WYATT TECHNOLOGY CORPORATION

DynaPro® Plate Reader II

High-throughput, automated Dynamic Light Scattering
from 96, 384, or 1536 well plates

Now with an onboard camera!

DynaPro® Plate Reader II

Where Quantity and Quality Meet

Dynamic Light Scattering (DLS) is an important tool in the research and development of proteins, nanoparticles, colloids and macromolecules with sizes between subnanometer and a few micrometers. DLS measures size and size distributions in solution or suspension, quickly and with a small amount of material, to help assess factors such as aggregation, stability and purity. There are plenty of DLS instruments available to make these measurements, but they all suffer from one big drawback: manual sample loading and "one-at-a-time" measurements.

Using either disposable or quartz cuvettes, traditional DLS requires significant operator time to make just a few measurements, even when the actual data acquisition only takes a few minutes. Scientists are deterred from full utilization of DLS including acquisition of multiple repeats and many different conditions. The scientist—and the science—get bogged down. Luckily, an elegant solution is at hand.

The DynaPro Plate Reader II is an automated DLS instrument that performs measurements on samples in standard 96, 384, or 1536 well plates. Think of each well as a fresh cuvette ready for the next condition or replicate, and you can immediately see the promise of high-throughput characterization.

The Plate Reader II offers enhanced performance and features to enable unprecedented productivity in:

- Development of biopharmaceuticals, including monoclonal antibodies, antibody-drug conjugates (ADCs), and novel therapeutics.
- Preparation of proteins for crystallization, NMR, SAXS, or chromatographic analysis.
- Optimization of drug-delivery or diagnostic nanoparticles.
- Formulation of virus-like particle vaccines.

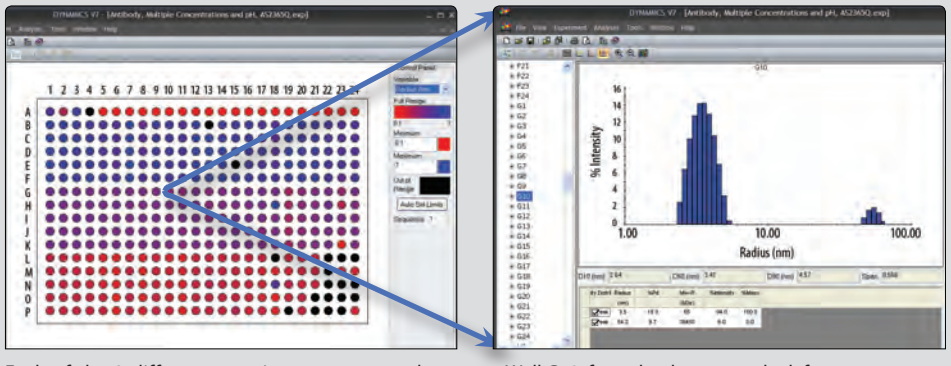
Imagine a quantum leap

Science experiences quantum leaps when the automation of previously slow and tedious processes leads to qualitatively new research. From massively parallel computing, to screening of compound libraries in drug discovery, to PCR and genome-sequencing, the benefits of high-throughput automation extend well beyond simply saving time. With high throughput, you can imagine—and carry out—novel studies previously inaccessible to most labs (unless they happened to have bench after bench of "one-at-a-time" instruments attended by legions of technicians).

The DynaPro Plate Reader II gives you the opportunity to dream big dreams for your DLS measurements. Acquire in one day the data that might otherwise take weeks. Test hundreds of samples and thousands of buffer/excipient/temperature conditions with no more effort than that required to load a single microwell plate. Analyze and visualize the entire dataset in one fell swoop using the DYNAMICS® software's SpectralView, then zoom in for a detailed study of the most promising conditions. Integrate with robotic liquid handling for even larger-scale automation.

Quantity without sacrificing quality

In the DynaPro Plate Reader II, all measurements are made *in situ* using industry-standard microwell plates. With no sample handling necessary, beyond pipetting into the plates, there is no fear of cross-contamination. Furthermore, to extend the analysis, the plates may be transferred seamlessly to spectroscopic plate readers or chromatographic well plate samplers for additional analyses.



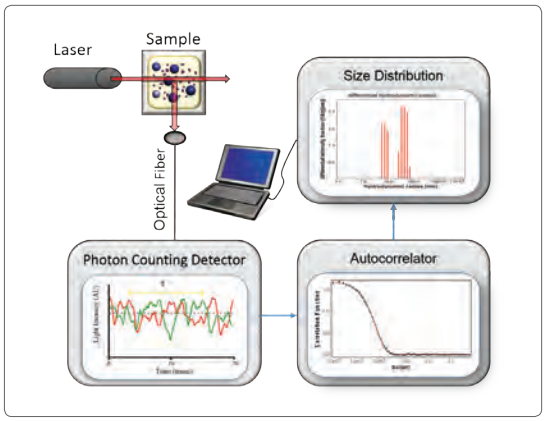
Each of the 3 different proteins was measured at 4 pH conditions with 6 concentrations at each pH, for a total of 72 different sample conditions with 5 replicates per condition!

Well G10, from the dataset on the left:

Here we show the results of a regularization analysis, which is used to determine the distribution of sizes present for this condition. In addition to a primary monomer peak, some large aggregates are evident.

Fortunately, the Plate Reader II's automation and high-throughput do not come at the expense of data quality. Even using inexpensive disposable plates, the sensitivity and robustness engineered into the instrument by Wyatt's uncompromising R&D team are similar to those achieved by other DLS instruments with precision-fabricated (aka expensive) quartz cuvettes.

You can collect more data, in less time, with less labor, and with greater accuracy, thanks to the ability to incorporate multiple replicates effortlessly into every experiment for robust statistical analyses.



So, what's new?

The first DynaPro Plate Readers offered many benefits to scientists including novel ways of looking at drug development, among many unique applications. However, Wyatt Technology doesn't believe in resting on its laurels. The Plate Reader II improves on the performance of the previous generations with an extended temperature range of 4 - 85°C, faster temperature ramp rates, and even better temperature uniformity across the plate.

The DynaPro Plate Reader II provides a comprehensive, multi-dimensional view of protein behavior under a range of solution conditions unmatched by any conventional DLS technology.

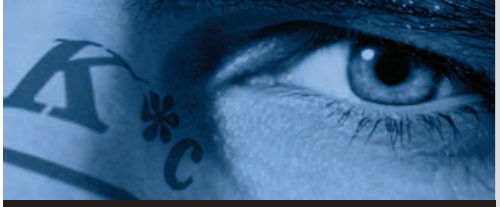
Perhaps one of the best features of the Plate Reader II is the onboard camera that captures an image of each well. Every scientist who has made DLS measurements has, at some point, run into odd data that resulted from dust, precipitation or a bubble in the sample. The only way to find the source of the bad data was to hold the cuvette up to the light and examine the sample with a jeweler's loupe… assuming, of course, that you hadn't yet discarded or emptied the cuvette. Thanks to the Plate Reader II's camera, when strange results show up, simply pull up the image that was taken automatically and verify if there was in fact some detritus in the well. No more guesswork, or squinting required.

Yet more benefits

Solid performance, versatility, and automation are central features of the DynaPro Plate Reader II. Here are a few more things you need to know:

- Auto-attenuation allows for measuring both very pure solutions, and turbid suspensions of large aggregates or particles, in the same plate without manually adjusting light levels.
- The DynaPro accepts industry-standard microwell plates in 96, 384, or 1536 well formats—at the cost of pennies per well.
- Sample volume per well can be as low as 4 µL using the 1536 wellplate.
- Plates need not be discarded when only some of the wells are used. Next time, just use different wells. When you have only a few samples at a time, one plate can last for months, and each instrument user can keep his or her own plate.

High-throughput DLS is just one of the tools in the Light Scattering Toolkit for Essential Biophysical and Nanoparticle Characterization. Please contact us to speak with a sales representative or an application scientist to learn how Wyatt instruments can solve your pressing analytical challenges.



How it works

Molecules and nanoparticles in a solution are subject to Brownian motion, an atomic-level, diffusive "random walk," first elucidated by Albert Einstein. Brownian motion is produced by the constant buffeting of the particle by solvent molecules. Smaller particles jitter around more rapidly, while larger particles diffuse more slowly. This random motion of many particles creates noise-like fluctuations in the light scattered by the solution. As it turns out, noise can contain important information!

Since the rate of fluctuation in the scattered signal correlates to the rate of diffusion, the fluctuating light scattering intensity can be analyzed to determine the diffusion rate of the particles and so identify their sizes. The mathematical transform applied to the signal in dynamic light scattering measurements is called autocorrelation analysis. Not only can DLS determine the diffusion rate of a single population in solution, it can actually determine a spectrum of diffusion rates to produce the distribution of translational diffusion constants D_T present in solution. Finally, the diffusion constants are converted to effective sizes known as hydrodynamic radii (R_h) according to the equation:

$$R_h = \frac{k_B T}{6\pi\eta D_T}$$

where k_B is Boltzmann's constant, T is the absolute temp. (in degrees Kelvin), and η is the solvent viscosity. The value of R_h corresponds to the radius of a sphere with the same diffusion constant as the particle being studied.

For more information:

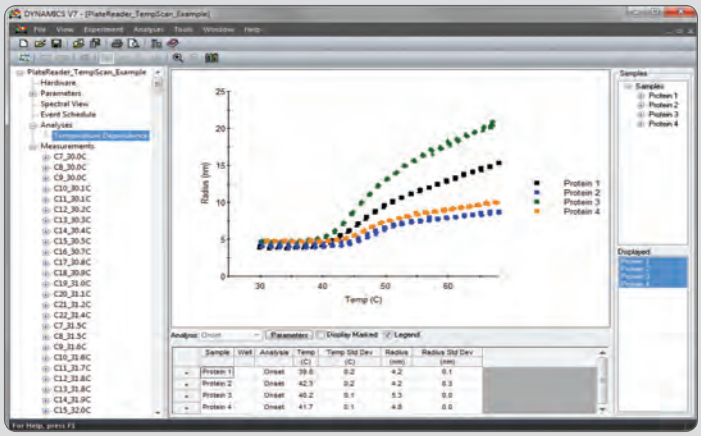
To learn more about DLS and it's uses in a variety of scientific endeavors, visit these resources:

www.wyatt.com/**Theory** - theory overview of static and dynamic light scattering

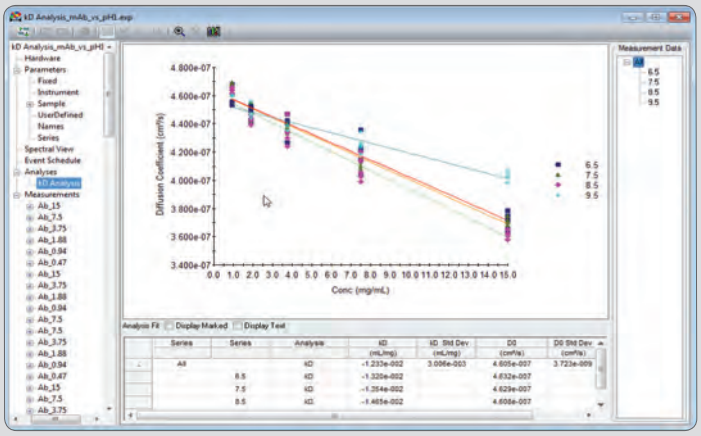
www.wyatt.com/**DLS** - introduction to DLS applications and instrumentation

www.wyatt.com/**PlateReader** - additional DynaPro Plate Reader II features

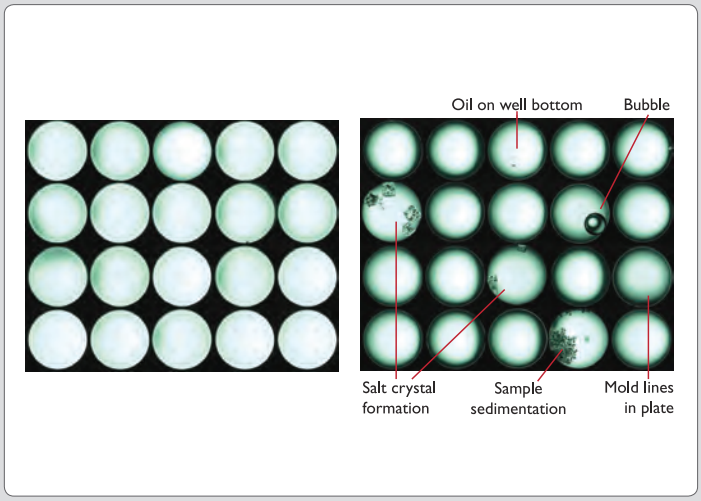
Weeks of experiments in just hours



Data may be both acquired and analyzed within the DYNAMICS software package or exported for your own detailed analyses. Shown above are melting/aggregation curve data and analyses for four proteins, each measured with three replicates. The standard deviation of the measured onset temperature was ≤0.2˚C or less for these samples, allowing subtle changes in protein stability to be observed.



Colloidal stability of proteins across a range of formulation conditions is captured in the concentration dependence of the diffusion coefficient, known as k_D. A k_D analysis is shown here for a typical monoclonal antibody in four different pH solutions. To measure k_D at each pH, the diffusion coefficient for the antibody was measured at five different concentrations with five replicate wells per condition. Measurement of all 100 samples is completely automated. In addition, sample preparation may be facilitated by a robotic liquid handling system.



The onboard camera in the DynaPro Plate Reader II can automatically acquire and store images of each well, giving scientists a new dimension of data acquisition, and instant insight into the behavior of their samples.

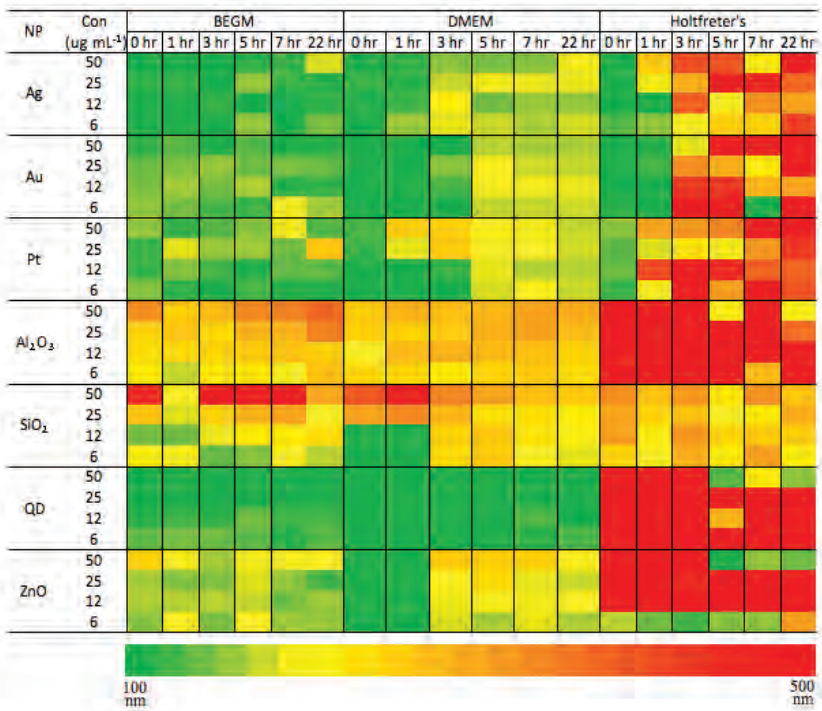
Integration of the DynaPro Plate Reader II with third-party robotic liquid handling equipment, such as Freeslate's Biologics Formulation System, allows for fully automated plate loading and data collection in the high-throughput lab.

Applications

Wyatt instruments are engineered to provide flexibility in hardware and software to meet a variety of specific application requirements.

With the DynaPro Plate Reader II you can:

- Characterize purified proteins for homogeneity, size, and aggregation over the widest range of solution conditions, automatically.
- Simultaneously assess the thermal, conformational and colloidal stability of monoclonal antibodies or ADCs.
- Determine the kinetics associated with macromolecular assemblies in parallel experiments.
- Screen biotherapeutics for self-association, hetero-association, or inhibitors of association.
- Measure the stability, size, and size distributions of liposomes, viral particles, nanoparticles, and drug delivery particles in a variety of formulations, quickly and easily.
- Determine melting and aggregation temperatures for a variety of solvent conditions and concentrations.
- Detect and analyze compound aggregates that may cause false positives or false negatives.
- Determine sample viscosity over a broad range of conditions in a fully automated fashion, using only several microliters of sample per condition.



Heat map showing color coded radius for a variety of nanoparticles as a function of time, concentration, and for several buffers and dispersing agents. These data were acquired in support of correlating the physical properties of nanoparticles in solutions to the toxicity of the nanoparticles. (Courtesy of Zhaoxia Ji)

PLATE READER BENEFITS

Experiment	Description	Batch (Cuvette) Operation	Plate Reader (Microplate)
Measurement of 1536 distinct samples	Loading samples	> 48 hrs (includes time to insert, remove, and clean each cuvette)	< 2 hrs (1536 well plate)
	Collecting data, personnel time required	> 24 hrs (personnel continuously required to change samples)	No personnel required
	Collecting data, measurement time	13 hrs	13 hrs
	Total personnel time required	> 72 hrs	< 2 hrs
Measurement of temperature dependence (42 different temperatures) of 384 samples	Loading samples	> 36 hrs (includes time to insert, remove, and clean each cuvette, and wait for temperature stability)	< 1 hr
	Collecting data, measurement time	634 hrs (> 26 days), with a person required to change samples every two hrs	45 hrs (no personnel required)
	Totals (personnel time only)	28 days (36 hrs)	3.25 days (< 1 hr)

With the DynaPro Plate Reader, a temperature-dependent experiment using a 384-well plate requires < 1 hr to fill the wells and 45 hrs (unattended) to acquire the data. The same data acquired with a single cuvette-based instrument would require personnel to change the sample every 2 hrs over > 28 days total run time.

Specifications

Supported well plate formats	96, 384, or 1536 (many industry standard well plates supported)
Size range (hydrodynamic radius, R_h)	0.5 to 1000 nm
Minimum concentration @14 kDa	0.125 mg/mL †
Optics	
Laser wavelength	830 nm
Scattering angle	158°
Laser power	Programmable 10% - 100%
Attenuation range	1 to 10^6
Temperature control	4 - 85°C ††
Fluidics	
Minimum sample volume	4 µL †††
Correlator	512 channels, 100 nsec sampling time in a multi-tau layout
Data acquisition time	1 - 3600 seconds
Read time per well	5 - 20 seconds
Electronics	
Onboard camera	3 megapixels
Digital communication	Ethernet (TCP/IP)
Dimensions	60 cm (l) x 36 cm (w) x 25 cm (h)

† Minimum concentration specified with 50 µL of lysozyme (14 kDa) sample in a 384 well plate, Wyatt P/N P8803-384, with 100 seconds acquisition.

†† Absolute accuracy of ± 0.5°C from 4 - 50°C, and ± 1°C from 50 - 85°C. Minimum temperature of 4°C requires a laboratory ambient temperature of 24°C or below. Camera operates with sample temperature in the range of 4 – 50°C, and automatically powers off above 50°C.

††† Minimum volume specified with 2 mg/mL lysozyme in a 1536 well plate, Wyatt P/N P8803-1536, with 25 seconds acquisition.

Wyatt Technology is committed to continual improvement. Specifications subject to change without notice.

Warranty: All Wyatt instruments are guaranteed against manufacturing defects for 1 year.

With installations in *more* than 65 countries, *more* than 10,000 refereed journal publications citing its instruments, and 18+ PhD scientists on site, Wyatt Technology is the **world's leading manufacturer of instruments** for absolute macromolecular characterization. It is the only company in the world focused exclusively on such systems, their design, and their applications.

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