An Overview of SEC and Light Scattering

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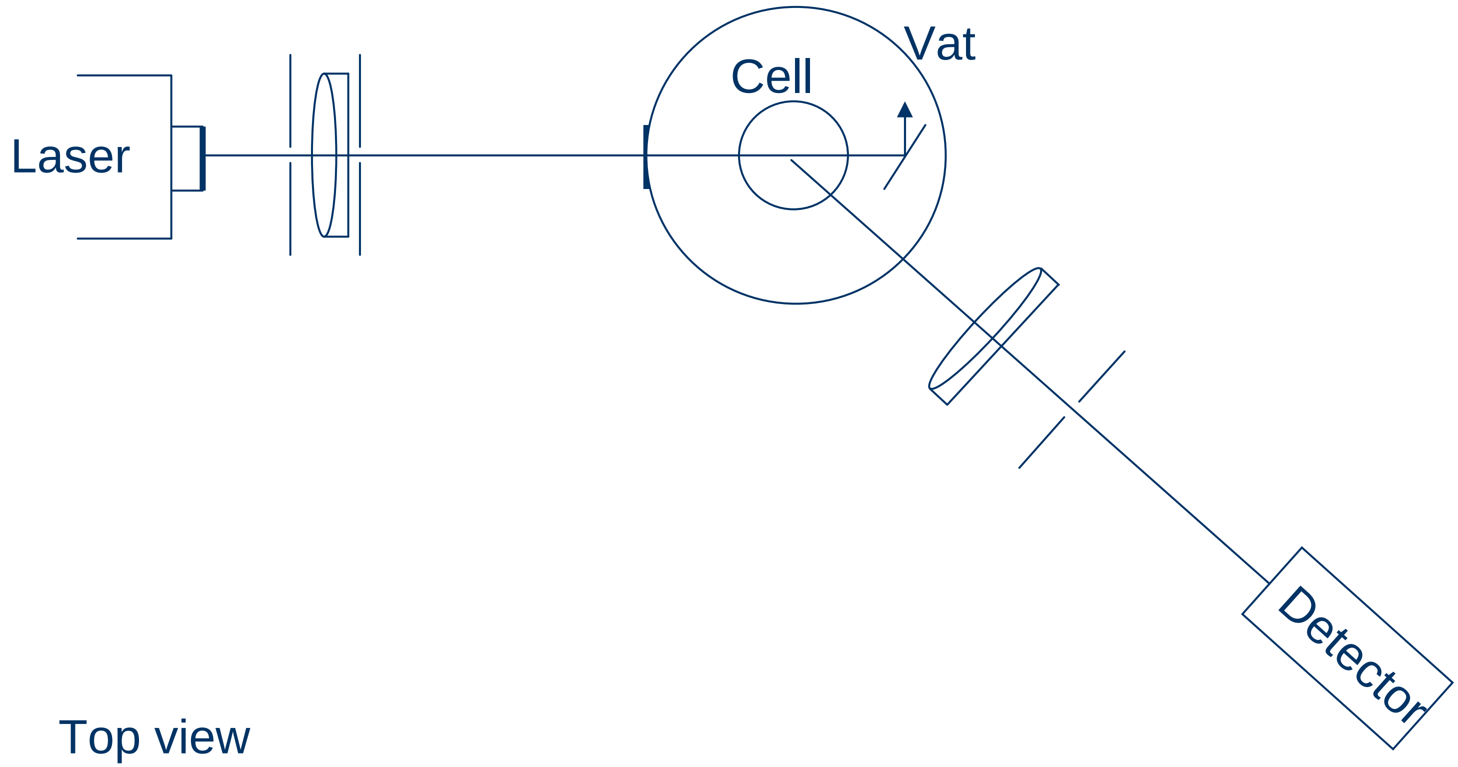
Overview

- Introduction to Static Light Scattering
- Combining Static Light Scattering with SEC
- DLS and SEC

Static Light Scattering, SLS

Dilute Polymer Solutions: M_w, R_g, A_2

Light Scattering in General



Top view

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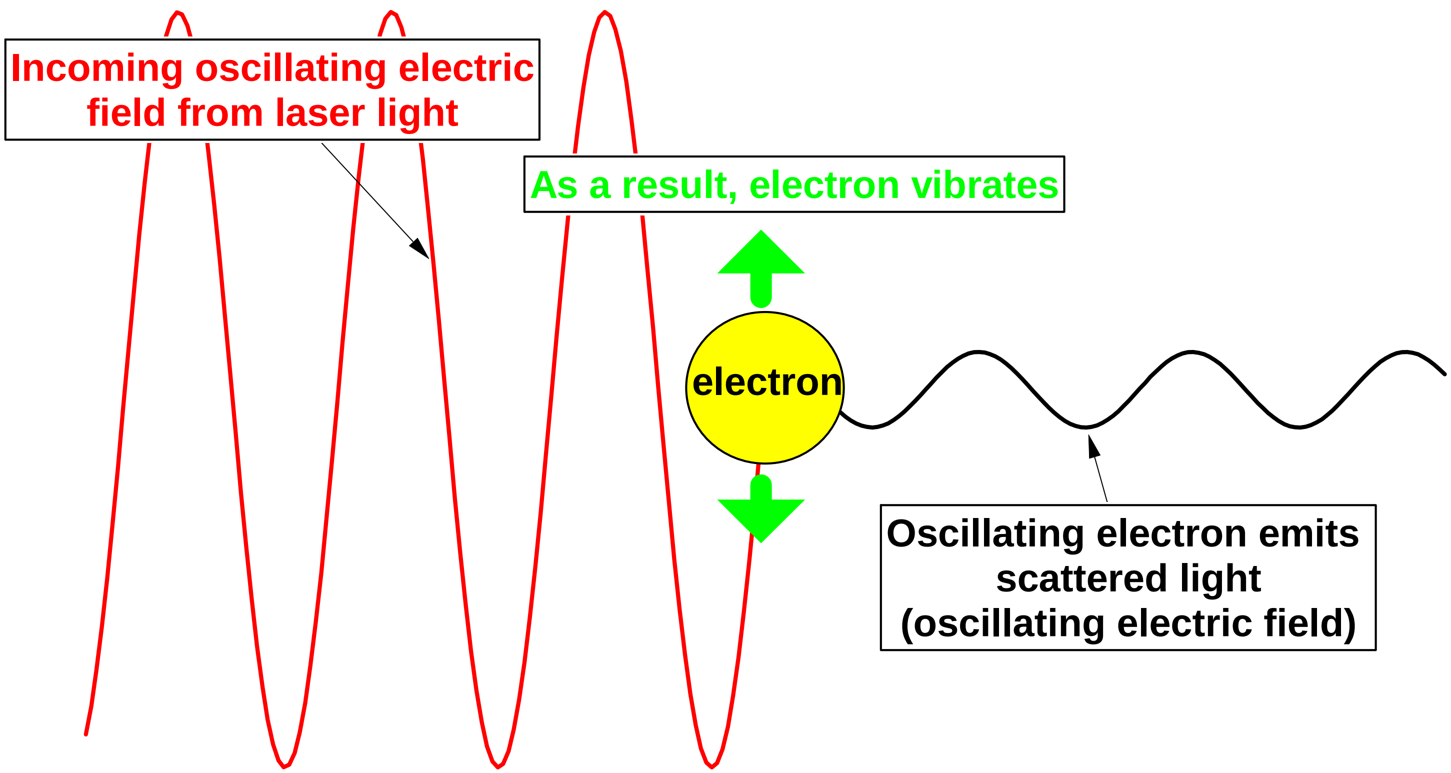
Why does light scattering occur?



- Scattering from a single object is proportional to the square of the object mass (~M^2)
- Proportional to concentration x MW

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Different Types of Light Scattering

Type	Measure	Determine
Static SLS	Intensity	M_w, R_g, A_2, Structure
Dynamic DLS	Intensity Fluctuations Correlation Function	Size Distribution, Relaxation Rates
Electrophoretic ELS	Doppler Shift	Mobility, Zeta Potential
Phase Analysis PALS	Phase	Mobility, Zeta Potential

SLS Measurements: Dilute Polymer Solutions

- M_w, Weight-average Molecular Weight
- R_g, Z-average Radius of Gyration (RMS Radius)
- A_2, Second Virial Coefficient

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SLS Basic Equation

- Homopolymers in single solvent, single contact approximation, dilute solutions:

$$Kc/\Delta R(\theta,c)=\frac{1}{\overline{M}_w}\left(1+\frac{R_g^2 q^2}{3}\right)+2A_2c$$

$$K=4\pi^2 n_o^2 (dn/dc)^2 / N_{avo}\lambda_o^4$$

$$q=\left[4\pi n_o \sin(\theta/2)\right]/\lambda_o$$

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Zimm Plots

$$Kc/\Delta R(\theta,c) = \frac{1}{\overline{\overline{M}}_w}\left(1+\frac{R_g^2 q^2}{3}\right) + 2A_2 c$$

Chromatographic data is primarily interpreted with this equation.

$$Kc/\Delta R(\theta,c \to 0) = \frac{1}{\overline{\overline{M}}_w} + \frac{R_g^2 q^2}{3\overline{\overline{M}}_w}$$

$$Kc/\Delta R(\theta \to 0,c) = \frac{1}{\overline{\overline{M}}_w} + 2A_2 c$$

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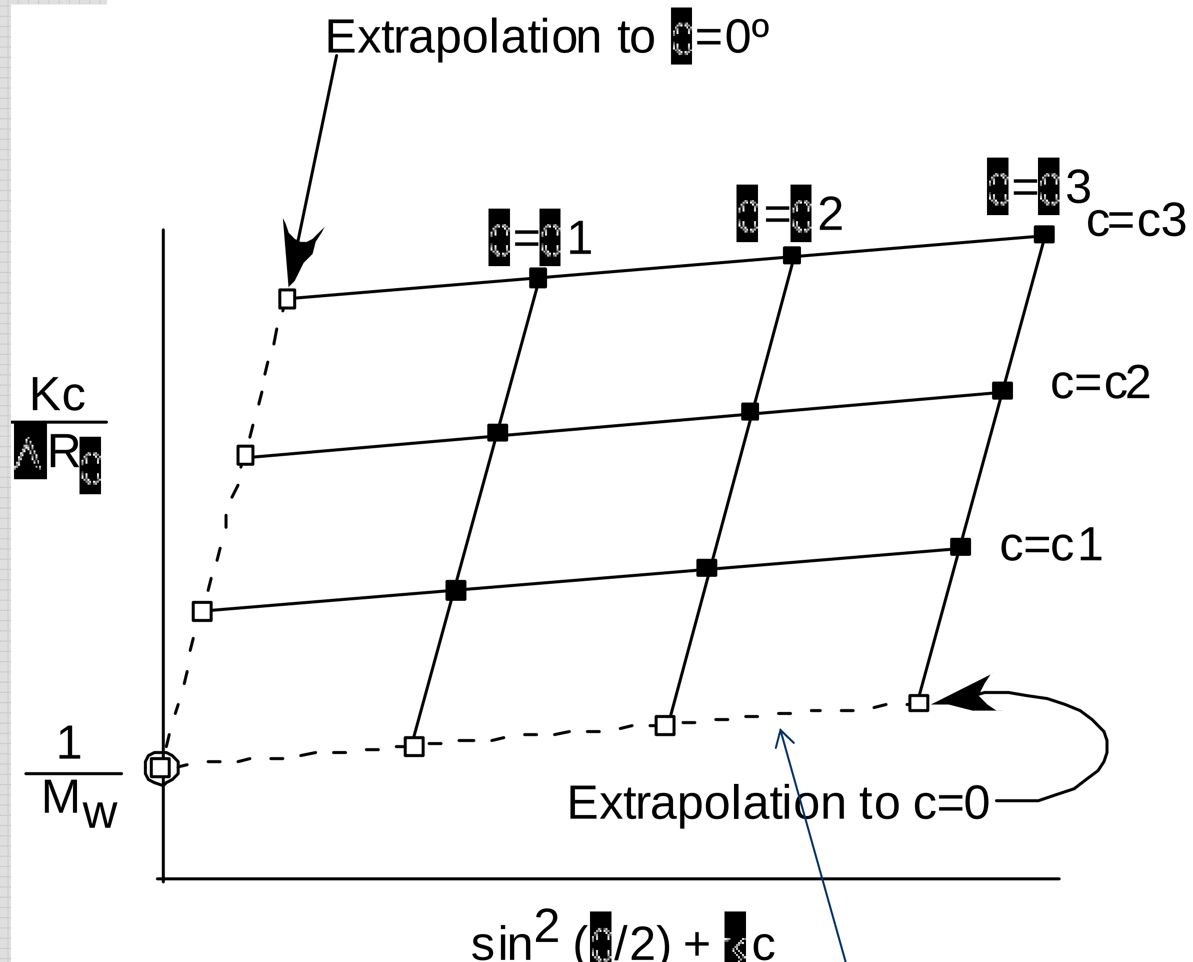
Details of a Zimm Plot



R_g is calculated from the c = 0 slope

A_2 is calculated from the $\theta = 0^o$ slope

$\theta = 0^o$ & c = 0 is $1/M_w$

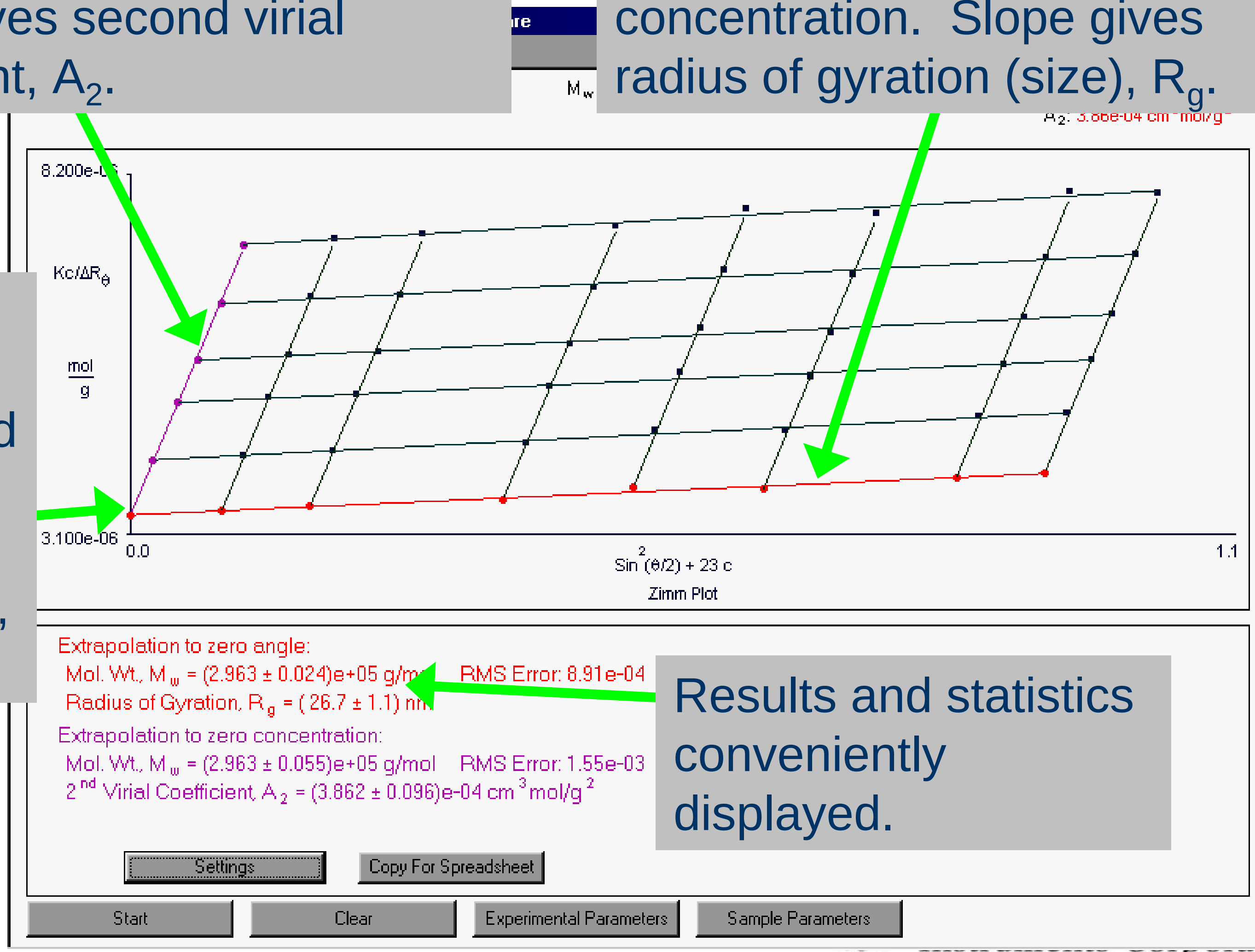
Chromatographic data is primarily interpreted along this line.

Batch-Mode: Zimm Plot

Extrapolate to zero angle. Slope gives second virial coefficient, A_2.

Extrapolate to zero concentration. Slope gives radius of gyration (size), R_g.

Extrapolate to zero concentration and zero angle. Intercept gives molecular weight, M_w.



Results and statistics conveniently displayed.

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The BI-MwA

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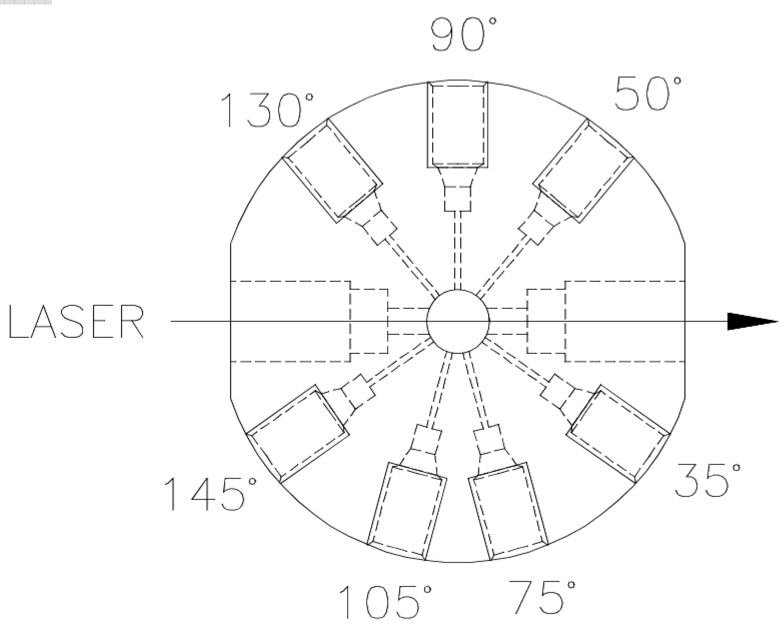
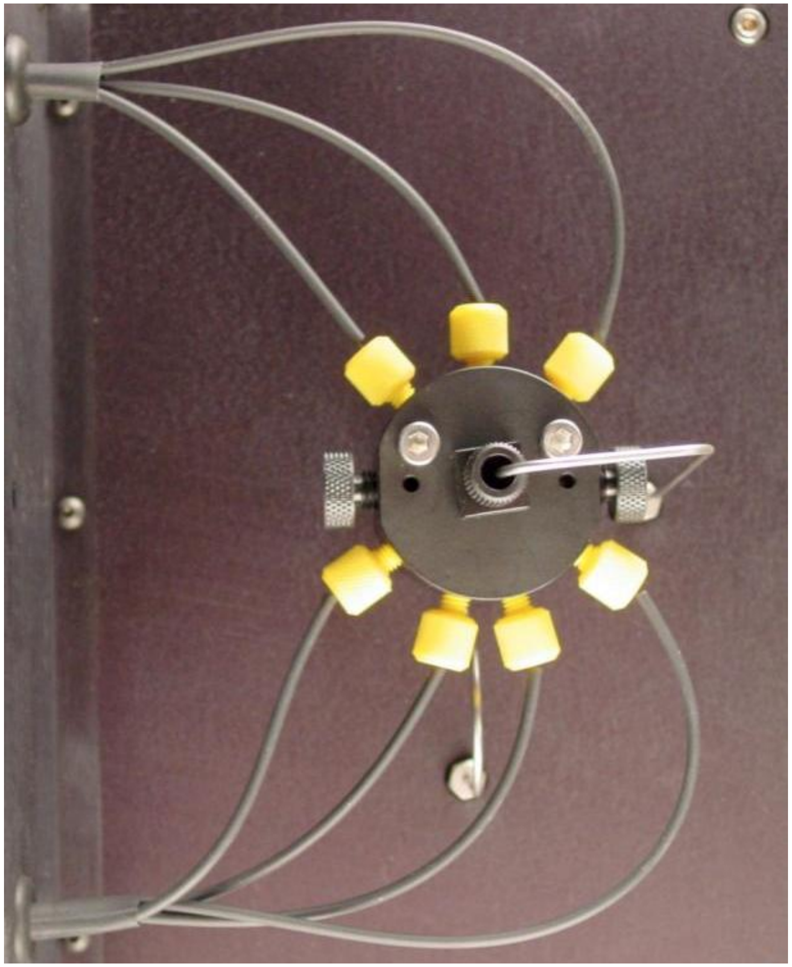
Flow Cell in the BI-MwA





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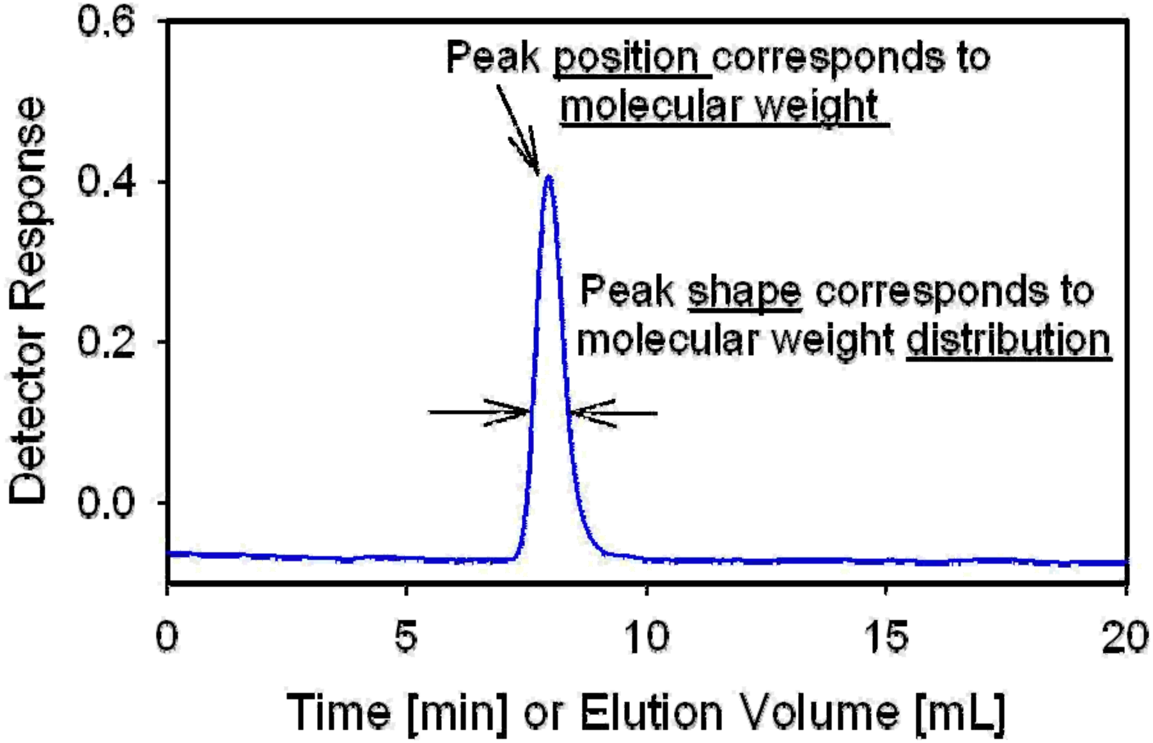
Combining Light Scattering and SEC

Why combine?

- SEC is a good fractionation technique, but it is often difficult to determine analyte molecular weight.
- Light scattering (LS) is a good technique for determining molecular weight, but gives no information about distribution.
- SEC and LS combined gives both molecular weight and distribution information.

Overview of SEC

- Location of peak gives information on molecular weight.
- Shape of peak gives information on molecular weight distribution.



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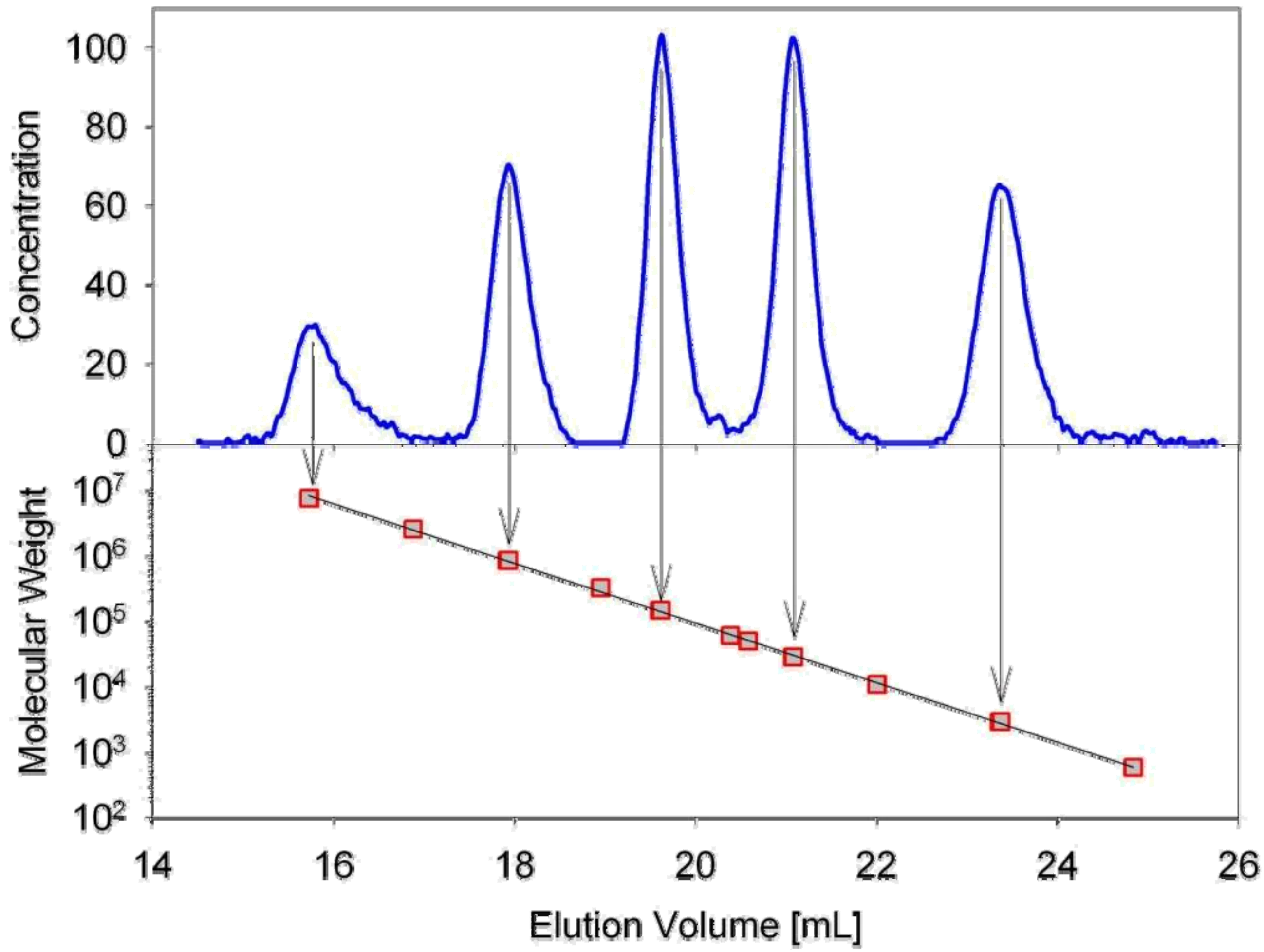
How to determine molecular weight?

- Standard Calibration (concentration detector only)
- Universal Calibration (concentration detector plus viscometer)
- Light Scattering (concentration detector plus LS) **ABSOLUTE**

Standard Calibration Graph



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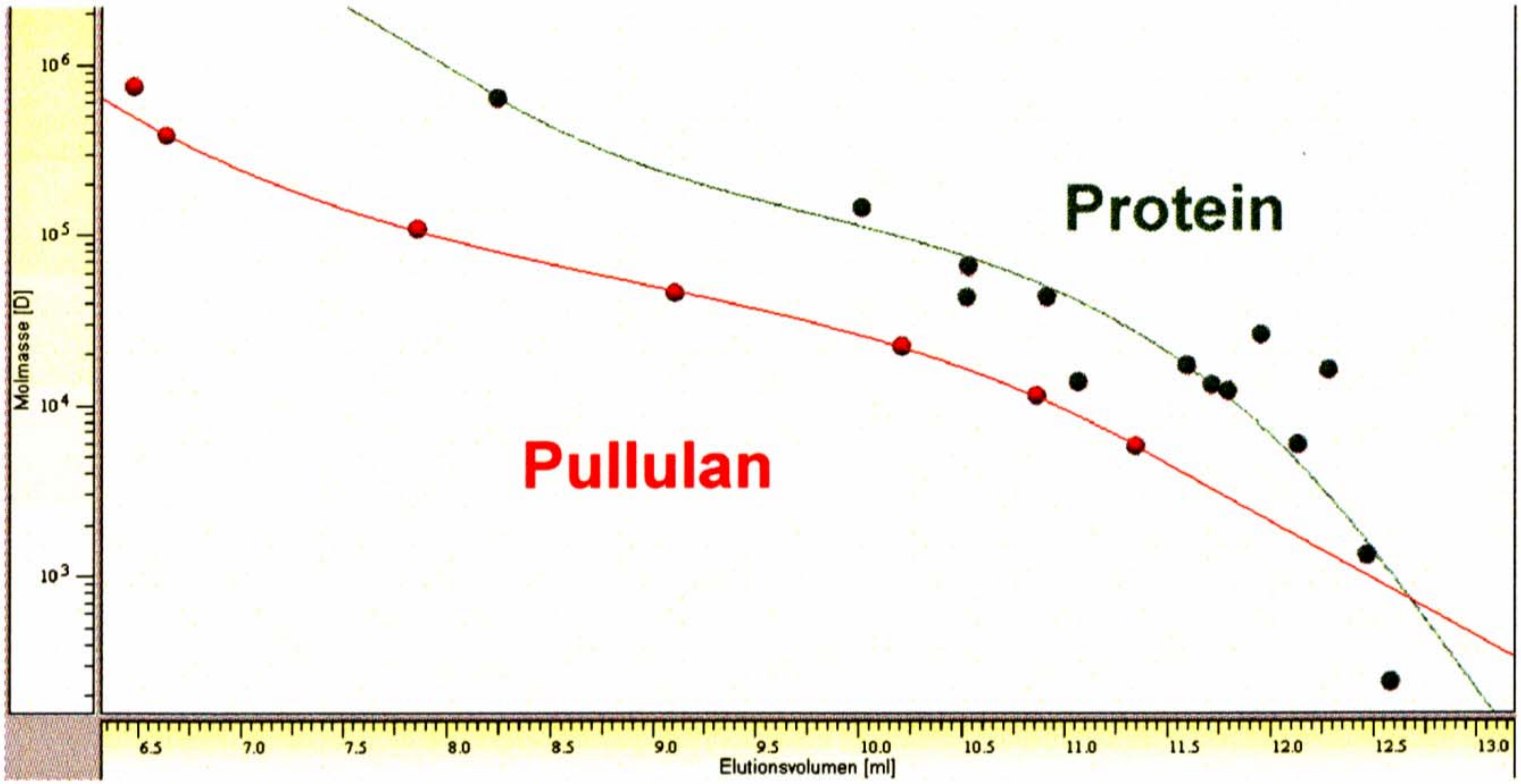
Some less ideal calibration curves



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Light Scattering and Absolute Molecular Weight

- With light scattering, molecular weight is measured *absolutely*.
- The BI-MwA is calibrated & normalized using a narrow, standard polymer in solution*.
- Column is used only to separate species.
- No need to know about the column as long as it separates polymers.
- **Light Scattering eliminates the need for column calibration (standard or universal).**

*The same standard can be used to calibrate concentration detectors and determine inter-detector delay.

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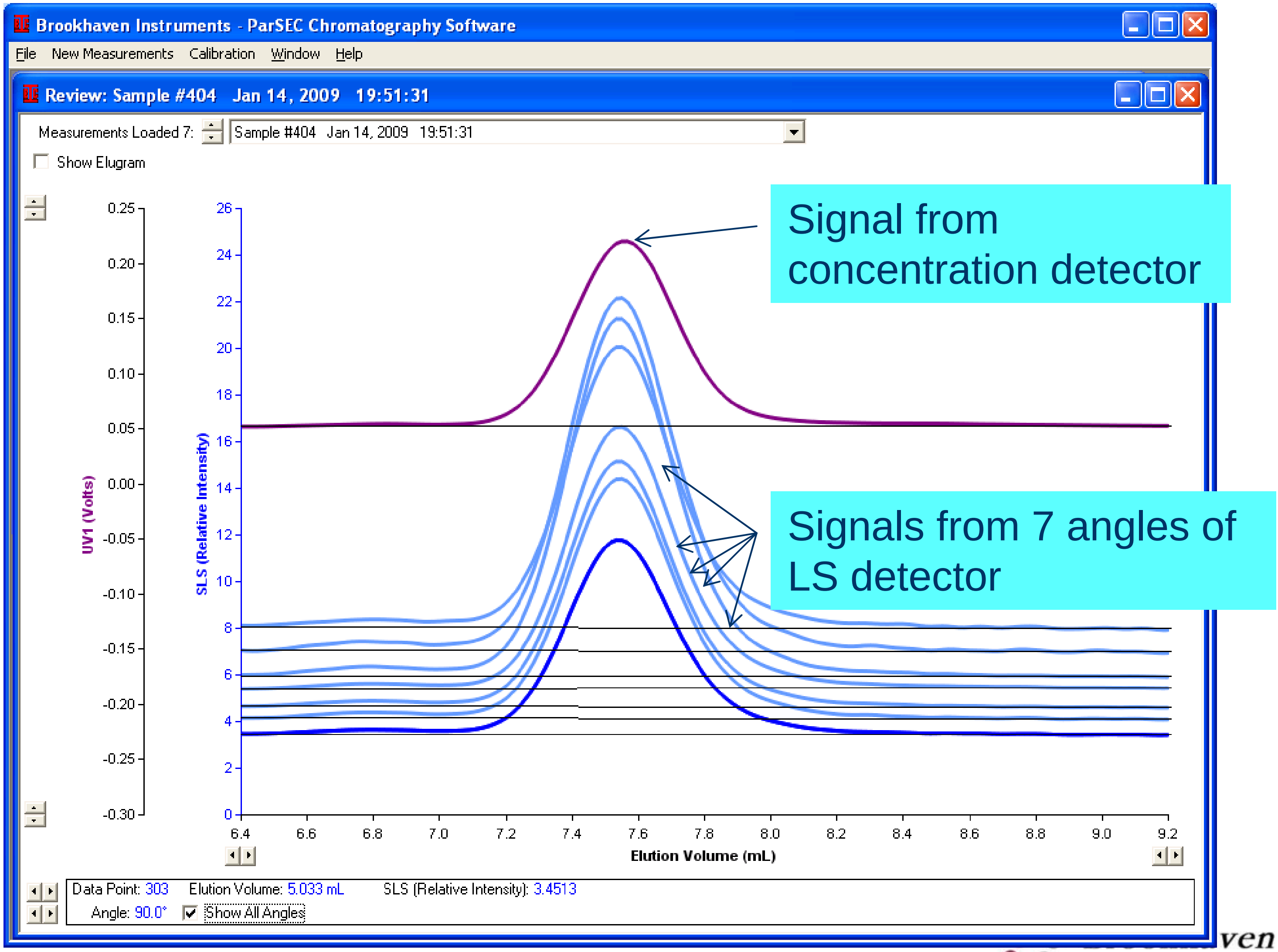
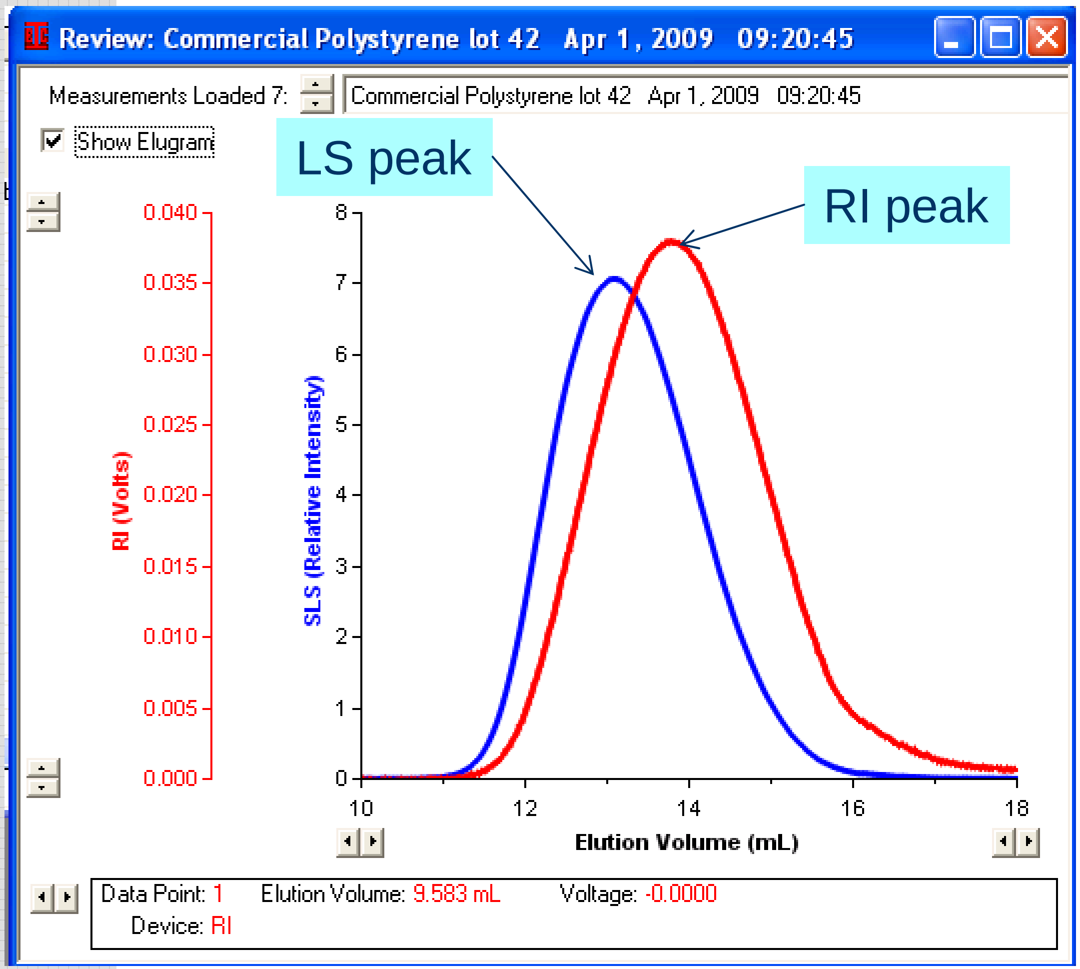
Signals from an SEC Measurement



LS signal (~c * M) vs. RI signal (~c)



- High molecular weight fraction has large LS signal and comparatively weak RI signal.
- Low molecular weight fraction has weak LS signal and comparatively strong RI signal.
- Peaks are shifted for polydisperse samples.

(this sample has Mw/Mn of ~3)

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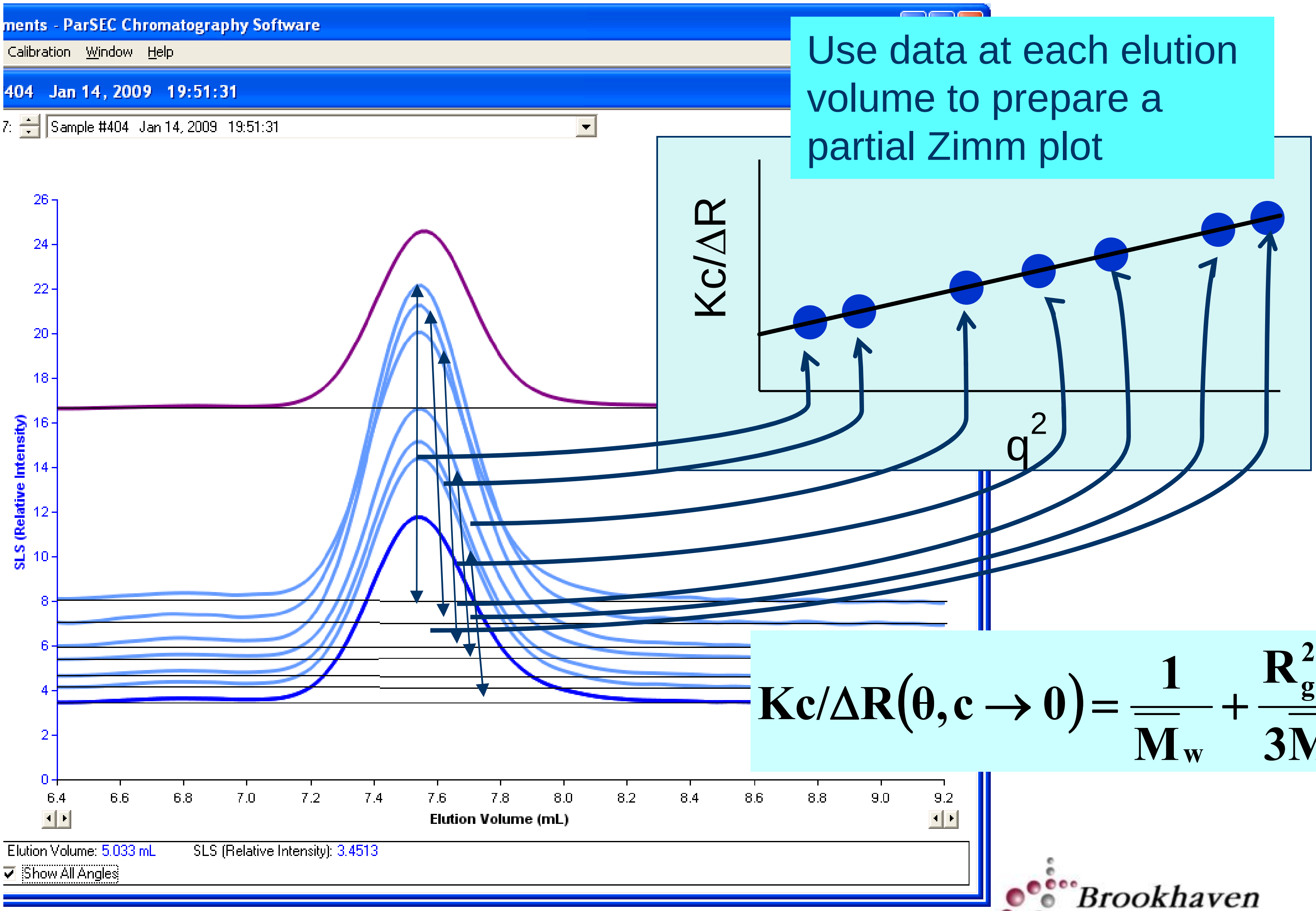
Signals from an SEC Measurement



Use data at each elution volume to prepare a partial Zimm plot

$$Kc/\Delta R(\theta, c \to 0) = \frac{1}{\overline{M}_w} + \frac{R_g^2 q^2}{3\overline{M}_w}$$

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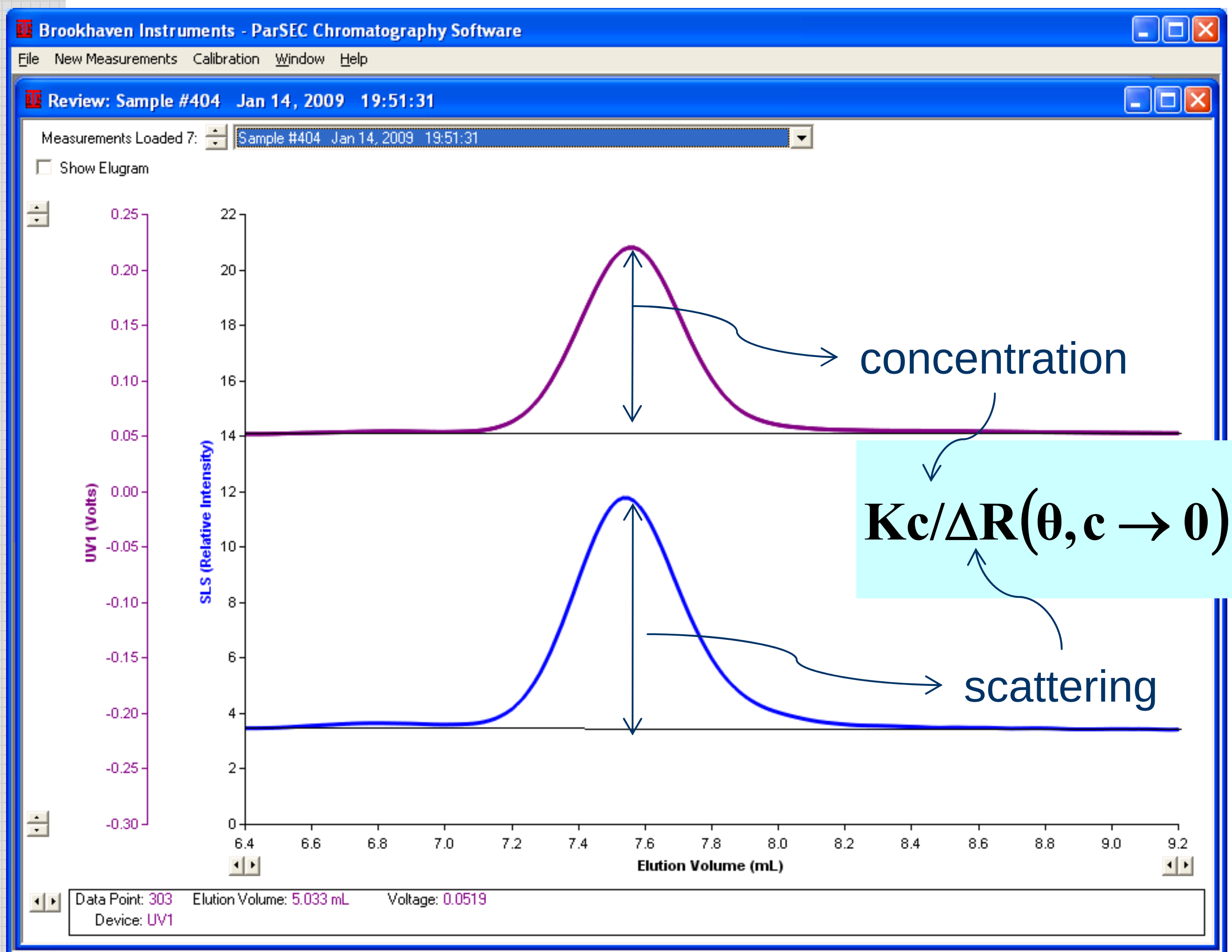
Using SEC signals (a simpler representation)



$$Kc/\Delta R(\theta, c \rightarrow 0) = \frac{1}{\overline{M}_w} + \frac{R_g^2 q^2}{3\overline{M}_w}$$

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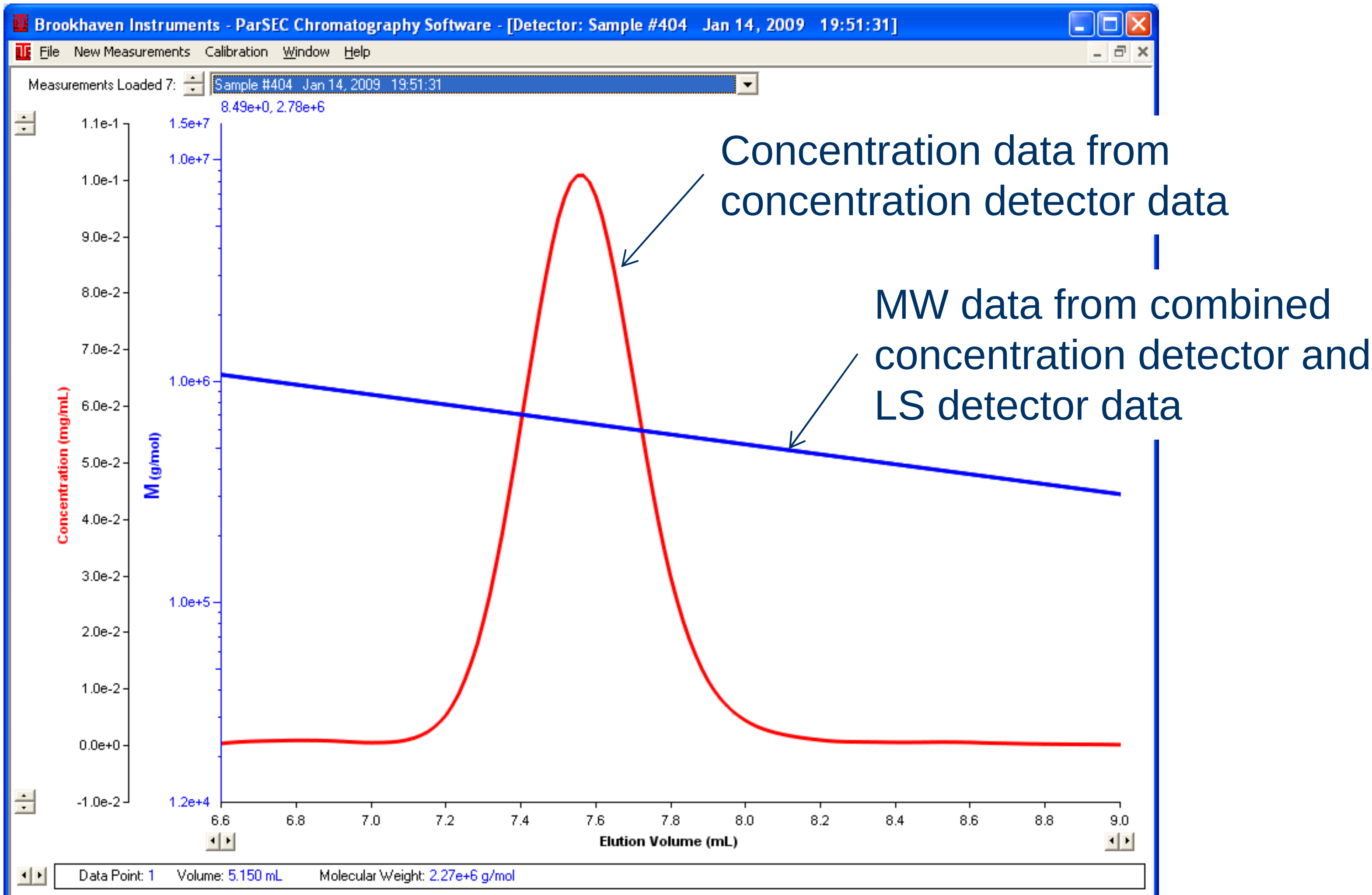
Software Calculates Concentration and Mw



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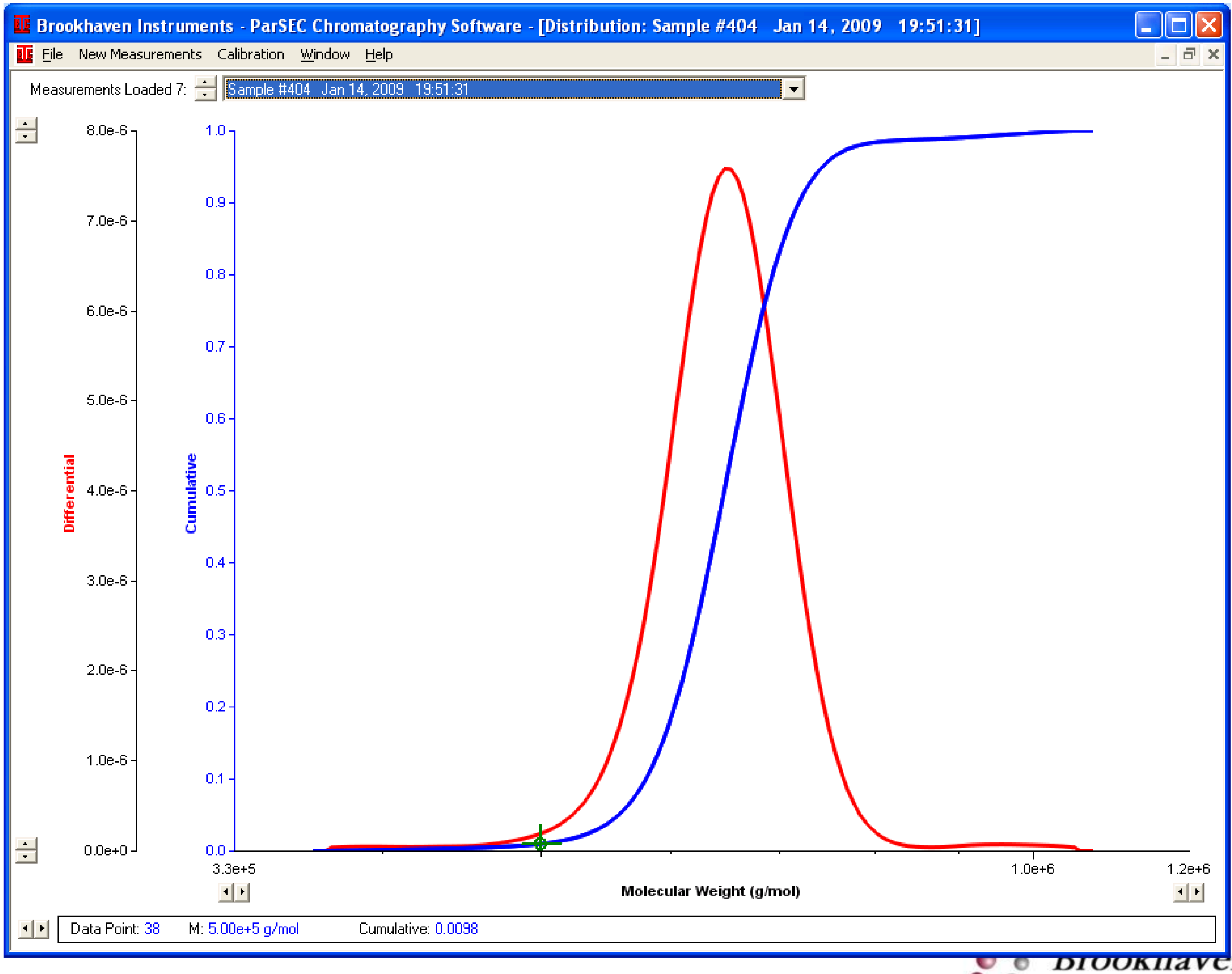
SEC/SLS: Absolute Distribution Data



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Some practical considerations

- Light scattering analysis requires quantitative evaluation of peak areas (heights).
- Ensure sample concentration is well known.
- Ensure that the injector is repeatable.
- For examples below, we assume that the sample concentration is known and the sample dn/dc is not.

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Effect of sample concentration accuracy

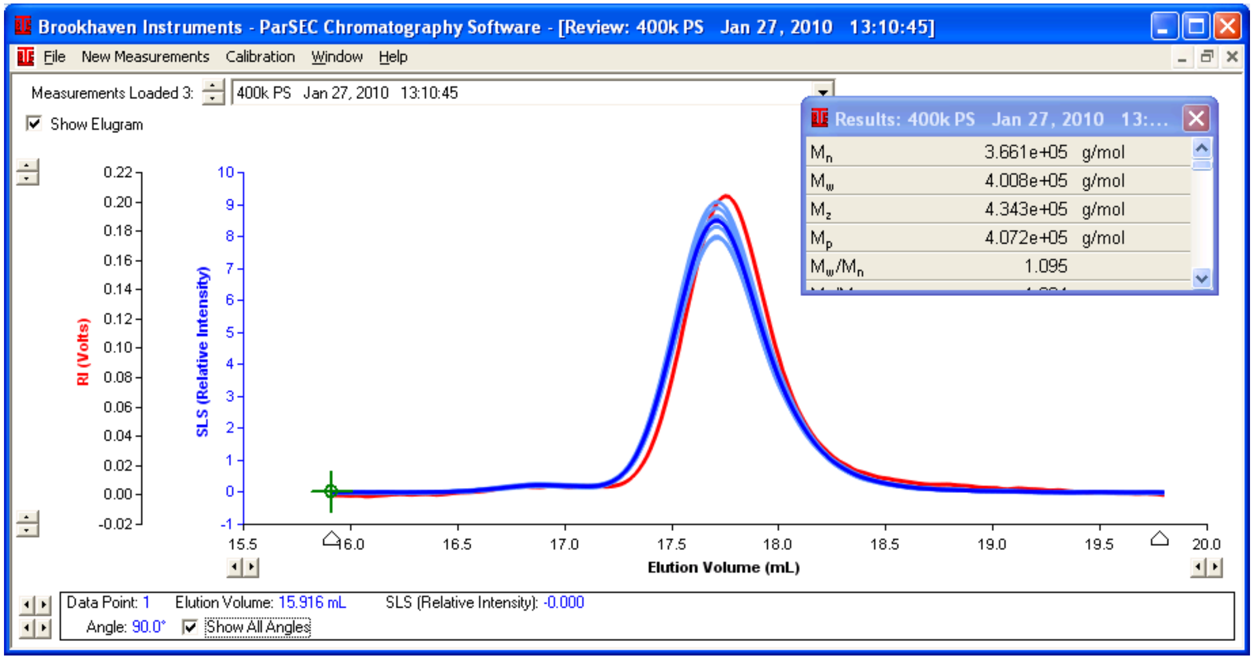
Stated sample concentration (mg/mL)	Determined M_w (g/mol)	Determined M_n (g/mol)	Determined Polydispersity (M_w/M_n)	Comments
2.208	313,200	108,600	2.89	Concentration 1% too low
2.230	316,600	109,700	2.89	Correct concentration
2.252	319,900	110,700	2.89	Concentration 1% too high

The sample concentration should be known to within 1%

For similar reasons, the injection volume should be repeatable to within 1%

Not Extrapolating to Zero-Angle

- Seven Angles, extrapolate to zero-angle: Mw = 400,800 g/mol



- Single angle (90 degrees), assume signal at 90 degrees equal to signal at zero-angle (Debye Plot): determined Mw = 369000 g/mol

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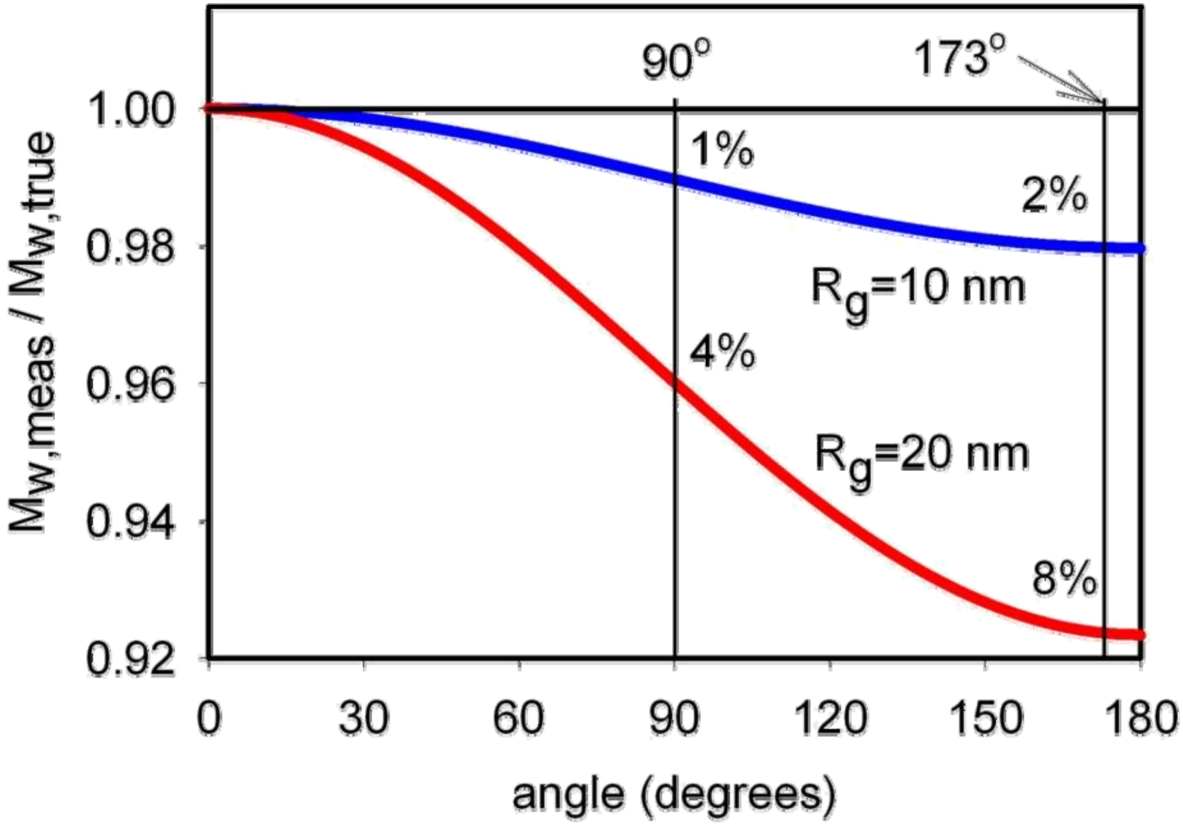
Effect of Angle Choice (Single Angle)



Here an angle of 0 refers to data obtained with a multi-angle instrument. The other angles refer to single angle instruments. For example, consider a polymer with a radius of gyration, R_g of 20 nm. An instrument operating at 90 degrees will systematically determine molecular weights that are 4% too low and an instrument operating at a "back-angle" of 173 degrees will systematically determine molecular weights that are 8% too low.

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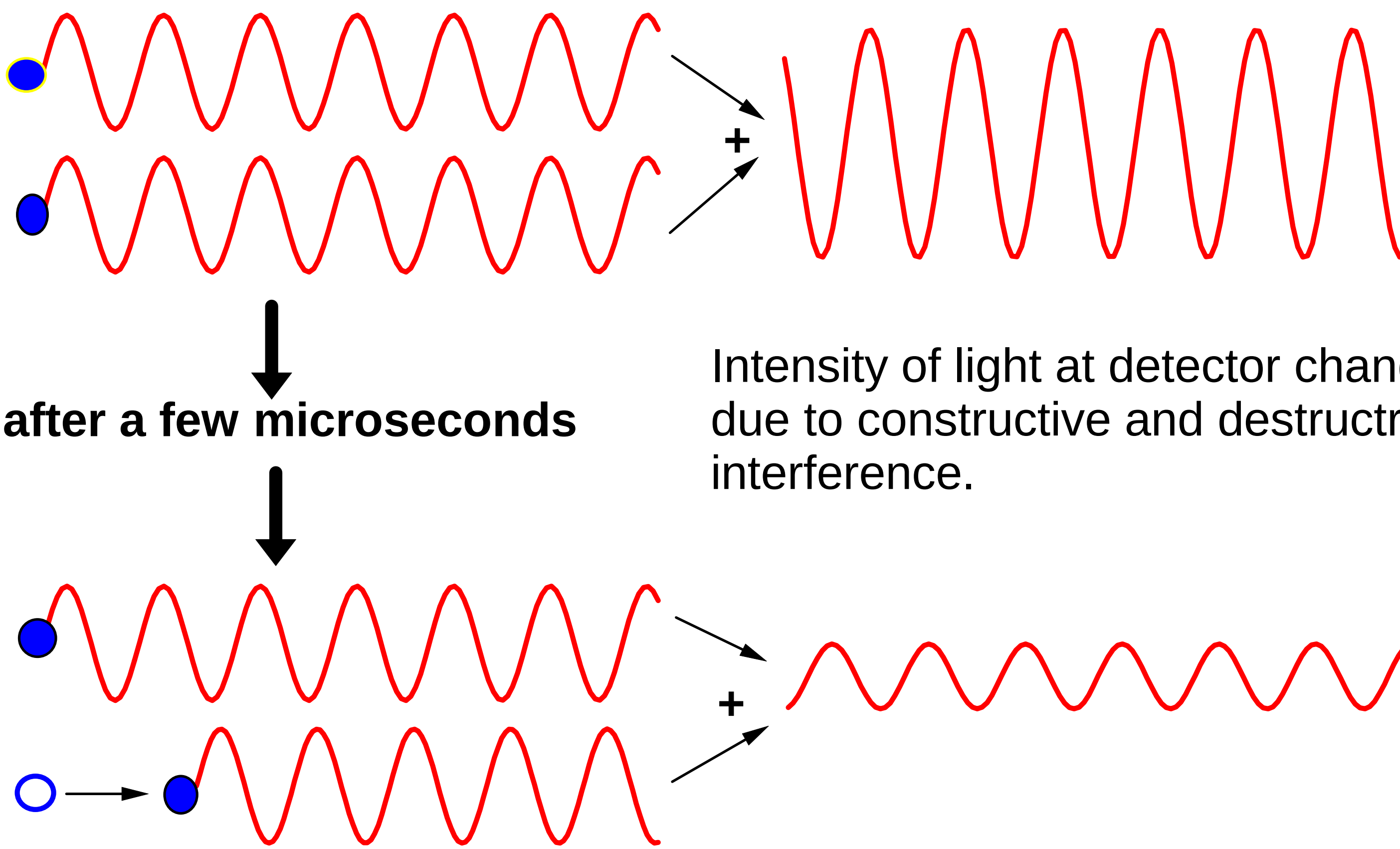
On-line Size Determination by DLS

Scattered intensity changes as particles move



Intensity of light at detector changes due to constructive and destructrive interference.

move due to Brownian motion

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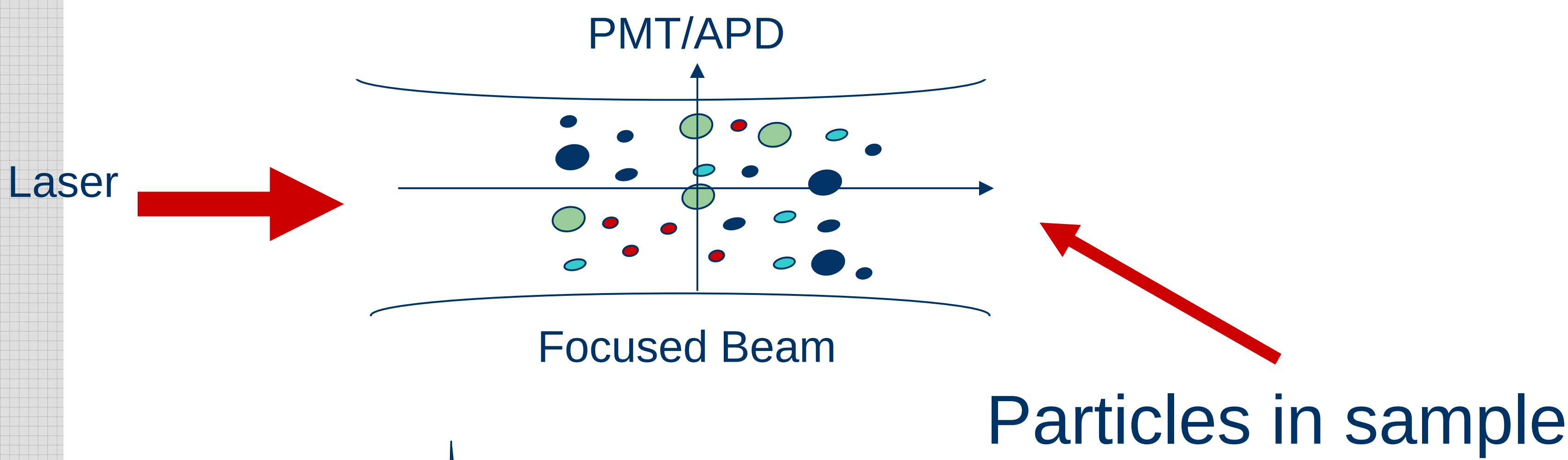
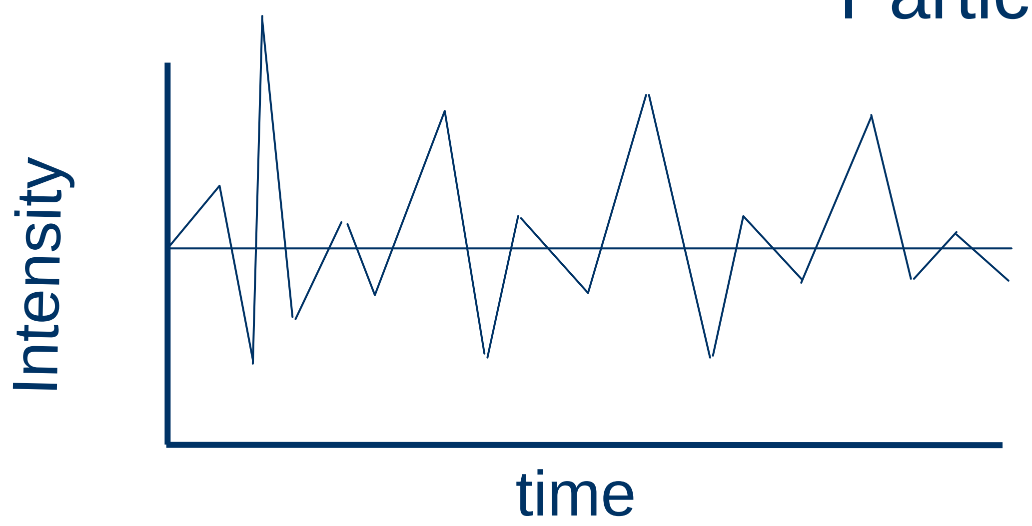
Intensity fluctuations





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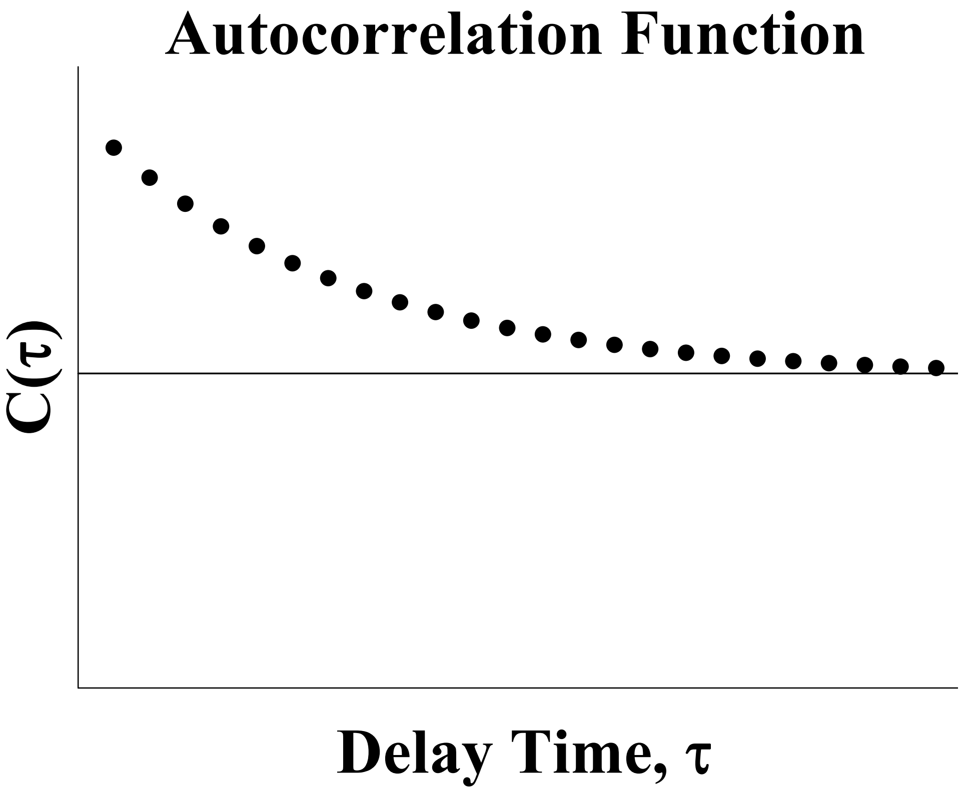
Correlation Function->Diffusion->Particle Size

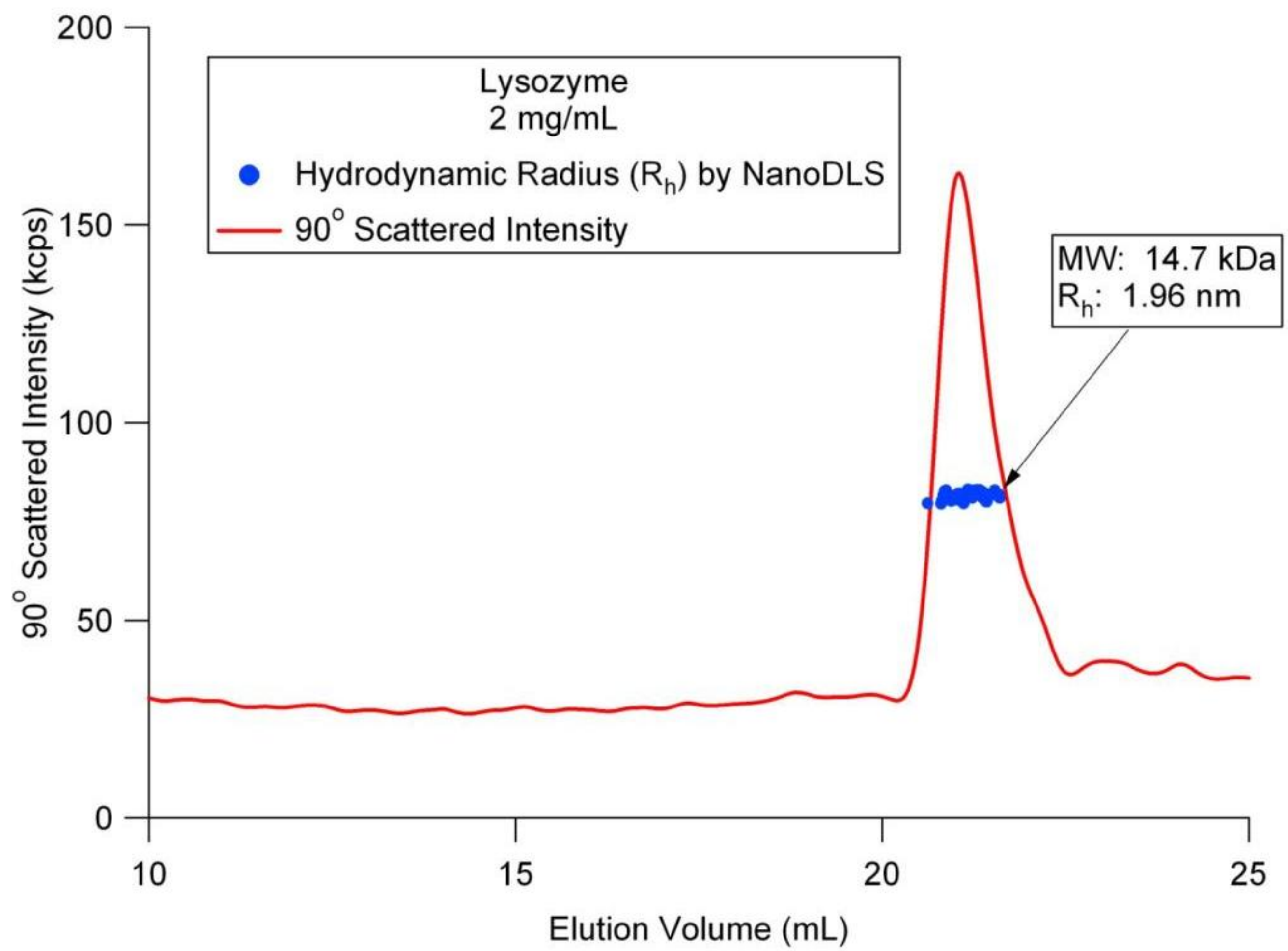


Fit exponential decay. Calculate translational diffusion coefficient,

$$D_t = \frac{k_B T}{3\pi\eta d_h}$$

d_h = Particle Size

DLS for particle size can be used on-line (with GPC)!



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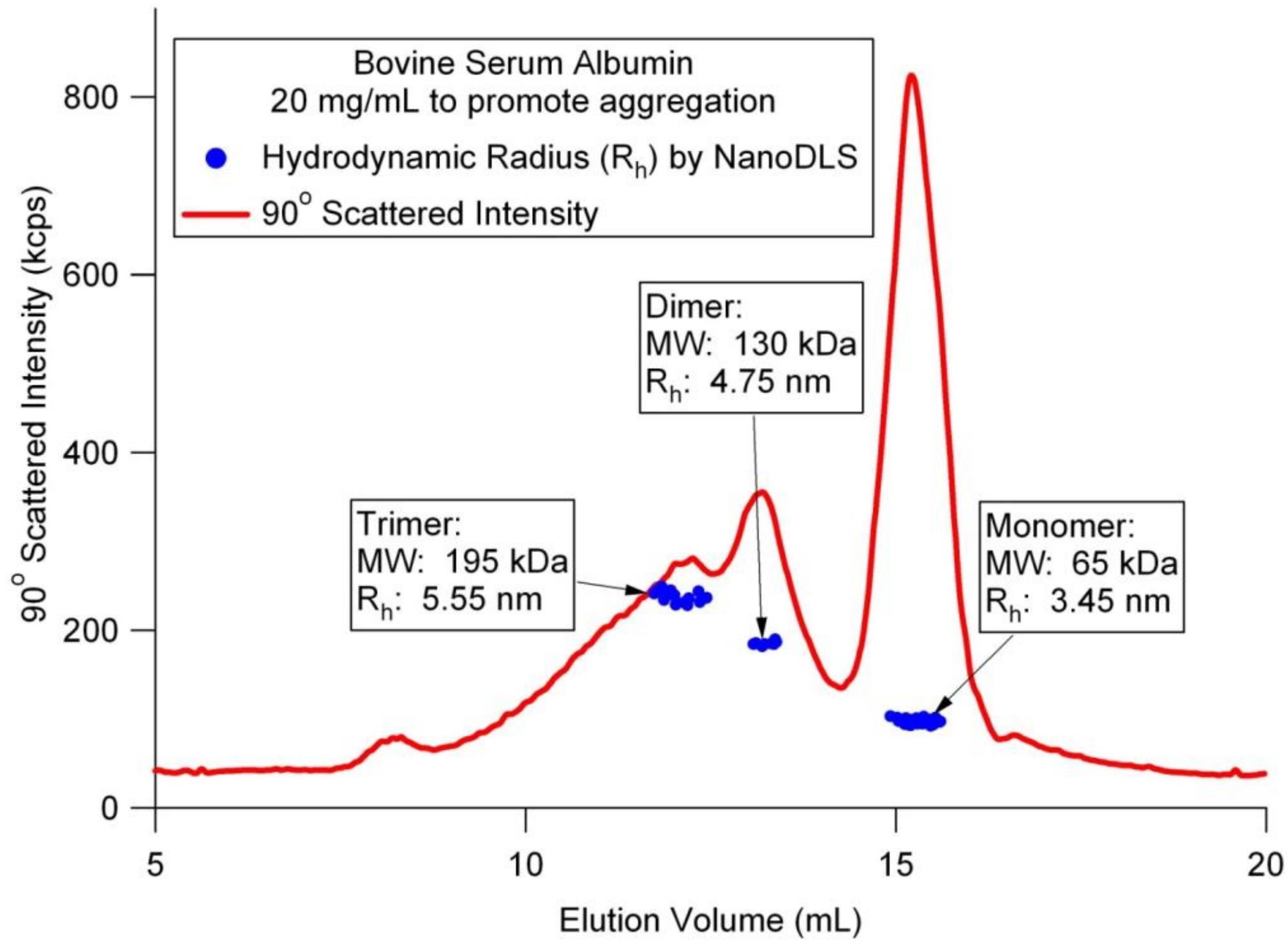
Use for evaluating aggregation



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