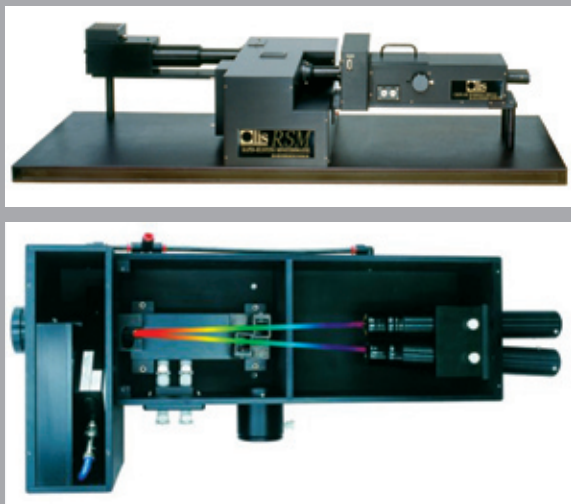




Digital Subtractive Method CD spectrophotometers

**Olis DSM CD**



## Simplify a Historically Challenging Measurement

DSM is the first direct means of collecting circular dichroism spectra. Methods used by all other non-DSM CDs require knowledge about the sample prior to the experiment. Settings for amplitude, sensitivity, and time constants can only be correct with experience. DSM totally eliminates these variables. DSM also eliminates the number one cause of variance in answers returned by CDs: calibration.<sup>1</sup>

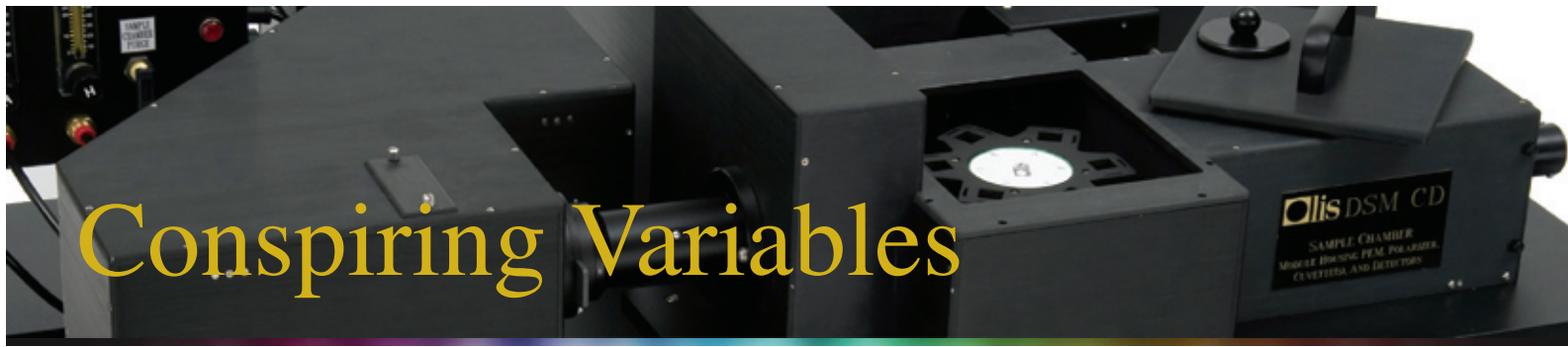
Olis, Inc. pioneered the digitization (computerization) of optical spectrophotometers in the 1970s.<sup>2</sup> When we introduced the Olis DSM 1000 CD in 1994, we had the expertise to employ sophisticated software, freeing our instruments from analog hardware that other companies continue to rely on, most notably the *circa 1964* lock-in amplifier.

Beyond reducing the variables and increasing the precision of the answer, Olis DSM CD spectrophotometers bring to your group reliability, modularity, and longevity.

We have six Olis DSM CD models to choose from, one certain to fit the needs of those new to the historically challenging technique and those who have built their career on it.

DSM is operationally simple, so your users can be successful.





## Olis DSM CD: Simple, direct, and error-free

What could be more direct and obvious than acquisition by definition?

Circular dichroism (CD) is an inherently difficult measurement to make. A large CD signal is 0.0001 AU. For the number one application, protein secondary structure, the spectral region of most interest is within 260-190 nm, where the light level is very low.



### Challenges inherent to CD

The absorbing nature of the buffers used, the critical need for an ideal sample concentration, and patience and attention required while handling the sample in short (0.1-2 mm) pathlength cuvettes are other physical realities challenging those who choose to use CD in their studies.

### Challenges unique to prior technology CDs

Historically designed CD spectrometers add other annoyances, including calibration variations plus lock-in amplifier settings for amplitude, time constants, and sensitivity. Other variables that contribute to the (in)accuracy of the measurements include baseline drift and finite linearity.

### Calibration

A manual process conducted by a trained technician and required by all non-DSM CDs. The answer is only as good as the calibration, which is perfectly temporarily or, if done poorly, never. Confounding the issue, one cannot determine from a CD spectrum whether it was collected under perfect calibration conditions. And, correcting the error is not possible. **Olis DSM requires no calibration. Ever.**

### Lock-in amplifier settings

Because we know how to find and collect the  $abs(L)$  and  $abs(R)$ , we have **eliminated the need for a lock-in amplifier** and its time constant, amplitude, and sensitivity settings. These settings can only be correct with experience. Eliminating them removes the need for any prior knowledge about the sample. This asset's value increases as the variety of samples increases (think shared equipment or core facility cases).

The alternative to DSM?

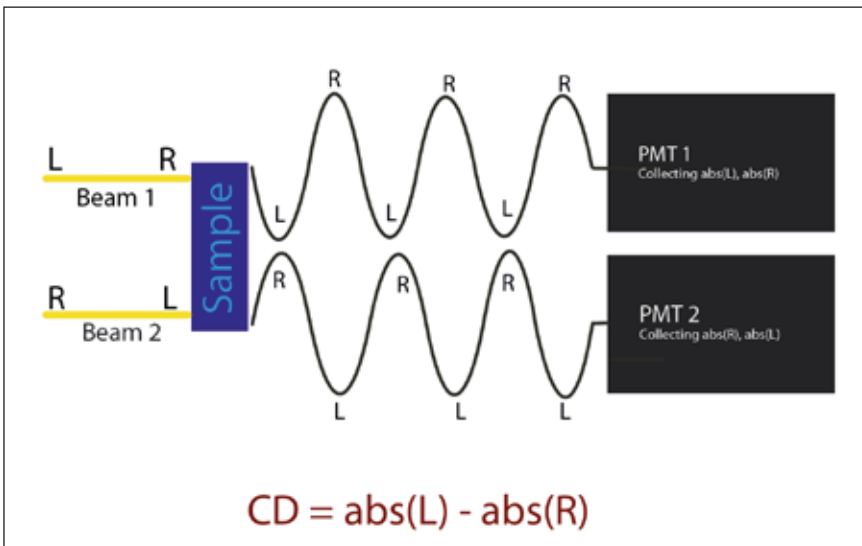
To quote W. Curtis Johnson in Fasman's, **Circular Dichroism and the Conformational Analysis of Biomolecules** (Plenum Press: 1996)<sup>3</sup>:

"The **most straight-forward** [method for measuring the CD of a sample] 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...

Inexpensive, high speed digital computers have made direct subtraction of left and right circularly polarizing 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 [DSM] 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 [K] to calibrate."

**DSM The correct answer is the only possible answer**



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

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, i.e., two scans in the time of one. And, each beam is sufficient of itself for  $CD/2$ .

**Your alternative technology CD**

With calibration and lock-in amplifiers, the correct answer is obtainable only if the electronic settings are correct and calibration is correct **because** CD is calculated as the ratio of intensities of the decoupled AC and DC components adjusted by the calibration constant (k), i.e., CD calculated as  $k(I_{AC}/I_{DC})$ .

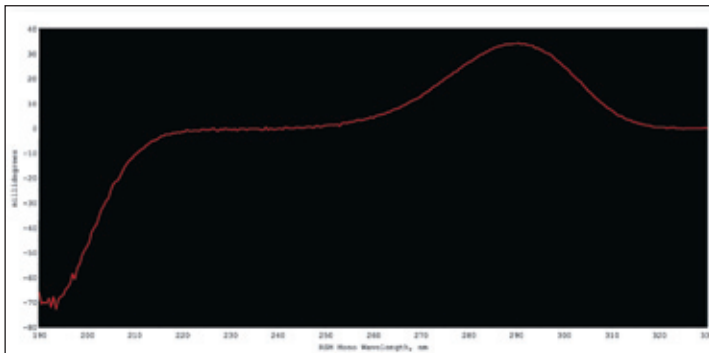


Direct acquisition CD frees you from choosing between time and spectral quality.

**Digital noise removal: Fast, easy, thorough**

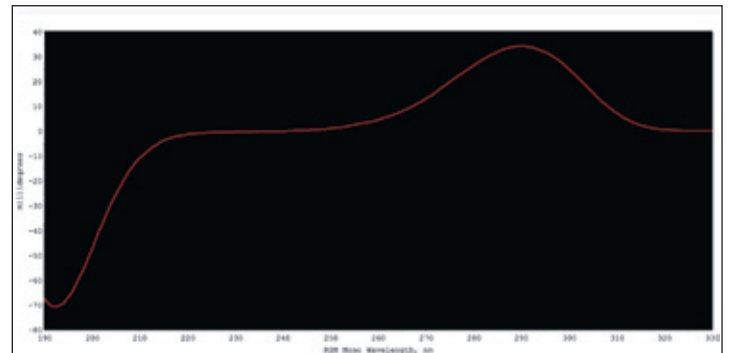
Alternative technology CDs use a lock-in amplifier to fix an electronic time constant prior to data acquisition. Our method for noise reduction or removal is mathematical (digital) after data acquisition.

Raw scan

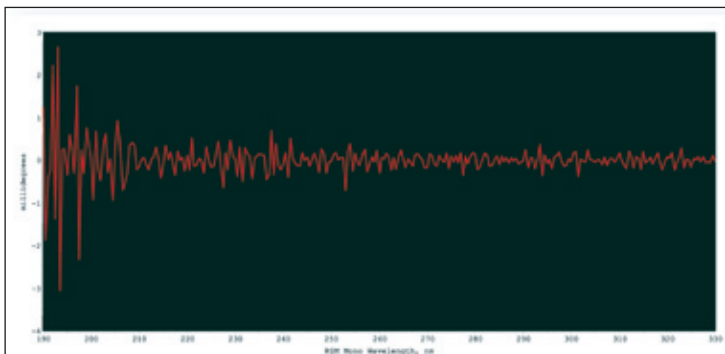


Notice the noise in the <200 nm region in this **un-filtered scan**. We were able to save time and nitrogen by not spending more time in this region because we have the algorithm to remove random noise in our data collection software.

Digitally filtered scan



After digital filtering, the noise in the lowest wavelength is gone. Noise removal by a lock-in amplifier is not verifiable or reversible.



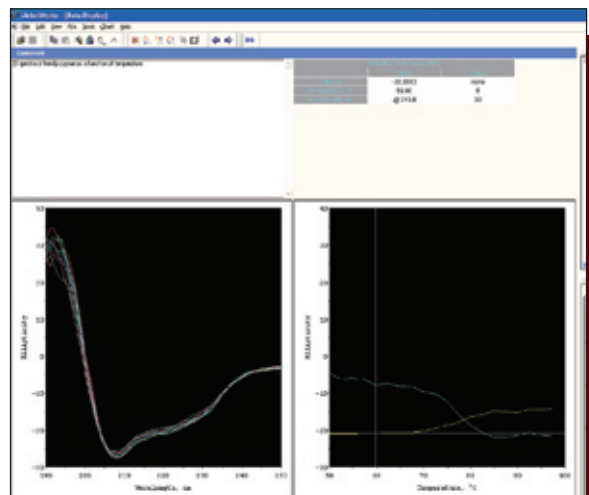
The difference between unfiltered and filtered, illustrating perfect removal of noise and **no structural distortion** of the spectrum.

## Analyzing temperature dependent scans<sup>4</sup>

For decades, thermal denaturation studies have been isolated to single wavelength traces because of the tremendous difference in time, energy, nitrogen, and data handling between one and many wavelengths. Scanning quickly without concern about noise makes 3D acquisition much more attractive.

The data analysis software that comes with every Olis DSM CD not only calculates the transition temperature and enthalpy from the raw data but includes another mathematical means of noise removal: singular value decomposition. (If you don't know about SVD, now is the time to learn!)

Temperature dependent scans shown in Olis SpectralWorks software; three selected wavelength traces in right panel.

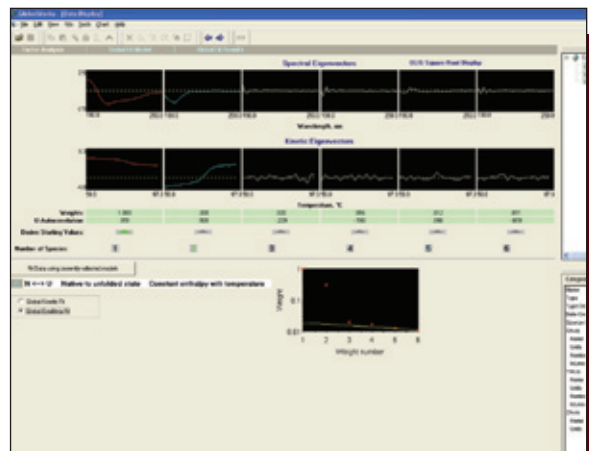


## Results of SVD

Eigenvector presentation, suggesting the presence of two species, as shown in the first two columns of the plots.

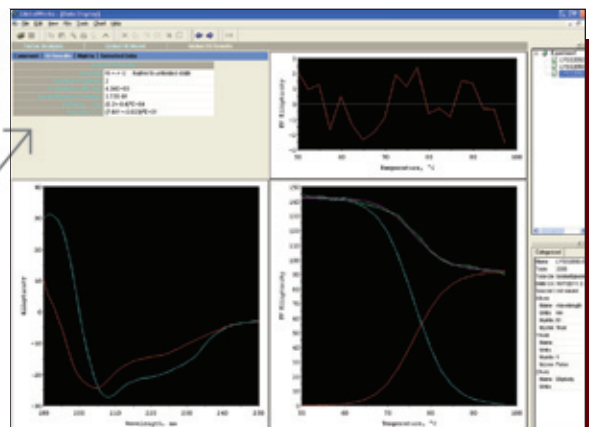
Non-structured data, noise, is isolated and removed in this step. Noise "scans" fill the remaining four columns of plots.

Numeric values beneath the graphic eigenvectors aid in analysis.



Results of spectral reconstruction fit: Starting and final form of protein (left) and temperature dependence (right).

Also returned are the transition temperature and enthalpy values, plus standard deviation values.





# Models & Configurations

## Olis DSM 1000 CD spectrophotometer

Highest light throughput of any Olis CD

- Built around the Olis RSM 1000, featuring the singular DeSa monochromator
- True dual beam detection
- Maximum modularity
- Upgradeable to FDCD, CPL, LD
- Spectral range from vacuum UV to NIR; default 170-540 nm
- Only rapid-scanning CD instrument available, up to 62 scans/sec are practical in the >220 nm regions, **including optional NIR**



## Olis DSM 20 CD spectrophotometer

Closest to the competition

- Built around the Olis Hummingbird subtractive double monochromator
- Smallest CD available
- Brilliant light throughput, 170 - 700 nm
- **Ideal for most common CD applications**



## Olis DSM 17 CD spectrophotometer

Dual beam, superior NIR performance

- Built around the classic Cary 14/17 UV/Vis [NIR] monochromator
- **Widest available spectral range, ≤185-2600 nm**
- Computer-controlled slit width
- Full use of Cary prism-grating monochromator for dual beam absorbance and CD



## Olis MultiScan, aka, mini "Protein Machine"

### Save time, sample, and energy

- Simultaneous CD, fluorescence emission and excitation spectra, light scatter, second derivative spectra, absorbance (and CD) spectra
- One software package
- Optimal collection time
- Minimal sample
- **High throughput means of capturing data on samples undergoing thermal denaturation**
- Automatic "Data Tree" data storage organizes all data into manageable folders for easy analysis



## Olis DSM 172 CD spectrophotometer

### Modular polarization hardware

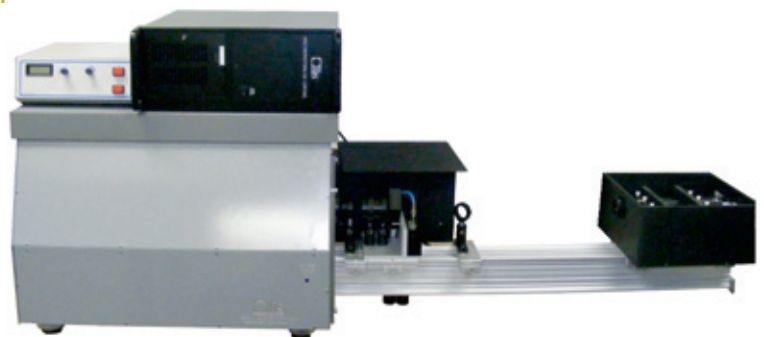
- Single beam detection
- Optically identical to Olis DSM 17 CD
- **Easily positioned polarizers and PEM**, extending uses to CD, CPL, FDCD, polarization of fluorescence, anisotropy, absorbance, ORD
- Scanning emission monochromator, optional



## Olis DSM 17 MCD spectrophotometer

### Optics, your cryogenic magnet, detector chamber

- Single beam detection
- **PEM external to the optical bench**
- Optically identical to the Olis DSM 172 CD
- Automatic detector changeover from Vis to NIR, optional
- Olis software for computerized control of magnet, optional

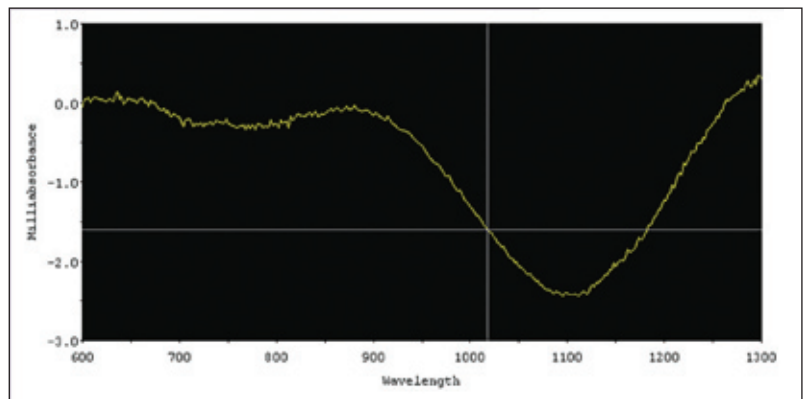




## NIR Circular Dichroism

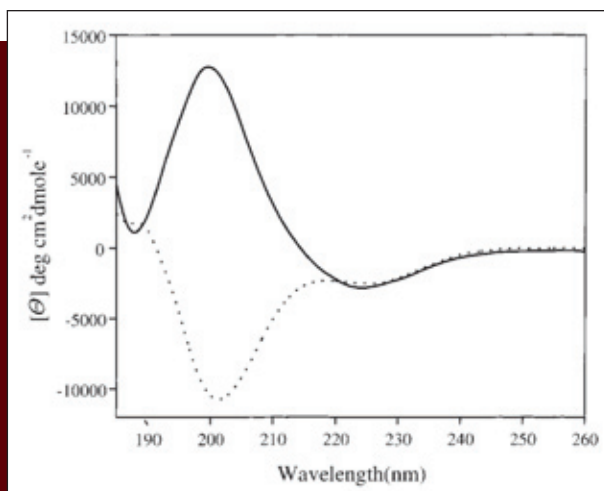
Non-DSM CD spectrophotometers have great difficulty dealing with the sharp and dramatic intensity variations in the NIR of the xenon arc lamp because they operate by decoupling the AC and DC components of the light. Thus, there are very few commercial NIR CDs and none which operate above 1100 nm.

The Olis DSM models – collecting CD not from an AC/DC ratio but from direct acquisition of  $abs(L)$  and  $abs(R)$  – handle these strong emission lines correctly and easily. And, because there is so much light at these wavelengths, the lowest noise regions in a NIR scan are actually where the normally confounding sharp intensity peaks are!



Olis CDs can be dedicated to NIR or configurable for both UV/Vis and NIR. In the model 17, the only change from one spectral region to the next is the detector: PMT for UV/Vis and InGaAs for NIR, with the changeover computerized or manual. In the model 20, the decision as to the spectral range must be made at time of manufacture. In the model 1000, both the detectors and the optical surfaces that determine the spectral range – the gratings – must be exchanged between UV/Vis and NIR studies. These changes are quick, simple, and tool-free.

## Success working with turbid samples



Without integrating spheres, moving sample stages, or other complication, the Olis DSM CD acquires credible spectra from particulate sample preparations. (Biochemistry 2002, 41, 7391-7399)

## Common Olis CD accessories

### DeSa 1.4 Tesla Permanent Magnet

#### MCD Made Economical and Accessible

Not every MCD experiment needs to be done with a high field \$100K magnet. In 2006, we introduced the DeSa Magnet, a 1.4 Tesla model which is perfect for many experimental cases. To quote one owner: "The data are wonderful and the magnet is certainly worth its weight ... it does a great job."<sup>4</sup> And, given that it is 1/20<sup>th</sup> the cost of a superconducting magnet, MCD in a pedagogical environment is now entirely practical. See the Olis DeSa Magnet brochure for more details.



### Olis Titrator

#### Short Pathlength Titrators

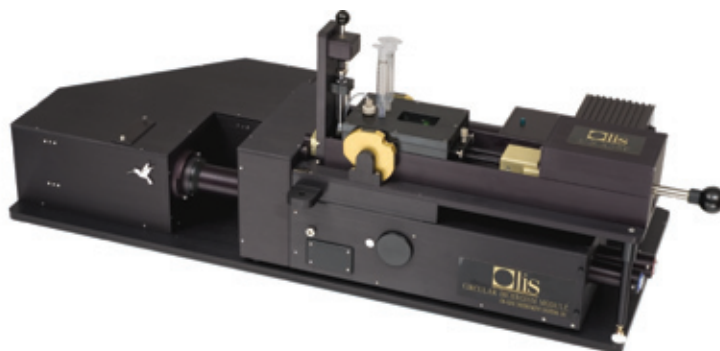
Pipetting into any dual beam Olis DSM CD can be done with the sample compartment lid off, because the ambient room light will be seen equally in the two beams and thus is subtracted out like the other non-CD specific information. But, when many titrations are anticipated and for use with the single beam Olis CDs, choose one of the four Olis titrators. Designed expressly for use in CD, these titrators can be used with all pathlength cuvettes, including < 2 mm. Mixing is done **within** the titrator, so mixing within the cuvette is not necessary. Many other benefits accrue; see the titrator brochure for more details.



### Olis Stopped-flow

#### Millisecond Kinetic Studies, aka Stopped-flow

Olis, Inc. was established on the founder's expertise in stopped-flow kinetics.<sup>2</sup> Our stopped-flow for dual beam CD employs a cylindrical 2 mm quartz window and dead time of under 5 ms. "Simple, Useful, and Affordable", this mixing device can be used with all Olis DSM CDs (shown here on the model 20). Algorithms within the Olis software are provided for fitting 2D and 3D data to exponential and poly-exponential equations.





## Algorithms for Determination of Protein Secondary Structure

### 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<sup>6</sup> and public domain site of Narashima Sreerama, CDPro<sup>7</sup>, and made them easily accessible under the 'Fits' menu of the Olis GlobalWorks and SpectralWorks programs.

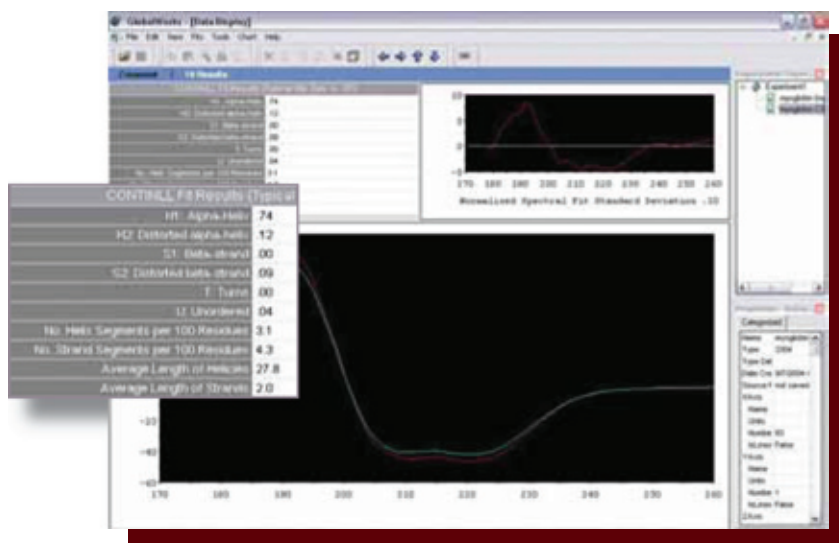
All three algorithms use variable selection of known secondary structure to achieve estimation of secondary structure composition from an unknown CD spectrum.

### 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
- unordered segments
- Graphical representations of the spectra and residuals



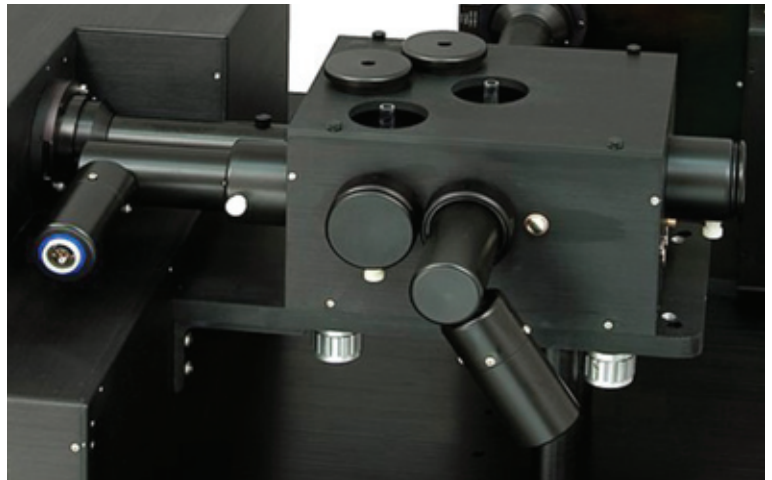
**Far more information** on the Olis DSM CD technology instrumentation is available on our very extensive and technically rich website, [www.olisweb.com](http://www.olisweb.com). And please, ask about any parameter that is not addressed in this brochure or website which interests you.

## Transform any Olis DSM CD spectrophotometer into Olis CLARiTY

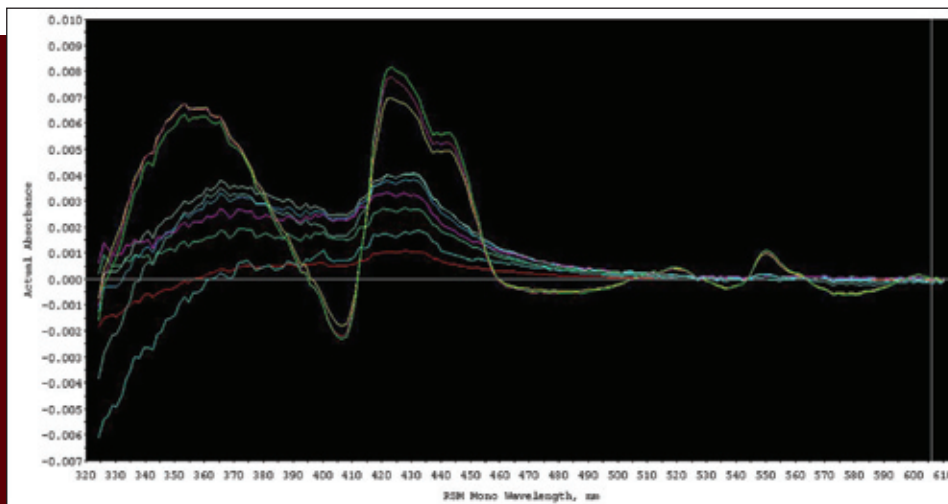
**No longer a CD, your Olis becomes a UV/Vis NIR for dynamic study of suspensions**

Exchange the CD sample compartment (see front cover, lower left photograph) for the CLARiTY sample compartment shown here, change to the appropriate software package, and you have created an absorbance spectrophotometer that is perfectly immune to scatter and thus useful for work with living cell suspensions, nanoparticles, and other "impossibly" turbid samples.

The Olis CLARiTY 1000, which employs the DeSa rapid-scanning monochromator, has the extraordinary ability to scan hundreds of nanometers each millisecond.



## Olis CLARiTY data



Difference spectra capturing the classic reduced-oxidized spectra, plus another 60 nm into the UV, showing the NADP peak, acquired from living yeast cells in suspension on an Olis CLARiTY 1000A.

See these and many other "first" experiments at <http://olisweb.com/Products/Absorbance/Clarity.html>

# “No calibration did it for me.”

Marc Pusey, NASA Flight Center



## Footnotes

1. Pinpointing calibration as #1 and inaccurate pathlength as #2 is the 2010 publication from NPL: "International comparability in spectroscopic measurements of protein structure by circular dichroism: CCQM-P59.1"
2. Olis founder, Dr. Richard J. DeSa, is recognized as the first to publish computerized data acquisition: "A Practical Automatic Data Acquisition System for Stopped-flow Spectrophotometry," R. J. DeSa and Q. H. Gibson, Computers and Biomedical Research, 2, 494-505 (1969).
3. Johnson, W. C. (1996). Circular dichroism instrumentation. In G. Fasman (Ed.), **Circular Dichroism and the Conformational Analysis of Biomolecules** (pp. 640, 644-645). New York: Plenum Press.
4. See these data in high resolution at <http://olisweb.com/Products/CircularDichroism/data/temperaturedata.html>.  
Also, these data are included as demonstration files when you download our free 30 day trial version of Olis GlobalWorks, <http://olisweb.com/Olis-Download-Software.html>
5. Written communication from Martin Stillman, February 2011. He continued, "As far as I can tell it doesn't interfere with the Jasco at all - as we normally put my 5.5T supercon magnet in, this is less hazardous!"
6. Reviews that are available in the literature compare these determinations.
7. <http://lamar.colostate.edu/~sreeram/CDPro>

For more details about Olis DSM CD spectrophotometers and other Olis products:

[www.olisweb.com](http://www.olisweb.com)  
[sales@olisweb.com](mailto:sales@olisweb.com)  
1-706-353-6547 (Worldwide)  
1-800-852-3504 (US & Canada)

