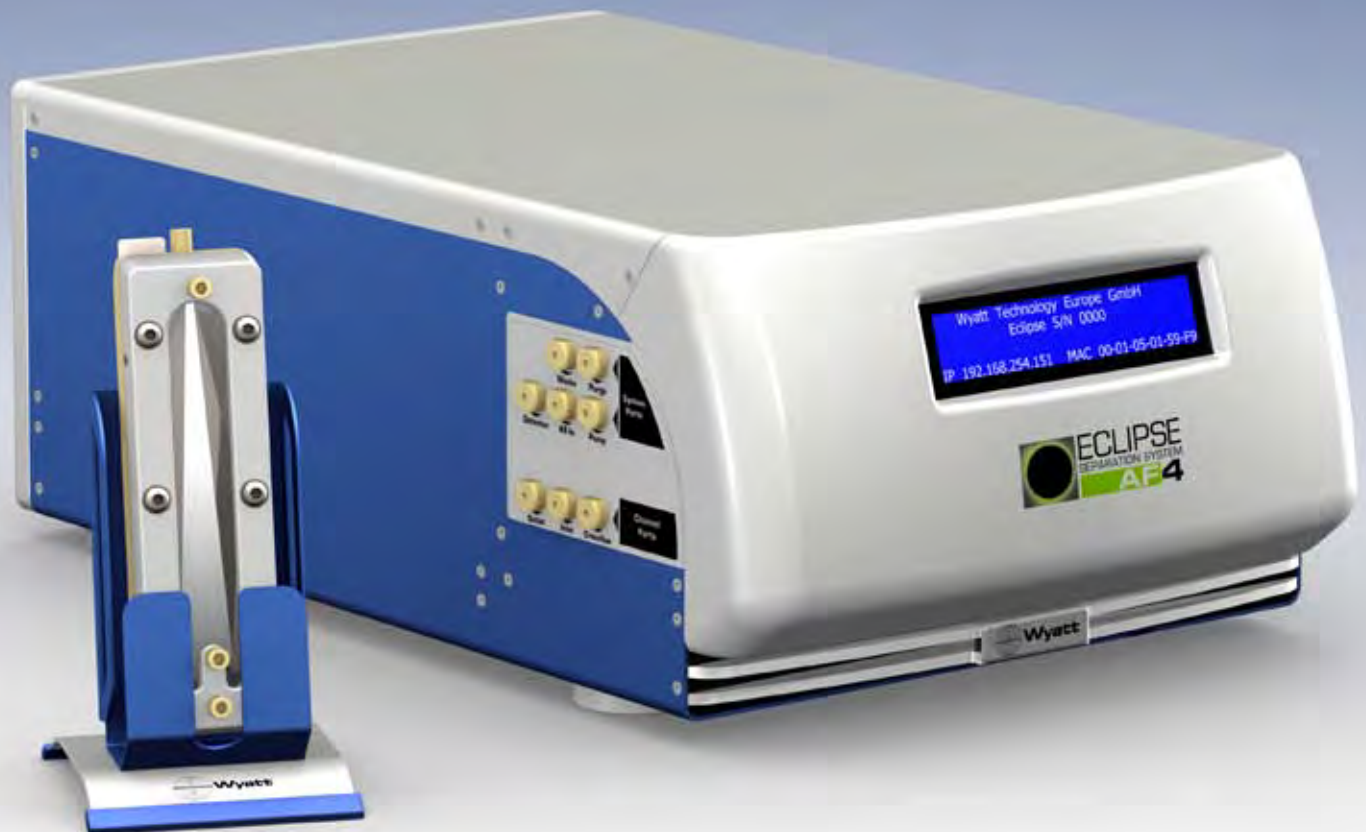


The Eclipse[®] AF4

**The ultimate system
for separating macromolecules,
proteins, colloids, and nanoparticles**



The Eclipse[®] AF4

The ultimate system for separating macromolecules, particles, and more...

The Eclipse AF4 system brings a powerful separation technique to your laboratory. It is based on Asymmetric-Flow Field-Flow-Fractionation (AF4). The advantage of AF4 over conventional column chromatography is the ability to separate both soluble and colloidal components over a wide size range including sensitive and “sticky” samples.

The analytical power of the Eclipse is comparable to complex analytical techniques that are far more laborious and expensive, e.g. analytical ultra centrifugation (AUC) or electron microscopy (EM).

The Eclipse separates numerous molecules and particles such as proteins and their aggregates, liposomes, viral particles, antibodies, as well as polymer latex particles, nanoparticles, colloidal suspensions, and a variety of other samples.

Due to the nature of this separation technique, three separate flow streams (detector flow, cross flow, and injection flow) are generated, and the flow paths are switched between two different modes (see AF4 explanation below). The complexity of solvent paths and the requirement to use several pumps has been historically a downside of the otherwise elegant method. Wyatt Technology’s innovative concept to generate all three flow streams from one main flow has been a breakthrough for the AF4 method.

Hardware and software have been created for maximum simplicity and automated operation. System safety has also been thoroughly addressed in the engineering of the Eclipse. Thus, the Eclipse represents the most versatile, robust, and stable system available.

The Eclipse is integrated with Wyatt Technology’s Multi-Angle Light Scattering (MALS) detectors, as well as the Optilab® T-rEX™ differential refractometer and

the ViscoStar™ differential viscometer. Dynamic Light Scattering (DLS) can also be added with the QELS option or the DynaPro™ NanoStar. A HPLC pump and UV detector complete the system.

Moreover, with the Eclipse AF4 Wyatt introduces a new software platform integrating the AF4 control into the market leading HPLC software like Agilent’s ChemStation and Shimadzu’s LC Solution.

The Eclipse offers the use of four different separation channels to accommodate any experimental requirement. The complete set of analytical instruments can be stacked on Wyatt’s newly constructed instrument rack to create a compact system with easy access to all components.

The Eclipse was launched 2001 and has made an immediate impact in popularizing AF4. Today the Eclipse AF4 represents Wyatt Technology’s fifth generation AF4 system. Continuous improvement and perfection of the instrumentation plus competent customer support make the Eclipse the market leading AF4 system worldwide.

The most convincing testimony for unsurpassed quality and performance is the fact that all major pharmaceutical companies rely on the Eclipse for research and quality control of biopharmaceuticals. In GLP and FDA regulated environments, the Eclipse has proven to meet the highest standards of instrument performance required in the pharmaceutical industry. Top research institutions and universities around the world use the Eclipse, producing publications that can be found in the best journals.



The advantages of AF4

- **Large dynamic range: from nanometers to micrometers**
- **Represents an orthogonal method to size exclusion chromatography**
- **Direct injection: minimal sample preparation necessary**
- **Rapid analysis: typically 10 to 30 minutes**
- **Fast equilibration after change of solvent, channel, or membrane.**
- **Absence of shearing forces**
- **High resolution separations, comparable to ultra centrifugation**
- **Adjustable and flexible separation ability: very efficient for complex samples**
- **Interfaces with modern analytical methods like MALS, MALDI-TOF, ICP-MS, etc.**
- **Ability to collect fractions for off-line analysis (electron microscopy, MS, ELISA, etc.)**
- **Robust and fully automated (auto sampler) operation for high sample throughput**
- **Experiments can be planned with a method development tool**

- 1 Compartment with fluid components can be pulled out of the chassis like a drawer without removing the cover. This allows quick access to the interior and makes it easy to replace plugged tubing or service other fluid components.
- 2 Rheodyne motorized 6-port switching valves.
- 3 Two proprietary pressure sensors: monitor pressure in the channel and beneath the membrane.
- 4 Needle valves: computer-controlled and motor-driven fine metering valves to adjust focusing and injection.
- 5 LiquiFlow flow meter to measure the injection flow rate.
- 6 CoriFlow cross flow controller: high precision regulating and measuring device for cross flow rates, universal for all solvents.
- 7 Drain pan with leakage sensor.
- 8 Electronics are fully isolated from fluid components and cannot get in touch with liquid even in the unlikely event of a severe leakage.



How AF4 works

The elegant one-phase separation

Field-Flow Fractionation is a one-phase chromatography technique, which was invented by Professor Calvin Giddings in 1966. High-resolution separation is achieved within a very thin flow against which a perpendicular force field is applied. The flow and sample are confined within a channel consisting of two plates that are separated by a spacer. The plates are then pressed together. The spacer has a typical thickness of 250 to 800 μm .

In the Asymmetric Flow-FFF, which is one technique among the family of FFF methods, the upper channel plate is impermeable. The bottom channel plate, however, is permeable, and made of a porous frit material. An ultra filtration membrane with a suitable cut-off covers the bottom plate to prevent the sample from permeating the bottom wall.

Within the flow channel a parabolic flow profile is created because of the laminar flow of the liquid. The stream moves slower at the boundary edges than it does in the center of the channel. When a perpendicular flow is applied to the laminar stream, the analytes are driven toward the boundary layer — the so-called “accumulation wall” — of the channel.

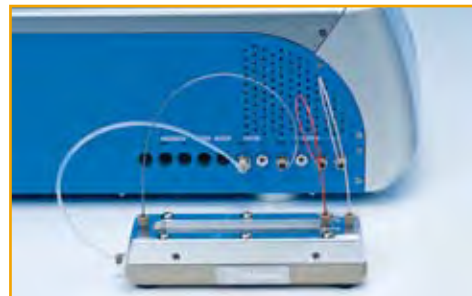
Diffusion (associated with Brownian motion) creates a counteracting motion. Smaller particles, which have higher diffusion rates, tend to reach an equilibrium position higher up in the channel, where the longitudinal flow is faster. Thus, the velocity gradient flowing inside the channel separates different sizes of particles, based on their diffusion rates.

The smaller particles are transported much faster along the channel than the larger particles. As a consequence, smaller particles elute before the larger ones. Therefore

the elution profile is the opposite of a Size Exclusion/Gel Permeation Chromatography (SEC/GPC) separation, in which the large molecules elute first.

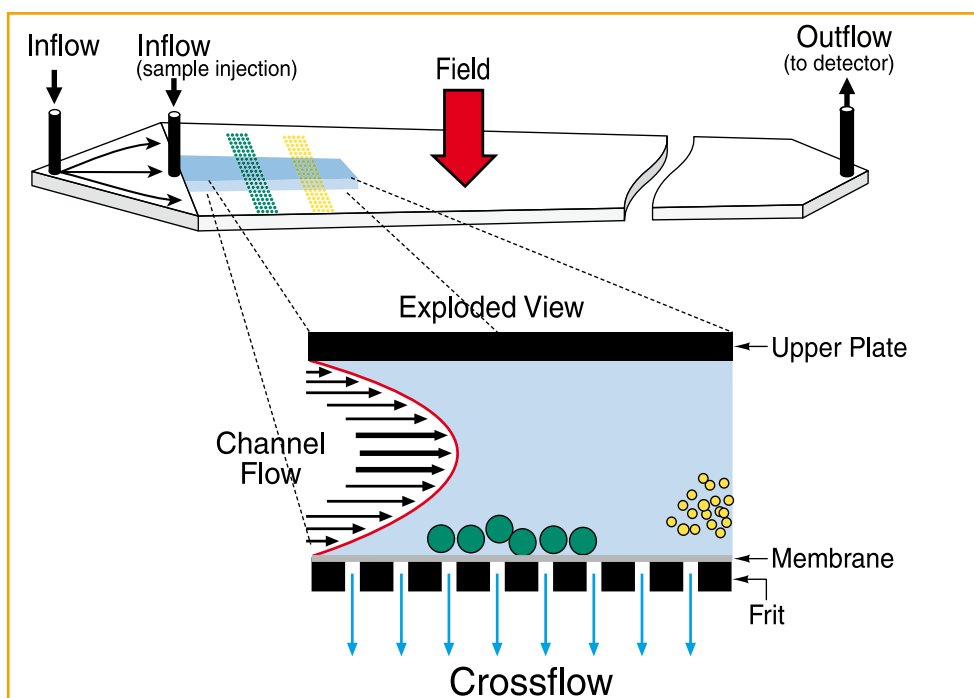
AF4 separation, requires no stationary phase or column packing, reducing unwanted sample interaction and shear forces. The entire separation is gentle, rapid, and non-destructive.

The separation process requires three steps. During the first two steps, injection and focusing, the main flow is split and enters the channel from both ends. The two flows are balanced to meet under the injection port. At this point the flow moves down and permeates through the membrane. Once the sample is injected, it focuses in a thin band and concentrates towards the membrane. After complete transfer of the sample volume, the injection



One of Wyatt's AF4 separation channels connected to the Eclipse controller chassis.

flow is stopped. One typically allows for another minute of focusing before the flow pattern is switched to the elution mode. Now the flow enters only from the inlet port and exits through the outlet, which is connected to the detectors. Sample constituents elute separated according to their size and are monitored by the array of detectors.



Principle of AF4 separation: the smaller particles elute first as they diffuse further into the parabolic flow stream.

The Eclipse - a Perfect Team Player

Options and integration with Wyatt Technology detectors and HPLC equipment from leading manufacturers

channel length with five possible spacer thicknesses and two different spacer widths. For the highest sensitivity and peak height the smallest channel is used. For high sample load, the semi-prep channel allows the injection of up to 5 mg (typical value depending on specific sample and fractionation requirements).

Option for organic solvents

For AF4 analysis in organic solvents such as THF or toluene, the Eclipse organic option is required. The organic option comes with stainless steel tubing inside the chassis, Kalrez o-rings, and a PEEK top plate for the channel. Additional safety features are also included. In case of a leakage, a vapor sensor triggers an audible alarm signal and activates a signal that can be used to shut down the main pump. Interface cables are provided in the delivery.

Metal-free option

For special applications the Eclipse can be delivered in a metal free version. This is advantageous especially when using high-salt buffers, which might exert corrosive effects on metal tubing. Certain other applications (e.g. water analysis) must also be performed in an environment that is free of metal ions. The metal-containing needle valves can be excluded from the flow path by switching a software-controlled PEEK valve.

SEC switching option

A switching option allows detector and HPLC equipment use of the Eclipse for both FFF and SEC analysis. Under software control, the separation mode can be switched from FFF to SEC. The change is even possible during a sequence

in unattended operation. Three motorized switching valves direct the flow alternately through the FFF channel or through the SEC columns. Two bulk head unions are provided to connect the columns to the Eclipse chassis.

Temperature control option

Running the separation under temperature regulated conditions is often beneficial for polymer and biopolymer applications. By placing the separation channel in an oven, temperature control from 5° to 85° C is achieved. Proprietary heat exchanger elements ensure precise temperature equilibration of the solvent entering the channel.

Integration with Wyatt Technology instruments and software

The Eclipse is designed to integrate with Wyatt Technology's range of detectors. Ensuring optimum baseline quality with these very sensitive detectors is at the core of the Eclipse design. Unsurpassed stability of flow and pressure is achieved by carefully optimizing the interaction of the main pump, flow regulators, and switching valves.

The Eclipse stacks with the DAWN® HELEOS™, the Optilab T-rEX, and the ViscoStar. Software integration is essential for usability of the system. The Eclipse software provides a control center for starting an experiment, either for a single run or for an unattended sequence of runs. All the parameters for the AF4 run and collection of light scattering data with the ASTRA software are entered in one menu. ASTRA operates in the background and collects the data, entirely under control of the Eclipse software.

For data processing the user switches to ASTRA, which is the powerful and versatile software tool for MALS data. Thus, the Eclipse user benefits from the specialized ASTRA software without compromising on the integrated control of the complete setup. The result is a seamless system for high resolution separation and cutting edge detection technology.

Integration with HPLC equipment

The job of controlling HPLC instruments is best done by the original vendor's own HPLC software. Therefore, the Eclipse does not use its own controller software, but integrates into various HPLC packages. There are many benefits of this approach. Users can stay with their HPLC software platform and run the Eclipse with the familiar interface they already know. Existing HPLC instruments can be integrated and local support and service from the original supplier is available. If the HPLC components need maintenance, the local service organization of the HPLC vendor takes care of this task.

Currently the integration is completed for Agilent, Dionex and Shimadzu HPLC. For Agilent OpenLab an add-in is installed, which allows access to the Eclipse as an instrument in OpenLab. The sample-set table has been extended to allow entering the AF4 method and all parameters required to start and control the ASTRA data collection. OpenLab data files store the FFF specific data traces, like pressure reading, cross flow, inject flow rates, etc.



Eclipse AF4 complete system shown with HPLC instruments. Detectors are placed on the Eclipse rack.

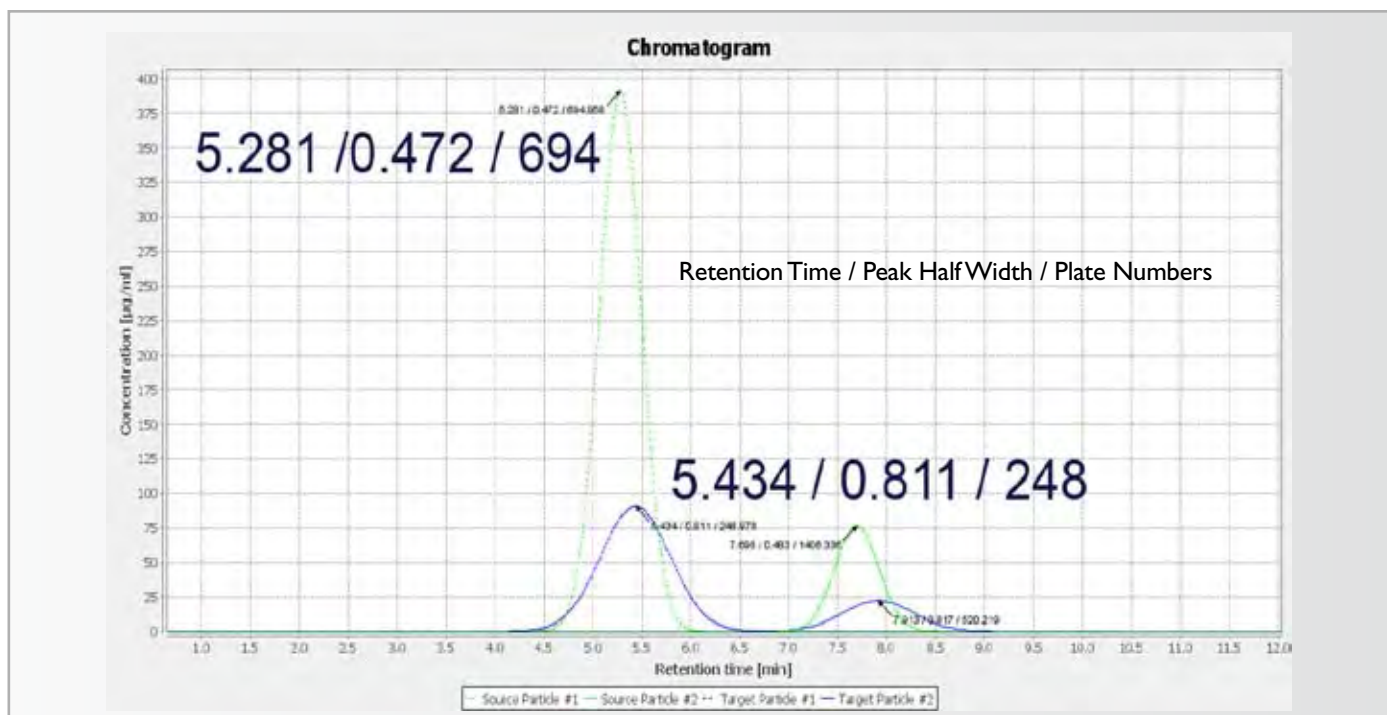
Eclipse ISIS

Interactive Separation Improvement System

Flexibility is one of AF4's main features, allowing to achieve optimal separation for most applications. Nevertheless the proper parameters for an optimal fractionation must be found. Wyatt Technology has developed an indispensable tool for method development, a software application called ISIS. Basis for ISIS is the FFF theory as developed by Giddings and Wahlund which has been implemented with a proprietary methodology (patent filed). The unique feature is to calculate the fractogram as displayed by the concentration detector resulting from a given set of experimental parameters. For the first time FFF users have a tool which predicts the result of the separation as a detector trace. The elution

time is calculated as well as the peak half-width and plate numbers for two components. The user can enter the size of two fractions which have to be resolved or the minimum and maximum size that should be eluted during the run. The influence of a higher cross-flow, different channel dimensions as well as different detector flow rates are displayed within a few seconds. Temperature and solvent viscosity are input parameters as well.

The example illustrates how ISIS predicts the experimental result of the separation and allows to plan the optimal fractionation based on experiments done on the computer instead of laborious method development in the laboratory.

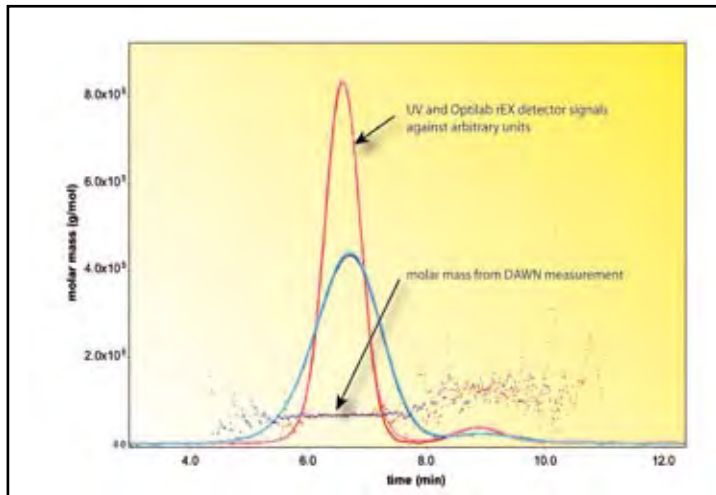


Colour	Green	Blue
Channel (Length mm)	SC (154)	LC (254)
Channel Height [µm]	250	350
Cross-Flow Rate [ml/min]	3	3
Channel Area [cm ²]	20,57	35,25
Cross-Flow Density [L/(h m ²)]	88	51
Detector Flow Rate [ml/min]	0.4	1.0

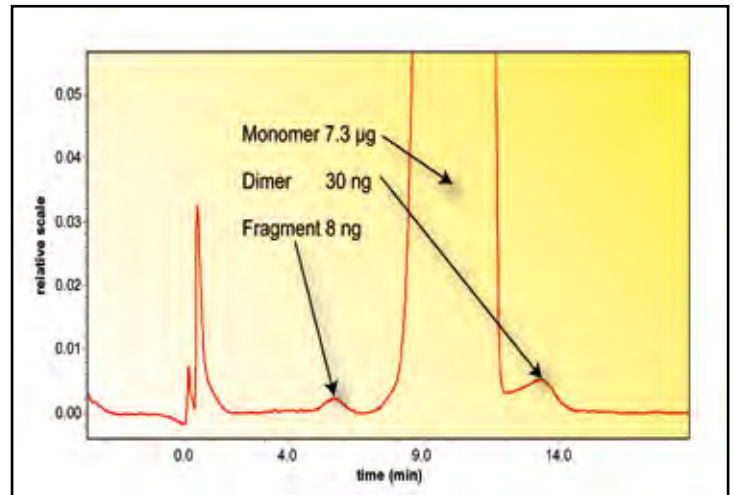
The figure above shows the overlay of two fractograms of BSA separation simulated with ISIS. The experimental conditions are listed in the table. In a quantitative way it can be seen how the higher cross-flow density and lower channel height increase the efficiency of the separation. The plate number for the monomer peak is 700 for the green trace versus 250 for the blue. Peak dilution is also given in quantitative numbers on the y-axis. The BSA monomer peak height differs by a factor of 4.75 with the same sample amount injected in both runs.

Applications

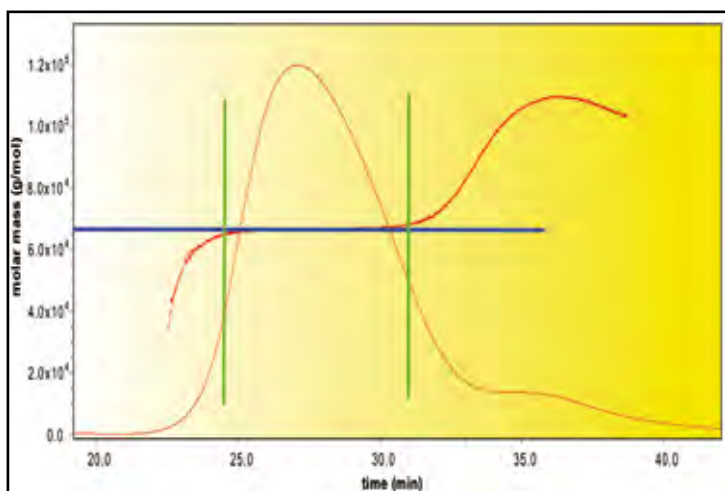
Proteins, biopolymers, particles, and more



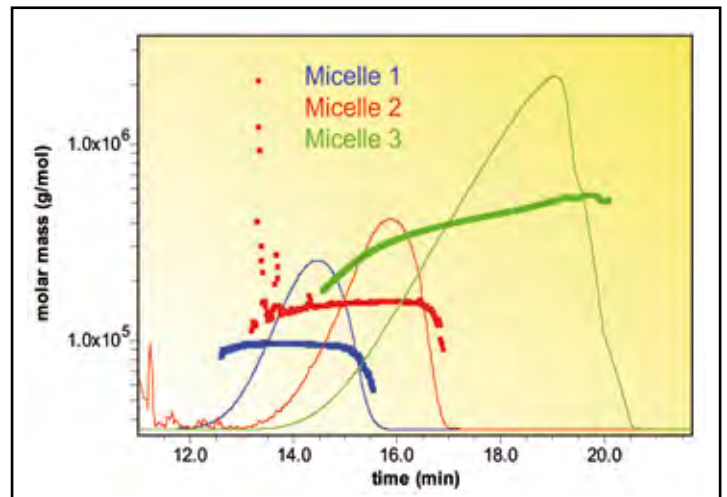
Separation of Bovine Serum Albumin (BSA) monomer and dimer compared on two different channel lengths. Shown in red is the chromatogram on channel SC (155 mm length) and blue on 240 mm length. A $490 \mu\text{m}$ spacer thickness was used and $10 \mu\text{g}$ protein injected. Using the SC channel, peaks are narrower, increasing signal to noise and therefore sensitivity. Signals shown are both UV 280 nm and Optilab rEX RI. Depending on requirements, the sample can be concentrated by choosing a short channel or sample load can be maximized by employing a larger channel geometry.



Separation of a monoclonal antibody (MAb), showing separation of the fragment, monomer, and dimer. Channel SC (155 mm length) with $490 \mu\text{m}$ spacer was used. The trace shown is UV at 220 nm with a blow-up concentrating on the dimer and fragment. Because of the extraordinary efficiency of the SC channel, the 8 ng of the fragment produce a clear peak, allowing precise quantification. It should also be noticed that there is a very narrow, small system peak, proving that there are no artifacts created in the detector signals.



Shown is separation of a BSA protein on the Semi-Preparative channel SP2. $1600 \mu\text{g}$ sample was injected using $800 \mu\text{m}$ channel height. The green bars indicate a fraction of pure monomer (molar mass $66.000 \text{ kD} \pm 10\%$). The amount of fractionated monomer is $1300 \mu\text{g}$ in one single injection. As the experiment is fully automated, it is possible to run multiple injections and pool the fractions.



Overlay of three micelle samples loaded with different active components. The Eclipse separation reveals how the molar mass of the micelles increase depending on which component is solubilized. This information can be used to calculate how many molecules are present in the micelle. The chromatogram shown is the 90° light scattering signal.

Service & Support

World-class service and support from the leading developer of light scattering and FFF instrumentation

Wyatt Technology has been working with FFF for over 20 years and started to develop its own instrumentation in 2001. Since then it has been very successful on the market, and Wyatt Technology now devotes far greater resources to the manufacture, service, and support of FFF systems than any other FFF developer. Besides a full-fledged R&D and production department for FFF, we have a group of post-graduate degreed scientists plus several engineers involved in the support of FFF systems and light scattering detectors. Building on a wealth of experience in FFF instrument development and application support, we deliver first class service and support for our customers.

We're proud to offer a unique FFF training seminar as an additional module to the Light Scattering University® course, which includes a total of four days of intensive hands-on training in Germany or Santa Barbara, California. The curriculum covers light scattering and FFF theory, data collection and analysis, troubleshooting, as well as maintenance of the Eclipse and light scattering detectors. During training, our customers interact with the people who write our software and create our hardware. Not only do they learn about Eclipse operation, but also about sample

preparation and data interpretation. Our internationally acclaimed immersion training also ensures that operating the Eclipse will be second nature by the time the course is completed.

If questions do arise, our superb staff answers telephone, fax, and e-mail inquiries quickly. By means of our interactive customer support, our service personnel are always just a phone call away, and a proprietary internet link can bring us directly into your lab for "hands-on" help and guidance with our Remote Assistant support feature.

Since the company has so many biotechnology, pharmaceutical, and polymer customers, we developed complete IQOQ service packages, which are available with on-site support.

An International Light Scattering Colloquium (ILSC) is held once a year to stimulate the exchange of information among our customers and to promote greater understanding of the capabilities of multi-angle light scattering. The FFF Focus Meeting takes place on the third day of the ILSC. All of Wyatt Technology's customers are eligible to attend and participate in this colloquium that is often called the most interesting scientific conference around.

The company also maintains an online Support Center where customers can view their instrument service status, download current software up-dates, read our Frequently Asked Questions, and order spare parts and supplies from the on-line store. Our Website provides frequent additions to its extensive bibliography of thousands of refereed scientific publications, training schedules, and Application Notes. You can find them all at www.wyatt.com and www.wyatt.eu



The Eclipse product is developed, produced, and supported by a highly qualified and motivated team of scientists and engineers.



WYATT.COM and WYATT.EU are the home of light scattering and FFF instrumentation. The sites include over 100 Application Notes, all upcoming Light Scattering and FFF University schedules, as well as the On-line Support Center, where customers may download software updates, read new FAQs, and order spare parts and supplies. A database with the largest compilation of dn/dc values as well as scientific literature can also be found in this section.

Additional Applications & Products

DAWN® HELEOS™ II

The DAWN HELEOS is the instrument of choice for applications where the greatest possible accuracy and precision are required. The HELEOS has 18 detectors for unparalleled multi-angle measurements (from a low angle of about 10° to a high angle of almost 180°), a 120 mW diode laser, and options for a variety of thermostatic-control systems to go up to 210° C or as low as -15° C.



The DAWN HELEOS is the ultimate research-oriented light scattering instrument. Its 18 angles of detection give it the widest angular range of any commercially-made light scattering detector, and its numerous options enable it to be customized for virtually any application.

miniDAWN™ TREOS™

The three-angle detector is designed specifically for the analysis of proteins, biopolymers, and polymers with a molar mass below 1000 kD.



The miniDAWN TREOS is the routine MALS detector, compact, with highest sensitivity.

Optilab® T-rEX™

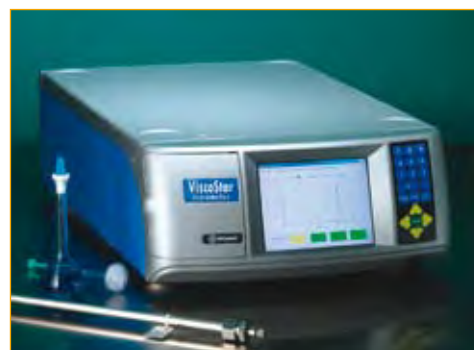
The Optilab T-rEX (refractometer with EXTended range) is the most sensitive differential refractive index (dRI) detector on with up to 50 times the dynamic range of any RI detector in existence today. The Optilab T-rEX may also be used to measure the absolute refractive index increment, dn/dc , at the same wavelength as the light scattering instrument. Furthermore, the Optilab can be used to measure the absolute refractive index of a solution.



Wyatt's Optilab T-rEX has 256 times the detection power and up to 5 times the dynamic range of any other RI detector on the market.

Wyatt ViscoStar™

The ViscoStar 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 state-of-the-art electronics. It achieves at least twice the signal-to-noise ratio of any commercial solution viscometer currently on the market, and it incorporates a high efficiency heat exchanger to provide extremely stable baselines.



The Wyatt ViscoStar represents a new generation of online differential viscometers.

Dynamic Light Scattering (DLS) Interface

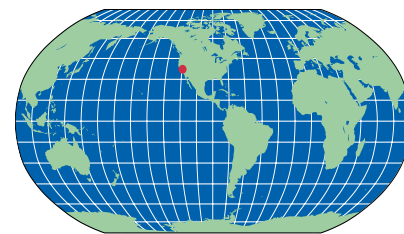
If you want to have the best of both worlds (classical and dynamic light scattering), our instruments can be ordered with the embedded WyattQELSTM, or they can interface with our DynaPro™ instruments. The combined instruments allow simultaneous static and dynamic light scattering measurements from which both absolute molar masses and hydrodynamic radii can be obtained. Or, if high throughput Dynamic Light Scattering is desired, the DynaPro Plate Reader™ system can automatically measure sizes of proteins and nanoparticles in 96 or 384 or 1536 well plates.



The DynaPro Plate Reader is the only DLS instrument that can measure directly in standard well plates of 96, 384 or 1536 format. Temperature control and temperature ramping from 4°C to 70°C is available.

Specifications

Separation range:	Approximately 1 nm to 10 μ m, depending on specific sample properties
Cross Flow rates:	Cross Flow ranges (channel dependent): 0.04 to 3.3 mL/min (standard), 0.07 to 6.6 mL/min or 0.1 to 8.3 mL/min, maximum total flow rate (cross flow plus detector flow rate) is 10 mL/min
Channel pressure:	Maximum 30 bar, software overpressure shut-down
Spacer thickness:	Set of spacers with 250, 350 and 490 μ m; each thickness comes in two different channel widths
Pre-cut membranes:	Polyether Sulfone: 1, 3, 5, 10, and 30 kDa cut off; Cellulose Triacetate: 10 kDa cut off; Regenerated cellulose: 5, 10, and 30 kDa cut off (data subject to change without notice)
Eclipse channels:	Micro MC: 114 mm effective length (e.l.); Short SC: 153 mm e.l.; Long LC: 246 mm e.l.; MC, SC, LC maximum spacer width 22 mm; Semi-Prep SP 1: 246 mm e.l., maximum spacer width 44 mm,
Eclipse chassis:	Two proprietary pressure sensors with range 0 to 30 bars, no dead-volume; LiquiFlow flow meter to monitor injection flow rate with range 0.01 to 1 mL/min; CoriFlow regulating and measuring device for cross flow control; drain-pan with leak sensor, two proprietary motorized needle valves for inject flow regulation and focus balance adjustment
Standard version:	Rated for aqueous solvents and alcohols; guaranteed corrosion resistant from pH 2 to 12; tubing PEEK; sample path free of any metal parts. Aqueous channel: lower block PEEK; cover: aluminum frame with poly carbonate inlay, Viton O-ring; stainless steel frit
Organic option:	Rated for organic solvents, e.g. THF, toluene, etc. tubing stainless steel, additional vapor sensor with interlock output, CoriFlow Flow meter for inject flow measurement. Organic channel: upper channel block made from PEEK; Kalrez O-ring
Metal free option:	Rated for aqueous solvents pH 1 to 14, configured to remove all metal parts from the flow path; three PEEK switching valves for elution/focus and replacing needle valves with PEEK tubing, metal free LiquiFlow Flow meter to measure the injection flow
SEC Switch option:	Three additional motorized switching valves for software controlled change of flow path from AF4 mode to SEC mode, two bulk head connectors for connection of SEC columns, one connector for an external rinse pump, one additional waste outlet
Dedicated electronic controller for all internal components and external interface connectors; front panel LCD display; Ethernet communication via TCP/IP with Eclipse controller. Eclipse Software is integrated in Chemstation®, Chromeleon®, and Shimadzu's LC Solution®.	
Software controlled electronic inputs/outputs: contact closure input from third party auto sampler, contact closure output to start ASTRA, contact closure output to trigger a detector zero function, detector analog IN and OUT, third party pump start signal, Agilent pump interlock (organic version only)	
Power supply:	110-240 V @ 50-60 Hz
Power:	100 W
Dimensions (cm):	64 (L) x 36 (W) x 25 (H)
Shipping Weight:	20 kg
Software:	MS-Windows 7



With installations in more than 50 countries, Wyatt Technology is the world's leading manufacturer of light scattering instruments for absolute macromolecular characterization. It is the only company in the world focused exclusively on such systems, their design, and their application.

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The Eclipse is manufactured in Germany.

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