Lab 3, Part 1: What does 'Random' motion look like? Describing Diffusion and Random Motion.

Overview:

So far in the laboratories, we have been exploring motion of objects traveling in one particular direction. We were able to connect this motion to forces because, in the cases we analyzed, the sum of the forces applied to the objects did not change significantly from frame to frame. This allowed us to apply Newton's laws and connect forces and observed motion. The objects we studied underwent what we call **directed motion.**

In contrast, for small objects inside a fluid, the pushes and pulls from the surrounding fluid change very rapidly, and thus the sum of the applied forces is changing magnitude and direction much faster than the fastest imaging frame rate (i.e., faster than our cameras can capture). On average, when the object is pushed to the right in one frame it will be pushed to the left in another frame. So, when looking at such small objects with our camera we no longer see directed motion, we see ***random*** **motion.** Such random motion is experienced by all microscopic objects and is an important attribute for living systems: cells—and the molecules, proteins, DNA and lipids within them—are always in seemingly chaotic motion, so **it is essential to understand and characterize this volatile behavior if we hope to make sense of the biological world!!**

For the remainder of the semester, you will have the chance to explore many aspects of random motion. During this three-week lab you will characterize some essential features of random motion and explore the dependence of random motion on particular experimental parameters. Following this lab, you will investigate motion that is random and directed at the same time (Lab 4) and then consider the analysis of intracellular motion in a living system, including the connections of this motion to work and energy (Lab 5).

For this 3-week Lab:

Your overall task for the next three weeks is to characterize the random motion of beads suspended in fluid and determine how the variation of experimental parameters (such as bead mass and size and the fluid viscosity) impacts the movement of the beads and their resulting diffusion. You will do this in multiple stages.

- **Collect three videos** (see chart)**;**
- **Analyze the first video to characterize the behavior of the random motion;**
- **Analyze all the videos to determine how the variation of parameters affects diffusion;** and
- **Write a lab report to synthesize the data gathered by you and the class and to summarize your findings regarding random motion and diffusion.**

The broad structure of this experiment is provided to you so that the smaller investigations piece together to give a unified picture of random motion and diffusion—but MANY decisions still need to be made by you in order for you to gather and interpret the data. You should be careful and thoughtful as you create and record your experimental protocol!

Since random motion is most easily measured for microscopic systems, we will be exploring it by studying the motion of microscopic beads in fluid under a microscope. Since the motion looks different for each bead, if we want to make statements about the group it will be crucial to measure the motion of many beads (say 15-20). It will be useful for you to measure averages over all beads, but also to create histograms to see the variability from bead to bead (just like you might be curious to know both the average grade and histogram of grades in an exam). When working with

histograms, it will be necessary to track EVEN MORE beads (say 30-50), so that there are sufficient representatives in each 'bin' of the histogram.

1. To be sure that you know how to use the microscope, and to *get a sense for scale*, start by taking a look at *yeast* under the microscope. The yeast cells are about 4 microns in diameter, so they are of a similar size to the beads we will be investigating. **Qualitatively describe the motion of yeast cells** for your report.

2. Gather your three videos (see the chart). Here are some **helpful hints**:
    a. As you prepare each slide, remember to shake the vial of solution before you extract a sample with the pipette. Your video should be collected fairly quickly after the sample is deposited on the slide. If it takes too much time (e.g., more than 10 minutes), you may want to make another preparation of the sample.
    b. Use the 40X objective for these bead videos.
    c. Each video should be approximately the same total time (about 5 seconds of video for each solution/sample).
    d. Be sure to note and RECORD the **video resolution** and **frame rate** for EACH video as soon as VirtualDub has captured the video. Most of this data cannot be retrieved once you leave VirtualDub/start a new video collection.

3. Harvest the data out of the video files. You might consider doing this ONLY for the first video during this first week of the lab.[1] Since all three videos have captured random motion, we need only look at one video to begin characterizing the behavior of random motion. (Also, you may learn tricks and ideas that can help you analyze the other videos in the coming weeks. Next week, we will teach you *automatic* tracking!) The method you use to harvest the data is a bit different from what you have done previously with ImageJ. Rather than tracking each object of interest through EVERY frame of the video, we can take very specific frames (reducing the number of clicks you need to make). So, for the beads that you are tracking, you want an initial position (at t=0, the first frame), a final position (at the last frame), and at least 4 other positions (at specific frames equally spaced between the initial and final frame). This will take some careful planning once you have the video in ImageJ and BEFORE you open the Manual Tracking plugin. **To compare these beads with each other (histogram-style), you need to be tracking these beads in the all of the SAME frames.**[2]

4. Characterize the random motion of the beads in your first video. Compare and contrast their motion to what you would expect for directed motion.

    a. Are the average total x- and y- displacements of the silica beads, $\langle \Delta x \rangle$ and $\langle \Delta y \rangle$, larger if you measure total displacements for larger time intervals? If the averages change, how do they change? If they don't change, why don't they change?
    b. Are the *individual* total x- and y- displacements of the silica beads, Δx and Δy, larger if you measure total displacements for larger time intervals? [*Hint: Make some histograms!*] If the individual displacements change, how do they change? If they don't change, why

[1] IF YOU DO THIS, YOU **MUST** have a copy of your remaining videos saved to your flashdrive/email/dropbox so that you can access the videos next week.

[2] **And for the first video ONLY, we suggest 30 -50 beads should be tracked (so that you can create good histograms). For videos 2 and 3, you can use only 15-20 beads (which should be sufficient for the RMS analysis).**

don't they change? How are these individual displacements linked to the average displacements?

c. How does the root mean square (RMS) total displacement, $r = \sqrt{(\Delta x)^2 + (\Delta y)^2}$, change as a function of the measurement time interval?

Equipment

Familiarize yourself with the ***Microscope Basics*** sheet before beginning any experimentation. If you do not know how a particular part of the microscope works, please ask a TA or LA—the equipment is expensive! Please be especially careful handling liquid samples near the microscope objectives.

The CCD camera attached to the microscope will allow us to capture video of what we observe. Using the same VirtualDub software we utilized in previous weeks, we can capture AVI videos of the motion we are trying to analyze. In VirtualDub, the microscope camera can be found under 'Device' and is named "UCMOS03100KPA."

The adjustment options for the microscope CCD camera are slightly less user friendly than the webcam options. However, they are still found in the same VirtualDub menus. The compression and output size options can still be found under 'Video' and 'Capture Pin.' Be sure to take note of at which resolution you record your videos; it will be important when determining the distance to pixel ratio. This can be done by taking a picture of the 1mm calibration slide at the same resolution and magnification level as your videos. If calibration slides are not available, pictures can be found on the lab computers.

Brightness, contrast, and other exposure settings can be found under 'Video' and 'Capture Filter.' The most important difference from the webcam cameras is that the frame rate cannot be directly set before capturing videos. It is necessary to control the frame rate by controlling the exposure time of the CCD camera. By telling it to expose the CCD to light for 100 ms intervals, for instance, you are telling it to take a picture every 0.1 seconds. This also means that you have to carefully control the amount of light through your sample using the iris and bulb power control. If you are having trouble getting the light settings correct, you can use the Auto Exposure option, but this will often result in very low frame rates.

Group # (Parameter) (each parameter will be investigated by two distinct groups, allowing you to 'double check' the results)	**Video 1** (condition for testing r^2 vs. t dependence)	**Video 2**	**Video 3**
1 & 2 (bead size)	2-micron silica beads in water	5-micron silica beads in water	1-micron silica beads in water
3 & 4 (fluid viscosity)	2-micron silica beads in water	2-micron silica beads in low viscosity glycerol/water mix	2-micron silica beads in high viscosity glycerol/water mix
5 & 6 (bead mass & viscosity)	2-micron polystyrene beads in water	2-micron polystyrene beads in low viscosity glycerol/water mix	2-micron polystyrene beads in high viscosity glycerol/water mix