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WHITE PAPER

WP9002: Fluorescent macromolecules and nanoparticles: characterization of molar mass, size and charge

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Summary

Fluorescent macromolecules and nanoparticles are found in many areas of biological, chemical and medical research and development, from natural polymers to engineered nanodiagnostic particles. Basic physical characterization of these materials is no less essential to carrying out R&D than for non-fluorescing materials, but it is definitely more challenging due to interference of the fluorescent emission with detectors designed to measure scattered light.

Instrumentation from Wyatt Technology™ offers multiple avenues to characterization of fluorescent samples. This article describes how Wyatt's SEC-MALS, DLS and PALS detectors overcome the challenges of fluorescence. Additional technologies that can deal with such samples are described, and several case studies presented.

Introduction

The amount of light scattered by a solution of nanometer-sized particles is typically on the order of 0.01-100 ppm relative to the incident light beam. Multi-angle light scattering (MALS), dynamic light scattering (DLS) and electrophoretic light scattering (ELS, a.k.a. phase analysis light scattering or PALS) require highly sensitive detectors in order to accurately characterize the molar mass, size and zeta potential of nanometer-sized entities such as polymers, protein and nanoparticles. While these instruments are engineered to eliminate interference from stray light scattered by the flow cell or cuvette walls, light emitted by the sample itself is generally detected as if it were scattered light.

Challenges of fluorescence in light scattering

Fluorescence is found in many types of macromolecules and nanoparticles, including:

- naturally occurring polymers such as lignin
- labeled proteins intended for research techniques such as ELISA, cellular super-resolution imaging, small-molecule binding screens or FRET studies
- tagged nanoparticles used for medical diagnostics, targeted cancer tissue imaging or environmental monitoring and remediation.

Each fluorescent material is characterized by a spectral range for fluorescent excitation between $\lambda_{exc,min}$ and $\lambda_{exc,max}$, and a range for fluorescent emission between $\lambda_{emiss,min}$ and $\lambda_{emiss,max}$. An example is presented in Figure 1. Depending on the position of λ_{exc} relative to the emission band, the actual emission spectrum may begin at λ_{exc} or at $\lambda_{emiss,min}$. The gap between the excitation wavelength λ_{exc} and the shortest emission wavelength is known as the Stokes shift Several key techniques for basic characterization of polymers, proteins and nanoparticles make use of analytical light scattering. For example, SEC-MALS combines separation by size-exclusion chromatography with absolute molar mass and size

analysis by MALS, to determine distributions of molecular weight, rms radius and conformation. DLS is used regularly to determine the hydrodynamic radius of proteins and nanoparticles, and PALS determines zeta potential which is closely related to charge and colloidal stability.

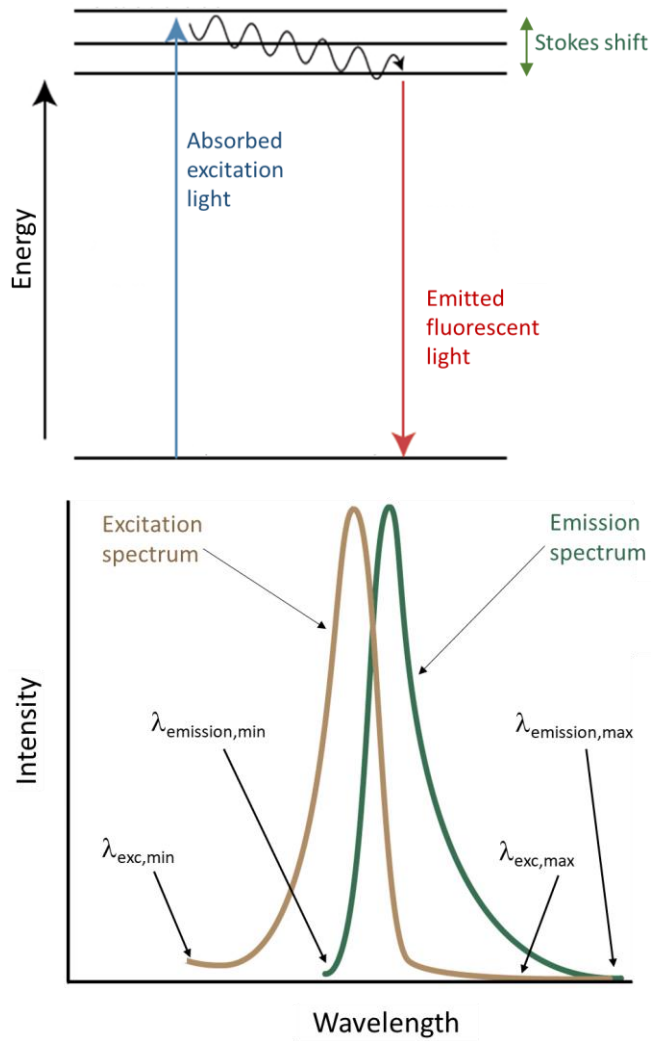

Figure 1. **Top**: Absorption takes place at higher energy (shorter wavelength) than fluorescent emission at lower energy (longer wavelength). **Bottom**: Illustration of a fluorescent molecules' excitation and emission spectra.

In light scattering measurements, an incident laser beam impinges on the sample with a specific wavelength, λ_{laser}. If λ_{laser} is within the fluorescent sample's excitation band, light will be absorbed and re-emitted as fluorescence; then $\lambda_{exc} = \lambda_{laser}$. If the detector is overwhelmed by light arising from fluorescence, or even if the signal amplitude is just increased arbitrarily by fluorescence relative to the purely scattered intensity without fully saturating the detector, the analysis will be disrupted or corrupted.

Specifically, MALS analysis is corrupted when the total measured intensity is skewed by detection of fluorescing photons, while DLS avalanche photodiodes (APDs) are saturated by fluorescence which prevents them from detecting individual photons, as is needed for DLS autocorrelation analysis.

The avenues to overcoming fluorescence and performing reliable characterization are:

1. Prevent the excitation of fluorescence
2. Prevent fluorescent radiation from reaching the detector
3. Utilize a light-scattering technology that is not adversely impacted by fluorescence
4. Utilize a technique that does not rely on light scattering

Each strategy has its pros and cons. Wyatt employs all four, in various configurations, to enable optimal characterization of fluorescent samples.

Wavelength Options

Since fluorescence only occurs when λ_{laser} is within the sample's excitation band, fluorescence can be avoided altogether if an appropriate laser wavelength is chosen.

1. MALS

Wyatt MALS detectors, including the DAWN™, miniDAWN™ and microDAWN™, are equipped with 660 nm lasers, with variability of a few nanometers between individual instruments. The 660 nm wavelength is well below the excitation band for the vast majority of macromolecules and nanoparticles. For these instruments, fluorescence is limited to a relatively small class of naturally fluorescent polymers, such as lignin, and a small number of the commonly used protein tags such as Cyanine 5 (see Figure 2) or Alexa 647.

In some of these cases a shorter wavelength will fall outside the excitation band, but for the most part it is preferable to go to a longer wavelength. Long-wavelength photons have lower energy than short-wavelength photons and therefore the probability of excitation is much lower at longer wavelengths.

The DAWN may be customized with a 785 nm laser, replacing the 660 nm laser; this replacement must be

performed at the factory. The longer wavelength is well below the excitation band of almost all fluorescent dyes used for protein labelling and FRET. Hence even if a dye molecule emits under 660 nm excitation, it is very unlikely that it will emit under 785 nm excitation. In fact, use of a 785-nm laser prevents fluorescence entirely for the vast majority of such molecules. When the 785-nm laser option is selected, an Optilab™ dRI concentration detector with compatible LED wavelength is paired to the DAWN®.

Because they are derived from diverse natural sources and may be processed in many different ways, the types and amounts of fluorophores in lignin samples provided for analysis may vary widely. Their excitation bands may exclude entirely 785 nm, or this wavelength may be in the very tail of the band. In any case, use of a 785 nm laser greatly suppresses fluorescence from lignin and similar polymers, and so is usually preferred for these applications. Additional suppression is provided by filters, explained below.

Benefits: Fluorescence suppression or elimination, enabling characterization of fluorescing samples.

Disadvantages: The 785 nm laser option adds cost to the system. Perhaps more importantly, it reduces the signal-to-noise ratio (SNR) for non-fluorescing samples: the 785 nm DAWN has about 1/3 the MALS sensitivity of the standard DAWN for non-fluorescing samples.

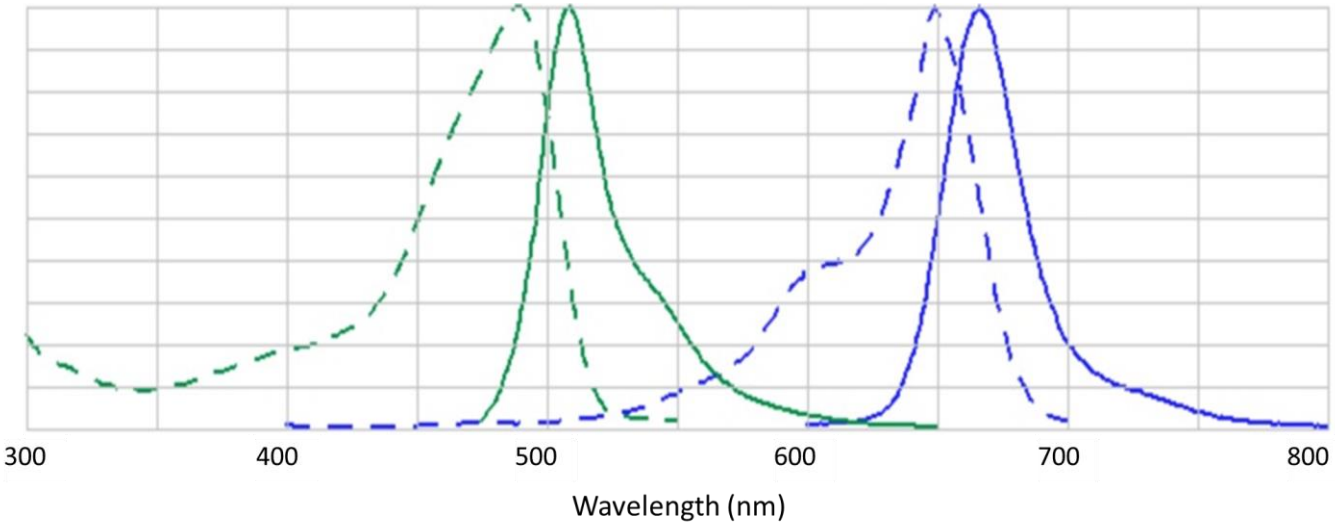

Figure 2. Excitation (dashed lines) and emission (unbroken lines) spectra of EGFP (green) and Cy5 (blue). EGFP is not excited by radiation of wavelength longer than about 550 nm, while Cy5 is not excited by wavelengths beyond 700 nm. 532-nm excitation of Cy5 would exhibit low fluorescent intensity and a large Stokes shift while 660-nm excitation would exhibit high fluorescent intensity and a negligible Stokes shift

2. DLS

The DynaPro™ NanoStar™ cuvette-based DLS instrument incorporates a 660 nm laser, while the DynaPro ZetaStar™ has a 785 nm laser. The pros and cons of these wavelengths for fluorescent samples is described above in the context of MALS.

Benefits: Fluorescence suppression or elimination, enabling characterization of fluorescing samples.

Disadvantages: The reduction in sensitivity at 785 nm for non-fluorescing samples is similar to MALS.

Further reduction in fluorescence for DLS measurements, if not complete prevention, is achieved by transitioning to the DynaPro Plate Reader, which uses an 830-nm laser. Very few fluorescent labels or polymers will emit under excitation by this near-infrared wavelength.

Benefits: Fluorescence elimination with no loss of sensitivity relative to non-fluorescing samples, plus all the benefits of automated DLS.

Disadvantages: The DynaPro Plate Reader offers about 25% less DLS sensitivity than the NanoStar for non-fluorescing samples. Since it uses microwell plates, it is generally not compatible with volatile organic solvents that may degrade the plate, corrode the instrument or

cause a spark. Some organic solvents may be measured using a special quartz plate, if the sealing tape is compatible with the solvent. For customers who do not need automation, the plate reader is more expensive than the cuvette-based DLS.

Fluorescence-blocking filters for MALS

Even if fluorescence is emitted, it is always subject to a Stokes shift, i.e. it occurs at a longer wavelength than the scattered light. Wyatt's fluorescence-blocking filters prevent all or most of the fluorescing photons from reaching the detector while allowing most of the scattered photons to pass. In many cases, this is sufficient to allow good molar mass and/or size measurements.

Fluorescence-blocking filters may be placed in any and all of the DAWN's angular positions. We usually recommend installing filters on eight or nine of the DAWN's 18 detector angles, to be placed in all the even-numbered positions, leaving the odd-numbered positions open. This interleaving of open and filtered angles allows for reliable measurements of non-fluorescing samples using half of the detector angles, and measurement of fluorescing samples using the other half which are calibrated separately.

The standard MALS fluorescence-blocking filters have a relatively wide passband of 20 nm. This means that photons emitted at wavelengths that differ by less than 10 nm from the central laser wavelength are not blocked, and reach the photodiodes. When the Stokes shift is large, i.e. the displacement from the λ_{exc} is large, then all the fluorescent photons are outside the passband and are effectively blocked. In order to fully block fluorescence with a smaller Stokes shift, a narrower passband is required. Wyatt can help you select the optimal filters.

Benefits: Fluorescence suppression or elimination, enabling characterization of fluorescing samples with little impact on analysis of non-fluorescing samples.

Disadvantages: If the Stokes shift is small then some fraction of the fluorescence is transmitted and corrupts the measured intensity. While Wyatt can help select narrow passband filters, it will not provide these filters.

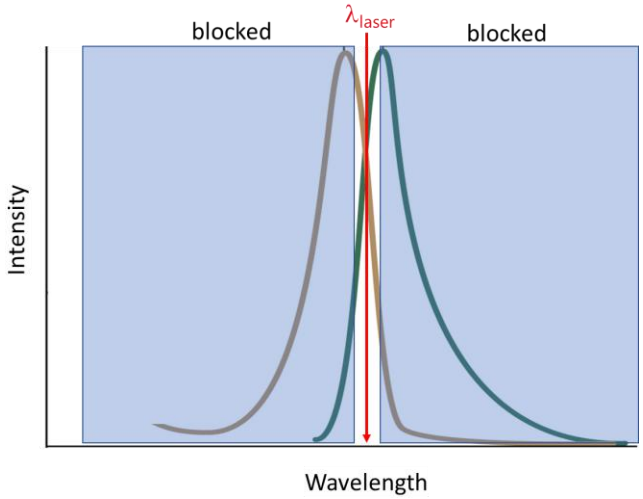

Figure 3. Fluorescence-blocking filters transmit only a narrow band of wavelengths centered on the laser wavelength λlaser.

Light scattering technologies not impacted by fluorescence

The phase of fluorescing photons is entirely uncorrelated with that of the laser used to excite their emission. When the light scattering technique relies on coherence, fluorescing photons cannot affect the results as long as the detector is not saturated and can respond.

Electrophoretic light scattering (ELS) is utilized in the ZetaStar to determine the zeta potential of particles. In Wyatt's unique fiber-interferometric Doppler electrophoretic light scattering (FIDELIS) technology, a portion of the beam is split off before the flow cell, guided by a fiber, phase shifted by tandem acousto-optic modulators and interfered with the scattered light at the detector array. The detectors are AC-coupled and hence only sensitive to the difference frequency between the scattered light and the initial beam. Since fluorescing photons have lost coherence to the incident beam, there is no interference, no difference frequency and the detector ignores them completely.

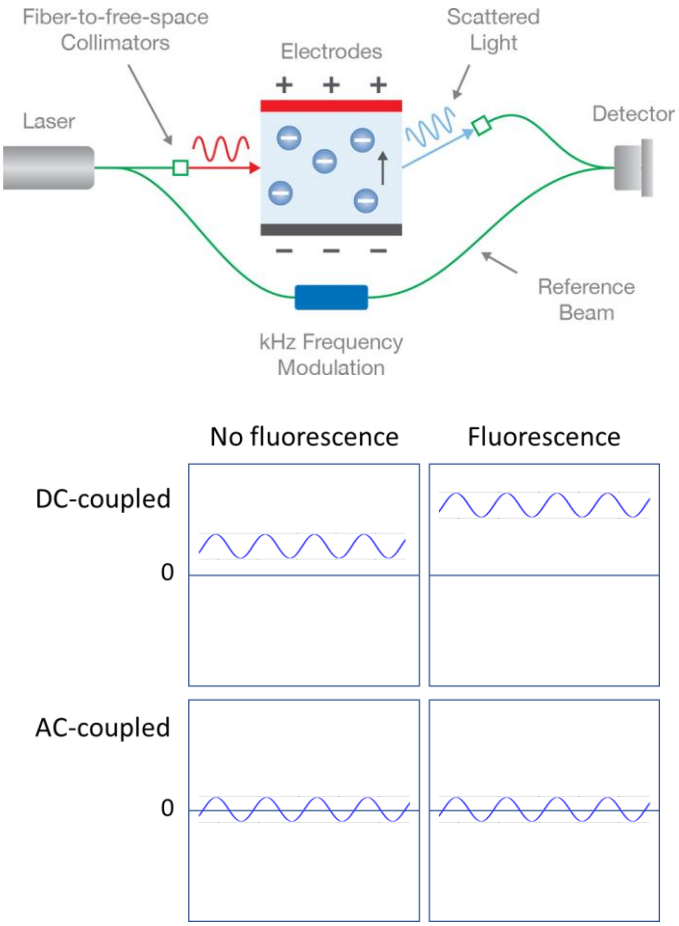

Figure 4. **Top**: FIDELIS technology implemented in the ZetaStar. **Bottom**: While fluorescence increases the overall DC signal in the MP-PALS detectors, they do not saturate and hence there is no effect on the AC-coupled signals.

Moreover, FIDELIS technology utilizes a high-dynamic-range detector array which does not saturate even when subjected to significant fluorescence intensity. Hence, unlike PALS instruments that rely on APDs or photomultipliers, zeta potential analysis in the ZetaStar is inherently unaffected by fluorescence.

Benefits: Measurements of zeta potential regardless of fluorescence, with all the benefits of high-sensitivity FIDELIS.

Disadvantages: The forward-scattering geometry of FIDELIS does not support zeta potential analysis of turbid samples.

Technologies that do not rely on light scattering

Wyatt offers two additional technologies for characterizing molar mass and size that do not rely on light scattering: differential viscometry and flow field-flow fractionation.

1. Viscometry

Differential viscometers such as the ViscoStar™ are highly sensitive to the small changes in solution viscosity arising from dissolved macromolecules. They are typically used with size-exclusion chromatography to characterize the distribution of intrinsic viscosity [η] of polymers (SEC-IV). SEC-IV can be applied to determination of molar mass in two ways:

- Universal Calibration, which relates elution volume from a column plus the measured intrinsic viscosity to molar mass, assuming that the polymer is a random coil
- Mark-Houwink-Sakurada (MHS) analysis, which relates molar mass to intrinsic viscosity via an empirical relationship $[\eta]=KM^{\alpha}$. The MHS parameters are taken from literature, characterization by more tedious methods, etc.

Examples of successful analysis of fluorescent particles by light scattering

Not every fluorescent sample can be analyzed correctly by light scattering or even by other, non-light scattering technologies. However, with careful optimization and validation of the instrumentation, most can be characterized successfully. Following are two examples.

Case study #1: Cy5-conjugated nanoparticles

Dynamic light scattering analyzes single-photon fluctuations over microseconds time scales. Hence, DLS instruments usually incorporate high-gain, high-speed photodetectors such as APDs or photomultiplier tubes. When exposed to moderately high light levels the DLS detector is saturated and can no longer track these rapid fluctuations.

Figure 5 compares the autocorrelation function (ACF) plots of three conjugated nanoparticles measured in a standard NanoStar vs. a ZetaStar. PGA-selumatinib does not fluoresce and exhibits a typical ACF with roll-off at an autocorrelation time corresponding to its diffusion coefficient. PGA-Cy5 and HPMA-Cy5, on the other hand, fluoresce strongly under 660 nm excitation and their ACFs are flat, as is typical for a saturated APD. Hence standard DLS cannot characterize the size of the Cy5-conjugated nanoparticles.

Solution A: ZetaStar

ACFs acquired in the ZetaStar for the three conjugated nanoparticles are presented in Figure 5. The Cy5-conjugated particles exhibit high-quality autocorrelations with typical amplitudes of ~ 0.3 and solid roll-offs. For selumatinib-conjugated PGA particles, the ZetaStar analysis highlights the presence of an additional species at short correlation times.

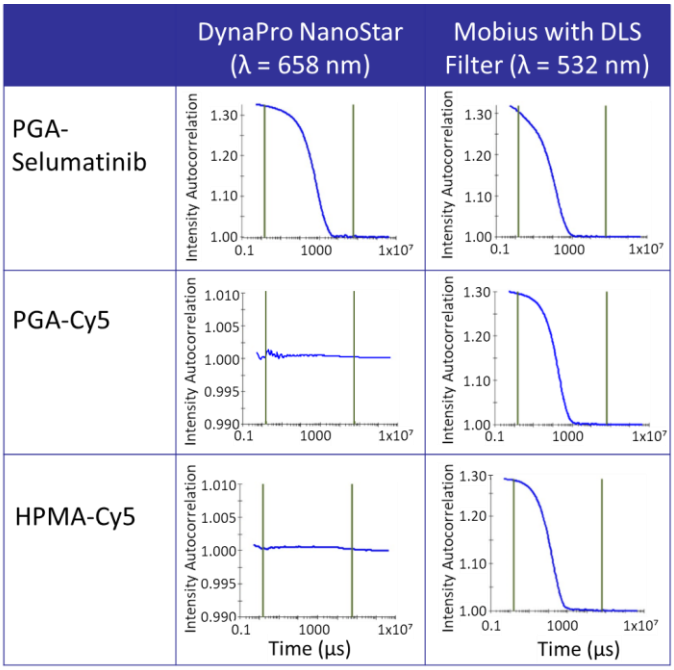

Figure 5. Comparison of autocorrelation functions from non-fluorescing nanoparticles (PGA-selumatinib) and fluorescent nanoparticles (PGA-Cy5, HPMA-Cy5) under 660-nm and 785-nm excitation.

Size distributions calculated by Regularization (NNLS) fitting of the ACFs are presented in Figure 6. All three particles appear in the size range r_h = 80-120 nm, with differing amounts of monomer in the range r_h = 3-5 nm.

The advantages of moving to a longer wavelength may not apply to all fluorescent particles. Still, the ZetaStar presents a viable solution for DLS analysis of the majority of dye-conjugated nanoparticles that are difficult to measure at 660 nm.

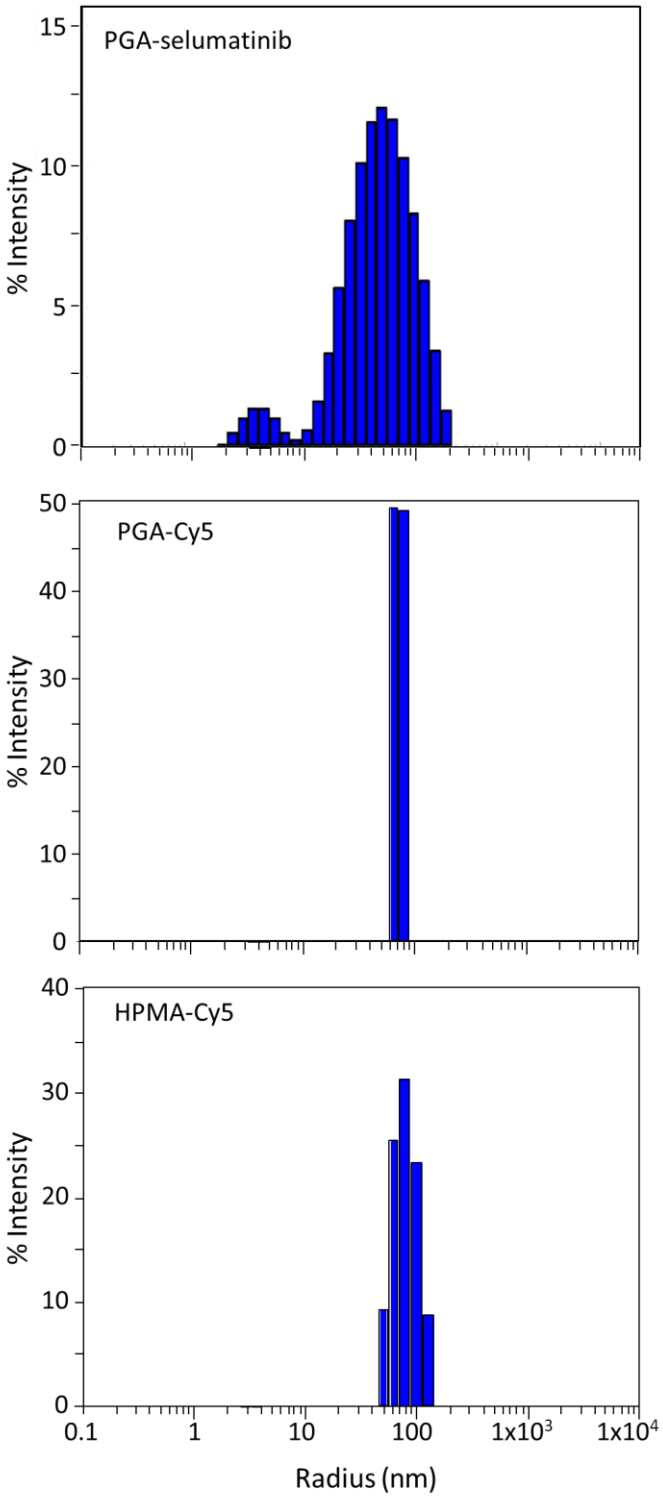

Figure 6. Size distributions obtained using the ZetaStar for three nanoparticles, two of which fluoresce.

Solution B: DynaPro Plate Reader

While the primary benefit of the DynaPro Plate Reader is its automation and plate-based workflow, it also provides advantages in the analysis of fluorescent samples due to its long-wavelength laser operating at 830 nm. Very few materials fluoresce under 830 nm excitation, and in particular Cy5 and similar dyes.

Figure 7 presents the ACFs and corresponding size distributions obtained for Cy5-conjugated HPMA and PGA particles. These samples were prepared differently than in the previous study and so the size distributions are not identical, but the challenges of DLS measurement at 660 nm remain.

ACFs obtained for these samples with the DynaPro Plate Reader are robust and clearly do not suffer from saturation effects. Certainly, this is not surprising, since the excitation spectrum for Cy5 does not extend beyond 700 nm; but our experimental confirmation does provide confidence that reliable size distribution analysis of such samples is essentially guaranteed by using the longer wavelength. The DynaPro Plate Reader can be expected to perform as well for just about all fluorescent nanoparticles within its range of measurement (R_h = 0.5 – 1000 nm).

Case study #2: lignin

Multi-angle light scattering determines molecular weight by measuring light scattering average intensity and concentration. When fluorescence arbitrarily increases the intensity at the MALS detector, the apparent molecular weight increases. For some materials the apparent molecular weight may increase several-fold. In addition, since fluorescent intensity is usually proportional to concentration while scattering intensity is proportional to the product of molecular weight and concentration, the relative effect of fluorescence is not uniform across the entire molecular weight distribution—it increases with decreasing molecular weight.

Successful suppression and elimination of fluorescence can be an iterative process. Since the correct molecular weights are usually not known *a priori*, how can we determine that we have achieved our goal? The answer is to apply successively more stringent suppression until there is little-to-no change in apparent molecular weight.

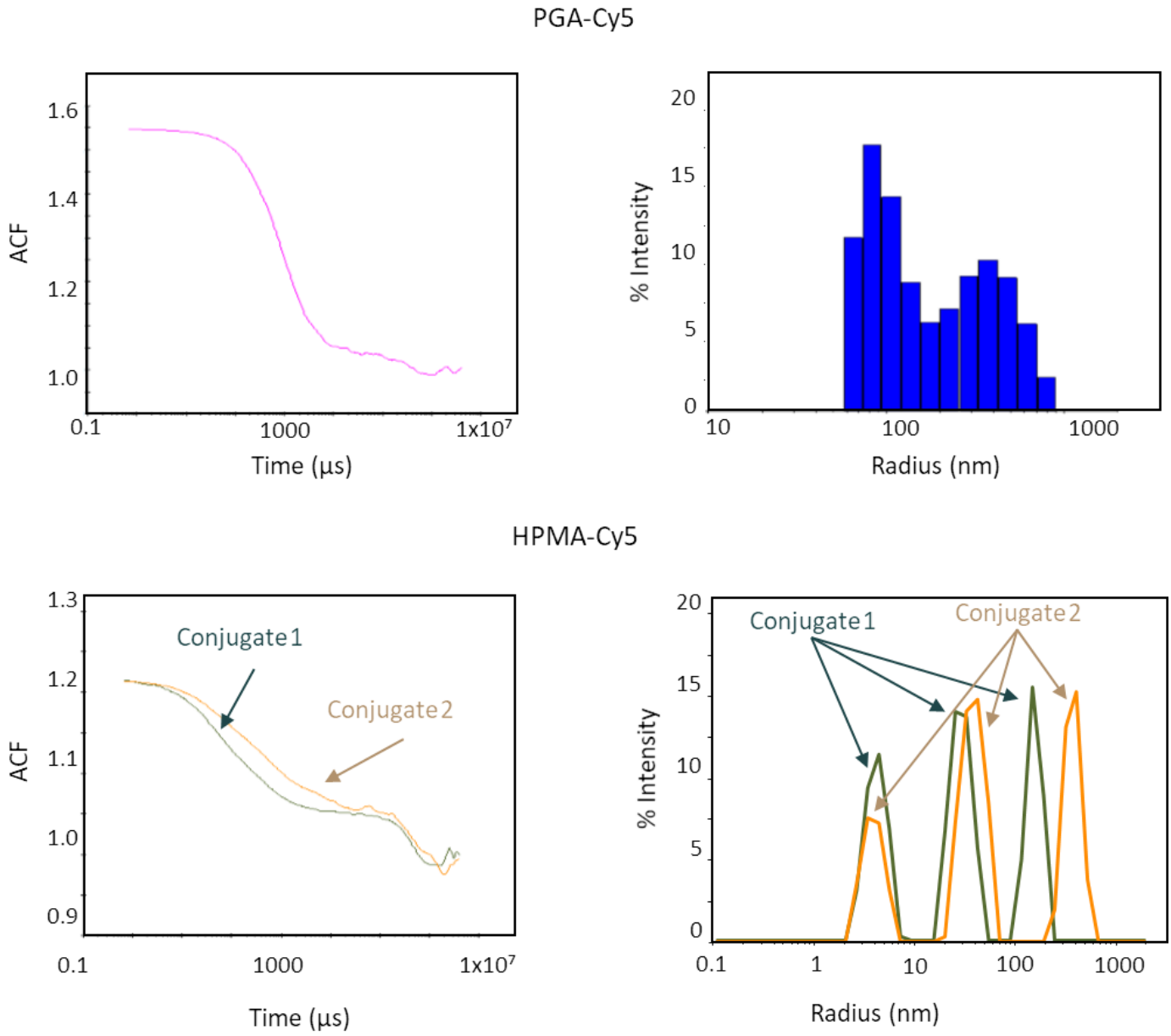

Figure 7. ACFs and size distributions for fluorescently-conjugated nanoparticles using the 830-nm DynaPro Plate Reader.

Comparison of 660 nm & 785 nm MALS, w/wo filters

For reasons described earlier, the 660 nm MALS system is more sensitive than the 785 nm system. If the 660 nm DAWN can be fitted with fluorescent blocking filters that sufficiently suppress the fluorescence signal then it is preferable over the 785 nm system, especially if other, non-fluorescing samples are to be measured.

Table 1 shows the outcome of a batch MALS study of two lignin samples. Batch MALS does not separate the sample on a GPC column, and therefore it determines the apparent weight-averaged molecular weight. Each of the samples was measured under a 2x2 matrix of experimental conditions: laser wavelength 665 nm or 786 nm, and with or without standard 20 nm passband filters. As we recall, the filters are installed in half of the angular positions so it is straightforward to make separate measurements, with and without the filters.

1. *Sample 1: Full evidence for fluorescence suppression*

For Sample 1, the threefold difference in apparent molecular weight at 665 nm with and without filters (89 kDa and 30 kDa, respectively) informs us that there is

significant fluorescence and the filters are absolutely necessary at 660 nm. However, it is insufficient to determine if the 20 nm filters are sufficient to obtain the correct molecular weight. At 786 nm without filters, the apparent MW is 30.7 kDa, just slightly more than the filtered 665-nm result, which means that the transition to 786 nm greatly suppressed the fluorescence.

At 786 nm with filters, the apparent MW of 27.2 kDa is only slightly less than without filters, indicating that in all likelihood they have succeeded in eliminating most of the remaining fluorescence and constitute a reliable configuration for characterizing this sample. However, the results are not very different from those obtained with the 665 nm system with filters, so a standard MALS detector configured with filters could be acceptable for this sample.

Laser (nm)	Interference Filters (20 nm)	Sample 1 App. MW (kDa)	Sample 2 App. MW (kDa)
665	No	89 ± 6	176 ± 6
665	Yes	30 ± 5	34.4 ± 5
786	No	30.7 ± 0.3	24.4 ± 0.1
786	Yes	27.2 ± 0.3	14.2 ± 0.1

Table 1. Apparent weight-averaged molar masses obtained for two lignins in batch mode, using two different laser wavelengths (665 and 786 nm) and two filter configurations (without filters, and with 20-nm passband filters).

2. *Sample 2: Incomplete evidence for fluorescence suppression*

For Sample 2 under 665 nm illumination, the difference in apparent weight-averaged molar mass with and without filters is even greater: five-fold (176 kDa and 34.4 kDa, respectively). Transition to the 786-nm laser without filters provides clear evidence that 665 nm + filters is insufficient for this sample: the apparent molecular weight is even lower at 24.4 nm, indicating the filters transmitted a fair amount of fluorescent intensity in the 665 nm system.

With filters added to the 786-nm system a further reduction in apparent molecular weight is evident, to 14.2 kDa. This difference is much smaller than was found under 665 nm illumination, proof that the longer wavelength is critical to suppressing fluorescence, but it is not clear if the filters have succeeded in eliminating it completely. For that we need to try narrower filter passbands.

Comparison of different filter bandwidths

In this example, the even detector angles were fitted with a succession of filters with varying bandwidths, as shown in Figure 8. Molar mass distributions were measured by SEC-MALS and analyzed for each filter bandwidth, as plotted in Figure 9.

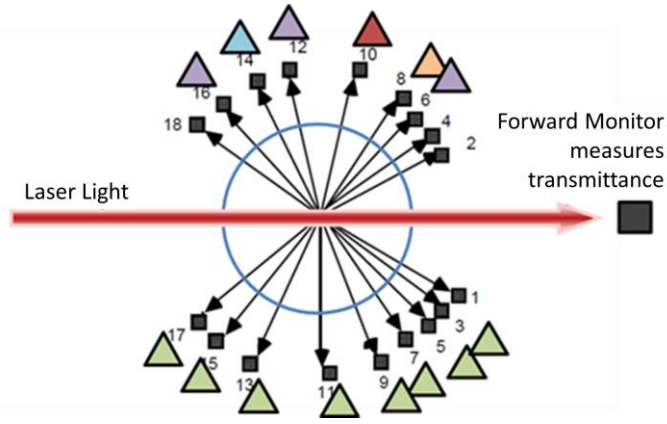

Figure 8. Positioning of fluorescence-blocking filters of different bandwidths in order to determine the optimal passband for the sample. Green – no filter; violet – 20 nm; blue – 10 nm; red – 6 nm; yellow – 4 nm. Numbers indicate the angular position in the DAWN® detector numbering sequence.

The MW distribution obtained with the 20-nm filters (cyan) exhibits an unlikely behavior: inconsistent and non-logarithmic variation of molecular weight with elution time. While this behavior is not impossible, it is suspicious. Application of a narrower, 6-nm filter leads to a large reduction in apparent MW (magenta) and retrieval of the expected, approximately logarithmic, molecular-weight/elution time behavior, indicating that the 20-nm results are still subject to large error due to fluorescence.

A yet narrower, 4-nm filter yields almost the same result as the 6-nm filter (blue), but the molecular weight plot is noisy, a result of greatly decreased SNR with the 4-nm filter which blocks a large fraction of the scattered light as well as the fluoresced photons. Hence, for this sample a 6-nm filter in conjunction with the long-wavelength laser is necessary and sufficient to obtain reliable molecular weight results, though they may still be slightly in error due to transmitting residual fluorescence.

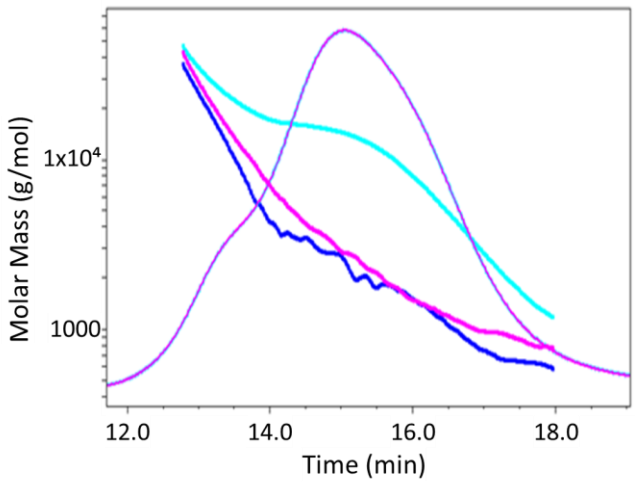

Figure 9. Apparent molar mass vs. elution time for a lignin sample, in a 786-nm DAWN MALS detector, using 20-nm (cyan), 6-nm (magenta) and 4-nm (blue) filters.

A word about transmittance

Fluorescent samples, of necessity, also absorb some of the incident laser light before it reaches the detection volume at the center of the flow cell. DLS detectors do not care about overall average intensity, just the rapid fluctuations due to Brownian motion, and are not overly affected by absorption unless it is very strong, causing heating and convection. On the other hand, MALS measures average scattered intensity and does care much about absorption.

When the sample absorbs, standard MALS analysis will misrepresent the amount of light that is scattered, and the apparent molar mass will be too low. While the effects of fluorescence (apparent molar mass too high) and absorption (apparent molar mass too low) can partially offset each other, to different degrees depending on sample, molecular weight fraction, etc., it is not good practice to rely on this 'bug' and turn it into a 'feature'… Wyatt MALS detectors all incorporate a Forward Monitor (FM) detector to measure the transmitted beam intensity. When significant absorption occurs, ASTRA can be set up to use this FM signal, shown in Figure 10, in order to correct for the change in intensity reaching the detection volume. The absorption is calculated at every point in the chromatogram and the apparent molar mass adjusted to take the absorption into account. The combination of FM detection, appropriate laser wavelength and fluorescence-blocking filters, ensures accurate measurements of these problematic samples.

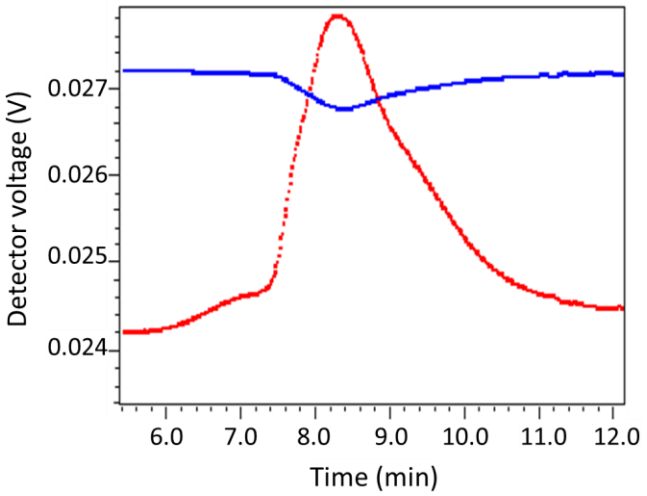

Figure 10. Forward Monitor signal (blue) exhibits a dip due to absorption by the sample, coinciding with peak scattering (red).

The FM correction is important for all kinds of samples that absorb at the laser wavelength, even if they do not fluoresce. For example, heme-containing proteins absorb the red laser beam, and without this feature, the measured molar mass would be too low by a significant degree. Hence the additional transmittance detector is essential for characterizing many types of macromolecules.

Lignin: an example from the literature

A good example of the application of narrow-passband filters to lignin characterization is described in Contreras et al., Propensity of Lignin to Associate: Light Scattering Photometry Study with Native Lignins. *Biomacromolecules* **9**(12), 3362-3369 (2008). In this study, 1-nm bandwidth filters were preferred over 10-nm filters in order to fully block fluorescence when used with a 633-nm laser. The narrow filters were feasible due to the high coherence, narrow bandwidth and reliable center wavelength of the HeNe laser used in the DAWN DSP, but are not applicable to later models that use diode lasers. Long-wavelength lasers with wider filters are a suitable solution for lignin.

Is fluorescence giving you bad data?

There are several behaviors in MALS and DLS that can indicate the presence of fluorescence and its adverse effects. It is important to note, though, that there may be other valid explanations for each of these behaviors, so if you are not sure, check with Wyatt Support at support@wyatt.com.

MALS clues

The two primary symptoms will be:

1. Absorption, identified as a dip in the FM signal that coincides with the peak, per Figure 10; be sure to check the value of the minimum FM signal (Shift + move cursor over the graph in ASTRA) to confirm that it is of any significance. *Absorption is not always accompanied by fluorescence.*
2. Abnormally high molar mass calculated by light scattering, relative to the elution position in the chromatogram. *Late elution can also be caused by branched or otherwise compact molecules compared to the column calibration standards, by anchoring or by sample sticking to the resin.*

DLS clues

The two primary symptoms will be:

1. Flat ACF, looks like pure buffer or very small particles. *Flat ACF can also be caused by complete precipitation or by turbid solutions.*
2. High count rate, in the tens of millions. *Anomalously high count rate can also be caused by turbid solutions or heavy precipitation.*

If these symptoms occur it might be worth checking your sample in a spectrofluorometer to determine the excitation and emission spectra.

Conclusions

Despite the challenges of performing light scattering measurements on fluorescent samples, there are solutions to characterizing these materials to determine absolute molar mass and size. When light scattering just will not do, other technologies are available to make these measurements albeit the analyses are no longer absolute and model-independent. Wyatt Technology offers adaptable instrumentation, and its application scientists stand ready to perform the tests needed to recommend an optimal configuration.

Detailed information on the use of fluorescence-blocking filters in MALS is available in Technical Note *TN3003 – Measuring Molar Mass for Fluorescing Samples*.

Detailed information on ASTRA's feature for absorbing samples is available in Technical Note *TN1010 - Correcting for Absorbance at the Laser Wavelength.* To learn more about these instruments and their uses for polymer, protein and nanoparticle characterization, contact info@wyatt.com.

Acknowledgements

The conjugated nanoparticle samples were kindly provided by the lab of Prof. Ronit Satchi-Fainero at the Tel-Aviv University Faculty of Medicine.

Click the button below to request information on Wyatt's instruments used in fluorescent macromolecule and nanoparticle analysis.

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