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Dynamic Light Scattering measurements

arpine.sokratian[1]

[1]Duke University

ABSTRACT

Brief instrument guide for Dynamic Light Scattering Wyatt DynaPro followed by specific instructions to measure the size of sonicated amyloid fibrils

PROTOCOL CITATION

arpine.sokratian 2022. Dynamic Light Scattering measurements. **protocols.io** https://protocols.io/view/dynamic-light-scattering-measurements-b9sdr6a6

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PARENT PROTOCOLS

In steps of

Real time-quaking induced conversion assay (RT-QUIC)

Preparation of α-synuclein fibrils amplified from clinical material

Preparation of α-synuclein fibrils amplified from clinical material

DLS overview

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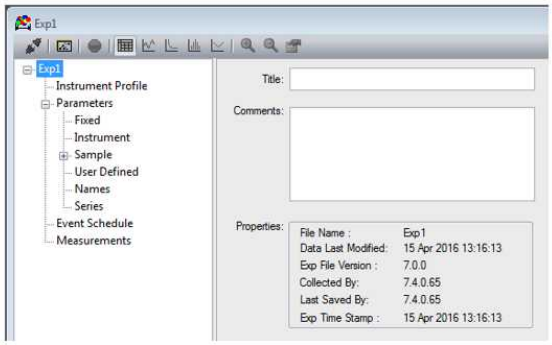
Outline: A DYNAMICS Experiment

Experiment tool bar

- Instrument controls
- Data display options

Experiment Nodes



- **Instrument Profile** contains hardware information.
- **Parameters** for data collection and analysis
- **Event Schedule** to automate experiments
- **Measurements** contains all data collected during the experiment.

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WYATT TECHNOLOGY

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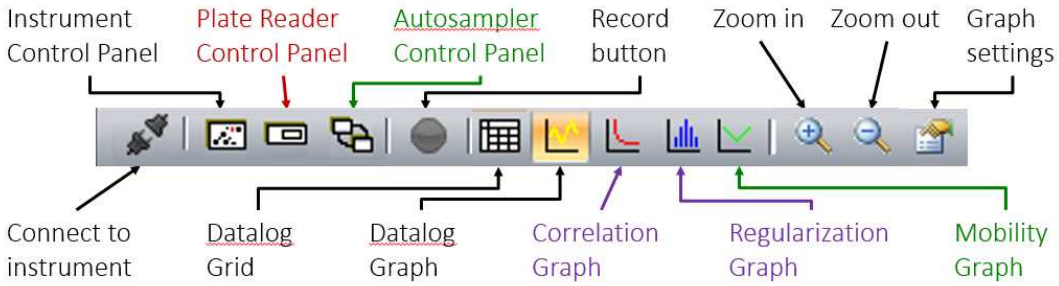
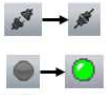
Experiment Toolbar



Buttons labeled in **black** apply to all instruments.

- In order to collect data, you must *Connect* to an instrument.
- When an instrument is connected, the *Record* button activates.



Buttons labeled in **purple** apply to DLS measurements on all instrument.

Buttons labeled in **red** are visible for Plate Reader instruments only.

Buttons labeled in **green** are visible for Möbiuζ instruments only.

- The Autosampler Control Panel is only visible if a supported autosampler is connected.

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WYATT TECHNOLOGY

Instrument Profile node

Identify the instrument

- Serial number, model, network name
- Firmware version
- Laser wavelength and detector angle

Instrument-specific information

- **Plate Reader:** Type of well plate (Well Count)
- **Möbiuζ:** Autosampler connection

Make any necessary changes prior to connecting.

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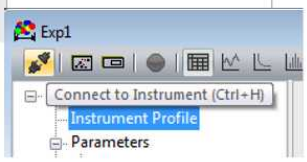
Instrument Profile Node: Plate Reader Example

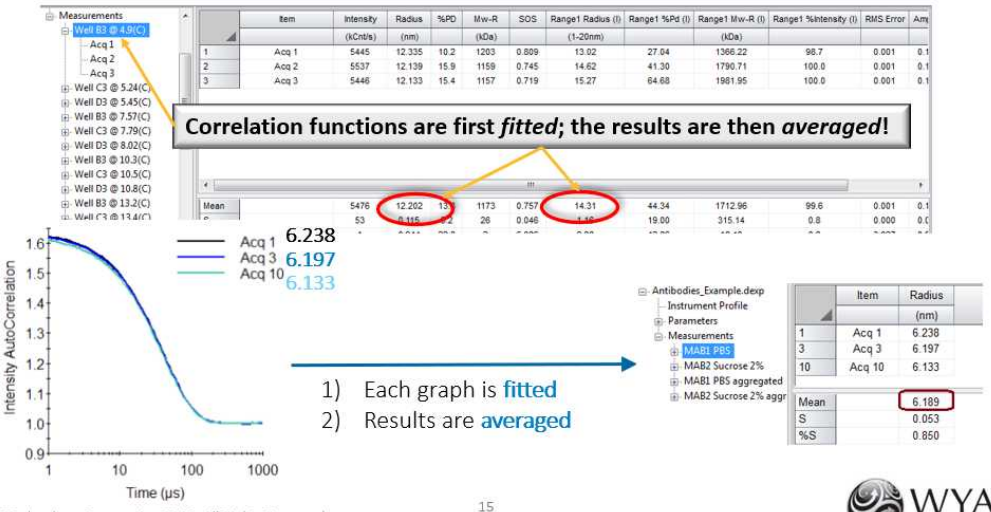
Property	Value
Instrument	
Serial Number	105-WPR2
Model	Plate Reader
Control Firmware Version	2.0.5.1
DLS Firmware Version	2.3.1.0
Plate Reader Firmware Version	2.0.2.0
Instrument Network Name	wyatt-105-wpr2
Temperature Controlled	Yes
Generation	2
Laser Wavelength (nm)	823.2
DLS Detector Angle (degrees)	150.0
Well Count	384



Inspect individual acquisitions or readings

Expand each Measurement to se results of individual acquisitions:

- Search for outliers or see how data filters have been applied



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Sample preparation

3 Add **4 µL** of diluted sample (ranges **0.1-0.5 mg/mL**) onto

NanoStar Disposable MicroCuvette Kit **Wyatt Catalog #WNDMC**

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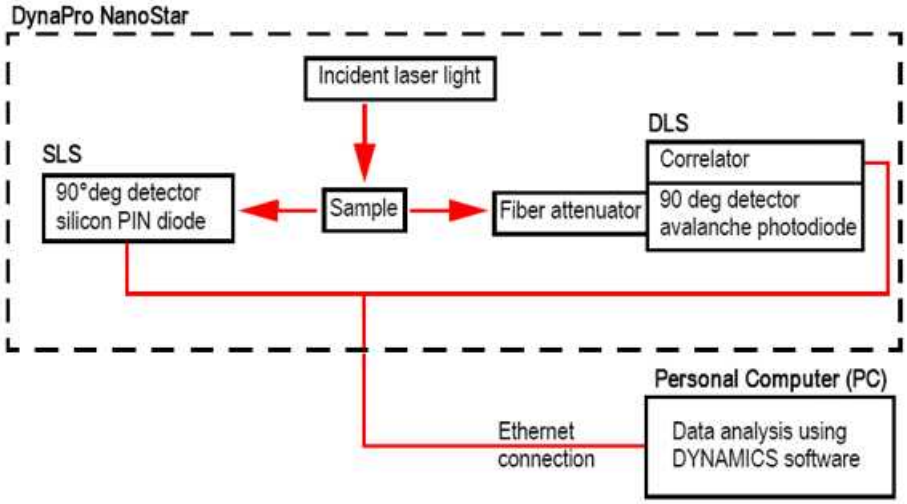
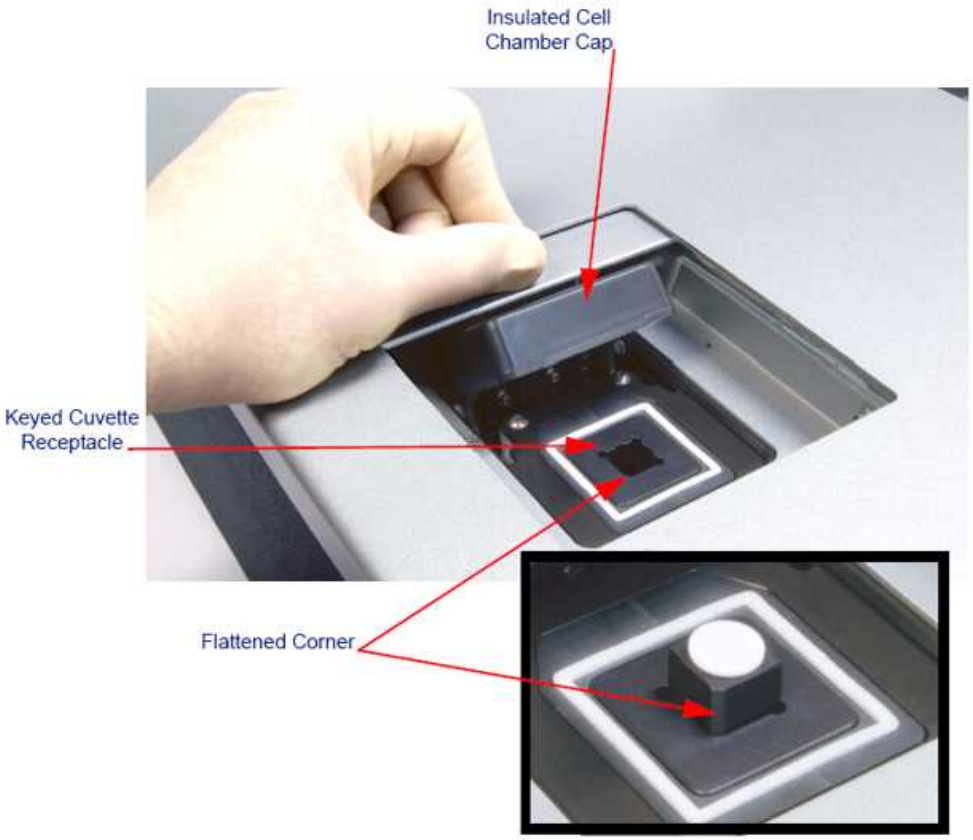
DynaPro NanoStar



Overview of DLS instrument (turn on DLS 30m prior the measurement)

5 Load the cuvette into the DLS machine

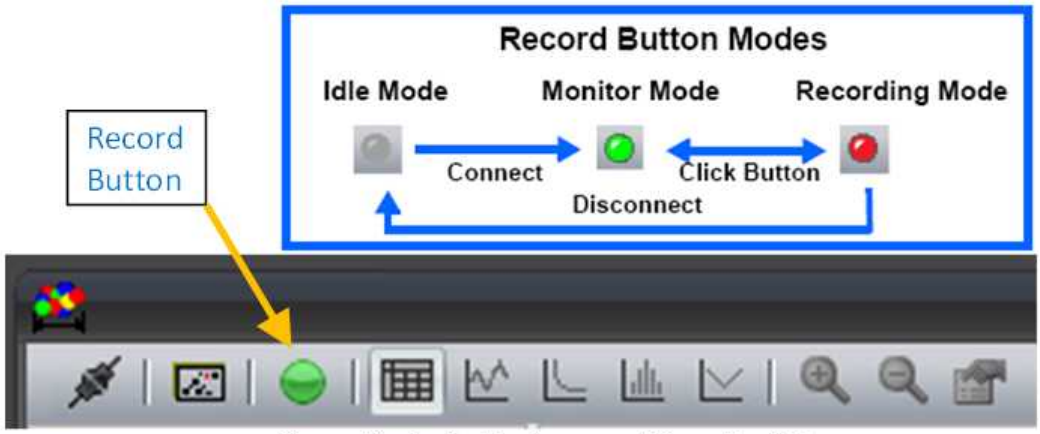


Wyatt disposable 4 µL cuvette

- Made of Cyclic olefin copolymer (COC), compatible with aqueous buffers and alcohols.
- Operates from 0 to 80 °C. When the cell is installed in the NanoStar, it actives a microswitch which allows the instrument to restrict the temperature range, as appropriate.
- Fits directly in the NanoStar cell cavity with no adapter required.
- Provided in individually sealed dust-free bags.

Recording the size distribution

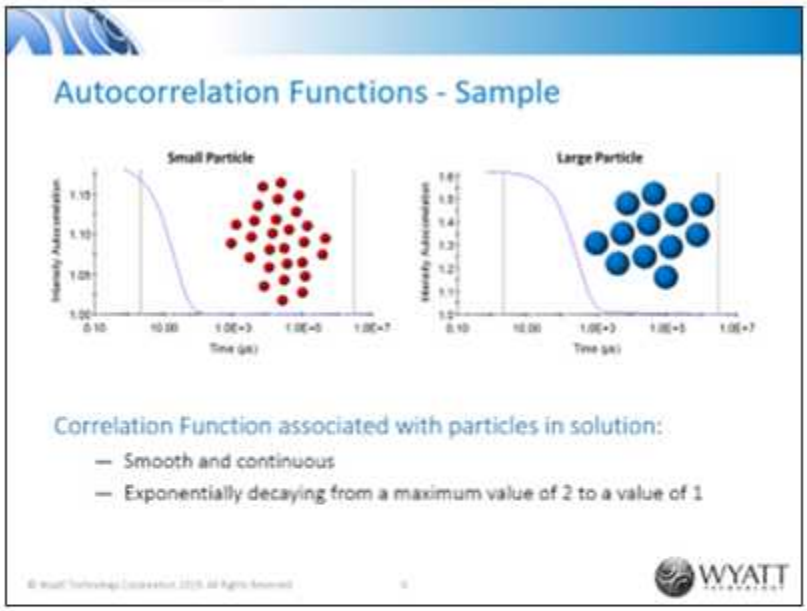
6 Connect the device and the detector software



Connecting to the Hardware and Recording Data

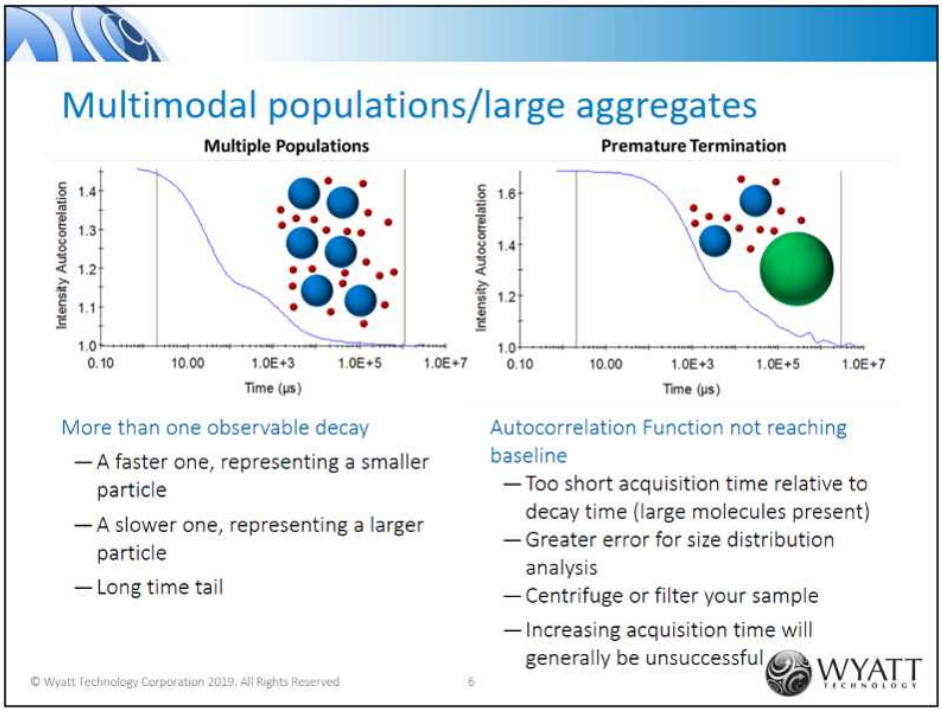
Press 'connect instrument' button (when plugged - connected)

7 Record the 10 acquisitions for each measurement; 3 measurements for each sample



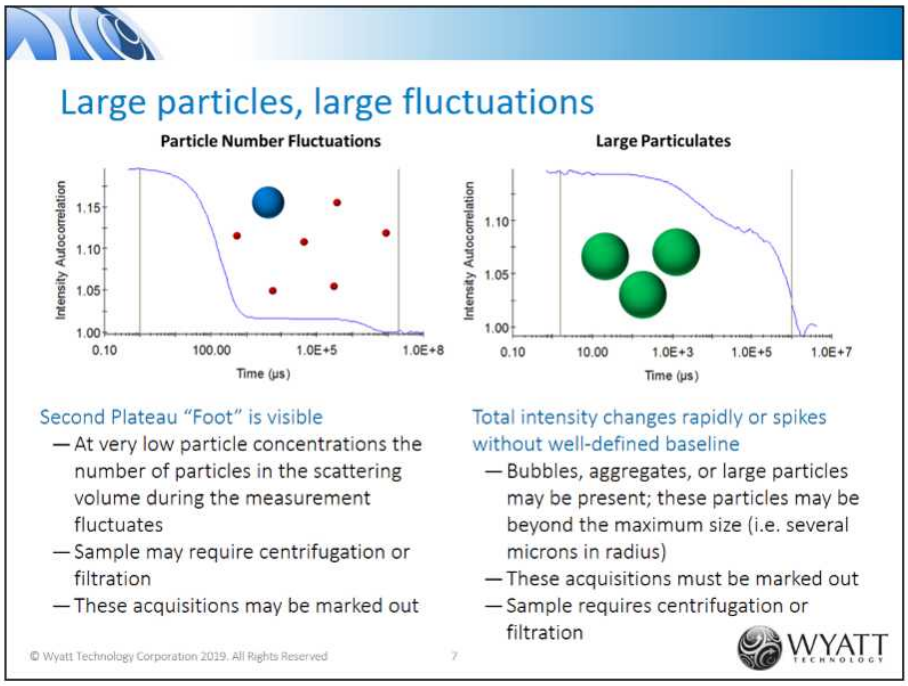
Notes:

- Measure pure (unfiltered) buffer if you suspect that aggregates are not formed by the sample itself.
- Some buffers containing larger molecules or micelles (e.g. sucrose, PEG, Tween, SDS,...) will have a decay as shown to the left (probably a lot more noisy than our example above) and small particle radii, typically around 0.3-0.5 nm for small molecules and 1-4 nm for micelles.
- Autocorrelation functions like the ones shown above are typical for a primarily monomodal size distribution.
- Given the same experimental conditions (same solvent, temperature, scattering angles), larger particles can be easily spotted by a longer decay of the autocorrelation function.



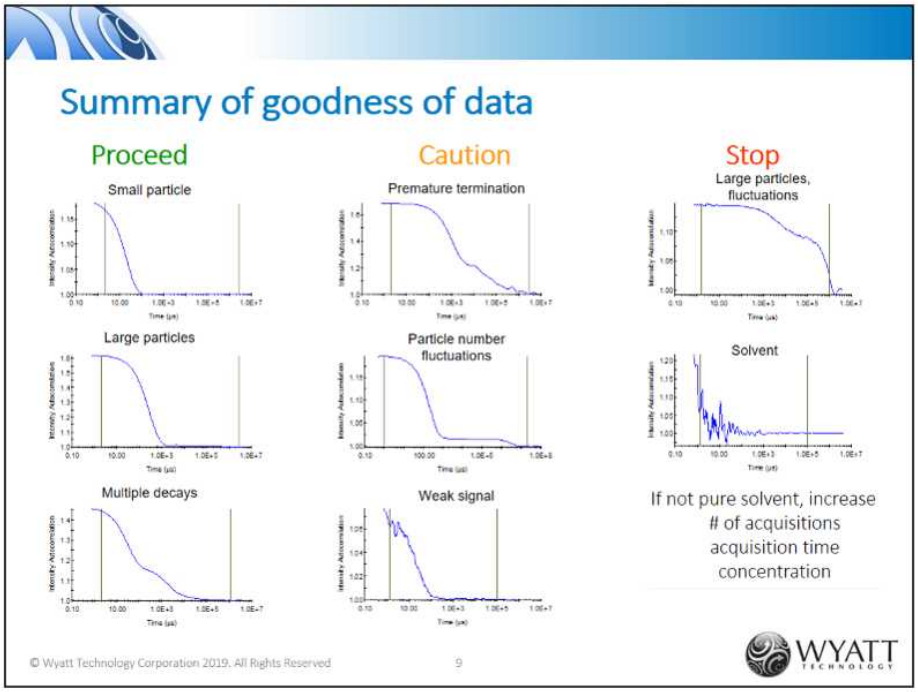
Notes:

- While the ACF ("Multiple Populations") on the left has a well defined baseline and can readily be analyzed, the ACF on the right ("Premature Termination") does not decay to baseline within the acquisition time.
  *NOTE: increasing the number of acquisitions will not capture additional coefficients in the longer time delays.
- In case of the "Premature Termination", it is best to centrifuge your sample to remove large aggregates (10-15 min on a benchtop centrifuge is a good starting point).
- For both scenarios above: If the measurement of subtle size changes of small molecules are desired (e.g. protein dimerization, unfolding), filter the sample with the smallest appropriate pore size. Fewer fit parameters (fewer decays to fit in the ACF) will increase the reproducibility and reliability of the results.



Notes:

- Particle number fluctuations: If the sample cannot be filtered or centrifuged, it is best to use a short acquisition time with a high number of acquisitions and mark out those acquisitions exhibiting the second plateau.
- Large particulates: If large particulates or bubbles are present in most acquisitions, the sample must be centrifuged or filtered. Also check the cleanliness of your cuvette or microwell plate. The example above was actually nanopure water in a very, very dirty Quartz cuvette!
- Bubbles in a quartz microcuvette usually indicate a dirty cuvette and cuvette cleaning is recommended.



QC of the sample includes recording polydispersity index (single size population); smooth autocorrelation function curve;
correct mean size distribution