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THE OLIS RSM 1000 Operating System Users' Manual

preliminary version V

prepared 3/31/94 by N. De Sa

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1 GETTING STARTED

Introduction to the Olis Rapid-Scanning Monochromator

The patented (# 5285254) spectroscopy system is the invention of Richard J. DeSa, Ph.D., creator of the first computerized stopped-flow (*Computers and Biomedical Research*, vol. 2, no. 5, October, 1969).

The *OLIS RSM* is a subtractive double monochromator with scanning rates of 3 x 10^5 nm/sec, immeasurably low stray light, and abundant features to collect and analyze spectral and kinetic data which are free from systematic error. The *OLIS* Rapid-Scanning Monochromator provides faster scanning speeds than all other dual beam or diode array spectrophotometers.

The *OLIS RSM* Spectroscopy Operating System is a powerful, inclusive, authoritative, and logical program. Since the introduction of the *OLIS RSM*, the program has been enhanced by the implementation of suggestions by programmers and scientists.

Opinions, special requests, and constructive criticisms by you can only help us to improve this elegant laboratory tool. Please share your ideas with us!

The *OLIS RSM* provides an exceptional degree of accuracy, minimal systematic error, and simplicity of data handling. These qualities, along with optional customization, allow you to "see your spectra in the best light."

Available procedure options are displayed on the screen as you progress from data collection to data storage or data manipulation, so the software is easy to master. This manual provides explanantions to expand your understanding of the program and to ease your transition from novice to pro-*OLIS* user!

Set aside time to read this manual. Some new users have lost data or gone through too many steps unnecessarily because they were not fully familiar with the capabilities of the software. If possible, read this manual while at the computer to directly observe the outlined procedures and note any discrepancies between the described and actual program (this manual might be a modification or two behind your current version of the software). Keep in mind that the RSM system is a powerful spectrophotometer: if you need some facility or capability for spectroscopy, the *OLIS RSM* probably has what you need.

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THE RSM-1000

The *OLIS RSM* package includes the scanning monochromator with ScanDisk and gratings, a light source, a dual-beam optical system (sample/reference) with two photomultiplier detectors, amplifiers and two 2MHz 12 bit A/D converters. The control unit includes a 486/66 MHz computer with at least 8 Megabytes RAM, a hard disk with at least 200 Megabytes, MS-DOS version 6.0 or higher, one RS-232 and one parallel port, a high resolution color graphics monitor, and a printer.

Options you may have chosen to include in your system are a color printer, red sensitive photomultipliers for scanning in 700-1100 nm, additional gratings or ScanDisks, an optic cable, a means to flash at your sample, etc. The system is intended to be all-inclusive; all you should need is your sample! The software is installed on the hard disk for you. If necessary, back-up floppies are provided should your system crash. System updates are provided on floppy diskettes, upon request.

The *OLIS RSM* System is a dual-beam UV/VIS recording spectrophotometer with high scan rate for collecting, displaying, and archiving absorbance or transmittance spectra. Facilities necessary to support these operations are included in the *OLIS RSM* software. The program can do most, if not all, that must be done with spectra.

CARE OF THE RSM-1000

The *OLIS RSM* should be treated with care to insure a long, useful life. You will receive the best performance for the longest time from your instrument if you follow a few guidelines:

When you are not collecting data, pull out the entrance slit by a few millimeters; this blocks the xenon light beam and reduces wear on the optical components.

We recommend that dry nitrogen be circulated through the optical system during operation of the RSM. This practice enhances the life of the optics, particularly in UV performance.

Keep lids and the ScanDisk cartridge securely in place to prevent airborne particles from entering the monochromator. Open the lid and trapdoors only when changing gratings.

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CALL OLIS WITH QUESTIONS!

Never hesitate to pick up the phone with questions, problems, or suggestions. We would much rather talk you through an issue than learn of your time spent struggling alone. Because of the software's design, you should have minimal problems; those which may occur are easily diagnosed. Call anytime — you'll never know if someone is working odd hours here, too, unless you ring. Use our toll-free number **(800-852-3504)** if you are in the United States or Canada; from other locations use **(706) 353-6547**. Please send us screen dumps illustrating your problem if appropriate, via FAX **(706) 353-1972**. If you have an emergency after hours and cannot reach anyone at these numbers, Dr. DeSa can be reached at (706) 367-9191.

HOW TO READ THIS MANUAL

The manual is divided into chapters according to main command menus in the program. The beginning of each chapter includes a path of keystrokes from the Command Mode to the present location in the program. This path is displayed in shaded ovals with CM standing for Command Mode. Each command description also includes a path of keystrokes from the menu in which that command is listed. If you get "lost" in unfamiliar territory, reference the present option in the manual's index and press "Escape" as many times as necessary to arrive back at the Command Mode. All options are listed alphabetically in the manual's index.



OLIS software is continually being improved. Names and descriptions of options may differ between this manual and your version of the program. If the option performs as defined, it's correct, even if it is labeled differently on your screen than in the manual. Please make note of discrepancies. If you are uncertain, check the index for cross-reference or call *OLIS*.

Until you are comfortable with the system, we recommend you explore the software using the demo files which are included for this purpose. See page 75 for explanation of the demonstration mode.

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THE COMPUTER SYSTEM AND HARDWARE SET-UP

Cautions on installing your computer:

Be sure that your computer is positioned so as to allow free air flow behind it. Never push the computer against another machine, wall, or other surface, because this will obstruct the computer's cooling system.

Do not plug your computer into an AC line which services devices that draw heavy current. Avoid AC lines which service devices that switch on and off regularly (such as freezers, vacuum pumps, etc.). In both cases, we are avoiding power surges which can cause damage to your hardware, software, and data.

Powering up/rebooting your computer:

Turn the computer ON and OFF: Most systems are equipped with a multiple outlet strip, usually located beneath the monitor. The switches on this outlet are labeled according to what they control. Use the "Master/Computer" switch to turn your system on and off. Your system might also have an *OLIS*-installed multiple outlet strip connected directly to the AC wall outlet. In case of an electrical storm, the outlet leading directly into the wall should be disconnected.

Reset/Reboot the system: Press the CTRL, ALT, and DEL keys simultaneously. Some computers, such as the GATEWAY 2000, have a RESET button on the front of their cabinet which can be used instead of these three keystrokes.

Full details about your computer hardware are available from the manufacturer. If you purchased the computer hardware from *OLIS*, all manuals were provided at installation — please refer to them as necessary.

** If you have a system with a xenon lamp, be sure the computer and all supporting electrical devices are turned off before you fire the lamp. **

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INPUTTING OLIS COMMANDS INTO YOUR COMPUTER KEYBOARD

All *numeric* inputs must be concluded by pressing the Enter key. You do not need to backspace over numbers highlighted in yellow which are followed by a blue block cursor before inputting new values.

Letter commands are accepted as single keystrokes -- the Enter key is not required, except in the Comment Editor section of the program where it is used as a carriage return on a typewriter.

The convention in this manual is to capitalize user keystrokes and enclose letter(s) in quotation marks to improve clarity. The program does not differentiate between upper and lower case inputs — both are accepted. Use the Escape key to exit routines which you entered inadvertently. Whenever you press the Escape key to exit a routine and it is the first key you press, the routine is exited with no changes recorded (unless, of course, the routine is a toggle, in which case, access it again to return to previous status).

INITIALIZING THE OLIS PROGRAM

Apply AC power to the computer:

Once the computer is turned on, the boot-up procedure begins. This boot-up procedure is defined by the AUTOEXEC.BAT and CONFIG.SYS files. (Refer to your MS-DOS manual for details on these essential files and to page 9 of this manual for the default RSM AUTOEXEC.BAT). Please do not change the boot-up procedure unless you are instructed to by *OLIS* personnel.

Since we set up hard disk systems with an AUTOEXEC.BAT file for entering your *OLIS* Operating System, usually turning the computer on is adequate to load and install the necessary files. Be certain that a floppy is NOT in any of your disk drives; you want the system to boot from the hard disk.

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Installing and Updating from Floppy Diskettes

Your RSM is installed with the software on hard disk. You will be provided with diskettes as back-up and when updates are made.

Insert the *OLIS* Operating System diskette into Drive A or Drive B: The *OLIS* diskette contains all the files necessary for using the system. Always keep a back-up version of the latest copy of your software in a safe place.

Type INSTALL or UPDATE: These commands refers to the directory of the floppy disk and to the provided instruction, type either INSTALL or UPDATE to initiate the batch file which *OLIS* provided. All files are copied to the appropriate subdirectories before the computer tells you what to type to initialize the program.

Para-files Essential for Your Spectrometer

The *OLIS* software must know the performance characteristics of the instrument it controls. This unique information is stored in two "para-files;" RSM-HDW.CFG is specific to hardware information and Parameter.RSM is specific to program information. Every time you exit the *OLIS* software with a 'Z' from the Command Mode, the Parameter.RSM para-file is updated, and, if appropriate changes have been made, the RSM-HDW para-file is also updated. Infrequently, deliberate adjustments to the para-files are necessary. Check with *OLIS* if your instrument performs erratically, especially after a software update is installed. Other hardware settings are stored in the para-files and might need to be adjusted after a new code is installed.

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AUTOEXEC.BAT

If you wish to enter the *OLIS* program from the system prompt, i.e., when "A:>," "B:>," or "C:>" is displayed, you may do so by typing in "RSM," or whatever else the executable batch file for calling up the program is named. The *OLIS* batch file will include the following string of commands:

c:
cd\olis-RSM
run87 olis-RSM

OLIS-RSM:	The subdirectory in which the *OLIS* program files are located, named for the instrument it controls.
RUN87:	The program to load the BASIC part of the software.
OLIS-RSM:	The instruction to load the primary *OLIS* program, again specific to the instrument being controlled.

If you have used the automatic installation, please notice that your original AUTOEXEC.BAT and CONFIG.SYS files have been copied to an \OLIS-RSM\ subdirectory, SETBAT, under the new names "AUTOEXEC.ORG" and "CONFIG.ORG," where "ORG " is shorthand for "original." Use these "original" files whenever you want to use your computer with non-*OLIS* software.

Type "SETNORM" when you want to use non-*OLIS* software;

Type "SET_OLIS" when you want to return to the *OLIS* software.

Executable programs in the form of batch files for toggling between the *OLIS* protocol and the "original" protocol have been added to your SETBAT subdirectory as well.

The last line in these two batch files is "REBOOT1." This command causes the computer to reboot. RAM requirements of some software, *OLIS* included, force you to reboot the computer to change from one memory intensive program to another. *OLIS software is very RAM intensive*; so are networks, SideKick and other such programs which are loaded at boot-up and retained in memory.

If you attempt to run RSM software without first clearing system RAM of non-OLIS programs such as Windows, no data collection or analysis will be possible even though sections of the program might function.

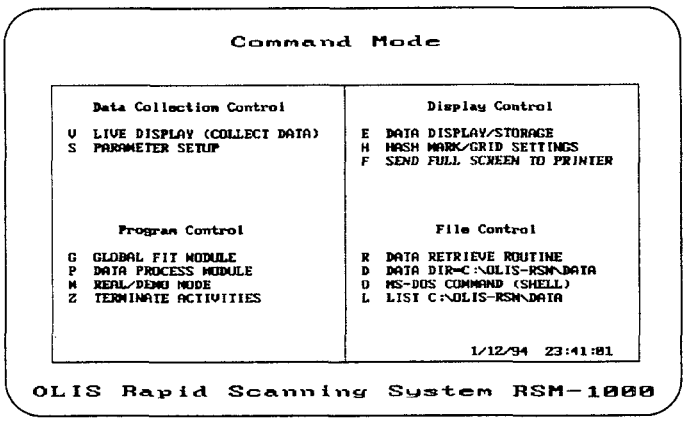
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Identifying the Sections in the Olis Operating System

The operations of the *OLIS* RSM-1000 Operating System are listed in four primary headings identified on the main menu (**Command Mode**) as **Data Collection Control**, **Display Control**, **File Control, and Program Control.** The menus within these headings list available commands.

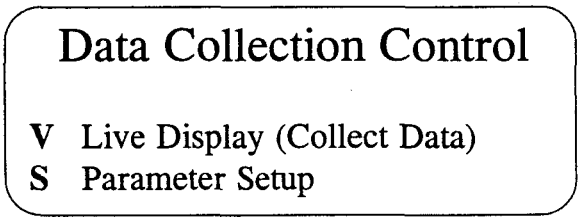
Command Mode

Upon start-up, the program displays the Command Mode menu which contains options listed under four headings. All data collection and fitting commands are accessible from the Command Mode menu. You can return to the Command Mode at any point in the program by hitting 'Escape' as many times as necessary.



Data Collection Control

The commands listed under this heading allow you to control the collection and display of your data.



Live Display (Collect Data):
Choose options for collecting data as well as controlling wavelength and PMT HV. Once the data are collected, the program proceeds to the DVM (Digital Volt Meter) Live where you can examine data in close detail and select other options for data manipulation.

Parameter Setup: Set up hardware specifications, basic data collection parameters, printer selection, and 2D and 3D display parameters. Functions are accessed and adjusted by keys located in the Parameter Setup Menu.

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Display Control

This heading lists options which allow you to manipulate and examine the display of selected scans from a record. You can observe single scans, scans derived by averaging multiple records, scans "cut" to include only part of the original scan, etc. Operation of these options does not affect the raw data permanently.

Display Control

E Data Display/Storage
H Hash Mark/Grid Settings
F Full Screen to Printer

Data Display/Storage: If data are in memory, this command directs the program to DVM Live.

Hash Mark/Grid Settings: You can choose between hashmark and grid graph settings for use throughout the program. This command also provides an opportunity to increase/decrease the number of X and Y hashmarks.

Send Full Screen to Printer. If data are in memory, this command enlarges the current data graph scans to full screen size, eliminating the menu boxes for print-out. Note: in the RSM-1000 software, input is Ctrl-P for a screen dump print, not the "print screen" key on the keyboard.

Printing with Pizazz Plus. Using the Pizzaz Plus software which you may have chosen as an option with your RSM software, you can capture any screen image. You can the import the captured images into word processing or desktop publishing programs. Pizazz Plus was created by Application Techniques, Inc. in Pepperell MA; it is not *OLIS* software. Application Techniques can be reached at 1-800-433-5201.

File Control

Use the options listed under this heading to enter MS-DOS commands without interrupting operation of the RSM program, and to retrieve previously stored files. See page 77.

File Control

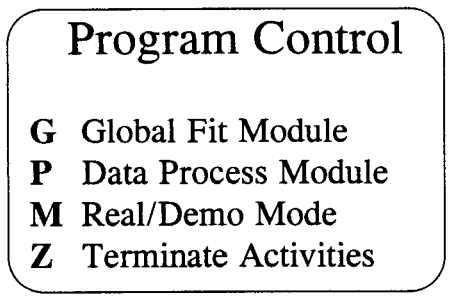
R Data Retrieve Routine
D Data Dir=C:\OLIS-RSM\Buffer
O MS-DOS Command (Shell)
L List C:\OLIS-RSM\Buffer

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Program Control

This heading lists options from which you can choose to examine your data files after collection. In addition, you can toggle between demo and real modes. The demo mode helps you familiarize yourself with the program.

Global Fit Module: Initiates Global Fit routine, which could be considered a raison d'etre of the RSM technology: rather than using 2D data, as in all conventional spectrometers, Robust Global Fitting uses 3D data arrays. Full functional descriptions are provided in chapter 7, further details about the math methodology are provided in appendix A, which includes recommended reading.



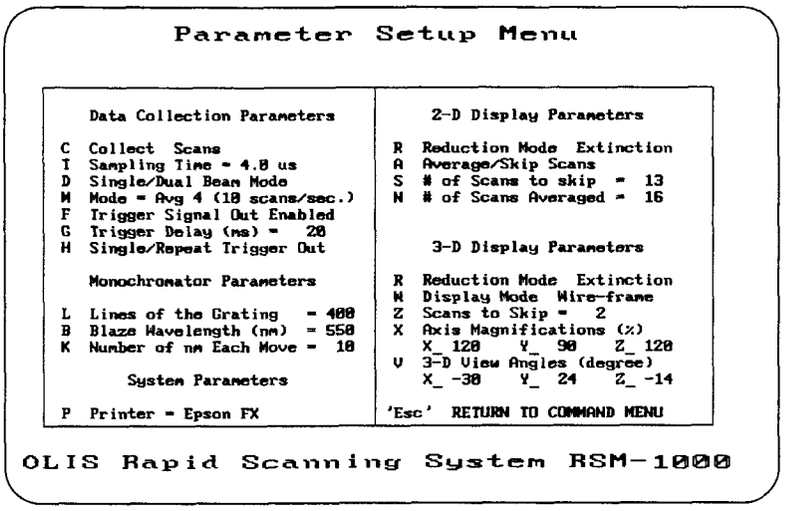
Data Process Module: Select options (add, subtract, reduce, multiply) for manipulation of data files. See page 73.

Real/Demo Mode: Toggle this command to choose between selecting a demo file for an introduction to the program, or setting the program for work with your data. The program functions with all of the demo data sets, but only the Holmium Oxide Filter data set allows you to simulate DVM Live mode and the options in it.. See page 76 for explanation of the demo mode.

Terminate Activities. This command allows you an orderly system shutdown when exiting the *OLIS* software. The operating parameters you have set will be written to record and reproduced at your next session. We recommend that you *always* conclude an *OLIS* session by "Z-ing out."

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2 PARAMETER SETUP



The Parameter Setup Menu lists commands which allow you to define most of the system variables for the collection and display of data. The 2D and 3D Display Parameters options can be set before or after data collection.
CM → S

DATA COLLECTION PARAMETERS

C

COLLECT

An *OLIS RSM* can be configured to collect rapid scan data, static spectrum, fixed (single wavelength) data, dual wavelength data, LD, and CD data. Toggle 'C' until the mode you desire is displayed. Selection of the Scan mode is recommended because data collected in this mode can be viewed using any other configuration. Keep in mind that data collected in the Static Spectrum or Fixed Wavelength modes can not be viewed as if they had been collected in the Scan or Dual Wavelength modes. Some additional modes may require the utilization of specific hardware. Please contact *OLIS* for details.

Scans

This is the default for most RSM systems, and presumes the presence of a ScanDisk with multiple slits and a scan rate of 1000 scans/second.

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Static Spectrum
This mode is used to collect data from a non-changing sample. The RSM collects 1000 scans/second and averages the incoming data to produce a data set.

The results from this mode are identical to those acheived by analyzing Scans with the Robust Global Fitting algorithm (see page 65). The Static Spectrum mode averages the data during data collection, so that the 'final' spectrum is available immediately after data collection. However, Robust Global Fitting returns statistical information and is the recommended means for obtaining high sensitivity data from the RSM. Averaging returns no statistics.

Fixed Wavelength
This mode presumes the presence of a static ScanDisk, as is often used in luminescence, fluorescence, and other data collection modes in which light intensity *from the sample* is measured. To 'create' kinetic traces from data collected as scans, use Extract Kinetic Trace ('K' in Cursor Scan Box, see page 48); this option creates kinetic traces of up to 1000 points from the collected scans.

Dual Wavelength
This mode should be used when your sample is extremely photosensitive and/or when you want to measure the effects of specific wavelengths of light.

Dual Wavelength spectrophotometry is used to study turbid samples which typically present falsely high absorbance readings because of light scattering by the sample. The dual wavelength method minimizes scatter effect by using one wavelength at which the sample under study exhibits a signal (due to chemistry) and another wavelength which the sample does not absorb, but at which the effects of scatter are similar. By subtracting the former from the latter, you isolate the effects of the chemistry and can measure the 'true' absorbance of the sample. This method is most effective if the two wavelengths are close together.

We recommend that you use this option judiciously, since collecting full spectral scans of 200 wavelengths provides more information than two wavelengths and you can use these spectral scans to create dual wavelength records for comparison with conventional dual wavelength records by extracting the two wavelengths from the scans using Extract Kinetic Trace ('K' in Cursor Scan Box, see page 48) and then subtracting these two scans from each other using option 'G' in the Data Process Module (see page 73). This method produces a single scan which ideally has no scatter, and which therefore, is correct.

If you wish to perform true dual wavelength scanning, your RSM must be fitted with a mask which only allows two wavelengths to illuminate the sample. The distance between the two openings on the masked ScanDisk is fixed; the mask may be moved to allow higher or lower wavelengths, but their distance from each other will always be the same.

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LD or CD

These modes presume that your RSM is configured for LD/CD spectroscopy. CD spectroscopy requires additional hardware. The Parameter Setup Menu will list the modes of CD spectroscopy which are available with your system.

C

T SAMPLING TIME

Toggle 'T' to switch between sampling times in microseconds for your data:

4.0 µs (250 KHz, there will be 201 data points per scan default.)
2.5 µs (400 KHz, there will be 321 data points per scan default.)

We recommend choosing the slower rate if you have a long collection time, since available memory RAM quickly fills when the higher rate is used. Additional sampling rates are possible with a RSM. Please contact *OLIS* for details on secondary modes which are not documented as of this publication.

T

D DATA COLLECTION MODE

Toggle 'D' to switch between Single and Dual Beam Mode. Single Beam Mode is used when the maximum light intensity is required or for test measurements where the beam splitter may be removed. In the Single Beam Mode, the sample and reference data must be collected before or after the data collection, i.e. sample and reference are separated in time. Dual Beam Mode uses sample and reference data simultaneously to collect true absorbance values and is the mode in which you will usually work.

D

M MODE

Toggle 'M' to cycle through the modes available with the RSM-1000 system: no average mode (1000 scans/second), and averaging 'on the fly' mode (Avg 1 to Avg 5). Since the maximum number of raw data scans which the Robust Global Fitting algorithms can fit is 1000 (or 5000 SVD scans), a collection time of five seconds produces five times more data than the fitting algorithms can handle. Averaging every other, or every fifth, scan enables you to collect data for a much longer time. The more time spent during data collection, the better the S/N. The mode you choose depends on whether you wish to optimize for time or sensitivity.

M

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F

TRIGGER SIGNAL OUT

Enable or disable the Trigger Signal Out by toggling 'F' to highlight your choice. The trigger signal is used to fire the stopped-flow, flash a lamp, or initiate another external device.

F

G

TRIGGER DELAY (ms)

When the previous command is Enabled, informing the program that an external device will be used, you can enter a time value for the Trigger Delay, the time from the beginning of data collection until the external device is fired. The data collected both before and after the trigger is fired are part of the total collection time.

For example, at the rate of 1000 scans/second, if the Trigger Delay equals 0.1 seconds, 100 scans will be collected before the trigger is fired, and 900 scans will be collected after the trigger is fired. Unless important data are anticipated before the external device is fired, the delay should brief, such as five to ten percent of the total collection time.

G → Input

H

SINGLE/REPEAT TRIGGER OUT

When the Trigger Signal Out is enabled, toggle 'H' to select either a single or repeated trigger signal. Select the single trigger out when you want to fire the external device only once. Repeat trigger out will fire the external device repeatedly until data collection is completed. The Trigger Delay (previous command) determines the time between signals.

H

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Monochromator Parameters

L	Lines of the Grating	= 400
B	Blaze Wavelength	= 550
K	Number of nm Each Move	= 20



LINES OF THE GRATING

Change this absolute number only when you change the gratings. The number of lines per mm on a grating determines its dispersive behavior and defines the available wavelength span and resolution of your RSM.

For example, to change the RSM from a specification with a span of 250 nm to one with a span of 150 nm, change the gratings from one with 600 lines to one with 400 lines. Gratings with a lower number of lines per mm produce a smaller dispersion of input light, and allow a broader wavelength span. A higher number of lines produces more dispersion, and narrows the span of wavelength available.

Whenever the gratings are changed, the monochromator must be calibrated. See page 28.

L

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B BLAZE WAVELENGTH (nm)

This value identifies the blaze of the grating, i.e., the wavelength for which the grating is optimized. "Blazing" is the process by which gratings are made optimally efficient by appropriate groove shaping.

As with 'L' above, change this setting when you change gratings.



K NUMBER OF NM EACH MOVE

This value determines the number of nanometers the wavelength at the center of the scan changes each time you move the monochromator (see the Command Mode menu, Live Display) via the arrow keys found in the Live Display menu (command is labeled Move Monochromator Up/Down). For example, an input of 20 causes the wavelength to change by 20 nanometers with each arrow keystroke. Use the arrow keys when you wish to move quickly through several values to arrive at one appropriate for your work.



System Parameters

P PRINTER

This command allows you to toggle among the supported printers including Epson FX, HP Laserjet, and other popular models. (If your model is not listed, use Pizazz Plus for screen dumps). Toggle 'P' until your printer is displayed.

Since so many modern printers have various emulation modes, you might not see the name, but the major type, of your printer listed. For instance, the Okidata Laserprinter which *OLIS* supplies for many installations uses the HP Laserjet driver.



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2D DISPLAY PARAMETERS

R REDUCTION MODE

Choosing this command provides you with a menu of options from which you can set the data reduction mode. The program enables you to set a new reduction mode before or after data collection. The option you select will be displayed on the Parameter Setup menu.

Set Data Reduction Mode	
A - Absorbance	T - %T
C - Concentration	V - Sample Voltage
E - Extinction	R - Ref Voltage

R

A ABSORBANCE

This mode is most commonly used with normal absorption spectroscopy. The computer calculates the Absorbance at any instant by:

$$\text{Absorbance} = -\log\left(\frac{\text{(Sample-Dark)}}{\text{(Reference-Dark)}}\right)$$

R → *A*

C CONCENTRATION

This mode allows you to input an extinction coefficient which causes the incoming absorbance data to be converted to concentration where the extinction coefficient is in liter/moles cm. This mode is not available for 3D display.

R → *C*

E EXTINCTION

Selection of this mode allows you to input a concentration value. Subsequent absorbance data will then be converted to extinction where concentration in is moles/liter. This mode is not available for 3D display.

R → *E*

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T %T

% Transmission is calculated as:

$$\%\text{Transmission} = \left(\frac{\text{(Sample-Dark)}}{\text{(Reference-Dark)}}\right) * 100$$

which is equivalent to $100/10^{A}$, where A is absorbance.

R → *T*

V SAMPLE VOLTAGE, REF VOLTAGE

R These modes report the raw signals. Use this mode to test new photomultiplier tubes or other hardware components or to see the uncorrected signal resulting from the whole optical system. This mode is recommended primarily for diagnostic purposes.

R → *V* *or* *R*

ADDITIONAL 2D DISPLAY PARAMETER OPTIONS

The following are strictly **display** options; changes are saved in the Parameter Setup but raw data are unaffected.

A AVERAGE/SKIP SCANS

Toggle 'A' to switch between Average and Skip Scans. If you choose to average, select the number of scans to average by changing the value of N, below. If you choose to skip, set the number of scans to skip with S, below.

A

```
A  Average/Skip Scans
S  # of Scans to Skip      = 13
N  # of Scans to Average   =  4
```

S # OF SCANS TO SKIP

Selection of this command provides a cursor at which you may indicate the number of scans to be skipped between the nine which are displayed on the screen.

S → *Input*

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N

OF SCANS AVERAGED

You must toggle 'A' to highlight "Average Scans" before you can input the number of scans to be averaged. Press Enter to set the value and choose the next command. Averaging is a means for improving the S/N ratio in your retained raw data. When scans are averaged, the "Number of Scans to Skip" is still valid. Note that the total number of scans to display is reduced by a factor of the number of scans averaged.

N → *Input*

3D Display Parameters

These commands allow you to set parameters for a new 3D file. To change parameters on an existing file, you must retrieve the file, make changes within the 3D menu and save it in the 3D program. Note that most of the reduction modes are interchangable, but once you switch from voltage and overwrite that file, you can not switch back.

3D Display Parameters

R	Reduction Mode		Absorbance
W	Display Mode		Wire-frame
Z	Scans to Skip		= 2
X	Axis Magnifications (x)		
	X_ 120	Y_ 90	Z_ 120
V	3D View Angles (degrees)		
	X_ -30	Y_ 24	Z_ -14

R

REDUCTION MODE

Selection of this command provides you with a menu of options from which you can set a new data reduction mode. See pages 19-20 for an explanation of these options.

R

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W DISPLAY MODE

This command allows you to choose your 3D display preference from six options: Wire-frame, Wire-frame², Surface, Wire-surface, Points, and Fat-Points. Toggle 'W' to cycle through the options until you reach the desired mode. The option you select is displayed in the Parameter Setup menu. To view the options as they are named, enter the 3D display. The display mode toggle is found at the bottom left corner of the 3D screen. By selecting "Auto R On" in the 3D Mode, the computer draws an example of each display option as it is selected. Consider the type of data you are dealing with and the type of printer you are using when making your selection.

W

Z SCANS TO SKIP

"Skipping" scans reduces the amount of data to be displayed to a convenient number for viewing without altering the raw scans in any way. Type in the number of scans to be skipped and press 'Enter.'

Z → Input

X AXIS MAGNIFICATIONS

This command allows you to set magnification percentages for the X, Y, and Z axes. Input the desired values when the cursor appears at each axis. Press 'Enter' after inputting the value for the Z-axis to move to the next command.

X → X or Y or Z

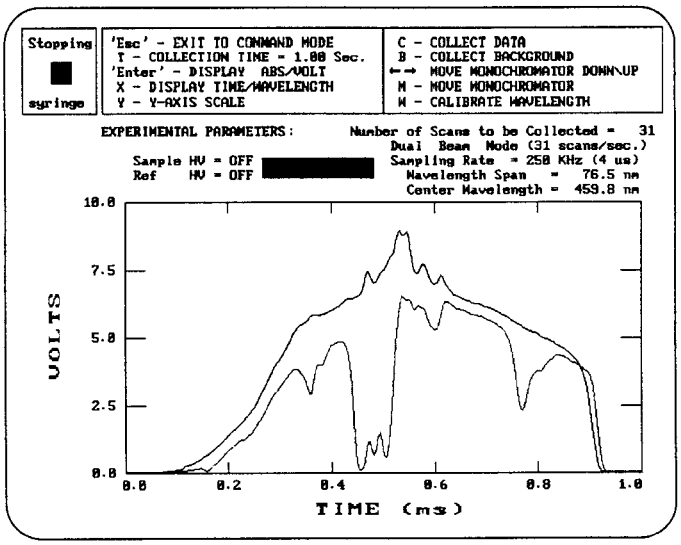
V 3-D VIEW ANGLES (DEGREE)

Using the same input process as above, enter desired degree values for X, Y, and Z axes for 3D view angles. Because there are no default values set by the program, the most recently entered values appear. There are no limits for the inputted values.

V → X or Y or Z

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3 LIVE DISPLAY



Live Display is where you can set up data acquisition parameters, observe incoming data, and collect data. This display is also referred to as "DVM Live," due to the intended similarity to a digital voltmeter live display.

If you are using the RSM with an OLIS U.S.A. stopped-flow and the trigger is enabled, the program activates safety interlock features. Among these features is a color-coded box in the upper-left of the menu screen. Green indicates that the stopping syringe is empty and you may proceed. Red indicates the syringe is full and must be emptied before the stopped-flow system can fire again.

CM → *V*

T

COLLECTION TIME

When you select this command, a box containing a cursor for inputting your data collection time appears. Please note the suggestion that appears to the left: **Choose the data collection time based on the time scale of the reactions that you are interested in.** Full speed data collection (1000 scans/second) fills approximately one megabyte of RAM per second, limiting most systems to seven to fifteen seconds of data. If the reaction time exceeds one second, you should choose one of the slower (data averaging) modes of collection in the Parameter Setup menu (see Chapter 2). Experiment with the system to determine the most effective time for data collection. If the reaction is exponential, you should collect data for four or five half-lives.

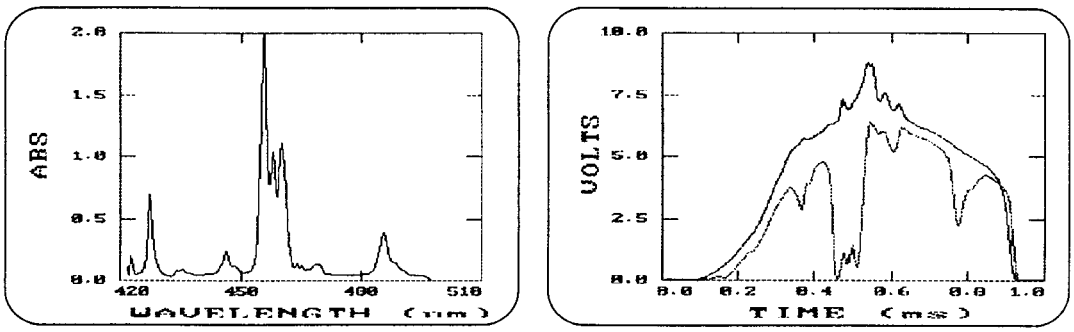
T → *Input*

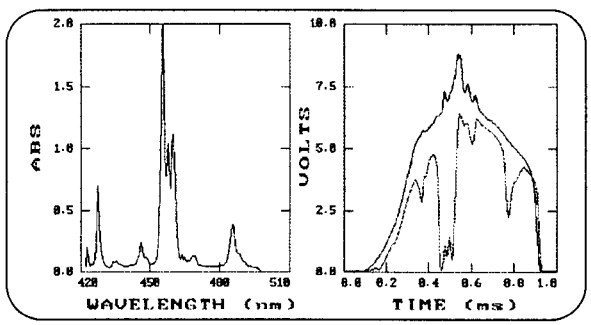
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ENTER DISPLAY ABS/VOLT

This command toggles the display screen among three display options: voltage, absorbance, or both. Toggle 'Enter' to obtain desired display.

Enter





X DISPLAY TIME/WAVELENGTH

This command toggles the X-axis between time and wavelength display.

X

Y Y-AXIS SCALE

Use this command to change the scale of the data displayed by entering minimum and maximum values for the Y-axis. Press 'Y' to replace the Live Display menu with a box containing a cursor at which to enter minimum and maximum values. Note that when both graphs are displayed on the screen, this option affects only the absorbance (or %T) display.

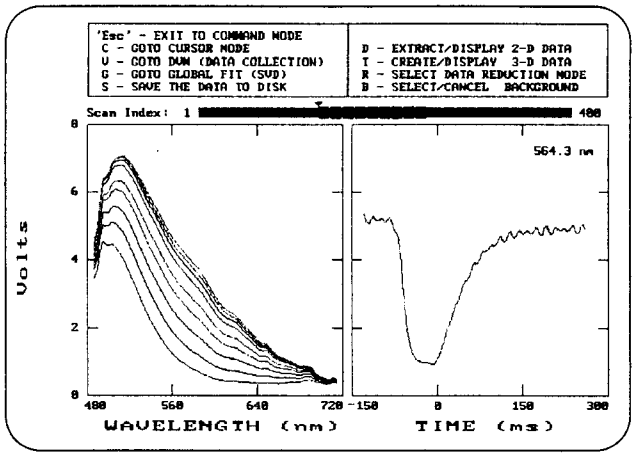
Y

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C

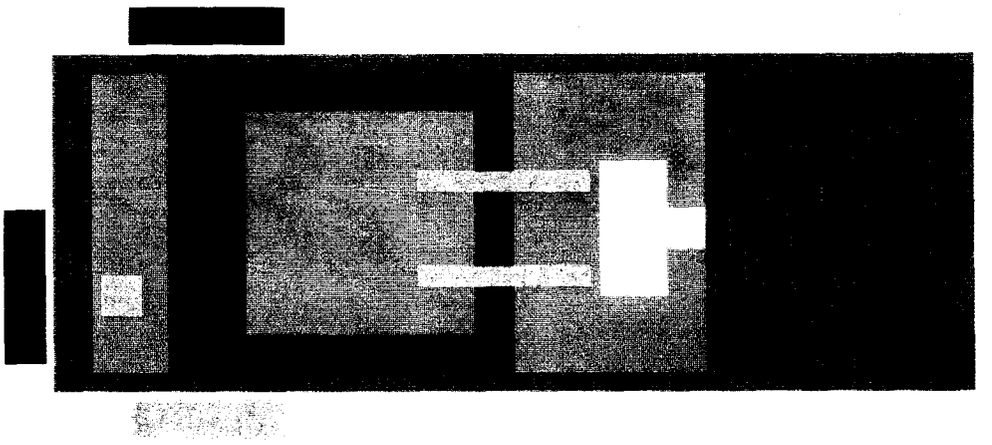
COLLECT DATA

Select this command to initiate data collection. A succession of messages will appear on screen, indicating the function being performed: "COLLECTING RAW DATA," "WRITE THE DATA TO DISK," and "DATA FILE O.K." Once data collection is completed, the program proceeds to the Data Display/Storage menu (see page 31) where data are extracted from the data file and displayed on the screen with scans in the left side box and a kinetic trace from one wavelength on the right side.



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If you are using the RSM with an OLIS U.S.A. stopped-flow, the program verifies that the stopped-flow settings are correct before firing is possible. This safety feature, referred to as the Interlock System, insures that your stopped-flow is triggered only when all components are positioned for fail-proof firing.



If the Interlock System detects a setting which is not optimized for firing when you press 'C,' the DVM screen clears and the program displays a diagram of the stopped-flow hardware. The colored display uses green bars to represent correctly positioned components and red bars for those which must be set to correct positions before the program will initiate firing. The program remains at this display until you have correctly positioned the incorrect setting(s). Once all settings are corrected, the program returns to the DVM screen, where you will be prompted to enter 'C' to initiate firing and data collection.

If the Interlock System *initially* confirms that all settings on the stopped-flow are correct, firing is initiated without further input.

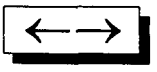
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SELECT/CANCEL BACKGROUND

This command toggles between 'collect' and 'cancel' background data. The background or 'baseline' data are used to correct for any differences between the sample and reference channels in the absence of a sample. Ideally, background correction results in 'zeroing' the absorbance signal. When "Collect Background" is displayed on the screen, press 'B' to initiate background data collection. The voltage reading is made from the sample (presumably empty) compartment. The cancel option is not available until the program finishes the data collection process. When "Cancel Background" appears, press 'B' to select this option. If you are working in the Single Beam Mode, this command appears as "Reference Cancel Background." You can collect another baseline or work without one, as appropriate.





MOVE MONOCHR UP/DOWN

Use the sideways arrow keys to move the monochromator up or down in increments that are set in the Parameter Setup Menu. The monochromator may not move in the exact increments that you have chosen; the program moves as close to the input value as possible (within a few tenths of a nanometer). By using this command to adjust the monochromator, you can move quickly to the desired wavelength. Use this option when you wish to move among wavelengths near the existing wavelength value. To make a large wavelength adjustment, use the next option, "Move Monochromator." View the effects your changes make on the "Center Wavelength" which is listed in the additional screen information box.



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M

MOVE MONOCHROMATOR

Use this command to move the gratings so that the wavelength span has a new center wavelength. After you press 'M,' the program provides a cursor at which you can enter the value (in nm) of your chosen wavelength setting. The program displays a "Please Wait" message while the monochromator adjusts to the new position. Use this option when you wish to make a large adjustment in the center wavelength. When you wish to move among values near the existing center wavelength, use the previous command, "Move Monochr Up/Down."

M

W

CALIBRATE WAVELENGTH

The RSM has an optical encoder device affixed to the grating movement system; this encoder produces a signal monitored by the system when the gratings are positioned at the *calibration wavelength.* When you select 'W,' a graph appears on the screen with a peak which represents the current calibration wavelength. On this graph, the most perfect calibration wavelength is represented when the peak is at zero.

The program provides a cursor at which you can enter a new calibration wavelength. Normally, you only need to adjust this value after changing gratings and periodically to confirm system accuracy. To confirm accuracy, find the calibration wavelength which centers the peak at zero on the graph, thus producing the correct wavelengh If the peak is not at zero, adjust the calibration wavelength according to the difference between the peak and zero. For example, if the peak is at 0.25 nm, enter "-0.25" as the new calibration wavelength in order to center the peak. After entering a new calibration wavelength, recalibrate and check the peak to confirm.

The peak should not be more than 10 nm from zero. If the difference between the peak and zero is more than 10 nm, the grating is probably not correctly installed. Open the trap door and confirm that the grating is seated securely.

Note that the program recalibrates the monochromator each time it starts-up.

W

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ADDITIONAL COMMANDS

The following commands *are not* listed in the Live Display menu but can be accessed at any time while data are displayed in this mode.

TAB This key toggles between **Sample HV** and **Ref HV**, which both appear beneath "Experimental Parameters." The selected option is highlighted. Use this command in conjunction with the arrow keys, below, for fine adjustment of either or both voltages, or use 'A,' below, for automatic adjustment of both voltages.

Tab

A Entering 'A' causes the program to adjust both HV automatically to the maximum photomultiplier voltage of 7.5. (This command can operate correctly only if the lamp is on and neither beam is blocked).

A

SPACE The space bar (or the center of Gateway cursor keys) toggles the HV on and off.

Space

↑↓ The photomultipler voltage can be raised or lowered in small increments using the up/down arrow keys, allowing you to override the selection of 'A,' above.

G DISPLAY WAVELENGTH RANGE

This command toggles among displays of the current, effective, and available wavelength range of the monochromator with the current gratings. This information appears above the graphs in three colored bars. The 'current' range measures the span from the center wavelength. The 'effective' range is the possible range of the current grating. The 'available' range is the possible range of the spectrophotometer which may require a change of gratings to actualize.

G

F FIRE STOPPED-FLOW

This command is used to clean the chamber, verify samples, or flush bubbles. This command fires the stopped-flow without storing the data. The Live Display reflects real-time changes in the sample chamber. Data are not captured or saved; use 'C' to collect stopped-flow or other data.

F

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Additional Screen Information

Number of Scans to be Collected = 3
Dual Beam Mode (31 scans/sec.)
Sampling Rate = 250 KHz (4μs)
Wavelength Span = 76.5 nm
Center Wavelength = 459.8 nm

This information is located on the Live Display screen throughout the mode, and is normally preset in the Parameter Setup (see Chapter 2). *Be certain these values are as you desire before beginning data collection.* Instructions for changing any of these values appear below:

Number of Scans to be Collected: This is the number of scans to be collected according to the designated collection time and mode, both of which are set from the Parameter Setup menu with 'T.'

Beam Mode: To change the beam mode, go to Parameter Setup and toggle 'D' to choose Single or Dual Beam Mode. 'Dual beam' means that a reference and sample signal are collected simultaneously; their ratio is used to compute true transmission/absorbance. 'Single beam' mode is used when no reference is needed, for example, when collecting uncorrected luminescence data.

Sampling Rate: This value is the current sampling rate. To change, go to Parameter Setup and toggle 'T' to choose a different sampling time. Usually, you will select 4 μs or a rate of 250 KHz. Usually, the higher sampling rate (400 or 500 KHz/ms) is not justified by the available resolution.

Wavelength Span: This is the wavelength span being scanned and is a function of the number of lines in the grating and the character of the ScanDisk. This is an empirical value which the program calculates based on the current grating.

Center Wavelength: Within the Live Display Menu, there are two commands for adjusting this value. Use 'M' to input any value, or use the left/right arrow keys to increase or decrease the center wavelength. The increases/decreases occur in increments you choose in the Parameter Setup menu. The center wavelength determines the position of the wavelength span within the available region.

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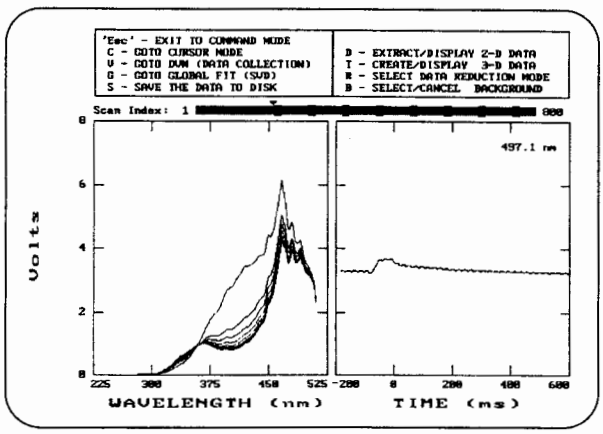
4 DISPLAY CONTROL

Display Control

E Data Display/Storage
H Hash Mark/Grid Settings
F Full Screen to Printer

The options listed under Display Control in the Command Mode allow you to customize the display of your data.

DATA DISPLAY/STORAGE



E DATA DISPLAY/STORAGE

Selection of this command brings you to the screen above. If data are in memory, messages appear on the screen informing you of the processes the computer is performing: "AVERAGING DATA," and "EXTRACTING KINETIC DATA." The data in memory are displayed, scaled according to the display parameters set in the Parameter Setup menu. If data are not in memory, the program can not proceed.

CM → E

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C

GO TO CURSOR MODE

This command takes the program to the Cursor Mode menu where you can inspect and study your data. See Chapter 5 for explanations of the commands in this mode.

C

V

GO TO DVM (DATA COLLECTION)

Selection of this command returns the program to DVM Live. See Chapter 3 for explanations of the commands in this mode.

V

G

GO TO GLOBAL FIT (SVD)

This command takes the program to the Global Fit module, where you can perform a fit on the data you are currently examining. The program creates a 3D file for the data (a screen message informs you of this--no 3D view is displayed) which will be used for the fit. See Chapter 7 for an in-depth explanation of the Global Fit procedure.

If the amount of data on which you want to perform a Global Fit is too large for the available memory, a message appears on the screen suggesting that you either average or cut your data to reduce the memory requirements. "Averaging" is explained on page 21; "cutting" is explained on page 44.

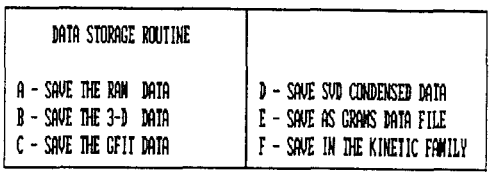
The Global Fit answers appear on the screen in place of the unfitted 2D scans when you exit this mode. These data can be saved using the "Save Data to Disk" command, below. To restore the original pre-fit data, press 'U,' which appears on the menu as "Restore Original Data."

G

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S

SAVE DATA TO DISK



Select this command to enter the data storage routine which provides six options for saving data: "Raw," "3D," "Gfit," "SVD condensed," "Grams data" or "kinetic family." See the Data Retrieve Routine, page 76?, for an explanation of how to retrieve files you have saved.

Raw Data

Raw data are data as they were collected before any mathematical or graphical manipulations were performed. Because the 'raw' form of the 3D matrices are extremely large (1 Mbyte) we recommend that you save data as 'raw' only when necessary.

The 'raw' file format is binary; the filename extension is *.raw.

3D Data

3D data are the data that are included in the 3D data file you created using the command "Extract 3D Data" (see page ??). All of the information necessary to reconstruct the 3D data file, exactly as it was saved, is included in this file. This information includes any averaging or fitting which was done. Like the 'raw' data file format, these are very large files so we recommend that you do not build multiple 3D files from a single experimental series without careful consideration.

3D format is binary; the filename extension is *.sc3.

Gfit (RGF) Data

RGF data files contains the answers, that is, the one, two, or three spectra and their associated kinetics returned at the completion of the global fit. You can not reconstruct raw data from these files, because none of the 'raw' information remains!

RGF files are ASCII for your convenience in reading them into other graphics software packages; the filename extension is *.gf.

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SVD Condensed Data

These files include all the information necessary to restore the spectra and kinetics of an experimental session. SVD condensed data retain all the information which is in the 'raw' format without the noise. As of February 1994, everything we have attempted in-house confirms that no useful data are lost in this condensation. These files are typically 40 fold smaller than 'raw' files. We strongly recommend this file storage option.

SVD Condensed format is binary; the filename extension is *.rdm (reduced data matrix).

Grams Data

Grams Data files are an example of *OLIS* file customization. This option was designed for one of our clients who wanted to retrieve the data from the RSM into a specific program. Upon request, *OLIS* will customize file formats to meet your particular needs.

You must supply a model for thr customized file format to be patterned after.

Kinetic Family Data

This file format allows you to store the displayed kinetic trace. Use this format if you collected data in the Single Wavelength data collection mode. Typically, Single Wavelength data collection is used when the reaction under study is too fast for the RSM scanning mode. By collecting multiple kinetic traces *over an equal wavelength interval*, (e.g., 320, 325, 330, etc.), a 'kinetic family' is built. RGF fitting treats this file exactly as a scan file. Currently, up to 28 traces can be saved as part of a family and displayed as a 'scan' series.

Kinetic Family files are ASCII for your convenience in reading them into other graphics software packages; the filename extension for each file is the wavelength of that file rounded to the nearest nanometer. The filename the program prompts for becomes the filename for each kinetic trace; the three letter extension is the wavelength of this family member. Thus, data stored with this option is "filename.320," "filename.325," etc. When you retrieve these records, the program picks up all the records with this filename, so do not use a name that is used for non-kinetic traces, e.g. "filename.new."



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D

EXTRACT/DISPLAY 2D DATA

This command allows you to extract and display specific scans for 2D examination. The Scan Index displays the total number of scans and highlights the nine displayed scans within the ensemble of scans at the end of the ruler. The carat above the ruler marks 'time zero' for stopped-flow records. The Scan Index bar is color-coordinated with the displayed scans.

To create a new 2D display, enter 'D,' and the program asks you to input the start scan to display and the number of scans you wish to skip. The number of scans to skip can also be set in the Parameter Setup (see page 20).



Thin color bars indicate that little or no averaging has been done.



Wide color bars indicate that multiple scans are averaged to result in the displayed scans.

These two example Scan Indexes illustrate parameters you can control when viewing data in 2D. In the first index, data collection started at scan #1; the carat is at time zero. In the second index, the carat indicates that time zero is now around scan #280, which shows that pre-trigger data were collected. Notice how thin the representative scans are in the first index compared to those in the second index which has the same number of total scans. The width of these representative scans corresponds to the amount of averaging which has been done. In the first index, the selected scans are centered in the collection time, about 280 to 500 scans into the series. The selected scans in the second index are dispersed from time zero to near the end of the data collection time.

Note that your selection of the numbers of scans to average, the number of scans to skip, and the scan at which to start, affect the 2D display only; raw data files remain intact and unaltered, so that you might access 'D' as many times necessary to create the most useful display for your purposes.

D

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T

CREATE/DISPLAY 3D DATA

Upon selection of this command, the program creates and displays a 3D data file on the screen. The program prompts you for the scan number for the display to begin and the number of scans you want displayed. If a 3D file already exists for the data with which you are working, the program states, "The 3D File Already Exists" and asks, "Do You Want to Create a New One?" Input of 'Y' or 'N' enables you to alter the existing file or create a new one.



R

SELECT DATA REDUCTION MODE

Selection of this command displays a menu which enables you to change the Reduction Mode without returning to the Parameter Setup Menu. Available modes include Absorbance, Concentration, %Transmission, Extinction, Sample Voltage, Reference Voltage, Fluorescence and/or CD, depending on your version of the hardware and software. This reduction mode does not affect data collection, but selects the manipulation to be made to the raw data for your visual presentation and analysis. Hardware specific options, such as Fluorescence or CD, should be selected only when appropriate.



B

SELECT/CANCEL BACKGROUND

This command is only applicable if you have previously collected background data using the command "Collect/Cancel Background" found in the Live Display menu (see page 27). Once collected, background data are a permanent part of the raw data file. You can display your data with or without correcting for background. "Background" is highlighted in yellow on the screen when background data have been collected. Cancel inclusion of the background data by pressing 'B'; "Background" is now not highlighted. This command only affects the display of the raw data file.



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HASH MARK/GRID SETTINGS

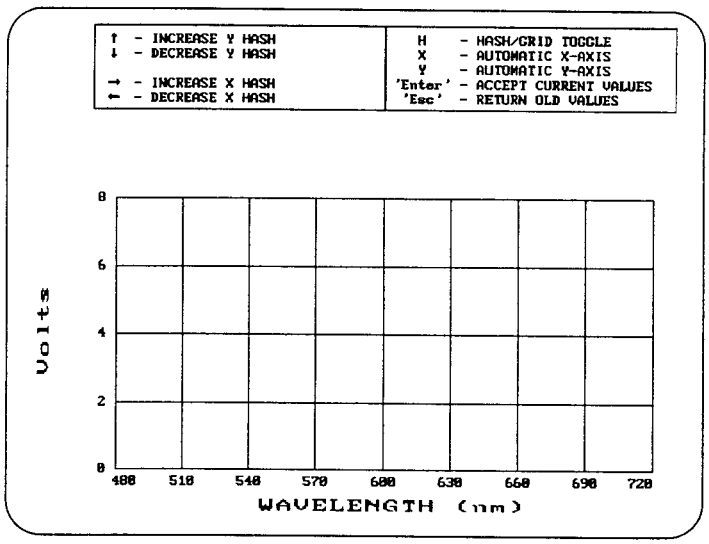


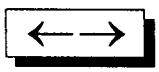
After selecting of 'H' from the Display Control menu in the Command Mode, you are presented with a series of options which allow you to adjust the number of subdivisions the program uses to display your data.

CM → H



INCREASE/
DECREASE Y HASH
The up/down arrow keys increase or decrease the number of hashmarks on the Y-axis. The program divides the range by the number of hashmarks to determine the best values for your graph. The numeric values at the hashmarks are selected by a special algorithm which forces 'rounded' values to provide a better display. Thus, the displayed range might vary depending upon the number of hashmarks, and using this option is the only way to 'force' a particular display range. For example, a graph might range from 300-600 nm when it has four or five hashmarks and 320-600 nm when it has six hashmarks.





INCREASE/DECREASE X HASH
As the above command, the left/right arrow keys increase or decrease the number of X-axis hashmarks.

←→



HASH/GRID TOGGLE
Press 'H' to toggle the graphs between hashmark and grid display.

H

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X AUTOMATIC X-AXIS

After you select 'X,' the program overrides any manual scaling, restoring the display so that all the data are presented. The Y-axis is not affected.

X

Y AUTOMATIC Y-AXIS

After you select 'Y,' the program scales the Y-axis so that all Y data are displayed. The X-axis scale is not altered.

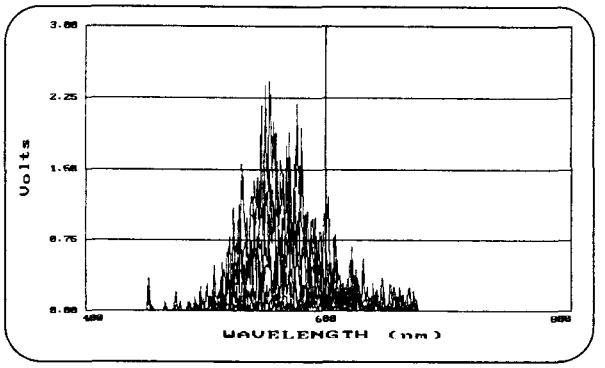
Y

Full Screen To Printer

F FULL SCREEN TO PRINTER

This command provides a printout of the current on-screen data without menus at the top of the screen.

CM → *F*

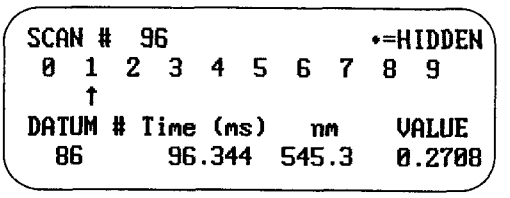


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5 CURSOR MODE

The Cursor Mode is where you can inspect and study your data using a tracking cursor to move within and among records. The boxes at the top of the screen contain numeric information about the datum on which the cursor rests.

The box in the upper left corner displays the information for the scan the cursor indicates. Note that the color of the numeric data corresponds to the color of the scan selected by the tracking cursor. The box in the upper right corner lists the available commands or functions.



Notice that the values are presented with different numbers of significant digits depending on the number of decimal places known for that value. For example, time is usually known to the microsecond, "nm" is presented to the finest step used by the monochromator, and OD values have one, two, three, or four significant digits, dependent upon the actual, known value.

Please familiarize yourself with cursor movement:

To move among scans in new or old data files, use the up and down arrow keys. The arrow in the numeric display in the upper left box indicates the scan on which the cursor rests. To move along an individual scan, use the right and left arrow keys.

You may set the step increments of the cursor (default is one) by typing in a digit from 1 to 9 at any time; the cursor steps that number of data points for each strike of a left/right arrow key. In this way the cursor can move rapidly or slowly. Return to single datum steps by entering a '1.' Move from one end of a scan to the other by 'wrapping around' - the left arrow key takes you from start to end, and the right arrow key takes you from end to start.

The cursor never floats on the screen. It is always on a datum. If the record is not visible beneath the cursor, the record has been hidden or erased. (Options 'H' and 'E' are explained below.)

CM → V → C → C or CM → E → C

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MENU SUBSETS

Toggling 'I' moves through the menus which contain the options available in the Cursor Mode. When the cursor rests in the scan box, there are four menus which 'I' toggles through; when the cursor rests in the kinetic box, there are three menus available. To move the cursor between the scan and kinetic boxes, toggle 'Tab.'

The two menu sets share a number of common options which differ only by labeling 'scan data' as **scans**, and 'kinetic traces' as **data**. For instance, when the cursor rests in the *scan* box, 'E/D' is listed as "Erase/Display Scan," and when the cursor rests in the *kinetic box*, the listing appears as "Erase/Display Data." If you can not find an option, the cursor is probably in the scan graph when the option is in the kinetic box menu or the cursor is in the kinetic graph when the option is in the scan box menu.

From within these sets of options, all are active; toggling 'I' to display the desired option is strictly optional.

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Scan and Kinetic Box Menus

Cursor in left scan box

SCAN MENU 1

E/D Erase/Display Scan
H Hide/Unhide Scan
S Swap Hidden/Unhidden Scan
F Save the Scan

SCAN MENU 2

C Crosshair
R ▭ Cursor
A Autoscale X/Y
X X-Axis Autoscale
Y Y-Axis Autoscale

SCAN MENU 3

W Cut Raw Data
B Little Boxes
L Enter/Edit Current Comment
M Data Manipulation Routines

SCAN MENU 4

K Extract Kinetic Trace
'Tab' Goto Kinetic Box
'Enter' Goto Big/Small Box
'Space' Show Scan Information

Cursor in right kinetic box

KINETIC MENU 1

E/D Erase/Display Data
H Hide/Unhide Data
F Save Kinetic Data

KINETIC MENU 2

C Crosshair
A Autoscale X/Y
X X-Axis Autoscale
Y Y-Axis Autoscale

KINETIC MENU 3

W Fit Kinetic Trace
'Tab' Goto Scan Box
'Enter' Goto Big/Small Box
'Space' Show Results of Fit

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SCAN BOX MENUS

Below are the options available when the cursor rests in the scan box. The options are contained in four menus which are cycled through with 'I.' Make sure the cursor rests in the scan box. To move the cursor to the scan graph from the kinetic graph, use the 'Tab' key.

Menu 1

'Esc'	Command Mode	**I**	Menu 1
E/D	Erase/Display Scan		
H	Hide/Unhide Scan		
S	Swap Hidden/Unhidden Scan		
F	Save the Scan		

E/D ERASE/DISPLAY SCAN

These commands instruct the program to erase or display the record (wavelength scan) indicated by the tracking cursor. 'E' erases individual records. Likewise, 'D' instructs the program to display the record indicated by the tracking cursor. Erasing data removes it from the screen temporarily. This function is often useful to verify identification of a scan on the screen; i.e., allowing you to identify a particular record or to isolate records for closer viewing and printouts.

I → *E* or *D*

H HIDE/UNHIDE SCAN

This command instructs the program to hide or unhide the record selected by the tracking cursor. Unlike data which are "erased," "hidden" data are transferred to the alternate display. "Hide/Unhide" is useful for grouping spectra together for analysis, storage, or printouts.

I → *H*

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S SWAP HIDDEN/UNHIDDEN SCAN

This command instructs the program to clear the current screen and display the alternate record set. The "alternate" record set is the collection of scans that have been *hidden* from the display. For example, if you start with memory slots 1-7, by hiding 1, 2, 4, and 6, you create a second display that contains records you removed from the first display. Thus, you have two independent images with scans 1, 2, 4, and 6 and with 3, 5, and 7, respectively. 'S' toggles between the two sets of scans.

I → S

F SAVE THE SCAN

This command allows you to save the scan indicated by the tracking cursor. You can save the scan in a two-column ASCII file, for which you must enter a filename extension, or, if you have performed a global fit on your data, you can save the data in a family of global fit results which can be used later with graphics programs. For a family of global fit results, the program saves a separate file for each species with the filename extension *.gf#, where # corresponds to the memory slot where each species resides. When you retrieve data, these records are restored to the same memory slots. Although saving as a family of global fit results might always be an available option, use it only if you have performed a global fit on the displayed data, to avoid future confusion.

A Save the Individual Trace as an ASCII File
B Save Global Fit Results as a Family of Files

To list the files in the current directory, press 'Enter.' The scan is stored in the active subdirectory. To save the scan to a floppy diskette, change the Data Directory to A: or B: before pressing 'F.'

I → F

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Menu 2

C CROSSHAIR

This command toggles between the display of a large or small tracking cursor on the graph. The large cursor is useful for relating the selected datum to the corresponding axis values.

I → C

'Esc'	Command Mode	**I**	Menu 2
C	Crosshair		
R	▭ Cursor		
A	Autoscale X/Y		
X	X-Axis Autoscale		
Y	Y-Axis Autoscale		

R RUBBER BAND (BOX) CURSOR

The option "▭ Cursor" is a graphic routine for selecting the horizontal and vertical scaling limits of your data. After you press 'R,' a small box appears within the graph. You can move this box, change its size, and change its proportions using the arrow and math symbol keys, as defined in the instruction boxes at the top of the screen. When the "rubber band" encloses the data of interest, press 'Enter.' The screen clears and redisplays with the new limits, as enclosed by the "rubber band." If you decide not to accept the new limits, prior to pressing 'Enter,' press 'Esc' to exit the routine. To return to the presentation of all the data in memory, use the "Autoscale X/Y" option, below.

I → R

A AUTOSCALE X/Y

This option returns X and Y-axis scaling to full-scale perspective, undoing any scaling changes made by the Rubber Band Cursor or X or Y-axis options, below.

I → A

X X-AXIS AUTOSCALE

This command allows you to input the upper and lower wavelengths of the X-axis scale. After pressing 'X,' you must input the upper and lower wavelengths, and then press 'Enter' to display the new X-axis scale. Press 'Esc' prior to 'Enter' to cancel the new limits.

I → X

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Y Y-AXIS AUTOSCALE

This command allows you to input the upper and lower wavelengths of the Y-axis scale. After pressing 'Y,' you must input the upper and lower wavelengths, and then press 'Enter' to display the new Y-axis scale. Press 'Esc' prior to 'Enter' to cancel the new limits.

I → Y

Menu 3

W CUT RAW DATA

This command allows you to "cut" sections of data, such as an unwanted spectral region, to achieve a representation of "good" data to fit. To "cut away" data, use the arrow keys to place the cursor at the starting point of the data you wish to keep. Press 'W,' and a vertical yellow line marks your chosen starting point. Place the cursor at the end point of the data selection and press 'W' a second time to frame the data you wish to keep. The computer recreates the file and extracts new kinetic data. The program uses the new "cut" data for display and fitting unless you press 'U - Restore Prior Data' which becomes available in the Data Display/Storage menu after you have cut data.

'Esc'	Command Mode	**I**	Menu 3
W	Cut Raw Data		
B	Little Boxes		
L	Enter/Edit Current Comment		
M	Data Manipulation Routines		

I → W

B LITTLE BOXES

You can view each of up to nine records in independently-scaled graphs. The nine records displayed are those in the nine memory slots. Any scaling adjustments you make with the Rubber Band Cursor will be retained for the Little Boxes display. Press 'Esc' to return to the Cursor Mode.

I → B

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L

ENTER/EDIT CURRENT COMMENT

This option allows you to enter notes (Comments) concerning the experimental variables to be saved with the data. Comments can be up to 47 characters long. When you enter the Comment Editor, begin typing, and your text will be displayed in the comment/information box. Use the arrow keys to move within your comment. Press 'Enter' to exit the Comment Editor and save your changes. Press 'Esc' prior to entering to exit the Comment Editor without saving your changes.

I → L

M

DATA MANIPULATION ROUTINES

The data manipulation routines are divided into single and dual operand routines. While we recommend you analyze your data in the 3D Global Fit Module, these routines are provided for routine 2D analysis and calculation of difference spectra.

I → M

Single Operand Manipulations:				Dual Operand Manipulations:	
L	Log	**S**	Smooth	+	Add Two Records
I	Invert	**D**	dA/dX	-	Subtract Two Records
C	Add a Constant			/	Divide Two Records
M	Multiply by a Constant			*	Multiply Two Records

Single Operand Manipulations

L

LOG

The program calculates the Logarithm of the 'source' and places the result in the 'destination,' where 'source' and 'destination' are memory slots. Switch between the calculation of the log base e (ln) and Log base 10 (log) by toggling 'L.' Notice you are also given the option of adding a numeric offset, useful whenever you need the logarithm of data otherwise inclusive of a zero. Finally, notice that Logs can be of the entire data set or of selected regions; use the brackets ("[,]") option to specify a given region of your set.

I → M → L

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I INVERT

The program inverts, i.e., takes the reciprocal of, a record (divide data into 1). Keep in mind that this is not the same as turning a record upside down.

I → M → I

C ADD A CONSTANT

This command allows you to add any positive or negative value to a record. The program requests the source, destination and the numeric constant to be added to the Y-axis.

I → M → C

M MULTIPLY BY A CONSTANT

After receiving the source and destination memory slot numbers, the program requests the constant by which you want the source multiplied.

I → M → M

S SMOOTH

The program calculates a least squares quartic smooth on the source and displays the resulting record in the destination slot. The window size can be from 5 to 25. This 1990 published algorithm by Gorry (*Anal. Chem.*, **1990**, *62*, 570) leaves *no* unsmoothed endpoints.

I → M → S

D DERIVATIVE dY/dX

The program calculates the derivative dY/d(nm) of a spectrum. After entering the source and destination, you will be prompted for the derivative order (1-4).

I → M → D

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Dual Operand Manipulations



ADD TWO RECORDS

The program adds the records you specify to each other; the result is put into the memory slot you specify.

I → M → +



SUBTRACT TWO RECORDS

The program subtracts one record from another and places the result into the current memory slot.

I → M → -



DIVIDE TWO RECORDS

The program divides one record by another and places the result into the current memory slot.

I → M → \



MULTIPLY TWO RECORDS

The program multiplies one record by another, and places the result into the current memory slot.

I → M → *

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Menu 4

'Esc'	Command Mode **I** Menu 4
K	Extract Kinetic Trace
'Tab'	Go to Kinetic Box
'Enter'	Go to Big/Small Box
'Space'	Show Scan Information

K EXTRACT KINETIC TRACE

Use this command to create a "conventional" kinetic trace from your ensemble of scans. After you press 'K,' the program provides a cursor at which you may enter the destination slot (1-3) for your kinetic data. The program then displays the kinetics of the wavelength specified by the position of the cursor. The new trace overwrites any trace which is currently in the destination slot you choose. Three memory slots are available for traces.

While we recommend that you use the Robust Global Fitting algorithms for all your kinetic analysis, we understand that you will be more comfortable accepting answers as correct if you use "conventional" methods with the new RGF, at least initially. Also, it is frequently easier to decide upon the kinetic model (one exponential, 2nd order, etc.) when fitting a "kinetic" trace. Certainly, it is reassuring to have double confirmation of the selection and accuracy of fit.

When choosing which rate constants to accept as "better," choose the results of RGF. Recall that RGF returns statistics to augment the rate constants and their respective errors. The single wavelength traces you create can be fitted with the exclusive OLIS SI-Fit algorithm (written by Dr. Iain Matheson, see Appendix A) which is available using option "W - Fit Kinetic Trace" in the kinetic box menu set (see page 52).

I → *K*

TAB GO TO KINETIC BOX

Press 'Tab' to toggle between scan and kinetic boxes. The majority of the commands are identical in both menus; indications are provided in this manual for those which are different.

I → *Tab*

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ENTER

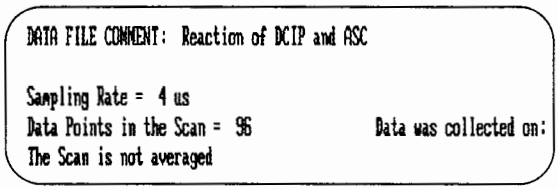
GO TO BIG/SMALL BOX

This command lets you toggle between viewing scans and kinetic trace graphs simultaneously or viewing an enlarged image of the scan box. Press 'Enter' to toggle between your choices. To toggle to a full screen kinetic trace box, have the cursor in the Kinetic Trace box when you press 'Enter.'

I → *Enter*

SPACE

SHOW SCAN INFORMATION



When the cursor is in the scan box, this command displays information on the sampling rate, data points in the scan, whether or not the scan has been averaged, and your comments. Press 'Space' to obtain this information. Press 'Space' a second time to return to the previous display. It is often appropriate to toggle to the Comment Display prior to making a screen dump to the printer.

I → *Space*

P

PEAK FINDER LABELER

This command is not listed in the Scan Box Menus but can be accessed when the cursor is in the scan box. After pressing 'P,' you must input the average counts and slope threshold before the program finds and labels peaks on a full screen display of the scan data.

I → *M* → *P*

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KINETIC BOX MENUS

Before you attempt to access these options on the screen, be certain that your cursor rests in the kinetic box. To move the cursor to the kinetic box, toggle the 'Tab' key.

For the options below which are not followed by a full description, refer to the option of the same name under Scan Box Menus.

Menu 1

E/D

ERASE/DISPLAY DATA
Erase or display the record (kinetic trace) indicated by the tracking cursor.
I → *E* or *D*

'Esc'	Command Mode	**I**	Menu 1
E/D	Erase/Display Data		
H	Hide/Unhide Data		
F	Save Kinetic Data		

H

HIDE/UNHIDE DATA
Hide or unhide the record indicated by tracking cursor.
I → *H*

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F

SAVE KINETIC DATA

This command allows you to save your single wavelength kinetic data. You can save the individual trace as a two-column ASCII file, for which you must enter a filename extension, or, if you have performed a global fit on your data, you can save it in a family of global fit results which can be used later with graphics programs.

For a family of global fit results, the program saves a separate file for each species with the filename extension *.gf#, where # corresponds to the memory slot where each species resides. When you retrieve data, these records are restored to the same memory slots. Although saving as a family of global fit results might always be an available option, use it only if you have previously performed a global fit.

To list the files in the current directory, press 'Enter.' The kinetic trace is stored in the active subdirectory. To save the kinetic trace to a floppy diskette, change the Data Directory to A: or B: before pressing 'F.'

I → *F*

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Menu 2

C CROSSHAIR
This input toggles between the display of the cursor as a small and a full-scale crosshair.
I → *C*

'Esc'	Command Mode	**I**	Menu 2
C	Crosshair		
A	Autoscale X/Y		
X	X Axis Autoscale		
Y	Y Axis Autoscale		

A AUTOSCALE X/Y
This option returns X and Y-axis scaling to full-scale perspective.
I → *A*

X X AXIS AUTOSCALE
This command allows you to input the upper and lower time for the X-axis scale.
I → *X*

Y Y AXIS AUTOSCALE
This command allows you to input the upper and lower range of the Y-axis scale.
I → *Y*

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Menu 3

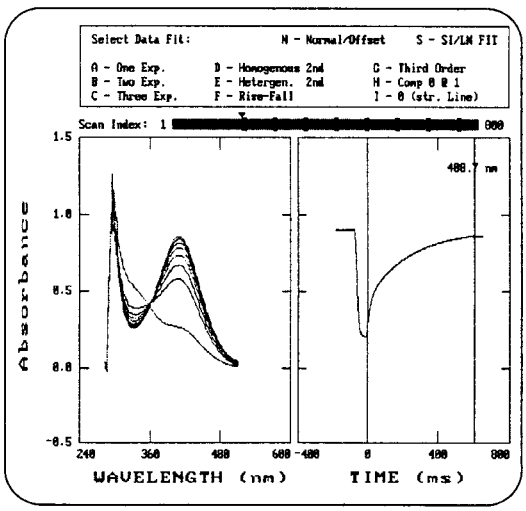
W

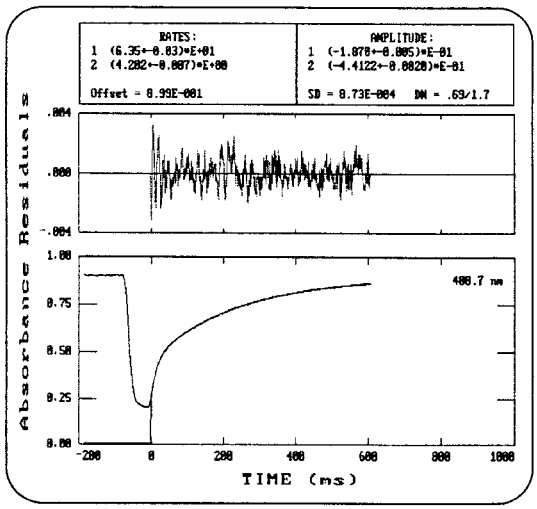
FIT KINETIC TRACE

'Esc'	Command Mode **I**	Menu 3
W	Fit Kinetic Trace	
'Tab'	Go To Scan Box	
'Enter'	Go To Big/Small Box	
'Space'	Show Results of Fit	

Use this command to fit a 2D trace with any of the available fit options. After the first 'W' input, a line appears which marks the start of the data in the record to be fit. The start is at time zero unless you force another starting point by moving the cursor along the kinetic trace before entering 'W.' With the start of the record marked, the program 'kicks' the cursor to the final datum on the trace. Accept this datum as the end of the reaction by pressing 'W' a second time, or move the cursor with the left arrow key to the preferred end point and then press 'W.'

After you indicate the region for fitting, the instruction box changes to list the supported fit models. Above the data fit menu, notice the options 'N' for Normal/Offset and 'S' for SI/LM FIT. Toggle 'N' to switch between fitting zeroing and non-zeroing data (data with or without offset). Toggle 'S' to switch between the exclusive *OLIS* method of of successive integration (SI) and the method of Levenburg-Marquardt (LM). We recommend you always use SI and trust its rate constant and amplitude information over values returned by LM. However, one can argue that using both is advisable for confirmation purposes.





The graphic information displayed after the fit includes the raw and fitted kinetic trace and the difference between the two (the residuals). The displayed information also includes rates, amplitude, standard deviation, and the Durbin/ Watson factor. With a good fit, no structure is discernable in the residuals, rather, the points are randomly distributed about zero. The DW ratio is ideally greater than one; see Appendix A for further details.

I → W

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TAB

GO TO SCAN BOX

This command toggles the cursor between the Scan Box and the Kinetic Box.

I → *Tab*

ENTER

GO TO BIG/SMALL BOX

This command toggles between viewing the scan and kinetic trace graphs simultaneously or viewing an enlarged image of the kinetic trace graph.

I → *Enter*

SPACE

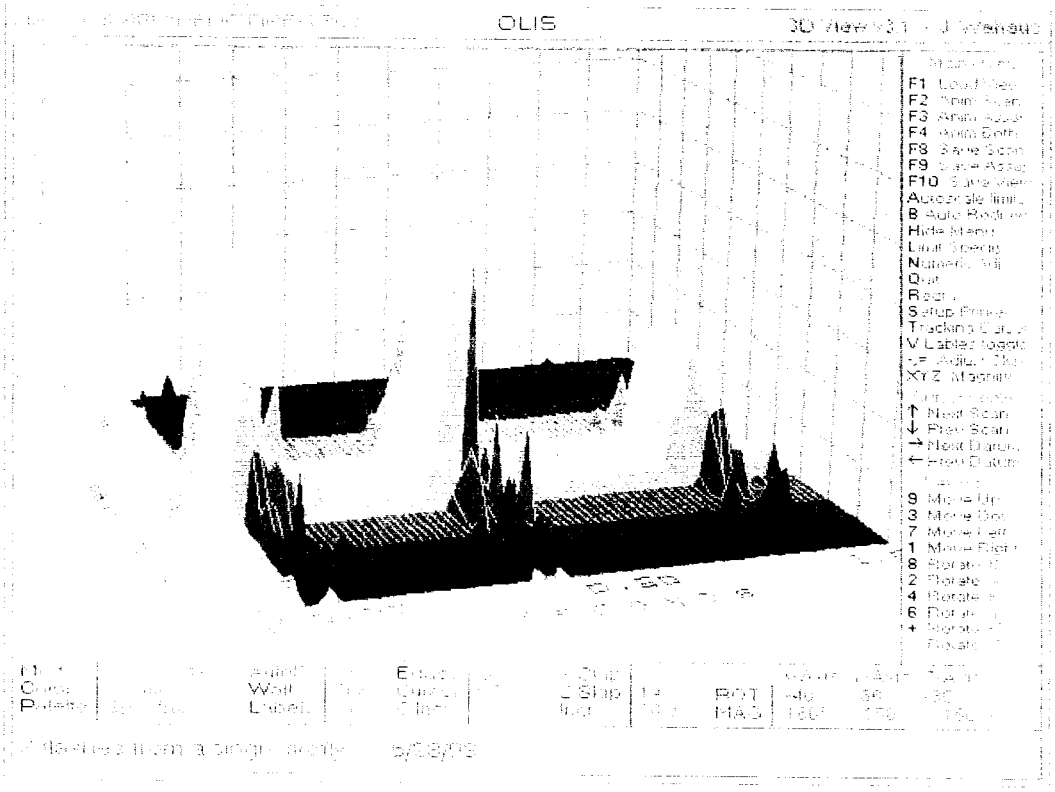
SHOW RESULTS OF FIT

This command provides graphs and numeric results of the last fit performed. The screen information reflects the type of fit you have chosen.

I → *Space*

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6 3D DATA DISPLAY



3D DATA DISPLAY

The 3D Data Display Mode is the mode for viewing all of the scans, up to 1000 in a data set. This mode contains its own specialized menu, wrapped around the right and bottom of the screen. You can choose commands that change the viewing perspective. As in the 2D Mode, a tracking cursor is available for moving through the data for careful scrutiny.

The bottom of the 3D View screen displays seven toggle commands which relate to the visual specifics of the 3D display. The additional information at the bottom of the screen relates to commands found on the Main Menu.

CM → E → T

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3D VIEW



Select 'T' from the Data Display/Storage menu to access the 3D Data Display Mode. The 3D display can include all your scans or selected ones. The program provides the opportunity of specifying which are included in the display immediately following the 'T' input.

To view the entire series of scans, type in a '1' for the first entry and press 'Enter' to accept the number the computer offers (the number of scans from the starting point to the ending point). To exclude some of the early scans from your 3D display, select a value deeper into the record; the second value the computer offers is the result of the total number minus those you skipped. That is, if you have 1000 scans and you want to view those from 200 on, the program returns 800 as the number of scans left to display. Once these values are entered, the screen displays the messages: "Creating 3D View Data" and "The Data File is O.K." These messages are presented to keep you informed that the computer is still active, since the steps can take several seconds. The 3D Data Display screen then appears, containing the data graph and menu. The input options and messages appear only when a new 3D file is being created. If you select 'T' after creating a 3D file, the program displays the message: "A 3D Data File Already Exists. Do You Wish to Create a New One? Y/N." If you input 'Y,' the program overwrites the existing 3D file with a new one. If you input 'N,' the program displays the existing 3D file.

The comment box at the bottom of the screen is useful for labelling your 3D data for displays and printouts. To change the text in the comment box, exit the 3D program by inputting 'Q.' Enter the Cursor Mode by inputting 'C,' then input 'L - Enter/Edit Comment' to change the comment. When you re-enter the 3D mode by selecting 'T,' you must recreate the data file for your new comment to be used.



Please familiarize yourself with the 3D menu commands, because they are unique and separate from other commands in the program.

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F1 LOAD VIEW
This command provides a cursor at the bottom of the screen at which you can enter the name of the previously saved data file you wish to view. We recommend only retrieving files from within the 3D program when you have saved files with parameters you have previously set in the 3D mode. If you are working with raw data files, we recommend that you retrieve your data using the Data Retrieve Routine in the File Control Menu (see page 78). Unlike the RSM program, the 3D program does not keep track of subdirectories and the likelihood of misplacing files is greater.
F1

F2 ANIM SCAN
This command causes highlighting of each scan in succession, along the absorbance or X-axis, "animating" the display.
F2

F3 ANIM ASSAY
As above, this command displays the continually tracking cursor as it animates the assay along the time, or Z-axis, display information.
F3

F4 ANIM BOTH
This command animates both scan and assay data simultaneously.
F4

F5 SELECT BACKGROUND
This command, which is not listed on the screen as of this printing, toggles the screen to a white background. The white background is provided so that you may create printouts for publications which require such a format.
F5

Main Menu
F1 Load View
F2 Anim Scan
F3 Anim Assay
F4 Anim Both
F8 Save Scan
F9 Save Assay
F10 Save View
Autoscale Limits
B Auto Redraw
Hide Menu
Limit Specify
Numeric Adj
Quit
Redraw
Setup Printer
Tracking Cursor
V Labels Toggle
-, = Adjust Skip
XYZ Magnify

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F8

SAVE SCAN

This command saves the 2D slice along the X-axis as a scan record.. Position the cursor on the wavelength you wish to save before pressing 'F8.' Upon executing the function, the program requires that you enter a file name, under which the program stores a two-column ASCII file containing the scan data. Keep in mind that the 3D program does not keep track of subdirectories.



F9

SAVE ASSAY

This command saves the 3D data as a 2D slice along the Z-axis, according to *time*, and performs exactly as the above "Save Scan" function. Position the cursor on a datum at the time of interest before pressing the 'F9' key. Keep in mind that the 3D program does not keep track of subdirectories.



F10

SAVE VIEW

Once you have designed a 3D graph with your preferred magnification, orientation, color selection, scan range, etc., you can use this command to store all display parameters with the data for later retrieval and viewing and/or printing. Keep in mind that the 3D program does not keep track of subdirectories.



A

AUTOSCALE LIMITS

This command displays the original limits of the X,Y, and Z axes on the displayed data graph after they have been changed using 'L' (see "Limit Specify," below).



B

AUTO REDRAW

This function toggles Auto Redraw on and off. When Auto Redraw is **On**, the program redraws the display whenever an adjustment to the 3D graph is made. When Auto Redraw is **Off**, the program only redraws the display when you press 'R' (see "Redraw," below). Turning "Auto R Off" allows you to make several adjustments to the graph without the program redrawing each time.



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H

HIDE MENU

This command displays the 3D data view without the wrap-around menu in order to obtain an optimal printout image. To return to the menu view, toggle 'H' a second time. The keystroke 'D' is also a toggle key to hide or display the menu on the screen. Notice that when you hide the menus the comment box remains at the bottom of the screen.



L

LIMIT SPECIFY

After pressing 'L' to access this command for setting display limits, type in X, Y, or Z to select the desired axis. Enter the low and high limits as prompted by the program.



N

NUMERIC ADJ

This command rounds the axes range for display purposes. For example, a scan with a wavelength range of 301 to 492 nm would appear as 300 to 500 nm on the screen. If this feature is disabled, the actual data range contained in the spectroscopic data file is used, with potentially awkward and undesirable values at the hashmarks, 301, 347, 393, etc., rather than 300, 350, 400, etc.



Q

QUIT

You are able to quit the 3D Data Display Mode at any point and return to the 2D Command Mode.



R

REDRAW

Erase and redraw data using this command to adjust the graph and clean up your screen image. Press any key to interrupt the redraw; the program immediately executes the command associated with the key which is pressed. The "Auto Redraw" function offers an automatic redraw option (see 'B,' above).



S

SETUP PRINTER

Though you can choose a printer through the 3D program, we recommend that select printers in the Parameter Setup in the RSM-program. Use Ctrl-P while within the 3D Display program to print screen dumps.



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TRACKING CURSOR

You can use the tracking cursor to move through the data for close inspection of values. By toggling 'T' you can choose from the four options: Point, Scan, Axis, and Off, which are displayed next to "Cursor" at the bottom of the screen.

Point selects an individual datum for inspection. Movement from that datum to the next is controlled by the sideways arrow keys.

Scan highlights an individual scan, and record movement is controlled by the up and down arrow keys.

Assay highlights an assay, and cursor movement is controlled with the sideways or up and down arrow keys.

Off removes the tracking cursor from the graph.

Step increments for the tracking cursor are indicated at the bottom of the screen next to "C Incr." The stepping movement can be 1, 3, 5, 7, and 10 steps with your input of the 1 through 5 keys on the keyboard (not the numeric pad).





LABELS TOGGLE

By toggling 'V' you can choose from the three options: On, Axis, and Off which are displayed next to "Labels" at the bottom of the screen.

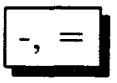
On identifies the time and wavelength axes with corresponding values.

Axis appropriately labels each axis as "X," "Y," or "Z," and includes the time and wavelength values without the corresponding labels.

Off displays your data and graph with no labels or information.



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ADJUST SKIP

These commands allow you to change the records skipped on the X-axis by toggling '-' (minus sign) and those on the Y-axis by toggling '=' (equals sign). The skips increase by *one* each time you press either the minus or equals sign. To reset to the default values (one and ten, respectively), you must use the 'Q' command to "Quit" the 3D view screen and then reselect 'T' from the Command Mode to return. You will not lose parameters that you have set since entering the 3D mode. In all cases but Adjust Skip, the most recent input serves as the default. The current skip adjustment values are presented at the lower right, labeled "X SKIP" and "Y SKIP."

-* or *=



MAGNIFY (AXES)

Magnify the 3D graph according to any axis by toggling the appropriate key (X, Y, or Z). Each keystroke magnifies the selected axis in increments of ten. By manipulating the various axes, you can view your graph from several perspectives. The axes values and magnifications are displayed in the lower right corner of the screen. Press 'shift' and 'X,' 'Y,' or 'Z' to unmagnify an axis.

X* or *Y* or *Z

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KEY PAD CHANGE VIEW

This set of commands is listed under "Key Pad Change View." Using the numeric keypad, move the 3D data and graph within the viewing box, rotate the data according to different axes, and zoom the view in or out. You have the opportunity to view your data from various perspectives, arriving at a view best-suited to your purposes. The numeric lock option on the keyboard can be on or off.

Key Pad	
9	Move Up
3	Move Down
7	Move Left
1	Move Right
8	Rotate +X
2	Rotate -X
4	Rotate +Y
6	Rotate -Y
+	Rotate +Z
-	Rotate -Z

The first four commands allow you to move the graph up, down, left, or right within the viewing box on the 3D screen. For easy reference, the **Move Up** and **Move Down** commands are accessed by keys 9 and 3, which are labeled PgUp and PgDn on the numeric key pad. These commands are useful if you have magnified an axis larger than the screen allows (using the "XYZ Magnify" option). You can position the graph on the screen so the scaling and labeling information is visible or hidden. If you require a move of more than one or two keystrokes, be certain that the Auto R option is Off, in order to avoid the tedious redrawing of the graph at each move increment. Check your progress by using the "Redraw" function to redraw at any point.

The second set of six commands allow you to rotate your data on the graph according to each of the three axes so you can find a perspective that is best for your purposes. Experiment with these options to take advantage of the possibilities. When you create the satisfactory perspective, note the parameters to document your preferences. Graphical parameters can not be saved independently of data.

The next set of commands (**Zoom In** and **Zoom Out**) magnifies and retracts your view of the graph. Each keystroke increases or decreases the zoom. These functions are useful for examining your data in close detail.

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LOWER SCREEN COMMANDS

Mode	Wire Surface	AutoR	ON	**E**dges	ON
Color	X Axis	**W**alls	FLB	Cursor	FLB
Palette	Spectrum	Labels	OFF	C Incr	OFF

The following commands are in a horizontal menu at the bottom of the screen. Each is a toggle command that affects the appearance of the 3D data. The first letter of each command serves as the toggle key. This box also shows the current settings for "Auto Redraw," "Labels," "Cursor," and "Cursor Increase," which can be changed by pressing 'B,' 'V,' 'T,' and '1-5,' respectively.



MODE

You can choose a preferred display mode for your 3D data from among six options: Wire Frame, Wire Frame^2, Surface, Wire Surface, Plot Points, and Fat Points. Press 'M' until the mode you wish to use is displayed. If the "Auto Redraw" (see 'B' above) command is on, the program draws the entire data set according to the various display modes as each is selected. Note that redrawing these enormous data sets can take several seconds. As you become familiar with the options, you may not wish to see each displayed as you make your selection. To save time, toggle "Auto Redraw" to off before making several or more changes which would otherwise force a redraw. Press 'M' repeatedly to move to the desired display—the display is interrupted with each keystroke. We recommend using Surface or Wire Surface for printouts.





COLOR

Control the color of the display data according to the axes. Press 'C' until the option you desire is displayed: X-axis, Y-axis, Z-axis, and Monochrome. We suggest using Monochrome for B/W printers.



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P

PALETTE

You can choose from among six color options for your display palette. Press 'P' to display the choices: Spectrum, Green to Red, Blue to Red, Blue to Green, Gray Scale, and Random. Consider the type of data you are examining and the type of printer you are using when making your choice.



W

WALLS

View your data with or without any or all of the graph walls. You may view your data with only its axes as guides, or you may view it with your selection of walls. Toggling 'W' displays your options (**F**loor, **L**eft, and **B**ack): **F, L, FL, B, FB, LB**, and **FLB.** Toggling to a blank space displays your data with no walls present.



E

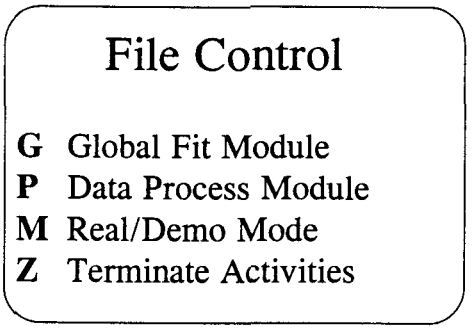
EDGES

You can display connecting lines which drop from the edges of the data to the graph, making it easier to gain the proper perspective on the data. The lines indicate the location of the data on the X-axis, and are available to ease your orientation with the 3D display view. You can place the edges on or off by toggling 'E.'



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7 PROGRAM CONTROL



The options listed under Program Control in the Command Mode allow you to access different programs within the *OLIS-RSM* operating system. These programs include Robust Global Fitting and the Data Process Module.

GLOBAL FIT MODULE

G

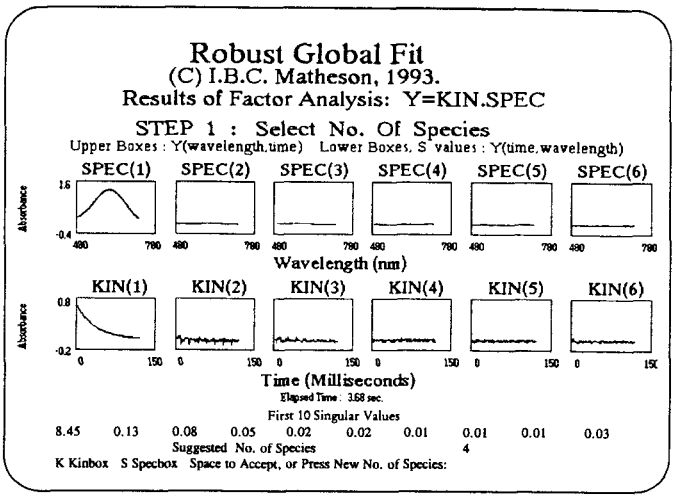
The Global Fit Module is a sophisticated data analysis scheme which allows you to examine the kinetics of all points of a given data set. Normally, when you use a rapid-scanning monochromator, absorbances are collected at hundreds of wavelengths and thousands of time increments, potentially producing hundreds of thousands of data points. The sheer amount of data necessitates an analysis system which can handle this enormous volume in a reasonable length of time using currently available computers. The *OLIS* fitting procedure used enlists the techniques of Factor Analysis and Global Analysis.

Factor Analysis takes a data set comprised of an unknown number of factors and separates out the minimum number of factors required to describe and reconstruct this data set. Global Analysis links the fitting of rate constants over the number of factors. The *OLIS* program uses a proprietory factor analysis algorithm which is two orders of magnitude faster and mathematically more robust than the normal SVD algorithm. Finally, when the kinetics have been determined, the spectra are obtained by a reconstruction process.

CM → G

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Select Species



When you select 'G,' a screen similiar to the one at the right appears. The program has already performed factor analysis to estimate the number of species present in the data. When data are collected with the rapid-scanning monochromator, where spectra vary with time, factor analysis produces the minimum number of spectra and decay processes needed to describe the original data. The first six wavelength factors are displayed in boxes labeled SPEC and the first six kinetic factors are displayed in boxes labeled KIN. Note that while boxes for six species are displayed on this screen, Robust Global Fitting currently supports fits for up to four species. The program displays likely species in color and noise conributions in gray.

First 10 Singular Values

8.45 0.13 0.08 0.05 0.02 0.02 0.01 0.01 0.01 0.03

Suggested No. of Species : 4

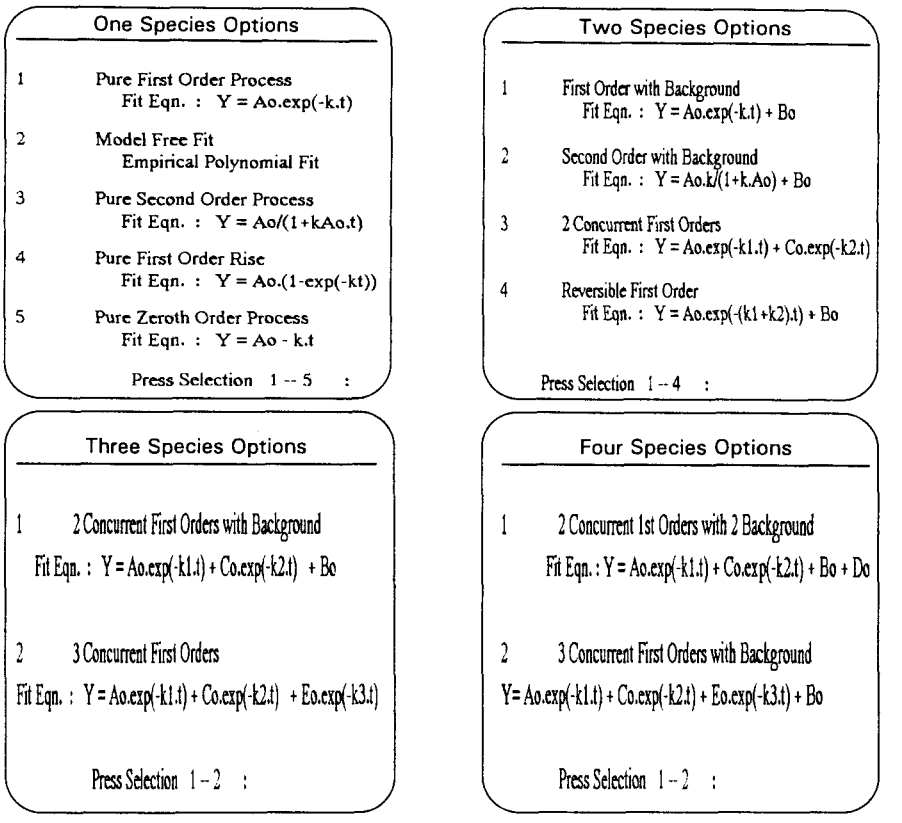
Based on the curvature of the displayed singular values, the program suggests the number of (colored) species necessary to describe the data set. However, you are free to make your own estimate by looking at the kinetics and spectra boxes and also by consulting the "First 10 Singular Values" listed at the bottom of the screen. By comparing these values to each other and to values for noise listed near the end, you can surmise the likely number of species present. Press 'K' or 'S' to view all the kinetic or spectral data on one graph. You can switch between the two enlarged graphs, or press 'Esc' and then 'R - Rerun Same Reduced Data' to review the first screen.

After you determine how many spectrally active species you believe to be present, either press the Space bar to accept the value the program suggests or enter a different value. Note that the algorithm seems to be consistant in over-estimating the number of species by one or more.

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Select Kinetic Fit

Depending on the number of species you decide are present, the program will display a set of kinetic fit models. Select the process which you believe best defines your data. We use a robust variation of the non-linear global analysis originally written by Levenberg-Marquardt and expanded upon by Dr. Matheson which minimizes the absolute deviation and thereby reduces the effects of assymetrically distributed deviations on the return of rate constants.



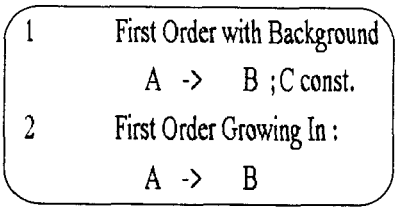
If you choose a process that does not readily fit the data, the program informs you that an error has been encountered at the successive integration level and a successful fit is unlikely. If you press 'P' after this message, the program forces the fit. If the program is still unable to fit the data after you press 'P,' the error message will appear again. Note that this error message might occur if the data are particulary noisy.

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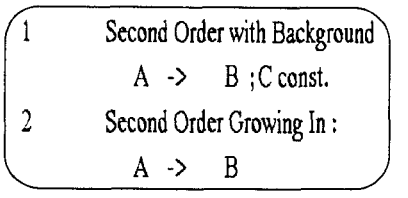
Select Mechanism (for Reconstruction)

When there is more than one reconstruction for the kinetic process you choose for your data, the next screen displays the available options. Notice that the program uses color to represent colored species and gray tones to indicate a bleached or non-colored species. For example, in the listing for a First Order with Background mechanism, A is blue, B is gray, and C is green, indicating that 'B' is the bleached species.

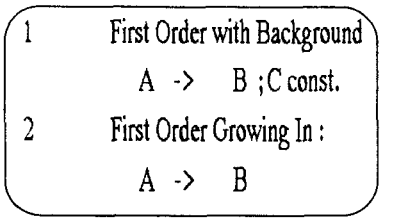
When you have two species and select a First Order equation with Background, the program offers the options on the right.



When you have two species and select a Second Order equation with Background, the program offers these options.

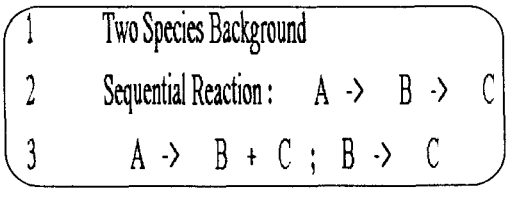


When you have two species and select Two Concurrent First Orders, the program offers these options.



When you have two species and select a Reversible First Order equation, the program asks you to input an equilibrium constant.

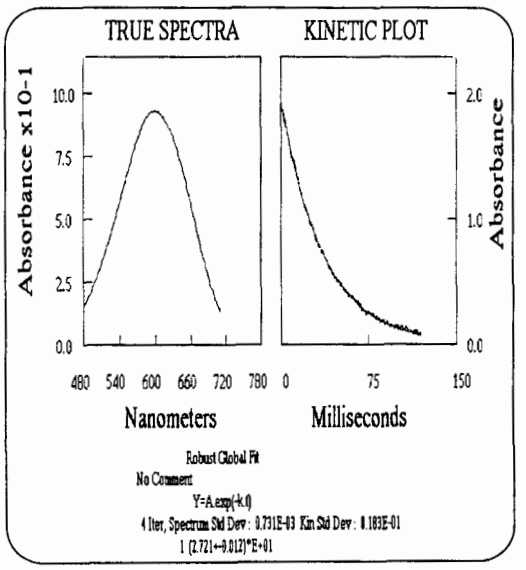
When you have three species and select Two Concurrent First Orders with Background, the program offers the options on the right.

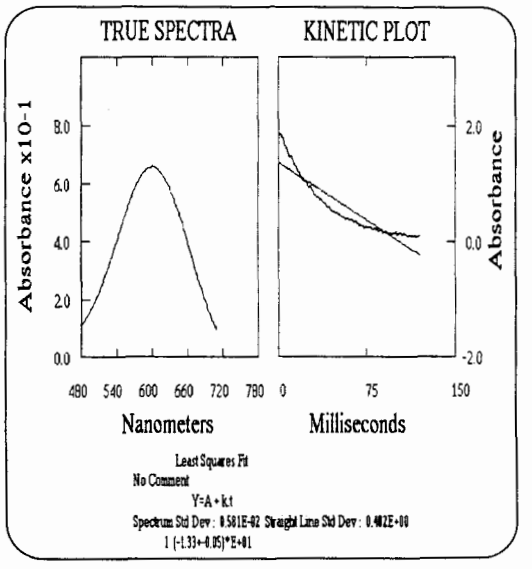


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Kinetic Fit

After you have selected the desired kinetic process and mechanism, the program fits the model globally, linking the rate constants over the chosen number of species. Because the number of species is typically small (much less than the number of wavelengths), the reduction in computation time due to factor analysis is great. The fitting is done robustly, minimizing the sum of absolute deviations rather than the sum of the squares. This process confers a resistance to the malign effects of the occasional outside point(s), also referred to as "outliers."

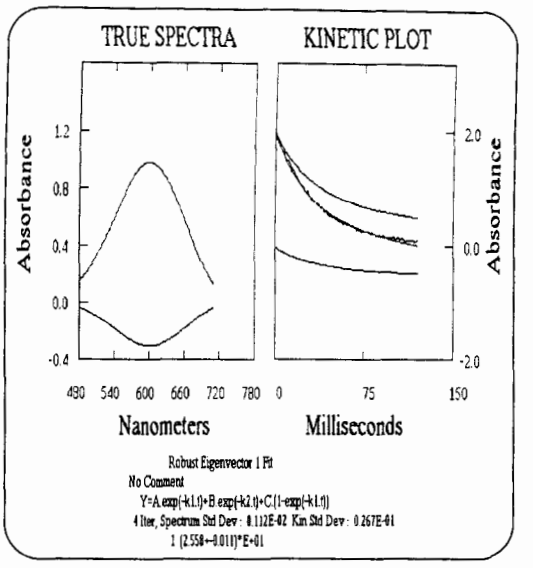




The program plots two graphs, one for the spectrum or spectra and one for the kinetics. The spectrum or spectra shown are those reconstructed by the software on the basis on the number of species changing and the kinetic mechanism chosen. On the Kinetic Plot, the red line represents the theoretical data while the yellow represents the reduced data. A kinetic fit is good if these two lines overlap each other, as in the graph on the left. This successful fit can also be seen in the statistics below the graphs. The graph on the right is an example of an unsuccessful kinetic fit; the theoretical and reduced data do not match since the wrong mechanism was chosen.

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Perfect kinetic fits do not necessarily provide you with unique spectra since kinetic data can often be fitted equally well with more than one mechanism. However, true spectra can be constructed only if the correct mechanism is used; usually only two choices are available. Anomolous spectra, such as negative ones, cannot exist, and if the fit produces such a spectrum, the mechanism must be incorrect. True spectra are only obtainable when you select the 'correct' mechanism. By selecting the 'wrong' mechanism, you obtain (possibly) negative spectra.



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Other Options

After the Global Fit results are shown, the program displays various options for refitting, reanalyzing or reviewing your data. The following pages provide information about these options.

R	Rerun Same Reduced Data
C	Reconstruct Same Kin. Fit
P	Replot Last Fit
D	Distribution Analyses
3	3D Plot
J	Joint Confidence Plot
G	Change Program Parameters
Q	Quit Program

Press Selection R, C, P, D, 3, J, G, Q

R

RERUN SAME REDUCED DATA

This command returns the program to the opening screen of Robust Global Fitting which displays the wavelength and kinetic eigenvectors which help you determine the number of species present.

R

C

RECONSTRUCT SAME KIN. FIT

This command allows you to select a new mechanism for the most recently fitted data. If your data only contains one species, the program returns to the most recently displayed answers.

C

P

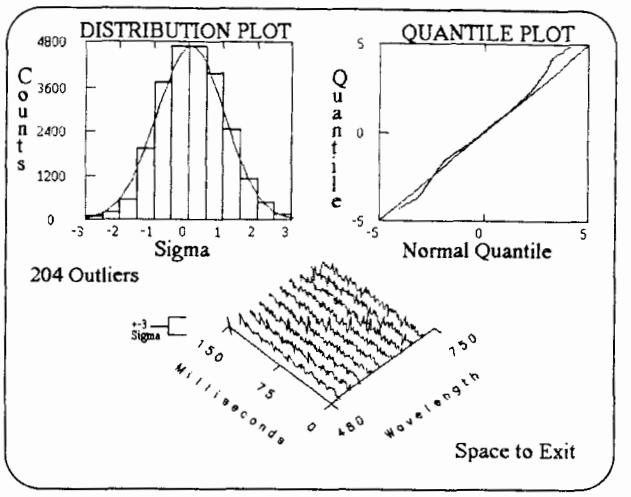
REPLOT LAST FIT

When you select this command, the program prompts you to enter 'S' to view a full-screen spectral graph, 'K' to view a full screen kinetic graph, or 'Space' to view both graphs simultaneously.

P

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D DISTRIBUTION ANALYSIS



Selection of this command brings the program to a screen which displays the fit results. The distribution plot consists of a histogram of the residuals in bins of 0.25 standard deviation width ranging from -3 sigma to +3 sigma. The number of outlying points is displayed beneath this plot ("204 Outliers"). The Gaussian curve is for display purposes; the bar graph is a representative of the noise distribution of your raw data. Many times, you will find that the noise is *not* random (i.e., Gaussian). Hence, had you fitted these non-Gaussian records using least squares methodology, your answer would be farther from correct than with our Robust methodology.

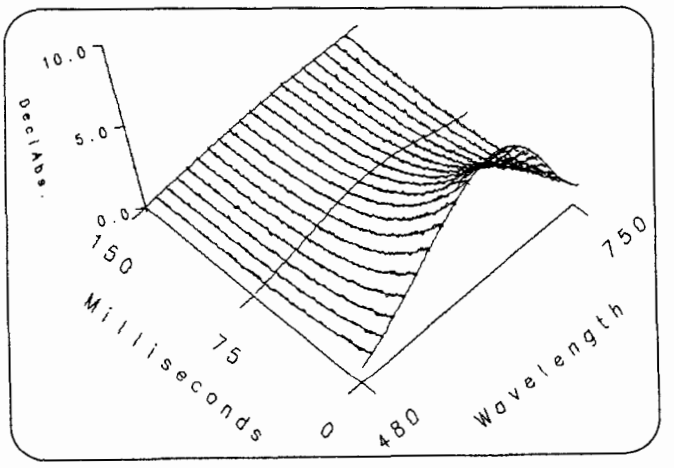
The second plot on this screen is a quantile/quantile plot which shows the sorted residuals plotted against a theoretical sequence. Normally distributed residuals produce a line with a slope of 1. Non-normally distributed residuals are indicated by points which are not on this 45 degree line.

The plot at the bottom of the screen is a 3D plot which shows the difference between the experimental data and the fitted spectra. A good fit produces a relatively flat display. A poor fit produces a shape which varies with time and/or wavelength.

D

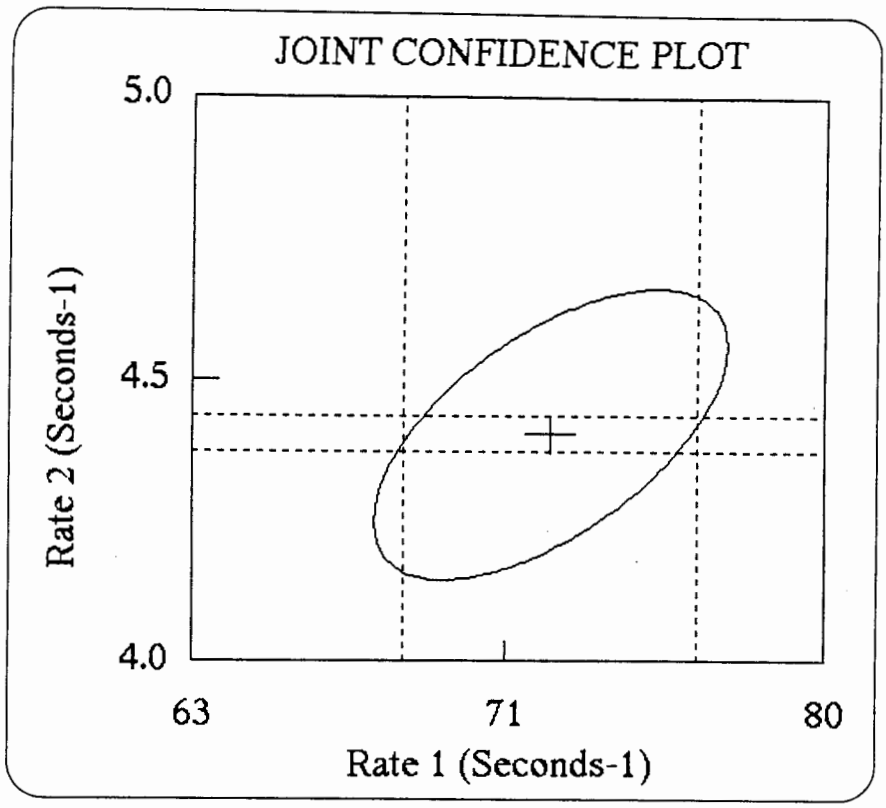
3 3D PLOT

This command allows you to view a 3D plot of the fitted spectra and several experimental curves as they change over time.



3

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J

JOINT CONFIDENCE PLOT

This option is available after only after global fits to data with two rate constants. The joint confidence plot is used to indicate the degree of confidence you can place upon the two rate constants. The enclosed region on the plot represents the approximate area of confidence for the two rate constants; the smaller the enclosed area, the smaller the errors that apply jointly to the rate constants. The statistically acceptable range of values for the two rate constants lie within the enclosed region. Any combination of the two rate constants which occurs within this region has an equal probability (95%) of being correct. The red lines which cross the region horizontally and vertically represent the standard deviation for the corresponding rate; the closer together the lines are, the more confidence you can place on the corresponding rate. Note the range of values for each rate when determining the confidence you place on that rate. For example, the enclosed region might range from 65 to 82 seconds^{-1} for Rate 1 and 4.25 to 4.6 seconds^{-1} for Rate 2.



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G CHANGE PROGRAM PARAMETERS

These options allow you to change some aspects of the calculation and display of the Global Fit results. You should not need to change these parameters often. The lowercase words in (some) of the command descriptions identify the active status of the toggles. To activate capitalized display options, enter the single keystroke command.

G

```
A    Select DIFFERENTIAL/True Spectra
B    Select EIGENVECTOR 1/Global Fits
C    DO NOT SHOW/Show Fit Components
D    Select STD DEV/Abs Dev Rate Errors
E    SHOW/Do not show Matrix Error Msg
G    Change Polynomial Degree (MFF)
H    Change Colors
P    Change Printer
R    Select ASCII/Binary Reduced Data File

Press Selection  A, B, C, D, E, G, H, P, R:
```

A SELECT DIFFERENTIAL/TRUE SPECTRA

This command switches between display of the differential and true spectra. When you choose to view differential spectra, the program will display a reference line at zero. True spectra are displayed exactly as they were collected; negative true spectra cannot exist. Difference spectra are the difference between two spectra and can be negative.

G → A

B SELECT EIGENVECTOR 1/GLOBAL FIT

This command toggles between two different ways to fit your data. The global fit is the normal fit and fits all the factors. The eigenvector 1 fit only fits the first factor or eigenvector; this fit is useful for records with very high noise.

G → B

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C

DO NOT SHOW/SHOW FIT COMPONENTS

This command toggles between displaying the components of the global fit and just the results of the fit on kinetic plot. The components of the fit are colored lines which correspond to the spectral data.

G → *C*

D

SELECT STD DEVIATION/ABS DEV RATE ERRORS

This command toggles between displaying the standard or absolute deviation error for the kinetic rate. This calculated rate error appears beneath the spectral and kinetic plots. The program uses the absolute deviation in fitting and calculates the standard deviation which you can display, as desired.

G → *D*

E

SHOW/DO NOT SHOW MATRIX ERROR MSG

This command toggles the program to display or not to display an error message when an error is encountered at a matrix solution step. If selected, matrix errors will be flagged. When you press Return, the program outputs the rate constants obtained prior to fitting. Note that these rates may not be fully optimized.

G → *E*

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G

CHANGE POLYNOMIAL DEGREE (MFF)

This option is provided for those cases where the kinetic model is not known, and therefore spectra based on a supported model are not possible. By using a polynomial equation to approximate the kinetic "shape" or behavior of the data, we can provide at least some indication of the spectra involved. Most cases have a polynomial degree of 4 or 5; use this option to change the value to any value between 1 and 9. There are no fast rules for this fit procedure; rather, we encourage you to experiment.

G → G

H

CHANGE COLORS

This command allows you to change the displayed color of different components of the Global fit program. After you select a component, i.e, text or background, the program presents a list from which you can choose a new color.

G → H

P

CHANGE PRINTER

Although this command allows you to select a printer, the printer you have chosen from within the RSM program in the Parameter Setup will override any selection you make from within the Global Fit program. Ctrl-P can be used at any point within the Global Fit to make a screen dump.

G → P

R

SELECT ASCII/BINARY REDUCED DATA FILE

This command allows you to choose between the program storing smooth spectral and kinetic data in ASCII or Binary reduced files. As of this date (3/94), this option is not yet active.

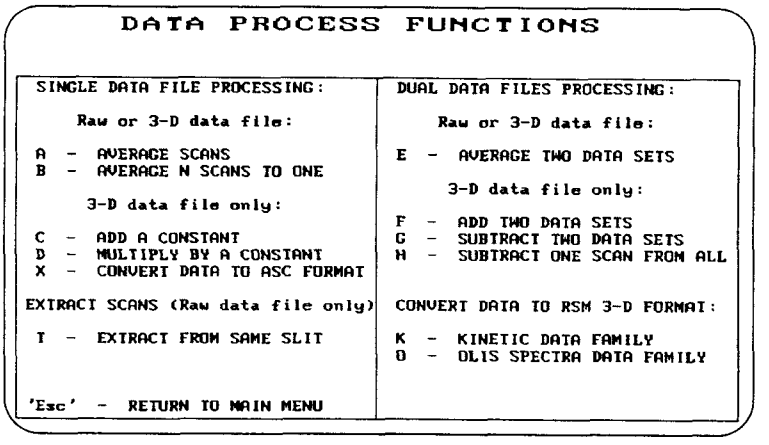
G → R

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P

This module supports various data processing options for raw and 3D data files. The available options are listed in the menu. The data are read from Input Data Files, processed, and written into the Output Data Files. The Input Data File is not changed. Note the division into routines to process one data set only and routines which require specification of two data sets.

CM → P



A

AVERAGE SCANS

This command reduces the number of scans in a file by averaging. After you select this option, a cursor appears for you to type in the Input Data File Name; you can press 'Enter' to list the data files. As protection against you permanently changing your raw data, the program will not fit the data you just collected until you enter a filename, saving the data to disk. When you enter the Input Data File Name, a second cursor appears for you to type in the complete Output File Name, including the extension. The program tells you the number of scans in the Input Data File before it asks you to enter the number of scans to be averaged.

This command is often used to reduce the number of scans in a data set so that Robust Global Fitting routines can be used. For example, 10,000 scans can not be fitted by Robust Global Fitting, but using this option, these scans can be averaged down to 1000, which can be fitted. 'Averaging' results in data with better S/N than the raw file, since the maxim "S/N improves as the square root of the number of data fitted" applies.

A

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B

AVERAGE N SCANS TO ONE

This command averages a given number of scans in a file to produce one resulting scan. After you enter the Input Data File Name and the Output Data File Name, the program asks you to specify the region of scans to be reduced. The program tells you how many scans are in the Input Data File. This command is usually appropriate when the sample was not changing during data collection. S/N improves as a function of the square root of the number of scans averaged.

B

C

ADD A CONSTANT

Only for use with 3D files, this command allows you to alter the data by adding a constant to all data points on the Y-axis. After entering the Input and Output File Names, the program asks you to enter the constant to be added. This value can be positive or negative.

C

D

MULTIPLY BY A CONSTANT

Also only for use with 3D files, this command allows you to multiply all data points on the Y-axis by a given constant. After entering the Input and Output File Names, the program asks you to enter the constant to be multiplied. This value can be positive or negative.

D

X

CONVERT DATA TO ASC FORMAT

This command allows you to convert 3D files (*.sc3) into ASCII files so you can read them into other graphics software programs. After you select 'X,' a cursor appears for you to input the data file you wish to convert.

X

T

EXTRACT FROM SAME SLIT

This command gives you the option of extracting specific scans from a sequences of scans, based on the slit in the ScanDisk that produced them. This function allows you to check the uniformity of the slits, as well as providing a alternate way of reducing the number of scans.

With the release of a ScanDisk with multiple sized slits, use this option to separate scans into groups containing records with identical resolutions. Check with *OLIS* if you are interested in this ScanDisk.

T

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E

AVERAGE TWO DATA SETS

This command allows you to average two stored data files into one. After you type in the two Input File Names, a cursor appears for you to type in the Output File Name. The averaging is done point by point.



F

ADD TWO DATA SETS

Only for use with 3D data files, this command allows you to add two data sets together, point by point. After you select this command, the program asks you to input the names of the two Input Data Files, and the name of the Output Data File.



G

SUBTRACT TWO DATA SETS

This command allows you to subtract one 3D data set from another, point by point. After you select this command, the program asks you to input the name of the data set to be subtracted from, the name of the data set to be subtracted, and an Output Data File Name in which to save the results.



H

SUBTRACT ONE SCAN FROM ALL

Only for use with 3D data files, this command allows you to subtract one scan from all the scans in the data file, point by point. After you select this command, the program asks you to enter the name of the data set to be subtracted from, the name of the scan file to subtract, and an Output Data File Name in which to save the results.



K

KINETIC DATA FAMILY

This command allows you to convert kinetic data which you have collected from another instrument or from the RSM in fixed wavelength mode into the RSM 3D format so that Robust Global Fitting can be used. These multiple kinetic shots must be over an evenly spaced wavelength range to result in a series of traces which can be converted to RSM format. See Appendix C for file format information.



O

OLIS SPECTRA DATA FAMILY

This command allows you to convert spectral data scans into RSM 3D format. See Appendix C for file format information.



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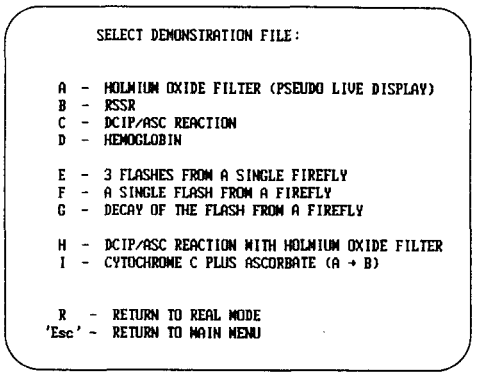
REAL/DEMO MODE

M

The Demo Mode is provided to help you become familiar with the software before attempting to work with your experimental data. We suggest that you move through the entire program in the Demo Mode, as virtually all features are active. Give yourself an adequate amount of time to explore the program and become comfortable with the various commands, so that when you begin working with your data, you can focus on the information it includes, rather than the next step to take.

In the Demo Mode, you will find nine examples of data files. These examples are representative types of data. Inspect each, and you will find one or more which are compatible with your type of research, and therefore most helpful to you. Selection A enables you to go to the Live Display menu.

CM → M



Please note: In order to enter the Demo Mode, you must select 'M,' then make a data file selection. To enter the Real Mode, press 'R' after selecting the Real/Demo Mode. If you inadvertantly place yourself in the Real Mode without turning on the RSM system, the program could lock up.

TERMINATE ACTIVITIES

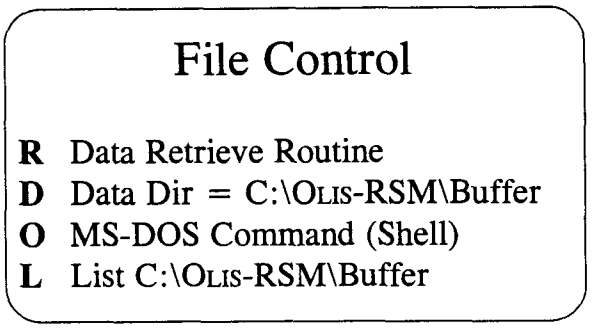
Z

You can leave the RSM program from the Command Mode. We strongly encourage you to always exit in this manner, as it leads to an "orderly shutdown," saving all parameters accurately for a successful reentry into the program. To avoid problems use 'Z' each time you shut down.

CM → Z

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8 FILE CONTROL



The commands listed under File Control in the Command Mode allow you to retrieve previously saved data and execute MS-DOS commands without exiting the program.

DATA RETRIEVE ROUTINE



This command allows you to retrieve the data you have collected and stored to disk. Type in the file name and then press 'Enter' to retrieve your file. Once the data are retrieved, the computer returns to the Data Display/Storage menu.

When the program asks you to input the file name, you can use the 'Enter' key to list the data files in the current directory. This allows you to review the contents of the active subdirectory as an aid in recalling the exact file name for retrieval. The 'Esc' command returns the program to the option menu before retrieving the file.

CM → R

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DATA DIR = C:\OLIS-RSM\BUFFER



This command allows you to change the data directory you are currently using. When you enter a directory name that does not yet exist, the program states, "Directory not found!" and ask, "Do you want to create it?" Press 'Y' to enter the new directory you have typed in. Press 'N' if you do not wish to create a new directory. Following the 'Y' or 'N' input, the program returns to the Command Mode. The default directory is **OLIS-RSM\DATA**. Any legal MS-DOS name is acceptable, i.e. ..\Newdir.

CM → ***D***

MS-DOS COMMAND (SHELL)



This command allows you to enter the MS-DOS command you want executed, i.e., "format a:," "copy *.* a:," etc, without exiting the program. Alternatively, you may press 'Enter' to enter the MS-DOS "shell." Entering the shell is suggested when more than one operation will be done. To exit the shell and return to the *OLIS* program, type 'EXIT.'

CM → ***O***

LIST C:\OLIS-RSM\BUFFER



Press 'L' to list the data files in the current data directory. Notice that this operation is available by pressing 'Enter' before entering the file name after selecting 'S'- 'Save Data to Disk' or 'R'-'Data Retrieve Routine.'

CM → ***L***

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Appendix A: Global Analysis

The Analysis of Multifunctional Data

Introduction

Global analysis is defined in the context of spectral data, collected as either absorbance or fluorescence, which is a function of both time and wavelength. The principal argument for global analysis has been made by Knuston et al. (1), who demonstrated that for fluorescence decay data, an analysis of the kinetics where the decay rates at several wavelengths are linked has an improved separation of decay rates when these wavelengths are closely spaced. A secondary argument for using global collection and analysis of data rather than separately collecting the data wavelength by wavelength is that by globally collecting and analyzing data, any systematic error contribution will have a constant effect, i.e., a baseline shift, whereas by separately collecting data, systematic errors have a variable effect, indistinguishable from real kinetic or spectral effects.

Features unique to the global analysis programs GFK16 and GFKS.

The data analysis is divided into three distinct steps which are followed by an optional analysis of the residuals and an optional plot of the joint confidence intervals of the returned rate constants where more than one is analyzed. The programs assume that the data consists of absorbance varying as a function of time and wavelength. The programs calculate the best fit for the rate constants and spectra for an user selected model.

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STEP 1: Select the number of absorbing species.

The program uses factor analysis to estimate the number of species, each with an associated decay process and spectrum, present in the data. This step is not essential to global analysis; the work of Knuston et. al. (1), does not incorporate this step. However, by identifying the number of species inherent in the data, the number of models to analyze is greatly reduced, allowing the rapid evaluation of several competing models. The algorithm for factor analysis is based upon the singular value decomposition (SVD) method of Golub and Reinsch (2-4) modified by Chan (5). Further proprietary modifications to the algorithm greatly increase its speed. The algorithm is used to decompose the data matrix, Y(time, wavelength), into U(time, wavelength), S(time, wavelength), and the transpose of V(wavelength, time). U, V, and S, are used in the global fit program. Graphical inspection of U, which consists of the eigenvectors, generally shows that only the first four columns are significantly above noise. Graphical inspection of S, which consists of the square roots of the eigenvalues, will also show only the first four values significantly above a noise trend line. The net effect of the SVD is to compress the wavelength data, which may consist of several hundred values, down to a small number N (1-4), which represents all the wavelength data as a number of species.

Even though the SVD process is computationally intensive, the overall reduction of the problem from several hundred wavelengths to N is considerable, and greatly reduces the scale of the following global analysis. This form of global analysis, where the wavelength data is, in effect, reduced to N significant values, is due to Maeder and Zuberbuhler (6).

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The next step is to generate the matrix KIN (time, N) used for the global analysis. Maeder and Zuberbuhler show that KIN is simply Y, scaled columnwise by the elements of S, i.e.., KIN = U.S. Since KIN is comprised of eigenvectors in another plane from the data, its scaling is not easily related to the original data. (For further explanation see (6)).

As an aid to the user, the first screen of the program shows 12 boxes. Inspection of the spectral information in the first set of boxes, V, labeled SPEC, the kinetic information in the second set of boxes, and the S values, enables N to be estimated. The second set of boxes, KIN(time,1-6), is obtained from factor analysis. With N selected and KIN created, step 1 is complete.

STEP 2: Kinetic Analysis.

The function of this step is for the user to select a kinetic process which is then fitted by non-linear global analysis. Consider the case where N = 2. This number of species could correspond to a first order decay in the presence of a constant background or a first order process with one species changing to another. These two different mechanisms are described by the same kinetic equation: KIN = A.exp(-k.t) +B, showing that one kinetic case may support more than one mechanisms.

This same argument for N=2 is valid for second order processes. Other possibilities when N = 2 include various pairs of concurrent zeroth, first and second order processes. The possibilities with three or more species include a variety of competing, concurrent, and consecutive processes which give rise to a much larger number of possible mechanisms. The non-linear algorithm used to fit the selected kinetic process is that of Levenberg and Marquardt, as given by Bevington (7). Matheson (8,9) modified this algorithm to be robust, or resistant

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to assymetrically distributed outlying points which are highly influential on least square fits, and cause incorrect return of parameters. For normally distributed data the robust fit returns parameters identical to those from least squares, thus robust fitting is often better and never worse than least squares. Although the robust routine minimizes the sum of absolute deviations and not the sum of squares, standard deviations of parameter estimates and the overall standard deviation are returned in order to increase the comfort level of those unfamiliar with robust fitting. Starting values for the Levenberg-Marquardt robust global fit are obtained by the method of successive intergration (10), so the kinetic fitting routine is fully automatic, and requires no user inputs of parameter estimates.

The method of GFKS differs slightly from Maeder and Zuberbuhler in that a simple global fit (a la Knuston et. al.) uses exact differentials of the function with respect to each parameter required for both the adjustment process and parameter error estimation. Maeder and Zuberbuhler use non-linear fitting for the rate constants only and reconstuct the amplitudes using linear matrix algebra, approximating the differentials. GFKS uses the simple global fit since it is no more complex and yields parameter error estimates based on exact differentials.

The best estimates for the rate constants and their standard deviation are obtained at the completion of the non-linear fit. At this point, an array, CONC(time,n), consisting of the time progress of each of the N species (normalized to vary between 0 and 1) is available for further use.

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STEP 3: Selection of a mechanism and data fit reconstruction.

Consider N = 2 for a first order decay with the background discussed above. The simpler mechanism case corresponds to the kinetic case and CONC (time,1) is a vector consisting of elements from 1 to 0, and CONC (time, 2) is a vector consisting of all 1s. For the growing in case, CONC (time,2) is (1 - CONC (time,1)) for all times. The user selects among the first order plus background case and growing in. For higher values of N, the number of choices is larger. After the user chooses a mechanism, the concentration array, CONC (time, N), is modified as necessary, and then its pseudo-inverse, sometimes called a Moore-Penrose inverse, is calculated. (details of this calculation may be found in any modern text on linear matrix algebra.)

To generate the spectra corresponding to the N species, SPEC (N, time), multiply the original data, Y (time,wavelength), by the pseudo-inverse of CONC (time, N), producing INVCONC (N, time). This calculation is processed by a matrix multiplication which has been optimized for speed.

The only remaining step is to generate the fit to the data by multiplication of CONC by SPEC. An array of residuals may be generated by subtracting the fit from the original data and the overall standard deviation generated from that. The fit is now complete and the resuls can be presented graphically.

In the general case where the data is collected as true absorbance spectra rather than difference spectra, it is possible to test the mechanism. Any fit and mechanism where all the spectral companents are non-zero for all wavelengths is an acceptable mechanism since all real extinction coefficients are positive.

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Analysis of residuals

The first display generated by global fitting shows a histogram of the residuals in bins of 0.25 standard deviation width ranging from -3 sigma to +3 sigma. The second display is a quantile/quantile plot which shows the sorted residuals plotted against a theoretical sequence, assuming a normal distribution for that number of points, Chambers et. al. (11). Normally distributed residuals give rise to a line with a slope of 1. Unnormality is indicated by points off of the 45 degree line. In a sense, the quantile plot is the 'derivative' of the distribution plot. The third display shows the residuals. A good fit is characterized by residuals that are evenly distributed about zero. Conversely, a poor fit will show a shape varying with time and/or wavelength. A final display is the joint confidence plot which is applicable for cases with two rate constants. The algorithm of Bates and Watts (12), is used to calculate a joint confidence area for the two rates, giving rise to an oval display which is an approximate representation of the combined limits for the two rates.

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Appendix A

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Additional References

SVD is one of several methods for extracting eigenvalues and eigenvectors from multifunctional data. One of the best arguments for the selection of SVD over various competitors has been made by E.R. Malinowski in his book 'Factor Analysis in Chemistry, 2nd Ed.' Wiley-Interscience, N.Y., 1991, pp 17 ff and pp 58 ff. This book offer the most accessible bridge between relatively arcane matrix mathematics and their application to problems of interest to experimentalists. An early application and explanation of SVD to biochemical problems is due to Shrager, R.I., Chemometrics Intell. Lab. Syst. (1986), 1 59-70. Brand and co-workers have applied global analysis to a variety of physical systems. These have been referenced by Maeder and Zuberbuhler, reference 6 above.

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Appendix B: Common Questions

Why is there difficulty in clicking the gratings into place?

The misalignment of gratings occurs when the new gratings are not identical to those previously in place. To check the alignment of the new gratings, move the wavelength to 0 nm (approximate). The reflection light should appear as a spot on the exit slit. Next, move the wavelength to a visible region (e.g. 500nm) to check the light spot again. If the light spot is not centered at the exit slit, the gratings are not aligned properly. To correct the discrepancy, place a small piece of tape behind the gratings.

Does the program need to be exited to calibrate the monochromotor?

No. You can calibrate the wavelength by pressing 'W' in the Live Display menu.

Does the absence of the exit slit influence the resolution?

In theory, the entrance and intermediate slit (scan disk) most directly determine the spectrum resolution. The entrance and exit slits, however, do have an affect on resolution and certainly on the light level through the RSM. The homium oxide filter is provided so that empirical estimation of the resolution can be made.

How do I obtain an accurate curve for the kinetic trace?

You must perform a kinetic extraction to see the kinetic trace correctly. The program memorizes the wavelength where the kinetics are extracted so that you do not have to input the information each time. Only three kinetic traces can be stored and displayed simultaneously. To perform the kinetic extraction, place your cursor in the scan box, move the cursor to the wavelength at which you want the kinetic trace to be extracted, and press 'K' to cause the program to perform the extraction. The trace can be stored in memory slot 1, 2, or 3.

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Appendix C: File Formats

When you save an ASCII file with option 'F' in the cursor mode, the resulting file contains two columns of x,y data. When the cursor rests in the scan box, the x column contains wavelengths in nanometers, and when the cursor rests in the kinetic box, the x column contains time in seconds. In both cases, the y column may contain volts, %T, Absorbance, etc., depending on the chosen data reduction mode.

When you save data in a Kinetic Family using option 'F' in the Save Data to Disk menu in the Data Display/Storage Menu, the resulting file is an ASCII file which contains four header lines followed by a single column of y values which depend upon the reduction mode (as above). The first header line identifies the data as an OLIS kinetic file. The second header line defines the time increment between each datum, The third describes the number of data points contained in the file. The fourth describes the units of the data to follow. For example:

```
OLIS KINETIC DATA
TIME INCREMENT  .001
NUMBER OF DATA  120
Absorbance
        0.9031
        0.8898
        0.8852
        0.8624
        0.8575
        0.8095
        0.7940
        0.7782
```

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