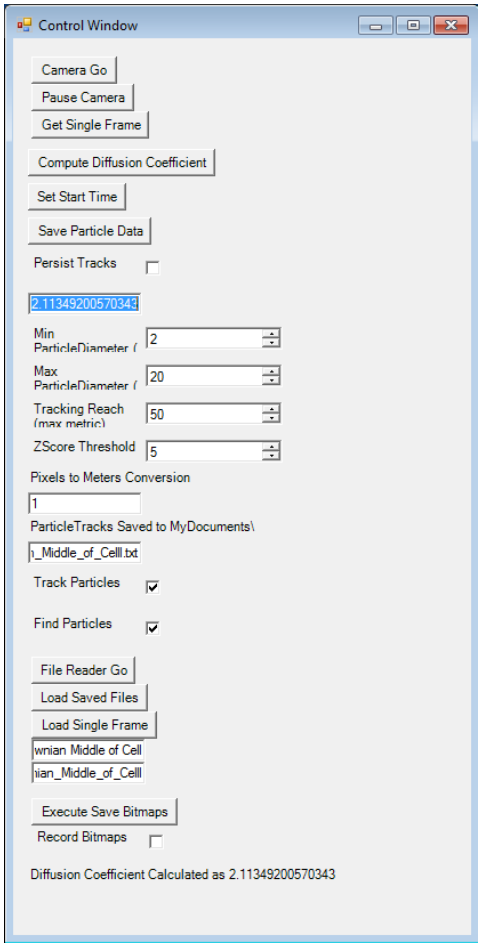
BMC Data collection Overview & Specifics

1 Software:

- Overview: Data was collected using the Brownian Application program described in the Brownian Software section of the lab. A screenshot of the control panel settings of the program is shown on the right:
- Min / Max Particle Diameter: These numbers are recorded in dimensions of pixels *not* micrometers. Different min/max values were entered depending on the expected particle sizes of the samples being observed. For the 0.47μm bead data sets, the minimum diameter is fixed at 1, and the maximum is fixed at 20 (corresponding to a range of about 0.25 - 5μm). For the 0.96 μm bead and the onion cell data sets, the min/max are set to 2 and 20 respectively, so about 0.5 - 5μm.
- ZScore Threshold & Post Processing: The Zscore threshold adjusted the contrast on the post-processing images, such that the particles we wanted to track were whiter / much brighter than their surroundings, making it easy to zero in on them. The way this was done in practice was by adjusting the number of standard deviations that a pixel and its nearest neighbors had to be brighter than their surroundings to classify as a particle. Typical ZScore values ranged between 4-8.
  It is also important to note that the particle tracking software was continually averaging over brightness as it ran. Thus, because we are collecting a running average, as time goes on and more data points are collected, noise is reduced according to $1/\sqrt{N}$ where N is the number of collected data frames. This means that whatever the software identified as a particle in the first 10 ms of it running a scan is less accurate than whatever it identified as a particle in the last 10 ms. Thus, if you have a choice, consider using data collected at later timestamps in the .txt files.



Control Panel Settings (Onion Cell)

• Pixels to Meters Conversion *__Important__*: Data in the .txt files are saved with information in the following columns: [x, y, time, dx, dy, dt, dr^2, TotalDisplacementSquared], where all of the positional data (x, y, dx, dy, dr^2, TotalDisplacementSquared) are saved in units of meters. The pixel to meters conversion box in the control panels allows the software to convert the original data stored in units of pixels into meters.
For the bead data sets, 0.00000025 m/pixel was input into the program. For the onion cell data sets, 1 m/pixel was input (thus, the positional data is stored in units of pixels NOT meters here). If you find a different pixel-to-meter conversion based on your calibration, you will need to make sure to take your calibration into account by converting your positional data arrays to the correct units.

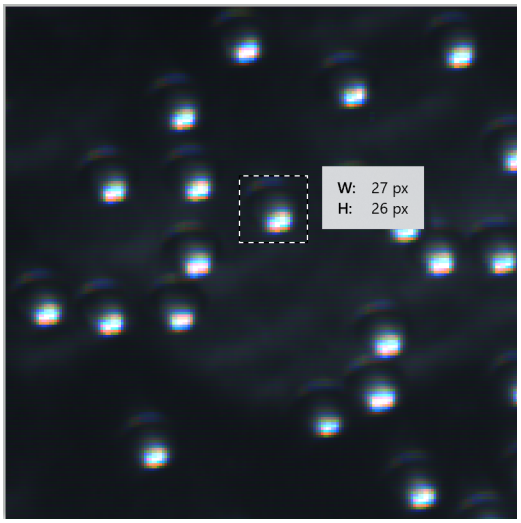
2 Sample Preparation & Data Collection Process:

- Beads: Beads were suspended in solutions of different viscosities (1.66cP, 4.65cP, and 13.2cP). Solutions were first prepared with PVP and then glycerol so that the relationship between solute type and Brownian motion can be analyzed. Each data set will have solute type and viscosity clearly labeled in the folder name.
  Every bead data set was observed using the 20× objective and dark field illumination. The only data sets taken at 40× were calibration data which was clearly marked as 40× and onion cell data.
  Dark field illumination was set up, the pre-processed image focused, and three full data sets per solute type per viscosity were collected. 100% of the light from the microscope was diverted to the CCD (thus none was diverted to the eyepiece) at the time of data collection. The Zscore threshold was also adjusted until a few particles were clearly visible in the post-processing images and background noise was filtered out as best as possible.

- Onion Cells: All of the samples used in the onion cell data sets were from the deepest layer of the onion. Every full data set was taken at 40× magnification using dark field illumination, unless otherwise stated (the 'Onion Images for Calibration' will have different illumination / magnifications specified in the file name). Different data sets were taken at different locations inside an onion cell, for example in the middle of the cell vs. close to the cell wall or nucleus. Particles exhibiting Brownian motion were more often located in the middles of cells while particles undergoing active transport were found closer to the cell walls.

3 Calibration - Pixels to μm:

- The folder containing the data meant to be used for calibration is labeled 'Calibration Data (20×, 40×).' Each sub-folder (20×, 40× folders) contains 6 different images that can be used for calibration. Each image was taken as part of a different data set (different area of the microscope slide or different zoomed-in portion of the pass through window). Every image was taken using dark field illumination



20× Calibration Using Paint3D

- Calibration should be done using a simple program like Paint, Paint3D, Preview, etc. The example shown to the right was done using Paint3D. A box can be drawn around each bead, the dimensions of which provide the diameter of the bead in pixels. The beads measure 9.51 μm across in diameter. Thus, by comparing the diameter of the beads in pixels and μm, the correct calibration for the data can be found. This calculation should be done several times and averaged over for accuracy. One calibration should be found per magnification (i.e you should have one conversion constant for 20× and another for 40×). These conversions should be used to scale the positional data such that the data being directly worked with is in meters (see section 1 - Pixels to Meters Conversion). Use the 20× conversion for the bead data sets and the 40× conversion for the onion cell data sets.

4 Bead Data Folders (0.47, 0.96 μm):

- 0.47μm Beads: contains data on 0.47μm diameter beads. There are 6 sub-folders, each of which is for a different solution / viscosity combination that is labeled in the folder name. I.e 0.47um, 1.66cP, PVP contains data on 0.47μm diameter beads suspended in a PVP solution of viscosity 1.66cP. This folder contains 3 complete data sets - 3 folders and 3 .txt files. For example, the folder `'pt47_1pt66cP_20x_PVP_1'` contains a movie of the acquired data (individual frames collected as .bmp files). Flipping through the ordered frames shows the particles experiencing Brownian motion. The `'pt47_1pt66cP_20x_PVP_1.txt'` file contains all of the relevant positional data (x, y, dx, dy, etc.). Each folder-txt pair is ONE data set. Between each data set being collected, the microscope was re-focused on a different part of the slide, Kohler and dark-field illumination were re-set up, the sample was replaced, or in some particularly stubborn cases, the solution was made from scratch again. Thus, each folder-txt pair should be treated as a unique data set.
- 0.96μm Beads: contains data on 0.96μm diameter beads. This folder follows the same format as the 0.47μm folder - there are 6 sub-folders, each for a different solution / viscosity combination. Within each sub-folder, a folder-txt pair contains all of the important data for one unique data set.

5 Onion Cell Data Folders:

- Onion Images - Bright, Dark, 20×, 40× (For Calibration): This folder contains screenshots of the pass-through window for onion cell data. Different screenshots show different experimental conditions and locations for data capture such as bright field illumination vs dark field illumination, 20× vs 40× magnification, and different locations in a cell where particle motion was observed (near the nucleus, in the middle of the cell, or close to the cell wall). Each image is appropriately labeled so you're able to discern what the experimental conditions for the data collection were. These images should give you a sense of what the onion cells and the particles we're observing inside them look like, as well as how changing the illumination type and magnification change the data quality.
  You can also use these images and an external program such as Paint, Paint3D, Preview, etc. to estimate what the size of the particles being observed is. This can be done by following the technique described in Section 3 (Calibration - Pixels to μm).
- Onion Brownian Close Up - Few Particles, Onion Brownian Middle of Cell, Onion Actin Near Cell Wall, Onion Actin Near Cell Wall 1:

  These folders all have the same main components. In each one, there should be 3 .txt data files, 1 .txt file called 'Diffusion coefficients' one folder full of .bmp files, a PNG called 'Control Panel Settings', and a PNG called 'Location of Data Capture.' **Note:** 'Onion Actin Near Cell Wall' and 'Onion Actin Near Cell Wall 1' have the same control panel settings.
  3 .txt data files: Each data set was taken under the same experimental conditions (same location on the microscope slide, same microscope focus, same amount of time for data collection - 10 seconds, etc). As soon as the first data set was done collecting, the second would start, and so on.
  'Diffusion Coefficients' .txt file: The Brownian Program has two options for what to do with data once it's collected - saving that data in a .txt file or using it to compute the diffusion coefficient of the sample. For each of these four folders, 15 additional data sets were collected,

but rather than being saved, they were used to compute diffusion coefficients. These coefficients were then manually recorded in the .txt file. Each coefficient was computed from data collected over the same 10 second window as the saved data files.
Folder of .bmp files: These bmp folders are movies recorded during the data collection process. Flipping through the ordered bmp files should give you an idea of how the particles inside the onion cell are moving and help you see the difference between active transport and Brownian motion.
'Control Panel Settings' .PNG file: This is just a screenshot showing you what the program settings were for each data collection. This shows how the ZScore thresholds changed at different locations in the cell (i.e where it was easiest and hardest to see distinct particles and where particles were more concentrated).
'Location of Data Capture' .PNG file: This is a screenshot showing you where we zoomed in on the Passthrough window to record and observe particle motion. This shows where the data collection was taking place zoomed out so you can see the data collection window in reference to the entire cell and other nearby ones. You can also see which specific particles are being tracked in the cell.

- Onion Brownian Close Up - Single Particle, Onion Cell movie - zoomed out active & Brownian:
'Onion Brownian Close Up - Single Particle': There are two unique data sets in this folder. `'Brownian_Close_Up'` is the first, and the particle location corresponds to the 'Location of Data Capture' PNG file. `'Close_Up_Single'` is the second, and the particle location corresponds to the 'Second Data Capture' PNG file. The two data sets were collected on completely different onion cells, with the data collection window located in the middle of each cell. These files are special because they track ONE particle experiencing Brownian motion. There may be outliers in the .txt files from other particles drifting past the one being observed. Because the data collection window was so small, this is unlikely but still something to look out for.
'Onion Cell Movie - Zoomed out, active & Brownian': This is just a zoomed out collection of .bmp files (a movie) that shows the different types of motion observed in the onion cells (so there are some particles undergoing active transport and some experiencing Brownian motion). This data was still taken at a magnification of 40x, but data was collected over a much larger window so that a larger area of cells could be seen. This is more to just give you an idea of what the different types of motion looked like rather than something that should be used for data analysis.