WYATT TECHNOLOGY

Ultimate Guide to light scattering instruments

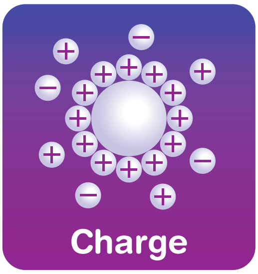
for absolute macromolecular and nanoparticle characterization

This booklet is both a brief introduction to the principles of light scattering and a guide for making an informed decision about the purchase of a light scattering instrument.

What Can Be Measured with a Light Scattering Instrument

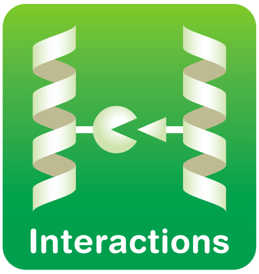
Absolute molar mass from 200 to 1,000,000,000 Da

RMS radius from 10 to 50 nm and hydrodynamic radius from .02 to 5,000 nm

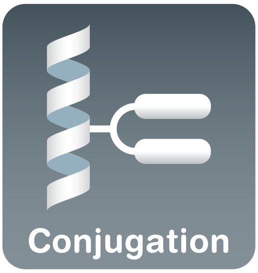


Zeta potential and net molecular charge for particles from 2 nm to 100 µm

Shape, structure and branching parameters



Binding affinity from pM to mM and absolute stoichiometry of complex interactions



Molar mass and fraction of each constituent in a binary conjugate

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WYATT TECHNOLOGY

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INTRODUCTION TO LIGHT SCATTERING

More and more scientists realize that characterizing proteins, polymers and biopolymers (and nanoparticles, too) with a Multi-Angle Light Scattering (MALS) instrument is the most practical and reliable way to determine the essential physical properties of their samples in solution. The combination of gel permeation or size exclusion chromatography with MALS provides distributions of molar mass, size and conformation unattainable by other means, without making assumptions or relying on questionable calibration standards.

In 1975, Dr. Philip Wyatt, the founder of Wyatt Technology, invented the first commercial MALS instruments to incorporate lasers as their light source. Since 1982, Wyatt Technology has been defining and redefining the state of the art in MALS hardware, software, service and support.

This booklet will explain and demystify key light scattering (LS) principles. For some, these concepts may be novel. Others, who are familiar with light scattering technology, will find a wealth of practical information to use in making the right choice when buying an instrument. If you are new to the field, we hope this booklet will help you to make MALS a common tool in your laboratory analysis.

Light Scattering Is Ubiquitous

Most of the light we see has been scattered as it passed from its source (the sun, a light bulb or a laser) to our eyes. Think about the sky or this booklet. The sky appears blue and you can discern the patterns that make up the letters on the page because of the way they scatter light.

We obtain virtually all of the visual information about our surroundings from light that has been scattered. We identify and differentiate objects by making deductions based on observing the wavelengths, spatial pattern and quantity of light they scatter. Such deductions are not necessarily limited to the objects we can see with the naked eye.

For very small objects, such as microscopic particles and molecules in solution or liquid suspension, various physical properties may be deduced from the precise measurement of the scattered light.

The quantitative measurement of light scattered from a solution begins by illuminating the sample with a fine beam of highly collimated and monochromatic light. The scattered light is detected and measured at one or more angular positions relative to the incident beam direction and the results analyzed via well-known physical equations. Measurements that are restricted to a single fixed angle—be it a low angle, a high angle or any angle in between—are rather limited in the information they provide. On the other hand, measurements can be made over a range of angles in order to provide additional information.

The Development of Light Scattering Technology

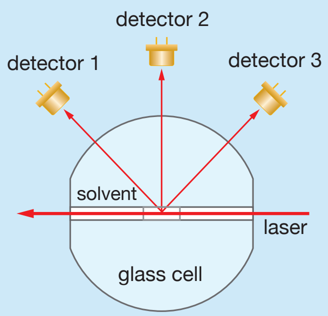
Early light scattering instruments, covering an appreciable angular range, incorporated a mercury arc lamp as a light source and a photomultiplier tube (PMT) as a detector. The PMT could be rotated around the sample vial by means of a goniometer. Measurements were performed in small angular increments across a wide range, giving rise to the term differential light scattering.† The first practical differential light scattering device was built by Bruno Zimm for academic research in the 1940s. The American-made Brice-Phoenix and the French-built SOFICA units, sold in the 1950s and 1960s, were successful commercial devices based on this design. In 1971, Philip Wyatt and David Phillips developed the first commercial light scattering instrument to incorporate a laser for greatly enhanced sensitivity, the Differential I. These instruments were capable of determining molar mass (M_w) and molecular size (root mean square radius, R_g).

The Brice-Phoenix was an early differential light scattering device used for academic research in the 1950s and 60s. It employed a mercury arc lamp as a light source and a photomultiplier tube as a detector.

The following year, Beckman Instruments introduced a laser-based system that measured scattered light at a single, low angle to determine molar mass alone. The Beckman optical design, which incorporated a plethora of mirrors, lenses and prisms in order to minimize stray light, became known by the acronym LALLS (Low Angle Laser Light Scattering). LALLS was further refined and commercialized by Chromatix and later purchased by LDC/Milton-Roy. Developed before the advent of the personal computer, LALLS could provide reasonably accurate molar mass values without the need to perform numerical curve fitting, making it suitable for on-line measurements with flowing samples. Another type of single-angle light scattering GPC detector is known as RALLS, for Right-Angle Laser Light Scattering. RALLS is only suitable for determining the molar mass of molecules smaller than about 10 nm in radius.

†The measurement is also called static, classical or total intensity LS to distinguish it from quasi-elastic light scattering or photon correlation spectroscopy, more commonly called dynamic light scattering (DLS).

In the late 1970's, Dr. Wyatt recognized the need to make measurements over a range of scattering angles, in order to measure size as well as molar mass for flowing samples. By 1983, after founding Wyatt Technology, Dr. Wyatt had completed the development of the first commercially viable MALS instruments for characterizing macromolecules and nanoparticles in solution, known by the trade name DAWN®. Scientists used to working with LALLS systems began to refer to the DAWN by the acronym MALS (Multi-Angle Light Scattering), which has endured to this day!



Typical geometry of the detectors in a multi-angle light scattering instrument

MALS instruments have since become indispensable tools in thousands of laboratories around the world, because they determine directly the molar mass and size of molecules, colloids and nanoparticles in solution, without depending upon reference-based calibration or physical assumptions about the sample. Wyatt Technology currently offers the DAWN, miniDAWN® and microDAWN™ MALS detectors for GPC/SEC/UHPLC.

The DAWN offers the widest range and highest sensitivity, while the miniDAWN is suitable for many proteins and small polymers. In addition, Wyatt's ASTRA® software provides comprehensive acquisition, analysis and reporting of MALS data.

The Two Types of Light Scattering: Static and Dynamic

There are two general types of analytical light scattering for characterizing macromolecules and nanoparticles and they provide complementary structural information.

Multi-angle light scattering instruments can determine absolute molar masses from 200 to 10^9 g/mol and rms radii from 10 nm to 500 nm.

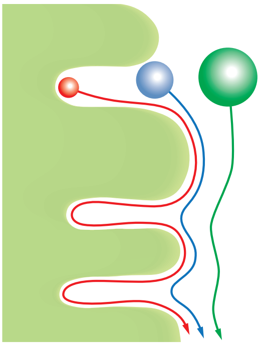
In static light scattering (SLS or MALS), the intensity of scattered light is measured as a function of the angle between the detector and the incident beam direction. Static light scattering measurements typically average the light intensity over a time of several tenths of a second to several seconds. These measurements are analyzed to determine the molar mass, molecular root mean square radius, conformation and intermolecular interactions of macromolecules and particles.

In dynamic light scattering (DLS)—also called quasi-elastic light scattering (QELS) or photon-correlation spectroscopy (PCS)—light intensity fluctuations taking place at microsecond or millisecond time scales are measured. Those fluctuations arise due to Brownian motion of the scattering particles and the rate of fluctuation is a measure of the diffusion constant. The diffusion constant, in turn, is related to the hydrodynamic radius of a molecule. While DLS provides direct information about the size of a particle, it is not a reliable means for determining molar mass. Assumptions about molecular conformation and specific volume may inform estimates of molar mass according to the measured size, but their accuracy depends on the accuracy of the assumption.

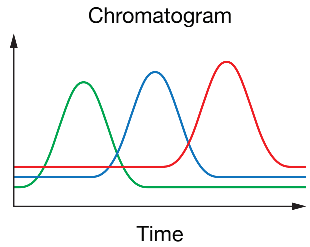
Determining Molar Mass and Size with MALS

MALS instruments enable you to determine absolutely (without assumptions) the molar masses of polymers and biopolymers from below 200 g/mol, up to hundreds of millions of g/mol, simply by measuring the intensity of scattered light along with concentration. And rather than relying on assumptions about your samples (whether they're rods, random coils or spheres), MALS instruments measure the molar mass directly—no matter what the structure. Thus, a MALS instrument is an ideal GPC/SEC detector for determining the number-, weight- and z-average molar masses of materials as diverse as peptides, polymers and proteins as well as the detailed distributions of molar mass and size. The chromatography equipment you already possess, plus a MALS detector, eliminate the need for column calibration or reference standards.

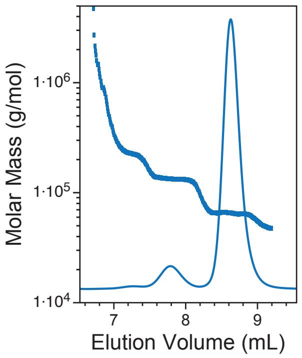
How Light Scattering Combined with Gel-Permeation Chromatography Determines Absolute Molar Mass



GPC column performs separation according to hydrodynamic volume.

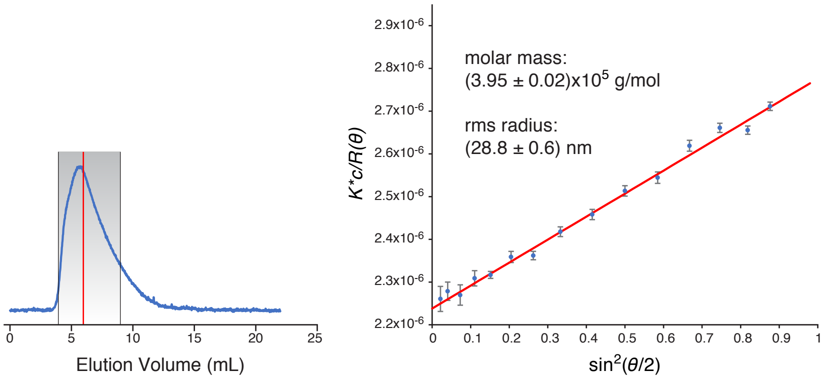


Particles of different size elute at different times.



Light scattering determines molar mass at each elution volume.

GPC separates particles by size, not by molar mass. The addition of light scattering analysis determines molar mass directly, independent of retention time and size.

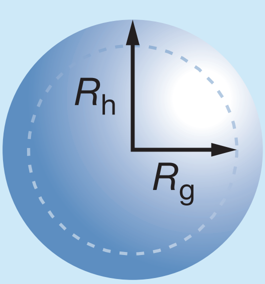


In SEC-MALS, the intensity measured at each scattering angle is analyzed to determine the molar mass and rms radius at each elution volume in the chromatogram.

Small, compact molecules possessing a radius below 10 nm have little discernible angular variation in scattered intensity, so MALS cannot determine their size (even though MALS has no problem determining their molar mass). On the other hand, for random-coil molecules above about 50,000 g/mol or globular proteins above about 500,000 g/mol, R_g is generally above 10 nm, so angular variation of the scattered light generally may be measured and size may be determined. This is a "rule of thumb," and the size cutoff may vary with different molecules.

If a light scattering instrument makes measurements at a single angle (such as a LALLS detector), the particle's size cannot be determined. Even a dual-angle scattering instrument is limited in the range of sizes it can analyze, since beyond a certain size the angular variation is non-linear in form. Hence MALS instruments must be used to determine R_g values.

At minimum, when R_g is between 10 and 50 nm, three angular scattering values spread over a wide angular range are required to accurately fit the data and return a reliable size. More angles are required to size larger molecules or particles. Depending on the wavelength and number of angles used, a MALS detector can determine molecular radii from about 10 nm to larger than a micrometer. And, MALS instruments never require *a priori* conformational assumptions; the size is read out directly from the angular scattering intensity distribution.



Light scattering provides two measures of molecular size. R_g, the rms radius, is determined from the angular scattering variation. R_h, the hydrodynamic radius, is determined from the rate of intensity fluctuations.

A key benefit of MALS is its ability to determine polymer branching properties directly, since branching calculations depend on the measurement of the molecule's molar mass and rms radius. For polymers below 10 nm in radius, a differential viscometer such as Wyatt's ViscoStar® is typically used with a MALS instrument to assess conformation and branching.

MALS detectors can also be used to detect protein aggregation that UV and RI detectors often miss completely or to determine the oligomeric state of conjugated proteins such as glycoproteins or surfactant-solubilized membrane proteins for which reference standards do not exist. In addition, MALS instruments can be used to study the homo- and hetero- association of proteins and other biological macromolecules.

The combination of online MALS and DLS creates a versatile system for characterizing molar mass, size and conformation over a very large range.

Determining Hydrodynamic Radius with Dynamic Light Scattering

Dynamic light scattering determines the diffusion coefficients of molecules in solution. The diffusion coefficient for a spherical particle is related directly to its radius. Thus, an equivalent radius may be associated with the diffusion coefficient measured for any molecule. This equivalent radius is called the hydrodynamic radius, R_h. DLS determines R_h of molecules from smaller than 0.5 nm to larger than a micrometer. Since 1982, Wyatt Technology has been building static and dynamic light scattering instruments, giving scientists the power to utilize these two complementary techniques simultaneously.

For GPC/SEC use, Wyatt Technology offers the WyattQELS™, a dynamic light scattering module that may be embedded in any of Wyatt's MALS detectors. The WyattQELS module comprises a single-photon-counting avalanche photodiode, a multimode optical fiber that connects to the optical read head of the DAWN, miniDAWN or microDAWN and a real time digital correlator that measures the autocorrelation of the intensity signal carried by the optical fiber. From the autocorrelation function, the software calculates the diffusion coefficient and hence, the hydrodynamic radius.

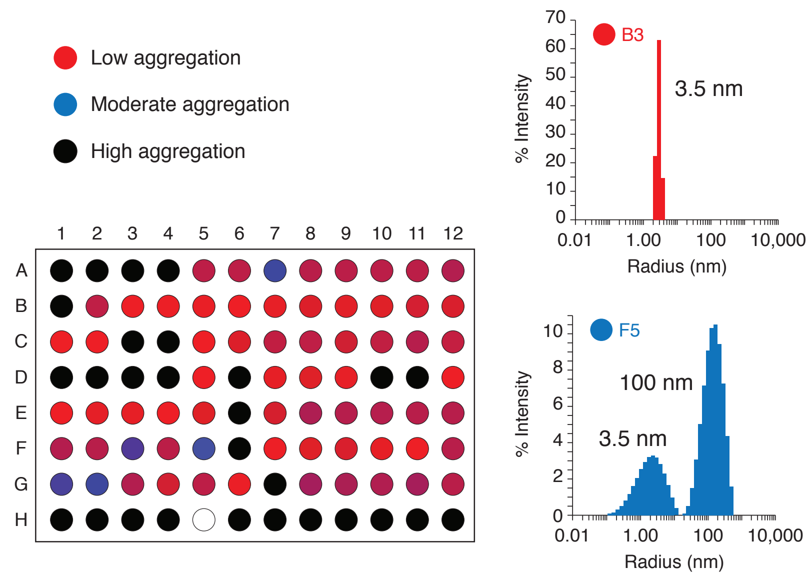
The WyattQELS may be used in batch (unfractionated) or on-line (fractionated) measurements. In batch mode, heterogeneous samples will produce complex correlation functions whose departure from pure exponential decay arises from the presence of heterodisperse components. Via a complex mathematical fitting process, the ASTRA software can obtain an approximate size distribution.

Dynamic light scattering instruments can determine hydrodynamic radius from 0.2 nm to 2500 nm. Inline with GPC, the size range is 0.5 nm to 300 nm.

In the on-line mode (connected to a GPC/SEC separation system), a MALS instrument incorporating a WyattQELS records, simultaneously and in the same flow cell, the dynamic and static light scattering data. The molar mass, rms radii (where possible) and hydrodynamic radii are calculated for each elution volume (a.k.a. slice).

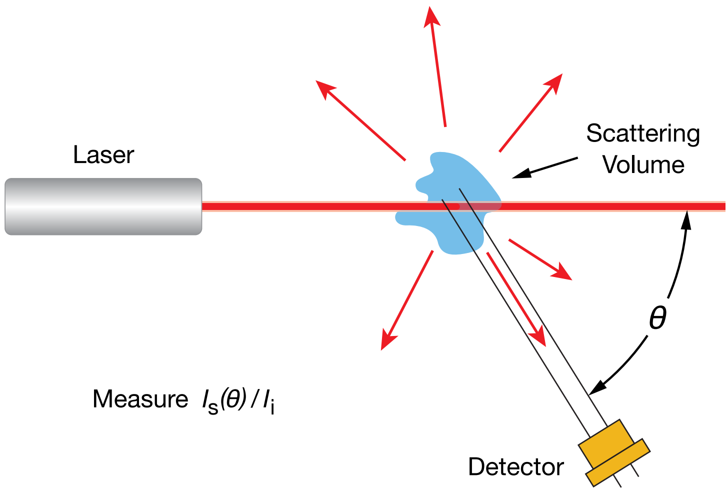
Combining the hydrodynamic radius data obtained from dynamic light scattering with the rms radius data obtained from MALS makes it possible to learn about the molecular conformation or nanoparticle shape, which may be difficult or impossible to achieve in any other way.

For off-line (batch) measurements, Wyatt also offers the DynaPro® line of cuvette- and microwell plate-based stand-alone DLS instruments and the Mobius™ electrophoretic mobility (zeta potential) instrument. Both the DynaPro and the Mobius can serve double duty: standard batch DLS or on-line. They mimic WyattQELS functionality by connecting via an optical fiber to the MALS flow cell.



Dynamic light scattering results summary showing the level of aggregation per well and the corresponding hydrodynamic radius for two wells, B3 and F5.

HOW STATIC LIGHT SCATTERING INSTRUMENTS WORK



The light scattering photometers of today use lasers because they are extremely reliable light sources with a few other advantages. They have superb beam collimation and purity. They can produce light at a single wavelength. They are relatively compact and their lifetimes are generally quite long (10,000 hours or more).

A MALS instrument uses its laser source to determine absolute molar mass and size in a pretty straightforward manner:

a) The laser beam passes through the sample—contained in either a flow cell or a cuvette.

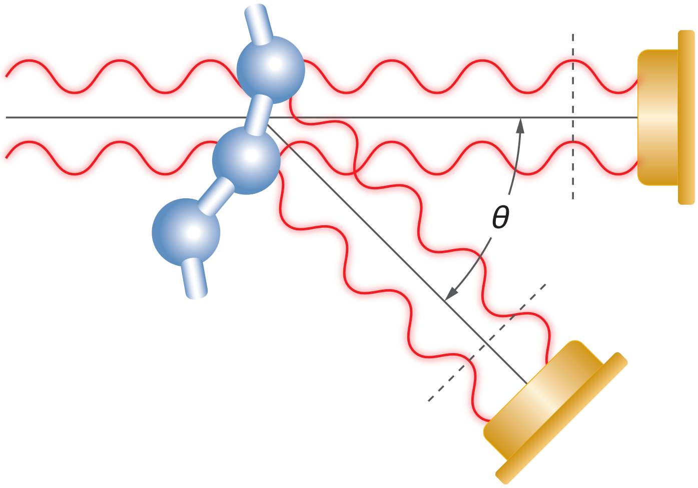
b) The sample scatters light at all angles. Each detector, which is placed at a different angular position around the sample, provides a response directly proportional to the intensity of the scattered light it receives.

c) The analog light scattering signals are digitized and transmitted to a computer for processing.

d) ASTRA performs the analyses necessary to extract the absolute molar mass and other parameters from the data. Eq. (1) is the heart of this analysis:

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2c + \cdots \qquad (1)$$

The angular variation of the scattered light is determined by the size and conformation of the molecule.



The excess Rayleigh ratio, $R(\theta)$, is the relative amount of light scattered by the solute at an angle θ; c is the solute's mass/volume concentration, usually in mg/mL; M_w is the weight-average molar mass; and K^* is a constant equal to $4\pi^2 n_0^2 (dn/dc)^2 / [\lambda_0^4 N_A]$, where n_0 is the refractive index of the solvent, dn/dc is related to the difference in refractive index of the solute and solvent at the laser wavelength, N_A is Avogadro's number and λ_0 is the vacuum wavelength of the incident light. Finally, $P(\theta)$ is the form factor, which describes the scattered light's angular dependence and depends on the size and structure of the scattering molecules. The mean square radius $< R_g^2 >$ of the molecules may be determined from the measured angular dependence.

That's all there is to it. The results come from fundamental measurements without any reference to so-called molar mass standards.

Wyatt's DAWN combined with ASTRA software represents one of the only absolute light scattering systems available.

So why go to all the trouble of multiple angles, when one might suffice? LALLS instruments certainly do minimize numerical calculations via the simplification of Eq. (1) by approximating $\theta \approx 0°$. At this value, $P(\theta) \approx 1$ and the molar mass is determined immediately, without fitting $P(\theta)$. Although the form of Eq. (1) becomes very simple in this low angle limit, the low angles utilized in LALLS are especially prone to the adverse scattering effects caused by particulate matter, like dust in the solvent or column shedding. The signals from such particulates often mask or distort the measurements of the solute of interest.

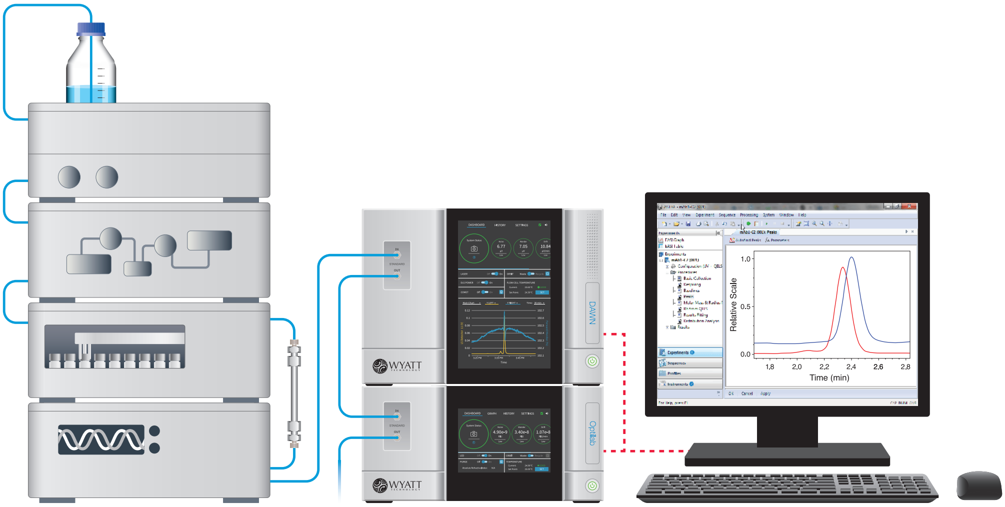
MALS eliminates the high susceptibility to particulate noise which can ruin a LALLS measurement.

Scattering measurements made at 90º are far less prone to noise from dust, but are only suitable for molar mass determination of molecules below the 10 nm limit for angular dependence. Analysis of larger particles is skewed due to the discrepancy between scattering intensities measured at 90º and the values relevant to molar mass near 0º.

With only one angle, both LALLS and RALLS instruments cannot determine size via angular dependence of the scattered light. Of particular concern is the fact that RALLS instruments do not offer any indication of the possibility that an incorrect molar mass is calculated due to angular dependence.

The determination of the molar mass from measurements made at many angles (MALS) overcomes the issues of noise at low angles and uncertainty regarding size at right angles. At the same time MALS provides accurate size determination, by performing a numerical fit to all of the angular data collected to Eq. (1). No data are discarded; since the scattering behavior at all angles is well described by the theory, the final molar mass determinations are far more precise than those obtained by other light scattering technologies.

When column shedding becomes problematic, vendors of LALLS instruments recommend using an in-line filter to remove the scattering effects of debris from the low angle data. Although such filtering may remove some of the sources of small angle noise, it leads to loss of chromatographic resolution and can remove much of the sample itself!



Wyatt's MALS detectors interface to most industry standard HPLC, GPC and FPLC systems.

Because of the intrinsic noise of a light scattering measurement—at all angles—due to dust and other debris, it is essential that any results (molar mass, rms radius, etc.) take these measurement fluctuations into account and present their precision. Besides the number and angular location of detectors, the method used to calibrate the detector response is another important factor in assessing a light scattering instrument. The two common methods are absolute calibration and calibration to reference molecules. The latter requires an accurate knowledge of the molecule's concentration in the light scattering cell and typically must be repeated in each solvent of interest. The former makes use of a well-characterized solvent such as pure toluene and is independent of concentration measurements or subsequent experimental solvents. Wyatt Technology's ASTRA software makes use of absolute calibration in order to ensure the most accurate results over the widest range of conditions and reports their experimental precision.

How Dynamic Light Scattering Instruments Work

The light scattered in the flow cell is collected by a special multimode optical fiber. The fiber detects wavelets of light, which interfere destructively or constructively depending on the positions of the illuminated molecules (or particles). As the molecules undergo Brownian motion, their relative positions change with time, resulting in transitions between constructive and destructive interference between the wavelets.

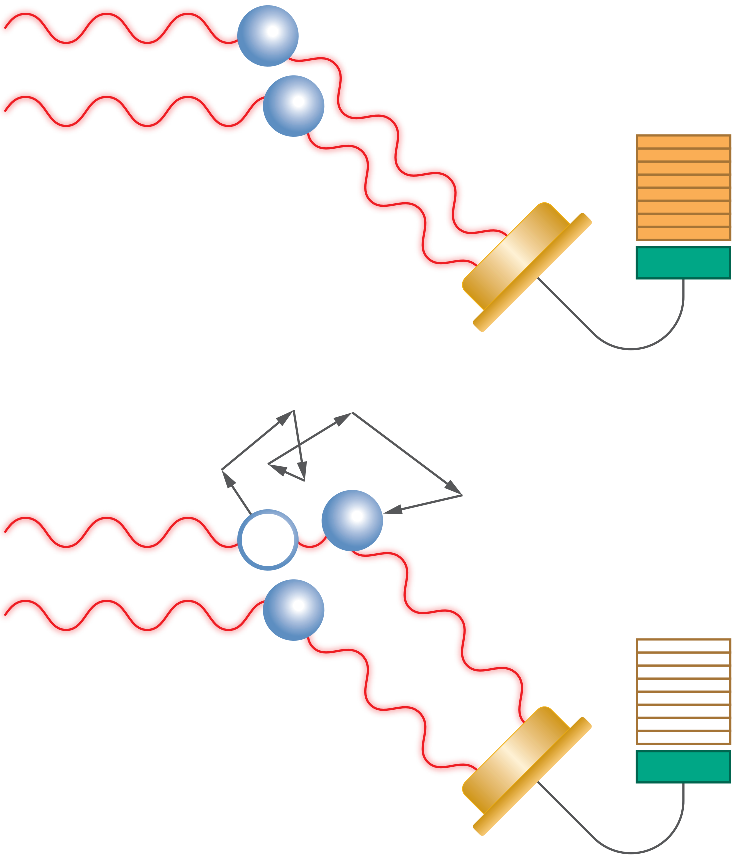
Small molecules—which diffuse quickly—generate signals that fluctuate rapidly. Conversely, large molecules generate signals that fluctuate slowly. The time dependence of these fluctuations is characterized by the intensity autocorrelation function. The autocorrelation function of a monodisperse sample is related to its diffusion constant by:

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

where τ is the delay time, β is the amplitude and $q = \frac{4\pi n_0}{\lambda_0} \sin\frac{\theta}{2}$ is the scattering vector. The Stokes-Einstein relation enables the molecule's hydrodynamic radius to be calculated:

$$R_h = \frac{k_B T}{6\pi\eta D_t} \qquad (3)$$

where k_B is Boltzman's constant, T is the absolute temperature (in degrees Kelvin) and η is the solvent viscosity.



Dynamic Light Scattering: Brownian motion of sub-micrometer particles gives rise to intensity fluctuations in the scattered light. The rate of fluctuation is analyzed to determine the diffusion coefficient and through the Stokes-Einstein relation enables the molecule's hydrodynamic radius to be calculated.

IMPORTANT QUESTIONS TO ASK BEFORE BUYING A MALS DETECTOR

The key to making an effective purchase is determining which light scattering instrument offers the features and support you need at a price that you can afford.

The cost of a light scattering instrument is not trivial, but in the world of scientific instrumentation the issues of service, support, instrument design and light scattering expertise are of far greater importance. We have compiled the following questions in order to help you assess your needs and evaluate the specific instruments from different manufacturers.

#1. What levels of technical expertise and support are available from the manufacturer?

This is undoubtedly the most important question of all. As with any discipline, light scattering has its own lore and its own experts. If light scattering is new to you, your learning will be accelerated significantly if you purchase an instrument from the most knowledgeable people in the field. Technical support, guidance and direction from a company whose leadership in light scattering is internationally recognized will help you to be successful with your measurements and their interpretation.

Wyatt Technology regularly hosts seminars to help advance the characterization and formulation of macromolecules and nanoparticles through light scattering technology.

Building a high-quality MALS detector requires years of experience and extensive technical qualifications. Intelligent circuit board design, state-of-the-art electronic components, superior knowledge of photodetection equipment—not to mention expertise in optical engineering and software development—all play critical roles in building all of Wyatt's light scattering instruments.

Dr. Wyatt commercialized the first MALS instruments many decades ago. Today, Wyatt Technology is the world leader in this field. No other company has more resources devoted to the manufacture, service and support of MALS. Wyatt employs dedicated hardware and software specialists and maintains the most comprehensive database of applications solutions in the field.

Wyatt Technology holds regular users meetings where customers can interact with leading scholars and industry practitioners (as well as an occasional Nobel Laureate or two) in order to make the most of their investment in light scattering equipment. Wyatt Technology's website has monthly updates to bibliographies of published papers that reference its DAWN and DynaPro equipment. As of March 2021, this searchable list includes more than 17,000 peer-reviewed references.

#2. How many angles do I need?

The number of angles is important since it determines the precision (reproducibility or absence of fluctuations) of the measurement, as well as the range and accuracy of the molar mass and size determination. More angles mean more data are collected; more data mean greater accuracy. In fact, the precision of a light scattering measurement is roughly proportional to the square root of the number of detectors.

Obviously, at least two angles are necessary in order to get any sense of the variation of scattering intensity. Yet in the presence of noise, a weighted linear fit of the data requires a minimum of three detectors covering a sufficient angular range. Wyatt Technology's miniDAWN and microDAWN instruments provide three angles, which is the minimum number recommended for all types of samples smaller than about 50 nm in radius.

The number and angular coverage of detectors in a MALS instrument determine its range of measurement. Large molecules produce non-linear angular scattering profiles. So they require many detectors, spread over a large angular range, in order to capture profiles and analyze both size and molar mass. For regulatory work, the redundancy of detectors built into Wyatt's DAWN systems is particularly useful. The precision of measurement is far superior to any one, two or ten-angle instrument and enables accurate molar mass determinations spanning a wide range.

Percent Error under Typical Chromatography Conditions
(smaller percentages are better)

Number of Angles	Errors in Molar Mass	Errors in Radius
15+	0.50%	3.0%
Low + 90°+ High	1.00%	5.0%
Very Low + 90°	14.00%	80.0%
90° Only	8.00+%	N/A

Wyatt Technology takes quantitative analysis seriously. That is why the DAWN instruments contain numerous detectors. This design ensures the range, precision and reproducibility of measurements over years of use.

At Wyatt, we have studied the effects of multi-angle technology on the precision of the calculated results for many years. The table on the previous page summarizes these results. Under a realistic set of chromatography conditions (neither perfect nor disastrous), the benefits of MALS are apparent: Even our miniDAWN will produce molar masses with over 8 times greater precision than a single or dual angle instrument.

However, simply counting the number of angles is not an end in itself. You may see other MALS instruments arbitrarily sporting 20 or 21 detection angles. Don't be fooled. Without proper design of the flow cell and optics, stray light (also known as flare) will blind the lowest and highest angles, distorting the data and leading to incorrect results. The innovative flow cell design pioneered by Dr. Wyatt in 1984 vastly reduces stray light in order to make full use of all the available angular detectors.

DAWN instruments are designed with numerous detectors to ensure the greatest range, precision and reproducibility of measurements.

#3. Can I clean the light scattering instrument's flow cell when it gets dirty?

Eventually, every flow cell in every light scattering instrument will get dirty. This is particularly true for aqueous chromatography. When time is of the essence and/or throughput is of paramount importance, the ability to access and clean your flow cell quickly and easily is essential. It is, therefore, vital to ascertain whether or not the instrument manufacturer employed these concerns in their product design.

Wyatt Technology designs all of its instruments with customer productivity in mind. The entire flow cell assembly can be removed from any DAWN instrument, cleaned and replaced—allowing you to be up and running quickly. You don't have to send the instrument back to the manufacturer or even replace it—as you must do with some vendors. And, if you're as meticulously clean with your chromatography as some of our customers are or if you have Wyatt's COMET™ ultrasonic cleaning accessory, you can run your instrument for years without having to remove the flow cell.

#4. Is the light scattering detector really absolute?

The DAWN instruments together with ASTRA software represent one of the only absolute light scattering systems available anywhere. By "absolute" we mean that our products determine molar mass directly from the scattered light intensity, without reference to a standard of "known" molar mass. We perform instrument calibration by measuring the scattered light from a pure solvent, such as toluene (which exists in the same form throughout the known universe).

Once our instruments are calibrated, they can analyze measurements made in all types of solvents without the need for recalibration. Our absolute calibration has a tremendous practical advantage over light scattering instruments supported by software that does not incorporate absolute calibration. Those units require recalibration for every solvent and then "compare" the light scattering to the scattering from reference standards in each solvent used. These systems then "adjust" their results to make them agree with the standard—with no direct proof that the standard is actually correct.

Many problems can result from trusting calibration standards. Some of these standards were made in large batches years ago and have aged. And, there are many solvent systems for which there are no standards. Even when suitable standards exist, the scientist must trust the "known" molar masses, even though they were often measured using other analytical techniques. In addition, the scientist must assume that the concentration in the light scattering cell is known with a high degree of accuracy.

The choice is whether or not you want to work in a calibration-dependent world, with column calibration and comparison to so-called standards? If you want to work in a world of absolute measurements, with a DAWN instrument and ASTRA software you can determine the absolute molar masses of the "standards" that other instruments require.

#5. How stable is the laser source?

This question may seem trivial since most commercially available solid state lasers contain power output monitors that can be used to ensure beam power stability. However, with age and/or temperature fluctuation, these lasers are prone to so-called "mode-hopping," which degrade their stability. The lasers used in DAWN products incorporate proprietary Wyatt stabilization technology in order to provide users with unequaled performance.

#6. Are there ample publications in professional refereed journals that reference the equipment?

The best surprise is no surprise when buying any kind of instrument. Procuring an instrument that has been used to collect publication-quality data is critical, since its performance will have been examined rigorously by peer review.

Wyatt Technology's website maintains a bibliography of peer-reviewed publications citing Wyatt instruments. There are currently more than 17,000 entries.

There are no 'NIST Standards' to validate light scattering instruments, so you have to rely on the scientific community for validation. Look carefully at the publications that have been generated using different light scattering equipment. Are they truly peer-reviewed publications or are they simply trade journals or abstracts of oral presentations?

Wyatt Technology's refereed bibliography citations total more than 17,000 as of this printing (2021). Each of these publications shows a Wyatt system at work, along with the results achieved by scientists who have had top-notch training and support.

From biotechnology to polymer production and nanoparticle research, manifold publications have substantiated the value and importance of the DAWN and DynaPro instruments in the modern laboratory. A regularly updated bibliography is available.

#7. How versatile is the software?

Software is what allows light scattering to address multiple application areas. If the software is not versatile, it will limit what you are able do with light scattering. Here are some of what Wyatt's ASTRA software can do:

1. Provide exceptional analytical power, where the analysis of each value reported includes its statistically derived standard deviation.
2. Support multiple instrument and analysis types (MALS, DLS, RI, UV and viscometry).
3. Give you control of third-party HPLC equipment (UV, pumps, injectors, etc.)
4. Support diverse applications from studies to batch measurements.
5. Provide remote access and control of instrumentation over a network.
6. Provide 21 CFR Part 11 compliance when needed (or a non-compliant version if desired).

7. Enable “one-click” molar mass by automating baseline settings, peak ID’s, etc.

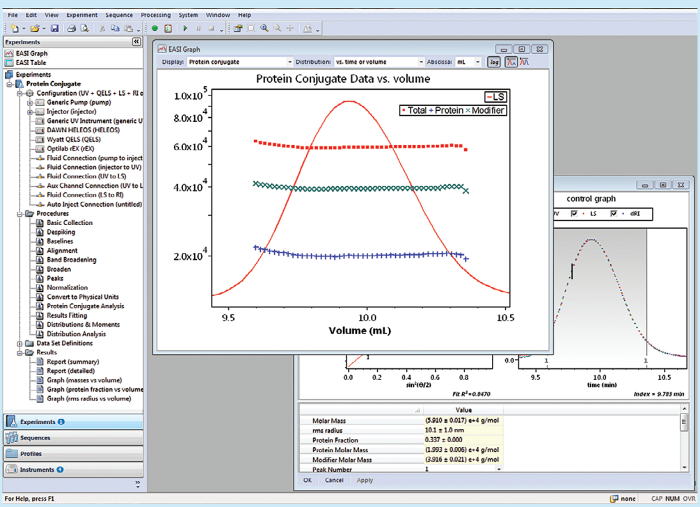
ASTRA has been built on Wyatt Technology’s decades of experience with light scattering analysis. It has also been built with an eye toward the future, with extensibility at its core. ASTRA is the most versatile light scattering software available and it is continually being improved.

#8. Does the software have built-in “fudge factors” or is it based on sound theoretical principles?

Most manufacturers know little about the physics of light scattering—let alone its limitations and its subtleties. As a result, inexperienced companies produce software containing large areas of uncertainty, which they prefer their customers never see!

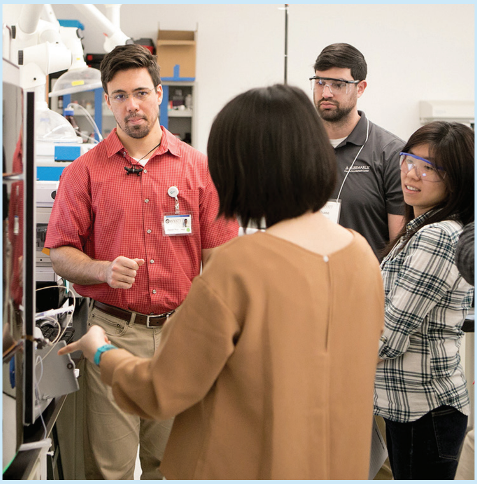
Make sure that your light scattering instrument vendor doesn’t sell you a house of cards. Before making a purchase, make sure that every quantity needed to determine molar masses and sizes can be measured and is available. These include: The excess Rayleigh ratio, *R(θ)*, the light scattering constant, *K** and the specific refractive index increment, *dn/dc*, at the same wavelength as the light scattering measurement. Also confirm that the temperature variation of refractive index and viscosity is fully accounted for.

It is also important to ascertain that the software you will be using was written by the same people who have developed the instrument. You can be at a significant disadvantage if you find yourself with software that was farmed out to an independent contractor. Wyatt Technology has more experience in software development for multi-angle light scattering



Wyatt’s ASTRA is the premier software for analyzing macromolecules and nanoparticles through multi-angle light scattering techniques. It integrates MALS, UV, refractive index, dynamic light scattering and intrinsic viscosity data for comprehensive characterization of the physical properties of materials in solution or suspension.

LSU



Light Scattering University

The purchase of every Wyatt light scattering instrument sold in North America includes a complimentary enrollment in Wyatt's Light Scattering University. This comprehensive training at Wyatt's Santa Barbara, California headquarters shows scientists how best to use light scattering instruments to meet their specific needs and applications. In addition to intensive hardware and software training, participants are treated to deluxe accommodations and gourmet meals during their stay.

Regular user meetings are scheduled throughout the United States and software and hardware trainings are also available at Wyatt Technology facilities in France and Germany.

than all other manufacturers in the world combined. It is simply a fact that Wyatt has more scientists involved in the development and refinement of its algorithms than anyone else in the business.

#9. Does the software report the degree of precision of the light scattering measurement?

The precision of a light scattering measurement depends on many factors, including the various sources of extraneous noise; such as solvent and sample debris, column shedding, pump pulsation, electrical noise, laser fluctuations, etc. Both the light scattering instrument and the concentration detector have their own detection characteristics as well. Top notch software and state-of-the-art electronic components, such as digital signal processing (DSP) chips, will result in reported measurements that take all these sources of signal degradation into account. Unless the software reports molecular properties (mass, size) measured with their standard deviations derived from the noise present, the results will have little meaning.

Wyatt Technology is the only analytical instrument manufacturer who reports the precision of the calculated results. Wouldn't it be better to know that a sample had a molar mass, for example, of 103,300 ± 1,500 than not knowing the level of uncertainty at all?

#10. Does the manufacturer provide training in light scattering and guidance for obtaining optimal results with their equipment?

Too often, instruments are sold by manufacturers without any technical support or knowledge of light scattering. When problems arise, you deserve expert assistance. Using a light scattering instrument successfully and with confidence requires training in light scattering theory, practice, sample preparation, data interpretation, as well as plenty of hands-on experience with the equipment and software.

The best customer training involves the people who actually design and build the instruments, write the software and handle service and support. Such training is essential in order to create confidence in the light scattering method and ensure that users aren't simply purchasing a "black box." A successful light scattering training session should make the user feel comfortable with the instrument they have just purchased and wonder why they didn't buy one sooner.

Wyatt Technology's training course—Light Scattering University® (LSU)—is included with the purchase of every MALS and DLS sold in North America. This multi-day instructional seminar provides comprehensive training materials, exercises, hands-on experience, question-and-answer sessions and meetings with the people who invented and popularized light scattering technology. Wyatt's scientists, customer service engineers and programmers interact with customers frequently during LSU sessions. For North American customers, admission to Light Scattering University includes round-trip airfare to the Wyatt training facility in Santa Barbara, California, as well as luxurious accommodations and gourmet meals.

#11. What accessories are available?

Some instruments are sold with features you don't need. Others come with incomplete hardware. A company that is focused on light scattering instruments will be able to provide instrumentation with a high degree of flexibility—an *à la carte* approach. This allows purchasers to select certain software and hardware options today and upgrade their system at a later date.

For example, different laser wavelength options can be important if your work requires the analysis of different classes of molecules. Studies with fluorescing molecules may require narrow band pass interference filters. Additional options are available for thermostatic control of the instruments both above and below ambient temperature.

The DLS instruments and the WyattQELS accessory can be interfaced with the MALS instruments to measure on-line or off-line static and dynamic properties of macromolecules and nanoparticles. In addition, spare parts, consumables and accessories are offered online through the Wyatt Technology Store that are all compatible with your Wyatt instrumentation.

#12. What service and support plans are available?

A comprehensive service or support plan protects your investment and takes the hassle out of keeping your instrument running at its best.

Wyatt Technology offers Platinum, Gold and Silver Instrument Service Plans to provide comprehensive service and support for all Wyatt instruments. Depending on the plan level, the service plans include annual preventative maintenance, onsite calibration, re-qualification, loaner instruments if available, training discounts at Light Scattering University and discounted spare parts and accessories.

The Software Support Plan provides all software upgrades, priority support and increased productivity through continual enhancements. If working with AAV applications, Wyatt has developed a personalized, on-site, application-specific training service to bring customers up to speed in a matter of days. The AAV Method Implementation and Training service saves significant time and effort in learning, method development and validation.

Service and Support Plans are the best way to ensure that an investment in light scattering technology pays a healthy dividend over many years of use.

#13. Are packages and services offered to aid in validation for government agencies like the FDA?

Often, agencies like the Food and Drug Administration (FDA) require that each instrument used in the production and analysis of a pharmaceutical substance be qualified according to well-defined procedures. Wyatt Technology has assisted customers with the development of procedures for its instruments' installation, operation, performance and maintenance qualifications. Wyatt offers an optional 21 CFR Part 11 compliance package for its software, including IQ/OQ documentation, procedures and services for the pharmaceutical and biotechnology industries, as well as any others that may require them.

The FDA has already received numerous submissions with DAWN, DynaPro and miniDAWN data, so new applications become more streamlined. The precision, redundancy and versatility built into Wyatt instruments make them especially powerful tools for validation purposes, since the FDA holds these three characteristics in high regard.

#14. Has the instrument been manufactured under a formal, validated quality control program?

With the ever-increasing scrutiny that regulatory agencies place on pharmaceutical and biotechnology industries, it is not only necessary that the light scattering instrument and software perform well, but essential that their performance be validated. The only way to ensure this is to develop and manufacture instruments and software under a formal quality system.

Wyatt Technology has always taken quality very seriously. No one is a tougher judge of our instrument and software performance than our own QC personnel. Wyatt's internal culture of quality has been incorporated into a formal ISO system. All aspects of instrument and software development, manufacturing and tests are documented. Customers working in regulated industries are welcome to audit us in order to aid in validating Wyatt Technology's light scattering instrumentation and software.

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LIGHT SCATTERING INSTRUMENT FEATURES

Features and Benefits	Wyatt DAWN
Molar mass range	200 to 10^9 Da
Molecular rms radius range	10 to 500 nm
Sensitivity	200 ng BSA in PBS or 10 ng 100 kDa polystyrene in THF
Available wavelength options	658, 785 nm
Heated/cooled option	-15º C to 150º C
Ultra-high temperature option	Ambient to 210º C
Output signal	Digital, Analog
Inputs	4 analog, auto-injector, alarm in, recycle in
Inline DLS (optional)	YES
Fluorescence-blocking interference filters (optional)	YES
Need for molecular calibration standards	NO
Model-dependent results	NO
Light Scattering University included	YES
PhD staff ready to answer technical questions	YES
Company dedicated to light scattering instrumentation	YES
Direct size determinations without assumptions or any additional equipment	YES
Sample cell easy to clean	YES
Software calculates uncertainty of each measurement	YES
Light scattering workshops and webinars	YES
Regular user meetings	YES

AND BENEFITS COMPARISON

Wyatt miniDAWN / microDAWN	Other Instrument
200 to 10^7Da	
10 to 50 nm	
70 or 500 ng BSA in PBS or 3.5 or 25 ng 100 kDa polystyrene in THF	
658 nm	
N/A	
N/A	
Digital, Analog	
2 or 4 analog, auto-injector, alarm in, recycle in	
YES	
NO	
NO	
NO	
YES	
YES	
YES	
YES	
YES	
YES	
YES	
YES	

Use this chart to compare light scattering instruments to other instruments you may be considering.

DAWN offers the highest sensitivity and is the premier SEC-MALS detector for determining absolute molar mass and size.

miniDAWN ranks as the best fundamental multi-angle light scattering instrument.

microDAWN offers superb sensitivity and is the only MALS detector uniquely designed for UHPLC.

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Woburn, Massachusetts, USA

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Taiwan
Systematic Instruments Co., Ltd.

Turkey
Bilim Lab.Cih.San.Tic.Ltd.Sti.

Left to right:
Geofrey K. Wyatt, Chief Executive Officer
Dr. Philip J. Wyatt, Chairman of the Board
Clifford D. Wyatt, President

For more than 40 years, we've operated as one of the very few remaining family-owned businesses in the analytical instrument industry. With installations in more than 65 countries, over 18,000 refereed journal publications citing our instruments and more than 25 PhD scientists on staff, we take great pride in the worldwide recognition that Wyatt Technology has received as a leading manufacturer of instruments and software for absolute macromolecular and nanoparticle characterization. Our dedication to providing customers with comprehensive training and personal support has made us the gold standard in this field.

WYATT TECHNOLOGY