# PHYSICS FOR LIFE SCIENCES 1- LAB

## PHY2131 - Lab Notes

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Some sections of these notes go into more detailed mathematical derivations and formulas. These are included to support your understanding. The data-analysis procedures and example graphs are provided so that you have a clear template to follow and to help avoid confusion when you work with your own experimental data.

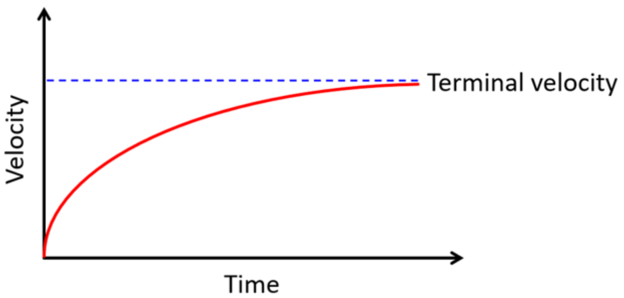
# Experiment 2

## Experiment 2 - Directed motion

In the coming weeks, your main lecture will cover terminal velocity and the role of resistive forces. To help you get a head start, this document provides a short introduction that outlines the basic concepts and includes a few equations showing how terminal velocity depends on various parameters.
This document is meant to give you a general idea; your own predictions about parameter dependence may differ, and your experimental results might not match the theory exactly.

### TERMINAL VELOCITY

When an object falls through a fluid (air, water, glycerin, etc.), several forces are at play. When you release the sphere, it falls toward the ground (toward the center of the Earth, to be more precise). This is due to the force of gravity, i.e., its weight. But since it is moving through a fluid (not a vacuum), it also experiences a resistive force that tries to slow the object down, much like friction.
So there is a downward gravitational force and upward resistive forces on the object. The resistive force depends on the velocity of the object, so it increases as the object accelerates. The downward gravitational force, however, remains the same. At some point, these forces balance out, making the net force zero, i.e., acceleration zero, which means the velocity becomes constant. This constant velocity is called the **terminal velocity**.




### Forces on a Falling Sphere

For a sphere of radius $r$, density $\rho_s$, and mass $m$ falling through a fluid of density $\rho_f$ and viscosity $\eta$:

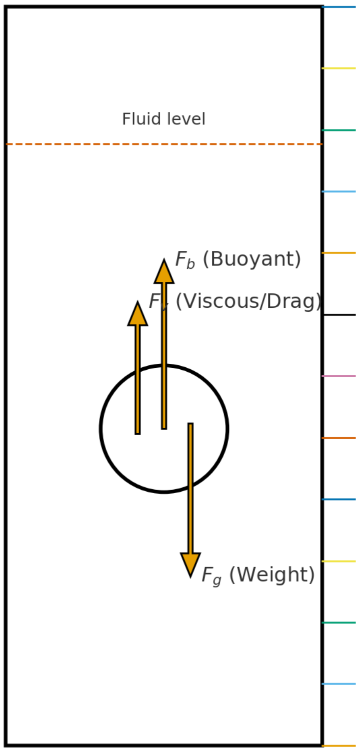
1. **Weight (downward)**

$$F_g = mg = \rho_s V g \tag{1}$$

   where $V = \frac{4}{3}\pi r^3$ is the volume of the sphere.

2. **Buoyant Force (upward, Archimedes' principle)**

$$F_b = \rho_f V g \tag{2}$$




3. **Velocity-dependent resistive forces (drag forces, upward)**
   At low speeds and for small spheres, viscosity dominates and Stokes' law applies:

$$F_v = 6\pi\eta r v \tag{3}$$

   At higher speeds/turbulent flow, drag depends on velocity squared:

$$F_d = \tfrac{1}{2} C_d \rho_f A v^2 \tag{4}$$

   where $A = \pi r^2$ is the cross-sectional area of the sphere and $C_d$ is the drag coefficient.

For the current experiment, you may neglect this turbulent contribution and only consider the viscous force.

## Condition for Terminal Velocity

At terminal velocity $v_t$, the net force is zero:

$$\text{Total downward force} = \text{Total upward force}$$

$$F_g = F_b + F_v.$$

Substituting and rearranging gives:

$$\begin{aligned} \rho_s V g &= \rho_f V g + 6\pi\eta r v_t \\ \frac{(\rho_s - \rho_f) V g}{6\pi r \eta} &= v_t. \end{aligned}$$

Thus, **Terminal velocity**

$$v_t = \frac{2\, r^2\, g\, (\rho_s - \rho_f)}{9\, \eta} \tag{5}$$

Where:

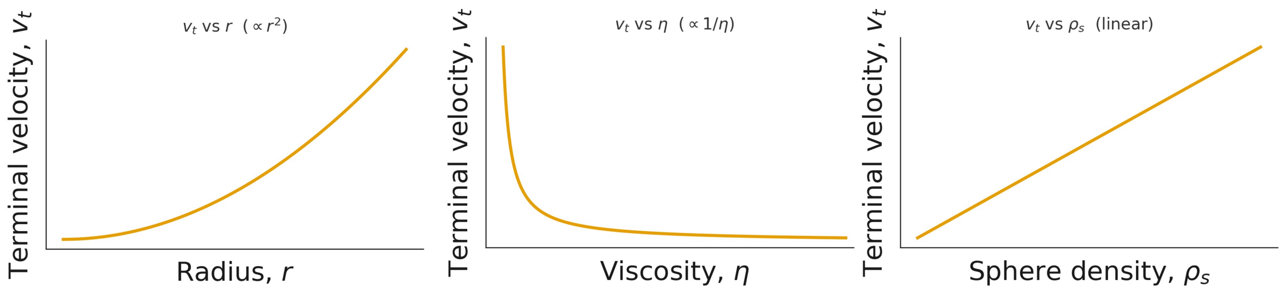
$$r = \text{radius of the sphere}$$
$$g = \text{acceleration due to gravity} \; = 9.8 \text{ m/s}^2$$
$$\rho_s = \text{density of the sphere}$$
$$\rho_f = \text{density of the fluid}$$
$$\eta = \text{coefficient of viscosity}$$

This experiment investigates the dependence of $v_t$ on the size of the sphere, i.e., $r$, the density of the sphere, i.e., $\rho_s$, and the viscosity of the fluid, i.e., $\eta$.

1. $v_t$ vs. radius $r$ – parabolic ($\propto r^2$)

2. $v_t$ vs. density $\rho_s$ – linear ($\propto (\rho_s - \rho_f)$)

3. $v_t$ vs. viscosity $\eta$ – inverse ($\propto 1/\eta$)



# Experiment 3

## Experiment 3 - Random motion

In this experiment, you will look at motion on the microscopic scale, known as Brownian motion. This was actually the first clear evidence for the existence of molecules. Robert Brown noticed that pollen grains under a microscope seemed to "wiggle" around, which is due to water molecules constantly bumping into the pollen, making it move randomly.
In the lab, you will replicate this idea by observing tiny silica beads suspended in water under a microscope. You will then study how the random motion (or diffusion) of these beads depends on two things: the radius of the bead and the viscosity of the liquid they are in.

### ROOT MEAN SQUARE (RMS) DISTANCE

Consider placing a drop of ink gently on the surface of water in a test tube (to minimize convection and other disturbances) and starting your clock. At time zero, the ink particles are all at the starting point. As time passes, they spread out. Eventually (after many hours), the entire column of water changes color, reaching a uniform concentration of ink particles throughout (see the figure).

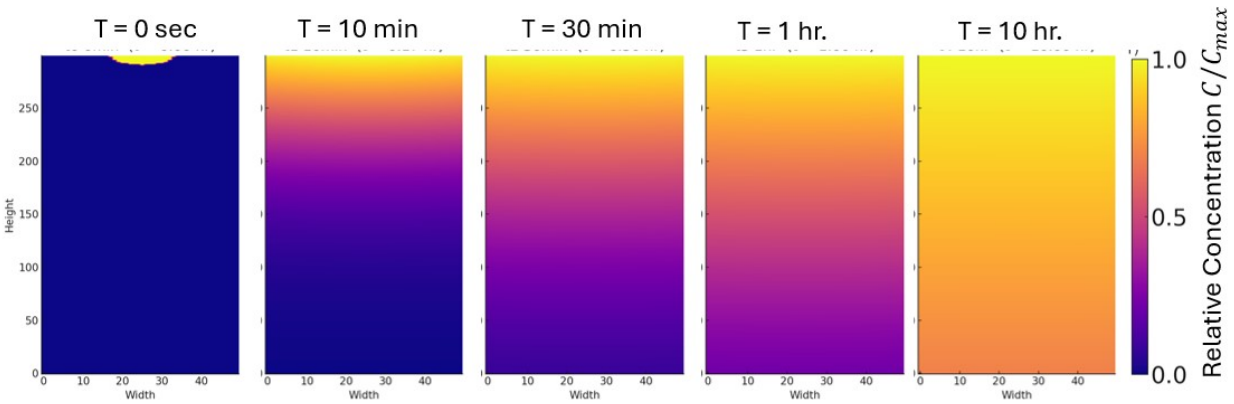



Figure 1: Time evolution of ink diffusion in a still water column. Panels show the normalized concentration $C/C_{\max}$ at 0 s, 10 min, 30 min, 1 h, and 10 h ( Molecular diffusion gradually spreads the ink and is a really slow process; in real-life examples like perfume dispersing through a room, bulk air currents (convection) are usually the primary mixing mechanism.)

This process is called diffusion: the transfer of particles from a region of higher concentration to a region of lower concentration. Diffusion occurs due to the underlying random, or Brownian, motion of the particles.
What would be an appropriate way to measure diffusion? Diffusion depends on time, so you need a measure that captures how far particles spread as time passes. At first, you might think of taking the average displacement of all the particles from the initial point. But since diffusion is a random walk, each particle has an equal chance of

moving left or right. When all these displacements are added up, they cancel out, and the average displacement is always close to zero. This makes the arithmetic mean a poor choice for describing diffusion.
Instead, you use the root mean square (RMS) displacement, which is a statistical mean:

1. Square each displacement (this removes the negative signs so left and right do not cancel).
2. Take the average (mean) of these squared displacements.
3. Finally, take the square root to return to distance units.

**RMS**

$$r_{rms}(t) = \sqrt{\frac{r_1(t)^2 + r_2(t)^2 + \cdots + r_i(t)^2 + \cdots + r_N(t)^2}{N}} \tag{6}$$

Where:

$$r_i(t) = \text{displacement of the } i^{\text{th}} \text{ particle at time } t$$
$$N = \text{total number of particles}$$

This RMS distance gives a meaningful measure of how far particles typically wander away from their starting point due to random motion.
For diffusive motion in two dimensions, the RMS distance in terms of the diffusion constant is given by
**RMS distance in 2D**

$$r_{rms}(t) = \sqrt{4\,D\,t} \tag{7}$$

Where:

$$D = \text{diffusion constant}$$
$$t = \text{time}$$

The reason for the random motion is the thermal agitation in the system. Einstein proposed that the diffusion constant depends on the temperature of the system and on a quantity called the **mobility**, defined as

$$\mu = \frac{v}{F} \tag{8}$$

which quantifies how easily an object moves under an applied force.
Einstein related diffusion to mobility through

$$D = \mu\, k_B\, T, \tag{9}$$

where $k_B$ is the Boltzmann constant and $T$ is the absolute temperature.
During random motion in a liquid, the dominant force experienced by the particle is the viscous drag, given by Stokes' law:

$$F_{\text{viscous}} = 6\pi\eta r v,$$

where $\eta$ is the viscosity of the fluid and $r$ is the radius of the particle. Therefore, the mobility is

$$\mu = \frac{v}{F_{\text{viscous}}} = \frac{1}{6\pi\eta r}. \tag{10}$$

Comparing the above two equations ( Eq. 9and 10), we obtain the famous Stokes–Einstein relation:
**Diffusion constant** ($D$)

$$D = \frac{k_B T}{6\pi\eta r} \tag{11}$$

Where:

$$T = \text{temperature}$$
$$\eta = \text{coefficient of viscosity}$$
$$r = \text{radius of the spherical particle}$$

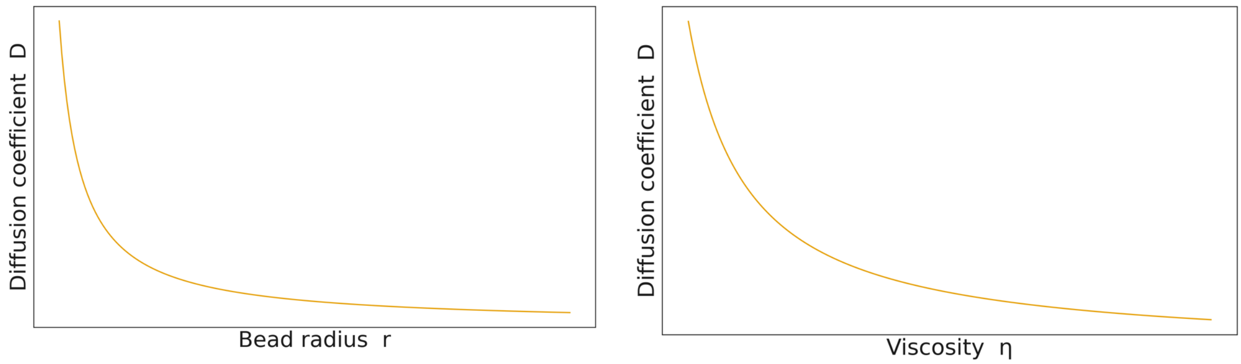



Figure 2: Shows the relationship of radius and viscosity on diffusion constant

## WEEK-2 DATA ANALYSIS

The aim of this experiment is to determine how bead radius and fluid viscosity affect the diffusion constant. Following the lab manual, you will record three videos of beads suspended in liquid and track at least 10 particles from each video.
From the ImageJ output, you will obtain the $x$ and $y$ positions of the tracked particles. From these, you can calculate the displacement at each time step, as follows:

| | A | B | C | D | E | F | G | H | AC | AD | AE | AF | AG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | time | particle 1 | | | particle 2 | | | ..... | particle 10 | | | | |
| 2 | | X1 | Y1 | R1^2 | X2 | Y2 | R2^2 | ..... | X10 | Y10 | R10^2 | R^2rms | |
| 3 | 0 | 19.2 | 1.75 | 0 | 573.225 | 3.8 | 0 | ..... | 688 | 12.5 | 0 | 0 | |
| 4 | 0.03333 | 19.5 | 1.804348 | 0.092953694 | 573.1316 | 3.657895 | 0.028917 | ..... | 687.9375 | 12.625 | 0.019531 | 0.047134 | |
| 5 | 0.06666 | 19.11905 | 1.833333 | 0.013497844 | 573.125 | 3.675 | 0.025625 | ..... | 688.1 | 12.5 | 0.01 | 0.016374 | |
| 6 | 0.09999 | 19.19565 | 2.021739 | 0.073861098 | 573.1923 | 3.705128 | 0.01007 | ..... | 687.9667 | 12.36667 | 0.018887 | 0.034273 | |
| 7 | 0.13332 | 19.31818 | 1.954546 | 0.055805847 | 573.0641 | 3.75641 | 0.027789 | ..... | 688.0294 | 12.67647 | 0.032006 | 0.038534 | |
| 8 | 0.16665 | 19.19231 | 2.307692 | 0.311079868 | 573.1 | 3.85 | 0.018125 | ..... | 688 | 12.5 | 0 | 0.109735 | |
| 9 | 0.19998 | 19.05556 | 2.240741 | 0.261690891 | 573.1842 | 3.605263 | 0.039587 | ..... | 688.125 | 12.6875 | 0.050781 | 0.117353 | |
| 10 | 0.23331 | 19 | 2.153846 | 0.203091834 | 573.0366 | 3.695122 | 0.046509 | ..... | 688.0294 | 12.67647 | 0.032006 | 0.093869 | |
| 11 | 0.26664 | 19.11539 | 2.153846 | 0.170251532 | 573.125 | 3.675 | 0.025625 | ..... | 687.7308 | 12.26923 | 0.125723 | 0.1072 | |

Figure 3: Example excel data sheet

## Steps

( refer to the example Excel sheet Fig. 3 )

1. **Define the Initial Position**

   At $t = 0$ s (first frame), define each particle's initial position

$$(x_0, y_0)$$

as the origin for that particle's motion. Therefore, the squared displacement values are

$$R^2 = 0$$

for the first row of data for all particles (for example: `D3`, `G3`, . . . ,`AE3`).

2. **Compute $R^2$ for Each Particle**

   For each time step, calculate the squared radial displacement relative to the initial position:

   $$R^2(t) = (x - x_0)^2 + (y - y_0)^2.$$

   *Example (Particle 1):*

   In Excel cell `D4`, enter:

   ```
   =(B4-B$3)^2 + (C4-C$3)^2
   ```

   The dollar signs ($) lock the reference to the initial position row ($t = 0$), ensuring that $x_0$ and $y_0$ remain fixed when the formula is copied.

   Drag this formula down to fill all time points (rows). Repeat the same process for each particle:

   - Particle 2 → Column `G`
   - Particle 3 → Column `J`
   - etc.

   (*Note: Exact cell references may differ depending on how your Excel worksheet is arranged.*)

3. **Compute the Mean-Squared Displacement**

   At each time step, calculate the mean of all squared displacements:

   $$R^2_{\text{rms}}(t) = \frac{R_1^2 + R_2^2 + \cdots + R_{10}^2}{10}.$$

   In Excel, use the `AVERAGE` function. For example, in the summary column (cell `AF4` in the Fig. 3) enter:

   ```
   =AVERAGE(D4, G4, J4, ...)
   ```

   Taking the square root of this quantity gives $R_{\text{rms}}$.

4. **Plot**

   Create a plot of:

   - $x$-axis: Time $t$
   - $y$-axis: $R^2_{\text{rms}}(t)$

   Add a **linear trendline** to the data and display its equation.

5. **Determine the Diffusion Rate**

   The slope of the trendline corresponds to the diffusion behavior. For Brownian motion analysis, the diffusion coefficient $D$ is obtained from:

   $$D = \frac{\text{slope}}{4}.$$

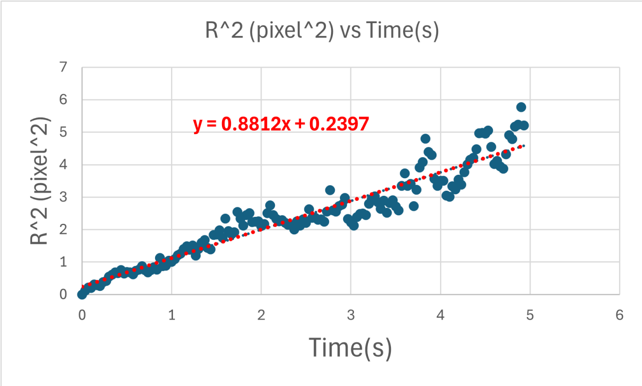



Figure 4: $R^2_{rms}$ vs $t$ graph with linear trendline.

**Example conversion of $D$ to SI units**

From the graph Fig. 4,

$$D = \frac{0.8812}{4} = 0.2203 \text{ pixel}^2/\text{s}.$$

Using the spatial calibration,

$$1\ \mu\text{m} = 5.179 \text{ pixels} \quad \Rightarrow \quad 1 \text{ pixel} = \frac{1}{5.179}\ \mu\text{m},$$

so

$$1 \text{ pixel}^2 = \left(\frac{1}{5.179}\right)^2 \mu\text{m}^2 = \frac{1}{5.179^2}\ \mu\text{m}^2 \approx \frac{1}{26.822}\ \mu\text{m}^2 \approx 0.0373\ \mu\text{m}^2.$$

Therefore,

$$D = 0.2203 \text{ pixel}^2/\text{s} = 0.2203 \times \frac{1}{5.179^2}\ \mu\text{m}^2/\text{s} \approx 8.2 \times 10^{-3}\ \mu\text{m}^2/\text{s}.$$

You should repeat the calculation for all the videos, and finally plot the parameter vs. diffusion constant graph.

# Experiment 4

## Experiment 4 - Random+Directed motion

In the previous experiment, you studied Brownian motion, the random motion of microscopic beads suspended in water, and observed how the radius of the bead and the viscosity of the fluid influence the diffusion constant. In that case, the motion was driven entirely by thermal agitation, so the particles had no preferred direction of movement.
In this experiment, you will perform a similar observation, but now the microscope is tilted, introducing a gravitational force component. This adds a directed force that tends to pull the beads in one direction.
Random motion (Brownian motion) causes particles to spread out in all directions.
Directed motion (due to gravity) causes particles to drift preferentially in one direction.
The motion you observe will be a combination of these two effects. The key objective of this experiment is to determine which type of motion dominates under different conditions, and specifically, how this depends on bead size. Smaller beads are strongly influenced by random motion, while larger beads are more affected by gravity.
To distinguish between these behaviors quantitatively, you will analyze the bead trajectories using log–log plots of mean-squared displacement versus time, which allows you to identify whether the motion is primarily diffusive, primarily directed, or a mixture of both.

### Purely Random (Diffusive) Motion

For Brownian motion in two dimensions, Einstein showed:

$$R_{rms}^2 = \langle r^2(t) \rangle = 4Dt,$$

where $D$ is the diffusion constant. This is a **linear** relationship in time.
Taking the logarithm of both sides:

$$\log(R_{rms}^2) = \log(4D) + \log(t).$$

If you plot $\log(R_{rms}^2)$ vs. $\log(t)$, the graph will be of the form $y = mx + b$ with slope $m = 1$.

**Diffusion-dominated motion: slope** $\approx 1$.

### Purely Directed Motion

If a constant force acts on the particle, it moves with a constant velocity $v$ (even though a constant force is present, microscopic particles in water do not accelerate; viscous drag is so strong that they reach terminal velocity almost instantly, so their displacement grows linearly in time rather than quadratically):

$$r(t) = vt \quad \Rightarrow \quad R_{rms}^2 = v^2 t^2.$$

Taking the logarithm:

$$\log(R_{rms}^2) = \log(v^2) + 2\log(t),$$

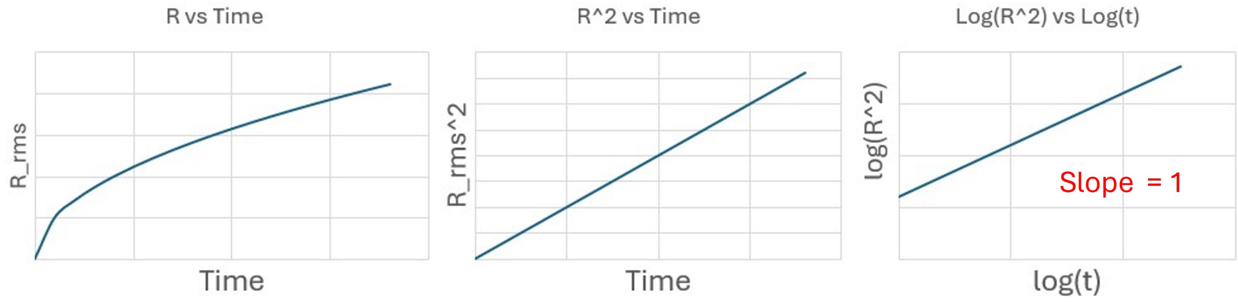



Figure 5: Graphs for the case with only diffusion.

which again matches $y = mx + b$, but now with slope:

$$\textbf{Directed motion: slope} \approx 2.$$

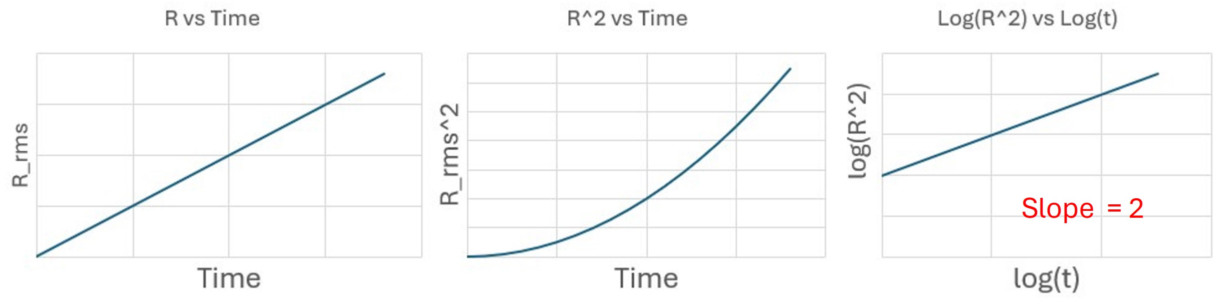



Figure 6: Graphs for the case with only directed motion.

## Mixed Motion

In many biological and experimental systems, both random and directed motion occur. In these cases:

$$\langle r^2(t) \rangle \propto t^{\alpha},$$

with $1 < \alpha < 2$. The value of $\alpha$ indicates the balance between Brownian motion and directed drift. If both effects are about equal, the slope will be around 1.5. In this experiment:

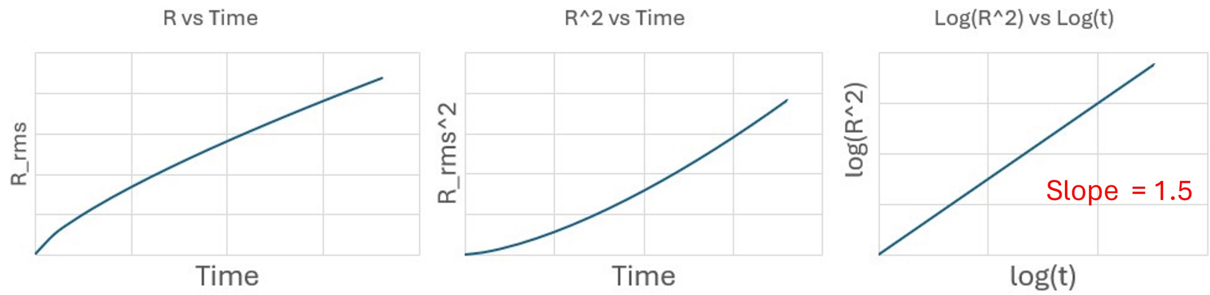



Figure 7: Graphs for the case of equal contribution of both motions.

- 2 $\mu$m beads are more affected by Brownian motion, so you expect their slope to be closer to 1 .
- 5 $\mu$m beads feel the directed motion more, so their slope should be closer to 2 .

The exact value depends on how much the microscope is tilted and how well the tracking is done.

## Week 2 - Data Analysis

| | A | B | C | D | E | F | G | H | AC | AD | AE | AF | AG | AH | AI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Time | X1 | Y1 | R1^2 | X2 | Y2 | R2^2 | .... | X10 | Y10 | R^10 | Rrms^2 | Rrms | log(t) | log(R^2) |
| 2 | | | | | | | | .... | | | | | | | |
| 3 | 0 | 982.2018 | 9.991228 | 0 | 66.34746 | 16.80508 | 0 | .... | 383 | 22.78572 | 0 | 0 | 0 | | |
| 4 | 0.0333 | 981.9182 | 11.20909 | 1.563648 | 67.14815 | 19.12963 | 6.044619 | .... | 383.9364 | 23.44545 | 1.312044 | 2.175224 | 1.474864 | -3.402198 | 0.777132 |
| 5 | 0.0666 | 982.0637 | 11.24546 | 1.592168 | 68.5678 | 20.02542 | 15.30047 | .... | 385.2895 | 25.00877 | 10.18361 | 6.462631 | 2.542171 | -2.709051 | 1.866037 |
| 6 | 0.0999 | 983.1724 | 11.37931 | 2.868839 | 68.03571 | 20.17857 | 14.23061 | .... | 385.7586 | 25.53448 | 15.1656 | 10.12251 | 3.181589 | -2.303586 | 2.314762 |
| 7 | 0.1332 | 983.5526 | 13.04386 | 11.14322 | 68.5678 | 20.02542 | 15.30047 | .... | 386.5 | 27 | 30.0102 | 16.92281 | 4.113735 | -2.015904 | 2.828663 |
| 8 | 0.1665 | 982.5 | 13.91818 | 15.50988 | 67.85714 | 19.46429 | 9.350484 | .... | 386.2931 | 29.55172 | 56.62337 | 28.87023 | 5.373103 | -1.79276 | 3.362811 |
| 9 | 0.1998 | 983.0273 | 12.97273 | 9.570787 | 67.16666 | 18.83333 | 4.784893 | .... | 386.8966 | 29.06897 | 54.66235 | 34.97697 | 5.914133 | -1.610438 | 3.55469 |
| 10 | 0.2331 | 982.0283 | 13.10377 | 9.718039 | 67.22881 | 18.87288 | 5.052558 | .... | 387.3491 | 29.53774 | 64.5041 | 33.38694 | 5.778143 | -1.456288 | 3.508165 |
| 11 | 0.2664 | 980.4245 | 13.40566 | 14.81715 | 68.39063 | 19.76563 | 12.93933 | .... | 387.3 | 30.08333 | 71.74524 | 38.21392 | 6.181741 | -1.322756 | 3.6432 |

Figure 8: Example excel data sheet

While choosing vesicles to track, **inspect the data carefully**. If you observe any **abrupt or unrealistic jumps** in the measured $x$ or $y$ positions (which typically indicate tracking errors), that particle should be **discarded** and replaced with a different particle.
Only particle exhibiting **continuous motion** should be included in the analysis ( you should look at the imageJ output image showing paths and labels, and pick the particles wisely).

## Excel Analysis Steps

( refer to the example Excel sheet, Fig. 8 )

1. **Convert Frame Number to Time**

   Change the Frame column (A) to Time by typing in 0, 0.033, and dragging down to all the cells. This corresponds to the frame interval

   $$\Delta t = 0.033 \text{ s}.$$

2. **Define the Origin**

   At $t = 0$ s (first frame), define each particle's initial position as the origin

   $$(x_0, y_0).$$

   Therefore,

   $$R^2 = 0$$

   for the first row of all particles (for example: `D3`, `G3`, . . . ).

3. **Compute $R^2$ for Each Particle**

   The squared displacement for each particle is defined as

   $$R^2(t) = (x - x_0)^2 + (y - y_0)^2.$$

   *Example (Particle 1):*

   In cell `D4`, enter:

```
=(B4-B$3)^2 + (C4-C$3)^2
```

The dollar sign ($) locks the reference to the initial position at $t = 0$ so it does not change when the formula is copied.

Drag this formula down to fill all time points (rows), and repeat the calculation for all tracked particles.

(*Note: Exact cell references for you may vary depending on the Excel layout.*)

4. **Compute the Mean-Squared Displacement**

   In the column following $R_{10}^2$ (for example `AF4`), calculate the average square displacement:

$$R_{\rm rms}^2(t) = \frac{R_1^2 + R_2^2 + \cdots + R_{10}^2}{10}.$$

   In Excel, use:

```
=AVERAGE(D4, G4, J4, ..., AE4)
```

5. **Compute the RMS Displacement**

   To obtain the RMS displacement,

$$R_{rms}(t) = \sqrt{R_{\rm rms}^2(t)}.$$

   In Excel, for example in cell `AG4`, enter:

```
=SQRT(AF4).
```

6. **Compute the Natural Logarithms**

   Excel's natural logarithm function is `LN()`.

   - For $\ln(t)$ (cell `AH4`), use:
     ```
     =LN(A4).
     ```
   - For $\ln(R^2)$ (cell `AI4`), use:
     ```
     =LN(AF4).
     ```

7. **Plot the Required Graphs**

   For each video, produce the following three plots:

   (a) $R$ vs. $t$

   (b) $R_{\rm rms}^2$ vs. $t$

   (c) $\ln(R_{\rm rms}^2)$ vs. $\ln(t)$

   The third plot **must include a linear trendline** and the displayed equation.

   Refer to the example graphs:

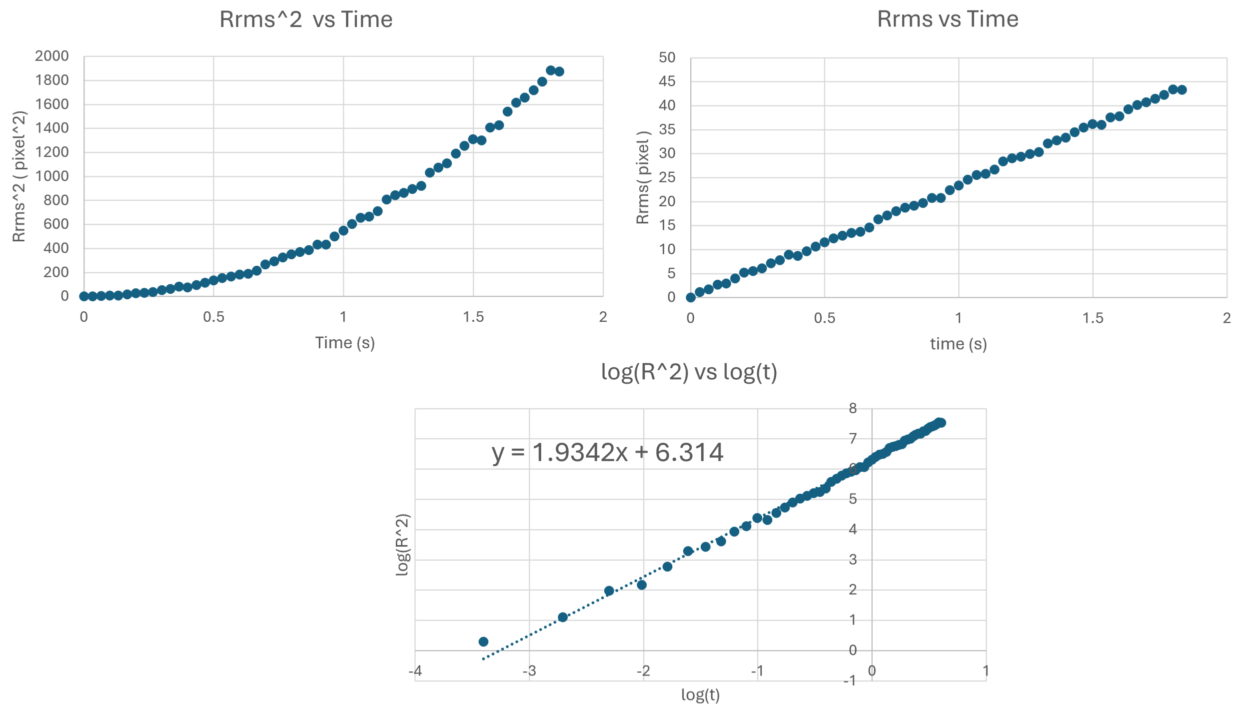



Figure 9: Graphs for a 5 $\mu$m silica bead in water. The log–log plot has a slope of 1.93, indicating that the motion is dominated by gravity.

# Experiment 5

## Experiment 5 - Motion in Living Cell

To calculate the **rate of ATP hydrolysis** $(R)$ and the **coefficient of viscosity** $(\eta)$, you will use the average radius $(r)$ and average speed $(v)$ obtained from ImageJ. With these values, you can proceed as follows:

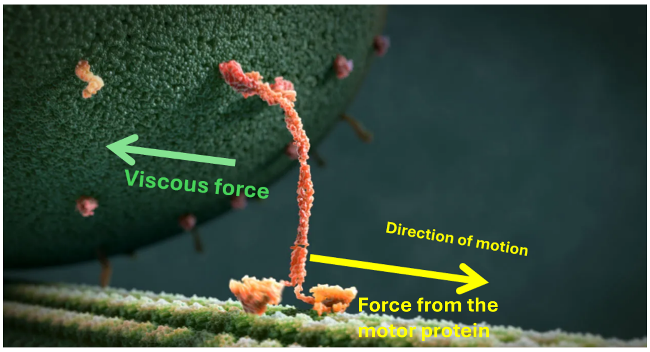



Figure 10: Schematic of a vesicle of radius $r$ moving with constant velocity $v$ through a viscous medium. The motor force is balanced by the viscous drag; therefore, the vesicle is traveling with a constant speed.

### Work and Power

Some definitions:
Work done on an object by a force **F** causing a displacement **d** is given by

$$W = \mathbf{F} \cdot \mathbf{d} = |\mathbf{F}|\,|\mathbf{d}| \cos\theta, \tag{12}$$

where $\theta$ is the angle between the force and displacement. In your case the viscous drag force is opposite to the direction of motion ($\theta = 180°$), so the work done by the viscous force is negative. In terms of magnitudes you will use

$$|W| = |\mathbf{F}|\,|\mathbf{d}|.$$

Power is defined as the rate of doing work:

$$P = \frac{W}{t}. \tag{13}$$

Useful biological numbers:

- Average size of a kinesin motor "step": $s_{\text{kin}} \approx 8$ nm (along a tubulin dimer).
- Average size of a myosin motor "step": $s_{\text{myo}} \approx 10$ nm (along an actin filament).
- One "step" $\approx$ one ATP hydrolysis cycle. The energy released per mole of ATP is about $E_{\text{mol}} = 23$ kJ/mol.

- To get the energy per ATP molecule in SI units (J), use

$$E = \frac{E_{\text{mol}}}{N_A},$$

where $N_A$ is Avogadro's number.

- The efficiency of the motor protein is approximately $e = 60\% = 0.6$.

The efficiency is defined as

$$\text{efficiency} = e = \frac{W_{\text{produced}}}{E_{\text{consumed}}} = \frac{P_{\text{produced}}}{P_{\text{consumed}}}. \tag{14}$$

The total work *consumed* comes from ATP hydrolysis. If $N$ is the number of ATP cycles in a time $t$ and $E$ is the energy released in a single ATP cycle (in J per molecule), then

$$P_{\text{consumed}} = \frac{W_{\text{consumed}}}{t} = \frac{NE}{t} = RE, \tag{15}$$

where

$$R = \frac{N}{t}$$

is the rate of ATP hydrolysis (ATP molecules per second).
Since the vesicle is moving with (approximately) constant velocity, the net force is zero: the forward motor force equals the viscous drag. The mechanical power *produced* by the vesicle (via the motor) is then

$$P_{\text{produced}} = \frac{W_{\text{produced}}}{t} = \frac{Fd}{t} = Fv, \tag{16}$$

where $F$ is the magnitude of the force exerted by the motor on the vesicle and $v$ is the speed.

## Rate of ATP hydrolysis $R$ from the average velocity $v$

The average velocity is given by

$$v = \frac{d}{t}. \tag{17}$$

Here $d$ is the total displacement in time $t$. If $N$ is the number of ATP cycles and $s$ is the step size of the motor, then the total distance is $d = Ns$. Therefore

$$v = \frac{Ns}{t} = Rs. \tag{18}$$

Thus the rate of ATP hydrolysis is

$$R = \frac{v}{s}. \tag{19}$$

**Important:** In your calculations, use SI units:

- Convert the step size from nm to m (1 nm = $10^{-9}$ m).
- Convert $v$ from $\mu$m/s (or similar) to m/s.

## Coefficient of viscosity $\eta$ from average radius $r$

The viscous drag force acting on a slowly moving spherical particle in a fluid is given by Stokes' law:

$$F_{\text{viscous}} = 6\pi\eta r v, \tag{20}$$

where

- $r$ is the average radius of the vesicle (from ImageJ),
- $v$ is the terminal (steady) velocity from your tracking,
- $\eta$ is the dynamic viscosity of the cytosol.

You can now relate this force to the motor energetics using the efficiency. Rearranging the efficiency relation,

$$e \times P_{\text{consumed}} = P_{\text{produced}}. \tag{21}$$

Substituting the expressions for $P_{\text{consumed}}$ and $P_{\text{produced}}$,

$$e \times R \times E = Fv. \tag{22}$$

Therefore the magnitude of the force is

$$F = \frac{eRE}{v}. \tag{23}$$

Using Stokes' law, the coefficient of viscosity is

$$\begin{aligned}\eta &= \frac{F}{6\pi r v} \\ &= \frac{eRE/v}{6\pi r v} \\ &= \frac{eRE}{6\pi r v^2} \\ &= \frac{e(v/s)E}{6\pi r v^2} \\ &= \frac{eE}{6\pi r v s}. \end{aligned} \tag{24}$$

Again, use SI units for all quantities:

- $r$ in meters,
- $v$ in meters per second,
- $s$ in meters,
- $E$ in joules per ATP molecule.

In this experiment, it is not possible to distinguish between kinesin and myosin motors, so you will compute $\eta$ for both:

- Kinesin: $s = 8$ nm,
- Myosin: $s = 10$ nm.

The calculated values can then be compared with the viscosity of water,

$$\eta_{\text{water}} \approx 8.6 \times 10^{-4}\ \text{Pa}\cdot\text{s}.$$

A significantly larger effective viscosity suggests that the cytosol behaves as a more crowded and structured medium than pure water.

## WEEK 1 - ANALYZING VESICLE MOTION

As a group, select **10 vesicles** that clearly exhibit **linear (directed) motion** (not random Brownian motion). Velocity data will be obtained using the **Manual Tracking** plugin in *ImageJ*.

## Reducing the Number of Frames

The original video is 5 seconds long and recorded at 30 frames per second (fps), giving a total of

$$150 \text{ frames.}$$

Tracking every frame would require 150 clicks per vesicle. To simplify the procedure, you will track every **third frame** instead (or You may consider recording the longer video at low fps).

## ImageJ Procedure

1. Open the AVI video file in ImageJ.
2. Navigate to:
   $$\text{Image} \longrightarrow \text{Stacks} \longrightarrow \text{Tools} \longrightarrow \text{Make Substack}$$
3. In the dialog box, enter:
   $$1 - 150 - 3$$
   This selects frames starting at frame 1, ending at frame 150, with a step size of 3. The resulting reduced stack will contain:
   $$\frac{150}{3} = 50 \text{ frames.}$$
4. Run:
   $$\text{Plugins} \longrightarrow \text{Tracking} \longrightarrow \text{Manual Tracking}$$
   on this reduced image stack.

## Time Step Calculation

The original frame interval is:

$$\Delta t_{\text{original}} = \frac{1}{30} \text{ s} \approx 0.03333 \text{ s.}$$

Since you are tracking every third frame, the effective time step becomes:

$$\Delta t = 3 \times \Delta t_{\text{original}} = 3 \times 0.03333 \text{ s} \approx 0.0999 \text{ s.}$$

## Calibration

In the Manual Tracking plugin:

- Keep the **x/y spatial calibration set to 1**.
- All distance data will therefore be recorded in **pixels**.

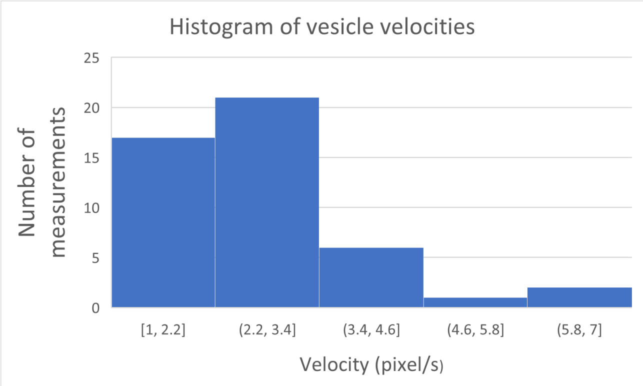
Conversion from pixels to meters will be performed later during the Excel data analysis stage.

# WEEK 2 - DATA ANALYSIS

Now that you have the data, do the following:

## 1. Combine Velocities

Put the velocity values for all vesicles into a single column. Remove any entries equal to $-1$ or 0.




## 2. Visualize

Plot a histogram of velocities and include it in your report.

## 3. Average Speed

Compute the average velocity of the cleaned list. This average will be your $v$ for the calculations.

## 4. Average Radius

Average the area measurements of the tracked vesicles, then convert the mean area $\bar{A}$ to a radius using

$$r = \sqrt{\frac{\bar{A}}{\pi}}.$$

## 5. Convert to SI Units

Convert $v$ from pixels/s (or $\mu$m/s) to m/s, and $r$ from pixels (or $\mu$m) to meters.
The scale factor is

$$1\ \mu\text{m} = 5.179 \text{ pixels} \quad \Rightarrow \quad 1 \text{ pixel} \approx 0.193\ \mu\text{m}.$$

## Example Calculation

Suppose the values for $v$ and $r$ are as follows (values are just an example):

$$v = 2.31 \text{ pixels/s}, \qquad r = 3.29 \text{ pixels},$$

and the scale

$$1\ \mu\text{m} = 5.179 \text{ pixels} \quad \Rightarrow \quad 1 \text{ pixel} = 0.193\ \mu\text{m},$$

and using

$$e = 0.6, \quad E_{\text{mol}} = 23 \text{ kJ/mol} = 23{,}000 \text{ J/mol}, \quad N_A = 6.022 \times 10^{23},$$

$$E = \frac{23{,}000}{6.022 \times 10^{23}} \approx 3.82 \times 10^{-20} \text{ J},$$

step sizes

$$\text{kinesin: } s = 8 \text{ nm} = 8 \times 10^{-9} \text{ m}, \qquad \text{myosin: } s = 10 \text{ nm} = 1.0 \times 10^{-8} \text{ m},$$

you proceed as follows.

**1) Convert to SI**

$$r = 3.29 \times 0.193\ \mu\text{m} = 0.635\ \mu\text{m} = 0.635 \times 10^{-6}\ \text{m} = 6.35 \times 10^{-7}\ \text{m},$$
$$v = 2.31 \times 0.193\ \mu\text{m/s} = 4.46 \times 10^{-7}\ \text{m/s}.$$

**2) Rate of ATP Hydrolysis $R$ (from $v = Rs$)**

Kinesin (8 nm):

$$R = \frac{v}{s} = \frac{4.46 \times 10^{-7}}{8 \times 10^{-9}} \approx 55.6\ \text{s}^{-1}.$$

Myosin (10 nm):

$$R = \frac{4.46 \times 10^{-7}}{1.0 \times 10^{-8}} \approx 44.6\ \text{s}^{-1}.$$

**3) Coefficient of Viscosity $\eta$**

Use

$$\eta = \frac{eE}{6\pi r v s}.$$

Kinesin (8 nm):

$$\eta \approx \frac{0.6 \times 3.82 \times 10^{-20}}{6\pi \times (6.35 \times 10^{-7}) \times (4.46 \times 10^{-7}) \times (8 \times 10^{-9})} \approx 0.54\ \text{Pa} \cdot \text{s}.$$

Myosin (10 nm):

$$\eta \approx 0.43\ \text{Pa} \cdot \text{s}.$$

**4) Compare to Water**

Compare these values with

$$\eta_{\text{water}} \approx 8.6 \times 10^{-4}\ \text{Pa} \cdot \text{s}.$$

If your effective viscosity is larger than that of water, this suggests that the cytosol behaves like a thicker, more crowded medium than pure water, which is realistic for a living cell.