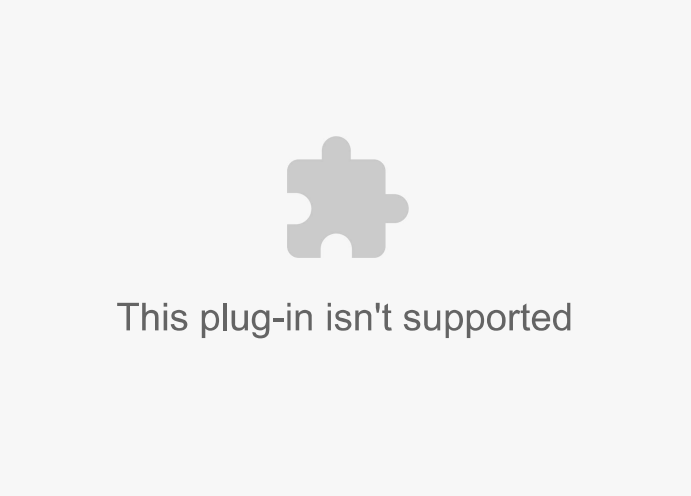
COS 126 Programming Assignment

The Atomic Nature of Matter

Re-affirm the atomic nature of matter by tracking the motion of particles undergoing Brownian motion, fitting this data to Einstein's model, and estimating Avogadro's number.

Historical perspective. The atom played a central role in 20th century physics and chemistry, but prior to 1908 the reality of atoms and molecules was not universally accepted. In 1827, the botanist Robert Brown observed the random erratic motion of particles found in wildflower pollen grains immersed in water using a microscope. This motion would later become known as *Brownian motion*. Einstein hypothesized that this Brownian motion was the result of millions of tiny water molecules colliding with the larger pollen grain particles.

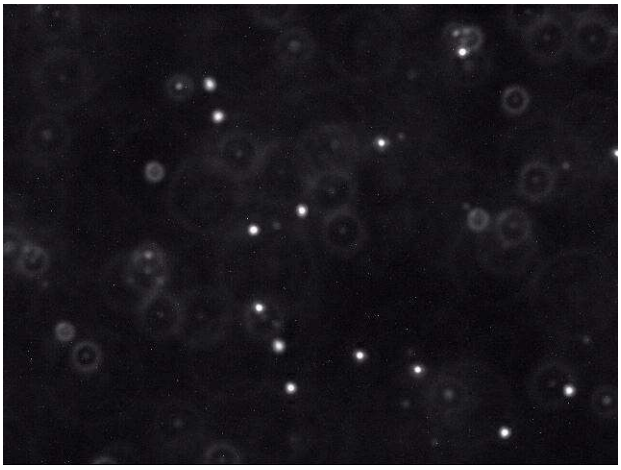
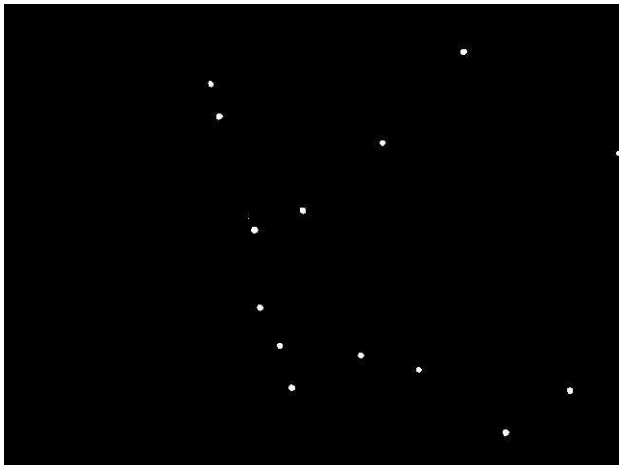


In one of his "miraculous year" (1905) papers, Einstein formulated a quantitative theory of Brownian motion in an attempt to justify the "existence of atoms of definite finite size." His theory provided experimentalists with a method to count molecules with an ordinary microscope by observing their collective effect on a larger immersed particle. In 1908 Jean Baptiste Perrin used the recently invented ultramicroscope to experimentally validate Einstein's kinetic theory of Brownian motion, thereby providing the first direct evidence supporting the atomic nature of matter. His experiment also provided one of the earliest estimates of Avogadro's number. For this work, Perrin won the 1926 Nobel Prize in physics.

The problem. In this assignment, you will redo a version of Perrin's experiment. Your job is greatly simplified because with modern video and computer technology (in conjunction with your programming skills), it is possible to accurately measure and track the motion of an immersed particle undergoing Brownian motion. We supply video microscopy data of polystyrene spheres ("beads") suspended in water, undergoing Brownian motion. Your task is to write a program to analyze this data, determine how much each bead moves between observations, fit this data to Einstein's model, and estimate Avogadro's number.

The data. We provide ten datasets, obtained by William Ryu using fluorescent imaging. Each run contains a sequence of two hundred 640-by-480 color JPEG images, `frame00000.jpg` through `frame00199.jpg` and is stored in a subdirectory `run_1` through `run_10`.

Here is a movie [avi · mov] of several beads undergoing Brownian motion. Below is a typical raw image (left) and a cleaned up version (right) using thresholding, as described below.

Each image shows a two-dimensional cross section of a microscope slide. The beads move in and out of the microscope's field of view (the *x*- and *y*-directions). Beads also move in the *z*-direction, so they can move in and out of the microscope's depth of focus; this results in halos, and it can also result in beads completely disappearing from the image.

Particle identification. The first challenge is to identify the beads amidst the noisy data. Each image is 640-by-480 pixels, and each pixel is represented by a `Color` object which needs to be converted to a luminance value ranging from 0.0 (black) to 255.0 (white). Whiter pixels correspond to beads (foreground) and blacker pixels to water (background). We break the problem into three pieces: (i) read in the picture, (ii) classify the pixels as foreground or background, and (iii) find the disc-shaped clumps of foreground pixels that constitute each bead.

- *Read in the image.* Use the `Picture` data type from Section 3.1 to read in the image.

- *Classify the pixels as foreground or background.* We use a simple, but effective, technique known as *thresholding* to separate the pixels into foreground and background components: all pixels with monochrome luminance values strictly below some threshold *tau* are considered background, and all others are considered foreground. The two pictures above illustrates the original frame (above left) and the same frame after thresholding (above right), using *tau* = 180.0. This value of *tau* results in an effective cut for the supplied data.

- *Find the blobs.* A polystyrene bead is typically represented by a disc-like shape of at least some minimum number *P* (typically 25) of connected foreground pixels. A *blob* or *connected component* is a maximal set of connected foreground pixels, regardless of its shape or size. We will refer to any blob containing at least *P* pixels as a *bead*. The *center-of-mass* of a blob (or bead) is the average of the *x*- and *y*-coordinates of its constituent pixels.

  Create a helper data type `Blob` that has the following API.

```
public class Blob
-----------------------------------------------------------------------------------------
public Blob()                    // construct an empty blob
public void add(int i, int j)    // add a pixel (i, j) to the blob
public int mass()                // return number of pixels added = its mass
public double distanceTo(Blob b) // return distance between centers of masses of this blob and b
public String toString()         // return string containing this blob's mass and center of mass
                                 // format center-of-mass coordinates with 4 digits to right
                                 // of decimal point
```

  Next, write a data type `BlobFinder` that has the following API. Use *depth-first search* to efficiently identify the blobs.

```
public class BlobFinder
-----------------------------------------------------------------------------------------
// find all blobs in the picture using the luminance threshold tau
public BlobFinder(Picture picture, double tau)
```

```
// return the number of beads with >= P pixels
public int countBeads(int P)

// return all beads with >= P pixels
public Blob[] getBeads(int P)
```

Write a `main()` method in `BlobFinder.java` that takes an integer *P*, a double value *tau*, and the name of a JPEG file as command-line arguments. It then creates a `BlobFinder` object using a luminance threshold of *tau*. Next, it prints out all of the beads with at least *P* pixels; finally, it prints out all of the blobs (beads with at least 1 pixel).

Here is a sample run of `BlobFinder`. Note: In Windows, you need to use backslashes `\` to refer to subdirectories, not the Mac/Linux forward slashes `/` that are used in all of our example commands. For executing your files, use the Terminal/Command Prompt since we'll need features not available in the DrJava console.

```
% java BlobFinder 25 180.0 run_1/frame00001.jpg
13 Beads:
29 (214.7241,  82.8276)
36 (223.6111, 116.6667)
42 (260.2381, 234.8571)
35 (266.0286, 315.7143)
31 (286.5806, 355.4516)
37 (299.0541, 399.1351)
35 (310.5143, 214.6000)
31 (370.9355, 365.4194)
28 (393.5000, 144.2143)
27 (431.2593, 380.4074)
36 (477.8611,  49.3889)
38 (521.7105, 445.8421)
35 (588.5714, 402.1143)

15 Blobs:
29 (214.7241,  82.8276)
36 (223.6111, 116.6667)
 1 (254.0000, 223.0000)
42 (260.2381, 234.8571)
35 (266.0286, 315.7143)
31 (286.5806, 355.4516)
37 (299.0541, 399.1351)
35 (310.5143, 214.6000)
31 (370.9355, 365.4194)
28 (393.5000, 144.2143)
27 (431.2593, 380.4074)
36 (477.8611,  49.3889)
38 (521.7105, 445.8421)
35 (588.5714, 402.1143)
13 (638.1538, 155.0000)
```

The program identifies 15 blobs in the sample frame, 13 of which are beads. Our string representation of a blob specifies its mass (number of pixels) and its center of mass (in the 640-by-480 picture). By convention, pixels are measured from left-to-right, and from top-to-bottom (instead of bottom-to-top).

Particle tracking. The next step is to determine how far a bead moved from one time step t to the next $t + \Delta t$. For our data, $\Delta t = 0.5$ seconds per frame. We assume the data is such that each bead moves a relatively small amount, and that two beads do not collide. (However, we must account for the possibility that the bead disappears from the frame, either by departing the microscope's field of view in the x- or y- direction, or moving out of the microscope's depth of focus in the z-direction.) *Thus, for each bead at time $t + \Delta t$, we calculate the closest bead at time t* (in Euclidean distance) and identify these two as the same beads. However, if the distance is too large (greater than *delta* pixels) we assume that one of the beads has either just begun or ended its journey. We record the displacement that each bead travels in the Δt units of time.

Write a `main()` method in `BeadTracker.java` that takes an integer *P*, a double value *tau*, a double value *delta*, and a sequence of JPEG filenames as command-line arguments, identifies the beads (using the specified values of *P* and *tau*) in each JPEG image (using `BlobFinder`), and prints out (one per line, formatted with 4 decimal places to the right

of decimal point) the radial displacement that each bead moves from one frame to the next (assuming it is no more than *delta*). Note that it is not necessary to explicitly track a bead through a sequence of frames—you only need to worry about identifying the same bead in two consecutive frames.

```
% java BeadTracker 25 180.0 25.0 run_1/*.jpg
 7.1833
 4.7932
 2.1693
 5.5287
 5.4292
 4.3962
...
```

Data analysis. Einstein's theory of Brownian motion connects microscopic properties (e.g., radius, diffusivity) of the beads to macroscopic properties (e.g., temperature, viscosity) of the fluid in which the beads are immersed. This amazing theory enables us to estimate Avogadro's number with an ordinary microscope by observing the collective effect of millions of water molecules on the beads.

- *Estimating the self-diffusion constant.* The *self-diffusion constant D* characterizes the stochastic movement of a molecule (bead) through a homogeneous medium (the water molecules) as a result of random thermal energy. The Einstein-Smoluchowski equation states that the random displacement of a bead in one dimension has a Gaussian distribution with mean zero and variance $\sigma^2 = 2\,D\,\Delta t$, where Δt is the time interval between position measurements. That is, a molecule's mean displacement is zero and its mean square displacement is proportional to the elapsed time between measurements, with the constant of proportionality $2D$. We estimate σ^2 by computing the variance of all observed bead displacements in the x and y directions. Let $(\Delta x_1, \Delta y_1)$, ..., $(\Delta x_n, \Delta y_n)$ be the n bead displacements, and let r_1, ..., r_n denote the radial displacements. Then

$$\begin{aligned} \hat{\sigma}^2 &= \frac{(\Delta x_1^2 + \ldots + \Delta x_n^2) \;+\; (\Delta y_1^2 + \ldots + \Delta y_n^2)}{2n} \\ &= \frac{r_1^2 + \ldots + r_n^2}{2n} \end{aligned}$$

  For our data, $\Delta t = 0.5$ so this is an estimate for D as well. The radial displacements r_i are measured in pixels: to convert to meters, multiply by $0.175 * 10^{-6}$ (meters per pixel).

- *Estimating the Boltzmann constant.* The Stokes-Einstein relation asserts that the self-diffusion constant of a spherical particle immersed in a fluid is given by

$$D = \frac{k\,T}{6\,\pi\,\eta\,\rho}$$

  where, for our data,

  - T = absolute temperature = 297 degrees Kelvin (room temperature)
  - η = viscosity of water = $9.135 * 10^{-4}$ N*s/m^2 (at room temperature)
  - ρ = radius of bead = $0.5 * 10^{-6}$ meters

  and k is the *Boltzmann constant*. All parameters are given in SI units. The Boltzmann constant is a fundamental physical constant that relates the average kinetic energy of a molecule to its temperature. We estimate k by measuring all of the parameters in the Stokes-Einstein equation, and solving for k.

- *Estimating Avogadro's number. Avogadro's number* N_A is defined to be the number of particles in a mole. By definition, $k = R / N_A$, where the universal gas constant R is approximately 8.31457 J K^{-1} mol^{-1}. Use R / k as an estimate of Avogadro's number.

For the final part, write a `main()` method in `Avogadro.java` that reads in the displacements from standard input and computes an estimate of Boltzmann's constant and Avogadro's number using the formulas described above.

```
% more displacements-run_1.txt
 7.1833
 4.7932
 2.1693
 5.5287
 5.4292
 4.3962
...

% java Avogadro < displacements-run_1.txt
Boltzmann = 1.2535e-23
Avogadro  = 6.6330e+23
```

```
% java BeadTracker 25 180.0 25.0 run_1/*.jpg | java Avogadro
Boltzmann = 1.2535e-23
Avogadro  = 6.6330e+23
```

Output formats. Use four digits to the right of the decimal place for all of your floating point outputs whether they are in floating point format or exponential format.

Running time analysis. Formulate a hypothesis for the running time (in seconds) of `BeadTracker` as a function of the input size N (total number of pixels read in across all frames being processed). Justify your hypothesis in your `readme.txt` file with empirical data.

Provided files. You can download the datasets and other files mentioned above from the course FTP site, or you can download them collected in this single zip file.

Submission. Submit `Blob.java`, `BlobFinder.java`, `BeadTracker.java` and `Avogadro.java`. the standard libraries and `Luminance.java`, submit them as well.
