

# Brownian Motion

## Experiment BM

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### Objective

A microscope is used to observe the motion of micron-sized spheres suspended in water. A digital video camera captures the motion which is then analyzed with a particle tracking program to determine the path of individual spheres. The sphere's random Brownian motion is analyzed with a spreadsheet to verify various theoretical predictions. The dependence of the particle's displacements on time as well as various physical parameters such as the temperature, the suspension liquid's viscosity and the sphere diameter is also explored. Diffusion of dye-labeled DNA molecules is also studied.

### References

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### Brownian motion

Brownian motion refers to the continuous, random motion of microscopic particles that are suspended in a fluid. Robert Brown in 1827 described such motion in micron-sized particles that were released from the pollen grains of *Clarkia pulchella*, a flower that had been discovered by Lewis and Clark a few years earlier. Although the biological origin of the particles at first suggested that this motion had something to do with life, Brown quickly realized that all sorts of inorganic particles ex-

<sup>1</sup><http://dx.doi.org/10.1119/1.4803529>

<sup>2</sup><http://dx.doi.org/10.1119/1.18210>

<sup>3</sup><http://dx.doi.org/10.1119/1.2386163>

<sup>4</sup><http://dx.doi.org/10.1119/1.1542619>

<sup>5</sup><http://dx.doi.org/10.1119/1.3475685>

<sup>6</sup><http://pubs.acs.org/doi/abs/10.1021/ma951455p>

hibited the same motion. Granules of carbon soot or ancient stone, when suspended in water, executed ceaseless motion with complex, apparently random trajectories. Although the rate of motion depends on the size of the particles and the temperature and viscosity of the fluid, the phenomenon is universal to particles in a fluid.

Brown himself did not understand the physical cause, but Einstein recognized that Brownian motion could be completely explained in terms of the atomic nature of matter and the kinetic nature of heat: the fluid is composed of molecules that are in continuous motion at any finite temperature. A larger particle in the fluid is subject to frequent collisions that deliver numerous small, random impulses, causing the larger particle to drift gradually but irregularly through the fluid. In one of his famous 1905 papers, Einstein showed that the rate of Brownian motion is directly related to the microscopic Boltzmann constant  $k_B$ , which sets the scale for the kinetic energy ( $\sim k_B T$ ) carried by a water molecule at temperature  $T$ .  $k_B$  is related to the macroscopic gas constant  $R = N_A k_B T$  by a factor of Avogadro's number  $N_A$ . As  $R$  is easily found in a benchtop experiment, the measurement of  $k_B$  reveals  $N_A$ . Brownian motion is a direct link between two very different size scales in physics: it originates in the microscopic motion of atoms, molecules and the tiny scale of their thermal energy, and it is observable as macroscopic motion that can be measured at the bench of any reasonably well equipped laboratory. Einstein's work paved the way for Perrin's measurements of Brownian motion, which provided compelling support for the atomic theory and earned the 1926 physics Nobel prize.

## Kinetic theory

Before considering Brownian motion, we should first recall certain aspects of the kinetic theory for the molecules of the fluid. These molecules are in constant interaction with all other molecules, which together form a heat bath at temperature  $T$ . The *equipartition theorem* of statistical physics requires that the kinetic energy in each spatial component of the molecular velocity has an ensemble average value of  $k_B T/2$ . For the  $x$ -direction this implies:

$$\frac{1}{2}m \langle v_x^2 \rangle = \frac{1}{2}k_B T \quad (1)$$

Here  $m$  is the molecular mass and  $T$  is the temperature. The angle brackets  $\langle \rangle$  indicate an ensemble average, i.e. the average over a large population of molecules. The ensemble average may be calculated if the probability distribution for  $v_x$  is known.

What is the probability distribution for the velocity components? Each of the three spatial components of the molecular velocity is distributed according to a Boltzmann distribution in the kinetic energy  $mv^2/2$  associated with that velocity component. This leads to the so-called Maxwell Boltzmann distribution for the probability that the molecule will have a velocity between  $v_x$  and  $v_x + dv_x$

$$dP(v_x) = \sqrt{\frac{m}{2\pi k_B T}} e^{-mv_x^2/2k_B T} dv_x \quad (2)$$

The square root prefactor is required for normalization

$$\int_{-\infty}^{\infty} dP(v_x) = 1 \quad (3)$$

Equation 2 is a Gaussian probability distribution with a mean of zero and a variance of  $k_B T/m$ . Of course, analogous expressions apply to the  $y$ - and  $z$ -components of velocity.

The Maxwell-Boltzmann probability distribution for  $v_x$  (Eq. 2) obeys the equipartition theorem because the average is

$$\langle v_x^2 \rangle = \sqrt{\frac{m}{2\pi k_B T}} \int_{-\infty}^{\infty} v_x^2 e^{-mv_x^2/2k_B T} dv_x \quad (4)$$

which leads to  $\langle v_x^2 \rangle = k_B T/m$ , consistent with Eq. 1.

For water molecules at room temperature the average molecular speed is roughly 600 m/s. Consequently there is plenty of momentum in the fluid molecules that collide with a small particle that is suspended in the fluid.

Consider a particle with mass  $M$ , suspended in the fluid at  $\mathbf{r}(t)$  and moving with velocity  $\mathbf{v}(t)$ . The particle is subject to a net force  $\mathbf{F}(t)$  from the fluid. We can analyze the motion over a time interval  $dt$  that is sufficiently short that  $\mathbf{r}(t)$  and  $\mathbf{v}(t)$  can both be considered very nearly constant. For a Brownian particle, it is convenient to analyze the motion by casting Newton's second law in the form:

$$d\mathbf{r}(t) = \mathbf{v}(t)dt \quad (5)$$

$$d\mathbf{v}(t) = \frac{1}{M}\mathbf{F}(t)dt \quad (6)$$

$\mathbf{F}(t)$ , which may depend on  $\mathbf{r}$  and  $\mathbf{v}$ , is evaluated and the right sides of Eqs. 5 and 6 are calculated. With the left sides defined by

$$d\mathbf{r}(t) = \mathbf{r}(t+dt) - \mathbf{r}(t) \quad (7)$$

$$d\mathbf{v}(t) = \mathbf{v}(t+dt) - \mathbf{v}(t) \quad (8)$$

the right-side values are then added to the values  $\mathbf{r}(t)$  and  $\mathbf{v}(t)$  to obtain updated values  $\mathbf{r}(t+dt)$  and  $\mathbf{v}(t+dt)$  at a time  $dt$  later. Starting from given initial conditions for  $\mathbf{r}(0) = \mathbf{r}_0$  and  $\mathbf{v}(0) = \mathbf{v}_0$  at  $t = 0$ , the process is repeated to obtain future values for  $\mathbf{r}(t)$  and  $\mathbf{v}(t)$  at discrete intervals.

The motion is said to be *deterministic* when  $\mathbf{F}(t)$  can be precisely determined from the val-

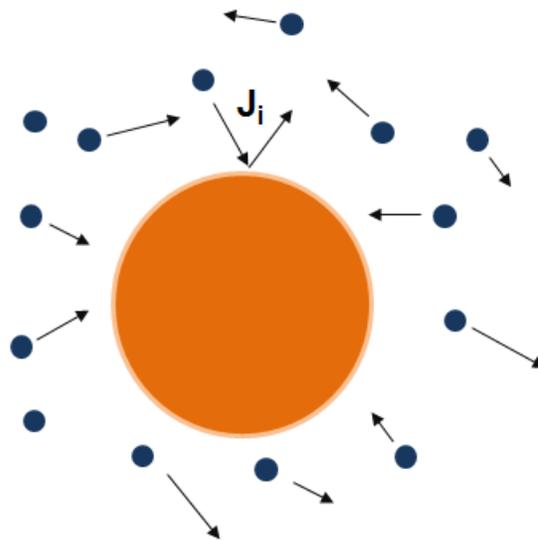


Figure 1: The molecules of the surrounding fluid undergo frequent collisions with a Brownian particle (shown as large circle), delivering small random impulses  $\mathbf{J}_i$ . There are many such impulses in even a short interval  $dt$ .

ues of  $\mathbf{r}(t)$ ,  $\mathbf{v}(t)$ , and  $t$ . For example, in a collision between two particles with a known interaction (such as the Coulomb or gravitational force)  $\mathbf{F}(t)$  is deterministic and the motion is quite predictable.<sup>7</sup>

In Brownian motion,  $\mathbf{F}(t)$  is created by the very frequent collisions between the suspended particle and the molecules of the surrounding medium, about  $10^{19}$  collisions per second for a  $1\ \mu\text{m}$  particle in water. Consider the force  $\mathbf{F}_i(t)$  that acts on the Brownian particle during its collision with one individual molecule from the medium. That one collision delivers an impulse to the Brownian particle

$$\mathbf{J}_i = \int \mathbf{F}_i(t)dt \quad (9)$$

where the integral extends over the dura-

<sup>7</sup>Deterministic does not always mean predictable. Some perfectly precise forms of  $\mathbf{F}(t)$  lead to chaotic solutions that cannot be predicted far into the future at all.

tion of the collision (Figure 1). The individual impulses  $\mathbf{J}_i$  vary in magnitude and direction because the velocities of molecules in the medium vary according to the Maxwell-Boltzmann distribution. Consequently the direction and magnitude of  $\mathbf{F}_i(t)$  are highly variable. This variable impulse adds a random component to the net force on the particle. Both the force and the particle's motion are said to be *stochastic*, and the motion of a single particle is unpredictable. Rather than focusing on predicting individual trajectories, we aim to understand the motion in terms of probabilities and average behavior.

## Dynamics of a Brownian particle

Because of the high collision frequency, we can choose a time interval  $dt$  short enough that  $\mathbf{r}(t)$  and  $\mathbf{v}(t)$  do not change significantly, yet long enough to include thousands of collisions. Over such an interval, the value of  $\mathbf{F}(t)dt$  in Eq. 6 would properly be the sum of all impulses delivered during the interval  $dt$

$$\mathbf{F}(t)dt = \sum_i \mathbf{J}_i \quad (10)$$

When the number of collisions during  $dt$  is large, we can use the *central limit theorem* to draw important conclusions about the form of  $\mathbf{F}(t)dt$  even though we lack detailed knowledge of individual impulses. The central limit theorem states that if we add together many random numbers drawn from the same probability distribution, the sum will be a Gaussian-distributed random number. This is true regardless of the distribution from which the random numbers are drawn (e.g. Gaussian or not). More precisely, the theorem states that if  $N$  individual random numbers  $x_i$  are drawn from *any* probability distribution that has mean  $\mu_i$  and variance  $\sigma_i^2$ , then the sum  $\sum x_i$  of those  $N$  numbers will itself be

a Gaussian-distributed random number with mean  $\mu = N\mu_i$  and variance  $\sigma^2 = N\sigma_i^2$ .

Each cartesian component of  $\mathbf{J}_i$  is a random number from an (unknown) distribution and thus the central limit theorem applies to each component of Eq. 10. Remember,  $\mathbf{v}(t)$  and  $\mathbf{r}(t)$  do not change significantly over the interval  $dt$ ; the probability distributions for the components of  $\mathbf{J}_i$  arise from the distribution of velocities for the colliding molecules and from the distribution of collision angles. Moreover, because the number of collisions  $N$  over a time interval  $dt$  will be proportional to  $dt$ , the central limit theorem implies that each component of  $\mathbf{F}(t)dt$  will be a random number from a Gaussian distribution whose mean and variance are both proportional to  $dt$ .

We do not expect that  $\mathbf{F}(t)$  necessarily has a mean value of zero. If the Brownian particle has a net velocity  $\mathbf{v}$  through the fluid, it will collide with more molecules on its leading side than on its trailing side, and so the force will be imbalanced. In fact we should expect that  $\mathbf{F}(t)$  will depend in part on the velocity  $\mathbf{v}(t)$  of the Brownian particle with respect to the bulk medium. Paul Langevin hypothesized that  $\mathbf{F}(t)dt$  can be expressed

$$\mathbf{F}(t)dt = -\alpha\mathbf{v}(t)dt + \mathbf{F}^{(r)}(t)dt \quad (11)$$

The term  $-\alpha\mathbf{v}$  describes a viscous drag force that is opposite in direction and proportional to the particle's velocity relative to the fluid. This had already been investigated by Stokes, who showed that the drag coefficient  $\alpha$  for a sphere of diameter  $d$  in a fluid of dynamic viscosity  $\eta$  is given by

$$\alpha = 3\pi\eta d \quad (12)$$

$\mathbf{F}^{(r)}(t)$  is the random part of the collisional force. Langevin successfully characterized this part and showed how it was responsible for Brownian motion.

We will use a shorthand notation

$$N(\mu, \sigma^2) \quad (13)$$

to refer to a Gaussian distribution of mean  $\mu$  and variance  $\sigma^2$ . For example,

$$v_x = N\left(0, \frac{k_B T}{m}\right) \quad (14)$$

will be shorthand for the statement that the  $x$ -component of velocity for a molecule of mass  $m$  is a random variable drawn from the Gaussian probability distribution of Eq. 2.

Any random number from a distribution with a mean  $\mu$  and variance  $\sigma^2$  can be considered as the sum of the mean and a zero-mean random number having a variance  $\sigma^2$

$$N(\mu, \sigma^2) = \mu + N(0, \sigma^2) \quad (15)$$

Then one may see how Eq. 11 is related to Eq. 10 and the central limit theorem. Each cartesian component of the  $-\alpha \mathbf{v} dt$  term in Eq. 11 is the mean of the sum in the central limit theorem applied to that component of Eq. 10. With the means accounted for by the  $-\alpha \mathbf{v} dt$  term, each component of the  $\mathbf{F}^{(r)}(t)dt$  term must be a zero-mean, Gaussian-distributed random number providing the random or distributed part of the central limit theorem.

Over time, the viscous drag force in Eq. 11 will tend to eliminate any initial velocity of the particle through the fluid; however the fluctuating force  $\mathbf{F}^r(t)$  will prevent the particle from ever coming to rest. Regardless of the initial velocity, random collisions with molecules in the environment will deliver kinetic energy to the suspended particle and ensure that it has mean kinetic energy as specified by the equipartition theorem and a velocity probability distribution of the Maxwell Boltzmann form.

**Exercise 1** Determine the room temperature rms velocities ( $\sqrt{\langle v^2 \rangle}$ ) of water molecules and of  $1 \mu\text{m}$  diameter spheres in water. Assume the spheres have the density of water.

Note that the  $\mathbf{F}^{(r)}(t) dt$  term (which drives the particle's motion) and the viscous drag force (which opposes it) will require a certain balance if the velocity at equilibrium is to follow the Maxwell Boltzmann distribution. The *fluctuation-dissipation theorem* describes this balance, relating the impulse delivered during an interval  $dt$  to the viscous drag coefficient and the temperature as follows:

$$F_x^{(r)}(t)dt = N(0, 2\alpha k_B T dt) \quad (16)$$

A similar equation holds for the  $y$  and  $z$ -components of  $\mathbf{F}^{(r)}(t)$ . As  $\alpha$  is the viscous drag coefficient, Eq. 16 makes a fundamental connection between the fluid's viscosity and temperature and the size of the fluctuating force.

Note that the mean, or  $-\alpha \mathbf{v} dt$  term, in Eq. 11 is proportional to  $dt$  as required by the central limit theorem. Note also that the random  $\mathbf{F}^{(r)}(t)dt$  term also satisfies the theorem in that its variance is proportional to  $dt$ . These two proportionalities are required if Eqs. 5 and 6 are to give self-consistent solutions as the step size  $dt$  is varied.

**Exercise 2** When solving differential equations numerically (i.e., on a computer), the time step  $dt$  must be chosen small enough that  $\mathbf{r}(t)$  and  $\mathbf{v}(t)$  undergo only small changes during the interval. However,  $dt$  must not be made too small because roundoff and other numerical errors occur with each step. Often, one looks at the numerical solutions for  $\mathbf{r}(t)$  and  $\mathbf{v}(t)$  as the step size  $dt$  is decreased, choosing a  $dt$  where there is little dependence on its size.

Why do the mean and variance of  $\mathbf{F} dt$  have to be proportional to  $dt$  in order for the equations of motion to be self consistent? Your

answer should take into account how the sum of two Gaussian random numbers behave (on average) and how  $\mathbf{v}(t)$  (on average) would change over one interval  $dt$  or over two intervals half as long.

We will take initial conditions at  $t = 0$  of  $\mathbf{r}(0) = \mathbf{r}_0$  and  $\mathbf{v}(0) = \mathbf{v}_0$ . Thus, the particle begins with a well defined position and velocity. However, the nature of the stochastic force implies that the particle position and velocity for  $t > 0$  will be probability distributions that change with time. The references<sup>8</sup> show how to integrate Eq. 11. Here we simply present the results without proof.

The solution for  $v_x(t)$  can be written

$$v_x(t) = N\left(v_{0x}e^{-t/\tau}, \frac{k_B T}{M}(1 - e^{-2t/\tau})\right) \quad (17)$$

where

$$\tau = \frac{M}{\alpha} \quad (18)$$

Analogous solutions are found for  $v_y$  and  $v_z$ . As required at  $t = 0$ , Eq. 17 has the value  $v_x(0) = N(v_{0x}, 0)$  (i.e., the velocity is  $v_{0x}$ ). At  $t \rightarrow \infty$  it has the solution  $v_x(\infty) = N(0, k_B T/M)$ , i.e., the Maxwell-Boltzmann distribution. Keep in mind that  $t \rightarrow \infty$  really means  $t \gg \tau$  where, for a particle of size  $1 \mu\text{m}$  in water,  $\tau \approx 100$  ns. Note how  $\tau$  in Eq. 17 describes the exponential decay of any initial velocity and (within a factor of two) the exponential approach to the equilibrium velocity distribution:  $\tau$  is such a short interval that the particle's velocity loses its initial value and becomes Maxwell-Boltzmann-like *very* rapidly.

The probability distribution for the position  $\mathbf{r}(t)$  is slightly more complicated. With analogous solutions for  $y(t)$  and  $z(t)$ , the result can be expressed

$$x(t) = N(\mu_x, \sigma^2) \quad (19)$$

<sup>8</sup>See especially D.T. Gillespie (1996).

where

$$\mu_x(t) = x_0 + v_{x0}\tau(1 - e^{-t/\tau}) \quad (20)$$

and

$$\sigma^2(t) = \frac{2k_B T}{\alpha} \left[ t - 2\tau(1 - e^{-t/\tau}) + \frac{\tau}{2}(1 - e^{-2t/\tau}) \right] \quad (21)$$

For a particle released from rest at the origin ( $\mathbf{r}_0 = 0$ ,  $\mathbf{v}_0 = 0$ ), the equilibrium position distribution then becomes

$$x(t) = N(0, \sigma^2) \quad (22)$$

Eq. 22 means that the probability for the particle to have an  $x$ -displacement between  $x$  and  $x + dx$  is given by

$$dP(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-x^2/2\sigma^2} dx \quad (23)$$

where

$$\sigma^2 = \frac{2k_B T t}{\alpha} = 2Dt \quad (24)$$

Here we have defined the *diffusion coefficient* of the particle,

$$D = \frac{k_B T}{\alpha} \quad (25)$$

The diffusion coefficient tells us how rapidly the *variance* in the particle's location grows with time.

The probability that the particle's displacement will fall within a volume element  $dV = dx dy dz$  around a particular value of  $\mathbf{r}$  is the product of three such distributions—one for each direction  $x$ ,  $y$  and  $z$ . Using  $r^2 = x^2 + y^2 + z^2$ , the product is

$$dP(\mathbf{r}) = \frac{1}{(2\pi\sigma^2)^{3/2}} \exp^{-r^2/2\sigma^2} dV \quad (26)$$

Consider a large number  $N$  of particles placed at the origin at  $t = 0$ . According to

Eq. 26, each will have the probability  $dP(\mathbf{r})$  to be in the volume element  $dV$  located at that  $\mathbf{r}$ . Consequently, the number of particles in that volume element will be  $NdP(\mathbf{r})$  and their number density would be given by  $\rho(\mathbf{r}) = NdP(\mathbf{r})/dV$  or

$$\rho(\mathbf{r}, t) = \frac{N}{(2\pi\sigma^2)^{3/2}} e^{-r^2/2\sigma^2} \quad (27)$$

where the (implicit) time dependence arises because  $\sigma^2$  grows linearly in time via Eq. 24. The concentration profile of the particles is a Gaussian function that becomes broader and flatter over time as the particles diffuse away from the starting point.

The particles spread according to Eq. 27 with Eq. 24 until hindered by the container walls. An observer might say that particles are being actively driven from regions of higher concentration to regions of lower concentration until they become uniformly distributed throughout the suspension, although of course the motion of individual particles is random.

Fick's second law of diffusion describes the flattening of  $\rho(\mathbf{r})$  over time.

$$\frac{d\rho}{dt} = D\nabla^2\rho \quad (28)$$

Einstein realized how Fick's second law is related to Brownian diffusion and was the first to relate  $D$  to  $\sigma$ .

**Exercise 3** Show that  $\rho(\mathbf{r}, t)$  satisfies Eq. 28 with

$$\sigma^2 = 2Dt \quad (29)$$

Note that if a spherical particle (diameter  $d$ ) moves in a fluid of viscosity  $\eta$ , we can combine Eq. 24 with Eq. 12 and obtain

$$D = \frac{k_B T}{3\pi\eta d} \quad (30)$$

This is the famous Stokes-Einstein expression for the diffusion coefficient of a spherical particle.

**Exercise 4** Eq. 29 says the width of the particle distribution increases with  $t$ . Qualitatively, this behavior is reasonable because with more time for the random Brownian motion, one would expect the values of  $\mathbf{r}$  to become more spread out. Explain in a similar qualitative way why the width of the distribution would be expected to increase with  $T$  and decrease with  $\eta$  and  $d$  as predicted by Eq. 30.

## Random walks and polymer chains

In this experiment you are going to study the Brownian motion of DNA molecules in water. The DNA strand coils up in a fairly random fashion in water, and the rate of its Brownian motion depends on the overall size of that random coil. Interestingly there is a close analogy between a Brownian motion trajectory and the configuration of a disordered chain molecule (polymer). Here we will examine a simple model known as the freely jointed chain (FJC). The FJC model will allow us to estimate the probability distribution for the distance between the two endpoints of a long, disordered polymer.

Imagine the polymer chain as consisting of a very large number  $N$  of links, each of length  $a$  (Figure 2). Suppose that the links are freely jointed so that the bond angle at the junction of two successive links  $i$  and  $i + 1$  can adopt any value, without bias. This may sound completely unrealistic, but for sufficiently long polymers such as nucleic acids (DNA, RNA) and large unfolded protein molecules it can be a reasonable approximation. The bonds that link the monomers of a real chain do have some intrinsic stiffness, but if we define a "link" as a sufficiently long segment of that chain (i.e. containing several monomer units), then successive links really can adopt nearly any orientation with respect to each other.

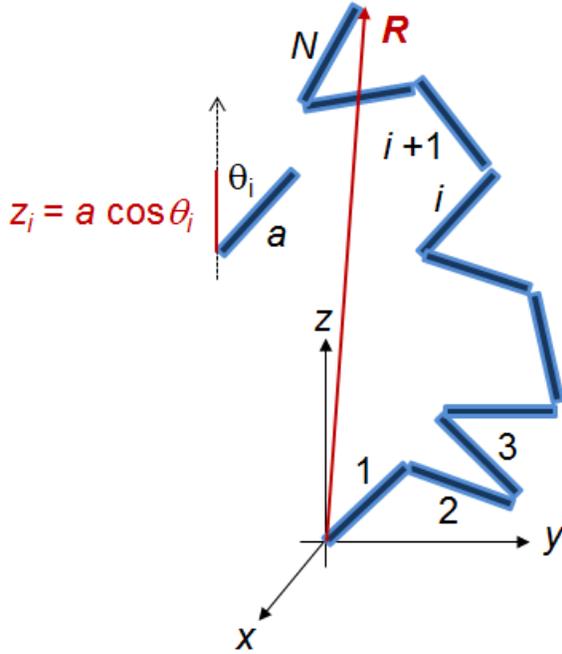


Figure 2: A freely jointed chain of  $N$  links, each of length  $a$ , adopts a random configuration. The total end to end displacement vector  $\mathbf{R}$  is the sum of the vector displacements of the links. Link  $i$  is oriented at angle  $\theta_i$  to the  $z$ -axis and contributes  $z_i = a \cos \theta_i$  to the total displacement along  $z$ .

We can easily find the average size of the random configurations adopted by such a chain. Suppose the first link is located at the origin ( $x = y = z = 0$ ). For  $i = 1 \rightarrow N$ ,  $\theta_i$  is the orientation of link  $i$  with respect to the  $z$  direction. Therefore each link contributes a small amount  $z_i = a \cos \theta_i$  toward the overall displacement  $Z$  of link  $N$ . The  $z$  component of the end-to-end displacement between link 1 and link  $N$  is

$$Z = \sum_{i=1}^N z_i = \sum_{i=1}^N a \cos \theta_i \quad (31)$$

Since the  $\theta_i$  are random,  $Z$  is the sum of  $N$  random variables (the  $z_i$ ). By the central limit theorem,  $Z$  must be a Gaussian distributed random variable. The mean and variance of

the probability distribution  $P(Z)$  are determined by the mean and variance of the individual  $z_i$ . The mean is

$$\langle z_i \rangle = a \langle \cos \theta_i \rangle = 0 \quad (32)$$

because the mean value of  $\cos \theta_i$  is zero. Then by the central limit theorem  $\langle Z \rangle = N \langle z_i \rangle = 0$ . The variance of  $z_i$  is

$$\langle z_i^2 \rangle - \langle z_i \rangle^2 = a^2 \langle \cos^2 \theta_i \rangle = \frac{a^2}{2} \quad (33)$$

Therefore the variance of  $Z$  is  $\sigma^2 = \frac{Na^2}{2}$ . This gives the probability distribution for  $Z$  as

$$P(Z) = \frac{1}{\sqrt{\pi Na^2}} \exp(-Z^2/Na^2) \quad (34)$$

where the prefactor ensures normalization

$$\int_{-\infty}^{\infty} P(Z) dZ = 1 \quad (35)$$

The total displacements  $X$  and  $Y$  in the  $x$  and  $y$  directions respectively must behave analogously, so

$$P(X) = \frac{1}{\sqrt{\pi Na^2}} \exp(-X^2/Na^2) \quad (36)$$

and

$$P(Y) = \frac{1}{\sqrt{\pi Na^2}} \exp(-Y^2/Na^2) \quad (37)$$

At this point it should be apparent that, since the distributions  $P(X)$  etc. have zero mean, the average of the vector displacement  $\mathbf{R}$  of link  $N$  with respect to the first link is zero. However the mean squared displacement  $\langle R^2 \rangle$  is not zero. From the Gaussian  $P(Z)$  we can see  $\langle Z^2 \rangle$  is equal to the variance of the distribution,  $\langle Z^2 \rangle = Na^2/2$ , and  $X$  and  $Y$  should behave similarly. Therefore the mean squared end-to-end displacement of the chain is

$$\langle R^2 \rangle = \langle X^2 + Y^2 + Z^2 \rangle = \frac{3Na^2}{2} \quad (38)$$

Eq. 38 is remarkable in itself. It says that as the length  $N$  of the polymer chain increases, it is not the mean displacement of the final link that grows in proportion to  $N$ . Rather it is the *mean squared* displacement that grows in proportion to  $N$ . This is very similar to the Brownian particle trajectories above, where the mean squared distance traveled by the particle grows in proportion to the duration  $t$  of the motion. Clearly the path taken by successive links of the chain is analogous to the irregular path taken by the Brownian particle. An important difference is that the links are all of the same length, whereas the “steps” taken by the Brownian particle are not. Still the result is essentially the same.

If you want to know what is the probability that the two ends of the chain are separated by a scalar distance  $R$  (i.e. irrespective of orientation) then you need to find the probability distribution for  $R$  as opposed to  $X$ ,  $Y$ ,  $Z$ . Start by multiplying the product  $P(X)P(Y)P(Z)dXdYdZ = P(\mathbf{R})dV$  to find the probability that link  $N$  terminates within a particular volume element of size  $dV = dXdYdZ$  located at  $\mathbf{R} = (X, Y, Z)$

$$P(\mathbf{R})dV = \frac{dV}{(\pi Na^2)^{3/2}} \exp(-R^2/Na^2) \quad (39)$$

In the exponent we have used the fact that  $R^2 = X^2 + Y^2 + Z^2$ . Then express  $dV$  in spherical polar coordinates  $(R, \theta, \phi)$  giving

$$P(\mathbf{R})dV = \frac{R^2 dR \sin \theta d\theta d\phi}{(\pi Na^2)^{3/2}} \exp(-R^2/Na^2) \quad (40)$$

If we are not interested in orientation then we can integrate over the angles and focus on the probability distribution for the magnitude  $R$ ,

$$P(R)dR = \int_{\phi=0}^{2\pi} \int_{\theta=0}^{\pi} P(\mathbf{R})dV \quad (41)$$

which gives

$$P(R)dR = \frac{4\pi R^2 dR}{(\pi Na^2)^{3/2}} \exp(-R^2/Na^2) \quad (42)$$

$R \geq 0$  is the scalar distance from one end of the polymer chain to the other. While the average  $\mathbf{R}$  is zero, the average  $R$  is clearly nonzero.

**Exercise 5** Note the difference between  $P(\mathbf{R})$  of Eq. 39 and  $P(R)$  of Eq. 42. Sketch  $P(\mathbf{R})$  and  $P(R)$  vs.  $R$ . Explain why the functions behave so differently near  $R = 0$ . That is, why does  $P(R)$  have to be zero at  $R = 0$  whereas  $P(\mathbf{R})$  does not?

## Effective dimensions of a polymer chain

We understand how the length of a polymer chain relates to the overall dimension  $R$  of the randomly coiled molecule. How does  $R$  relate to the rate of its Brownian motion in a fluid? We have already seen the Stokes-Einstein relation Eq. 30 for diffusion of a spherical particle. Given the diffusion rate of any other particle, we could characterize its motion by saying that it diffuses with  $D = k_B T / 6\pi\eta R_h$  where  $R_h$  is an “effective” radius that depends on the size and shape of the particle. That is,  $R_h$  (known as the *hydrodynamic radius*) is the radius of the sphere that would exhibit the same diffusion coefficient. For biopolymers  $R_h$  is difficult to calculate, but it is often well approximated by a quantity known as the radius of gyration,  $R_G$ .  $R_G$  describes<sup>9</sup> the mass distribution of the chain relative to the chain’s center of mass. For the freely jointed chain you can show that

$$R_G^2 \rightarrow \frac{1}{6} \langle R^2 \rangle = \frac{Na^2}{4} \quad (43)$$

<sup>9</sup>Specifically,  $R_G^2$  is the mean (over the whole chain) of the squared distance from each link to the chain’s center of mass.

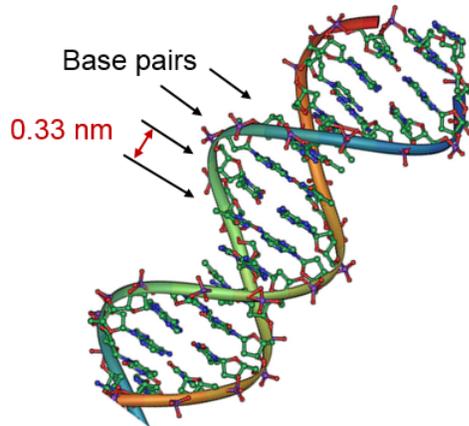


Figure 3: The length of the DNA double helix is determined by the number of base pairs. Each base pair consists of two nucleotide bases (seen from the side in this drawing) which are attached to the two backbone strands. The bases extend to the center of the helix to form hydrogen bonded pairs. Each base pair contributes about 0.33 nm to the length of the helix.

if the number  $N$  of links is sufficiently large. We will be dealing with some long DNA molecules, so we expect their diffusion coefficients to be  $D = k_B T / 6\pi\eta R_G$ . Note that  $R_G^2 \propto N$ , so that we expect  $D \propto N^{-1/2}$ . Increasing the length of a polymer chain by  $2\times$  slows its diffusion by a factor  $1/\sqrt{2}$ .

Finally we note that the length of a DNA molecule is measured in base pairs (bp), where each base pair contributes about 0.33 nm length to the double helix. However the number of base pairs is not the number of fundamental links  $N$  in the chain, because while the backbone of the DNA double helix is flexible, it is not so flexible that we can model the individual base pairs as freely jointed links. A property known as the “Kuhn length”  $b$  of a chain tells us the size of the “effective” links. The Kuhn length is the minimum length of actual physical chain that is truly free (as in an FJC) to take virtually any orientation with respect to an adjacent, similar length of chain.

If a polymer chain has physical length  $L$ , then we can model it as a FJC of  $N = L/b$  effective freely jointed links where each link has length  $b$ . For a DNA double helix in a typical aqueous environment the Kuhn length is about 100 nm.

## Samples

In this experiment you will study the Brownian motion of several types of microscopic particles. The first are fluorescent latex beads (FluoSpheres) with diameters in the range of a few hundred nanometers up to about  $1\ \mu\text{m}$ . Using these beads, you will learn to record the Brownian trajectories using a fluorescence microscope and camera, and analyze the images to determine the diffusion coefficient  $D$  of the particles. You can then study the diffusion of DNA molecules of known size, stained with a fluorescent dye in order to make them visible. From these studies you can determine the physical dimension  $\langle R^2 \rangle = 3Na^2/2$  that is associated with a particular length (basepairs) of DNA. Dilute the beads at least  $1000\times$  into water before attempting to observe the Brownian motion.

You can also study the diffusion of fragments of  $\lambda$  DNA. The  $\lambda$  phage is a sort of virus that attacks *E. coli* bacteria; its DNA is used as a standard in molecular biology labs because it can be cut into fragments of precisely known lengths. These standard fragments can serve as calibration markers in gel electrophoresis (DNA separation) work. Their length is measured in units of DNA base pairs or, if you like, microns. Our  $\lambda$  DNA kit is a so-called HindIII digest, containing fragments with the following lengths: 125, 564, 2027, 2322, 4361, 6557, 9416 and 23130 base pairs. Given that the DNA double helix has a length of about 0.33 nm per base pair, the length of these fragments runs from  $L \simeq 40\ \mu\text{m}$  to

$L \simeq 7.7$  mm. Such long DNA strands adopt randomly coiled configurations just like the freely jointed chain discussed above. Consequently they diffuse at a rate  $D$  that should vary inversely as the  $R_G$  of the strand, i.e., inversely proportional to  $L^{1/2}$ .

**Exercise 6** From the known lengths of the *HindIII* fragments, the Kuhn length of the DNA double helix, and the Stokes Einstein relation, estimate the diffusion coefficients  $D$  of the different  $\lambda$  DNA fragment lengths.

### Analyzing trajectories

The goal is to observe the trajectory of the Brownian particle and, from the particle's displacement vs. time, determine the diffusion coefficient  $D$ . Eq. 19 and Eq. 29 together state that the particle's mean squared displacement after a time  $t$  is

$$\langle x^2 \rangle = \sigma^2 = 2Dt \quad (44)$$

(Remember that  $\langle x \rangle = 0$ .) Therefore we can find  $D$  from the slope of the average squared displacement vs. time:  $D = \langle x^2 \rangle / 2t$ . You might expect that a reasonable way to find  $D$  is then to allow a particle (starting at  $x = 0$ ) to move for a very long period  $t$ , and then divide its final squared displacement  $x^2$  by the time period  $t$ . In fact this technique gives poor results<sup>10</sup>, because the relation  $x^2 \propto t$  is only true as an ensemble average: in practice you need to measure a very large number of individual trajectories before their average  $\langle x^2 \rangle / t$  gives  $D$  to satisfactory precision. This can be very time consuming.

There are good alternatives however. Using a microscope equipped with a scientific camera you can collect a series of  $N$  image frames at

<sup>10</sup>Error and uncertainty in the analysis of Brownian trajectories is discussed in the article by M. Catipovic et al. (2013)

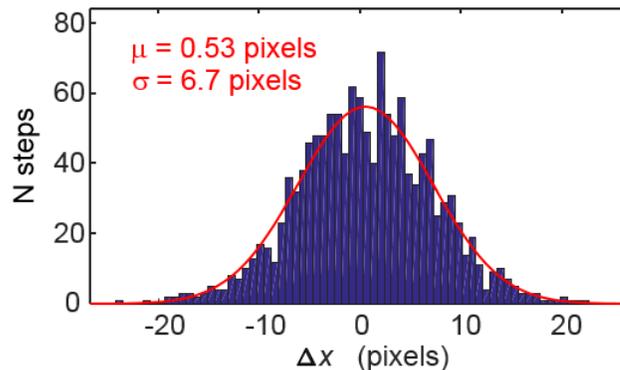


Figure 4: Histogram of step sizes  $\Delta x$  from observation of  $0.38 \mu\text{m}$  spheres diffusing in water. Displacements are measured in camera pixels, where one pixel is equivalent to a displacement of  $0.162 \mu\text{m}$  at the  $40\times$  optical magnification that was used. Over 1300 individual frame-to-frame displacements were observed. The red curve is a Gaussian function with the same mean ( $\mu = 0.53$  pixels) and variance ( $\sigma^2 = (6.7 \text{ pixel})^2$ ) as the data.

regular time intervals  $\Delta t$ . Within each image frame  $i$  ( $i = 1 \rightarrow N$ ), identify the location  $\mathbf{r}_i$  of the particle. Then calculate the individual frame-to-frame displacements  $\Delta x_i = x_{i+1} - x_i$ . There will be many of these displacements in your dataset, and so one approach is to generate the histogram of the  $\Delta x_i$ . By Eq. 19, the histogram should be a Gaussian with a mean of zero and variance  $\sigma^2 = 2D\Delta t$ . As the number of frames, multiplied by the number particles tracked in each frame, can be made large (see Figure 4), the statistics of this method are significantly improved. As the observed trajectories will be two-dimensional, estimates of  $D$  can be obtained for both the  $x$  and  $y$  directions.

Another approach is simply to calculate, from the set of  $N$  frames, the mean squared displacement over many short intervals, e.g.

$$\Delta x_n^2 = \langle (x_{i+n} - x_i)^2 \rangle \quad (45)$$

for different values of  $n$ . You can then plot

$\Delta x_n^2$  vs  $n$ , which should obey  $\Delta x_n^2 = 2n\Delta tD$ .

Of course, since the data contain fewer values of  $(x_{i+n} - x_i)^2$  for larger values of  $n$ , the averaging is less effective at large  $n$  and the proportionality  $\Delta x_n^2 \propto n$  will be less well-determined at large  $n$ . In practice it may be sufficient just to find the slope using the first two points,  $n = 0, 1$ .

## Hardware

The apparatus for this experiment is simple. Although it is entirely possible to observe Brownian motion in white light, the image analysis is a little easier (the particles are easier to track) if they are observed in fluorescence instead of white light. Hence you will use fluorescently dyed (stained) particles. These absorb blue light and reemit it as green (fluorescent) light. Using the appropriate color filters in the base of the microscope, the particles then appear in the images as bright dots against a dark background. DNA is not naturally fluorescent in the visible spectrum. Therefore, in order to make the DNA fluoresce, we add a small amount of dye to the solution containing the DNA. The dye, SYTO 9, binds strongly to the DNA molecules and causes them to fluoresce bright green under blue light illumination.

You will use an inverted fluorescence microscope (Nikon Eclipse TS100-F model), to study the diffusing particles. The fluorescence of the particles is excited by a mercury arc lamp and the fluorescent emission passes through a filter set in the base of the microscope before going to either the eyepiece or the camera.

The fluorescence of the particles will be only dimly visible (if at all) to your eye. However a sensitive camera will have no difficulty recording the fluorescence. Therefore, for data collection the fluorescent light will be sent di-

rectly to a camera. We use a cooled charge-coupled-device camera (“CCD camera”, Andor Clara model) which provides an array of  $1360 \times 1024$  pixels in the focal plane of the microscope. The pixels are square in shape, with dimensions  $4.65 \times 4.65 \mu\text{m}$ . Particle displacements can be measured in camera pixels, at least during data collection and preliminary analysis. Later you can convert from pixel units to  $\mu\text{m}$  based on the magnification of the microscope objective. The microscope provides magnifications of  $10\times$ ,  $20\times$ , or  $40\times$ . You will need to collect a series of image frames showing the same group of particles, and then analyse these images (see below) to identify the particle trajectories. You will probably find it convenient to collect the images at a rate  $\sim 0.5 - 1$  frame per second, using higher magnification for smaller particles only. For example, image at  $40\times$  for  $0.38 \mu\text{m}$  diameter particles.

## Software

The PC attached to the microscope has software that controls the CCD camera, including frame rate, exposure time, number of exposures, and other settings. You will need to experiment with it to learn how to obtain good fluorescence images of the fluorescent beads. To adjust physical parameters such as the microscope magnification and focus you will need to physically adjust the microscope itself. Note that you will need to determine the spatial calibration of the camera image (pixels per micron), which will depend on the microscope magnification. The physical size of an image in the camera focal plane is simply related to the size of the real object by a factor of the microscope magnification (i.e. the number that is inscribed on the microscope objective). You can calculate the size calibration of the images by using the pixel size data above, and

then check it by imaging an object of known size such as a reticle.

The PC also has a popular software package known as ImageJ that will allow you to track the motion of particles between frames. ImageJ contains a particle detection and tracking plugin (Mosaic) that will locate bright particles against a dark background in your images, find their coordinates in the image (in units of pixels) and then track the same particles from frame to frame, generating a list of coordinates (frame-to-frame) for each particle that is tracked. This analysis includes some important parameters that determine how far the particle is assumed capable of moving from one frame to the next, as well as setting the threshold for how clearly the particle should be visible in the image before it is tracked. Once a particle moves more than a few  $\mu\text{m}$  in the axial ( $z$ -direction) it will be out of focus and cannot be tracked accurately.

Finally you can import your trajectory data into software such as Excel or Matlab in order to further analyze the trajectory, as described below.

## Error sources

There are a few sources of error that you need to consider. First, it is very important that the fluid be stationary. If there is even a small bulk motion of the fluid, such that the particle drifts with a net speed  $v$  in the  $x$ -direction, then the mean frame-to-frame displacement will be  $v\Delta t$ . This drift will affect the variance, especially if the number of frames is small<sup>11</sup>. Be sure to allow your sample sufficient time after preparation to equilibrate so that the drift

<sup>11</sup>Consider for example the case where  $N = 2$  and only two frames are studied. Then you cannot know whether to ascribe the particle's motion during  $\Delta t$  to Brownian motion or to drift. For larger  $N$  the situation is less dire, but still requires caution.

velocity  $v = 0$ .

Through the Stokes Einstein formula the diameter  $d$  of the particles and the solvent viscosity  $\eta$  are both potential sources of error. However the manufacturer of the particles (FluoSpheres, Molecular Probes) characterizes the diameter of the particles to less than  $0.02 \mu\text{m}$ , so the size uncertainty contributes an error of no more than 2-5% for particles in the size range of  $0.5 - 1 \mu\text{m}$ . The viscosity of water is highly temperature sensitive, but you can measure room temperature to an accuracy better than  $1^\circ\text{C}$  and then look up the viscosity at that temperature.

An important source of error arises in the determination of particle positions and frame-to-frame displacements. The ImageJ/Mosaic particle-tracking algorithm offers parameter settings that adjust how well it recognizes the same particle at different locations in adjacent image frames. Although it is tempting to adjust those settings so that the algorithm aggressively seeks and identifies lengthy (many-frame) trajectories of an individual particle, you do not need such long trajectories to perform your data analysis. In fact they are likely to introduce errors such as poor guesses of the location of out-of-focus particles, and mix-ups over which of the particles in one frame was seen at a similar location in the previous frame. These errors lead e.g. to unphysically long  $\Delta x_i$  values. It is perfectly acceptable to employ more conservative settings that occasionally lose track of some particles, in exchange for more accurate location data when those particles are seen.

## Procedure

- First prepare a sample of spheres by diluting the sphere suspension into deionized water. A large dilution of  $1000\times$  or greater is appropriate.

- Use a pipette or syringe to load the sample into the observation channel of the slide. We use the Ibidi type 81121 microfluidic slide. This is a plastic slide containing a closed channel that is a few millimeters long and 0.1 mm deep. The slide is equipped with Luer fittings so that you can load it with a standard syringe and then cap the ends to prevent the fluid from evaporating. Be sure to fill the slide evenly so that the fluid comes to the same height in the Luer fittings at the two ends of the channel. Otherwise there will be a pressure gradient in the channel and fluid will tend to drift for some time, interfering with your measurements.
- Allow the slide to equilibrate on the microscope stage for 15-30 minutes before you begin measurements in earnest.
- Use the multi-frame imaging function of the camera software to collect the trajectories of several Brownian particles at once. You can set a frame rate (e.g. 2 frames per second,  $\Delta t = 0.5$  s) on the camera and collect a series of images.
- Load the images into the ImageJ and run the particle tracking plugin, and then transfer the results to Excel or Matlab. Generate the  $\Delta x$  and  $\Delta y$  displacements and analyze as described above to obtain  $D$  for your particles.
- Based on the known size of the particles, the measured temperature in the room, and the tabulated dynamic viscosity  $\eta$  of water, you should be able to obtain an estimate for Boltzmann's constant  $k_B$ .
- To observe the diffusion of DNA, load a dilute solution of the  $\lambda$ -DNA containing the SYTO dye into an Ibidi slide channel. You will notice that if you use a

bright fluorescence lamp intensity, the fluorescence of the DNA will diminish very rapidly, making it impossible to observe Brownian motion. This loss of fluorescent emission under illumination is known as "photobleaching". However if you use low light intensity and long exposures you can collect several seconds' worth of images at any one location, and thus observe the Brownian motion of the DNA molecules.