



## Diffusion in Fibrin Clots

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The formation, function, and lysis of blood clots is largely governed by the transport of nano- and micro-scale particles. A physiological clot's environment includes blood, pathogens, cells, and drugs, which move through the fibrin scaffolding by various mechanisms, including diffusion, advection, and active motility. Clot formation relies on transport processes: the geometry of thrombin generation has an impact on the subsequent polymerization of fibrin gel. Similarly, fibrinolysis is regulated by the transport of fibrinolytics, as determined by the mobility of the enzymes and the structure of the clot. Diffusion within a formed clot presents an attractive system to make such a measurement. Diffusion, which is a local transport phenomenon, can also be measured in small specimen volumes ( $\sim 10\mu\text{L}$ ) and requires only moderate data collection time.

## Procedure

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### *Sample Prep*

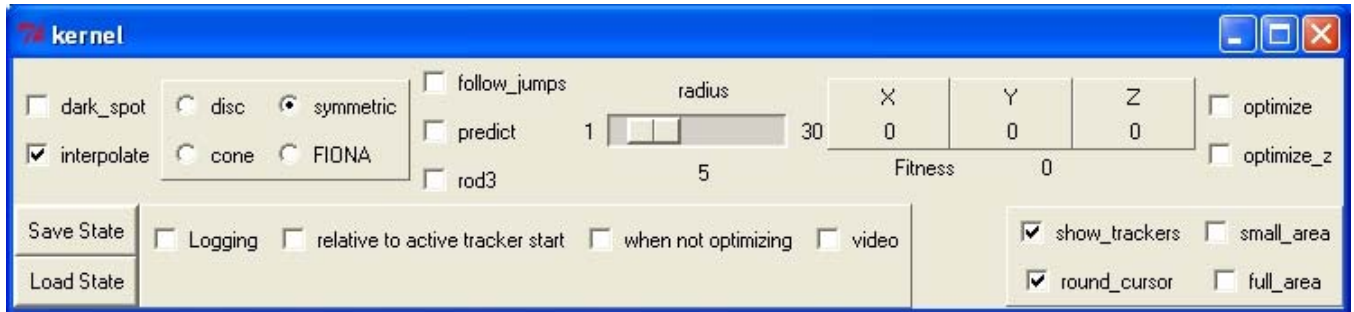
**Fibrin clot:** Clots are formed in a glass-bottom 96-well microplate (NUNC, Rochester NY), which is plasma cleaned to ensure uniform clotting and meniscus in all wells. They are formed mainly from the protein fibrinogen, and caused to assemble or polymerize by addition of the enzyme thrombin. The microplate has three different clot types, each with a different concentration of fibrinogen. Changes in fibrinogen concentration affect the structure of the clot. In this lab we will look for changes in nanoparticle diffusion due to the variation in clot structure. Clots are allowed to incubate for two hours, which was the time before the level section of the slowest turbidity curve (meaning all clots should be finished polymerizing). Clots are sealed to prevent evaporation and left at room temperature.

**Nanoparticles:** Carboxylate nanoparticles were functionalized with polyethylene glycol (PEG). This is to prevent particle adhesion to the fibrin, which is notoriously sticky.

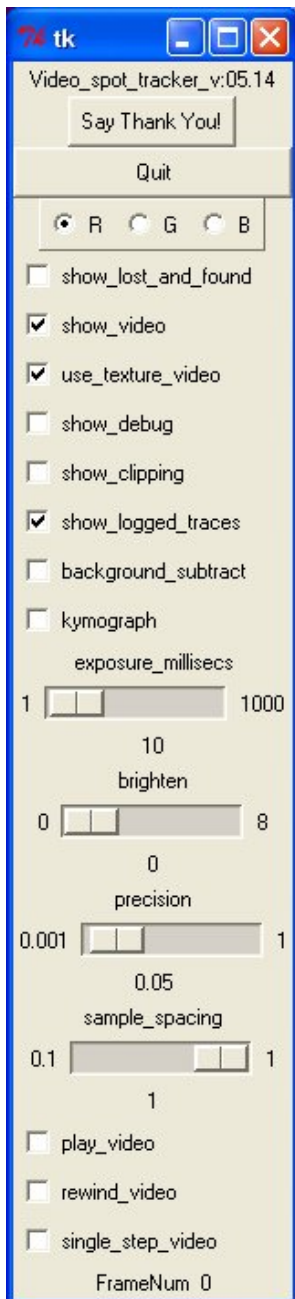
### *Diffusion Protocol, step by step*

1. Place the sample on the microscope and get a camera image of the nanoparticles.
2. Choose a field of view in the center of the well, roughly  $60\mu\text{m}$  from the substrate.
3. **Acquire 60 seconds of video with the diffusing particles.** Save this file to disk. Congratulations: you're already done with data collection!
4. Open the file in Video Spot Tracker by dragging it onto the VST desktop shortcut. (If you acquired images through IPLab, you may first need to convert the movie to an AVI using ImageJ.)
5. Familiarize yourself with the VST interface. First, note the top bar. The parameters that we'll be using are the tracker radius, the logging button, the optimize button, and the "save" and "load" state buttons.

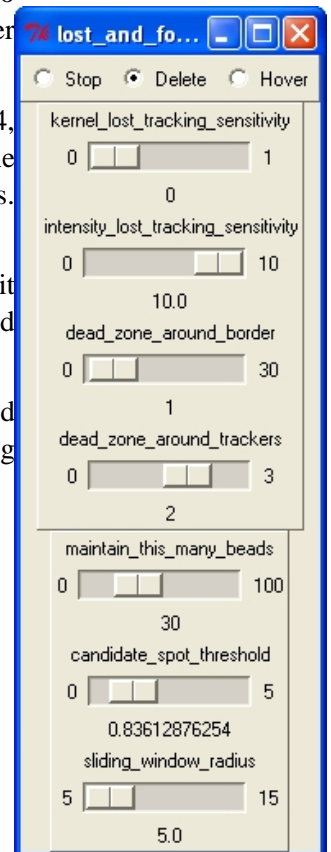




6. Next, take a look at the sidebar to the left of the screen. For now, the only two buttons we'll use here are show\_lost\_and\_found at the top, and the play checkbox at the bottom. It's worth noting that the "sample spacing" and "sensitivity" sliders increase the resolution of the tracking, which can be useful in situations where particles do not move more than a few pixels.



7. The show\_lost\_and\_found checkbox will reveal a variety of additional parameters. Among these, the most interesting are "kernel lost tracking sensitivity" and "maintain this many beads." Make sure "Optimize," in the top pane, is checked on. Set "maintain this many beads" at 100 and hit "play." Notice how VST can find particles, but when the trackers loose the particle, they stay on the screen. Now stop the video, increase the "kernel lost tracking sensitivity" to 0.05, select the "delete" button at the top of the window, and hit play again. Notice how the bad trackers get deleted. Finally, check if the trackers appear to be the right size (or slightly larger) than the particles on the screen. If the trackers are too big or too small, adjust the "radius" slider (top pane) to set the right tracker size.



8. Now play with the "candidate spot threshold," lowering it first to 4, then to 3, then to 2. Notice that the algorithm finds more of the particles, but also begins generating more false positive trackers. Find a good threshold.

9. Time to track the whole video. Hit "rewind video" (left pane), hit "Logging," enter the filename (the extension should be .vrpn), and press "play."

10. Finally, find the .vrpn file you generated, and right click. Select Send To...>vrpnLogToMatlab 04.01. You're now ready to begin reviewing your data.

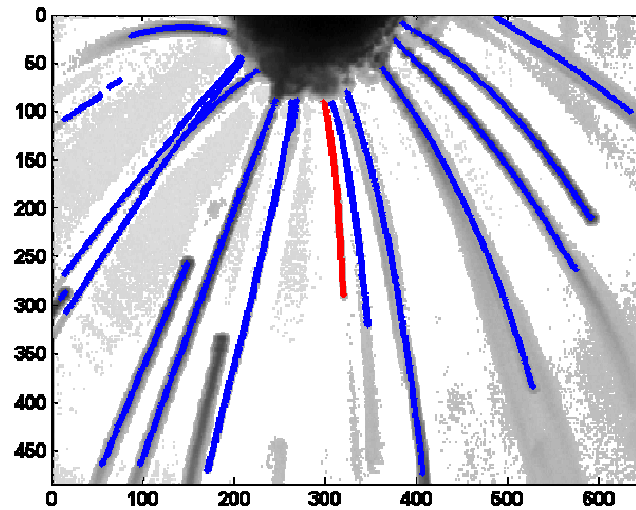




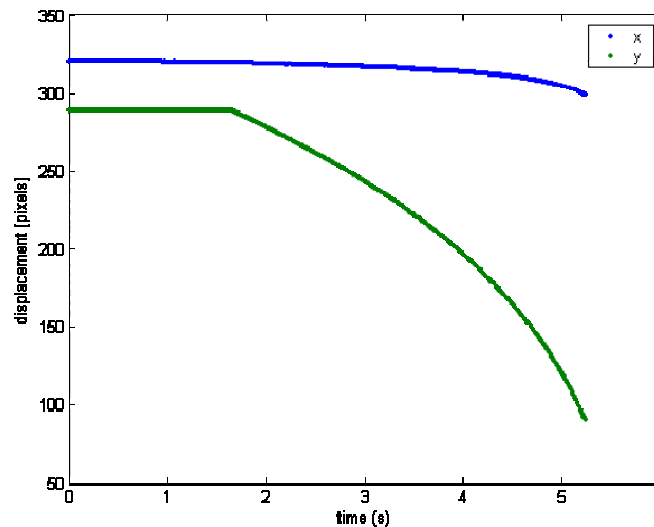
## Reviewing and filtering data in evt\_GUI

1. To look at the data you collected, open Matlab 2007b.
2. Type evt\_GUI to open CISMM's video tracking editing environment.
3. Press the "Load" button to select the .vrpn.mat file with your tracking data.
4. After loading a dataset, two figures will appear on the screen...

**XY traces:** The first figure contains a spatial representation of the data contained in the \*.vrpn.mat file. If a MIP file is found in the same directory as the data, it will also load and display behind the spot\_tracker data. Intensity projection images aid in determining the validity of video tracking information. **The active tracker path is red, while inactive trackers are blue.**

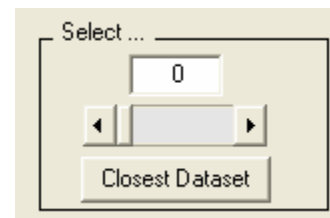


**XT trace:** The second figure depicts a temporal representation of the data contained in the active tracker (displayed in red in the first figure).



5. Play with the controls to browse your dataset.

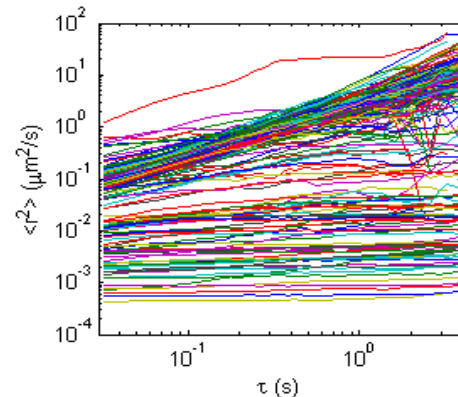
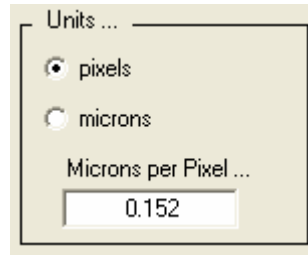
**Selecting tracks:** There are three ways to navigate through multiple trackers: the select textbox, the select slider, and the "Closest dataset" button. Pressing this button activates the *x-y* plot, and waits for a single mouse click within the viewing area of that plot. Clicking on or near a path will cause the closest path to become the active tracker.





**Switching units between pixels and microns:** To transform the units on the plot axes from pixels to microns and vice versa, click on the radio button containing the desired unit. This requires a calibration factor that defines the microns per pixel value. Enter this value into the textbox labeled “Microns per Pixel...” to adjust this parameter as desired. (Today’s experiment will be .547 microns per pixel)

**Plotting the mean squared displacement (MSD):** In the “aux plot” popup menu, select “MSD.” This will bring up a third figure containing the average MSD of the trackers. To see the MSD for each tracker, select “Plot Individual.” Depending on how much data you collected, and in what type of plot, your plot might look something like the one at right.



- Next we'll filter out some of the lower-quality tracking data. In the “aux plot” menu, select “tracker availability.” Notice that some trackers are visible only for a few frames. In order to eliminate them, we'll use the “minFrames” filtering option. Check the “Filter by minFrames” box on the far right and enter the minimum tracker length you want to preserve. Now reload the tracker file. Notice that the number of trackers has decreased, and that the MSD vs. tau plot should look cleaner. Press save when you are satisfied with the filtering results.





## Data analysis

1. Follow the diffusion protocol above for several different clot types. You should generate a few videos for each type of clot.
2. Calculate the diffusion coefficient. For this step, you will use the function:

```
[DATA, bufferD] = AnalyzeDiffusionLinearAlpha(filenamees, ...  
  
        framerate, calibum, beadrad, window, ...  
  
        minTAU, maxTAU, ...  
  
        D_eff_binres, colors)
```

The inputs and outputs of this function are:

|                |   |
|----------------|---|
| filenames      | An array where rows refer to clot types. Wildcards are okay. For example: '*_1mg_10U*.evt.mat'            |
| calibum        | Number of microns per pixel. This depends on the objective and camera settings. Today, use 0.547          |
| beadrad        | The radius of the diffusing particles.  |
| window         | A vector of frame spacings. These are typically log-spaced. For example: [1:10 20 50 100 200 500...]      |
| MinTAU, maxTAU | These values specify over what range the powerlaw <i>alpha</i> will be determined                         |
| D_eff_binres   | This parameter simply specifies how finely the histogram of D_eff will be binned. 50 is typical.          |
| colors         | This is a matrix that specifies the colors of each clot type for plotting. Leave this empty for defaults. |
| DATA           | This function returns a structure that captures the MSD and diffusion coefficient of each tracker.        |
| bufferD        | This value is the theoretical diffusion coefficient expected for a particle of this size                  |

3. Find the average diffusion coefficients, with error bars, for each clot type. Hint: use `nanmean(DATA(i).D_avg)` and `nanstd(DATA(i).D_avg)/numel(DATA(i).D_avg)`.

