**Etude on the limitations of DLS**

Introduction: This is a section out of a proposal by several of the Macro faculty to obtain a Nanosight particle tracking instrument. To justify it, we work through some of the limitations of DLS, choosing as an example some work we did for Prof. Monroe in Biological & Agricultural Engineering. This should not be misconstrued: DLS will remain a vibrant technique. I think, though, that routine particle sizing will often be conducted by its competing methods. These include the particle tracking methods discussed here, DOSY NMR (especially for small molecules), and methods that separate particles by size (GPC or AF4) that also feature on-line analysis (including DLS and, soon, SAXS). DLS will be used for complex fluids, gels and turbid suspensions. Ne variants of the method remove a lot of limitations for these systems.

So, you can read this document and revel in the troubles DLS has showing anything useful for Prof. Monroe’s particles, but lurking in the near future is new power for DLS.

Now….only some of the proposal is presented. The rest is an embarrassing collection of BS we are required to produce while groveling for money.

***Before this, the proposal has some introductory stuff about LSU, the Departments involved, etc.***

**1.b. Rationale for Project (5 points).** LSU operates one of the most complete laboratories in American academics for the conventional characterization of polymers and particles in dilute solution. Included are static and dynamic light scattering (SLS/DLS), small- and wide-angle X-ray scattering (SWAXS), a full complement of thermal analysis tools, analytical ultracentrifugation, viscosity, surface tension equipment, rheology and optical microscopy. Unfortunately, the small size of the program and the correspondingly limited opportunities for new faculty hires (especially since the cancellation or postponement of the Multidisciplinary Hiring Initiative due to budget pressures) means that we have fallen behind the competition in adding young faculty experts in the latest image-based methods for dilute solution and complex fluid characterization. Two significant missing elements are particle tracking and diffusing wave spectroscopy with its powerful extensions to optical rheology. Both technologies have recently become commercially available at modest cost. The major focus of this proposal is on the first of these, particle tracking, where we can leverage Board of Regents seed money support to the PI (through the Pilot Fund program) that has established sufficient expertise by enabling travel to established labortories.

*This proposal will add modern particle tracking capabilities to LSU’s Polymer Analysis Laboratory, heavily used by ~70 researchers in 10 LSU units and 9 off-campus academic and industrial clients. The expected payoff is higher productivity on existing research awards, leading to more successful graduates and new proposals to study systems hitherto beyond the capabilities of traditional methods.*

The preferred particle tracking device, a NanoSight LM10, is a robust, turnkey instrument for the non-expert user interested in particle counting, aggregation and sizing. This instrument, with its stunning visual display of Brownian motion, will serve teaching, outreach and recruiting purposes, too. The NanoSight will be purchased with a second, more sensitive camera. This will enable us to begin development of more versatile particle tracking options on existing microscopes, thereby providing an integrated teaching/research challenge to graduate students and postdocs. The long-term strategy adheres to the successful approach adopted by PAL to make commercial equipment available to non-specialists while retaining the ability to advance measurement science by building custom instruments.4-7 Graduate student users of the NanoSight will be able to step into industrial laboratories and hit the ground running, while a few will learn to advance polymer/particle measurement science. The turnkey system ensures, for example, that a biomedical engineer from Prof. Monroe’s group can accurately obtain particle size distributions without the long learning curve associated with inverse Laplace transformation of DLS data. Preliminary results (below) suggest a huge increase in productivity, the essential foundation of successful new proposals. The same device, combined with the existing instruments, also enables polymer analysis research to explore the limits of particle tracking as a sizing method for objects of unusual shape, such as carbon nanotubes.8 The second camera, mounted on existing microscopes, reflects that particle tracking technology is rapidly advancing and can be applied beyond the very dilute solutions required by the turnkey instrument. Particles can be studied in polymeric fluids, including liquid crystals and materials for photonic bandgap applications. Detailed flow for lab-on-a-chip problems can be investigated as well. The development platform may contribute to dilute solution work, too. It is known that the size limits can be pushed to lower values using fast cameras and appropriate optics; see, for example Ref. 9.

No laboratory on the LSU campus, or elsewhere in the vicinity, has a NanoSight instrument. The closest option is a device specialized for velocimetry, located in a single-investigator laboratory where the anticipated workload would prove disruptive. The request is particularly appropriate for the BOR Enhancement program because the equipment cost falls into a gap between major instrumentation programs and individual support mechanisms. The NanoSight is too inexpensive for NSF’s MRI program (minimum $100,000 and the agency-mandated institutional limit on the number of submissions almost always ensures larger proposals are chosen) or even the on-again/off-again IMR program (minimum $100,000 for Ph.D.-granting institutions) but it would consume too large a portion of most types of individual Federal awards.

**1.c. Impact on Existing Resources (5 points).** The main competitor for the NanoSight in the PAL arsenal is a Malvern Zetasizer Nano. This apparatus normally operates as a DLS instrument at a single, fixed scattering angle (173°). When the distribution contains large particles, they are under-represented in the intensity autocorrelation function measured at such a high scattering angle because scattering decreases with angle for large particles. As promised in the successful 2005 NSF IMR proposal which purchased the Zetasizer Nano, we guard against such undercounting by screening new samples with conventional multi-angle scattering. If these tedious and slow screening measurements show an apparent size that does not depend on scattering angle, the Zetasizer Nano is satisfactory. *The NanoSight will improve and modernize PAL’s response when the screening measurements indicate the Zetasizer Nano is not an appropriate tool.* In the next few paragraphs, this will be illustrated by direct example.

It is appropriate to begin with a short review of how DLS works. Contrary to popular belief, a monochromatic laser light source is not necessary, but a laser is usually selected as the most convenient way to illuminate the solution, which is scrupulously prepared to be free of dust or other contaminants, in a spatially coherent manner. The intensity of light scattered by the solution to a finite scattering angle fluctuates whenever the detector optics maintain spatial coherence. This is achieved either by a set of pinholes or by use of a single-mode (or nearly single-mode) fiber optic. LSU’s six (6) DLS instruments include samples of both types, both commercial and custom-built. In all cases, the light is correlated using a digital autocorrelator, and the resulting signal is a decaying exponential (monodisperse particles that are spherical or small) or multiple-exponential decay (distributions of small or spherical particles and even monodisperse, nonspherical particles under some circumstances). With the exception of various techniques for suppression of multiple scattering (see, for example Refs. 10, 11) almost any DLS experiment can be conducted at LSU. This includes high-temperature measurements12 and zero-angle depolarized measurements.13 An argon-krypton mixed gas laser provides a number of wavelengths to help with highly colored samples. Scientists from two separate national laboratories have recently come to LSU to learn DLS. There may be more ardent proponents of the DLS method than the PI, but not many. Yet it is time to develop particle tracking technology because of its potential to accelerate research.

To detect large particles well, DLS must operate at very low scattering angles where stray light reduction becomes difficult. Although the PI once constructed an instrument for scattering angles of less than 1°, such experiments are not an everyday thing. Even if they were, they would be painfully slow. The distance required for molecules to diffuse far enough to relax the correlation function increases as the angle falls, resulting in long acquire times (without dust contamination, and dust is always more problematic at low scattering angles). The decay rate associated with a single diffuser is *q*2*D*, where *q* is the scattering vector magnitude and *D* the mutual diffusion coefficient, which must be extrapolated to zero concentration to obtain the self-diffusion coefficient that can be inverted via the Stokes-Einstein relation (*Dc=*0= *kT*/6*R*h) to yield the hydrodynamic particle radius, *R*h (*kT* is the molecular thermal energy and  is the viscosity). When large particles are mixed with small, as happens frequently during aggregation and self-assembly processes studied by several PAL users, DLS tends to see the large ones preferentially at low scattering angles, but it may miss them entirely at higher angles (the scattering of a sufficiently large particle goes through a null point at some angle). **Figure 1** shows an intermediate result of a short collaboration between the PI and co-PI on DNA-coated silver particles to assess the severity of the problem. As described above, this is in keeping with our policy to test any new sample by multi-angle DLS before using the Malvern Zetasizer Nano.

Prof. Monroe’s sample had to be run under very dilute (for DLS) conditions, resulting in weak scattering signals. Concern for number fluctuations14 should always accompany measurements at low concentrations, and this dictated a “fairly incoherent” detector setup, meaning large scattering volumes. A multiple-run collection protocol was selected to avoid the potential dust contamination that comes with large measuring volumes. In the conventional pinhole setup selected for these measurements, a large scattering volume implies the laser beam will strike a fairly large face of the scattering cell, increasing the potential for “splash” (stray light) due to imperfections or scratches. A tilted square fluorimeter cuvette with polished windows was chosen to reduce the splash and to guide the back-reflected incident beam away from the detection volume (better in this case than absorbing it). These special setups are easy and fast on our custom-built instrument, which can use simple test tubes for many samples, but it still took about two hours to acquire the data (not including the tedious cleansing of a sample cell, which was taught to Prof. Monroe’s student). Two hours is nothing compared to the analysis, as shown next.

In the semilogarithmic representation chosen for **Figure 1**, a monodisperse diffuser would give precisely straight lines. Clearly, the particles are not monodisperse! The next step was inverse Laplace transformation of the multiple-exponential signals using the program CONTIN.15 This is now an easy and fast procedure, but the same cannot be said of the learning curve. Inverse Laplace transformation of “noisy” data—meaning any real data—is justifiably called an “ill-posed problem”.15 The amplitude associated with each decaying exponential is given by the proportionality *A* ~ *c·M·P*, where *c* is the mass/volume concentration, *M* the molecular weight and *P* the particle form factor (0<*P*<1) which depends on shape, scattering vector magnitude and size. The upshot in the case of Prof. Monroe’s samples was that the polydisperse particles could not be measured well at the single scattering angle provided by the Zetasizer Nano. For purposes of guiding the particle preparation effort or getting a rough idea of dispersion stability, that “black box” instrument *might* be pressed into service. But doing so would reflect a conscious decision to live with the risk that reviewers might reject any manuscript based on the results, especially since the advent of particle tracking methods we now seek. The best alternative available to us now is to put one of Monroe’s students through a month-long learning program and analyze all the samples by multi-angle DLS. Although the PI was willing to “loan” a student to the co-PI for the purpose of measuring these particles, that was motivated partly by the training opportunity the exercise provided. No funds exist to do that on a regular basis, and attempts to obtain such funds today one would likely run afoul of some reviewer who asks: “Why don’t they just do this with a NanoSight?”



**Figure 1**. DLS correlation functions at five scattering angles in semiloga-rithmic form to highlight the curva-ture that reveals size polydispersity.

|  |  |  |
| --- | --- | --- |
| lm10hs  A | MilkRingsNegative  B | C |

**Figure 2.** **A:** Nanosight LM10 (video monitor not shown). **B:** Careful inspection of this video snapshot will reveal six (6) diffraction ring patterns seen by the NanoSight during a demonstration on the LSU campus July 2010. The sample is diluted milk (a request from a potential industrial partner in Australia) and this image is a negative; the NanoSight normally sees particles as bright images on a dark background. **C:** Size distributions for four samples measured at LSU during a demonstration of the requested instrument. A logarithmic scale has been selected to demonstrate the range of concentrations that can be counted, but this scale highlights minor features and may give the impression of false detail; for example, the bumps in the blue and green curves may disappear with multiple measurements, which is the sort of thing to be determined during instrument commissioning. Black curve: bubbles made from the fungal hydrophobin *Cerato ulmin* are surprisingly uniform with average size about 320 nm; Red curve: an *unsuccessful* preparation of silica is not very uniform; Green curve: milk poses a severe challenge for DLS sizing due to its very broad size distribution; Blue curve: commercial latex standard.

As shown in **Figure 2**, the NanoSight ( [http://www.NanoSight.com/](http://www.nanosight.com/) ) is an upright microscope equipped with a flow cell into which the user injects samples. A slit-like ray of light travels *across* the flow cell (perpendicular to the direction of observation) and a camera captures the diffraction pattern of each particle against a dark background. Like DLS, the size is based on diffusion coefficient, *D*, but rather than correlate the light scattered by tens of thousands of particles and then, ironically, extrapolate the results to low concentrations, the NanoSight directly observes the scatter from several hundred individually resolved (one hopes) particles at vanishing concentration. Not only the average intensity, but the *location* of each scattering source, is recorded. As the particles undergo Brownian motion, the NanoSight tracks their positions (the position resolution is smaller than the optical resolution, but that’s a long story). Once it has mean square displacements <*x*2> as a function of time, *t*, *D* is computed as <*x*2>/6*t* = *kT*/(6*R*h). Solving for radius yields the size of each detected particle.

Because only the positions matter, not the intensities, the method does not require a particle form factor correction. Unfortunately, small particles with low contrast are harder to detect. The practical limit depends on optical contrast, but reasonable results can be expected for particles exceeding 10 to 20 nm. If the particles fluoresce, the situation ought to be better. Because each particle is separately and absolutely tracked, there are no multiple exponential signals to unbundle. Thus, the “band broadening” associated with the finite “grid set” of typical Inverse Laplace Transform algorithms is eliminated, as is the very long learning curve. Because the NanoSight operates at very low concentrations, the extrapolation to zero concentration is not needed: the user directly gets the self diffusion at nearly infinite dilution. The NanoSight can also count particles, which is valuable if they are aggregating. The particles are easily visualized on a computer monitor, which makes for a good teaching opportunity.

It is easy to imagine the problems with the NanoSight approach (overlapping particles, undersampling, drop-outs of a particular size due to a scattering null at the primary angle of observation) and these will morph into one leg of the research described below, but preliminary results obtained during two on-site demonstrations suggest it is often an effective tool. When the particle tracking approach works, it is speedy and intuitive compared to DLS.

***The remainder of the proposal consists of what a Nanosight costs, who will manage it and how, where we will put it, and some individual research projects that will use it.***

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2) The SEALs program has received national attention in Chemical & Engineering News (May 19, 2008). See also: <http://www.selu.edu/acad_research/depts/chem_phy/news/articles/SEAL.html>

3) To celebrate its independence from state government, APTEC is transitioning to a new website. Meanwhile, the old one remains on an LSU server: http://macro.lsu.edu/aptec/

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22) A former Honors Chemistry Lab student frequently stops by the PI's office to show off his latest laser acquisition; this guy, a Petroleum Engineering major, spends a significant portion of his income on lasers that light matches or burn holes in leather...all because he thinks they're cool.