

Data Analysis Software User Manual

Version 1.1

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Chapter 1: About the Data Analysis Software

Program Description

The C&L Data Analysis package is an easy-to-use 32-bit software application designed for use with the C&L Dye Fluorometer. It offers powerful data analysis features using a standard Windows[®] interface. The software allows the user to:

- View data from your experiments, collected with the C&L Dye Fluorometer and the Data Acquisition software
- Analyze the fluorescence and analog data from your experiments
- Export data to other software programs such as Microsoft Excel[®]
- Run concurrently with the C&L Instruments Data Acquisition Software, in another program window, so you can analyze one experiment while taking data from another

System Requirements

The Dye Fluorometer software requires the following minimum configuration:

- Pentium[®] 133 MHz computer
- Windows[®] 95 or Windows[®] 98 operating system
- 16 MB of RAM (32 MB recommended)
- Mouse input device

Screen resolution of 600 x 800 pixels (or greater) with 256 colors (or greater) is recommended.

Technical Support

If you run into problems while using Data Analysis, first check *Chapter 7: Troubleshooting* for solutions to common problems. For additional support, and to report software bugs or documentation errors, contact C&L Instruments. Contact information is listed on the front cover of this manual.

About this Manual

What's Covered

This manual covers the installation and operation of C&L's Data Analysis software. It is assumed that the user is already familiar with the following:

Fluorescence spectroscopy concepts and procedures

How to use Windows[®] 95 or Windows[®] 98

The other hardware components of the C&L Dye Fluorometer. The operation of these components is covered in separate user manuals. It is recommended that the user become familiar with operating the C&L Dye Fluorometer hardware prior to using this software.

Obtaining Current Documentation

The most current version of the Data Analysis User Manual is available for downloading at the C&L Instruments, Inc. Web site. See the front cover of this manual for the URL.

Chapter 2: Software and Hardware Installation

Overview

This chapter explains how to install the PC card into the host computer and install the Data Acquisition software.

Installing the Hardware

Installing the PC-DAQ Controller Card

If you did *not* purchase a computer from C&L Instruments along with the C&L Dye Fluorometer, you will need to install the PC-DAQ Controller card and software in your computer.

The PC-DAQ Controller card is a 16-bit ISA card. It can operate in two modes: Plug and Play (PnP) and non-Plug and Play (non-PnP). Non-PnP is sometimes called legacy mode. In PnP mode, the installation process is automatic, in that the Windows operating system will assign the necessary hardware interrupt (IRQ) and base address to the PC-DAQ Controller card without user intervention. In non-Plug and Play mode, the user must set jumpers and switches on the Controller card to manually set the hardware interrupt and base address. The non-PnP mode is generally reserved for special cases when a resource conflict in the computer must be resolved by manual intervention by the user.

It is recommended that the user install the PC-DAQ Controller card in PnP mode. In this mode, the installation is considerably easier, and the operating system automatically assigns resources to the card. The following instructions outline the procedure to install the PC-DAQ Controller card in the PnP mode. Contact C&L Instruments for instructions for installing the card in non-PnP mode.

To install the PC-DAQ Controller card in PnP mode, follow these steps:

1. Turn off all electrical power to the computer.
2. Open the computer case. Refer to the manual that came with your computer if you do not know how to open the computer case.
3. Install the PC-DAQ controller card in an empty ISA slot. Note that the Controller card has one single jumper in the lower left side of the card (Jumper J11). This jumper must be **off** (i.e., open position) to select PnP mode. Secure the card in the chassis using the hardware provided by the computer manufacturer. Usually one screw is used to hold an ISA card in place.
4. Close the computer case.
5. Start the computer.

When the computer restarts for the first time after installing the PC-DAQ Controller card, Windows should find the new hardware and report:

“New Hardware Found - ISA PnP S48C Data Acquisition”

Follow the instructions by Windows. When prompted for location of the driver, insert the floppy disk comes with the PC-DAQ Controller card and direct Windows to install the driver from the floppy disk.

To make sure the card is installed correctly, open the Control Panel (in the Start menu, under Settings / Control Panel). Select System, and then the Hardware Manager tab. There should be an entry indicating “Virtual Motor Driver” under the “C&L Instruments”. Select the device with the left mouse button and check the Properties of the device. Windows should have assigned resources to the PnP Controller card. Windows may report something similar to the following:

```
Interrupt Request      05
Input/Output Range    0280-02BF
```

The Interrupt Request and Input/Output Range assigned to the PC-DAQ Controller in your computer may differ from the above example.

Note: The current device driver supplied with your PC-DAQ Controller card has been fully tested with Windows 95 version 4.00.950, Windows 95 version 4.00.950 B (OSR2), Windows 98, and Windows 98 Second Edition. If you upgrade or install a new operating system, it is recommended that you open the computer case and remove the card from the computer before you start the upgrade or installation. After the upgrade or installation is complete, reinstall the PC-DAQ card.

Installing the Data Acquisition Software

After installing the PC-DAQ Controller card in your computer, you need to install the Data Acquisition Software. To install the Data Acquisition software, follow these steps:

Insert the diskette provided by C&L Instruments.

Using the **Run...** command in the Windows **Start** menu, launch the Setup.exe program provided on the diskette.

Follow the installation directions on the screen. Read the License Agreement that is displayed by Setup.

When the software installation is complete, several system files will be stored in the Windows/System directory. The main C&L Dye Fluorometer software files and the default configuration file will be placed in subdirectory structure under either the C:\Program Files\CandL directory created by Setup or the directory you specified during the installation process. Reboot the computer before using Data Acquisition for the first time.

Chapter 3: Getting Started

Overview

This chapter will quickly familiarize the user with the basic operating steps required for analyzing data collected with the C&L Instruments Dye Fluorometer. It is recommended that the user be familiar with the data acquisition procedures and C&L Instruments Dye Fluorometer operation described in the Data Acquisition software manual.

Further details about viewing and analyzing data are provided in the following chapters, which describe all available software features and explain how they are used to view and analyze data. The user is encouraged to read these chapters so that the many powerful features of the data analysis software can be utilized to their fullest potential.

Basic Steps in Data Analysis

To begin a data analysis session, there are only four basic steps to follow.

1. Turn on your PC and Monitor
2. Launch the Data Analysis software.
3. Open the data files from a previously recorded data acquisition session.
4. Apply data analysis functions to interpret your data.

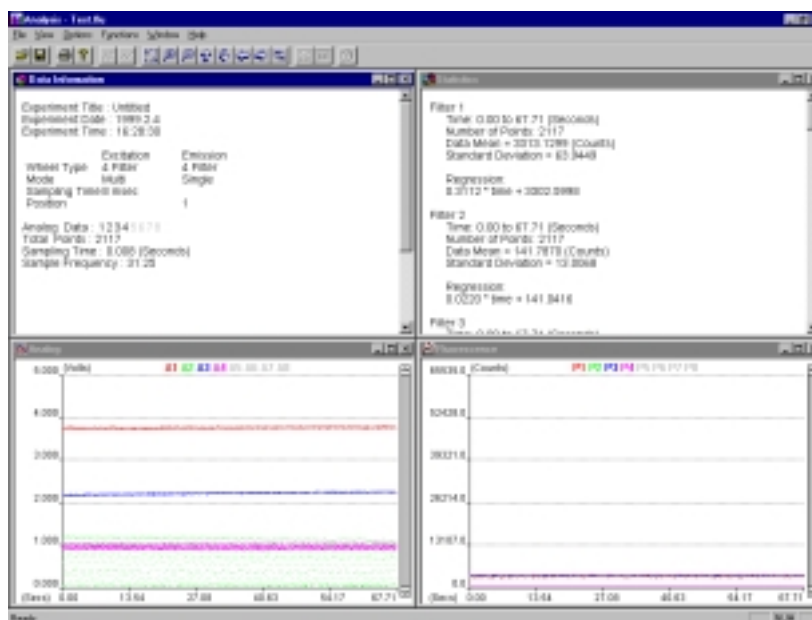


Figure 1. Program window as it appears when you open the file Test.flu, showing the toolbar, status bar, and 4 child windows.

Chapter 4: Command Reference

Overview

This chapter describes in detail all features of the Data Analysis software that are available to the user. The user has control over how the Dye Fluorometer views and analyzes data by specifying various **F**ile, **E**dit, **O**ptions, **V**iew, **W**indow, **F**unction and **D**isplay options in the Data Analysis software.

The following features are covered, in the listed order:

- Main program window and child windows
- Drop-down menu bar and individual drop-down menu items
- Toolbar
- Status bar

Main Program Window and Child Windows

The Data Analysis program opens as one main program window containing a typical Windows title bar and a drop-down menu bar.

When the Data Analysis program is first started, the Window is in ready mode for you to load a data file of a previously recorded session from disc. Included with the program files is a file named “Test.flu”. This file is available in the “data files” folder to demonstrate the features of the analysis software. You may access the file from the **F**ile menu or the “open file” icon.

Drop-down Menu Bar

Under the program title bar is the drop-down menu bar, which contains the following menus: **F**ile, **V**iew, **W**indow and **H**elp. When you open a data file, the **O**ptions and **F**unctions menus are activated. Through these drop-down menus, all features of the Data Analysis software can be accessed. In addition, some menu options are also available through icons displayed in the toolbar.

The drop-down menus serve the following functions. Each menu is described in greater detail in the following sections.

- **F**ile - Allows the user to **O**pen and **S**ave files, **P**rint the contents of data windows, and **E**xit the program.
- **E**dit – Allows the user to **C**opy the viewed data into the Windows® clipboard. Edit is only available when Fluorescence Counts, Fluorescence Ratio, Analog, Ion Concentration, or Interlace child windows are active.
- **V**iew - Allows the user to toggle the main program window’s **T**oolbar and **S**tatus Bar on and off. Also allows the user to perform various actions to manipulate the presentation of data in the active Fluorescence, Fluorescence Ratio, or Analog

windows. These actions include **Z**oom, **U**nzoom or **M**ove the scale of the Y-axis, and change the **S**ettings... of the zoom function, **S**elect **X** to select a region of interest, and **S**ync **T**ime. When an FFT window is active, you can choose to include or exclude the DC component with the **D**C command.

- **O**ptions - Allows the user to specify the display **C**olor of various channels and text.
- **F**unction – Allows the user to access data analysis functions which are detailed later in this chapter. These include: Downsample, Filter, Interlace, Savitzky-Golay analysis, Fluorescence Ratio and Ion Concentration calculation. The **S**ettings command gives access to the parameters used in the respective functions.
- **W**indow - Allows the user to open **N**ew **F**luorescence, **N**ew **A**nalog, or **N**ew **F**luorescence **R**atio, **N**ew **D**ata **S**tatistics, or **N**ew **S**preadsheet data windows. Also allows the user to select the active window and to arrange the child windows within the main program window using the **C**ascade, **T**ile and **A**rrange **I**cons options.
- **H**elp - Allows the user to access on-line information **A**bout **A**nalysis.

File Menu

The **File** menu contains the following options:

- **O**pen...,
- **S**ave,
- **S**ave **A**s...,
- **P**rint...,
- **P**rint **P**review,
- **P**rint **S**etup...,
- **1** [filename] (etc.)
- **E**xit

These options are described in detail below.

File / Open...

Opens a dialog box in which the user can locate and open a specific data file. Data files are stored with the .flu extension.

File / Save

Saves the data file using the same file name.

File / **Save **A**s...**

Opens a dialog box in which the user can save the data file using a new file name.

File / Print...

Prints the contents of the active window.

File / Print Preview

Allows the user to preview the output of the Print command on the monitor screen prior to printing.

File / Print Setup...

Allows the user to change the printer device and to specify other print options available through the printer driver.

File / 1. [file name] (Etc.)

Displays the list of the most recently used data files. Selecting one of these file names provides a shortcut for opening previously used C&L Dye Fluorometer data files.

File / Exit

Terminates the Data Analysis program.

Edit Menu

Allows the user to **Copy** the viewed data into the Windows[®] clipboard. Edit is only available when Fluorescence Counts, Fluorescence Ratio, Analog, Ion Concentration, or Interlace child windows are active.

View Menu

The **View** menu allows the user to control how data is viewed in the main program window and data windows. The **View** menu options vary depending on the type of window that is currently active.

If an information window is active, such as Data Information or Statistics, the following menu options are available in the **View** menu.

- **Tool Bar**
- **Status Bar**

If an Analog window is active, the following menu options are available in the **View** menu.

- **Tool Bar**
- **Status Bar**
- **Zoom >Range, In, Out**
- **Move >Up, Down, Left, Right**
- **Unzoom**

- **Settings**
- **Circles**
- **Lines**
- **Sync Time**

If a Fluorescence Ratio window is active, the following menu options are available in the **View** menu.

- **Tool Bar**
- **Status Bar**
- **Zoom >Range, In, Out**
- **Move >Up, Down, Left, Right**
- **Unzoom**
- **Settings**
- **Circles**
- **Lines**
- **Sync Time**

If a Fluorescence window is active, the following menu options are available in the **View** menu.

- **Tool Bar**
- **Status Bar**
- **Circles**
- **Lines**
- **Zoom >Range, In, Out**
- **Move >Up, Down, Left, Right**
- **Select X**
- **Unzoom**
- **Settings**
- **Sync Time**
- **Counts**
- **Counts / sec**

Accessing the Filter function generates a window displaying a Fast Fourier Transform (FFT). If an FFT window is active, the following menu options are available in the **View** menu.

- **Tool Bar**
- **Status Bar**
- **Circles**
- **Lines**
- **DC**
- **Zoom >Range, In, Out**
- **Move >Up, Down, Left, Right**
- **Select X**
- **Unzoom**
- **Settings**
- **Sync Time**
- **Counts**
- **Counts / sec**

If an Interlace window is active, the following menu items are available in the **View** menu.

- **Tool Bar**
- **Status Bar**
- **Circles**
- **Lines**

View Menu Commands:

View / Tool Bar

Displays or hides the Tool Bar.

View / Status Bar

Displays or hides the Status Bar.

View / Counts and View / Counts/Sec

Changes the units on the Y-axis in the active Fluorescence data window.

- Selecting **Counts** changes the units to (Fluorescence) Counts. This represents the number of photon counts collected by the detector within the sampling period.

- Selecting **Counts/Sec** changes the units to Counts/Second. This represents the number of photon counts collected by the detector within a sampling period, but expressed on a per second basis.

View / Zoom Range, Zoom / In, Zoom / Out, / Move / Up, / Move / Down, / Move / Left, / Move / Right, and / Select X

The **Zoom Range, Zoom In, Zoom Out, Move Up, Move Down, Move Left, Move Right, and Select X** options are used to control the display of data in the Fluorescence, Analog and Fluorescence Ratio windows. When one of these menu options is selected, the mouse cursor changes from the standard pointer to an icon indicating the selected option. The new mouse cursor is visible only when the mouse is moved over the Fluorescence, Analog or Fluorescence Ratio windows.

- When the **Zoom Range** command is selected, the cursor changes to a rectangle. With this tool the left mouse button can then be used to select a rectangular window to zoom in to.
- When **Zoom In** is selected, the mouse cursor changes to a magnifying glass containing a plus sign. The left mouse button can then be used to zoom in on any area of interest in Fluorescence, Analog and Fluorescence Ratio windows.
- When **Zoom Out** is selected, the mouse cursor changes to a magnifying glass containing a minus sign. The left mouse button can then be used to zoom out of any area of interest in Fluorescence, Analog and Fluorescence Ratio windows.
- When **Move Up** is selected, the mouse cursor changes to an upward pointing arrow. The left mouse button can then be used to move the data displayed in the Fluorescence, Analog and Fluorescence Ratio windows in an upward direction. The data can only be moved upward after the **Zoom In** function has been performed and only if the display is not already at its upper limit.
- When **Move Down** is selected, the mouse cursor changes to a downward pointing arrow. The left mouse button can then be used to move the data displayed in the Fluorescence, Analog and Fluorescence Ratio windows in a downward direction. The data can only be moved downward after the **Zoom In** function has been performed and only if the display is not already at its lower limit.
- When **Select X** is selected, a Region Of Interest (ROI) can be selected using the left mouse button. The selected ROI is indicated as a red bar that is visible under the X axis of each data window. This feature is used to select a ROI for the available functions.
- The ROI data will be analyzed and displayed in the Statistics window. The Statistics window indicates the range of points selected in the ROI, the mean of each channel and the equation for the best fit line through the data of each channel. In the Analog or Fluorescence windows, the ROI selected will be the data range used for frequency analysis in the FFT window and the data range used in the Interlace window.

- If the ROI is selected in the FFT window, then the Analog and Fluorescence windows are updated with the results of the selected filter. Depending in the settings of this function, the ROI is used to select either a passband or notch region in the frequency spectrum.

View / **U**nzoom

Unzoom restores the full view of the data in the window.

Shortcuts to the Zoom **R**ange, Zoom **I**n, Zoom **O**ut, Move **U**p, Move **D**own, Move **L**eft, Move **R**ight, Select **X** and **U**nzoom features are also available in the icon bar.

View / **S**ettings...

The **S**ettings command gives access to dialog boxes which set the parameters for Zoom, and allows the user to specify which values are displayed on each axis. The user may enter values for each axis and the Region Of Interest (Select **X**) directly in the dialog boxes instead of designating the positions with the cursor. The Zoom ratio is the percent increase in magnification scale when a zoom command is issued, and may be specified independently for the X and Y-axes. The Zoom ratio on top is the X-axis and the one on the bottom is the Y-axis. The ratio settings may be saved by clicking the **S**ave **c**urrent **r**atio button.

- **C**ircles

Displays data points as circles.

- **L**ines

Connects data points with lines.

Shortcuts to the **C**ircles and **L**ines commands are available in the toolbar.

- **D**C

The **D**C command toggles the DC component in the FFT window on or off. This is useful when you want to display an FFT without the usually large DC component that would otherwise distort the scale of the frequencies. Turning **D**C off will cause the FFT window to display only the AC component of the signal.

- **S**ync **T**ime

The **S**ync **T**ime command is used to synchronize the time axes for all windows so that changes in one will be reflected in the others.

A shortcut to **S**ync **T**ime is available in the toolbar.

- **S**ync **F**requency

The **S**ync **F**requency command is used to synchronize the frequency axes of multiple FFT windows.

Options Menu

The Options menu contains the following menu items:

- **C**olor...
- **S**equential
- **A**ligned
- **A**cquisition
- **C**ompact

These options are described in detail below.

Options / Color... and the Color Dialog Box

Opens the Color Dialog box.

The **Color Dialog** box is used to change the color of the data points and text displayed in the Fluorescence, Fluorescence Ratio and Analog data windows. Up to eight channels of data can be displayed in each Fluorescence and Analog data window.

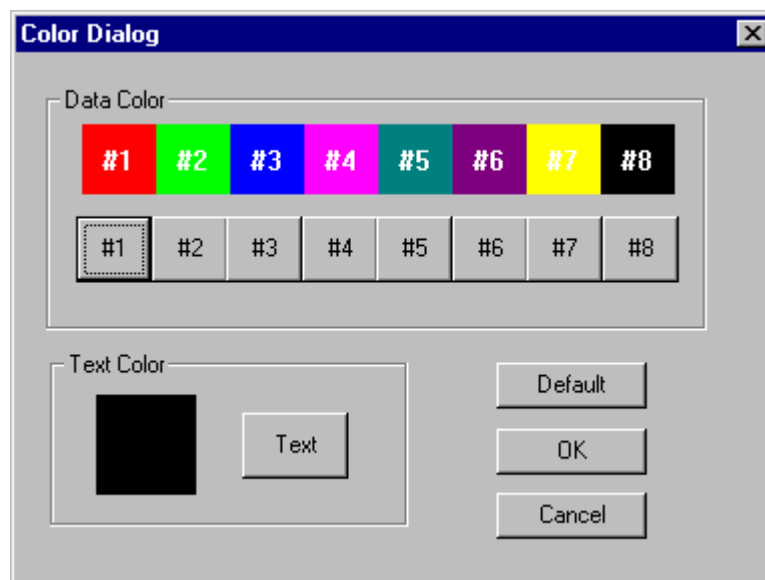


Figure 2. The Color Dialog box.

Selecting Channel Color

Clicking on the button for the desired channel in the **Channel Color** section displays a standard Windows® **Color** selection dialog box. The same Channel colors are used in both the Fluorescence and Analog data windows. The color selected for **#1** (i.e., Channel 1) is used for the display of the fluorescence ratio in the Fluorescence Ratio window. The default setting assigns unique colors to each channel.

Selecting Text Color

Clicking on **Text** allows the user to change the color of the text that appears in the data windows. The same text color is used in the Fluorescence, Analog and Fluorescence Ratio windows.

Accepting and Canceling Changes

Clicking on the **Default** button will revert all color changes to their default setting.

Clicking on **OK** closes the Color Dialog Box and saves all changes.

Clicking on **Cancel** cancels all changes and causes the colors to remain the way they were prior to opening the Color Dialog box.

If desired, the user can also change the background color of the data windows. This is accomplished by editing the Properties of the Windows[®] desktop. Further instructions can be found in the Windows[®] 95 user manual.

Sequential, **A**ligned, **A**cquisition, and **C**ompact options are available when a file is loaded that was collected under Mixed (random access filter positions) Mode. These selections work together to allow you to change the manner that the data is displayed. These selections do not alter the data, only their display. **S**equential and **A**ligned change the alignment of the data with respect to the time axis. **A**cquisition, and **C**ompact change the display between two representations to illustrate the data either as acquired or as an average.

The **S**equential command shows the Mixed Mode data with an advancing time index such that each filter wheel position is rendered sequentially. Along the time axis, at any time, there will be counts for only one filter position displayed. This display method illustrates an accurate temporal representation of the data as it was originally acquired.

The **A**ligned command is the alternate for the **S**equential command. In Aligned mode, the data are displayed with the first data point of each filter position aligned at the same time index, the time of the first channel selected. The counts may be superimposed if the count level is the same in two or more filter positions,.

The **A**cquisition command displays all the data for each filter position.

The **C**ompact command is the alternate for the **A**cquisition command. **C**ompact takes the data for each filter position and averages them and then displays the average as one point only.

These commands may be used in a total of four combinations, Sequential with Acquisition or Compact and Aligned with Acquisition or Compact.

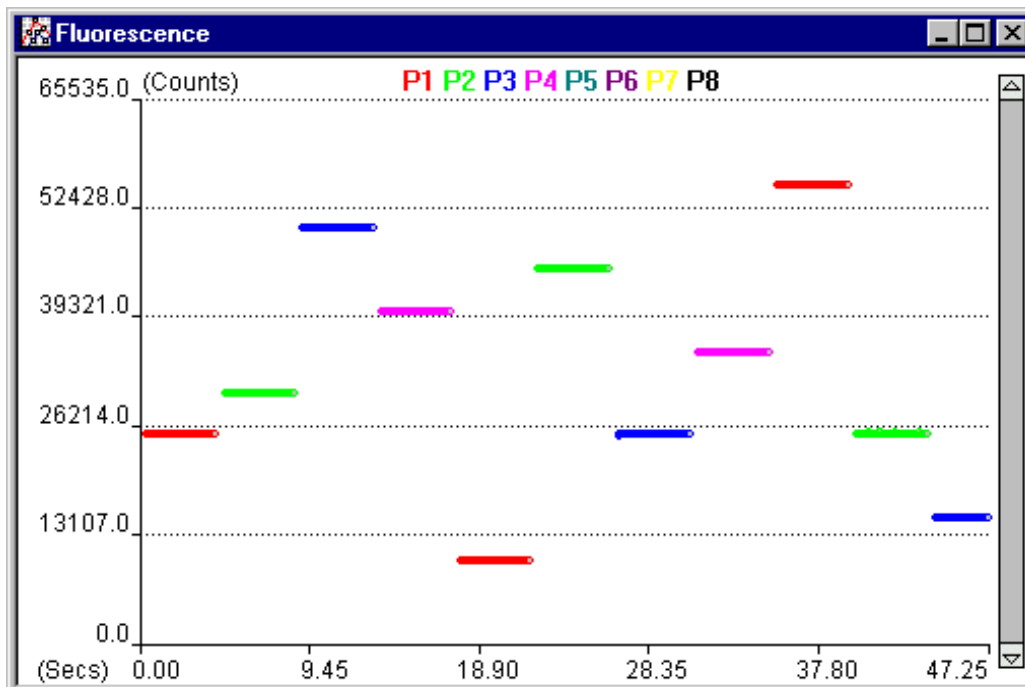


Figure 3. A fluorescence window with data displayed in the Sequential and Acquisition mode. Data points are displayed as Circles as chosen in the View menu.

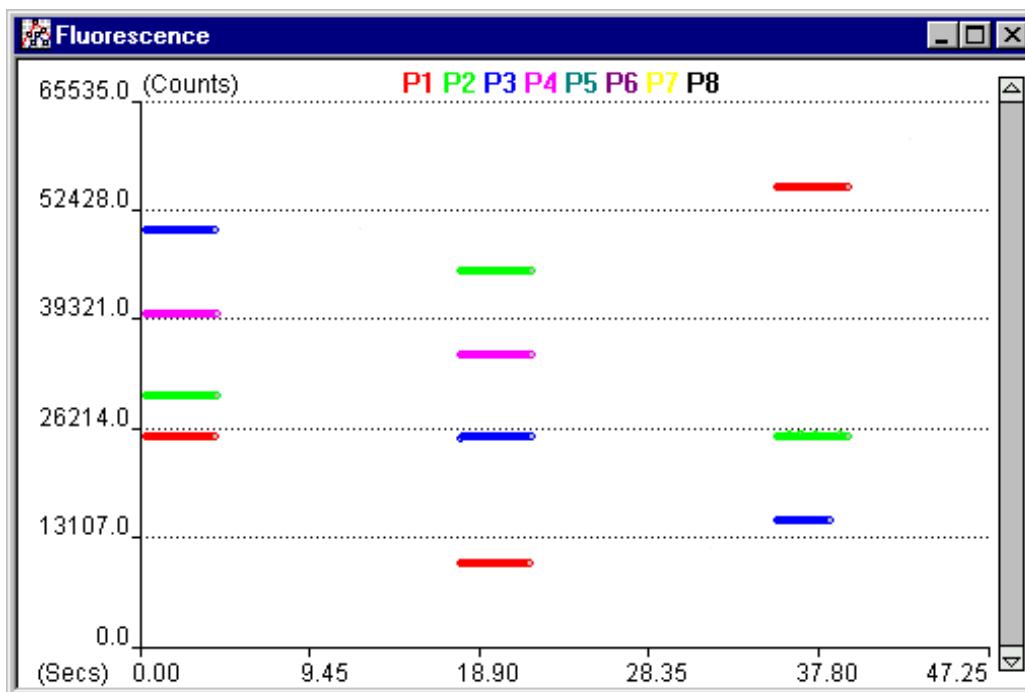


Figure 4. A fluorescence window with data displayed in Aligned and Acquisition mode. Data points are displayed as Circles as chosen in the View menu.

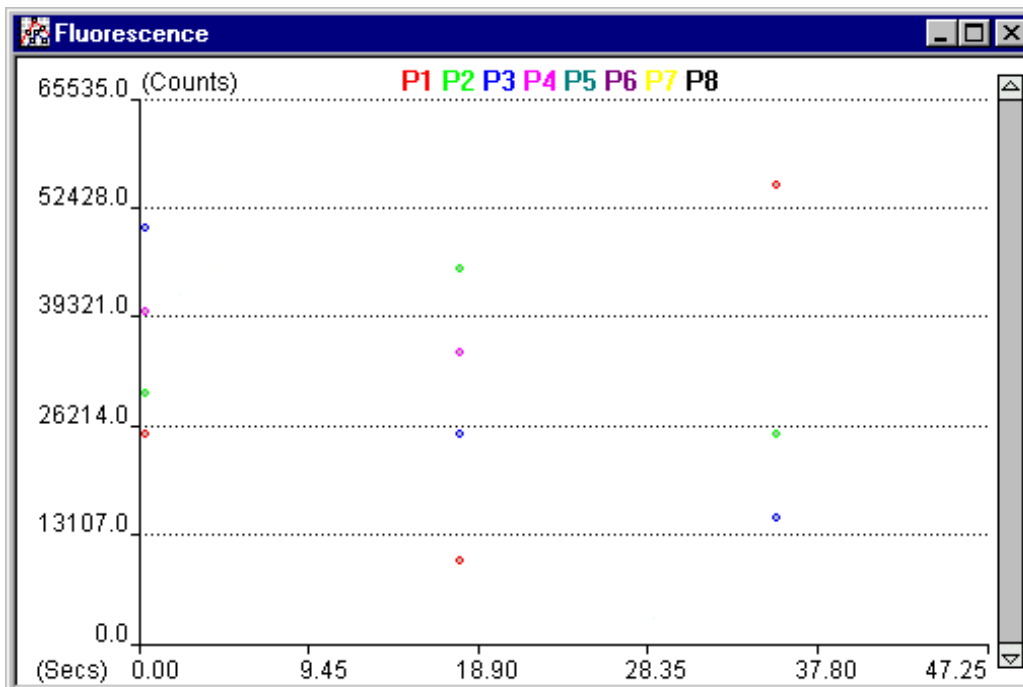


Figure 5. A Fluorescence window with data displayed in Aligned and Compact mode. Data points are displayed as Circles as chosen in the View menu.

Functions Menu

The **Functions** menu allows the user to access data analysis functions for curve fitting and smoothing as well as Fluorescence Ratio and Ion Concentration calculations. In order to access the parameters used in the functions, select the **Settings** command.

The Functions menu contains the following items:

- **Downsample**
- **Filter**
- **Interlace**
- **Savitzky-Golay**
- **Fluorescence Ratio**
- **Ion Concentration**
- **Settings**
- **Save Settings**

These options are described in detail below.

Downsample

The **Downsample** Function is a data smoothing routine, which is similar to a running average. It is generally used when the data is slowly varying, and you wish to consolidate

the display and data file by eliminating redundant information. The Downsample factor, which is selected in the **Settings** menu, determines the number of points which are taken as an average. The averages are then plotted as the new display. A downsample factor of 1 would leave the display unchanged, while a downsample factor of 10 would reduce the number of points displayed by a factor of 10.

Once the data has been downsample by a specific factor, reapplying the function will not change the display unless the factor is changed. Applying a downsample factor of 1 will restore the original data.

The **Downsample** function is disabled when data files collected with the Single Sample Mode are loaded into the Data Analysis program. See *Chapter 6: Viewing, Analyzing and Saving Data* for further discussion of the Single Sample Mode.

Filter

The **Filter** command performs an FFT (Fast Fourier Transform) on the data and displays a new window with the results. The results are the data displayed in the frequency domain from zero Hertz (i.e., the DC component) up to the Nyquist frequency. The Nyquist frequency is determined by the rate of data acquisition and it cannot be changed after the data has been collected.

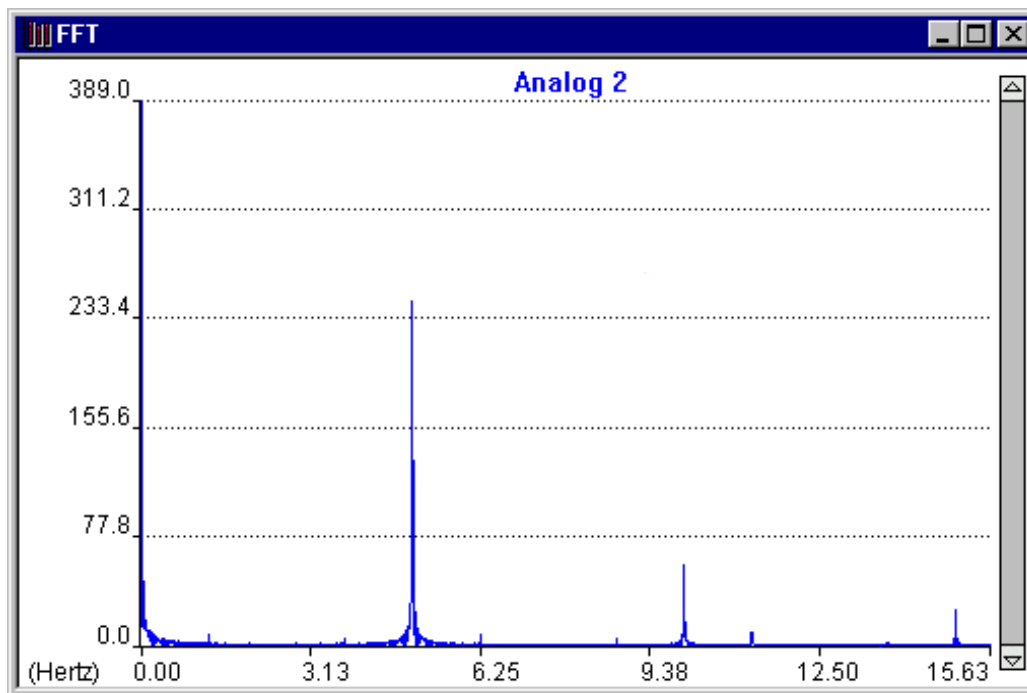


Figure 6. FFT window of the data in Analog 2 channel using the Test.flu data file displayed with the DC component and Lines turned on.

You may choose a pass filter or notch filter from the **Settings** dialog box. In the FFT display window, the bandpass or notch region is chosen with the **Select X** command from

the **V**iew menu. When the FFT window is active, you can select to include or exclude the DC component from the **V**iew menu with the **D**C command.

The Region of Interest in the FFT window is selected using the **S**elect **X** command in the same manner as used in other data windows. In the FFT window, the data segment illustrating the selected ROI is shown with the chosen display color while the remaining range is “grayed” out. After the ROI is selected in the FFT window, the data in the Fluorescence or Analog window is updated in accordance with the frequency range selected in the FFT window.

The **F**ilter function is disabled when data files collected with the Single Sample Mode are loaded into the Data Analysis program. See *Chapter 6: Viewing, Analyzing and Saving Data* for further discussion of the Single Sample Mode.

Interlace

The **I**nterlace function is applied as a means of data analysis that can be used when data from a repetitive event has been collected. To use this function, the data must be acquired in synchrony with the timing of the repetitive event. The Dye Fluorometer allows you to synchronize the multiwavelength data acquisition sequence (Multiwavelength mode) of the fluorometer with an external stimulus, such as a cardiac cycle or nerve depolarization. The user is referred to the Data Acquisition User Manual for instructions of how to set up the Dye Fluorometer for synchronous data acquisition.

Since the Dye Fluorometer is a time-sharing device, data points collected at different wavelength setting cannot be obtained at the same instant in time. As a result, a ratio between the fluorescence intensities measured at two wavelengths may be in error because the numerator and denominator of the ratio were measured at different times. The extent of this error depends on the rate of data acquisition and the rate in which the data is changing. These errors are insignificant at high rates of data acquisition or when the fluorescence of the sample is changing slowly. One method of circumventing this error is by collecting the data in a synchronous fashion and applying the **I**nterlace signal processing function.

The **I**nterlace function allows you to obtain the data from the same point in time of all measured channels as if the data from all channels were actually collected instantaneously. The **I**nterlace function uses a sampling technique to obtain these data from different cycles of the repetitive event in order to reconstruct the repetitive cycle at all wavelengths. This can be applied, for example, if you want a fluorescence ratio from a cyclically varying event.

In the **S**ettings for the **I**nterlace function, you enter the Repetition Frequency (in cycles/second) of the event when the data was originally acquired. The Sample Frequency is calculated by the software and is based on the rate of data acquisition used when the file was collected. Selecting the Apply button will return the Number of Samples that will be derived within each cycle. The Number of Cycles indicates the number of repetitive events that are required to calculate one data point at each time point in the repetitive cycle. This represents the minimal number of cycles that must be acquired during the data acquisition process and selected using the Region of Interest function in the Data Analysis program.

Applying the **Interlace** function will derive the fluorescence data and standard deviation for each wavelength during one cycle of the repetitive event. The reader may reference an article by Scott, et al. "Ratiometric measurement of rat heart NAD(P)H by Surface Fluorescence," *American Journal of Physiology* **267**: H636-H644, 1994, for a further discussion of this technique.

The **Interlace** function is disabled when data files collected with the Single Sample Mode are loaded into the Data Analysis program. See **Chapter 6: Viewing, Analyzing and Saving Data** for further discussion of the Single Sample Mode.

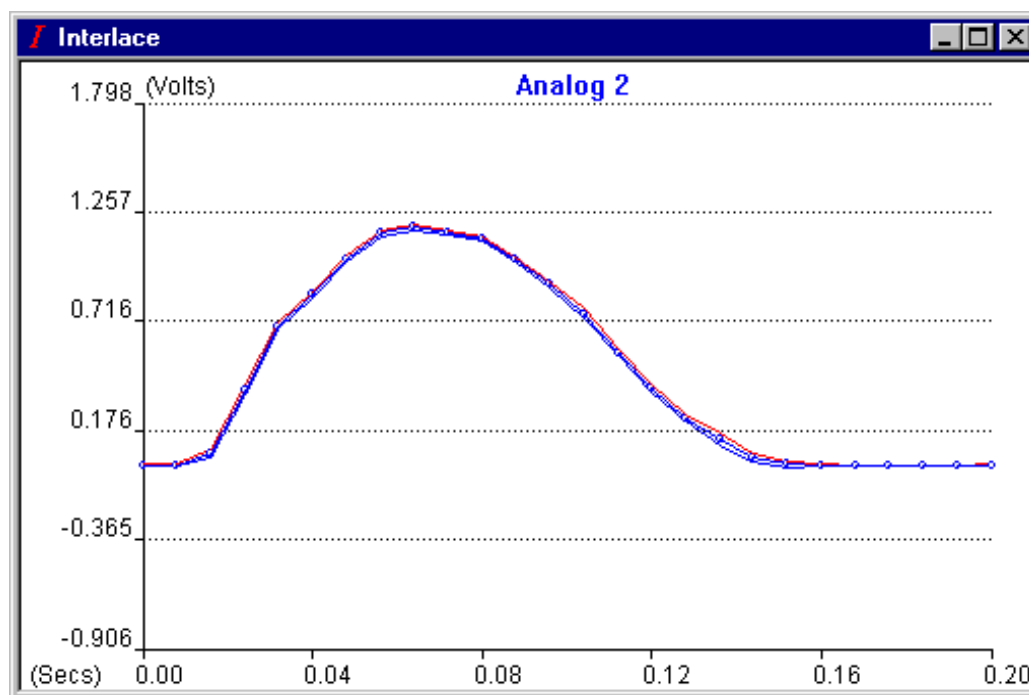


Figure 7. Results of the Interlace function when applied to a data segment of the Test.flu file using the Repetitive Frequency of 5. Each cycle lasts 0.2 seconds. Shown is the mean and standard deviation of the data in Analog channel 2.

Savitzky-Golay

The **Savitzky-Golay** function invokes a data smoothing routine. The function returns a new data set of smoothed data using a least-squares procedure. It is designed to remove extraneous noise and erroneous data points while minimizing the distortion of the actual shape of the data profile. The technique involves convoluting a quadratic/cubic or quartic/quintic function with the data. The selection of quadratic/cubic or quartic/quintic and the number of points to include in the calculation is accessed from the **Settings** command. Interested readers may reference A. Savitzky and M. Golay, "Smoothing and Differentiation of Data by Simplified Least Squares Procedures," *Analytical Chemistry*, **36**(8): 1627-1639, 1964.

The **Savitzky-Golay** function is disabled when data files collected with the Single Sample Mode are loaded into the Data Analysis program. See **Chapter 6: Viewing, Analyzing and Saving Data** for further discussion of the Single Sample Mode.

Fluorescence Ratio

The **Fluorescence Ratio** command calculates a fluorescence ratio according to the parameters chosen in **Settings**. You select the filter positions of interest used to derive the ratio and a background value, if desired. You may also enter a scaling factor in the Settings dialog box. Select between Counts or Counts/Second to indicate the form of the background counts entered.

Ion Concentration

The **Ion Concentration** command calculates an ion concentration based upon the parameters entered in the Settings dialog box and the data. You may choose a calculation based upon one fluorescence wavelength according to the formula:

$$[\text{Ion}] = K_d(F - F_{\min}) / (F_{\max} - F)$$

where K_d is the dissociation constant, F is the fluorescence intensity, F_{\min} is the fluorescence count at zero ion concentration, and F_{\max} is the fluorescence count at saturating ion concentration.

You may choose a calculation based upon two wavelengths according to the formula:

$$[\text{Ion}] = B(R - R_{\min}) / (R_{\max} - R)$$

where B is a constant $K_d(S_{f2}/S_{b2})$, R is the fluorescence ratio of wavelength pairs, R_{\min} is the limiting value R can have at zero ion concentration and R_{\max} is the R at saturating ion concentration.

Provision is made in the **Functions / Settings** menu to enter the fluorescence background before the ratios are calculated. Select between Counts or Counts/Second to indicate the form of the background counts entered.

The reader may find further information about these formulae and methods in the publication:

Grynkiewicz G., Poenie M., and Tsien R., "A new generation of Ca^{2+} indicators with greatly improved fluorescence properties," *Journal of Biological Chemistry* **260**(6): 3440-3450, 1985.

Settings

The **Settings** command provides access to a dialog box, which contains parameters for each of the various functions described above. You can select or change parameters as desired.

A shortcut to the **Settings** command is available in the toolbar.

Save Settings

The **Save Settings** command saves the choices made for the various functions, with the data file. Settings are saved with the Data Analysis software for later use and are available the next time the program is used.

Display Menu

The **Display** menu allows the user to specify the data channels that will be displayed in the Fluorescence, Analog or Fluorescence Ratio data windows. The **Display** menu is available on the menu bar *only* when the active window is one of these three data window types. Furthermore, the **Display** menu options vary for each of the three data window types. The menu items displayed when **Display** is selected depend on which data child window is active.

Fluorescence and Analog Window Display Options

If a Fluorescence or Analog window or is active when **Display** is selected, then the following options are available for selection in the **Display** drop-down menu.

- **Channel 1**
- **Channel 2**
- **Channel 3**
- **Channel 4**
- **Channel 5**
- **Channel 6**
- **Channel 7**
- **Channel 8**
- **Select All**
- **Deselect All**

The number of channels available depends on the number of channels available in the data file. This depends on how the data was collected in the Data Acquisition program.

Selecting and Deselecting Channels

Channels can be selected (indicated by a checkmark) or deselected individually by clicking on the appropriate options.

Selecting **Select All** selects all Fluorescence or Analog channels.

Selecting **Deselect All** removes the selection from all Fluorescence or Analog channels.

Fluorescence Ratio Window Display Options

If a Fluorescence Ratio window is active when **D**isplay is selected, a dialog box appears that allows you to set the numerator and denominator used to calculate the ratio. This is the same dialog box you access from the **F**unctions / **S**ettings command.

Ion Concentration Window Display Options

If an Ion Concentration Window is active when **D**isplay is selected, a dialog box appears that allows you to choose wavelengths pairs and background information. This is the same dialog box you access from the **F**unctions / **S**ettings command.

Window Menu

The following menu items are available in the **Window** menu:

- **New (Fluorescence) Counts**
- **New Analog**
- **New Fluorescence Ratio**
- **New Data Information**
- **New Statistics**
- **New Spreadsheet**
- **Cascade**
- **Tile**
- **Arrange Icons**
- **1... (etc.)**

Window / New Counts, / New Analog, / New Data Information, New Statistics, and / New Spreadsheet

Selecting **New (Fluorescence) Counts**, **New Analog**, **New Data Information**, **New Statistics**, or **New Spreadsheet**, opens an additional Fluorescence, Analog, Fluorescence Ratio, Data Information, Statistics or Spreadsheet window, respectively. The newly added window initially inherits default attributes for this type of window. The additional window, however, can be customized to display data in a fashion that is different from and independent of other windows of the same type. For instance, two separate Fluorescence windows can be used to view fluorescence from different filter wheel positions and the data can be scaled differently in each window. A discussion of the use of Statistics and Spreadsheet windows can be found in *Chapter 6: Viewing, Analyzing and Saving Data*.

Window / Cascade

Displays all child windows in a cascade.

Window / Tile

Displays all child windows in a tile display.

Window / Arrange Icons

Arranges at the bottom of the screen, above the status bar, the icons of all child windows that have been minimized.

Window / 1... (Etc.)

Changes the active window to the particular window selected from the **Window** menu list. This action is analogous to clicking on the title bar of an inactive child window to make it the active window.

Help Menu

The following options are available in the **Help** menu.

- **Online Manual – PDF**
- **Online Manual – HTML**
- **C&L Instruments Website**
- **About Analysis...**

Help / Online Manual – PDF

This option opens a help file as a PDF (Portable Document Format) document. The PDF file contains bookmarks to facilitate navigation through the document. To open this file, you must have Adobe[®] Acrobat[®] or the Adobe[®] Acrobat Reader[®] (version 3.0 or greater) loaded on your computer. Information about Adobe Acrobat can be obtained from the Adobe website (www.adobe.com). This version of the help file is essentially the same as the HTML version.

Help / Online Manual – HTML

This option opens a help file as an HTML (Hypertext Markup Language) document in your default web browser application. This version of the help file is essentially the same as the PDF version.

Help / C&L Instruments Website

Selecting this option opens your default web browser and loads the home page of the C&L Instruments Internet website.

Help / About Analysis...

Selecting **About Analysis** displays on-line information about C&L Instruments, Inc. and the version information about the Analysis software.

Toolbar

The Toolbar provides shortcut icons to some common functions such as: Open File, Save, Print, Circles, Lines, Zoom Range, Zoom In/Out, Move Up/Down and Left/Right, and Select X, Unzoom, Zoom Settings, and Sync Time. The use of these icons as shortcuts to specific menu options is discussed in *Chapter 5: Using the Icons in the Toolbar*.

Status Bar

The status bar at the bottom of the main program window displays two types of status messages:

Ready - This is the default status message, which is generally displayed when the program is ready and able to accept input from the user.

Option description - When the cursor is moved over a drop-down menu option, the status area displays a brief description of that option.

Chapter 5: Using the Icons in the Toolbar

Overview

This chapter describes how the icons in the Toolbar can be used as short cuts to save time when operating the Data Analysis software. Many of the program selections available in the main Menu Bar are also available as icons. The reader is referred to the previous chapter, *Chapter 4: Command Reference* for an explanation of the functions represented by these icons.

The Icons

The following is a list of the icons that are available in the Toolbar, together with the function they represent. These functions can also be found in main Menu Bar.

 <u>F</u>ile / <u>O</u>pen	 <u>F</u>ile / <u>S</u>ave
 <u>F</u>ile / <u>P</u>rint...	 <u>H</u>elp / <u>A</u>bout C&L...
 <u>V</u>iew / <u>C</u>ircles	 <u>V</u>iew / <u>L</u>ines
 <u>V</u>iew / <u>Z</u>oom > <u>R</u>ange	 <u>V</u>iew / <u>Z</u>oom > <u>I</u>n
 <u>V</u>iew / <u>Z</u>oom > <u>O</u>ut	 <u>V</u>iew / <u>M</u>ove > <u>U</u>p
 <u>V</u>iew / <u>M</u>ove > <u>D</u>own	 <u>V</u>iew / <u>M</u>ove > <u>L</u>eft
 <u>V</u>iew / <u>M</u>ove > <u>R</u>ight	 <u>V</u>iew / <u>S</u>elect <u>X</u>
 <u>V</u>iew / <u>U</u>nzoom	 <u>V</u>iew / <u>S</u>ettings...
 <u>V</u>iew / <u>S</u>ync Time	

Chapter 6: Viewing, Analyzing and Saving Data

Use of Right Mouse Button

When a window with a graph is displayed, such as Fluorescence, Fluorescence Ratio, Interlace, Analog, or FFT, you can use the right mouse button as a shortcut to get to pull down menus. When you position the mouse within the graph area and then right click, you will access the View menu functions that pertain to that window. When you position the mouse outside the graph area, but still inside the window, and then right click, you will access the Display menu functions that pertain to that window. These are the same functions available from the toolbar when the relevant window is active.

Data Windows

The Analysis program can display several types of data windows to facilitate the viewing and analysis of your data. These windows are the Fluorescence, Analog, Statistics, Data Information, FFT, Fluorescence Ratio, and Ion Concentration. A discussion of the Fluorescence, Analog, FFT, Fluorescence Ratio, and Ion Concentration windows can be found in *Chapter 4: Command Reference*.

The Data Information Window

The data information window illustrates information about how and when the data file was collected using the Acquisition program. This feature alleviates the user from manually recording this information about each data file when it is initially recorded. These data fields cannot be edited by the user, and are presented for informational purposes only.

The Spreadsheet Window

The Spreadsheet window can be used to visualize and/or change numerical values of the data. This window is available by selecting **W**indows / **N**ew **S**pread **S**heet in the menu bar. Shown are the values selected in the ROI data range.

To change a particular data value, double click on a cell containing a data value using the left mouse button. This will cause a Change Value dialog box to appear in which you can change a value. To permanently change the data value in the file, you must save the data using either **F**ile / **S**ave or the **F**ile / **S**ave **A**s command. This new value will then permanently replace the prior value in the data file after the file is saved. You may save the data in a new file to avoid overwriting the original file using the **F**ile / **S**ave **A**s command.

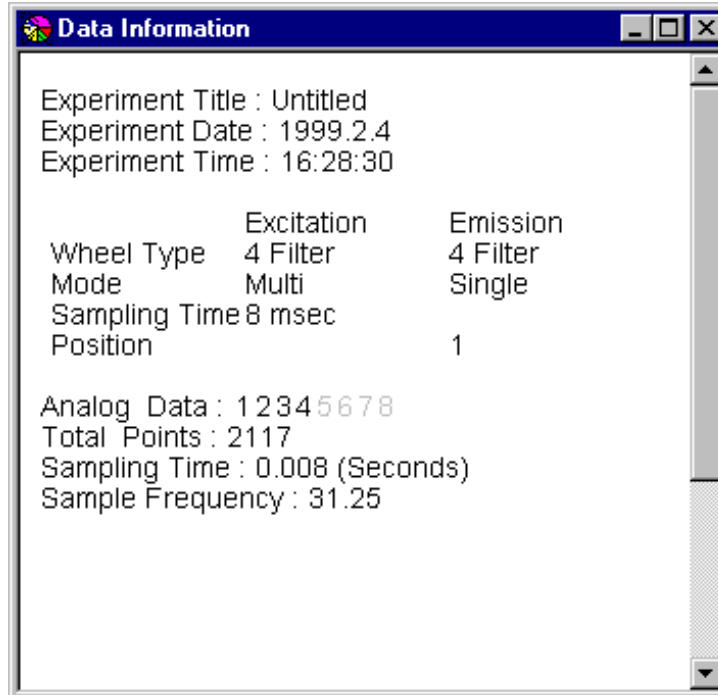


Figure 8. The Data Information window illustrating information about the data file currently loaded into the Analysis program.

The Spread Sheet window displays the following data values:

Time	Count1	Count2	Count3	Count4	Analog1	Analog2	Analc
16.35	3353.0	143.0	3820.0	10.0	3.74634	0.00000	2.18
16.38	3270.0	123.0	3823.0	12.0	3.73657	0.67871	2.18
16.42	3390.0	174.0	3677.0	16.0	3.74512	1.19263	2.18
16.45	3212.0	134.0	3856.0	14.0	3.73047	0.91797	2.18
16.48	3322.0	136.0	3706.0	10.0	3.74146	0.23804	2.19
16.51	3328.0	121.0	3805.0	11.0	3.75122	0.00000	2.19
16.54	3309.0	131.0	3779.0	13.0	3.74634	0.00488	2.19
16.58	3376.0	149.0	3795.0	15.0	3.74512	0.37354	2.19
16.61	3324.0	125.0	3760.0	14.0	3.74756	1.15479	2.20
16.64	3205.0	154.0	3677.0	12.0	3.75488	1.02417	2.20
16.67	3409.0	111.0	3666.0	13.0	3.75854	0.37354	2.20
16.70	3244.0	142.0	3793.0	12.0	3.75488	0.00000	2.20
16.74	3363.0	157.0	3769.0	11.0	3.75732	0.00000	2.19
16.77	3342.0	133.0	3870.0	12.0	3.74268	0.03174	2.18

Figure 9. The Spread Sheet window illustrating the data values within the Region of Interest.

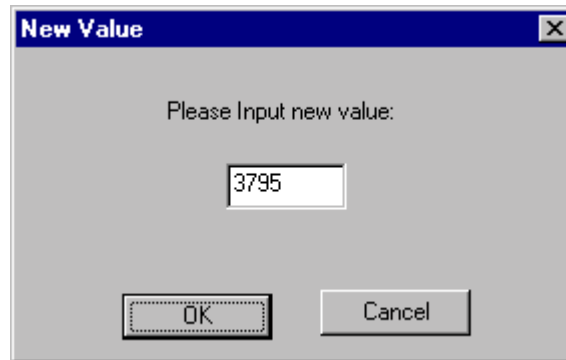


Figure 10. The New Value dialog box that appears after double-clicking on a data value in the Spread Sheet window.

The Statistics Window

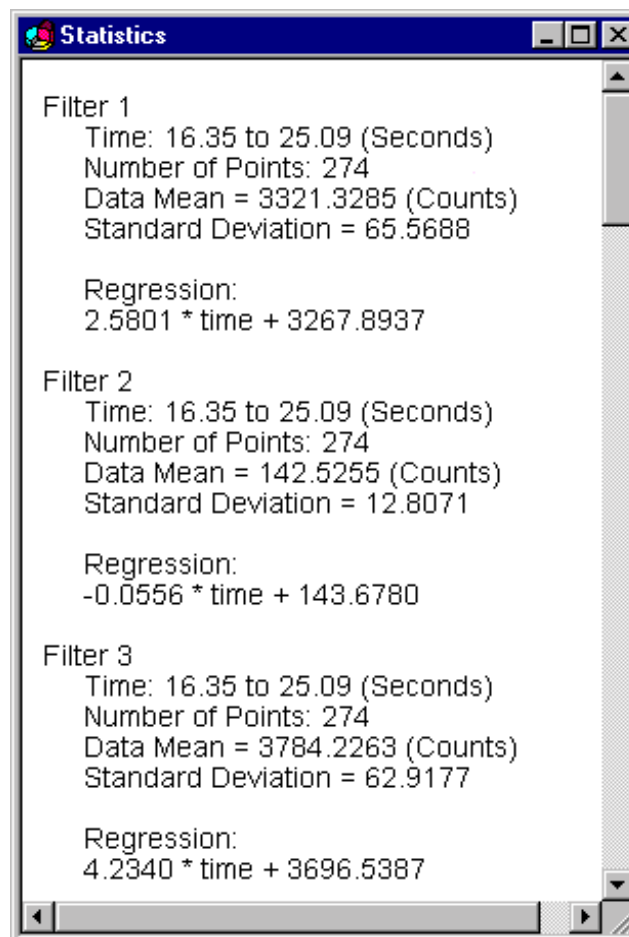


Figure 11. The Statistics window illustrates information about the data within the Region of Interest.

The Statistics window automatically opens as a default child window when a data file is opened in the Analysis program. It can also be opened using the **Window / New Statistics** command. Shown in the Statistics window is information about the range of data selected as the Region of Interest (ROI). For each Fluorescence and Analog channel, the time interval, the number of data points selected, the data mean and data standard deviation are displayed. This feature can be used to quickly obtain the mean value of data over a given time range. In addition, the regression best-fit line, calculated by least squares analysis, fitting the ROI region is displayed. This equation can be used to extrapolate the data to any point in time or to obtain the rate of change (i.e., the slope) of the data values.

Exporting Data

Several options are available to export data from the Analysis program. Data displayed in any active data window (i.e., Fluorescence, Analog, Ion Concentration, FFT, Fluorescence Ratio, and Interlace) can be exported.

Exporting Data using the Copy Command

To export data from a data window, click on the title bar of the data window to make the window active. Then access the **Edit / Copy** command. There are three **Copy** choices, **Copy All**, **Copy View** and **Copy ROI** (Region of Interest) **Copy All** copies all the data pertaining to the active window to the Windows® clipboard, whether or not all the data is currently displayed. **Copy View** copies only the data that is displayed in the current view (i.e., the data that is visible). **Copy ROI** copies the data from the selected ROI. These three choices are available for all windows except for the Interlace window, in which only **Copy All** is available.

To select an ROI for exporting data, use the **View** menu and **Select X** to select a range of values using the mouse. The ROI selected is indicated as a red bar under the data window. Information about the selected ROI will automatically appear in the Statistics Window (time range, number of points, data mean, etc.). This option is also available as an icon in the tool bar (icon with double horizontal arrows). You may deselect the ROI by clicking the left mouse button once, inside the window. This function makes the entire range of data the ROI.

These functions copy the data to the Windows® clipboard where it may be pasted into and used by other programs such as Excel® or a text editor such as Notepad® or Word®. Data is presented as columns of either Fluorescence Intensities or Voltages for each channel. The data is copied to the clipboard in ASCII CSV (comma separated value) format.

Exporting Data in ASCII Format

The entire data file can be exported as an ASCII file by selecting **File / Save As**. This will display the Save As dialog box. Click on the **Save as type:** drop down selection and chose the CSV format. Type in a file name and click on the **Save** button.

Exporting Data from Files Collected in the Single Sample Mode

The Data Acquisition software program allows the user to collect data in a Single Sample Mode. In this mode, single data points may be collected which are not separated by a constant time interval. In this case, data displayed in the Fluorescence, and Analog windows will not contain a time value along the X axis. In addition, all time related functions (Downsample, Filter, Interlace and Savitzky-Golay) are disabled. When these data are exported using either the **C**opy command or by saving the file as a CSV file, the data in the first column will represent the point number rather than a unit of time.

When the Single Sample mode is used to collect data in an automated fashion, in which data are collected in intervals of 1 minute or more, the time related functions (Downsample, Filter, Interlace and Savitzky-Golay) are also disabled. When these data are exported using either the **C**opy command or by saving the file as a CSV file, the data in the first column will be in units of minutes rather than seconds.

Saving Processed Data

You may save the entire data file data by using either the **File / Save** or **File / Save As** command. The **S**ave command overwrites the .flu file with the data in use by the Analysis program. The **S**ave **A**s command gives you the option of saving the data in .flu format or .csv (comma separated value) format, as described above. It also allows you to change the filename so that you do not overwrite your original file. Note that the Analysis program can only open data files saved using the .flu format.

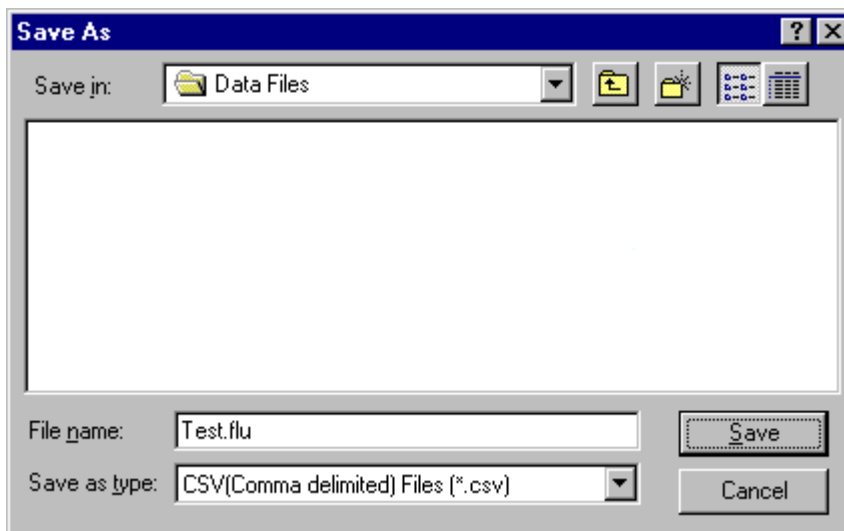


Figure 12. The Save As dialog box illustrating the “cvs” file type.

Chapter 7: Troubleshooting

If you have trouble operating the C&L Instruments Dye Fluorometer or Data Analysis software, please follow these steps.

1. Consult this chapter for answers to common problems.
2. Contact C&L Instrument, Inc. Contact information is given in the front of this manual.

<u>Symptom</u>	<u>Possible Problem</u>	<u>Possible Solution</u>
Main program window opens without any child windows and most menu items are disabled.	A data file has not been loaded.	Use the File / Open command to open a data file.
Fluorescence and/or Analog data is not visible in the Fluorescence window.	Viewing of Fluorescence data is not enabled	Make sure the Fluorescence or Analog window is active, and then enable viewing of data using the View menu.
	Data is off scale	Use the Unzoom feature or either the Move Up or Move Down command.
	Colors of data point(s) are the same as the graph background color.	Change the color of the data points using the Color... menu item under Options .
The Zoom In or Zoom Out feature does not work.	The Zoom setting is set to zero percent.	Select View / Settings... when a data window is active and set the percent zoom setting to a number above 10 percent.

Chapter 8: Agreement, License and Warranty

Agreement and License

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