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Tracking Brownian Motion Through Video Microscopy

Syed Hassan Abbas Bukhari* ~~and Muhammad Sabieh Anwar†~~
LUMS, School of Science and Engineering

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Boltzmann's constant ~~k_B~~ was measured by observing the Brownian motion of polystyrene spheres in water. An inexpensive monochrome CCD camera and video card were used to create a video of the spheres's motion. After preprocessing the images, custom routines were used to examine the video and to identify and track the particles from one frame to the next. From the mean squared displacement of the particles versus time, we extracted the value of k_B from the slope, assuming that the drag force on an individual sphere is well modeled by Stokes' law. By averaging over ~~7~~ seven particles, we obtained $k_B = (1.49 \pm 0.07) \times 10^{-23} J/K$.

I. INTRODUCTION

fluid [1].

Brownian motion is the random motion of particles suspended in a fluid ~~(a liquid or a gas)~~ resulting from their collision with the fast-moving molecules in the fluid.[1] This pattern describes a fluid at thermal equilibrium, defined by a given temperature. Within such a fluid there exists no preferential direction of flow ~~as in~~ characteristic transport phenomena. More specifically the fluid's overall linear and angular momenta remain null over time. It is important also to note that the kinetic energies of the molecular Brownian motions, together with those of molecular rotations and vibrations sum up to the caloric component of a fluid's internal energy.

$\boxed{\text{indent}}$ or space

This motion is named after the botanist Robert Brown, who was the most eminent microscopist of his time. In 1827, while looking through a microscope at pollen of the plant *Clarkia pulchella* immersed in water, the triangular shaped pollen burst at the corners, emitting particles which he noted jiggled around in the water in random fashion. He was not able to determine the mechanisms ~~that caused~~ causing this motion. Atoms and molecules had long been theorized as the constituents of matter, and Albert Einstein published a paper in 1905 that explained in precise detail how the motion that Brown had observed was a result of the pollen being moved by individual water molecules, making one of his first big contributions to science. This explanation of Brownian motion served as convincing evidence that atoms and molecules exist, and was further verified experimentally by Jean Perrin in 1908. The direction of the force of atomic bombardment is constantly changing, and at different times the particle is hit more on one side than another, leading to the seemingly random nature of the motion.

II. THEORY

There are two parts of theory: the first part consists in the formulation of a diffusion equation for Brownian particles, in which the diffusion coefficient is related to the mean squared displacement of a Brownian particle, while the second part consists in relating the diffusion coefficient to measurable physical quantities.[2] Classical mechanics is unable to determine this distance because of the enormous number of bombardments a Brownian particle will undergo, roughly of the order of 10^{14} collisions per second. Einstein regarded the increment of particle positions in time τ in a one dimensional (x) space (with the coordinates chosen so that the origin lies at the initial position of the particle) as a random variable (x') with some probability density function $\varphi(x')$. Further, assuming conservation of particle number, he expanded the density (number of particles per unit volume) at time $t+\tau$ ~~in~~ as a Taylor series,

$$\rho(x,t) + \tau \frac{\partial \rho(x)}{\partial t} + \cdots = \rho(x, t+\tau) \tag{1}$$

$$= \int_{-\infty}^{+\infty} \rho(x - x', t) \cdot \varphi(x') \, dx' = \mathbb{E}_{x'}[\rho(x, t+\tau)] \tag{2}$$

$$= \rho(x,t) \cdot \int_{-\infty}^{+\infty} \varphi(x') \, dx' - \frac{\partial \rho}{\partial x} \cdot \int_{-\infty}^{+\infty} x' \cdot \varphi(x') \, dx'$$

align "=" signs in the equation

$$+ \frac{\partial^2 \rho}{\partial x^2} \int_{-\infty}^{+\infty} \frac{x'^2}{2} \cdot \varphi(x') \, dx' + \cdots \tag{3}$$

use $\boxed{\backslash\text{cdot}}$

$$= \rho(x,t) \, 1 + 0 + \frac{\partial^2 \rho}{\partial x^2} \cdot \int_{-\infty}^{+\infty} \frac{x'^2}{2} \cdot \varphi(x') \, dx' + \cdots . \tag{4}$$

here the Eq 2. is by definition of φ. The integral in the first term of which eq is equal to one by the definition of probability, and the second and other even terms (i.e. first and other odd moments) vanish because of ~~space~~ spatial symmetry. What is left gives rise to the following relation:

$$\frac{\partial \rho}{\partial t} = \frac{\partial^2 \rho}{\partial x^2} \int_{-\infty}^{+\infty} \frac{x'^2}{2\tau} \varphi(x') \, dx' + \text{higher-order moments} \tag{5}$$

x

* Electronic mail: 18120009@lums.edu.pk
† Electronic mail: sabieh@lums.edu.pk

Where the coefficient before the Laplacian, the second moment of probability of displacement x', is interpreted as mass diffusivity D:

$$D = \int_{-\infty}^{+\infty} \frac{x'^2}{2\tau} \varphi(x')\,\mathrm{d}x' . \qquad (6)$$

Thus ~~Then~~ the density of Brownian particles ρ at point x at time t satisfies the diffusion equation:

$$\frac{\partial \rho}{\partial t} = D \frac{\partial^2 \rho}{\partial x^2} , \qquad (7)$$

A ~~a~~ssuming that N particles start from the origin at the initial time $t = 0$, the diffusion equation has the solution

$$\rho(x,t) = \frac{N}{\sqrt{4\pi D t}} e^{-\frac{x^2}{4Dt}} . \qquad (8)$$

This expression (which is a normal distribution with the mean $\mu = 0$ and variance $\sigma^2 = 2Dt$ usually called Brownian motion B_t) allowed Einstein to calculate the moments directly. The first moment is seen to vanish, meaning that the Brownian particle is equally likely to move to the left as it is to move to the right. The second moment is, however, non-vanishing, being given by

$$\overline{x^2} = 2\,D\,t . \qquad (9)$$

We can also write the mean squared displacement in two dimensions,

$$\overline{r^2} = 4\,D\,t , \qquad (10)$$

expressing ~~This expresses~~ the mean squared displacement in terms of the time elapsed and the diffusivity. From this expression Einstein argued that the displacement of a Brownian particle is not proportional to the elapsed time, but rather to its square root.[2] His argument is based on a conceptual switch from the "ensemble" of Brownian particles to the "single" Brownian particle: we can speak of the relative number of particles at a single instant just as well as of the time it takes a Brownian particle to reach a given point.[3] The second part of theory relates the diffusion constant to physically measurable quantities, such as the mean squared displacement of a particle in a given time interval. This result enables the experimental determination of Avogadro's number. Einstein analyzed a dynamic equilibrium being established between opposing forces. The beauty of his argument is that the final result does not depend upon which forces are involved in setting up the dynamic equilibrium.

cit [?]. (P) new para please

Consider, for instance, particles suspended in a viscous fluid in a gravitational field. Gravity tends to make the particles settle, whereas diffusion acts to homogenize them, driving them into regions of ~~smaller~~ concentration. Under the action of gravity, a particle acquires a downward speed of $v = \mu m g$, where m is the mass of the particle, g is the acceleration due to gravity, and μ is the particle's mobility in the fluid. George Stokes had already shown that the mobility for a spherical particle with radius r is $\mu = 1/6\pi\eta r$, where η is the dynamic viscosity of the fluid. In a state of dynamic equilibrium, and under the hypothesis of an isothermal fluid, the particles are distributed according to the barometric distribution

$$\rho = \rho_0 e^{-\frac{mgh}{k_B T}} , \qquad (11)$$

where $\rho - \rho_0$ is the difference in density of particles separated by a height difference of h, k_B is Boltzmann's constant (namely, the ratio of the universal gas constant, R, to Avogadro's number, N_A), and T is the absolute temperature. Avogadro's number is to be determined. Dynamic equilibrium is established because the more that particles are pulled down by gravity, the greater the tendency for the particles to migrate to regions of lower concentration, ~~The flux~~ forming a flux which is given by Fick's law,

$$J = -D \frac{d\rho}{dh} , \qquad (12)$$

where $J = \rho v$. Introducing the formula for ρ, we find that

$$v = \frac{Dmg}{k_B T} . \qquad (13)$$

In a state of dynamical equilibrium, this speed must also be equal to $v = \mu m g$. Notice that both expressions for v are proportional to mg, reflecting that the derivation is independent of the type of forces considered. Similarly, one can derive an equivalent formula for identical charged particles of charge q in a uniform electric field of magnitude E, where mg is replaced with the electrostatic force qE. Equating these two expressions yields a formula for the diffusivity, independent of mg or qE or other such forces:

$$\frac{\overline{r^2}}{4t} = D = \mu k_B T = \frac{\mu R T}{N} = \frac{RT}{6\pi\eta r N} . \qquad (14)$$

Here the first equality follows from the first part of the theory, the third equality follows from the definition of Boltzmann's constant, ~~as~~ $k_B = R/N_A$, and the fourth equality follows from Stokes's formula for the mobility. By measuring the mean squared displacement over a time interval along with the universal gas constant R, the temperature T, the viscosity η, and the particle radius r, Avogadro's number N_A can therefore be determined.

III. EXPERIMENTAL SETUP

We used an inexpensive American Optical Spencer Microscope. The image from the objective lens is directly imaged onto the CCD. We calibrated the image using a Motic calibrating slide and obtained values of 0.207101 $\mu m/pixel$ horizontal and 0.208955 $\mu m/pixel$ vertical. To observe Brownian motion, we used 0.75μm diameter polystyrene micro-spheres. One drop of the micro-sphere solution was placed on a cover slip and the cover

(company name)

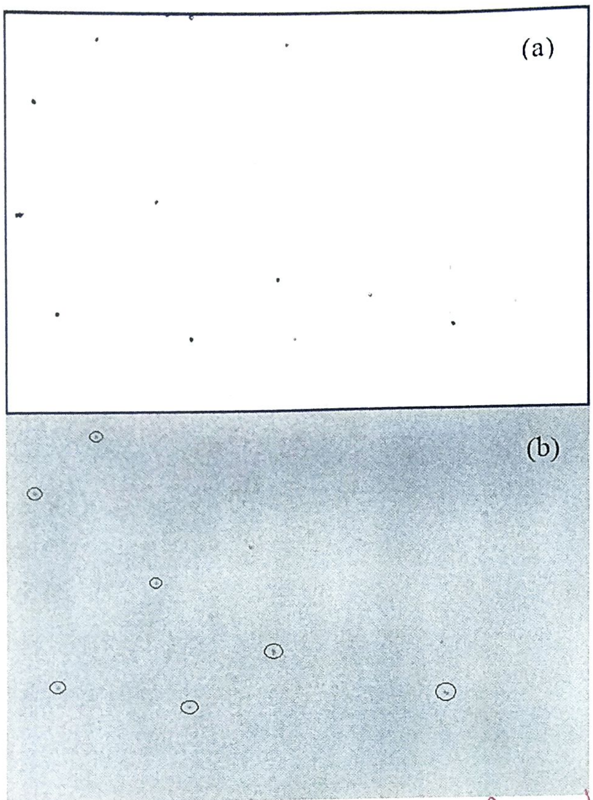

Figure 1. Raw image (a) resulting from subtracting the background, smoothing, and adjusting the contrast. (b) of 750 nm spheres and corresponding spheres are marked by circle.

Processed image

P?

which resulted in

slip was inverted, the drop ~~adheres~~ adhere via cohesive forces to the glass! and placed on the glass slide in the center of the plastic reinforcement. We observed the slide on the computer using the Motic live imaging module. If the sample exhibited any evidence of a coherent macroscopic oscillation or flow, we discarded the slide and ~~make~~ made a new one. Our experience is that if an air bubble is visible in the central circular area, there will be visible flow which will make the data from such a slide unusable. Although we have not quantitatively studied the motion in such a slide, our qualitative observation is that the fluid appears to oscillate. The data ~~in~~ for this report was sampled at 20 Hz for a total of 9.9 seconds. Then the sampled image is then converted to grayscale and each pixel's ~~lightness~~ brightness is compared with a threshold variable. The pixels lighter than the threshold ~~have greater lightness value than the threshold and~~ are considered as the background. All remaining pixels are considered to be the micro-spheres (the dark objects). The Matlab script[7] ~~will allow~~ allowed to manually mark the objects to be tracked, after marking the script will start tracking the objects. Once the mean square displacement of all particles ~~is~~ was known after processing the video, some more variables ~~are~~ were collected from the user using message boxes. These variables included the

$20~$HZ

Don't switch tense from past to future. Use only one tense.

See Fig. (1) for a typical frame and the result after the thresholding operation.

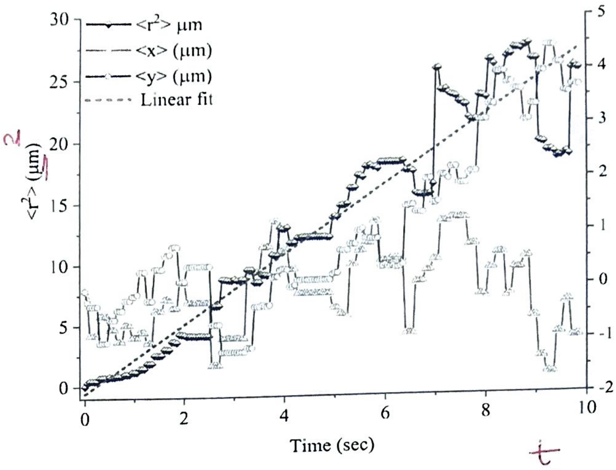

Figure 2. $\langle r^2 \rangle$, $\langle x \rangle$ and $\langle y \rangle$ of the objects vs ~~Time~~ time.

sample temperature, viscosity and average sphere diameter. At the end of a successful run, the script will leave some variables (k_B, N_A and D) in the main workspace.

IV. RESULTS AND DISCUSSION

We read, analyze, and plot the data from Matlab, ~~and~~ while subtract ~~off~~ ex the initial x and y positions for each particle, so that for the ith particle at time t, we have $\Delta x_i(t) = x_i(t) - x_i(0)$ and $\Delta y_i(t) = y_i(t) - y_i(0)$. The square displacement of the ith particle at time t is therefore

$$\langle r(t)^2 \rangle = \frac{1}{N} \sum_{i=1}^{N} [\Delta r_i(t)^2 = x_i(t)^2 + y_i(t)^2]. \tag{15}$$

To obtain Boltzmann's constant, we need to plot the mean squared displacement versus time, so we calculate the time dependent quantity where N is the number of particles tracked (7 in our case). From the slope ($(2.8 \pm 0.1) \times 10^{-12}$) in Fig. 2, we use Eq. 14 to obtain Boltzmann's constant, k_B. The coefficient of viscosity is a nonlinear function of temperature, so we used data from the Lab manual[5] and used an exponential fit[8] ~~and~~ interpolated ~~data~~ to approximate the viscosity $\eta = 9.0084 \times 10^{-4}\ Nsm^{-2}$ for $(24.48 \pm 0.03\ °C)$[9].

units not in math mode

$$k_b = (1.5 \pm 0.1) \times 10^{-23}\ J/K \text{ (uncorrected)}.$$

Error in k_B is because the viscosity of water is a sensitive function of temperature, the value we obtained for k_B is ~~very~~ highly dependent on both temperature and viscosity. The most difficult aspect of our experiment is creating an observation cell with no noticeable drift and with a sufficient and quantifiable depth to allow us to ignore sphere-wall interactions. ~~Although the value k_B is acceptable.~~ The values of N_A and D are ~~given as with uncertainties~~ computed.

$$N_A = (5.5 \pm 0.3) \times 10^{23}$$

Avoid "very!"

$$D = (7.1 \pm 0.3) \times 10^{-13}\ m^2 s^{-1}$$

Some care must be taken when calculating the spheres's self-diffusivity D, due to the effects of the walls. For a sphere far from two parallel walls, the modified self-diffusivity is given by[4]

$$D' = D\Big[1 - \frac{9r}{16}\Big(\frac{1}{x_1} + \frac{1}{x_2}\Big)\Big] \quad (16)$$

where x_1 and x_2[10] are the distances from the sphere to the two walls. The distance d between the wall is $d = 0.11 \pm 0.01\ mm$ (by measuring the width of tape which is used between cover slip and glass slide measured by Screw gauge) and $d = 0.116\ mm$ (measured using fine focusing knob of Microscope)[11] Applying Eq. 16 yields a wall-corrected value which is 0.95% smaller:

$$D' = (6.99 \pm 0.30) \times 10^{-13}\ m^2 s^{-1}$$

which also give us the corrected value of k_b as:

$$k_b = (1.49 \pm 0.07) \times 10^{-23}\ J/K.$$

Error in the value of Boltzmann's constant is 8% which is good in agreement with the sensitivity of our experimentation.

Conclude. Conclusion is missing

[1] Feynman, R. (1964). *"The Brownian Movement". The Feynman Lectures of Physics*, Volume I. pp. 41&45, 1.

[2] Einstein, Albert (1956) [1926]. *"Investigations on the Theory of the Brownian Movement"*. Dover Publications. Retrieved 2013-12-25.

[3] Lavenda, Bernard H. (1985). *"Non-equilibrium Statistical Thermodynamics. John Wiley & Sons."* p. 20. ISBN 978-0-471-90670-4.

[4] W. B. Russel, D. A. Saville, and W. R. Schowalter, *Colloidal Dispersions* Cambridge University Press, Cambridge, 1989.

[5] Asma Khalid, Muhammad Umar Hasan and Muhammad Sabieh Anwar's Lab manual *"Tracking Brownian Motion Through Video Microscop"*.

[6] John C. Crocker *Measurement of the hydrodynamic corrections to the Brownian motion of two colloidal spheres.* Chem. Phys. 106 (7), 15 February 1997.

[7] PhysLab's video tracking library"PhysTrack.

[8] Two-phase exponential association equation.

[9] By using Thermocouple via DAQ of the sample. (100 Samples).

[10] $x_1 = 30\ \mu m$ and $x_2 = 86\ \mu m$.

[11] Measured by changing the height of the stage from 34 to 150 grating of fine focusing knob and the distance between the grating is 2 μm.