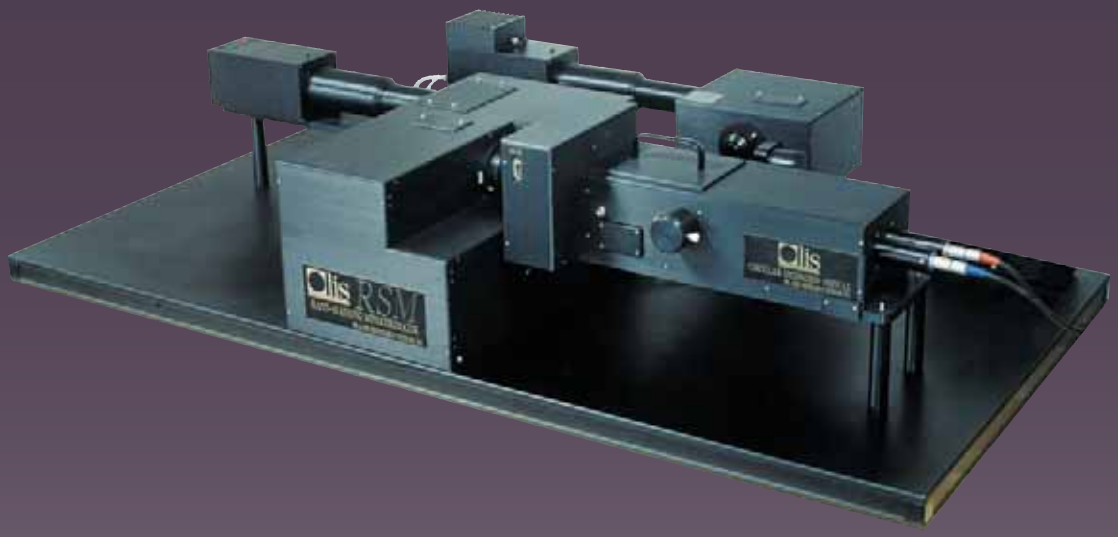
The Olis® CD Spectrophotometers



Digital Data Acquisition Instruments

Traditionally, CD spectroscopy has been difficult, time-consuming, and error-prone. Now, there are better instruments, designed for simplicity, directness, and error-free answers.

Today's modern Olis DSM CD spectrophotometers are small, fast, direct, and digital. These "CDs designed by definition" have no reliance upon calibration or user settings, so they offer no opportunity for uncorrectable user-introduced error.

CD = abs(L) - abs(R)

Brilliant Breakthroughs in CD!

Three models support your protein secondary structure work, NIR CD, and rapid-scanning CD. Accessories support automatic titration, temperature ramping, sample preparation (titration), stopped-flow mixing, MCD, LD, FDCD, and other processes.

Digital Measurement Now Replaces Indirect Calculation

Stay with the traditional or move to the modern. With the modern, enjoy perfectly reliable and reproducible results, quickly, easily, and always correctly.

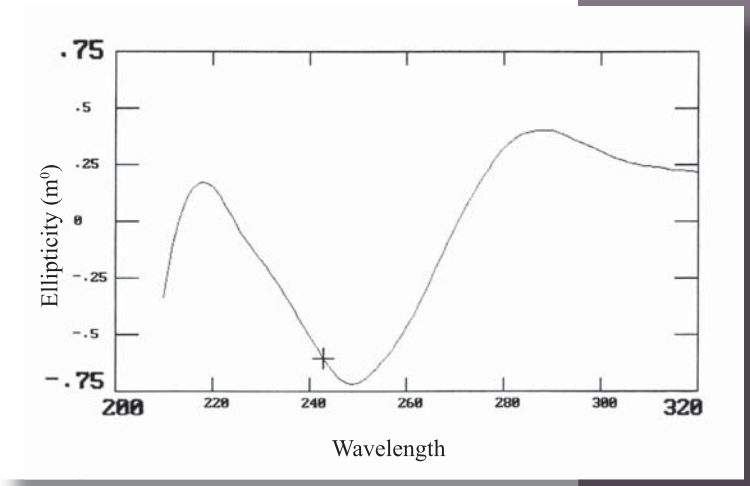
We open with a quotation from W. Curtis Johnson's chapter in Fasman's 1996 book, **Circular Dichroism and the Conformational Analysis of Biomolecules** (Plenum Press): "***There are three methods for measuring the CD of a sample. The most straight-forward is to measure the absorption for each rotation of light and subtract directly the measurement for right circularly polarized light from the measurement for left circularly polarized light. However, this requires making each measurement to great accuracy, which was not practical until the recent availability of powerful and inexpensive computers... Inexpensive, high speed digital computers have made direct subtraction of left and right circularly polarized beams a practical method of measuring CD. This method is pioneered in a commercial instrument for electronic CD by On-Line Instrument Systems (Olis, Bogart, Georgia)...This dual beam collection and*** **direct subtraction method** ***has two advantages in addition to the flat baseline. First … CD signals are measured correctly. Second, the instrument is measuring absorbance directly, so there is no constant of proportionality to calibrate.*** *"* [Quoted from pages 640 and 644-5, underlining added] (The other two means of measuring CD are *"to measure the ellipticity imparted on linearly polarized light that passes through the sample. This method is limited by the quality of the linearly polarized light and the mechanics of measuring the small perpendicular component of the elliptically polarized light. The third method is to modulate the light between the two rotations, and measure the difference at each wavelength. This method is well suited to analog electronics, and is the method used by most commercial instrumentation.")*

W. Curtis Johnson Coined "DSM"

The Olis DSM CD Spectrophotometers are the first and only new CD spectrometers in decades. These are not me-too solutions to an old problem. The Olis DSM CD spectrophotometers are fundamentally different. They are **simple, direct, and error-free.**

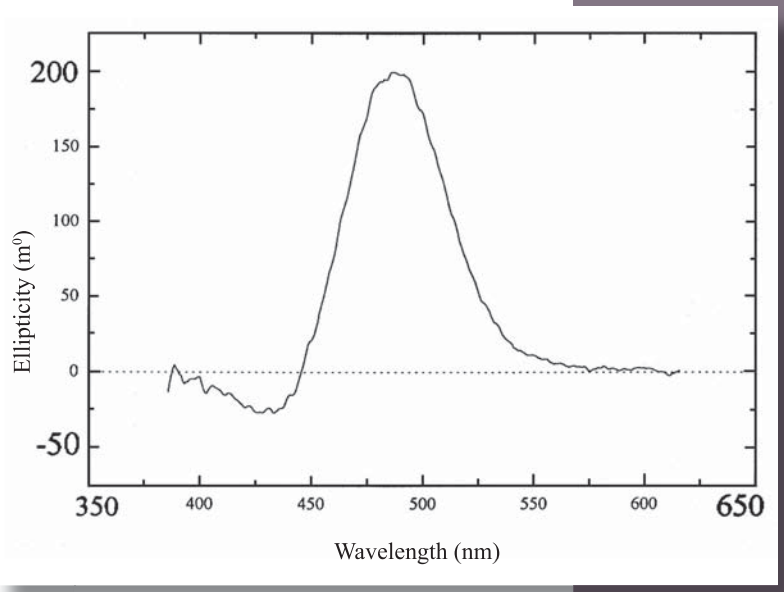
Exquisite Sensitivity to CD/LD Signal

Large Signal or Small, Fast Acquisition or Slow, the Data are Always Right



Olis DSM 1000 CD
DNA complexed with polymer

90 minute collection time



Olis DSM 1000 CD
Spectrum of (+)Co(en)3+

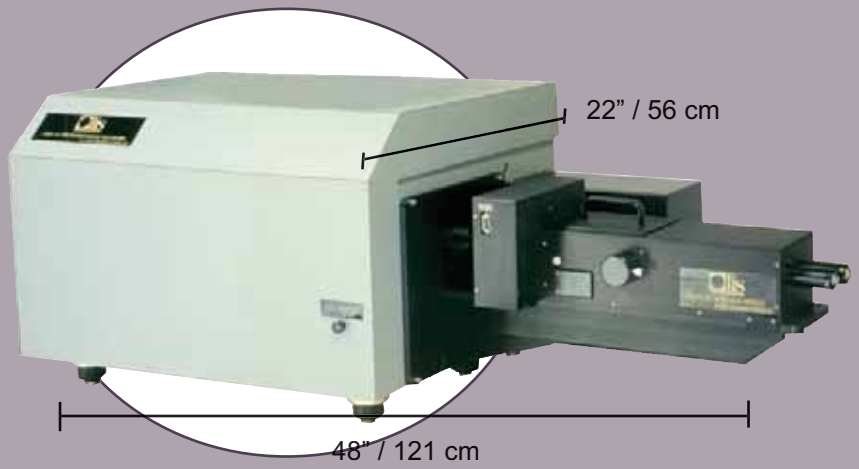
32 milliseconds collection time

Only the Monochromators Differ

DSM 20 CD

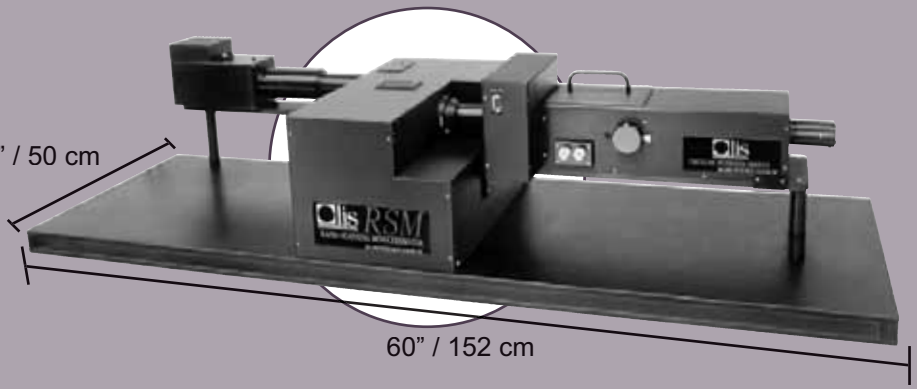
Smallest CD made

DSM 17 CD



Best for NIR & Absorbance

DSM 1000 CD



Best for Light Throughput, Modularity & Kinetics

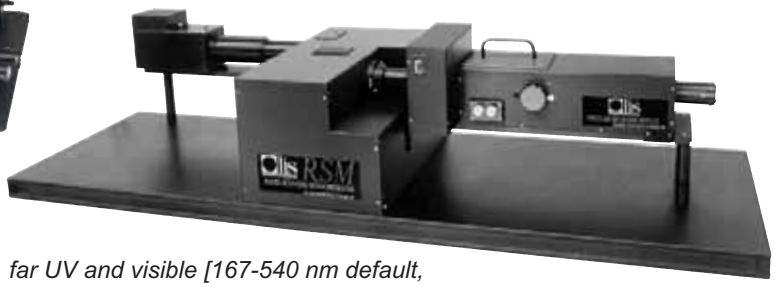
The Olis® DSM 17 CD

UV, visible, extendible to NIR [185-800 nm default, extendable to 1700 and 2500 nm]

The Olis® DSM 20 CD

far UV to visible, extendible to NIR [167-2500 nm]

The Olis® DSM 1000 CD



far UV and visible [167-540 nm default, extendable to 1100, 1700 and 2500 nm]

The Olis™ DSM 17 CD Spectrophotometer The model 17 uses the Cary 17 prism + grating monochromator. Only this model of the three DSM models provides the option of computerized slit width adjustment during scanning (as opposed to automatic high voltage adjustment and fixed slit width). This monochromator has a NIR potential to 2600 nm and this potential can be used in absorbance measurements when the PbS detector is used in place of the photomultiplier tubes. When enhanced for fluorescence applications, the model 17 is usable as a scanning excitation, fixed wavelength emission system. This model is capable of angstrom resolution throughout most of its range.

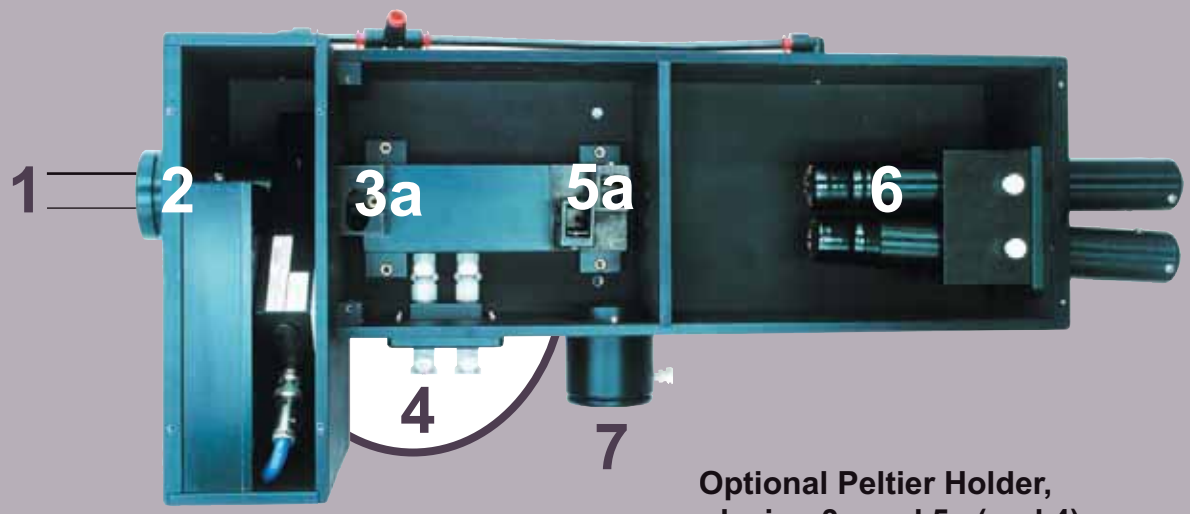
The Olis™ DSM 20 CD Spectrophotometer The model 20 uses the the new Hummingbird subtractive double grating monochromator. This tiny 7-sided monochromator uses two Olis 40 x 45 mm gratings for outstanding light throughput, even down to the lowest wavelengths. These holographically blazed gratings can be for ultraviolet, UV/Vis, visible, Vis-NIR, or NIR. The spectral resolution is 0.5-5 nm, based on the slit width used; slits are interchangeable fixed slits of 0.5, 0.6, 1.24, and 2 mm, with other sizes available by special request. Like the Olis DSM 17, it can be enhanced to support scanning (or fixed wavelength) excitation and fixed wavelength emission; it is slightly superior to the model 17 in its excitation intensity performance.

The Olis™ DSM 1000 CD Spectrophotometer The model 1000 uses the patented DeSa subtractive double grating monochromator. This model provides you with everything: broadest spectral range, most light throughput, easiest modularity, and rapid-scanning potential[1]. For Olis clients who want the most from their spectrophotometer, this is the model of choice.

Footnotes

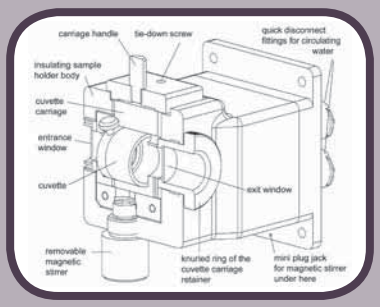
[1)] Rapid-scanning CD is possible when the spectral range to be scanned has approximately equivalent light levels from one end of the scan to the other, as is true from 500-350 nm. Rapid-scanning CD is impractical across a range such as 250-170 nm due to the enormous differences in the wavelength levels across such a range.

Physical Layout of CD Module



Optional Peltier Holder, replacing 3a and 5a (and 4)

3b



5b

1 Incoming beam from monochromator.

2 Magnesium fluoride polarizer/beam splitter splits the light beam (1) into two linearly polarized beams each orthogonal to the other. Angle of divergence can be as small as 1° and as wide as 5°; standard divergence is 3° putting beams of light 3.5 mm apart at the center of the CD sample position (3). Also, the 50 kHz Hinds PEM-90, which modulates between RCP and LCP 50 times per millisecond.

3 Under most circumstances, the CD sample will be positioned near the PEM and beam splitter. The sample holder will be the default jacketed model, compatible with cylindrical and rectangular cuvettes (3a) or the optional QNW TLC-250 Peltier model (3b).

4 Water lines to cuvettes 3a & 5a, so that a digital or manual temperature can be achieved using a water bath.

5 Pair of 1 cm^2 rectangular cell holders for dual beam absorbance, single beam fluorescence, and running two CD samples simultaneously. Default pair, 5a, is water jacketed and is provided with 3a. Optional Peltier holder, 5b, is provided in addition to or instead of 3b.

6 Two detectors optimized for the desired UV/Vis or Vis/NIR region (see pages 26 and 27). Each detector has its own amplifier circuitry and its own high precision A/D converter.

7 Port for optional detection of fluorescence or FDCD, or for flash lamp to photolyze sample, in position 5.

Other Hardware Involved

1 Computer

OLIS Control Unit includes an OLIS verified and supported computer with the Intel 2.8 GHz Pentium 4 microprocessor. Features 512 Mbytes RAM, a 512KB Pipeline Burst SRAM cache, a 40 GB IDE 7200 RPM harddisk with a 3.5" high density disk drive and CD-RW drive, four USB ports, dual port RS 232 card, and one parallel port, Windows XP, custom OLIS 16 bit A/D, high-resolution 17" LCD, and HP® Deskjet printer.

2 Electronic Boxes

Both the Hinds PEM box (tan) and the Olis control box (black) are external to the spectrophotometer for greatest ease in access for diagnosis and repair.

3 Lamp Power Supply

4 Closed-Cycle Cooling Box

An integrated closed cycle cooling system for lamp cooling which guarantees instantaneous lamp power supply shut down if cooling becomes compromised and runs lamp below ambient for long life.

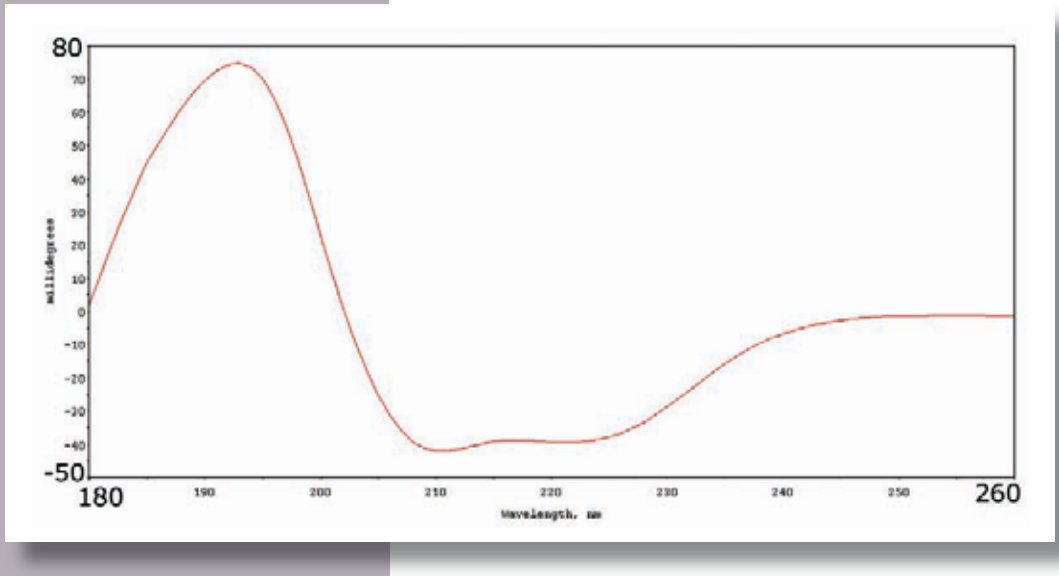
5 Cooling Box for the (optional) Peltier Cell Holder

6 Cooling Box for the 450 Watt lamp and Peltier

(infrequently required)

Acquisition Modes for Three Olis DSM CD

Computer Calculated Time per Datum with Scanning



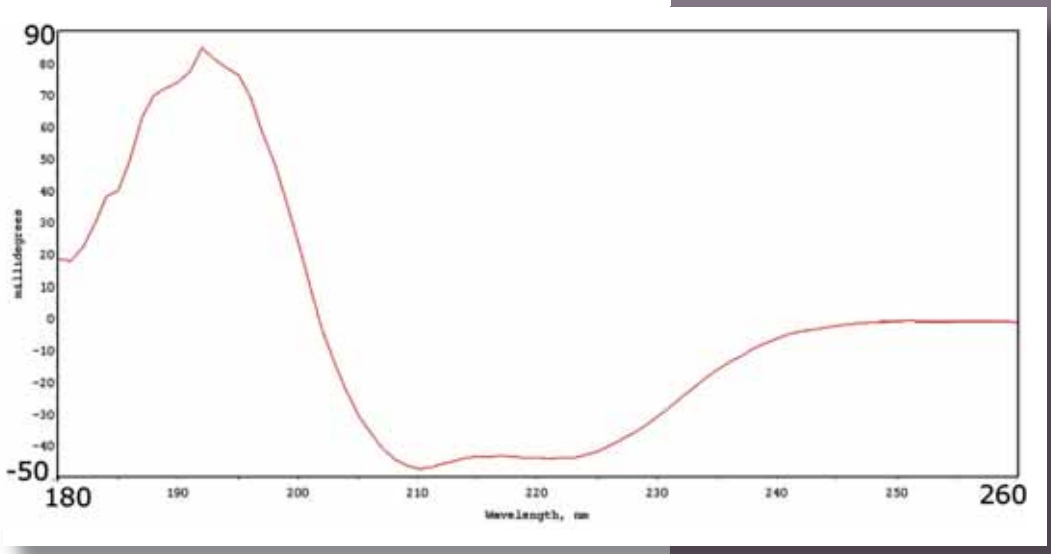
Variable Time/Datum Equivalent S/N

~30 minute total time, 1 to 200 sec/datum

Bovine Serum Albumin

- **All three Olis DSM CD spectrophotometers** support 'variable time per datum' where the computer is allowed to determine how much signal averaging is needed per datum.
- The advantage of this mode is that the best S/N is obtained in the minimal length of time, since the computer collects and signal averages at each datum for exactly the optimal length of time.
- **Using the common protein BSA spectrum of 260 nm to 180 nm as the example,** the computer will determine that 0.5, 1, or 2 seconds is sufficient in the wavelength region above around 200 nm.
- As the light level drops in the 200-180 nm region, the computer will calculate increasingly longer periods of time per datum, such that tens of seconds will be spent collect the final data points.
- The scan will be collected with effectively identical noise levels from one end of the scan to the other.
- The maximum time per point can be any number of seconds.

Operator-Specified Time Per Datum with Scanning



Bovine Serum Albumin

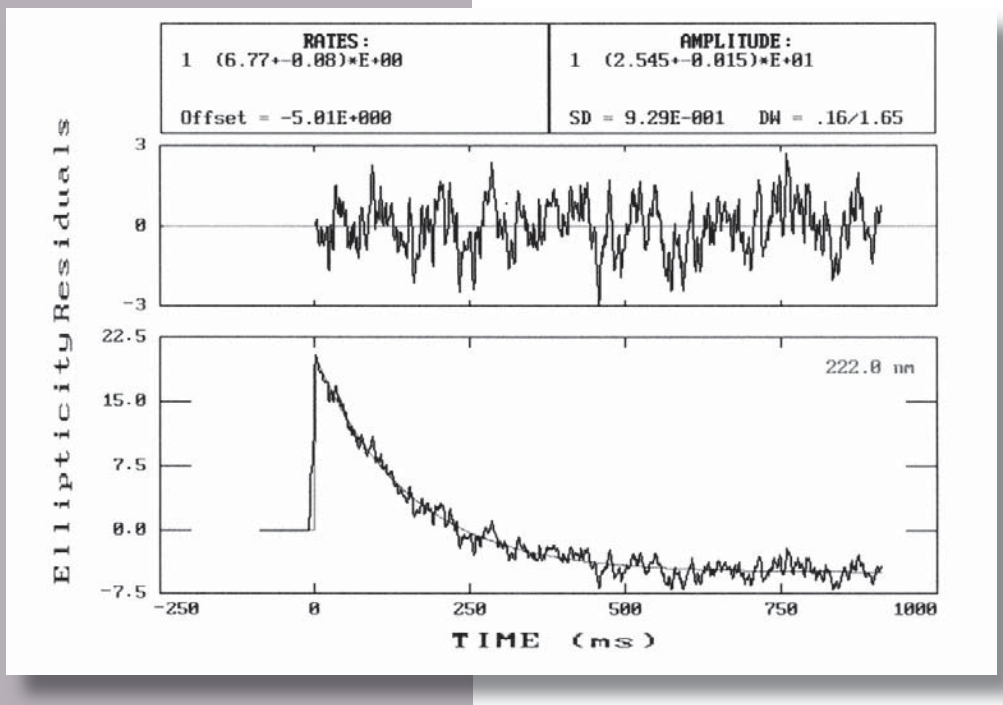
Constant Time/Datum
Variable S/N

~1 minute total time,
0.5 sec/datum

- **All three Olis DSM CD spectrophotometers** can support fixed time per datum readings up to 1000 reading per second (1 kHz); these points are signal averaged per retained datum.
- This time per point can be any number of seconds from one millisecond to hundreds of seconds.
- How much time is **between** data points (wavelengths) is variable based on the speed at which the gratings/prisms move to the next wavelength, but this time is under one second in all three models.
- The advantage of this mode is an exactly known length of time to make the measurement.
- This mode works best when scanning a region across which there is approximately equal light levels.
- This mode is recommended when survey scans are being done, and the best signal to noise at the lowest wavelengths is of less interest than getting an informative scan quickly.

Kinetics Mode

Operator-specified time per datum *without Scanning*.



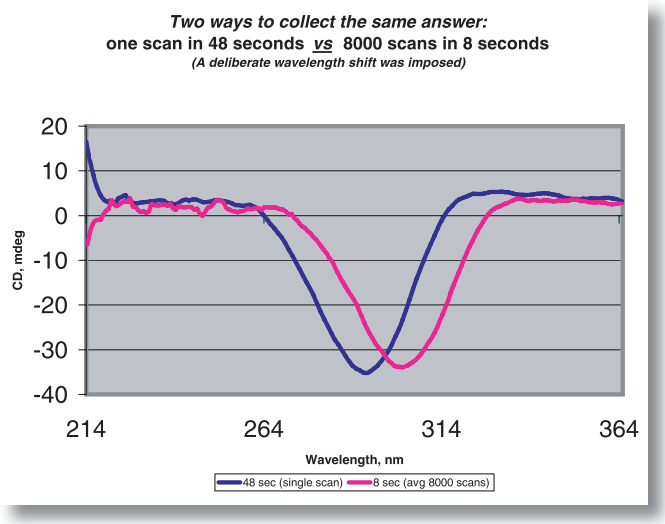
One exponential fit and residuals for a single stopped-flow shot. Data acquired at 220 nm.

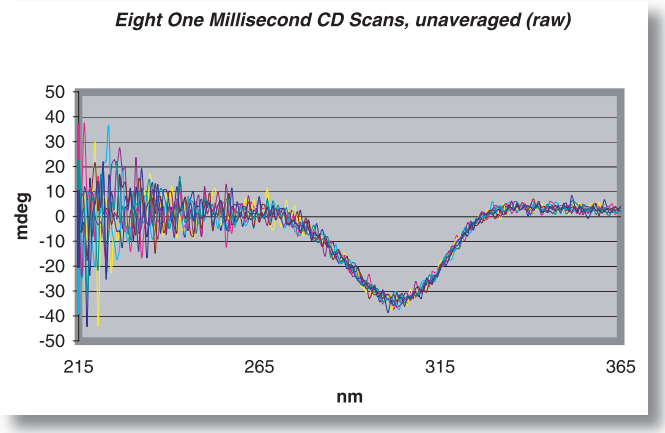
Bovine Serum Albumin

All three Olis DSM CD spectrophotometers can support high-speed data acquisition in a kinetic mode, such that 1000 reading per second (1 kHz) are made and signal averaged per retained datum. Up to three “fixed times per datum” are available across a time course.

Rapid Scanning to 1000 CD Scans per Second!

This is not a typo! The Olis DSM 1000 CD employs the same monochromator and fast electronics as the Olis RSM 1000 rapid-scanning spectrophotometer. It can collect 1,000 CD scans per second!



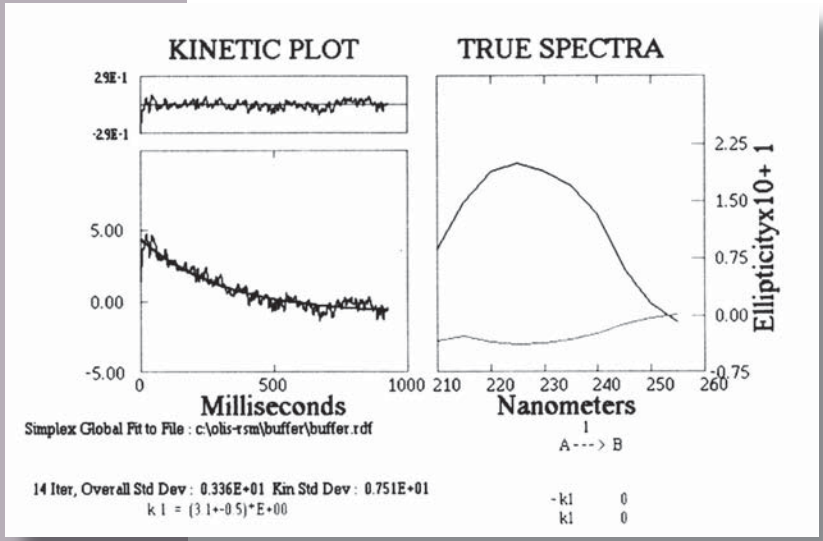


- Unique to the Olis DSM 1000 CD is the ability to collect a CD scan every millisecond.
- One can use the 1000 scans per second to monitor a changing sample (kinetics).
- In this mode, each one millisecond scan is a two dimensional kinetic data point.
- Data analysis is performed using SVD and Global Analysis.
- The tens, hundreds, or thousands of CD scans allow you to calculate starting and ending species and any transient spectral intermediates.
- Global analysis returns better rates constants than fixed wavelength kinetic analysis. And, removes RMS noise. And, supports calculation of more precise rate constants.
- Rapid-scanning is also useful for making quick scans.
- When conditions are right, one can collect useful CD spectra in fractional seconds.
- With rapid-scanning, one is able to collect the true spectrum of an unstable CD sample. Any changes which occur over time will be captured as a change in the entire spectrum, not as change in the shape of the spectrum which was caused by deterioration of the sample during the seconds or minutes which elapse from the start to the end of the (non-rapid-scanning) CD scan.
- For best rapid-scanning CD results, the scan should be above 190 nm, so that there is useful light available, and the CD signal should be larger than a few millidegrees.

Kinetics & Absorbance Utility

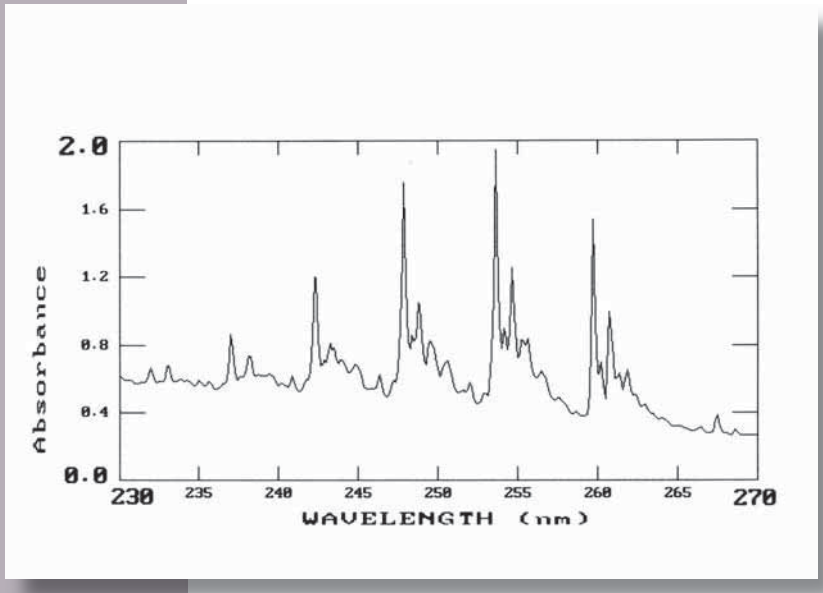
Millisecond Kinetics

Fitted 3D results of 10 CD stopped-flow shots of glucone lactone, each collected at one of ten wavelengths.



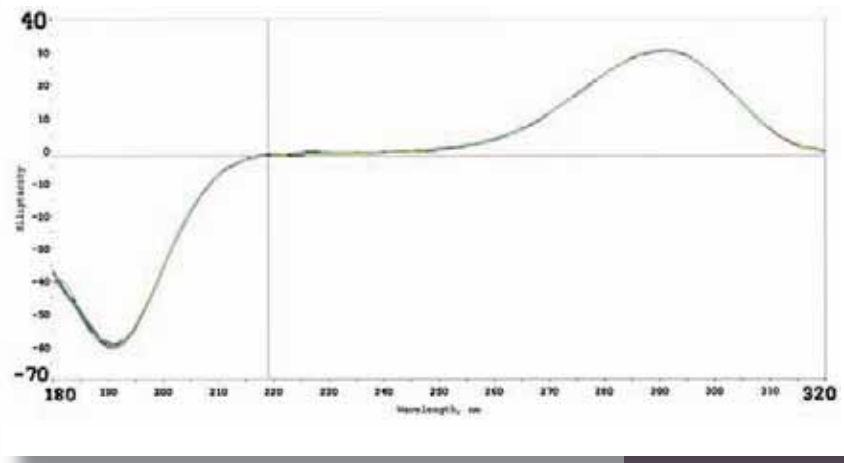
Absorbance Scanning

True sample/ reference acquisition in absorbance for dual beam acquisition



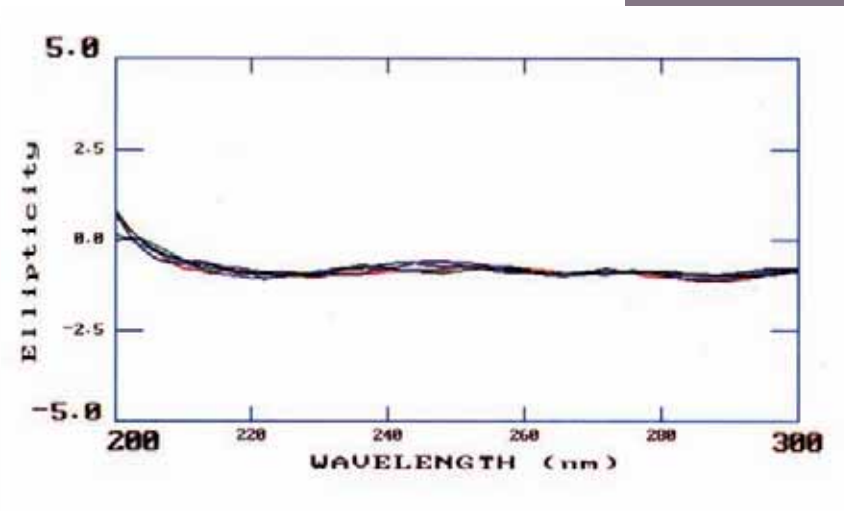
Wavelength & Electronic Stability

Stable & Reproducible Spectral Scanning



10 scans in 2400 seconds

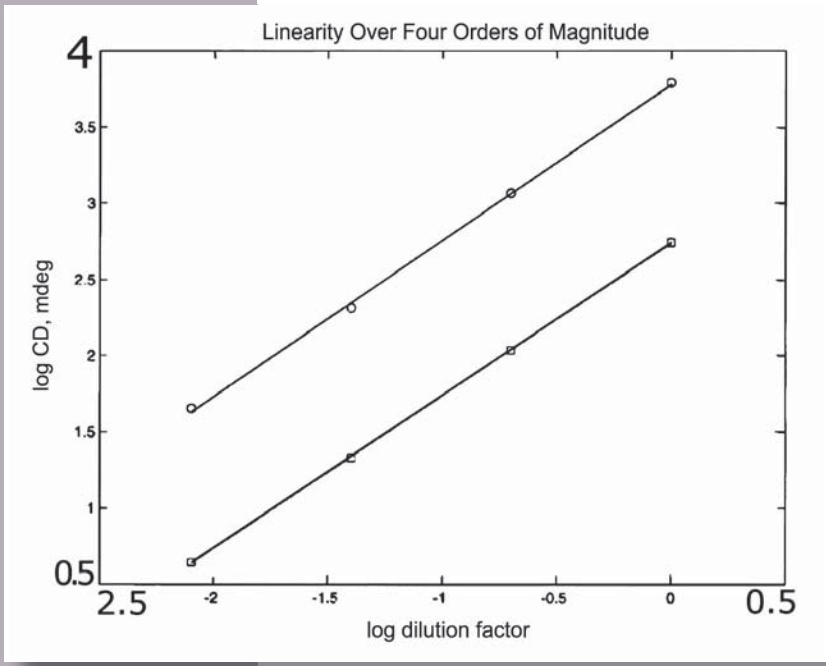
Stable & Reproducible Flat baseline



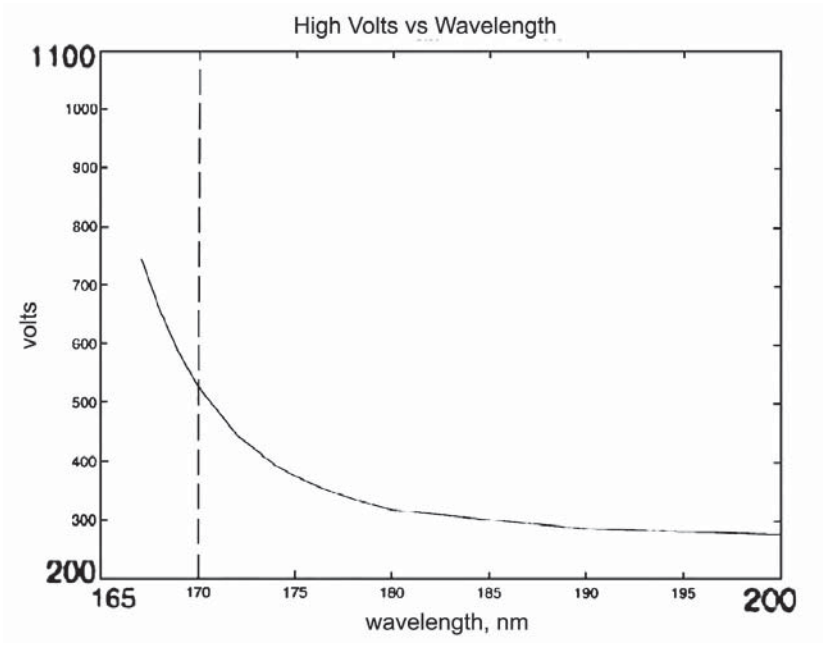
5 scans at 20° after temperature run; on for 36 hours

DSM Means Widest Useful Ranges

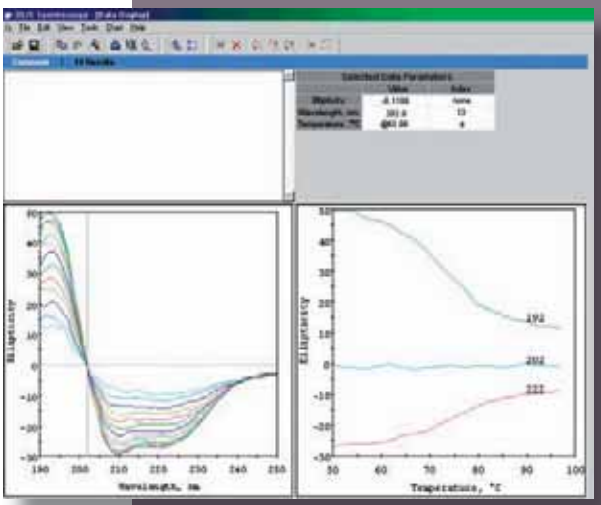
Linear over orders of magnitude.



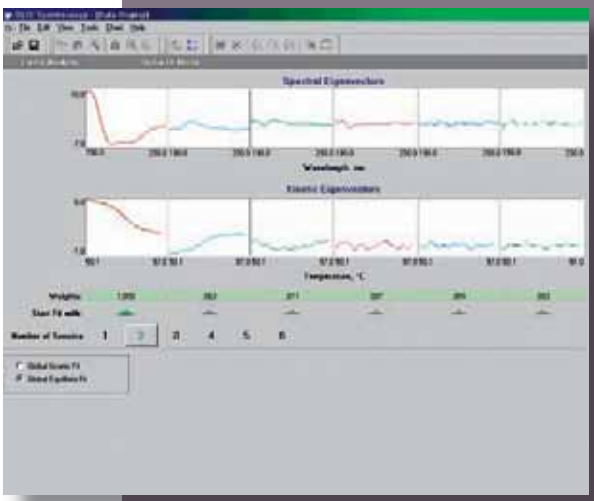
Useful light to 170 nm and beyond.



Analyzing Temperature Dependent Scans

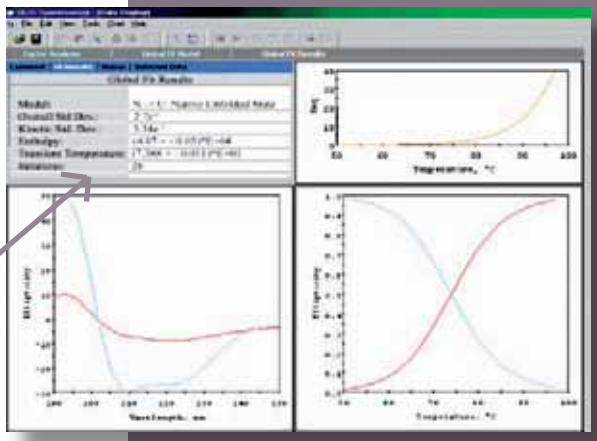


Temperature dependent data shown in SpectralWorks program with temperature dependence at three selected wavelengths in right panel.



Eigenvector presentation of GlobalWorks, suggesting the presence of two species.

Non-structured data, noise, is isolated and removed in this step Noise of individual scans has minimal impact on final answer, below.



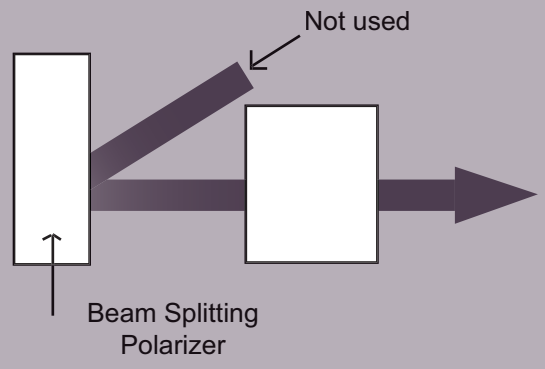
Results of 3D fit: Starting and final form of protein (left) and temperature dependence (right). Also shown are the transition temperature and enthalpy values.

Traditional vs. Modern

Complex electronics find, isolate, and amplify CD information from a single, non-referenced beam of light.

The correct answer is obtainable ***only*** if the electronic settings are correct ***and*** calibration is correct ***and*** the lamp is stable ***and*** the signal is ≤ 2 degrees.

Old: Traditional, Single Beam



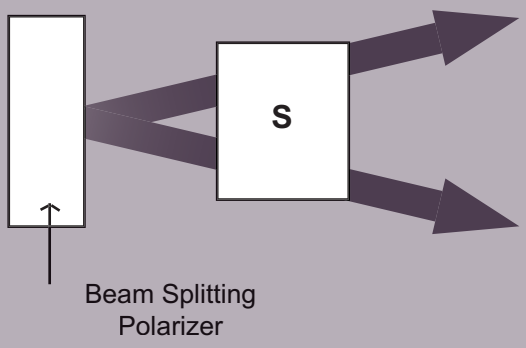
CD is proportional to (I_{AC}/ I_{DC})

Dates to 1960

Powerful software collects raw data from two referenced beams producing abs(L) and abs(R) directly.

The correct answer is the only answer possible!

Olis, Inc.: Modern Dual Beam



CD = abs(L) – abs(R)

Premiered 1994

Every non-Olis CD spectrophotometer is a single beam instrument. Every one.

Single vs. Dual Beam

Single Beam: Old-Fashioned Way

- The spectrophotometer must be calibrated correctly, or the wrong answer is obtained.
- The operator must know the approximate CD answer in advance to set the electronics correctly or the wrong answer is obtained.
- There is no real-time reference, so external influences cannot be isolated as such, making the wrong answer possible.
- The CD is calculated from decoupled and modified AC and DC signals, so all data available to the user is already influenced by the electronic settings.
- Half of the light from the lamp is not used, so the device's sensitivity is less than its potential.

Old: CD = (I_{AC}/ I_{DC}) adjusted by calibration constant

Dual Beam: Olis Digital Way

- Calibration is not required.
- No sensitivity settings are used; response is linear from at least 0.5 to 5,000 m0.
- Raw data are acquired and used in the direct calculation of abs(L) - abs(R).
- Each beam is a reference for the other, so all non-CD information is identified as such and is kept from influencing the answer.
- All of the light from the lamp is used. No potential sensitivity is wasted.
- Each beam contributes 50% of abs (L) and 50% of the abs(R) information. Using both beams results in twice the intensity of using one, but each beam is sufficient of itself for CD/2.

Olis: CD = abs(L) - abs(R)

Single vs. Dual Beam

	Traditional "market leader"	Modern Olis DSM 17/ 20/ 1000 CDs
Means of acquiring CD information	**Analog:** modulate polarization of one beam of light, isolate and amplify signal with lock-in amplifier, measure also DC level. Calculate, including 'k,' the calibration constant, as $CD = k(I_{AC}/I_{DC})$	**Digital:** the absorption for each rotation of light is measured; the measurement for right circularly polarized light is directly subtracted from the measurement for left circular polarized light. **CD = abs(L) - abs(R)**
Calibration against a standard	**Required, and if calibration is off, answer is wrong**	**A dual beam system provides the correct answer, obviating the need for calibration against a standard.**
Lock-In Amplifier	Required, and if settings are wrong, answer is wrong	Not Used
Time Constants	Fixed prior to running sample; if too fast, spectra will be too noisy; if too slow, time and nitrogen will be wasted	Digital filtering is done after the raw data are acquired, so that one is assured of the exactly optimal noise reduction while collecting at the fastest possible rate.
Dynamic Range	0-2000 millidegrees	0-10,000 millidegrees and perhaps beyond
Light Source	Xe, 150 watt air cooled	Xe, 150 watt with self-contained circulating fluid to operate lamp at coolest temperature, **extending life of lamp. Includes secure automatic shutoff.**
Spectral Range[1]	165-900 nm	**Default for protein studies, model 17, 185-800 nm; model 20, 167-720 nm; and model 1000, 167-540 nm.** Extendible to 1700 nm and 2500 nm. Wavelength range determined by accessible gratings and detectors (see below for detector information).
Mechanical Range	Most quote 165-900 or 1100 nm	**Models 20 and 1000**: default 0-700 nm; or 0-1700 nm, or 0-2600 nm **Model 17**, 185-2600 nm
Interrogation Method	**Single channel**	Dual beam, constantly modulating left/right, right/left, with 2 beams 180° out of phase with each other ("phase coherent").
Mode of detection	**Single detector**	Two UV/Vis optimized PMTs, or two Vis-NIR InGaAs detectors, or two extended range NIR InGaAs detectors.

Footnotes

[1)] Let us remember that the wavelength to which light can be detected and the wavelength to which useful data can be acquired might be quite disparate. Sample, solvent, and cuvette absorbance will inevitably limit useful data to 175 nm in every CD spectrophotometer, except, perhaps, those in a synchrotron.

Dispersive elements	Prisms; or prism and grating	**Model 17**: prism and grating. **Model 20**: Two 40 x 45 mm holographic gratings, default for protein studies are blazed at 200 nm with 2400 lines per millimeter. **Model 1000**: Two 50 mm^2 gratings, default for protein studies are blazed at 200 nm with 2400 lines/ mm.
Number of scans per second	Less than 13	Less than 1 for all three models. **Model 1000** also has unique rapid-scanning mode, wherein up to 62 CD scans per second can be collected.
Slew rate	Most quote fixed rates of up to 5000 nm/minute	**Models 17 and 20**: up to 40 nm/second or 2400 nm/ minute. **Model 1000**, full range of grating takes 10 seconds, so that for a 2400 line grating, 40 nm/sec = 2400 nm/ minute, and for a 400 line grating, this results in 240 nm/sec = 14,400 nm/ minute.
Scan Rate	continuous wavelength scan, step-scan, auto-response scan	**Entirely variable** with the speed being determined by the difficulty of the measurement; or variable with upper limit; or fixed, 0.1 to 20 nm.
Wavelenth Accuracy	180-300 nm, ±0.1 or 0.2 nm 300-400 nm, ±0.3 to 0.5 nm	For **models 20 and 1000**, 0.125 nm. For **model 17**, <0.05 nm to 800 nm
Slits	variable	For **models 20 and 1000**, selectable from 0.12-6 mm. For **model 17,** automatic and continuously variable to provide constant bandpass (or fixed).
Spectral bandpass	variable	For **models 20 and 1000**, fixed setting, based on chosen slit width and gratings, 0.1 to 20 nm. For **model 17,** automatic and continuously variable (or fixed)
Modulator	18.5 or 50 kHz	50 kHz
Autoscale	Up to some limit ranging from 1000 to 3300 m°	Arbitrary
RMS noise[1]	Measured without sample, 1 nm bandpass, 4 sec integration time: commonly quoted values, 0.04 m° at 185 nm, 0.003 m° at 500 nm	Measured with empty chamber, 3 nm bandpass, ~3 sec integration time without digital filtering which will ordinarily be applied. 0.7 m° at 180 nm — 0.2 m° at 200 nm 0.2 m° at 185-190 nm — 0.1 m° at 220 and up
Baseline stability	**Typically quoted per hour**	**<1 m° per day**

Footnotes

[1)] Recall that S/N improves as a function of the square root of the number of data fitted; and that 1 m^o is equivilent to $3x10^{-5}$ AU.

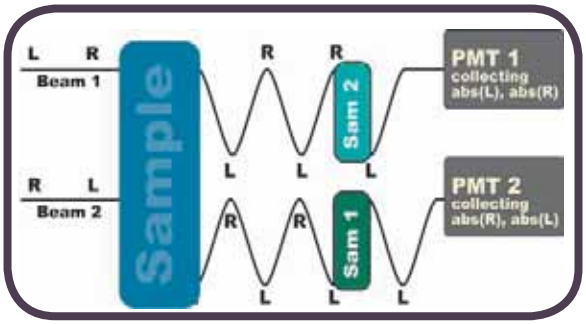
Comparison Chart Continued...

Single vs. Dual Beam Continued...

Integration time	**Response time, usually given as 0.5 (or 1) msec to 15 (or 60) seconds**	**0.001 to 64,000 seconds per datum**
Absorption range	Quote as 0-4 or 0-5 OD	0-3 OD, without additional filters
Absorbance mode	**Single beam**	**Dual beam**
Upgradable to fluorescence	Unknown	Yes, including options of millisecond emission scanning, fixed wavelength photon counting and others
Upgradable to LD	Unknown	Yes
Nitrogen consumption	Quoted from 55 to 22 l/m at startup, down to 3 l/m maintenance level in visible	Full flow to purge (10 to 20 liters/min), 5 l/m above 190 nm for models 17 and 1000 and 2 l/m for the model 20. Estimates are easily adjustable with separate valves for lamp housing, monochromator, and sample chamber.
3D Fitting Methodology	Not available	Since all Olis systems can acquire spectral scans as a function of any process (time, temperature, etc.), they can acquire 3D data files. Our 3D data analysis software provided with these systems is fast, robust, and useful in counting or rejecting chemical models. After SVD, to find the number of species and remove noise, this software uses Downhill Simplex and Matrix Exponentiation to solve the chemical equation describing the reaction. **These 59 algorithms** do not employ any outmoded methods such as Levenberg-Marquardt, Savitsky-Golay, Least Squares Fitting, or numerical integration techniques.
Secondary Structure Determination Algorithms	Unknown	**Four methods** from the public domain are standard fitting options in the Olis software. Methods include CDSSTR, CONTINLL, SELCON3, and Inverse Spectrum (Compton)
Data Processing Algorithms for 2D Data Files	**Unknown**	**19 models for kinetic data, plus 22 fits for 2D analysis**
Data File Output:	**Unknown**	Right click to JPG, ASCII, and Excel™, as well as copy to clipboard, Olis binary, and print

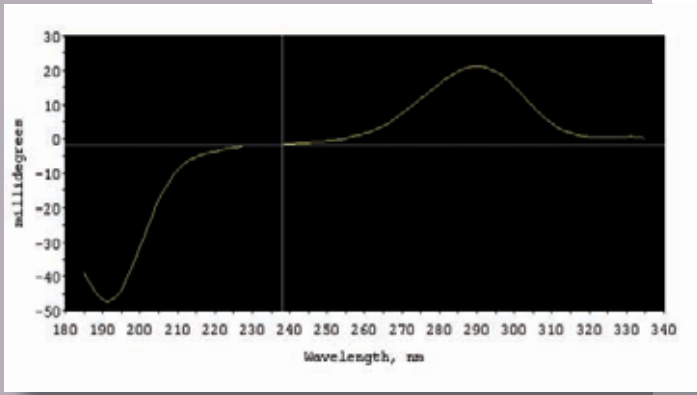
Perfect Experimental Recall	Unknown	Protocol files, containing 100% of the instrumentation hardware settings and 100% of the software settings, can be produced by management and retrieved by technicians, so that guaranteed duplication of experimental settings is made.
Cuvette Holder(s)	variety available	Default includes one holder for cylindrical or rectangular cell (standard CD sample position) and two holders for rectangular cells (use for dual beam absorbance, or fluorescence, or FDCD), all jacketed for ambient or thermal control with a water bath. Optional choices include 1, 2, 4, 6, and 10 position cell holders for cylindrical or rectanglar cuvettes, Peltier or jacketed.
Access to Company President	Unknown	Yes
Access to Programming Staff	Unknown	Yes
Immediacy of Technical Assistance	Unknown	Upon receipt of call for help
Availability of Repair Parts	Unknown	Typically in-stock and shipped by 24-hour courier, worldwide
Availability of Customization	Unknown	Encouraged and practically standard practice
Country of Origin	Japan	USA (Athens, Georgia)
Years in Business	Unknown	Founded 1976, so 2006 is our 31st year
LifeSpan of the Instrument	Unknown	Decades and counting

An Olis DSM CD Should Be Your Next CD Because:



- Two CD samples can be scanned simultaneously, which is used mainly for long thermal melts; samples are in a twin CD/2 rectangular cell holder.
- Kinetic CD acquisition supports up to 2,000 readings per second.
- The CD spectrophotometer sample compartment includes up to three jacketed cuvette holders, one for cylindrical and rectangular dual beam CD cuvette use and two for 1 cm^2 and microvolume single beam CD and dual beam absorbance cuvette use. Alternatively, and at higher cost, the CD spectrophotometer sample compartment can be fitted with 1, 2, 3, or 6 thermoelectric heating/cooling Peltier cells for electronic temperature ramping.
- There are three independent nitrogen regulator settings for the monochromator, sample compartment, and lamp housing, allowing for most judicious nitrogen use.
- The CD spectrophotometer is delivered with software for collecting and analyzing data as a function of wavelength, time, concentration, and/ or temperature (3D global analysis, as well as 2D fits).

- The CD detection is the “Digital Subtractive Method” (“DSM”) of acquiring absolute CD. “DSM” detection is the only means of collecting (not calculating) circular dichroism (“CD”). The answer can only be correct!
- The CD spectrophotometer requires no calibration. Acquisition by definition is superior to calculation after (fallible) calibration.
- The CD is linear to 10,000 millidegrees, opening up use with a huge choice of sample concentrations.
- The CD has zero drift. Experiments can be indefinitely long and the last scan will be as correct as the first.
- Use of the CD spectrophotometer does not require any prior knowledge about the sample by the operator. There are no amplitude, sensitivity, or time constant settings to make prior to scanning the sample.



- CD spectrophotometer uses 100% of the light from the lamp rather than the 50% used by a single beam CD spectrometer. More light is always good.
- The CD supports variable data collection rates, so that the minimum length of time will be used to scan a spectrum, saving time, nitrogen, and the need for repetitive scanning.

Nitty-Gritty Details of DSM CD

The CD measurement begins with the digitization of the voltage signals generated by the detectors. Once digitized, the CD information is extracted from these signals in an entirely digital manner using mathematical manipulations derived by rigorous analysis of the optical system.

The digitized signal consists of a DC level, a 50 kHz signal, and noise. The digital processing derives the CD amplitude from the DC and 50 kHz signals while repressing the noise.

Step one is to measure the DC component of each signal by computing the time average of each signal. This DC level is derived from the same data as the 50 kHz signal, so there is no need to deal with independent amplification of each component and thus no need for a photometric calibration using a chemical standard.

With the DC amplitudes known, step two is to normalize each signal by dividing each by their respective DC level. The resulting signals have DC amplitudes of unity with normalized 50 kHz components. The CD information is derived from these normalized signals; thus, the absolute DC amplitude of either signal before normalization is of no consequence to obtaining the right answer. That is, the two beams of light do not need to be identical (although they happen to be very nearly identical). The absolute DC amplitudes prior to normalization do not contain information about the CD signal[1].

Step three is the subtraction of one normalized signal from the other. The results are (1) the constructive interference between the 50 kHz signals in the two channels and (2) the destructive interference of all remaining components. In detail, the 50 kHz modulation which contains the CD information is phase shifted exactly π radians between the two channels. This phase difference is a consequence of the orthogonal polarization states produced by the beam splitter and, as such, is absolute and unchanging. The subtraction of the two channels is essentially equivalent to a phase shift followed by an addition. As a consequence, the two 'out of phase' signals are brought 'into phase' and then added.

The destructive interference is achieved in exactly the same manner, except here, the two signals are originally 'in phase' and are brought 'out of phase' and then added; their sum is zero. These originally in-phase signals includes noise produced by sources before the detectors (i.e., lamp fluctuations).

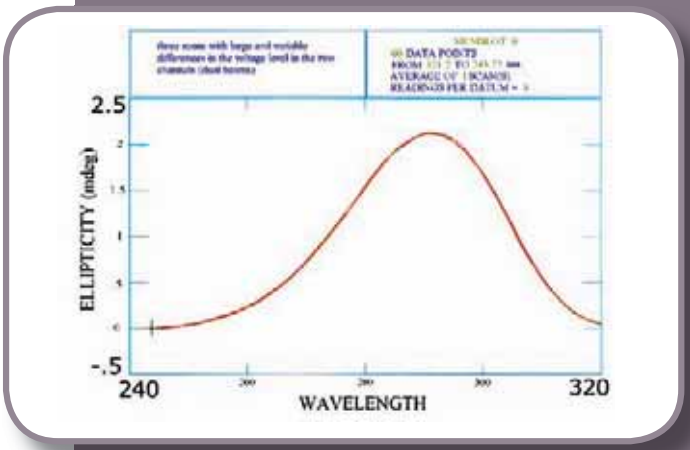
At this point, we have a single data set which is the result of the subtraction of the two normalized signals. These data can be visualized as having the 50 kHz modulation and noise. The time average of these components is zero. If we now apply a 50 kHz rectification to the data, we are effectively multiplying the data by a 50 kHz square wave which varies between +1 and -1 and has the same phase as the 50 kHz modulation in the signal. After this operation, the 'rectified' 50 kHz component has a non-zero time average, whereas the time average of the noise is unaffected, remaining zero. The final step is to average the data. Again, the noise will average to zero and the result is the CD signal[1].

Identical Light Levels are Unnecessary

Scan 1: **Beams Identical**

Scan 2: **Beam 1 Fixed, Beam 2 Variable**

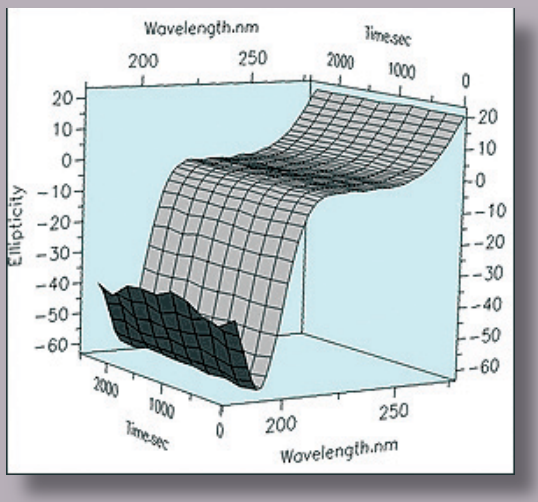
Scan 3: **Beam 1 Variable, Beam 2 Fixed**



- The absolute intensity of the light has no effect on the answer in any single beam or dual beam CD instrument.
- The dual beam CD makes use of no relationship between the beams except the relative phase of polarization, which is set absolutely by the polarizing beamsplitter and which never changes.
- A change in light intensity or detector gain will effect each component in the same proportionate amount, leaving the relationship unchanged.

Footnotes

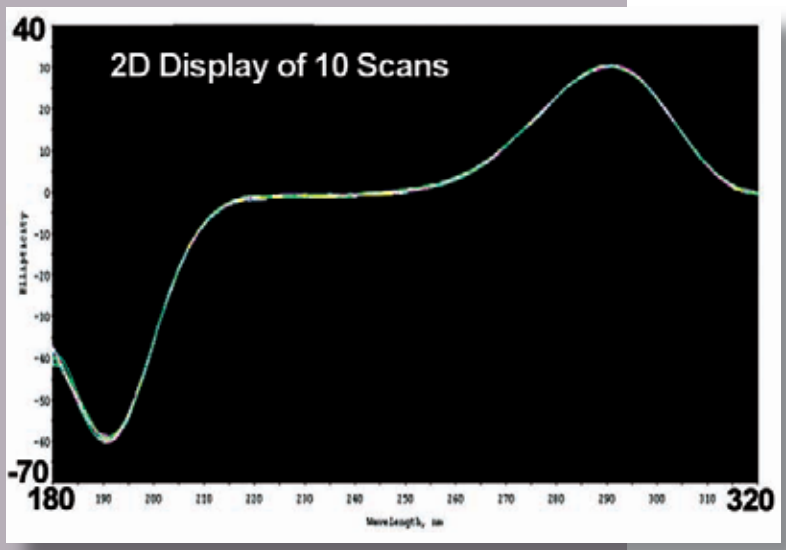
[1)] Contact Olis to request the Mueller calculus paper.

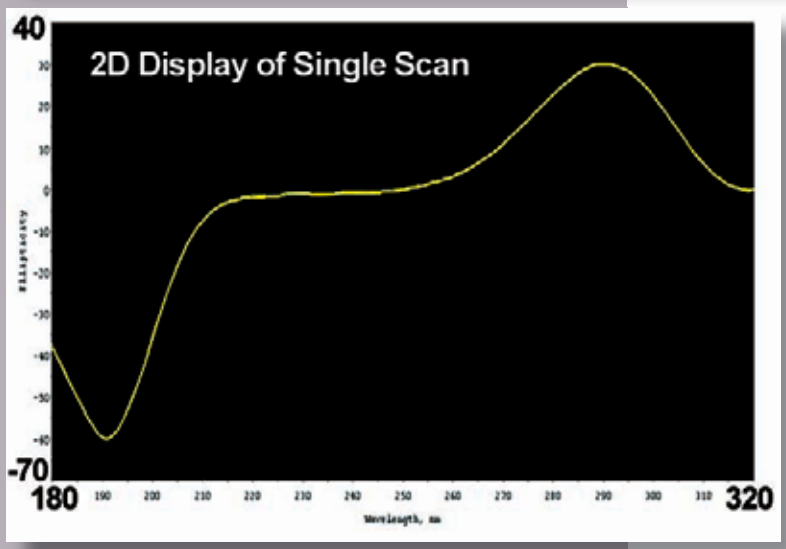


Spectra of Standard

Client Data

Shown are 10 raw CD scans of d-camphor-sulfonic acid, a common standard for CD, acquired by Olis DSM CD owner, Dr. Henrik Ipsen. Henrik sent us these data so we could confirm that he had installed a replacement lamp correctly. These data, 10 scans acquired in 2400 seconds (<4 minutes per scan), illustrate well the reproducibility, low noise, and general quality of the DSM method.





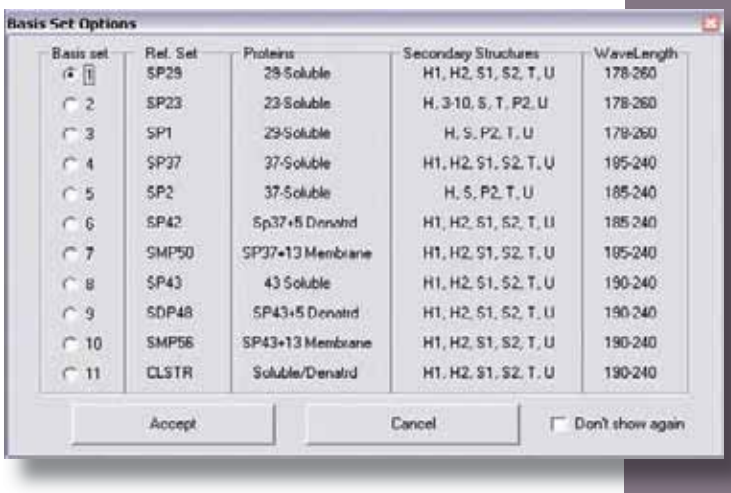
"The single scan that was baseline corrected looked every bit as good as the average of 5 scans that was baseline corrected, which is what I sent you... Needless to say, the results were very reproducible but you already know that."

Michael Sehorn, LSUMC
April 2001

Four Algorithms for Determination of Protein Secondary Structure

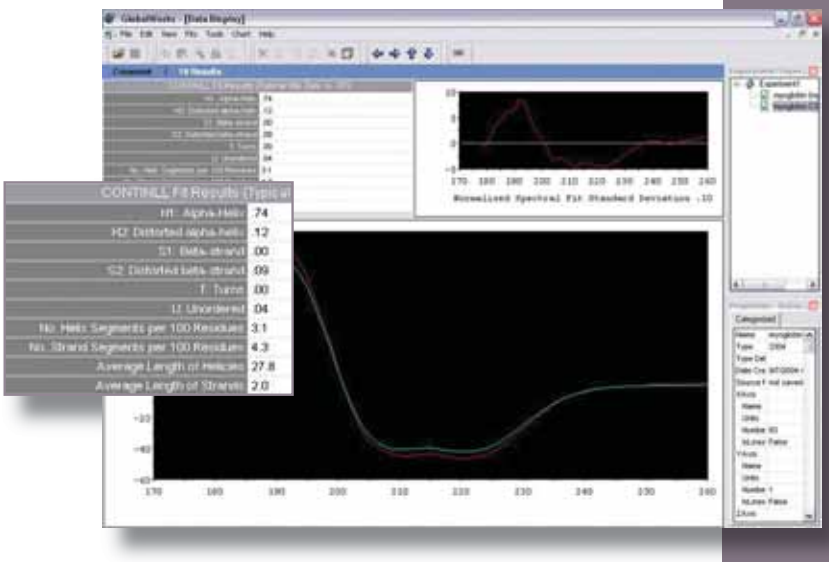
Inverse Spectrum, CDSSTR, CONTINLL, *and* SELCON3, as pull-down fits

These secondary structure determination algorithms are mathematically as their authors wrote them. Olis, Inc. reproduced them from the public literature[1] and public domain site of Narasimha Sreerama, CDPro[2], and made them easily accessible under the 'Fits' menu of GlobalWorks and SpectralWorks programs.



BASIS SETS

All of the secondary structure fits require a basis set, which contains CD spectra of proteins of known secondary structure. Secondary structures of these model proteins were obtained from X-ray crystal structures. The data are acquired at 1 nm interval.



SECONDARY STRUCTURE FIT RESULTS

The results of a protein secondary structure determination are presented as fractional composition of secondary structure motifs. These include alpha-helix, beta-strand, turns, proline turns, and unordered segments.

Footnotes

1) Reviews available in the literature compare these determinations
2) http://lamar.colostate.edu/~sreeram/CDPro

Near IR with the DSM CD

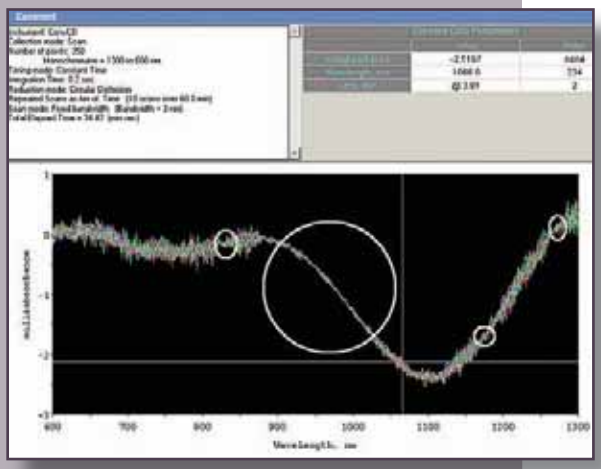


Figure 1.

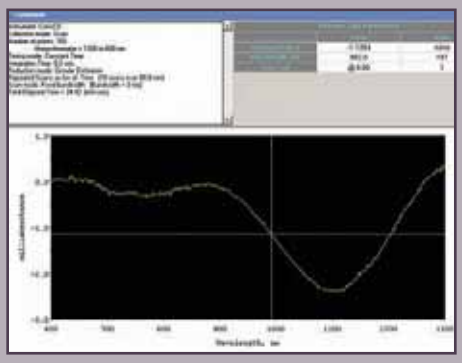


Figure 2.

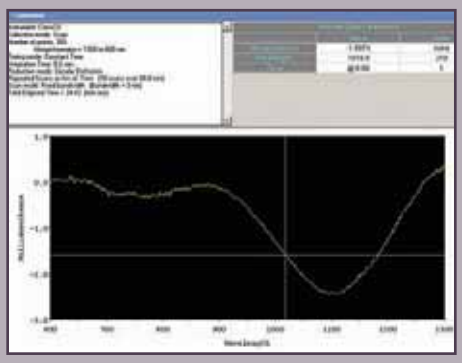


Figure 3.

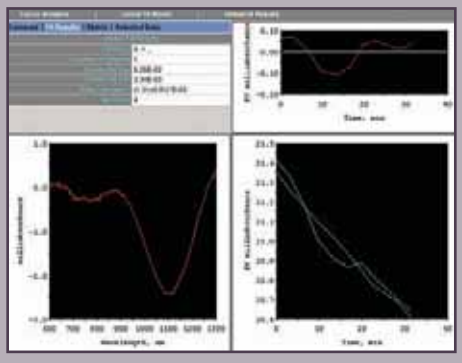


Figure 4.

The Olis® DSM CD 17 and 1000 support NIR detection to 1700 nm (optionally to 2500 nm) using large area (12.5 mm²) InGaAs detectors with unique and innovative Olis preamplifier circuitry. An exclusive photoelastic modulator (PEM) made for Olis, Inc. provides the left and right circularly polarized to 2500 nm.

Unlike all other CD spectrophotometers, the Olis DSM 17 and 1000 CD spectrophotometers produce useful low noise CD results deep into the NIR.

These 10 scans of 0.12 Molar nickel tartrate (Figure 1) were collected in 34 minutes, 42 seconds using an integration time of 0.2 seconds per datum across the 1300-600 nm span.

The variable noise levels along this wide span are reminders of the sharp lines of the 150 watt xenon arc lamp. The emission in the lamp output varies by about 30 fold over this range, with the sharpest peaks being in the regions with the least noise (circled). Double beam spectrophotometers, like all Olis CD instruments, deal with this high variance correctly, such that only the noise level is affected.

Single beam spectrophotometers, such as all non-Olis CDs, which must form the ratio of the CD signal and the DC signal, are considerably troubled by the sharp and dramatic intensity variations in the NIR. To quote Mr. Castigliono, an internationally respected CD expert who publishes applications notes for Jasco Europe, “Xe lamps, while showing a continuous spectra in most of the UV-Vis range, over 750 nm have strong emission lines, which may create problems in obtaining proper CD signals (which is coming from AC/DC ratio).[1]” The Olis DSM CD spectrophotometers—collecting CD not from an AC/DC ratio, but from a direct dual beam acquisition of ABS(L) and ABS(R)—deal with these strong emission lines correctly and easily.

With the Olis software, these ten scans might be averaged (Figure 2). The Olis software also supports applying global analysis (SVD, etc.) to remove the noise (Figure 3). And, if these ten scans were changing because the sample was undergoing a chemical or thermal reaction, you could use the Olis global fitting software to calculate rate constants, enthalpy values, transition wavelengths, and other equilibrium and kinetic values associated with changing spectra (Figure 4).

Footnotes

1) ECS Technical Report No. 19 July 2000: “Near NIR-CD”

Changing from PMT Detectors to InGaAs

Open-architecture and good design result in fail-safe and tool-free modularity when exchanging detectors (shown), gratings, cuvette holders, and all accessories.

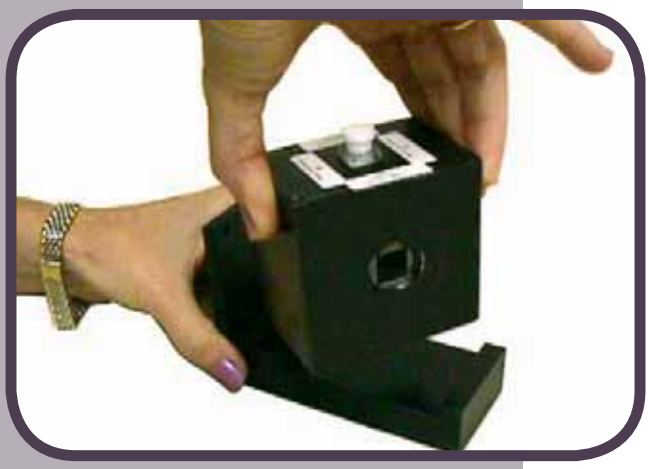
First, the thumbscrews holding the panel in place are removed.

Then, the PMT cables are released by pressing the small lever on the side of each.

Remove the detector panel as shown. Replace with the new panel by reversing these steps.

Two Exclusive Accessories

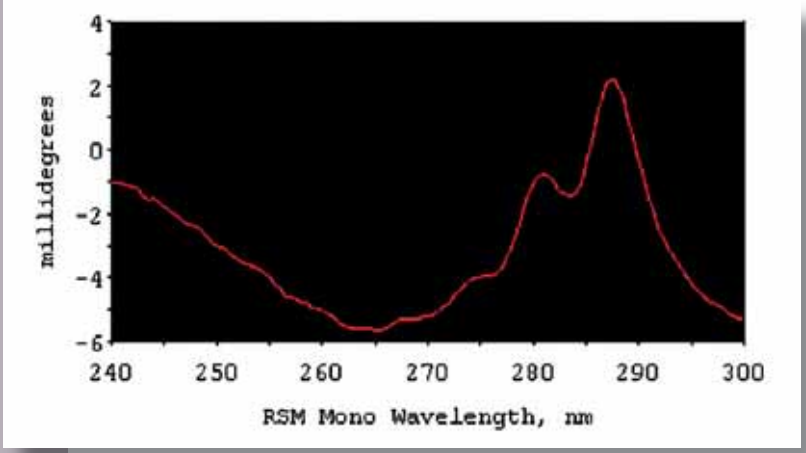
Reversing the Olis Magnet
The relationship of the lines of magnetic force relative to the measuring beams can be reversed by simply turning the magnet on its base 180°.



Cuvette Holder with 1.4 Tesla Magnetic Field

The DeSa magnet is a powerful permanent magnet which requires no water or electricity. The magnet is easily reversible for doing reverse field MCD. The accessory weighs only 1.9 kg, yet produces a useful 1.4 T magnetic field.

Shape Where there Was None
Subtracting the reverse MCD data from the forward MCD data cancels any normal CD and produces a 2MCD spectrum. This indole plus d-camphosulfonic acid mixture shows minimal structure in MCD, but the 2MCD spectrum has a distinctive spectrum, shown.



Four Syringe Titrator with Peltier Thermal Regulation

Joining three other Olis titrators is this most ambitious one, which allows microliter precision mixing of up to four samples. All mixing is achieved within the titrator, so that temperature stability, anaerobic operation, and photolabile stability are maintainable. All pathlength and dimension cuvettes can be used with this mixing accessory.

The four syringe model is useful for simple protein + titrant (two syringes), for maintaining sample concentration (3 syringes), for enzyme assay or other complex titration (4 syringes).

Papers Referencing DSM CD Spectrophotometers

Download these and other papers citing use of the Olis CDs from our website at http://olisweb.com/products/cd/papers.php

- Andrews, D., Mattatall, N. R., Arnold, D., Hill, B. C.2005. Expression, puriWcation, and characterization of the CuA–cytochrome c domain from subunit II of the Bacillus subtilis cytochrome caa3 complex in Escherichia coli. Protein Expression and Purification, 42: 227–235.
- Brzezinska, K. R., Curtin, S. A., Deming, T. J. 2002. Polypeptide End-Capping Using Functionalized Isocyanates: Preparation of Pentablock Copolymers. Macromolecules, 35: 2970-2976.
- Chen, S., Ferrone, F. A., Wetzel, R., 2002. Huntington's disease age-of-onset linked to polyglutamine aggregation nucleation. Proceedings of the National Academy of Sciences, 99: 11884–11889.
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- Crawley, S. W., de la Roche, M. A., Lee, S. F., Li, Z., Chitayat, S. Smith, S. P., Cote, G. P. 2006. Identification and Characterization of an 8 kDa Light Chain Associated with Dictyostelium discoideum MyoB, a Class I Myosin. Journal of Biological Chemistry: 1-14.
- Gorski, A., Kohler, T., Seidel, D., Lee, J. T., Orzanowska, G., Sessler, J. L., Waluk, J. 2005 Electronic Structure, Spectra, and Magnetic Circular Dichroism of Cyclohexa-, Cyclohepta-, and Cyclooctapyrrole. Chemistry- A European Journal, 11: 4179–4184.
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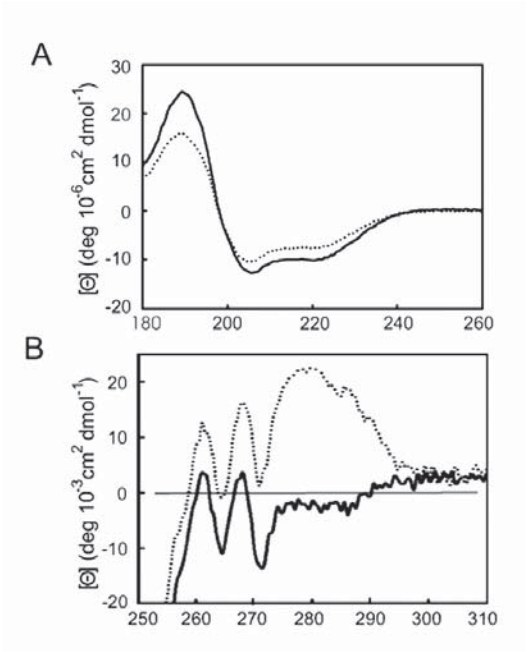
- Ybarra, J., Bhattacharyya, A. M., Panda, M., Horowitz, P. M. 2003. Active Rhodanese Lacking Nonessential Sulfhydryl Groups Contains an Unstable C-terminal Domain and Can Be Bound, Inactivated, and Reactivated by GroEL. Journal of Biological Chemistry, 278: 1693–1699.



Crawley, etal, JBC 2006
Identification and Characterization of an 8 kDa Light Chain Associated with Dictyostelium discoideum MyoB, a Class I Myosin

Comparing the Modern Olis DSM CD with the 'market leader' in CD

We hear a lot that we are competing against "The Market Leader in CD." What can we say? This is true. **But** …. Ours is better for all the reasons we state in this **"CD by Definition or by Tradition"** brochure.

They continue to use a technique invented in 1960.
We use a new technique invented in 1994.
(Computers and software are tools of the modern era, which, when used to their best advantage, obviate the need for complex electronics and circuitry.)

They can only make an indirect calculation.
We make direct measurements.
(Direct always preferable to indirect in the context of collecting experimental results, right?)

They rely on complex electronics.
We wrote sophisticated software instead.
(Hardware breaks. Software runs forever.)

They have only manipulated results to show you.
We have the unadulterated raw data, right down to Abs (L) and Abs (R), as well as their difference (CD).
(If there is error in the manipulated results, one cannot know nor correct it.)

Their instrument requires calibration.
Our instrument does not.
(Calibration is an arbitrary thing ... who does it? when? is it done correctly?)

They have no reference channel.
Our instrument's two beams are each a reference for the other.
(A reference reading is hugely advantageous, especially when working with xenon arc lamps, which all CDs do.)

They use 50% of the light from the lamp.
We use 100% of the light from the lamp.
(More light means more photons which means less time.)

They can only look at one sample at a time.
We can look at two samples at a time.
(We don't advertise this feature much, because one loses "100% of the light" and "reference channel" benefits, above, but if you have a lot of samples and their signals are reasonably large, you can get twice the throughput with an Olis DSM CD.)

They collect ? points per millisecond.
We can collect 2,000 points per millisecond.
(If you are doing kinetics, you will appreciate our fast acquisition rate. If you are doing routine scanning, you will not notice that each returned datum is the fitted result of hundreds of thousands of dual beam readings … only that the noise level is low.)

They have minimal (if any) useful data handling software.
We have the best data handling software available.
(In addition to the correct answer and reliability, what you will care about most is excellent software, and we have it!)

They have no 3D data handling functions.
We have the most powerful 3D global analysis software available. Global (multiple wavelength) data and analysis of it is always superior to single wavelength measurements and fits.
(A movie is always superior to a snapshot, even if it is only to prove that the subject did not move; 3D is always superior to fixed wavelength, as only with spectra can one know something of the chemistry.)

Our instrument is smaller, so it uses less nitrogen, takes up less bench space, and is easier to move.

Our instrument has no electronic drift, so you can collect for hours on end without concern about increasingly incorrect answers.

Our instrument is linear to more orders of magnitude, so you can work with samples with unusually large CD signals.

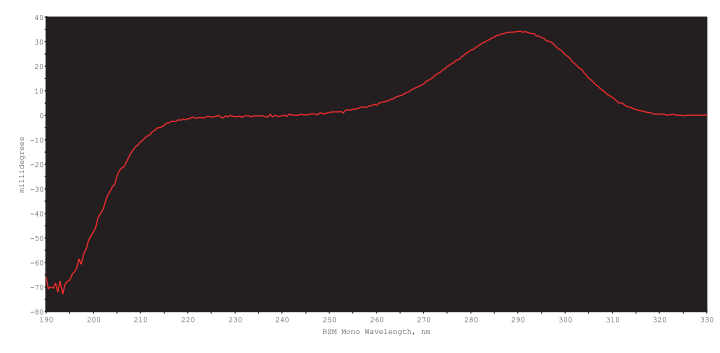
Not all of these points are critical, but combined, **they make a powerful case for today's new technology CD spectrophotometers**. Your decision today is one you will live with for years... Make it for the Olis DSM CD!

Welcome to the Digital Age!

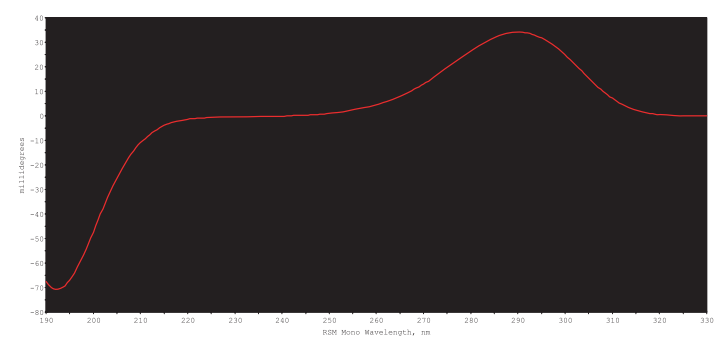
Digital Noise Removal: Fast, Easy, Thorough

Traditional CDs use a lock-in amplifier to fix an electronic time constant prior to data acquisition. Our method for noise reduction/ removal is mathematical (digital) after data acquisition.

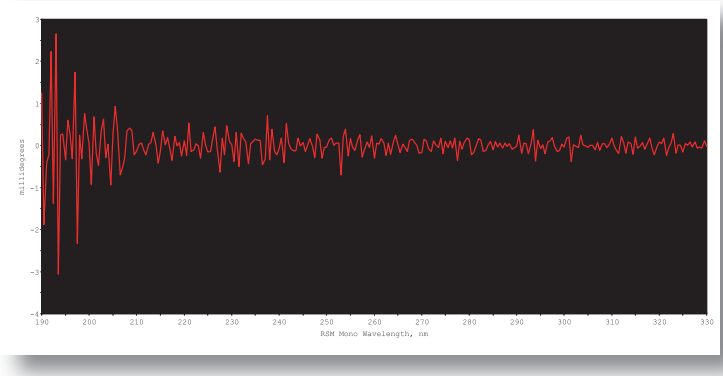
Notice the higher noise in the <200 nm region in this un-filtered scan.



Here, after the digital filtering, we achieve a spectrum with low noise levels along the entire span.



The difference between unfiltered and filtered, illustrating perfect removal of noise and no structural distortion of the spectrum.



Exclusive to the Mathematical (Digital) Method:

- Allows maximum data acquisition rates under all conditions
- Allows reversible noise filtering after raw data are collected
- The shape of the spectrum with the noise removed by the filtering can be confirmed as unaffected (not distorted).
- Useful information about the noise itself can be found in the difference between the raw unfiltered data and the final filtered data.

Whereas, with the Traditional Electronic Method:

- There are no raw data to return to, so there is no means of adjusting the data once they are acquired.
- There is no means of determining whether the spectrum has been distorted other than to run the experiment again with a different time constant and hope to conclude which of the two is ‘more’ correct.
- An accepted way of noise reduction is to signal average multiple scans. Of course, each scan will have high noise in the same region as the other scan. We include this method, too, for those people who choose to use it.

"You can go ahead and use my last e-mail which is basically the highest praise the tech-support in any company can get. I have never seen an instrument getting fixed within 24 h including diagnosis of the problem and sending the replacement part."

Kai Griebenow, Univeristy of Puerto Rico, April 2002

"In their bid the vendor claimed, 'There is no experimentally justifiable reason to have dual beam CD.' To which I replied that it was like saying there was no reason to have two lenses in a pair of glasses or a dual beam spectrophotometer."

John Schloss, Kuwait University, July 2001

"The [Olis DSM 1000 CD] machine is really good … the performance of the instrument is usually not appreciated by the average users, because they don't realize the difficulty of measuring small CD signals and because they don't have experience with other instruments."

Wim Jiskoot, University of Utrecht, April 2000

"Gorgeous data!" **Catherine Murphy, University of South Carolina, February 1999**

"A never-be-satisfied attitude is what's brought OLIS to where it is, and it's what will bring you much success in the future. … I hope you guys never change that way of thinking. … I've been using the CD quite a bit, and am quite happy with it. … options for SF CD are several, but OLIS is my 1st (and second) choice …"

Wilfredo Colon, RPI, May 1998

Olis

For more information on this and other Olis products:

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