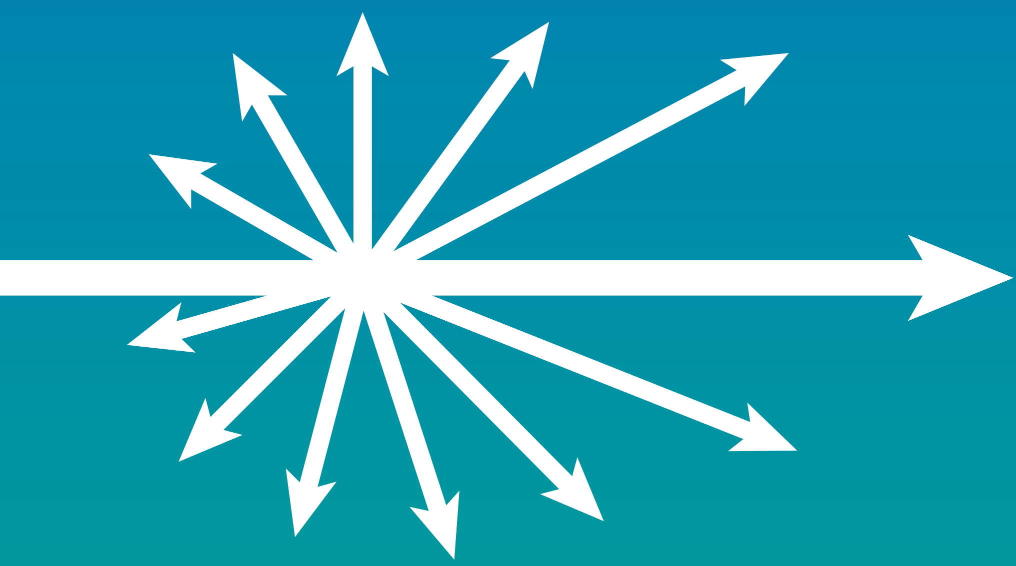
Wyatt Technology Corporation

The Ultimate Guide

Buying a SEC-MALS Detector



Absolute Macromolecular Characterization

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Introduction

So, you're thinking of buying a MALS detector for GPC/SEC?

More and more scientists realize that characterizing proteins, polymers and biopolymers (and nanoparticles, too!) with a Multi-Angle Light Scattering (MALS) instrument is the only 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 reliable distributions of molar mass, size and conformation unattainable by other means, *without* making assumptions or relying on questionable calibration standards.

This booklet serves as a brief introduction to the principles of light scattering and as a guide to help you make the right choice when buying an instrument.

As long ago as 1975, Wyatt Technology's founder invented the very first commercial MALS instruments incorporating lasers as their light source. And since 1982, Wyatt Technology has been defining and redefining the state-of-the-art in MALS hardware, software, service, and support.

In this booklet we'll explain the key light scattering (LS) principles. For some, these concepts may be novel; on the other hand, those already familiar with light scattering technology should find a wealth of practical information for making the right choice when buying an instrument. If you are new to the field, we hope that this booklet will demystify the subject and help you to make MALS a common tool in your laboratory analysis.

What is Light Scattering?

Light scattering is ubiquitous. Most of the light we see has been scattered in its passage from its source (the sun, a light bulb, or a laser) to our eyes. Notice the blue sky, or the booklet you are now reading: the sky appears blue, and you can discern the patterns making up letters on the page, because of the way they scatter light.

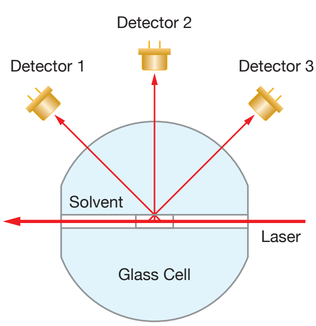
Indeed, we obtain virtually *all* of the visual information about our surroundings from light that has been scattered. We identify and differentiate objects by making deductions based on observing the *wavelengths, spatial pattern* and *quantity* of light that they scatter. Such deductions are not necessarily limited to 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, you could make measurements over a range of angles in order to obtain additional information, as described below.

A Historical Perspective

Early light scattering instruments, covering an appreciable angular range, incorporated a mercury arc lamp as light source and a photomultiplier tube (PMT) as detector. The PMT could be rotated around the central platform (holding the sample in a 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 1940's. The American-made Brice-Phoenix and the French-built SOFICA units, sold in the 1950's and 1960's, 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).

Detector Geometry



†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* LS.

The following year, Beckman Instruments introduced a laser-based gel-permeation chromatography (GPC) detector that measured scattered light at a single, low angle to determine molar mass alone. The Beckman optical design, incorporating 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 still 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 online measurements with rapidly evolving 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.

In the late 1970's, Dr. Wyatt recognized the need to make measurements *simultaneously* over a range of scattering angles, in order to measure size in GPC as well as molar mass, and to overcome some of the inherent limitations of LALLS and RALLS. This first multi-angle light scattering (MALS) instrument became known as the DLS 800. By 1984, 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/RALLS systems began to refer to the DAWN by the acronym MALS (Multi-Angle Light Scattering), which has endured to this day!

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 18-angle and miniDAWN™ 3-angle MALS detectors for HPLC/GPC, and the µDAWN™ 3-angle MALS detector for UHPLC. The DAWN offers the widest range and highest sensitivity while the miniDAWN is suitable for most proteins and small polymers. In addition, Wyatt's ASTRA® software provides comprehensive acquisition, analysis and reporting of MALS data.

Two Types of Light Scattering: Static and Dynamic

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

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.

The Molar Mass and Size From 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

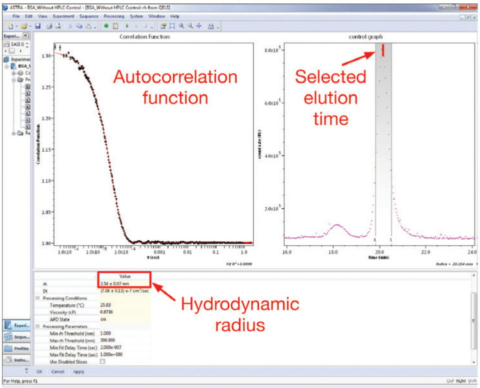
Light scattering instruments can determine absolute molar masses from less than 10^3 to 10^9 grams/mol.

proteins as well as the *detailed distributions* of molar mass and size. The chromatography equipment you *already* possess, plus a MALS detector, obviate the need for column calibration or reference standards.

The *size* of a molecule—its root-mean-square radius, or r_g—affects the angular variation of the scattered light intensity. 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.

Small, compact molecules possessing a root mean square (rms) 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).

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, of course, MALS instruments never require *a priori* conformational assumptions; the size is read out directly from the angular scattering intensity distribution.



Integrating a WyattQELS™ with SEC-MALS characterization, the hydrodynamic radius is determined at each elution volume in the chromatogram.

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 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. In addition, MALS instruments can be used to study the homo- and hetero-association of proteins and other biological macromolecules.

Hydrodynamic Radius from 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 < 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 manufactures the WyattQELS, a dynamic light scattering module that integrates with 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 µDAWN, 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 thence, the hydrodynamic radius.

The WyattQELS may be used in batch (unfractionated) or on-line (fractionated) measurements. In the 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.

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). By separating the particles into homogeneous slices and measuring the contents of each slice, ASTRA produces a highly accurate distribution of molar mass and size.

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 offline (batch) measurements, Wyatt also offers the venerable DynaPro® line of cuvette- and microwell plate-based stand-alone DLS instruments, and the Möbius® electrophoretic mobility (zeta potential) instrument. The DynaPro NanoStar® cuvette-based DLS detector as well as the Möbius may be connected via optical fiber to a DAWN or miniDAWN in-line with GPC/SEC, or to a µDAWN in-line with UHPLC-SEC, to serve double-duty: either batch *or* online DLS instruments.

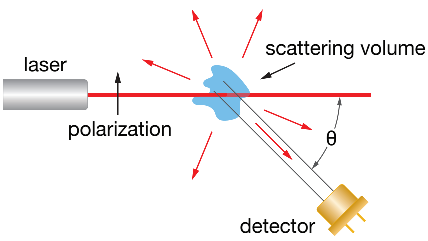
How MALS Instruments Work

The light scattering photometers of today use lasers because they are extremely reliable "light bulbs", 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 and more).

How, then, does a MALS instrument use its laser source to determine an absolute molar mass and size? It's pretty straightforward:

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

$$(1) \quad \frac{K^*c}{R(\theta)} = \frac{1}{[\, M_w P(\theta) \,]} + 2A_2 c + O(c^2)$$

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 $\langle r_g^2 \rangle$ 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.

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.

The existence of mirrors, lenses, and prisms in LALLS instrumentation adds to their expense and maintenance.

Scattering measurements made at 90° 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 90° and the values relevant to molar mass near 0°.

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. Signals at multiple, noise-free angles are *extrapolated* to zero for maximum accuracy. At the same time MALS provides accurate size determination, by performing a numerical fit to all of the angular data collected to Eq. (1), thanks to the now-ubiquitous personal computer. 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 removes much of the sample itself!

A few dual-angle instruments have evolved, although these are merely low-cost, low-quality derivatives of the MALS approach Wyatt has pioneered and popularized. Unfortunately for users of dual-angle instruments, calculations that depend on determining the slope of a line through only two experimentally measured points (the two detection angles) are mathematically unsound, especially in the presence of column noise that predominantly affects the low-angle detector. Dual-angle instruments are of no help for larger particles where the $P(\theta)$ is non-linear.

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.

How Dynamic Light Scattering Instruments Work

The light scattered in the DAWN 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 that is calculated from the measured intensity fluctuations by:

$$G(\tau) = \int_{-\infty}^{\infty} I(t)\, I(t-\tau)\, dt,$$

where $I(t)$ is the intensity as a function of time, and τ is a delay time. The autocorrelation function of a monodisperse sample is related to its diffusion constant by:

$$G(\tau) = \langle I(t) \rangle^2 \left(1+\alpha e^{-2D_T q^{2\tau}}\right)$$

where $\langle I(t) \rangle^2$ is the average intensity squared, α is an instrument constant, D_T is the translational diffusion constant, $q = (4\pi n/\lambda_0)\sin(\theta/2)$, n is the index of refraction of the solvent, λ_0 is the wavelength of light in vacuum, and θ is the angle of detection with respect to the incident beam direction.

By fitting the theoretical expression of equation 3 to the measured autocorrelation function of equation 2, one can directly calculate the diffusion constant of the molecule. Furthermore, the Stokes-Einstein relation enables the molecule's hydrodynamic radius to be calculated:

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

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

Questions to Ask Before Buying a MALS Detector

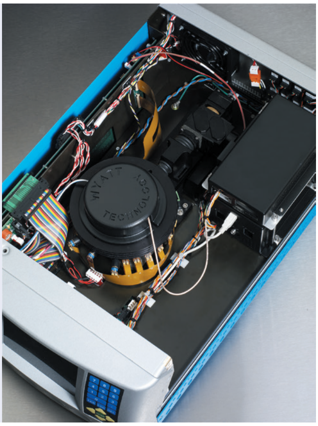
The key to making an effective purchase decision is determining which light scattering instrument offers the features you need – no more and no less – at a price you can afford. And while prices are not trivial, in the world of scientific instrumentation, service, support, instrument design, and light scattering expertise are of far greater importance. In order to assist you, we have compiled the following questions to help you assess your needs as well as to evaluate specific instrument vendors. We hope that they help you make an informed decision.

1. What technical expertise and support are available?

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

DAWN HELEOS® features and benefits:

- DSP chips process the light scattering and auxiliary signals with 24 bit analog to digital converters for maximum noise rejection without signal distortion.
- The flow cell, read head, and laser are integrated into a rigid optical system to provide maximum electro-optical stability.
- Custom-made hybrid photodiodes spaced around the read head provide for rapid data collection and superb reproducibility.
- The 120 mW diode laser provides unparalleled signal-to-noise benefits.



Building a quality MALS detector requires years of experience and 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 Wyatt's exceptional and versatile light scattering instruments.

Dr. Wyatt himself commercialized the first MALS instruments decades ago. It's no surprise, then, that Wyatt Technology is the world's leader in this field. No company has *more* resources devoted to the manufacture, service, and support of MALS than we do. Wyatt employs dedicated hardware and software specialists and maintains the most comprehensive database of applications solutions in the field.

The company also holds annual users' meetings so that customers can interact with the leading scholars and industry practitioners (as well as an occasional Nobel Laureate, or two) in order to make the most of their investment in their light scattering equipment. Wyatt Technology's web site has almost daily updates to the bibliography of papers that have been published, which reference the DAWN and DynaPro equipment. This searchable list now numbers *well above* 11,000 known 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. Indeed, 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. The miniDAWN instruments provide exactly three angles which is the minimum recommended number 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-monotonic angular scattering profiles and so require many detectors, spread over a large angular range, in order to capture that profile and analyze both size and molar mass. For regulatory work, the redundancy of detectors built into the DAWN systems is particularly useful. The precision of the measurement is far superior to any one, two, or ten-angle instrument and enables accurate molar mass determinations spanning a wide range.

At Wyatt, we take quantitative analysis seriously so Wyatt Technology's DAWN instruments contain 18 angles. This design ensures the range, precision, and reproducibility of your measurements over years of use.

For many years we have studied the effects of multi-angles on the precision of the calculated results. The table below 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 *14 times* greater precision than a single or dual angle instrument.

However, simply counting the number angles is not an end in itself. 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.

Typical Chromatography Conditions
Errors in Percent (Smaller numbers are better)

Angles	Molar Mass (%)	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

3. Can I clean my 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 other vendors. But, if you're as meticulously clean with your chromatography as some of our customers, or if you have our COMET™ ultrasonic cleaning accessory, you can run your instrument for years without having to remove the flow cell!

4. Is my light scattering detector really absolute?

The DAWN instruments together with ASTRA software represent one of the *only* absolute light scattering systems available anywhere. But just what do we mean 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 universe).

The DAWN instruments plus ASTRA software represent one of the *only* absolute light scattering systems available anywhere.

Once calibrated, we 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. Such 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.

There are many problems that may result from trusting calibration standards. Some of these standards were made in large batches years ago and have aged. 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.

So the choice is yours; do you want to work in a calibration-dependent world, with column calibration and comparison to so-called standards? Or, do you want to work in a world of *absolute* measurements? With a DAWN and ASTRA *you* can determine the absolute molar masses of the “standards” other instruments require!

5. How stable is the laser source?

This may seem a trivial question since most commercially available solid state lasers contain power output monitors that may be used to ensure beam power stability. Unfortunately with age and/or temperature fluctuation, such lasers are prone to so-called “mode-hopping”, which degrade their stability. The lasers used in DAWN products incorporate proprietary Wyatt stabilization technology providing our customers with unequaled performance.

6. Are there ample professional publications (from refereed journals) that used the equipment?

The best surprise is no surprise when buying any kind of instrument. And 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.

Look carefully at the publications that have been generated using different light scattering equipment. Are the publications truly peer reviewed, or are they all just “trade journals” or abstracts of oral presentations?

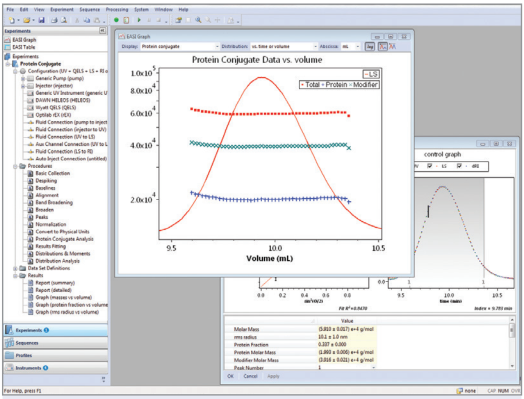
Wyatt Technology’s refereed bibliography citations total more than 11,000 as of this printing (2016). Each of these publications shows another DAWN, miniDAWN, µDAWN, Mobius or DynaPro system at work. From biotechnology to polymer production to nanoparticle research, manifold publications substantiate the value and importance of the DAWN and DynaPro instruments in the modern laboratory. Wyatt's website contains the latest bibliography, so the current list of publications is accessible 24 hours a day, from anywhere in the world.

7. Just how versatile is the software?

Undoubtedly, software is behind the power of light scattering to address multiple application areas. If the software is not versatile, how will this limit what you can do with light scattering?

Examine this short list of the things that 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. Provide customizable reports and access to all data.
4. Support diverse applications from high-throughput studies to batch measurements.
5. Provide remote access and control of instrumentation over a network.
6. Provide 21 CFR Part 11 compliance for those who need it (or a non-compliant version for those who don't).



The ASTRA workspace has rich features to analyze your samples. One is the triple-detection Protein Conjugate Analysis which yields absolute protein and modifier masses for PEGylated proteins, glycoproteins and other binary protein or polymer complexes.

ASTRA has been built on the decades of experience with light scattering analysis that only Wyatt Technology Corporation can provide. In addition, it has been built with an eye toward the future, with extensibility at its core. ASTRA is the most versatile light scattering software around, and it keeps getting better.

8. Does the software I buy have "fudge factors", or is it based upon sound theoretical and experimental principles?

Unless the manufacturer is experienced and familiar with light scattering instruments, he will probably 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 saw.

Make sure that your light scattering instrument vendor doesn't sell you a house of cards; determine beforehand that every quantity needed to determine molar masses and sizes can be measured and is available. These include: the excess Rayleigh ratio, $R(\theta)$, the light scattering constant, K^*, and the specific refractive index increment, dn/dc, at the *same* wavelength as the light scattering measurement. Make sure that the temperature variation of refractive index and viscosity is fully accounted for.

In fact, it is important that you make sure the software has been written by the people who have developed the instrument. Don't settle for software that was "farmed out" to a nameless contractor. Wyatt Technology has more experience in software development for multi-angle light scattering than all other manufacturers *combined*. We have more scientists involved in the development and refinement of our algorithms than anyone in the business.

9. Does the software I buy report the degree of precision of a 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 have taken all of 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 have little meaning.

Wyatt Technology is the only analytical instrument manufacturer who reports the precision of the calculated results. Wouldn't you rather know that you had a molar mass, for example, of 100,000 ± 1,500 than not knowing the uncertainty at all?

10. Does the instrument manufacturer provide training in light scattering and guidance in obtaining optimal results from the instrument?

Too often, instruments are sold by manufacturers without any technical support or knowledge of light scattering. Some vendors refer you to third parties for support. When problems do arise with your equipment, you deserve expert assistance. Building customer confidence requires superb training in light scattering theory, practice, sample preparation, data interpretation, as well as plenty of hands-on use of the equipment and software.

At Wyatt's LSU, students learn how to utilize our instruments to meet their specific needs and applications.

The best customer training classes should introduce you to the people who design and build the instruments, write the software, and handle your service and support questions. Such training is essential in order to reinforce your confidence in the light scattering method and ensure that you aren't purchasing a "black box". Quite the contrary! After a successful light scattering training session, you should feel comfortable with the instrument you have just purchased and wonder why you didn't buy one before!

Wyatt Technology includes its acclaimed "Light Scattering University®" (LSU) training course with every MALS and DLS we sell (US and Canadian customers only). The course provides comprehensive training materials, exercises, hands-on experience, question-and-answer sessions, and exposure to the people who invented and popularized this technology. Our scientists, customer service engineers and programmers interact with our customers frequently during LSU. For U.S. and

Canadian-based customers, LSU includes round-trip airfare to our training facility in Santa Barbara, CA, as well as award-winning accommodations and gourmet meals to reward you after a hard day's work.

11. Are accessories and service contracts available that may be purchased separately?

Some instruments are sold with features you don't need. Others come with incomplete hardware. A company focused on light scattering instruments will be able to provide you with instrumentation featuring the greatest amount of flexibility – an *à la carte* approach. This way you can purchase certain software and hardware options today and upgrade the system at a later time. Pages 24-27 contain a select list of Wyatt accessories currently available.

For example, different laser wavelength options can be important if your work requires you to analyze different classes of molecules. Polarization fixtures can be useful for experiments on anisotropy. 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.

Our DynaPro NanoStar and WyattQELS (Quasi-Elastic Light Scattering) instruments can be interfaced with Wyatt MALS instruments to measure on-line or off-line static and dynamic properties of macromolecules and nanoparticles.

Furthermore, Wyatt Technology is the only instrument vendor to offer a complete SEC-MALS solution with the Optilab® T-rEX™ refractometer. The extended range of the T-rEX makes it easy to measure differential refractive index both on-line (for determination of molar mass) and off-line (for determination of *dn/dc* at the MALS wavelength). We know how important *dn/dc* is, and we build our Optilab at the wavelength of the MALS laser for optimal synchronization!

Our Critical Care™ program provides comprehensive service contract support for *all* of our instruments. It makes loaner instruments available and gives additional training credit discounts at LSU, as well as significant discounts on spares and accessories so that your investment in this technology continues to reward you after years of use.

12. Does the vendor offer consulting services to aid in validation for different 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 its customers with the development of procedures for our instruments' installation, operation, performance, and maintenance qualifications, and the number of our successful clients reads like a *Fortune 500* list. Probably because it is!

The FDA has already received numerous submissions with DAWN, DynaPro, and miniDAWN data, so new applications are even more streamlined. And the precision, redundancy, and versatility built into our instruments make them especially powerful tools for validation purposes – since the FDA holds those three characteristics in high regard. We also offer IQ-OQ services for pharmaceutical, biotechnology, or any other industries requiring them.

13. Is the instrument manufacturer under a formal quality program with hardware *and* software that can be validated?

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 this performance be validated. The only way to ensure this is to develop and manufacture instruments and software under a formal quality system.

Wyatt Technology Corporation has always taken quality very seriously – no one is tougher on our instrument and software performance than our own QC personnel. This internal culture of quality is incorporated into a formal system aligned with the ISO-9001 standard. All aspects of instrument and software development, manufacturing, and tests are documented. Customers working in regulated industries are welcome to audit Wyatt Technology Corporation to aid in validating light scattering instrumentation and software.

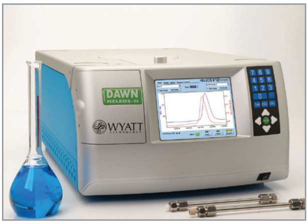
Laser Light Scattering Features and Benefits Comparison Chart

Fill in this chart to compare the instrument you are considering.

FEATURES/BENEFITS	DAWN HELEOS	miniDAWN/ µDAWN	BRAND X
Molar mass range	~100 to > 10^9*	~100 to > 10^7*	
Molecular rms range	10 to 500 nm*	10 to 50 nm*	
Sensitivity	0.2 µg BSA	0.5 µg/70 ng BSA	
Number of angles detected	18	3	
Light source (highly stabilized)	120 mW semiconductor laser, 658 nm	50 mW semiconductor laser, 658 nm	
Available laser options	658, 785 nm	658 nm	
Polarization analyzers (option)	YES	N/A	
Ultra High temperature (option)	Ambient to 210ºC	N/A	
Heated/cooled temp. (option)	-15ºC to 150ºC	N/A	
Output signal	Digital, analog	Digital, analog	
Inputs	4 analog, auto-injector, alarm in, recycle in	4 analog, auto-injector, alarm in, recycle in	
Autocorrelator interfacing (option)	YES	YES	
Fluorescence-blocking interference filters (option)	YES	N/A	
Need for molecular calibration standards	NO	NO	
Model dependent results	NO	NO	
Light scattering training included	YES	YES	
PhD staff ready to answer technical questions	YES	YES	
Company dedicated to light scattering instrumentation	YES	YES	
Direct size determinations without assumptions or any additional equipment	YES	YES	
Sample cell easy to clean	YES	YES	
Software that calculates the uncertainty of each measurement	YES	YES	
Light scattering workshops and webinars	YES	YES	
Annual Users' meeting	YES	YES	

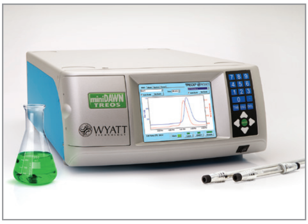
** Depending on sample structure, sample concentration, and chromatography conditions.*

Ultimate Instruments & Accessories



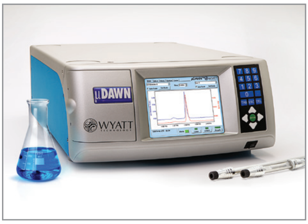
DAWN HELEOS

The ultimate in a research-oriented light scattering instrument. Its 18 angles of detection and its numerous options – from lasers to temperature control – enable it to be customized for practically any application you can imagine for SEC-MALS detection, FFF-MALS or CG-MALS.



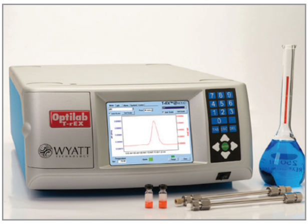
miniDAWN TREOS®

A triple-angle light scattering instrument incorporating the best-selling features of the DAWN. The miniDAWN TREOS can be used as a SEC-MALS "plug-and-play" detector to determine absolute molar masses from just a few hundred to several million Daltons—without column calibration or reference standards.



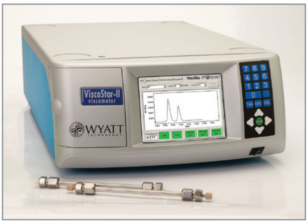
µDAWN

The first (and, as of this printing, only) MALS detector for UHPLC, the triple-angle µDAWN offers all the benefits of SEC-MALS such as absolute molar mass and size, WyattQELS compatibility for DLS, and the COMET cell-cleaning device (included with every µDAWN).



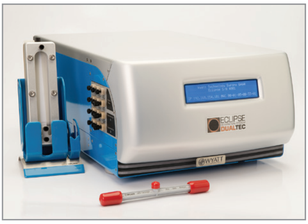
Optilab T-rEX

The only RI detector customizable at a variety of wavelengths. It can be used for off-line *dn/dc* determinations as well as on-line for high-sensitivity RI detection in GPC/SEC (we even have a special model—the Optilab UT-rEX — just for UHPLC). Covering a temperature range from 4°C to 65°C, the Optilab can also determine the *absolute* refractive index of a solvent.



ViscoStar Viscometer

The ViscoStar is the newest generation of online differential viscometers. The uniqueness of the ViscoStar springs from its high accuracy and contemporary electronics by which it achieves at least two times the signal-to-noise ratio of any commercial viscometer currently on the market.

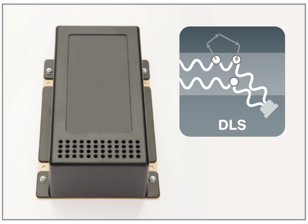


Eclipse Field Flow Fractionation

The Eclipse DualTec brings powerful sub-micron particle separation to your lab for macromolecules and nanoparticles. The Eclipse DualTec also supports, disposable hollow-fiber cartridges for sizing virus particles, liposomes, or protein aggregates. And if you'd like to go back to HP-SEC work, it's a only a switch away!

Calypso® CG-MALS

The Calypso composition-gradient system and software work in conjunction with a MALS detector to characterize the affinity and stoichiometry of macromolecular interactions without labeling or immobilization.



WyattQELS

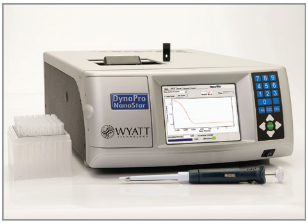
The WyattQELS embeds seamlessly inside the miniDAWN or the DAWN to provide you with on-line dynamic light scattering for determining radii down to 0.5 nm. It fits perfectly inside the instrument, so there's no wasted bench space.

SEC Protein Columns

Wyatt's silica-based columns are specifically designed for SEC-MALS protein applications. The columns provide exceptionally low light scattering noise, high shock resistance, and excellent lot-to-lot reproducibility, and are individually tested by Wyatt's QC lab to ensure light scattering performance.

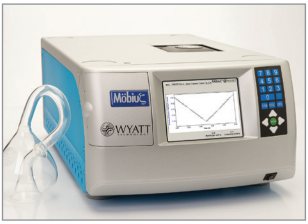
DynaPro Plate Reader

The remarkable Plate Reader for automated, high-throughput dynamic light scattering in 96 or 384 or 1536 well plates uses as little as 4 µL of sample per well. The Plate Reader comes with an on-board camera for capturing a high-resolution photograph of every well to reveal contamination, crystallization, and even bubbles.



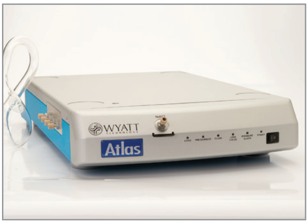
DynaPro NanoStar

The DynaPro NanoStar determines nanoparticle and protein size with sample volumes as small as 1 µL and a temperature range from -15°C - 150°C. A dedicated static light scattering channel measures absolute molar masses simultaneously with DLS.



Möbiuζ®

The Möbiuζ is the world's first and only light scattering instrument for reliable, non-destructive electrophoretic mobility measurements of proteins as well as nanoparticles via an autosampler, extending the measurable molecular size range of zeta potential down to 2.0 nm.

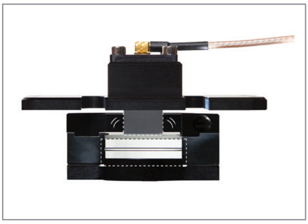


Atlas

Zeta potential measurements of proteins in high-salt buffers was always problematic due to electrolysis-induced bubble formation until the advent of the Atlas cell pressurization accessory for the Mobius.

NanoFilter Kit

The NanoFilter makes low sample volume filtration possible, with a hold up volume of less than 5 µL. Designed for ease of use, both in filtration and recovery of a sample.



COMET Cell Cleaning Device

The patented COMET (Cell Operation and Maintenance Enhancing Technology) integrates with the flow cell manifold of any Wyatt light scattering instrument. It applies ultrasonic fields specially tuned to the flow cell structure itself to shake loose particulates and keep the cell clean.

Orbit™ Recycling System

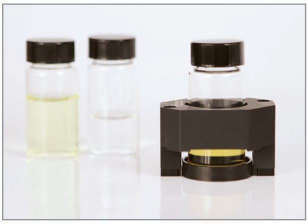
The Orbit Solvent Recycler integrates with Wyatt instruments to direct the eluent from your flowing system to a waste bottle or back to the solvent reservoir. This automated recycling functionality is critical for chromatography systems whose mobile phases are costly to purchase/prepare/dispose.

WISH

The WISH (Wyatt Injection System High-Pressure) manual injector for high-pressure HPLC pumps is designed specifically for use with Wyatt online detectors. It provides an autoinject signal to begin data acquisition upon injection.

MicroCUVETTE™

The MicroCUVETTE option is available with the DAWN HELEOS and miniDAWN TREOS instruments, allowing static and dynamic light scattering measurements to be made simultaneously in a quartz cuvette requiring only 10 µL of solution.



Flow-to-Batch Conversion Kit

The Flow-to-Batch Conversion Kit is like having two instruments in one. Within minutes the DAWN's flow cell can be removed from the read head and the vial adapter of the instrument installed. Inexpensive scintillation vials are perfect when sample volumes aren't an issue.

With installations in more than 65 countries, more than 11,000 refereed journal publications citing its instruments, and over 25 PhD scientists, Wyatt Technology is the world's leading manufacturer of instruments and software for absolute macro-molecular and nanoparticle characterization. Our dedication to providing customers with comprehensive training and personal support make us the gold standard in this field.

The Solution is Light™

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