



Live Webinar Q&A Sheet:

An Introduction to the Next Generation in Light Scattering

The recorded webinar may be viewed from the [SEC-MALS](#) webinars page. These questions were submitted by live viewers. Additional information on FFF-MALS, DLS, CG-MALS, and ELS may be found on the Wyatt web [Library](#) under Webinars, Application Notes, Featured Publications and Bibliography, as well as on the corresponding [Product page](#) and [Theory](#) page of our web site.

Please contact info@wyatt.com with any additional questions.

On the new design

Q: Can you please comment on the enhancements made to the performance of the instruments? Specifically interested with the improvements made to data obtained from the microDAWN.

What are the overall benefits of the newer models of the miniDAWN and Optilab?

A: The performance specifications have not been changed for the new instruments relative to the last model of each one. The enhancements of the Neon models have focused on user experience, user guidance and serviceability. Please see www.wyatt.com/Products/MALS for an overview of MALS performance specifications and www.wyatt.com/microDAWN for microDAWN performance specifications. See www.wyatt.com/NextGen (as well as the webinar itself) for details of the enhancements.

Q: Can you access the instrument control panels remotely?

A: Yes, using VNC software.

Q: Is there no more computer on the new set-up?

A: Use of ASTRA on a host computer is required, just like in previous models, in order to control the instruments, acquire and analyze data, and report results.



Q: Is there a key pad option for the new generation of detectors? Will the touch screen be robust enough?

Are all controls done via the touch screen only or via ASTRA as well?

A: There is no key pad, and no need for one! The new touch screen is robust and sensitive, similar to a modern tablet. Our application scientists have confirmed that it can be navigated quite easily even with gloved fingers.

The majority of controls, settings and history are accessed via the touch screen. ASTRA can, of course, control the instruments as needed for the method: it sets temperatures and collection intervals, turns the laser and COMET on and off, sets the Orbit to recycle or waste, etc.

Q: Do you have an estimated life of the laser (perhaps in hours of use)? If the laser were to reach its end of life, how could that be solved? Would the instrument need to be sent in?

A: The laser is rated for 10,000 hours. Since it can be automatically turned off after your experiments have completed, and in any case automatically goes into laser-saving “idle mode” when data acquisition has not been in effect for some time, the actual lifetime is practically many years. If the laser does fail, there is no need to send the instrument in for service—a replacement optical bench can be shipped out and installed, on-site, by a trained field service technician.

MALS – general questions

Q: How many SEC columns are necessary to get a good separation and signal to noise ratio with the different detectors?

A: The answer is very dependent on the specific samples and chromatography system, not on the detectors. For example, for proteins or polymers with low polydispersity, usually only one column is needed. For heterogeneous polymers with broad distributions covering multiple orders of magnitude in molar mass, between two and four columns may be needed to fully resolve the sample. As long as you can find the right separation conditions (columns, mobile phase, etc.), Wyatt’s MALS and dRI detectors will maintain the resolution and (within reason) provide excellent signal-to-noise. Please see www.wyatt.com/Products/MALS to understand the range and sensitivity of the detectors.

Q: Some recent publications used chromatography methods other than SEC. Did you try to use hydrophobic-interaction chromatography or ion-exchange chromatography?

A: Use of MALS with ion-exchange chromatography has been published in Nature’s *Scientific Reports* by Amartely et al. in 2018 – see <https://doi.org/10.1038/s41598-018-25246-6>. For some Wyatt application notes describing the use of MALS with ion-exchange and reverse-phase



chromatography, see <https://www.wyatt.com/library/application-notes/by-technique/rpc-mals.html>.

MALS has also been combined with preparative hydrophobic interaction chromatography, as described in <https://doi.org/10.1080/19420862.2018.1505178>. We have not yet seen MALS combined with analytical HIC, but there is no reason it cannot be accomplished in the same manner as reversed-phase or ion-exchange chromatography. We do recommend a high-concentration Optilab if RI data are needed over the mobile-phase gradient.

Applications

Q: *Can multi-angle light scattering technology be used to identify and quantify antibody-antigen (protein or whole bacterial/virus) interactions?*

A: Light scattering is appropriate for label-free, solution-based (*in vitro*) characterization of antibody-antigen and many other biomolecular interactions including protein-protein, protein-nucleic acid and self-association. In some cases, it may be useful for antibody-virus interactions too.

- SEC-MALS uses a DAWN, miniDAWN or microDAWN MALS instrument, in conjunction with size-exclusion chromatography (FPLC, HPLC or UHPLC) and ASTRA software, to identify the formation of complexes and their absolute stoichiometry. The analysis is based on determining the molar masses of the monomers and complexes. Usually antibody-antigen complexes have high affinity and do not dissociate while eluting from the SEC column, making this a straightforward indicator of complex formation, but not a means of quantifying affinity.
- CG-MALS uses a DAWN or miniDAWN, in conjunction with a Calypso composition-gradient device and CALYPSO software, to identify the formation of complexes, quantifying affinity as well as absolute stoichiometry. Here, too, the analysis is based on determining the molar masses of the monomers and complexes, but in the case of CG-MALS there is no separation, and the measurement is made over a series of concentrations or compositions in order to fully understand the interactions.

Q: *What is Calypso?*

A: Calypso implements composition-gradient light scattering (CG-MALS), a label-free and immobilization-free technique for characterizing biomolecular interactions. Calypso itself is a composition-gradient device that combines up to three different solutions. It degasses, filters, mixes and injects the aliquots into the MALS and concentration detector, stopping the flow after each injection in order to allow the complexes to reach equilibrium.



The CALYPSO software acquires the MALS and concentration signals and provides various algorithms for analyzing the data, to determine which complexes are formed as well as the equilibrium association or dissociation constants for each one. This technique is appropriate for simple and complex interactions ranging from homodimers or heterodimers to quite complex association schemes, including simultaneous self- and hetero-association, multivalent-multivalent interactions, and interactions at high concentration where thermodynamic non-ideality is an important factor.

The system is quite useful for automating batch measurements such as Zimm plots or dn/dc measurement. It can also be used to study the kinetics of self-assembly or degradation of supramolecular assemblies on time scales of seconds to hours. As a closed system with built-in filtration and computer-controlled precision syringe pumps, it provides accurate and highly repeatable results.

Q: How is the protein conformation identified by any of the instruments?

A: Protein conformation is generally identified from the combination of molar mass and hydrodynamic radius R_h . In order to measure both, dynamic light scattering must be added to whichever MALS detector is most appropriate (DAWN or miniDAWN for HPLC, microDAWN for UHPLC, etc). For DLS, either a WyattQELS DLS module is embedded in the MALS detector, or an external DynaPro NanoStar or Mobius is connected via the DLS Compatibility Kit to the MALS flow cell.

Q: If one protein has two conformations, the hydrodynamic radius R_h could identify the difference. What's the detection limit for R_h difference for two conformations of one protein?

A: The accuracy of R_h measurement is $\sim 5\%$ and the precision $\sim 2\%$, which is much better than the ability of most SEC columns to resolve two species. If the two conformations are in fact separated by SEC, it should be straightforward to distinguish between them by DLS while using MALS to confirm that they have the same molecular mass. If they are not resolved by SEC then it should be possible to combine MALS and DLS with IEX or RPC to separate and analyze the two conformations.