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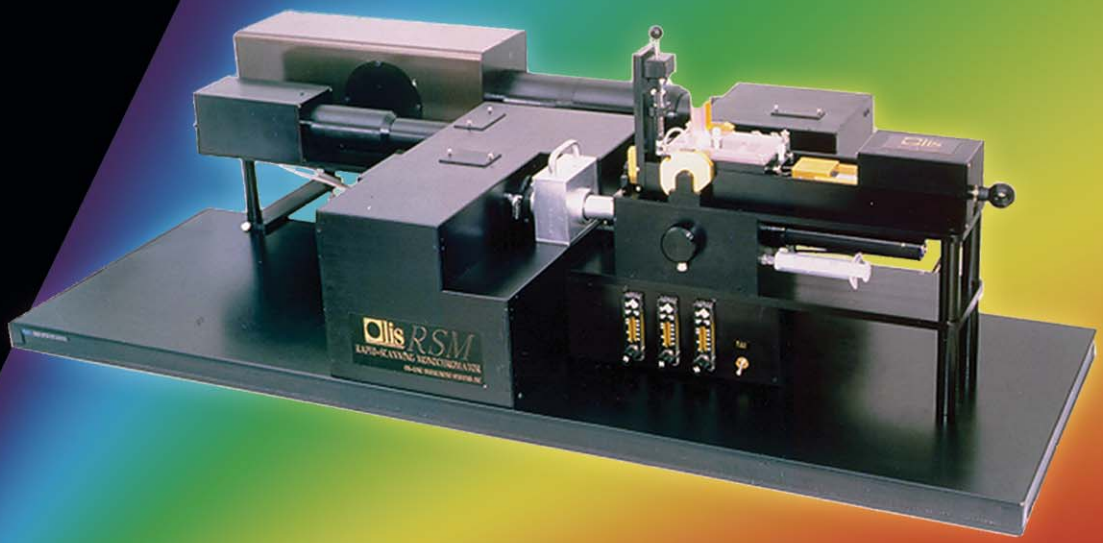
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Olis RSM 1000 Software Manual



Olis SpectralWorks™

Software for Instrument Control and Data Analysis

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On-Line Instrument Systems, Inc.
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(800) 852-3504 • (706) 353-6547
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Olis RSM 1000 Software Manual

Utilizing SpectralWorks Software for Instrument Control and Data Analysis

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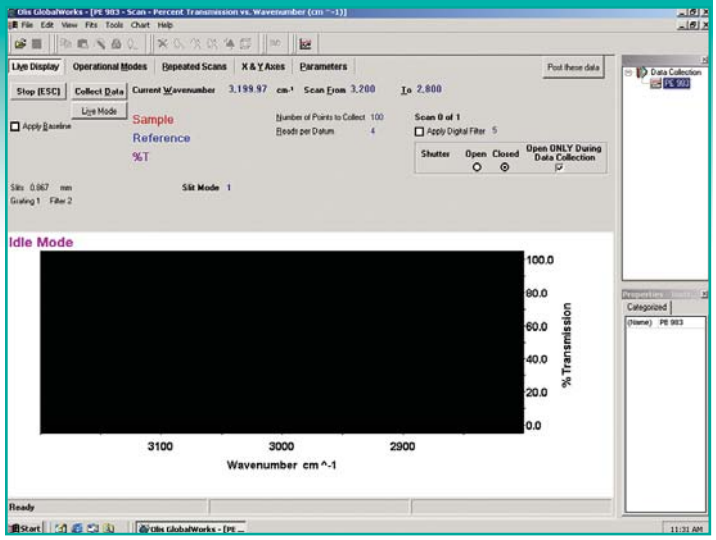
APPENDIX II: ADMINISTRATION FORM 40
Information on how to use the Administration Form to access information in the patameters.xml file.

This manual references screenshots from the control software for Olis RSM Spectrophotometer. The appearance of these screenshots may vary slightly in different versions of the software.

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Software Overview

The screenshot at right is a typical SpectralWorks screen. The elements of the application window are described below and are exploded and labeled on the next page.



TITLE BAR
The **Title Bar** contains the program name as well as the current data collection modes (if an instrument is present). The control buttons on the right hand side of this bar will close, minimize or maximize the program.

MENU BAR
Menus located in the **Menu Bar** are used in data analysis. Changes in how data are viewed, fit or printed are located here. The control buttons on the right hand side of this bar will close, minimize or maximize the dataset currently being viewed.

TOOL BAR
The **Toolbar** contains shortcuts from the menu bar. This bar contains several smaller toolbars, which indicate from which menu the shortcut is derived from. From left to right these are **File** (open and saving files), **Edit** (cutting & pasting, as well as dynamic slicing), **View** (Hiding data and 3D views), **Fits** (SVD fit), and **Data Collection** (Initiate data collection if an instrument is present).

DATA COLLECTION TABS (DATA COLLECTION ONLY)
The data collection portion of the software is divided into five (or more) tabs. Each of these tabs displays information about an aspect of data collection. The content of these tabs is dependent on the type of instrument that the software is running. The content of these tabs is described in detail later in this manual.

INSTRUMENT SETTINGS (DATA COLLECTION ONLY)
All user changeable instrument settings are located here in this region of the screen. The contents will vary with instrument type and what tab is currently displayed. Detailed information on these settings is located later in this manual.

DATA DISPLAY (DATA COLLECTION ONLY)
Current data are displayed graphically in this area. After data are collected they are transferred to the **Experiment Window** for analysis. The current status of the instrument is updated in the upper left hand corner of this window.

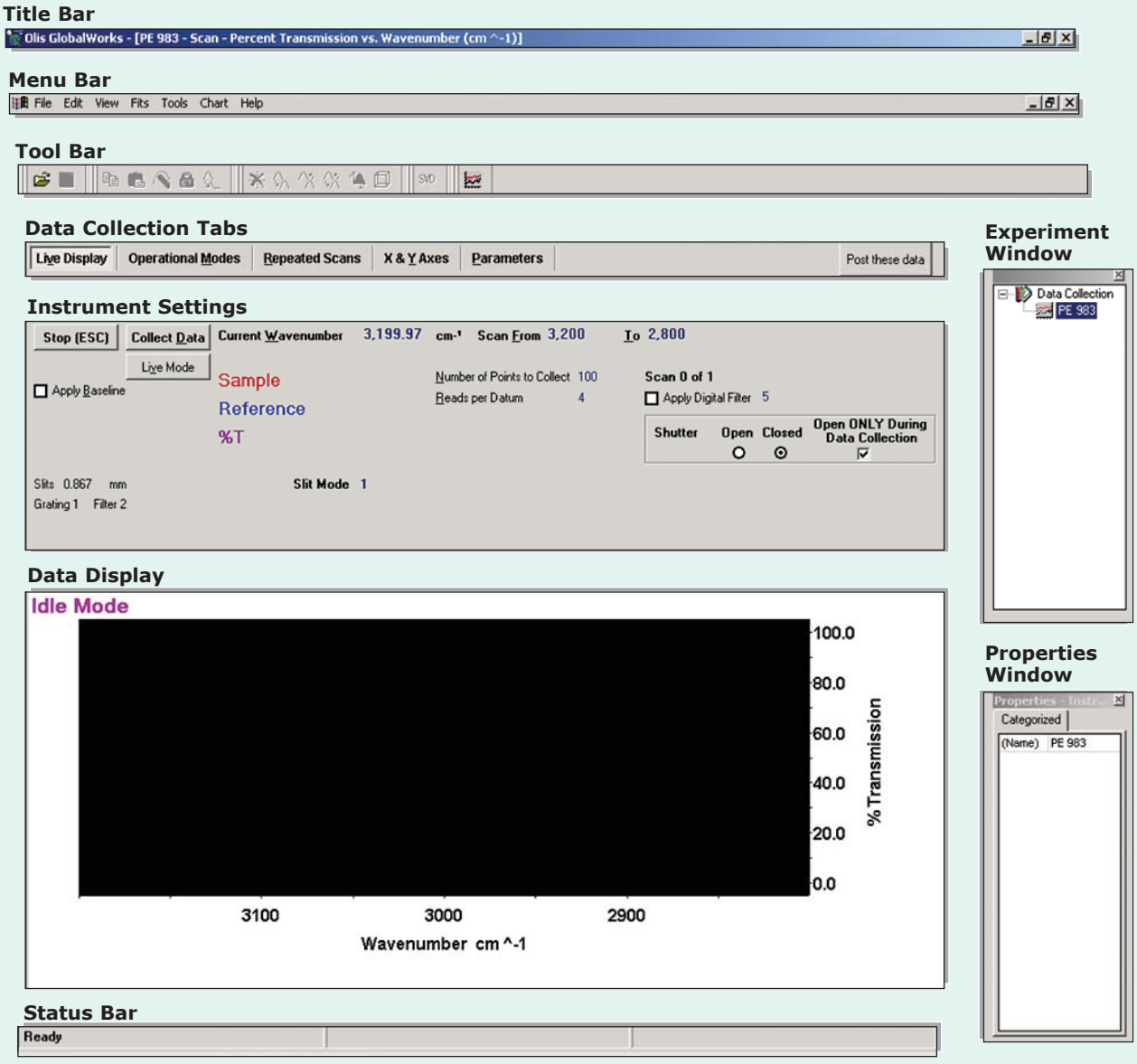
STATUS BAR (DATA COLLECTION ONLY)
In this region information such as elapsed time of current scan and current scan speed are displayed.

EXPERIMENT WINDOW
Every open window, including the data collection window, is listed here. When a dataset or fit is generated, an icon is created in this window. Move between windows by clicking on the icons in this window ("Ctrl+tab" is the keyboard shortcut). The highlighted dataset is the one currently being displayed.

PROPERTIES WINDOW
This window lists properties of the current dataset being displayed, such as number of data points, data collected, and dataset name. The dataset name can be changed here by double clicking on the name property and entering the new name.

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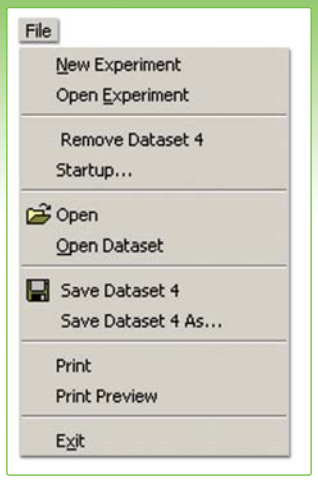
Exploded View of SpectralWorks Screen



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Menu Bar Descriptions

File



NEW EXPERIMENT
This option allows you to create a new experiment, which will consist of protocol and dataset files.

OPEN EXPERIMENT
Open a previously saved experiment (*.ole or *.olg).

REMOVE DATASET
This option allows you to remove/close the active dataset. The name of the active dataset in this case was PROTFOLD. The active window is also indicated in the experiment window as well as in the windows title bar along the top margin of the window.

STARTUP
Select this option to view the initial startup. From here convenient windows exist which allow access to existing files, recently opened files, and tutorials.

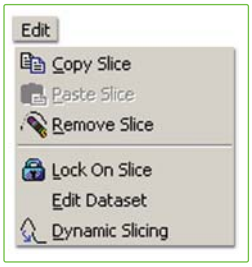
OPEN / OPEN DATASET
This option allows you to open a previously saved dataset.

SAVE DATASET
Select this option to save the currently active dataset.

SAVA DATASET AS...
This option allows for the current dataset to be saved under a different name or in a different directory.

EXIT
Choose this option to exit the GlobalWorks program.

Edit



COPY SLICE
Use this option to select a slice from among many in an active 2D dataset. Once the cursor is active, selecting copy slice from the edit menu with designate that slice as the one to be copied.
Once a slice has been selected for copying, the selected slice can be pasted into a different dataset with the same collection mode (i.e. absorbance vs. wavelength, absorbance vs. time, etc.)

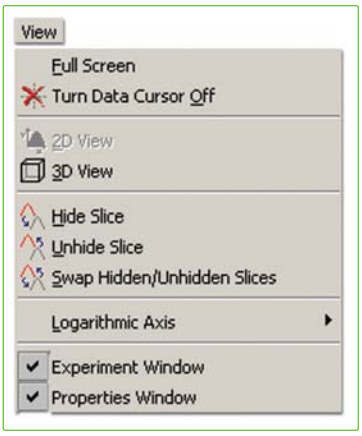
PASTE SLICE
This option becomes active only after you have selected a slice and applied to "Copy Slice" option from the edit menu. The slice is then available from the clipboard to be pasted into a dataset with the same collection mode.

REMOVE SLICE
This option is used to remove any slice from an active dataset. *NOTE: In order to make a removed slice visible again, the scan's number must be entered into the third box of the index column of the Selected Data Parameters. To activate this table, click the cursor on any slice.*

LOCK ON SLICE
Once the cursor is active you may select this option from the Edit Menu and the cursor will lock on this slice. Moving the cursor allows you to travel along the selected scan exclusively. Information about the 'locked on' slice will appear dynamically in the parameter table.

DYNAMIC SLICING
This option is available only in the 2D view. By selecting a scan from the collected data and then performing dynamic slicing, a slice of the scan at the selected wavelength will be displayed in the right graph.

View



TURN DATA CURSOR OFF
Once a slice is selected and the cursor (crosshairs) appears deactivation requires the selection of this option.

2D VIEW
This options allows the viewing of a three dimensional file (typically an *.sc3 file) as a two dimensional file with selected scans on the left and a slice thru the data at some wavelength on the right.

3D VIEW
This option allows for the viewing of a 2D dataset as a three dimensional image. The 3D graph created can then be manipulated (rotated, rescaled, etc.).

HIDE SLICE
When the data cursor is active on a slice, this option allows you to hide (remove from display) the selected slice.

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UNHIDE SLICE
When this option is selected all previously hidden slices will reappear. If multiple scans were hidden, all will reappear simultaneously.

SWAP HIDDEN/UNHIDDEN SLICES
This feature allows you to swap visible slices with slices that you have previously hidden with the hide slice option.

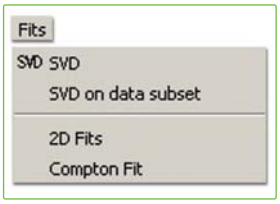
LOGARITHMIC AXIS (X-AXIS)
When you select this option the program calculates and displays the Log to the base 10 (Log) of the x-axis.

LOGARITHMIC AXIS (Y-AXIS)
When you select this option the program calculates and displays the Log to the base 10 (Log) of the y-axis.

EXPERIMENT WINDOW
All open windows for the active experiment(s) are listed in this window. Selecting a file from the list makes this selection the active window. This option allows you to view/hide the experiment window.

PROPERTIES WINDOW
The parameters of the active window are displayed. The parameters include name, data, type, full path, etc. This option allows you to view/hide the experiment window.

Fits



SVD
Factor analysis is a multivariate statistical method for extracting the information content from a dataset. In the Olis case we have a large matrix consisting of absorbance, etc. as a function of both wavelength and time. Factor analysis, in the Olis case singular value decomposition (SVD), reduces this to 3 smaller matrices that contain all the information present in the data. The kinetic information is contained in a kinetic eigenvector matrix. Experience has shown that only a small number of eigenvectors are needed to describe the data. Olis uses 6. The various rate processes are stored over a small number of eigenvectors and a kinetic model may be fitted to those to extract the rate constants. Similarly the spectral information is stored in a set of 6 eigenvectors. Finally a vector of singular values is produced. Typically these start off at a high value and fall off rapidly to a value near zero. The number of singular values above zero is an indication of the number of species significantly present in the data and is useful for model selection.

A representation of the original data may be obtained by a suitable matrix multiplication of the 2 eigenvector sets of vectors and the singular values. This reconstruction is the original less most of the noise. Thus, the processes of SVD and reconstruction smooth the data. The 3 matrices are commonly used to store the information in the data in a much more compact form than the original; about 30 times smaller. It is also much easier to do the global kinetic fit on the compact kinetic set than the original large data set. Thus factor analysis cleans up the data, affords more compact storage, and greatly reduces the scale of the global fitting problem.

SVD is one of several methods for factor analysis and is generally recognized as being the mathematically most robust. The Olis modifications to SVD in no way modify the way in which it is used or the conclusions drawn from it. The only difference is a large gain in speed.

SVD ON DATA SUBSET
Use this option to perform SVD on a data subset.

2D FITS
Olis GlobalWorks software provides multiple mechanisms for fitting single kinetic traces.

COMPTON FIT
Choosing this option after clicking on a circular dichroism scan allows for prediction of protein secondary structure based on the algorithm described in Compton, L. A., and Johnson, C. W. (1986) Anal. Biochem. 155, 155-167.

In order for this option to provide valid results data must be collected in the prescribed way shown below.

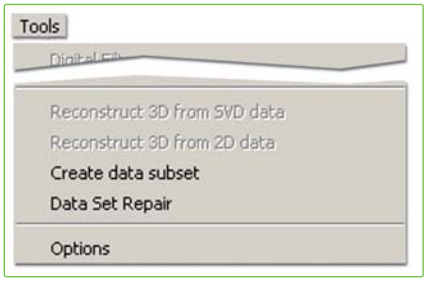
Four variants are possible:

41 pts	260 to 178 nm	39 pts	260 to 182 nm
40 pts	260 to 180 nm	38 pts	260 to 184 nm

VARIABLE SELECTION FIT
Choosing this option after clicking on a circular dichroism scan allows for prediction of protein secondary structure based on the algorithm described in Johnson, C. W. (1999) Proteins: Structure, Function, and Genetics 35, 307-312. This method differs from the Compton fit in that it uses eight basis spectra which are extracted from a set of twenty-six spectra of known proteins. The spectra are selected to be those which most resemble the spectrum to be fit, which must be from 178 nm to 260 nm and contain one point per nm (83 points).

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Tools



DATA PROCESSES
The data processes listed under the **Tools** menu are identical to those described on the **Data Processing Menu** page.

RECONSTRUCT 3D FROM SVD DATA
This option utilizes the eigenvectors to produce a 3D dataset similar to the original data (source of the SVD eigenvectors) but with much of the noise removed. Click here to see an illustrated example.

RECONSTRUCT 3D FROM 2D DATA
This option allows for the conversion of a 3D data file (*.sc3) to a 2D data file.

CREATE DATA SUBSET
By entering new wavelength and time values, a subset suitable for fitting can be created from the original data; this facility will be used if one wishes to 'trim' a dataset which may contain data deemed unsuitable for fitting.

DATASET REPAIR
This option is used to remove an errant data point, which may be affecting a kinetic fit. Once a questionable data point (or region) has been selected, the data will be replaced by an average of a user-selected adjacent data region.

OPTIONS MENU
Choosing this option opens a form with the following three tabs:



Report Options: Use this page to change the default color for printing to black and white or to choose whether or not to print the data cursor on the graph.

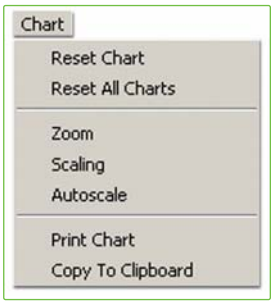


SVD Type: Choose between the Matheson SVD algorithm, which utilizes wavelength compression, or the original Golub-Reinsch (Golub G., Reinsch C. Singular Value Decomposition and Least Squares Solutions, in Wilkinson J.H. and Reinsch C.(editors) Handbook for Automatic Computation, vol II.Springer. 1971.) algorithm. The Matheson method uses matrix exponentiation (Zamis, T. M., Parkhurst, L. J., and Gallup, G. A. (1989) Computers in Chemistry, 13, 165-171.) and is significantly faster than the original algorithm. The default type uses a either algorithm depending on dataset size. The Matheson technique is more appropriate for large datasets (>10,000 points).



SVD Data Display Options: The two options available for SVD data display are the Olis square root display and the conventional display. This option changes the SVD Eigenvector display only, and has not effect on the data itself. In the Olis display, spectral data are presented as the square root of the eigenvector value, which serves to de-emphasize the spectral component of the eigenvector and reduce the potential or inclusion of spectral artifacts into the fit. For this reason, this display is the default display.

Chart



RESET CHART
This option allows the restoration of the active chart to its original condition (i.e., unhides hidden slices, resets zoom or scaling, etc.).

RESET ALL CHARTS
This option allows for the restoration of all active data charts to their original condition (i.e., unhides hidden slices, resets zoom or scaling, etc.).

ZOOM
To activate the zoom function you must be viewing 2D data and the cursor must be active on a slice. Clicking and dragging your mouse cursor allows you to create an outline of the data which once zoomed in on will consume the entire area designate to the data screen. To undo this function select reset chart from the chart menu.

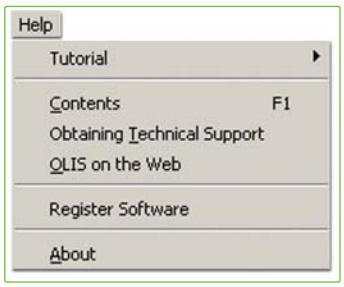
SCALING
When this option is activated the active chart can be rescaled in its window using mouse movements.

PRINT CHART
This option allows for the printing of the chart actively selected by the cursor.

COPY TO CLIPBOARD
Once this option is selected a copy of the active dataset is saved to the clipboard which can then be pasted as an image file to any program which allows the importation/pasting of an image file from the clipboard.

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Help



TUTORIAL
MATHESON-DESA FITTING TUTORIAL
This option allows you to activate an interactive tutorial guiding you step by step through applying global fit to your data set.

CONTENTS
Access the help index.

OBTAINING TECHNICAL SUPPORT
This option allows you to email your technical concerns directly to Olis' technical support department at techsupport@olisweb.com.

OLIS, INC. ON THE WEB
If you have an internet connection, this option sends you to our website, www.olisweb.com.

REGISTER SOFTWARE
After your trial has ended you can select this option to purchase GlobalWorks software and receive your registration number and unlock key.

ABOUT
This screen contains information about the software including your version number, trial expiration date, and registration information.

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Start-Up Display

This display appears at startup and contains shortcuts for opening files and instrument setup. Identical on all Olis programs.



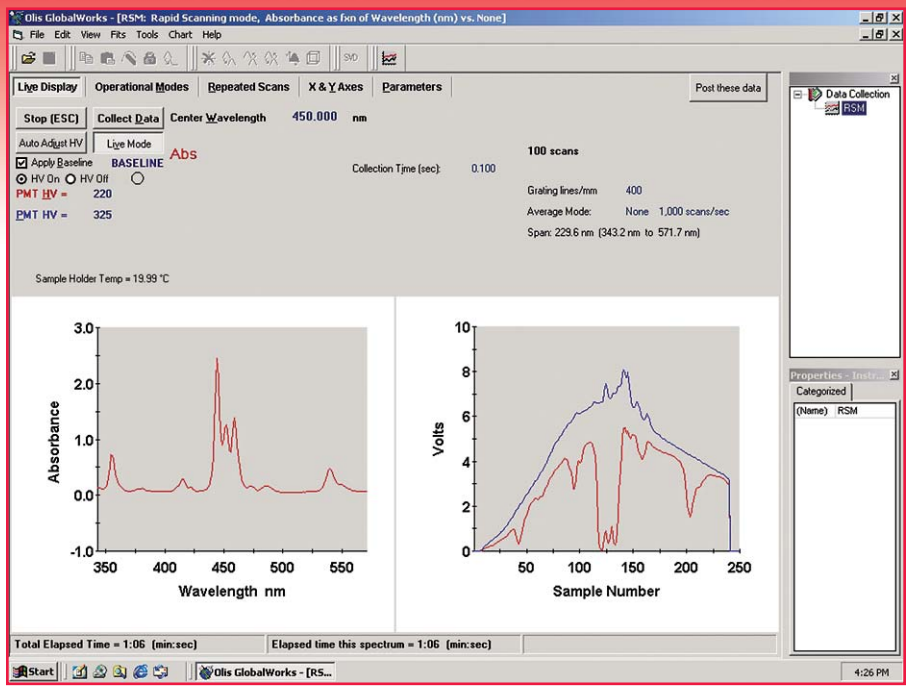
EXISTING
Select this tab to open a dataset or experiment already acquired and stored.

DATA COLLECTION
Select this tab to open a data acquisition mode to start using the spectrophotometer.

TUTORIALS
Select this tab to open the tutorial on Global Fitting.

Live Display - Rapid Scanning Mode

Displays current instrument settings and collection data. Instrument specific, yet largely identical on all Olis programs.



EXCITATION WAVELENGTH
(Not shown) The current wavelength of the excitation monochromator is displayed here and can be changed by clicking on the text box and entering in the desired wavelength.

*The keystroke shortcut for entering the Excitation Wavelength is **W**.*



CENTER WAVELENGTH
The current center wavelength of the RSM, determined by the position of the gratings, is displayed here. The RSM center wavelength determines where the center of the scan will be. The span is determined by the number of lines per millimeter of the grating. For example, a 400 line grating and a center wavelength of 450 nm will give a scan from 343 to 572. This value can be changed by clicking on the text box and entering the desired wavelength.

*The keystroke shortcut for entering RSM Center Wavelength is **A**.*



COLLECTION TIME
The time spent collecting data is entered here. The number of scans collected is dependent on the averaging mode and is displayed in the **Live Display** tab as well. The data collection time does not include the pre-trigger data, collected before stopped flow reaction is mixed.

The units for collection time can be changed to milliseconds, seconds, minutes, or hours. See **page 18** for more information on changing the time units.

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NUMBER OF SCANS TO COLLECT

The number displayed here is the number of scans to be collected using the current collection time and average mode. This value should usually be limited to less than 5000, but larger numbers may be used. The limit is determined by the amount of memory in the computer, but is typically greater than 20,000 scans. If global fitting is to be employed on the dataset, the number of scans must be less than 5000.

To reduce the number of scans to be collected, decrease **Collection Time** or increase **Scan Average Mode**.



POST THESE DATA

Clicking this button stores current data only in memory and not on the hard disk. Posted data are transferred to data processing, where they can be manipulated, fitted, or saved to a disk. Data that are not posted will be overwritten when new data are collected.

The option to post data will also be given in a prompt at the end of data collection.



GRATING LINES/MM

The lines per millimeter of the gratings in the RSM is entered here. *This value should always match the lines per grating in the instrument, and both gratings should be identical.* If the desired number is not on the drop-down list, the number can be entered directly. When this value is changed, the RSM will recalibrate automatically. This value is not to be confused with the blaze wavelength of the grating (given in nm), which determines the useful range of the grating. A rule of thumb for calculating the useful wavelength range of a grating is from 2/3*W to 3/2*W, where W is the blaze wavelength. For example, a grating blazed at 500 nm would be useful from 330 to 750 nm.



AVERAGE MODE

Seven averaging modes are available on the RSM. They are 62, 31, 20, 10, 5, 2, and 1 scan per second. No averaging is 1000 scans per second. The desired averaging mode will depend on the collection time and the required signal to noise ratio. Higher averaging modes will result in fewer scans and a higher signal to noise ratio, which increases as the square root of the number of scans averaged. For longer collection times, higher averaging modes should be used to limit the number of scans to less than 5,000.

SHOW PRE-TRIGGER DATA

(Not shown) For stopped-flow only. Checking this box allows the user to view data collected before a stopped-flow has fired. There is a variable delay between when the **Collect Data** button is clicked and when the stopped flow is triggered. There is also a fairly constant period of around 20-30 msec when reagents are being mixed, not to be confused with the dead time of the instrument. These data will be reported as negative time relative to the stop time.

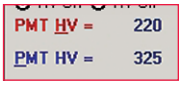
See **page 22** for more information on setting TTL Delays in the Parameters tab.



CURRENT SAMPLE TEMPERATURE

The sample temperature is displayed here if the instrument is equipped with an active temperature controller. This display is updated continuously when the instrument is in **Live Mode** or during data collection. This is the bath or Peltier cell temperature unless the controller is equipped with an external temperature probe.

See **page 34** for more information on Temperature Controller options.



CURRENT PMT HV

The current PMT high volts (HV) are shown here. These values are adjusted automatically in **Live Mode** and during data collection to keep the PMT signals near 2 volts, where optimal signal/noise is achieved. These values are directly related to the intensity of light hitting the detectors; higher voltages indicate low light levels. This value will range from 200-1100 V, and will read 0 when PMTs are off.

Warning Note: *To avoid damage to PMTs, make sure they are turned off when the sample compartment lid is removed or in any case that the PMTs are exposed to an undesirably high level of light.*



MANUAL PMT HIGH VOLTS CONTROL

This feature turns on or off the high volts (HV) to the photomultiplier tubes when **Manual High Volts** is selected as a detector option in the **Operations Mode** tab. In this mode, the PMTs must be turned off with this switch before they are exposed to room light.

See **page 16** for more information on the Operational Modes tab.

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APPLY BASELINE

This box is checked when a baseline is applied to the data. Once a baseline is selected the number of data points and scan range cannot be changed without deselecting the baseline. To undo a baseline, uncheck this box by clicking on it.

*The keystroke shortcut for selecting Apply Baseline is **B**.*



LIVE MODE

Click on this button to enter the instrument into **Live Mode**. In this mode all read values are continuously updated and data are collected but not stored. In addition, displays such as current signals and sample temperature are updated continuously.

Exit **Live Mode** by pressing the Escape key on the keyboard or clicking the **Stop** button. It is recommended that this mode be exited before performing data analysis or exiting the program.

*The keystroke shortcut for entering Live Mode is **V**.*



COLLECT DATA

Click on this button to begin data collection. In rapid scanning mode, scans will be collected as a function of time. The number of scans indicated in the **Live Display** tab will be collected.

In stopped-flow mode (Rapid Scanning + SF or Stopped-Flow) this button will be red or green. When this button is red, a sensor is out of position and the stopped-flow will not fire.

In flash or rapid scanning mode, a TTL pulse is sent out of the electronics box when data collection is initiated.

*The keystroke shortcut for Collect Data is **D**.*



AUTO ADJUST HV

This button causes the PMTs to adjust high volts to give a maximum PMT signal of 7.5 volts. It is important that a signal be present when this button is clicked (for fluorescence, ensure that shutters are open, sample is in place, slits are appropriate). No data are collected.

*The keystroke shortcut for Auto Adjust HV is **U**.*



STOP (ESC)

Clicking on this button or pressing the Escape key exits from **Live Mode**. If pressed during data collection, the current assay or scan is terminated and the instrument goes to **Idle Mode**, in which case the truncated dataset can be retained.

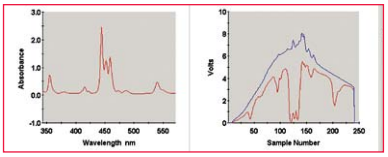
*Note: If the **Manual HV Shut Off** check box in the **Operational Modes** tab is not selected, then the PMT HV will automatically shut off when exiting **Live Mode**.*

See **page 16** for more information on the Operational Modes tab.



SPAN

This display shows the calculated spectral span (i.e., the scan range) of the rapid scan. This value is dependent on the number of grating lines and also is corrected slightly for the effects of wavelength on bandwidth. The actual scan range is displayed in parentheses, and will shift as the center wavelength is adjusted.



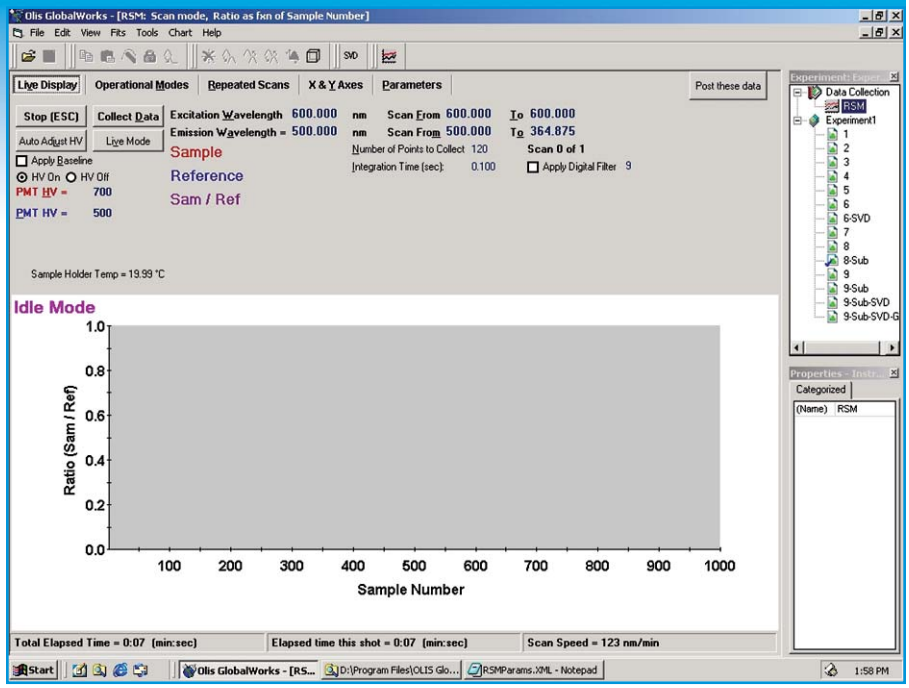
DISPLAY

If only one graph is visible, double click on the graph to make both visible. The raw signal to the PMT detectors is displayed on the right graph. This is a measure of the PMT output as the wavelength changes across the scan range. The traces are color coded to match the red and blue cables on the PMTs. The left graph contains the calculated data from the raw signals. This calculation will depend on the **Data Reduction Mode** as it is set in the **Operational Modes** tab.

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Live Display - Scan Mode

Displays current instrument settings and collection data in the conventional scanning mode.





EXCITATION WAVELENGTH
The current wavelength of the excitation monochromator is displayed here and can be changed by clicking on the text box and entering the desired wavelength.

*The keystroke shortcut for changing the Excitation Wavelength is **W**.*



SCAN RANGE (EXCITATION)
Starting and ending wavelengths for the excitation monochromator are displayed here. To take an excitation scan, enter the starting and ending excitation wavelengths, then enter the desired emission wavelength, a constant value, as both the starting and ending emission wavelengths. The X-axis on the graph will read **Excitation Wavelength**.

If synchronous scanning is desired (both excitation and emission monochromators scanning), then enter the desired scan range for both monochromators.

*The keystroke shortcuts for entering the starting and ending wavelengths for the excitation monochromator are **F** and **T**, respectively.*



POST THESE DATA
Clicking this button stores current data only in memory and not on the hard disk. Posted data are transferred to data processing, where they can be manipulated, fitted, or saved to a disk. Data that are not posted will be overwritten when new data are collected. *The option to post data will also be given in a prompt at the end of data collection.*



EMISSION WAVELENGTH
The current wavelength of the emission monochromator is displayed here and can be changed by clicking on the text box and entering in the desired wavelength. If more than one monochromator is present, both monochromators will move to this value.

*The keystroke shortcut for entering the Emission Wavelength is **A**.*



SCAN RANGE (EMISSION)
Starting and ending wavelengths for the emission monochromator are displayed here. To take an emission scan, enter the starting and ending emission wavelengths, then enter the desired excitation wavelength, a constant value, as both the starting and ending excitation wavelengths. The X-axis on the graph will read **Emission Wavelength**.

If synchronous scanning is desired (both excitation and emission monochromators scanning), then enter the desired scan range for both monochromators.

*The keystroke shortcuts for entering the starting and ending wavelengths for the emission monochromator are **M** and **O**, respectively.*

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NUMBER OF INCREMENTS TO COLLECT

The number of increments to be collected in each scan or assay is entered here.

*The keystroke shortcut for entering the Number of Intervals to Collect is **N**.*

NUMBER OF POINTS	= # of INCREMENTS +1



CURRENT SCAN NUMBER

If multiple scans are to be collected, the current scan and total scan numbers are displayed here.

See **page 18** for details on setting up repetitive scans or assays.



INTEGRATION TIME

The time spent collecting data per each data point is shown here. This value is user specified and is limited to 10 minutes/datum with a PMT.

*The keystroke shortcut for entering Integration Time is **I**.*



APPLY DIGITAL FILTER

Checking this box automatically applies a digital filter to the data after it has been collected. Filtering is available from 5 to 25 points and follows a *Svitsky-Golay* algorithm.

Take care not to over-filter, which can distort spectral features. To test for distortion, data can be collected without the filter. Comparing data before and after filtering will make distortion of spectra apparent. Alternatively, subtracting filtered data from the unfiltered data should result in a difference spectrum containing only random noise. This can be done by posting both the filtered and unfiltered data using the **Post Data** button, The data can then be subtracted using the **Subtract Datasets** option in the **Data Processing** menu.

See **page 26** for more information about Subtract Datasets in the Data Processing menu.



RAW PMT SIGNALS

The raw PMT signals (in volts) are shown in real time when in **Live Mode**. They are color coded red and blue to match the corresponding PMTs. Generally, the blue PMT is used in the reference position.

If the PMT is saturated, this reading will be 10.000V in **Live Mode**. When this is the case, the light intensity reaching the PMT must be reduced by either reducing sample concentration, monochromator slit width, or **PMT High Voltage**.



CALCULATED DATA

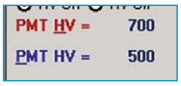
The current calculated fluorescence data is presented here. This value will depend on the **Data Reduction Mode**.

See **page 16** for more information on Data Reduction Mode.

For example, when **Ratio** is chosen, the data provided in this location will be Sam/Ref, the ratio of the red PMT signal to the blue PMT signal.

Polarization or anisotropy data is also presented here.

See **page 16** and **Appendix I** for more information on anisotropy data.



CURRENT PMT HIGH VOLTS

The current PMT high volts are shown here. To change this voltage, click on the number and enter the desired voltage. To automatically adjust high volts, use the **Auto Adjust HV** button.

*The keystroke shortcuts for setting the PMT high volts are **H** for the red PMT and **P** for the blue PMT.*

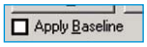


MANUAL PMT HIGH VOLTS CONTROL

This option turns on or off the high volts (HV) to the photomultiplier tubes and is only available when **Manual High Volts** is selected in the **Operational Modes** tab.

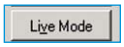
See **page 16** for more information on the Operational Modes tab.

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APPLY BASELINE
This box is checked when a baseline is applied to the data. Once a baseline is selected, the number of data points and scan range cannot be changed without deselecting the baseline. To undo a baseline, uncheck this box by clicking on it.

See **page 25** for details on how to assign a baseline.



LIVE MODE
Click on this button to enter the instrument into **Live Mode**. In this mode, data is collected but not stored. In addition displays such as current signals and sample temperature are updated continuously.

Exit **Live Mode** by pressing the Escape key on the keyboard. It is recommended that this mode be exited before performing data analysis or exiting the program.

*The keystroke shortcut for entering Live Mode is **V**.*



COLLECT DATA
Clicking on this button will begin the data collection process. With the **Data Collection Mode** set to **Scan**, the instrument will scan the number of points from start to finish. With the **Data Collection Mode** set to **Assay**, the data will be collected at a fixed wavelength as a function of time.

See **page 17** for details on changing the Data Collection Mode.

*The keystroke shortcut for Collect Data is **D**.*



AUTO ADJUST HV
This button causes the PMTs to adjust high volts to give a PMT signal in the range of 6-8 volts. It is important that a signal be present when this button is clicked (shutters open, sample in place, slits appropriate). No data is collected.

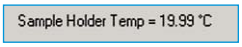
*The keystroke shortcut for Auto Adjust HV is **U**.*



STOP (ESC)
Clicking on this button or pressing the **Escape** key exits from **Live Mode**. If pressed during data collection, the current assay or scan is terminated and the instrument goes to **Idle Mode**, in which case the truncated dataset can be retained.

*Note: If the **Manual HV Shut Off** check box in the **Operational Modes** tab is not selected, then the PMT HV will automatically shut off when exiting **Live Mode**.*

See **page 16** for more information on the Operational Modes tab.



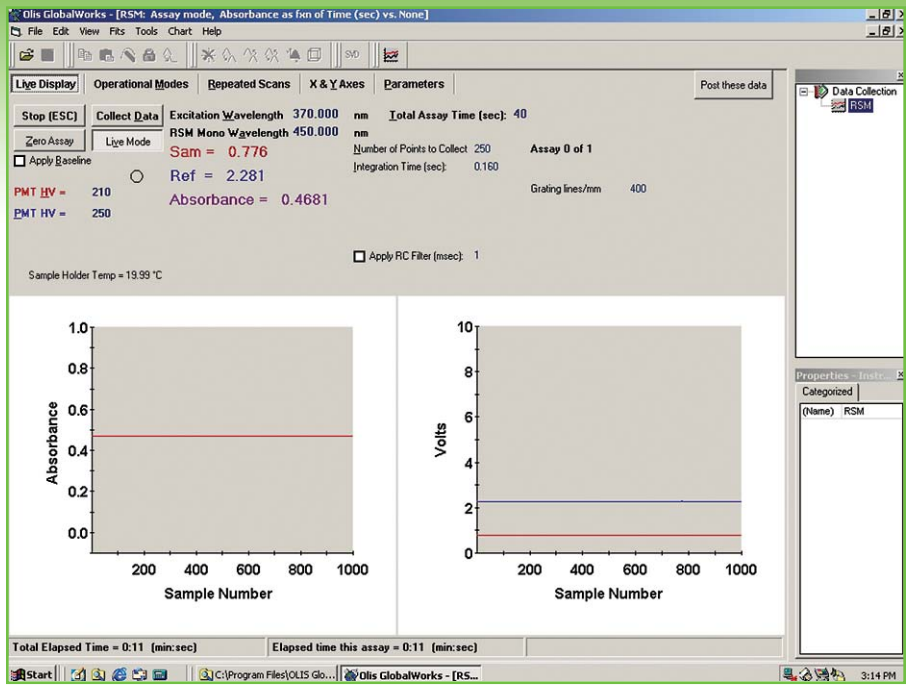
CURRENT SAMPLE TEMPERATURE
If the instrument is equipped with an active, Olis-recognized temperature controller, the **Current Sample Temperature** is displayed here and is updated when the instrument is in **Live Mode** or during data collection. This is the bath or Peltier cell temperature unless the controller is equipped with an external temperature probe.

See **page 34** for temperature controller options.

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Live Display - Assay Mode

Displays current instrument settings and collection data in assay mode.



Excitation Wavelength 370.000 nm

EXCITATION WAVELENGTH

The current wavelength of the excitation monochromator is displayed here and can be changed by clicking on the text box and entering in the desired wavelength.

*The keystroke shortcut for entering the Excitation Wavelength is **W**.*

RSM Mono Wavelength 450.000 nm

RSM MONO WAVELENGTH

The current center wavelength of the RSM, determined by the position of the gratings, is displayed here. This value can be changed by clicking on the text box and entering in the desired wavelength. When **Collect Data** is clicked, a single wavelength time trace at this wavelength will be collected.

If an excitation monochromator is not present, this option will be called **Assay Wavelength**.

Total Assay Time (sec): 40

TOTAL ASSAY TIME

The time spent collecting an assay is entered here. The integration time will automatically be calculated to give the maximum possible collection time during the assay. Time units can be changed in the **Operational Modes** tab.

See **page 16** for more information on the Operational Modes tab.

*The keystroke shortcut for changing the Total Assay Time is **T**.*

Post these data

POST THESE DATA

Clicking this button stores current data only in memory and not on the hard disk. Posted data are transferred to data processing, where they can be manipulated, fitted, or saved to a disk. Data that are not posted will be overwritten when new data are collected. *The option to post data will also be given in a prompt at the end of data collection.*

Number of Points to Collect 250

NUMBER OF INCREMENTS TO COLLECT

The number of increments to be collected in each scan or assay is entered here.

*The keystroke shortcut for entering the Number of Intervals to Collect is **N**.*

NUMBER OF POINTS	= # of INCREMENTS +1

Assay 0 of 1

CURRENT ASSAY NUMBER

If multiple assays are to be collected, the current assay and total assay numbers are displayed here.

See **page 18** for details in setting up repetitive scans or assays.

Integration Time (sec): 0.160

INTEGRATION TIME

The time spent collecting data per each data point is shown here. This value is user specified and is limited to 10 minutes/datum.

*The keystroke shortcut for entering Integration Time is **I**.*

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APPLY RC FILTER

When this box is checked, a digital time constant is applied to all time course data. The filtered data which results is not revertible. A time constant is required (in msec) and cannot exceed the length of the dataset. A rule of thumb for the maximum time constant without distorting the data is 5-10% of the total time.

This algorithm can also be applied to normally collected data after collection.

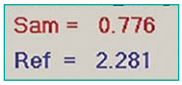
See **page 26** for more information on the Data Processing Menu.



CURRENT SAMPLE TEMPERATURE

If the instrument is equipped with an active, Olis-recognized temperature controller, the **Current Sample Temperature** is displayed here and is updated when the instrument is in **Live Mode** or during data collection. This is the bath or Peltier cell temperature unless the controller is equipped with an external temperature probe.

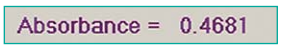
See **page 34** for Temperature Controller options.



RAW PMT SIGNALS

The raw PMT signals (in volts) are shown in real time here when in **Live Mode**. They are color coded red and blue to match the corresponding PMTs. Generally, the blue PMT is used in the reference position.

If the PMT is saturated, this reading will be 10.000V in **Live Mode**. When this is the case, the light intensity reaching the PMT must be reduced by either reducing sample concentration, monochromator slit width, or **PMT High Voltage**.



CALCULATED DATA

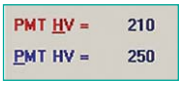
The current calculated fluorescence data is presented here. This value will depend on the **Data Reduction Mode**

See **page 16** for more information on Data Reduction Mode.

For example, when **Ratio** is chosen, the data provided in this location will be Sam/Ref, the ratio of the red PMT signal to the blue PMT signal.

Polarization or anisotropy data is also presented here.

See **page 16** and **Appendix I** for more information on anisotropy data.



CURRENT PMT HIGH VOLTS

The current PMT high volts are shown here. To change this voltage, click on the number and enter the desired voltage. To automatically adjust high volts, use the **Auto Adjust HV** button.

*The keystroke shortcuts for setting the PMT high volts are **H** for the red PMT and **P** for the blue PMT.*

MANUAL PMT HIGH VOLTS CONTROL

Not shown. This option turns on or off the high volts (HV) to the photomultiplier tubes and is only available when **Manual High Volts** is selected in the **Operational Modes** tab.

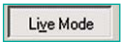
See **page 16** for more information on the Operational Modes tab.



APPLY BASELINE

This box is checked when a baseline is applied to the data. Once a baseline is selected, the number of data points and scan range cannot be changed without deselecting the baseline. To undo a baseline, uncheck this box by clicking on it.

See **page 25** for details on how to assign a baseline.



LIVE MODE

Click on this button to enter the instrument into **Live Mode**. In this mode, data is collected but not stored. In addition displays such as current signals and sample temperature are updated continuously.

Exit **Live Mode** by pressing the Escape key on the keyboard. It is recommended that this mode be exited before performing data analysis or exiting the program.

*The keystroke shortcut for entering Live Mode is **V**.*



COLLECT DATA

Clicking on this button will begin the data collection process. With the **Data Collection Mode** set to **Scan**, the instrument will scan the number of points from start to finish. With the **Data Collection Mode** set to **Assay**, the data will be collected at a fixed wavelength as a function of time.

See **page 17** for details on changing the Data Collection Mode.

*The keystroke shortcut for Collect Data is **D**.*

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STOP (ESC)
Clicking on this button or pressing the Escape key exits from **Live Mode**. If pressed during data collection, the current assay or scan is terminated and the instrument goes to **Idle Mode**, in which case the truncated dataset can be retained.

*Note: If the **Manual HV Shut Off** check box in the **Operational Modes** tab is not selected, then the PMT HV will automatically shut off when exiting **Live Mode**.*

Operational Modes

Displays current data collection and data reduction modes. Contains additional options.



DATA REDUCTION MODE
This option chooses the mode in which data will be calculated from raw signals. The options available are dependent on the number of polarizers and detectors available.

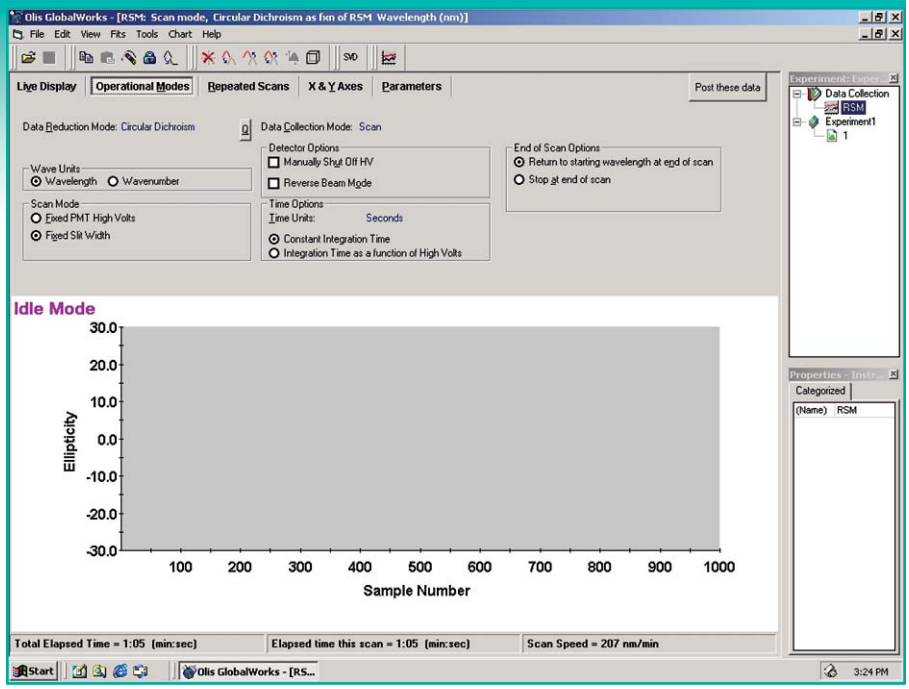
See **page 21** for more information on how to change the number of active detectors.

For one PMT the following options are available:

Signal: Signal is reported directly with no mathematical manipulation of the data. The signal is reported in volts for a PMT and counts for a photon counter.

L-Format Anisotropy: This option is available with at least two polarizers. Data is collected as fluorescence intensity for both vertical and horizontal emission polarizers and computed to give anisotropy. See **Appendix I** for more information on polarization and anisotropy.

L-Format Polarization: This option is available with at least two polarizers. Data is collected as fluorescence intensity for both vertical and horizontal emission polarizers and computed to give polarization. See **Appendix I** for more information on polarization and anisotropy.



For two PMTs the following options are available:

Absorbance (A.U.): Reports data as the log of the ratio of reference to sample signal, corrected for the dark signal. Abs.=log[(reference-dark)/(sample-dark)].

Transmittance: Reports data as the ratio of sample signal and reference signal, calculated as T=(sample-dark)/(reference-dark).

%Transmission: Reports data as a percentage of the ratio of sample signal to reference signal (%T=(sample-dark)/(reference-dark)*100%).

Ratio: In this mode, the fluorescence emission signal is reported as a ratio of sample signal to reference signal. Fluctuations in lamp intensity are corrected for by this method.

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Single Beam: This mode reports the raw signal from the sample and reference channels.

Circular Dichroism: Reports circular dichroism in units specified in the Parameters tab. A circular dichroism module is required to operate in the data reduction mode. This option is only available if the RSM is equipped with a CD module.

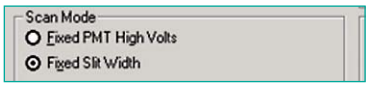
T-Format Anisotropy (fluorescence): This mode is available with two detectors, and three polarizers. Data is collected as a ratio of detector signals (one channel vertically polarized, the other horizontally). These data are converted automatically to anisotropy.

T-Format Polarization (fluorescence): This mode is available with two detectors, and three polarizers. Data is collected as a ratio of detector signals (one channel vertically polarized, the other horizontally). This data is converted automatically to polarization. See **Appendix I** for more information on polarization and anisotropy.



WAVE UNITS

Choose between data being reported in wavelength (nm) or wave number (cm-1).



SCAN MODE

In the RSM, there are two possible scan options for collection of scans.

Fixed Slit Width: In this mode, the PMT HV are changed to compensate for changes in light intensity. This mode is highly recommended for collection of circular dichroism data.

Fixed PMT HV: In this mode the PMT HV remain at a user-specified, fixed value. This mode is necessary for absorbance or fluorescence measurements.



Q

This button is used to convert the current data to that of a different data reduction. For example if data are collected in absorbance reduction mode, the raw data (sample and reference) can be extracted by selecting a data reduction mode of single beam, and pressing the **Q** button. The data will then be presented as sample and reference rather than a calculated absorbance. This data will remain in memory until more data are collected, or **Live Mode** is entered.



DATA COLLECTION MODE

Several data collection modes are available on the RSM. Note that different data collection modes may require changes in the instrument configuration.

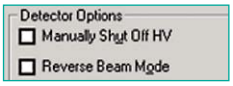
Rapid Scanning: In this mode, data are collected as a series of rapid scans (from 1 to 1000 scans/second). A scan disk is required to collect these data. Typically a 0.2 mm scan disk is used to collect absorbance data (see **Data Reduction Mode**) and a 1.0 mm disk is used for fluorescence data. This collection mode can be used for both fluorescence and absorbance configurations. This mode can be used to collect absorbance or fluorescence data. A stopped-flow accessory can be present but will not be fired during data collection.

Rapid Scanning + SF(Stopped Flow): This collection mode is identical to Rapid Scanning except the stopped flow is fired during data collection. To run this mode, a stopped flow accessory must be present on the instrument.

Scan: This is the "conventional" scanning mode in which scans are collected by scanning wavelength by moving the gratings. A fixed scan cartridge is required for this mode. Scans are collected on the order of 1 per minute, depending on wavelength range and integration time. This mode can be used to collect absorbance, fluorescence, or circular dichroism data.

Assay: In this mode wavelength, wavelength is constant and data is collected over time. A fixed cartridge is required for this mode, and it can be used to collect absorbance, fluorescence, or circular dichroism data.

Stopped Flow: In this mode, the stopped flow data are collected at a fixed wavelength. A stopped flow accessory and a fixed scan cartridge are required to operate in this mode. Absorbance, fluorescence, or circular dichroism can be collected in this mode.



DETECTOR OPTIONS

Turn this option **On** in order to have control over turning the high volts on and off when in **Live Mode**. Turn this option **off** to keep high volts under computer control. With the option off, the PMT high volts will be turned off whenever data is not collected. This is the safest mode for the PMTs.

If the user prefers that the PMTs remain on when the instrument is not collecting data, this option should be **On**. In this mode, however, care must be taken to avoid exposing the PMTs to room light while a PMT HV is applied.

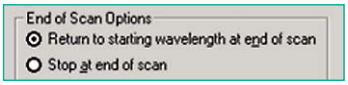
Reverse Beam Mode inverts the red and blue signals for calculations. This has the effect of inverting the signal and is used for specific applications in which the blue PMT is used to detect the sample signal.

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TIME UNITS

This option changes time units for data collected as a function of time. Hours, minutes, seconds, or milliseconds are available.



END OF SCAN OPTIONS

This choice determines whether the monochromator returns to the start position after a scan or remains at the end position. **Stop at the end of scan** is used when the sample is photosensitive.

Repeated Scans

Allows collection of multiple scans or assays in a single dataset.

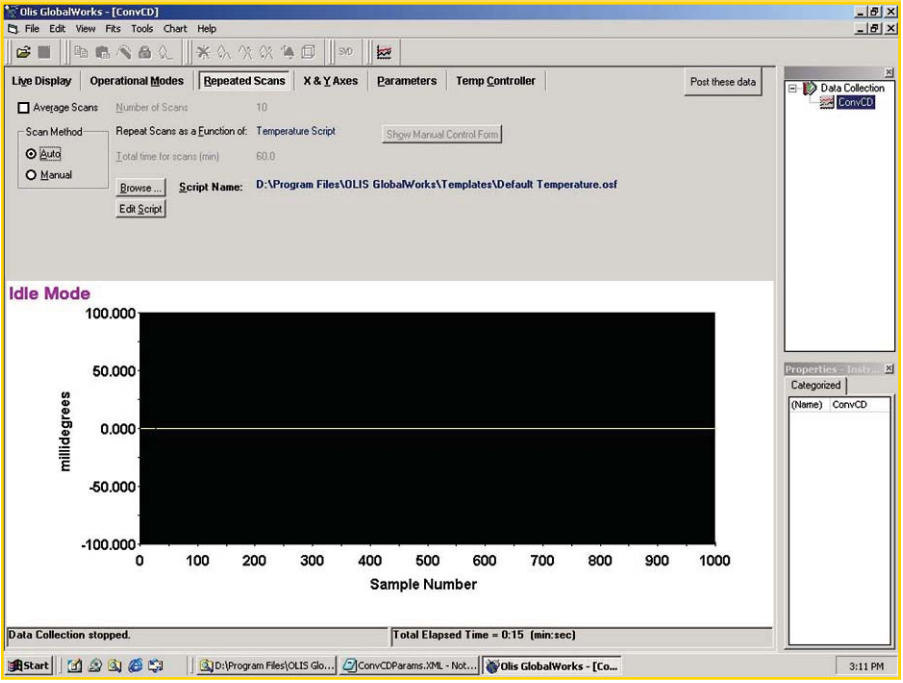


NUMBER OF SCANS

Enter the number of scans or assays to be repeated here. After they are acquired, all of these scans will be included in a single 3-D dataset for storage and subsequent handling. When data is collected in rapid scanning mode, this number will reflect the number of scans to be included in one dataset. When a script is enabled in **Repeat Scans as a Function of**, this value is obtained from the script. See **page 36** for a sample script file.

*The keystroke shortcut for entering the number of scans is **N**.*

See **page 17** for more information on rapid scanning mode.





REPEAT SCANS AS A FUNCTION OF

Repetitive scans can be collected as a function of **None**, **Time**, **Temperature Script**, or **Titration Script**. In rapid scanning mode these options are not available.

None: Scans are collected consecutively, as quickly as possible, without pause. This is a useful mode for averaging several scans, or if scans will be collected as a function of another parameter. In this case, this information can be added to the dataset using **Edit Dataset**. See **page 31** for more information on Edit Dataset.

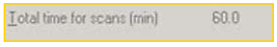
Time: Scans are equally spaced out over the designated amount of time. The incubation period between scans can be skipped by pressing the spacebar, with the remaining scans being spaced out over the remaining time.

Temperature Script: When repeated scans are acquired as a function of temperature (either by water bath or Peltier cell), an Olis script is used. The instrument will automatically adjust the temperature, incubate a prescribed amount of time and collect data when **Collect Data** is clicked in the **Live Display** tab. The resulting scans will be presented in a 3-D dataset with wavelength, temperature, and signal intensity as the three axes. The script is selected and edited as described below. The instrument must be equipped with a water bath or Peltier cell that is compatible with Olis software.

Titration Script: To use this option the instrument must be equipped with an Olis titrator. In this mode additions of titrant are made automatically between scans or assays. The resulting dataset is a 3-D dataset with wavelength, concentration, and signal intensity as axes. The **Titrator Control Panel** also becomes active when this mode is selected. See **page 36** for a sample titration script.

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Ezyme Assay Script: If the RSM is equipped with an enzyme assay device, assays may be collected as a function of concentration. As opposed to the titrator, a fresh solution is pushed into the cuvette as prescribed in a script. The enzyme assay control panel becomes active when this mode is selected.



TOTAL TIME FOR SCANS (UNITS)

This option is active when **Collect Scans as a Function of Time** is selected. The program will compute the required wait time between scans, so as to give equal intervals between scans or assays. If a time is entered that is less than the amount of time required to complete the scans, they will be collected as fast as possible (i.e., no wait time between scans). This option is disabled when collecting in rapid scanning mode.

See **page 18** for more information on changing the time units in the Operational Modes tab.

*The keystroke shortcut for changing the total time for scans is **T**.*



SCRIPT NAME

This display shows the location of the current active script file. A new file can be selected by clicking on the filename and entering the new file. Alternatively, pressing 'Browse' allows the user to open the new script file. Script files have the extension *.ols*.

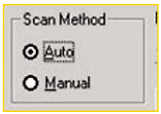
See **page 36** for a sample titration script file.



EDIT SCRIPT

The current active script can be edited by clicking on this button. Changes in the script must be saved (under the **File** menu) before exiting. The script can also be edited using the program Notepad, which is included in Microsoft Windows. *Note: Do NOT use Wordpad or any other word processing software to change the script. This may corrupt the script file format.*

See **page 36** for a sample titration script file.



SCAN METHOD

Choosing **Auto** causes the instrument to operate entirely without user intervention. When a script file is being run, this option is automatically set to **Auto**.

*The keystroke shortcut for setting the Scan Method to Auto is **A**.*



AVERAGE SCANS

Selecting this option will automatically average all scans into one 2-D scan as the final result. If this option is not checked, the data can be averaged later as a data process. Choosing to average scans cannot be undone, as only the average is saved, not the raw data.

See **page 26** for more information on the Data Processing Menu.

*The keystroke shortcut to activate Average Scans is **R**.*



SHOW MANUAL CONTROL FORM

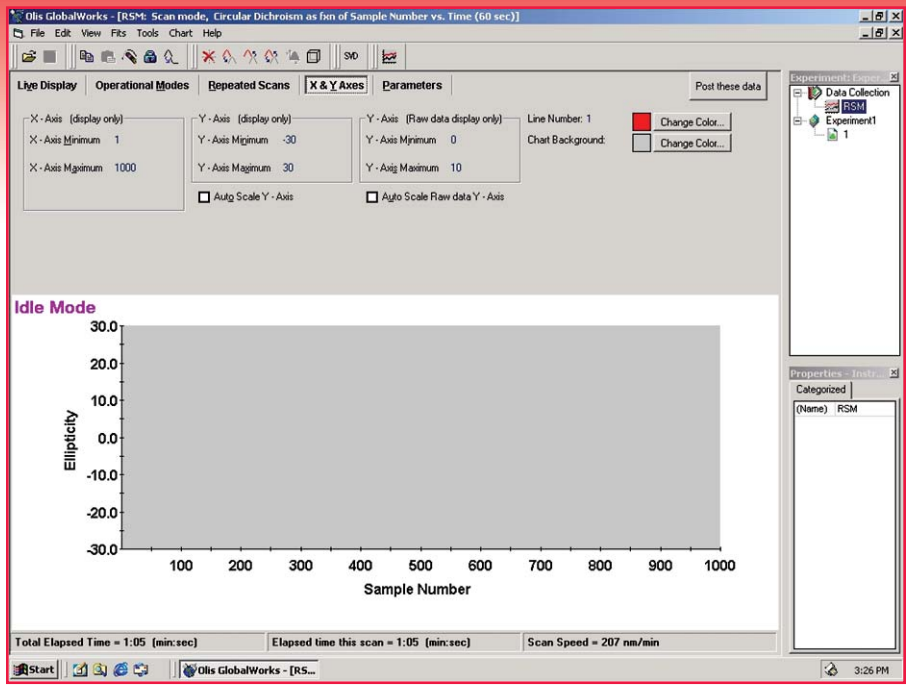
This button is enabled when repeat scans or assays are collected as a function of titrator or enzyme assay script. Pressing this button shows manual control form to control the syringes. Note: the titrator will calibrate when this button is pressed.

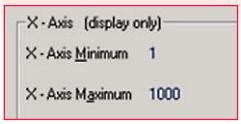
See **page 35** for more information on the Titrator Control Panel.

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X & Y Axes

Options for editing the axis scale and display colors.

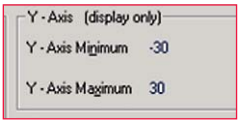




X-AXIS

This option changes the scale of the X-axis during data collection. The data are not affected by changing this scale; only the display is affected.

*The keystroke shortcuts to access the X-axis minimum and maximum values are **M** and **A**, respectively.*



Y-AXIS

This option changes the scale of the Y-axis (the fluorescence intensity) during data collection. The data are not affected by changing this scale; only the display is affected.

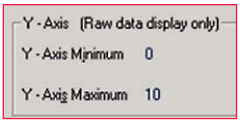
*The keystroke shortcuts to access the Y-axis minimum and maximum values are **N** and **X**, respectively.*



AUTO SCALE Y-AXIS

This option will allow the graphic to be automatically scaled continuously during data collection. This is particularly useful if the user does not know the amplitude of the expected signal.

*The keystroke shortcut for selecting Auto Scale Y-axis is **O**.*



Y-AXIS (RAW DATA)

This option changes the scale of the raw signal of the PMTs. This signal can be accessed by double clicking on the graph, during data collection. The data are not affected by changing this scale; only the display is affected.

*The keystroke shortcuts to access the Y-axis minimum and maximum value are **I** and **S**, respectively.*



AUTO-SCALE (RAW DATA)

This option will allow the raw signal graphic to be automatically scaled continuously during data collection. This is particularly useful if the user does not know the amplitude of the expected signal.

Pressing keystroke for activating Auto Scale Raw Data is **U**.



CHANGE LINE COLOR

This control allows the user to change the line color during data collection. Up to nine different colors can be used from a palette of hundreds of colors.



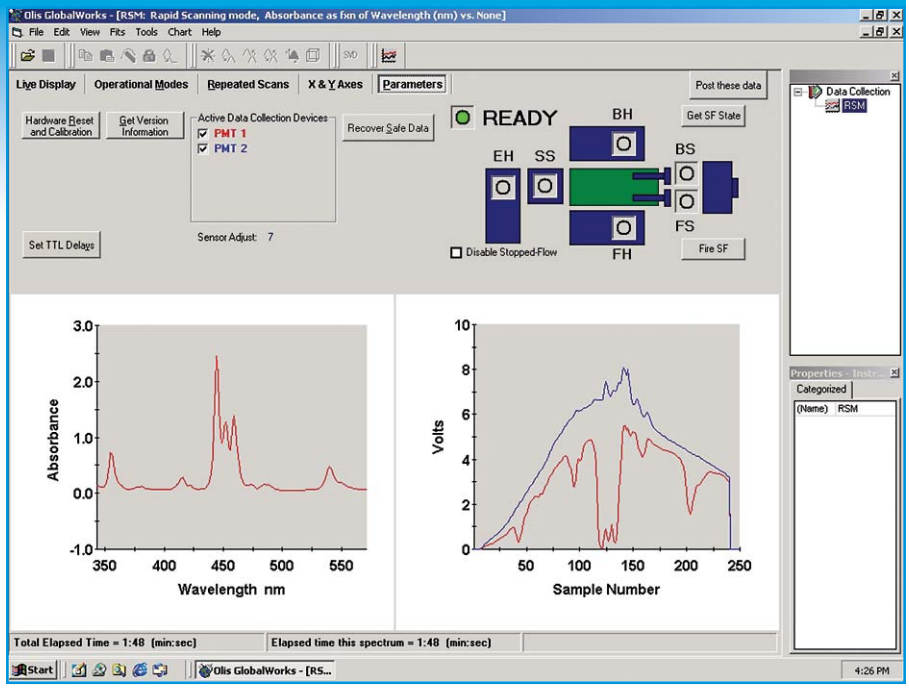
CHANGE BACKGROUND COLOR

This control allows the user to change the chart background color during data collection. Up to nine different colors can be used from a palette of hundreds.

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Parameters

Contains controls for checking calibration, checking stopped-flow sensors, and changing active detectors.





HARDWARE RESET AND CALIBRATION

Clicking this button will result in calibration of the monochromator and any other associated motors. Recalibrating should be performed if any problems are encountered. If problems persist, contact Olis.

*The keystroke shortcut for Hardware Reset and Calibration is **R**.*



GET VERSION INFORMATION

This button will allow for reading of the current hardware versions on your instrument. This information can be very useful when receiving technical support from Olis.

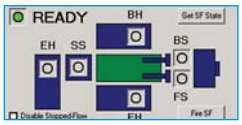
*The keystroke shortcut for Get Version Information is **G**.*



ACTIVE DATA COLLECTION DEVICES

This is a list of all possible detectors on the instrument. Check the detectors that will be active here. Be sure to update the current data in reduction mode to reflect any change in number of detectors. The available modes will depend on the number of active detectors.

See **page 16** for more information on changing the Data Reduction Mode.



STOPPED FLOW SENSOR MAP

This display shows the current readings of the stopped-flow sensors and will only be present if instrument is equipped with a stopped-flow apparatus. When in **Live Mode**, this map will continually update. A similar display is shown on the front of the Electronics box. In order for the stopped flow to fire, no sensors can be showing red as its sensor state.

FS and **BS** refer to the front and back syringes, respectively. The syringes must be in contact with the pneumatic drive block in order for these sensor states to not be red.

BH and **FH** are the respective front and back handles. These move the syringe valves from **Fill** to **Flow**. These valves must be on **Flow** to fire the stopped flow, and **Fill** to fill the syringes.

SS refers to the stop syringe. The plunger in the stop syringe must be down for the stopped-flow to fire. This can be pressed down by moving the exhaust valve to empty and pushing down on the shaft. The syringe will be in the up position after each firing of the stopped-flow.

EH refers to the exhaust handle. This valve must be in **Flow** position for the stopped flow to fire.

When all valves and syringes are in their proper positions for firing, the **Ready** light will become green. If data collection is attempted when the **Ready** light is not green, the stopped flow will not fire, and the program will go to this map to tell the user to move the offending valve or syringe.



GET SF STATE

If the instrument is not in **Live Mode**, click on this button to update the sensor map. This will detect any valves or syringes, which are in an inappropriate position.



FIRE STOPPED-FLOW

Clicking this button will cause the stopped-flow to fire without collecting data as long as the **Ready** light on the **Stopped-Flow Sensor Map** is green.

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DISABLE STOPPED-FLOW

Clicking on this checkbox disables the stopped flow such that when **Data Collection** is chosen, data are collected, but the stopped-flow does not fire. This mode is especially useful for diagnostic purposes and is not available in Rapid Scanning Stopped Flow.

To disable stopped flow in Rapid Scanning Mode, change the data collection mode to Rapid Scanning rather than Rapid Scanning + SF. This will result in identical data collection, but no firing of the stopped-flow.



SENSOR ADJUST

This value is an adjustment parameter unique to each scan disk. When a different scan disk is installed, this number should be changed to match the number printed on the inside of the disk. If there is no number, contact Olis for assistance in determining this adjustment value.



SET TTL DELAY

For Stopped flow only. Click this button to change the delay between the start of data collection and the firing of the stopped flow. This allows for collection of background signal before shot is fired and is limited to 65 milliseconds.



RECOVER SAFE DATA

A backup of each dataset is created after each data collection. Upon normal exiting of the program, these files are erased. In the event of a power failure or other disruption of data collection, previously collected data can be restored by clicking on this button and selecting the desired dataset to restore.

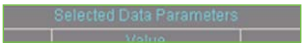
2-D Data Display

Data display for 2-D datasets.



COMMENTS

This section allows for the user to add comments that will remain with the file when it is saved. After data collection, useful instrument settings will be reported here.

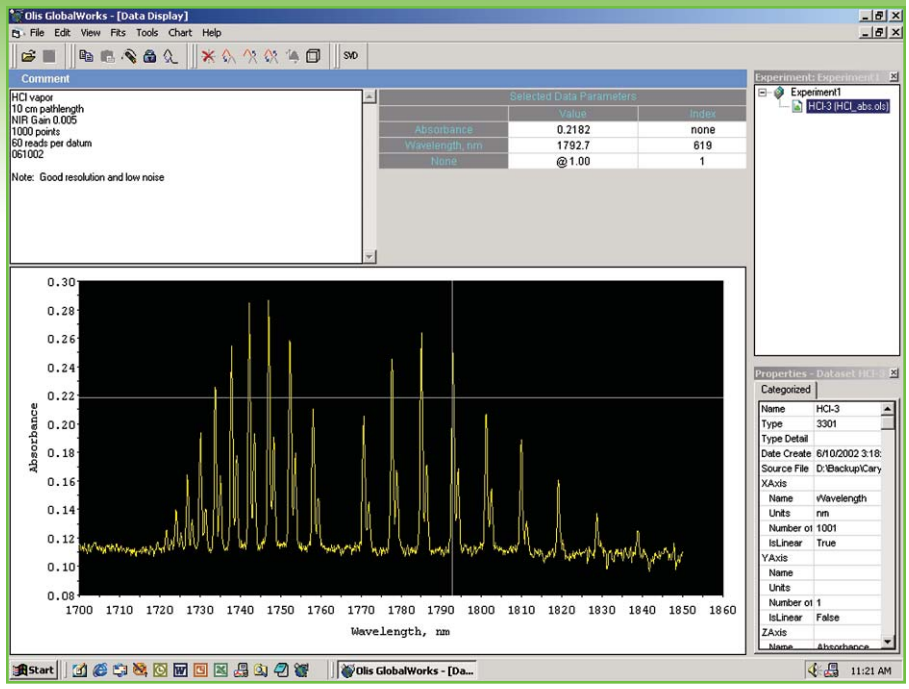


SELECTED DATA PARAMETERS

This table indicates the location of the cursor, which can be activated by clicking on the data.

The X and Y values are recorded under **Absorbance** and **Wavelength**. None refers to the fact that there is no third axis (i.e. the data is 2-dimensional).

The **Index** column refers to the datum number of the data set. Only independent variables will have an index value (e.g., time, scan number, wavelength).





EXPERIMENT WINDOW

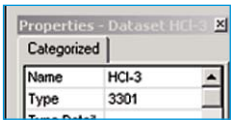
Every open window, including the data collection window (not shown above) is listed here. When a new data set or fit is generated, a new entry is added to this window.

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Move between windows by clicking on the data sets in this window.

To delete a window, make it the current window, and click in the lower X in the right corner.

Data can also be selected for data processing here by right clicking and choosing Select. The data processing options become active in the right click menu. Among these options are arithmetic, smoothing, and averaging of data.



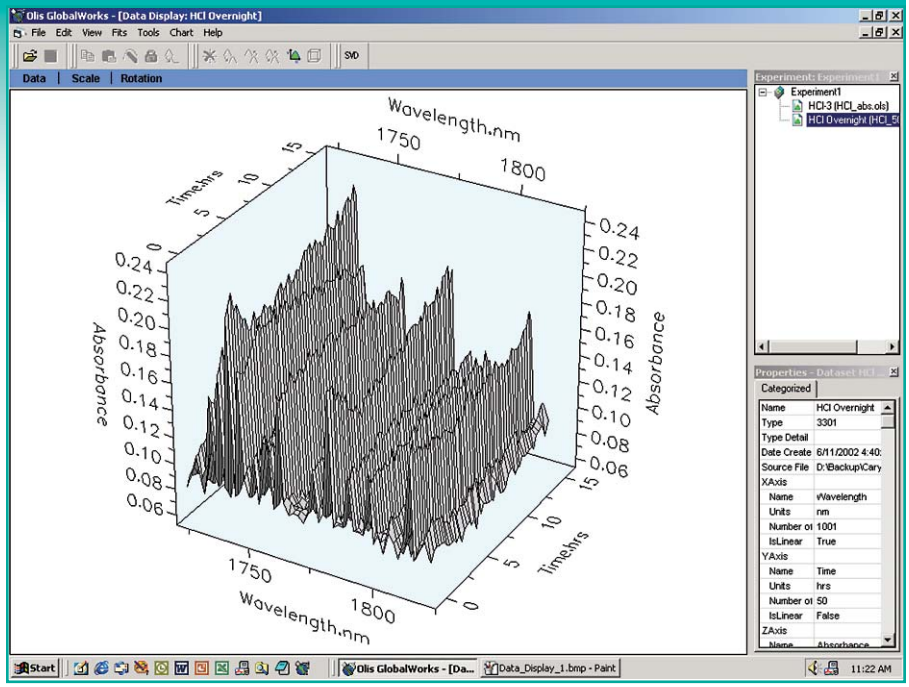
PROPERTIES WINDOW
This window lists properties of the file being displayed, such as number of data points, date collected, and data set name.

The data set name can be changed here by double clicking on Name and entering in a new name followed by the return key. This is not the file name, but only a name for display that will be saved with the data.

Data can be saved by selecting Save As under the **File** menu.

3-D Data Display

Data display for 3-D datasets.





2-D DATA VIEW
Pressing this button displays the 3-D data as two 2-D graphs. In this case one graph would have overlayed spectra and the other has the kinetic trace at one wavelength.

See **page 24** for more information on 2-D Data Display.



SVD
This button initiates analysis of the data using the Singular Value Decomposition algorithm. This process can be used to globally fit 3-D data or as a noise filter for the data. A tutorial on SVD is available in the **Startup Menu**.



DATA
The number of scans to be displayed are changed here. A step of "1" shows all data up to 200 scans.



SCALE
Clicking this option allows the axis scales to be changed. Autoscale is also available.

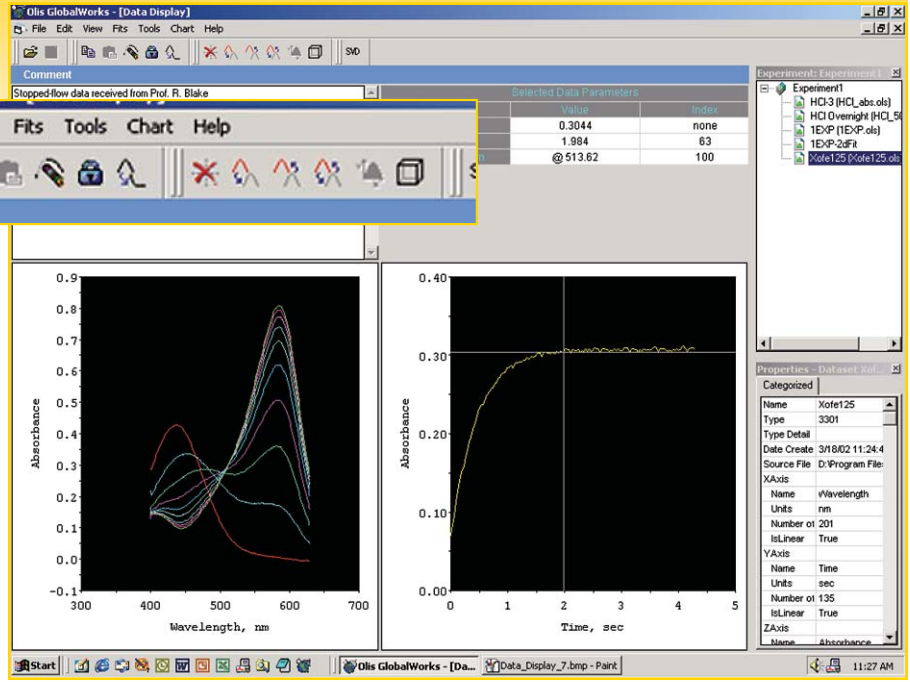


ROTATION
Clicking this option allows the view to be changed. Degree rotation of axes can be entered or the graph can be dragged after checking the **Rotation** box.

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2D Data Display (Multiple Trace)

Displays datasets as 2-D slices. Allows simultaneous view of overlayed spectra and kinetic trace.





LOCK ON SLICE
Once the cursor is active by clicking on data, you may select this option to lock on a particular slice of data. Moving the cursor allows you to travel along the selected scan exclusively. Information on the "locked on" slice will appear dynamically in the **Selected Data Parameters** window.



DYNAMIC SLICING
Clicking on this button after selecting a scan from the collected data will dynamically display slices at the selected wavelength in the other graph. This option is available only in the 2-D view.



REMOVE CURSOR
Once a slice is selected and the cursor appears, this button will deactivate the cursor.



HIDE SLICE
When the data cursor is active on a slice, this option allows you to remove from display to a hidden clipboard.



UNHIDE SLICE
When this option is selected all previously hidden slices will reappear. If multiple scans are hidden, all will reappear simultaneously.



SWAP SLICES
This feature allows you to swap visible slices with slices that have been hidden with the **Hide Slice** option.



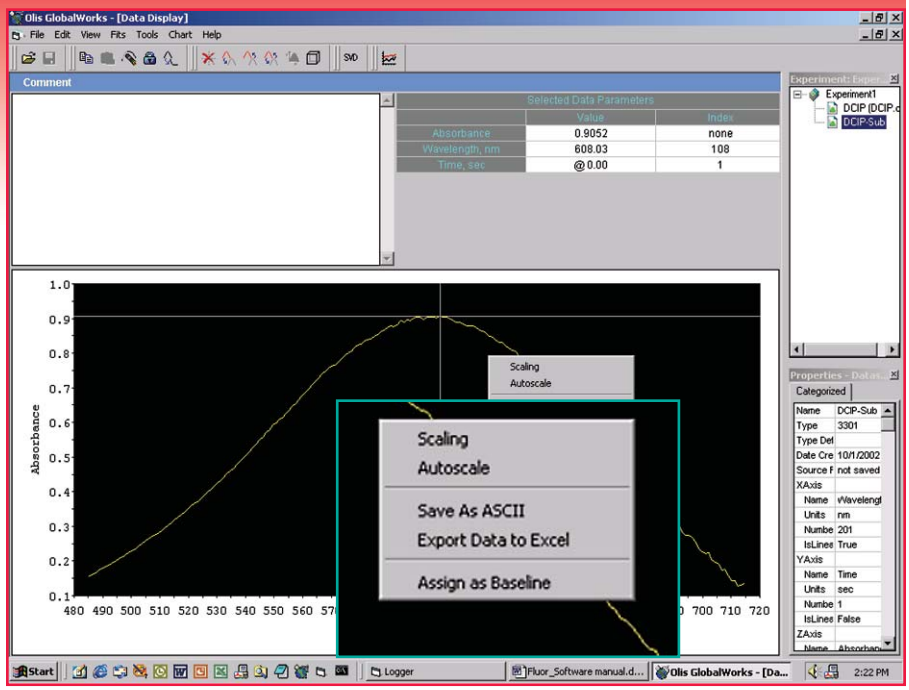
3-D VIEW
This option allows viewing of the 3-D data sets a 3-D image. The 3-D image can then be manipulated.

See **page 23** for more information on 3-D Data Display.

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Data Display Options menu

This menu can be accessed by right-clicking on the data.



SCALING
To change the scale of either the X or Y axis, select this option. A window will appear to prompt the new scaling values.

AUTOSCALE
Choosing this option causes the X and Y axis to be autoscaled.

SAVE AS ASCII
This option allows for a 2-D dataset to be exported as a two column, tab-delimited ASCII text file with no headers or footers.

If this option is selected for a 3-D dataset, the highlighted 2-D dataset will be exported.

EXPORT DATA TO EXCEL
This feature will open the current data set (2-D or 3-D) into Microsoft Excel as a spreadsheet. Micrososft Excel must be installed on the computer for this feature to work.

ASSIGN AS OPEN
Clicking this option assigns the highlighted scan as the "open" scan for scan correction. The "open" scan should be a scan with no sample in the beam and should ideally give a 100% transmission.

ASSIGN AS BLOCKED
Selecting this option assigns the highlighted scan as the "blocked" scan for scan correction. This scan is taken with the shutter closed and should ideally give 0% transmission.

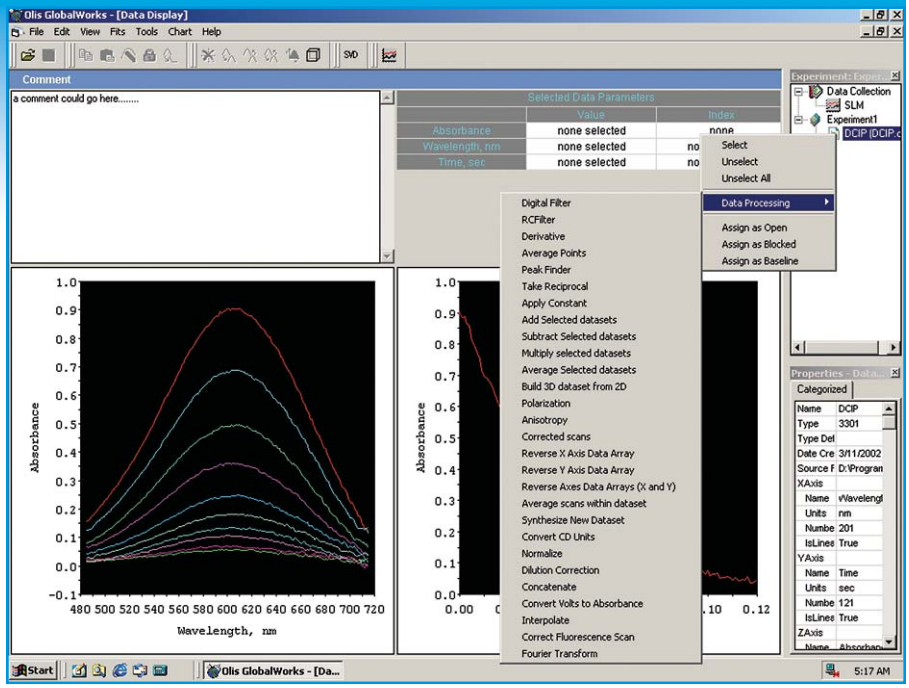
ASSIGN AS BASELINE
Selecting this option will cause the highlighted scan to be assigned as a baseline, causing this dataset to be subtracted from subsequent measurements. "Baseline" will appear in the **Live Display** and the **Apply Baseline** checkbox will be checked. Data collected must be equal to the baseline in number of points and scan range in the **Properties** window.

See **page 10** for more information on the Apply Baseline checkbox.

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Data Processing Menu

All data processing steps are given in this menu. The highlighted dataset must be selected for this menu to become activated. This menu is also available under the Tools menu.



SELECT

To select a dataset:

- Highlight the desired dataset by left-clicking on it.
- Right-click on the dataset.
- Choose **Select** to make the dataset available for processing, A red checkmark will appear beside the dataset when it is selected.

UNSELECT

This option will unselect the highlighted dataset.

UNSELECT ALL

This option will unselect all datasets.

APPLY CONSTANT

To add, subtract, multiply or divide by a constant choose **Apply Constant**. You will then be prompted to choose the appropriate operation and constant. The resulting data set will be added to the **Experiment** window.

ADD SELECTED DATASETS

This option will sum all datasets which are selected. The resulting dataset will be added to the **Experiment** window.

Datasets must be of the same type and contain identical x-axes, as well as the same number of data points to be added. An error message will be displayed if an inappropriate data set is selected.

SUBTRACT SELECTED DATASETS

Choosing this option with two data sets selected will subtract the second data set from the first. The difference dataset will be added to the **Experiment** window.

As with Adding, the datasets must possess identical x-axes. An error message will be displayed if an inappropriate data set is selected.

MULTIPLY SELECTED DATASETS

This option will cause the selected datasets to be multiplied. The product dataset will be displayed in the **Experiment** window.

The datasets must have identical x-axes to be multiplied. An error message will be displayed if an inappropriate data set is selected.

AVERAGE SELECTED DATASETS

This option will average two or more sets of data with identical x-axes. The resulting averaged data is added to the **Experiment** window.

SMOOTHING

This option allows for a *Savitzky-Golay* smooth of the selected data and prompts the user to select from a 3-25 point smooth. The smoothed dataset will be added to the **Experiment** window.

Note: It is important that care is taken to avoid over filtering the data.

DERIVATIVE

This option calculates the derivative of the dataset and adds it to the **Experiment** window. First through fourth derivatives are available.

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RC FILTER
This option applies a simulated time constant to time course data. The time constant must be specified (in sec or msec) and cannot exceed the length of the dataset. *A rule of thumb for the maximum time constant without distorting the data is 5-10% of the total time.*

Note: It is important that care is taken to avoid over filtering the data.

REVERSE DATA ARRAYS
This option changes the order in which the data is processed. This has no effect on the data but may be necessary for performing SVD on some datasets.

AVERAGE SCANS WITHIN A DATASET
This option averages multiple scans within a dataset. The resulting dataset will be a 2-D dataset added to the **Experiment** window.

There is also an option to perform this operation during data collection. See **page 19** for more information on averaging scans during data collection.

BUILD A 3-D DATASET FROM 2-D
This option will convert all selected datasets to a 3-D file. The selected datasets must be 2-D, and each dataset must contain consistent values for Y-axis name, units, and a value for each scan.

In addition, the X-axes must be compatible: they must have the same range and number of data points.

SYNTHESIZE NEW DATASET
This option prepares a synthetic dataset. After selecting this option enter the relevant information in the prompt and click on "Synthesize". *The only algorithm currently available is "linear."*

POLARIZATION
This option converts two selected polarization of fluorescence spectra to polarization units.

The selected data sets must be one spectra calculated with vertical emission polarizer and one with a horizontal emission polarizer. The resulting polarization spectrum will be reported in the **Experiment** window.

ANISOTROPY
This option converts two selected polarization of fluorescence spectra to anisotropy units.

The selected datasets must be one spectra calculated with vertical emission polarizer and one with a horizontal emission polarizer. The resulting spectrum will be reported in the **Experiment** window.

CORRECTED SCANS
Choosing this option corrects transmission scans for intensity variations. To correct a spectrum, transmission spectra obtained with the beam blocked and beam open must be selected, along with the spectrum to be corrected. The resulting corrected spectrum will be added to the **Experiment** window.

ASSIGN AS OPEN
Clicking this button assigns the selected scan as the "open" scan for scan correction. This is defined as a scan with no sample in the beam and should ideally give 100% transmission.

ASSIGN AS BLOCKED
This option assigns the selected scan as the blocked scan for scan correction. This scan is taken with the shutter closed and should ideally result in 0% transmission.

CONVERT CD UNITS
This option allows circular dichroism spectra to be converted to other units. The available units are **degrees**, **millidegrees**, **absorbance units**, **milliabsorbance units**, **molar extinction**, and **molar ellipticity**.

See **Paul's Practical CD Guide** for more detailed information about circular dichroism units.

NORMALIZE
This button results in division of the selected dataset by the largest intensity in the dataset. The result is a dataset which has been internally normalized. This is useful for comparing shapes of spectra of different intensities.

DILUTION CORRECTION
This option corrects the selected dataset for effects of dilution and is only available for datasets collected with an Olis titration accessory.

Each scan is corrected by the factor:

$$\frac{V_{current}}{V_{initial}}$$

where Vcurrent is the total volume of the sample for the current scan and Vinitial is the starting sample volume.

CONCATENATE
This option allows two selected spectra from assays to be merged into one dataset. If the datasets overlap, the software will prompt to either choose a dataset or average the overlapping datasets.

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CURRENT VOLTS TO ABS
This function is used to convert a single beam time trace into an absorbance trace using any region of the curve as a reference value.

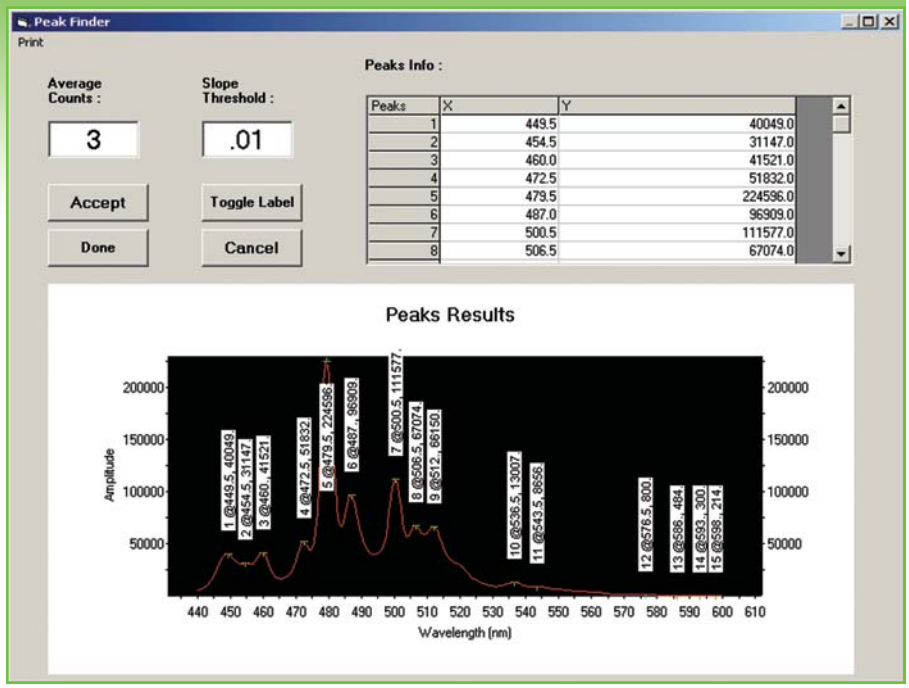
INTERPOLATE
This function is used to change the number of data points or interval between data points in the selected dataset. A new dataset range and number of intervals are chosen and the data is fit by a cubic spline. The new data is computed and added to the **experiment** window.

CORRECT FLUORESCENCE SCAN
This function is used to correct a fluorescence scan for effects of wavelength changes on the sensitivity of the instrument. A correction file is chosen to be applied to the data. *Contact an Olis representative for more information on generating a correction file.*

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Peak Finder

This data process determines the location of peaks along a dataset by averaging the slope in given point counts and determining when that slope shifts from positive to negative.



Average Counts :

AVERAGE COUNTS
The number of points to be averaged at a time in order to calculate the slope of the data is entered here. Increasing this number has the effect of decreasing the sensitivity to sharp peaks. Thus, a dataset which contains broad peaks punctuated by noise would require a relatively large number of average counts. Conversely, a small number of average counts is useful for identifying numerous sharp peaks.

Slope Threshold :

SLOPE THRESHOLD
This is the criterion for determining if a change in slope constitutes a peak or noise. The lower this value, the lower the intensity of the peaks that will be identified. Typical values will range from 0.01 to 1.

Peaks Info :

PEAKS INFO
The identified peaks will be listed in this table after the **Average Counts** and **Slope Threshold** have been accepted.

Accept

ACCEPT
Click this button to accept the current **Average Counts** and **Slope Threshold**. This will result in the identified peaks being reported in the **Peaks Info** graph.

Toggle Label

TOGGLE LABEL
Click this button to add or remove labels for the peaks on the **Peaks Results** graph.

Done

DONE
Click this button when you are finished. The peak values in the **Peaks Results** graph will be included in the data set comments, and **Peak Finder** will be exited.

Cancel

CANCEL
Clicking the **Cancel** button exits peak finder without performing a peak fit.

Peaks Results

PEAKS RESULTS
The location of the peaks which are identified are shown graphically here. The labels can be turned on or off by clicking on **Toggle Label** button.

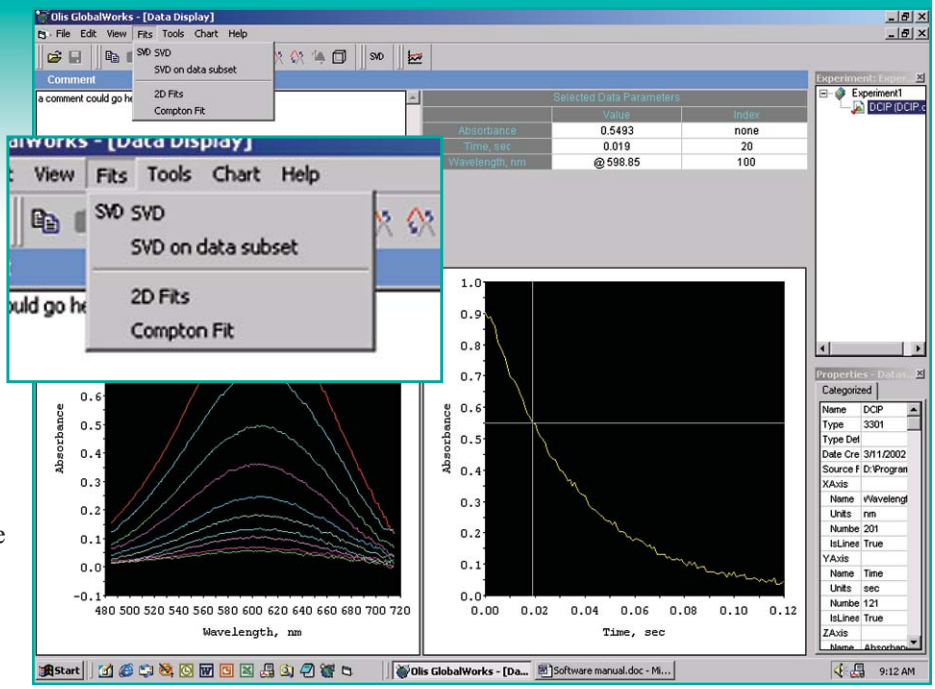
Print

PRINT
Choosing this option prints a report containing the identified peak values and the graph in the **Peaks Results**.

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Data Fits Menu

2-D or 3-D datasets can be fitted by one of many mechanisms.



SINGULAR VALUE DECOMPOSITION (SVD)
Choosing this option initiates the process of singular value decomposition (SVD). The highlighted dataset must be 3D and contain at least ten points in each axis. Please see the SVD tutorial in the startup screen (page 2) for more detailed instructions on performing SVD.

SVD ON DATA SUBSET
This process initiates the SVD process on only a portion of the highlighted dataset. The desired data ranges will be prompted by the program. This option is useful if a portion of the collected data is unusable or contains little information.

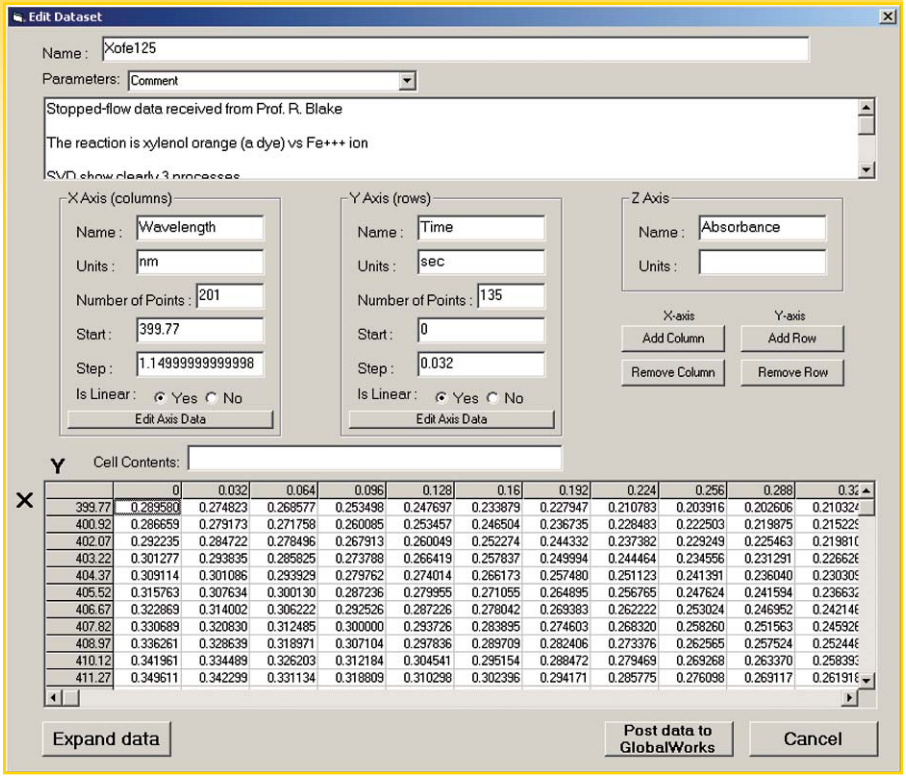
2D FITS
Two dimensional kinetic data can be fitted by selecting the kinetic trace by clicking on the curve, and choosing this option. A list of possible kinetic mechanisms will be displayed. Choose the appropriate kinetic mechanism, and the resulting kinetic fit will be added to the Experiment Window. This window contains kinetic parameters, residuals, and the calculated data.

COMPTON FIT
This menu item initiates the Compton secondary structure analysis algorithm for analysis of a protein circular dichroism spectrum. The highlighted spectrum must be from 260 nm to 178, 180, 182, or 184 nm and be 2 nm/point. After this option is chosen, the fit results will be shown in place of the comments. The fractional contribution of five secondary structure motifs will be displayed. These motifs are alpha helix, antiparallel beta sheets, parallel beta sheet, beta turn, and other structure. See ‘Paul's Practical CD Guide’ for more information about the Compton fit.

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Edit Dataset

Allows for data to be viewed in speadsheet form.



NAME
Displays the dataset name. This is not the filename and can be changed without affecting the filename.

PARAMETERS
This contains a list of options to display in the text box below. The options are **Comment**, **Date of File Creation**, and **Sourcefile Name**. Only **Comment** can be edited here.

All data text can be edited in the text box; however, only **comment** text will be changed in the dataset.

AXIS NAME
Displays the axis name, which can be edited. *To build 2-D scans into a 3-D scan they must have the same axis names in all axes.*

AXIS UNITS
Displays the axis units, which can be edited. *To build 2-D scans into a 3-D scan they must have the same axis units in all axes.*

NUMBER OF POINTS
This reports the number of points along this axis. This value cannot be edited. Rows or columns can be deleted as described in "Remove Column" and "Remove Row."

START
Indicates the value of the first data point along this axis.

STEP
Indicates the interval between data points if the axis is linear (i.e., has equal spacing between points).

IS LINEAR
Indicates whether there is equal spacing between points along an axis. If **Is Linear** is "Yes" then the axis can be described by the number of points, start, and step. `Value=Start+(N-1)*Step`, where `N` is the current data point.

EDIT AXIS DATA
Clicking on this button allows the X or Y axis data to be changed.

See **page 33** for more information on the Edit Axis Data window.

ADD COLUMN
Use this button to add an additional column (Y data point) to the end of the dataset. To enter the Y value, choose **Edit Axis Data**.

See **page 33** for more information on the Edit Axis Data window.

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REMOVE COLUMN
Use this button to remove a column from the end of the dataset. To remove a column or columns other than the end column, select data to be removed, right-click on the data, select **Axis Options**, and choose **Remove Axis Point(s)**.

ADD ROW
Use this button to add an additional row (X data point) to the end of the dataset. To enter the X value, choose **Edit Axis Data**. Enter or paste data using cut and paste.

See **page 33** for more information on the Edit Axis Data window.

REMOVE ROW
Use this button to remove a row from the end of the dataset. To remove a row or rows other than the end column, select data to be removed, right-click on the data, select **Axis Options**, and choose **Remove Axis Point(s).**

CELL CONTENTS
This box displays the contents of the selected cell. Data can be edited in this box. Pressing the Enter key or clicking on another portion of the screen will enter this data in the dataset.

X-AXIS
Data in the first column constitutes the X-axis of the dataset. This data can be edited by clicking on **Edit Axis Data** in the X-axis window.

See **page 33** for more information on the Edit Axis Data window.

Y-AXIS
Data in the first row constitutes the Y-axis of the dataset. This data can be edited by clicking on **Edit Axis Data** in the Y-axis window.

See **page 33** for more information on the Edit Axis Data window.

EXPAND DATA
Clicking on this button enlarges the data window to fit the entire **Edit Dataset** window. Clicking on **Shrink** returns to the original condition.

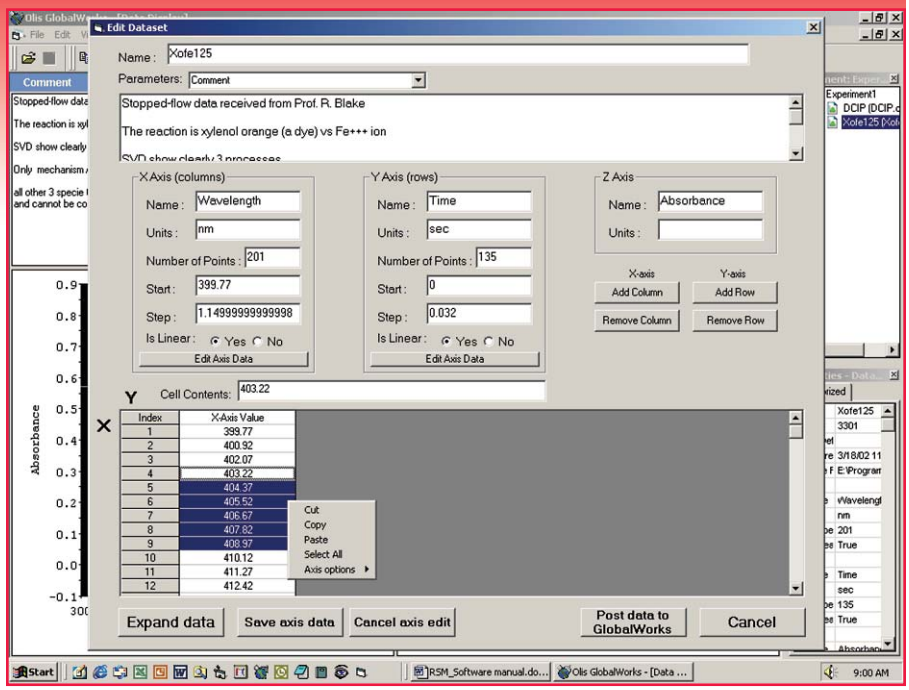
POST DATA TO GLOBALWORKS
Clicking this button stores the data in the **Experiment** window as a new dataset. The dataset can be saved, viewed in the chart window, fit, or discarded.

CANCEL
Quits **Edit Dataset** without saving any changes.

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Edit Axis Data

Allows axis values to be changed.



CUT
Copies selected data to Windows clipboard and deletes it from the data set. The data are then available to paste into other Windows applications.

COPY
Copies selected data to Windows clipboard without removing it from the data set. The data are then available to paste into other Windows applications.

PASTE
Inserts data from Windows clipboard into selected data window. This will overwrite any data that are already present.

SELECT ALL
This option selects the entire dataset.

AXIS OPTIONS
This option allows the selected data to be deleted. This option is only enabled while in **Edit Axis Data**.

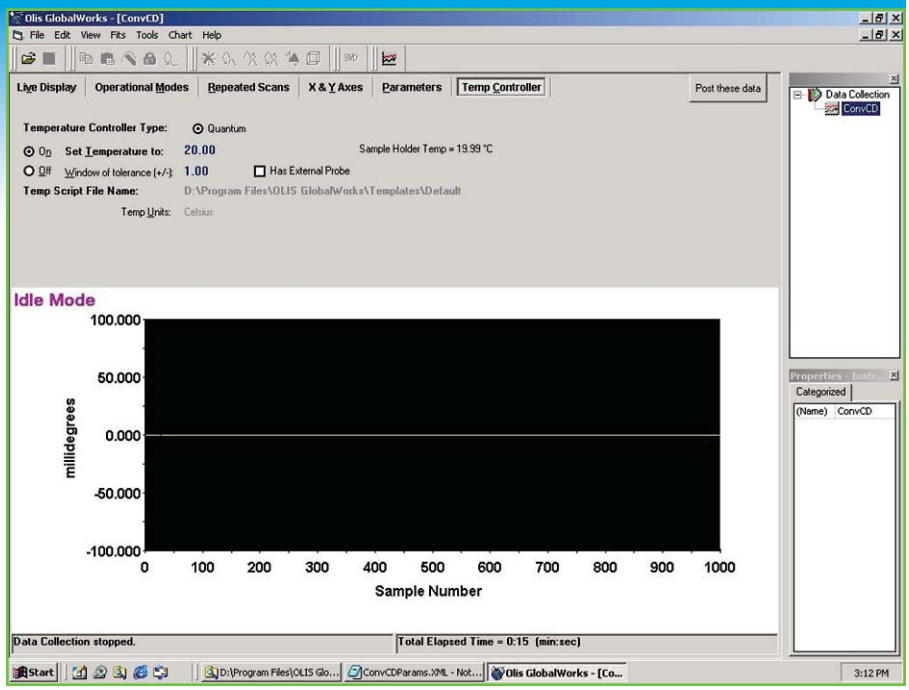
SAVE AXIS DATA
Clicking this button will save any changes made to the axis data and return to the full data set view. This must be done before clicking **Post data to Globalworks** if changes in axis data are to be retained.

CANCEL AXIS EDIT
This button returns the **Edit Dataset** form to the full data view without saving any changes made to the axis.

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Temperature Controller

Provides software control for Olis-compatible temperature controllers.





TEMPERATURE CONTROLLER TYPE

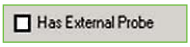
If the instrument is equipped with more than one type of temperature controller, the active temperature controller is chosen here. The current choices possible are Quantum (Peltier controlled cell) and Julabo (water bath). Only one choice will be available if the instrument is only equipped with one.



SAMPLE HOLDER TEMPERATURE

This is the current reading of the sample holder and is only updated if the instrument is in **Live Mode**. With the Peltier (Quantum Northwest) this temperature represents than inside the cell holder. In the case of the water bath (Julabo), this temperature is the temperature of the bath.

See **page 10** for more information about Live Mode.



HAS EXTERNAL PROBE

This checkbox indicates whether the temperature controller is equipped with an external temperature probe. This probe would be inserted into the cuvette holder, or into the sample itself, to give a more accurate temperature reading of the sample. An additional field will report the probe temperature.



ON/OFF

This switch controls whether the temperature controller is active. If **Off** is selected, no communication will be attempted between the software and the temperature controller. If **On** is selected the temperature controller unit must be on, or errors will result.



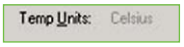
SET TEMPERATURE

This is where the set point temperature on the bath or Peltier cell is set. The temperature should immediately approach this value if the controller is active. The only time this is not the set point is when a temperature script is being run.



WINDOW OF TOLERANCE

The window of tolerance is the range of temperatures in which the current temperature is equal to the set point temperature. For example, if during the running of a temperature script with the current temp set at 40°C and a window of tolerance of ±1°C, the set temperature will be achieved when the current temperature reaches 39-41°C. The actual temperature, however, will be recorded with the data.



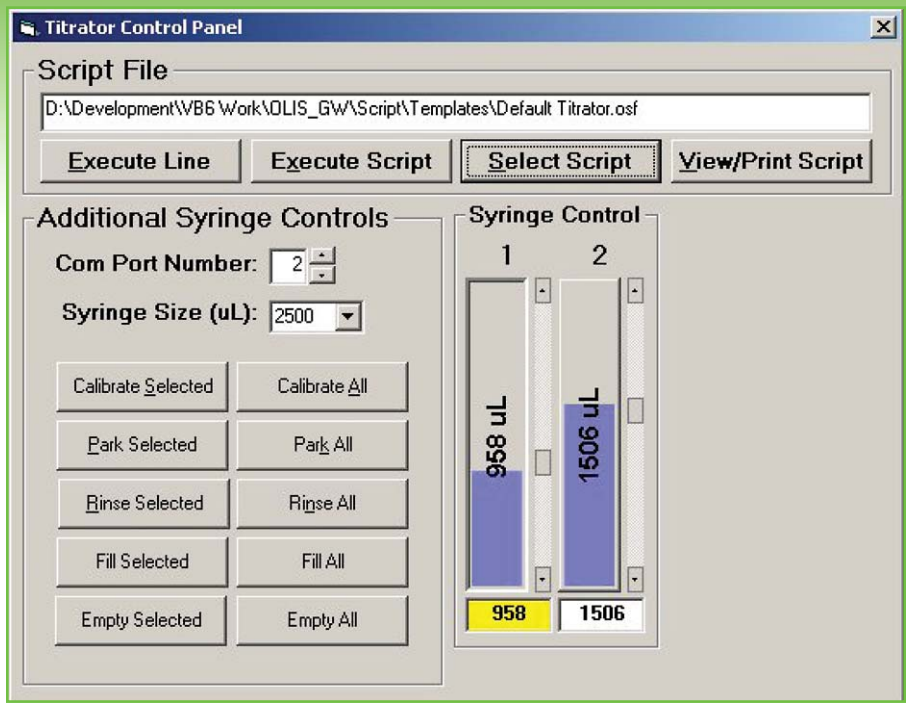
TEMPERATURE UNITS

Only Celsius units are available.

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Titrator Control Panel

Allows for software control of the Olis titrator. Access to this screen is in the repeated scans page.





SCRIPT FILE
This designates the current script file name. It is identical to that shown in the Repeated Scans tab.

See **page 18** for more information on the Repeated Scans tab.



EXECUTE LINE
Click on this button to execute a line of the script *without collecting data*. The software will prompt for which line to execute. The syringes will move, but no data will be collected.



EXECUTE SCRIPT
Click on this button to execute an entire script *without collecting data*. The syringes will move, but no data will be collected.



SELECT SCRIPT
This button allows the user to select a valid titration script. Changes in the script in this window will change it in the others as well.

See **page 36** for a sample titratrion script file.



VIEW/PRINT SCRIPT
This button allows the user to open the script for editing or printing. *Note: Do not attempt to change the "Default Titrator.osf" script as it is a read only file and is necessary as a backup script file.*

See **page 19** for details on editing script files.



COM PORT NUMBER
Ensure that this number matches the com port number that the titrator is actually using.



SYRINGE SIZE (uL)
Ensure that the syringe size in this display matches the actual syringe size for the selected syringe.

To select a syringe, click on the desired syringe in **Syringe Control**. The text box below the syringe will become yellow, indicating it has been selected. Changing the syringe size changes the size of only the selected syringe.



CALIBRATE SYRINGE
These buttons initiate a calibration procedure. During calibration, the syringe fills completely until a sensor is reached, whereupon the syringes empty. It is important to have the valve in “Fill” position and have enough sample in the fill syringe. If samples are precious, it is important that calibration is done using water or buffer.

Calibrate Selected only calibrates the selected syringe, while **Calibrate All** initiates the calibration procedure for all syringes.

*The keystroke shortcuts for Calibrate Selected and Calibrate all are **S** and **A**, respectively.*

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PARK SYRINGE

When one of these buttons is pressed, one or more of the syringes fills or empties to half full.

Park Selected parks only the selected syringe, while **Park All** moves all of them.



RINSE SYRINGE

Rinsing syringes involves alternating filling and emptying them. When clicking one of these buttons, the software will prompt to ask the number of desired rinses (1-99). It is important to have the valve in "Fill" position and have enough sample in the fill syringe. If samples are precious, it is important that calibration is done using water or buffer.

Rinse Selected rinses only the selected syringe, while **Rinse All** initiates the procedure for all of them.

*The keystroke shortcuts for Rinse Selected and Rinse all are **R** and **N**, respectively.*



FILL SYRINGE

These buttons cause syringes to completely fill. It is important to have the valve in "Fill" position and have enough sample in the fill syringe. If samples are precious, it is important that calibration is done using water or buffer.

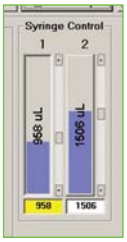
Fill Selected rinses only the selected syringe, while **Fill All** initiates the procedure for all of them.



EMPTY SYRINGE

These buttons cause syringes to completely empty. It is important to have the valve in the desired position so the contents of the syringe are not inappropriately expelled. If samples are precious, it is important that calibration is done using water or buffer.

Empty Selected rinses only the selected syringe, while **Empty All** initiates the procedure for all of them.



SYRINGE CONTROL

This control allows the user to manually manipulate the syringe volume. The current volume is displayed in the box at the bottom of each virtual syringe. This volume can be changed by entering in a new value (in uL) or dragging the sidebar control to the desired approximate volume. Filling of the syringe is graphically illustrated by the blue shading.

Sample Titration Script

```
[Type]
Titrator

[Comment]
Comment Section.

Any amount of text and/or white space may be put into the Comment
Section.  Any comments outside of the comment section (line
comments) MUST be preceded with a semi-colon, and all text
following a semi-colon on a single line is ignored.
```


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Script files are divided into different sections by section headers (header names enclosed in square brackets). Section headers must start in the first column, and no other data, including comments, can follow them. Different types of scripts will contain different sections. The only sections guaranteed to be in every script file are Type and Scans. A comment section is optional but can also be placed in any script file.

```
[Scans]
10    ;Number of scans/assays to take

[Cuvette Volume]
;This is the volume (in uL) that is put into the cuvette to make
;the measurement
500

[Starting Volumes]
;    Syringe
; 1     2
1000 1000  ;These are the volumes (in uL) that will be drawn into
           ;the syringes prior to the beginning of data collection.
           ;You must supply a volume for each syringe to be used
           ;and this volume must  equal to or greater than the sum
           ;of all additions to be made from each syringe and less
           ;than or equal to the maximum volume of the syringe.

[Scan Data]
;Data for each scan consists of Titrant to be added (in uL),
;Incubation time (in minutes) and mix cycles. There is always at
;least one mix cycle, any specified here are in addition to the
;mandatory one mix cycle.
;  Titrant    Incubation   Mix Cycles
     50           1           1
     50           1           1
    100           0           0
    100           0           0
    100           0           0
    100           0           0
    100           0           0
    100           0           0
    100           0           0
    200           0           0
```


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Sample Temperature Script

```
[Type]
Temperature

[Comment]
Comment Section.
```

Any amount of text and/or white space may be put into the Comment Section. Any comments outside of the comment section (line comments) MUST be preceded with a semi-colon, and all text following a semi-colon on a single line is ignored.

Script files are divided into different sections by section headers (header names are enclosed in square brackets). Section headers must start in the first column and be alone on the line. Different script types will contain different sections. The only sections guaranteed to be in every script file are Type and Scans. A comment section is optional but can also be placed in any script file.

```
[Scans]
10    ;Number of scans/assays to take

[Scan Data]
;Data for each scan consists of a temperature (in degrees C) and
;an incubation time (in minutes) for the machine to wait before
;collecting data after the temperature control device has reached
;the desired temperature.

;    Temp   Incubation
      20          1
      25          0
      30          0.5
      35          2
      40          0
      45          1
      50          0
      55          0
      60          0.5
      65          0
```


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Appendix I: Polarization and Anisotropy

Polarization and Anisotropy measurements on Olis instruments are based on those described by Lakowicz[1]. In L-format polarization measurements, only a single emission channel is used. The instrument consists of a light source, excitation monochromator, excitation polarizer, sample chamber, emission polarizer, emission monochromator (possibly replaced by a filter), and a detector. To make the polarization measurement, the emission channel must be corrected for effects of polarization on the detected signal with a gain-factor (G-factor). This is collected with the excitation polarizer in the horizontal position and intensity is measured with the emission polarizer in horizontal (IHH) , then the vertical (IHV) position. The G-factor is defined as the ratio of vertical to horizontal intensities (Equation 1).

$$G = IHV/IHH \qquad (1)$$

Once the G-factor has been obtained, the polarization or anisotropy measurements are made with the excitation polarizer in the vertical position. As with the G-factor, data is collected as a ratio of signal with a vertical emission polarizer to data with a horizontally positioned polarizer (IVH and IVV). This is corrected for the G-factor to give $I_{\parallel}/I_{\wedge}$ as described by Equation 2.

$$\frac{I_{\parallel}}{I_{\wedge}} = \left(\frac{1}{G}\right)\left(\frac{IVV}{IVH}\right) \qquad (2)$$

From $I_{\parallel}/I_{\wedge}$, the polarization (p) and anisotropy (r) can be obtained from Equations 3 and 4.

$$p = \frac{\frac{I_{\parallel}}{I_{\wedge}} - 1}{\frac{I_{\parallel}}{I_{\wedge}} + 1} \qquad (3)$$

$$r = \frac{\frac{I_{\parallel}}{I_{\wedge}} - 1}{\frac{I_{\parallel}}{I_{\wedge}} + 2} \qquad (4)$$

In T-format polarization, two emission channels are used (with monochromators or filters). The emission polarizers are fixed with one channel in the vertical position and the other in the horizontal position. The G-factor is collected as described for the L-format, except that IVV and IVH are collected simultaneously. Thus, the G-factor is collected as the ratio of emission channel 1 (vertical emission polarizer) to emission channel 2 (horizontal polarizer) with the excitation polarizer in the horizontal position. The polarization data is collected as the same ratio with the excitation polarizer in the vertical position, corrected for the G-factor (Equation 2). Polarization and anisotropy are calculated as described in Equations 3 and 4.

[1]Lakowicz, J. R. (1999) Principles of Fluorescence Spectroscopy, Second Edition, Kluwer Academic/Plenum Publishers, New York, pp. 291-319.

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Appendix II: A Bit about the Administration Form

Every Olis instrument uses a parameters.xml file to store information. This file is located in the Olis Globalworks\ParaXML\ directory. Included in this file are all of the necessary information for the Spectralworks program to operate your instrument. These include calibration numbers, number of detectors, number of monochromators, number of accessories, delay times, and previous instrument settings. This is an important file and the instrument will not run correctly if this file is corrupted. Because of this, you should not edit this file directly unless directed to do so by Olis. There are some values of this file, however, that you may need to access occasionally. This can be achieved from the data collection program by simultaneously pressing 'Shift'+'Ctrl'+'Alt'+'a' to open the administration form. This form is divided into several links, each of which contains information about a device on the instrument. For example, clicking on the 'Monochromators' link contains information such as calibration wavelength, motor delay, and motor direction for the monochromators. Use extreme care in editing this form as hardware damage can result from running the instrument with incorrect settings. The following table describes some parameters which may at one time or another be changed.

Parameter	Location (link)	Description
Calibration wavelength	Monochromator	Determines the wavelength assigned to the sensor position on the monochromator. This should be adjusted if the wavelength is believed to be off.
Stop Adjustment time	PCI416	An adjustment to the time in which the stopped flow calls zero time. The units are milliseconds after the stop syringe has stopped. This should be adjusted if the zero time of the stopped flow reaction is not correct.
A/D sampling rate	PCI416	The rate in which the A/D board streams in data. The upper limits are 2 and 1 MHz for 12 and 14-bit A/D boards, respectively.
Number of Points (Stopped flow)	PCI416	The number of points after zero time for stopped flow. This value is typically about 1000.
Response delay time	PMT	This is the time the instrument waits after turning on (or changing HV) a detector before collecting data

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On-Line Instrument Systems, Inc.
130 Conway Drive, Suites A & B, Bogart, GA 30622
(800) 852-3504 • (706) 353-6547
techsupport@olisweb.com • www.olisweb.com

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