

## **Physics Department Report on Use of HHMI Equipment in 2006-07**

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### **Introduction**

The physics department has recently purchased a high quality, research grade light scattering apparatus as part of the last year's department-specific round of HHMI funding. The instrument allows students to perform a variety of basic experiments which demonstrate physical principles at work in inter-disciplinary fields such as molecular biology, biotechnology, biophysics and physical chemistry. The emphasis is placed on demonstrating physical phenomena at a molecular level. The apparatus has been used for experiments at both the introductory level (PHYS110 – First Year Seminar in Physics: Biological Physics) and advanced level (PHYS 480 and 481 – Research Methods for Experimental Physics and Experimental Physics).

Light scattering techniques are most commonly used for determining properties of small particles in solution: their concentration, distribution of sizes, shapes, and orientation. Practical applications are found in a variety of industries: in biotechnology for the study of protein-protein interactions, stoichiometry of protein-protein complexes, and protein aggregation phenomena; in pharmaceuticals for study of protein-ligand interactions and binding, characterization of pre-crystallization parameters in macromolecular crystal growth prior to x-ray structure determination; in petrochemicals for study of the molecular weights, sizes and branching properties of olefins, synthetic rubber, polyethylene, and synthetic lubricants, in colloidal chemistry; and in other industries.

### **The Light Scattering Instrument**

Our instrument is capable of both static light scattering and dynamic light scattering, allowing students to measure not only parameters such as molecular weight of an unknown macromolecule (biological molecule or a polymer), but also its hydrodynamic radius, the translation diffusion coefficient, and conformational stability. An emphasis is placed on demonstrating the basic principles behind the laws of probability, friction, and diffusion that are central to understanding Brownian motion and are so fundamental to understanding many aspects of the molecular machinery of living cells and other biological systems. In addition, upper level students can learn about the light scattering theory, easily extendable to other scattering methods (x-ray, neutron, etc.) and about photon correlation spectroscopy, involving mathematical skills ubiquitous in statistical methods.

### **Use of the Instrument in First-Year Courses**

The Physics Department's first-year and intermediate seminars provide potential physics majors and other students with an early opportunity to acquaint themselves with cutting edge topics in physics. This year's focus is on biological physics. In these courses, students learn about the role of physical principles in the world of biology. We aim to understand the elaborate machinery of a living cell and other amazing biological systems in terms of structure, forces, energy, and system design. We discuss topics in current research on protein folding and nucleotide conformations, biopolymers, biomembranes, membrane transport processes, diffusion of molecules in liquids, chemical forces and self-assembly, propagation of nerve impulses and briefly survey topics in nanotechnology and soft materials. The courses are a combination of

lectures, discussion of assigned readings, small group problem-solving sessions, demonstrations, and experimental work with biophysical techniques.

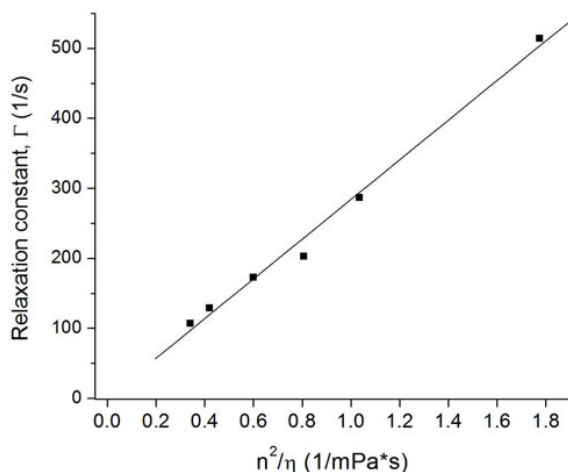
The light scattering apparatus is used in experiments that test the elementary concepts of probability theory, entropy, random walks, fluid dynamics and Boltzmann statistics; these physical concepts are particularly relevant in biological systems. Early in the semester, the students perform basic experiments on ideal gas law, osmosis across semi-permeable membranes, motion of particles due to thermal fluctuations and drift under constant force (e.g. electrophoretic mobility in electric fields and sedimentation under gravity). Toward the end of the semester, their experience culminates in performing experiments using the state-of-the-art light scattering instrument to confirm some of their previous results involving macroscopic variables (e.g. temperature, pressure, viscosity, diffusion, etc.) with data gained from measurements on microscopic scale (e.g. direct measurement of size via scattering and diffusion constants via photon counting).

In the first part of the experiment, students measure the size of latex spheres in a colloidal solution by simple observation under a microscope. Although they cannot resolve the nano-scaled size of the spheres, they can see speckles of scattered (nanowatt) laser light that passes through the solution. By recording the motion of speckles with a camera, they can analyze the trajectory of individual spheres and determine their diffusion constants from the two-dimensional diffusion law:  $\langle \Delta r^2 \rangle = 4Dt$ . Using the Stoke's formula,  $\zeta = 6\pi\eta R$ , and the Einstein relation,  $\zeta D = k_B T$ , they can calculate the sphere's radius  $R$  (see Appendix). These measurements setup the conceptual framework needed to understand the basic physical principles behind the operation of the dynamic light scattering later.

In the second part of the experiment, the students measure the size of the spheres using the dynamic light scattering (DLS). While the full mathematical treatment of the correlation-fluctuation data analysis obtained from the statistics of photon counting is beyond the scope of the introductory course, we outline the process diagrammatically. The students pick up the physical ideas with ease, partly because they have worked through the concrete set of conceptually related exercises using the microscope, and partly because they are allowed to get their "hands on" the experiment, from preparing the colloidal solutions to operating the instrument. Once they insert their samples and open the shutter, the instrument reports the relaxation constant  $\Gamma$  that is related to the translational diffusion constant,  $D$ , via the relation  $\Gamma = Dq^2$ , where  $q$  is the magnitude of the scattering vector (see Appendix). The experience of determining the sub-micron particle size almost "instantly" lies in contrast to the laborious (often imprecise, impractical and sometimes impossible) task of analyzing data obtained by the microscope.

In the final part of the lab using the DLS, students determine the Boltzmann constant, a fundamental constant of physics stemming from nearly all statistical formulations of physics. This experiment closely mimics the conceptually related experiments suggested by Einstein in his 1905 Nobel prize-winning result. The product  $\zeta D$  is a falsifiable prediction of the hypothesis that heat is disordered molecular motion, incidentally, first observed in a jiggly motion of a pollen particle by biologist Robert Brown. The experimental determination of the Boltzmann constant finally settled the size scales for molecules. The students prepare colloidal solution of

latex spheres and systematically vary the viscosity,  $\eta$ , of their solutions by adding sugar. Measuring the spheres' diffusion constants and utilizing the Stoke and Einstein relations, they construct a plot of  $\Gamma$  vs.  $n^2/\eta$  ( $n$  is the solution's index of refraction) and determine the Boltzmann constant (see Appendix). A representative plot of actual data collected by a group of students is shown below.

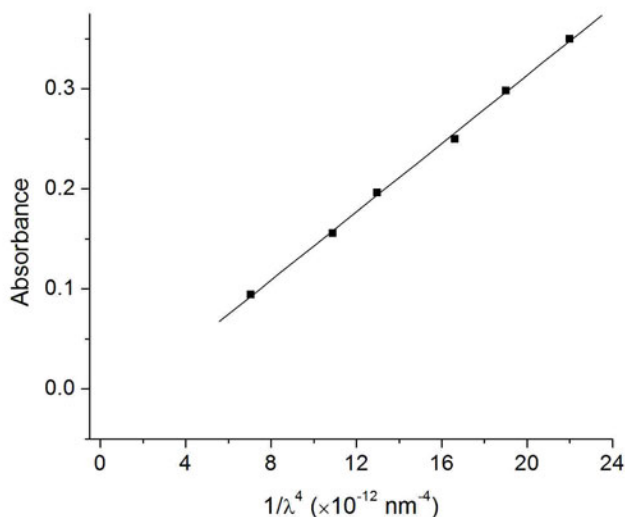


**Figure 1:** Data collected by students using DLS. The relaxation constant  $\Gamma$  is measured by the instrument. The Boltzmann constant can be obtained from the slope and is measured to be  $1.20 \times 10^{-23}$  J/K, a little lower than the accepted value.

### Use of the Instrument in Upper Level Courses

In the advanced labs, the objectives run parallel to those in the first-year courses but the analysis is performed with greater mathematical sophistication. In these experiments, students measure the scattering intensity of several particles of different diameters, as a function of both wavelength and scattering angles (the so called static light scattering, SLS). They model these experimental spectra using Rayleigh and Mie's scattering theories and verify that Rayleigh theory works well for the case where the particle dimension is much smaller than the wavelength of light. The concepts of scattering cross section is extended to other types of scattering (e.g. Compton and Rutherford scattering), ubiquitous in the undergraduate physics curriculum.

In the first part of the experiment, students use a spectrophotometer (so that they can vary the wavelength) to measure the total cross section by monitoring the beam attenuation as a function of wavelength. They scan the photon energy of the beam passing through colloidal solutions of varying sphere size and analyze the spectra using both, the approximate Rayleigh and the full Mie theories (see Appendix). Their results confirm that the cross section is approximately proportional to  $\lambda^{-4}$  for the small size particles, an effect responsible for both the blue sky and red sunset. A representative plot of actual data collected by a group of students is shown below.



**Figure 2:** Data collected by students. Absorbance (related to the experimental cross section) follows the  $\lambda^{-4}$  law obtained from Rayleigh approximation for particle radius  $a = 280$  nm.

In the second part of the experiment, students collect intensity of scattered light as a function of angle. The students can obtain from static light scattering measurement the size of the submicron spheres when their concentration in a solvent is known. They prepare colloidal solutions of varying concentration, and measure the dependence of scattered intensity on the scattering angle for each solution. A formula relating the scattered intensity to the scattering angle is derived using principles of electromagnetic theory and the connection between the index of refraction and the polarizability for a transparent dielectric. In subsequent advanced exercise, the theoretical treatment is extended to spheres whose size is greater than the wavelength of light, modeling the large particle as a collection of dipoles.

### References

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**Appendix – Lab Handouts:**

PHYS110 First Year Seminar in Physics  
Biological Physics

**Lab 6: Diffusion, Size, and the Boltzmann Constant**

In the early 20<sup>th</sup> century, Einstein outlined a testable theory that would make the connection between molecular theory and macroscopic quantities that could have been measured at that time. As we have discussed in class, finding a way to measure the Boltzmann constant  $k_B$  establishes the size scale for molecules. In the first part, we will measure the size scales of latex spheres by observing their random jiggling under a microscope. In the second part, we will measure the Boltzmann constant from the Einstein relation, but we will be using modern instrumentation to determine the diffusion constants.

Form groups of 3 students each, and begin to work on “Preparation for Experiment 2” and “Experiment 1”. While you work on Experiment 1, I will call two groups (6 students) at a time to use the light scattering apparatus, in ½ hour intervals.

**Preparation for Experiment 2:** prepare colloidal solutions of various viscosities. Use sugar to control the viscosity.

- 1) Dissolve 65g of sugar in 100g of water – This will be your stock solution of 65% sugar.
- 2) The instructor will add 2 $\mu$ L of latex spheres of 260 nm diameter in size into your stock solution.
- 3) Make dilutions of the stock to obtain 25mL of 60%, 50%, 40%, and 30% concentrations in the small plastic beakers.
- 4) Pour straight 25ml of stock solution into 1 small beaker and a solution of spheres with no sugar into another (provided as a separate stock).
- 5) You should now have 6 small beakers labeled 65%, 60%, 50%, 40%, 30%, and 0%.
- 6) Before you leave to use the light scattering apparatus, transfer the solutions of the small beakers into the 6 scintillation vials (these will be shared, so you may need to wait until the previous group returns.)
- 7) As you wait your turn for the DLS (Dynamic Light Scattering), proceed to Experiment 1.

**Experiment 1:** Measure the size of latex spheres by observing their random motion under a microscope.

- 1) When available, go to the demo station and observe the random motion of the spheres under the microscope, and enjoy for a moment.
- 2) The instructor has video captured the motion and saved it onto P:\temp\phys110\diff01.avi – place this file into your folder.
- 3) Use Video point to track the motion. There are many ways to do this, and if you figure out a better way, that is fine, or you may follow these directions.
- 4) Open the file and specify 10 objects.
- 5) “Double” the view size for the frame.

- 6) The frames advance in 150ms increments. Track the particles in a time increment of 300ms.
- 7) Here is the general approach, but see the next step for details: Select 10 specs in a frame; advance the frames by 2 (e.g. skip one frame), and select the same 10 specs. Video point will thus give you the coordinates for each spec before and after 300ms.
- 8) Because the spheres can move in 3D, some of them will leave the field of focus by going upward or downward and you will not be able to track those. So you must select only those specs that remain in the field of view after the 300ms. So before you select any spec, use the keyboard arrows to advance the frames by 2 to check whether your spec is still on the screen. If so, go back to your original frame and select your spec. Do this for 10 specs.
- 9) Advance the frames by 2 and select the same 10 specs as on your original frame. The numbering matters; each spec must have the same number on both frames. If you forget the order, you can use the keyboard arrows to backtrack 2 frames to check which spec is which. Video point will double circle an active selection for easy identification.
- 10) Click on the “table” icon in the left column. Click on “File” and export the table as an “xls” file.
- 11) Close the file (do not save).
- 12) Repeat the steps 4 through 11 two more times, but choose different frames in the movie.
- 13) You should have 3 sets of data, each set with x-y coordinates for 10 specs for the two frames separated by 300ms.

The analysis: For each spec, calculate the change of its position  $\Delta x$  and  $\Delta y$ , that is,  $\Delta x = x_f - x_i$  and  $\Delta y = y_f - y_i$ . For each spec, calculate the displacement  $\Delta r$  it has made in 2D, that is,  $\Delta r = \sqrt{\Delta x^2 + \Delta y^2}$ . Make a table in Origin showing the spec number, and its  $x_i$ ,  $y_i$ ,  $x_f$ ,  $y_f$ ,  $\Delta x$ ,  $\Delta y$  and  $\Delta r$ . Use the unit of “pixel” instead of meters for now. Your table should have 30 rows.

Right click on the column  $\Delta r$  and select “Frequency count.” For the step size input “2”. Origin will generate a table containing frequency data for your histogram. Plot “Count” as a function of “BinCtr” to plot your histogram. If everything went well, you should obtain a nice Gaussian distribution for your  $\Delta r$ .

Fit the distribution with a Gaussian – Click “Analysis”->“Fit Gaussian.” You must improve on this fit. Click “Analysis”->“Non-linear Curve Fit”->“Advanced Fitting Tool”. The Gaussian fitting should be the default when the fitting tool opens, if not, click on the icon with the green check mark and choose “Gauss” from “Origin Basic Function.” Unclick “y0” and set it to 0. Start the fitting process, visually inspecting the quality of the fit. When happy, click “finished” and read off the value for  $w$ . Note: Notice, the average of  $\Delta r$  is not zero (the Gaussian is not centered on zero). Why?

The width of the Gaussian is related to the RMS for  $\Delta r$  as follows:  $\Delta r_{\text{RMS}} = w/2$ . This comes about from the difference in the way we define variance and Origin defines  $w$ .

From the diffusion equation  $\langle \Delta r^2 \rangle = 4Dt$ , determine the diffusion constant for these spheres. Convert it to unit of  $\text{m}^2/\text{s}$ ., given that 1 pixel =  $2.65 \times 10^{-7}$  m. Using the Einstein-Stokes relations,

determine the size of the spheres. Report the diameter in Kenyon Form. The temperature was  $T=20\text{ }^{\circ}\text{C}$ . Look up the viscosity of water for that temperature in the appendix of this write-up.

**Experiment 2:** Measure the Boltzmann constant using the dynamic light scattering apparatus.

We pass a laser through a colloidal suspension and set our photon detector at  $90^{\circ}$  with respect to the direction of the incident beam. As the incident photons scatter, once in a while one will make it to the detector. Suppose for a moment that the laser sends out photons in equal intervals and the spheres are frozen in time. In that scenario, we would expect the time of arrival at the detector between two successive photons to be the same for all photons. Now suppose that we allow the spheres to meander for a while and freeze them again. Two consecutive photons arriving on the detector would now be separated by a different time interval, corresponding to the new probability of scattering (maybe the concentration has changed locally in the scattering volume). Now imagine a scenario where the spheres diffuse at the same time as we make our measurements of photon arrivals. The arrival time continually changes depending on how fast the spheres are diffusing – for slow diffusion, we can expect almost no fluctuations in the arrival time, and for fast diffusion we expect large fluctuations in the arrival time. Making the statistical analysis of fluctuation in the arrival time, we can figure out the diffusion constant. (How this is done mathematically you will study in your later courses).

The instrument reports the relaxation constant  $\Gamma$  (obtained from the analysis of arrival statistics), in units of  $1/\text{s}$ . This constant is related to the translational diffusion constant as:

$$\Gamma = Dq^2 \text{ where } q \text{ is the magnitude of the scattering vector given as } q = \frac{4\pi n}{\lambda} \sin \frac{\theta}{2}. \text{ The}$$

wavelength for our laser  $\lambda = 633\text{nm}$  and the index of refraction,  $n$ , depends on the concentration of sugar; please look it up in the appendix of this write up. The viscosity  $\eta$  is also reported there for the various concentration of sugar.

Make a table with the following 5 columns: Concentration,  $n$ ,  $\eta$ ,  $\frac{n^2}{\eta}$ , and  $\Gamma$ .

Make a plot of  $\Gamma$  (on the y-axis) versus  $\frac{n^2}{\eta}$  (on the x-axis). From the Einstein and Stokes'

relations, figure out how the slope of your plot is related to the Boltzmann constant. Report the Boltzmann Constant in Kenyon Form.

From  $\Gamma$  obtained at 0% concentration of sugar, determine the diffusion constant  $D$  of the spheres in pure water.

Do not forget to include discussion and conclusions, and also address the following questions.

1. Why is the average displacement  $\Delta r$  not zero in Experiment 1?
2. You have determined  $\Delta r_{\text{RMS}}$  from the fit to the Gaussian. Suppose you did not want to plot a histogram for your  $\Delta r$ . In that case, how else could you calculate  $\Delta r_{\text{RMS}}$ ?
3. Is the diffusion constant of the spheres in Experiment 1 and 2 roughly the same? Do you expect that it should be the same? Why or why not?

PHYS 481; Fall 2006

## Light Scattering

### Objective

The objective of this experiment is to investigate the light scattering methods associated with spherical particles dispersed in solvents. First you will measure the scattering intensity of several particles of different diameters, as a function of both wavelength and scattering angles. Then you will learn to model these experimental spectra using Rayleigh and Mie's scattering theories. This experiment will also help you to verify that the Rayleigh theory works well for the case where the particle dimension is much smaller than the wavelength of light.

### Theory

As shown in class some time back, interaction of EM waves with matter can be modeled using a driven harmonic oscillator. This formulation leads us to conclude that the scattering intensity is proportional to the  $\omega^4$ , which is the famous result derived by Rayleigh. A more formal treatment of the problem in which spheres (with index of refraction  $n_{sp}$ ) are dispersed in a medium (with index of refraction of  $n_{med}$ ) leads the following result for the cross section for Rayleigh scattering:

$$\sigma_{Rayleigh} = \frac{8\pi}{3} \left( \frac{2\pi m_{med}}{\lambda_0} \right)^4 a^6 \left( \frac{m^2 - 1}{m^2 + 2} \right)^2$$

Where  $a$  is the particle radius,  $\lambda_0$  is wavelength of light in vacuum, and  $m = n_{sp}/n_{med}$ .

However, the above derivation is valid for problems in which the sphere diameter is much smaller than the wavelength of light. As the particle dimensions approach the size of the wavelength of light, one has to use the theory developed by Mie, which in fact is valid for all situations, including Rayleigh scattering. The cross section derived for Mie scattering as follows:

$$\sigma_{Mie} = \left( \frac{2\pi}{k_{med}^2} \right) \sum_{n=1}^{\infty} (2n+1) (|a_n|^2 + |b_n|^2)$$

Where  $k_{med} = 2\pi n_{med}/\lambda_0$ , and  $a_n$  and  $b_n$  are coefficients that are functions of spherical Bessel functions and Hankel functions.

### Experimental Details

You will work with 5 different solutions. Four of these have polystyrene particles with different diameters (30 nm, 60 nm, 260 nm, and 1500 nm) suspended in water. The fourth solution has silica particles (diameter  $\sim$ 250 nm) suspended in ethanol. You have to perform two types of measurement on each of these four solutions, and model the experimental data with either Mie or Rayleigh theories.

You will have to prepare dilute solutions of the particles by taking small quantities from the stock solution, and mixing it with water and ethanol. It is extremely important to keep things as clean as possible because if dust particles get into the cuvette, their contribution to the scattering intensity will at times be higher than the contribution from the particles in solution.

### *Wavelength Dependence*

Using the spectrophotometer, measure the absorption of the unpolarized light as it passes through each of the solutions. In this case, one can assume that the absorption in this case is actually the scattered intensity that no longer arrives at the detectors.

1. Obtain the initial intensity of a blank, either of water or ethanol, depending on the specific solution that you are using.
2. Fill the cuvette with the solution and obtain a spectrum. If the optical density is higher than 1, dilute the solution further and retake the data. This step is important to avoid multiple scattering associated with concentrated samples.
3. Select the particular spectrum and save it as an Excel file.

### *Scattering Angle Dependence*

Using the light scattering instrument, obtain scattered intensity as a function of scattering angle for all of the five solutions. In this experiment, the incident light is fixed at a specific wavelength (632.8 nm).

1. Fill a scintillation vial with the dilute concentration of particles (completely full), and place it in the sample holder which is filled with index-matching liquid. Make sure to fill the liquid to the appropriate level if it is empty
2. Put the pump on to filter out the dust particles in the index-matching liquid. Leave it on for about 15 minutes.
3. Turn the laser on. CAUTION: This is a 25 mW laser, so be sure to take precautions.
4. Using safety glasses, observe the footprint of the laser/vial, to confirm that you do not see scattering from dust as well as to verify that the solution is properly diluted.
5. Make sure the filter is on the 'C' (closed) position on the detector. Put the detector on.
6. Start up the Brookhaven Static Light Scattering program. Follow the directions specified on the manual to obtain a "Dark Count Rate", to see the "Duration/Repeats", to specify the "Number of Repeats" by selecting the "Experimental Parameters" in the main menu. You will have to open the detector and also change the positions of its filter to perform these tasks.
7. Select "Sample Parameters" and defined the various parameters.
8. Specify the angles at which you want the data to be taken, and obtain a scan of the scattered intensity versus the scattering angle.
9. Export the data into a XY-type worksheet so that you can use it to compare with theory.