

## DynaPro Plate Reader III: CMI Getting Started Guide to Dynamic Light Scattering

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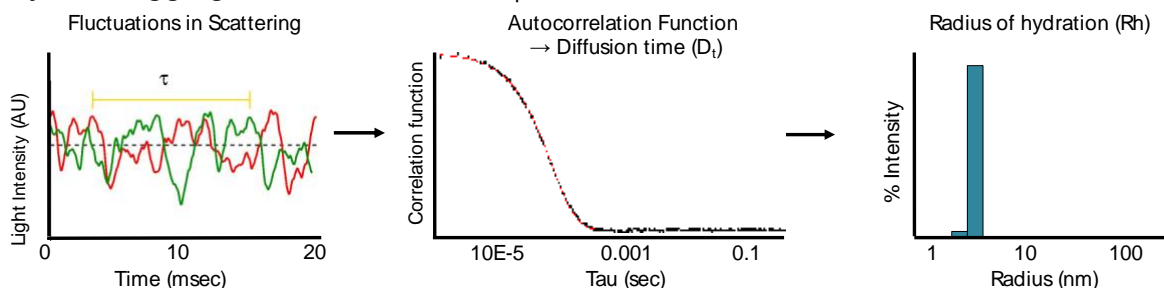
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### Introduction

[Dynamic Light Scattering \(DLS\)](#) measures time-dependent fluctuations in scattered light, which are directly related to the rate of diffusion of the molecule through the solvent and can be used to measure the hydrodynamic radius ( $R_h$ ) of particles in solution. Particle monodispersity and aggregation state of a sample in solution can be assessed in minutes by DLS measurements of  $R_h$ . DLS can be used to monitor sample quality during any (or all) stages of a protein purification project, or after storage. DLS can assess the size of a variety of nanoparticles including proteins, liposomes, micelles, bicelles, and nanodiscs, and analyze the aggregation of chemical compounds.



### Instrument Overview

The CMI has a **DynaPro Plate Reader III** instrument from Wyatt Technologies, which measures dynamic and static light scattering at high-throughput for many sample types using standard microplates.

### Applications

- $R_h$  and  $M_w$  of a wide range of particles (proteins, liposomes, nanodiscs, and other particles)
- Monodispersity for rapid protein quality test
- Protein stability from thermal denaturation
- Aggregation analysis of samples, including chemical compounds
- Multi-peak Resolution
  - Peak resolution limit in dynamic light scattering is ~5X in size
  - Highly unlikely to resolve oligomers, such as dimers and trimers, from the monomer
  - Mixtures appear as a single broad peak (polydisperse)
  - High-order oligomerization can be detected (>5-10mer)

### Key Features

- Measure  $R_h$  from 0.5 nm to 1000 nm
- Measure  $M_w$  from 1 to 1000 kDa
- Sensitivity for size as low as 12.5  $\mu\text{g/mL}$  IgG, 0.125 mg/ml Lysozyme
- Measure in 96-1536 well microplates
- Temperature ramps from 20 to 85 C (option for low temp, 4 C, with nitrogen gas)

### **Required Supplies**

- DLS-compatible clear-bottom black microplate (plates must be tested by Wyatt for compatibility and to determine the proper plate insert to use) We have a 9 mm plate insert, which works for the recommended plates below.
- (optional) 0.02-0.1  $\mu\text{m}$  filters, e.g. Whatman Anotop 10, 0.02  $\mu\text{m}$ , 10 mm i.d., # 6809-1002

Recommended clear-bottom black microplates:

number of wells	384	384	96
Manufacturer	Aurora Microplates	Greiner Bio-One	Greiner Bio-One
Part Number	ABM2-10100A	781 892	655 892
base material	188 $\mu\text{m}$ film	glass	glass
Minumum Volume	25 $\mu\text{l}$	50 $\mu\text{l}$	100 $\mu\text{l}$
Temperature Range	20-85 C	20-37 C	20-37 C
SLS Compatible	yes	yes	yes
Recommended For	high temperature/ thermal stability	Best performance Room Temp	Best performance Room Temp

### **Sample Preparation**

#### **Assay Buffers**

- DLS is compatible with a range of buffers
- Use filtered buffers
- Measure scattering of buffer control
  - Detergents frequently have micelle size similar to that of proteins and scatter significantly

#### **Samples**

- DLS is very sensitive to small amounts of aggregates.
  - Significant aggregates will make accurate size measurements difficult.
  - Samples should be filtered or centrifuged (6000g, 10-30 min) prior to transfer to the clear bottom plate to remove large aggregates and precipitates.
- The minimum amount of sample required depends on the size of the molecule, as large molecules scatter more light than small molecules.
  - For most protein samples, concentrations of 0.2 mg/ml or higher are recommended.
  - As little as 0.125 mg/ml of lysozyme or 12.5  $\mu\text{g/ml}$  of IgG can be detected.
- Small molecule aggregation analysis should be done at working concentrations in and working solvents (e.g. 5% DMSO).
  - Solvent controls are essential. DMSO itself is prone to aggregation.
- When working with membrane proteins, note that empty detergent micelles will not resolve from protein-filled micelles (except when empty micelles are 5-10X smaller than filled micelles).
  - Avoid excess detergent by purifying membrane proteins by affinity and/or size-exclusion chromatography and avoid concentrating after purification

## **Getting Started**

### **Resources**

Additional resources are available at the instrument, including:

[Wyatt DynaPro Plate Reader III User Guide](#) and [Dynamics 7.9 User Guide](#), for additional information about data collection and analysis.

[Wyatt Technical Note TN7004, Performing Static Light Scattering Measurements with DynaPro Plate Reader III](#), for instructions on batch static light scattering (SLS) measurements.

### **Experimental Tips**

- The measurement acquisition time and number of acquisitions needed will depend on the sample concentration and size. Try several options to find the best conditions for your samples.
- Static Light Scattering (SLS) experiments are most reliable for monodisperse samples.
  - SLS measure weight-averaged molar mass ( $M_w$ ).
  - Even a small amount of aggregates will shift the weight-averaged mass significantly.
  - A SEC-MALS experiment allows for more accurate mass measurements than SLS alone, especially for polydisperse samples or samples with any amount of aggregate.

### **General Care and Maintenance**

- The DynaPro Plate Reader instrument will generally be powered off.
- Allow 30 min to equilibrate the temperature after turning on the power.
- Keep the door (located on the top of the instrument) closed to minimize dust in the instrument.

### **Startup**

1. Before you start, book time on the PPMS calendar.
2. Turn on the instrument power, located on the front of the instrument.
3. Login to the computer using your PPMS credentials (eCommons ID and password).
4. Open Dynamics software for DynaPro Plate Reader III data collection and analysis.
5. Load microplate with samples.
  - a. Prepare samples according to guidelines above.
  - b. Fill wells using at least the recommended minimum volume guidelines for the plate
  - c. Minimize air bubbles in the well by:
    - i) using a narrow gel loading tip to fill wells
    - ii) Spinning the plate after loading
  - d. For high-temperature measurements or thermal denaturation experiments.
    - i) Load 1.5-2X more than the plate minimum to reduce deleterious effects of evaporation.
    - ii) Cover the plate with sealing tape or oil appropriate for the plate type.
  - e. Open the door on the DynaPro using the button on instrument LED panel or in Dynamics.
  - f. Insert plate in the instrument with A1 position toward the front of the instrument.
    - i) DO NOT leave plastic plate covers on the plate while in instrument.

## Data Collection

1. Start a new experiment in Dynamics software
2. go to File → New or to File → Open Preset
3. **Parameters**, expand to set
  - a. Sample (Optional).
    - i) Define new sample names by clicking new and entering sample names
    - ii) Assign sample parameters by clicking template button and choosing value(s) and assigning them to wells. Options include:
      - (1) Sample (must be in sample list)
        - (a) "Sample" is typically treated as a *Sample Type* and **for most samples, the default Sample may be used.**
        - (b) Define a new sample type if the dn/dc or polymer model for globular proteins is not appropriate for your samples
      - (2) Solvent Name
        - (a) If solvent viscosity is significantly different from water or PBS, please create a custom solvent profile, as this will affect DLS calculations
      - (3) Concentration
      - (4) User Specified
  - b. Plate
    - i) All plate types must be tested by Wyatt to assess compatibility and determine the appropriate plate insert, which determines the read position in the well.
      - (1) We have a 9mm insert installed.
      - (2) Talk to Kelly if you wish to add additional plate types not listed or if a plate you are considering needs a different plate insert.
    - ii) Select Plate from the Pull-down list.
      - (1) For DLS, use a predefined general plate name
      - (2) For SLS, you'll need to calibrate each plate and define it with a unique identifier
4. **Experiment Designer**, click to design experiment
  - a. If you do not see "Experiment Designer" in the side panel, right-click on "Event Schedule" and switch to Experiment Designer.
  - b. Click Edit Experiment.
  - c. Select an experiment type:
    - i) Fixed Temperature, for standard dynamic light scattering measurements.
    - ii) Continuous Temperature Ramp, for constant temperature ramp rate (best for very small number of samples).
    - iii) Discrete Temperature Increment, for experiments in which all samples are measured at defined temperature before ramping to next (generally best for thermal stability measurements, especially with multiple samples).
  - d. Static Light Scattering, choose Yes or No. Note that SLS requires plate calibration with Dextran standards and solvent offset measurements.
    - i) Click Next to proceed.
  - e. **Well Selection**

- i) Click on Select Wells (or on the plate)
- ii) Select wells to be collected
- iii) Click Input Template (optional) to edit sample names, solvents, and other well values
- iv) Adjust Replicate measurements to read wells multiple times
  - (1) Within each scan... number of times a well is read before moving to next well
  - (2) For multiple scans... number of times scan of all wells is repeated
- v) Click Next to proceed.
- f. **Properties**
  - i) Enable Auto-attenuation                      Yes
  - ii) Acquire an Image of Each Well      Yes
  - iii) DLS Acquisition Time(s)                      5 (default) - 20 (more for low conc. and large size)
  - iv) Number of DLS Acquisitions                      5 (default) - 20
  - v) Optimization Calculator can be used to optimize Acquisition time and number.
  - vi) Label Measurements
    - (1) Click [...] to add measurement labels.
    - (2) Recommended minimal labeling: Well, TimeStamp
  - vii) Set temperature
  - viii) Click next to proceed.
- g. **Review**
  - i) View experimental summary.
  - ii) Save as Preset (optional) to save parameters as a template.
  - iii) Click Finish when experiment editing is complete.
- h. **Start Experiment**
  - i) Click Connect to Instrument Icon, top left (if not already connected).
  - ii) Click on Green Start button to begin acquisition.
  - iii) You will be prompted to name your experiment and data will be saved automatically.
  - iv) You may collect additional data in the same file by:
    - (1) rerunning the same experiment
    - (2) editing experimental parameters (wells, acquisition time, number of acquisitions, etc.) and running again.

## **Data Analysis**

Data can be examined in various ways:

1. Datalog Grid
2. Datalog Graph
3. Correlation Graph
4. Regularization Graph
5. Spectral View

## 1. Datalog Grid

- Displays summary data table of measurements.
- Selecting data to display:
  - Select individual measurement in the Left Side Pane will show data from all acquisitions.
  - Select Measurements to show summary table of all data.
- To change display data, Right-click in the table and select Table Settings.
- To Filter Data:
  - Right-click the table and select Data Filter to omit data by criteria.
  - Right-click on an acquisition or measurement and select Mark to exclude from analysis.

## 2. Datalog Graph

- Displays experimental data in graphical form.
- Simple graphs of data can be created for reports.

## 3. Correlation Graph

- Displays the intensity auto-correlation function (ACF), the raw dynamic light scattering data.
- Displays fit for both cumulants (a single species fit) and regularization (multi-species fit).
- Correlation data should be examined carefully to assess data quality.
  - See **Assessing Data Quality** below for tips on evaluating data.

## 4. Regularization Graph

- Displays the calculated hydrodynamic radius distribution using regularization fit of the autocorrelation function (Regularization is a multi-modal fit).
- Select measurement(s) to be displayed.
- Lower panel displays peak results.
  - Radius (nm)
  - %PD, polydispersity
    - PD < 15% is considered a narrow peak and monodisperse.
    - PD > 30% is considered a broad peak and polydisperse.
  - Mw-R is mass calculated from Radius and shape model.
  - %Intensity is size distribution as a function of the measured scattered light intensity.
  - %Mass is the size distribution in terms of the mass of the particles.
    - Note: these peaks are distinct from the user-defined Ranges in the Datalog Grid, which are an arbitrary binning of the data.

## 5. Spectral View

- Graphical view of the data in a well plate format.
- Used to quickly screen large sample sets.
- Use Sequence to toggle different measurements taken in the same well.

## 6. Exporting

- Right-click on tables or graphs to export.
- Export Values or Graph to clipboard or to file.

### **Assessing Data Quality**

- Examine the **Correlation Graph** to assess data quality (see [dls\\_evaluation\\_criteria.pdf](#) for examples).
- Proceed** with analysis if:
  - Correlation function show a single decay.

- b. Correlation function shows multiple decays that are resolved in the evaluation time window.
- 3. **Use Caution** when analyzing if:
  - a. Correlation function shows premature termination (noisy at long times).
    - i) Increase acquisition time.
  - b. Correlation shows a foot at long delay times indicating particle number fluctuations.
    - i) Decrease acquisition time.
    - ii) Increase number of acquisitions.
    - iii) Use data filtering.
  - c. Correlation function is not smooth indicating a weak signal.
    - i) Increase acquisition time.
    - ii) Increase number of acquisitions.
    - iii) Increase laser power (of auto-attenuation disable).
    - iv) Increase concentration.
- 4. **Do not analyze** data if:
  - a. Correlation function shows evidence of large particles.
    - i) Centrifuge or filter sample.
  - b. Correlation function shows no evidence of particles (looks like solvent).

## Shutdown

1. Remove your plate from the instrument.
  - a. Plates can be reused.
    - i) Mark used wells (black marker on the clear bottom works well).
    - ii) Cover your plate and store in a clean, safe place for future use. (Remember: Avoid Dust!)
2. **Close the door** using the button on instrument LED panel or in Dynamics.
3. **Turn off the DynaPro III laser.**
4. Quit Dynamics Software.
5. **Logoff from PPMS!**

## Data Management

Technology	Dynamic Light Scattering (DLS)
Instrument	Wyatt Dynapro Plate Reader III
Recommended Repository	<a href="#">Generalist Repository</a>

### **Data Collection & Analysis Software**

Current Version	Dynamics, Version 7.10.1.21		
Data Files (Type, ~size)	experiment file	.dexp	~500KB/measurement
Readable Exports	Autocorrelation functions (all)	.csv	20-100 KB/experiment
	Autocorrelation functions (indiv)	.csv	10 KB/measurement
	Autocorrelation errors (indiv)	.csv	10 KB/measurement

individual acquisitions	.csv	30-50 KB/measurement
Well image	.jpeg	350 KB/measurement
Regularization fit (all)	.csv	10 KB/measurement
Regularization fit (indiv)	.csv	10 KB/measurement
peaks tables	.csv	10 KB/experiment
results tables	.csv	5 KB/experiment

Book time and Report Problems through the PPMS system: <https://ppms.us/hms-cmi>

- rates are based on booked and real time usage

Contact [cmi@hms.harvard.edu](mailto:cmi@hms.harvard.edu) with questions.

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