SMS VIS User's Guide

Kevin Claytor
Dr. Link's Group, Rice University
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Introduction

SMS_VIS was developed as a program for following the movement of nanocars, however, it can be generalized to a number of applications. Regardless of what it is being used for, this document outlines how the program works, both on a user level and on the more extensive level of the programming. If you are simply a user, feel free to skip to Section 3; Operation. If you feel that you would like to have a better grasp of how the program works, read through Section 2: Hierarchy of Functions. If for some reason you do not think that SMS_VIS suits your needs as it is, you probably want to start diving into the Appendices to get a better understanding of what is going on at the level of the programming. Whatever your interests, I hope this file may help.

Operation

SMS_VIS should be fairly straightforward to use. I would recommend storing it and all of the subfunctions in the same folder for easy reference. In any case, once you have that folder in the Matlab path directory, or the working directory, simply type in SMS_VIS into the command prompt to launch the program. This will bring up a window similar to that in Figure 1.

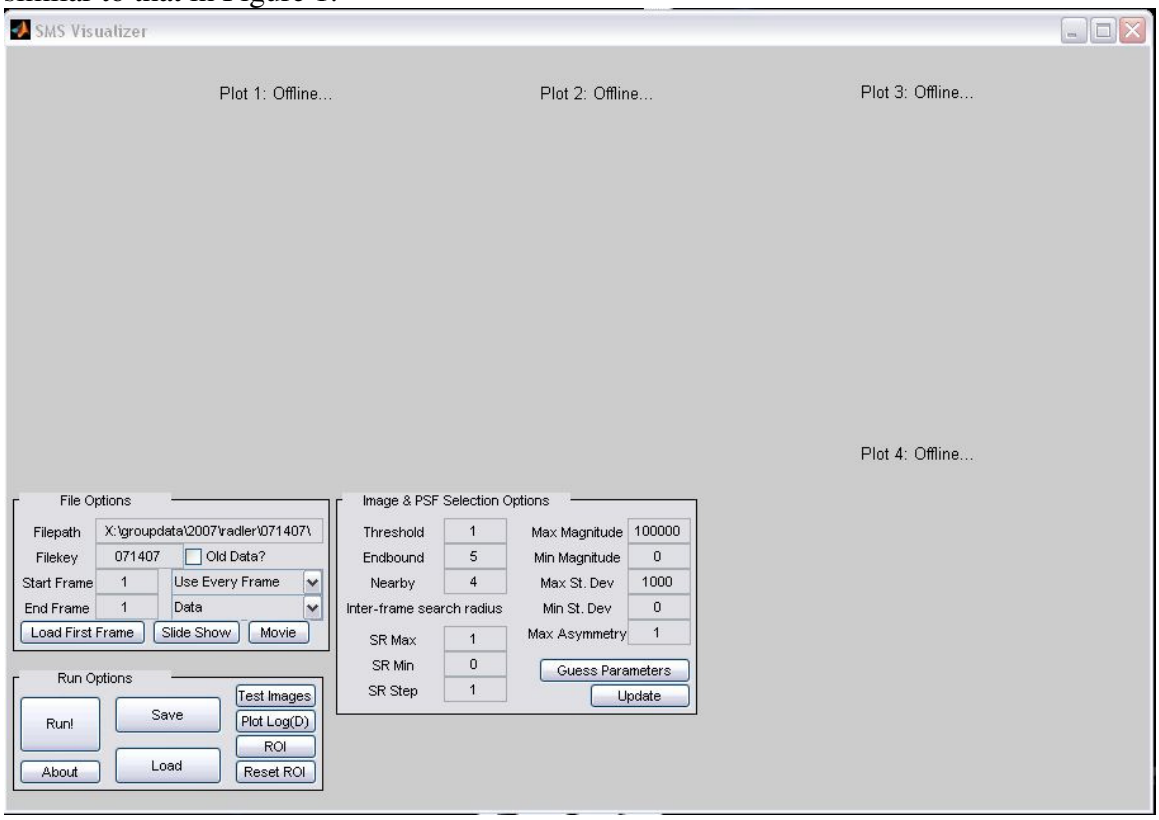

Figure 1: The SMS_VIS main screen upon launch.

The input is divided into three sections, and the output into four graph subplots. Each of these will be explained below.

Program Control Box (unlabeled)

Run! – The important button. This runs the program on the images specified in File Options, and displays the results (see Figure 3). It will update the window with an XY movement scatterplot, a Radius plot, a Search Radius efficiency plot, and a Trajectory plot.

Save – This is only a valid option after a data set has been run. It will save the variables used the create the XY, Radius, Search Radius, and Trajectory plots, as well as the settings used to obtain that data.

Load – This loads the information stored upon a save.

Test Images – This will launch another window allowing you to generate a jpg sequence of diffusive molecules.

PlotLogD – This allows you to analyze the results of the diffusion calculator.

ROI – This button brings up a new window in which the user can select any two opposite corners of the region of interest (ROI).

Reset ROI – Resets the ROI back to the full image.

About – Brings up an information dialog that gives version number, revision history, and license information about the program.

File Options

This area contains the information for loading the files for analysis. It can operate in one of three modes. These can be accessed using the drop-down menu labeled "Data" in Figure 1.

Mode 1: Data

This is the normal operation mode. In this mode the data stored in filepath with the filename "Filekey + Image Number" is used. If the data was taken BEFORE the RHK upgrade check the Old Data checkbox. The number of the first image you want to analyze should be input into the Start Frame box, and the number of the last image you want to analyze should be put into the End Frame box. If you want to skip no, one, two, or three frames you can select this with the Use Every Frame drop-down box.

Mode 2: Intentional Shift

All input boxes in this mode operate the same as Mode 1. However, half of the frames will contain a shift in the x direction by s pixels and in the y direction by s pixels. "s" is a variable that must be set by hand in DVISd (line 121 or thereabouts).

Mode 3: Test Images

In this mode, real data is not loaded and only test images are used. To generate test images run MakeTestImagesGUI. When using this mode, the buttons Load First Frame, Slide Show, Movie, Guess parameters, and Update should not be used as they will not give the expected results. The ROI button will still give the proper results, however, it may not function correctly (as it usually loads up a sample image from the data).

The value in Filekey now corresponds to the value specified in Generic Name set in MakeTestImagesGUI. The value in End Frame corresponds to the value set in Number of Images in MakeTestImageGUI. The Use Every Frame, etc. drop down box is still active.

The values in the other boxes, namely Filepath, Start Frame, and the Old Data checkbox are not used and will be ignored by the program. All the numerical value boxes in Image & PSF Selection Options will still be used as per normal operation.

Load First Frame – This button will load the first frame and display it in the first subplot (see Figure 2).

Slide Show – This button will run a slide show of the selected frames (from Start Frame to End Frame, skipping the set number of frames (0, 1, 2, 3)).

Movie – This button opens a new window and runs through the slide show (again from Start Frame to End Frame, skipping 0, 1, 2, or 3 frames), saving it as a .avi movie at the end.

Image & PSF Selection Options

This section sets the cutoff values. Threshold sets the value above which the data must be to be considered as part of a molecule. Endbound sets the area around the image or region of interest (ROI) that will be analyzed. Nearby sets the distance that the central point finder uses to find the center point of a group. Eg; if there is a point of higher value within a radius of "Nearby" it will be assigned to be the center point rather than the current point.

The four boxes below Inter-frame Search Radius determine the size of the association circle. DVISd will change the search radius from the value in SR Min to SR Max in intervals of SR Step.

The remaining options set the cutoff values. Max Magnitude sets the maximum magnitude that the normalized Gaussian magnitude can be, while Min Magnitude sets the minimum value that will be kept. A point that has magnitude outside of these bounds will not be considered for association. Max std and Min std set the standard deviation cutoff values, while asymmetry sets the asymmetry in the standard deviations that will be accepted.

Guess Parameters – This button will run PSFF with wide cutoff values (the defaults) on the first frame. It then looks at the statistics of the particles found and suggests values such that particles with 2σ around the mean are kept. Because of a usually low threshold value (set as 1σ above the background level) this process may take a few seconds and user confirmation takes place. Clicking No or Cancel in the querry window that appears will cancel this process. Yes will proceed with finding the statistical cutoffs. Clicking Discard in the PGuess window (Figure 3) will dismiss the suggestions, while clicking Save will update the parameters in the SMS_VIS window.

Update – This button runs PSFF on the first frame, applies the cutoffs and displays the results in subplots 2 and 3 (see Figure 3).

Figure 2. The PGuess window brought up by guessparams.m.

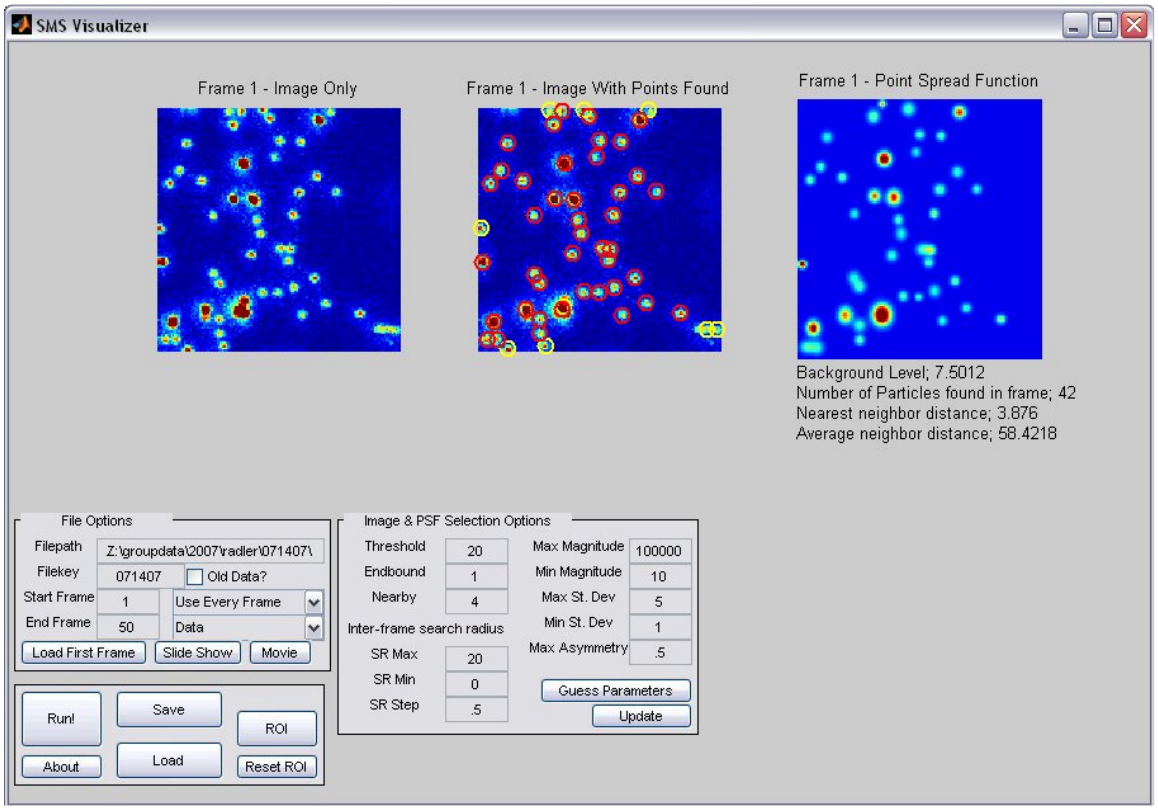

Figure 3. The result of the Update button. Subplot 1 is the left most image in the window. This shows the image as it was loaded up from the .sm3 file. Subplot 2 is the middle image and shows the centroid locations overlaid. A red circle indicates a centroid that satisfies the cutoff values and is kept for later analysis, while a yellow circle indicates a centroid that was eliminated from later analysis based on the cutoff values. Subplot 3, the right most image, is a Gaussian point spread function based on the values from the points kept. Below subplot 3 are some values based on the first image, namely the background level, the number of particles, and if this last value is greater than 1, the

distance between the two closest points and the average distance between points (the latter value should be on the order of ½ the image size).

Options after a Run

After the program is run, the main window will change to appear similar to Figure 4. Here we see that the subplots have changed to show the shift, and that below each is a button allowing the graph to be enlarged and which may bring up other options (see Figures 5, 6, and 7). Subplot 1 shows the X-Y scatterplot, Subplot 2 shows a Radius histogram (bin size is 1, centered between the integers (.5, 1.5, 2.5, etc.)) of the same data.

In the main SMS_VIS window, the histogram and scatterplot show the results for all search radii and between all frames, the number of particles displayed in the title corresponds to this value. For example, if you examine 20 search radii (SR Min = 0 SR Max = 20, SR Step = 1) and there are 10 frames, each with 5 particles per frame we would expect to see on the order of 5*10*20 = 1000 particles.

Clicking the Step Through… button will bring up a new window allowing the user finer control over what result is displayed. Below each of these graphs is a button allowing the user to step through the search radii and between frames (Figure 5). The Next and Previous search radius change the search radius for which the shift is displayed, while the Next and Previous Image Pair buttons allow one to compare the shift between one frame and the next and are only valid when the Sum… box is unchecked, otherwise the shift between all frames is displayed. This functionality is the same for the XY scatter plot or the radius histogram.

The search radius efficiency plot can also be enlarged (Figure 6) by using the button below it. This plot shows the number of molecules associated normalized to the number of molecules that could be associated. Thus for a value of 1, perfect association was achieved – every molecule had a corresponding molecule in the next frame. Concentration will change the shape of this curve – samples lower in concentration will have less overlap and excluded molecules as the search radius increases, and therefore will have a shallower tail. Blinking will also force down the maximum value of the peak – if a molecule blinks off, it cannot be associated, and therefore the peak will be lower.

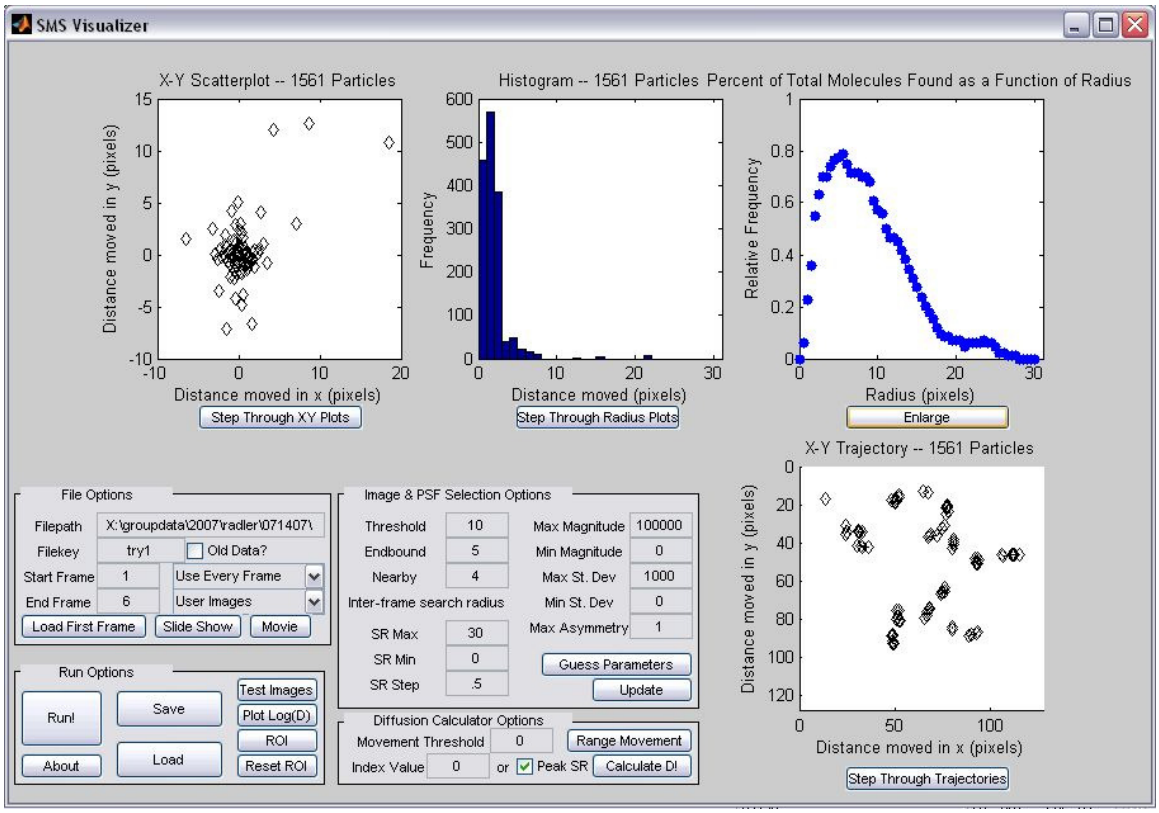

Figure 4. The SMS_VIS window after a data set was run.

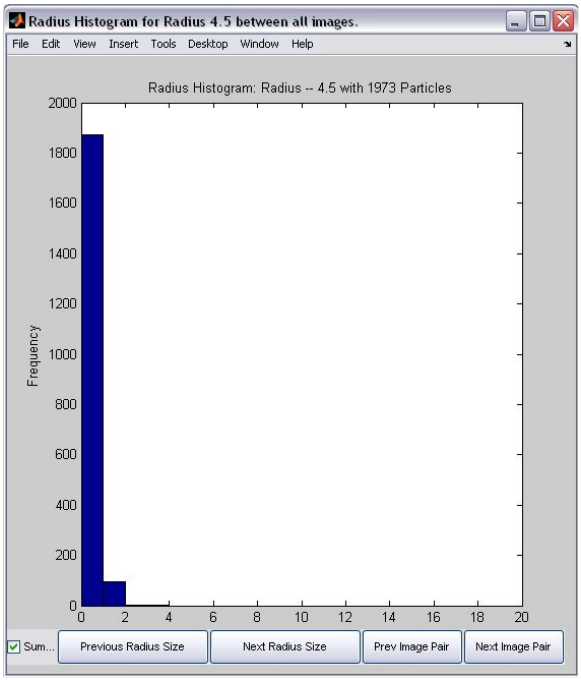

Figure 5. The radius step figure. This figure allows one to examine a specific subsection of the data. With the box checked, the shift between all images at a certain search radius will be displayed. An unchecked box will display the shift between specific images at a certain search radius.

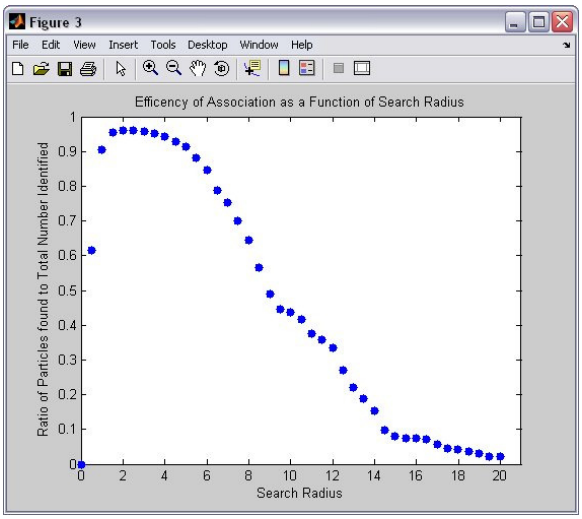

Figure 6. The search radius efficiency plot enlarged.

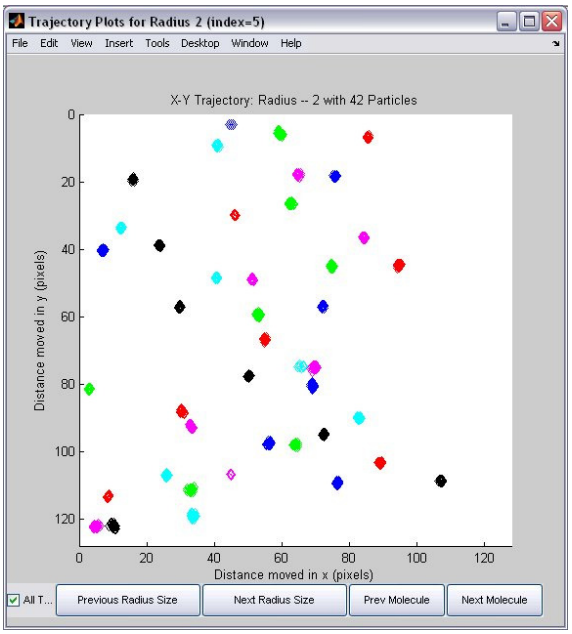

Figure 7. The trajectory step figure. This allows one to see all, or single molecule trajectories for a certain search radius. Checking the box will display all molecules, while having the box unchecked will display only one molecule.

The trajectories can also be analyzed separately by clicking the button below that image. As before, the Next and Previous Radius Size buttons will change the trajectories to those determined for the labeled search radius. When the All… box is checked, all trajectories will be displayed, while when it is not only one will be. The current trajectory displayed can be changed by the use of the Previous and Next Molecule buttons.

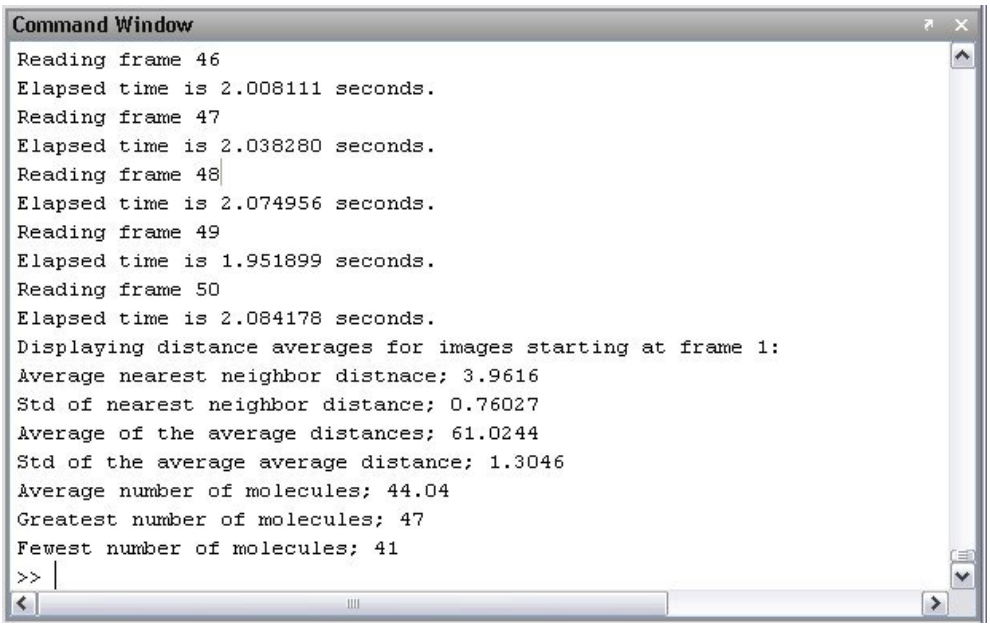

Figure 8. The Command Window after SMS_VIS was run. Additional information is displayed as the program is running (eg; what frame is being analyzed, and after it is completed how long the analysis took). Additionally nearest neighbor information for all frames are displayed.

Additionally, after the program is run on a sample set, nearest neighbor information appears in the command window. The value after "Average nearest neighbor distance" shows the average of the distances of the closest two particles. The "Std of nearest neighbor distance" gives the standard deviation of the nearest neighbor distances found in all the frames. In each frame there is also an average neighbor distance, this is displayed after "Average of the average distances," while the standard deviation of this value is displayed after "Std of the average average distance." There are also a certain number of particles found and kept (again based on the cutoffs) in each frame. The average value of this is displayed after; "Average number of molecules" and the two extremes are subsequently displayed.

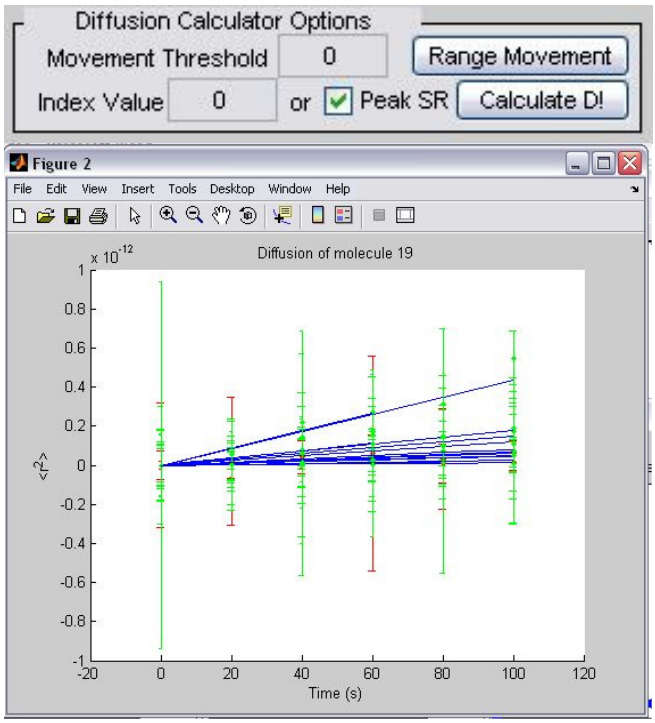

Figure 9: Diffusion options, and the results of running Calculate D!.

Diffusion Calculator Options

These options allow you to calculate the diffusion.

Range Movement – puts the mean of the movement values into the threshold box.

Calculate D! – will calculate the diffusion for all the molecules. If the movement is above that in the threshold box it will be colored green, otherwise red when plotted. The diffusion constants will then be saved to a .mat file (if you want another format, e-mail us for the code and you can make the changes yourself) of the same name as "filekey."

MakeTestImagesGUI

This button will launch another window (Figure 10) which will allow you to generate a sequence of jpg images simulating diffusion. "Nanocars" can also be simulated in the sequence. These will default to moving in a straight line unless an angular deviation off of that line that can is set. Their magnitude can also be set separately from the diffusive particles. The time between frames and the scale is saved in a .mat file under the name "SMSTest_myname1.mat," where myname1 is the corresponding text in Figure 10. You can also include noise (defined as +/- 1/sqrt(signal)).

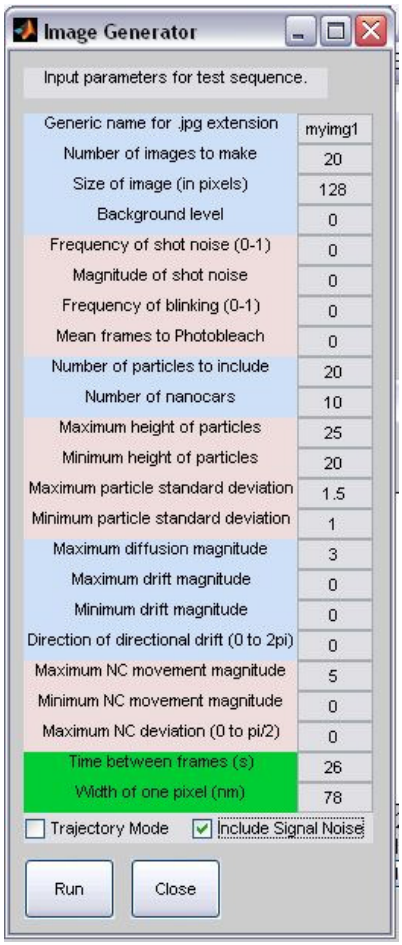

Figure 10: The MakeTestImagesGUI interface.

PlotLogD

This button allows you to take the diffusion values saved by running the diffusion calculator ("Caculate D!") and merge them into one cohesive histogram.

You will navigate and select multiple files for each type of file. For example, if you have two samples (tritc and nanocars), and fifteen image sequences for each. First you would need to run SMS_VIS on each image sequence, being sure to run the diffusion calculator at the end of the run. Then run PlotLotD – you would enter 2 distributions to compare, then when asked to find the files, select ALL 15 files, enter the name for the legend, and then do the same for the other type. See Figure 11 for what this would look like in the command window. When done, you will see a new figure appear similar to Figure 12.

```
Number of Distributions to Compare: 3
Minimum value (zero for auto): 0
Merging data, this may take a moment...
Sample Name: Beads
Merging data, this may take a moment...
Sample Name: Nanocar
Merging data, this may take a moment...
Sample Name: Tritc
Done!
Figure Title: Comparison of Three Samples
```

Figure 11: Text input to PlotLogD

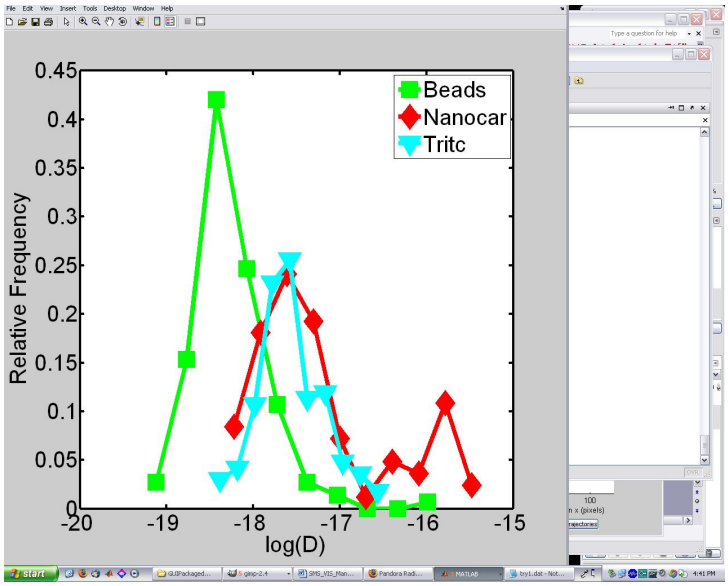

Figure 12: The results of PlotLogD.

I think that covers the functionality of SMS_VIS at this point. As additional functions are added, this section will be updated, however, as is you should now have a working idea of what each field and button does. My strongest suggestion would be to play around with each in turn. Perhaps, if you're feeling really daring put some breakpoints in the code to see how the variables change with each change of a drop down menu, text field, etc.

Hierarchy of Functions

All the functions and their purposes are listed in Table 1, and a graphic illustrating how they interact is presented in Figure 1.

Function	Use
SMS_VIS	Allows GUI interface to DVISd and plots the results
DVISd	Calculates the shift between all frames for all search radii
DiffV2	Calculates the shift between only two frames
DiffV3	Calculates the shift between all frames and adds to a trajectory matrix
PSFF	Finds the centroid location and applies cutoff values
ReadImageP	Reads in pre RHK-upgrade data
ReadImageL	Reads in post RHK-upgrade data
guessparams	Takes a shot at figuring cutoff values based on the spread of values observed for a large cutoff initial value
radstepfig	Opens up a new window and allows the

	user to step through the radius plot for different search radii and between images
xystepfig	Opens up a new window for stepping through the XY plots
trajstepfig	Opens up a new window for stepping through the trajectories
nearestneighbor	Finds a number of parameters based on how far the molecules are apart in the frame
MakeTestImageGUI	A standalone program that creates images to use with testing SMS_VIS

Table 1: A table of all .m files used by SMS_VIS, or relating to it.

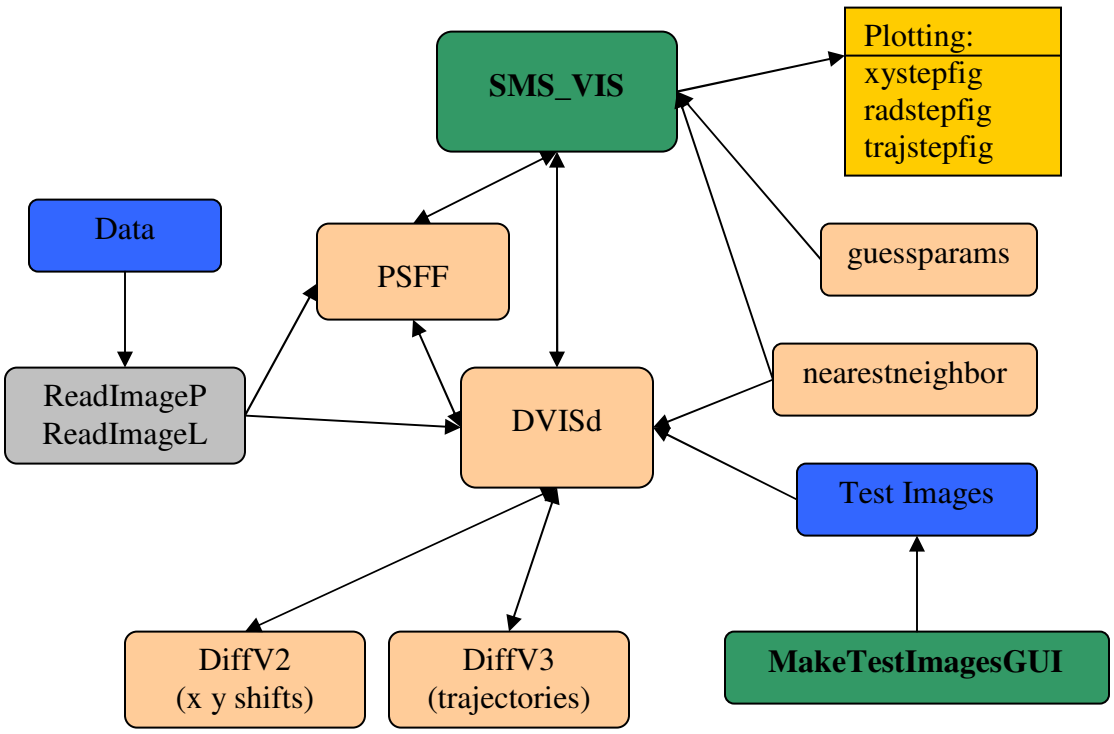

Figure 13: A graphic explaining how the different functions go together. In green are the standalone GUI programs. Blue are data sources, gray are data interpretation, tan are subfunctions, and gold are plotting routines. The lines indicate connections. In general arrows on the end of the lines indicate the flow of data (not necessarily information).

As can be seen from Figure 13 the critical program is DVISd, which is used to access the shift programs and gives back to SMS_VIS the results of the shift programs for plotting. SMS_VIS also accesses some programs alone to make life easier, such as finding the proper cutoff values.

Appendix 1. Flexibility

So say that the idea of SMS_VIS is what you would like to have. But perhaps you don't use .sm3 files, how should SMS_VIS be modified? This question actually has

a really simple answer. As long as you'll be looking at an image-like file, I would recommend replacing ReadImageL with a different data loader. This is, in effect, done in DVISd for test images. Opening up that code you'll see that I load up the .jpg file directly, then flatten it and split it into two channels. This is essentially what your data loader will do. I would rename ReadImageL.m to, say ReadImageL_old.m. Then make a new ReadImageL.m file that loads your data, splits it equally into two channels (SMS_VIS will recombine the channels anyway) and has that as its output. Specifically the output of your file will need to have the same format;

```
[Data,Data2,m_nWidth,m_nHeight,time,scaleout,scaleout2,unitsout,unitsout2]
```

Where Data is half of your image matrix, Data2 is the other half, m_nWidth is the width of the image, m_nHeight is the height, time is the time between frames, scaleout and scaleout2 are length 2 column vectors relating the size of the pixel to physical units (element 1 is the x direction, and element 2 is the y direction), and unitsout and unitsout2 are the units for the physical units in the x and y direction, again these are two column vectors of length 2. The program should accept these parameters as if they were given from a .sm3 file.

Appendix B. Variables

Within SMS_VIS there are a few critical variables that you should understand before you start messing with them. These are e, RadiusShift, and Trajectory.

e is a structure that holds all the parameters that we will be used EVERYWHERE. It is a structure that has 15 fields described in Table 2.

Field	Use	Where it is Used
threshold	The threshold value, set from the threshold text entry box	PSFF.m
endbound	The endbound value, set from the textfield box.	PSFF.m
nearby	The nearby value, set from its textfield box	PSFF.m
magCutoffmax	The maximum value that the Gaussian height can be.	PSFF.m
magCutoffmin	The minimum value that the Gaussian height can be.	PSFF.m
sCutofflarge	The maximum value of standard deviation that the Gaussian can have.	PSFF.m
sCutoffsmall	The minimum value of standard deviation that the Gaussian can have.	PSFF.m
aCutoff	The maximum asymmetry that the standard deviations can have. This value is in pixels and is defined as absolute value of sigma x – sigma y.	PSFF.m
searchradiusmin	The lower bound on the search	DVISd.m

	radius that we will use.	
searchradiusmax	The upper bound on the search radius that we will use.	DVISd.m
searchradiussetp	The step size that we will take to get from the lower bound to the upper bound of the search radius.	DVISd.m
startframe	The first frame to be included in the analysis. This is set from Start Frame.	DVISd.m, SMS_VIS.m, guessparams.m
stopframe	The final frame to be included in the analysis. This is set from End Frame.	DVISd.m
frames	how many frames do we skip? 1 – skip zero frames, 2 – skip 1 frame…. n – skip n-1 frames. The max value of n is determined by how many fields there are for the popup menu "Use Every Frame…"	DVISd.m
old	switch between data loaders. 0 (Old Data box is unchecked) – use new loader (default), 1 (Old Data box checked) – use old loader	DVISd.m, SMS_VIS.m, guessparams.m

Table 1. The fields of e and how they are used.

RadiusShift is another structure, and it is the structure which is used to store the results. It is a structure which is

Another less important variable is imageStorage. This is a

Appendix C. Known Errors / Bugs

1. The interface is not user-proofed: If one loads up data and then puts in different parameters (images, frames to skip, etc), then you HAVE to re-run the data, even if the diffusion calculator shows up, otherwise, the data displayed will be of the saved data, and not of what is labeled in the box. Basically run the data again after making ANY changes.
2. Also the "region of interest" or ROI, may not work correctly – I never really used it, so I didn't put much effort into making it work correctly.