Tracking Nanocars Using Single Molecule Spectroscopic Methods

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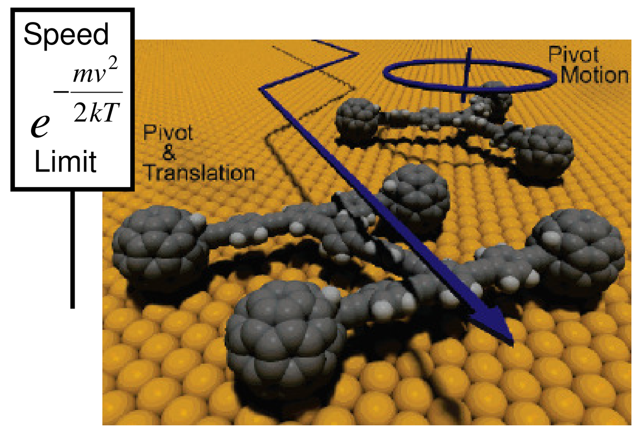
Introduction

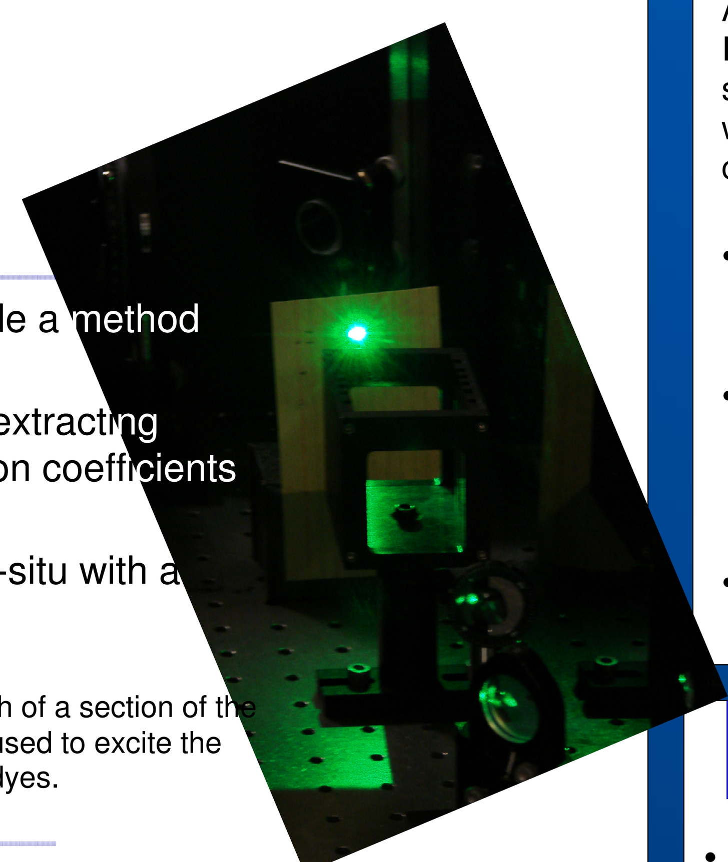
- Nanocars are molecular machines designed to roll over surfaces.
- Rotational motion of the nanocar wheels is thought to result in directional motion.
- The ultimate goal is to use them to transport cargo (which may be as small as a single molecule!) from place to place.



A computer render of the fullerene nanocar, courtesy reference 1.

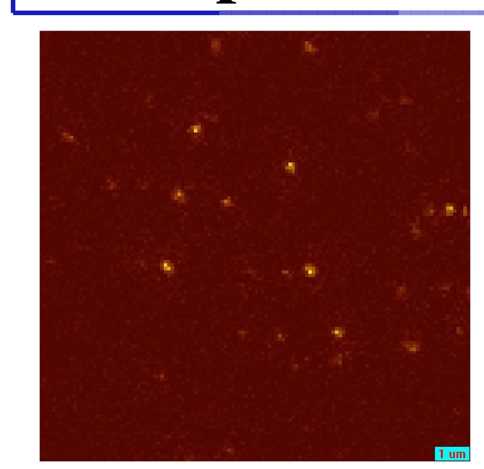
Goals

- The goal of this project is to provide a method of optically tracking nanocars.
- Useful to have a quick method of extracting useful information, such as diffusion coefficients and trajectories.
- Also useful to be able to do this in-situ with a non-invasive technique.



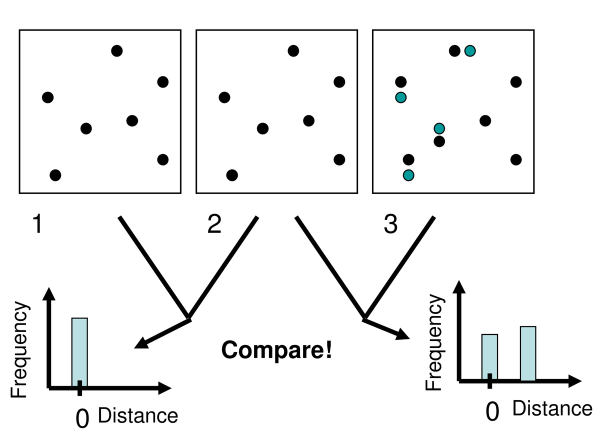
A photograph of a section of the laser setup used to excite the fluorescent dyes.

Concept

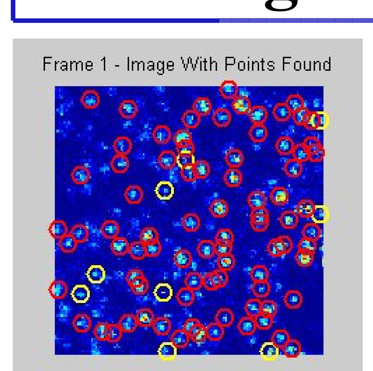


An image from the microscope.

- Take images (via a scanning confocal microscope), and fit a point spread function to each molecule (allows us to get position beyond the diffraction limit).
- Compare consecutive frames for movement.

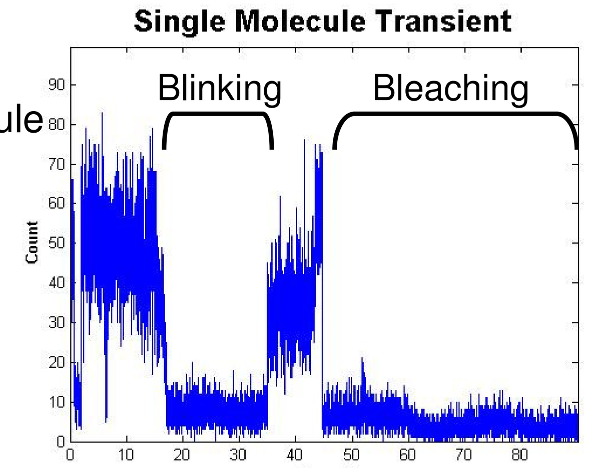


Challenges



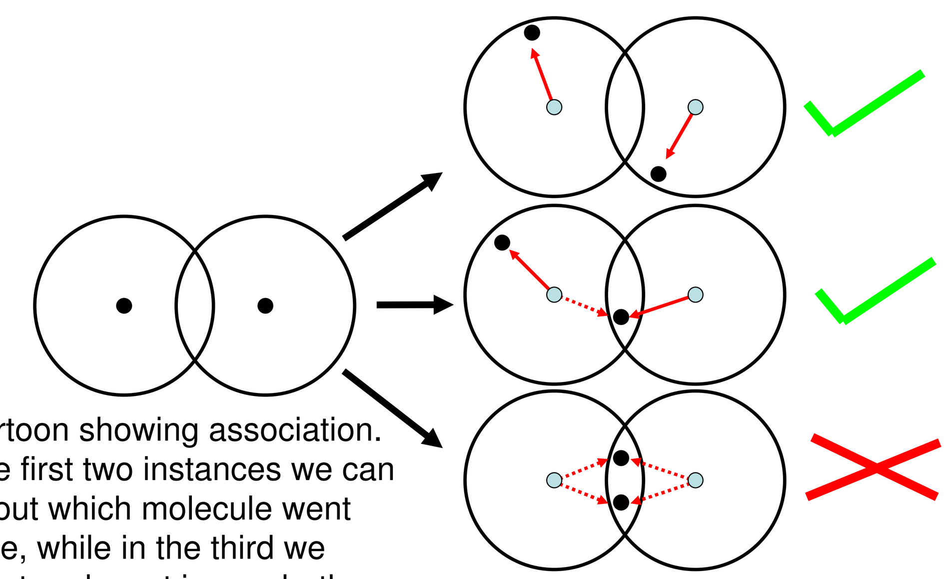
- The nanocars are a few nanometers large, however we can only resolve ~260 nm.
- To pinpoint the location of the nanocar more accurately, we use a centroid fit.
- Parameters from this fit are used to eliminate aggregates of single molecules.

- Photoblinking is a reversible phenomenon where the dye molecule will enter into a triplet state, temporarily not emitting any light.
- Photobleaching is a process where the dye undergoes irreversible photochemistry and stops emitting light.



Algorithm

- In order to detect movement we have to identify molecules in consecutive frames as the same.

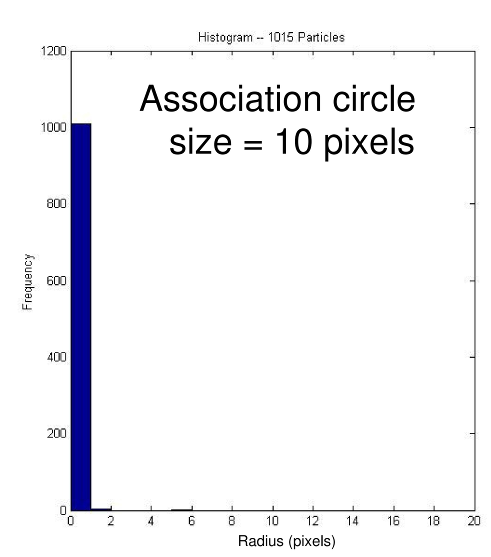


A cartoon showing association. In the first two instances we can sort out which molecule went where, while in the third we cannot and must ignore both.

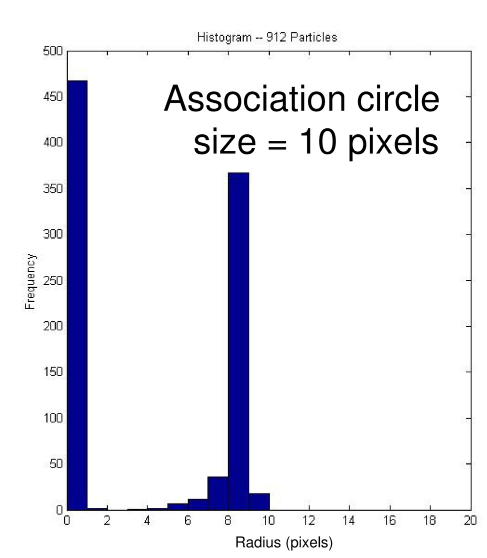
- By looking for molecules a certain distance from the ones we see in the first frame we make potential matches.
- If there is more than one match per molecule we can use a pruning technique to remove known matches to eliminate possible matches, allowing us to associate more molecules between frames.
- If we still have more than one match we must remove both, as we don't want wrongly associated molecules.

Verification

- To make sure the algorithm works we can intentionally shift frames.
- The data below show no shift compared with *every other* frame shifted by 8.486 pixels (6x6). So we expect to see half the molecules showing a shift and half showing no shift.



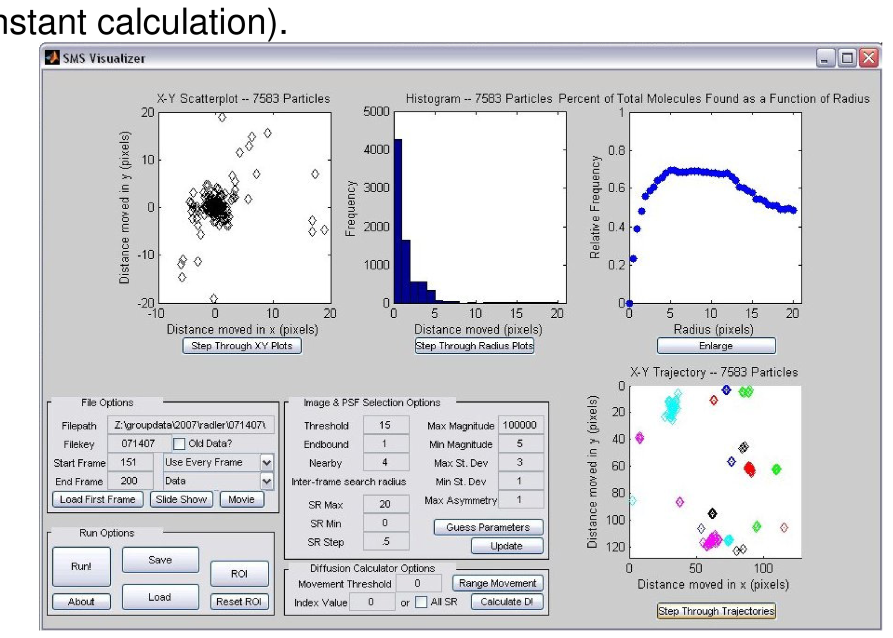
Original Data



Data with a 6x6 pixel shift

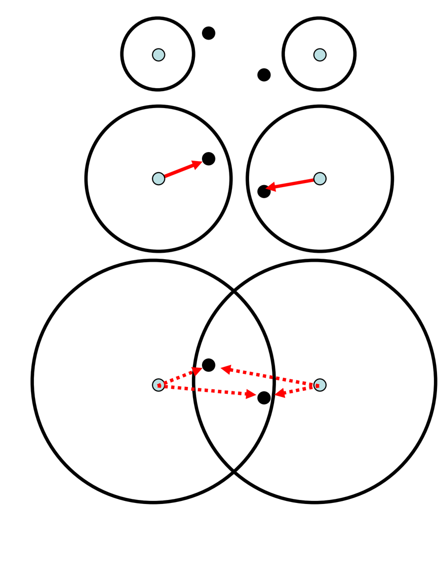
Program

- A MATLAB program was written to perform all of the tests (search radius, histogram, scatterplot, trajectory, and diffusion constant calculation).



Data – Search Radius

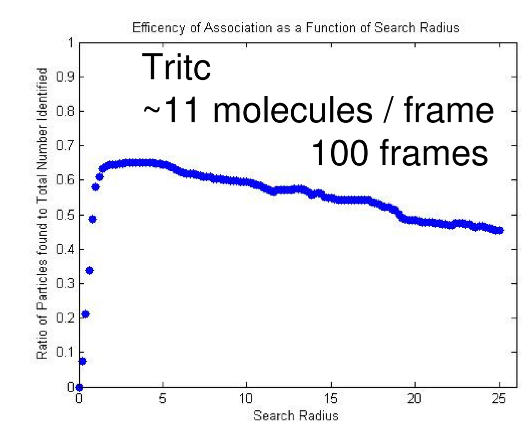
- By varying the size of the radius of the circle that we draw to look for molecules we can vary the number of particles associated.

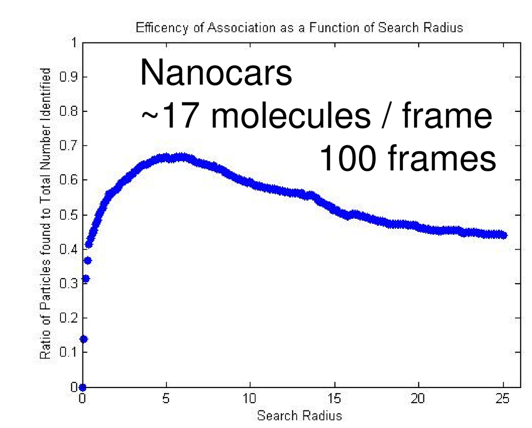


Too Small: The second molecule might not be found.

Optimum: All, or most of the particles are associated.

Too Large: Molecules fall into overlap, and are discarded.

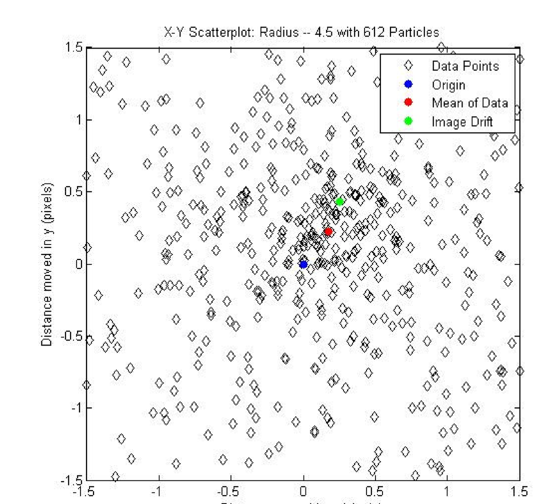




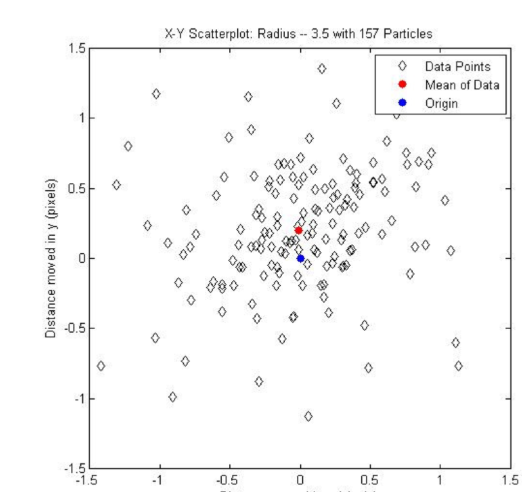
- The peak association efficency for the nanocars occurs farther out indicating that the molecules are moving a greater amount than the Tritc sample.

Data – X-Y Scatter Plots

- Scatterplots can be used to detect drift in the system, as opposed to random diffusion.

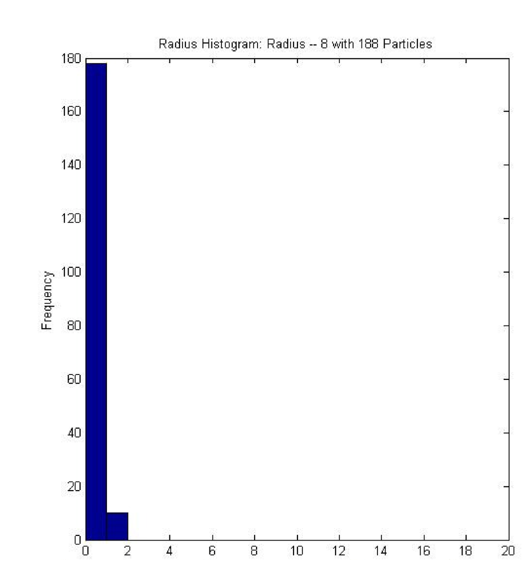


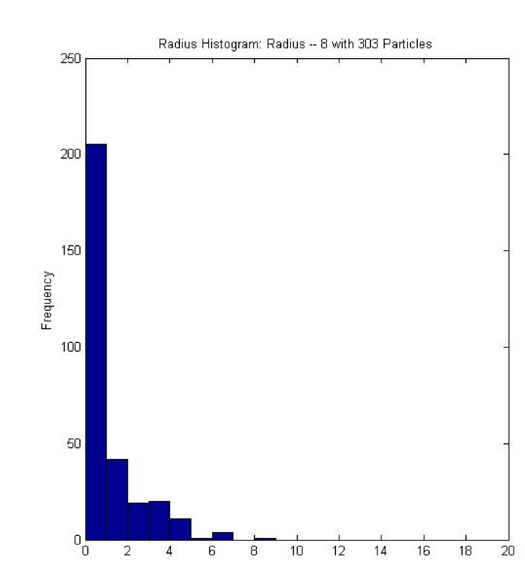
Test data with an imposed drift



Data that appeared to be drifting

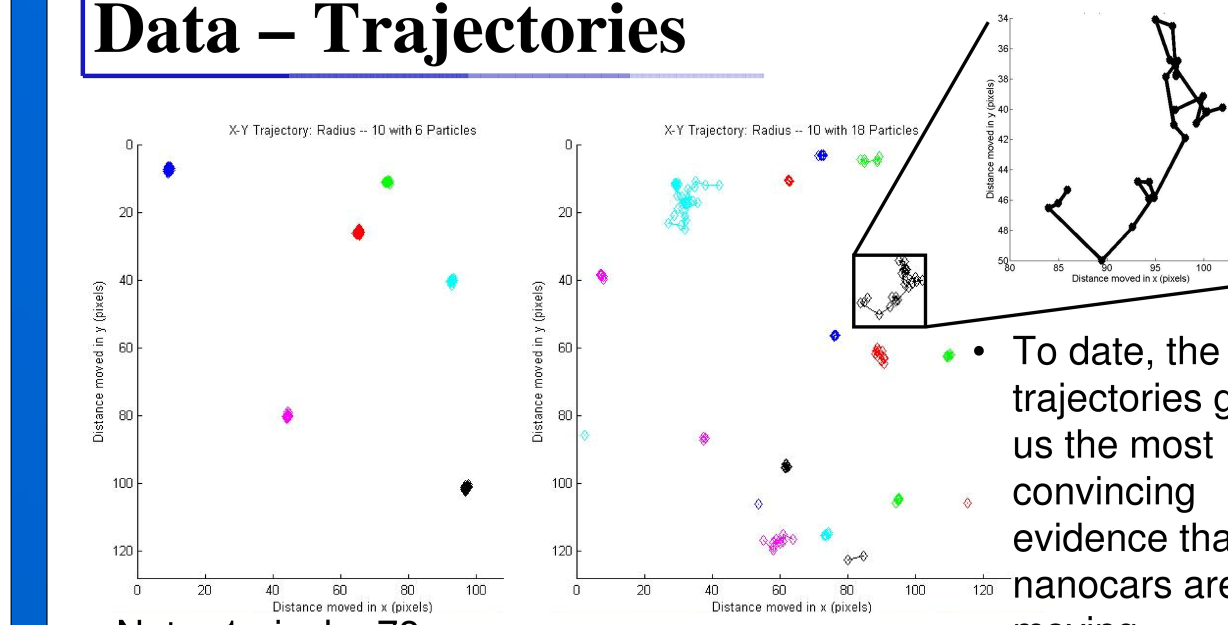
Data – Radius Histograms





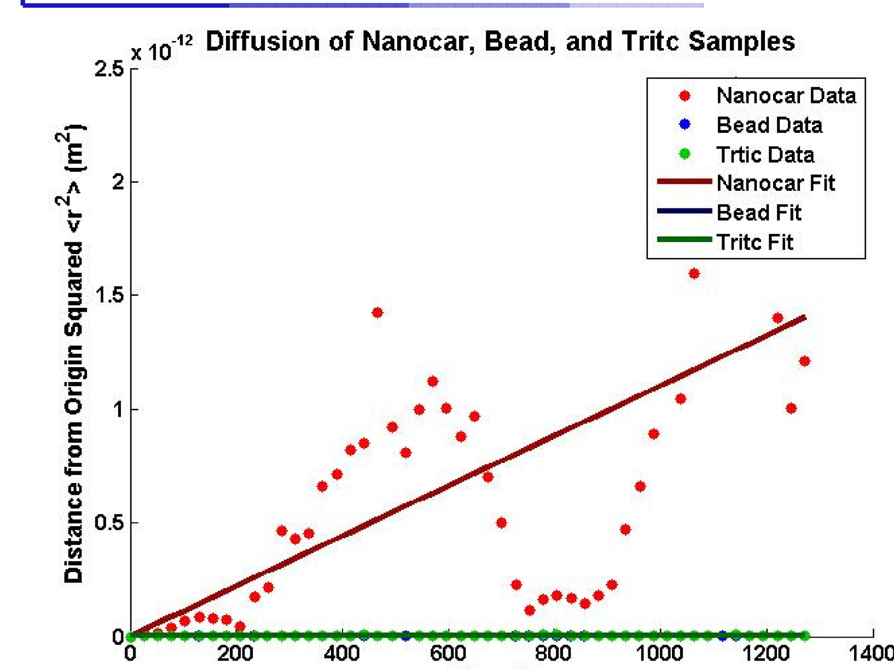
- From the histograms we can see that more nanocars move a greater amount for a given association radius size.

Data – Trajectories



- To date, the trajectories give us the most convincing evidence that the nanocars are moving.
- Note: 1 pixel = 78 nm

Data – Diffusion Constants



- The nanocars can be seen to move a greater distance than either the Bead or Tritc samples.
- The fit for each is a linear fit as if it were diffusing.

Conclusions & Future Work

- Nanocars, or what are thought to be nanocars can be identified quantitatively using the methods presented here.
- The program can successfully track single molecules in a field of single molecules. It also behaves as we would expect for all different test conditions.
- We are on the verge of obtaining diffusion constants from the trajectory information, these will play a major role in any temperature dependence studies.
- From studies using artificial data we have a better idea of the qualities of the sample we are looking for, and can model some parameters.
- The method of nanocar movement remains to be investigated, however, we believe we now have all the tools we need developed.
- To this extent, we plan on investigating different temperatures (we have recently developed a cryostat for this purpose).
- Alternate coverslip materials will also be used, for example, atomically flat mica instead of glass, or on silicon with and without grooves etched in.

References & Acknowledgements

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