The Ultimate Guide to Buying a Light Scattering Instrument for Absolute Macromolecular Characterization
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We encourage you to visit www.wyatt.com for the latest news and developments in light scattering instrumentation.

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Introduction

So, you’re thinking of buying a light scattering instrument?

More and more scientists realize that characterizing polymers and biopolymers (and particles, too!) with a multi-angle light scattering instrument is the only practical way to determine absolute molar masses, sizes, and conformations without making assumptions.

More than 40 years ago, Wyatt Technology Corporation’s (WTC) scientists invented the very first commercial light scattering instruments incorporating lasers as their light source. Since then, we’ve been defining and redefining the state-of-the-art in laser light scattering hardware, software, service, and support.

In this booklet we explain the key light scattering 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 multi-angle light scattering a routine part of your laboratory analysis.

What is Light Scattering?

Only a very small fraction of the light that enters our eyes does so directly from its source. Most of the light we see has been scattered in its passage from its source (e.g., the sun, a light bulb, a computer monitor, a laser,...) to our eyes. Witness the blue sky, the booklet you are now reading, or a smoke filled room. 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 from the differences and quantity of light scattered from them. Such deductions are not necessarily limited to objects we can see. For very small objects, such as microscopic particles and molecules, many of their physical properties may be deduced from the measurement of the light they scatter.

The quantitative measurement of light scattered from a solution usually begins by illuminating the sample with a fine beam of highly collimated and monochromatic light produced by a laser. The scattered light is then detected and measured as a function of the angle between the detector and the incident beam direction.

This measurement may be restricted to a single fixed angle, a low angle, a high angle, or any angle in between. On the other hand, one could make measurements over a range of angles. Some early examples of such light scattering instruments were scanning devices incorporating a photomultiplier tube (PMT) as a detector, a mercury arc lamp with filters, and a central stage upon which the sample could be positioned to make measurements at specific angles. The PMT subtended a small solid angle about the direction of scattering giving rise to the term differential light scattering.†

The first practical scanning device was built by Bruno Zimm (until his death, he was an active member of WTC’s Scientific Advisory Board) in the 1940’s, while the most successful commercial units were the American-made Brice-Phoenix and the French-built SOFICA in the 1950’s and 1960’s.

In 1971, Dr. Philip Wyatt and Dr. David Phillips introduced the first commercial laser-based device, the Differential I. The next year, Beckman Instruments introduced a laser-based instrument detecting light at a single, low angle. Over the years, the Beckman design became known by the acronym LALLS (Low Angle Laser Light

†More recently, the measurement has been called “classical” or “total intensity” LS to distinguish it from quasi-elastic light scattering or photon correlation spectroscopy (sometimes called “dynamic” LS).
Scattering); it was further refined and commercialized by Chromatix and later still by LDC/Milton-Roy.

In the late 1970’s, Wyatt recognized the need to make measurements *simultaneously* over a range of scattering angles. This first multi-angle development became known as the DLS 800. By 1984, after founding Wyatt Technology, Dr. Wyatt directed the development of the first *commercially* viable, simultaneous multi-angle instruments known by the trade name DAWN®. Scientists used to working with LALLS systems frequently refer to the DAWN by the acronym MALS (Multi-Angle Light Scattering).

Regardless of what they are called, however, these instruments have become indispensable tools of the polymer chemist and biochemist because they determine *directly* the molar mass and size of molecules in solution *without* depending upon reference-based calibration or physical assumptions about the sample.

**Two Types of Light Scattering: Static and Dynamic**

There are two general types of light scattering, which provide complementary information.

In *static* light scattering (also called classical, or total intensity scattering), the scattered light intensity 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 hundredths to several tenths of a second. Static light scattering measurements give information concerning the molar mass, molecular root mean square radius, conformation, and intermolecular interactions.

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 scales are measured. Those *fluctuations* are a measure of the diffusion constant (Brownian motion) of the molecules and are related to the hydrodynamic radius of a molecule.
The Molar Mass and Size From Static Light Scattering

Multi-angle laser light scattering instruments enable you to determine absolute molar masses of polymers and biopolymers from below 200 g/mol (daltons) to hundreds of millions of daltons. And rather than being based on assumptions about your samples (whether they’re rods, random coils, or spheres), multi-angle light scattering instruments measure the molar mass directly—no matter what the structure. Thus, a multi-angle instrument is an ideal additional HPLC detector to determine the Number, Weight, and Z-average molar mass of polymers or biopolymers. The chromatography equipment you already possess, plus a MALS detector, obviates the need for column calibration or reference standards.

The size of a molecule affects the angular variation of the scattered light intensity. If a light scattering instrument makes measurements at a single angle only, the angular dependence of scattered light cannot be determined. Only multi-angle light scattering instruments can be used to determine molecular sizes directly. Small, compact molecules (<10nm) have little—or no—angular variation in scattered light. For a random coil molecule above about 50,000 g/mol (which is really a “rule of thumb”; there is no exact cutoff, and there are many exceptions), an angular variation of the scattered light generally may be detected. Depending on the number of angles used, a multi-angle light scattering instrument can determine molecular root mean square (rms) radii from about 8–10 nm to larger than a micrometer! And, of course, multi-angle light scattering instruments never require a priori conformational assumptions. Since the mean square radius is determined from the slope of the angular variation, a minimum of three angles is needed.

Other benefits of multi-angle light scattering involve the abil-
ity to determine branching properties directly, since branching calculations depend on the measurement of the molecule’s mean square radius. Multi-angle light scattering instruments also can be used to detect protein aggregation that UV and RI detectors often miss completely or to determine the oligomeric state of conjugated proteins like 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 can determine the diffusion coefficients of molecules in solution. The diffusion coefficient for a spherical particle may be related directly to its radius. Thus, an equivalent radius (the so-called “hydrodynamic radius”) may be associated with the diffusion coefficient measured for any molecule. This equivalent radius, which assumes the molecule is a sphere, is called the molecule’s hydrodynamic radius. Dynamic Light Scattering (DLS) determines, therefore, the hydrodynamic radius of a molecule from 1 nm to larger than a micrometer. For more than 25 years, Wyatt Technology has been building static and dynamic light scattering instruments, giving scientists the power to utilize these two complementary techniques simultaneously. Wyatt Technology manufactures the WyattQELS™ as well as the venerable DynaPro™ line.

The WyattQELS and DynaPro instruments contain a single photon-counting avalanche photodiode, a multimode optical fiber that can be connected to the read head of the DAWN or mini-DAWN™, 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 dif-

Using the WyattQELS online, one can view a slice of the chromatograph and the corresponding $r_h$ value.
fusion coefficient and thence, the hydrodynamic radius.

The WyattQELS instrument may be used in batch or on-line mode. In the batch mode, unfractionated samples will produce complex correlation functions whose departure from pure exponential decay arises from the presence of heterodisperse components. The user can then select among a set of distribution assumptions on which basis the software will obtain a size distribution.

The DynaPro, too, may be used in a batch mode or can also be connected to a DAWN or miniDAWN. In addition, Wyatt offers the DynaPro Plate Reader for automatic DLS analyses in standard well plate formats.

In the flow mode (connected to a chromatographic separation), the instruments may be used to collect, simultaneously, the dynamic and classical light scattering data from which the molar mass, $rms$ radii (where possible), and hydrodynamic radius are calculated for each slice. The inset figure on page 7 illustrates how, at a given slice of the chromatograph, the ASTRA® software will provide the WyattQELS hydrodynamic radius value.

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

**How Static Light Scattering Instruments Work**

The light scattering photometers of today use lasers because they are an extremely reliable light source, 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 light scattering instrument use its laser source to determine an absolute molar mass and size? It’s actually quite straightforward:
a) The vertically polarized laser beam passes through the sample—either a flow-through cell or a static (batch) container.

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 from the data. Solving the following Eq. (1) is the heart of this analysis:

\[
\frac{K^*c}{R(\theta)} = \frac{1}{[M_W P(\theta)]} + 2A_2c
\]

The excess Rayleigh ratio, \( R(\theta) \) is the light scattered per unit solid angle by the solution at an angle \( \theta \) in excess of that scattered by the pure solvent, divided by the incident light intensity; \( c \) is the molecular concentration, usually in g/mL; \( M_W \) is the weight-average molar mass; \( A_2 \) is the second virial coefficient; \( K^* \) is a constant equal to \( \frac{4\pi^2n_0^2(dn/dc)^2/\lambda_0^4N_A}{\lambda_0^4} \); \( n_0 \) is the refractive index of the solvent, \( dn/dc \) is the specific refractive index increment of the dissolved molecules at the same wavelength of light as the light scattering measurement; \( N_A \) is Avogadro’s number; and \( \lambda_0 \) is the vacuum wavelength of the incident light. Finally, \( P(\theta) \) is the form factor, which depends on the structure of the scattering molecules and describes the scattered light’s angular dependence, from which the mean square radius \( <r_g^2> \) of the molecules may be determined.
Note that the left hand side of equation (1) is comprised of the fundamental quantities measured, at various angles and concentrations. From these measurements, the analytical software finds the best values of the three unknowns on the right hand side: $M_W$, $A_2$, and the mean square radius, $<r_g^2>$, which is extracted from the measured variation of $P(\theta)$. That’s all there is to it! The results are derived from fundamental measurements without any reference to so-called molar mass standards.

Light scattering instruments vary widely in their features and capabilities. At one extreme are those detecting scattered light only at a single low angle (LALLS). These instruments were developed in the early 1970’s based on the simplification of Eq. (1) or when $\sin^2 \theta/2 = 0$. At this value, $P(\theta) = 1$ and an immediate determination could be made of the molar mass in the limit as $c$ goes to zero. Indeed, the weight average molar mass is equal to the value of $R(\theta)/K^*c$ in the limit as $\theta$ and $c$ go to zero. If data were available at several concentrations, the second virial coefficient, $A_2$, could be determined as well. Since no angular variation of scattered light is measured in LALLS systems, no molecular size information can be derived.

Although the form of Eq. (1) becomes very simple in this low angle limit, the LALLS instrumentation becomes very complex because it requires an abundance of lenses, mirrors, and prisms. These add expense, complexity, and maintenance to the instrument. Furthermore, low angles are especially prone to the scattering effects caused by particulate matter, like dust in the solvent, or shedding columns, which often mask or distort the measurements of the solute itself.

Because the advent of LALLS devices came long before the ubiquity of low-cost personal computers, and since LALLS’ primary objective was to simplify data analysis, the LALLS instrument design never changed, and its operational and structural complexity was never rationalized. Moreover, the entire principle of LALLS became flawed as personal computers evolved.
Since low angles are always noisier than higher angles, and since personal computers can solve the equations more accurately using a plurality of angles—instead of just one—low angle measurements ceased to be emphasized.

Somehow, the belief that the molar mass is obtained entirely by extrapolation to zero scattering angle still persists. For many years, before the advent of personal computers and especially in view of Zimm’s classic method, this was true. Nevertheless, in the past few years, the determination of the molar mass from measurements made at many angles (MALS) and a plurality of concentrations has been achieved by making a global fit to all of the data collected. No data are discarded and, 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 any other means.

Since low angle data are notoriously noisy, they are often ignored, but this is no longer the case since global fitting uses all data (suitably weighted by their associated statistical uncertainties). Despite the dramatic analytical advancement of global fitting, there is still an active belief that the LALLS approach should be the method of choice. In order to use such an old (and long repudiated) technique, some people have resorted to 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 also removes much of the sample itself!

A few dual angle instruments have evolved, although these are merely derivatives of the multi-angle approach. Like the single angle devices, they require molar mass calibration standards for each solvent used. In addition, trying to determine the slope of a line through only two points (whose errors are not reported) is mathematically unsound, especially in the presence of noise. 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.
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 scatter destructively or constructively depending on the positions of the illuminated molecules. As the molecules undergo Brownian motion, their relative positions change with time. 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 defined as:

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

where \( I(t) \) is the intensity as a function of time, and \( \tau \) 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, \( \alpha \) is an instrument constant, \( D_T \) is the translational diffusion constant, \( q = \left( \frac{4 \pi n}{\lambda_0} \right) \sin \left( \frac{\theta}{2} \right) \) is the scattering vector, \( n \) is the index of refraction of the solvent, \( \lambda_0 \) is the wavelength of light in vacuum, and \( \theta \) is the angle of detection with respect to the incident beam direction.

By analyzing the correlation function, one can directly measure the diffusion constant of the molecule. Furthermore, if the molecule is assumed to be a uniform sphere, the Stokes-Einstein relation enables the molecule’s hydrodynamic radius to be determined:

\[ r_b = \frac{k_B T}{6\pi \eta D_T}, \]

where \( k_B \) is Boltzmann’s constant, \( T \) is the absolute temperature (in degrees Kelvin), and \( \eta \) is the solvent viscosity.
Questions to Ask Before Buying a Laser Light Scattering Instrument

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. Prices are important, but so are service, support, instrument design, and light scattering expertise. 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 practical light scattering instrument requires years of experience and technical qualifications. Intelligent circuit board design, state-of-the-art electronic components, superior knowl-
edge of photodetection equipment—not to mention expertise in optical engineering and software development—all play critical roles in building exceptional and versatile light scattering instruments.

The principals of Wyatt Technology commercialized the first classical laser light scattering instruments almost 45 years ago. It is 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 light scattering than we do. WTC employs dedicated hardware and software specialists and maintains the most comprehensive database of applications solutions in the field.

The company also holds its 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 frequent updates to the bibliography of papers that have been published, which reference the DAWN and DynaPro equipment. This searchable list now numbers more than 6000 references.

2. How many angles are measured?

The importance of the number of angles cannot be overemphasized and is critical since it determines the precision (reproducibility or absence of fluctuations) of the measurement, as well as the 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.

![Typical Chromatography Conditions](image)

<table>
<thead>
<tr>
<th>Angles</th>
<th>Molar Mass (%)</th>
<th>Radius (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15+</td>
<td>0.50</td>
<td>3.0</td>
</tr>
<tr>
<td>Low + 90° + High</td>
<td>1.00</td>
<td>5.0</td>
</tr>
<tr>
<td>Very Low + 90°</td>
<td>14.00</td>
<td>80.0</td>
</tr>
<tr>
<td>90° Only</td>
<td>8.00 → ?</td>
<td>N/A</td>
</tr>
</tbody>
</table>
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 angles.

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.

Wyatt Technology’s DAWN instruments contain far more angles than any other instruments on the market. This design ensures the 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 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 14 times greater precision than a single or dual angle instrument.

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 ideas in their product design.

Wyatt Technology builds all of their 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 represent one of the only absolute light scattering instruments available anywhere. But just what do we mean by “absolute”? We mean that our instruments measure 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).

Once calibrated, we can change solvents without the need for recalibration. Our absolute calibration has a tremendous practical advantage over pseudo-light scattering instruments. Such units require recalibration for every solvent and then “compare” the light scattering to the scattering from reference standards in each solvent used. These other instruments 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.

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 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 WTC stabilization technology providing our customers with unequalled 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 questioned rigorously by peer review.

Look carefully at the publications that have been generated using different light scattering equipment and in what disciplines. 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 6000. Each of these publications shows another DAWN or DynaPro system at work. From biotechnology to polymer production, manifold publications substantiate the value and importance of the DAWN and DynaPro instruments in the modern laboratory. WTC’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?
More and more, software is adding the power to light scattering to move it into new 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 WTC’s ASTRA software can do:
1. Provide exceptional analytical power, where the analysis of each value reported includes its statistically derived standard deviation. This is the only LC software in the world that provides such information.

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. Run on standard Windows platforms.

7. Provide 21 CFR Part 11 compliance for those who need it (or a non-compliant version for those who don’t).

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” in it, 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 con-
taining large areas of uncertainty, which they prefer their customers never saw.

Make sure that your light scattering instrument vendor doesn’t sell you “a bill of goods”; 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. And don’t forget the second virial coefficient $A_2$, which is critical for the study of protein crystallization and solubility.

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 using software that was “farmed out” to someone else. Wyatt Technology has more experience in software development for classical 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 classical light scattering. Whenever problems arise with your equipment, you deserve expert assistance. Building customer confidence requires good training in light scattering instrument theory and troubleshooting software and hardware operation.

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 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 years ago!

Wyatt Technology includes its acclaimed “Light Scattering University®” training course with every instrument we sell. We provide comprehensive training materials, lectures, hands-on experience, question-and-answer sessions, and exposure to the people who have popularized this technology. Our scientists, customer service engineers, and programmers interact with our customers frequently during “LSU”. For U.S.-based customers, we provide round-trip airfare from anywhere in the continental U.S. 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
The technology of light™

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 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 fluorescent 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 and WyattQELS (Quasi-Elastic Light Scattering) instruments can be interfaced with existing MALS instruments to measure on-line or off-line static and dynamic properties of macromolecules and particles.

Furthermore, Wyatt Technology is the only instrument vendor to offer a complete solution with the Optilab® T-rEX™ refractometer. We know how important $dn/dc$ is, and we build our Optilab at 514, 633, 658, 690nm or whatever wavelength your work requires! You can even change the wavelength yourself if your work requires it.

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 your organization 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.

The FDA has already received numerous submissions with DAWN, DynaPro, and miniDAWN data, so new applications are even more streamlined than the very first. 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 even have IQ-OQ services available for the pharmaceutical and biotechnology industries.

**13. Is your company 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. Now we have moved this internal culture of quality 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.

So whether you need IQ-OQ procedures or compliance with 21 CFR Part 11 or electrical safety certification compliance from TUV, the quality system at Wyatt Technology Corporation will help make sure that light scattering can be used for your application.

We hope that this booklet has been valuable to you in your search for a light scattering instrument. On the next page is a matrix of light scattering features and benefits. We have filled them in for our DAWN and miniDAWN products and invite you to use the blank column to compare other brands.
**Laser Light Scattering Features and Benefits Comparison Chart**

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

<table>
<thead>
<tr>
<th>FEATURES/BENEFITS</th>
<th>DAWN HELEOS®</th>
<th>MINIDAWN™</th>
<th>BRAND-X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simultaneous multi-angle detection</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Number of angles detected</td>
<td>18</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Light source (highly stabilized)</td>
<td>120mW semiconductor laser, 658 nm</td>
<td>60mW semiconductor laser, 658 nm</td>
<td></td>
</tr>
<tr>
<td>Molar mass range</td>
<td>~100 to &gt;10^7 *</td>
<td>~100 to &gt;10^6 *</td>
<td></td>
</tr>
<tr>
<td>Molecular rms radius range</td>
<td>10 to 500nm *</td>
<td>10 to 50nm *</td>
<td></td>
</tr>
<tr>
<td>Output signal</td>
<td>Digital, analog</td>
<td>Digital, analog</td>
<td></td>
</tr>
<tr>
<td>Inputs</td>
<td>4 analog auto-injector, alarm in, recycle in</td>
<td>4 analog auto-injector, alarm in, recycle in</td>
<td></td>
</tr>
<tr>
<td>Available laser options</td>
<td>658, 685, 785nm†</td>
<td>658 nm</td>
<td></td>
</tr>
<tr>
<td>Polarization analyzers (option)</td>
<td>YES</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Ultra High temperature (option)</td>
<td>Ambient to 210° C</td>
<td>[High Temperature miniDAWN]</td>
<td></td>
</tr>
<tr>
<td>Low temperature (option)</td>
<td>-15° C to +150° C</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Autocorrelator interfacing (option)</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Interference filters (option)</td>
<td>YES</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Infrared filters (option)</td>
<td>YES</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Need for molecular calibration standards</td>
<td>NO</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Model dependent results</td>
<td>NO</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Light scattering training included</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>PhD staff ready to answer technical questions</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Company dedicated to light scattering instrumentation</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Direct size determinations without assumptions or any additional equipment</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Sample cell easy to clean</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Software that calculates the uncertainty of each measurement</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Light scattering workshops</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Annual Users’ meetings</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
</tbody>
</table>

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

† Custom wavelengths available.
Ultimate Instruments & Accessories

The ultimate in a research-oriented light scattering instrument. Its 18 angles of detection give it the widest angular range of any on-line commercially-made light scattering detectors, and its numerous options enable it to be customized for practically any application you can imagine.

A triple-angle light scattering instrument incorporating many of the best selling features of the DAWN (including a high-temperature option), the miniDAWN TREOS can be used to determine absolute molar masses from just a few hundred to several million Daltons—without column calibration or reference standards.

The only RI detector available at any wavelength. The Optilab can be used for dedicated off-line \( dn/dc \) determinations as well as on-line for high-sensitivity refractive index detection. The instrument can be operated from \( 4^\circ\text{C} \) to \( 50^\circ\text{C} \) using its on-board temperature control functions. It can also determine the absolute refractive index of a solvent.

The Eclipse system brings a powerful sub-micron particle separation technology to your lab for separating macromolecules and nano-particles. The Eclipse matches the level of sophistication of current HPLC technology to give you hardware and software that are easy to use, whether you’re sizing virus particles, liposomes, or protein aggregates.
The WyattQELS interfaces seamlessly inside the miniDAWN TREOS or the DAWN HELEOS to provide you with the best of both worlds: simultaneous on-line masses and sizes down to 1 nm. It also stacks perfectly, so there’s no wasted bench space.

The Calypso combines multiple syringe pumps, degassers, mixers, and filters to measure the composition and concentration dependence of light scattering measurements as well as the analysis of self- and hetero-associations and the measurement of association constants.

The ViscoStar II represents a new generation of online differential viscometers that is designed with a traditional four-arm “bridge” arrangement. The uniqueness of the ViscoStar springs from its high accuracy and contemporary electronics. It achieves at least 2 times the signal-to-noise ratio of any commercial viscometer currently on the market, and it incorporates a high efficiency heat exchanger to provide extremely stable baselines.

The DynaPro line of instruments includes the remarkable DLS Plate Reader for automated high throughput DLS screening of 96 or 384 or 1536 well plates using as little as 1.5 µL of sample per well.
The NanoStar can be used for nanoparticle and protein size determination, with sample volumes down to 1 µL and a temperature range from 4-150°C. A dedicated Static Light Scattering detector has also been added to the NanoStar so that it can also measure absolute molar mass.

The Möbiuζ is the world’s first and only laser-based light scattering instrument for reliable, reproducible, and non-destructive electrophoretic mobility measurements of nanoparticles (including proteins and small macromolecules), extending the measurable molecular size range of zeta potential down to 1.0 nm.

Wyatt’s new silica-based columns, specifically designed for SEC-MALS protein applications, are made with well-controlled pore size and highly reproducible surface chemistry. The columns provide high resolution, exceptionally low light scattering baseline noise, better shock resistance, and excellent lot-to-lot reproducibility. Offered in a full range of pore size selections.

The Orbit Solvent Recycler integrates with Wyatt and certain third party 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 may be costly to purchase/prepare/dispose of, and whose column equilibrations could take hours.
The patented COMET (Cell Operation and Maintenance Enhancing Technology) may be integrated with the flow cell manifold of any Wyatt light scattering instrument. It applies radio frequency ultrasonic fields specially tuned to the flow cell structure itself to keep particulates to a minimum.

The MicroCUVETTE option is available with the DAWN HELEOS and mini-DAWN TREOS instruments and allows static and dynamic light scattering measurements to be made simultaneously in a quartz cuvette requiring only 10µL. Perfect for cases where only a small amount of sample is available.

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

The NanoFilter makes low sample volume filtration possible, with a dead volume (or hold up volume) of less than 5µL. Designed for ease of use, both in filtration and recovery of a sample. Filter membranes are used in a proprietary design that minimizes the dead volume.
With installations in more than 50 countries, Wyatt Technology is the world’s leading manufacturer of instruments for absolute macro-molecular characterization. It is the only company in the world focused exclusively on such systems, their design, and their application.

*Light Scattering for the Masses®*