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Molecular-Scale Biophysics Research Infrastructure

Dynamic Light Scattering

Bertrand Raynal

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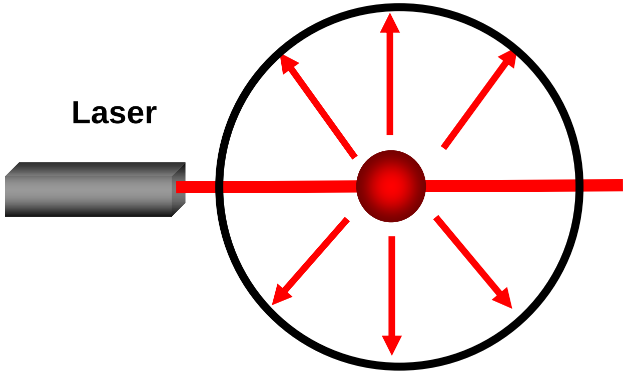
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

Scattering Principal

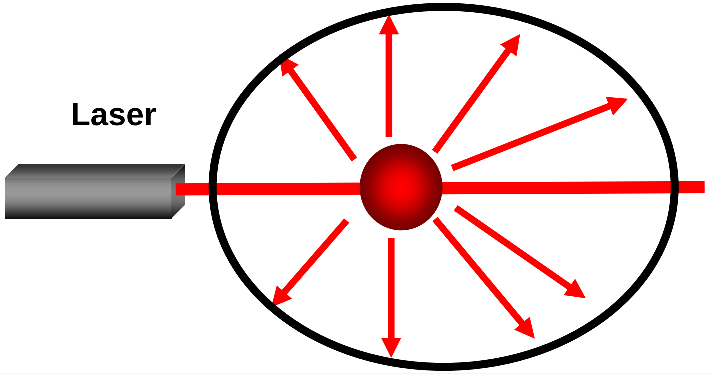
If radius <15nm



$I_\theta = I_0$

$$I_0 \propto k(dn/dc)^2 CM$$

If Radius >15nm



$I_\theta \neq I_0$

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Scattering Principal

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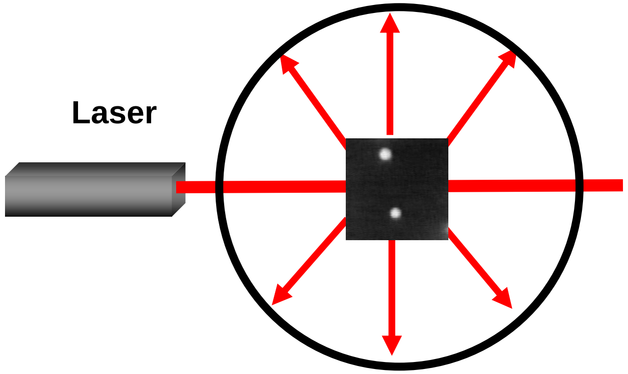
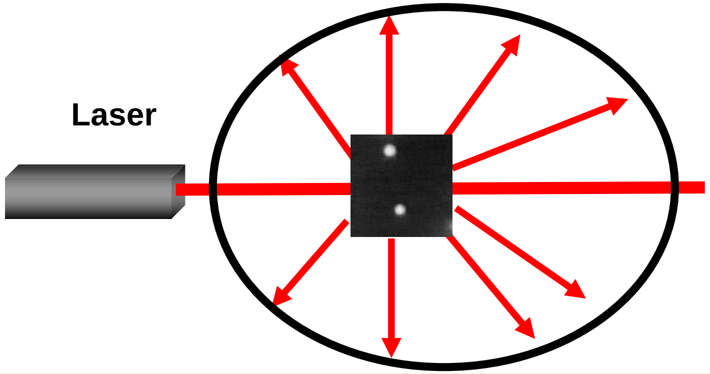


$I_\theta = I_0$

If Radius >15nm



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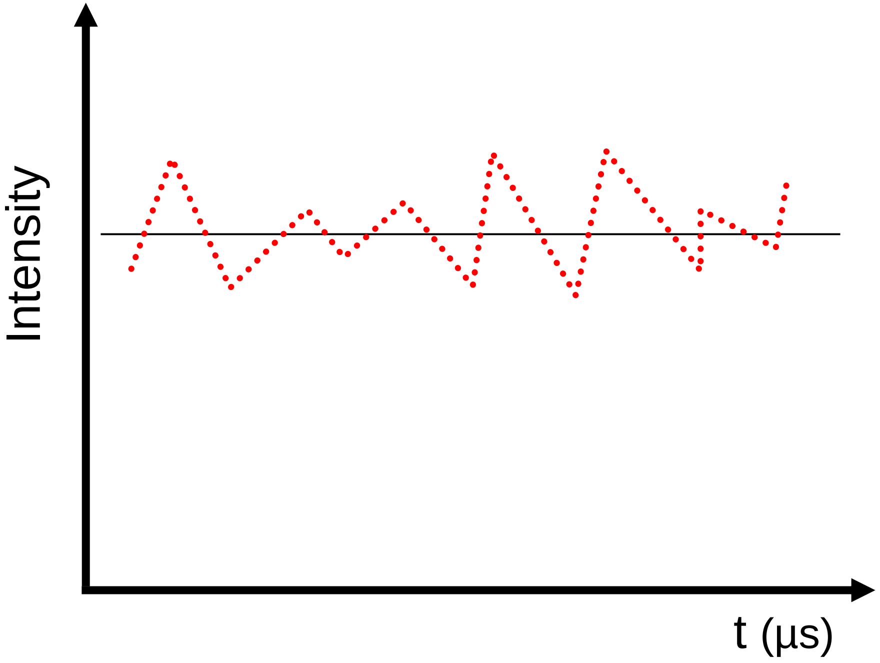

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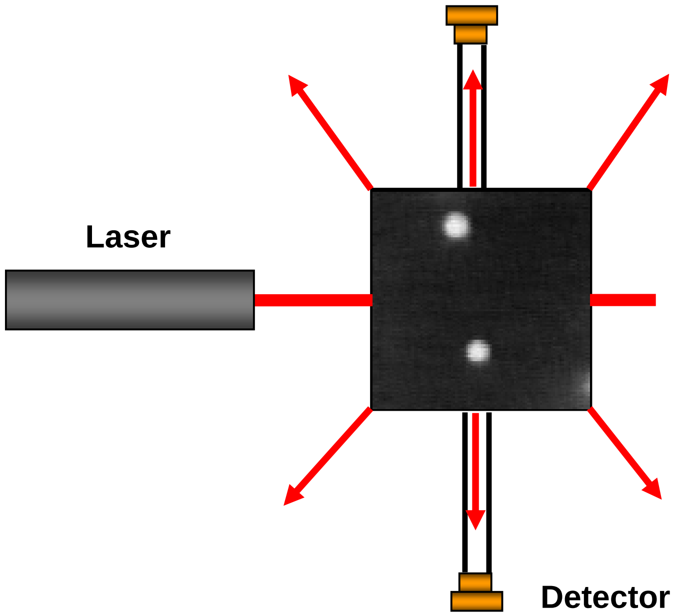
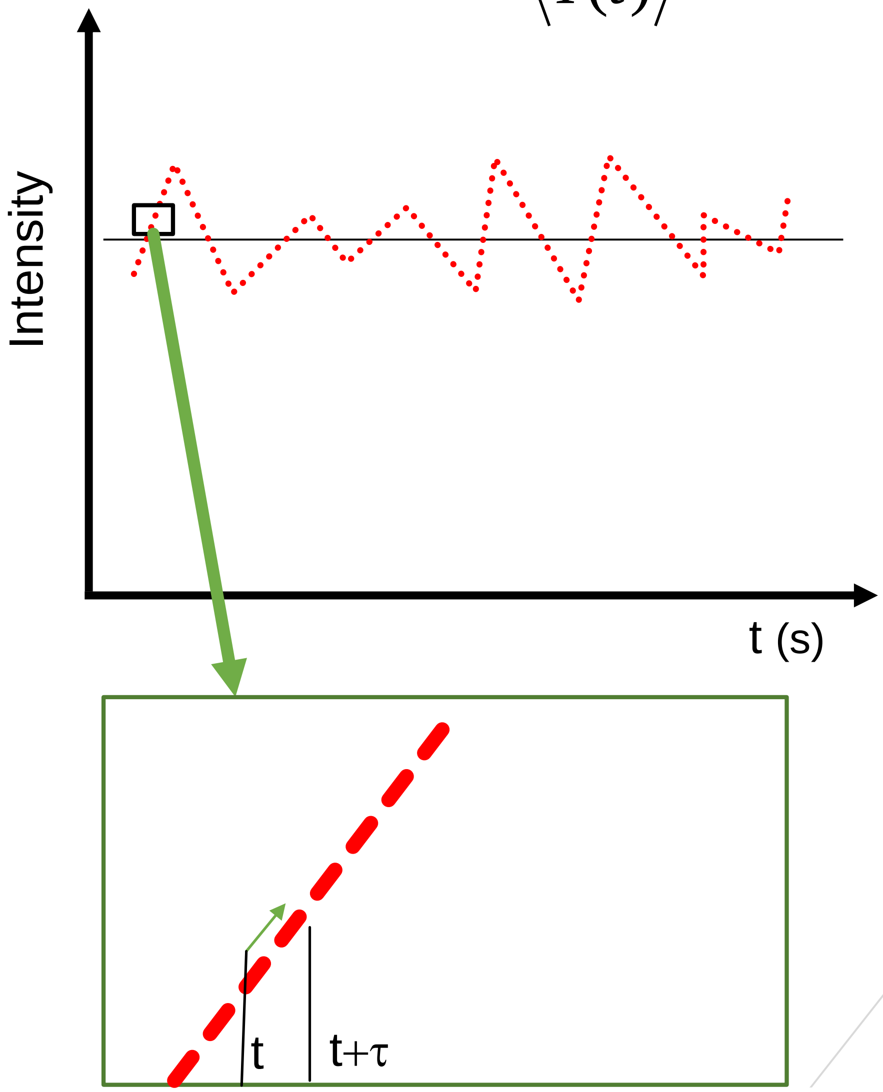
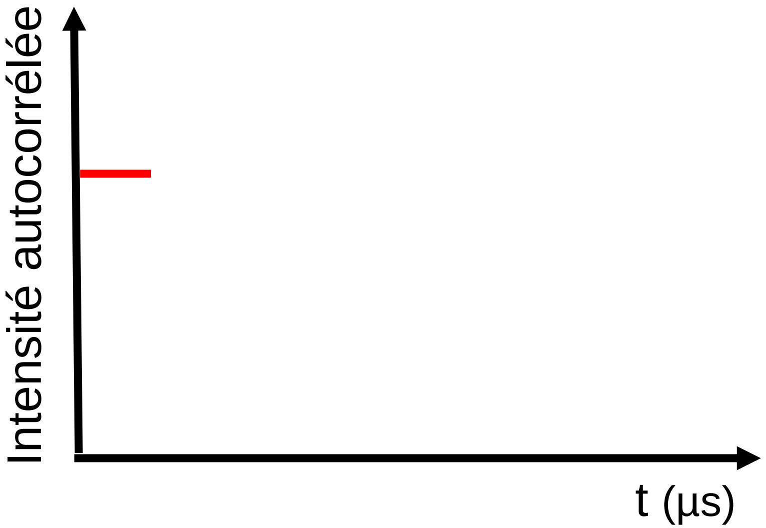
$$g^{(2)}(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2}$$







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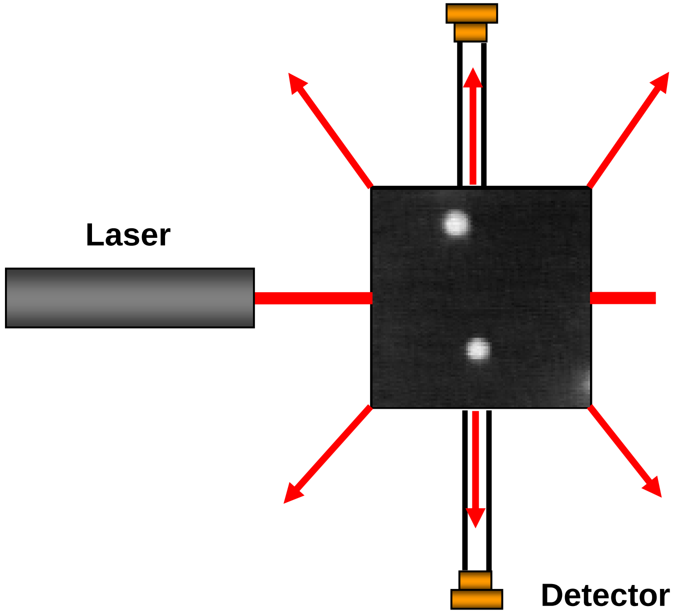
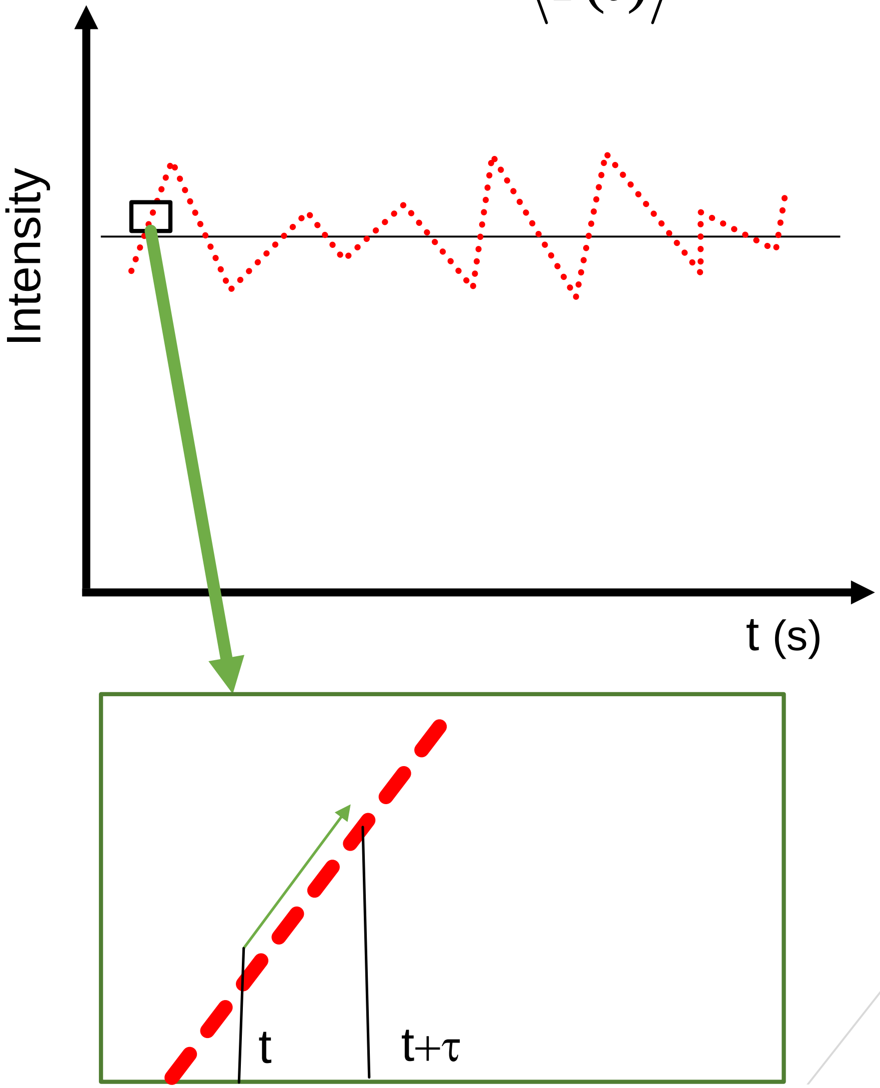
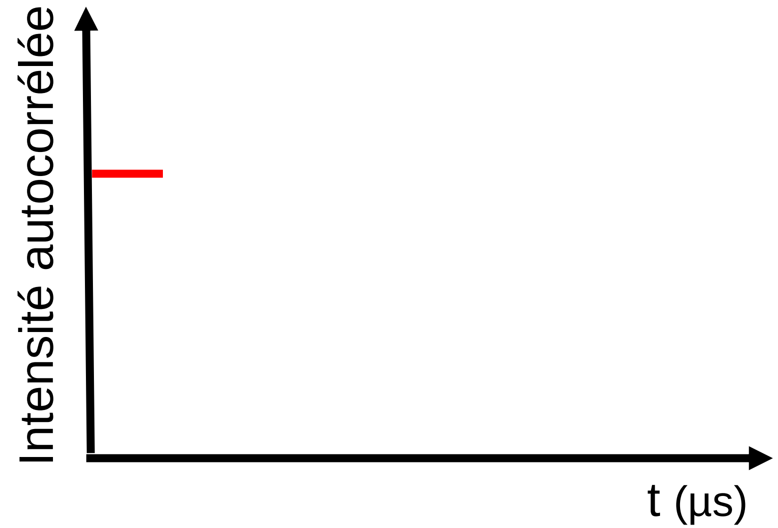
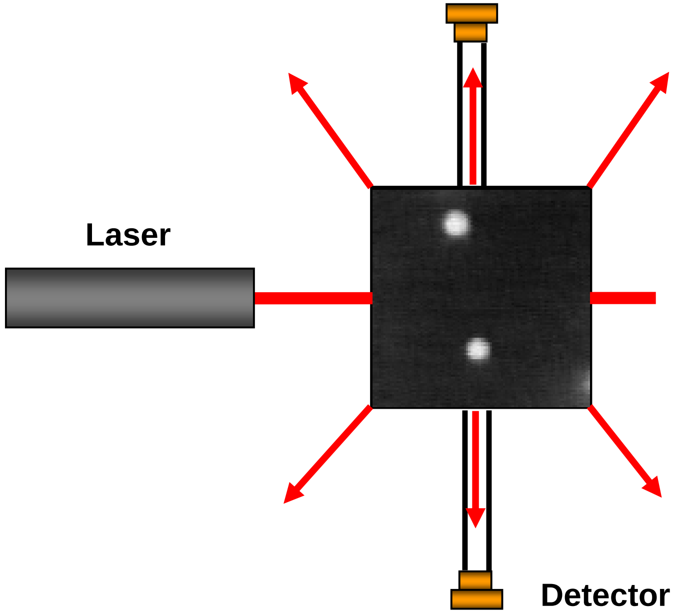
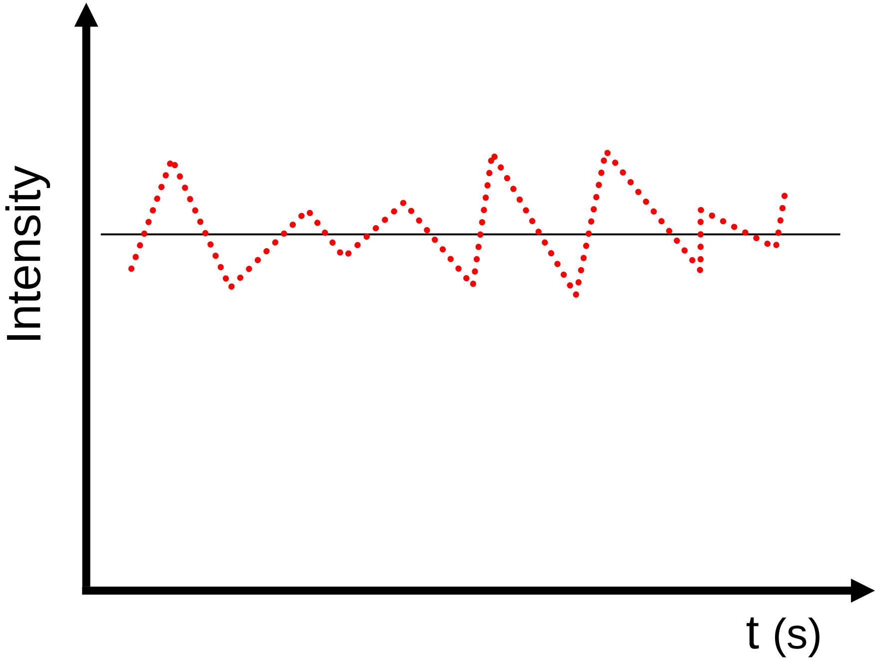
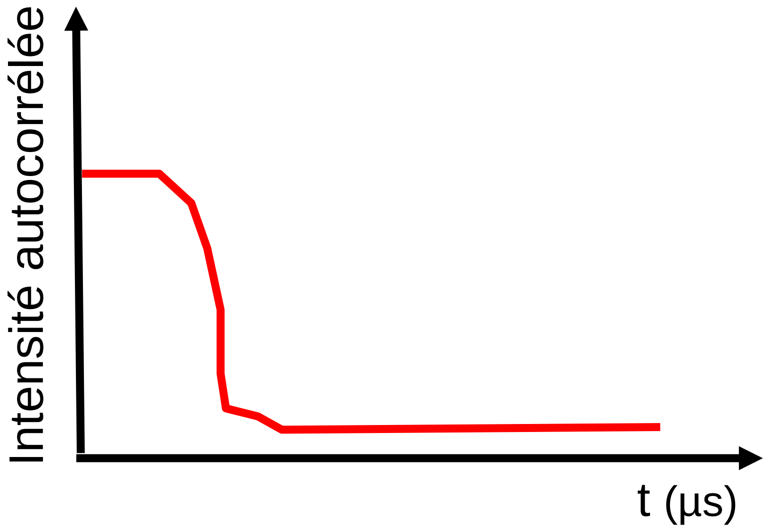












Mondisperse sample

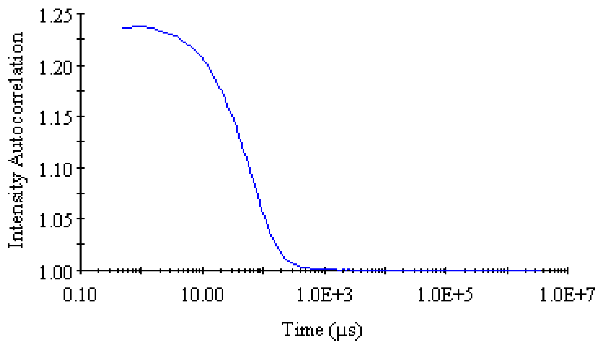
$$g^{(2)}(\tau) = B + \beta \exp\left(-2D \left(\frac{4\pi n_0}{\lambda_0} \sin\left(\theta/2\right) \right)^2 \tau \right)$$

Analysis of Polydisperse Samples

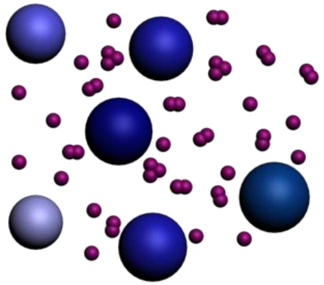
- For a *monodisperse* particle, the normalized intensity autocorrelation function is described by an exponential with a decay rate Γ:

$$g^{(2)}(\tau) = 1 + \beta e^{-2\Gamma\tau}$$

$$\Gamma = D_t q^2 \propto \frac{1}{R_h}$$



- For a *polydisperse* particle size distribution, the normalized intensity autocorrelation function is described by a sum of the autocorrelation functions of all particle species, weighed by their normalized intensities p_j:



$$g^{(2)}(\tau) = 1 + \beta \left| \sum_j p_j e^{-\Gamma_j \tau} \right|^2$$

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Cumulant Expansion

- One solution to the problem is to assume amonomodal size distribution and compute the moments of the distribution:

1st cumulant — 2nd cumulant — 3rd cumulant

$$g^{(1)}(\tau) = 1 + \beta e^{-\kappa_1 \tau} + \frac{\kappa_2}{2!}\tau^2 - \frac{\kappa_3}{3!}\tau^3 + \ldots$$

mean — width — skewness

- The first cumulant describes the mean decay rate $\bar{\Gamma}$;
- Adding the second cumulant describes the width of distribution of decay rates;
- The third cumulant describes the asymmetry of the distribution of decay rates.

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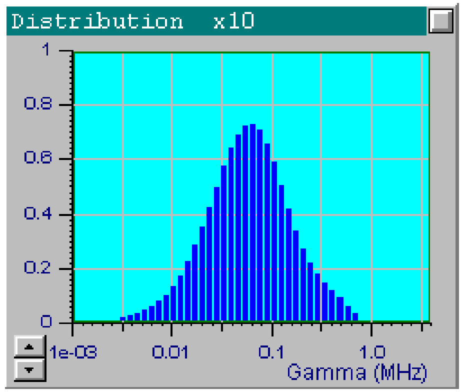
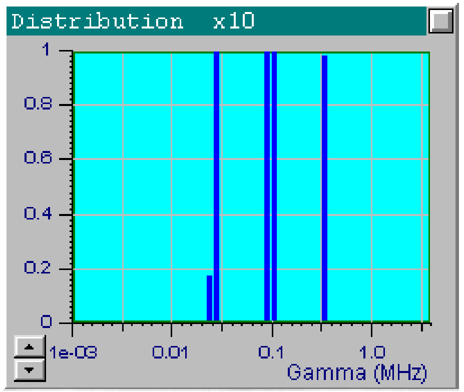
Distribution

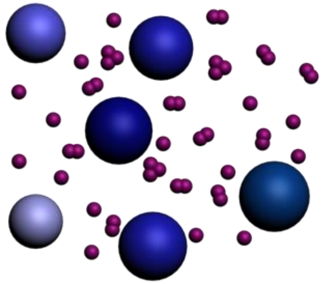
- Make assumptions about the distribution:
  - All scattering amplitudes are positive: the number of particles cannot be negative.
  - Choose the "smoothest" solution: the decay rate (and particle size) distribution is rather continuous than discrete.



continuous



discrete



  - Mathematically this means the software adds a term to the least squares fit:
  - α is called the *regularizer*

$$\alpha \sum_n (f_n - f_{n+1})^2$$

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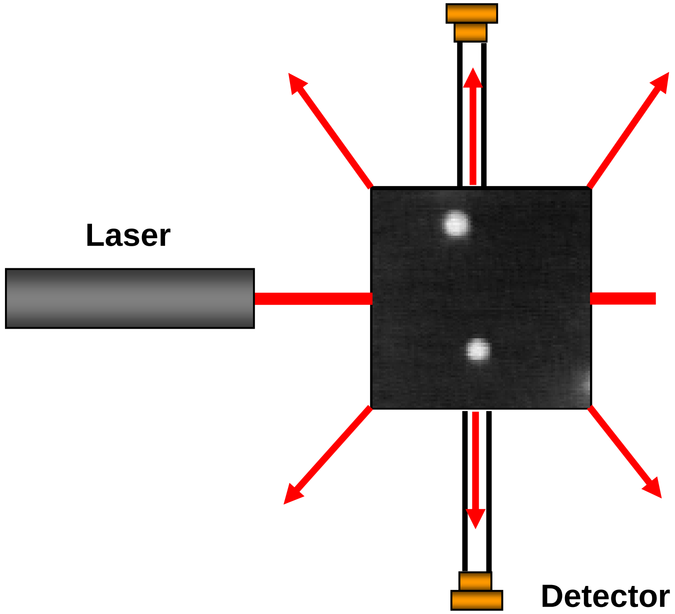

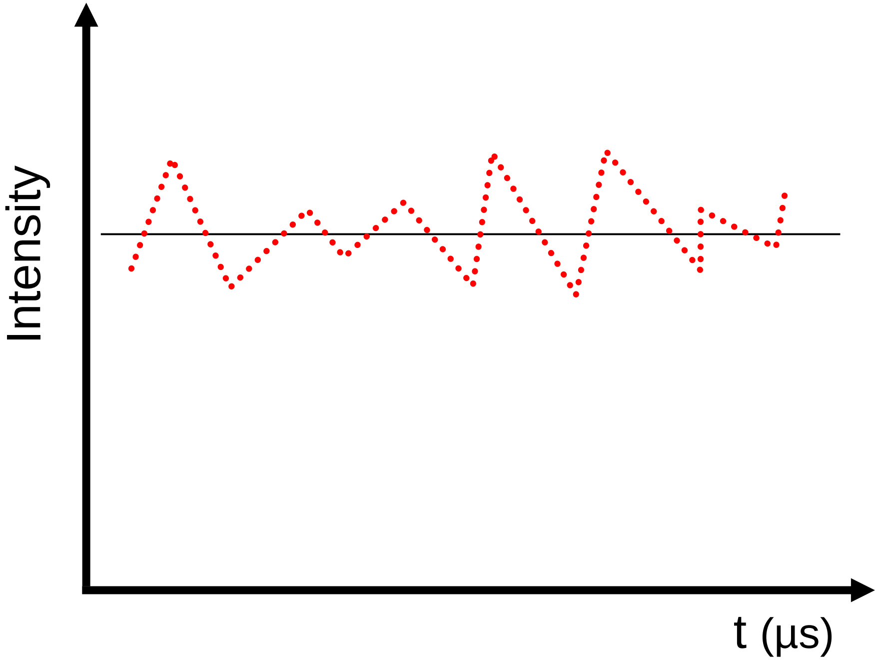

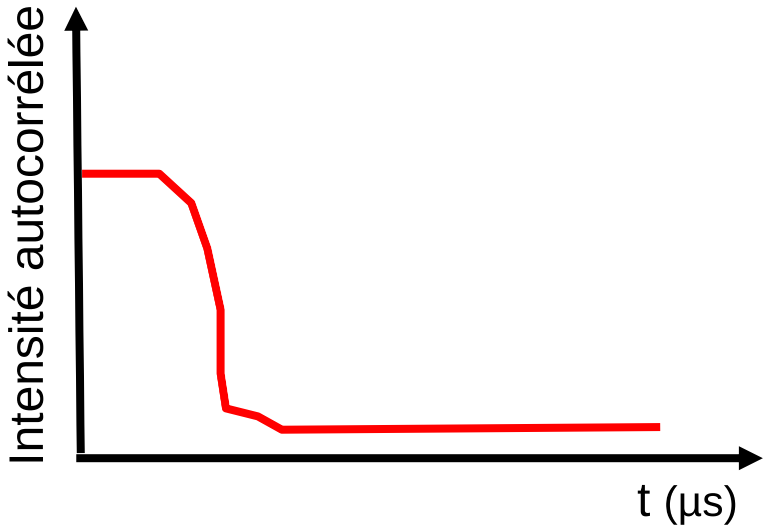

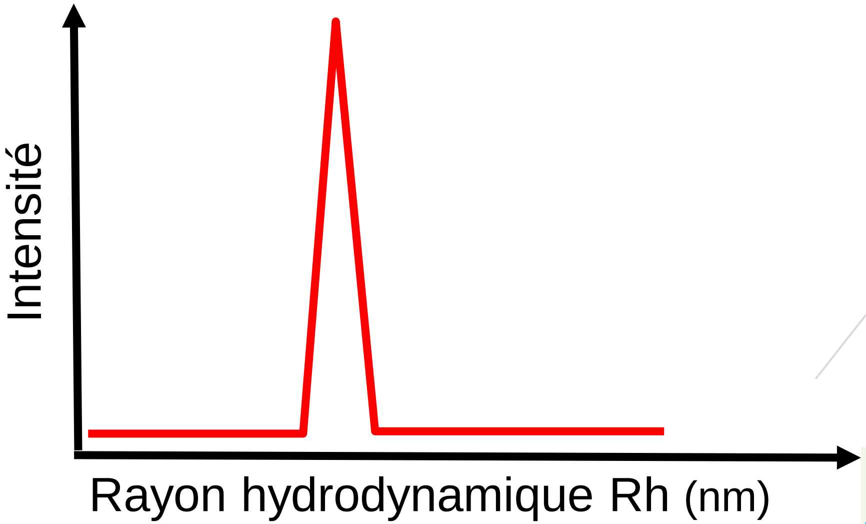

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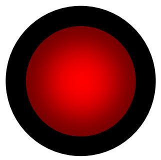
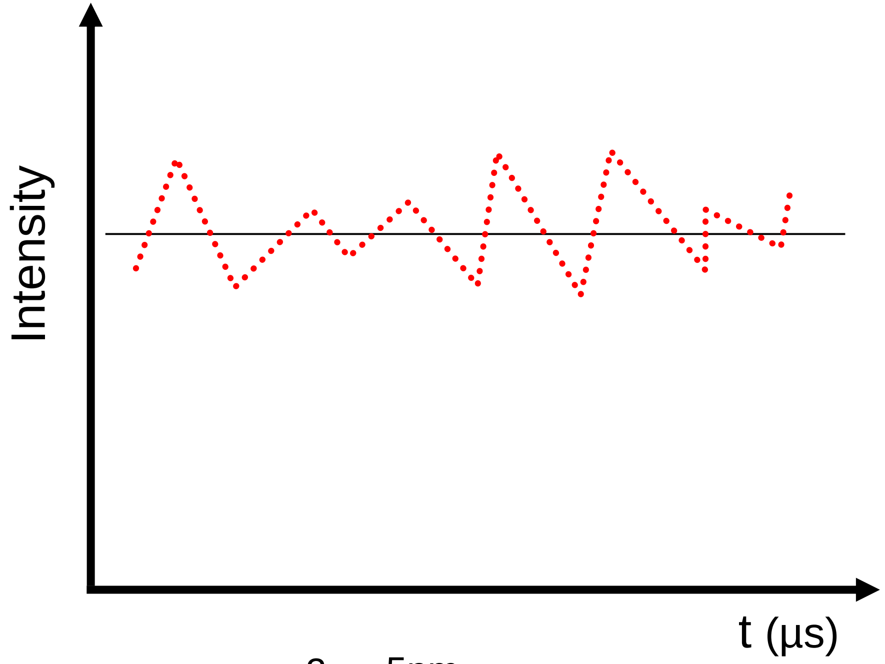
The hydrodynamic radius Rh is the radius of the sphere that diffuse at the same speed than molecule of interest.

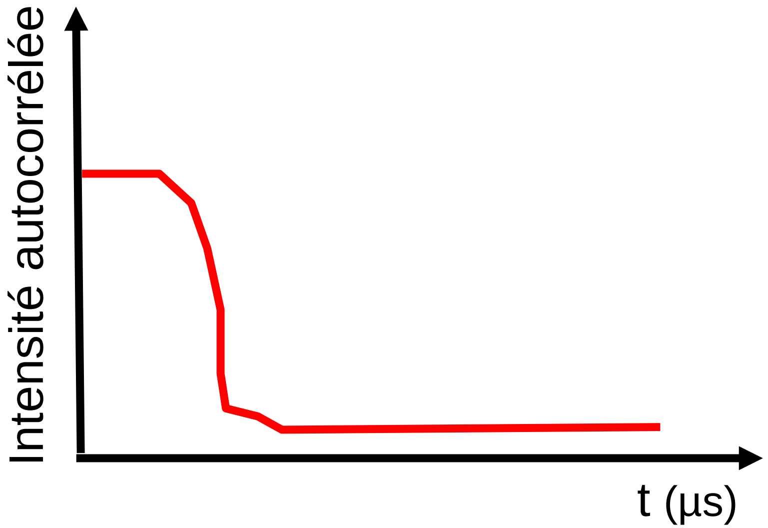


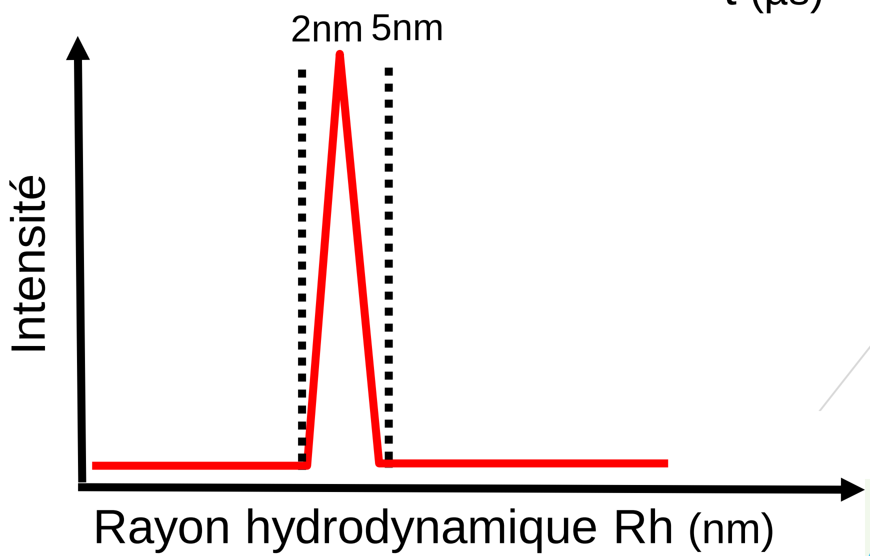
BSA

Monomer 3.4nm

Dimer 4.5nm







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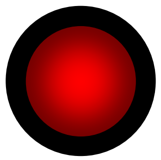
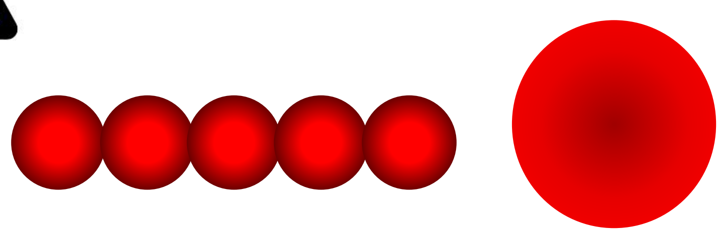


BSA

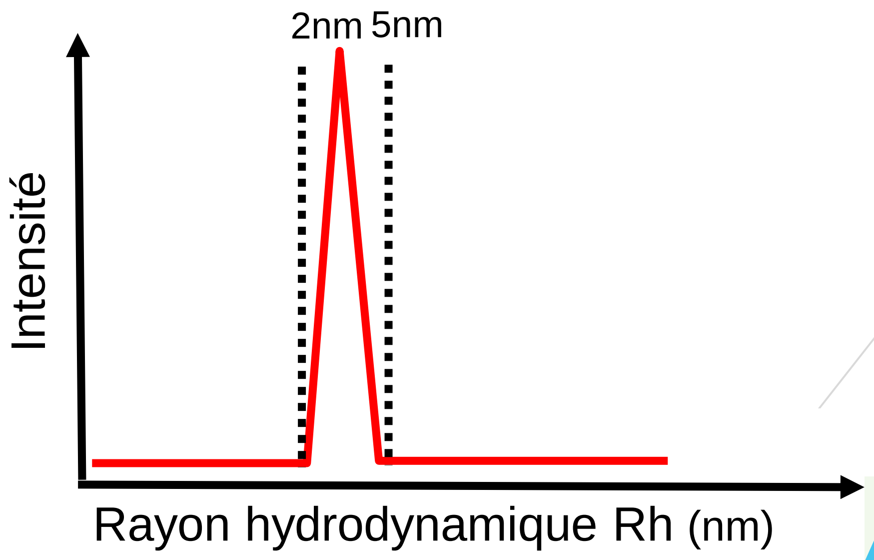
Monomer 3.4nm

Dimer 4.5nm

DLS does not measure mass



Rh	5.5nm	5.5nm
M	150kDa	300kDa



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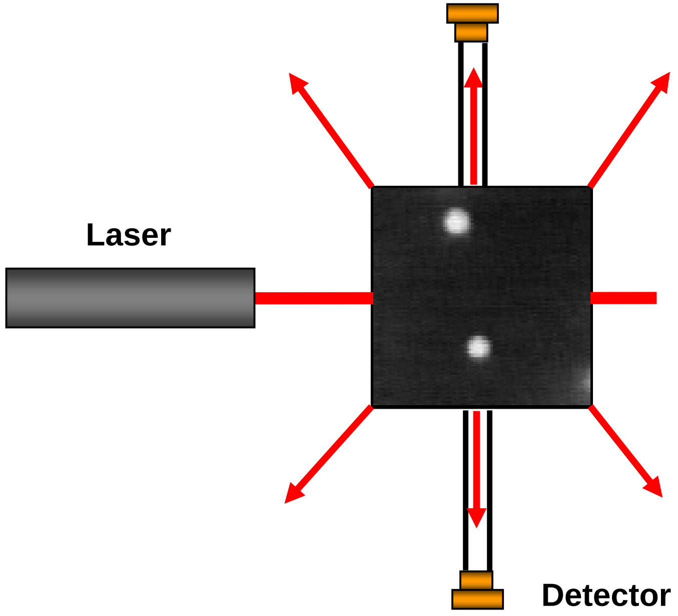
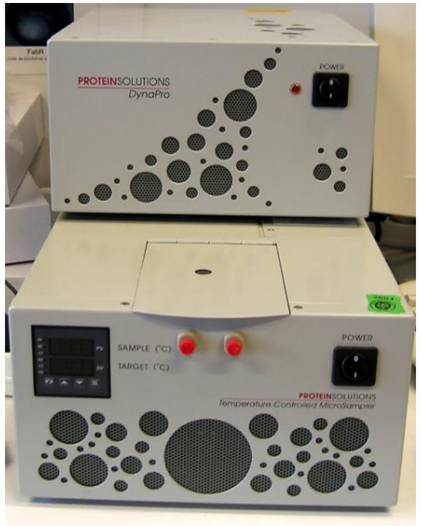
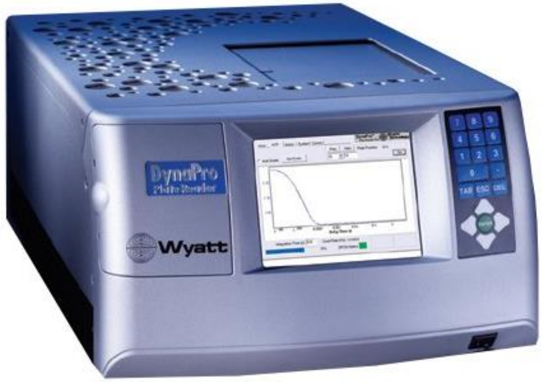
Dynamic Light Scattering Instruments





DynaPro MS-800 *(Wyatt)*

Cuvette One by one



DynaPro plate reader III *(Wyatt)*

Plate up to 1536 wells

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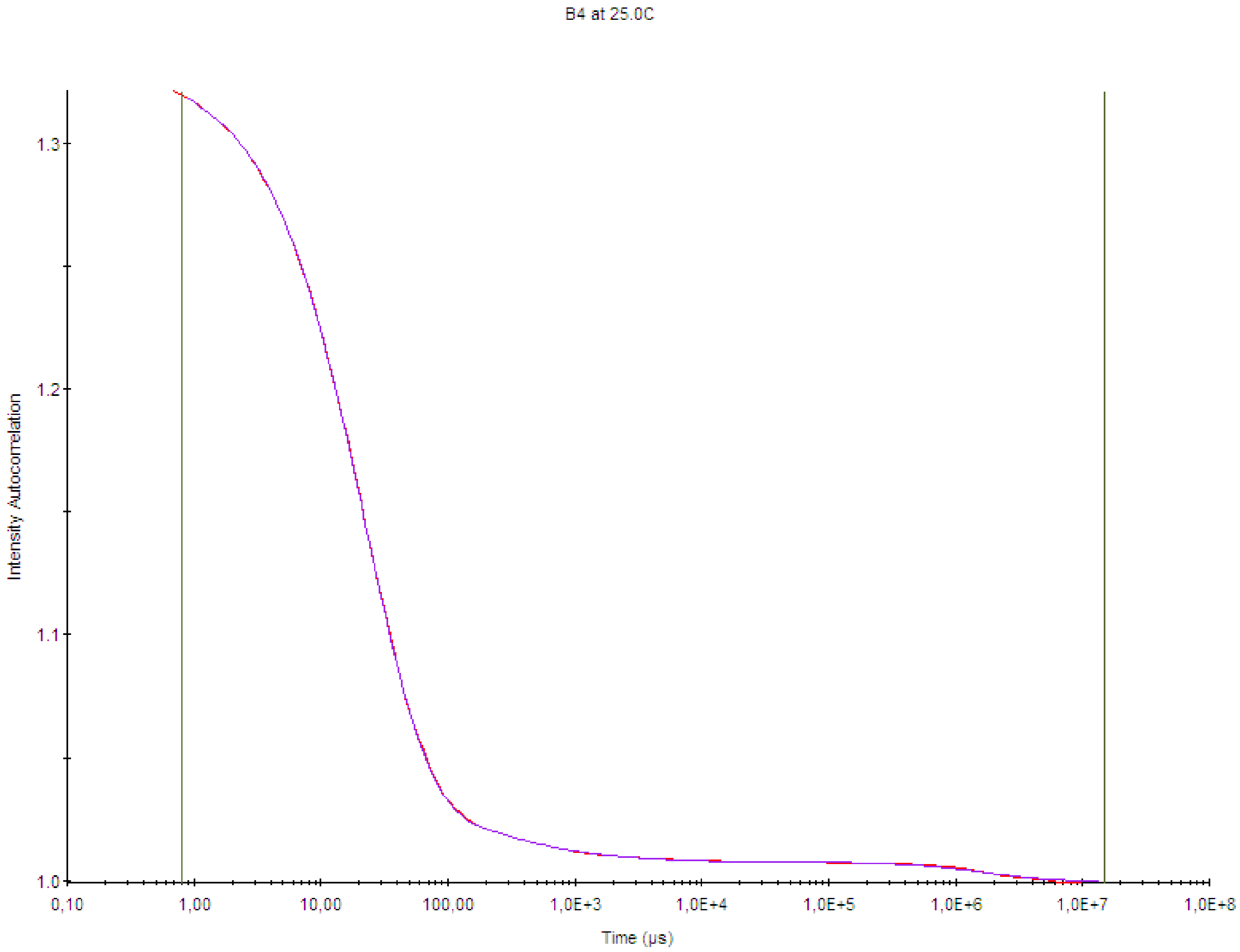
Real Data



This project has re

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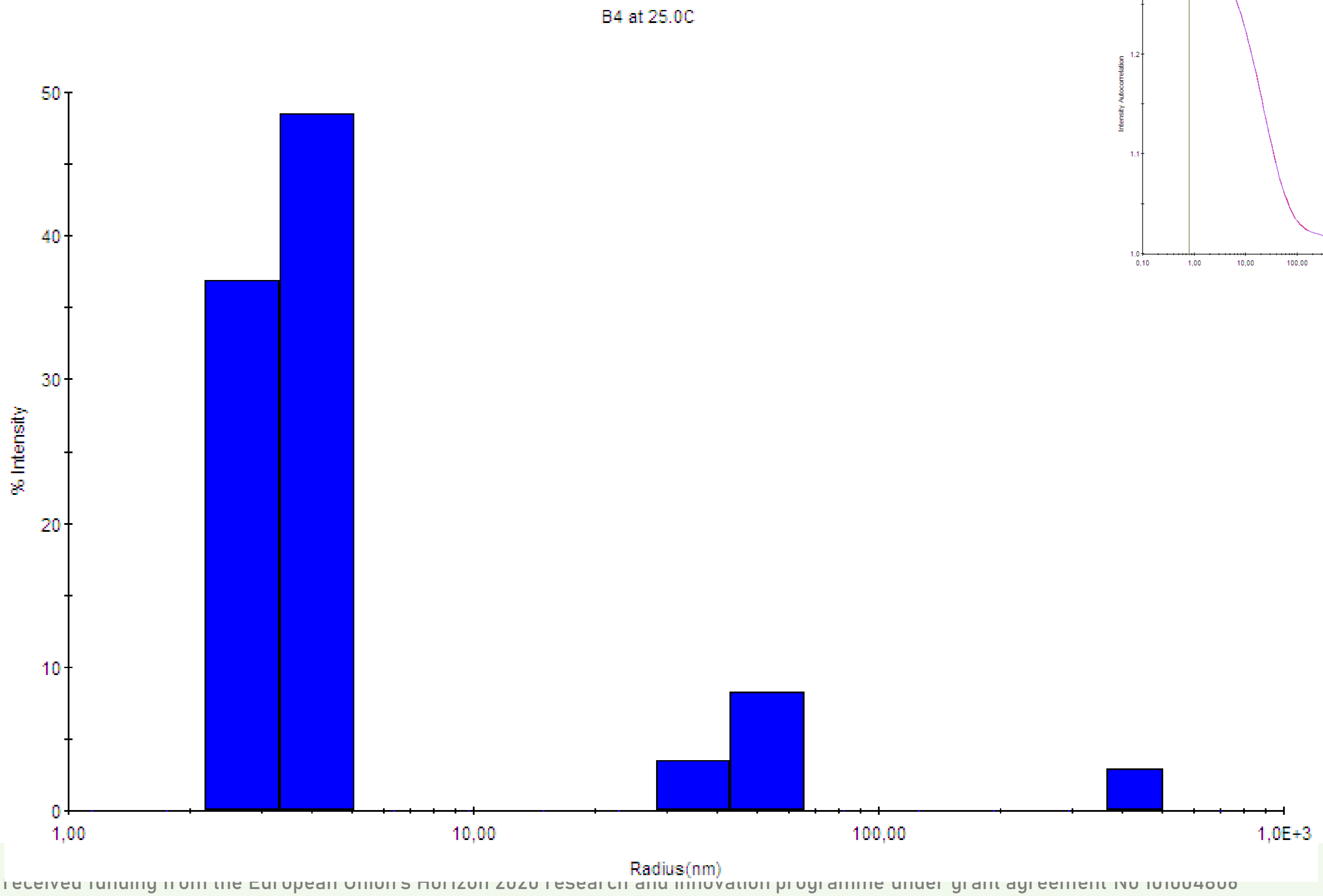
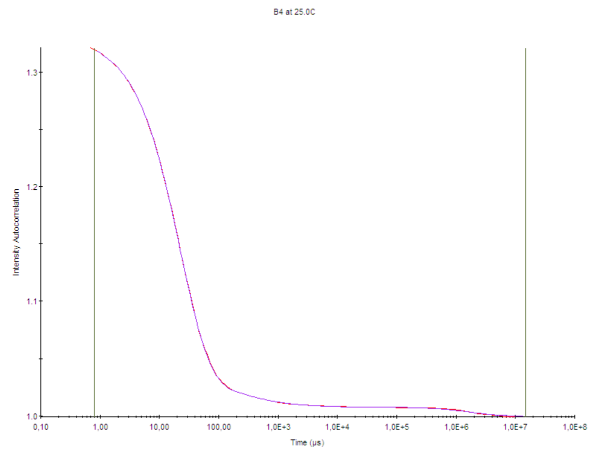
Real Data





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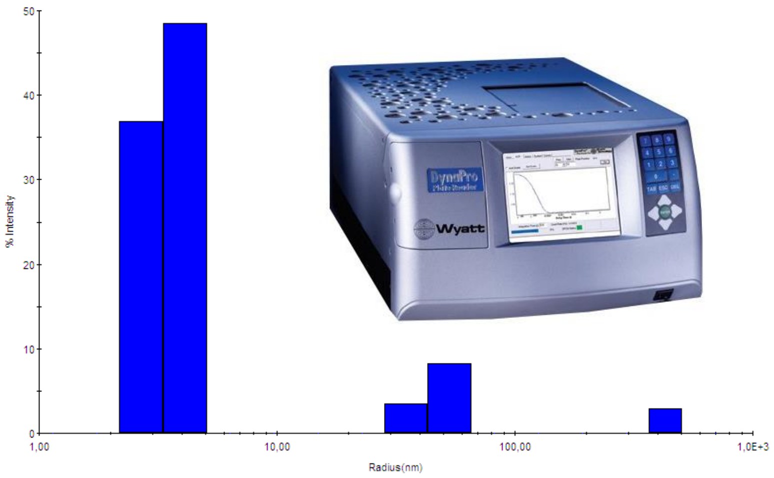
Conclusion on the technique

Dynamic light scattering (DLS)



$$I_0 \propto k(dn/dc)^2 CM$$

- Detect low amount of aggregates
- Low resolution does not distinguish between monomer and dimer

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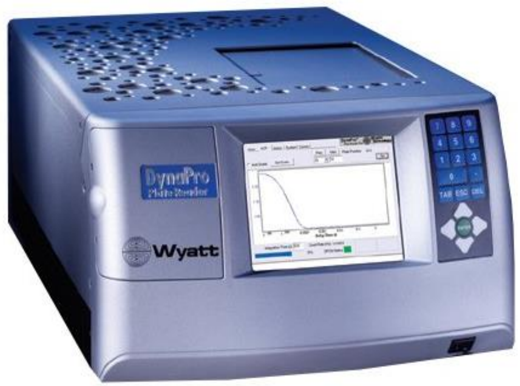
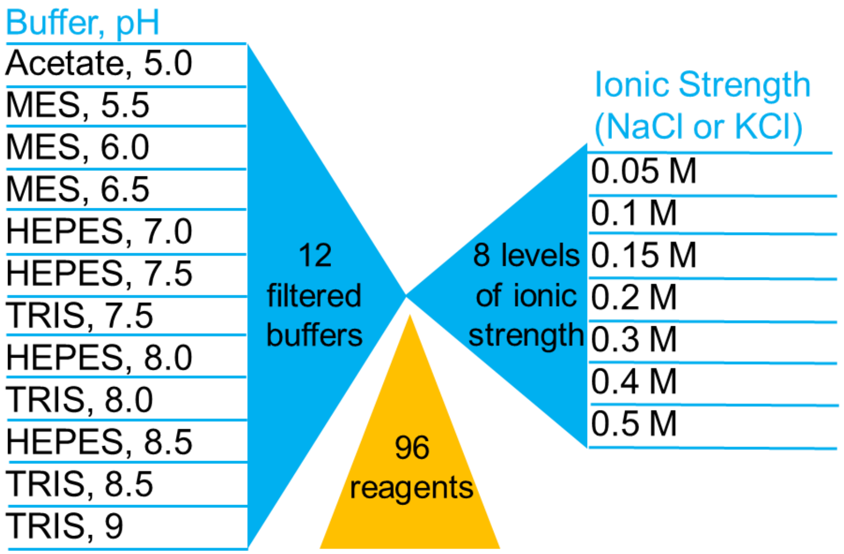
Long term stability, experimental condition, storage condition

Dynamic light scattering (DLS)

- Can detect low amounts of aggregates
- Plate of different buffer solutions (pH, salt type and amount, buffer type)
- Fully automated plate reader can measure 96 conditions in 6 hours





2ul of sample in 18ul of buffers

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Questions ?

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