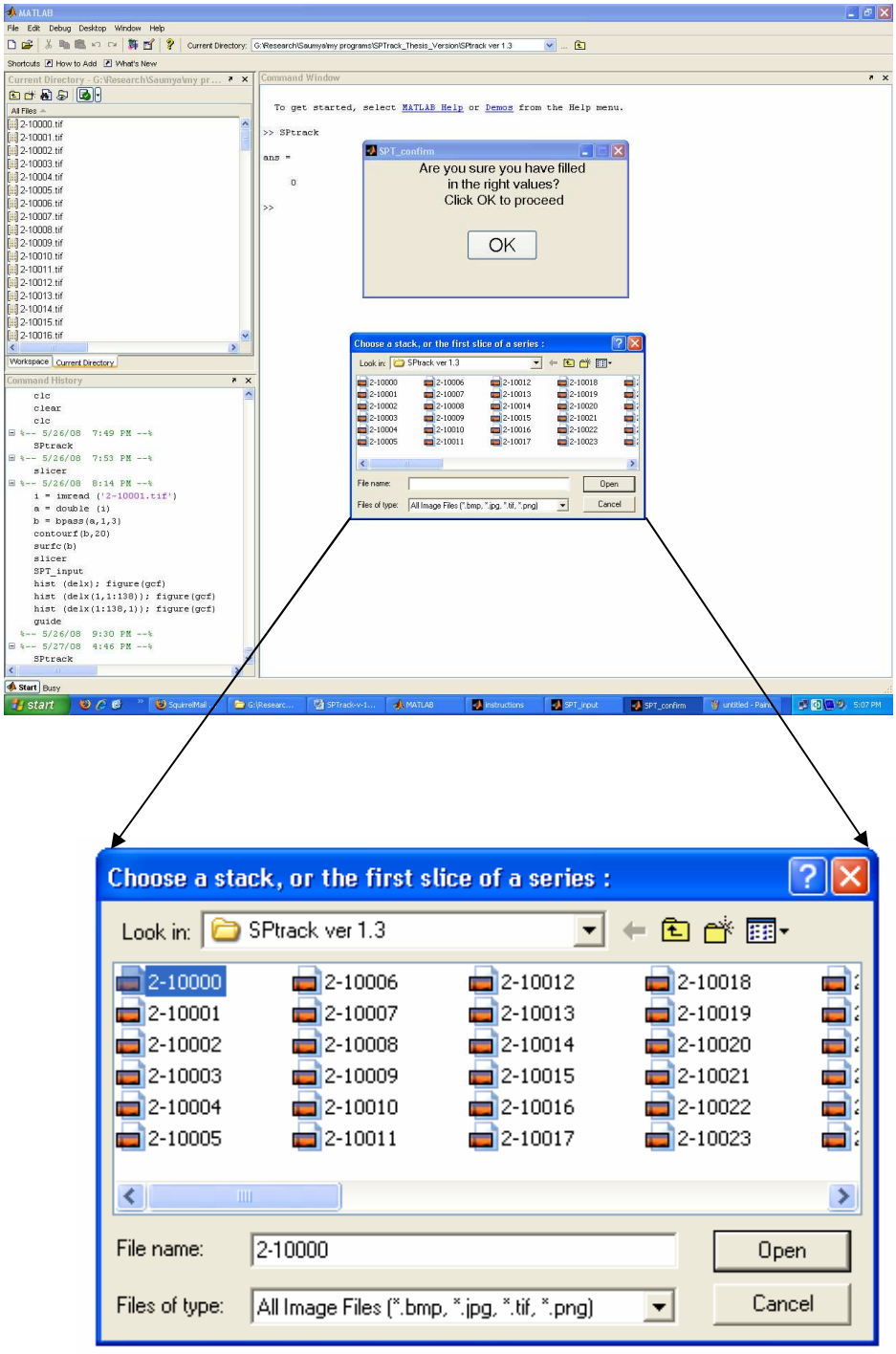
This window gives the instructions regarding the usage of the program. Since the program can be started anytime the program files x.txt, y.txt, xy-data.txt, RMSD.dat and StepTime.dat should be empty. Advance users may change the output type to the one where these files are emptied before every tracking operation by making modifications in the *readstack.m* subroutine. Once the user is ready she/he has to press the **Push Button**. This action will enable the user to fill in parameters for the tracking through a window that will look like:



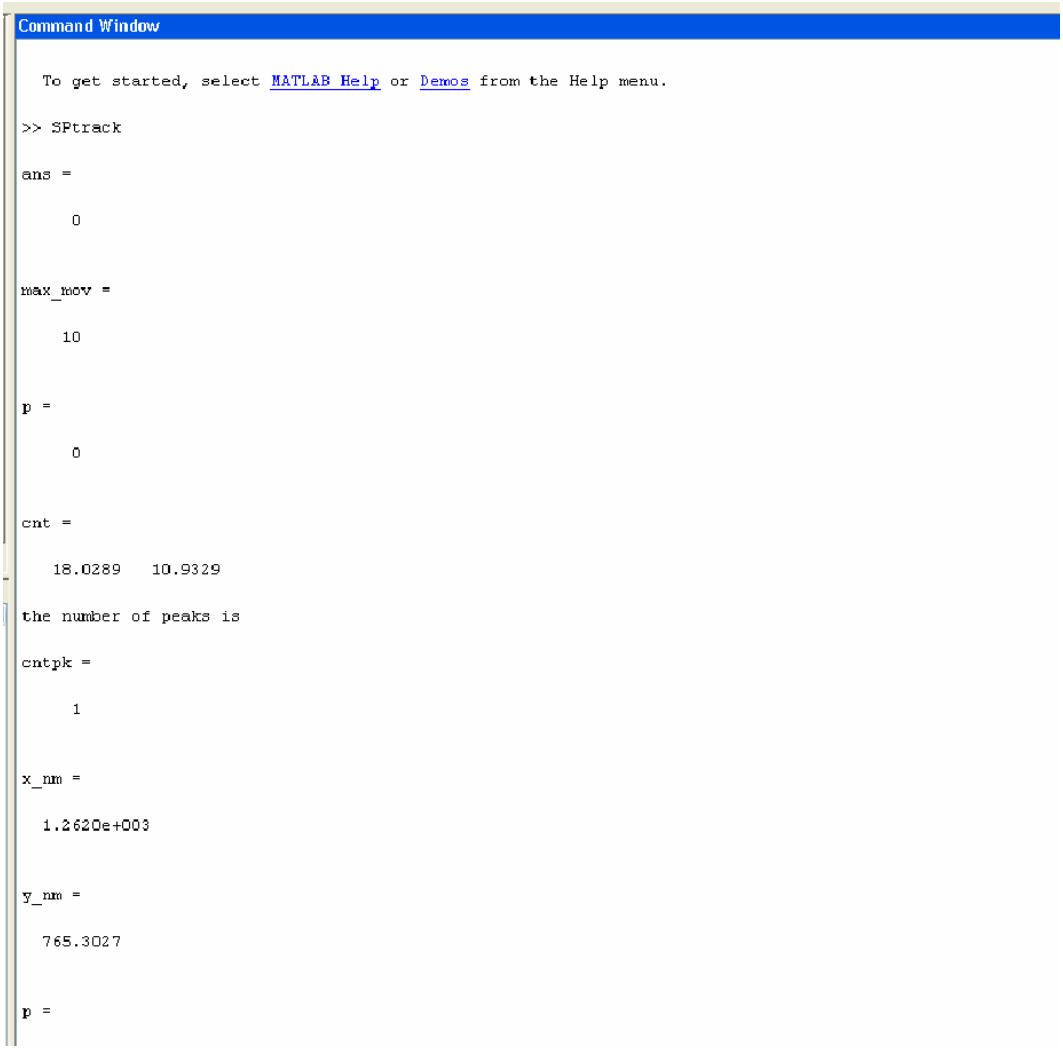
In this window the user is supposed to fill in the parameters as follows:
FWHM: Full width at half maxima of the single molecules.
Threshold Intensity: The minimum signal that the user wants to be detected by the program.
Number of Frames: The number of frames that you are working upon.
Magnification: The magnification that is used in the experiments.
Pixel Length: Length of a pixel in Nanometers.
FPS: Frames Per Second
Max_X_or_Y_Move: Maximum move allowed in either direction for a single particle. This parameter sets a limit on a single jump of a single particle. The number should be chosen based on experience and changes with the experimental conditions etc.
Similarly, threshold intensity is also a parameter that is based on experience. So normally the user has to play around with the value of Threshold intensity so as to obtain maximum track points with single peaks rather than multiple peaks.
For a good estimate of the threshold parameter, it is also important that the image sequence which is made after processing the image in some software like ImageJ, is free

from multiple particle frames as far as possible and are adjusted for brightness/contrast. Also if the size of the images is being changed, care has to be taken to adjust the pixel length as per that.
The formula for determining the pixel length is discussed in the Thesis report by the author.

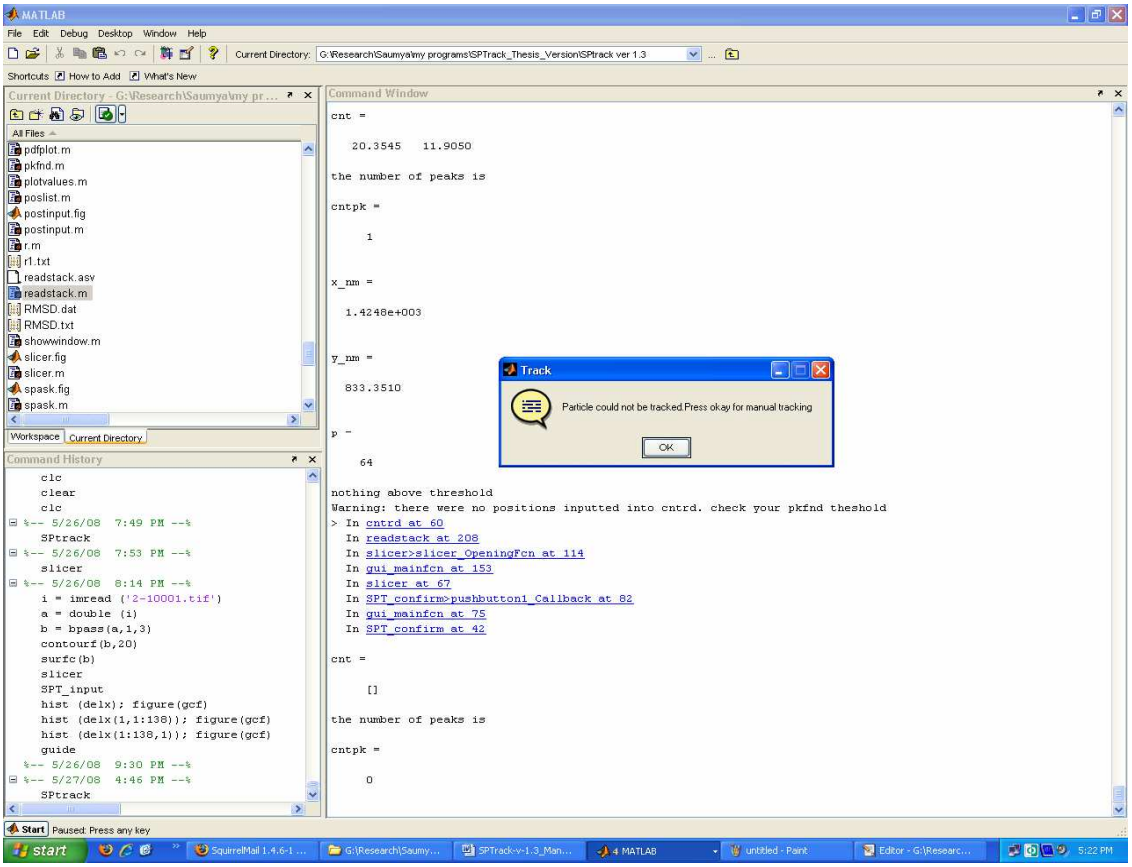
Once the parameters have been filled the user has to click on **Input Parameters**. Once that is done, the following windows pop up:



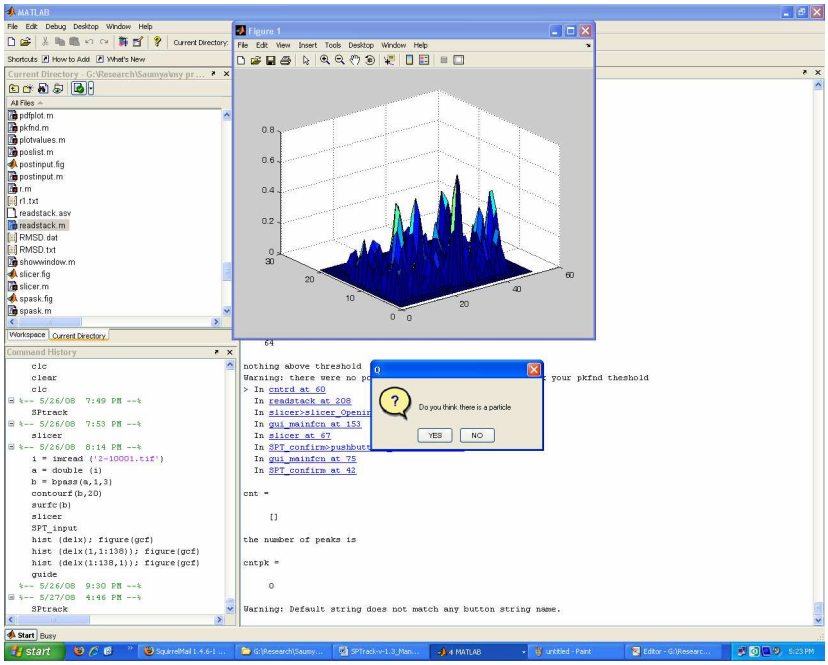
The user can press OK first after which a new window will open from which the stack or the first slice of the image sequence has to be chosen. Click on the first image and press Open / double click on the first image. The command line will look like:



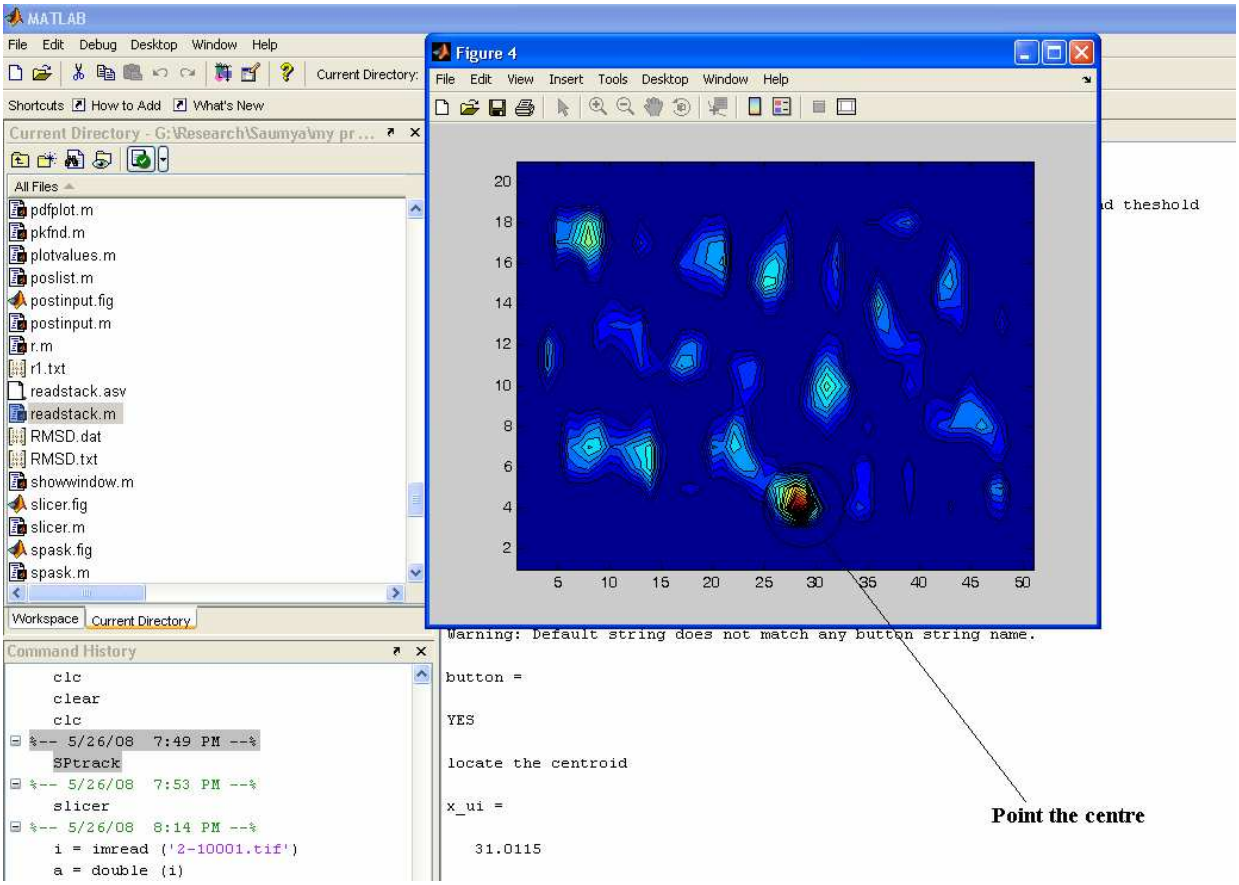
You can see the value of the centroid located on the CLI. At the same time the output is also fed into the files x.txt, y.txt, xy-data.txt. The default mode of the program is the stop and go mode in which the user will have to press enter for the program to track the next frame. This mode can be turned off by making a small change in the subroutine *readstack.m* by uncommenting line 215 and executing the command pause off.
Whenever the program fails to track any particle in a frame of tracks more than 1 particle it asks the user for manual tracking. The use can take this option upto her/his discretion. In such a situation the CLI looks like:



On pressing OK for manual tracking, the following window pops up:

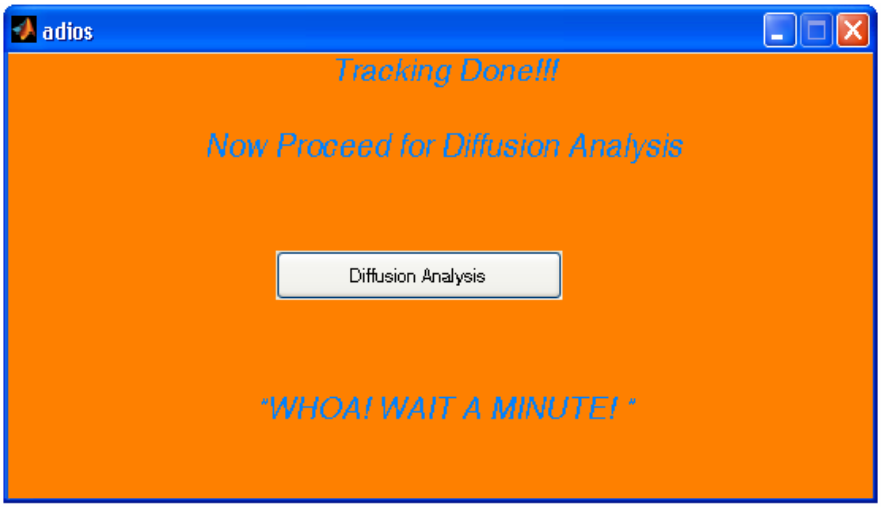


In this window, the user can see a surface plot of the image and from the height of the signal can say whether there is a particle or not. This discretion comes from experience of tracking more and more particles. Also SPtrack can track particles with Signal to Noise Ratio of as low as 3:1. So by looking at the surface plots one can say whether what they are looking at is a signal or noise.
If the user thinks that there is a particle, a window showing the contour plot will appear where the user has to manually pin point the centroid.



Once the centroid is located, the coordinates are fed into the output files as usual. After all the frames are tracked the diffusion analysis begins in the following pop up window:

```
x_nm =

   1.2661e+003


y_nm =

   854.5605


p =

     77


cnt =

    18.3259    12.1461
```



There are two methods of Diffusion analysis from which the user cam choose from. The details of the analyses are contained in the author's Masters' Thesis. After this the user can just follow instructions as they appear in the GUI.

Limitations:
1) Bug in slicer due to which the user can not see the contour plot automatically after every frame is tracked.
2) Computer Memory usage is very high.

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