

# Tracking Brownian motion through video microscopy

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August 23, 2010

Brownian motion is the random motion of colloidal particles suspended in water, air or any other solvent. In 1905, Einstein argued that this motion is a direct evidence for the atomic nature of matter. Einstein's and Perrin's efforts helped raise the status of atoms from useful hypothetical objects to objects whose existence could no longer be denied.

**KEYWORDS:** Brownian motion · Colloidal particles · Viscosity · Boltzmann constant · Image processing · Kinetic theory

**APPROXIMATE PERFORMANCE TIME:** 1 week

## 1 Objectives

In this experiment, we will,

1. observe Brownian motion of microparticles,
2. calibrate a compound microscope,
3. use the microscope's built-in camera to record Brownian motion,
4. learn how to extract images and frames from a movie using MATLAB,
5. use some basic and simple commands for image processing,
6. locate and track microparticulate motion,
7. plot Brownian motion in 2-D,
8. observe how the mean square displacement of particles helps calculate Boltzmann's and Avogadro's constants.

## References and Essential Reading

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## 2 Theoretical Introduction

In 1827, Robert Brown observed the random motion of micro-particles suspended in gases and liquids. He called this random or jiggling motion 'Brownian motion'. However, it was only in 1905 that Einstein first explained this phenomenon on the basis of kinetic theory of molecules. In a quantitative manner, Einstein connected quantities of kinetic theory such as viscosity and mobility with the Brownian motion.

Einstein performed a statistical analysis of molecular motion and its effect on particles suspended in a liquid. As a result, he calculated the mean square displacement

of these particles. He argued that an observation of this displacement would allow an exact determination of atomic dimensions and prove the existence of atoms and verify the molecular kinetic theory of heat.

Perrin, a brilliant experimentalist, performed a series of experiments in the first decade of the twentieth century, one of which depended on Einstein's calculation of the mean square displacement of suspended particles. His results confirmed Einstein's relation and thus the molecular-kinetic theory. Eventually a physical explanation of the phenomenon of Brownian motion led to the acceptance of the atomic or molecular-kinetic theory [1].

## 2.1 Brownian motion and kinetic theory

Brownian motion can be explained using the kinetic theory of matter and the kinetic molecular theory of heat. The kinetic theory of matter posits the existence of atoms and molecules, and their constant motion due to which they elastically collide with one another. The kinetic molecular theory describes temperature as the constant motion of atoms and molecules in matter.

Brownian motion which is characterized by the constant and erratic movement of minute particles in a liquid or a gas is thus due to the inherently random motions of the atoms or molecules that make up the fluid in which the particles are suspended. The fluidic atoms or molecules collide with the larger suspended particles at random, making them move randomly.

Einstein described that Brownian motion actually arises from the agitation of individual molecules due to the thermal energy  $k_B T$  they possess at a specific temperature. The collective impact of these molecules against the suspended particle yields enough momentum to create movement of the particles.

## 2.2 Mathematical picture

The origin of Brownian motion can be understood on the basis of the theorem of equipartition of energy [2]. Each colloidal micro particle, possessing a mass  $m$  is free to exhibit translational motion. The mean kinetic energy of the particle in three dimension is,

$$\frac{1}{2}mv^2 = \frac{3}{2}k_B T.$$

This energy, though small in value, leads to a measurable amplitude of vibration for a small micro particle. It is worth noticing that in addition to the random fluctuating force, the particles also experience a drag force (frictional force) as they are pulled through the solvent.

To find a solution to the motion of the particles, we will use the **Langevin** equation

for a particle of mass  $m$  and velocity  $\mathbf{v}$

$$m \frac{d\mathbf{v}}{dt} = -\alpha \mathbf{v} + \mathbf{F}(t). \quad (1)$$

From Equation (1), we can see that each colloidal particle is subject to two forces:

1. the random molecular bombardment  $\mathbf{F}(t)$  that causes the Brownian motion, and the
2. resistive force  $\alpha \mathbf{v}$ , where  $\alpha$  is the damping coefficient related to viscosity of the fluid or solvent.

In one dimension, the scalar form of Equation (1) is written as,

$$m \frac{d^2x}{dt^2} + \alpha \frac{dx}{dt} - F(t) = 0.$$

Multiplying both sides of the above equation by  $x$ , yields,

$$mx \frac{d^2x}{dt^2} + \alpha x \frac{dx}{dt} - xF(t) = 0. \quad (2)$$

To simplify, we use the expansion of the expression  $\frac{d^2}{dt^2}(x^2)$ . The modified Equation (2) becomes,

$$\frac{m}{2} \frac{d^2x^2}{dt^2} - m \left( \frac{dx}{dt} \right)^2 + \frac{\alpha}{2} \frac{dx^2}{dt} - xF(t) = 0. \quad (3)$$

**Q 1.** Using chain rule, expand the derivative  $\frac{d^2}{dt^2}(x^2)$  to obtain Equation (3).

Now we use the theorem of equipartition of energy to find the average energy of single particle for one degree of freedom, which is given by

$$\begin{aligned} \frac{1}{2} m \langle v^2 \rangle &= \frac{1}{2} k_B T, \\ \Rightarrow \frac{m}{2} \langle \left( \frac{dx}{dt} \right)^2 \rangle &= \frac{1}{2} k_B T. \end{aligned} \quad (4)$$

We average Equation (3) over time and recognizing that since  $F$  is a random force, hence  $\langle xF \rangle = \langle x \rangle \langle F \rangle = 0$ . Defining  $\beta = \langle \frac{dx^2}{dt} \rangle$  and substituting Equation (4) into (3), we obtain,

$$\frac{m}{2} \frac{d\beta}{dt} - k_B T + \frac{\alpha}{2} \beta = 0. \quad (5)$$

**Q 2.** Derive Equation (5) (refer to [4] and [5] for help) with respect to time and show that the solution is,

$$\beta = \frac{2k_B T}{\alpha} + A \exp\left(\frac{-t\alpha}{m}\right), \quad (6)$$

where  $A$  is the integration constant. For a reasonably long observation time ( $t = \tau$ ), the factor  $-t\alpha/m$  will be very small and hence we can ignore the second term on the right hand side. Finally integrating the modified Equation (6) over the observation time  $\tau$ , we get

$$\int_0^\tau \left\langle \frac{dx^2}{dt} \right\rangle dt = \int_0^\tau \frac{2k_B T}{\alpha} dt$$

$$\langle x^2 \rangle = \frac{2k_B T \tau}{\alpha}. \quad (7)$$

For spherical particles, each of radius  $a$ , Stokes law can be used to write  $\alpha$ ,

$$\alpha = 6\pi \eta a,$$

where  $\eta$  is the viscosity of the fluid. Equation (7) hence takes the form,

$$\langle x^2 \rangle = \frac{2k_B T \tau}{6\pi \eta a}.$$

Using above Equation, we can write the mean squared displacement in two dimensions,

$$\langle r^2 \rangle = \frac{4k_B T}{6\pi \eta a} \tau. \quad (8)$$

Hence, by plotting  $\langle r^2 \rangle$  as a function of time, we expect a straight line through the origin whose slope can be used to obtain Boltzmann's constant  $k_B$ . Equation (8) is traditionally written as,

$$\langle r^2 \rangle = 4DT, \quad (9)$$

where  $D = k_B T / (6\pi \eta a) = k_B T / \alpha$  is the self-diffusion constant [1], [2].

Using the relation between Boltzmann's constant and molar gas constant  $R$ , we can also use Equation (8) to find Avogadro's number  $N_A$ ,

$$N_A = \frac{1}{\langle r^2 \rangle} \frac{2RT}{3\pi \eta a} \tau. \quad (10)$$

**Q 3.** Explain the dependence of the diffusion constant  $D$  on the damping factor  $\alpha$ .

### 2.3 Significance of Brownian motion

The theory of Brownian motion has come a long way since its humble beginnings in the nineteenth century. There now exist a large number of applications that have evolved. Some of the applications are listed below.

- In electronic devices, the discussion of Brownian motion is specifically important in understanding the effects of thermal motion of electrons contributing to Johnson noise [6].
- Researches in the field of biomedicine have shown that Brownian motion plays a critical role in the transport of enzymes and chemicals both into and out of cells in the human body. Scientists have subsequently discovered that many fundamental processes in living cells are driven by Brownian motion which also shows potential for use as probes at the nanoscale. For example it is possible to obtain detailed information about a particle's environment by analyzing its Brownian trajectory [7].
- In environmental sciences, the application of Brownian motion enables the prediction of extreme floods and droughts. By modeling volumetric flow of rivers mathematically, the relation can define a Brownian walk [8].

### 3 Apparatus

1. Motic microscope with built-in 3.0 megapixel camera and data transfer cable USB 2.0
2. Motic calibration slide
3. 76 mm x 25 mm glass slides
4. 18 mm x 18 mm cover slips
5. polystyrene microspheres (by Polysciences) diluted in water
6. 20 $\mu$ L micro pippete with tips

#### 3.1 Apparatus handling

The two main components of the experiment are the microscope and the polystyrene microspheres solution. The Motic microscope is a biological compound microscope, model BA210 with a 3.0 mega pixel built in camera. Live image resolution of the camera is 2048X1536 and data transfer rate is 480 MB/sec. The scope has four different objectives i.e., 4X, 10X, 40X and an oil immersion 100X objective. This range of different objective lenses allows us to select a suitable magnification to view the sample under observation. Microscope's objectives are parfocal, which means that when the objective lenses are changes the sample stays in focus. The light source in the scope is a 30 Watt halogen bulb whose intensity can be varied with the intensity control knob. Different parts of the microscope are labeled in Figure 1. The microspheres which will be used in the experiment are made of polystyrene and are available in five different sizes (diameters), i.e., 0.75  $\mu$ m, 1  $\mu$ m,

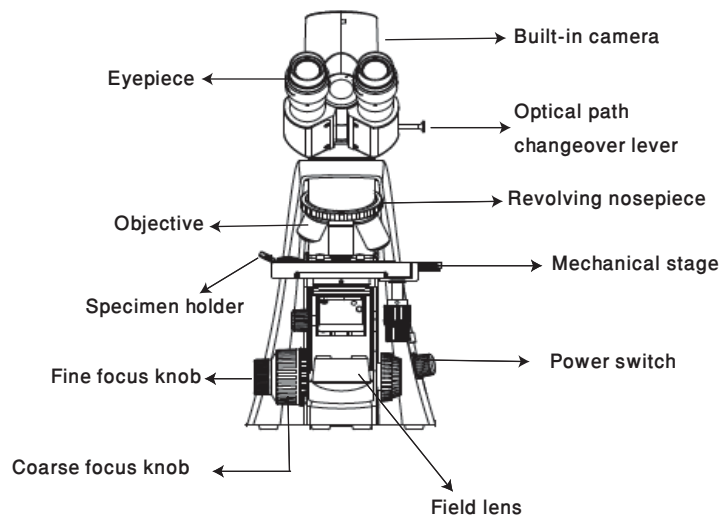


Figure 1: A diagram of the microscope.

1.5  $\mu\text{m}$ , 2.0  $\mu\text{m}$ , 4.0  $\mu\text{m}$ . These microparticles are harmless and should be used with normal precautions and care. However, special care should be taken for their storage. They should be stored at 4°C and in no case be permitted to freeze.

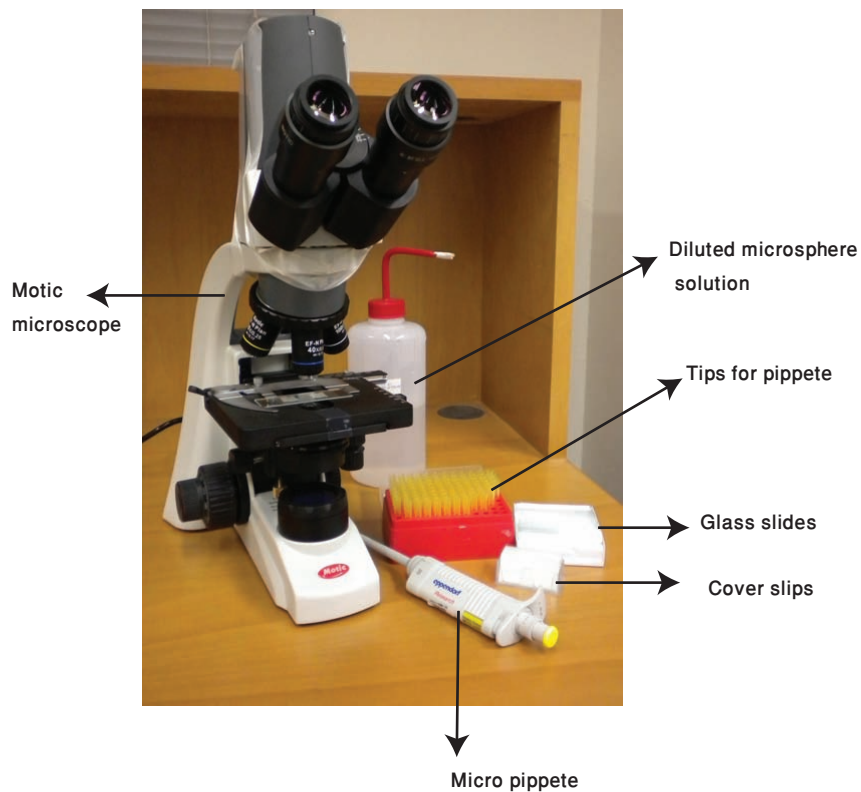


Figure 2: Microscope with slide preparation components.

## 4 Experimental method

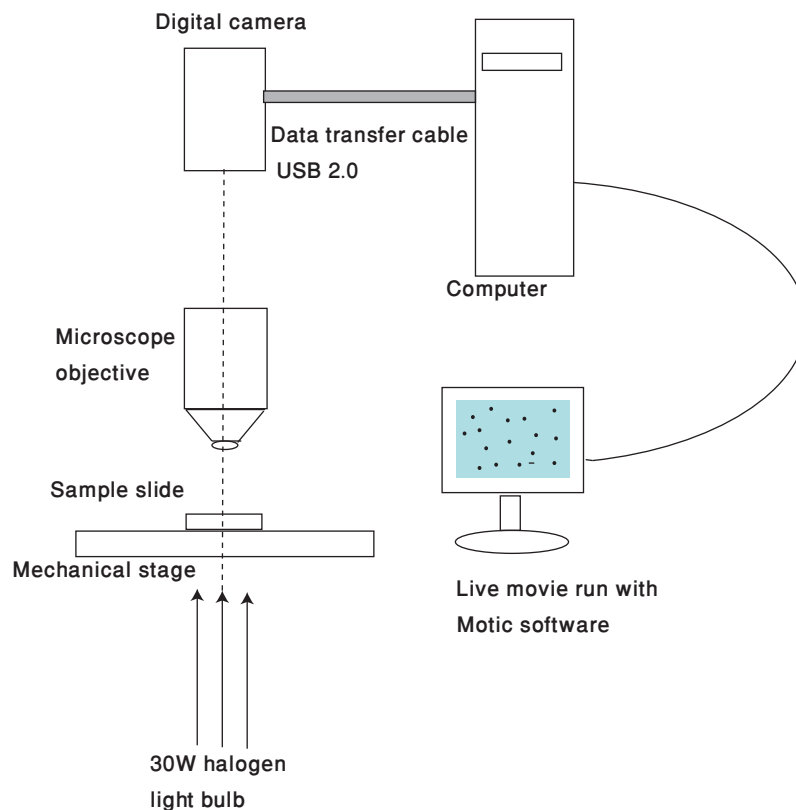


Figure 3: Apparatus for viewing Brownian motion.

### 4.1 Microscope adjustment and Image viewing software

To start the experiment, the **Motic Image Plus 2.0** application is run, and the **Capture** window is opened. The built-in camera will execute a live image in the window. One of the three resolutions is selected, the white balance is applied and the image is fit to the window.

### 4.2 Obtaining and Focusing the Image

1. Foremost, the calibration slide is fixed in the **specimen holder**.
2. An objective lens is selected with which calibration and all other measurements and recordings are to be taken.
3. Focusing is carried out with the **Coarse** and **Fine** focusing knobs. These knobs are present at the bottom left and right of the microscope. The direction of vertical movement of the stage corresponds to the turning direction of the focus knobs and one rotation of this knob moves the stage by 0.2 mm.



For more details on setting up the microscope and obtaining a sharp focused image, refer to [9].

### 4.3 Image calibration

Calibration is a very important step to be performed prior to the recording of any data, since the image processing and particle tracking algorithm is strongly dependent on the particle size, hence any carelessness in this step can lead to erroneous data. Better the calibration, easier would it be to record the particles' trajectories. Another important thing is to record the movie using the same objective and image resolution with which the calibration was done since each combination of objective and image resolution give different calibration values. The 10X and the 40X objectives are most suitable for our microparticles. Refer to [10] and [11] for detailed calibration methods using the **Motic** calibration slide. The calibrated values are in terms of  $\mu\text{m}$  per pixel and using this we can acquire the microspheres' diameter in microns.

### 4.4 Microsphere solution

To observe Brownian motion we use polystyrene microbeads. These microbeads (or microspheres) are available in the lab in a range of different diameters (0.75 to 4.00  $\mu\text{m}$ ). Refer to [13] for the microspheres' safety data sheets and physical properties. When originally packaged, the beads are suspended in 5 or 10 mL of water. Their concentration is very high. The estimated mass of microbeads in 1  $\text{cm}^3$  is 1.05 grams. Due to the high sphere density the solution is diluted  $10^4$  times. This increases the average distance between the beads so that the spheres do not frequently collide with each other and can be independently visualized and tracked.

A diluted solution has already been prepared for you and provided in the squeezed bottle. This solution should be shaken well before use, since the spheres start to settle down at the bottom with the passage of time.

### 4.5 Observation slide preparation

1. A custom observation slide of dimensions 76 mm  $\times$  25 mm and coverslip 18 mm  $\times$  18 mm is used for sample preparation. Before use both the slide and the cover slip are washed with acetone to remove any grease or dust on the surface.
2. The rotating knob of micro pipette is set to obtain a volume between 2–5  $\mu\text{L}$ . A tip is fixed to the pipette's narrower end. A drop of the diluted microsphere solution is sucked by pressing the head of the pipette gently and released by pressing the head with a little force of thumb. The drop is then placed on the slide.

3. Now the cover slip is placed on the slide and gently pressed on to it to form a seal between the slide and the coverslip.
4. Two thin slices of plastic tape or paper are stuck to the slide in order to prevent the adhesion of liquid to the glass surface as shown in Figure 4. This unnecessary adhesion can stick the microspheres to the surface of glass, hindering their free movement and leaving them immobile.
5. The glass slide is placed on the microscope's specimen stage and fixed using the specimen holder. The image is viewed on the computer screen. If there is a coherent macroscopic flow in the liquid, the slide should be discarded and a new one should be prepared.

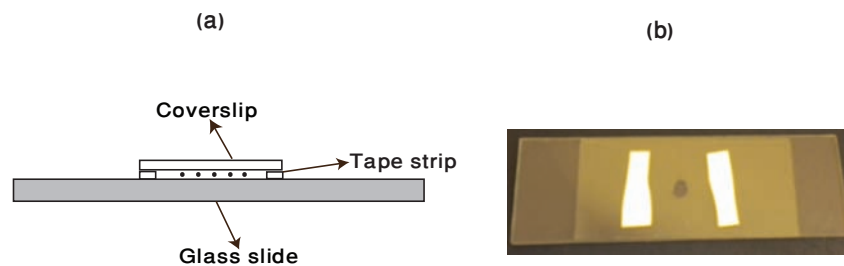


Figure 4: (a) Illustration of slide mounting system, (b) An observation glass slide with microsphere solution. A cover slip is used to prevent the liquid flow due to air currents..

Figure 5 shows different steps involved in preparation and mounting of the sample slide. Figure 5 (a) shows pouring a 5  $\mu\text{L}$  diluted solution drop on the slide, (b) illustrates placing the glass cover slip on the slide whereas (c) shows the slide being mounted on the microscope's stage.

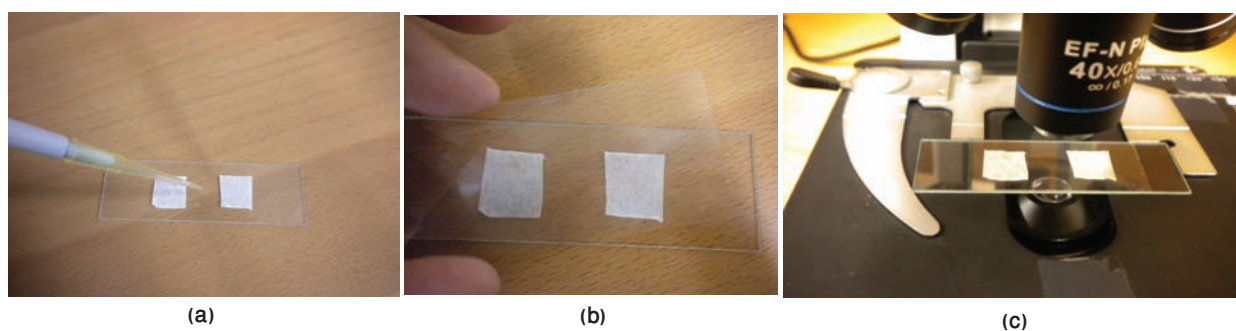


Figure 5: (a) Using micro pipette to place the solution of a known volume at the center of the slide, (b) covering the slide with cover slip, (c) mounting the slide onto the specimen stage.

## 4.6 Recording Brownian motion

Once an acceptable sample slide is obtained, the **Record** button in the *Capture* window is pressed, and the destination for saving the file is selected (preferably **MATLAB's work** folder). The software will start recording a movie of the Brownian motion. While recording data, the following precautions must be followed,

1. The microscope or slide should not be subjected to any movement.
2. The stage and the slide shouldn't be exposed to any air currents.
3. The intensity of surrounding light and light coming from the lamp of the microscope must not change.
4. Avoid storing movies longer than 15-20 seconds interval. Adjust the time length according to the resolution of image and frames per second. Number of frames per second is large for smaller resolution and is lower for higher resolutions.
5. Take at least five movies from a single slide. These movies are of course saved with different names.

## 4.7 Particle tracking through MATLAB

For data extraction from the movie, image processing and particle locating, the *MATLAB* file **tracker.m** is run. The following sections give a description of this program file. You are encouraged to study the source code and convince yourself of the various data extraction, image processing and particle tracking tasks described here.

### 4.7.1 Extracting movie frames

1. The recorded Brownian motion movie saved in the **MATLAB's work** folder is loaded into MATLAB file using the command

$$obj = mmreader('filename.avi').$$

This command can read in video data from a multimedia file and save it in an object '**obj**'. This object will be specified by the duration of the movie, frame rate, total number of frames, height, width and video format of the movie.

2. The number of frames per second (fps) is usually large, normally greater than 20 fps. In this case the built-in camera's resolution is not fast enough to detect the particle's movement every (1/20)th of a second, hence, a suitable **step size** needs to be input. The step size can be any integer lying in the

range 5-20, depending on the number of frames available. If the total number frames are 100, the frame rate is 10 fps and we select the step size to be 5, for example, then our program will select every fifth frame, thereby providing only 2 frames per second and a total of 20 frames. The step size helps discard similar frames and ensures that MATLAB does not run **OUT OF MEMORY**.

3. **obj** is an  $H \times W \times B \times F$  matrix where  $H$  is the image frame height,  $W$  is the image frame width,  $B$  is the number of bands in the image (e.g. 3 for RGB), and  $F$  is the number of frames read in. The following command

$$images(:,:, :, count) = read(obj, [n])$$

is used to read in video frames from **obj** one by one and save them as separate images. Here **count** specifies the serial number of the frames to be saved and **n** is the index running from first to last frame with the specified step size. For example, if  $n = 1 : 5 : 200$ , then the first frame will be saved as  $images(:,:, :, 1)$ , sixth frame as  $images(:,:, :, 2)$ , eleventh frame as  $images(:,:, :, 3)$  and so on. The type of data returned is always *UINT8* data representing RGB24 video frames.

4. The images are cropped if required.

#### 4.7.2 Image processing

To differentiate the background, dust particles and water droplets from the microspheres, the following steps are performed on images to make the microsphere tracking more accurate and reliable.

1. The `RGB2GRAY` command is used to convert RGB images to grayscale by eliminating the hue and saturation information while retaining the luminance.
2. `IMCOMPLEMENT` computes the complement of the images. This converts the dark particles to bright white particles with maximum brightness.
3. `IM2BW(image, LEVEL)` produces binary images by converting the gray images to binary by thresholding. The output binary image has values of 1 (white) for all pixels in the input image with luminance greater than **LEVEL** and 0 (black) for all other pixels.
4. Selecting **LEVEL**: A threshold level is necessary to choose for converting gray images to binary. The value of this variable lies preferably in the range 0.4 to 0.7. To select a threshold level, following steps are performed
  - A value of **LEVEL** (lying in the range 0.4–0.7) is entered when prompted by the program.
  - The result of applying the **LEVEL** to a typical frame will be displayed on the screen.

- The process is repeated until a suitable threshold level has been applied.
- To exit from the process, a random value lying outside the specified range is selected and the most suitable last value of **LEVEL** will be saved and will be used for all subsequent images.

**Note:** The value of **LEVEL** should be very carefully selected in a manner that the binary image so obtained shows maximum number of microspheres appearing as bright and well defined particles. Values with a difference of 0.01 can greatly improve the quality of the data. The original *RGB* image and its modification to binary images using three different **LEVEL** values are shown in Figure 6.

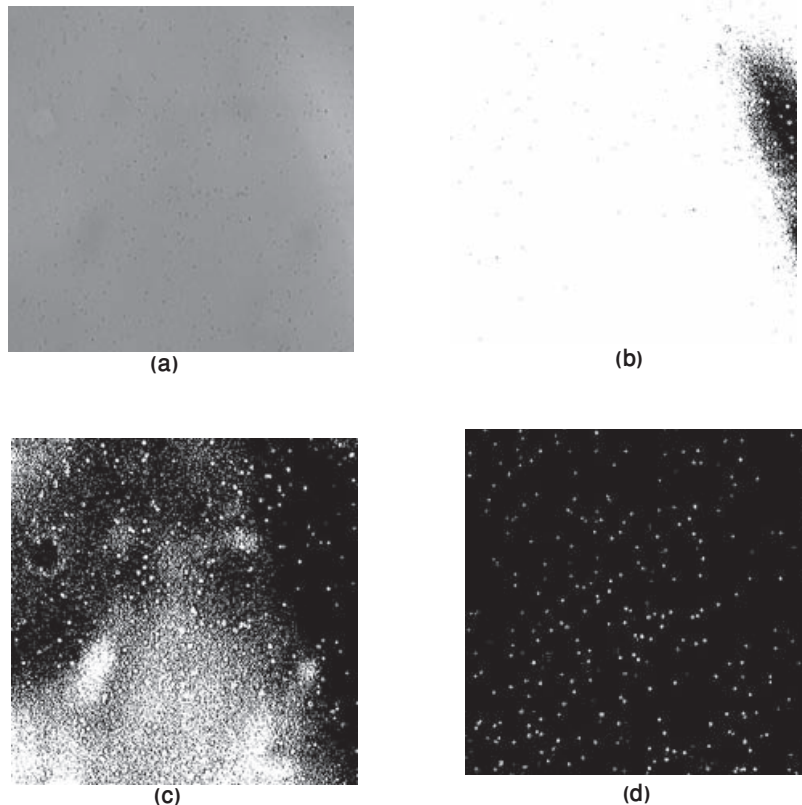


Figure 6: (a) Original image, in contrast to its binary forms with levels of threshold at (b) 0.4, (c) 0.5 and (d) 0.56.

**Q 4.** Why is Figure 6 (b) the most undesired binary counterpart of the original image?

5. A real-space bandpass filter **bpass.m** [14] is implemented on the binary images that suppresses pixel level noise and long-wavelength image variations while retaining information of a characteristic size. The function is called by the following command

$$filtered\_images = bpass(binary\_images, lnoise, lobject)$$

- **Inoise** is the characteristic length scale of noise in pixels and all the noise of this length would vanish from the image. One may assume any positive floating value, preferably in the range 0-1. This parameter is carefully selected in a way that all the noise lying in the neighborhood of a sphere vanishes and microspheres of a certain diameter are distinguished as spherical objects with sharp boundaries.
- **lobject** is the microspheres' length in pixels and should be somewhat larger than the actual sphere's diameter so that all particles with diameters in that range are counted. This value is taken from the image calibration and sphere size calculation performed in Section 4.3. All the particles that lie out of this range will be considered as background noise and will be subtracted from the final filtered image [14].

Figure 7 shows different bandpass filtered images with different values of noise parameter.

### 4.7.3 Particle tracking and data extraction

Once we have obtained filtered images in which microspheres appear as smooth, spherical and bright particles, the next step is to locate these bright intensity particles in every frame and then link the trajectory of each microsphere.

**Particle Finder** The program used for locating particles in a frame is named **pkfnd.m** [14] which finds local maxima in an image to pixel level accuracy and provides a rough guess of the particle's center. The command to call this function is as follows

$$position = pkfnd(filtered\_images, int, d)$$

- **filtered\_images** can be any of the frames processed in Section 4.7.2.
- **int** is the minimum brightness of a pixel that might be a local maxima. Approximate this parameter by setting a lower limit for the maximum intensity value, which is provided by the command **max(max(filtered\_images))** for any one of the frame.
- **d** is the diameter of the particles in pixels, this input helps get rid of any particle found within a radius of  $d/2$ , or agglomerated particles, and the code will keep only the brightest.

The output **position** is an  $N \times 2$  matrix containing as many rows  $N$  as there are particles in the image. The first column gives the x-coordinates and the second gives the y-coordinates of the positions of particles.

**Centroid Finder** After finding the positions of particles in a specific frame using the particle finder program, we then have to find the exact location of the centroid

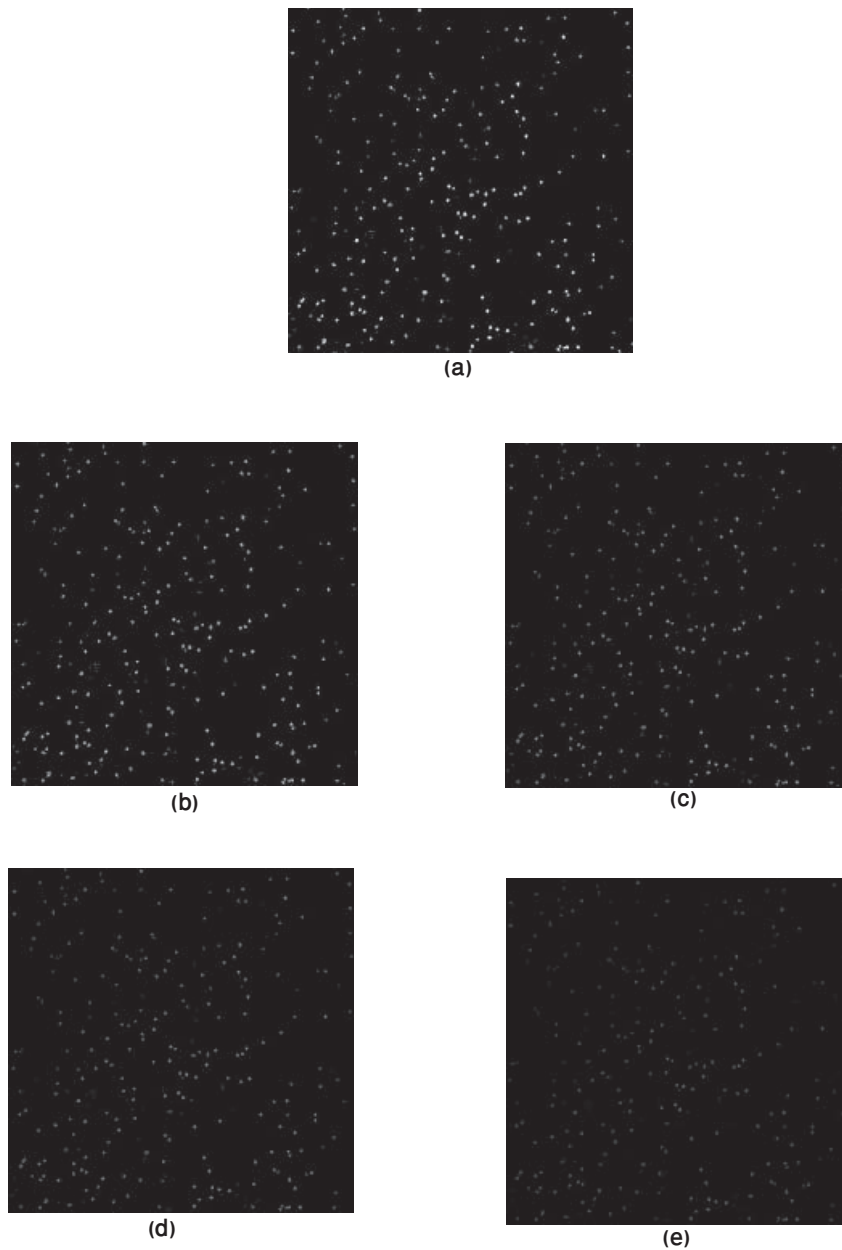


Figure 7: (a) Binary image, in contrast to its filtered forms with lengths of noise, noise at (b) 0, (c) 0.5 and (d) 0.7 and (e) 1. The value of `lobject` for all of these images is 3. The boundaries of particles become more smooth and circular as the noise length is increased.

of each particle in all the frames. The program `cntrd.m` [14] is called within a loop that runs from the first to the last frame. The following command is used

$$centroid\_location = cntrd(filtered\_images, position, dim)$$

which runs for all the frames and calculates the centroid of all bright spots in the frame to sub-pixel accuracy.

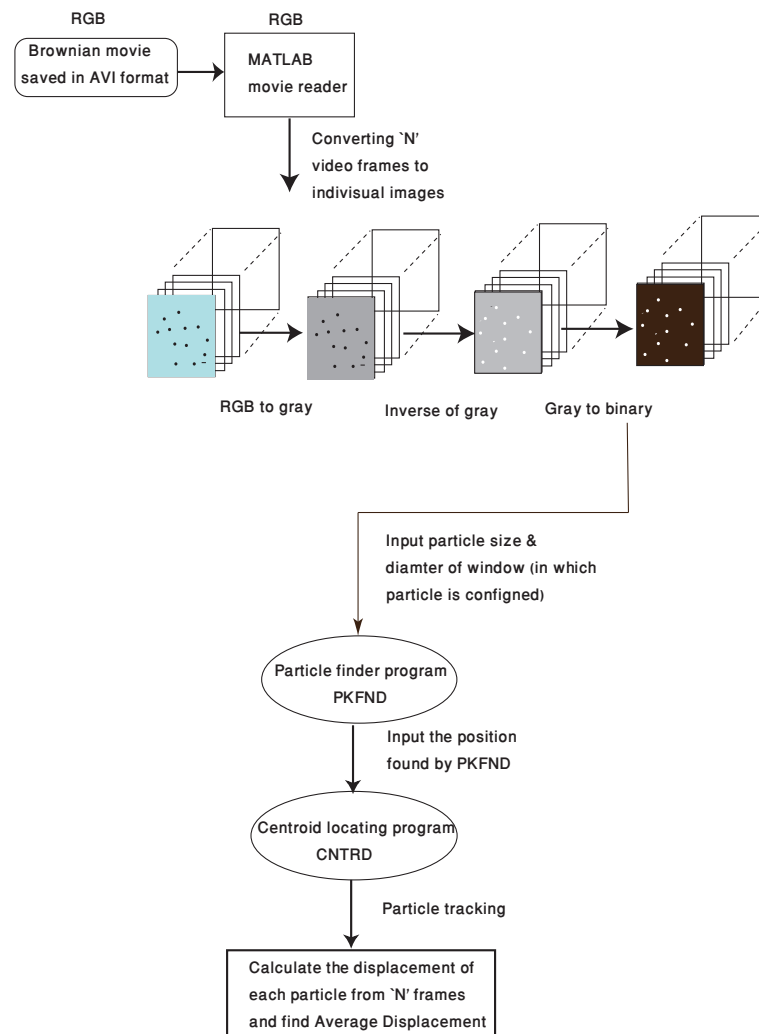


Figure 8: A flow chart outlining the various operations in the particle tracking software.

- **position** is the locations of local maxima obtained from **pkfnd.m**.
- **dim** is the diameter of the window over which to average to calculate the centroid. This value should be big enough to capture the whole particle but not so big that it captures other particles too.

Output of this function is an  $N \times 4$  array containing x-coordinates, y-coordinates and brightness for each feature.

- `centroid_location(:,1)` are the x-coordinates.
- `centroid_location(:,2)` are the y-coordinates.
- `centroid_location(:,3)` are the brightness.
- `centroid_location(:,4)` are the square of the radius of gyration (radius of gyration is the root mean square distance of the microsphere's parts from either its center of gravity or from an axis).



Now after having the coordinates of all the particles in all the frames, the only task left for you, for which you have to write your own program is to subtract the initial position of each particle in the first frame from the positions in the successive frames, to find the displacement. The displacement is then squared and averaged over the number of particles  $N$  in each frame and then plotted against time.

The initial positions of all particles in first frame are taken as the origin  $O(x_{0p}, y_{0p})$ , where  $p$  runs from 1 to  $N$ .

The square of the distance of the particle from the origin to a point  $P(x_{np}, y_{np})$  in the  $n$ 'th frame is given by

$$r_p^2 = (x_{np} - x_{0p})^2 + (y_{np} - y_{0p})^2,$$

where  $n$  runs from first to last frame. The mean square displacement of all particles in the  $n$ 'th frame is

$$\langle r_n^2 \rangle = \frac{\sum_{p=1}^N r_p^2}{N}.$$

The final task is to plot  $\langle r_n^2 \rangle$  for each frame against time.

The image processing, particle locating, centroid finding and particle tracking are shown as a sequence of steps in the flow chart of Figure 8.

**Q 5.** Plot the average  $\langle r_n^2 \rangle$  against time.

**Q 6.** Plot  $r_n^2$  against time for a representative microparticle.

**Q 7.** Plot a graph of Brownian motion exhibited by the particles in the 2 dimensional  $xy$ -plane. Does the plot looks like the random motion of actual particles in the movie?

## 4.8 Calculation of Boltzmann constant $k_B$ and Diffusion constant $D$

Boltzmann's constant  $k_B$  is calculated using the slope of the  $\langle r_n^2 \rangle$  vs *time* curve. The slope value after conversion of units of displacement from  $\text{pixel}^2$  to  $\mu\text{m}^2$  is substituted into Equation (8). The diffusion constant is calculated using Equation (9).

**Q 8.** Calculate  $k_B$  and  $D$  for your data. Refer to [15] to find the value of viscosity at the temperature of laboratory.

**Q 9.** Find the uncertainty in the value of  $D$ .

**Q 10.** Calculate Avogadro's number  $N_A$  using the value of slope and Equation (10). What is the uncertainty in  $N_A$ ?

## 5 Precautions in microscope handling

1. The light source in the microscope which is a halogen bulb is provided with the intensity control knob. Always try to focus the objective starting with the lowest values by rotating the knob clockwise. Never turn the knob abruptly to its extreme position of maximum intensity. This can damage the objective lens of the microscope.
2. While switching the viewing mode from eyepiece to camera using the optical path changeover lever, always minimize the intensity first.
3. Always switch off the microscope at the least intensity value i.e., zero.
4. Never rotate the left and right **Coarse** or **Fine** knob while holding the other.
5. Never turn the **Coarse** and **Fine** focus knobs further than their limit.