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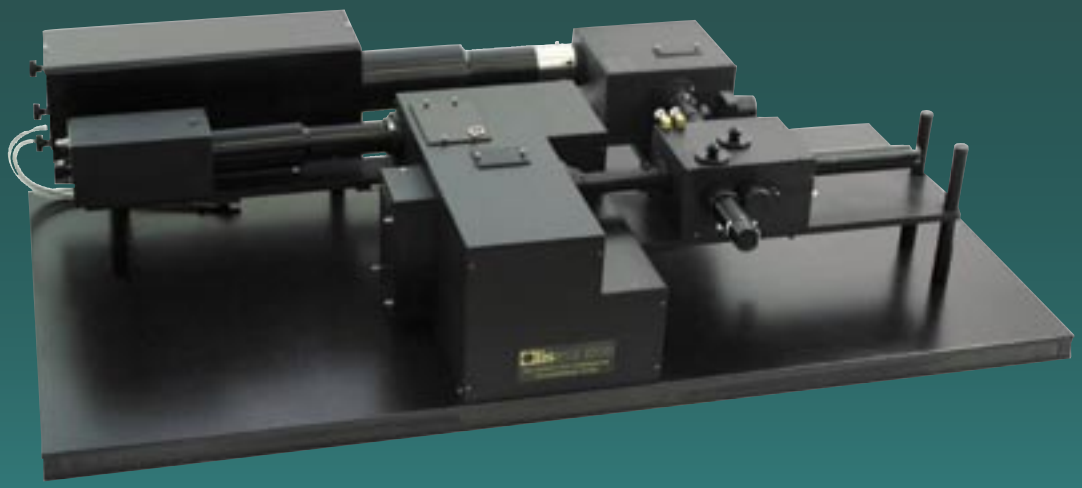
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The Olis® RSM 1000 Spectrophotometers



1000 Scans Per Second is Just the Beginning!

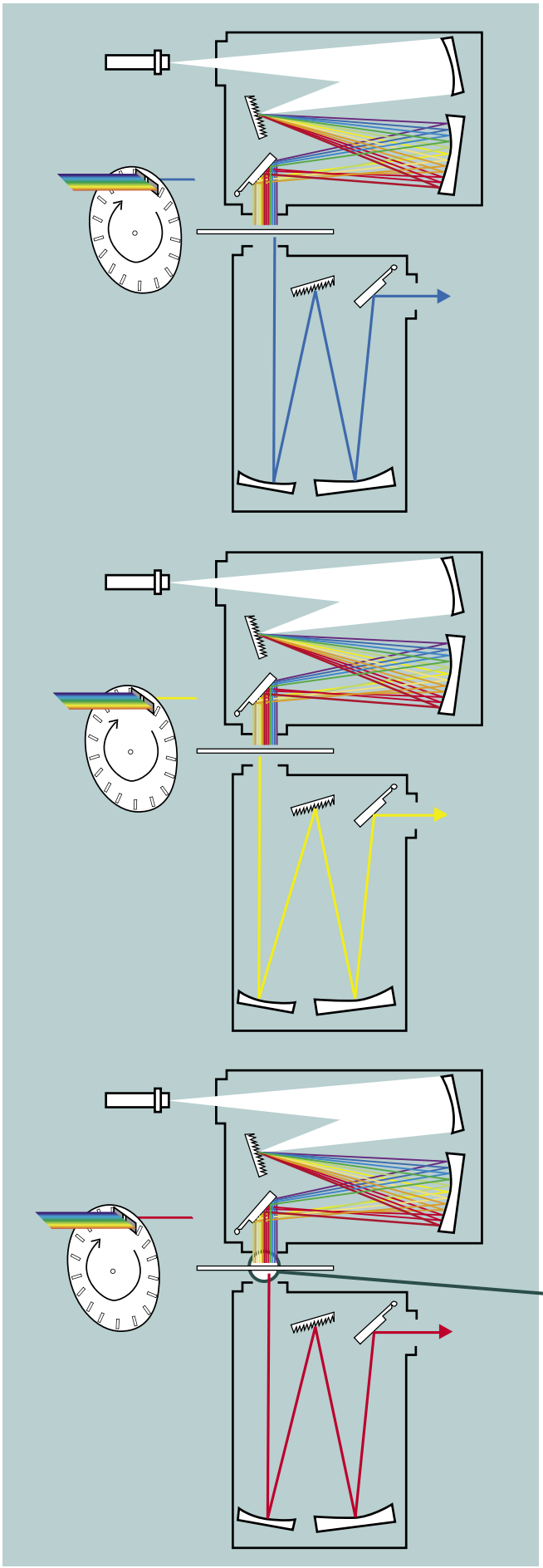
Since 1994, Olis RSM 1000 spectrophotometers have been selected by top research laboratories involved in stopped-flow, flash photolysis, and other challenging data acquisition projects. With the Olis RSM 1000, high speed kinetic studies can be followed with millisecond scans, not just fixed wavelength traces. Rate constants calculated from 3D data are more precise than those calculated from 2D data, and chemical models can be confirmed or rejected by examination of the same kinetic spectral data set.

Today, many Olis RSM 1000 spectrophotometers are configured for steady-state absorbance, fluorescence, and circular dichroism spectroscopy. Here, all of the high speed electronics are used with extensive over-sampling, resulting in excellent sensitivity and low noise.

The open-architecture design allows sensible and effective modularity, so that one RSM can be used for rapid-scanning absorbance stopped-flow in the morning and steady-state circular polarized luminescence with only minutes of hardware rearrangement in between.

Whatever the measurement, be confident that you are using the premier optical bench available for microsecond and slower data acquisition modes, **optimized for 1,000 scans per second and ideal for so much more.**

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A Breakthrough Monochromator is the Center of all Olis RSM 1000 Spectrophotometers

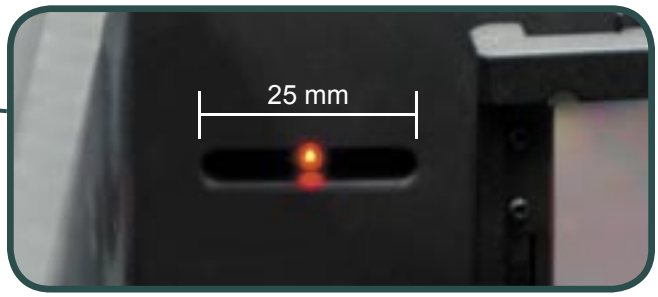
The patented DeSa[2] monochromator spends all day long scanning 1,000 times per second over a spectral range in the UV, Visible, or NIR region with milliabsorbance sensitivity and absolute reproducibility.

All optics are fixed in position. Scanning is effected by moving a (narrow) slit across an (25 mm) aperture at the intermediate plane of the double grating monochromator.

This mode of scanning is possible only with a "subtractive" double grating monochromator.

In addition to millisecond spectral scanning, the DeSa "Subtractive Double Grating Monochromator with Moving Intermediate Slit"[1] produces a homogeneous output beam, very low stray light, very high photometric precision and accuracy, and a dark reading between every scan.

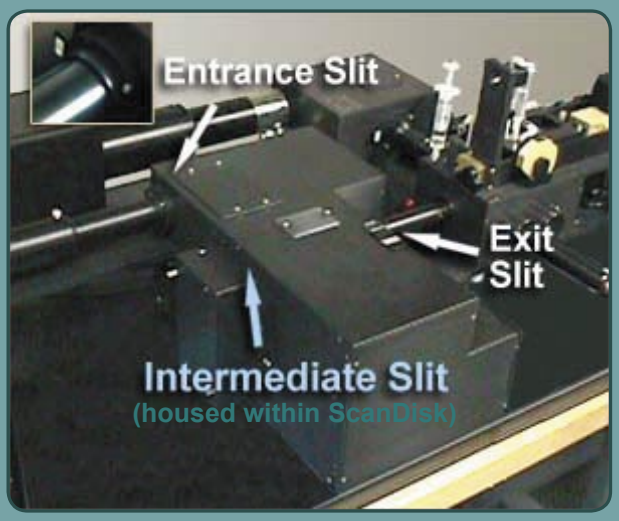
The ScanDisk has 16 slits and spins at 62.5 Hz to achieve the 1000 scans per second.
(16 * 62.5 = 1000)
Slits are spaced to allow a 50 microsecond dark period between each scan.



[1] US Patent 5,285,254, issued February 8, 1994.
[2] DeSa, originally de Sá, is the family name of Dr. Richard J. DeSa, and retains the Portuguese pronunciation (approximately, di Sah or day Sah).

Unique Scanning Mechanism is Responsible

As a double monochromator, the DeSa has three slits: an entrance, intermediate, and exit. Entrance and exit slits could be any width, often 1.24 mm or less.



Our innovation is to house the intermediate slit in a motorized “ScanDisk.” The 0.2 or 1 mm slit version is on the left; the spoke version is shown at right. A SpokeDisk is available to realize the Fellgett effect.



With the lid of the monochromator removed, one can see the 25 mm aperture at the midplane of the monochromator where the intermediate slit blocks all of the light except that behind the slit itself (photo on facing page shows the light passing through the intermediate slit).

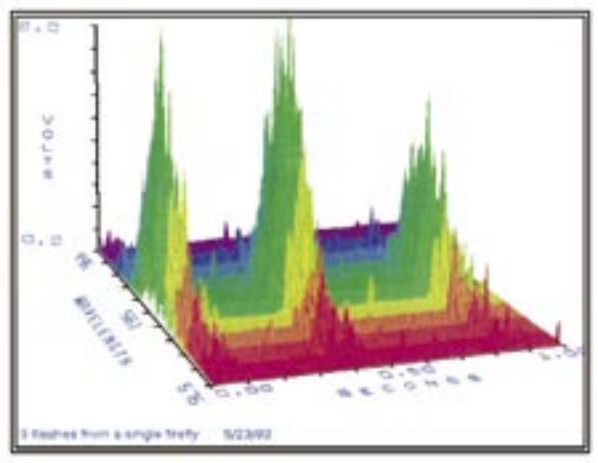


When rapid-scanning is not needed, one inserts a ‘SlitDisk’ which does not spin but which houses a single slit of chosen width, fixed in position. Now 50 microsecond and slower kinetic traces can be collected at a single wavelength,[1] wherein ‘scanning’ is simulated and/or done by instructing the monochromator to move the wavelength between collection of successive fixed wavelength traces.[2]



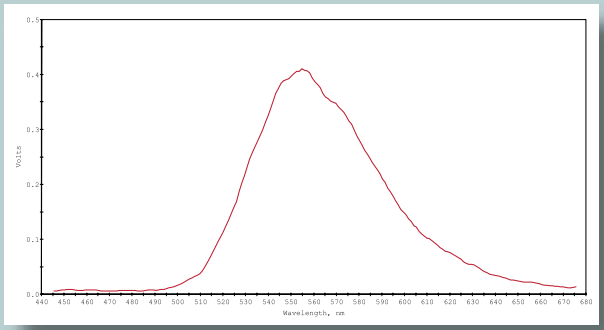
[1] Acquisition rates to 20 MHz can be handled by the Olis RSM 1000, making it ideal for microsecond acquisition following laser flash photolysis, for instance.

[2] Such cases are most common in circular dichroism, where one scans from 260 nm, where there are many photons, to below 180 nm, where photons are few.



One firefly, one second of data collection, and a little computation to produce an excellent emission spectrum from raw data comprised of three different amplitude flashes by the animal.

The firefly was placed in the sample cuvette of the rapid-scan fluorimeter; it was considerably annoyed by this and flashed 3 times in rapid succession. 1000 scans captured the flashes.



The kinetics of the flashes cannot be fitted, but one can do SVD and a simple fit (e.g., A→....) to construct of the emission spectrum.

Add the step digital filtering (modern smoothing) to achieve the correct emission spectrum shown above.

These data illustrate millisecond spectral scanning, in a low light situation, with sharp spectral resolution. There is no other technology instrument which can match this performance.

Only Desirable Optical Characteristics

- The Olis RSM 1000 is a premium grade double monochromator for high resolution, high speed, high precision data acquisition.
- All readings are dual beam, ensuring greatest noise reduction.
- The measuring beam is monochromatic, so that the sample sees only the light intended, protecting against unintentional sample photolysis. Rapid-scanning of highly photolabile samples such as B12 is successful.
- The detectors are positioned millimeters from the sample, so that accurate readings on light scattering samples are achieved.
- Detectors are chosen to optimize for the spectral range and data acquisition rate. Choices are photomultiplier tubes (PMTs), red-sensitive PMTs, InGaAs detectors, and photon counting detectors.
- Xenon arc lamps of 75, 150, and 450 watt provide brilliant light throughput across the entire UV/Vis/ NIR regions.
- Gratings are chosen to optimize for any spectral range and spectral resolution. Gratings are easily accessed, making it practical to change them between measurements.
- Millisecond emissions scans of low light level signals are exclusively possible with the fluorescence-optimized Olis RSM 1000 series.
- The entrance and exit positions of the DeSa monochromator are reversible, such that the sample can be the source of illumination.
- Ports are available to mount a laser, flash lamp, or other actinic source at the sample.
- Effectively any sample holder can be added directly or with a custom fit.

Research Spectrophotometers with the DeSa Monochromator

Absorbance, fluorescence, and circular dichroism spectrophotometers are created around the DeSa monochromator. Spectral range, spectral resolution, sensitivity, and other parameters differ for the three experimental cases. Mirrors, gratings, lamps, intermediate slits, detectors, and sample chambers are selected to optimize each Olis RSM 1000 for its mode of operation.

Accessories for stopped-flow are common, since the speed of a stopped-flow reaction is ideally suited to the millisecond spectral scan rate of the RSM 1000. Flash photolysis, using lasers, LEDs, and pulse sources are equally suited to add to the spectrophotometer, with acquisition of microsecond data as successful as millisecond scans.

The designed-in modularity means that one “model” can be reconfigured by any interested party.

A model in development in late 2006 has two DeSa monochromators. One serves as the excitation monochromator and is equipped with a SweepDisk, which sweeps a 250 nm spectral width in one second (or slower). The other monochromator is configured to collect 1000 emission scans per second. By scanning both monochromators simultaneously, one obtains a matrix of 1000 emission scans, collected while the excitation wavelength is varied. This process can be repeated at rates to several Hz.



Olis RSM 1000 for UV/Vis absorbance



Olis DSM 1000 CD with scanning emission module

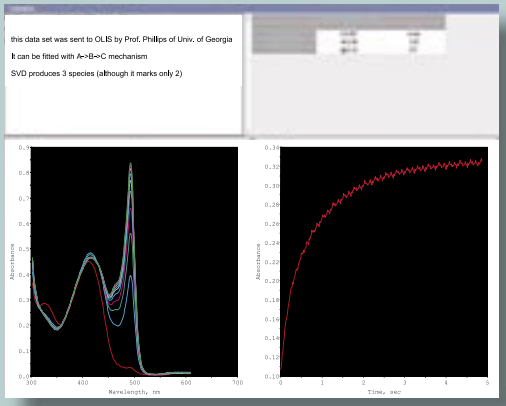


Olis RSM 1000 F4 with stopped-flow

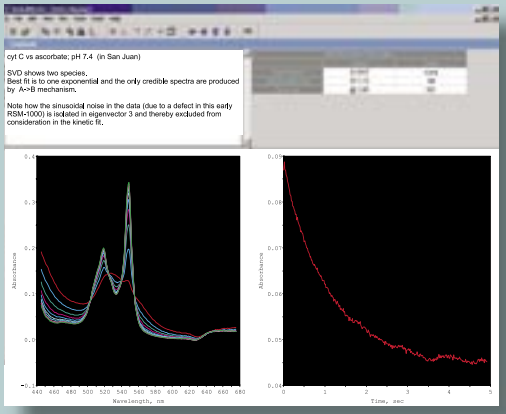
Absorbance

Absorbance is the easiest of the modes, due to the size of the signal. Millisecond scanning is nearly always practical. An intermediate slit of 0.2 or 1 mm is common. Gratings optimized for the UV, visible, or NIR are selected. Photomultiplier tubes provide sensitivity and high-speed detection in the UV/Vis ranges. InGaAs detectors expand utility into the NIR region.

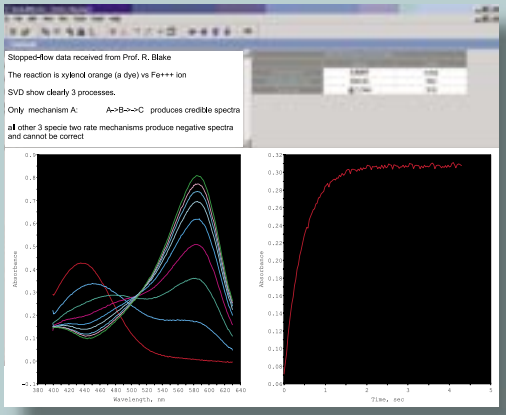
All Olis RSM systems are mounted on 2” thick honeycombed breadboards. These anodized aluminum surfaces provide static and dynamic rigidity. The boards are 20 x 60” or 30 x 60”, depending on the hardware mounted.



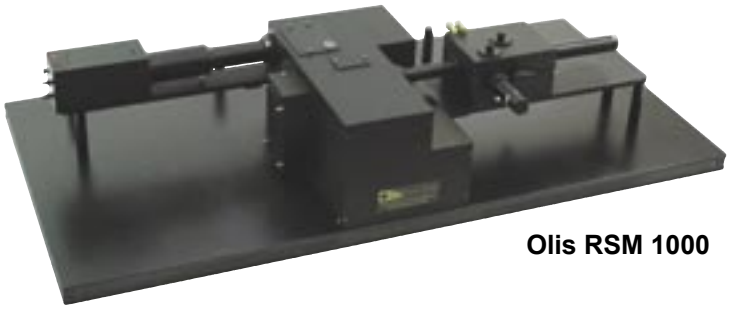
Results provided by Dr. Robert Phillips of the University of Georgia. Shown above, nine scans of hundreds collected during 5 second stopped-flow reaction. Any wavelength from 600-300 nm can be extracted from scans; kinetics at 455 nm shown at right.



Results provided by Dr. Jorge Colon of the University of Puerto Rico. Shown above, nine scans of hundreds collected during 5 second stopped-flow reaction. Any wavelength from 675-445 nm can be extracted from scans; kinetics at 560 nm shown at right.



Results provided by Dr. Robert Blake of Xavier University. Shown above, nine scans of hundreds collected during 5 second stopped-flow reaction. Any wavelength from 625-400 nm can be extracted from scans; kinetics at 514 nm shown at right.



Olis RSM 1000



Olis RSM 1000 with scanning emission module

The first four Olis RSM 1000s were delivered in 1994, one for laser photolysis and three for stopped-flow studies.

Fluorescence

Fluorescence systems are the most variable of the three models, as there are more choices in lamps, detectors and detector positions, and excitation/ emission monochromator combinations. “Fluorescence optimization” can involve as little as a different detector, perhaps a photon counter, to a second lamp, second monochromator, and additional detector. Often, two pairs of gratings are provided, one for broad resolution fluorescence and a second for high resolution absorbance. Shown are three configurations of the Olis RSM 1000 as a spectrofluorimeter.

The Olis RSM 1000 F1 uses a 150 watt Xenon arc lamp as the excitation source for millisecond emission scanning. Absorbance readings are possible, but do not utilize the DeSa monochromator so rapid-scanning is not supported.



Olis RSM 1000 F1

The Olis RSM 1000 F4 is identical to the model F1, except the large 450 watt Xenon arc lamp replaces the 150 as the excitation source for millisecond emission scanning. One does realize the expected 3-fold higher light throughput with this 3x larger excitation source.



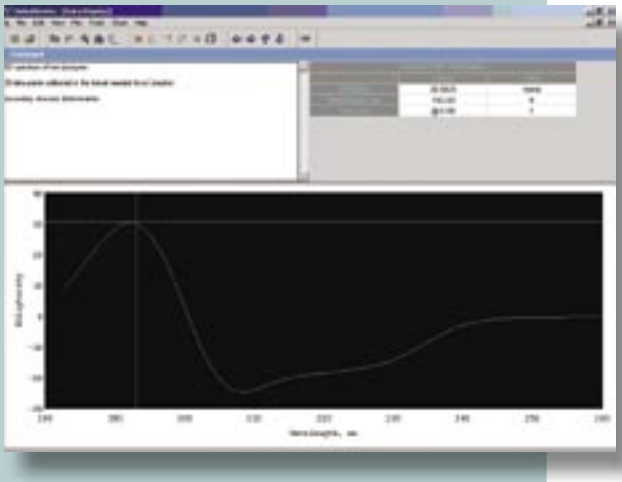
Olis RSM 1000 F4

This version of an Olis RSM 1000 F4 has an additional lamp, which enters the DeSa monochromator so that millisecond absorbance scanning is possible. One will use one lamp and the appropriate detector(s) to collect either rapid-scanning absorbance or rapid-scanning fluorescence with this model.

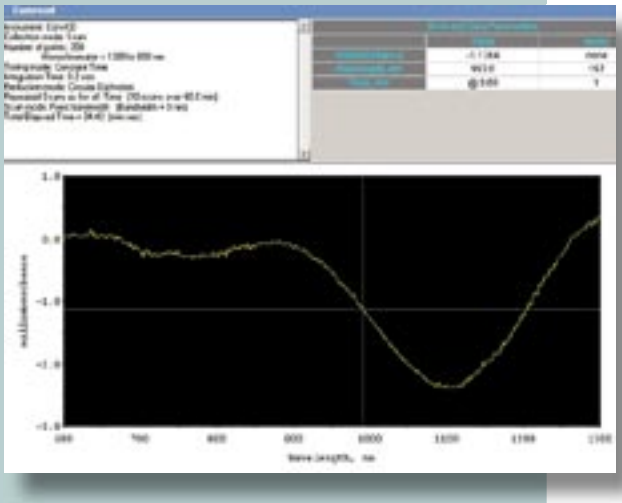


Olis RSM 1000 F4 with dual beam absorbance module

Circular Dichroism



Protein spectrum ready for secondary structure determination



NIR CD scan of nickel tartrate

This model is generally identified as the Olis DSM 1000 CD, as it rarely retains the “rapid-scanning mode” but instead becomes a ‘Digital Subtractive Method’ CD spectrophotometer.

Circular dichroism of proteins is done in the 260-180 nm regions and rapid-scanning across this range is impractical. There are too few photons. And, even more critically, there is much more light at one end of the span than the other.[1] Sweeping a span of ‘bright’ to ‘very dim’ in a millisecond is less than optimal. Thus, effectively all CDs utilizing the DeSa monochromator use the SlitDisk.

Everything about the system is ideal for CD: highest sensitivity, highest light throughput, and broadest dynamic range of any CD yet made. The high-speed electronics are used to collect one million dual beam data points per second. Typically, under 0.5 seconds per point is adequate for low noise data above 195 nm; below this threshold, the software progressively spends more time per point to maintain best RMS noise.

“Digital Subtractive Method” CD spectroscopy is exclusive to Olis, Inc., and secures error-proof CD data acquisition: no calibration and no lock-in amplifier settings mean no potential for uncorrectable systematic error.[2]



The Olis DSM 1000 CD is one of three DSM CDs offered by Olis, Inc., and is the premium model, offering higher light throughput and faster scanning than all CDs by any manufacturer. Under ideal conditions, up to 62 CD scans per second can be acquired!

[1] While this ‘very bright’ to ‘very dim’ situation is most egregious in CD, it might occur in fluorescence and absorbance, too. ‘Normal’ spectrophotometers handle this situation by adjusting the high voltages to the detectors or the slit widths durring the scan. In one millisecond, there is time for neither adjustment. However, when the RSM is fitted with the SlitDisk, adjusting the high volts is done, and the RSM acts like a ‘normal’ spectrophotometer.

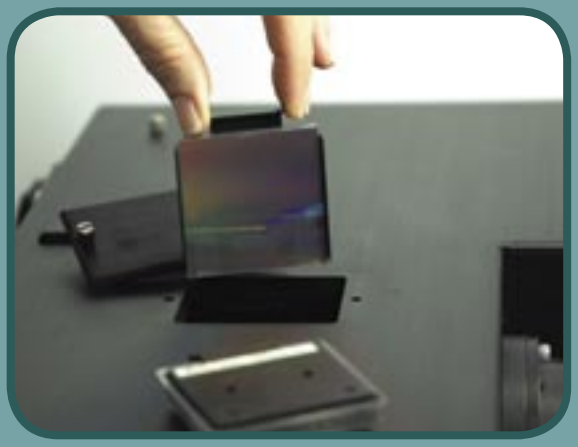
[2] Request the DSM CD brochure for details.

Spectral and Detection Ranges

The spectral range of the DeSa monochromator is fixed by the blaze wavelength of the 50 mm^2 gratings.[1] The detection range of the spectrophotometer is determined by the detectors, either photomultiplier tubes, useful from the lowest wavelengths to approximately 1100 nm, or InGaAs detectors, useful from around 600 nm to as far as 2500 nm.

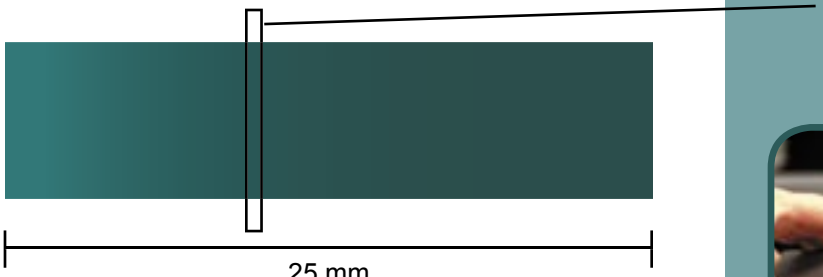
Gratings are accessed through ‘trapdoors’ on the lid of the DeSa monochromator. With the lid removed, the grating can be lifted out and replaced with another. The mountings are cemented in place. The gratings are locked into position with a simple mechanical arm which swings into position.

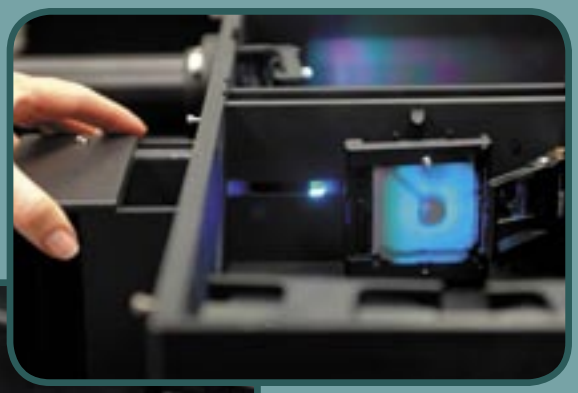


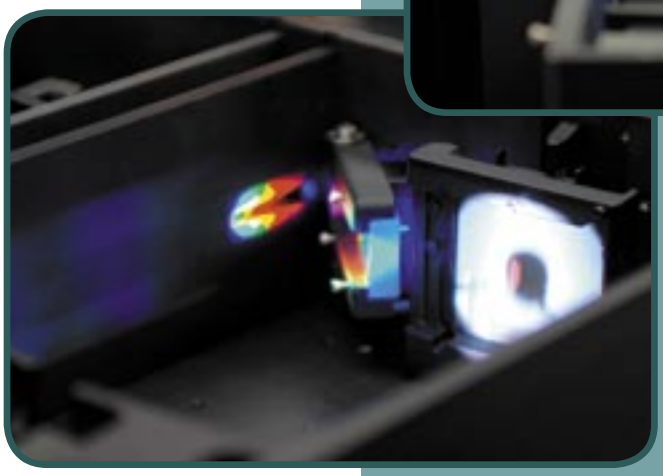


Considerations For Choosing Gratings For The RSM

The span provided by the gratings you choose is spread across the 25-mm ScanDisk aperture. The greater the number of lines in the grating, the fewer the number of wavelengths that will be projected at the aperture, i.e., the better the resolution.







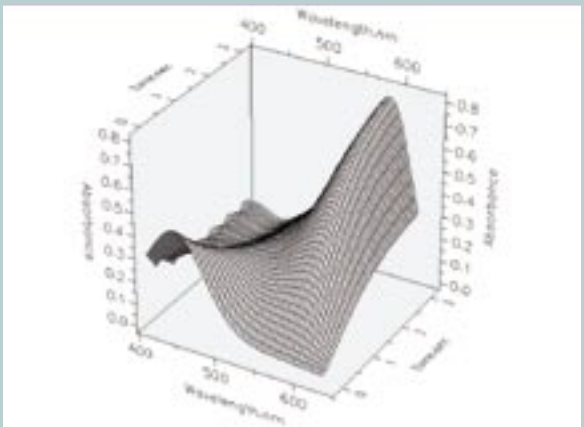
White light enters the first mono and is dispersed into a spectrum, which is then directed into mono 2 through the 25 mm aperture.

View through the 25 mm aperture as the SlitDisk is being withdrawn. (See page 3 for view from mono 2 without a Disk in place.)

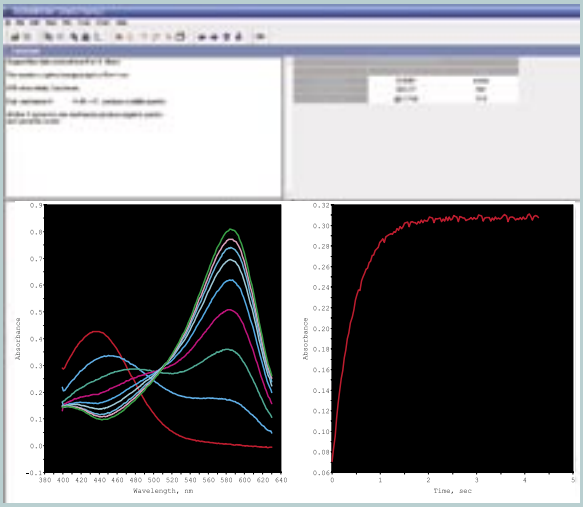
[1] Refer to page 15 for a selection of commonly used gratings.

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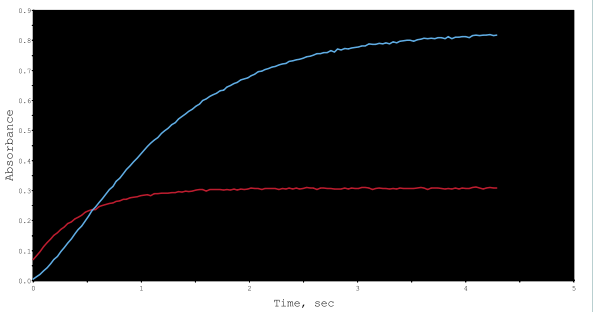
What Benefits Does Rapid-scanning Bring to Kinetic Experiments?



3D presentation of all raw data from one experiment



2D presentation of select raw data from same experiment



2D presentation of results from two fixed wavelength experiments

Single wavelength analysis is often incomplete and incorrect. With multiple wavelength data, one has a “movie” of a kinetic process with ‘frames’ every one millisecond. Having this real-time record of the reaction allows one to make conclusions about the chemistry as well as the rates. And the rate constants will be known with higher precision.

But collecting sufficient data for multi-wavelength analysis with a fixed wavelength kinetic spectrophotometer can take considerable effort and sample.

At left, we see what benefits rapid-scanning brings. The first graph shows the raw data from one stopped-flow shot. The second graph shows the same data in a 2D display. A kinetic trace, as shown in the right-hand graph, can be pulled from any of the hundreds of wavelengths of the kinetically acquired scans. One places his cursor on the wavelength in the spectral plot and out pops the kinetic trace at that wavelength. And, using the ‘dynamic’ setting, one can scan along the kinetic trace and watch the chemistry change with time.

The third graph shows the raw data from two stopped-flow shots taken in a traditional fixed wavelength mode. Twice as much sample was used for these two kinetic traces as was used for the far more informative multiple wavelength data!

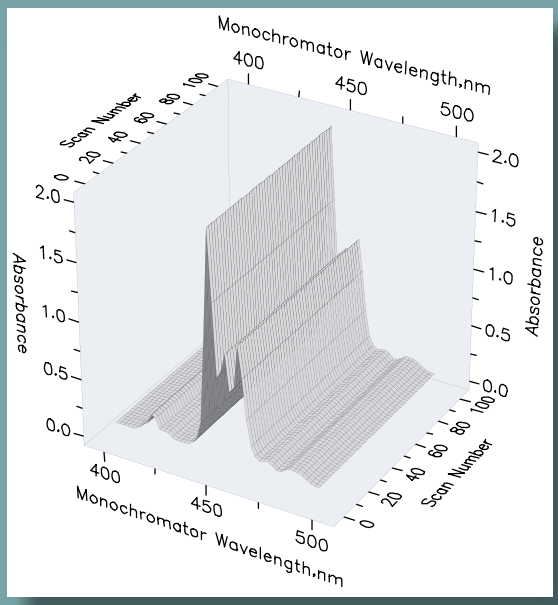
Olis RSM 1000 systems can achieve precisely such millisecond results in fluorescence, too, as well as NIR absorbance and fluorescence, and (under certain conditions) in CD, CPL, and FDCD.

What Alternatives to the Olis RSM 1000 Are There?

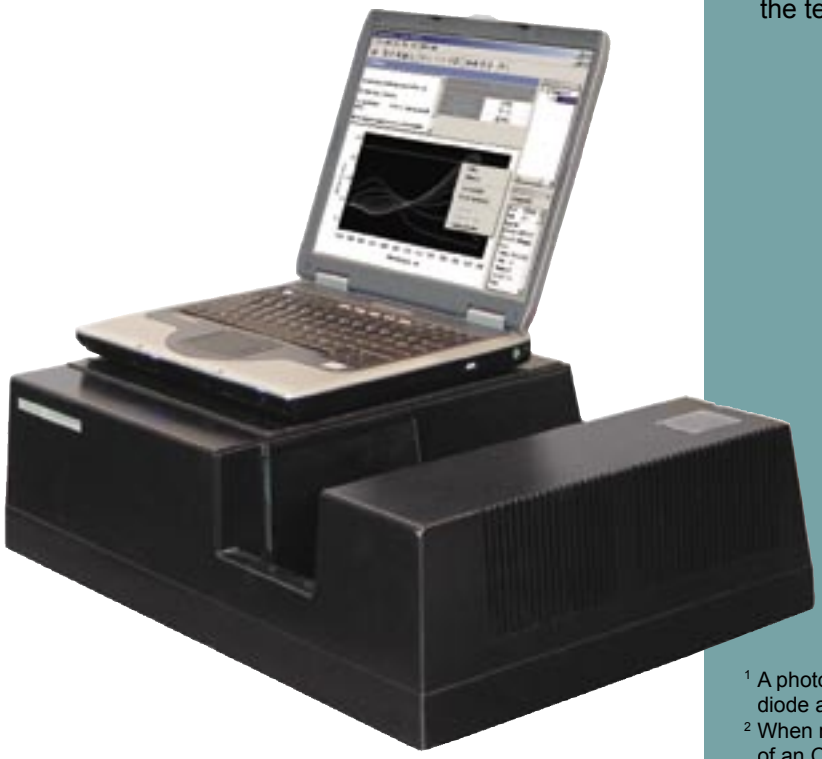
The alternative equipment for multiple wavelength acquisition are diode array and CCD systems.

These systems are inherently less sensitive[1] and often slower[2] than the Olis RSM 1000 line. They have limited utility in the UV and NIR, and they are useless in low light level situations. They cannot work with turbid nor photolabile samples. And diode array and CCD systems cannot be enhanced to support fluorescence or circular dichroism.

Diode array spectrophotometers are heralded as modern, neat, all-electronic, no-moving-parts, etc. They are. However, use of them is limited to absorbance readings on non-photolabile samples within an approximate 200-900 nm range, wherein under ten scans per second is sufficient. And even in these cases, one will have less confidence in the photometric accuracy of the collected spectral information than with the premium performance Olis RSM 1000 spectrophotometer.



Scans collected at the fastest acquisition rate from an Olis computerized HP 8452 diode array. 100 scans over a 100 nm span with 2 nm resolution took 108.3 seconds to collect. During this time, the Olis RSM 1000 could have acquired 1000 * 108.3, or 108,300 scans of a 50, 230, or even a 500 nm span with 0.5 or better resolution. (This reminder helps to explain the cost difference between the technologies, too!)



[1] A photomultiplier tube is 100-fold more sensitive than a diode array detector

[2] When rapid-scanning, the maximum data acquisition rate of an Olis RSM 1000 is one scan per one millisecond. When rapid-scanning is not employed, the maximum data acquisition is limited by the 20 MHz A/D card or the optional digital oscilloscope, which could have nanosecond acquisition rates.

How Does One Handle Megabyte Data Files?

Extraordinary effort and years of cutting-edge software development was undertaken to “deal with all the data your machine will vomit at the poor scientist.”[1]

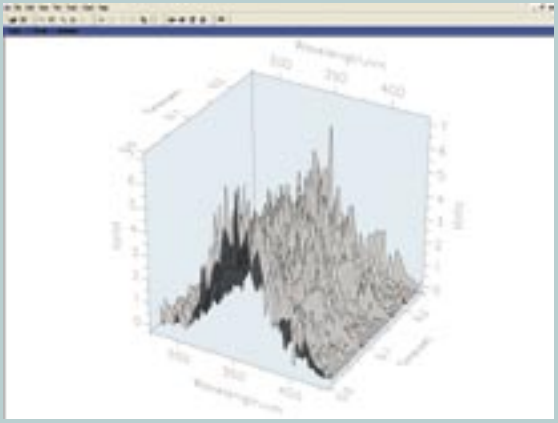
Today’s Olis SpectralWorks software handles megabyte data files as effortlessly as the best alternative programs handle a single scan or trace. Fitting of millions of data points takes the Olis software a second. Plotting of hundreds of thousand points takes a second. Truly, the number crunching and popping up 3D data sets happens effectively instantaneously.

Moving from the raw data to the final answer takes only two steps: SVD and Spectral Reconstruction using Matrix Exponentiation. The second panel, “Apply SVD,” shows the “eigenvectors” which are returned by Singular Value Decomposition. SVD finds the number of species in the raw data (here, two) and isolates the remaining information, i.e., random noise. The third panel shows the chemical models for a two species fit. An A → B mechanism was chosen. The final panel is the fitted results, giving the investigator not only rate constants but spectral information about his reaction.

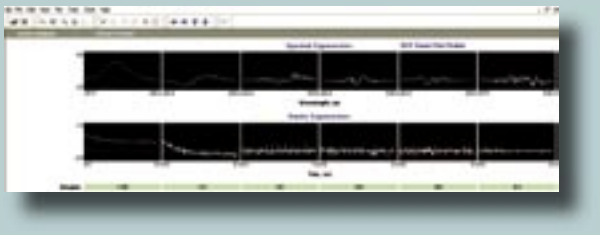
This 250 millisecond protein unfolding study used data from one stopped-flow shot.

The unique Olis software executes these steps as quickly as the investigator makes his selections, that is, effectively instantaneously.

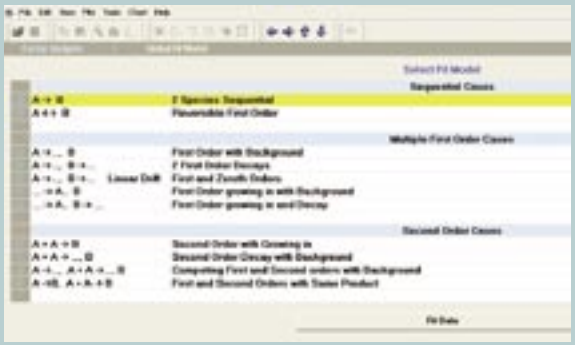
Collect Data



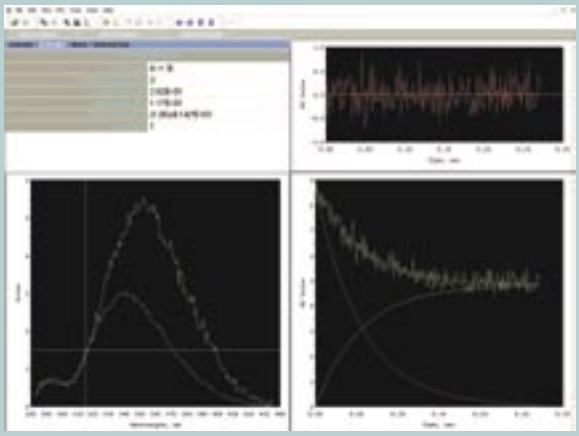
Apply SVD



Choose Fit From Two Species Mechanisms



Evaluate Results



[1] This comment made to Richard DeSa by his post-doctoral advisor, Prof Quentin H. Gibson, during a personal correspondence in the early 1990s. As DeSa loves to point out, part of Gibson’s genius is the ability to see through issues to their kernel. Without this software, an Olis RSM 1000 would instantly overwhelm both the hard-drive and the investigator! (Along with being the first step in global analysis, SVD reduces the size of the file 40-fold.)

Using a Standard to Illustrate the Absence of Bias in the Fitting Algorithms.

Using the Olis RSM 1000 in its fluorescence mode, 50 scans were collected in 0.05 seconds from light emitted by overhead fluorescent lamps. The mercury lines and the 120 Hz modulation of the lamp are easily identified in the eigenvectors (second panel) and the answer (final graphs).

SVD identified the changes in the light caused by the lamp's on/off operation at 60 Hz, and the spectrum of the light (a continuum with sharp Hg emission lines). SVD acts with no bias about the source of the data, chemical, kinetic, thermal, or other process.
SVD: Single species

Since user starting values are only used for the most complicated cases, bias cannot be entered during the fit, either.

Kinetic Fit: Select 1 species and then fit single rate so as to construct the spectrum

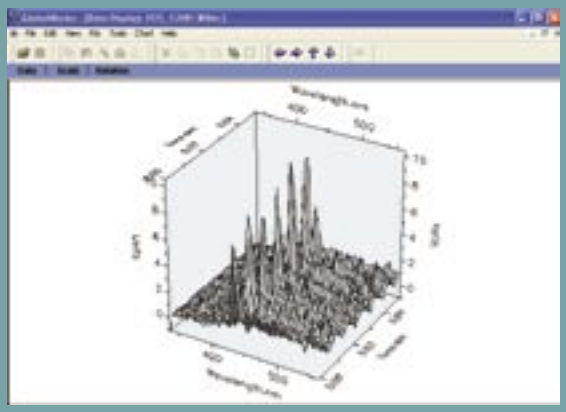
Mechanism: Not a kinetic process

Comments: Fluorescent lights switching on and off at 120 Hz, demonstration of a particular shape (the spectrum) varying in a particular way (sinusoidal). The eigenvectors are presented in the way shown to emphasize that the kinetic and spectral eigenvectors are related. One reads the display by noting that the kinetic eigenvectors show a particular time course and that it is the corresponding spectral eigenvector which varies in that way.

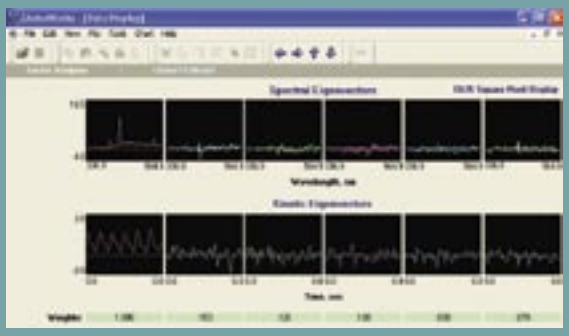
File: p05_120h.sc3[1]

[1]These data, as with all data shown in this brochure, are provided for pedagogical, demonstration, and testing purposes in the Olis GlobalWorks software.

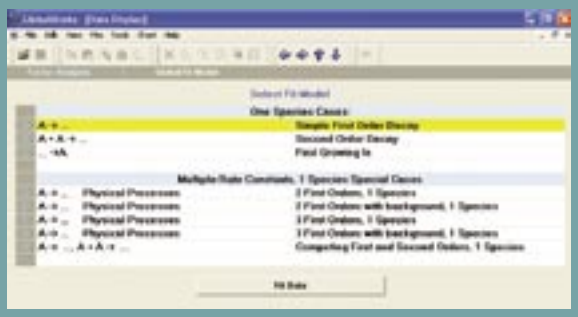
Collect Data



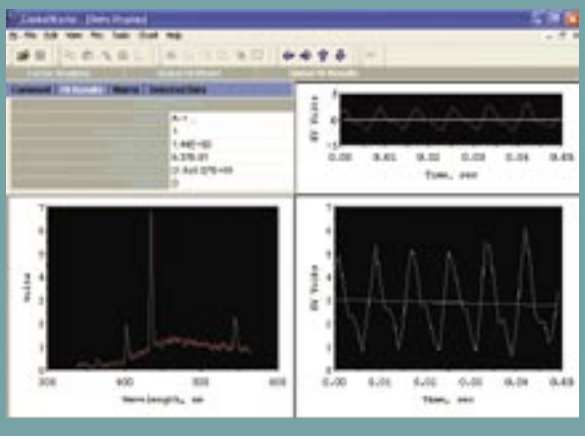
Apply SVD



Choose Fit From One Species Mechanisms

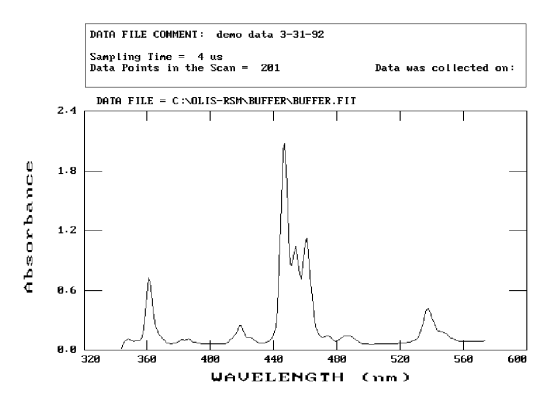


Evaluate Results

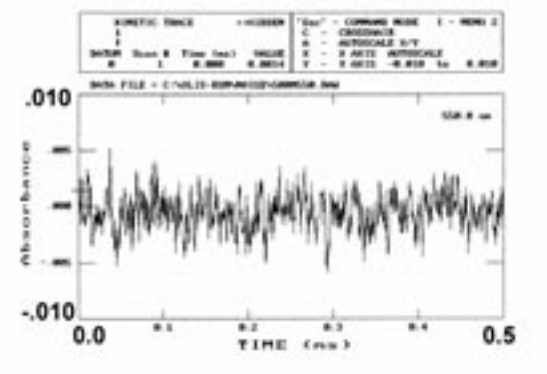


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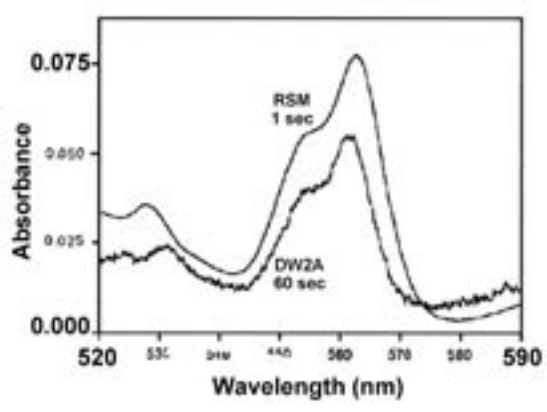
With an Olis® RSM 1000, you will get perfectly reliable and reproducible results, quickly, easily, economically, and in a wide range of experimental cases.



Holmium Oxide Filter



Air-to-Air Baseline



Turbid Sample Reading

One Millisecond Per Scan
While the Olis RSM 1000 is collecting 1,000 scans per second, its dual beam, double grating hardware is supporting milliabsorbance sensitivity. This one millisecond scan of a holmium oxide filter can be compared with the data on page 11. Notice that the RSM can scan a wider spectral range and still maintain better spectral resolution.

And, the RSM spent 0.001 seconds, not 0.1 second. The RSM is "orders of magnitude" better than a diode array detector.

0.001 AU RMS Noise in 0.5 Milliseconds
Recall that S/N improves as a square root of the number of data fitted. Noise in the fourth and fifth decimal places, as is required for circular dichroism work (for instance) is obtainable.

Better for So Many Cases
Collect accurate absorbance readings on cytochromes, mitochrondia, tissue suspensions, and other scattering samples. (Dual wavelength is a subset of hundreds of wavelengths.) Use with photolabile samples, microvolume samples, changing samples, and always get better results than with alternative technologies.

Optics, Detection, and Mathematics: the Complete Package

Subnanometer resolution for highest resolution samples

Select gratings for the DeSa monochromator to optimize for spectral range, resolution, and span. Gratings are accessed without tools or optical alignment, so that moving from one pair to another is a 2-3 minute operation.

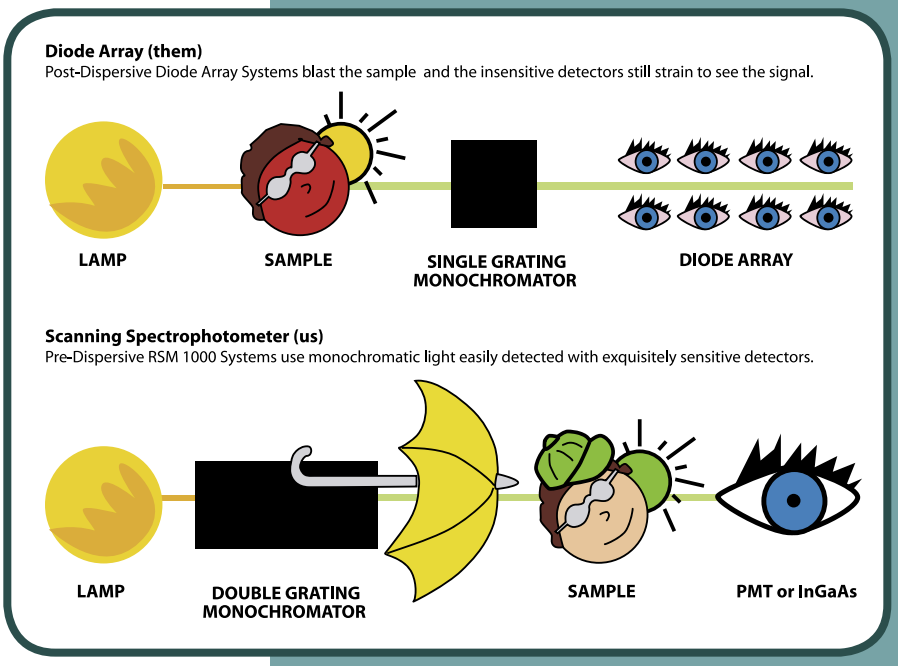
MOST POPULAR GRATINGS FOR THE DESA MONOCHROMATOR[2]

Blaze Wavelength	Ruling Density lines/mm	Linear Dispersion nm/mm	Resolution[1] (0.2 mm slit) nm	Wavelength Span nm	Wavelength Range[2] nm
230	2400	1.54	0.3	38	150-350
300	600	6.15	1.2	154	200-450
500	400	9.22	1.8	231	330-750
500	1200	3.07	0.6	77	330-750
1000	600	6.15	1.2	154	670-1800

[1] Resolution values based on nominal intermediate slit width of 0.2 mm.
[2] Wavelength range based on a 2/3 blaze to 3/2 blaze.

Monochromatic Illumination

Gentle monochromatic light is used for all measurements, so that photolabile samples can be studied safely and successfully. Sample is measured only with the appropriate wavelengths: broadband light and stray light—inherent in diode array systems—are never a concern.



Powerful Mathematics easily handle MB data files

The software provided with each Olis spectrophotometer handles up to 4000 scans of up to 500 points per second more adroitly than commercial programs handle single scans. Equations available: 60+ models for multiple wavelength (3D) data, 25+ for 2D. The key behind this power is the trio of SVD, Downhill Simplex, and Matrix Exponentiation.[1]

The Matrix for $A \rightarrow B$

$$\begin{array}{c} \\ dA/dt \\ dB/dt \end{array} \begin{array}{c} \begin{array}{cc} A & B \end{array} \\ \begin{bmatrix} -k1 & 0 \\ +k1 & 0 \end{bmatrix} \end{array}$$

The Matrix for $A \xrightarrow{k_1} B \xrightarrow{k_2} C$

$$\begin{array}{c} \\ dA/dt \\ dB/dt \\ dC/dt \end{array} \begin{array}{c} \begin{array}{ccc} A & B & C \end{array} \\ \begin{bmatrix} -k1 & 0 & 0 \\ k1 & -k2 & 0 \\ 0 & k2 & 0 \end{bmatrix} \end{array}$$

[1] These remarkable algorithms are discussed in Methods in Enzymology, volume 384, 2004, chapters 1, 2, and 3.
[2] See a much longer list of suitable gratings on our web site at http://olisweb.com/products/rsm/gratings.php.

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The OLIS RSM 1000 is a unique kinetics spectrometer that is capable of obtaining spectra over the visible range with millisecond time resolution using sensitive photomultipliers that permits the intensity of sample illumination to be minimal. Furthermore, extraction of meaningful kinetic data from the spectral noise through singular value decomposition is built into data collection and analysis and will allow us to monitor the very small optical absorption changes association with the oxidation and reduction of the monolayers of synthetic redox proteins that are a principal object of study of the MRSEC grant. There is no other commercial instrument that comes close to this combination of capabilities.

Christopher Moser, Ph.D.
Associate Director, Johnson Research Foundation
January 22, 2003

Your instrument, although designed for absorption spectra, was quickly modified to measure the rapid flash produced. In our first experiments we took a few micrograms of the modified aequorin and triggered it. The individual 1 msec spectra looked awful, but the cumulative data showed the two emission peaks very clearly, with the correct positions for both aequorin and fluorescein emission. Our subsequent rapid scanned stopped flow emission spectra of this transient signal showed how good the detection capabilities of the instrument are. We were looking at very small amounts of the protein and obtained individual 1 msec emission spectra with excellent S/N. In a few hours we obtained quality data and spectra that are publication quality. To anyone looking at low light level chemiluminescent or bioluminescent reactions, or wishing to obtain spectral and kinetic data on transient or precious biological samples the rapid scanner is a must. A wonderful instrument!!

Russell Hart, D. Phil.
Director of Clinical Assays
March 31, 1993

Olis

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Write **sales@olisweb.com**

Call **1-800-852-3504** in the US & Canada
1-706-353-6547 worldwide

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