

Note: this document borrows from an older one on DLS, which is still on the HowTo website (<http://macro.lsu.edu/howto>) for historical purposes. Details of an old-style correlator, based on shift register and add command generator technology, are now relegated to the final appendix. The present document is also recast as a minicourse with a “tour guide”—e.g., recently trained student to lead it. Eventually, we may present it as a YouTube video.

A Practical Minicourse in Dynamic Light Scattering *¹

Learning objectives:

- Explain why/when use DLS.
- Place photon correlation in context of other DLS experiments.
- Understand origin and behavior of speckle pattern.
- Write a formal expression for correlation function from memory.
- Write a practical expression for correlation function for homodyne DLS.
- Be able to estimate decay time from various plot representations of correlation functions.
- Analyze a DLS correlation function using a cumulants approach to estimate particle size (Excel or other computational aid OK).
- Prove the first cumulant is the weighted average of the decay times for a multi-exponential correlation function.
- Understand noise on correlation functions, at least at a rudimentary level.
- Execute an Excel Solver fit for a DLS correlogram.
- Understand error propagation in DLS.
- In a bimodal mixture of differently sized but otherwise similar particles (example: latex spheres) estimate the relative amounts of those particles by mass and by number.
- Know when to apply inverse Laplace transform algorithms such as CONTIN.
- Understand ILT algorithms, at least the more transparent ones such as exponential sampling.

¹ This is an attempt at a 5E approach: Engage, explore, explain, extend, evaluate: <http://enhancinged.wgbh.org/research/eeeeee.html> Probably a few e’s are missing or weak, but major sections are marked with an E. Who can see the flaw in this ‘constructivist’ educational approach?

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Part I. Guided Tour

Orientation

The reader is assumed to possess a working knowledge of particle and/or polymer properties. Terms like ‘virial coefficient’, ‘osmotic compressibility’, ‘Zimm plot’, and ‘radius of gyration’ should be understood prior to attempting this minicourse. Readers lacking this kind of background material may still be able to benefit from the minicourse, but should be especially careful not to form strong impressions that will be difficult to erase when and if their study of polymer/particle science improves. For such readers, this document may be more about forming questions than answers.

Why and when do we use DLS: making connections to other experiments you may already know.^E

DLS is used across the spectrum of polymer and particle science: synthetic and preparative chemists may apply it for routine characterization; polymer analytical chemists try to improve DLS and need it as a benchmark for other alternatives; experimental physicists and physical chemists use it to study complex fluids such as gels, transient networks and even liquid crystals; theorists publish dynamic structure factors on systems such as concentrated dispersions undergoing hydrodynamic interaction; and, even pure academics use it as a touchstone to optical design, the nature of time correlation functions, and computer programming. Without question, though, the main application is obtaining highly precise size information. We shall see that high precision does not guarantee ease of interpretation, but that does not diminish the nobility of making a good attempt at particle sizing. Even students ultimately intending other applications will benefit from an understanding of polymer and particle sizing by DLS.

Alternatives for size determination include static light scattering (SLS), small-angle X-ray or neutron scattering (SAXS and SANS respectively), analytical ultracentrifugation (AUC), and various forms of microscopy. Newer alternatives are fluorescence photobleaching recovery (FPR, also known as fluorescence recovery after photobleaching or FRAP), fluorescence correlation spectroscopy (FCS), forced Rayleigh scattering (FRS), pulsed field gradient NMR (PFGNMR) or the related diffusion ordered spectroscopy (DOSY), particle tracking (PT), and differential dynamic light scattering (DDLS). Despite the name, this last alternative is really a form of microscopy. Finally, certain new variants of DLS could be considered alternatives for particle sizing; these include DWS (diffusing wave spectroscopy), two-color cross-correlation DLS, depolarized DLS, zero-angle depolarized DLS, and gated cross-correlation DLS. Some characteristics of these methods appear in Table 1.

Table 1. Particle Sizing Methods

Method	Basis	Range.Å	Extrapolations	Requirements	Precision
SLS	Angle dependence of scattered light.	>50	Almost always	No dust	1% ???
DLS	Time or frequency dependence of scattered light	5 - 20000	Depends	Even less dust	1%
SAXS	Same as SLS, shorter wavelengths	>5?			
USAXS	Same as SAXS, even lower angles	<50000?			
SANS	Same as SAXS, neutrons instead of light	>5?			
PFGNMR	Spin echo amplitude reduction	<1000?			
PT	Microscope observation	>300?			
LD	Similar to SLS	>10000			
FPR	Optical tracer self diffusion	5 to >10000			
FCS	Optical tracer self diffusion	Similar to FPR?			
AUC	Sedimenting particles	Depends on density			

An important characteristic of DLS is its ability to measure particles *as they exist in solution*. Like any light scattering method, DLS is highly sensitive to aggregation. This is good if you want to know about aggregation, but many users have been disappointed to find that the highly precise, monodisperse 200-nm particles they see inside on a transmission electron microscopy (TEM) grid are dispersed as cruddy accretions in suspension! Without DLS, one might falsely hope the accretions form during sample preparation for TEM. Sometimes, it goes the other way: DLS confirms the desired particles are dispersed and the accretions did form during TEM sample preparation. An attribute of DLS is speed; if the sample is easily cleaned of dust, an approximate size can be obtained in just a few minutes or even less; however, SLS can be even faster, as and so can SAXS if a powerful synchrotron source is readily available. Still another important DLS characteristic is low cost and wide availability; this feature is certainly not shared by SAXS (and even less by SANS). Pulsed Field Gradient NMR (PFGNMR) is also closely related to Diffusion Ordered Spectroscopy (DOSY). In these diffusion-based sizing methods, the particles are “tagged” by alignment of their nuclear spins in an NMR spectrometer; although the tag only lasts

a short time, the particles can be observed as they move in a magnetic field gradient. Like DLS/SLS, no chemical tag needs be applied. Multicomponent diffusion can sometimes be sorted out, and the principle difficulty of the method is sizing of very large, slow diffusers. Particle tracking methods have the opposite limitation. PT follows diffusers by taking (possibly blurry) images of the particles; even species smaller the size resolution limit of optical microscopy can be followed. These methods are extremely promising. Analytical Ultracentrifugation (AUC) is a classic method that has seen a strong resurgence in the past two decades. The friction coefficient of particles is obtained as a combination of their sedimentation and diffusion when the particles sink in a high centrifugal field.

Two fluorescence methods deserve mention, even if they do require (usually) the attachment of a dye to render the particles visible. These are Fluorescence Photobleaching Recovery (FPR) and Fluorescence Correlation Spectroscopy (FCS). In an FPR measurement, a region of a sample has its fluorescence permanently erased, ideally without causing damage to the diffusers; the return of still-fluorescent particles to the region yields the diffusion coefficient. In FCS, the fluorescent intensity from a small volume of an extremely dilute solution of fluorescent diffusers is monitored. As the number of fluorescent particles in the viewing volume rises and falls, so does the intensity; autocorrelating that signal yields a diffusion coefficient if the volume, shape and illumination profile are known. A common misconception about DLS is that it also relies on variation of the number of scatterers in the viewing volume...ideally, this should NOT be the case.

To summarize this section, you should use DLS if an easily de-dusted particle or polymer scatters light but is too small for SLS, too soft for TEM or even Cryo-TEM, and not worth the effort of SAXS or SANS. Even then, it might make sense to consider FPR or FCS if the particle is easily labeled, without much potential to be damaged by the label, and if it is to be studied at very low concentrations. PT, PFGNMR, DOSY and AUC might be chosen if the scattering signal is hopelessly weak (low differential refractive index increment, dn/dc). Often, it makes sense to use DLS in addition to other methods; in particular, knowing the shape of the particles (e.g., from TEM) helps. Then use DLS to improve the statistics through the high number of particles it naturally observes.

A walking tour of a highly visual DLS apparatus.^E

DLS takes a variety of forms. There are, for example, heterodyne measurement systems in which a little of the laser light is added deliberately to the scattered signal (Figure 1). This method, rarely used, is very powerful for complex systems where one cannot be certain that the Siegert relation (see below) is not guaranteed to hold.

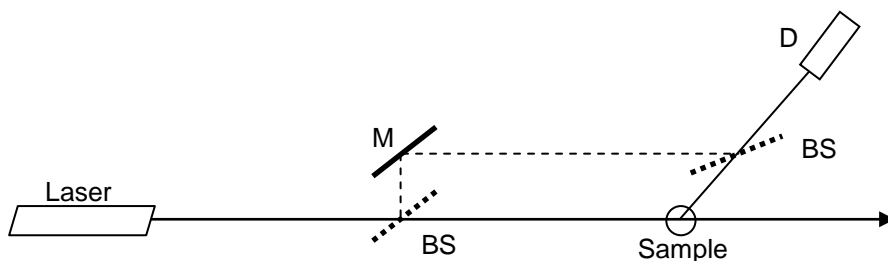


Figure 1. Heterodyne DLS apparatus. BS = beamsplitter; M = Mirror; D = detector. For a homodyne apparatus, just remove the two beamsplitters and mirror.

Detection can be by photomultiplier tube (PMT) or single photon avalanche photodiode detector (SPAD). These light collectors may feed autocorrelators, cross-correlators, structurators, spectral analyzers, Fabry-Perot interferometers or even just a computer card equipped for A/D conversion or photon counting. (Shoemaker PChem Lab Text) The most common way to do the measurement is a ‘homodyne’ arrangement of just the scattered light (no added light directly from the beam) combined with photon correlation spectroscopy (PCS). This section walks the student through observations that can be made on a high-quality instrument that uses a homodyne PCS detector mounted on a rotating arm to define the scattering angle. The light reaches that detector through a classical lens/aperture/pinhole arrangement, as shown in Figure 2. Much of what is learned here applies to other homodyne optical schemes, the main competitor being single-mode or nearly single-mode fiber optic detectors; XXXRicka despite some

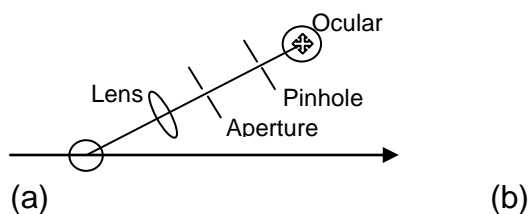


Figure 2. Classical lens-aperture-pinhole setup for DLS detection (a); and, photograph of the instrument (b).

interesting and useful characteristics, those systems do not readily provide for a visual approach to learning DLS.

- **Safety:** You will have a “tour guide” for these initial observations. *Do not attempt them without help, as permanent eye damage may result.* Your tour guide will explain:

1. How to avoid directly looking into the beam.
 2. How to avoid exposure to reflected light.
 3. How to use the instrument's viewer (ocular) safely.
 4. How to prevent damage to the DLS detector (this is a safety issue because then the laboratory personnel will kill you).
- **Speckle pattern for latex scatterers**
 1. The photomultiplier (or other) detectors will be turned off.
 2. Your tour guide will show you the flight path as the laser goes through the sample and how it gets through two "holes" (aperture and pinhole, in that order) on the way to the detector (or up through the ocular, via a movable mirror).
 3. It will be apparent that we can easily change angle, aperture and pinhole.
 4. Your guide will have prepared (or taken from our "DLS Fun" sample holders) two strongly scattering latex samples, one "small" (typically 0.1 μm) and one large (ca. 1 μm). They will be very slightly bluish in tinge, but normally DLS samples are quite clear.
 5. Begin by inserting the cell containing the larger latex into the beam. Observe the beam hitting a screen several feet away from the instrument. With the room lights down, what do you see in the area *surrounding* the beam? (inserting the beamstop may help if there seems to be nothing to observe).
 6. Your tour guide will adjust the scattering angle to about 20 degrees and ask you to observe the sample's scattering through the ocular, using "open" settings for aperture and pinhole.
 7. If the detector recorded the total scattered intensity from *all* the image you now see, how would that single intensity plot out as a function of time?
 8. Following instructions from the guide, adjust the front aperture. What do you see as you go to smaller and smaller aperture sizes?
 9. Now close down the pinhole setting. You should see a pinpoint of light.
 10. NOW we repeat the question 7 above: if the detector recorded the intensity from the tiny spot you now see, how would that single intensity plot out as a function of time?
 11. Open the aperture and pinhole, then take your detector for a walk! The tour guide will show you how to observe the speckle pattern as you walk the detector from low angles to high.
 - **The tour guide will now explain some of what you saw and place it in the context of the experiment.**

Explanation^E

1. The speckle patterns we observed by looking into the instrument ocular are the result of the high spatial coherence of laser light sources.* These speckle patterns were moving because molecules in the system move.
2. The speed at which the speckle patterns move can tell us how fast individual molecules diffuse.
3. The latex particles whose speckle patterns we observed are huge. For smaller particles, the rates of motion easily exceed what can be followed by eye. Particularly, it would be difficult to capture the whole image at the necessary speed. So the whole speckle pattern is not measured, but only the intensity of a single speckle. Thanks to Einstein, we are able to convert the fluctuations in intensity at this one point into the size of the molecules that cause the scattering...but only after extrapolation to zero concentration.
4. Selecting a smaller aperture made bigger speckles: you are squeezing light through an orifice, so it diffracts.
5. Selecting a smaller pinhole lets you look at just one speckle, which will fluctuate in intensity if the laser light has sufficiently high spatial coherence. For a very tiny hole and highly coherent laser source, the light through the pinhole may appear to switch on and off and do this randomly (almost...we shall see that nothing is truly random).
6. Walking the rotating arm detector around as a function of angle resulted in faster-moving speckle patterns (they might have gotten dimmer, too).
7. Preview: run the correlator for small particle and large particle at the same angle; notice the difference—for the smaller of the two particles, the displayed function lies to the left, corresponding to shorter times.
8. Preview: run the correlator to demonstrate the difference between low angle and high angles for the same sample.

*Actually, a laser is not needed—only spatial coherence. An Israeli group once set up a DLS using sunlight through two widely separated pinholes. It worked great at noontime on sunny days, even though the light was not monochromatic!

Periodic boundary conditions for diffusion and how DLS enforces them.

In the Chem 4011 class (and in many other places, such as the Cantor & Schimmel textbook) it is discussed that if someone could somehow make a sinusoidal oscillation in concentration at a particular time $t = 0$, then Fick's 2nd law predicts exponential decay of that oscillation with a characteristic time τ_c . This is the basis of fringe pattern FPR and fringe pattern FRS. Stripe-pattern FPR as practiced in the Russo lab and elsewhere relies on this behavior, too: the stripe can be expressed as a Fourier sum of sine terms and leads to multiple exponential decays, which are filtered out in the Russo instrument, except for the lowest, or fundamental, term. When initial conditions follow a periodic pattern expressible in such a sum, the problem is said to have periodic boundary conditions.

What has this got to do with DLS? As seen during the visual exploration of the instrument, we merely insert a solution and turn on an instrument called an autocorrelator. There is no initial step to create a special concentration gradient. *It turns out that DLS automatically selects a sinusoidal concentration gradient.* The figure below shows the incident and scattered light vectors, along with the difference q .

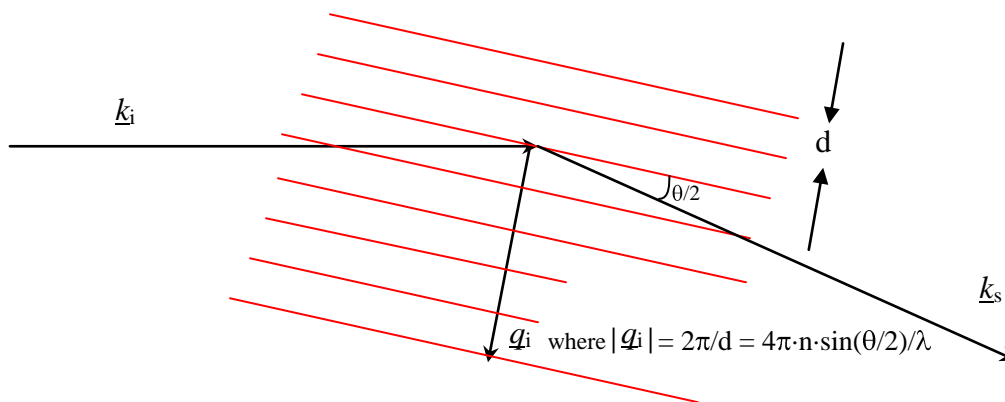


Figure 3. Scattering explained as diffraction from Fourier components selected by the scattering geometry.

The red lines in the figure, drawn at half the scattering angle θ , represent peaks in a particular sinusoidal Fourier concentration component. The actual concentration is the sum of many waves with different orientations and wavelengths, but scattering only couples to the one wave shown, oriented as it is, and with a period which satisfies the first-order Bragg's law expression $d = \lambda / [2 \cdot \sin(\theta/2)]$.

Correlation Functions

Unlike FRS or FPR, there is no “start pulse” to create the sinusoidal concentration profile. DLS works by making an almost endless series of “comparisons” separated by time. Ideally, one might observe all the speckles and watch how they change over time. This could be done, for example, using a frame grabber to capture the images. Each pixel could be “compared” to itself over a period of time to see how that pixel was evolving. In practice, as we saw when looking at the scattered light through the pinhole via the ocular of the DLS instrument, only a small area of the speckle pattern is observed—usually one to several “coherence areas”. The intensity at a given time $I(t')$ is “compared” to that at a later time, $I(t'+t)$. “Compared” means “multiplied into”. The separation in time, t , is called the “lag time” and it is the independent variable of the DLS experiment. The dependent variable is the correlation function, $G^{(2)}(t)$, which will be developed now.

You observed the twinkling light, but those intensity fluctuations *were not really random!* In fact, almost nothing is completely random---everything that seems random is “correlated” (i.e., not random) on some sufficiently short time scale. For example, the Dow Jones industrial average produces a “signal” that fluctuates randomly about its mean value over some long enough time scale. (For simplicity, pretend that the average value does not change—i.e., that the

economy is a zero-sum game; in reality stocks go up as new wealth is generated, not to mention inflation.) But if the market is high at some time t' , then it is also likely to still be high at some time $t' + \varepsilon$, provided that ε is a very short time (the reason for the prime— t' instead of just t —will be apparent soon; it does *not* mean time derivative). Thus, if I put all my money into stocks today, I will *probably* not lose everything by tomorrow. On the other hand, at some time $t' + \delta$, where δ is very large, there is no telling what the stock market will be like. It could be higher or lower; stock value becomes uncorrelated with the initial value after a long time. Investors and politicians would both love to know how long a bull market will last. Light scatterers are luckier; as you will see, our measurements of how long the signal remains correlated are simple and precise.

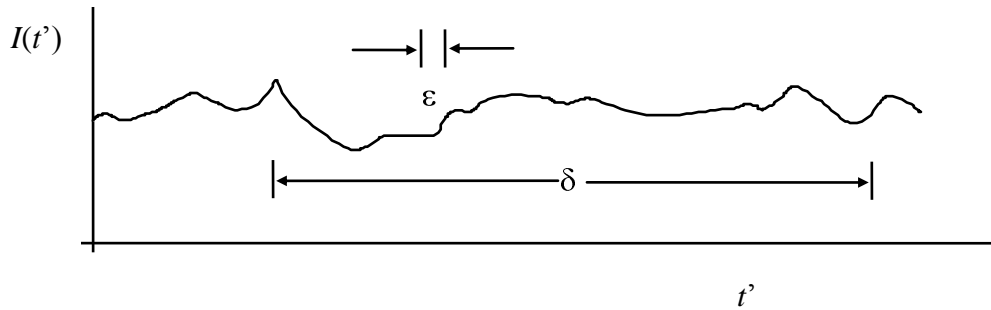


Figure 4. Intensity fluctuations are “random” on a time scale δ but “correlated” on a time scale ε .

Quasi-random phenomena are followed theoretically and experimentally with the aid of *correlation functions*. A correlation function is a mathematical construct designed to help determine how long a given signal stays the same. For heavily damped phenomena such as diffusion in a periodic boundary condition, correlation functions decay with some characteristic time constant τ , the "correlation time." In the foregoing, the time $\varepsilon < \tau$ while $\delta > \tau$.

The autocorrelation function we measure will be that of the scattered light intensity:

$$G^{(2)}(t) = \langle I(0)I(t) \rangle = \lim_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^T I(t') \cdot I(t'+t) dt' \quad \langle 1 \rangle$$

The ⁽²⁾ superscript indicates that $G^{(2)}$ is a *second-order* autocorrelation function--i.e., one involving intensities, which are the *squares* of electric fields. The use of a capital G indicates that the data are *not* normalized. Later, we will see correlation functions like $g^{(1)}$ which represents a normalized electric field autocorrelation function and $g^{(2)}$ which is a second order function whose baseline is normalized. DLS notation is confusing; you will eventually get used to it.

You should commit Eq. 1 to memory—it is the essence of what a correlation function does. The integral in Eq. 1 is a recipe. It instructs you to do the following: 1. Record the intensity vs. time for an infinitely long period of time $2T$; and, 2. Take any and all pairs separated by the lag time interval t and "compare" them. The comparison is done by forming the products. When t is very short, $G^{(2)}(t)$ approximates the average of the squares because $I(t') \approx I(t'+t)$: $G^{(2)}(0) = \langle I^2 \rangle$.

At very long t , one obtains the square of the average: $G^{(2)}(\infty) = \langle I \rangle^2$. Do you see the difference? Mathematically, $\langle I^2 \rangle$ is guaranteed to be larger than or equal to $\langle I \rangle^2$. The “equal to” situation corresponds to “no fluctuations”—i.e., $I = \text{constant}$. So $G^{(2)}$ decreases with t if there are fluctuations or stays unchanged if there are no fluctuations; you already saw the optical steps we take to ensure there are fluctuations—do you remember them?

The process of getting $G^{(2)}(t)$ is repeated for many values of t until $G^{(2)}$ is known over a wide range of t . (Now you can see why we have t and t' ; t is the independent variable for building the correlation function—the so-called *lag time*—but t' is the controlling variable for recording the light intensity over a very long time, $2T$.) Actual digital correlators construct $G^{(2)}$ at many t values simultaneously, but it's easier for humans to imagine doing it for one value of t at a time. Operational details of *how* correlators work are discussed in an appendix for old-style correlators. New correlators are a mystery, and may combine custom-designed, fast chips with the central processor in a personal computer to “build” and display the correlation function.

Correlation functions are approximated, never measured, because the infinite time limits of the integral in Eq. 1 can never be achieved in practice (infinite time being impractical, the actual requirement on T is that it must be very many correlation times; as a bare minimum, we might require that $T > 10^4$).

After some time, the signal in the correlator is well approximated² by:

$$G^{(2)}(t) = B(1 + f |g^{(1)}(t)|^2) \quad \langle 2 \rangle$$

In this expression, B and f are experimental parameters that will be discussed soon. The quantity of main importance is $g^{(1)}(t)$, the electric field autocorrelation function. It has to be extracted from the measurable $G^{(2)}(t)$ by solving Eq. 2 at each value of t measured. Rather than write that equation now, we wait to introduce a normalized version of $G^{(2)}$; see below. Meanwhile, just know that in many cases, $g^{(1)}(t)$ is a simple exponential decay:

$$g^{(1)}(t) = e^{-\Gamma t} \quad \langle 3 \rangle$$

where Γ is the decay rate (the inverse of the correlation time). So we can write $\langle 2 \rangle$ as:

$$G^{(2)}(t) = B(1 + f \cdot e^{-2\Gamma t}) \quad \langle 3.5 \rangle$$

For simple translational diffusion, the decay rate Γ is:

$$\Gamma = \tau^{-1} = q^2 D_m \quad \langle 4 \rangle$$

where D_m is the mutual diffusion coefficient. The scattering vector magnitude, q , is:

²In addition to the usual approximations having to do with finite acquisition time, another important approximation is that the scattering is *homodyne* and a random Gaussian process, and Eq. 2 relating intensity and electric field autocorrelation functions is sometimes referred to as the Siegert relationship.

$$q = 4\pi \cdot n \cdot \sin(\theta/2) / \lambda_0 \quad <5>$$

where λ_0 is the laser wavelength *in vacuo*, n is the refractive index of the solution, and θ is the scattering angle. Thus, the decay rate is lower (correlation time is longer) at low scattering angles than at high ones. You can also slow the fluctuations by shifting to a longer wavelength. The physical reason is that the molecules must diffuse *farther* to change the speckle pattern at low q . The *distance scale* associated with any scattering experiment is *inversely* proportional to q :

$$\text{Scattering Distance Scale} = 2\pi/q \quad <6>$$

You can think of q as the “fineness” or “spatial frequency” of a ruler. A ruler that is divided into very fine marks—that is, a high- q ruler—permits you to determine details of an object. For example, you could measure the length of a pencil as 8.2 cm with a good ruler, but with one that lacks fine markings, you could only guess 8. Thus it is with DLS: at high q (high θ , short λ_0) you can see short-range details of the motion. The DLS ruler has about 10^5 tick marks per centimeter (for typical angles and wavelengths). The distance between tick marks is therefore about 6300 Å.

The quantity f in Eq. 2 is an instrumental parameter ($0 < f < 1$) related to how many speckles the detector sees (fewer speckles correspond to higher f values—i.e., “taller” correlation functions as you saw during the observation period). We can change the value of f by pinhole and aperture settings. Other factors that affect it are solution scattering level compared to solvent scattering (see below), laser design, PMT dark count, and the degree to which the incident beam has been focused.

The parameter B is a baseline. It is proportional to the square of the average intensity, but some modern correlators cover this up by pre-normalizing the baseline such that the values at long sample times are about 1 (give or take a little, due to noise). Each channel reported is the instrument’s latest determination of the quotient

$$g^{(2)}(t) = \frac{G^{(2)}(t)}{G^{(2)}(t = \infty)} = \frac{G^{(2)}(t)}{B}. \text{ This is called the normalized second-order autocorrelation function}$$

or homodyne correlation function. Putting it all together, we see that the correlation function has the form of an exponential decay on top of a baseline, as shown in Figure 5.

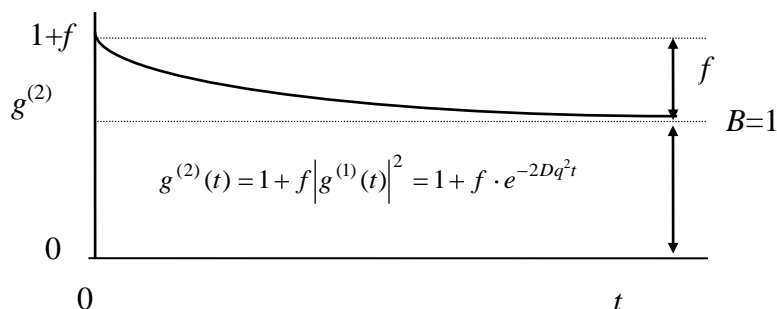


Figure 5. The measured correlation function is an exponential sitting atop a baseline.

Channels representing large lag times have a value near 1; early channels will have a value near $1+f$. The usable, interesting quantity is $\frac{g^{(2)}(t)-1}{g^{(2)}(0)-1}$ or its square root,

$$\sqrt{\frac{g^{(2)}(t)-1}{g^{(2)}(0)-1}} \equiv \sqrt{\frac{g^{(2)}(t)-1}{f}} \equiv g^{(1)}(t). \quad <6.5>$$

Correlator Design

There are two basic types of correlator, *linear* and *log-time*. The Langley-Ford 1096 shown in Figure XXX was almost the epitome of the classic linear design. A linear correlator measures the correlation function at discrete “lag times” according to $t = i\Delta$ where $i = 1, 2, 3, \dots, NCHAN$ and Δ is called variously the channel time, sample time, or “sampling time.” The LFI-1096 could actually operate with three separate values of Δ simultaneously, but normally it just had $NCHAN = 272$ linearly spaced channels. The limitations of such a device for multi-exponential signals are apparent from the rule of thumb that, in order to measure a decay process with characteristic time t_1 , you must devote about 64 channels before t_1 and about 80 channels after t_1 . So...you would normally set Δ to $t_1/32$ and use about 96 channels to capture that decay process. If another, slower, decay were located at longer times, you are quickly running out of channels with which to capture it. Thus log-time correlators were invented in the 1980s. In these, the lag times are spaced like this: $\Delta, 2\Delta, 4\Delta, 8\Delta, 16\Delta, 32\Delta, 64\Delta$ etc. The early log-time correlators actually did not give enough channels for measurement of samples containing almost monodisperse particles; thus, a modern instrument typically uses a mixed quasi-logarithmic approach: a block of 16 channels at D , a block of 8 channels at $2D$, a block of 8 channels at $4D$, a block of 8 channels at $16D$, 8 channels at $32D$, etc. Our ALV correlators have this architecture, which you can see by inspecting the file named `alvsing.lag` (usually in `c:\` on our DLS computers).

The actual appearance of correlation functions

Probably, the correlation function on your computer screen will not look like the one in Figure 5. That is because various scale choices are available. In the good old days of linear correlators, all correlation functions really did look like Figure 5, but with the development of log-time correlators (see box, above) the time range became too large for linear scales. So, people started showing a log-time scale. About the same time, correlators were integrated into computers. Then it became very easy to show $\log(g)$ vs. t (semilog) and $\log(g)$ vs $\log(t)$ (log-log) representations. The four popular representations are shown below, along with how you use them to estimate the correlation time, τ . The representations involving logarithmic y-axes have considerable noise at long lag times. This is because the correlation function approaches zero (after baseline subtraction) and $\log(0) = -\infty$. Noise has a huge effect then and, indeed, some channels cannot be computed at all because, due to noise, the baseline-subtracted, normalized value drops below 0 and $\log(\text{negative})$ cannot be computed. The noise is less evident on the linear y-scale of the top two correlation representations. In fact, on a linear y-scale, you will ordinarily see more noise at *low* sample times due to a phenomenon known as photon starvation (not discussed here).

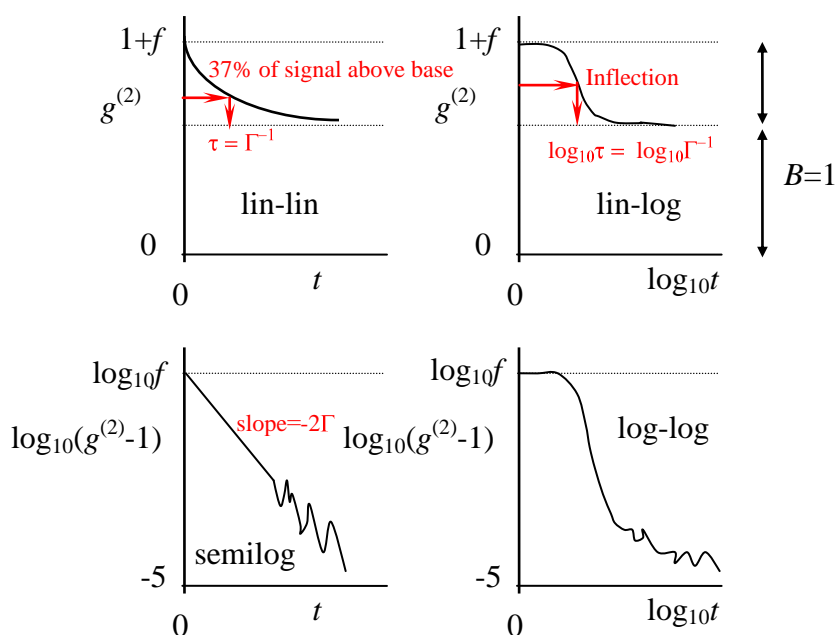


Figure 6. Various forms of autocorrelation function available on most correlators.

Using the equations to get the results out of your data

From Figure 5 and Eq. 3 - 3.5, it is possible to see how to obtain the mutual diffusion coefficient. There are two options:

- 1) Linearization followed by linear regression
 - subtract off the baseline (i.e., 1.0)
 - take the natural logarithm of what's left

- fit that to a straight line using, for example, Excel Analysis Toolpack (better yet, do it manually with a ruler).
- The negative of the slope of that line is 2Γ . Divide 2Γ by $2q^2$ to get D .

2) Nonlinear fitting. In this method, you try to fit Eq. 3.5 using a nonlinear fit algorithm, such as Excel's Solver (see HowTo guide for Solver).

Either way, the resulting diffusion coefficient is D_m , which differs in principle from the diffusion coefficient extrapolated to infinite dilution, D_o . For a dilute sample, it will turn out that $D_m \approx D_o$. Once you know D_o , the hydrodynamic radius is given by:

$$R_h = \frac{kT}{6\pi\eta_o D_o} \quad \langle 7 \rangle$$

where kT is the thermal energy ($k =$ Boltzmann's constant, $1.38 \times 10^{-16} \text{ g}\cdot\text{cm}^2\cdot\text{s}^{-2}\cdot\text{K}^{-1}$, T is the Kelvin temperature, and η_o is the viscosity). And that's it—that's particle sizing!

What does R_h mean?

Sometimes newcomers to DLS do not know what R_h really means, so let's be very clear about that.

1. If your object is a solid sphere of radius R , then $R_h = R$.
2. If your object is spherical "bubble" (e.g., liposome) with *outer* radius R , then $R_h = R$ (exception: some liposomes may "wobble" and that could alter R_h).
3. If your object is a sphere on the outside, but has inclusions of any shape inside, then $R_h=R$.
4. If your object has some other shape—such as cylinder, cube, polymer chain or star—then R_h is the radius of some hypothetical sphere that diffuses as fast as your object does.
5. If your object is a semidilute solution, gel, etc., all bets are off.

Practice

Now it is time to analyze some data to see how this goes. Your tour guide will choose one of the latex samples observed during the visual tour and obtain a correlation function. These steps should be followed:

1. The temperature bath will be set to some convenient value, usually 20°C to 25°C.
2. Pinholes will be adjusted to give some reasonable value of f (usually >0.1).
3. After a short run, the approximate value of f will be estimated by inspecting the display screen. The tour guide will point how this is done, but it is evident from Figures 5 and 6.
4. The approximate value of τ will be estimated by inspecting the display screen. The tour guide will point how this is done, but it is evident from Figure 3.
5. The acquisition time will be set to at least $10^4 \tau$.

6. The data will be saved on the computer drive and on a portable USB drive provided by the student.
7. The student will be told T , λ_o , θ , η_o and n .
8. The student can now go home and use the data and the equations above to obtain R_h . The student should obtain R_h by linearizing the data as described above AND by using a nonlinear least squares fitting algorithm (see Solver HowTo guide and/or ask tour guide). For normal latex standards, the sizes obtained by these separate methods should be very close.

Data handling options

After the student has obtained a hydrodynamic by linear regression and by nonlinear least squares fitting, obtaining essentially identical results, the outcome can be checked against other software, such as that provided by the vendor of the correlator. These always include “cumulant” fits and “multiexponential” fits.

Cumulant fitting. The cumulant fit is much like the linear regression the student used, but it permits the drawing of a polynomial, rather than a straight line, after the subtraction and log-taking steps. These equations are followed:

$$\text{First-order cumulant: } \ln[g^{(1)}(t)] = K_o - \Gamma t \quad \langle 8a \rangle$$

$$\text{Second-order cumulant: } \ln[g^{(1)}(t)] = K_o - \Gamma t + \frac{\mu_2}{2} t^2 \quad \langle 8b \rangle$$

$$\text{Third-order cumulant: } \ln[g^{(1)}(t)] = K_o - \Gamma t + \frac{\mu_2}{2} t^2 - \frac{\mu_3}{3 \cdot 2} t^3 + \dots \quad \langle 8c \rangle$$

The intercept K_o should be zero, but may not be due to slight baseline issues.

The jargon of DLS is confusing. In particular, Γ is called the “first cumulant” no matter what order of cumulant fit was used to get it. But why would you ever need higher-order polynomial fits in the first place? The answer is that many samples contain diffusers of different sizes, so the plot of $\ln[g^{(1)}(t)]$ vs. t exhibits curvature instead of being a straight line. In this case, Γ is obtained as the *initial* slope of a plot of $\ln[g^{(1)}(t)]$ vs. t and it represents a particular average decay rate, $\bar{\Gamma}$.

$$\bar{\Gamma} = \lim_{t \rightarrow 0} \frac{d \ln g^{(1)}(t)}{dt} \quad \langle 9 \rangle$$

To pursue this a bit, we write the actual correlation function as a sum of exponentials, each with amplitude A_i and decay rate Γ_i .

$$g^{(1)}(t) = A_1 e^{-\Gamma_1 t} + A_2 e^{-\Gamma_2 t} + A_3 e^{-\Gamma_3 t} + \dots \quad \langle 10 \rangle$$

Do this exercise: Put Eq. 10 into Eq. 9 and try to show that Γ becomes the average decay rate,

$$\bar{\Gamma} = \frac{A_1\Gamma_1 + A_2\Gamma_2 + A_3\Gamma_3}{A_1 + A_2 + A_3} \quad <11>$$

Polydispersity Parameter. As a simple measure of the polydispersity, DLS people often form the dimensionless quotient $\mu_2 / \bar{\Gamma}^2$. This is sometimes called PDI, but it should not be equated with M_w/M_n in polymer science. The parameter $\mu_2 / \bar{\Gamma}^2$ would be zero for monodisperse samples. For typical polymer latex, it might be about 0.03. When $\mu_2 / \bar{\Gamma}^2 > 0.3$, it is time to think about other ways to fit the data besides cumulants.

Problems with cumulant fitting.

1. According to Eqs. 6.5 and 9, you have to subtract the signal, take a square root and take a logarithm. This gets ugly as the signal decays into the baseline because noise will sometimes drive the signal negative. We cannot take the square root of a negative number (complex numbers notwithstanding), nor can we take its logarithm.

Solution 1: you can take just the top 90% of the signal above baseline—e.g., include those channels where $g^{(2)}(t)$ runs from about $1+f$ to about $1 + 0.1f$.

Solution 2: Another solution is to cut channels off when the computed noise in that channel rises to one-third of the channel value.

Solution 3: Another solution is to locate the inflection point in the $g^{(2)}(t)$ vs. $\log(t)$ plot, then move halfway from that point to the baseline...this is qualitative.

Solution 4: you can avoid the square-root taking—e.g., replace Eq. 8b with something like this:

$$\ln[g^{(2)}(t) - 1] = 2(K_0 - \Gamma t + \frac{\mu_2}{2} t^2) \quad <12>$$

2. What's the initial part of the curve? In strongly non-exponential correlation functions, following the suggestions of the previous paragraph leaves enough long-time data points that you cannot fit cumulants without quite a lot of terms. In this case, you can just fit the data points at lower times. But how low?
 - a. Solution: Long ago, Ken Schmitz suggested to keep reducing the number of points, compute $\bar{\Gamma}$, and plot the result against the number of fitted points. Such "asymptotic analysis" may identify the initial decay rate.
3. Who says the baseline really ought to be 1? A unity baseline is appropriate for perfect signals. Any number of things can mess up the signals—dust, laser drift, dust in your index matching bath, etc.
 - a. Solution 1: re-perform the cumulants fit using a baseline other than 1.0; for example, you might try 0.9995 or 1.0005. In some cases (low-f measurements) it really matters. Keep track of $\bar{\Gamma}$ variation and use it as part of your error estimate.
 - b. Some groups have gone after this problem by using a nonlinear least squares version of cumulant-like fitting:

$$g^{(2)}(t) = B[1 + f \cdot [\exp(K_0 - \Gamma t + \frac{\mu_2}{2} t^2 - \frac{\mu_3}{3 \cdot 2} t^3 + \dots)]^2] \quad <13>$$

An Excel spreadsheet is available from us to fit data to this form using Solver, Excel's nonlinear least squares routine.

Nonlinear least squares fitting. In addition to trying to linearize the data and then patch up the minor curvature with a polynomial fit as the cumulant method does, we can fit the exponential decay directly. For a hypothetical perfectly single exponential decay, our equation is:

$$g^{(2)}(t) = B + Bfe^{-2\Gamma t} \quad <14>$$

This equation can be described by three parameters, B , f and Γ . We can fit it using Excel Solver, but correlator vendors typically supply programs to do this, too. Next, consider a bimodal mixture—two diffusers with amplitudes A_1 and A_2 and decay rates G_1 and G_2 .

$$g^{(2)}(t) = B + (A_1e^{-\Gamma_1 t} + A_2e^{-\Gamma_2 t})^2 \quad <15>$$

Now, we have 5 parameters to fit and one important thing to realize: ***there is no uniquely best fit to those parameters!*** That's right. . .unlike cumulants/polynomial fitting, where every necessary term (Γ , μ_2 , etc.) appears as a *coefficient* of t , t^2 , t^3 , etc., now we find that the decay rates appear in transcendental functions, such as the exponential. When this happens, the best parameters cannot be found by an analytical expression. Instead, computer algorithms have been designed to find parameters that closely approximate the data. The SOLVER algorithm you probably used to analyze the data provided by your tour guide is an example, and so is whatever vendor-supplied algorithm you are practicing now. All these are a trial-and-error processes, and it sometimes matters where you begin the search. You must ***guess*** the beginning parameters of the search. Once the algorithm provides its best answers, you should try several different guesses to ensure that the algorithm is not getting fooled by a bad initial guess.

Part II. Solo Experiments

Ia. Your First Solo Experiments: Mixed latex spheres.

Goals:

1. Measure R_h and R_g of two different latex spheres.
2. Mix them at known intensity ratios.
3. See if you can get the correct R_h and R_g values after mixing.

Hints:

1. DLS cannot resolve spherical particles very well unless they are separated in size by more than about 1.2 (multiexponential fits) or 2 (CONTIN).
2. It will be hard to get R_g unless the spheres are bigger than about 100Å (depends on wavelength, quality of instrument alignment, dust, etc.)

Time Required: Maybe 1 or 2 days the first time

Recommended procedure:

Monodisperse Stock Solutions

1. Measure the scattering level for toluene and for water, as always, to establish a Rayleigh reference point.
2. Choose 2 latex spheres with big enough size separation (example: diameter = 250Å and 750Å)
3. Prepare two stock solutions... they might just barely show a slight bluish tinge in a 1-cm pathlength cell. You do this by adding one drop (or less) of latex to a plastic cell and adding dust-free water.
4. Remove dust by filtering if necessary (may also remove aggregates). Choose filters according to the size—you don't want to remove the unaggregated particles.
5. Try to get the scattering intensities of the two solutions about the same at some reasonable angle (perhaps 60 degrees).
6. Set the apparatus for low coherence and measure the I vs. q dependence.
7. Re-set the apparatus for high coherence and measure correlation functions as a function of angle.
8. Analyze (Guinier plots or nonlinear fit for SLS, Γ vs q^2 for DLS, etc.)
9. For each stock solution, does $R_h = R_g \cdot (5/3)^{1/2}$? It should! If not, get help (however, if one latex is too small to measure by SLS, don't worry about it much).

Bidisperse Test Samples:

1. Make a 50:50 mixture by volume. Because you know the scattering powers of each solution at any angle, you will know the scattering amplitudes A_1 and A_2 to expect at any angle.
2. Measure SLS and DLS at various angles.
3. Analyze by Guinier (or nonlinear fit) for SLS and Γ vs q^2 for DLS. You will probably note that 3CUMU and 1EXP fits are not in great agreement anymore—the effect of polydispersity (the two algorithms respond differently to it). What is the average R_h and R_g ?

The first is the inverse of the Z-average of the radii, while the second is the square root of the Z-average of the squares of the radii. Write equations and see if this works!

4. Now analyze the DLS data for $A_1, \Gamma_1, A_2, \Gamma_2$ using a bi-exponential fit. Plot the Γ_1 and Γ_2 results vs. q^2 . Still looks OK? Gives the same sizes as for particles measured separately?
5. Plot the A_2 and A_1 data a'la Guinier (or use a nonlinear fit). Do you get the same values you measured for the particles individually? Why or why not?
6. Try to place the A_1 and A_2 values on an absolute scale. That is, associate the total scattering intensity ($A_1 + A_2$) with a Rayleigh factor, R (See the Zimm plot HowTo for this: <http://macro.lsu.edu/howto/#SCATTERING> -- specifically, the file called GuiDe Manual; an equation in here gets you from measured intensities to Rayleigh factors). Hint 1: In most cases, A_1 and A_2 are effectively isolated from the solvent scattering—you do not have to subtract that. Hint 2: the coherence parameter f may vary with angle—I think you have to take this into consideration. Try it and see!
7. Now split your Rayleigh factors into components R_1 and R_2 .
8. Plot against q^2 and/or try a nonlinear fit for spheres. Do you get the same R_g values as you measured for the particles separately?

IIb. A Second Solo Experiment: Dynamic Light Scattering Of Dilute Polymer Solutions....including Tips on Preparing Clean Samples, Expected vs. Actual Coherence, and Concentration Dependence

Goals: Try to get a molecular weight distribution for a polystyrene sample in some convenient solvent, e.g. toluene or THF.

Hints: Be patient—this ain't easy.

- Weak polymer solutions will be measured. For the first time, we have to be concerned with the adequacy of the signal over solvent level.
- In order to make measurements at all, the solutions must sometimes be more concentrated than one would wish. Then the results have to be extrapolated in to zero concentration in order to be meaningful.
- Much greater care has to be paid to cleanliness.

Time required: several days for the first time...you get faster later.

Plan:

- We will use a commercial polystyrene (ideally, the same one selected during some simple GPC experiment)
- We will try to make 4 dust-free samples with different concentrations
- We will get their diffusion coefficients correctly, and extrapolate the diffusion coefficients to zero concentration.
- Extend to zero angle (if required).
- We will study the effect of dilution on the coherent scattering amplitude.
- We will perform inverse Laplace transform operations to get Amplitude vs Gamma distributions.
- We will convert these distributions to Concentration vs Molecular weight distributions, making appropriate assumptions.
- Later, we can use these same samples for conventional Zimm plot analysis and, perhaps, GPC/light scattering.

Preparation of Clean Samples:

We cannot hide it any longer; the worst part of light scattering is preparing clean samples. With the latex samples we measured earlier, it was very easy: latex particles are almost as big as some dust particles and they can almost “defend” themselves from dust. In measuring most polymer solutions, you will not be so lucky!

Therefore, the *first* step is to prepare clean solvent! Yup....just solvent, no polymer. Polymer analysts live by these words:

Measure Nothing First!

If you cannot do solvent well, forget the rest. Once you are measuring nothing well, measure something unimportant next, then measure the important perfectly (MNF \Rightarrow MSUN \Rightarrow MIP) But nevermind.....what will you put the clean solvent in? Clean Cells!

Clean Cells:

In our lab, we use three cell-cleaning strategies:

1. Water Cleaning

- Clean cell with soap, water, Chromerge or Alcoholic KOH
- rinse
- TEST every cell
- dry
- store in aluminum foil somewhere clean

2. Acetone Percolator Cleaning

- Clean cell with soap & water
- Rinse extensively
- pop on Acetone Percolation device
- Cells often dry quickly
- TEST some cells, dry and hope for the best.

3. Cell surface modification (beyond the scope of this course)

What's Clean?

We define a clean sample as one in which no dust appears when the cell is inserted in the instrument and observed *using the Argon ion laser at some fairly low angle, like 30°*. This is a stringent test: the instrument's optical magnification is about 40-100 \times , and you can view a very large volume in the instrument, even though the measured volume is usually set to much less. Particles with sizes $< 0.1 \mu\text{m}$ can be detected (not resolved, but detected). Over the years, many samples have been prepared that can be observed for many minutes without a trace of dust. While such samples do exist (and if you don't believe it, some have been retained in a kind of dustless "Hall of Fame") it is more common that *one or two* dusts will be observed eventually. Such samples often can be measured (maybe after centrifugation). If you're seeing something all the time, forget it and start over. Stay patient; some systems and cell types are harder to clean than other others, but I have never seen a system that cannot be cleaned---we always win! (Sometimes, winning means preparing just a few samples a week, but it's worth it).

Clean Solvent:

- Distilling can help (but the glassware should be tested with clean water; for example, pour water from the collection flask into a clean plastic cell and see if it comes out OK)
- Filtering can help (*always* check the chemical compatibility tables)
- Centrifugation (the last resort)

As a first attempt, see if you can just filter a great grade of solvent into one of your clean cells. For most solvents, the pore size should be 0.1 μm . There isn't much "dust" smaller than 0.1 μm . We have occasionally found smaller filters useful, but the commonly available 0.02 μm Anotop filters must be tested carefully; not all these filters work. The ones that work at all work very well. We have large batch filtration devices for making lots of clean solvent if that helps. Ordinarily, we make it in small quantities that can be prepared on a syringe filter.

Clean Polymer:

Yup.....sometimes, it really helps to "preclean" the polymer. This is particularly true of high-surface area polymers (e.g., any powdery or fibrous polymer). Your average organic chemist (and is there any other kind?) likes to pad his or her yield with lots of crud from filter paper. Pelletized solid polymers (polyolefins, for example) are sometimes fairly clean. To clean your polymer:

- Dissolve it in clean, tested solvent (at 1-2% typically)
- Prepare a clean nonsolvent (test by holding it in the laser beam) in a clean beaker
- Filter the polymer solution into the nonsolvent, using the smallest filter that does not plug or require excessive pressure.
- Vacuum dry or freeze dry. Caution: some polymers misbehave on drying.

Filter Advice:

- Never force a solution rapidly through a small filter. Polymers can be degraded by shear forces in filters. This is particularly true of large polymers--molecular weights above one million or perhaps even less for rigid, extended polymers.
- It should *almost never* be necessary to filter a polymer solution more than once! If you have to do this, it means the collection vessel is not sufficiently clean or that you picked too large a filter size. You will often see "frequent filterers" in the literature: these are people who cannot clean their cells.

Clean Solutions:

FINALLY! Take your clean polymer, weigh it, and add the clean solvent, weigh that, to obtain.....***a dusty solution!*** Oh well, the best-laid plans of mice and men fail. Usually, you will make your solutions in a volumetric or other large, screw-top glassware that will be imperfectly clean. With any luck (and you should not be in the light scattering game unless you are lucky) the dust will be of the easily removed, large variety.

Strategies vary for making a series of clean solutions, each with a different concentration:

1. Direct: If you have a large amount of polymer and many clean volumetrics, then just go ahead and make the solutions directly in the volumetrics. This is the most accurate way--especially if you are sampling the output of a large factory without regard to preserving some precious polymer.

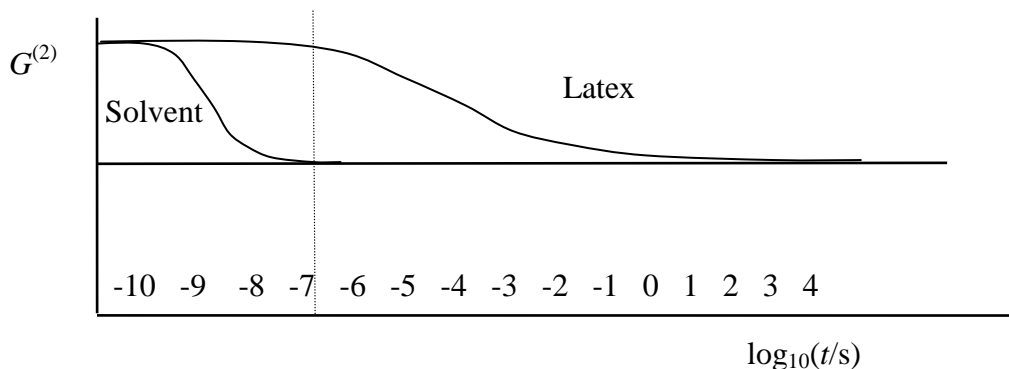
However, each solution must be separately cleaned....and you have to test that the concentration of each solution is not affected by the cleaning.

2. SuperClean Stock: Make a stock solution in a volumetric and *clean it to perfection*. You will sometimes find that the stock solution has changed its concentration during cleaning. It is possible to compensate for this by measuring the concentration gravimetrically (or spectroscopically) after cleaning. With a clean stock solution available, you can make dilutions-- either in clean volumetrics (correct) or directly in clean cells (may involve approximations).
3. Preparing dilutions in cells. The polyethylene tips on Pipetman type adjustable pipets are often quite clean (and they can be cleaned easily just in case). These adjustable pipets can simplify preparation of diluted polymer solutions from the precleaned stock. Sometimes the error of assuming volumetric addition is small. Other times, you may wish to actually obtain proper concentrations, as follows (example is for 20% c_{stock}):
 - 0) determine the stock solution concentration as c (g/mL) and w (g/g)
 - 1) weigh a clean cell
 - 2) deliver 0.200 mL of stock solution to a clean cell
 - 3) weigh
 - 4) add 0.800 mL of clean solvent
 - 5) reweigh
 - 6) compute c from the known weights

Expected vs. actual coherence values:

Still with us? If so, you have finally earned the right to measure your samples. Let's start by bracketing the problem: quickly measure the lowest concentration at lowest and highest angles; do the same with the highest concentration. You aren't going to keep these runs—just look at the data. You are looking for the coherence parameter and, roughly, the decay times. In an ocular-equipped system, you are also literally looking at the scattering from the samples to see if there is stray light, rapid twinkling or sluggish twinkling, etc.

The coherence parameter has to be compared to the *expected* value, which is what you would get with a very strong scatterer in your same solvent. Example: if you are measuring a very low-M polystyrene in toluene, try to get the coherence parameter for some high-M polystyrene in toluene (or a crosslinked latex in toluene). Let f_{max} = the f value you would measure with a *very strongly scattering* sample that exhibits no long-term decay anomalies. Examples are latex, microemulsion, and silica sphere solutions. The sample should also exhibit no very fast, short-time decays. The value of f_{max} depends on the aperture and pinhole settings, laser wavelength, beam focusing, photomultiplier dark count rate and, to lesser extent, scattering angle. The *expected* f value is reduced from f_{max} due to incoherent (on the time scale of the autocorrelator) scattering from the solvent. The figure below schematically shows the correlation functions of latex spheres and pure solvent (e.g., water or toluene). A linear y-scale and log x-scale results in sigmoidally shaped plots for a normal exponential decay. The plot is drawn for high f values (i.e., the low-time y-intercept is almost twice the baseline).



The scattering from rapidly diffusing solvent molecules only remains correlated for *very* short times, inaccessible to correlators. The limit for the LFI-1096 is 10^{-7} s, as shown; a stock ALV-5000 is limited to 2×10^{-7} s. An ALV-5000 *with the fast-card option* and the new Brookhaven BI-9000 can reach about 1×10^{-8} s. However, the solvent decay time lies still further *below* this for most normal solvents. Thus, the expected f value for a solvent is usually zero. The expected f value of weakly scattering polymer solutions will lie in between zero and f_{\max} . It can be computed from:

$$f_{\text{expected}} = f_{\max} \left(\frac{A_p^2}{A_s^2 + 2A_s A_p + A_p^2} \right) \quad <16>$$

where A_p is the scattering amplitude associated with the polymer and A_s is the scattering associated with solvent (i.e., the solution scattering is $A_{\text{total}} = A_s + A_p$). Thus, if the solution scattering is twice the solvent scattering, expect f to be decreased to 25% of its maximum value.

What if f is actually *less than* f_{expected} ? This is very valuable information! It can mean two things:

- 1) The experiment is not being conducted in the homodyne mode---i.e., there is stray light or a deliberately added local oscillator to force the heterodyne condition. Check carefully for stray light.
- 2) The polymer dynamics are too fast to capture with the correlator.

Conversely, if the measured f value is equal to the expected, it means that the correlation function has been collected correctly in the homodyne limit and that all the decay modes present have been captured.

4) The *total* acquisition time $2T$ should be something like $10^6 - 10^9 \tau$. It could be even longer in cases where very quiet data are required for the initial part of the decay (where the noise is determined by photon starvation--i.e., there are few photons per sample time for very short times). Since about 1990, when correlators became able to measure ridiculously long lag times, many investigators have relaxed the requirements on $2T$. At a bare minimum, $2T$ should be $>10^4 \tau$ (in the case of several τ values, $2T$ should exceed the *longest* τ by $10^4 \times$).

Establish Concentration Dependence:

First, we will need a parameter called k_D describing the concentration dependence of diffusion. The ALVAN program we use and/or the ALV stock routines perform a so-called “cumulants” analysis to obtain the average decay rate $\bar{\Gamma} = q^2 D_m$. The diffusion coefficient is given by:

$$D_m = D_o(1 + k_D c) \quad <17>$$

where D_o is the zero-concentration extrapolated value, and k_D is concentration dependence, which contains thermodynamic and frictional parameters. Sometimes this expression is written with more detail:

$$D = M(1 - v_2 c) \cdot \frac{\left(\frac{\partial \pi}{\partial c}\right)_T}{N_a f_o (1 + k_f c)} \quad <18>$$

where $(\partial\pi/\partial c)_T$ is the osmotic susceptibility, N_a is Avogadro’s number, M is the molecular weight, v_2 is partial specific volume of the polymer, f_o is the friction coefficient for the polymer, extrapolated to zero concentration. For a derivation, see Yamakawa: "Modern Theory of Polymer Solutions". For still another form of this equation, see Eq. 17 of Varma et al., which is derived using an expression for $(\partial\pi/\partial c)_T$ which you should look up in, for example, Yamakawa's book or Tanford's.

In any case, our goal here is not to *worry* about the dependence of diffusion with concentration; this is a research project. Our goal is to verify that the effect really exists and then to "extrapolate it away," which is the standard approach in polymer analysis. By measuring the diffusion coefficient at several concentrations, the value extrapolated to zero concentration (where there is no thermodynamic interaction or interference from neighboring polymer molecules in solution) contains just information about the molecule. Specifically, the zero-concentration diffusion coefficient is related to molecular weight by:

$$D_o = \alpha M^{-\beta} \quad <19>$$

where α and β reflect the dimensions and scaling properties of the polymer, similar to the Mark-Houwink intrinsic viscosity parameters. It should be noted that these parameters are not very well-determined in the literature at the low molecular weights of present concern.

Conversion of Decay Rate Distributions to c vs M results:

This step is somewhat optional. You can try it if your μ_2/Γ^2 parameters exceed about 0.3. In that case, we recommend you do the Milk exercise that follows. Then go to the Appendix on Laplace Inversion. Following steps similar to those for the milk experiment, you can come up with your own c vs M distribution. You have the necessary k_D value. You can assume stuff about shape and the X factor relating R_g to R_h . It comes down to an Excel operation on the Amplitude vs Γ data after that.

IIIc. A Third Solo Experiment: Dynamic Light Scattering Of Milk—DIY Data Fitting for Multiexponentials

It is not always realized, especially by chemists, that a really important thing about macromolecules is that they are *big*. Colloids are also big, but they are not necessarily molecular. In this exercise, we are using DLS to measure the size distribution of a common colloid: milk. Materials safety data sheets are not required, except if you suffer milk allergy and plan to drink the supplies.

What we are going to do is measure an autocorrelation function, as we already have for latex spheres and for polystyrene. Whereas latex particles are very uniform in size, yielding a single exponential decay, and commercial polystyrene is reasonably uniform, yielding a correlation function that might be handled by cumulants, colloidal milk contains broadly polydisperse particles of fat bound up with proteins and other molecules. The correlation functions will have pronounced nonexponentiality. Two “Laplace inversion” routines will be used to sort out the decay spectrum, and then you will be asked to make a conversion to a size spectrum on your own, making the appropriate corrections for particle form factor, assuming a spherical particle shape. Your answer can be tested against the “crude but effective” software that we use in our own research.

You are reminded that the main quantity of interest in DLS is the electric field autocorrelation function, $g^{(1)}(t)$ which for a polydisperse sample consists of a sum of exponential decays:

$$g^{(1)}(t) = \sum A_i e^{-\Gamma_i t} \quad <20>$$

An amplitude A_i is proportional to molecular weight and concentration of species i , modified by the particle form factor for sufficiently large particles:

$$A_i \sim c_i M_i P(qR_{g,i}) \quad <21>$$

At infinite dilution, a decay rate Γ_i is related to the diffusion coefficient (hence, hydrodynamic radius) of that particle and the scattering vector:

$$\Gamma_i = q^2 D_o = \frac{q^2 kT}{6\pi\eta_o R_{h,i}} \quad <22>$$

where Stokes' law was used in the last equation to relate D_o and hydrodynamic radius R_h through the viscosity η_o . In the present experiment, we will assume we are at infinite dilution.³

The particle form factor depends on shape; you must know it (or assume it) and then refer to tables (for example, in Kratochvil chapter in the famous book by Huglin). Also, in order to obtain $P(qR_{g,i})$ one must convert from $R_{h,i}$ to $R_{g,i}$. This conversion also requires the assumption of a shape. For solid, spherical particles ($R_h = R$) of uniform density, we can write:

³A possible “cure” if one is not really at low enough concentration would be to make a k_D correction, assuming that k_D does not depend strongly on molecular weight and that the overall polymer concentration can be used.

$$R_{g,i} = XR_{h,i} \quad <23>$$

where

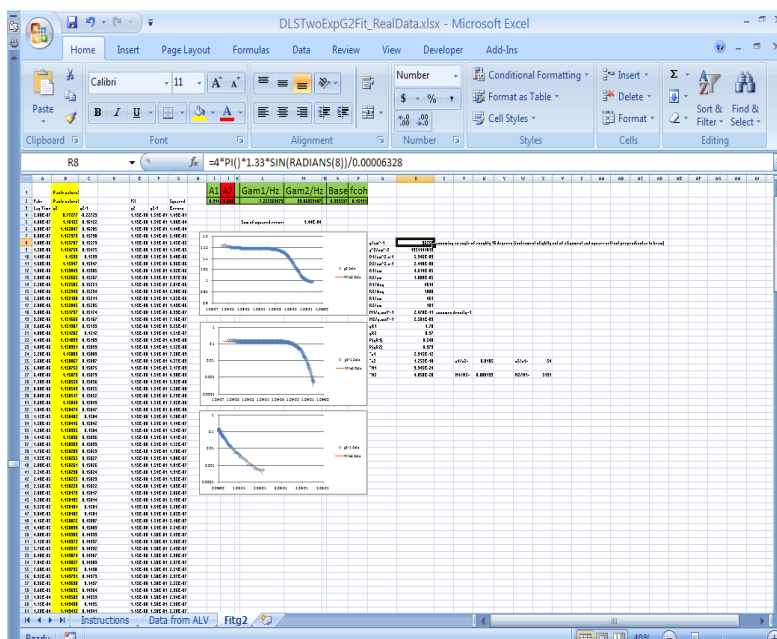
$$X = \sqrt{3/5} \quad <24>$$

You will prepare a milk suspension, reasonably free of dust, and measure it, probably at $\theta = 90^\circ$ (depending somewhat on wavelength). We will supply $g^{(2)}(t)$ vs t data by e-mail or USB key.

WHAT YOU MUST DO

1. Do It Yourself Data Fitting

You should try to obtain the distribution c vs. R_h yourself. One way to do this is to write a short program that first reads and then plots the experimental $g^{(1)}(t)$ data on screen. Add a little routine that generates a fitted data set according to Eq. 1, where you supply the values A_i and Γ_i manually. You could plot this fitted line in a different color easily enough (see programming examples from beginning of course). In a Visual BASIC implementation, it would be simple enough to use menubars to adjust the A 's and Γ 's. It is also quite possible to use Origin or a spreadsheet to good advantage for these same purposes. In Origin, look under the Fit/Parameters-Simulate option. For day-to-day use, this is slow compared to a purpose-built program, but it's OK for what you are doing in this exercise. I encourage everyone to at least try to write their own program. "Canned" graphing packages are great until you have to use them again and again. Then their general purpose baggage becomes too much. An intermediate solution is using the scripting option of Origin or Excel. A screenshot from an Excel worksheet using Solver to fit two exponentials is shown below. We can provide a copy of this, but it's a better challenge to write your own, starting from the Solver HowTo (<http://macro.lsu.edu/HowTo>).



The best approach is to fit A vs. Γ first. There is no way to tell *a priori* how many exponentials this will take. Make a good guess at Γ by inverting the lag time at which the

correlation function is actively decaying and try to fit the function with just one exponential. Semi-log plots ($\ln(g^{(1)}(t)$ vs. t) can be very helpful. Then keep adding exponential decay terms until the randomness of the residuals no longer improves. More than 3 or 4 exponentials will probably not be required.

After you are satisfied with the fit quality, you can worry about converting the Γ 's to R_h 's and, subsequently, R_g 's. Then you can convert the A 's to c 's. Well, actually you cannot directly obtain the c 's: you lack the constant of proportionality in Eq. 14. So, what you will do is divide through the amplitude A_i by $M_i P(qR_{g,i})$:

$$c_{i,\text{pseudo}} = A_i / M_i P(qR_{g,i}) \quad <25>$$

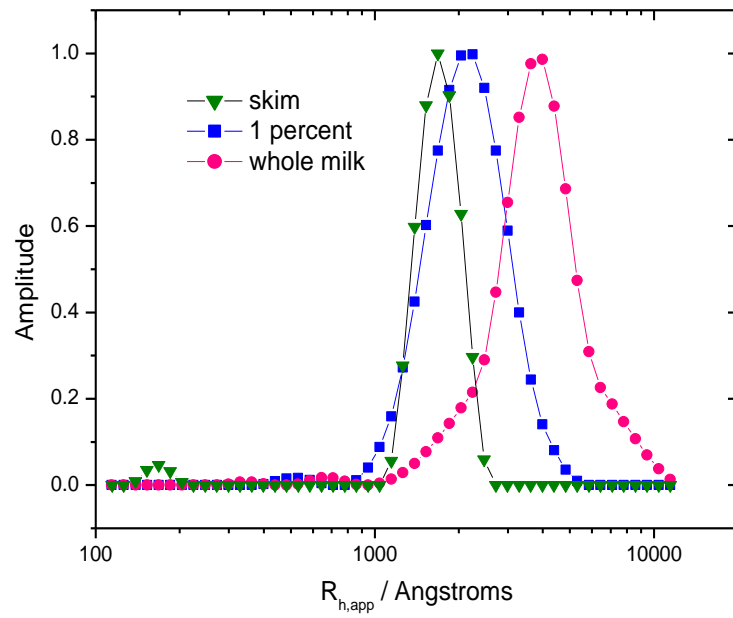
where $c_{i,\text{pseudo}}$ is proportional to the concentration. But you must do something to estimate M_i from R_h . To really do this right, you would need the density. But on the assumption that the density of all particles is the same, you could just replace M_i in Eq. 18 with $R_{h,i}^3$. However you do it, you should discover that fitting data this way is a very dicey business! It will be interesting to see how close you can all come to each other. A student will be appointed to gather the results and make this comparison.

2. Use our Data Fitting

We have several programs that reliably give fits to polydisperse data. *After you have successfully fit the data and demonstrated your own method to me*, you can use our software to see how close you came to the right answer. Appendix 1 tells a little about how our software works, and it gives good advice for the use of inverse Laplace transforms to fit DLS data. It is *not* as simple as pushing the CONTIN button on some commercial instrument.

3. Milk Results

In June 2009, an old friend asked me to look into the distribution of milk. Here are some results based on scattering at a single angle (always a bad idea) of 30 degrees from diluted milk in 3 grades. This is an easy experiment to do; comparisons to the Malvern zetasizer, which measures at 173 degrees, showed somewhat smaller sizes. An advantage of the Malvern, though, is that you do not have to dilute the milk at all. It's clear that DLS can detect the difference in grades of milk; how accurate these distributions are is another matter entirely.



Appendix 1: Data Fitting in DLS—Getting a Decay Rate Distribution

Overview: Some of this is covered in the main part of the document; here we attempt a little extra detail...and inverse Laplace transformation.

Linear Fitting: The simplest cases are latexes or other nearly monodisperse scatterers. These are easily fitted using the usual linear fitting routines found in the classic book, *Data Reduction in the Physical Sciences*, by Bevington, or in the more modern but not necessarily better book entitled *Numerical Recipes* by Press, Flannery and Vetterling. Our programs use Fortran code from Bevington, converted into QuickBASIC or PASCAL in some cases.

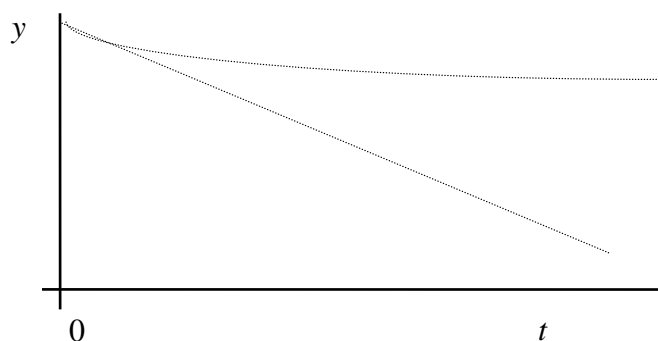
Equations 2 and 3 can be combined to read:

$$G^{(2)}(t) = B(1 + f e^{-2\Gamma t}) \quad <26>$$

This is linearized to:

$$y = \ln(G^{(2)}(t) - B) = \ln(Bf) - 2\Gamma t \quad <27>$$

Thus, a plot of y vs. t has slope -2Γ , from which D_m can be extracted, since $\Gamma = q^2 D_m$.



The figure shows the ideal, linear behavior as a straight line. Polydisperse samples look like the curved line instead. The initial slope is called the “first cumulant” and given the symbol $\bar{\Gamma}$ because it represents the average decay rate (You should try to convince yourself that $\bar{\Gamma}$ really does represent the average decay rate). In order to get the first cumulant, a polynomial fit is performed on the y vs. t curve. This is called cumulants analysis; the *order* of the cumulants analysis means how many terms: a first order cumulant analysis just fits a straight line, a second order analysis a quadratic, and the third order analysis a cubic, etc. In practice in our laboratory, we commonly take the first cumulant from third cumulants analysis (confusing, isn’t it?). If the third cumulant analysis does not well represent the data, it is time for something more sophisticated (see below).

Cumulants were introduced by Koppel, who showed that $\bar{\Gamma}$ was proportional to the z-average of the diffusion coefficient:

$$\bar{\Gamma} = q^2 D_z \quad <28>$$

where

$$D_z = \frac{\sum n_i M_i^2 D_i}{\sum n_i M_i^2} \quad <29>$$

I will leave it to you as an exercise to figure out which average of hydrodynamic radius is obtained! Note: it is not the simple z-average as is often claimed in the literature by people who don't know better. People write Koppel's expression differently; I write it like this:

$$\ln(g^{(1)}(t)) = -\bar{\Gamma}t + \frac{1}{2!} \mu_2 t^2 + \dots \quad <30>$$

(As of this writing—March 2012—a Wikipedia article has an error in its cumulants expression). The term μ_2 is called the second cumulant. For a perfect single exponential decay, it would be near zero. Like the first cumulant, the value one obtains for the second cumulant of nonexponential recoveries varies a little bit with which order of fit you are using. (One of the disappointments of cumulants fitting is how sensitive the second cumulant can be for weird decays). A measure of the polydispersity of the distribution can be given in terms of the unitless quotient $\mu_2/\bar{\Gamma}^2$. DLS people sometimes call this the polydispersity parameter or “normalized variance.” In most of our programs, it is called POLYD

$$\text{POLYD} = \mu_2/\bar{\Gamma}^2 \quad <31>$$

POLYD is hard to measure well, involving as it does a ratio of two quantities that themselves depend a bit on the order of fit. As a rule of thumb, if $\text{POLYD} > 0.3$ it is time to consider another approach. Cumulants analysis should be reserved for data screening (e.g., the program ALVAN) and for nearly single exponential decays.

We have been doing cumulants analysis for a very long time, but we still continue to learn. The arrival of the ALV correlator, with its very wide ranges of lag times t , has obliged us to be more careful about how data are weighted for noise while doing cumulants. Also, with any cumulants package, it is essential to delete some channels near the tail: some of these will go negative when the baseline is subtracted, and log(negative) operations are not well liked by computers. For this reason, some people choose to fit a baseline, which converts the cumulants approach into a nonlinear problem. Plusses and minuses to that—surely, the baseline will be well fit, but other artifacts of fitting may pop up.

Nonlinear Fitting: In cumulants analysis, the parameters of interest ($\mu_2, \bar{\Gamma}$) appear as linear coefficients of the independent parameter, t , in eq. <30>. Cumulants fitting is like a small perturbation applied after a huge linearization operation. More generally, we might try to fit $G^{(2)}$

or $g^{(1)}$ directly. If $g^{(1)} = \sum A_i e^{-\Gamma_i t}$ then we could look for A_i and Γ_i by trial and error. The

Marquardt algorithm (see Bevington) makes this process as rational as possible. In this algorithm, one makes initial guesses at the A 's and Γ 's. The program looks in a semi-intelligent fashion for better parameters--i.e., ones that reduce (but perhaps do not really minimize) the difference between fitted and actual data. The parameter χ^2 is monitored to assess the progress:

$$\chi^2 = \frac{1}{\nu} \sum_1^N w_i (y_i - y_{fit,i})^2 \quad <32>$$

The function y_{fit} is a multiple exponential whose amplitudes and decay rates are adjusted. It is evaluated at precisely the same t_i where the experimental data were evaluated. The symbol ν represents the number of degrees of freedom, approximately the same as the number of data points, N . The statistical weight w_i is the inverse, squared uncertainty of each data point: $w_i = \sigma_i^{-2}$. The meaning of χ^2 is this: when it is unity, the errors of fitting are comparable to the uncertainty in the measured data. The data have been fit to within their reliability, using the model function y_{fit} . The simplest trial function y_{fit} to produce this desired result is preferred.

Be careful interpreting χ^2 ! A lot of students think that high χ^2 means something is wrong with the data. This is one possibility, but not the only one! The χ^2 parameter is the result of data quality, data noise and the adequacy of the fitting function. Suppose you have a genuinely nonexponential decay, but are fitting with just a single exponential term. Then, a high χ^2 value doesn't necessarily mean anything is wrong with the data. It may mean you are just using the wrong fitting function. In that case, better data (lower σ) will increase χ^2 . If $\chi^2=1$ the data are fit by the model to within the noise. A legitimate use of χ^2 is when comparing multiple runs of similar quality. If a particular run has a χ^2 much higher than the others, then that run may be defective. If all the multiple runs have similar χ^2 values, but these values are high, it possibly means that a better fitting function must be selected.

One should never forget that nonlinear fitting is prone to give false minima. There is actually a hyperspace where χ^2 could be plotted against many parameters (for example, two A 's and two Γ 's in a two-exponential fit). There is some particular combination of A 's and Γ 's that really produce a minimum χ^2 ---but there could be lots of *local* minima. To avoid getting stuck in a local minimum, the initial guesses are varied and one sees if the Marquardt algorithm will steadfastly return the same "best" values. If it does, then it is assumed that these fitted parameters really do describe the data. *This is quite a different situation than linear fitting, where the best parameters of fit are determined analytically!*

The raw data $G^{(2)}$ may have lots of decaying exponential terms, in general. For example, if $g^{(1)}$ has two terms ($g^{(1)} = A_1 e^{\Gamma_1 t} + A_2 e^{\Gamma_2 t}$) then the active part of $G^{(2)}$ (which is just $|g^{(1)}|^2$) must contain *three* exponentially decaying terms. They have amplitudes A_1^2 , $2A_1 A_2$ and A_2^2 with decay rates, respectively, of $2\Gamma_1$, $\Gamma_1 + \Gamma_2$ and $2\Gamma_2$. However, the three decay rates are not independent. If $g^{(1)}$ contains just two exponentials, and you fit $G^{(2)}$ to three exponentials, then the decay rates of those three exponentials *should be* tied together. Sometimes, they will not be: this either

indicates that $g^{(1)}$ does not contain just two exponentials, or that some error in measurement has occurred. A good test for two exponentials (and no more) is to fit $g^{(1)}$ to two exponentials and $G^{(2)}$ to three exponentials. A consistent set of Γ_1 and Γ_2 should result.

Our home-brew multi-exponential, nonlinear fitting software is called MARLIN (for the ALV, MARLINA). Nevermind why. It's a slight adaptation of the routine CURFIT found in Bevington. Since it only fits sums of exponentials, one must specify a baseline if one wishes to fit $g^{(1)}$. The program easily handles fitting $G^{(2)}$ but, except possibly in the case of a bimodal sample, the decay rates from $G^{(2)}$ are difficult to interpret. We have not been using these routines lately, as the ones supplied by ALV itself seem good. These routines, or variants of them, are used in the FPR apparatus.

Laplace Inversions:

In many cases, a near-continuum of scatterers is present. For example, in a polycondensate with $M_w = 200,000$, the individual species may differ in molecular weight by, say, 100. Instead of writing $g^{(1)}(t) = \sum A_i e^{-\Gamma_i t}$ we can write, to a good approximation,

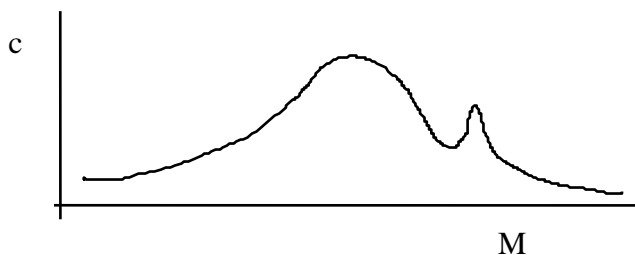
$$g^{(1)}(t) \approx \int_0^{\infty} d\Gamma A(\Gamma) e^{-\Gamma t} \quad <33>$$

The big question is: knowing $g^{(1)}(t)$, can we obtain $A(\Gamma)$? The two functions are Laplace transform pairs, and the process of inverting $g^{(1)}(t)$ to get $A(\Gamma)$ is called Laplace transformation. The *idea* is similar to Fourier transformation in that functions in reciprocal variables are involved, but the actual process is *not* like Fourier transformation because uniquely best answers are very difficult to obtain. Fourier transforms are easily computed using the famous Fast Fourier Transform algorithm. (In a modern programming language like LabView, just wire your signal to the FT icon—voila!) Provided that sampling theorem considerations were respected when the measured data were taken, the FFT is not very sensitive to noise and consistent results are obtained. The Laplace transform process fares worse: noise, even a very small amount of noise, really leads to problems. Also, unlike Fourier transformations, no single, universally-accepted, fast, efficient algorithm exists for Laplace inversion. Indeed, there are still a few DLS researchers who think Laplace inversion is a black art and even statistically unsound! This view is probably extreme, but Laplace inversions really should be scrutinized and double-checked.

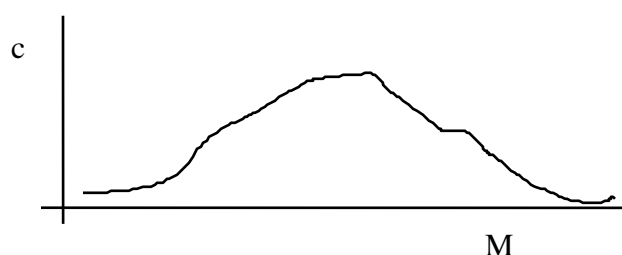
Despite the frequent existence of multiple exponential decays in a variety of natural and measurement processes, it was not until the late 1970's that the situation was understood. The paper by Ostrowski *et al.* contains a nice discussion of the original work of McWhirter and Pike. The essential discovery of these workers can be summarized:

If the data in t -space (the correlation function we can measure) contain any noise, then the information available in Γ space (i.e., the function $A(\Gamma)$ that we desire) is limited to low resolution: fine details of $A(\Gamma)$ will be extremely difficult to obtain.

Remember, $A(\Gamma)$ is really $c(M)$ because A converts to c and Γ converts to M , with a series of approximations. The implication is that, if the *true* distribution looks like this:



we might be very lucky to measure something instead like this:



If you think in Fourier series terms, the sharp little “blip” in the high- M side of the true distribution requires some *high frequency* components. In this context, frequency means that lots of M values would have to be included in our sampling of the distribution: we would have to know the difference between $c(M)$ and $c(M+\delta M)$ where δM is small. Although the basis set of Laplace inversion is not simple sine or cosine terms, the frequency analogy is still apt: the high-frequency terms required to correctly describe the “blip” are simply not available if $g^{(1)}(t)$ is “noisy”---and it doesn’t have to be that noisy!

Pike and McWhirter liken the information retrieval process to image formation by a lens. Bob Detenbeck (U. Vermont) makes a wonderful analogy to audio reproduction. Trying to determine $A(\Gamma)$ from $g^{(1)}(t)$ is like trying to decide the type of violin being played by a gifted soloist from an AM radio broadcast: the high-harmonic overtones that you require to distinguish one violin from another are *simply not present* at any level above the noise (static) of the broadcast. The reason is that AM radio has a severely limited bandwidth: frequencies above, say, 5000 Hz are not reproduced. However, noise is present--including some components above 5000 Hz. The correlation function is like a bad radio or amplifier: it just cannot transmit the high-frequency components with any significant amplitude. They get buried in the noise. If you try to guess the high-frequency components (i.e., finer details of the distribution) you will often get the wrong answer--because your “guessing” is based on the noise as much as the real signal. Similarly, if you try to guess whether Yitzakh Perlman is playing his Stradivarius or some other violin, while listening to an AM radio broadcast, you are likely to get the wrong answer. You may be able to discern that a violin, and not a viola, is being played. In DLS/Laplace transform, you will be able to tell that molecules are “big” or “little.” Perhaps more sometimes. Don’t expect much and you won’t be disappointed.

In Fourier analysis, one obtains the amplitudes of sine or cosine functions that, added together, give the waveform of interest. The sine functions have frequencies like γ , 2γ , 3γ , etc.--i.e., the frequency of the functions to be superposed linearly increases...but it is discrete. The essential bit of information for the Pike-McWhirter analysis of the Laplace inversion operation is that there is just no point seeking such detailed information. It is still possible to represent the distribution using a discrete set of (exponential) functions. But the decay rates of the exponential functions whose amplitudes we seek should be spaced farther apart. Instead of looking for $A(\Gamma)$, $A(2\Gamma)$, $A(3\Gamma)$, we should space the decay rates evenly *in a logarithmic space*. This can be expressed:

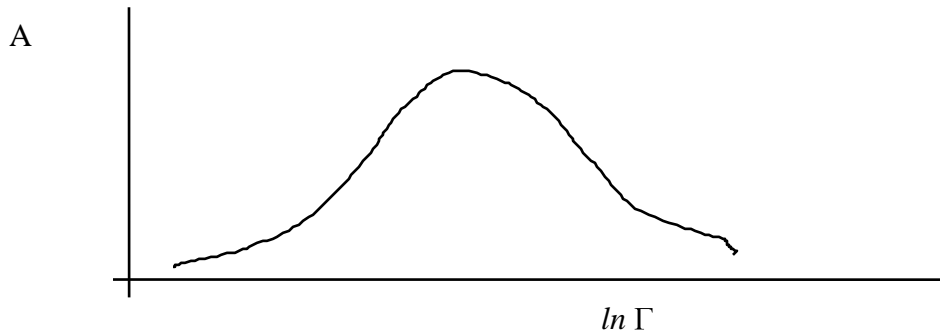
$$\Gamma_{i+1} = \Gamma_i e^{\pi/\omega_{\max}} \quad \langle 34 \rangle$$

or, equivalently:

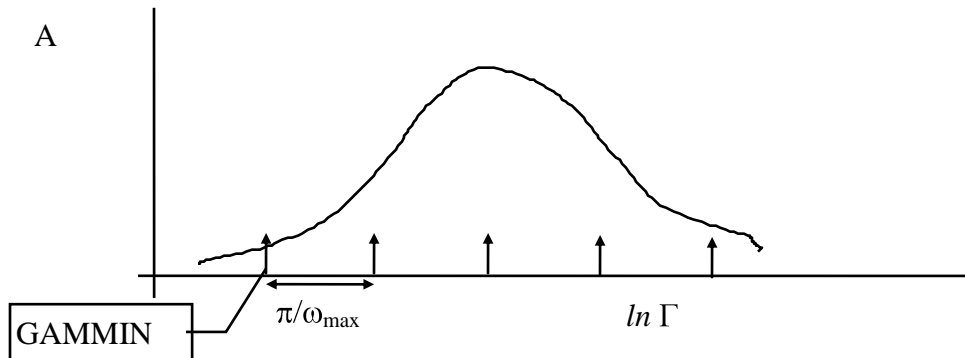
$$\ln \Gamma_{i+1} = \ln \Gamma_i + \pi/\omega_{\max} \quad \langle 35 \rangle$$

The parameter ω_{\max} is set empirically according to the noise level: for less noise, use a higher ω_{\max} so that the distance, in log space, decreases. This corresponds to more resolution. For noisier data, do not attempt such resolutions. Decrease ω_{\max} so that the distance between functions (they are called “grid points” as you will see below) increases.

The suggestion of McWhirter-Pike is to *sample* the true distribution, using a discrete number of grid points (exponentially decaying functions) whose decay rates are *exponentially* related. They called this *exponential sampling*. Suppose the *true* distribution looks like this:



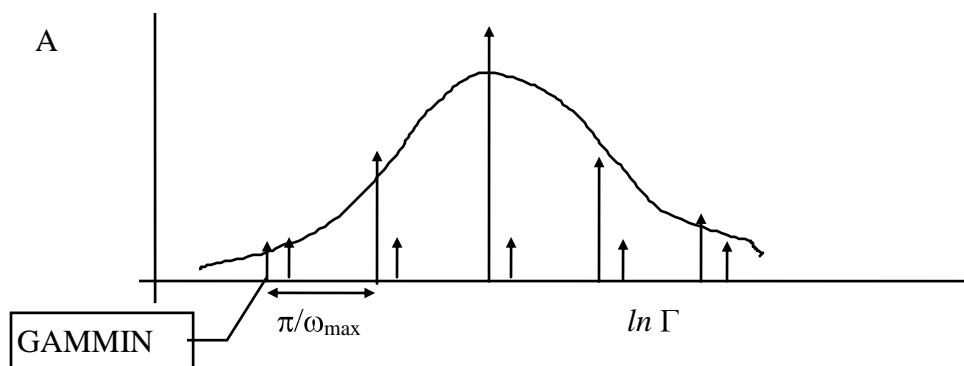
How would you sample this function? First, you would have to define the minimum and maximum ranges over which to seek solutions. Call these GAMMIN and GAMMAX; they set the RANGE of the inversion process. Next, you would select ω_{\max} and select some “grid points” that fall in-between GAMMAX and GAMMIN. The number will usually be something like five.



The vertical arrows show the evenly spaced grid points. The next step is to determine how long the arrows should be, according to the only information we have about the true distribution, which comes in the form of the imperfectly measured correlation function. This is done by taking the function $\sum A_i e^{-\Gamma_i t}$ where the Γ 's are now specified by Eq. 27 and fitting it to the data

by finding the best A 's. Since the decay rates are fixed, this is a *linear* fitting problem. There would be no point trying to float five decay rates and five amplitudes; that should just about fit any decaying function and might violate the McWhirter-Pike guideline, since floating decay rates could float very close to each other.

One thing may bother you. Five little grid points do not look very much like a continuous distribution. There are two solutions to this. The first is to use an interpolation formula (see Ostrowski paper). The second is to *shift the grid points*. In practice, we use the latter option. The whole set of grid points is shifted by a distance $\pi/5\omega_{\max}$ (there is nothing magical about the number 5). The new grid is shown below, and the lengths of the original grid point arrows have been extended according to the best fit obtained. Note that the arrow lengths do not exactly match the true distribution--that's the result of noise. But remember, in practice, you do not know the true distribution!



Now the process is repeated. The new grid point arrows are lengthened, according to the best fit for that grid. Then there occurs another shift by $\pi/5\omega_{\max}$, another linear best fit, etc. Eventually, the true distribution is approximated.

But how do you select ω_{\max} and thereby control the number of grid points? And, for that matter, how do you set GAMMAX and GAMMIN? The answer is to set GAMMAX and GAMMIN very liberally at first---include decay rates that are too fast and too slow. Set ω_{\max} to some low value, like 3. Try to fit the data. Probably, the best fit will contain some negative amplitudes. This is physically unrealistic! Molecules cannot have negative molecular weights or concentrations, so we enforce a *nonnegativity constraint*. To do so, delete those grid points from the fit and repeat (some exponential sampling algorithms do a bit better and re-arrange the grid). In this way, the physically meaningful RANGE will be identified. Then try to add as many grid points as possible by raising ω_{\max} . If ω_{\max} is too high, your fit will again respond to the noise, not the real $g^{(1)}$ signal, and you will have to back off and/or decrease your range.

If this process sounds laborious, it is! We may still use it, however, to “scope out” an inversion in preparation for *automated Laplace inversion routines*. The most important of these is the Fortran program CONTIN, written by S. Provencher, an American emigre’ to Germany. Provencher’s program is a standard for the DLS crowd, and it is used elsewhere too (e.g., fluorescence and DOSY NMR communities). It does not rely on sequential stepping as does exponential sampling and, therefore, is capable of returning narrower distributions. Actually, CONTIN generates up to 12 different answers and then automatically chooses the one it thinks is best. To CONTIN, “best” means the *least detailed* distribution that is consistent with the data. It makes this choice based on statistical estimates (pretty vague statement, eh?). Some more detailed distributions will be obtained and some less detailed ones too. The user should always inspect all of them. Our software makes this easy to do. CONTIN competitors include exponential sampling (but there is no standard, and everyone writes their own program; ours is quite good). Other CONTIN competitors are based on the Maximum Entropy approach; these have received mixed reviews.

Another thing should be mentioned. As usually used, CONTIN and also our own version of exponential sampling, called EXSAMP, do not just minimize χ^2 . Rather, they minimize a modified χ^2 where a term has been added to penalize fits where adjacent grid points produce dramatically different A values. Thus, unrealistically sharp variations in amplitudes are reduced. This is called enforcing *parsimony*. This is discussed in two articles from our lab, and in many other places.

As already mentioned, Laplace inversion has its detractors. Most people who look at the problem are amazed that Laplace inversion cannot be done with excellent resolution. For example, 32 grid points is a commonly used CONTIN configuration. The number of data points collected is usually much more---perhaps 272. To the uninitiated, it may seem that 32 parameters might be fitted to 272 points without so much trouble. Well, it ain't so. On the other side of the spectrum are experienced dynamic light scatterers who think that CONTIN, exponential sampling, etc. are all *too much* detail, and that one should stop with cumulants, double or perhaps triple exponentials, or stretched exponentials (we haven't discussed this option). These people claim those 32 functions give way too many adjustable parameters, that it defies logic and statistics to use so many, etc. I think that position is extreme, too. CONTIN and exponential sampling algorithms attempt to take advantage of the solid theoretical work of McWhirter-Pike, which defines about how much you can expect (i.e., not much!). The programs attempt to construct a logical, repeatable method to take advantage of what is available. With parsimony, these functions do not really overfit the data as badly as it may seem; the amplitudes are tied together because the objective is to minimize the *modified* χ^2 . As sometimes happen, the intermediate position is best: use Laplace inversion programs, but use them with great caution and respect for the fact that resolution is inherently poor.

Some guidelines (from experience)

- Exponential decays cannot be resolved unless the two decay rates differ by more than a factor of about 2.
- Low-amplitude peaks are especially suspect.
- Always confirm by applying two inversion routines.
- Always apply multiple exponential fits in addition to Laplace inversion.
- Don't feed these programs bad data!
- Always investigate the effects of modest baseline changes on the distribution.

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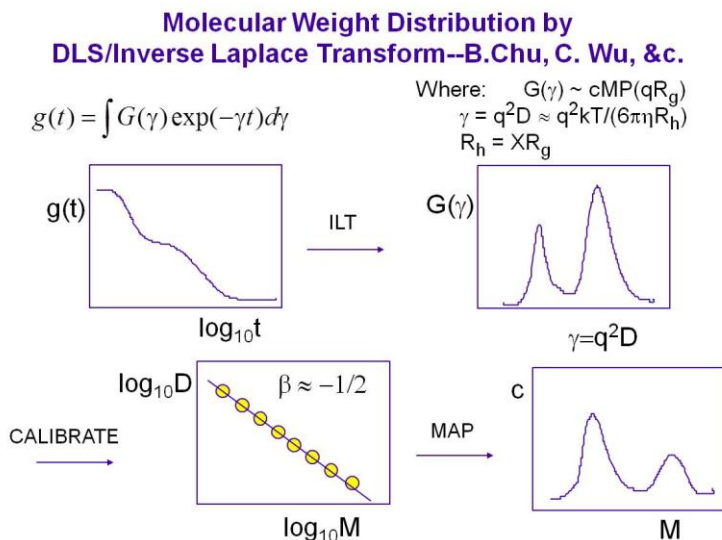
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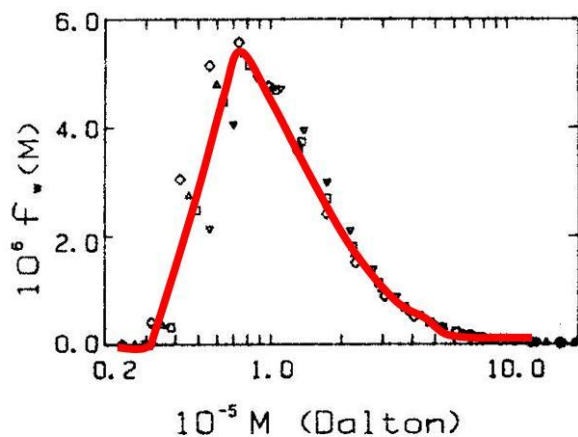
Appendix 2: Extracting Molecular Information from the Decay Rate Distribution

This section under development—at the moment, a drop point for resources from PowerPoints etc.



Hot Ben Chu / Chi Wu Example

Macromolecules, 21, 397-402 (1988)



MWD of PTFE
Special solvents
at $\sim 330^\circ\text{C}$

Problems:

- Only “works” because MWD is broad
- Poor resolution.
- Low M part goofy.
- Some assumptions required.

Appendix 3. Description of Available Software

This section under development.

ALVAN Suite

Laplace Suite: Laplace & Pltagam

ANSCAN (for FPR, but....)

2EXP simulate Excel

2EXP real data Excel

Appendix 4. Functions and Settings on the LFI-1096 Correlator

This section is mainly of historical interest, but it may help students nail down what an autocorrelation function is and how it could be measured...oops, *estimated!*

We discuss the LFI-1096 because it's simpler than the more modern ALV correlator or the correlator.com multicorrelator, which work by actually performing multiplications (I think) while the LFI1096 and similar BI9000AT perform multiple additions using an add command generator and shift register. Many of the same principles will apply to all instruments, so reading this section is important even if you won't use the LFI-1096 (you'd have to *find* it first!). The LFI-1096 is a *linear* correlator (it has some weird modes, too, but we ignore these for now). As already discussed, its function is to approximate the integral:

$$G^{(2)}(t) = \langle I(0)I(t) \rangle = \lim_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^T I(t') \cdot I(t'+t) dt' \quad \langle 9 \rangle$$

First, you must understand that the LFI is usually used as a *digital* correlator: it detects and counts discrete photon events. You connect the output of the photomultiplier tube (PMT) first to a preamplifier/discriminator (PAD), and then to the correlator:

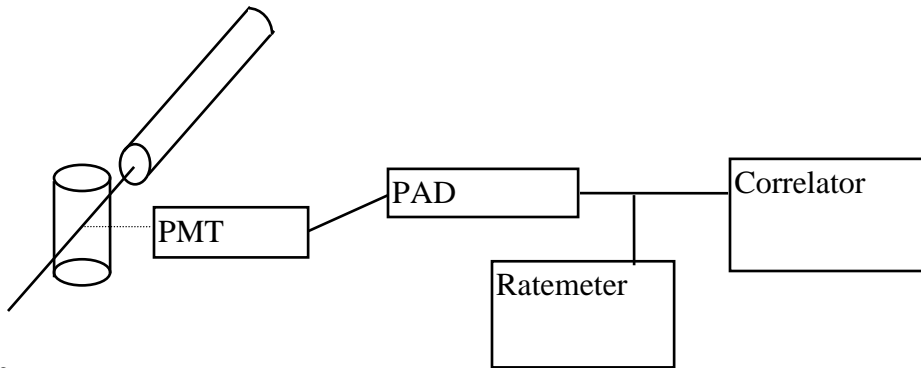
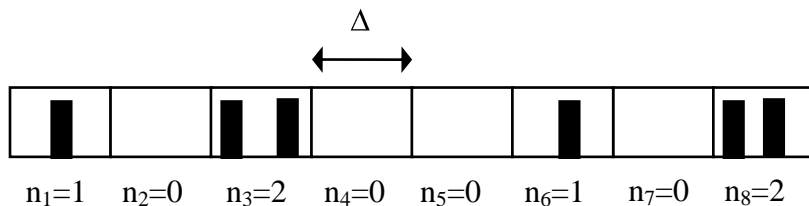
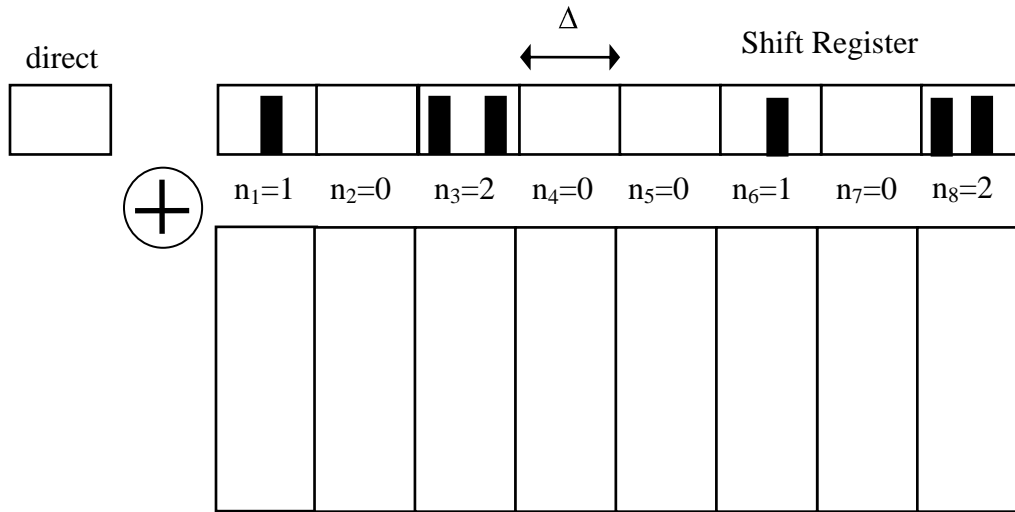


Figure 3

So, Figure 1, showing a continuous variation of intensity, fails to represent the true situation. If we divide the time axis into discrete packets, all separated by a time interval Δ then we really measure a pulse train coming out of the PAD.



When building the average correlation function at lag time $i\Delta$, products of the photocount samples of all times separated by $i\Delta$ are generated. The natural way to do this would seem to be to collect data for awhile, store it in a big array and then use a software algorithm to create the correlation function. This is called “batch processing” and no good correlator actually works this way (it is too inefficient; too much time devoted to computing and not enough to measuring). Instead, *real time correlators* are used, in which the data stream is pipelined down a shift register. The shift register contents move one channel to the right with each clock cycle, Δ . The contents of each shift register element can be added to memory *every time* a new pulse comes into a direct channel (the direct channel represents the “present time”). In the figure below, we have added the direct channel and memory to the time diagram already shown, which now represents the shift register.



Also shown in the figure is the add command generator (the circle with the + sign in it). Whenever a pulse is detected in the direct channel, the each element in the shift register is added to the appropriate channel. For example, a pulse detected in the direct channel will cause the number “two” to be added to the memory associated with channels and 8. The memories of channels 1 and 6 would be increased by one. All the other channels do not change. If *another* pulse comes into the direct channel, the memories are again increased. Each time a new pulse arrives in the direct channel, the memories are increased. You can see this corresponds to multiplication of pulse counts, separated by times. For example, suppose 3 pulses arrive in the direct channel during some time period of duration Δ . The memory contents of channel 3 would be incremented by $3 \times 2 = 6$. This would be done by three separate additions. After a time Δ has expired, the data are clocked down the shift register, which now looks like this:



Now, with each new pulse in the direct channel, these data will be added to their respective memories. Thus, the products in the correlation function $\langle I(0)I(t) \rangle$ are built by successive additions $n(0)n(t)$. The process is approximate because Δ is finite, because not all photons

produce pulses (PMT's are not that efficient), because some pulses are false (arising from within the PMT and not having to do with light). For a detailed account of the approximations made in assembling a digital photocount correlation function in this way, see the textbook by Chu. A few points are all we need to stress.

- It is clear that the ability of the direct channel to detect a rapid stream of pulses and the ability of the adders to add them to memory must be very high.
- The “shift” operation of the register must be fast and free of losses.
- Ideally, the shift register should hold very large numbers, but in practice it is limited. For example, the LFI-1096 uses a 4-bit shift register: numbers in each element can range from 0 to 15. Thus, if Δ were set to 0.1 s, the maximum average count rate would have to be much less than $15/0.1 \text{ s} = 150 \text{ Hz}$.
- Since some photomultiplier tubes produce dark count rates this high, “prescaling” may be required: only count every other pulse, every fourth pulse, every eighth pulse, etc.
- The total “time window” is the number of channels times Δ . Extending the time window to handle five decades of time would require 100,000 channels---i.e., prohibitively expensive.
- You must be sure to set Δ to capture the decaying process, and hope that you can capture all decaying processes in the available number of channels (which is 272 on the LFI-1096).

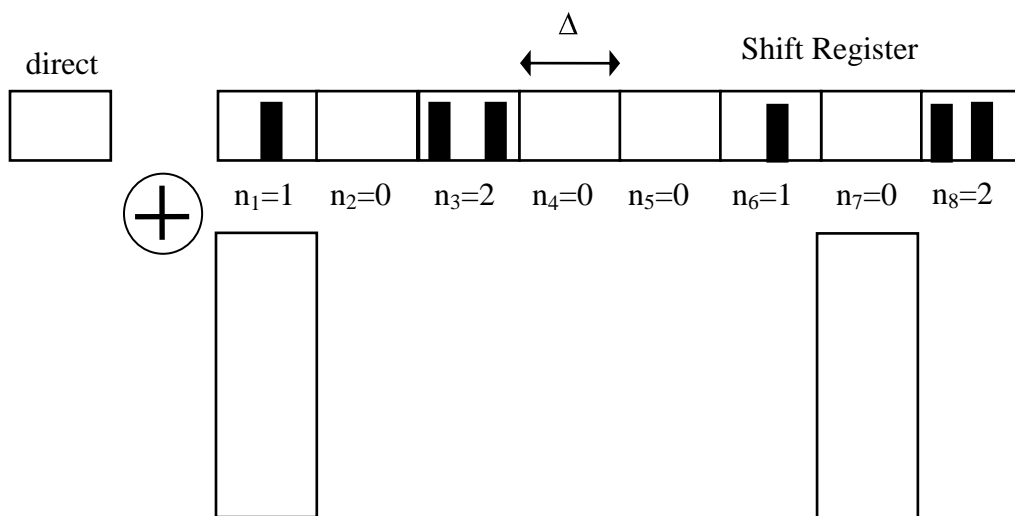
These guidelines make for decent correlation functions with the 1096 or similar correlators.

- Set the decay time first! Try to make sure you can capture all the processes. If the baseline is not laying down flat, use a different correlator (e.g., the ALV).
- Try to keep the photocount rate to about $1/\Delta$. If the count rate greatly exceeds $1/\Delta$ the natural fluctuations in the intensity will ensure that the count during some sample times will exceed 15--i.e., you will overflow the shift register. This can cause great problems with analysis, so avoid it! If the coherence factor f is small, you can exceed this rate somewhat because the fluctuations in intensity will be small then.
- We usually keep the average count rate under about 400,000/s, even if this is not yet $1/\Delta$.
-
- There are quite a few other tips to LFI-1096 operation, but these are best learned with practice. In the bad old days, you had to learn all the tricks, since linear correlators were commonly pushed beyond their limits. Now you would only use a linear correlator for fairly easy measurements. Log-time correlators such as the ALV-5000 are now used for almost any difficult case. It is beyond the scope of this document to explain the mighty ALV (and its main competitor, the Brookhaven BI-9000). However, a little information is provided, just so you can see that the limitations of the linear correlator can be dealt with--using radically different design.

Log-time correlators were suggested by the theoretical work of McWhirter and Pike, which will be discussed in greater detail later. These authors demonstrated that there was no advantage to having lots of linear spaced points if one wants to measure exponential decays (or multi-exponential decays, as in polydisperse samples). A correlator that would work well could

be constructed with just a few channels, whose lag times were exponentially spaced: $t_i = 2t_{i-1}$. Alternately, you could use the same number of channels as a typical linear correlator (e.g., 256 or 272) and cover *much* greater time windows. About this same time, a number of workers discovered that wide time windows were really necessary to capture all the physics of many important processes (not usually in dilute solutions, however).

There were several attempts to stretch out the time window. The LFI-1096 has an optional mode, called *multi-tau*, that divides system resources up so that *three* correlation functions can be measured simultaneously. The first uses a “base time” Δ between each channel; the second uses an extended time $m\Delta$ (m is called the divisor, never mind why) and the third uses $m^2\Delta$. The width of the shift registers expands so that, if the intensity is set so that overflows do not occur in the first correlation function, they also do not occur in the other two, even though the effective Δ is there very long. Operating in this mode, the window of the LFI-1096 is 0 - 8192Δ . Thus, almost four decades of time can be spanned. Not bad, but not ALV either! Another patched-up attempt to extend the time window was made by Malvern, who made a correlator that looks kind of like this:



Note that memory only exists for some shift register locations. Thus, the data were delayed large amounts to generate the log-time spacing, but lots of data are being “wasted” in the shift registers. The performance features of the Brookhaven BI-9000 suggest that it may operate this way, too--but I do not know for sure. There is not time to discuss “the ultimate solution” which is the ALV-5000, developed by the late Klaus Schätzl, and discussed in the Wyn Brown book on dynamic light scattering. The ALV-5000 has a completely different architecture, using (I think) multiple 8-bit processors and the CPU on a host IBM-PC computer to actually multiply the lagged intensities together very rapidly instead of add them repeatedly. Data are stored in memories, and precision varies from 8-bit to 16-bit, depending on the lag time. There are a

number of other “tricks” to making the data come out very accurate, but the main point of the ALV is the same as other new correlators: it achieves very wide sample times with little hardware. Also, you don’t have to “aim” the available channels at the actively decaying part of the correlation function. If it moves, the ALV will likely capture it. There is no decay time increment to set.