Modern Particle Characterization Techniques Series

Dr. Mike Pohl
I: Introduction

Julie Chen Nguyen
II: Laser Diffraction

Dr. Jeff Bodycomb
III: DLS

Dr. Sean Travers
IV: Multi-laser NTA

Carl Lundstedt
Series V: BET

Dr. David Fairhurst
VI: Zeta Potential

Darren McHugh
VII: Image Analysis
Modern Particle Characterization Techniques Series III: Dynamic Light Scattering (DLS)

July 8, 2020
Outline

- Introduction
- What is DLS and what, exactly, does it measure?
- Method Development
A word from our sponsor

Did you sign up for our newsletter?

Receive regular updates and news on the world of particle analysis.

Send us a chat or e-mail your desire to labinfo@horiba.com
What is dynamic light scattering?

• Dynamic light scattering refers to measurement and interpretation of light scattering data on a microsecond time scale.

• Dynamic light scattering can be used to determine
  – Particle/molecular size
  – Size distribution
  – Relaxations in complex fluids
Other light scattering techniques

- **Static Light Scattering**: over a duration of ~1 second. Used for determining particle size (diameters greater than 10 nm), polymer molecular weight, 2\textsuperscript{nd} virial coefficient, $R_g$.

- **Electrophoretic Light Scattering**: use Doppler shift in scattered light to probe motion of particles due to an applied electric field. Used for determining electrophoretic mobility, zeta potential.

- **Nanoparticle Tracking Analysis (NTA)**: use scattering to track particle location as a function of time, that is, particle motion. Use motion to determine particle size.
Sizing techniques
Laser diffraction

Laser Diffraction

• Particle size 0.01 – 3000 µm
• Converts scattered light to particle size distribution
• Quick, repeatable
• Most common technique
• Suspensions & powders
Laser diffraction

Suspension

Silica

~ 30 nm

- S.P. Area: 2.0183E+6 (cm²/cm³)
- Mean Size: 0.02990 (µm)
- Variance: 5.0313E-6 (µm²)
- Median Size: 0.03013 (µm)
- Mode Size: 0.0302 (µm)
- Skewness: -0.2301

Powders

Coffee Results

0.3 – 1 mm
Nanoparticle tracking analysis (NTA)
Nanoparticle tracking analysis (NTA)

- **light source**
- **light sheet**
- **investigated volume**
- **light sheet thickness**
- **scattered light**
- **microscope + camera**
Outline

• Introduction
• What is DLS and what, exactly, does it measure?
• Method Development
DLS optics

Backscatter (173°)
(High concentration)

Particles moving due to Brownian motion

Laser 532nm, 10mW
Attenuator

90° for size and MW, A2

Particles

PD
For T%

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Brownian motion

Particles in suspension undergo Brownian motion (random thermal motion).

Brownian Motion
- Random
- Related to Size
- Related to viscosity
- Related to temperature
DLS signal

Random motion of particles leads to random fluctuations in signal (due to changing constructive/destructive interference of scattered light).
Correlation function

Random fluctuations are interpreted in terms of the autocorrelation function (ACF), \( C(\tau) \).

\[
C(\tau) = 1 + \beta \exp(-2\Gamma \tau)
\]

\[
C(\tau) = \frac{\int_{0}^{\tau} I(t)I(t+\tau)dt}{\langle I(t)I(t) \rangle}
\]
Gamma to size

\[ q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right) \]

\[ \Gamma = D_m q^2 \]

\[ D_h = \frac{k_B T}{3\pi \eta(T) D_m} \]

Note effect of temperature!

- \( \Gamma \): decay constant
- \( D_m \): diffusion coefficient
- \( q \): scattering vector
- \( n \): refractive index
- \( \lambda \): wavelength
- \( \theta \): scattering angle
- \( D_h \): hydrodynamic diameter
- \( \eta \): viscosity
- \( k_B \): Boltzman’s constant

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What is hydrodynamic size?

DLS gives the diameter of a sphere that moves (diffuses) the same way as your sample.
Hydrodynamic size

Gold Colloids

<table>
<thead>
<tr>
<th>Technique</th>
<th>Size nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic Force Microscopy</td>
<td>8.5 ± 0.3</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td>9.9 ± 0.1</td>
</tr>
<tr>
<td>Transmission Electron Microscopy</td>
<td>8.9 ± 0.1</td>
</tr>
<tr>
<td>Dynamic Light Scattering</td>
<td>13.5 ± 0.1</td>
</tr>
</tbody>
</table>

SEM (above) and TEM (below) images for NIST RM 8011
Nanogold data

<table>
<thead>
<tr>
<th></th>
<th>Z-average Diameter, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>50.5</td>
</tr>
<tr>
<td>Run 2</td>
<td>51.1</td>
</tr>
<tr>
<td>Run 3</td>
<td>49.2</td>
</tr>
<tr>
<td>Run 4</td>
<td>51.5</td>
</tr>
<tr>
<td>Run 5</td>
<td>49.7</td>
</tr>
<tr>
<td>Run 6</td>
<td>50.9</td>
</tr>
<tr>
<td>Avg.</td>
<td>50.5</td>
</tr>
<tr>
<td>St. Dev.</td>
<td>0.9</td>
</tr>
<tr>
<td>COV</td>
<td>1.7 %</td>
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<tr>
<th>Run</th>
<th>Z-average Diameter, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>10.5</td>
</tr>
<tr>
<td>Run 2</td>
<td>10.6</td>
</tr>
<tr>
<td>Run 3</td>
<td>10.2</td>
</tr>
<tr>
<td>Run 4</td>
<td>10.5</td>
</tr>
<tr>
<td>Run 5</td>
<td>10.3</td>
</tr>
<tr>
<td>Avg.</td>
<td>10.4</td>
</tr>
<tr>
<td>St. Dev.</td>
<td>0.2</td>
</tr>
<tr>
<td>COV</td>
<td>1.9 %</td>
</tr>
</tbody>
</table>
## Lab to lab comparison

### Colloidal Silica

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean determined Z-average size (nm)</th>
<th>COV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic Light Scattering with SZ-100, laboratory 1</td>
<td>34.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Dynamic Light Scattering with SZ-100, laboratory 2</td>
<td>34.6</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Polystyrene latex
Polydisperse samples: cumulants

For a mixture of sizes, the autocorrelation function can be interpreted in terms of cumulants. This is the most robust method of analyzing DLS data.

\[ C(\tau) = 1 + \beta \exp(-2\Gamma \tau) \]

\[ C(\tau) = 1 + \beta \exp\left[ 2\left( -\bar{\Gamma} \tau + \left( \frac{\mu_2}{2!} \right) \tau^2 - \cdots \right) \right] \]

\[ \bar{\Gamma} = D_m q^2 \]

“z-average size”

\[ D_{z.h} = \frac{k_B T}{3\pi \eta(T) D_m} \]

Polydispersity = \[ \mu_2 / \bar{\Gamma}^2 \]
Z-average

Size determined from intensity weight diffusion coefficient ~1/D

Intensity weighted harmonic mean size

\[
\frac{1}{D_z} = \frac{\sum D_i S_i}{\sum S_i}
\]

\(D_z = \) z-average
\(S_i = \) total scattering from all of species \(i\)
\(D_i = \) Diameter of species all

As size goes up, so does \(D_z\).
<table>
<thead>
<tr>
<th>Run</th>
<th>Z-average Diameter (nm)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>473.2</td>
<td>0.127</td>
</tr>
<tr>
<td>2</td>
<td>479.5</td>
<td>0.066</td>
</tr>
<tr>
<td>3</td>
<td>478.8</td>
<td>0.077</td>
</tr>
<tr>
<td>4</td>
<td>487.7</td>
<td>0.039</td>
</tr>
<tr>
<td>Avg.</td>
<td>479.8</td>
<td>0.077</td>
</tr>
</tbody>
</table>
Mixtures of particles

Sum the autocorrelation functions
Polydisperse sample: ILT

A more general relationship can be given between the autocorrelation function and the size distribution. Let each size have a relation constant $\Gamma$. The scattering from each population is then given by $S(\Gamma)$. Now we have an integral equation. Solving for $S(\Gamma)$ gives us size distribution.

\[
C(\tau) = 1 + \beta \left| g^{(1)}(\tau) \right|^2
\]

\[
g^{(1)}(\tau) = \int S(\Gamma) \exp(-\Gamma \tau) d\Gamma
\]
Bimodal sample

Nominal 20 nm and 500 nm latex run individually
Bimodal sample

Mixed sample (in black)
Colloidal Silica

- Standard off-the-shelf Ludox
  - Colloidal Silica
  - Used to clarify beer, wine, and juice

- Matches data from the LA-960 (laser diffraction)
BSA

- BSA - well characterized protein
- DLS – Can be used to determine the aggregation state of the protein
Protein aggregation

- Unstabilized 10mg/ml lysozyme at pH 2
- Lisa Cole and Ben Burnett at the Florida Institute of Technology
- Can also be done with ViewSizer (NTA)

Protein size in nm vs. time in hours
Liposomes

- Liposomes to target tumor growth
- Size is critical to how the liposome
  - Encapsulates protein
  - Functions within body
  - Remains stable over time
  - Delivers the protein
Outline

• Introduction
• What is DLS and what, exactly, does it measure?
• Method Development
• Dust: large, rare particles in the sample
• Generally not really part of the sample
• Since they are rare cannot get good statistics
Filters are your friend

- Filter to remove dust. If particles are too large (D >50 nm for 0.1 \( \mu \text{m} \) filter), at least filter diluent.

- Filters available in sizes 20nm to 2\( \mu \text{m} \)

- We can also centrifuge the sample and extract the supernatant.
Suspension liquid

• Choose a liquid that
  • does not dissolve the particles
  • prevents loose agglomerates

• Add energy to break up loose agglomerates
  • stirring
  • ultrasound
Surfactants

Enable wetting
Prevent agglomeration

**Common concentration: 0.01-0.1%**

example:

Tetrasodium pyrophosphate (TSPP)
Triton X
Wetting

- Many dry particle samples will never form a nanoparticle suspension without significant effort.
- Sprinkle particles on top of target dispersant. If the particles float on top and do not penetrate the water surface, they are not wetted. This is usually a bad sign.
- If the particles break through surface and sink, they are a) wetted or b) so big that gravity is more important than surface tension. If it is case (a), you are in luck.
Working with aqueous systems is usually easier for many reasons.

But don’t forget to try a less polar solvent such as isopropyl alcohol.

And, don’t forget that organic solvents are more difficult to handle due to fire and health hazards.
Try a series of options

Make a series of suspensions and check them by eye, then measure.
Concentration

Make a plot like this to learn range of concentrations for your sample

![Concentration graph]

- Concentration too low (noisy result)
- Concentration getting high (shift in result)
Estimate concentration by eye

Read a newspaper through it

Best range for this material
Effect of salt concentration

• Note that when we suppress effect of charges by adding salt, the effect of concentration is suppressed.

• Concentration effects are due to changes in particle motion, not just multiple scattering.
Hints Summary

- Web search
- Consider solvent and surfactant
- Consider ultrasound
- Expect to filter
- Choose largest cell you can
- Optimize concentration
Settling and DLS

Not all motion is Brownian motion 😞

<table>
<thead>
<tr>
<th>Particle Diameter (µm)</th>
<th>Movement due to Brownian Motion</th>
<th>Movement due to Gravitational Settling</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>2.36</td>
<td>&gt;&gt; 0.005</td>
</tr>
<tr>
<td>0.25</td>
<td>1.49</td>
<td>&gt; 0.0346</td>
</tr>
<tr>
<td>0.50</td>
<td>1.052</td>
<td>&gt; 0.1384</td>
</tr>
<tr>
<td>1.0</td>
<td>0.745</td>
<td>~ 0.554</td>
</tr>
<tr>
<td>2.5</td>
<td>0.334</td>
<td>&lt; 13.84</td>
</tr>
<tr>
<td>10.0</td>
<td>0.236</td>
<td>&lt;&lt; 55.4</td>
</tr>
</tbody>
</table>

The Natural limit for Dynamic Light Scattering: Gravitational Settling

Gravitational Settling occurs at about 1-3µm
DLS disadvantages

- Sensitive to large particles
- Poor resolution of distribution
- Not appropriate where settling is significant (use laser diffraction)
DLS Advantages

- Noninvasive
- Requires only small quantities of sample
- Good for detecting trace amounts of aggregate
- Good for macromolecular sizing
- Reaches smallest particle sizes
SZ-100

Single compact unit that performs size, zeta potential, and molecular weight measurements.
Summary

• Fast, repeatable nanoparticle sizing
• Think about suspension chemistry in method development
• Reports hydrodynamic size:

\[ D_h \]
Thank you