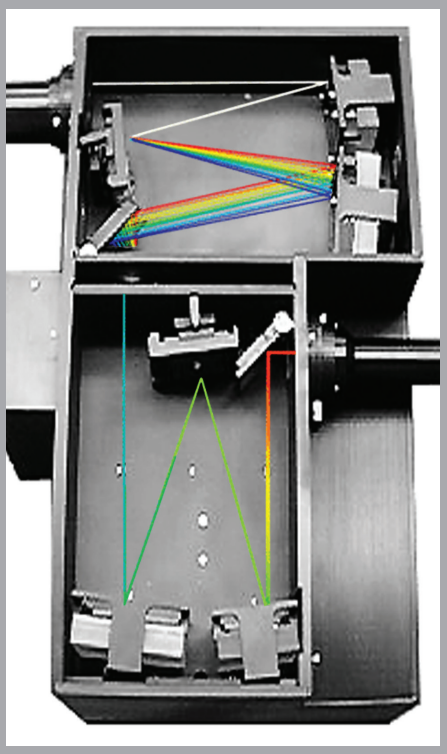
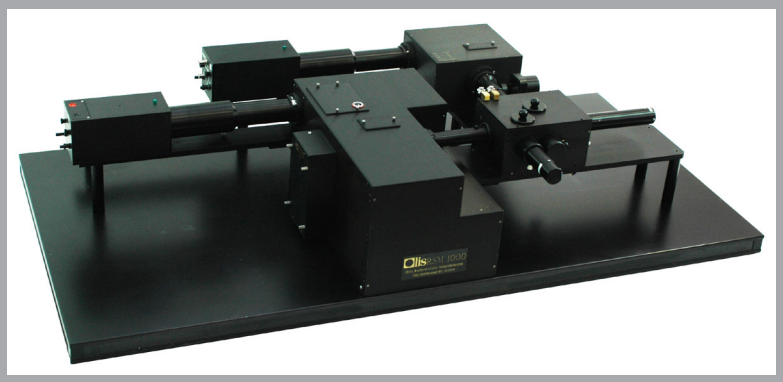
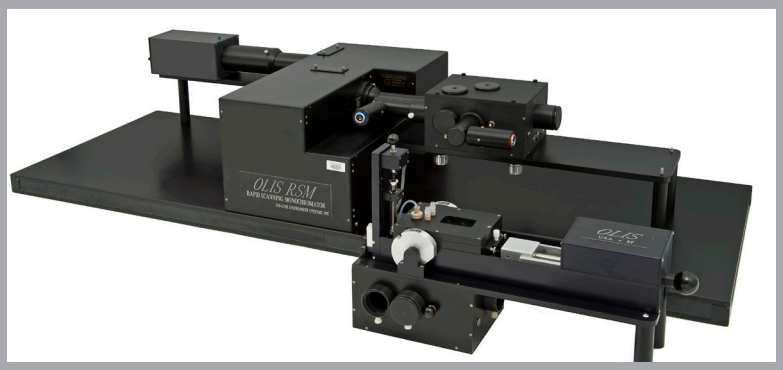
Olis

Rapid-Scanning Monochromator *
UV/Vis [NIR] spectrophotometer series

Olis RSM 1000







From the Classic to the Visionary: the Olis RSM 1000 series

Since its introduction in 1994, the Olis RSM 1000 UV/Vis [NIR] spectrophotometer has been the first choice of top research labs involved in kinetic studies such as stopped-flow and laser/flash photolysis. With a routine scan rate of 1,000 scans per second, this premium quality instrument makes simple challenging studies that would otherwise require tens or hundreds of experiments to duplicate.

The Olis RSM 1000 can also be used in slow scanning and fixed wavelength modes, opening up its brilliant light throughput and other superb characteristics for non-kinetic measurements. This brochure introduces two exciting ones: circular dichroism and scatter-immune absorbance spectroscopy.

Olis®

*US Patent 5,285,254: "Subtractive Double Grating Monochromator with Moving Intermediate Slit"

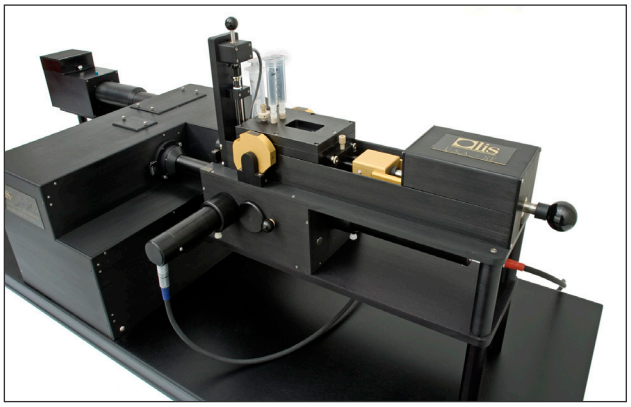
Applications & Techniques

Kinetics, from millisecond stopped-flow to sub-microsecond photolysis

"1000 scans per second is just the beginning."

With millisecond scan rates, microsecond fixed wavelength, nanometer resolution, milliabsorbance sensitivity, perfect reproducibility, and easy modularity, the Olis RSM 1000 has every optical, photometric, and operational parameter optimized for ambitious kinetic and spectrophotometric research.

Stopped-flow is the most common, primary application.



Stopped Flow

Stopped-flow: Olis RSM 1000 + Olis USA stopped-flow mixer

Single wavelength analysis is often incomplete and misleading. With multiple wavelength data, one has a 3D movie of a kinetic process with 2D 'frames' every one millisecond (i.e. 2D frames vs. time). Having this spectral movie of the reaction allows you to obtain **far more precise rate constants** and allows you to make **conclusions about the chemistry** that are impossible to make without spectra.

Designed to scan, detect, and collect up to 1000 scans/second, the RSM 1000 eliminates the need for redundant experiments and the resulting high volume of reactants & associated systematic error.

From your perspective: "Very revealing...comprehensive"

Prof Fred Guengerich of Vanderbilt University shared his experiences as the principal investigator whose large group has used an Olis RSM 1000 with stopped-flow for over a decade.

He ranks this spectrophotometer as ideal for his lab's range of research pursuits, including kinetic studies involving DNA polymerase and cytochrome P450.

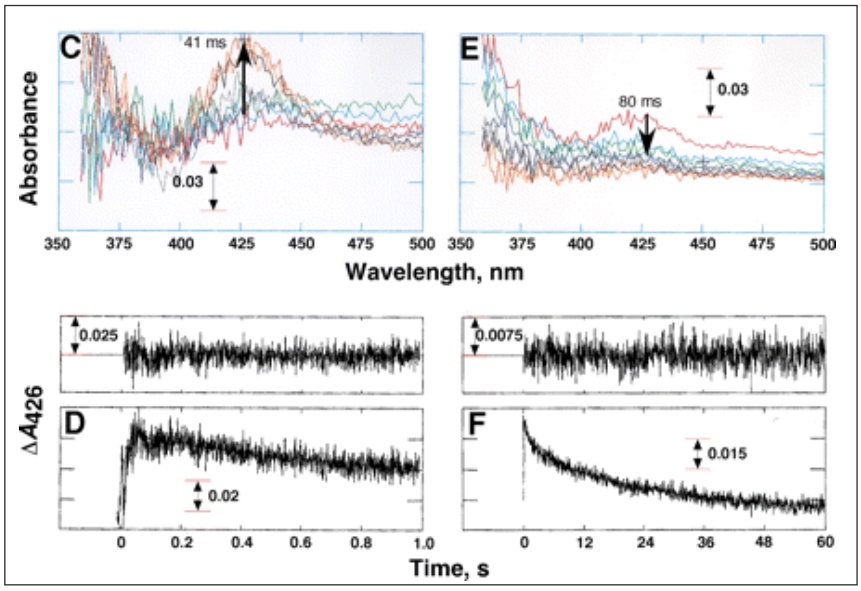
Dr. Guengerich values being "able to collect all the scans" because "[they] reveal such a comprehensive picture of the reaction process." Speaking of one of his 2006 publications, he recalls a multi-phasic substrate binding with P450 as "something we had been trying to do over 10 years and the results were very revealing."

"Very reliable and robust."

Binding of pyrene to ferrous P450 1A2

(C) P450 1A2 (5 μM) was mixed with 1 μM NADPH-P450 reductase (and 75 μML-α-dilauroyl-sn-glycero-3-phosphocholine) and reduced anaerobically with an NADPH-generating system. In the experiments shown, the final concentration of pyrene (after mixing) was 20 μM. Difference spectra were collected every 1 ms and are shown for 5-ms intervals up to 41 ms. (D) ΔA426 data were fit to a biexponential expression (absorbance increase, then decrease) with k1= 106 ± 11 s-1 and k2 = 1.0 ± 0.3 s-1. (E) latter phases of reaction after mixing. The first spectrum shown was collected at 80 ms, and subsequent spectra were collected every 2.4 s. (F) data from E were fit to a biexponential decrease in A426 with k2 (corresponding to second part in B) = 0.66 ± 0.11 s-1 and k3 = 0.041 ± 0.002 s-1.

Published results from the lab of Prof Fred Guengerich, Director of the Vanderbilt University Center in Molecular Toxicology



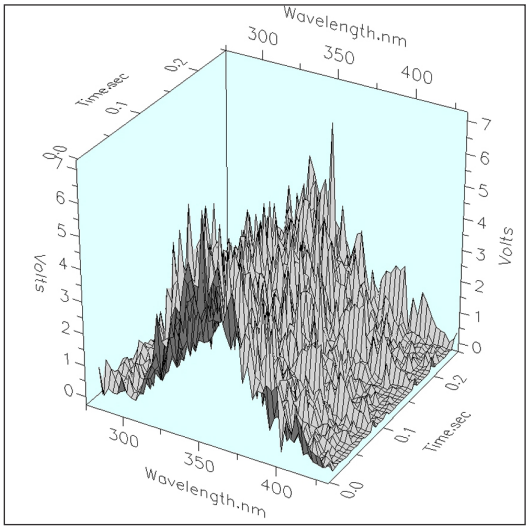
Journal of Biological Chemistry. (2008) Mar 14, 283(11):7293-308.

Fluorescence stopped-flow

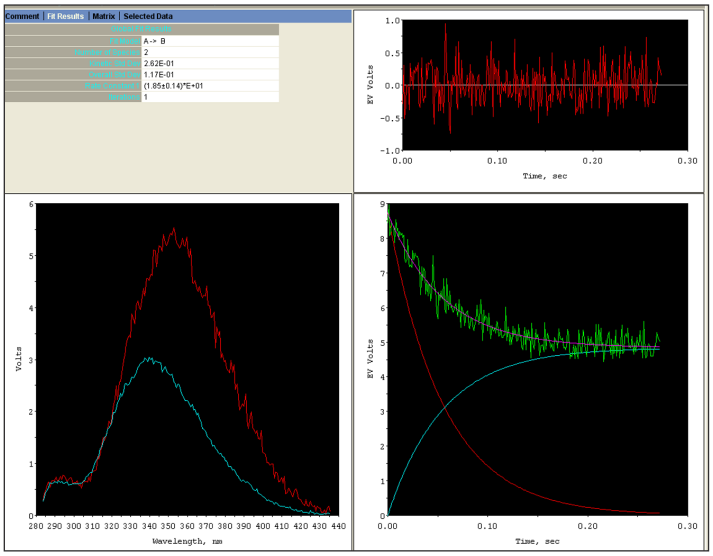
Shown here are data we acquired in house back in the early 1990s using an absorbance model Olis RSM 1000. We retain these emission spectra as illustrative because they highlight a very fast stopped-flow case, they show how effective global data analysis is in reducing noise, and they remind us that even "simple proteins should be studied with multiple wavelength data." (As noted by Celia Bonaventura)

Notice the wavelength shift as well as the intensity shift from the original form to the final product. We can make a very satisfactory conclusion about the folding of this protein and the shift in fluorescence emission as the protein shape changes. This information could not have been deduced from fixed wavelength results.

One stopped-flow shot. 250 milliseconds worth of spectral data acquisition. An instant of data fitting.



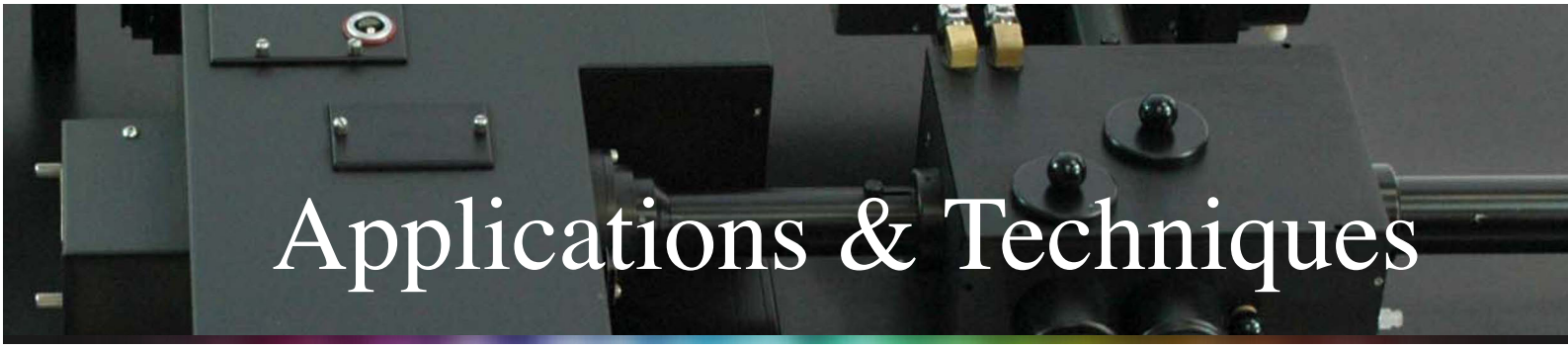
Raw data from 250 ms reaction



Starting and final spectra

Three-dimensional data at left shows the full 250 ms movie.

In the second image, information in the upper right-hand box includes the overall residuals, and kinetics of the appearance and disappearance of the folded and unfolded forms of the protein are plotted in the other charts.



Applications & Techniques

Kinetics continued, Flash Photolysis

Laser/ Flash Photolysis: Olis RSM 1000 + Actinic Source

When the moving intermediate slit is exchanged for a static intermediate slit, the RSM changes from continuously scanning a spectrum every one millisecond to working much like other monochromators, but its fast electronics and detectors can now support sub-microsecond acquisition rates, perfect for laser/ flash photolysis experiments.*

From your perspective: "The RSM 1000 excels"

The research group of Prof Andrew Pacheco, University of Wisconsin, Milwaukee, uses laser photo-initiation to catalyze some of their reactions. They follow kinetics with durations of 20 microseconds to tens of seconds.

In his own words, Dr. Pacheco explains how the RSM's design benefits his research: "Unlike most commercial laser photolysis systems, the RSM 1000 is a dual-beam instrument, so that the baseline stays stable over the longer timescales. More specialized systems are better for very short timescales (less than 20 microseconds), but produce poor results above the ms range because of baseline drift.... the RSM 1000 excels in situations where one must go out to tens of seconds in addition to collecting data on the microsecond timescale."

Dr. Pacheco uses the full range of acquisition modes the RSM offers. About the data at right, he says:

Figure 1 shows nitric oxide generated from a photoactive precursor using a laser pulse. The reaction was monitored using Olis rapid-scanning mode. Spectral changes show the reaction of nitric oxide with Mb over 10 ms as well as oxidation of Mb to metMb over 100 ms. Data were analyzed using singular value decomposition (SVD), which is featured in Olis GlobalWorks software [see page 11].

Figure 2 shows nitric oxide generation from the same precursor as Fig. 1. Spectral changes show the reaction of nitric oxide with MbO_2, collected on the μs timescale. Spectra were generated by combining single-wavelength absorbance vs. time traces collected in μs.

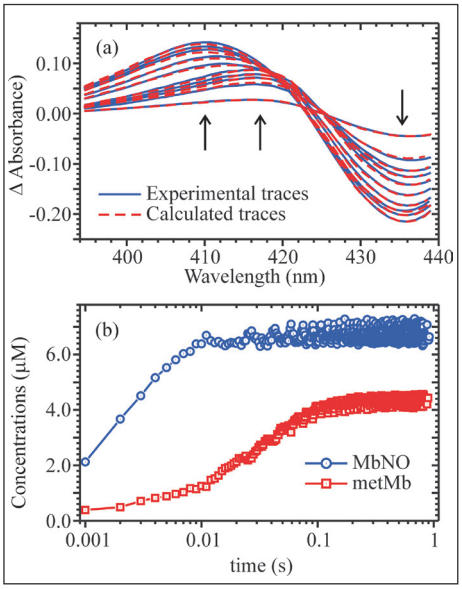


Figure 1, Cabail et al, J. Phys. Chem. A (2007) Vol 111, pp 1207 – 1213.

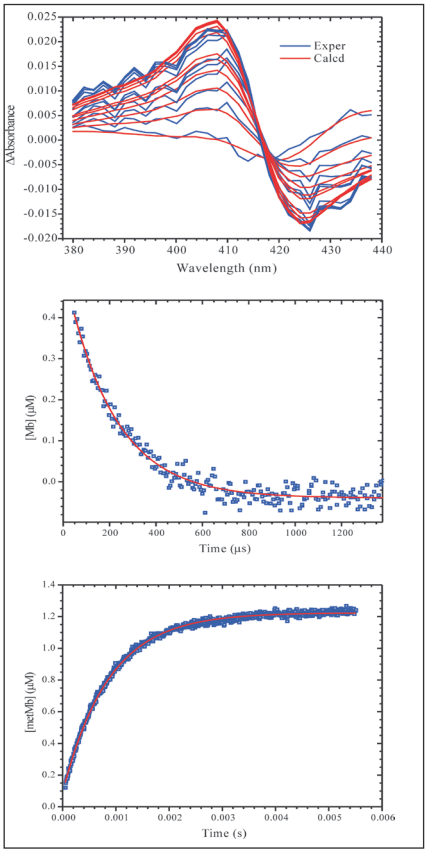


Figure 2, Data yet unpublished

*In order to protect the detectors from bright actinic sources, certain precautions are advised. See, for instance, page10 and the ShutterDisk introduction.

Circular Dichroism

Circular Dichroism (CD): Olis RSM 1000 + DSM* CD sample compartment

The same microsecond fixed wavelength detection rate that makes the DeSa monochromator perfect for laser/ flash photolysis is what makes it perfect in a modern, digital CD spectrometer.

The high-speed electronics support collection of one million dual beam data points per second. Half of these data points are acquired while left circularly polarized light passes through the sample and half when right circularly polarized light does.

This direct digital acquisition of abs(L) and abs(R) data allows true "Digital Subtractive Method" (DSM) CD collection. This method is preferred over the common modulation method because **DSM requires no calibration (correction) factor**, and the modulation method does. Inaccurate calibration is one of two major contributions to error in CD data acquisition. Variable cuvette pathlength is the other.

*DSM is exclusive to Olis, Inc. This method alone returns the exactly correct answer.

Under some conditions, rapid-scanning rates up to 62 CD scans per second makes sense with the Olis DSM 1000 CD. Such cases include work in the visible and NIR.

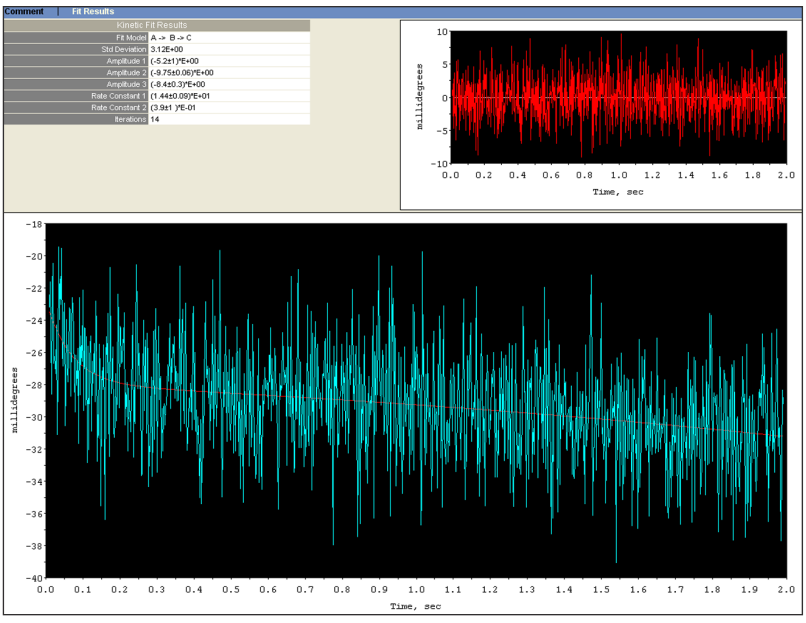
From your perspective: "I've always been impressed with the quality of data"

Prof Andrew Mehl, Knox College, uses the Olis DSM 1000 to monitor protein folding. His lab is interested in investigating the pathway of protein folding for oligomeric proteins.

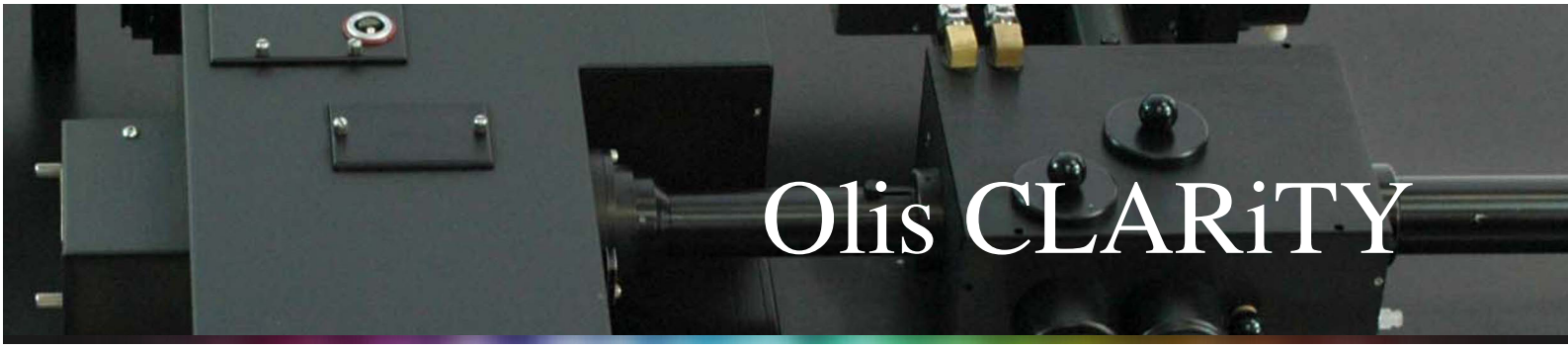
Dr. Mehl notes that he has "always been impressed with the quality of the data" that the Olis DSM 1000 produces as well as with "the software [Olis SpectralWorks] that allows for data analysis."

The figure at right shows the fitted results and residuals of CD stopped-flow spectra Dr. Mehl acquired using the Olis DSM 1000. The data reflect the average of 6 stopped-flow shots taken at 220 nm. The data "suggests a two-step process for the refolding of the [GrpE1-112 deletion mutant protein]."

These results are preliminary. The data are yet unpublished.



Six CD stopped-flow shots, 2-second acquisition time each. Rates returned are (14.4±0.9)s-1 and (.39±1)s-1.



Olis CLARiTY

Study living, whole cells

Scatter Immune Absorbance: Olis RSM 1000 + CLARiTY sample compartment

The use of the Olis RSM 1000 with the CLARiTY chamber creates a workstation of stunning performance.

Now, for the first time, one can work with organelles and whole living cells, watching as these suspensions interact with internal and external stimuli.

No longer are you limited to reduced and purified samples isolated from their natural environment. Now, you can work with intact living cells, aggregating systems, tissue suspensions, nanoparticles, and other historically 'impossibly' turbid suspensions.

The optical characteristics of the RSM 1000 – true dual beam detection, low stray light, high photometric precision, and high speed scanning – make it perfectly suited for 'real time' studies of cell metabolism and other dynamic processes on living intact cells and organelles.

The CLARiTY handles non-biological, static turbid samples equally well.

Unique to the Olis CLARiTY

The measurement beam is diffused into a "gas of photons" in the DeSa Suspension Presentation Cavity (DSPC). The DSPC includes a quartz cavity, suspended in a highly reflective material.

Illustrated at right, the quartz cavities can be sized and shaped specific to the suspension or solid sample.

Second to 100% immunity to scatter is the tremendous effective pathlength enhancement of the DSPC versus a common 1 cm^2 cuvette. The 4 mL volume vesicle achieves 20 cm; the 8 mL, >30 cm!

Four quartz cavities shown here, outside of DSPC holder

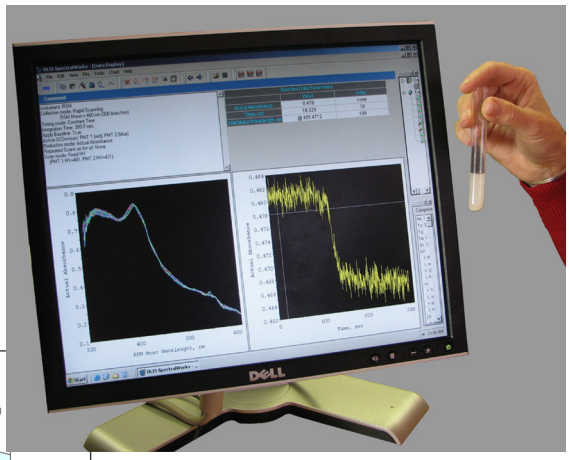
For the first time, you can collect accurate, actual absorbance of highly turbid samples

"This system is an almost incredible advance on the best Britton Chance was ever able to do for turbid suspensions."
-Prof Em Quentin Gibson, July 2010

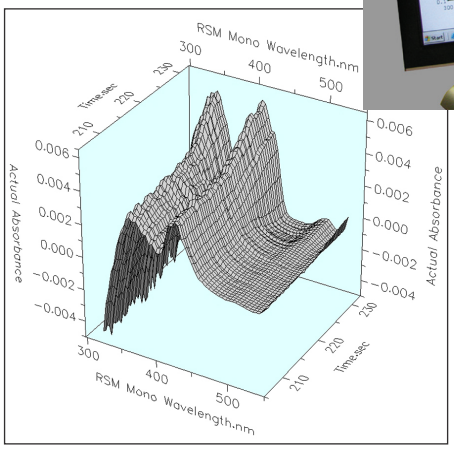
These spectra were captured with an Olis CLARiTY 1000 using the sample in the test tube, which is a 5 mg/mL suspension of living, intact *S. cerevisiae* cells in water.

The electron transport chain – captured here from the UV into the visible – was captured using subsecond scan rates of the cells.

Such spectra are not possible on any other existing absorbance UV/Vis, NIR spectrophotometer.



The 3D display shows the 20 second transition period in the approximate midpoint of the full 5 minute study as the cells became anaerobic.



Three seconds, correct spectrum

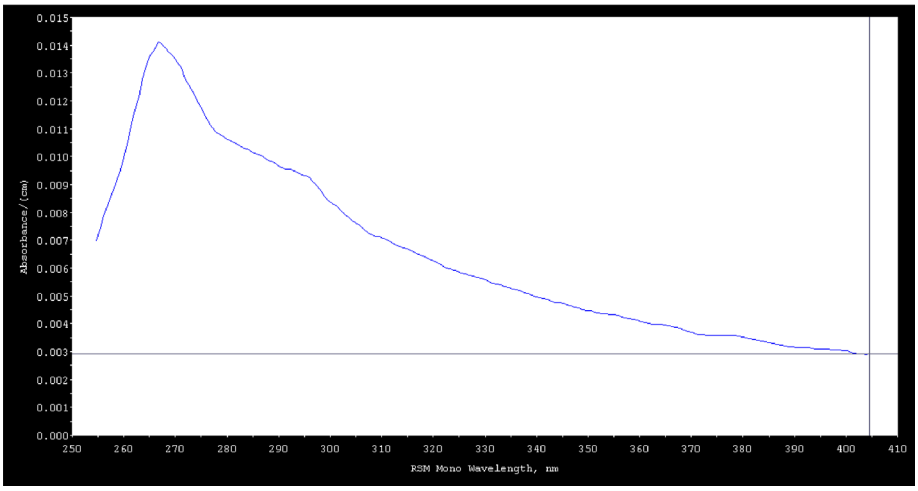
The CLARiTY produces beautiful results for steady-state studies, too.

The CLARiTY returned the correct absorbance and a very descriptive spectral shape on a milky protein suspension, given to us by Prof David Lynn's group (Emory University).

Their conclusion:

"Wow! I was not expecting to see a UV maximum at 265 and 295!!...Definitely not something we could see with our UV."

Only an Olis CLARiTY can find the structure hidden behind the scatter



Comparing Alternatives

RSM vs. Other Multiwavelength Detection Choices

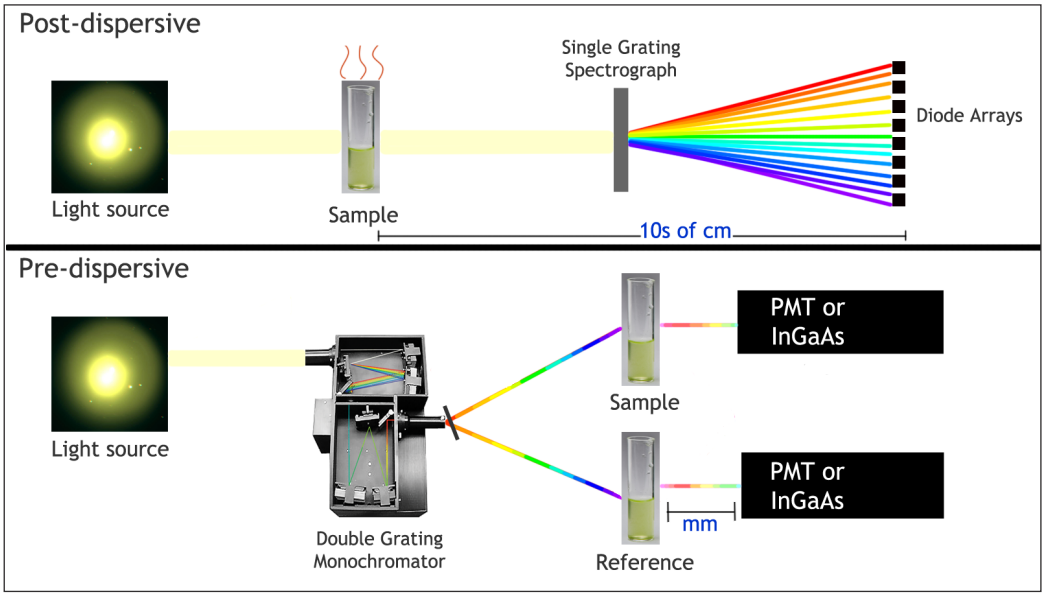
The alternatives to a rapid-scanning monochromator for multiple wavelength data acquisition are diode array detection and CCD systems. Diode array systems are praised for being modern, neat, all-electronic, and having standard, non-movable parts. This is all true. However, diode array systems are only effective when the light levels are high, acquisition rates are modest, and photometric precision is not a priority.

Critically, the intense broadband measurement beam of a diode array causes undesirable and irreversible photolysis in many biological & other photosensitive samples.

Pre-dispersive spectrometers have fewer limitations

The RSM 1000 uses gentle monochromatic light for all measurements, so that photolabile samples can be studied safely and successfully. Each sample is measured with, and only with, exactly the wavelengths desired. Broadband light and stray light – i.e., light beyond what you want to use – are never a concern with pre-dispersive rapid scanning systems.

Diode Array vs. RSM



Why monochromatic illumination is important:

- Molecules might be very sensitive to certain wavelengths and be caused to change in undesirable and irreversible ways.
- Molecules respond to precise wavelengths. Measuring them with additional wavelengths confounds the experiment's exactness.

"One of the reviewers said there is no way that you can do optical stopped-flow studies with a B12 compound, yet there it is, because unlike a diode array, you are not blasting the hell out of the sample with white light in the RSM."

–George Reed of U. Wisc. After obtaining beautiful data on a photolabile B12 compound [Data published in Biochemistry (2000), 39(39): 12069-75.]

Comparing Alternatives, continued

Higher sensitivity

Photomultiplier tubes (PMTs) are the first and best choice used in all premium UV/Vis spectrophotometers.

- Unlike small and insensitive diodes, which must be used in an array, PMTs and InGaAs detectors have large detection surfaces
- Milliabsorbance sensitivity in milliseconds is routine
- Controllable gain settings for maximum dynamic range utility

- Useful deep into the UV (≤180 nm) and to the NIR (> 850 nm)
- Useful to sub-microsecond acquisition rates with Olis electronics; to nanoseconds when used with digital oscilloscopes

Broader spectral range utility

A diode array is useful for UV/Vis absorbance. Only.

An Olis RSM can be configured for use in the far UV – as far as <170 nm for CD – through the NIR, to 1700 nm and even 2500 nm.

Primary applications:

- Stopped-flow mixing
- Flash photolysis
- Circular dichroism
- CLARiTY

RSM configured for absorbance and fluorescence

Other applications not addressed in this brochure:

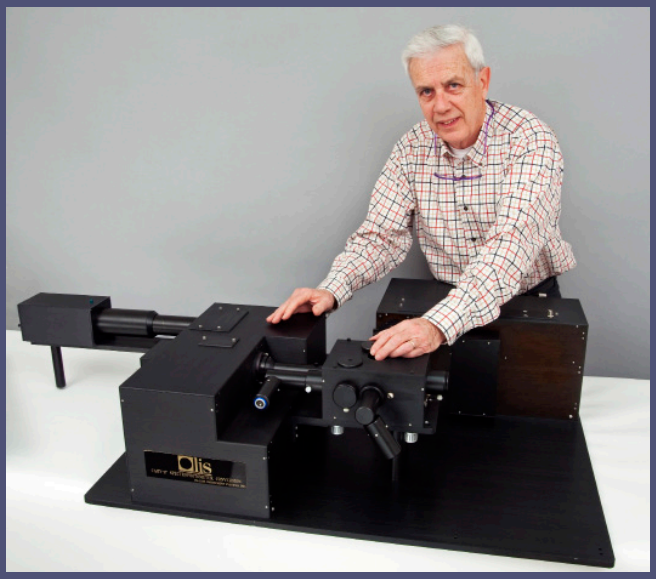
- Polarization of fluorescence
- Anisotropy (r)
- Circularly polarized luminescence (CPL)
- Linear dichroism (LD)
- Specular reflectance
- Diffuse reflectance

Employing Two RSMs

The most ambitious configuration of the RSM series is the TWIN, which employs two rapid-scanning monochromators. Installations thus far were for Prof Ross Middaugh (University of Kansas), who has the first Protein Machine, and Dr. Roger Prince (ExxonMobil), who will use his TWIN in a CLARiTY mode.

The two DeSa monochromators can be scanned synchronously or asynchronously to produce extraordinarily special spectrophotometers.

- Scanning is slowed to a top rate of 250 scans/second
- Interlaced absorbance and fluorescence scans at rates up to 100 scans/second
- Absorbance scanning to 8 AU
- Resonance light scattering (RLS)



Richard DeSa (Chief) with Twin RSM and CLARiTY detector

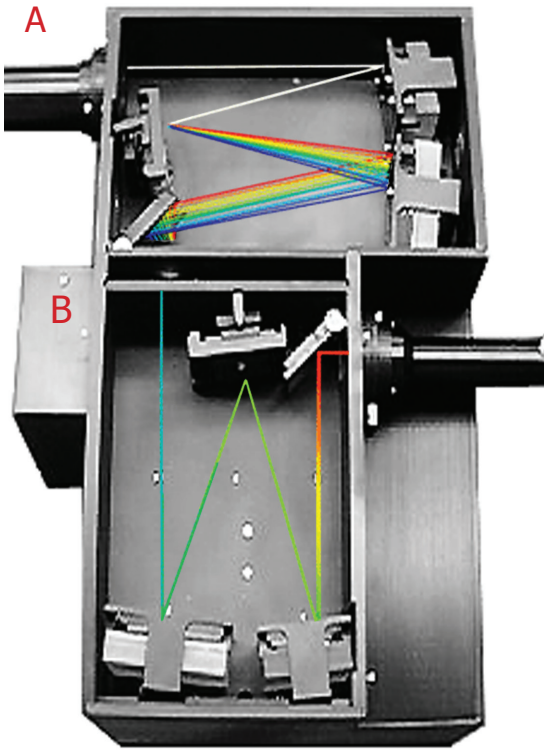
Components & Software

Standard components of an Olis RSM 1000

- DeSa "Subtractive Double Grating Monochromator with Moving Intermediate Slit"*
- 75 or 150 watt Xenon arc lamp
- Two 50 mm gratings, blazed at the wavelength to suit your spectral range requirements
- ScanDisk, interchangeable with StepDisk, useful for fixed wavelength and normal scan rates
- Sample chamber, choose stopped-flow, CD, CLARiTY, or other
- Two photomultiplier tubes, two InGaAs, or both pairs
- Olis SpectralWorks software (see facing page)

A DeSa "Subtractive Double Grating Monochromator with Moving Intermediate Slit"

This patented monochromator features stationary optics during rapid-scanning, low stray light, high light throughput, a homogeneous output beam, and bandwidth determined by the entrance, exit, and moving intermediate slit (options below). All optics are usable throughout the UV/Vis/NIR range.



B 3 intermediate slit cartridge options:

ScanDisk

- Flywheel motor for highest speed
- 1000 scans/sec
- Useful for millisecond absorbance & fluorescence

StepDisk

Stepping motor for highest precision stepping between wavelengths
Useful for:

- CLARiTY measurements
- Flash photolysis
- Conventional slow scanning

In a novel application, a large aperture ScanDisk, also called a ShutterDisk, is positioned to protect the detectors from the bright actinic source during a laser/ flash photolysis experiment. The sample alternately sees the measurement beam or the actinic source, and the detectors are not blasted with the bright actinic beam because they are shuttered during the flash period.

*US Patent 5,285,254

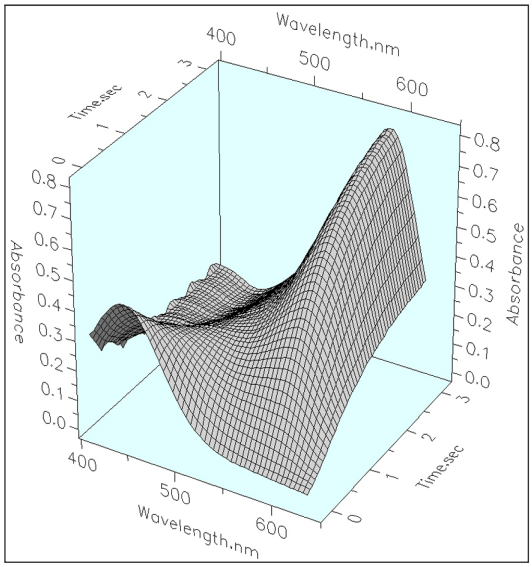
SpectralWorks Software

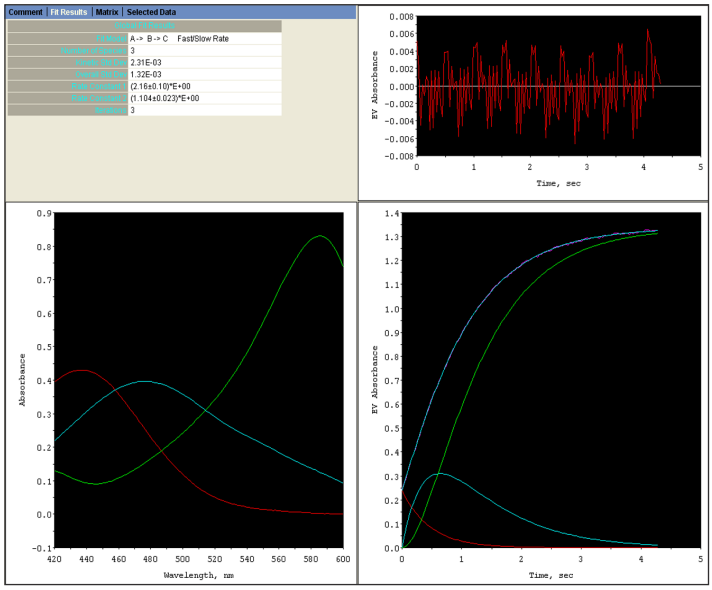
Olis SpectralWorks is our proprietary and ever-evolving program for the dynamic display and analysis of 2D and 3D data files collected as a function of time, temperature, titration, pressure, or other process.

The analysis portion of the software employs Singular Value Decomposition (SVD), Downhill Simplex, and Matrix Exponentiation, a combination of algorithms that allow the researcher to make accurate conclusions from multidimensional data instantly.

In the two examples below, we show 3D raw data (left) collected with SpectralWorks and the calculated residuals, kinetics and spectra produced after applying SVD (right).

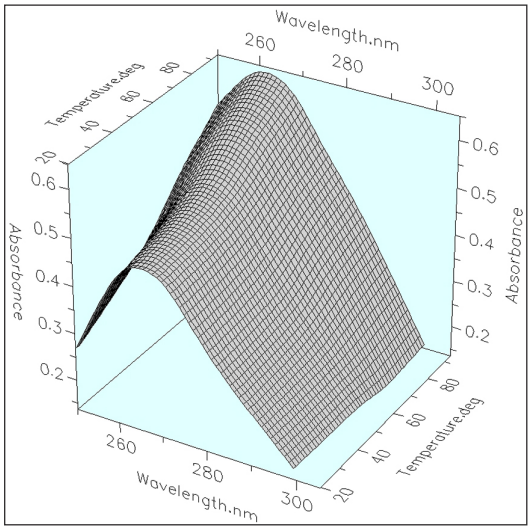
Kinetic fit to single stopped-flow shot

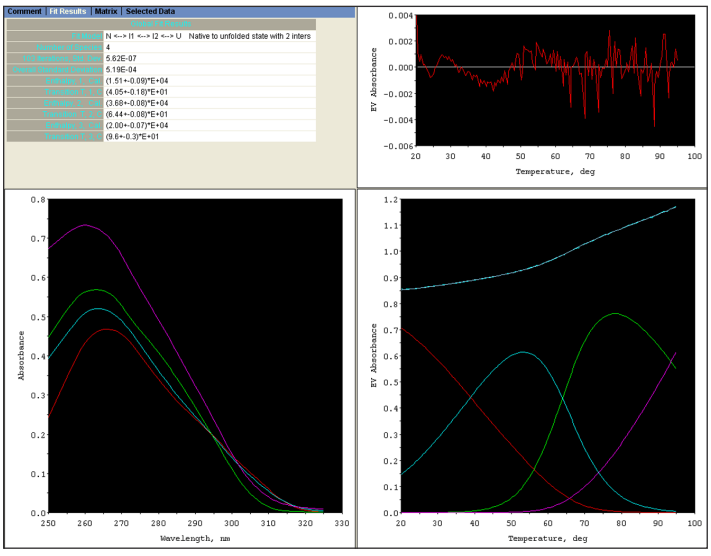




Spectra acquired by Prof Robert Blake, Xavier University using an Olis RSM 1000 with stopped-flow.

Equilibrium fit to thermal denaturation of nucleic acid

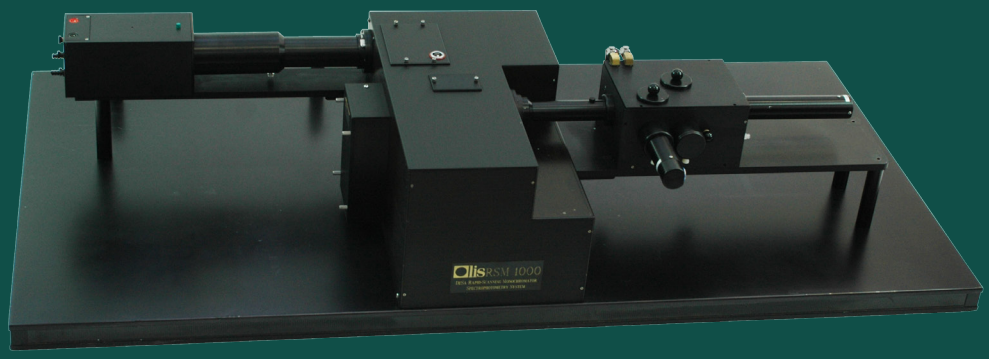




Spectra acquired by Prof Brad Chaires, University of Louisville Medical School, using an Olis computerized HP 8452 diode array.

"You have created a marvel of simplicity, functionality, and versatility, all in one."

Brian G. Fox, University of Wisconsin



"You did everything right" -Bettie Sue Masters, UTHSC, San Antonio

- Millisecond scan rates over hundreds of nanometers with 1 nm resolution
- Sub-microsecond fixed wavelength, optional
- Exquisite sensitivity throughout chosen UV/Vis, Vis, or NIR spectral range
- Superb stray light rejection (< 0.0001%)
- Photomultiplier tube detection (InGaAs detection for NIR)
- Dark current acquisition between every dual beam scan
- Homogeneous output beam(s)
- Easy optimization for many different experiments
- The best algorithms available for 2D & 3D data handling and analysis

For more details about the Olis RSM 1000 spectrophotometer series and other Olis products:

www.olisweb.com
sales@olisweb.com
1-706-353-6547 (Worldwide)
1-800-852-3504 (US & Canada)

Olis, Inc. | 130 Conway Drive | Suites A, B, & C | Bogart, GA 30622 | USA