

The Olis[®] Modernized DW-2 & DW-2000 Spectrophotometers



Dual Wavelength Spectroscopy: Still the Method of Choice for Acquiring Correct Absorbance Readings on Turbid Solutions!

When investigators at the University of Pennsylvania¹ designed an absorbance spectrophotometer which sent two wavelengths of light through one sample, they created a spectrophotometer which investigators working on P450, mitochondria, plant and tissue suspensions, and other particulate samples would trust for decades to produce accurate absorbance readings on scattering samples.

Turbid samples have large to overwhelmingly-large backgrounds, which make obtaining accurate absorbance readings very difficult. The more turbid the sample, the more incorrect the answer obtained by an ordinary scanning spectrophotometer will be.

And, one cannot know that the results are incorrect with the ordinary spectrophotometer without further quantitative measurements.

Scatter is a function of $1/\lambda^4$, so the deleterious effects of scatter increase dramatically as the wavelength goes towards shorter wavelengths (the UV).

With a highly scattering sample, the measuring light is deflected (scattered) away from the detector, so close positioning of a detector to the sample is critical. Most absorbance spectrophotometers use small face detectors positioned many centimeters from the sample. The DW-2 uses a large face detector a few millimeters from the sample.

¹ Britton Chance and Ron Estabrook

Technical Specifications of the Aminco DW-2 and DW-2000

Monochromators	Holographic, dual gratings, Czerny-Turner mountings with 1200 grooves/mm. Five-phase step motor control with minimum step size of 0.05 nm.
PMT	Hamamatsu R562HA or equivalent
Wavelength Readability	< ± 0.05 nm
Wavelength Range	190 - 900 nm
Wavelength Accuracy	< ± 0.2 nm
Wavelength Precision	< ± 0.1 nm
Wavelength Resolution	< 0.3 nm (measured at the half-peak height of 486 nm deuterium emission line)
Slit Width Range	< 0.01 nm to 15.0 nm, continuously variable
Stray Light	at 200 nm: < 0.02%; at 300 nm: < 0.03%; at 700 nm: < 0.01%
Photometric Ranges	Absorbance Range: -3.0 to 3.0 AU; Maximum Sensitivity: 0.001 Au full scale; Baseline Offset: -3.0 to 3.0 AU; Transmittance Range: 0 to 100%; Maximum Sensitivity: 0 to 0.1% full scale; Baseline Offset: 0 to 100%
Photometric Accuracy	Deuterium Lamp Supply: current-regulated pulse width modulator with full line isolation. Automatic warmup cycle and regulated two-stage DC filament source for use with 10 V filament, 35 watt Hamamatsu deuterium lamps. RMS ripple at 60 Hz: 0.01%; RMS noise at 29 kHz: 0.03% Tungsten-Iodide Lamp Supply: High stability linear element regulator with soft-start capability. Voltage sensed regulation. RMS ripple at 60 Hz: 0.008%; RMS noise at <60 Hz: 0.008%
Signal to Noise Ratio	at 3 nm slit, 450 nm, slow filter; at 0 Å: < ±0.0005 AU; at 2 Å: < ±0.001 AU peak to peak
Photometric Stability	double beam mode at 550 nm, 3 nm slit; < 0.004 AU/hour
Wavelength Scanning Speed	0.1 nm/sec to 20 nm/sec
Repetitive Scanning	Arbitrary number of repetitions
Baseline Flatness	in double beam mode, < ±0.001 AU throughout the wavelength range, computer corrected
Power Requirements	120V, 50/60 Hz; 240V, 50/60 Hz; 1000 watts
Dimensions	Length: 44 inches (112 cm); Width: 22 inches (56 cm); Height: 21.5 inches (55 cm);
Weight:	approximately 300 pounds (136 kg)

Continued from cover...

However, the "DW-2" gets its name from its more obvious difference from normal spectrophotometers: its use of two monochromators to produce a reference wavelength which is subtracted from the (artificially high) absorbance returned by the sample.

One monochromator is motorized, so that it can scan wavelengths. This "Mono 1" is used to scan the spectrum of interest, i.e., 580-520 nm. "Mono 2" is set to the reference wavelength. When the difference between the returned values is taken, one has a

"difference spectrum" which is as close to "correct" as one can obtain from a scattering sample. (One is still contending with two effects: the reference beam is not at the same wavelength as the measuring beam, and, without extraordinary measures, one cannot collect all the scattered light and therefore cannot get a perfect answer.)

When the absorbance value is calculated using the Mono 2 reference, in effect, one produces a "Difference Spectrum."

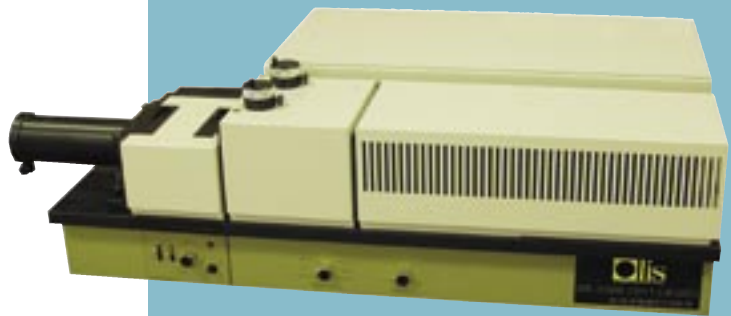
Three Choices from Olis, Inc.

Choice 1: We will modernize your Aminco DW-2 or Aminco DW-2000 for modern computerized operation and years of reliable performance.



The Olis® DW-2 Spectrophotometer

Choice 2: We will modernize an Aminco DW-2 or Aminco DW-2000 which we have in stock, creating a system identical to choice 1.



The Olis® DW-2000 Spectrophotometer

Choice 3: Move from dual wavelength detection to full spectral data acquisition using the Olis RSM 1000 spectrophotometer. The Olis RSM 1000 obtains superior results on turbid samples and obtains correct answers on clear solutions (the DW-2 is not particularly good on clear solutions). See pages 8 and 9 for details.



The Olis® RSM 1000 Spectrophotometer

**Difference spectrum =
ABS of mono1 spectrum (containing chemistry + scatter) -
ABS of mono 2 reference wavelength (containing scatter) = chemistry**

Why modernize your Aminco DW-2 or DW-2000 Spectrophotometer?

Choices 1 and 2



The instrument produces the desired optical performance, notably for turbid samples such as suspensions of soil, tissue, yeast, mitochondria, etc.

The software we add is trouble-free. The polished Windows®-compatible software supports everything from setting wavelengths to fitting 3D results. The software can be used by all skill-level users and provides full control over the monochromators, supports spectral and kinetic data collection, data analysis, and data synthesis. If a facility is needed in the research or pedagogical environment, it will be found in this comprehensive software package.

The hardware Olis adds includes the most simple, modern, and dependable electromechanical components, plus the newest Pentium computer, laser or color printer, and interface hardware. These new components require minimal maintenance and will extend the life of the optical bench's use for decades.

Sample handling accessories can be computerized and controlled, too. If accessories are not available, but are needed, Olis offers a complete line for stopped-flow mixing, flash/ laser photolysis, automatic titration, temperature ramping, etc.

The procedure to modernize the Aminco

Whether the Aminco belongs to you or Olis, the modernization procedure is the same. The original strip chart recorder and power supply assemblies are disconnected from the optical bench and are discarded. The chopper motor is replaced with a similar sized synchronous motor operated from the 60 Hz AC supply. A small stepping motor is coupled to the lead screw of the monochromator 1; this motor provides wavelength drive with 20 steps per nanometer.

The various toothed belts coupling the monochromator 1 lead screw to the indicator dials are replaced. A new power supply assembly is connected to the optical unit, which includes power supplies for the tungsten halide lamp and the new deuterium lamp. Finally, a photometer box containing the photocurrent amplifier is placed near the large end-on photomultiplier tube (PMT) and is connected to the PMT via the original HV and signal leads coming from the PMT.

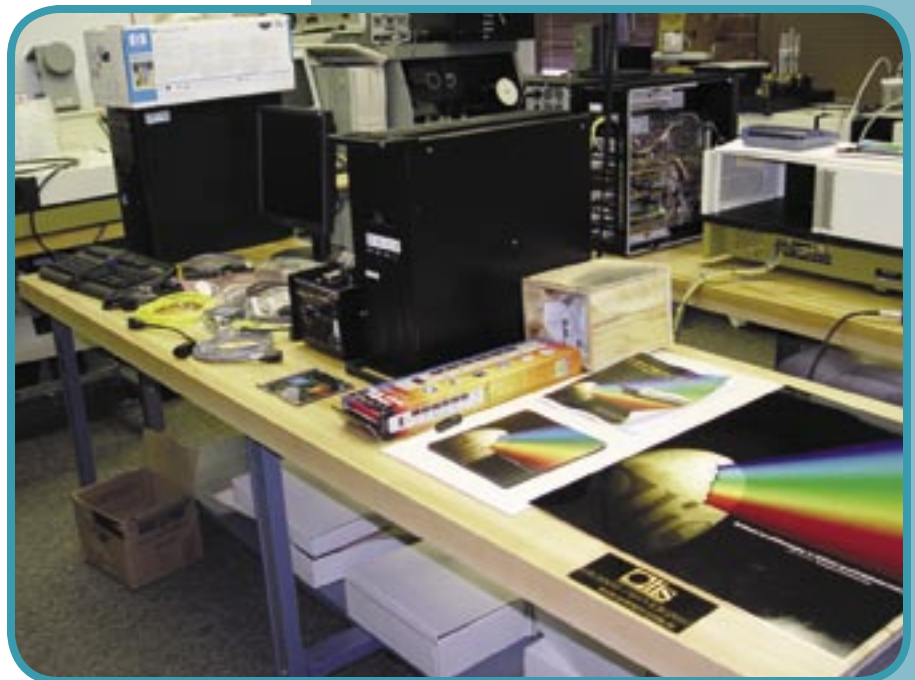


The original Aminco instrument.



Dr. Richard DeSa and chief engineer, Rich Hantus, at an Olis DW-2 system.

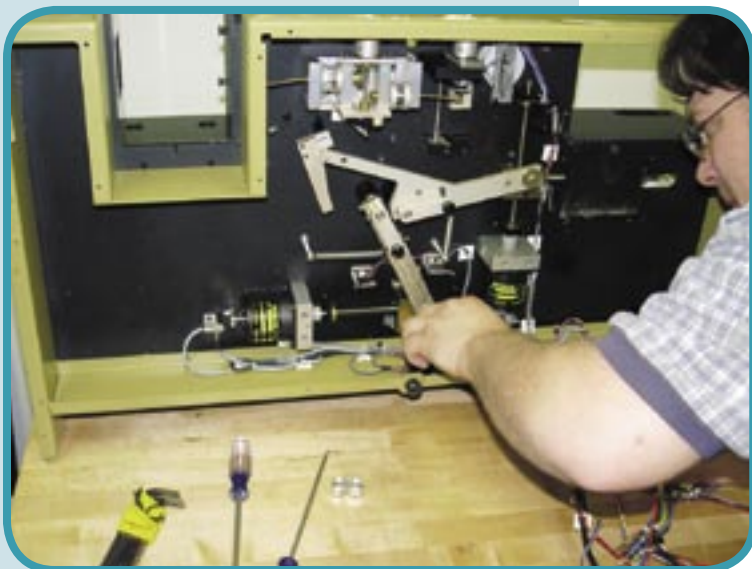
Cables, manuals and other components gathered for shipment.



Modes of Operation

Two functions of the original DW-2 are not supported after this modernization. There is no longer an automated wavelength scanning by monochromator 2 to provide synchronous scanning of the two monochromators, and there is no longer a high-speed kinetics mode. Synchronous scanning was used to obtain derivative results, more correctly done today mathematically. The high-speed kinetic mode was a very poorly designed feature which few, if any, labs used.

Otherwise, everything which the original could do is retained or enhanced. The spectral range of ~200-850 nm is retained; the spectral resolution is 0.2 nm, readable to 0.1 nm. Modes of operation remain **split-beam, dual wavelength, and dual wavelength scanning**; the derivative mode, known to result in the wrong answer, is not retained. Light sources are **deuterium and tungsten** lamps that can be turned on simultaneously; a selector switch controls which source enters the monochromator(s). Two continuously variable optical attenuators are used for balancing the two beams in the dual wavelength and the split beam modes. **Drift is < 0.0005 AU/ hr, stray light is better than 0.2% at 200 nm, maximum noise level is 0.0002 AU, and optical resolution is better than 3 Å.**



Technician Brent Corbitt works on mechanics of the converted DW-2000.

Optional Accessories

1



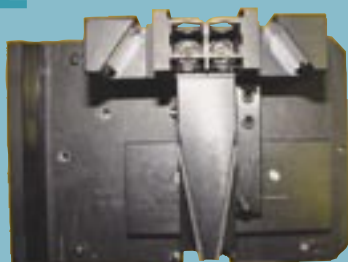
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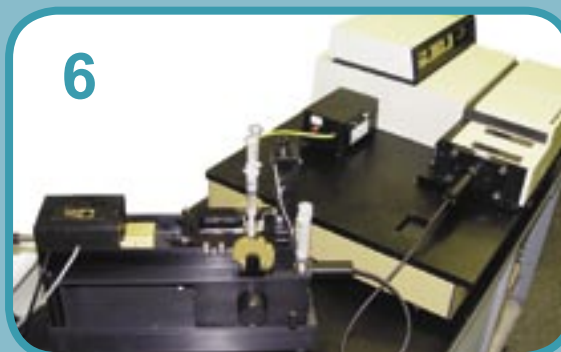
5



2



6



3



7



8



1

Jacketed 4 position cell holder

5

Assortment of masks used at cuvette

2

Fluorescence emission module

6

Fiber optically linked Olis USA stopped-flow

3

Digital waterbath for use with 1, 6, and 7

7

Directly mounted Aminco-Morrow stopped-flow

4

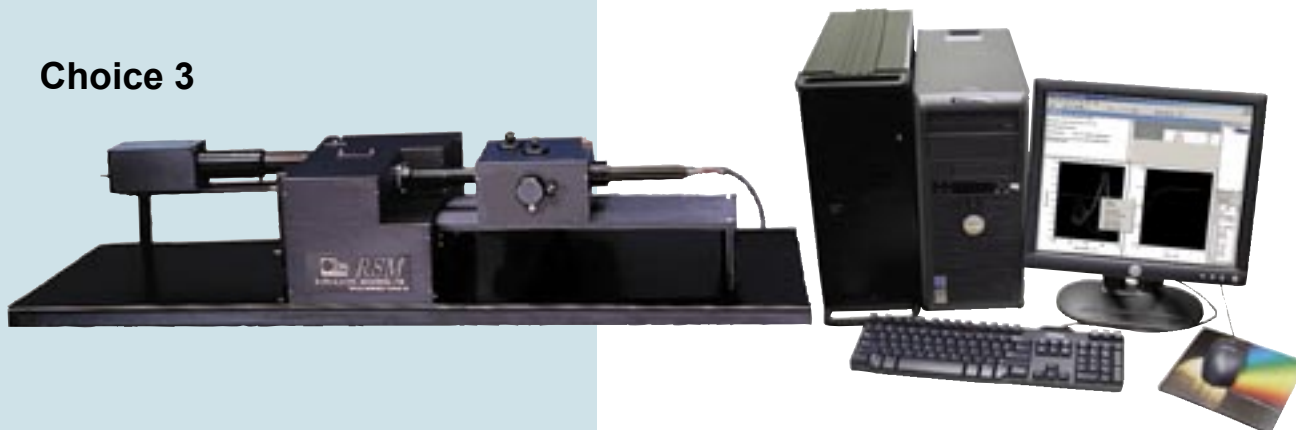
Twin Cuvette Holder with Peltier thermal regulation and magnetic stirring

8

High-pressure cell

The Olis® RSM 1000 Spectrophotometer

Choice 3



When compared with the DW-2, the RSM 1000 is superior for turbid samples because,

1. Optics which provide nearly 3.5 times greater reflective surface are used, providing dramatically higher light throughput.
2. Two end-on photomultiplier tubes are used rather than one.
3. Both detectors are within millimeters of the sample to capture the maximum signal and scatter.
4. The detectors have enormous gain range and can be adjusted to measure light of very widely varying intensities.
5. A Dark Current reading is acquired between each scan.
6. Dual wavelength handling is a fractional subset of the hundreds of wavelengths collected every one millisecond by the RSM rapid-scanning spectrophotometer.

Working with turbid samples is one of many experimental opportunities for the unique technology Olis RSM 1000 to show its strengths. Commonly, the RSM is used in high speed kinetic studies, such as stopped-flow and flash photolysis, where its ability to collect 1,000 scans per second with milliabsorbance sensitivity can be fully utilized. Slow studies are easily done, too, as a function of absorbance, fluorescence, circular dichroism, and many variations.

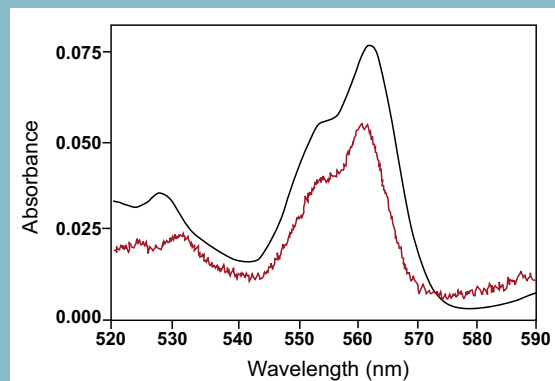
The base price of the highly versatile RSM is just over twice that of the application specific DW2.

Better Answers with the RSM 1000

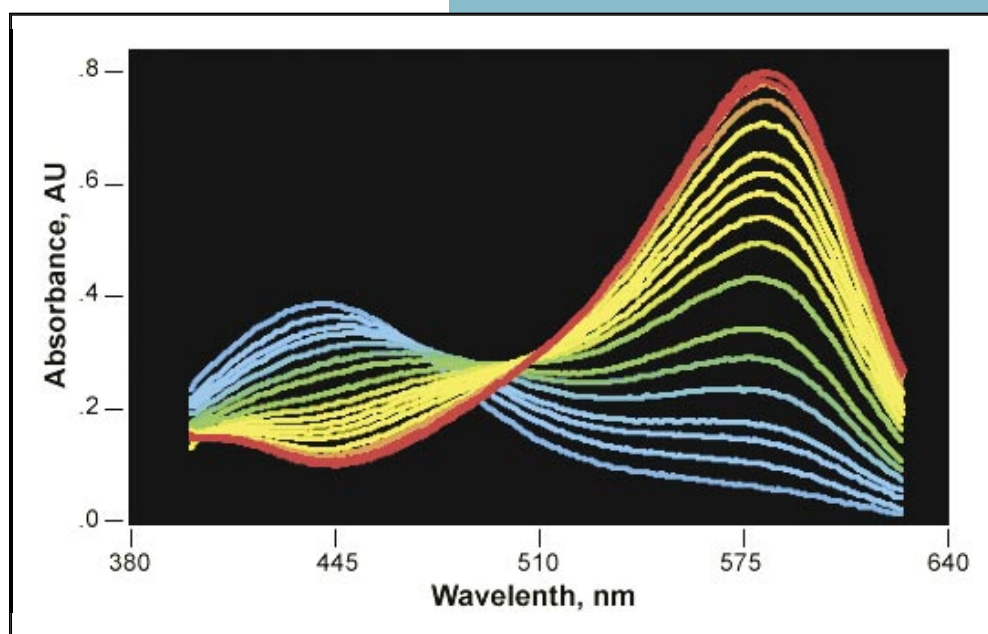
The only spectrophotometer known to collect better data on highly scattering samples than the Aminco DW-2 is the Olis® RSM 1000.

The spectra shown to the right were acquired by an owner of both, Bernard Trumpower. One sees clearly that the RSM spectrum, in black, has a higher signal strength, lower noise, and greater precision than the spectrum acquired by the Olis modernized Aminco DW-2, in red. Amazingly, the RSM spectrum was acquired in one second.

One can understand how the designer of the DW-2 instrument, Prof Britton Chance,¹ once wrote to the designer of the RSM, Dr. Richard DeSa, "You know, you really do provide excitement and significant technological advance in your product line and move in a way that is quite impossible for our bulkier colleagues to do ..."



One second RSM spectrum (black) and multiple minute DW-2 spectrum (red)



A scan from the RSM is a 2D data point. Here, 17 scans (17 2D data points) from 400 to 600 nm are shown; data from Dr. Robert C. Blake.

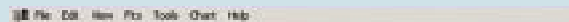
¹ Britton Chance, Ph.D., former director of the Johnson Foundation at the University of Pennsylvania; member of the National Academy of Sciences and the Royal Society. Designer of the Aminco-Chance dual wavelength spectrophotometer.

Exploded View of SpectralWorks Screen

Title Bar



Menu Bar



Tool Bar



Data Collection Tabs



Instrument Settings



Data Display



Status Bar



Experiment Window



Properties Window



Olis[®]

SpectralWorks[™]

Every step of setting up the experiment through final storage of the results is done with the Olis SpectralWorks software package.

Through this Windows XP compatible program, one sets the spectral range, scan speed, and accessories to be controlled. Parameters can be changed individually or collectively with easily produced Protocol files.

Raw data from both sample and reference channels can be viewed at any time; processing of these signals is available as abs, Δ abs, %T, volts, mdeg, or other unit.

A split screen is often employed so that both spectra and kinetics can be viewed side by side; a dynamic slicing option allows viewing of 'scan at time x' as well as 'kinetics at wavelength y.' Within this comprehensive data acquisition/instrument control program is all of the functionality of GlobalWorks, our data analysis program. Here, you will find all data processing algorithms for simple 2D analysis (e.g., smoothing, derivation, peak finder, integration, secondary structure determination) as well as algorithms for fitting kinetic and equilibrium 3D data sets.

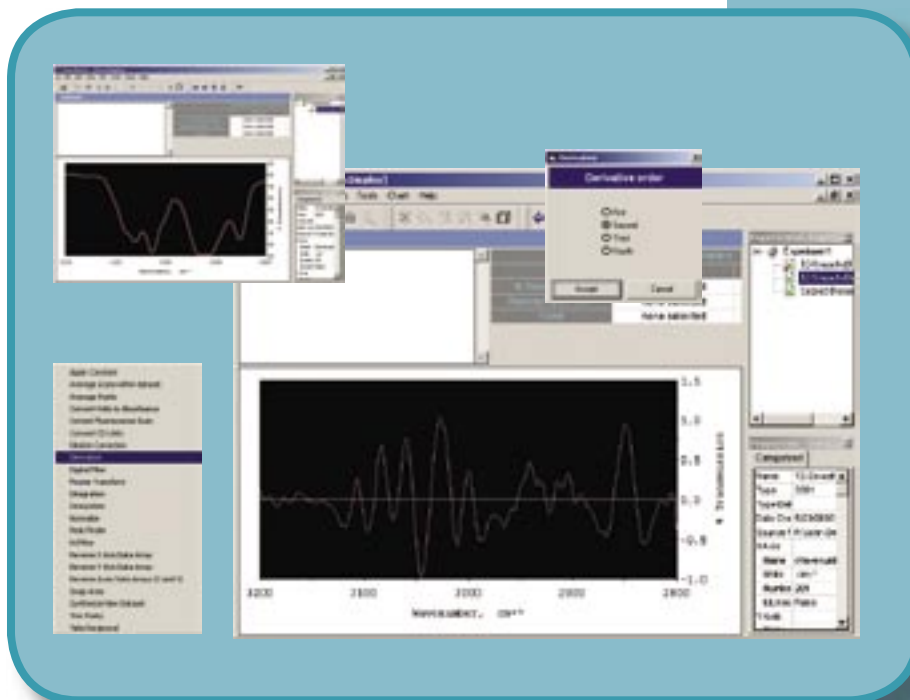
Data are saved as Olis binary files; with a right click of the mouse, ASCII, Excel, and JPG files can be produced.

Both the Pentium PC (laptop or tower) and the Olis SpectralWorks software are included in the purchase price of an Olis spectrophotometer.

SpectralWorks™

All Olis Instruments operate with extremely similar versions of Olis SpectralWorks™ software

This composite image shows the spectrum (upper left), the data processing choices (lower left), selection of 1-4 order derivative (upper right), and the final screen with the second derivative calculated and plotted.



Data Processing with Olis SpectralWorks Software

Olis SpectralWorks software includes a long list of useful data processes for single data-sets including:

- Apply Constant
- Average Scans Within Dataset
- Average Points
- Convert Volts to Absorbance
- Correct Fluorescence
- Convert CD Units
- Dilution Correction
- Derivative
- Digital Filter
- Fourier Transform
- Integration
- Interpolate
- Normalize
- Peak Finder
- RC Filter
- Reverse X Axis Data Array
- Reverse Y Axis Data Array
- Reverse Axes Data Array
- Swap Axes
- Synthesize New Dataset
- Trim Points
- Take Reciprocal
- CDSSTR
- CONTINLL
- SELCON3
- Inverse Spectrum

Olis SpectralWorks software also includes data processes for working with multiple datasets:

- Add Selected Datasets
- Subtract Selected Datasets
- Average Selected Datasets
- Multiply Selected Datasets
- Apply Constant
- Normalize
- Concatenate
- Anisotropy
- Polarization
- Corrected Scans
- Build 3D Dataset from 2D

“The [Olis modernized Aminco DW2] spectrophotometer has been working perfectly ever since [modernization was done]. It is fair to say that I do share the opinion of my students and colleagues about your system, it is excellent!!! We have been using routinely the instrument in the scanning mode and it is performing flawlessly.”

Roberto Iglesias Prieto, UNAM, Cancun

“As we expected we are delighted with the Olis [Aminco] DW2 upgrade.”

Roberto A. Bogomolni, UC Santa Cruz

“I should have done this years ago!”

Patrick Dansette, University of Rene Descartes

See names of many owners of this dual wavelength instrument at
<http://olisweb.com/products/upgrades/dw2owners.php>



For more information on this and other Olis products:

Visit **www.olisweb.com**

Write **sales@olisweb.com**

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