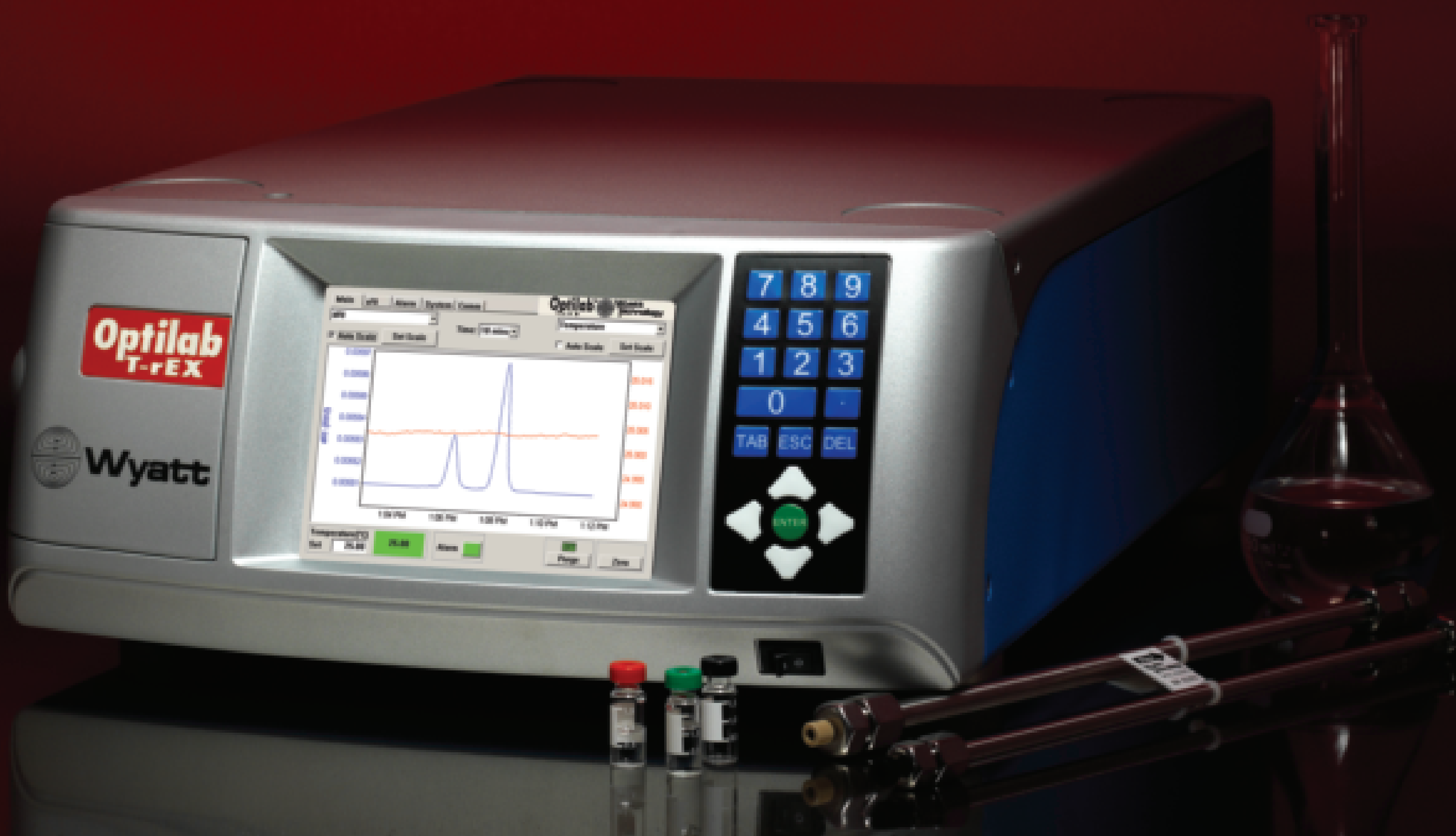
WYATT TECHNOLOGY CORPORATION

Optilab® T-rEX™

Differential Refractometer with Extended Range

256 Times the Detection Power
50 Times the Dynamic Range

Absolute Refractive Index Determination



Wyatt's Optilab® T-rEX™

The Evolution Continues!

In 2004 Wyatt Technology Corporation (WTC) revolutionized Refractive Index (RI) detection with the introduction of the Optilab® rEX™, which went on to win the R&D100 Award and the Pittcon Editors' Silver Award. Prior to the rEX, virtually every RI detector in the world employed a two photodetector deflection-based design to measure the change of refractive index.

The old two photodetector design had been utilized for the previous 40 years and could be manufactured using simple analog circuitry available long ago. However, developments in nanoscale semiconductor fabrication techniques, NMOS linear photodiode array technology, high speed data acquisition, and raw computational power allowed WTC's scientists to conceive of new ways to make this traditional measurement better. Combining cutting edge technology with measurement and processing techniques from the field of particle physics resulted in the creation of the Optilab rEX refractometer, a first in liquid chromatography detection.

Since 2004, technology has continued to evolve, enabling Wyatt Technology to improve the award-winning Optilab rEX into a higher form—the T-rEX™. Recent developments in heterojunction light emitting diodes (LEDs) allow the T-rEX to have a light source 50x brighter than the rEX. This superior light source results in the detector array "filling" with light much faster than before. Concurrent developments in high speed data acquisition allow the array to be scanned correspondingly fast, with 1 million measurements made *every second*. As described herein, all of those data require analysis by an onboard computer—fortunately, advancements in computational power have kept pace. The Optilab T-rEX is the combination of all of these advances.

The Optilab T-rEX is an RI detector that stands so far apart from other RI detectors that it is almost misleading to call it one. It has 256 times the detection power and up to 50 times the dynamic range of any RI detector in existence today. The most extraordinary aspect of the T-rEX is that it has *no range or gain settings*. The full range of instrument detection is always present, and the full sensitivity exists across the entire range.

With all other RI instruments, one has the choice of extreme sensitivity over a very limited range or a greater range but with reduced sensitivity. With the T-rEX, there is no compromise. Extreme sensitivity exists over its entire, extraordinary range. Tiny signals and huge signals may be measured within the same data run. This unique ability—to see both large and small signals—may be quantified by the instrument's dynamic range (defined as range divided by noise). The dynamic range of the T-rEX system is more than 6.25 million—literally orders of magnitude greater than any other RI detector.

The T-rEX also has a flow cell with a total volume of only 7.4 µL—25% less than the leading refractometers available today. This translates into minimized band broadening and better temperature stability.

Old-fashioned Deflection

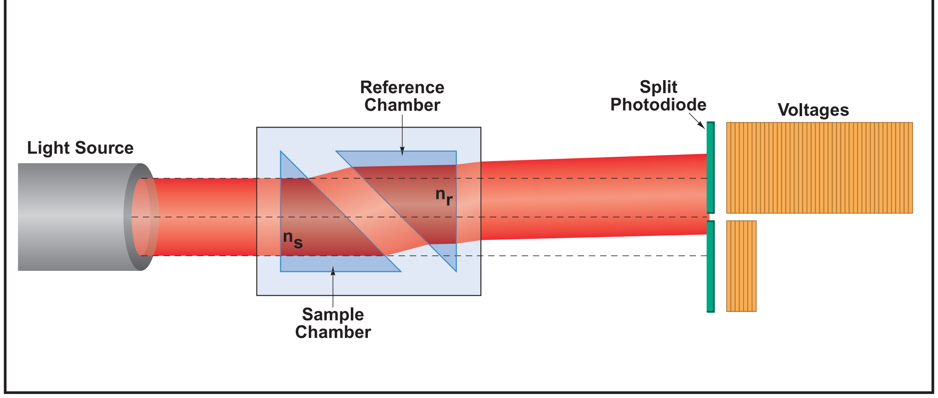
Measurement of the deflection of the light beam after it has passed through the flow cell is the basic measurement made in a deflection-type refractometer. This deflection is proportional to the differential refractive index of the two fluids being measured. For the past 40 years, a split photodiode has been used to measure the light beam's angular deflection, as pictured in Figure 1.

The split photodiode contains *two* detectors placed side-to-side. As the light beam changes angle, the beam moves away from one photodetector and onto the other. The voltage difference between the two detectors gives a measure of the light beam's position, proportional to the light beam's angular deflection, which is itself proportional to the differential refractive index.

The precision of a split photodiode detector based instrument is limited by the precision with which the voltages may be read from the two photodetectors *and* the intensity stability of the light source. The

Figure 1

The old-fashioned method for measuring the dRI between two fluids uses only two photodetectors. When the light beam undergoes an angular deflection due to a refractive index difference between the sample and reference fluids, the beam deflects more onto one detector than the other, giving a larger signal on one detector than the other. This technique was developed four decades ago.



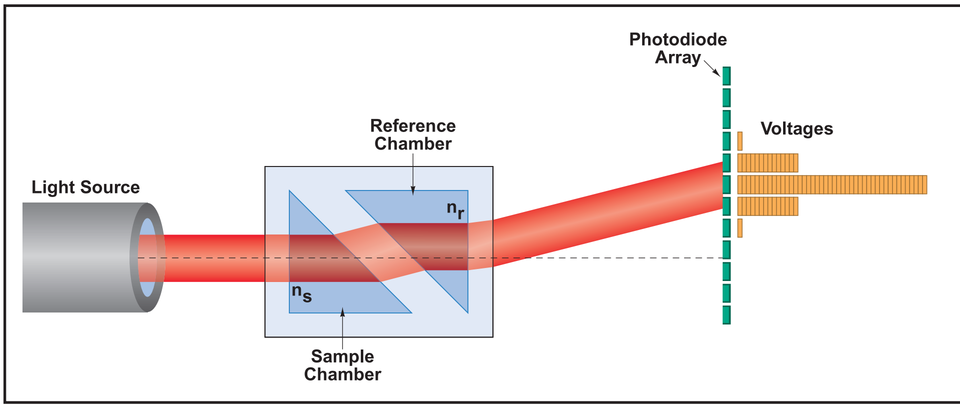


Figure 2 T-rEX Technology

The Optilab T-rEX uses an array of 512 photodetectors. The signal from the photodetectors is greatest where the light beam impacts upon the array. Using advanced data acquisition methods, mathematical processing techniques, and fitting algorithms, it is possible to determine the position of the light beam to extreme accuracy over a very great distance, achieving an unprecedented combination of sensitivity and range.

maximum signal a split photodiode detector may observe depends upon the width of the light beam. Once the light beam has "walked" off one photodetector and onto the other photodetector, there is no way to determine the beam position.

Wyatt's T-rEX Technology

The Optilab T-rEX contains 512 light measuring elements in one photodiode array. But how does it work?

Each element of the photodiode array, called a pixel, precisely measures the intensity of light impacting upon it (Figure 2). The light beam is made to have a Gaussian intensity profile: the brightest point of which is at the *middle* of the beam and the dimmest part is *furthest* from the center. The voltages measured from the photodiodes reveal the intensity profile. Fitting the measured voltages to a Gaussian function and so finding the center of the Gaussian beam determines the precise beam position anywhere on the array. And, rather than use just one Gaussian beam, the Optilab T-rEX uses 10 beams in parallel, averaging the positions of all beams together in order to obtain even greater accuracy.

The Optilab T-rEX records the voltages from each of the 512 elements of the photodiode array 80 times *per second*. To obtain the most precise possible measurements, the signals from each element are measured many times and the results averaged together. One million voltage measurements per second are required for the precision of the T-rEX. The computational power to perform these mathmatically-intensive operations is matched by the 2 GHz on-board computer "under the hood" of the T-rEX.

With a photodiode array, extraordinary light source, 10 Gaussian profile light beams, 1 million measurements a second, and a 2 GHz CPU, it is possible to determine the average light beam position with a precision of less than 10 Ångstroms. This corresponds to the width of 6 carbon atoms in a row. The photodiode array is 1.3 cm long, and the beam may move more than a centimeter of travel before it slides off the end.

To put this into more human proportions, consider if the photodiode array were 1 mile (1.6 km) long. The precision of the T-rEX is equivalent to locating the beam of light to within 1.5 widths of a human hair over that entire 1 mile distance. This combination of sensitivity *and* range has never before been seen or used routinely in a single bench top laboratory instrument.

High Concentration T-rEX

The range of a standard Optilab T-rEX is so great that the instrument will almost never go "off scale" with any standard isocratic chromatographic conditions, and the instrument may even be used for many gradient applications. The *maximum* RI signal measurable by the T-rEX corresponds to a concentration of 25 mg/mL of a protein, or 32 mg/mL of a sugar or starch. However, it is advantageous to be able to measure to even higher concentrations for a number of "batch" (*i.e.* unfractionated) applications, *e.g.* high concentration protein product applications.

To support these important formulations, Wyatt has developed a high concentration option for the T-rEX. With this high concentration option, the T-rEX can measure concentrations up to 180 mg/mL of a protein, or 220 mg/mL of a sugar or starch. This range is more than 7x the range of the standard T-rEX, which itself already has extraordinary range, and is accomplished at the expense of a very slight decrease in sensitivity (see specifications on the back page).

Absolute Refractive Index Measurements

The Optilab T-rEX can also do something no other on-line RI detector can: it can measure the *absolute* refractive index of a solution. Among other uses such as assessing general solvent purity and measuring extremely high concentrations, the absolute refractive index of a solvent is a required input parameter in light scattering measurements for determining the molecular mass of molecules in solution. Not only must the solvent's refractive index be known, but it must be known at the same wavelength as the light scattering detector being used.

A refractometer with the ability to measure both the solvent's *absolute* refractive index (to 3 decimal places) and the sample/reference *differential* refractive index to 9 decimal places provides users with the tools to produce molar mass measurements of unprecedented accuracy. The Optilab T-rEX comes with both of those capabilities built-in.

Off-line *dn/dc* Measurement

For absolute molar mass determinations via light scattering, the differential refractive index increment, *dn/dc*, must be measured at the same wavelength as the light scattering measurement. Since the Optilab T-rEX operates at the same wavelength as Wyatt Technology's DAWN® instruments, no corrections or "fudge factors" need to be used. Furthermore, the flow cell is thousands of times *smaller* than off-line differential refractometers, meaning that a vastly smaller volume of sample is required for measurement.

Light Source

The T-rEX may be ordered at almost any wavelength, from near-ultraviolet, across the visible spectrum, and into the infrared. The light source itself is an extremely high-powered heterojunction LED to which is coupled a special optical fiber. This "pigtail" configuration means that if the light source ever malfunctions, or burns out, it can be replaced easily, in just minutes. There's no expensive service appointment to organize. Moreover, if one wants to change the wavelength, installing a "pigtail" with a different wavelength LED can be done in minutes, as well.

Temperature Control

The Optilab T-rEX features dual temperature control feedback loops, with an inner temperature control system nested within an outer control environment, resulting in exquisite temperature control and maximum immunity from changes in the environmental temperature. This leads to extraordinarily stable baseline signals.

The T-rEX features Peltier thermoelectric driven temperature control, allowing programmed regulation of temperature from as low as 4°C to as high as 50°C. If you elect to work at cooler temperatures (below 20°C), you may need dry nitrogen gas to be bled into the system to prevent condensation on the optics. You simply attach a 20-80 psi nitrogen line to a port on the back of the instrument, and you're ready to go. Almost all other RI detectors have only internal heaters for temperature control, and as a result, they can only regulate temperatures 10°C above ambient and higher. The T-rEX is one of the very few RI instruments in the world with the ability to regulate *at* or *below* ambient temperature.

On-Board Computer Controls

The on-board 2 GHz CPU controls the temperature, keeps track of the illumination source power, as well as safety checking for leaks and vapors. The computer also checks the stability of the instrument, purges it, or changes its operating parameters. All system parameters may be set locally via the instrument front panel computer display, or remotely from a desktop computer connected to the instrument via Ethernet.

The large, intensely bright 16-bit (64,000 color) display (LCD) is driven by the CPU to give you the maximum amount of information in an ergonomic fashion. It is easily legible and can be read from virtually any angle between 10° and 150°.

Safety Sensors

Sensors onboard the Optilab T-rEX detect organic vapors or liquid leaks from within the instrument or from external fittings. In the event of a leak or the detection of organic vapors, visual and audio alarms are activated, and a port on the instrument back panel sets a voltage which may be used to disable a pump. The back panel output may be set by the user to be "Active High" or "Active Low".

A gas pressure sensor monitors nitrogen pressure when operating at sub-dew-point temperatures. When operating below 20°C, an onboard safety system alerts the user if the nitrogen pressure drops below a safe level (20 psi) and automatically warms the system to a safe temperature.

Orbit Automated Recycle/ Waste Selection

The Wyatt Orbit™ integrates with the T-rEX to direct the eluent from your flowing system to a waste bottle or back to the solvent reservoir. Program the Orbit (using Wyatt's ASTRA® Software) to place your system in "recycle" mode at the end of a sample set, and your flowing system will be

The T-rEX was built with maximum laboratory flexibility in mind. It can be controlled in a variety of ways using Ethernet connection. It has TTL output and inputs, autoinject signals, as well as analog input and outputs.

equilibrated and ready upon your return without wasting large quantities of your mobile phase. 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 Orbit may be controlled manually via the T-rEX front panel interface, or it may be automated within an ASTRA sample set.

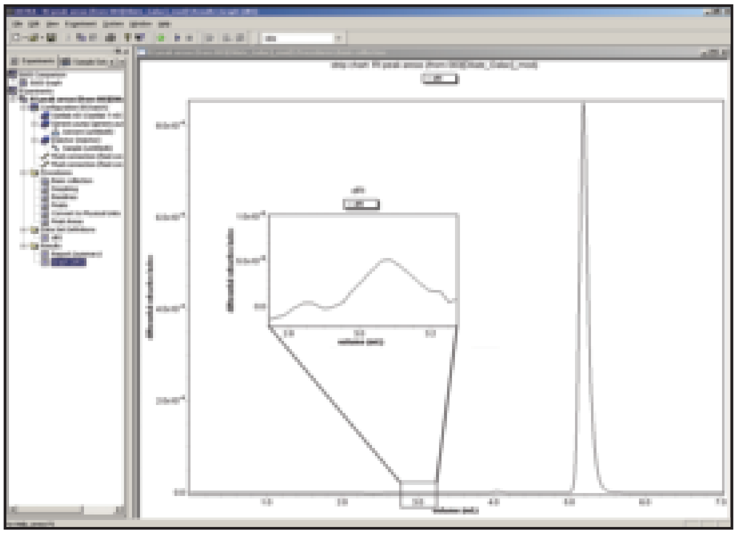
Instrument Control

Rather than controlling the instrument via the front panel, or via Wyatt's ASTRA software, some features of the T-rEX may be controlled via digital TTL inputs on its back panel. A signal on the "Zero" input causes the last measured dRI value to be considered zero. A signal on the "Purge" input causes the internal solenoid valve to actuate, flushing fluid through the reference chamber of the flow cell. A signal on the "Recycle" input causes power to be sent to an external solenoid valve after some time delay set by the user via the front panel interface (from zero delay up to as long a delay as is desired). This allows switching the fluid path, from waste to recycling, after a chromatography run is completed.

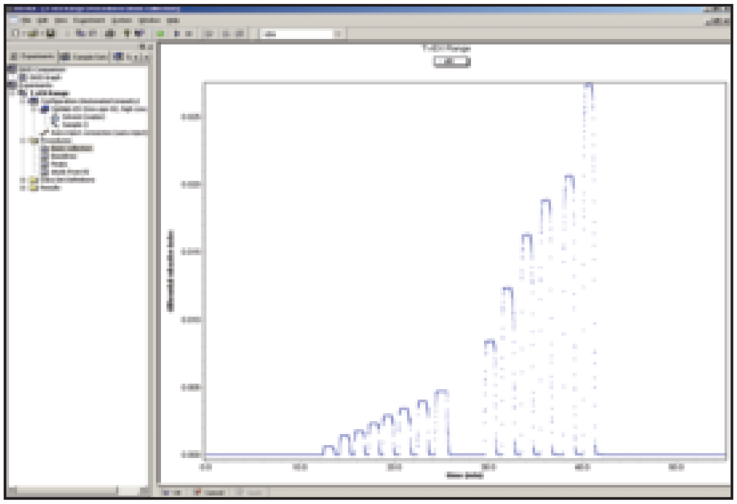
Computer Interfacing

The T-rEX can be interfaced to a local desktop computer via Ethernet, and the network may be configured so that the instrument is "seen" only by a single desktop computer, or so that any computer on the local network can see it.

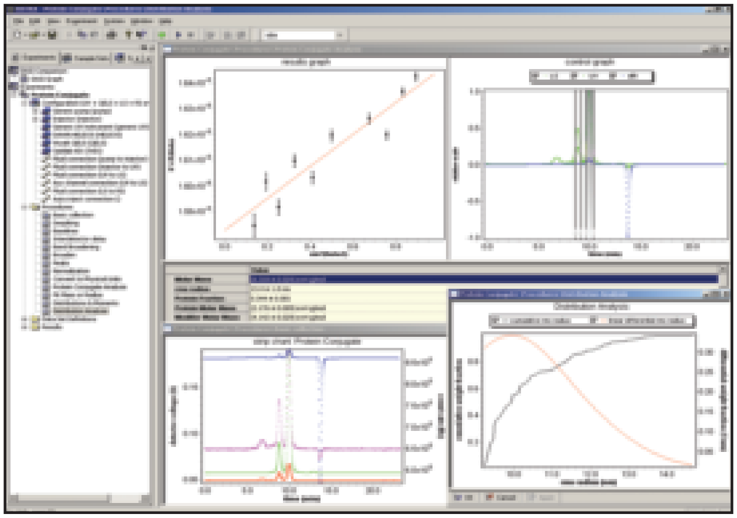
T-rEX Features & Benefits at a Glance



A portion (one-sixth) of the enormous range of the T-rEX is illustrated here. In the main graph a large sugar peak is shown, and upon magnification of the baseline an impurity species is observed. The quantity (injected mass) of the impurity is 125,000 times less than that of the main sugar peak.



For ***dn/dc*** determinations, at any wavelength, the T-rEX's vast range is superior to any instrument on the market. The result: more accurate measurements mean more precise molecular weights.



The T-rEX can be combined with a miniDAWN™ or DAWN®, plus a UV detector, as well as a viscometer (ViscoStar®), in order to perform triple or quadruple detector measurements for conjugated protein and copolymer analysis.

• Novel micro detector array technology

Allows for a combination of maximum sensitivity & dynamic range

Measures accurately the concentration of absorbing samples

• State-of-the-art fiber light source technology

User changeable light source to any wavelength

dn/dc measurements at any wavelength

• Air Cooled Peltier Temperature Control

Ambient, sub ambient temperature control (4° – 50°C) eliminates need for external water bath

• Unique flow cell design

Absolute refractive index may be measured

Small cell volume at 7.4 µL

Easy on-line or off-line operation

• Other T-rEX Benefits:

Rapid warm-up time of less than 1 hour

Autozero in 0.1 seconds

Stable baseline with minimum noise

Accepts external signals for zero, purge, and recycle

Ethernet

Full color LCD display conveys data at a glance

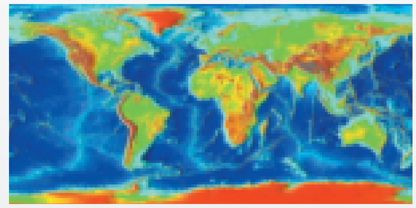
Specifications

Differential Refractive Index Range & Sensitivity:	
Range (RIU)	-0.0047 — + 0.0047
Peak-to-peak noise (RIU)*	$\pm 7.5 \times 10^{-10}$
High Concentration Option T-rEX:	
Range (RIU)	-0.0026 — + 0.034
Peak-to-peak noise (RIU)*	$\pm 1.5 \times 10^{-9}$
Fluid Volumes:	
Flow cell	7.4 µL
Inlet tubing (0.010" ID)	44 µL
Outlet tubing (0.030" ID)	370 µL
Dynamic Range:	22+ bits only via digital communication
Response Time:	0.1 - 5 seconds, user selectable
Absolute Refractive Index Range and Sensitivity:	1.2 - 1.8 RIU (sensitivity of ± 0.002 RIU)
Light Source:	660 nm standard; Other wavelengths available
Sample Temperature Control Range:	4°C - 50°C, temperature regulated
Temperature Regulation:	± 0.005°C
Maximum System Pressure:	30 psi (2 bar)
Maximum Cell Pressure:	100 psi (7 bar)
Safety Sensors:	Vapor and liquid (leak)
CPU:	2GHz VIA C7 Processor
Display / User Interface:	6.4" 16-bit color high resolution LCD display
Inputs and Outputs:	
Analog In	16 bit ± 10V A/D for auxiliary user data
Analog Out	18 bit D/A for dRI data, ± 10V or ± 1V output
Inputs:	Zero, Purge, Recycle, Autoinject, Alarm In
Output:	Autoinject Retransmit, Alarm Out, External Solenoid Recycle Valve. 12VDC solenoid valve (sold separately, or user supplied) to switch automatically between waste and recycle
Digital Communication:	Ethernet
Dimensions:	168 mm x 357 mm x 595 mm (H x W x D)
Weight:	11.5 kg
Power Requirements:	Universal input, AC 100/115/220/240 V, 50/60 Hz
Power:	480 W max, 86 W typical

Warranty: All Optilab instruments are guaranteed against fabrication defects for twelve months. Should any unit become defective due to normal use within the warranty period, we will repair or replace it at no charge.

Wyatt Technology has a policy of continual improvement. Specifications are subject to change without notice.

*Short term peak-to-peak noise measured as per ASTM-E1303-95(2000) using a 4 second time constant and temperature control at 25°C.



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

Wyatt Technology CORPORATION

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