Brownian Motion in Optical Tweezers

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Brownian motion is the apparently random evolution of an object’s position due to collisions with the molecules of the media it is immersed in. All currently accepted mathematical models of Brownian motion determine that an objects average distance from its original starting point will change linearly over time. By using video tracking software to quantify Brownian motion of micron-sized polystyrene spheres, we observed motion in quantitative disagreement with this model. One possible source of this disagreement is that our video tracking has a significantly faster frame rate than previous experiments. We were also able to qualitatively limit the Brownian motion of the spheres by using a Helium-Neon laser to trap them, known as optical tweezers, which we hope to use in further experiments such as exploring the manipulation of proteins and DNA.
It has long been known that if an object of a comparable mass to that of the molecules in the fluid is immersed in that fluid, the collisions between molecules will result in random motion for the object. [3, 4, 6, 8] This is known as Brownian motion. In order to investigate this phenomenon, we have made improvements to previous microscope designs [7, 9, 13, 14] and gathered video data in which we tracked Brownian motion of micron-sized polystyrene spheres. We have found that the motion of these particles quantitatively disagrees with the accepted model.

In order to observe the motion of the particle, we use the microscope setup illustrated in Figure 1. This setup is designed to accommodate a fiber laser input to trap the particles as explained below. The microscope uses a 100x oil-immersion objective. Two lenses of focal length 5 cm magnify the image virtually, and an Edmund Optics adjustable focal length lens focuses the light so that it can be recorded by a Guppy CCD camera. The image is recorded via firewire with a computer.

We placed a dilute solution of polystyrene spheres (radius $a = 545 \pm 27$ nm) in deionized water on a microscope slide as shown in Figure 2, making sure that the objective did not come in contact with the cover slip and that the slide mount was level. We took videos approximately three minutes in length of the motion of the microspheres as viewed through the microscope. We determined whether the particles exhibited Brownian motion by measuring the average magnitude of the particle’s displacement after $N$ frames of video

$$
\langle r^2 \rangle \equiv \frac{\sum_{n=1}^{N} r_n^2}{N},
$$

(1)

where $r_n$ is the the magnitude of the displacement in the $n$th frame [6]. In two dimensions, Brownian theory says that this average value at a time $t$ is given by

$$
\langle r^2 \rangle = \frac{2k_BT}{3\pi \eta a} t,
$$

(2)

where $k_B$ is Boltzmann’s constant, $T$ is the temperature of the sample, $\eta$ is the viscosity of the fluid (in this case, water), and $a$ is the radius of the sphere [6]. More simply, given the constraints of constant $T$ and consistent $a$, we can say that

$$
\langle r^2 \rangle = At + C,
$$

(3)

for a constant $A$ and a vertical offset $C$ that takes into account any short-term drag effects [6].
FIG. 1. The setup of our microscope. Light is projected up through the device using a light source. The visible light can pass through the dichroic mirror. A set of lenses magnify the light which is then recorded by a Guppy CCD Camera. We also can send a laser through this device through a fiber input in order to trap the microspheres. The laser exits the fiber and passes through two converging lenses—one to reduce the divergence angle and the second to focus the beam. Then, the IR light is reflected down to the objective. It is focused by the 100X objective down through the oil drop and into the microspheres. There is also a blue dichroic filter to prevent the CCD from becoming saturated.

To make sense of Eq. (3), we need to know the values of all of the constants that make up $A$. As stated above, we know that the radius of the spheres is $a = 545 \pm 27$ nm. We also measured the lab temperature $T = 301 \pm 1$ K. At that temperature, water’s viscosity is known [12] to be $\eta = 0.000833 \pm 0.000018$ kg/m \cdot s. Thus, we expect that $A = 1.94 \pm .11 \mu m^2/s$. (Note that all uncertainties here and below are given to a 95 % confidence interval.)

We also need a conversion scale between pixels on the computer movie and the actual distances between objects. To do this, we filmed a picture of a 20$\mu$m pinhole using the Guppy CCD Camera. After taking a short video of the pinhole through the microscope, we isolated the frames of the video and used ImageJ to scale the number of pixels across the pinhole (about 120 pixels). This allowed us to find the scale factor of $\chi = 6.332 \pm .046$ pixels per micrometer.
FIG. 2. We prepared a slide to view our microsphere solution as illustrated here. We used a heat source to melt a strip of parafilm to a microscope slide and then used a razor to cut a hole in the parafilm layer. We placed a drop of microsphere solution in this hole, being careful that the solution did not touch the parafilm. We then put the cover slip on top of the drop and placed a drop of immersion oil on top of the coverslip so that we could use the microscope to view the drop.

![Diagram of slide setup](image)

FIG. 3. An illustration of the background subtraction we performed in order to more easily locate the particle. (a) A “blank background” with no spheres in the field of view. (b) A frame of the movie from the CCD camera in which we see a particle. (c) The result of background subtraction. The particle is visible but the background has been replaced by black. This is easier for ImageJ to read.
To properly track the spheres that were exhibiting Brownian motion, we prepared a solution that would have a high enough concentration such that it was easy to find the spheres, but not so dense that it would be hard for us to focus on one at a time. We created a solution at a concentration of 137 mL of de-ionized water and one drop of the 5\% weight per volume concentration of spheres. Using this concentration, we placed a small drop onto the slide in the middle of the parafilm square, as illustrated in Figure 2, being sure the fluid did not touch the film. We then placed a cover slip over the sample with an oil drop on top. The sample was then placed under the microscope and we used the focus to find the edge of the parafilm on the slide. When we focused on the edge of the film, it was approximately the focus needed to view the spheres in the sample, which we found when we panned over the sample and used the fine focus to bring them into a more clear view. Once we found the spheres in the solution, we used the CCD camera to track their motion, actively adjusting the focus as the spheres moved vertically through the solution with respect to the microscope. Because the sphere’s moved in three dimensions, the video did not always have the spheres in clearest focus, but the picture was clear enough for data analysis.

When we tracked the spheres using the procedure above, we saw obvious qualitative examples of Brownian motion. We used Adobe Photoshop to subtract the background out of the frames in the videos, giving a more clear picture of the spheres in each frame, by making the background black and the outline of the sphere white (see Figure 3). We also used Photoshop to crop the pictures to the area of interest in the video. By tracking a single point on the sphere, the ImageJ software provided a clear representation of the sphere’s motion with a complete picture of the spheres interaction with the fluid. The tracking, shown in Figure 4, shows small jumps in the particle’s position per frame, that add up over time to create an overall displacement from the sphere’s original position. For more information on video data processing, see ref. [8].

We used Wolfram Mathematica to analyze the $\langle r^2 \rangle$ value based on the ImageJ trajectory output. The graph of $\langle r^2 \rangle$ versus time of the average of the data sets is shown in Figure 5. As stated above, the model would predict $A = 1.94 \pm .11 \mu m^2/s$, but this does not agree any of our three data sets presented in that figure.

The model cited above is used for 300 frames of data in ref. [6]. Thus, we decided to check to see if the model worked for our data in shorter frame lengths. We parceled the data
FIG. 4. The path taken by the microsphere plotted in blue in Figure 5. The green lines represent the path taken by the microsphere, the white circle, over a time period of 90 seconds. The red lines in the path of the sphere are fillers by ImageJ when the particle was not clearly defined in the given frame. The program attempts to complete the trajectory by connecting the points before and after the missed frame.

into equal segments of around 300 frames so that our frame count is comparable to that of the data used to determine the model in ref. [6]. We created 300 frame segments of three data sets, and then plotted the segments of each set together. Then we averaged each group of segments, and plotted the averages of the set as shown for one of the sets in Figure 6. For all three sets the found value of $A$ does not agree with the model.

By establishing a way to view and track microspheres in water, we have qualitatively
FIG. 5. The $\langle r^2 \rangle$ value versus time with the best linear fit for the data sets using untrapped particles. The model, showed by the dashed green line, gives us $A = (1.94 \pm 11) \mu m^2/s$, which is not in agreement with any of our data sets.

identified Brownian motion. However, further data is needed to confirm the model of Brownian motion quantitatively. We have recognized significant drift of the spheres in the video’s we have analyzed, resulting in a net displacement from the original position, rather than moving randomly around its starting position like previous models suggest. By analyzing segmented sets of the data, as well as full sets of the data, we have shown that the currently accepted models for Brownian motion do not completely describe the phenomena. A more complex model may be needed to better explain Brownian motion as observed with higher-resolution data collection.

Aside from looking at Brownian motion, we have also made a few strides towards establishing our optical tweezers. Optical tweezers use a laser to trap microscopic dielectric particles. Easily-trapped particles are polystyrene beads and hollow glass spheres [1, 2, 5, 7, 9, 10, 14]. Optical tweezers are often used in the field of biophysics, particularly in DNA manipulation [2, 5, 9, 10, 14]. Originally very large and expensive, optical tweezers can now be constructed at much lower costs and without creating a hazardous work environment. We have shown qualitatively the trapping ability of our optical tweezers.

Optical tweezers work on the principle of refraction. A sphere illuminated by a laser can
FIG. 6. The $\langle r^2 \rangle$ value versus time for the segmented data. The dashed purple line represents the average of all the data sets at a given time along the horizontal axis. The model, showed by the dashed blue line, gives us a linear fit slope $A = 0.8349 \pm 0.0177 \mu m^2/s$, which is not in agreement with the model. The full, unsegmented data is shown in maroon in Figure 5 and has a fit parameter $A = 1.218 \pm 0.010 \mu m^2/s$.

become trapped because of a change in momentum of rays of light as they refract through the sphere. Newton’s third law dictates that the particle will have an equal and opposite momentum, which forces the particle to migrate toward the center of the laser beam. The beam’s focus is the location of zero net refractive force, and is subsequently where the particle will become trapped [9] as illustrated in Figure 7.

Our optical tweezers use a Helium-Neon laser from Melles Griot at 632.8 nm with 30 mW of power. The beam is coupled into a 10 m fiber cable using a collimation set from Thorlabs with the first collimator having a focal length of 220 nm. As the beam exits the cable and enters the side of the microscope setup explained above and illustrated in Figure 1, it passes through a lens in order to dramatically reduce the divergence angle of the beam. The beam then travels through a 5-cm converging lens that can be moved in order to adjust the focus of the laser. After this, the beam bounces off a green Thorlabs FDIG additive dichroic color filter that acts as a mirror to reflect the beam while passing through other wavelengths. The beam is then focused through the 100x microscope objective. An additional blue dichroic
FIG. 7. An illustration of the principle behind optical tweezers based off of a figure in ref. [11]. The Gaussian on the left is a graph of the intensity of the beam. Light from the higher-intensity portion of the beam is refracted through the microsphere resulting in a momentum shift and corresponding force $\vec{F}_{\text{higher intensity}}$. Similarly, light from the lower-intensity portion of the beam is refracted resulting in a force $\vec{F}_{\text{lower intensity}}$. Adding up all of these beams gives a net force directed toward the focus of the beam. This is the equilibrium point of the trap.

FIG. 8. Two video frames separated by 5 seconds that show that particles have been trapped. There is a bunch of particles that are located at the beam focus that remain stationary as the reference particles in the surrounding solution move.
filter is placed above the mirror to further filter the high-intensity beam so as not to saturate
the CCD.

We have gained initial qualitative confirmation of the trapping ability of the tweezers. We have been able to trap a bunch of particles and have them remain stationary even as we slowly move the slide and surrounding solution relative to these trapped particles. This is shown in Figure 8. The next step is to do a quantitative analysis of the trapped spheres. We expect that the optical tweezers will diminish the Brownian motion of the spheres since they are trapped. By improving the optical tweezers procedure we hope to move objects using the power of the laser, such as DNA manipulation.