DLS Protocol

Written 05-09-11 by M. Refaei

Instrument specifications: Protein Solutions; DynaPro-801 TC molecular sizing instrument

Software: Dynamics(tm) version 3.27 (1997).

Background: The directly measured quantity is the amount of light scattered at a 90° angle. Changes in the intensity of scattered light are used to calculate the translational diffusion coefficient through an autocorrelation function (1). The hydrodynamic radius (R_H) can be calculated from the translational diffusion coefficient, and by assuming a basic shape, the approximate molecular weight can be calculated (2). This method also gives information on the monodispersity, or range of apparent sizes, in the sample. Generally, a well-behaved, well-purified protein sample will be monodisperse, and a sample containing impurities or aggregates will not be.

Protocol:

Prepping the sample: Any sample that goes into the DLS needs to be filtered in order to screen large particles that will interfere with measurements. This can be accomplished by a standard syringe filter (0.2μm or smaller) or by spin-filtering (SpinX® Cat. # 8160). Approximately 30μL of sample is needed for the cuvette, but it helps to have about twice that for the spin-filter, and at least 100μL for the syringe filter.

NOTE: *Spin-filters will need to be well-rinsed before use. Anecdotal evidence in the lab suggests that 3x rinse with 0.5 ml water will do the trick.*

Prepping the cuvette: It helps to soak the cuvette in 10% nitric acid overnight after heavy use with protein solutions. The sides need to be absolutely clear of gunk (that's the scientific term). Use a long pipette tip to rinse with distilled, filtered water, and "scrub" the sides of the cuvette after soaking. A less rigorous cleaning method involves rinsing with water, ethanol and water, about three times each. To get remaining liquid out of the cuvette, blast with canned air.

Starting the instrument: Turn on the DLS first, then open Dyna-Pro and wait for the software to load. The software will show an interface with two sides, the left measures the count number and the right side shows calculated data (Figure 1).

The count number will need to be determined before the experiment can be started. It's helpful to check cuvette cleanliness by measuring the counts of filtered buffer alone. Expect values ranging from 8,000 to 12,000. For a protein solution, ideal values range from 150-200. Counts that seem erratic, e.g. jumping from 100k to 500k and back, and counts that are too low, e.g. below 100k will not yield any data (See figure 2).

Once the sample has been established to have a good count number that is stable and between 150k and 250k, the experiment can begin. Click stop to end count measurement and start the experiment. Average time is about 10 minutes.

Once enough points have been collected, e.g. 14-20 points, stop the DLS experiment and click on the seventh button on the taskbar. This will bring up your DLS data (figure 3).
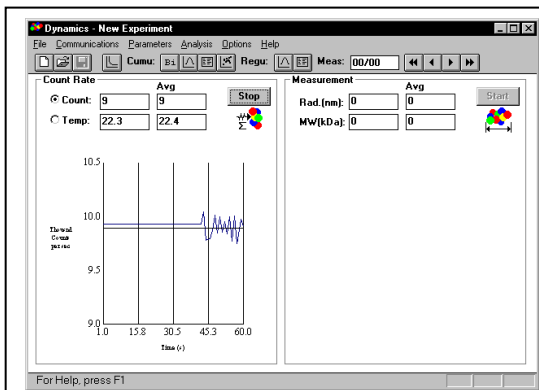
Figure 1: Measuring count rate. This needs to be done prior to starting the DLS experiment.
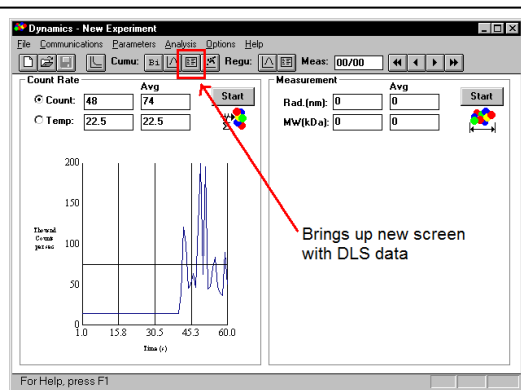
Figure 2: Once data has been collected, use highlighted button to bring up data screen.
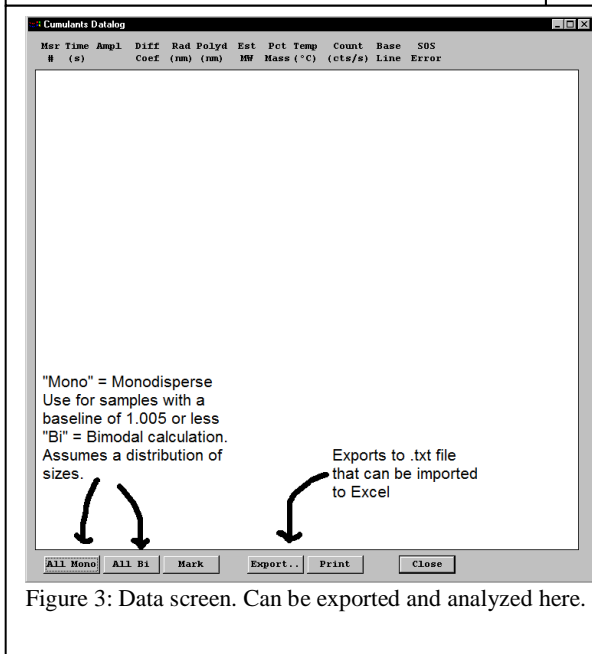
Figure 3: Data screen. Can be exported and analyzed here.

References:
1. *Principles of Physical Biochemistry*. 1st ed. K. E. van Holde, W.C. Johnson, P.S. Ho. pg: 319-325
2. "How to Use Dynamic Light Scattering to Improve the Likelihood of Growing Macromolecular Crystals" G.E.O. Borgstahl. *Methods in Molecular Biology vol. 363*. Edited by: S. Doublié Humana Press Inc., Totowa, NJ