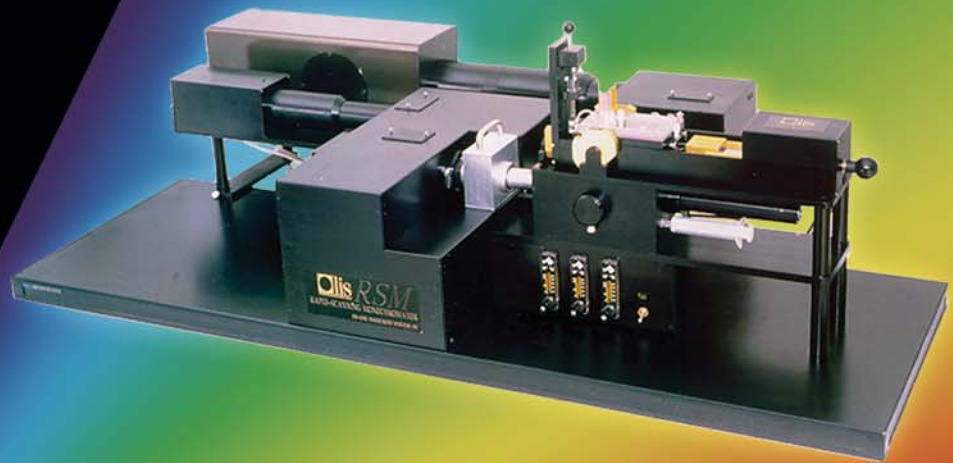


PAUL'S* PRACTICAL RSM GUIDE



A Practical Guide to Using the Olis RSM

* Dr. Paul Boxrud, *Olis Staff Scientist*



Where brilliant breakthroughs *do* happen,
and where results are always foremost!

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What Makes the RSM Unique?

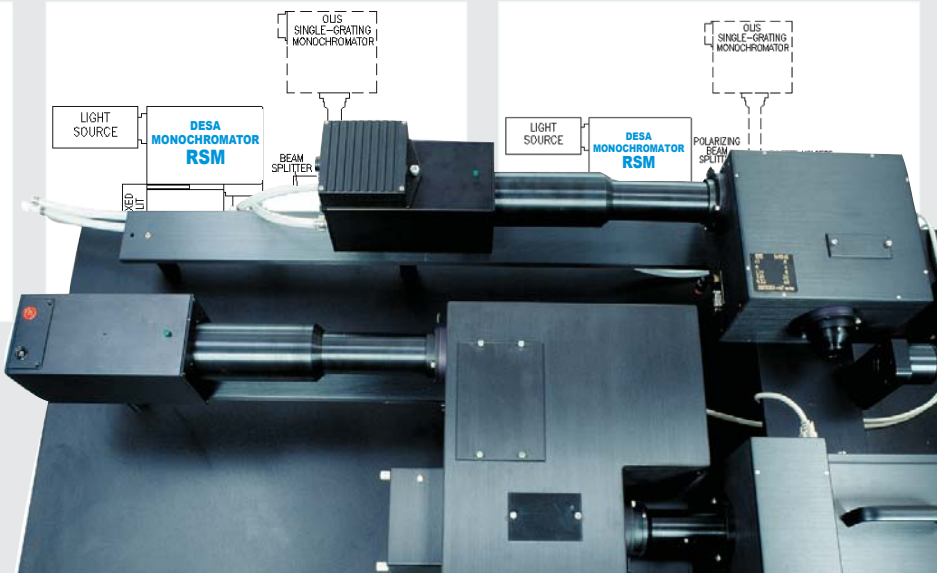
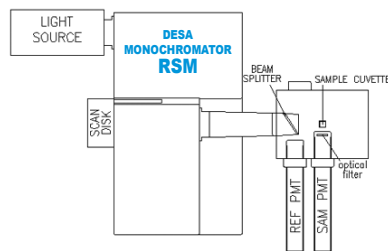
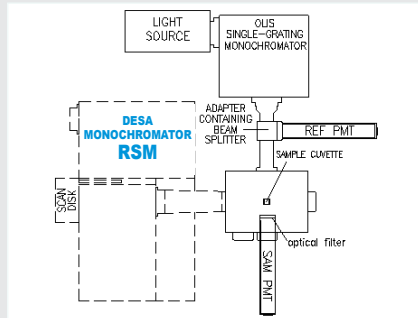
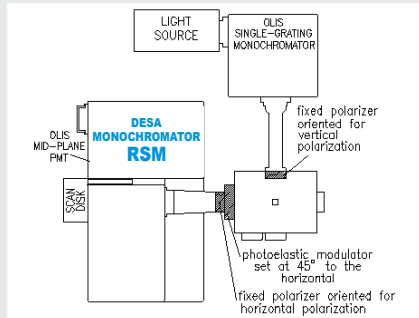
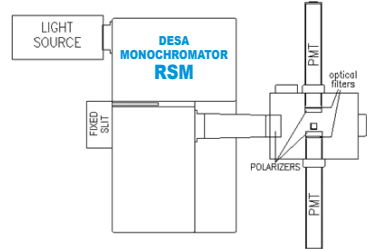
Two fundamental properties of the RSM which make it unique among other instruments are its ability to collect spectra at rates up to 1000 scans/second and its modularity. While several spectrophotometers use diode arrays or charge coupled detectors to collect simultaneous data over a spectral range, none can match the speed or sensitivity of the RSM to achieve milliabsorbance sensitivity in milliseconds. The modularity of the RSM allows for the instrument to be used to collect absorbance, fluorescence, or circular dichroism data with minimal hardware and software changes.

The RSM configured for fluorescence.



The RSM configured for absorbance.

The RSM configured for CD and fluorescence.



Unique Modularity:

These diagrams show just a few of the dozens of possible configurations for the RSM 1000.

Hardware Descriptions

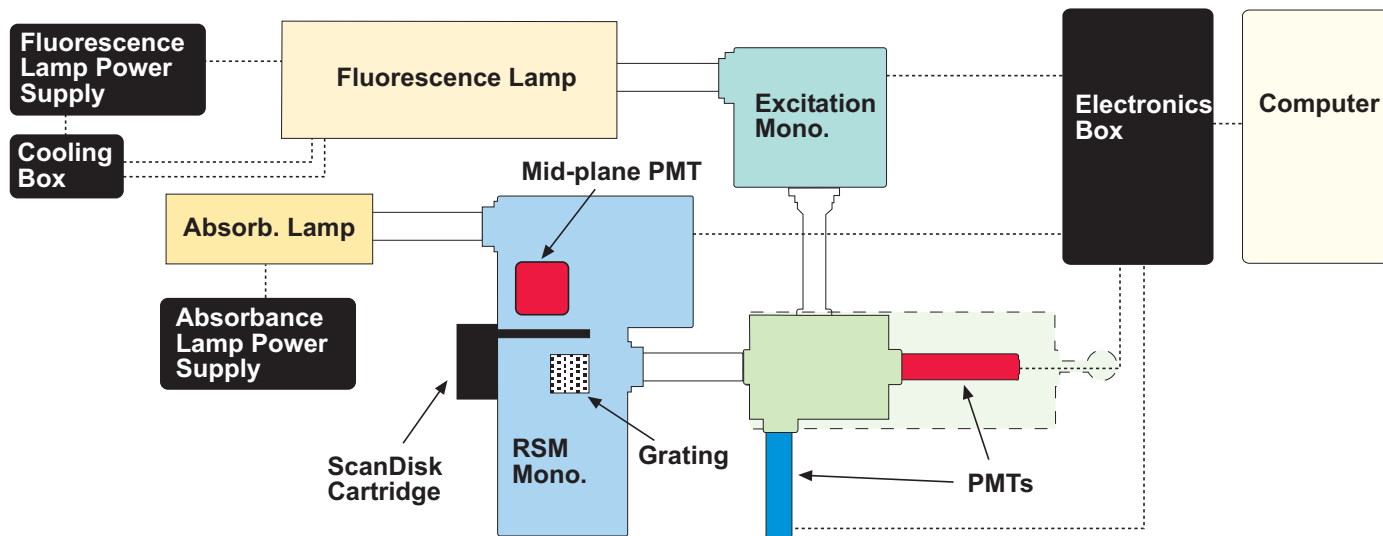


Figure 1 - RSM Components

DESA SUBTRACTIVE DOUBLE GRATING MONOCHROMATOR

The heart of the RSM 1000 line of spectrophotometers is the DeSa monochromator, which is a subtractive double grating monochromator capable of up to 1000 scans/second. Rapid-scanning is accomplished through the use of a moving intermediate slit which moves through the dispersed light produced by the grating (Fig. 2), resulting in millisecond spectral scanning without movement of any optical components. The intermediate slit is housed within a ScanDisk. The ScanDisk is described in more detail below. The gratings of the RSM are removable and the choice of gratings to use depends on the scan range required for the application. See below for a more in depth discussion of RSM gratings. It is important to the operation of the RSM that when gratings are changed that the software settings are updated to include this change. The RSM can also be operated in conventional mode, in which a fixed slit cartridge replaces the rotating scan disk and wavelength variation is achieved by movement of the gratings. This designation is made in the software.

SCANDISK

The ScanDisk cartridge contains a metal disk with 16 slits radially dispersed with a synchronous motor spinning at 62.5 Hz ($16 \times 62.5 = 1000$) supporting the collection of 1000 scans per second. The three ScanDisks available for the RSM monochromator: The 16 x 0.2 mm disk contains 0.2 mm slits and is used for absorbance measurements, in which plenty of light is available and spectral resolution is important. The 16 x 1.0 mm scan disk is similar to the 0.2 mm, but with wider slits. This disk is typically used in fluorescence mode, where light intensity is of more importance than spectral resolution. Finally, a fixed ScanDisk is used fixed wavelength work and slow scanning. Any size slit may be used, 0.1-20 mm.

ScanDisks are changed by removing the four thumbscrews on the outside of the cartridge and sliding the unit out. The ScanDisk is electrically connected through a cable inside the monochromator. Care must be taken to plug this cable in the correct orientation and, if the instrument is a DOS operating system, to turn the control box off before connecting or disconnecting the scan disk.

Hardware Descriptions

GRATINGS

Gratings are chosen for the monochromator using two criteria. The first is the wavelength to be used. The useful wavelength range of a grating is determined by its blaze wavelength. A good rule of thumb for determining the useful range of a grating is $2/3*B$ to $3/2*B$ where B is the blaze wavelength. For example, a grating blazed at 500 nm would be useful from 330 nm to 750 nm. A second criterion is the scan range of the RSM in the rapid scanning mode, which is determined by the dispersion of the light that the slit passes through. Gratings with higher density lines result in rapid spectra of narrow wavelength span, whereas the opposite is true for gratings with lower density of lines (broad ranges with broad spectral resolution). For example a grating with 400 lines per mm provides a scan range of 230 nm whereas one with 2400 lines per mm exhibits a scan range of only 25 nm.

Care must be taken not to touch the grating surfaces as they are nearly impossible to clean without damaging the grooves. The typical gratings provided with the RSM are 400 line/ 500 nm blaze (for visible wavelengths), 600 line/300 nm blaze (for ultraviolet wavelengths), and 2400 line/230 nm blaze (for high resolution deep ultraviolet). Other gratings are available from Olis, Inc..

LAMP

All lamps can be used with the DeSa monochromator. Lamps we found best for rapid scanning are xenon arcs for their brightness and broad spectral utility. Three sizes available are 75, 150, and 450 watt. The 75 watt lamp is useful for absorbance measurements and requires no cooling. The 150 watt lamp is twice as bright, and will be ozone producing or not (ozone produces light well into the deep UV (<170 nm)). [A consequence of this is that ozone will form in the presence of oxygen. The oxygen must be removed by purging the lamp and monochromator with at least 8 L/min of nitrogen to displace the oxygen whenever the lamp is running. Typically ozone producing lamps are used for circular dichroism so if an RSM is equipped with a circular dichroism module, it has an ozone producing lamp.] The 450 watt lamp is used for fluorescence and requires water cooling; a beam cooler, which absorbs infrared radiation and dissipates the absorbed heat, is located between the lamp and excitation monochromator and uses flow from the cooling box.

As with all xenon arc lamps, the RSM lamps should be started before the electronics and computer are turned on. Additionally, the lamp should be allowed to cool at least 30 minutes with the cooling box on before restarting.

COOLING BOX

Xenon arc lamps of 150 watt or more require water cooling when operating. The RSM will be equipped with a lamp cooling box if a xenon lamp of 150 watt or greater is present. The function of the cooling box is to provide circulating ambient temperature water to the lamp. The power to the lamp runs through the cooling box so that the lamp will not run without the cooling box on. Additionally, a flow sensor in the box will automatically shut off power to the lamp to prevent it from overheating if flow is interrupted. Because of this, it is important to remove any bubbles from the flow system to ensure that the lamp is not shut off due to an air bubble in the sensor. The indicator light on the front of the cooling box will be on if sufficient flow is detected. A bottle is included with the cooling box to facilitate the removal of air bubbles. Hook the bottle in series as with the lamp as shown. It is also a good idea to periodically change the water in the cooling box and add a few drops of algicide as recommended by the manufacturer.

Hardware Descriptions

SAMPLE CHAMBER/STOPPED-FLOW

A lens tube connects the DeSa monochromator to a sample chamber. Inside the sample chamber, the beam passes through a beam splitter which reflects ~20% of the beam to the reference photomultiplier tube (blue PMT); this signal is used for the reference in absorbance measurements. The light passing through the sample is detected by the red PMT placed just after the sample. If fluorescence is to be detected, this beam splitter should be removed. The possible PMT configurations are described in the RSM Configurations Section. One of the distinct advantages of the RSM 1000 is that the sample chamber allows for the sample PMT to be placed very close to the sample. This improves performance for samples which are highly scattering.

If stopped flow collection is to be employed, the stopped flow apparatus replaces the hanging cuvette cover such that the beam passes through the stopped flow cuvette. There are six screws along the top of the stopped flow which hold it in place in the sample chamber. Two syringes, which contain the reagents, are present on the stopped flow apparatus. These are rapidly emptied by a pneumatically driven block. The applied gas pressure should be between 75 and 90 psi (5-7 atmospheres). The reagents are pushed through a Berger-Ball mixer and into the cuvette. The contents of the cuvette are pushed out into the stopping syringe, filling it until a physical stop is reached. The position of this stop determines the total volume delivered per stopped flow shot. The stopping syringe is emptied by putting the exhaust valve into the EMPTY position and pushing down on the syringe. The stopped flow is equipped with sensors on all three valves and at the contacts of the syringe plungers with the drive block. These sensors demand that all valves be in the correct positions before the stopped flow is fired. The positions of the stopped flow valves are displayed on the control box.

CONTROL BOX

The control box contains the electronics to run all motor movements (e.g. monochromator movements) and voltage settings (e.g. application of High Volts to the PMT dynode chain). And all data comes through this box before entering the computer.

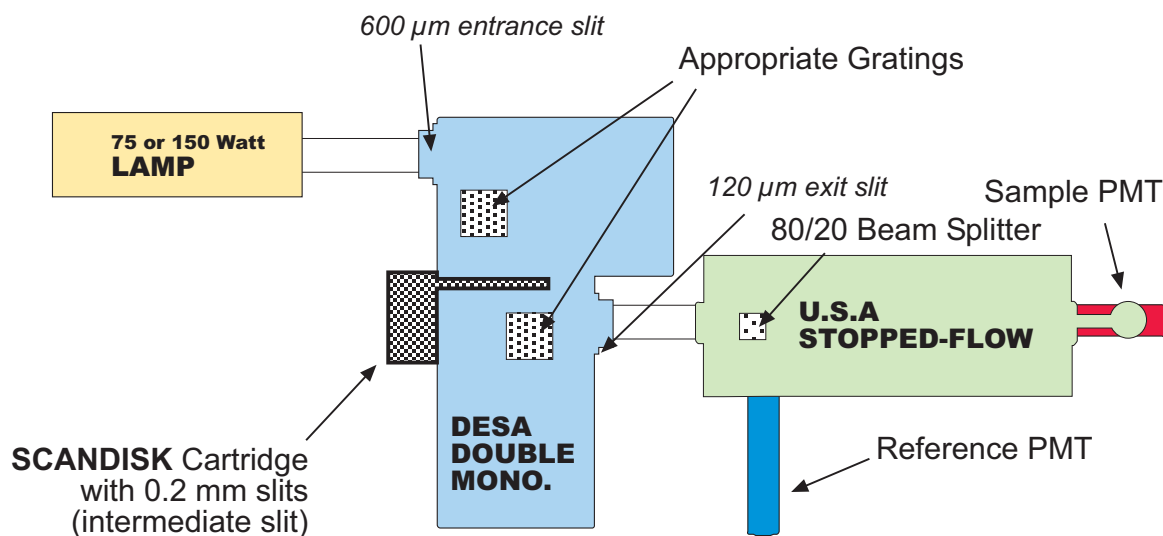
The control box for Windows controlled systems has several indicator lights. 5V DC, which is power indicator, should be on. The Sync lights S1 and S16 are indicative of a spinning ScanDisk and should be off, unless a fixed scan cartridge is being used.

EXCITATION MONOCHROMATOR

If the RSM is optimized for a fluorescence accessory, it will have an excitation monochromator between the lamp and sample and detector (PMT or PCM) at right angles to this excitation beam. This is a single grating monochromator in which the grating is not removable. A filter holder is present which will accommodate a standard, 25 mm diameter filter. Slits are placed in both the entrance and exit positions. The dispersion of the excitation monochromator is 4 nm per mm. Thus, the standard slit sizes of 0.12, 0.60, 1.24, 3.16, and 6.32 mm result in spectral bandwidths of 0.5, 2.4, 5.0, 12.6, 25.3 nm, respectively.

Rapid-Scanning Absorbance Stopped-Flow

HARDWARE SETTINGS



SOFTWARE SETTINGS

WINDOWS

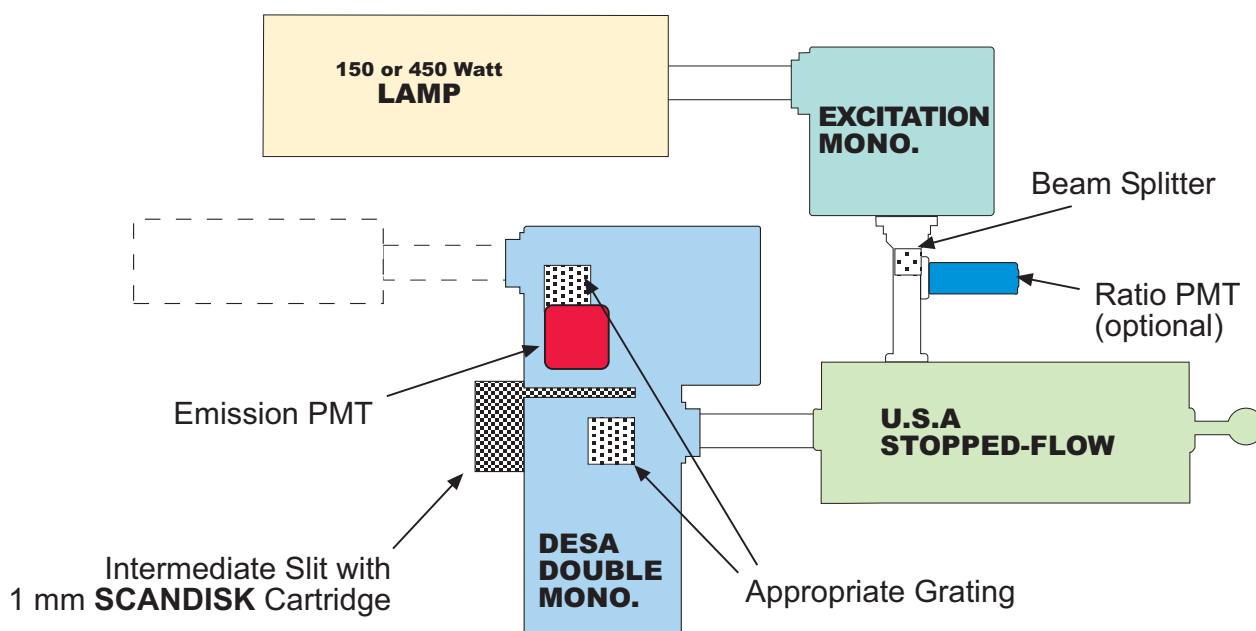
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Active Data Collection Device	2 PMTs	Parameters Tab
Sensor Position	As printed on inside of ScanDisk cartridge	Parameters
Data Reduction Mode	Absorbance	Operational Modes Tab
Data Collection Mode	Rapid Scanning + SF	Operational Modes Tab
Scan Mode	Fix Everything	Operational Modes Tab
Grating Lines	Same as those in RSM	Live Display

DOS

SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Program	rsm-16 or rsm-1000	C:\olis-rsm
C Collect	Scans	S Parameters page
D Beam Mode	Dual	S Parameters page
F Stopped-Flow	Enabled	S Parameters page
L Grating Lines	Same as those in instrument	S Parameters page

Rapid-Scanning Fluorescence Stopped-Flow

HARDWARE SETTINGS



SOFTWARE SETTINGS

WINDOWS

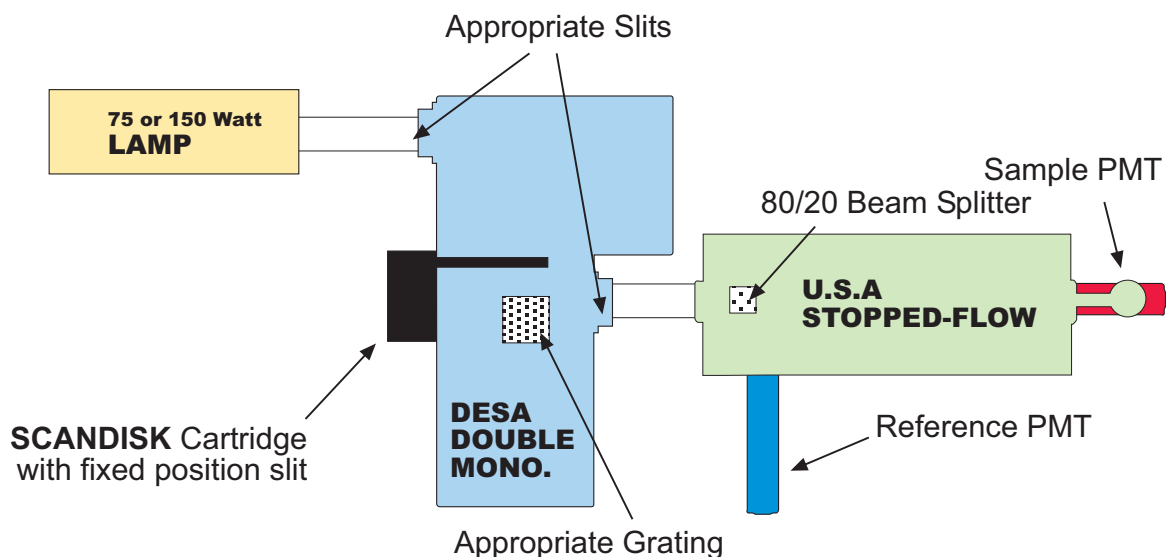
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Active Data Collection Device	2 PMTs (blue is optional)	Parameters Tab
Sensor Position	As printed on inside of ScanDisk cartridge	Parameters
Data Reduction Mode	Ratio (Signal if only red PMT)	Operational Modes Tab
Data Collection Mode	Rapid Scanning + SF	Operational Modes Tab
Scan Mode	Fix Everything	Operational Modes Tab
Grating Lines	Same as those in RSM	Live Display

DOS

SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Program	rsm-16 or rsm-1000	C:\olis-rsm
C Collect	Scans	S Parameters page
D Beam Mode	Ratio	S Parameters page
F Stopped-Flow	Enabled	S Parameters page
L Grating Lines	Same as those in RSM	S Parameters page

Single-Wavelength Absorbance Stopped-Flow

HARDWARE SETTINGS



SOFTWARE SETTINGS

WINDOWS

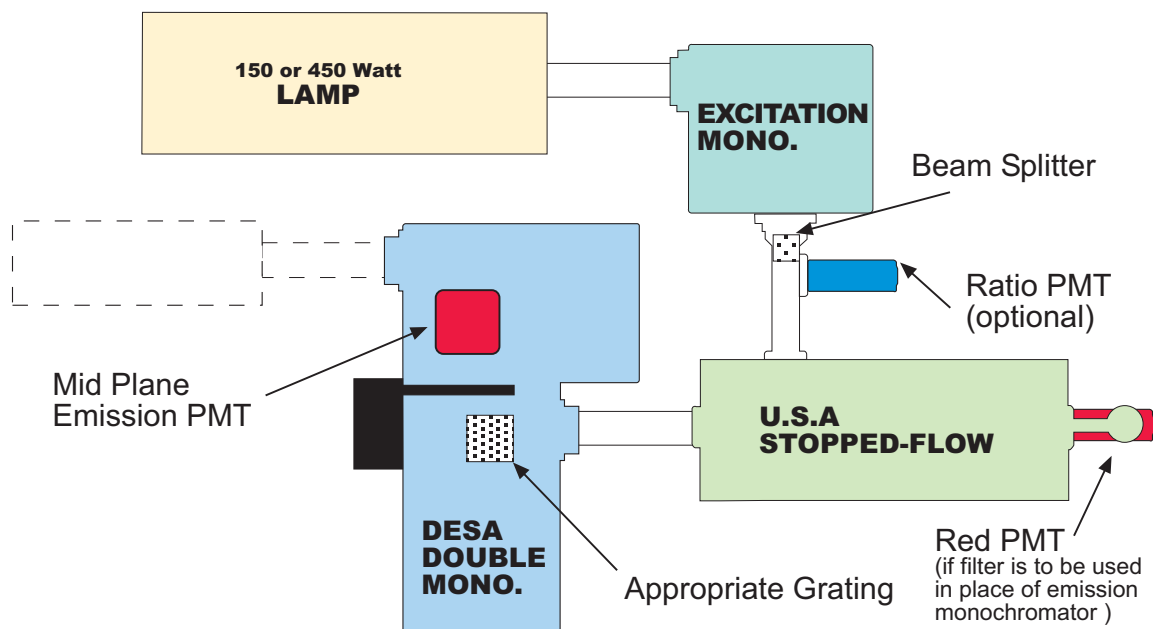
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Active Data Collection Device	2 PMTs	Parameters Tab
Data Reduction Mode	Absorbance	Operational Modes Tab
Data Collection Mode	Stopped-Flow	Operational Modes Tab
Scan Mode	Fix Everything	Operational Modes Tab
Grating Lines	Same as those in RSM	Live Display

DOS

SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Program	rsm-16 or rsm-1000	C:\olis-rsm
C Collect	Fixed Wavelength	S Parameters page
D Beam Mode	Dual	S Parameters page
F Stopped-Flow	Enabled	S Parameters page
L Grating Lines	Same as those in RSM	S Parameters page

Single-Wavelength Fluorescence Stopped-Flow

HARDWARE SETTINGS



SOFTWARE SETTINGS

WINDOWS

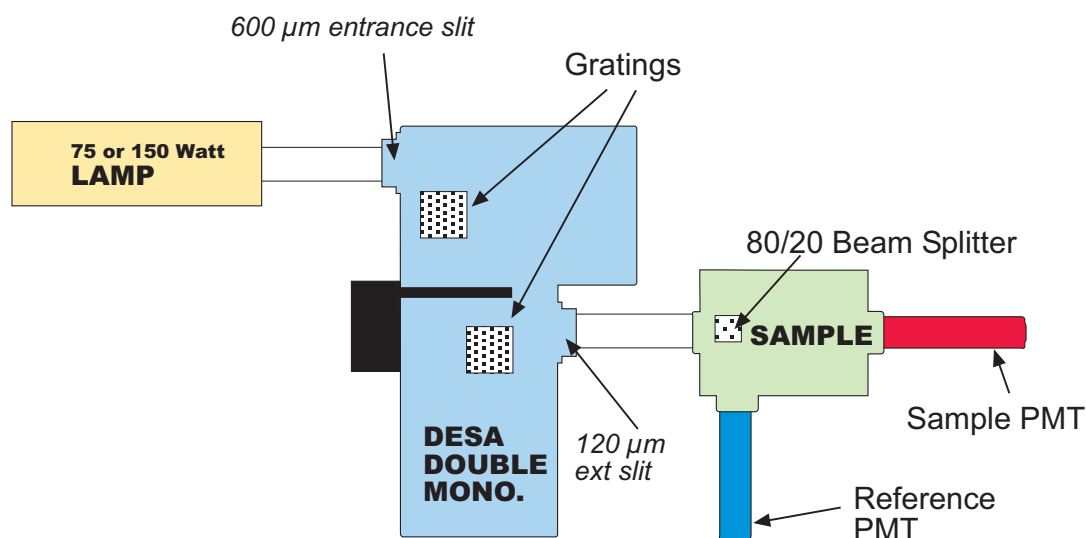
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Active Data Collection Device	2 PMTs (blue is optional)	Parameters Tab
Data Reduction Mode	Ratio (or signal)	Operational Modes Tab
Data Collection Mode	Stopped-Flow	Operational Modes Tab
Scan Mode	Fix Everything	Operational Modes Tab
Grating Lines	Same as those in RSM	Live Display

DOS

SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Program	rsm-16 or rsm-1000	C:\olis-rsm
C Collect	Fixed Wavelength	S Parameters page
D Beam Mode	Ratio	S Parameters page
F Stopped-Flow	Enabled	S Parameters page
L Grating Lines	Same as those in RSM	S Parameters page

Conventional Absorbance Scans (or assays)

HARDWARE SETTINGS



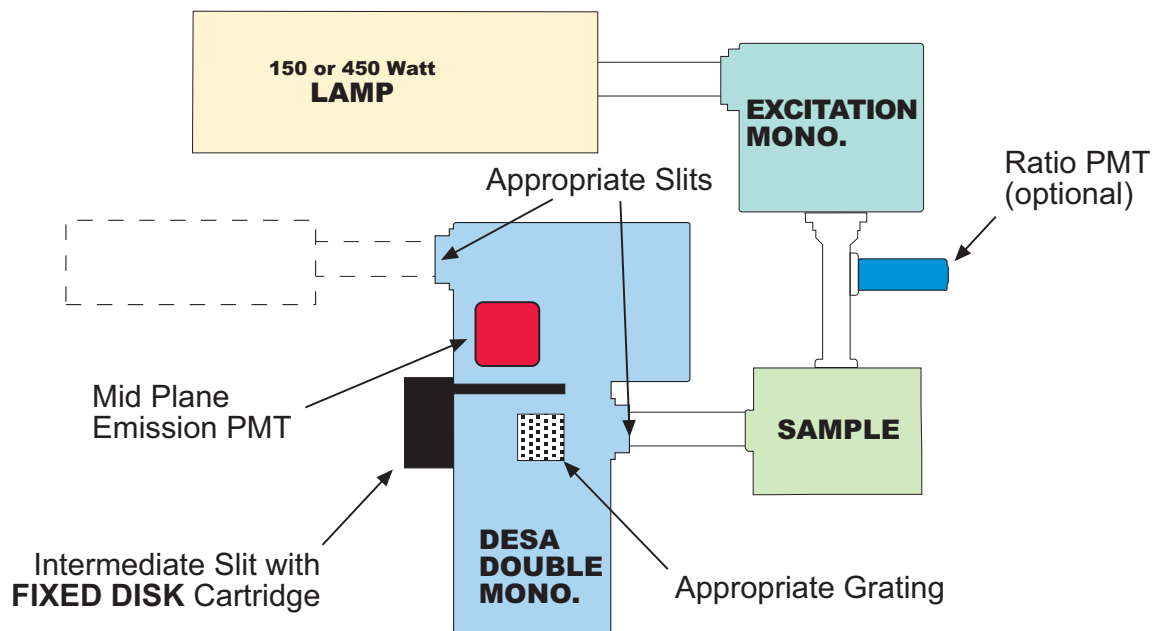
SOFTWARE SETTINGS

WINDOWS		
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Active Data Collection Device	2 PMTs	Parameters Tab
Data Reduction Mode	Absorbance	Operational Modes Tab
Data Collection Mode	Scan or Assay	Operational Modes Tab
Scan Mode	Fix Everything	Operational Modes Tab
Grating Lines	Same as those in RSM	Live Display

DOS		
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Program	Conv_abs	C:\olis-rsm
L Grating Lines	Same as those in RSM	S Parameters page

Conventional Fluorescence Scans

HARDWARE SETTINGS



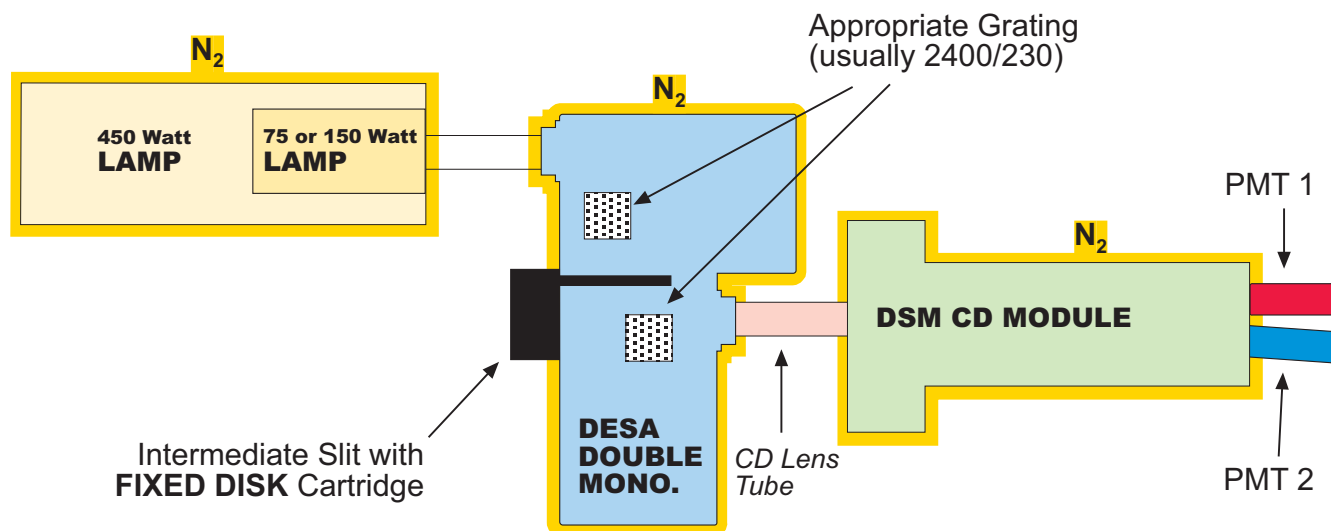
SOFTWARE SETTINGS

WINDOWS		
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Active Data Collection Device	2 PMTs (blue is optional)	Parameters Tab
Data Reduction Mode	Ratio (or signal)	Operational Modes Tab
Data Collection Mode	Scan or Assay	Operational Modes Tab
Scan Mode	Fix Everything	Operational Modes Tab
Grating Lines	Same as those in RSM	Live Display

DOS		
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Program	Conv_fluor	C:\olis-rsm
L Grating Lines	Same as those in RSM	S Parameters page

Circular Dichroism

HARDWARE SETTINGS



SOFTWARE SETTINGS

WINDOWS		
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Active Data Collection Device	2 PMTs	Parameters Tab
Data Reduction Mode	Circular Dichroism	Operational Modes Tab
Data Collection Mode	Scan or Assay	Operational Modes Tab
Scan Mode	Fixed Slits	Operational Modes Tab
Grating Lines	Same as those in RSM	Live Display

DOS		
SCREEN SHOWS:	SHOULD BE:	SELECT FEATURE:
Program	Conv_CD	C:\olis-rsm
L Grating Lines	Same as those in RSM	S Parameters page

Spectral Bandpass and Triggering Data Collection

SPECTRAL BANDPASS

The spectral bandpass of the RSM is dependent on the slit size of the entrance, exit, and intermediate slits, and the line density of the gratings. A complete table of bandwidth calculations is available in reference 1. The common settings are listed in the table below:

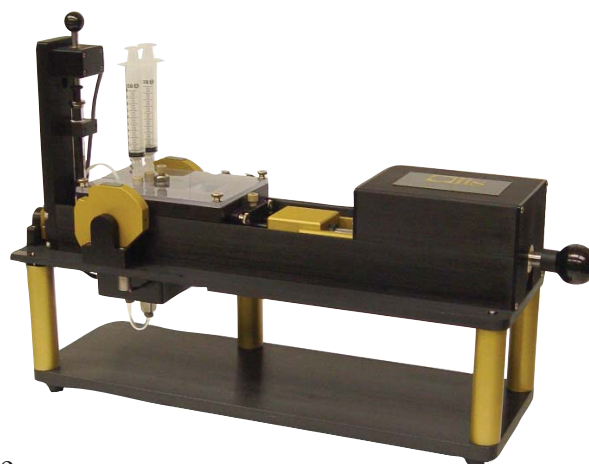
Gratings (lines/mm)	Intermediate ScanDisk (mm)	Entrance slit (mm)	Exit slit (mm)	Resulting Bandpass (nm)
400	0.2	0.60	0.12	1.9
600	1.0	1.24	1.24	7.9
600	1.24 (fixed)	1.24	1.24	7.9
2400	1.24 (fixed)	1.24	1.24	1.7

TRIGGERING DATA COLLECTION

By far the most common method of triggering data collection is the stopped flow attachment, which is discussed below in more detail. The stopped flow apparatus simply acts as a fast mixer in which two reagents are rapidly mixed and pushed into a cuvette. The mixture is then monitored by the RSM (absorbance, fluorescence, or circular dichroism) for a specified amount of time. Data collection can also be triggered by a flash lamp or laser flash. In the Windows RSM software, this is referred to as the 'Flash mode.' In the DOS operating system the external TTL pulse is enabled in the Setup menu. In the flash mode, a TTL pulse is sent from the box when data collection is initiated. This pulse is used to fire a flash lamp or laser aimed at the sample. The effect of the flash on the sample is monitored by the RSM. The TTL can be used to trigger other perturbations to the sample such as changing the voltage potential of an electrode. An absorbance spectrum of holmium oxide gives prominent peaks at 360.9

U.S.A. Stopped-Flow

The stopped flow accessory is used on an RSM to rapidly mix two reagents for analysis of fast reactions. The dead time (the time between reagent mixing and data measurement) on the RSM is typically on the order of 1-2 msec. References 4 and 8 describe procedures for measuring dead time. Use of the stopped flow is appropriate for reactions from a few msec to hundreds of seconds.



The stopped-flow fits within the sample compartment so that the observation window takes the place of the cuvette. The apparatus is held in place by six screws. The flow is actuated by high pressure gas (air, nitrogen, other) at 75 psi to 90 psi. The stopped flow apparatus contains three valves to control flow. Each syringe attaches to a valve (labeled 'BH' and 'FH'). These valves should be in the 'Fill' position when filling the drive syringes and in the 'Flow' position which performing stopped flow reactions. The third valve is the exhaust valve ('EH'). This should be placed in the 'Flow' position for stopped flow reactions, the 'Empty' position to empty the stop syringe, and the 'Flush' position to flush the cell. The positions of these valves are reported on the control box. In addition to the valve positions, the position of the drive block (which must make contact with the syringe plungers), and the stop syringe (which must be down) are displayed. The stopped flow will not fire unless all of these sensors indicate that the stopped flow is ready. The green ready light will be on when this is true. To collect stopped flow data, the stopped flow must be enabled in the software. For the DOS system, enabling the stopped flow is accomplished by changing the 'Stopped Flow' (F) control in the Parameter setup menu ('S' from the Command mode) to enabled. In the Windows system, the stopped flow is enabled by choosing a 'Data Collection Mode' in the 'Operational Modes' tab that includes stopped flow ('Stopped Flow' or 'Rapid Scanning + SF'). **After the software has been updated, the stopped flow will fire whenever data are collected.**

After data collection is initiated, data are collected for a period of time equal to the specified delay (for DOS 'Stopped Flow Delay' in 'Parameter Setup' or in Windows 'TTL Delays' in 'Parameters' tab) during which time nothing happens to the sample. Following this delay termed (Delay 1) the stopped flow is fired. During this time (Delay 2), the stopped flow fires and the reagents are mixed. Delay 2 ends when the stopping syringe sensor is triggered. The stop time (the point in which the reaction is fully mixed) occurs slightly after the stop sensor is triggered and is designated as zero time in the software. The stop is computed in the dataset by applying a stop time adjustment to the data. This is the time after the stop sensor is triggered that the real stop has occurred. This adjustment time is measured from a known test reaction (see next page). Contact Olis if you feel this stop time adjustment needs to be changed. All data collected after stop are processed as reaction data and are subject to kinetic fitting (5-7, 9-10).

Test Reactions

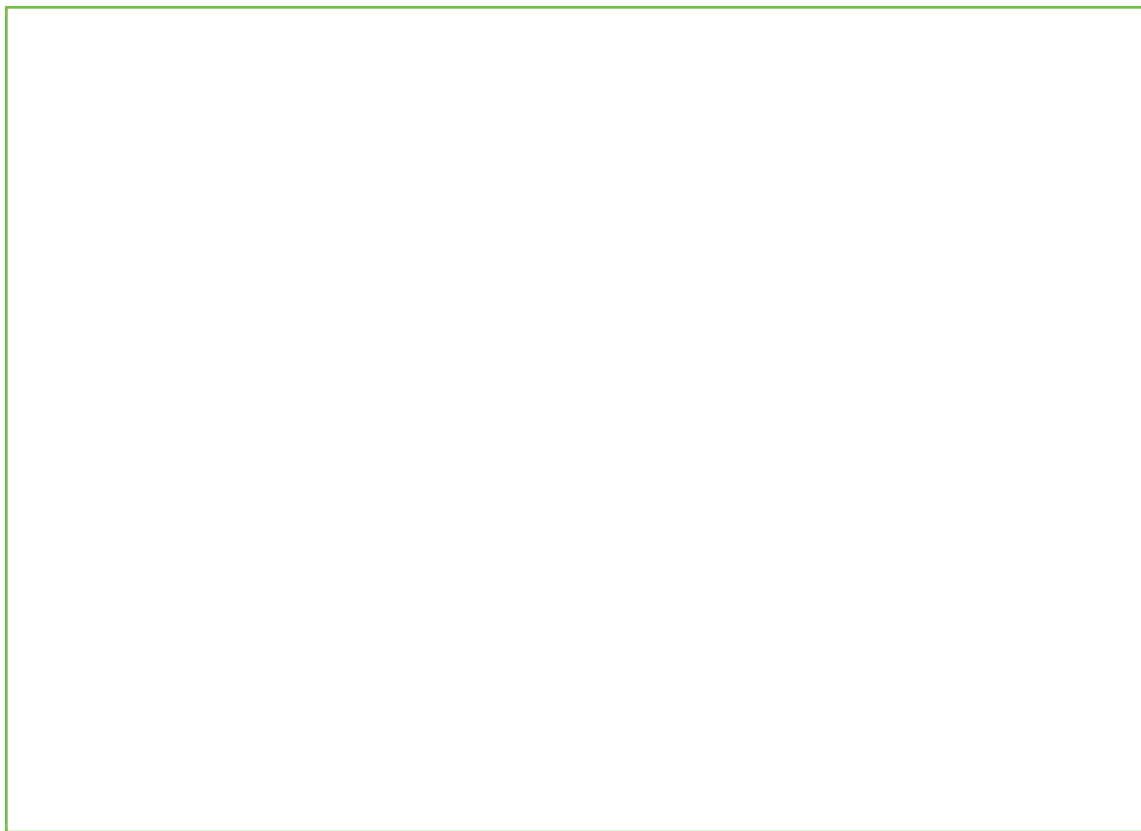


Figure 2

HOLMIUM OXIDE - ABSORBANCE

A holmium oxide filter, a cuvette shaped piece containing a yellow glass, is provided with all RSM instruments. It is typically mounted on a shaft in the sample chamber. Pushing in this shaft moves the filter into the sample beam. The absorbance spectrum of holmium is shown in Figure 2. The holmium oxide spectrum can be acquired in conventional or rapid scanning collection mode (See RSM Configurations). An absorbance spectrum of holmium oxide gives prominent peaks at 360.9, 418.7, 453.2, 450.0, and 536.2 nm.

Test Reactions

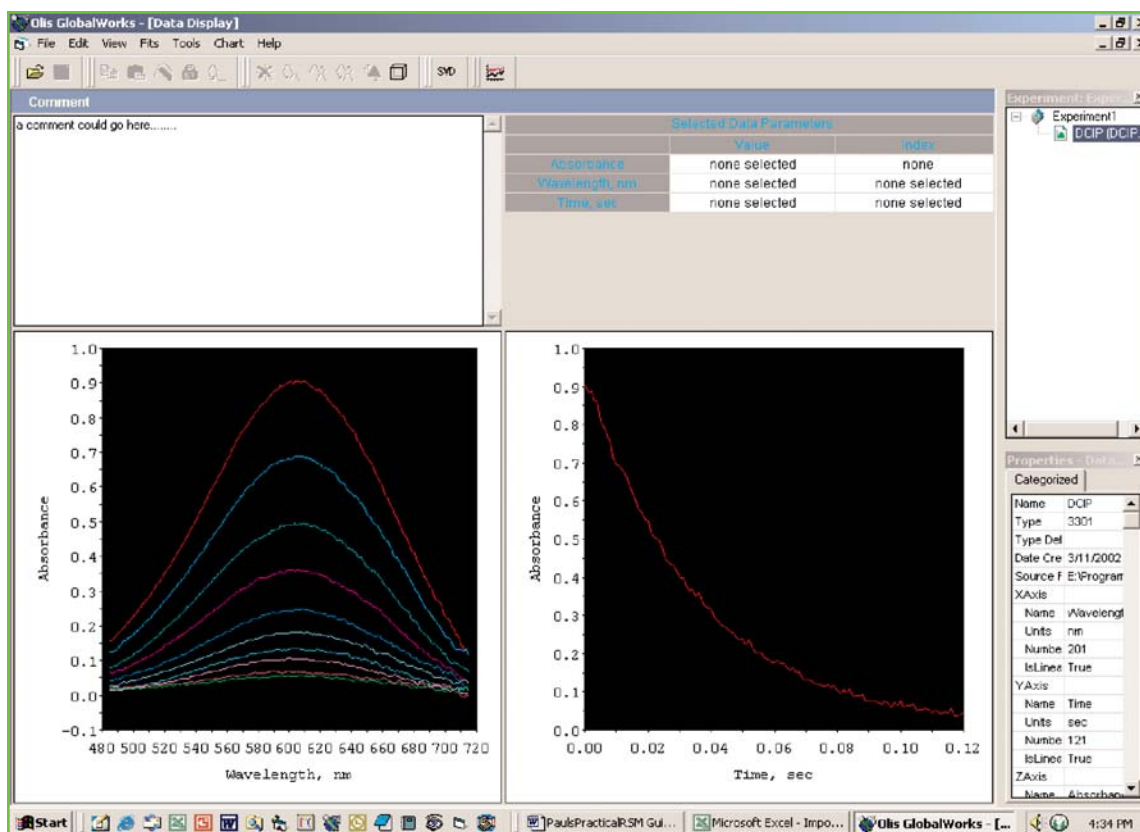


Figure 3

STOPPED-FLOW - ABSORBANCE

The reaction of 2,6-Dichloroindolphenol (DCIP) with ascorbate provides an excellent test reaction for stopped flow absorbance (Ref 3). In the reaction, DCIP, which exhibits a maximum absorbance at 600 nm, is reduced with ascorbate. The resulting bleaching follows pseudo first order kinetics. The optimal pH is about 7.0, and at low pH the maximum will shift to 525 nm and greatly increase the rate of bleaching. A good concentration range to use is 0.05-0.5 mM DCIP and excess of 10 mM sodium ascorbate. The reaction can be done in water or most pH 7 buffers. A typical result is shown in Figure 3. Note that the reaction rate is dramatically increased at low pH (<5) and high pH (>10). Spectral shifts are also exhibited during changes in pH.

Test Reactions

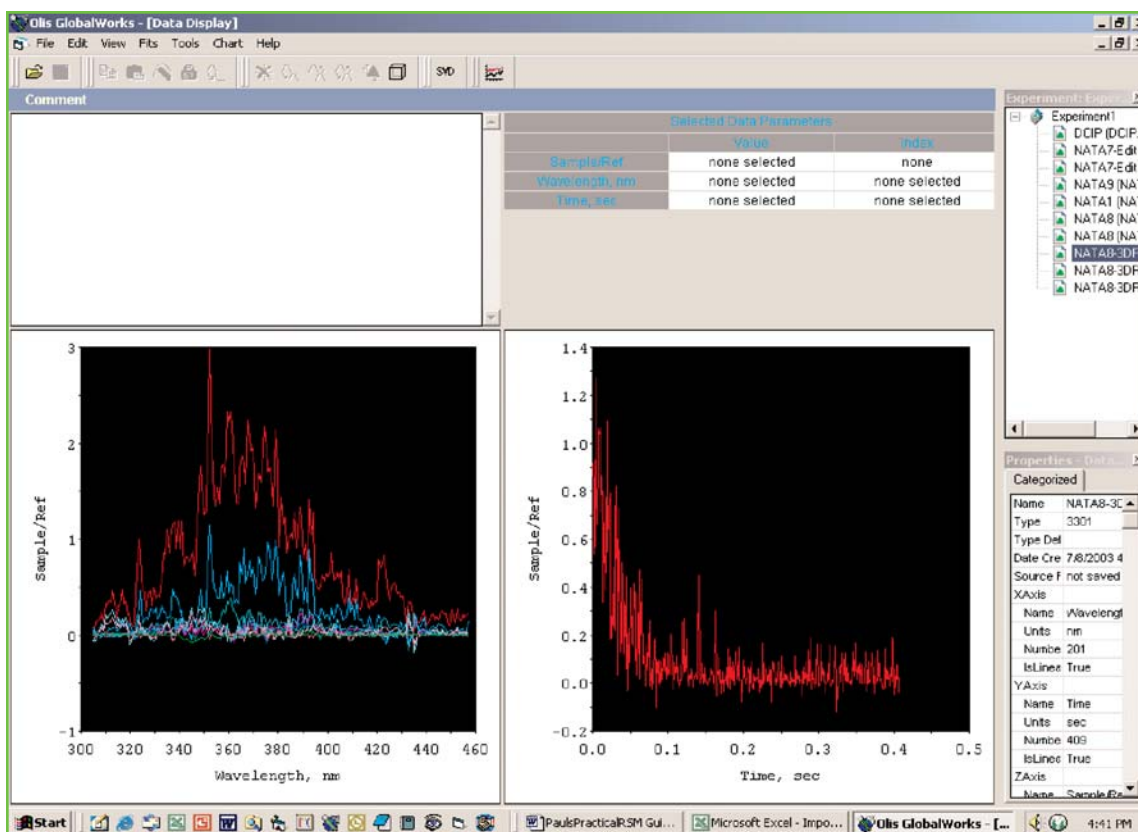


Figure 4

STOPPED-FLOW - FLUORESCENCE

The reaction of N-acetyl tryptaphanamide (NATA) with N-bromosuccinimide (NBS) provides a reasonable test reaction for fluorescence stopped flow setups (Ref. 4). The reaction is a bromination of the indole ring resulting in quenched fluorescence of the NATA. Reaction is maintained by fluorescence signal using an excitation wavelength of 290 nm. The emission wavelength is 350 nm. The kinetics follow pseudo first order behavior. A good concentration range to use is 0.2 - 5.0 mM NATA and NBS. One should use a small excess concentration of NBS in a pH 7 aqueous buffer. A typical dataset is shown in Figure 4

Test Reactions

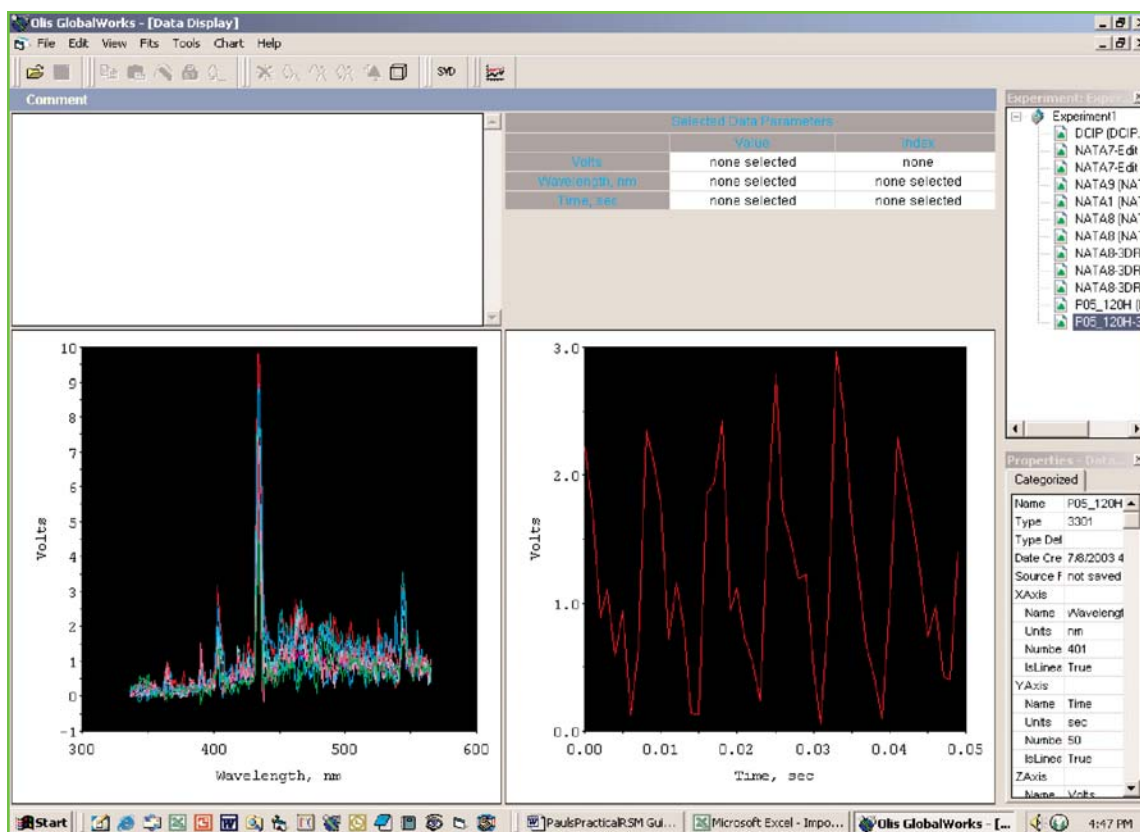


Figure 5

OVERHEAD LIGHTS (FLUORESCENCE)

Overhead fluorescent lights provide an excellent fluorescence 'sample' because the lights are spiked with mercury (and sometimes argon) which provides bright emission lines. By removing the red PMT and replacing it with a piece of white paper to gather the overhead lights, the overhead light spectrum can be easily obtained. The overhead light spectrum should appear as shown in Figure 5. Prominent lines should appear at 365.0, 404.7, 435.8, 488.0 (if argon is present in the lights), 546.1, and 615.0 nm. In addition the fluorescence intensity should oscillate at a frequency twice that of the AC current (60 Hz in the United States).

Data Fitting

Kinetic data collected with the RSM will either be in a 2-Dimensional dataset (e.g. signal vs. time) or a 3-dimensional dataset (e.g. scans vs. time). The former are typically collected in fixed wavelength mode (i.e. collecting a single wavelength at a time) or extracted from a 3D dataset. These data can be fit using the '2D fits' under the Fits menu in the Windows program or 'Fit Kinetic Trace' in the cursor mode of the DOS program. Both programs list a variety of kinetic fits with which to fit the data.

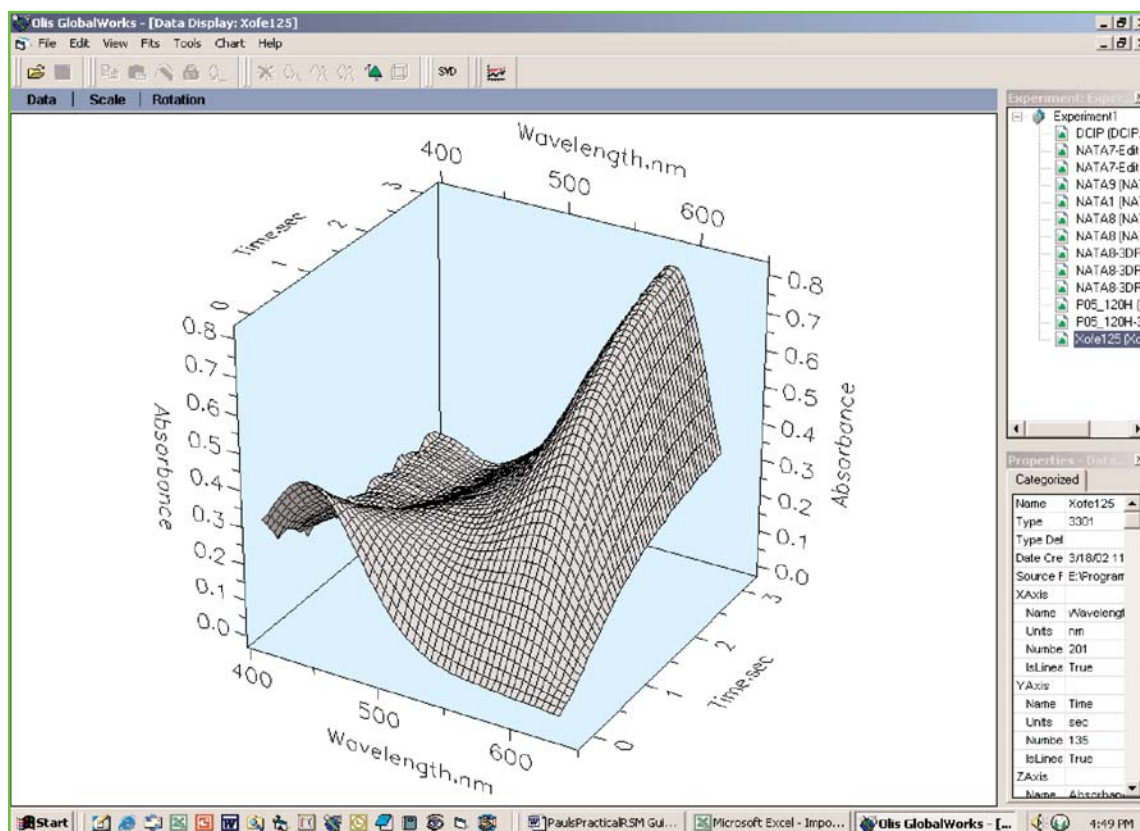


Figure 6

3D kinetic data should be processed first using the Singular Value Decomposition algorithm provided with the RSM software. SVD analysis of a dataset requires it to contain at least 10 scans each containing at least 10 points. A sample dataset is shown in Figure 6. This dataset is a collection of scans of a reaction of xylenol orange with Fe^{3+} over three seconds (xofe125.ols in the Demo Data). A 3D dataset can be collected directly using rapid scanning mode. Alternatively, single wavelength traces measured at different wavelengths can be combined into a single dataset using 'Build 3D Dataset from 2D' in the Data processing menus (in the Windows software). In the Windows program SVD analysis is initiated by clicking on the SVD button, in the DOS pressing G serves this function. The mathematics behind the SVD process are described in detail in several references (ref 5-8), including an excellent overall view of the SVD process (ref 5). The SVD results of analysis of the xofe125 dataset are shown in Figure 6. SVD analysis shows that the dataset can be broken down (decomposed) into a linear combination of six components, also called eigenvectors. The top row of graphs show the spectral component of each eigenvector, while the lower set of graphs refer to the kinetic component. Clearly from this figure, some of the eigenvectors are not real (i.e. they contain only noise and not any information about chemical species involved in the reaction). It is the user's responsibility to determine how many of these eigenvectors

represent real chemical species. This decision is based on a number of criteria, the most important being the look of the kinetic component of the eigenvector in question. Eigenvectors representing only noise will have a flat random appearance to the kinetic eigenvectors. The second most important criterion for this evaluation is the weight percentage. This value is shown directly below the kinetic components and is expressed relative to the first eigenvector. A large drop off in weight percentage is indicative of an insignificant eigenvector. The graph in the lower right is a log plot of weight percentage versus eigenvector number. Deviations from a linear plot are suggestive of significant eigenvectors. This is the criterion the program uses when it predicts the number of species, as indicated by the highlighted number of species. In the example shown in Figure 7, we can safely choose three as the number of species.

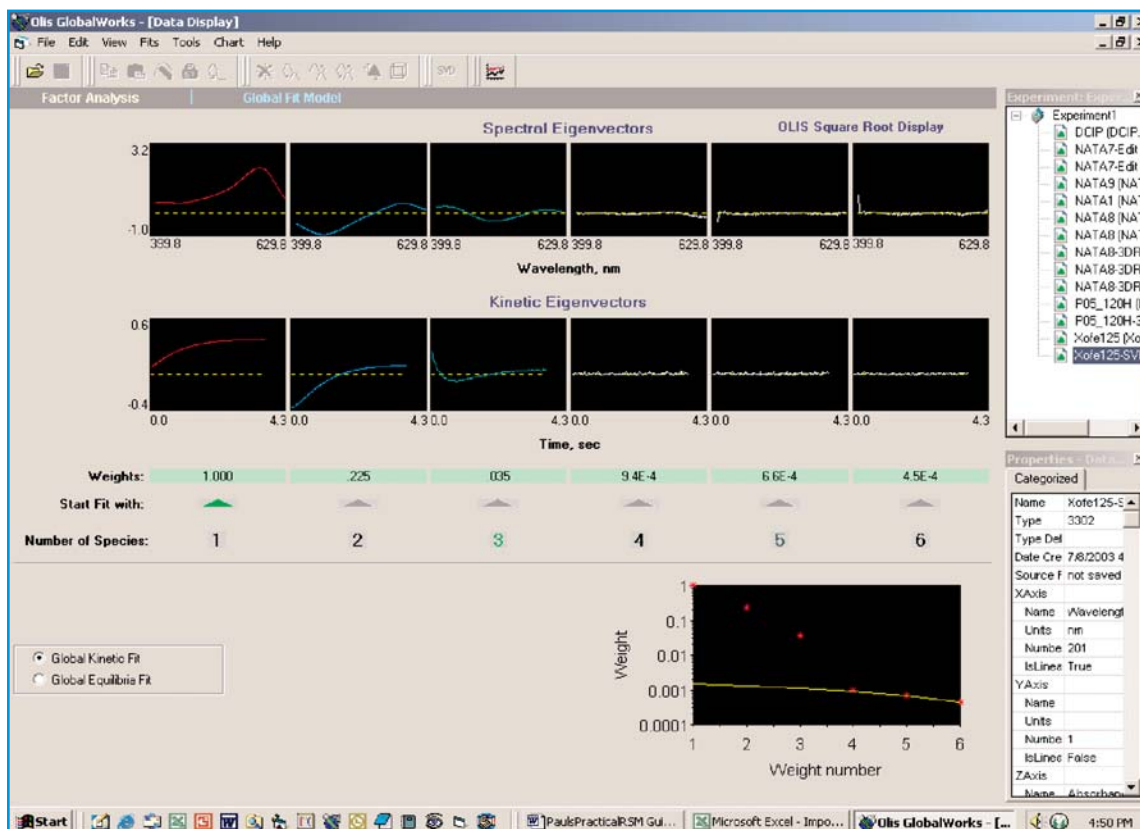


Figure 7

Once the number of chemical species is chosen, the program will present a number of kinetic fits available for the selected number of species. The fits available for a three species fit are shown in Figure 8. To fit the kinetic data to a particular model simply select the model and choose 'Fit Model.' In the DOS system, simply enter the corresponding letter of the model of choice. For the xofe125 dataset, a model of $A \rightleftharpoons B \rightleftharpoons C$ is chosen. Note that two different $A \rightleftharpoons B \rightleftharpoons C$ models are present; one with a soft $A \rightleftharpoons B$ rate and the other a slow $A \rightleftharpoons B$ rate. As described below, these two models can be differentiated.

The results of the fit are shown in Figure 9. The resulting rate constants, amplitudes, and standard deviations are given on this page (upper left). The page also contains three graphs. The left graph shows a calculated spectrum of each kinetic component. Figure 9 shows spectra for A (red line), B (cyan line), and C (yellow line). The right graph shows the contribution of each of these species to the total as a function of time. Note that A (red line) decreases as B (cyan line) increases then decreases, followed by the formation of C (yellow line). The total calculated eigenvector contribution is given by the white line. Finally, the blue line shows the experimental data. The third graph (top) is a plot of the residuals, which are obtained by subtracting the experimental data from the total calculated contribution.

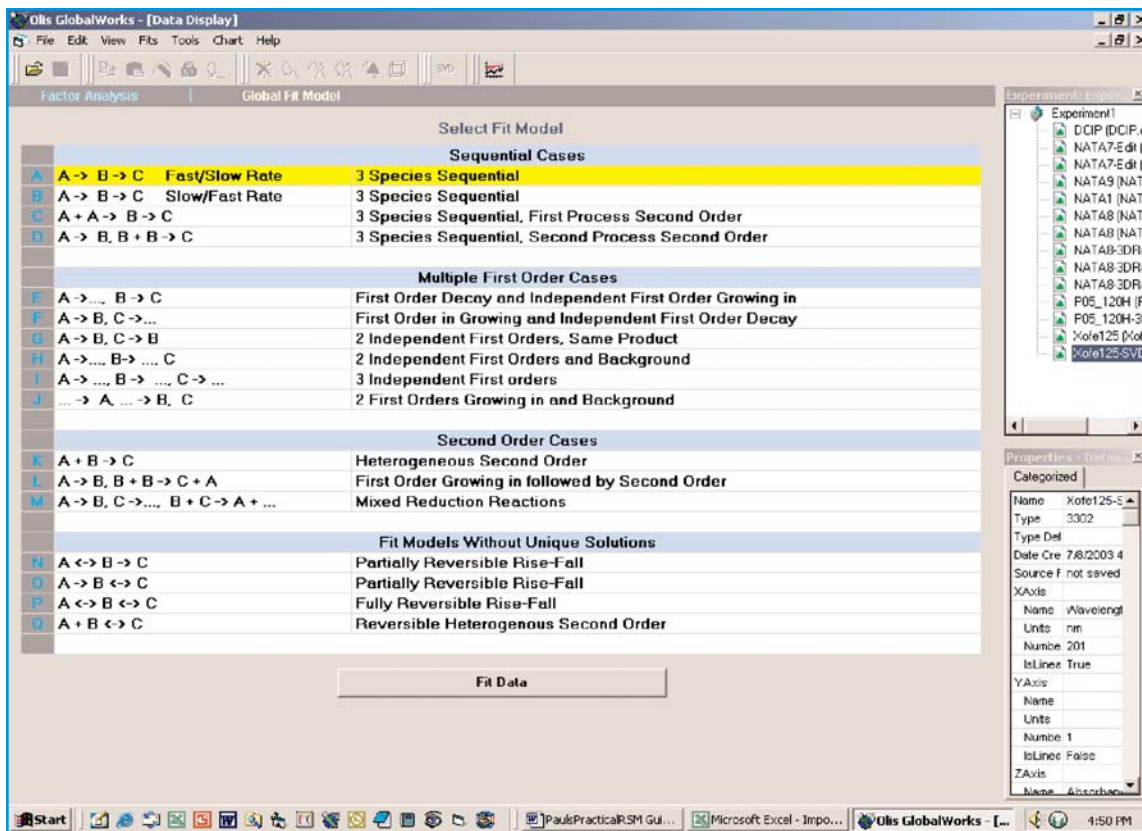


Figure 8

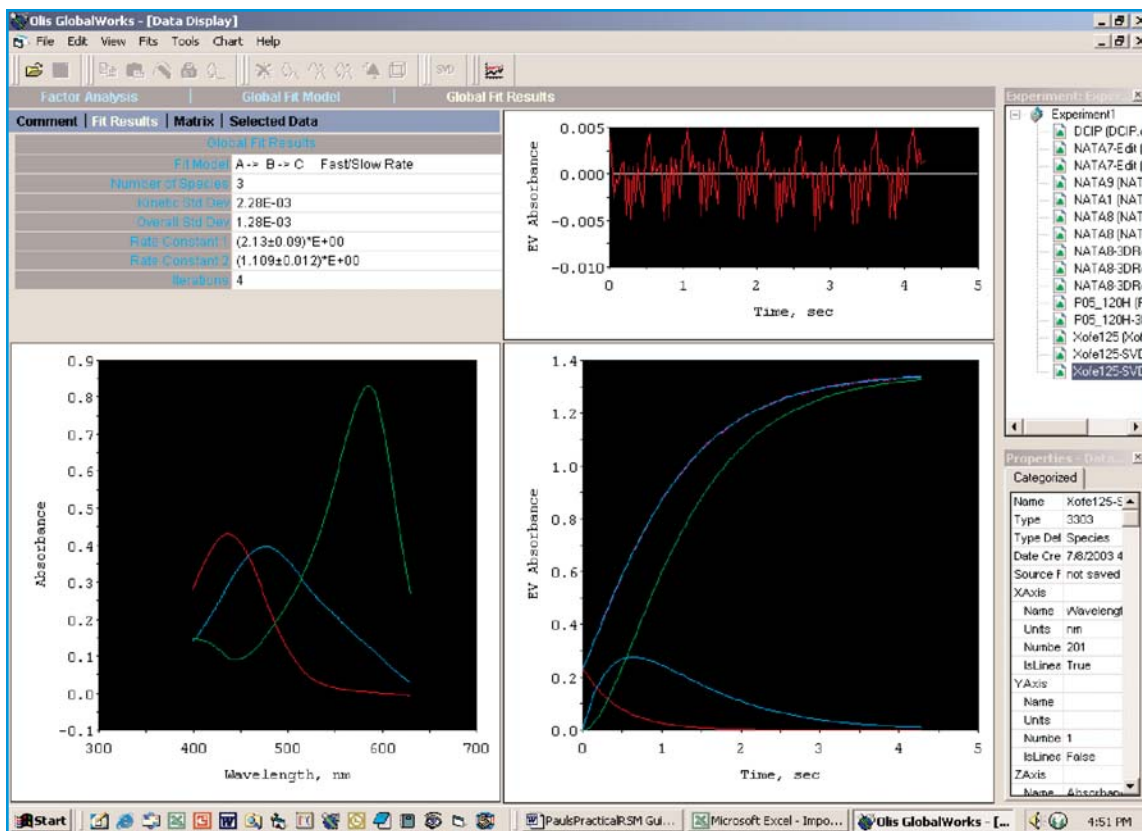


Figure 9

There are several criteria for global fit. The kinetic fit can be evaluated by the residuals. A good fit shows a residuals plot which is featureless (i.e. linear around zero with only random noise). A second criterion is the overall standard deviation. This value should be as low as possible. Finally, the calculated spectra themselves can be evaluated. Do they look real? For example, an absorbance spectrum (not a difference spectrum) cannot be negative. So a global fit which results in negative spectra likely is not correct, even if the kinetic fit appears to be good. Spectra can also be used to differentiate models that appear to fit. For example, a fit of xofe125 data using AŠ BŠ C fast/slow kinetics gives slightly different spectra from the AŠ BŠ C slow/fast kinetics model. If sufficient information is available about these spectra, these two models can be differentiated, based on the calculated spectra.

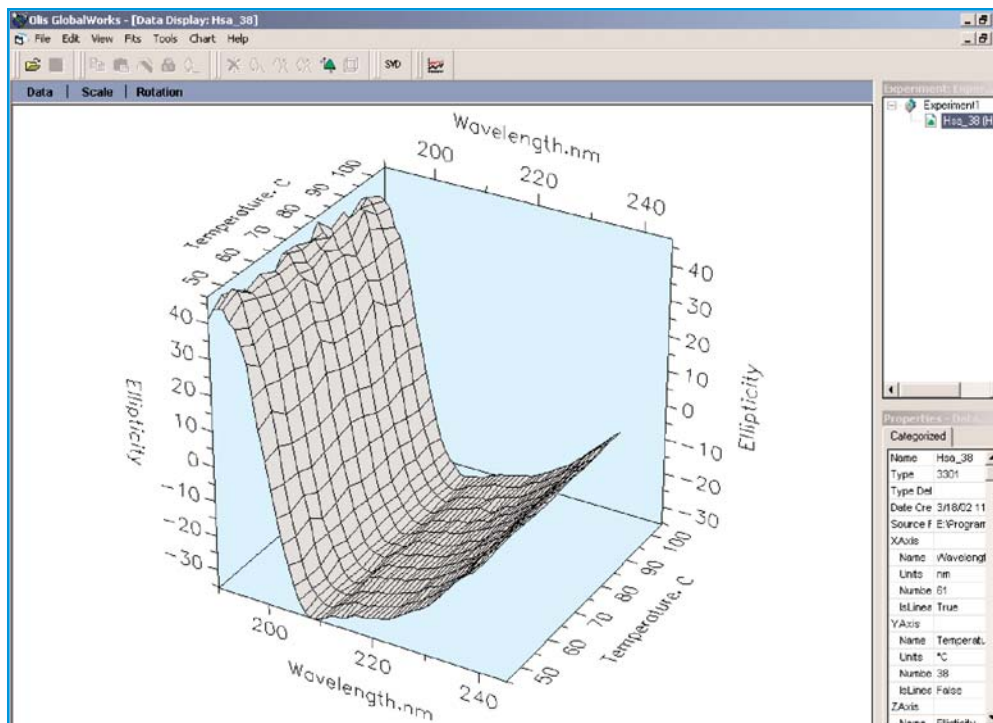


Figure 10

SVD analysis is not limited to kinetic datasets.

Figure 10 provides an example of circular dichroism spectra of human serum albumin taken as a function of temperature. SVD analysis of this data shows that two species can be differentiated which change as a function of temperature, as shown in Figure 11. Selecting the number of species provides a number of equilibrium fits which may be employed. The resulting fit, after starting parameters have been given, is shown in Figure 12. Note that this page is

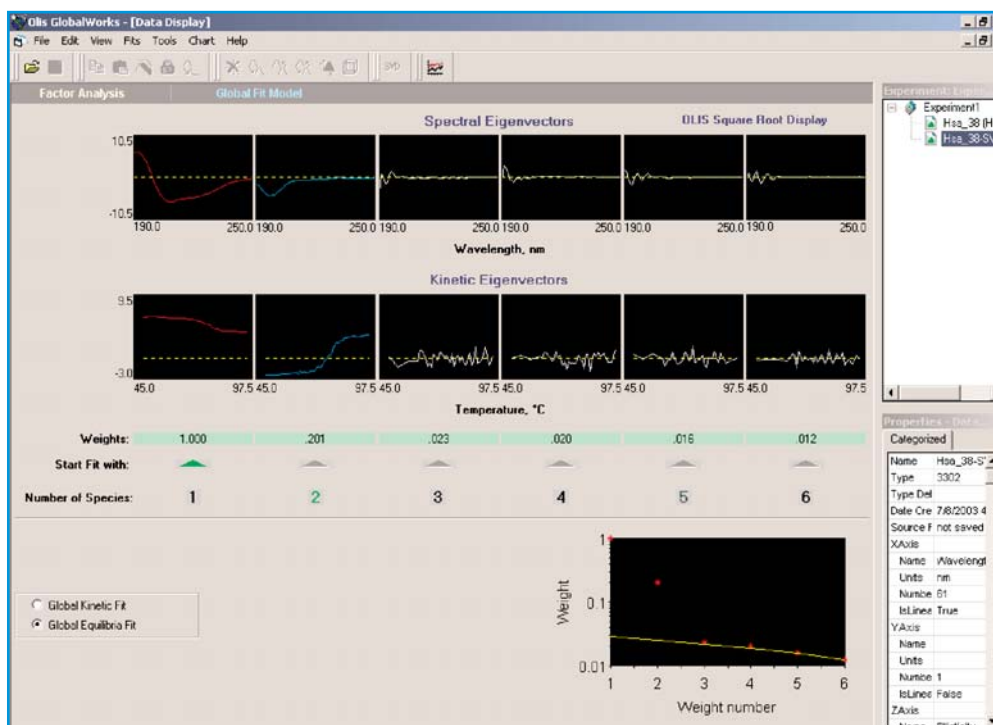


Figure 11

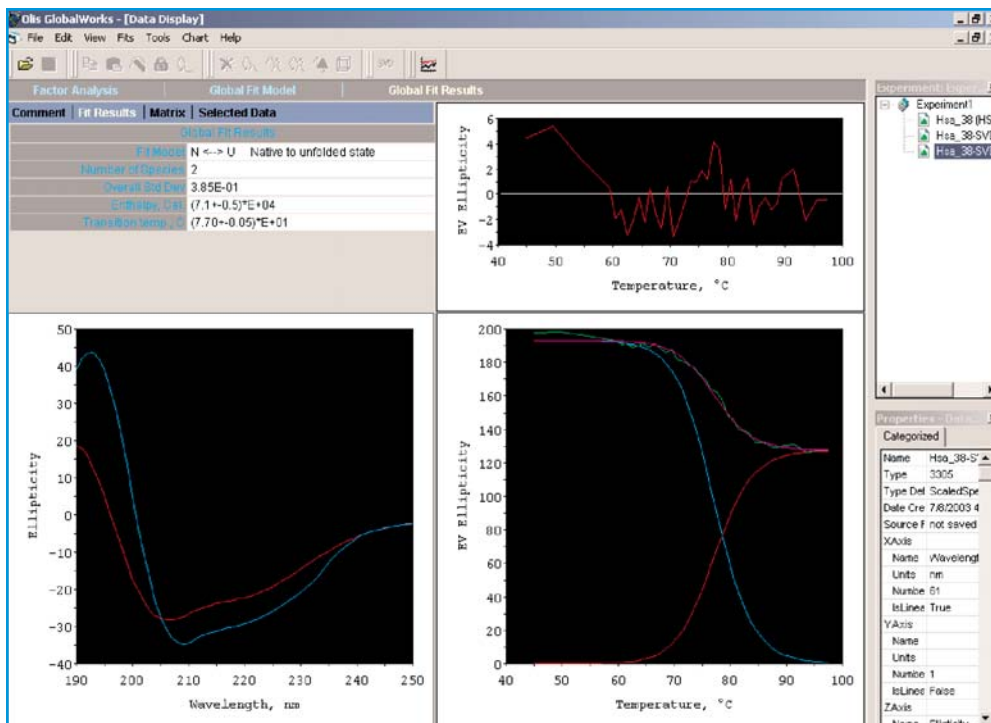


Figure 12

analogous to the kinetic page with the exception that the right graph is shown as a function of temperature rather than time. The advantage to global fitting (fitting scans rather than single wavelength points) is that spectral information is maintained in the fit. Less temperature points are required because more information is provided for each temperature.

SVD also has the effect of removing noise from a dataset. By recombining a given number of eigenvectors a dataset can be recombined. A consequence of this is that all information contained in eigenvectors not used in the recombination are lost. Thus, when eigenvectors containing noise are removed the resulting dataset is dramatically noise reduced. A dataset can be synthesized from six eigenvectors by selecting Build 3D dataset from SVD data in the TOOLS menu.

Demo Data

Demo data is provided with all RSM software and is located in \Program Files\Olis Globalworks\Demo Data in the Windows software and can be accessed in the Demo data option from the Command Mode in the DOS software. Information on the data files is as follows:

Data File*	Number of Species	Correct Model	Comments
xofe125.ols	3	Aš Bš C	Oxidation of xylenol orange with Fe ³⁺
protfold.ols	2	Aš B	Unfolding of T4 lysozyme, very noisy raw data
1EXP.kin	2	Aš ..., B	Synthetic single exponential
2EXP.kin	3	Aš ..., Bš ..., C	Synthetic double exponential
3EXP.kin	3	Aš ..., Bš ..., Cš ...	Synthetic triple exponential
3expneg.kin	3	Aš ..., Bš ..., Cš ...	Synthetic triple exponential, one negative amplitude
3flashes.ols	-	-	3 flashes of a firefly
3species2rates.ols	3	Aš ..., Bš C	Reduction of cytochrome C with ascorbate
4species3rates.ols	4	Aš Bš Cš D	Synthetic data
5species4rates.ols	5	Aš Bš Cš Dš E	Synthetic data
6species5rates.rdf6	6	Aš Bš Cš Dš Eš F	Synthetic data
Cyto_ascorbate.ols	2	Aš B	Reduction of cytochrome C with ascorbate
DCIP.ols	2	Aš ..., B	Reduction of DCIP with ascorbate
DCIPHOLM.ols	2	Aš B	Reduction of DCIP with ascorbate with holmium filter background
HSA_38_CD.ols	2	Aš B	CD Spectra of human serum albumin vs. temperature
Lyso2050_CD.ols	2	Aš B	CD Spectra of hen egg white lysozyme vs. temperature
Lyso260182_CD.ols	1	Spectrum	CD Spectrum of hen egg white lysozyme
P05_120H.rdm	1	-	Overhead lights
TPLASP1.ols	3	Aš Bš C	

*.ols - Olis Windows data file,

.rdf, .rdm - DOS reduced data file. Data file must be created when opening in Windows.

Create data file by selecting Create 3D from SVD Data under the Tools menu.

.kin - Synthetic kinetic file

Data Handling Tools

There are several data handling tools available in GlobalWorks for dealing with 3-dimensional data files.

DYNAMIC SLICING

Viewing kinetic traces in a 3 dimensional dataset is accomplished using the 'Dynamic Slicing' feature located in 'Edit' menu. Once this mode is activated, the kinetic trace corresponding to the current wavelength is shown in the kinetic graph. If this wavelength is changed by dragging the cursor on the spectral graph, the corresponding kinetic trace will change dynamically. Thus, one is able to quickly view the effect of wavelength on the observed kinetic traces. If the dynamic slicing is turned off by selecting it again in the menu, the kinetic trace at the last wavelength is left on the kinetics graph. A kinetic trace at a desired wavelength can be extracted in this manner. Obviously, this feature is not limited to kinetic traces as 3D datasets taken as a function of temperature, concentration, pressure, etc. can be viewed. Also, by clicking on the kinetic graph rather than the spectral one, the change in spectra can be observed by dragging the cursor through the time domain and watching the spectrum change. It is often useful to watch a 'movie' of your spectral change.

EDIT DATASET

A dataset can be viewed in a spreadsheet format by selecting 'Edit Dataset' from the Edit menu. From this screen, the data can be viewed, axis values added, or axis labels changed. This page is especially useful for adding a third axis to data which has been collected individually.

RECONSTRUCT 3D FROM SVD DATA

The process of singular value decomposition (SVD) can be reversed in order to obtain a 3D dataset from a set of SVD eigenvectors. This is accomplished using the

'Reconstruct 3D from SVD Data' selection in the 'Tools' menu. The advantage to this is that reconstructing data from the eigenvectors is an effective way to reduce noise in a dataset without smoothing. Thus there is no danger of over-smoothing and distorting data. The only limitation to this is that your data of interest must be contained in the first six eigenvectors. The reason behind the noise removal is that the recalculation uses only six eigenvectors so all noise contained in any higher eigenvectors is lost. Thus, if the data of interest is contained in the first six eigenvectors (which it almost always is), it will be constructed with a significant decrease in noise. A second advantage to this feature was much more important in the DOS system. That is that the data can be stored as a set of eigenvectors which take up significantly less storage space than the raw data. Because data storage devices have advanced tremendously in recent years, storage space for datasets is no longer a real issue in the Windows systems.

CREATE DATA SUBSET

To truncate a dataset, use the 'Create Data Subset' option in the 'Tools' menu. Choosing this allows one to enter a new range for both axes (if a 3D dataset) and create a dataset with new axis limits. This is particularly useful for extracting a single scan from a multi-scan dataset (Dynamic Slicing can also be used as well). Also datasets in which data was collected beyond the useful range of the grating or detectors can

Data Handling Tools

be trimmed to give more valid SVD fits.

DATA PROCESSES

Many data processes are available for processing datasets. These are available by right-clicking on the desired dataset(s) in the experiment window and choosing 'Select' from this menu. This activates the 'Data Processes' menu. Located in this menu are processes such as averaging scans, arithmetic on datasets, applying a constant, digital filters, building 3D datasets from 2D, peak finder, and interpolation.

IMPORTING/EXPORTING DATA

Data can be exported as an ASCII text file in one of two ways. Right-clicking on the data (on the graph) reveals a menu from which 'Save as ASCII' or 'Export to Excel' can be selected. Choosing 'Save as ASCII' saves the current 2D slice as a two column tab delimited ASCII file. If 'Export to Excel' is chosen, data is exported into an Excel spreadsheet for further processing. Two dimensional ASCII data files can be imported directly as a column of XY pairs if the file contains no header or footer. In addition, GlobalWorks will directly read a number of different manufacturers file types. Other file types can be added to this list if Olis is provided with detailed file specifications.

Olis Website

The Olis website (ref 2) contains extensive information about using and troubleshooting the RSM 1000. Included on this website are sample data, lists of available accessories, technical information such as how to calculate bandpass and instrument specs, detailed descriptions of components and accessories, and technical support information. The website is continuously updated so visit it regularly.

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