

Data Acquisition Software User Manual
for the PC-DAQ Controller
Version 1.0a

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Chapter 1: About the Data Acquisition Software

Program Description

C&L Data Acquisition is an easy-to-use 32-bit software application designed for use with the C&L Dye Fluorometer. This version of the software applies to the data acquisition system that uses the Model PC-DAQ controller card. A separate application is used with the RS-DAQ controller. C&L Data Acquisition offers powerful data collection features using a standard Windows[®] interface. The software allows the user to:

- Control the operation of all the individual hardware components of the C&L Dye Fluorometer
- Make multiwavelength fluorescence measurements
- Make analog measurements along with the fluorescence measurements in a simultaneous fashion
- View data from all fluorescence and analog channels on the computer monitor, in real time, as it is acquired.

System Requirements

The Dye Fluorometer software requires the following minimum configuration:

- Pentium[®] 133 MHz computer
- At least one free 16-bit ISA slot in the 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 Acquisition, first check **Chapter 10: 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 Acquisition 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 this Data Acquisition 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:

1. Insert the diskette provided by C&L Instruments.
2. Using the **Run...** command in the Windows **Start** menu, launch the Setup.exe program provided on the diskette.
3. 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: Fundamentals of Data Acquisition

Overview

This chapter describes some of the basic ways in which the C&L Dye Fluorometer hardware and Data Acquisition software work together to monitor and save data obtained from your samples. Understanding the fundamentals of hardware and software functioning will enable you to control the data acquisition process effectively.

The use of specific software functions is covered in greater detail in *Chapter 7: Acquiring Data* and *Chapter 8: Viewing Data*.

Filter Wheels

Filter Wheel Type

The Dye Fluorometer uses two filter wheels, one in the excitation path and one in the emission path, to select the excitation and emission wavelengths. Depending on the particular type(s) of filter wheels purchased with your instrument, the filter wheels can contain either four 1-inch diameter filters or eight ½-inch filters.

Filter Wheel Operation Modes

The Data Acquisition software allows the user to control the operation of the excitation and emission filter wheels. The features for each of the wheels can be controlled separately. Both the excitation and emission filter wheels can be operated in three different operation modes: Multi, Single and Mixed.

Multi Mode

In Multi Mode, the filter wheel revolves at a constant velocity. In this mode, the period of time in which the sample is exposed to a specific wavelength of light is determined by the speed of revolution. This period of time is referred to as the **sampling time**. The exposure of the sample to specific excitation wavelengths and the measurement of light emission from the sample are in a constant ordered sequence, determined by the placement of the individual filters in the filter wheels.

Single Mode

In Single Mode, the filter wheel is held in a stationary position, permitting light to pass through one position in the filter wheel. Both filter wheels can be placed in the Single mode for measurement of fluorescence using a single excitation and emission wavelength pair. Alternately, either the excitation filter wheel or the emission filter wheel can be placed in the Single Mode while the other filter wheel is operated in the Multi Mode. This setting would be used for either multiwavelength excitation with a single emission wavelength or multiwavelength emission with a single excitation wavelength. When either the excitation or emission wheel is operated in the Multi mode, the sampling time is determined by the speed of revolution.

Mixed Mode

In the Mixed Mode, the excitation and emission filter wheels can each be programmed to go to any filter in any sequence. For instance, the excitation filter wheel can be set to cycle through the filter sequence of 2,1,4 while the emission wheel cycles through the filter sequence of 3,2,1. In this case, the excitation-emission pairs would be 2-3, 1-2, and 4-1. The specific **dwel time** can be adjusted. The **dwel time** is the duration of time that the filter wheels remain stationary in one position prior to moving to the next position programmed in the sequence. This feature provides a programming ring, capable of up to eight excitation wavelength pair combinations. In the Mixed Mode, the sampling time is user defined. Data is acquired after the filter wheel is stationed at one excitation-emission wavelength pair. The number of data points acquired for one excitation-emission wavelength setting is dependent on the sampling time and the dwell time.

Data Acquisition Modes

Data is acquired with the excitation and emission filter wheels operating in the three modes described above (i.e., Single, Multi and Mixed). Although the setting for each filter wheel can be specified separately, in combination the user can select from 5 data acquisition modes. These are Single-Single, Multi-Multi, Multi-Single, Single-Multi and Mixed-Mixed. Further details of these 5 modes of data acquisition and how they are used are a topic of *Chapter 7: Acquiring Data*.

Shutters

The C&L Dye Fluorometer contains two shutters, one in the excitation path (within the Model S48D Illumination Source) and one in the emission path (within the Detection Module). The shutters can be controlled through the Data Acquisition software to limit either the sample's or the photomultiplier tube's exposure to excessive light. Moreover, the shutters can be controlled through software either manually or automatically. Through the software, the user can specify that the sample be exposed only during active data collection and that light be blocked when the filter wheel is repositioning during operation in the Mixed Mode.

Fluorescence and Photon Counting

All fluorometers detect and quantitate the intensity of light emitted from fluorescence samples when excited by excitation energy. The C&L Dye Fluorometer uses a highly sensitive end-on photomultiplier tube (PMT) as the light detection device. The PMT captures single photons and converts the photon energy into pulses of electric current. These pulses, which are proportional to the intensity of light impinging on the PMT, are counted by the data acquisition system. The photon-counting method of light detection is unparalleled for high sensitivity and accurate detection of low light levels. The C&L Dye Fluorometer counts these pulses in discrete time intervals. The count rate is proportional to the intensity of the light emitted from the sample.

The Data Acquisition software can display these data as either “counts” or “counts per second”, depending on the preference of the user, in a separate program window

designated as the Fluorescence window. One or more fluorescence windows can be opened to view the data and each window can be separately customized with different display options. These acquired “counts” represent the number of photon counts accumulated within the specified acquisition period. Throughout this manual, the acquisition period is referred to as the **sampling time**.

Depending on the data acquisition mode, the sampling time is set either by the speed of the filter wheel revolution or by a keyed entry in a dialog box. In the Single-Single mode, the sampling time is determined by a value keyed into a dialog box. In Multi-Multi, Single-Multi, and Multi-Single modes, the sampling time is determined by the revolution speed of the filter wheel. In the Mixed-Mixed mode, the sampling time is set by a value keyed into a dialog box. These dialog boxes are in the Setup Options.

Single Sample Mode

The C&L Dye Fluorometer Data Acquisition software contains a Single Sample Mode that is designed for short data acquisition sequences. These sequences can be acquired one at a time, or in an automated fashion using a programmed time interval. The Single Sample Mode feature is designed for use of the Dye Fluorometer with the Model CV1 Cuvette Accessory, but it can be used with any fluorescence hardware setup.

The Single Sample Mode allows the user to acquire a brief data sequence. Acquisition will then wait for either a key entry from the user or a specified time period before acquiring a second data set. This is repeated until the user decides to write the data to a file. This feature saves the user from having to collect multiple files for short-duration data acquisition sequences.

Fluorescence and Fluorescence Ratios

Newer fluorescent dyes, which are commonly used for measurement of cation concentrations, produce a signal in which a fluorescence ratio is proportional to the cation concentration. For the convenience of the user, the Data Acquisition software provides separate data display windows where the user can monitor a fluorescence intensity ratio in real time. Multiple Fluorescence Ratio windows can be opened and set to monitor any fluorescence ratio pair.

Excitation Light Intensity and The Neutral Density Wheel

The C&L Dye Fluorometer uses interference filters for excitation and emission wavelength selection. The design of the filters determines the bandpass and percent light transmission of the filter. As a result, the performance of the filter wheels used in the Dye Fluorometer has a strong influence on the performance of the fluorometer. Contact C&L Instruments if you have questions about selecting filters for your Dye Fluorometer.

In measurements of fluorescence using several wavelengths, it is sometimes desirable to use several filters having different transmission and/or bandpass characteristics. The bandpass is determined solely by the design of the interference filter. The C&L Dye Fluorometer, however, contains a system for attenuating the intensity of the excitation light. Depending on the model number of your illuminator, the illumination source

contains either a computer-controlled light attenuation wheel or neutral density wheel to attenuate the output light intensity. Throughout this manual and in the dialog boxes of the software, this system will be referred to as a Neutral Density wheel, even though your illuminator may contain a different type of light attenuation wheel. Refer to the hardware user manual for details of the attenuation system in your illuminator.

This feature is used to set the light intensity that is output by the illuminator. Light transmission can be precisely adjusted over a 50-fold range for exquisite control of illumination intensity. This method of light control is preferable over manipulation of the lamp current, since it is rapid and reproducible and it does not affect the color of the illumination source.

Measuring Fluorescence by Balancing Factors

Several factors influence the intensity of the acquired fluorescence signal. Obviously, the concentration of the fluorescent dye and its quantum yield affect the observed intensity. But there are additional factors that can be controlled by the fluorometer that affect the number of fluorescence counts detected within the sampling time. These are the excitation lamp intensity, the specifications of the excitation and emission interference filters, and the sampling time setting.

Excitation Lamp Intensity

The PMT used in the C&L Dye Fluorometer responds in a linear fashion to light intensity up to a count rate of 10×10^6 counts/second. The user should adjust the excitation intensity using the neutral density wheel in order to maintain the PMT in the linear range. The count rate can be altered by either adjusting the characteristics of the sample or changing the excitation energy or emission collection efficiency. The latter can be adjusted by either resetting the neutral density filter or by using a different excitation and/or emission filter.

Specification of Excitation and Emission Interference Filters

These specifications are determined by the design of the specific interference filter. Consult C&L Instruments or the manufacturer of the interference filter for questions regarding the optical characteristics of the filters used in your instrument.

Sampling Time Setting

The number of counts collected within the sampling period is dependent on the count rate and the sampling time. A long sampling time can be used to collect the fluorescence emission from a weakly fluorescing sample. A long sampling time, however, may cause saturation of the counter in the C&L Dye Fluorometer if the sample is strongly fluorescent. In the latter case, the user can either adjust the count rate, as described above, or use a shorter sampling time. The C&L Dye Fluorometer uses several 16 bit counters and can count up to 2^{16} (i.e., 65,536) counts within a sampling period before saturation of the counter. The feature in the software indicates to the user if this saturation occurs. See *Data Overflow* in *Chapter 8: Viewing Data* for a discussion of this topic. The setting of

the sampling time and neutral density filter wheel are discussed in more detail in *Chapter 7: Acquiring Data*.

The C&L Dye Fluorometer can display the observed fluorescence in the Fluorescence window in units of Counts or Counts/second. In the Counts mode, the number of counts accumulated within the sampling time is displayed. In the Counts/second mode, the count rate is displayed as the accumulated counts divided by the sampling time in seconds. Further details about viewing fluorescence data are discussed in *Chapter 8: Viewing Data*.

Acquisition of Analog Data

Along with the acquisition of fluorescence data, the C&L Dye Fluorometer is able to monitor and record external events through acquisition of analog data. Inputs are provided for acquisition of up to eight analog channels. Data can be acquired within an input range between 0 and 5 Volts. The Model PC-DAQ Controller is equipped with a 12 bit analog-to-digital converter, providing a resolution of 1.2 mVolt. This feature gives the user the ability to record events that may be occurring simultaneously with the recording of fluorescence data. This may include, but is not limited to, the recording of data from patch clamp instruments, flow meters, and other sensors.

Time-sharing

The C&L Dye Fluorometer uses a single detector for measuring the intensity of light at up to eight wavelengths. Since these measurements are made in a sequential fashion and not simultaneously, this instrument can be considered a time sharing device. This must be borne in mind when setting up the fluorometer for a data acquisition sequence. Generally, the data sampling frequency should be at least 3-fold faster than the rate of change of the fluorescence signal in order to faithfully record the signal. This is especially important when the data of interest is derived from a wavelength ratio. In this instance, data should be acquired at a fast enough rate to insure that the sample intensity has not changed appreciably in the time interval between two sequential measurements. The C&L Dye Fluorometer is capable of high-speed operation. Up to eight separate wavelength measurements can be made at the rate of 1 sample per millisecond.

A further discussion of the timing relation between the acquisition of fluorescence and analog data can be found in *Chapter 9: Timing Considerations*.

Chapter 4: Getting Started

Overview

This chapter will quickly familiarize the user with the basic operating steps required for collecting data with the C&L Dye Fluorometer.

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

The Data Acquisition Process

The user starts the data acquisition process by setting up the software parameters to collect data under specified conditions. The user then starts and stops a data acquisition session. After data has been acquired, it is written to a file for later analysis. In between periods of data acquisition, the fluorescence and analog signal can be monitored.

Basic Steps in Data Acquisition

To begin and end a data acquisition session, there are only five basic steps to follow.

1. Turn on all external hardware, such as the Model S48D Illumination Source and the Detection Module.
2. Launch the Data Acquisition software.
3. Specify and/or load previously specified **Setup Options** for data acquisition.
4. **Run** the filter wheels to engage them in the desired operation **Mode(s)**.
5. **Start** and **Stop** a recording session.

Chapter 5: Command Reference

Overview

This chapter describes in detail all features of the Data Acquisition software that are available to the user. The user has control over how the Dye Fluorometer acquires data by specifying various **Setup Options** in the Data Acquisition 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, including the Setup Options dialog box
- Toolbar
- Status bar

Before reading this chapter, it is recommended that the user become familiar with the concepts introduced in the preceding chapters. The upcoming two chapters, **Chapter 7: Acquiring Data** and **Chapter 8: Viewing Data**, explain how the software functions discussed in the present chapter can be used to acquire data in a specific fashion.

Main Program Window and Child Windows

The Data Acquisition program opens as one main program window containing a typical Windows title bar and a drop-down menu bar. The title bar displays the name of the configuration file that is currently loaded into memory. The configuration file is discussed in greater detail later in this chapter and in **Chapter 7: Acquiring Data**.

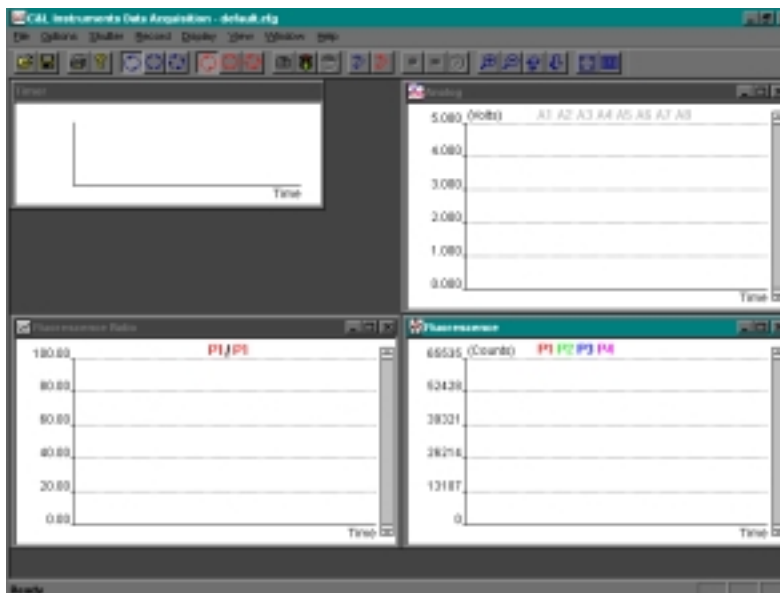


Figure 1. The main program window.

When the Data Acquisition program is first started, the main program window contains either three or four “child” windows, as shown in Figure 1. If the child windows are not observed, it is because a configuration file has not been loaded. Open a configuration file using the **F**ile / **O**pen command. Data Acquisition will automatically open the last configuration file used.

The child windows—Timer, Fluorescence, Analog and Fluorescence Ratio—are used to view the data as monitored by the C&L Dye Fluorometer. The Fluorescence Ratio window is not available when the C&L Dye Fluorometer is operated in the Mixed-Mixed Mode. For further details about the use of these windows to display data, see *Chapter 8: Viewing Data*.

Drop-down Menu Bar

Under the program title bar is the drop-down menu bar, which contains the following menus: **F**ile, **O**ptions, **S**hutter, **R**ecord, **D**isplay, **V**iew, **W**indow and **H**elp. Through these drop-down menus, all features of the Data Acquisition software can be controlled. In addition, some menu options are also available using the icons that are available in the Toolbar. The use of these icons as shortcuts to specific menu options is discussed in *Chapter 6: Using the Icons 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 configuration files, specify **S**etup **O**ptions, control the fluorometer hardware components, print the contents of data windows, and exit the program.
- **O**ptions - Allows the user to specify the **C**olor of various channels and text, set the **O**ffset of the excitation filter, emission filter, and/or neutral density filter, and check the interface settings for the PC-DAQ controller PC **B**oard.
- **S**hutter - Allows the user to open and close the excitation and emission shutters manually.
- **R**ecord - Allows the user to **S**tart and **S**top the data acquisition process or used the **T**imed feature for fixed duration data acquisition.
- **D**isplay - Allows the user to specify the data channels to be displayed in the Fluorescence and Analog windows and the channels to be used for the numerator and denominator for display of data in the Fluorescence Ratio window.
- **V**iew - Allows the user to toggle the main program window's **T**oolbar and **S**tatus **B**ar 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 **I**n, **Z**oom **O**ut, **U**nzoom or **M**ove **U**p or **M**ove **D**own the scale of the Y-axis, and change the **S**ettings... of the zoom function.
- **W**indow - Allows the user to open **N**ew **F**luorescence, **N**ew **A**nalog, or **N**ew **F**luorescence **R**atio 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 C&L Instruments.

File Menu

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

- **O**pen...,
- **S**ave,
- Save **A**s...,
- **S**etup Options...,
- **R**un,
- **M**ove ND Wheel...,
- **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 configuration file. The configuration file is used to store and recall all C&L Dye Fluorometer settings. Configuration files are stored with the .cfg extension. This feature is generally used to save all system setting for a particular experiment so that the settings can be easily recalled at a later date.

File / **Save**

Saves the current settings in the currently loaded configuration file using the same file name.

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

Opens a dialog box in which the user can save the current settings as a new configuration file using a new file name.

File / **Setup **O**ptions...**

Opens the tabbed Setup Options dialog box that is used to set up most hardware and software features.

The Setup Options dialog box is covered in detail later in this chapter.

File / Run

Activating **Run** causes the filter wheels to index and start running in the operating mode specified in the Setup Options dialog box. After the filter wheels begin operating, data is displayed in the Fluorescence, Analog and Fluorescence Ratio windows, provided that data channels have been enabled for viewing using the **Display** menu item.

File / Move ND Wheel... and ND Moving Dialog Box

Selecting **Move ND Wheel...** opens the **ND Moving** dialog box to allow the user to control the setting of the neutral density wheel for one to eight excitation-emission filter wheel positions used in the data acquisition sequence. This feature is disabled during active data collection and prior to selecting **Run**.

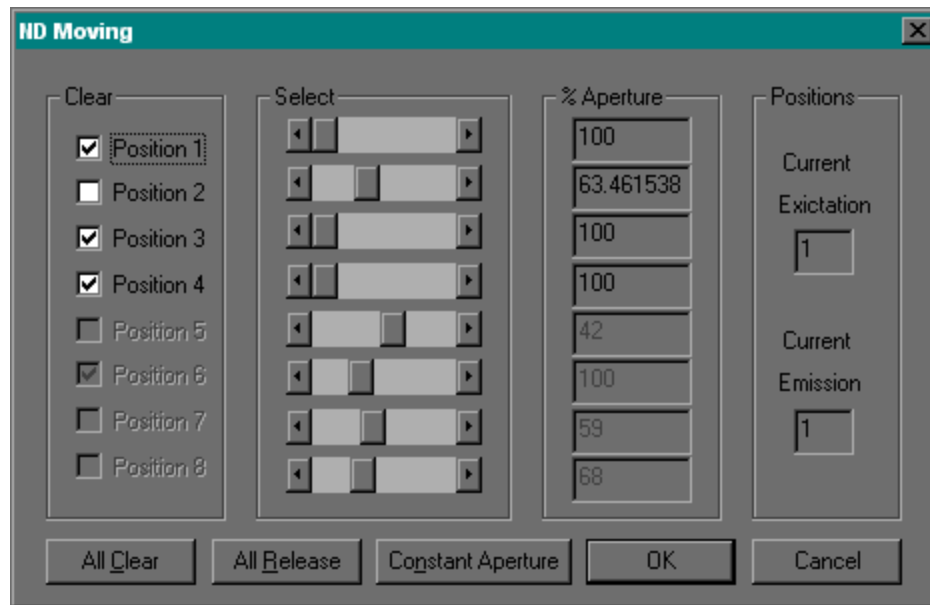


Figure 2. The Set ND Dialog Box

The **Set ND Wheel** dialog box contains five sections—**Clear**, **Select**, **% Aperture**, and **Positions**—as well as a set of buttons along the bottom. There are eight rows, labeled Position 1 through Position 8, corresponding to a maximum of eight possible excitation-emission filter wheel positions used in data acquisition. The data acquisition mode (see **File / Setup Options**), determines the number of Position rows that are enabled. Figure 2 illustrates that the first four positions are enabled.

- When the excitation or emission filter wheel is operated in the Multi or Single mode only Position 1 is enabled in this dialog box. In these two modes, the setting entered into Position 1 is used for all Positions.
- In the Mixed mode, individual ND wheel settings can be made for *each* Position. When multiple Positions are enabled, the user has the choice of specifying the neutral density wheel settings either *individually* for each excitation-emission Position used in the data acquisition sequence or for *all* Positions at once.

Fluorescence data can be monitored in the Fluorescence and Fluorescence Ratio windows during adjustment of the neutral density wheel settings using the **Set ND Wheel** dialog box.

Specifying No Attenuation

Placing a check in the **Clear** field for a particular Position will automatically set the ND filter to the 100% transmission setting (i.e., Clear) for that Position.

The **All Clear** button is used to quickly set *all* the Positions to the **Clear** setting. A checkmark will be displayed in the Clear column for all Positions.

The **All Release** button reverses the All Clear action by removing the checkmark from the Clear column for all Positions. This allows the user to adjust settings individually for each Position.

Specifying Attenuation

When the **Clear** check box is left unchecked, the slide bar in the **Select** column is enabled and can be used to set the percent aperture for each Position to anywhere within the usable range between 1 and 99. Light intensity is decreased as the setting is changed from a value 99 to 1.

To specify the same aperture for all active Positions (when more than one are enabled), select the desired aperture for Position 1, then click on the **Constant Aperture** button.

Viewing the Current Excitation and Emission Positions

The last column, labeled **Positions**, indicates the current Excitation and Emission filter positions. If the Dye Fluorometer is in the Run mode using the Mixed mode of data acquisition, this field is updated to illustrate the current wheel position. This information is especially useful when the ND wheel is set while in the Mixed mode.

Accepting or Rejecting ND Wheel Settings

The **OK** button accepts the new settings in this dialog box and closes the dialog box.

The **Cancel** button cancels any changes the user may have entered into the dialog box and returns the C&L Dye Fluorometer to the state prior to opening the dialog box.

A similar dialog box is also available as a Tab in the **Setup Options** dialog box.

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 configuration files. Selecting one of these file names provides a shortcut for opening previously used C&L Dye Fluorometer configuration settings.

File / Exit

Terminates the Data Acquisition program.

Setup Options Dialog Box

Clicking on **Setup Options...** in the **File** menu opens the tabbed **Setup Options** dialog box.

The tabs, from left to right, are **Mode**, **Multi**, **Single**, **Mixed**, **ND**, **EX**, **EM**, **Record**, **Analog** and **Wheels**. Each tab is described in greater detail below.

Once you have specified the desired settings on the appropriate tabs, choose **OK** to accept them or **Cancel** to return to the previous settings.

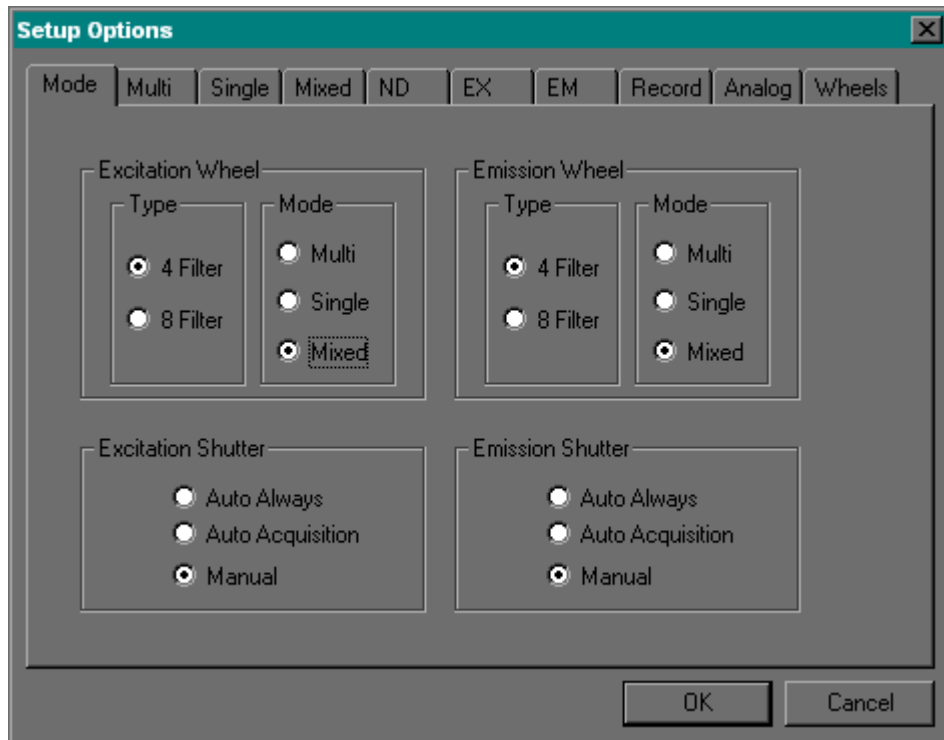
Mode Tab

Figure 3. The Mode tab in the Setup Options dialog box.

The Mode tab displays the *major* Setup Options available for the C&L Dye Fluorometer. Using the selections available in the Mode tab, the user can specify the filter wheel type and operation mode for the excitation and emission wheels, and the shutter control mode for the excitation and emission shutters. This dialog box has four sections: Excitation Wheel, Emission Wheel, Excitation Shutter and Emission Shutter.

Specifying Filter Wheel Type

The **Type** field in the **Excitation Wheel** and **Emission Wheel** sections is used to indicate the filter wheel type (4- or 8-position) installed in the excitation and emission light paths. The Type setting determines which filter wheel operation modes (**Multi**, **Single** and **Mixed**) are available for selection.

Specifying Filter Wheel Mode

The **Mode** field in the **Excitation Wheel** and **Emission Wheel** sections is used to indicate the filter wheel operation mode (**Multi**, **Single**, or **Mixed**) for the excitation and emission filter wheels. The excitation and emission filter wheels can be operated in either separate or similar modes. The filter wheel **Type** settings determine which filter wheel operation modes (**Multi**, **Single** and **Mixed**) are available for selection. Some combinations of excitation and emission filter wheel operation modes are not available, depending on the type of filter wheels installed in the C&L Dye Fluorometer.

About Filter Wheel Operation Modes

The excitation and emission filter wheels can each be set to operate in one of three modes:

- **Multi Mode** - The filter wheel rotates at a constant speed and all the filter positions that are occupied by interference filters are available for measurements. The **Multi** tab (see below) is used to set the sampling time for data acquisition using this mode.
- **Single Mode** - The filter wheel is held at a stationary filter position. Either the excitation or emission filter wheel can be placed in the **Single Mode** while the other is in the **Multi Mode**. Alternatively, both the excitation and emission filter wheels can be placed in the **Single** mode. The **Single** tab (see below) is used to specify the excitation and emission filter position when the filter wheel is set to **Single Mode**.
- **Mixed Mode** - The excitation and emission filter wheels can go to any filter position in a sequence programmed by the user. The **Mixed** tab (see below) is used to specify the excitation and emission filter positions when both filter wheels are set to **Mixed Mode**.

The filter wheel operation modes available for selection depend on the type of filter wheels installed in the C&L Dye Fluorometer. The chart below indicates the five possible mode combinations (i.e., data acquisition modes) available for selection.

The numeric designation in this chart indicates the number of positions in the excitation and emission filter wheels. For instance, 4-8 indicates that a 4-position filter wheel is installed in the excitation and an 8-position filter wheel is installed in the emission. With this filter wheel combination, the four data acquisition modes available for the excitation-emission combination are **Single-Multi**, **Multi-Single**, **Single-Single**, or **Mixed-Mixed**, respectively. If the same type of filter wheel is installed in both the excitation and emission (4 or 8-position), five data acquisition modes are possible: **Single-Single**, **Single-Multi**, **Multi-Single**, **Multi-Multi** and **Mixed-Mixed**.

Filter Wheel Types (Excitation-Emission)	Possible Mode Combinations (Excitation-Emission)
Same Type (4-4 or 8-8)	Multi-Multi Multi-Single Single-Multi Single-Single Mixed-Mixed
Different Type (4-8 or 8-4)	Multi-Single Single-Multi Single-Single Mixed-Mixed

The Single-Mixed and Mixed-Single modes are not available. However, this type of operation is available using the Mixed-Mixed mode in the **Setup Options...** dialog box and setting and specifying a constant filter wheel position in the **Mixed** tab of either the excitation or emission filter wheel.

Specifying Excitation Shutter and Emission Shutter Control Mode

The **Mode** field in the **Excitation Shutter** and **Emission Shutter** sections is used to indicate the shutter control mode for the excitation and emission shutters. The excitation and emission shutters can each be controlled in three ways—**Auto Always**, **Auto Acquisition**, and **Manual**—which are described below.

- **Auto Always** mode - the shutter(s) will always remain in an automatic control mode. In this shutter mode, manual control for the shutter using the **Shutter** menu is disabled. **Auto Always** indicates that the shutter(s) are always linked to the **Start**, **Timed** and **Stop** options (in the **Record** menu) used to control data acquisition. When either **Start** or **Timed** is selected to begin data acquisition, the Data Acquisition software checks the state of the shutter. If the shutter is open, it remains open. If it is closed, the shutter is opened. When either the **Stop** feature is selected or the period of **Timed** data acquisition expires, the software closes the

shutter. This shutter mode insures that the sample and/or the detector are exposed to light only during data acquisition.

- **Auto Acquisition** mode - the shutter(s) are controlled by data acquisition, as discussed above for the **Auto Always** mode, except that manual control is also permitted. That is, automatic control of the shutter occurs only after the **Start**, **Stop** and **Timed** commands are used to control data acquisition. When either **Start** or **Timed** is selected to begin data acquisition, the Data Acquisition software checks the state of the shutter. If the shutter is open, it remains open. If it is closed, the shutter is opened. When either the **Stop** feature is selected or the period of **Timed** data acquisition expires, the software checks the state of the shutter. If the shutter is open, the software closes the shutter. If it is closed, the shutter remains closed.
- **Manual** mode - the shutter(s) are controlled only by the options in the Shutter menu. In the **Manual** mode, selecting **Start**, **Stop** or **Timed** does not change the state of the shutter(s).

Multi Tab

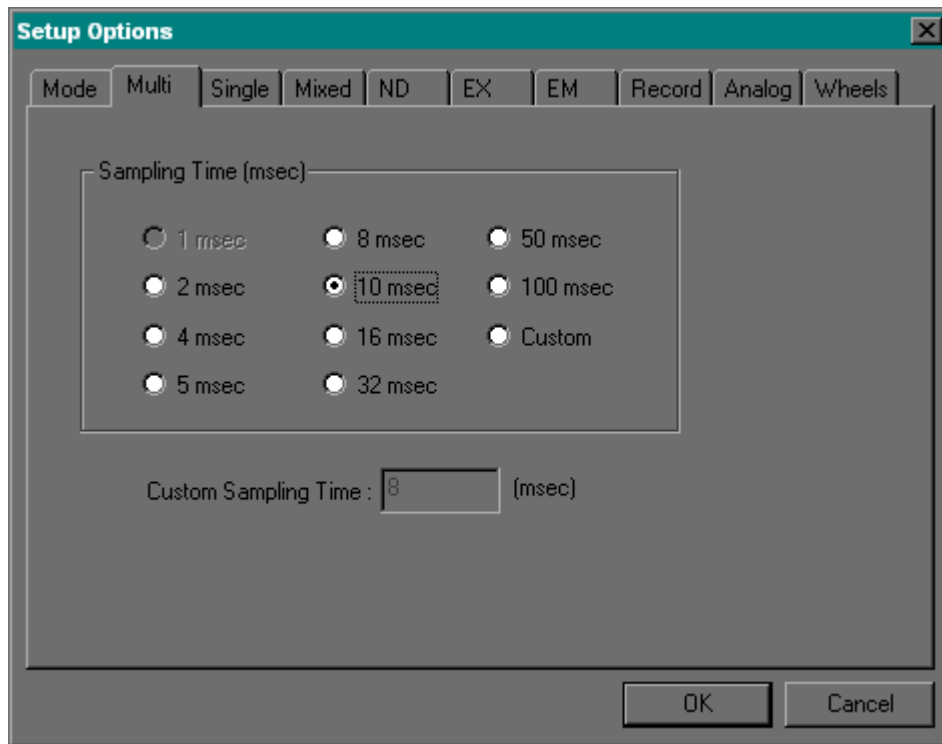


Figure 4. The Multi tab in the Setup Options dialog box.

The **Multi** tab allows the user to specify sampling times for any filter wheel(s) which are set to operate in the **Multi Mode**, as specified in the **Mode** tab. If the **Multi Mode** is not selected in the Mode tab for either the excitation or emission filter wheel, the Multi

Sampling Time selections are disabled. This dialog box has two sections: Sampling Time and Custom Sampling Time.

In the Multi Mode, the Sampling Time is the duration of time that one filter is in the optical path. For any filter wheel(s) operating in the Multi Mode, the setting on this tab determines the duration of time that fluorescence counts are accumulated and the rate of data acquisition. Fluorescence counts are accumulated to derive one data point for the period of time indicated by the Sampling Time setting. For instance, at a sampling time setting of 10 milliseconds, data is acquired at a rate of one point per 10 milliseconds, or 100 points per second.

Selecting a Standard Sampling Time

The **Sampling Time** section lists a choice of standard sampling times for selection. The specific sampling times that are enabled on this tab depends on the type of filter wheel selected in the **Mode** tab (i.e., 4 or 8-position). The setting of 1 millisecond is only available using an 8-position filter wheel. Using a 4-position filter wheel, this selection is disabled, as shown in Figure 4.

Specifying a Custom Sampling Time

The **Custom Sampling Time** field is used to enter a sampling time (in milliseconds) that is not available in the standard sampling times in the previous section.

Single Tab

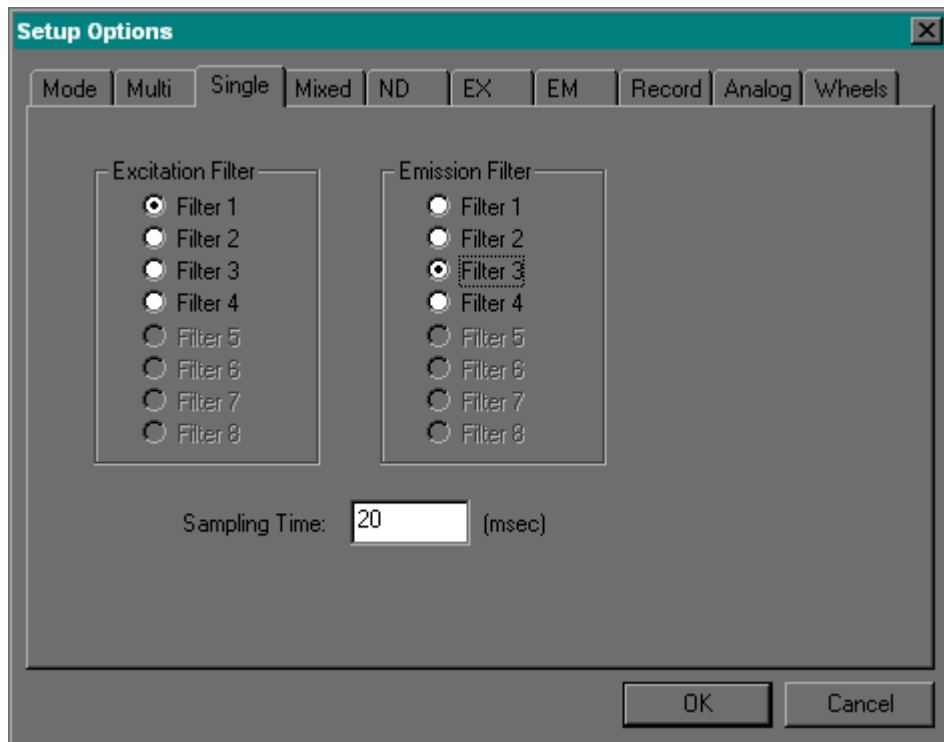


Figure 5. The Single tab in the Setup Options dialog box.

The settings in the **Single** tab of the **Setup Options** dialog box determine the operation of the **Excitation Filter** wheel and/or **Emission Filter** wheel when in the **Single Mode**. This dialog box has three sections: Excitation Filter, Emission Filter, and Sampling Time.

Specifying the Excitation and Emission Filter Position

The Excitation Filter and Emission Filter selections allow the user to specify the filter to be used for data acquisition in any wheel(s) which are set to operate in the **Single Mode**, as specified in the Mode tab. The type of filter wheel selected in the **Mode** tab dialog box (i.e., 4 or 8-position) determines how many Filter positions in the **Single** tab are enabled. If the **Single Mode** is not selected in the **Mode** tab for either the excitation or emission filter wheel, the Excitation Wheel and Emission Wheel Filter selections are disabled.

Specifying the Sampling Time

When *both* filter wheels are in the **Single Mode**, the **Sampling Time** field is enabled to allow the user to specify a sampling time (in milliseconds). If only one filter wheel is set to the **Single Mode**, then the sampling time is determined by the sampling time setting of the filter wheel that is in the **Multi Mode**.

Mixed Tab

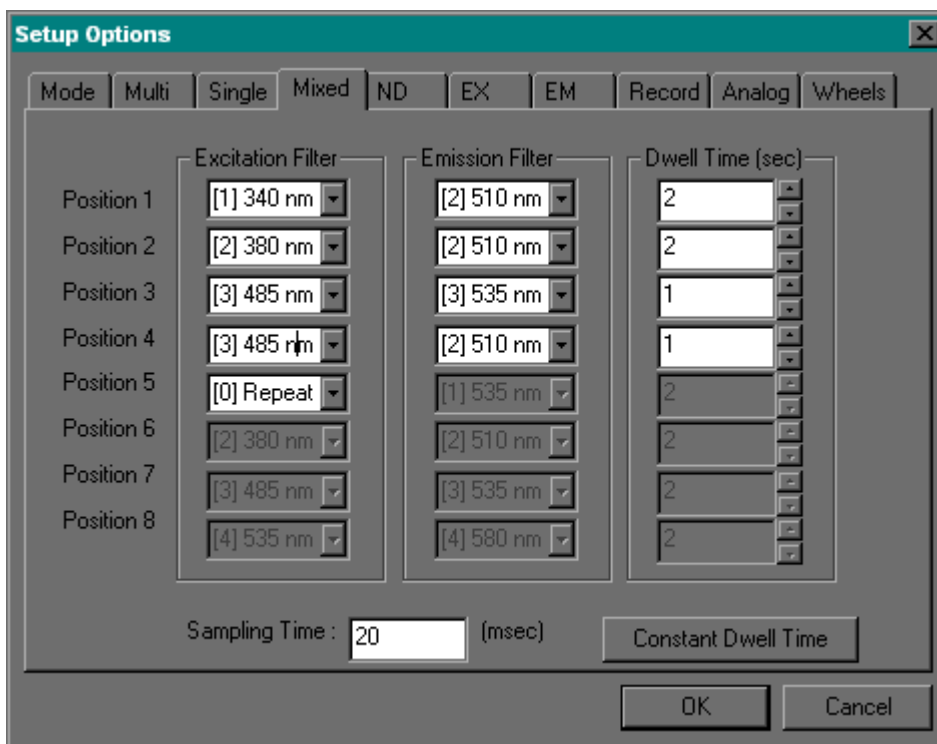


Figure 6. The Multi tab in the Setup Options dialog box.

When *both* filter wheels are set to operate in the **Mixed Mode**, the **Mixed** tab fields are enabled. The Mixed tab allows the user to program the operation of the emission and excitation filter wheels for up to eight excitation-emission filter combinations, Positions 1 through 8, which are accessed during data acquisition. The following settings can be specified on the Mixed tab:

- The specific sequence of **Excitation Filter** and **Emission Filter** combinations to be accessed during data acquisition. Any filter can be programmed to be accessed in any one of the eight positions.
- The **Dwell Time** for each Excitation-Emission filter combination. The **Dwell Time** is the duration of time that the filter wheels remain in a specified position during the period of data acquisition and prior to repositioning to the next entry in the program sequence.
- The **Sampling Time** – The duration of time in which photon counts are accumulated within the specified **Dwell Time**. The number of sample points that are acquired within the **Dwell Time** is determined by the setting of both the **Sample Time** and the **Dwell Time**. The **Sample Time** specifies the rate of data collection and the **Dwell Time** determines the duration of time that data is acquired at this rate.

Programming the Excitation Filter and Emission Filter Positions

The user can program a sequence of up to eight Excitation-Emission filter combinations by selecting the desired filters from drop-down menus in the **Excitation Filter** and **Emission Filter** columns.

For each wheel, the drop-down menus list the available filters by number, based on the wheel type specified in the **Mode** tab. Filters are designated [1] through [8] for an 8-position filter wheel, or [1] through [4] for a 4-position filter wheel.

In addition, the user can specify names for individual filters on the **EX** and **EM** tabs (described below), which are then appended to the filter number in the drop-down menus. Entering names for individual filters helps the user remember which filter type is in a given filter position in each filter wheel.

At some point in the sequence, the user may want the C&L Dye Fluorometer to repeat the filter combinations entered in the preceding rows. To do this, the user selects the Repeat option from the drop-down menu in either column, at the desired point (row) in the sequence. For instance, entering Repeat in Position 3 will cause the excitation and emission filter wheels to oscillate between the first two filter positions, entered in the two rows above the Repeat entry. Figure 6 illustrates a setting in which the filter sequence will cycle through 4 settings.

If the user wants *both* filter wheels to remain in a single stationary position, the Single-Single data acquisition mode should be used instead of the Mixed-Mixed mode. (For more information, see **Mode Tab**, above.)

Specifying the Dwell Time

On the right side of the **Mixed** tab, the user can specify the **Dwell Time** for each Position. The Dwell Time is the duration of time that the filter wheels remain in the specified position prior to repositioning to the next entry in the program loop. This entry is in units of seconds. If desired, a different dwell time can be specified for each position entry.

The button labeled **Constant Dwell Time** can be used to program a constant Dwell Time for all positions. In this case, the Dwell Time specified in the entry associated with **Position 1** will be applied to all Excitation and Emission filter positions.

Specifying the Sampling Time

The **Sampling Time** field is used to specify the duration of time in which fluorescence counts are accumulated within each **Dwell Time** period.

Shutter considerations: In the **Mixed Mode**, the excitation and emission shutters can be used to block unwanted light from passing through the filter wheels during the time in which the filter wheels are changing position. This can be used to protect either the sample and/or the PMT from excessive illumination. The sequence of events is that the shutters are closed, the filter wheels are repositioned to the next programmed position and then the shutters are opened. It takes approximately 300 milliseconds for the shutters to cycle and the filter wheels to reposition in between filter wheel positions. Data is not collected during this transition period.

Further discussion of the Sampling Time and the relation between the Dwell Time and Sampling Time can be found in **Chapter 9: Timing Considerations**.

ND Tab

The **ND** tab is used to control the setting of the neutral density wheel (i.e., the excitation light attenuation) for one to eight excitation-emission filter wheel positions used in the data acquisition sequence. The amount of attenuation is displayed as **% Aperture**.

The **ND** tab has four sections; **Clear**, **Select**, **Optical Density** and **% Aperture**, as well as a set of buttons along the bottom. There are eight rows, labeled **Position 1** through **Position 8**, corresponding to a maximum of eight possible excitation-emission filter wheel positions used in data acquisition.

The data acquisition mode (see **File / Setup Options**) determines the number of Position rows that are enabled.

- If the C&L Dye Fluorometer is set to operate in the Mixed-Mixed Mode, the user can select a different **% Aperture** setting for each filter wheel Position.
- If the C&L Dye Fluorometer is operated in any other mode (Single-Single, Multi-Multi, Single-Multi, or Multi-Single), the **% Aperture** setting remains at one position for all filter wheel positions. Under these conditions, the position of the neutral density filter is set using the **Position 1** row.

For each Position, either *no* attenuation (using the **Clear** field for a 100% aperture) or a specific degree of attenuation (using the slider in the **Select** field) can be specified.

Specifying No Attenuation

Placing a check in the **Clear** field for a particular Position will automatically set the ND filter to the 100% aperture setting (i.e., Clear) for that Position.

The **All Clear** button at the bottom of the tab is used to set *all* Positions to the Clear setting. A checkmark will be displayed in the Clear column for all Positions.

The **All Release** button reverses the All Clear action by removing the checkmark from the Clear column for all Positions. This allows the user to adjust settings individually for each Position.

Specifying Attenuation

To specify the degree of attenuation for individual Positions, use the **Select** sliders.

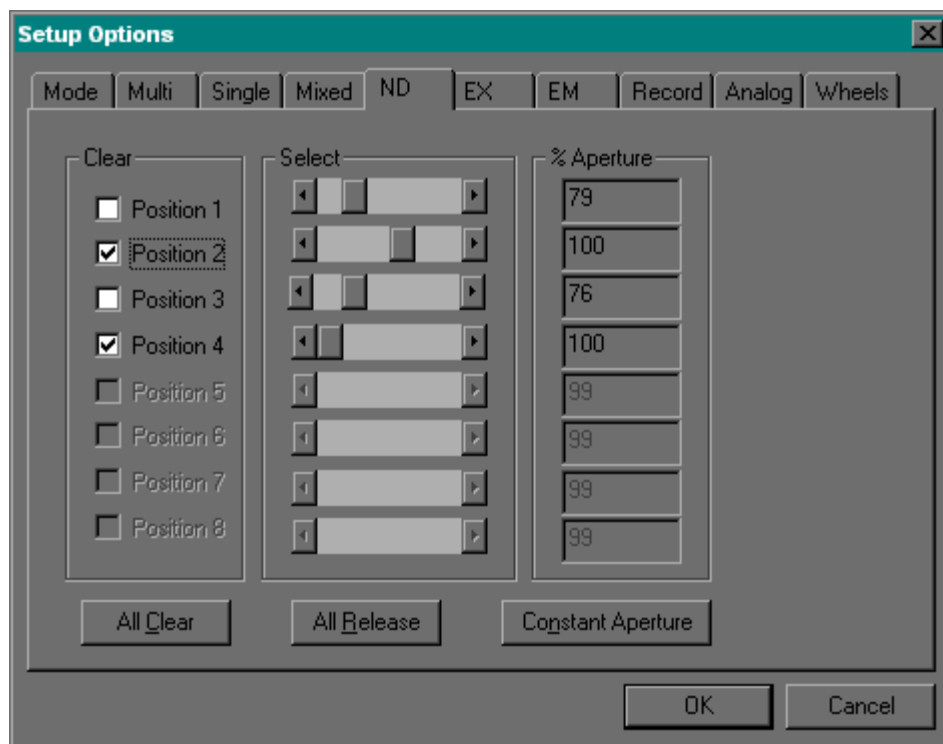


Figure 7. The ND tab in the Setup Options dialog box.

When the **Clear** check box is left unchecked, the slide bar in the **Select** column is enabled and can be used to set the **% Aperture** for each Position to anywhere within the usable range between 1 and 99.

To specify the same attenuation for all active Positions (when more than one are enabled), select the desired attenuation for Position 1, then click on the **Constant OD** button.

To move the ND wheel while monitoring data, use either the **Move ND Wheel...** option in the **File** menu or the ND toolbar icon to open the **Set ND Wheel** dialog box. Changes made in the **Set ND Wheel** dialog box are reflected in the **ND** tab in the **Setup Options** dialog box.

EX Tab

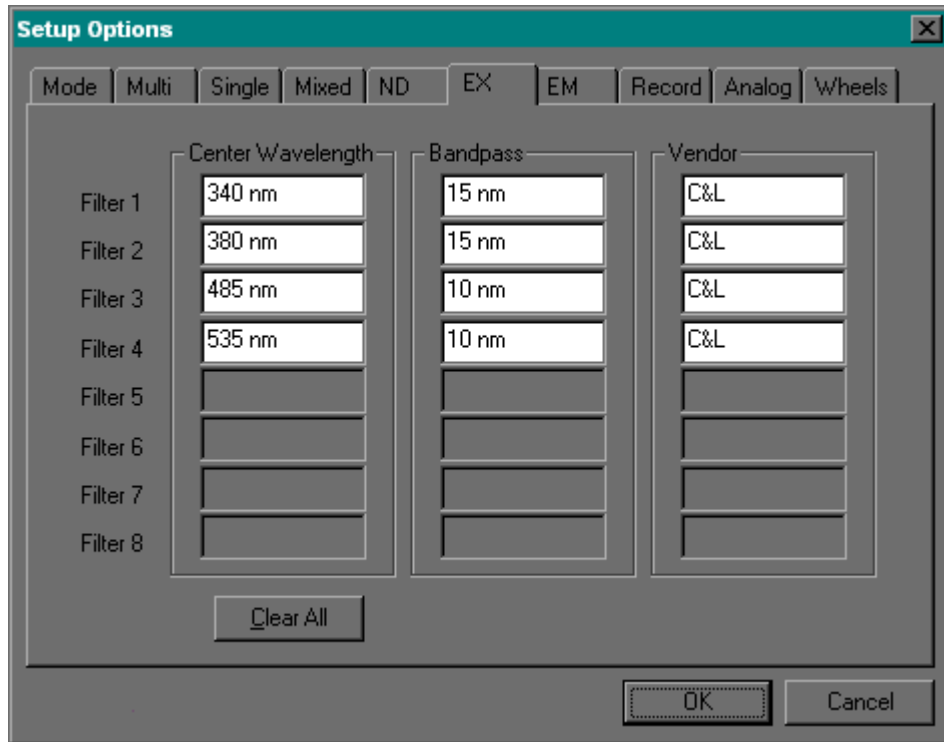


Figure 8. The EX tab in the Setup Options dialog box.

The EX tab is used to enter optional descriptive information about each filter in the excitation filter wheel. This information includes the **Center Wavelength**, **Bandpass** and **Vendor**.

The **Clear All** button at the bottom of the tab deletes any entries made in these fields.

The entries made in these fields are used to identify filter positions in other dialog boxes. These entries are saved along with other settings in the configuration file.

EM Tab

The EM tab is used to enter optional descriptive information about each filter in the excitation filter wheel. This information includes the **Center Wavelength**, **Bandpass** and **Vendor**.

The **Clear All** button at the bottom of the tab deletes any entries made in these fields.

The entries made in these fields are used to identify filter positions in other dialog boxes. These entries are saved along with other settings in the configuration file.

This dialog box is identical to the EX dialog box discussed above and shown in Figure 8.

Record Tab

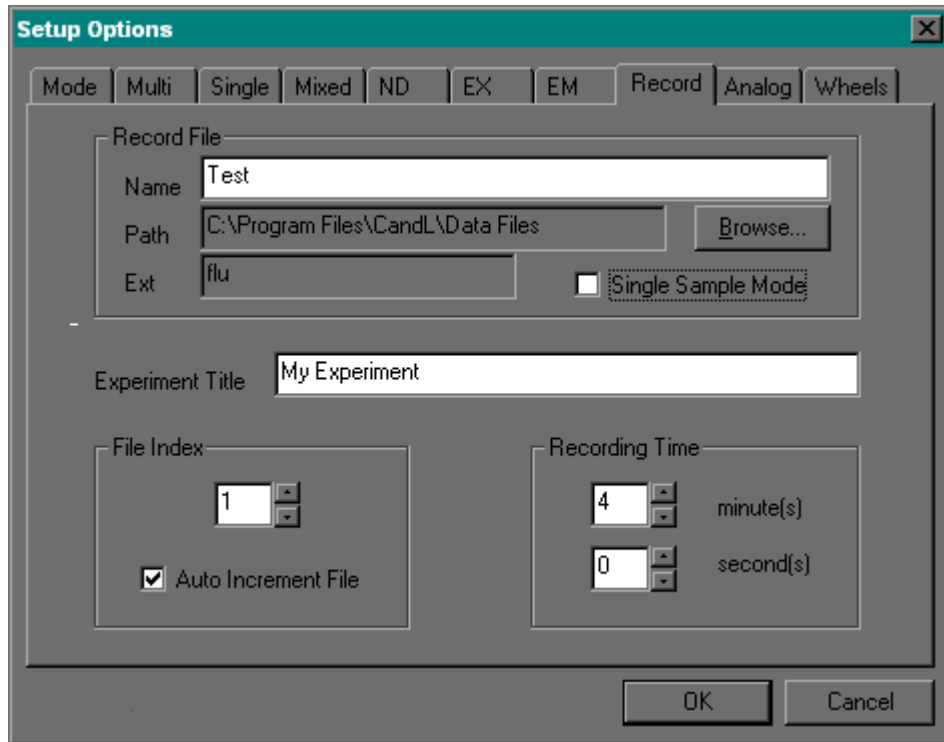


Figure 9. The Record tab in the Setup Options dialog box.

The Record tab has three sections in which the user can specify details of how data files are to be recorded: Record File, Experiment Title, File Index, and Recording Time. There is also a check box to enable the operation of the Single Sample Mode.

Specifying the Data File Name and Path

In the **Record File** section, the user specifies the **Name** and **Path** of the data file that is saved after completion of a data acquisition sequence. The **Browse** button is used to open a dialog box in which the user can specify the path to the directory used to store the data file. The data file is automatically given the extension of “.flu,” as indicated in the **Ext** field.

Specifying an Experiment Title

In the **Experiment Title** field, the user can enter an optional name for the experiment title. This title will be recorded along with the data in the data file.

Specifying Auto Incrementing

The Auto Increment feature is used to automate the file naming process so that files can be named in a simple numbered sequence without further intervention by the user.

This feature is enabled with the **Auto Increment File** check box. When enabled, the indicated file name is appended with the number indicated in the **File Index** field. If desired, the user can use the up and down arrows to change the index number. The name of each successive file is then incremented by 1 after each data acquisition session. For instance, if the index “1” is used for the first data file called “Test,” files will be saved sequentially as “Test1.flu”, “Test2.flu”, “Test3.flu”, etc.

Specifying the Recording Time

The **Recording Time** section is used to specify the duration of time that data is recorded when using the **Timed** recording feature. In **Timed** recording, data is recorded for the time interval indicated in the Recording Time field. (See the **Record Menu** section, later in this chapter, for more information on recording modes.)

Using the Single Sample Mode

The Single Sample Mode is used to collect data in either short bursts, or a series of short bursts separated by a time interval. When this feature is disabled, data is acquired continuously at the specified sampling rate. In this case, the data is collected as a continuous sequence that is stored as one file.

In some instances, however, it may be desirable to collect shorter period of data separated by one or more waiting periods and have all the data saved as one file. This feature is most useful when using the Model CV1 Cuvette Accessory. This is to allow the user to make additions to the cuvette or to change the cuvette. This feature, however, can be used in any data acquisition protocol. With the Single Sample Mode, data can be collected for each sample and the entire data set can be saved as one file.

This sample mode can also be used to collect data intermittently from one sample in order to avoid exposing the specimen to excessive illumination light. In this instance, data can be collected for brief periods that are separated by a longer time interval.

Data acquisition in the Single Sample Mode can be synchronized to the operation of the excitation and/or emission shutter(s) to restrict illumination of the specimen and/or the detector to light in between periods of data acquisition. See **Specifying Excitation Shutter and Emission Shutter Control Mode**, described above, for more detail.

For more information about recording fluorescence data, see **Chapter 7: Acquiring Data**.

Analog Tab

The Analog tab allows the user to enable or disable the recording of analog data on a channel by channel basis.

In the **Enable and Record Analog Data** section, channels are listed as **Analog 1** through **Analog 8**. Placing a check in a given check box enables recording of analog data on the indicated channel.

When an analog channel is enabled, the user can display the data in the Analog data window. If a specific analog data channel is disabled, it is not available for display. (For more information about viewing data, see **Chapter 8: Viewing Data**.)

The **All Select** button enables (checks) all of the analog channels.

The **All Release** button disables (unchecks) all of the analog channels.

For more information about recording analog data, see *Chapter 7: Acquiring Data*.

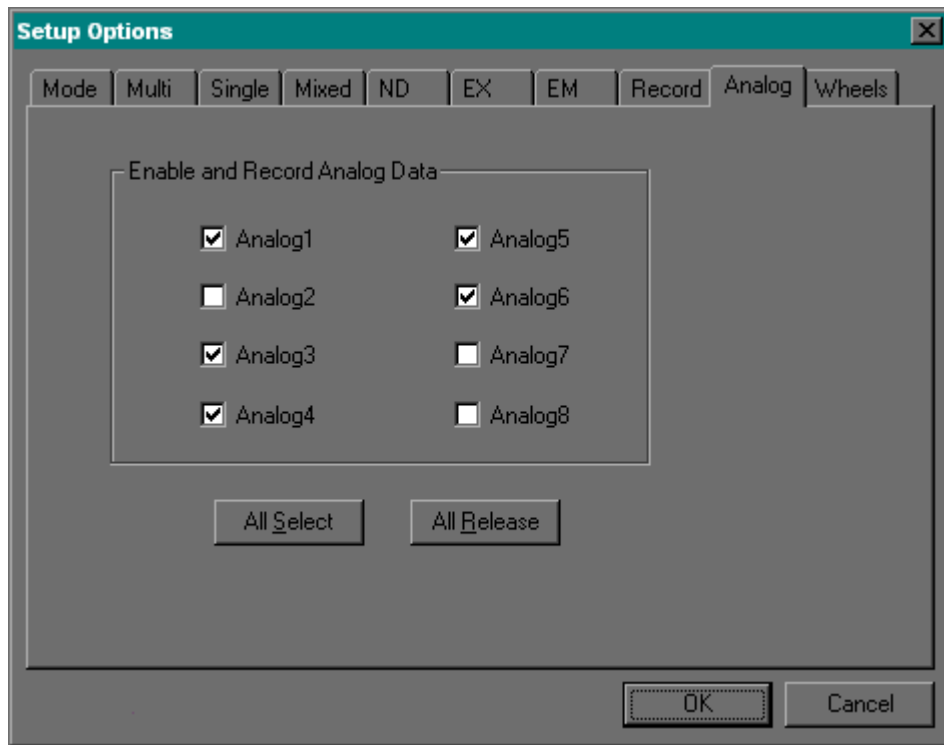


Figure 10. The Analog tab in the Setup Options dialog box.

Wheels Tab

The wheels tab is used to indicate to the Data Acquisition program what hardware has been installed in the fluorometer. The Data Acquisition program can operate any combination of filter wheels shown in this dialog box. Three check boxes are used to indicate the presence of the Excitation Filter Wheel, the Emission Filter Wheel and the Neutral Density Wheel.

When the Dye Fluorometer is set to operate using the Run command. Data Acquisition checks for the presence of the enabled devices. If the enabled devices are not found, a warning dialog box is displayed.

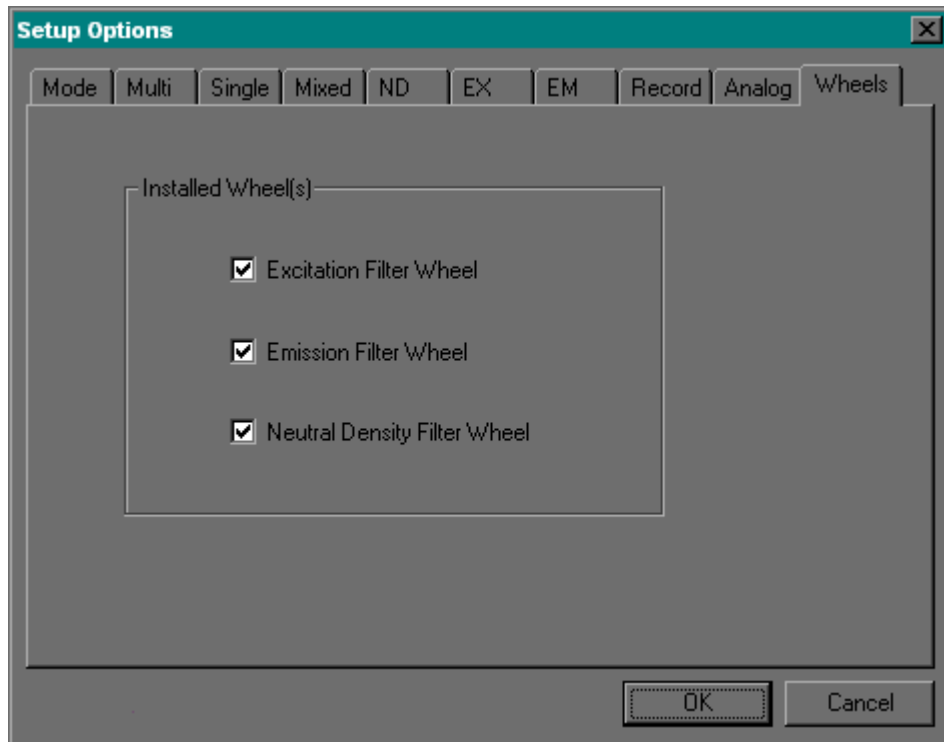


Figure 11. The Wheels tab in the Setup Options dialog box.

Options Menu

The Options menu contains the following menu items:

- **Color...**
- **Offset...**
- **Board...**

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. Using different colors allows you to more easily visualize the data in each channel.

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.

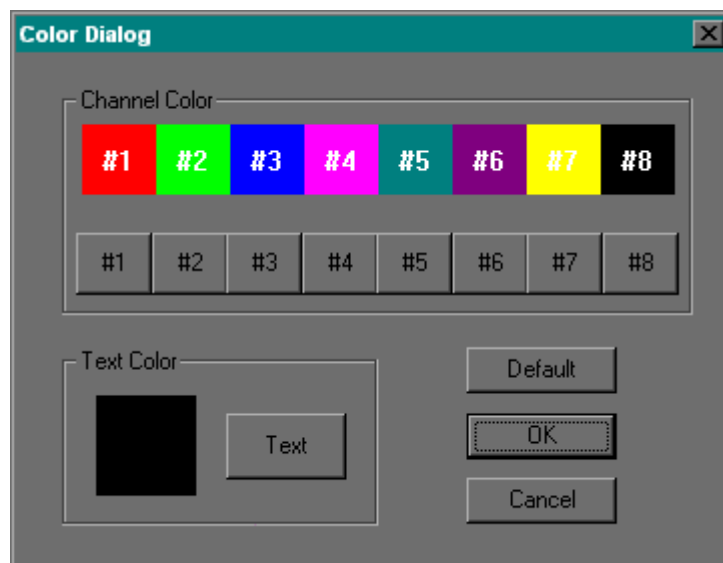


Figure 12. The Color Dialog Box.

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.

Options / Offset...

Offset... opens the Offset dialog box.

The Offset dialog box contains three entry fields. These entries are used to set the amount of offset error in the motors used by the C&L Dye Fluorometer in order to properly position the excitation, emission and neutral density wheels. Refer to the hardware user manuals that came with the illuminator and detector used with your Dye Fluorometer for these settings. *The entries in these fields should not need adjustment and any changes by the user may cause improper operation of the C&L Dye Fluorometer.*

If it is suspected that the excitation filter wheel, emission filter wheel or neutral density filter wheel are not positioning properly, contact C&L Instruments. Contact information is available in the front of this manual.

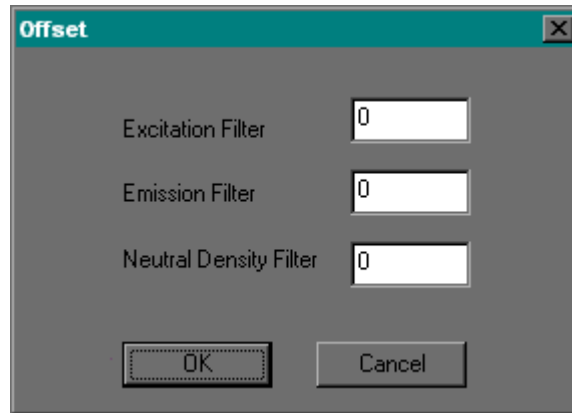


Figure 13. The Offset dialog box.

Options / Board

Board...

Selecting Board will open a dialog box that indicates the hardware interrupt and the base address used by the PC-DAQ Controller card. This dialog box is only used for diagnostic purposes should the PC-DAQ controller card not respond to user commands. If this is observed to occur, refer to the Help section at the end of this manual for a troubleshooting guide.

Shutter Menu

The Shutter menu options allow the user to control the shutters manually when the shutter control mode has been set to either **Auto Acquisition** or **Manual**. When the shutter control mode is set to **Auto Always**, the Shutter menu options are disabled. For details about the shutter control mode, see the *Mode Tab* of the **Setup Options** dialog box, under *File / Setup Options*.

The Shutter menu options are:

- **Open EX-Shutter** - Opens the excitation shutter (if closed) or closes it (if open).
- **Open EM-Shutter** - Opens the emission shutter (if closed) or closes it (if open).

Record Menu

The **Record** menu options are used to start and stop the recording of data. The length of a data recording session can be controlled manually or on a timed basis. The **Record** menu options are:

- **Start**
- **Stop**
- **Timed**

These options are described in detail below.

Record / Start

Starts the recording of data to be saved to a file. The **Start** option is only enabled after the **Run** operation is initiated to begin operation of the filter wheels (see **File / Run**, above).

Using **Start** to initiate data recording will cause the Data Acquisition software to begin recording data. After selecting **Start**, data will continue to be recorded until either the data buffer is full or the user selects the **Stop** command, whichever occurs first. The data buffer becomes full when any fluorescence channel has accumulated 10,000 data points. The duration of time required to fill the data buffer is dependent on the speed of data acquisition. For instance, using a sampling period of 100 milliseconds allows the user to collect a data file over a period of 1,000 seconds, or about 16.6 minutes.

Record / Stop

The **Stop** command stops the recording of data and tells the user when the data in the computer RAM has been written to a file. **Stop** will stop the recording of data when either the **Start** or **Timed** option was used to initiate recording. The name of the data file is specified in the **Record** tab of the **Setup Options** dialog box (see the **Record Tab** section, above). When **Stop** is selected, recording is stopped and a message box appears to inform the user that the data file has been saved.

Record / Timed

This option starts the recording of data for a fixed period of time. The time duration for data collection with the **Timed** option is set in the **Record** tab of the **Setup Options** dialog box. The **Timed** option is only enabled after the **Run** operation is initiated to begin operation of the filter wheels (see **File / Run**, above).

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 the three data window types. Furthermore, the **Display** menu options vary for each of the three data window types, depending on which data window is active when the **Display** command is selected.

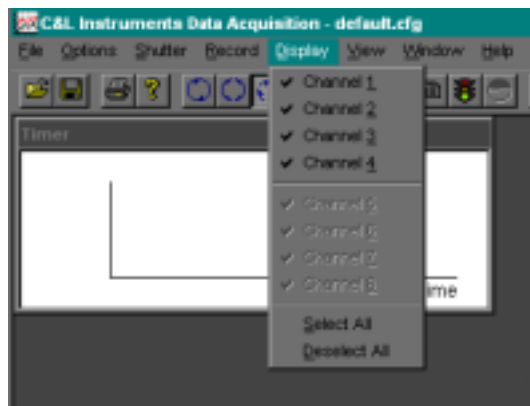


Figure 14. The **Display** menu items available when the Fluorescence window is active.

Fluorescence Window Display Options

If a Fluorescence window 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**

Number of Channels Enabled

The number of fluorescence channels that are enabled on this menu depends on the data acquisition mode and the filter wheel type selected in the **Setup Options**.

- If the C&L Dye Fluorometer is equipped with two 4-position filter wheels, only the first four channels can be enabled in the Multi-Multi, Multi-Single or Single-Multi Modes of data acquisition.
- If the C&L Dye Fluorometer is equipped with either one or two 8-position filter wheels, then all eight channels can be enabled.
- If the C&L Dye Fluorometer is operated in the **Mixed-Mixed Mode**, the number of channels that are enabled is determined by the number of discrete positions programmed in the **Mixed** tab of the **Setup Options** dialog box.

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 enabled channels.

Selecting **Deselect All** removes the selection from all enabled channels.

Analog Window Display Options

If an Analog window is active when **D**isplay is selected, then the following options are available for selection in the **D**isplay drop-down menu. These channels can be enabled for display in the same manner as described above for the Fluorescence channels.

- **A**nalog 1
- **A**nalog 2
- **A**nalog 3
- **A**nalog 4
- **A**nalog 5
- **A**nalog 6
- **A**nalog 7
- **A**nalog 8
- **S**elect All
- **D**eselect All

Number of Channels Enabled

The number of analog channels that are enabled on this menu depends on the setting in the **A**nalog tab of the **S**etup **O**ptions dialog box. Only the analog channels that have been enabled in the **A**nalog tab are available for selection in the **D**isplay menu. The analog channels that are enabled in the **D**isplay menu are *independent of* the data acquisition mode and the filter wheel types specified in the **S**etup **O**ptions.

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 enabled analog channels.

Selecting **Deselect All** removes the selection from all enabled analog channels.

Fluorescence Ratio Window Display Options

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

- **N**umerator
- **D**enominator

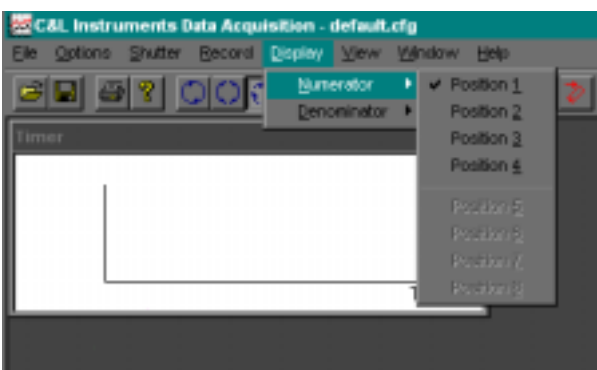


Figure 15. An example of the **Display** menu items that are available when the Fluorescence Ratio window is active.

Selecting a Position for the Numerator and Denominator

To select a Position for the **N**umerator or **D**enominator, click on **N**umerator or **D**enominator to display the list of available Positions. Then select the desired position.

Number of Channels Enabled

The number of fluorescence channels that are enabled on the Numerator and Denominator menus depends on the data acquisition mode and the filter wheel type selected in the **Setup Options**.

- If the C&L Dye Fluorometer is equipped with two 4-position filter wheels, only the first four channels are enabled for selection in the Multi-Multi, Multi-Single or Single-Multi Modes. (The **Mode** settings of the excitation and emission filter wheels are specified in the **Mode** tab of the **Setup Options** dialog box.) The fluorescence ratio cannot be monitored in Mixed-Mixed Mode.
- If the C&L Dye Fluorometer is equipped with either one or two 8-position filter wheels, then all channels are enabled in all available data acquisition modes.
- If the C&L Dye Fluorometer is operated in the **Mixed-Mixed Mode**, the number of channels that are enabled is determined by the number of discrete positions programmed in the **Mixed** tab of the **Setup Options** dialog box.

View Menu

The **V**iew menu allows the user to control certain aspects of the appearance of the main program window and data windows. In similar fashion to the **Display** menu, the **V**iew menu options vary depending on the type of window that is currently active.

If either the main program window or the Timer window is active, the following menu options are available in the **V**iew menu.

- **T**oolbar
- **S**tatus Bar

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

- **Toolbar**
- **Status Bar**
- **Lines**
- **Zoom In**
- **Zoom Ot**
- **Move Up**
- **Move Down**
- **Unzoom**
- **Settings...**

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

- **Toolbar**
- **Status Bar**
- **Zoom In**
- **Zoom Ot**
- **Move Up**
- **Move Down**
- **Unzoom**
- **Settings...**

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

- **Toolbar**
- **Status Bar**
- **Counts**
- **Counts/sec**
- **Zoom In**
- **Zoom Ot**
- **Move Up**
- **Move Down**
- **Unzoom**
- **Settings**

View / Toolbar

Displays or hides the Toolbar.

View / Status Bar

Displays or hides the Status Bar.

View / Lines

Enables the display of lines between the data points in the Analog window. The display of lines is only available in the Analog data window.

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 Counts.
- Selecting **Counts/Sec** changes the units to Counts/Second.

View / Zoom In, / Zoom Out, / Move Up, and / Move Down

The **Zoom In**, **Zoom Out**, **Move Up**, and **Move Down** 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 indicative of the selected option. The new mouse cursor is visible only when the mouse is moved over the Fluorescence, Analog or Fluorescence Ratio windows.

- 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. The extent that the **Zoom In** selection will zoom in on the data displayed in the active window depends on the setting of **Zoom In**. This is set using the **View / Settings...** command, as discussed below.
- 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.

Shortcuts to the **Zoom In**, **Zoom Out**, **Move Up**, and **Move Down** features are also available as icons in the Toolbar. For more information on viewing data, see **Chapter 8: Viewing Data**. For more information on using the icons in the Toolbar, see **Chapter 6: Using the Icons in the Toolbar**.

View / Unzoom

Unzoom returns the scale of the Y axis of the active window to the original completely unzoomed value.

In the Fluorescence window, the maximal value of the value indicated on the Y axis after **Unzoom** has been selected depends on the rate of data acquisition. If the scale is set to display the fluorescence intensity in counts, then the maximum value will be 65,535 counts (e.g., $2^{16}-1$). If the scale is set to display the fluorescence intensity in Counts/Sec, then the maximum value will be 65,535 divided by the sampling time in seconds. In either case, the maximal value displayed on the Y axis after selecting **Unzoom** is the fluorescence intensity that will cause saturation of the counter.

In the Analog window, selecting Unzoom will set the Y axis scale to display from 0 to +5 Volts.

In the Fluorescence Ratio window, selecting Unzoom will set the Y axis scale to display from 0 to 100.

View / Settings...

View / Settings... is used to manually set the Y axis scale of active window and to set the magnitude of the zoom when **Zoom In** is selected.

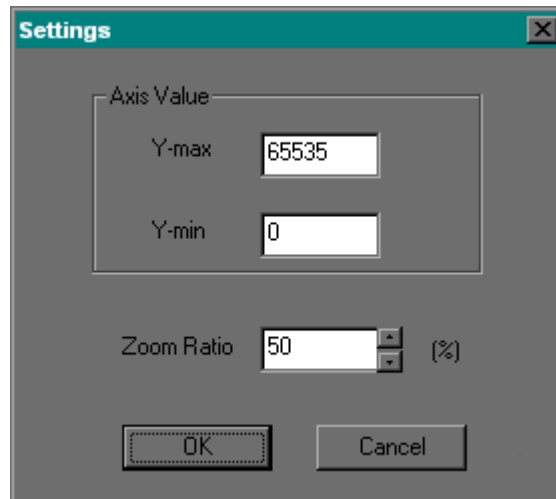


Figure 16. The Settings dialog box.

The maximum and minimum values of the Y axis are entered in the Axis Value section of the Dialog box. The value entered for Zoom Ratio sets the extent that the Zoom In feature will zoom in the active data window. This value can range from 0 to 100%. A value of 50% will decrease the scale of the Y axis by 50% (a factor of 2).

Accepting and Canceling Changes

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

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

Window Menu

The following menu items are available in the Window menu:

- **New Fluorescence**
- **New Analog**
- **New Fluorescence Ratio**
- **Cascade**
- **Tile**
- **Arrange Icons**
- **1... (etc.)**

Window / New Fluorescence, / New Analog, and / New Fluorescence Ratio

Selecting **New Fluorescence**, **New Analog**, or **New Fluorescence Ratio** opens an additional Fluorescence, Analog or Fluorescence Ratio 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 monitor the fluorescence from different filter wheel positions and the data can be scaled differently in each window.

Any number of additional data windows can be opened to customize the viewing of your data. The presentation of the data, however, may appear sluggish if too many data windows are open. The ability of Data Acquisition to display data in multiple windows depends on the speed of your computer and computer graphics.

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 Acquisition...**

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 Acquisition...

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

Toolbar

The Toolbar displays icons that can be used as quick shortcuts to many of the features available in the Drop Down Menu Bar. The Toolbar in Data Acquisition has “Bubble Help”. Moving the cursor over the icon will cause the display to indicate a brief explanation of the function of the particular icon. The use of these icons as shortcuts to specific menu options is discussed in *Chapter 6: 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. The program is usually in this state. For example, the software is in a “ready” state during data acquisition, since it allows you to perform software functions using the drop-down menus or toolbar icons.
- **Option description** - When the cursor is moved over a drop-down menu option, the status area displays a brief description of that option.


Chapter 6: 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 Acquisition 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 5: 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 either the main Menu Bar or the Setup Options dialog box.

 File / <u>O</u>pen	 File / <u>S</u>ave
 File / <u>P</u>rint...	 Help / <u>A</u>bout C&L...
 Sets the Excitation filter wheel to the Multi Mode	 Sets the Excitation filter wheel to the Single Mode
 Sets the Excitation filter wheel to the Mixed Mode	 Sets the Emission filter wheel to the Multi Mode
 Sets the Emission filter wheel to the Single Mode	 Sets the Emission filter wheel to the Mixed Mode
 File / <u>S</u>etup Option...	 File / <u>R</u>un
 File / <u>M</u>ove ND Wheel	 Shutter / <u>O</u>pen <u>E</u>X-Shutter
 Shutter / <u>O</u>pen <u>E</u>M-Shutter	 Record / <u>S</u>tart
 Record / <u>S</u>top	 Record / <u>T</u>imed
 View / <u>Z</u>oom <u>I</u>n	 View / <u>Z</u>oom <u>O</u>ut
 View / <u>M</u>ove <u>U</u>p	 View / <u>M</u>ove <u>D</u>own
 View / <u>U</u>nzoom	 View / <u>S</u>etting...

Chapter 7: Acquiring Data

Overview

This chapter describes how to use the C&L Dye Fluorometer to acquire fluorescence and analog data. A brief overview of the key features discussed in the present chapter can be found in *Chapter 3: Fundamentals of Data Acquisition*. Detailed explanations of the various software functions available in the menu bar can be found in *Chapter 5: Command Reference*. The user is encouraged to first become familiar with these functions prior to operation of the C&L Dye Fluorometer.

Data and Configuration Files

The C&L Dye Fluorometer uses two types of files: data files and configuration files.

Data Files

The Data Acquisition software creates data files and saves them with the extension of “.flu”. The C&L Instruments Dye Fluorometer creates these data files, which are used by the Data Analysis software. The Data Acquisition software does not read or use these files in any fashion for operation of the C&L Dye Fluorometer. Once saved by Data Acquisition, the files must be opened by Data Analysis to either view or analyze the data.

Configuration Files

A configuration file, which has the extension of “.cfg”, is used to store all operating parameters of the C&L Dye Fluorometer. One or more configuration files can be stored for recalling previously saved setting of the Dye Fluorometer. Configuration files are used to save operation settings so that they can be quickly retrieved at a later date. The **Open** and **Save** menu options under the **File** menu are used to open and save configuration files.

Configuration files can be used for specific data acquisition tasks that may become repetitive. For instance, if a particular experiment requires the measurement of fluorescence from only a few fluorescence and analog channels, a new configuration file can be saved for later recall after the fluorometer is set up the first time. It is recommended that the user should save the current configuration file using a new name if the previous setup will be used at a later date.

Using the Setup Options

All of the major features of the C&L Dye Fluorometer can be set for the measurement of fluorescence and analog data using the tabbed **Setup Options** dialog box.

Usually, the first step in specifying the Setup Options is to decide on the data acquisition mode to be used for data collection. The filter wheel and shutter operation mode selections are available in the Mode tab.

Data Acquisition Modes

The selection of the data acquisition mode depends on several factors and considerations. If only one excitation and emission filter position is required for data collection, the Single-Single mode is probably the desired selection. This corresponds to using a single excitation and emission wavelength pair. In this mode, both filter wheels are maintained in a stationary filter position. An advantage of using this mode is that the movement of the filter wheel(s) does not limit the maximal rate of data acquisition. In the Single-Single mode, data can be acquired a rate of up to 0.1 millisecond per point.

When a filter wheel is set in Multi mode, the filter wheel will spin and all the filters in the wheel are available for either Excitation or Emission. In this mode, it is important to remember that the filters in the wheel are positioned in the light path in the order that they were mechanically mounted in the wheel. Thus, some forethought is required to plan a complicated multiwavelength protocol when both the excitation and emission filter wheels are set to Multi (e.g., Multi-Multi mode).

The combination of Multi-Single is used for multiple wavelength excitation and single wavelength emission. Single-Multi is used for single excitation and multiple wavelength emission.

Mixed-Mixed mode can be thought of as a combination of the Single and Multi modes. In the Mixed-Mixed mode, both filter wheels are set to a stationary position (as in the Single mode) for a period of data acquisition. Then the filter wheels are repositioned to the next desired setting for the next period of data acquisition. The duration of time that the filters wheels are held stationary for data collection is the Dwell Time. Up to eight excitation and emission wavelength pairs can be programmed to access these wavelengths in a continuous sequence.

The operation of the Mixed-Mixed mode can be synchronized with the automated operation of the shutters. This automated process will close either one or both shutters during the time it take to reposition the filter wheels for the next period of data collection.

Combinations of the Multi mode (e.g., Multi-Mixed, etc.) other than Multi-Multi are disallowed. The operation of the Multi-Single and Single-Multi, where one filter wheel is stationary while the other moves in a programmed sequence, can be accomplished using the Mixed-Mixed mode. This is performed by selecting the same filter position for one filter wheel and programming the other to operate in a desired sequence.

For the measurement of fluorescence using more than one excitation and/or emission wavelength, the user can choose between Multi-Multi, Multi-Single, Single-Multi or Mixed-Mixed data acquisition modes. These modes would be applicable for measuring the fluorescence of a dye that exhibits a wavelength shift in either its excitation and/or emission spectrum. The Multi-Single and Single-Multi modes are generally used for conditions in which the user wants *either* multiple excitation or multiple emission wavelengths, but not both. The Multi-Multi mode is generally used for conditions in which the user wants both multiple excitation and multiple emission wavelengths.

The following table summaries some of the features of the five data collection modes. Considerations in Selecting a Data Acquisition Mode.

<i>Mode</i>	<i>Purpose</i>	<i>Pros</i>	<i>Cons</i>
Single-Single	Single excitation and single emission wavelength	Fastest rates of acquisition.	Only one wavelength pair measured.
Single-Multi	Single wavelength excitation, multiwavelength emission.	Fast method of data acquisition to obtain fluorescence ratios.	Only one excitation wavelength permissible. One Neutral Density filter setting for all filter positions.
Multi-Single	Multiwavelength excitation, single wavelength emission.	Fast method of data acquisition to obtain fluorescence ratios.	Only one emission wavelength permissible. One Neutral Density filter setting for all filter positions.
Multi-Multi	Multiwavelength excitation and multiwavelength emission.	Fast method of data acquisition to obtain fluorescence ratios.	One Neutral Density filter setting for all filter positions.
Mixed-Mixed	User selectable use of more than one filter wheel position in excitation and emission	Most flexible data acquisition mode. A unique Neutral Density filter setting for each filter positions. Any excitation filter position can be paired with any emission filter wheel position.	More time is required to change filter wheel positions. Fluorescence ratios cannot be monitored during data acquisition.

Using Run

The **Run** command is used to initiate the filter wheels and the neutral density wheel and to place them in the data acquisition mode specified in the Setup Options. **Run** also allows the data to be viewed in the data windows. When the Data Acquisition program is first started, data cannot be viewed (i.e., monitored) in the data windows (Fluorescence, Analog, Fluorescence Ratio) until the **Run** command is issued. Likewise, data acquisition cannot be started, using either the **Start** or **Timed** options, without first initiating the **Run** command. Until the user issues the **Run** command, the Start and Timed options are disabled.

Issuing the **Run** command a second time reverses the action of the **Run** command. It stops all activity of the filter wheels. This feature can be used prior to setting the filter wheels to new acquisition mode. The **Run** command is also available as an icon in the Toolbar. This icon appears “pushed” when run has been selected and “flush” when it has been depressed after **Run** is selected.

Using the Neutral Density Wheel and Avoiding Peak Pile Up

The Neutral Density Wheel

The Neutral Density Wheel is used to attenuate the intensity of the excitation light. The position of this wheel can be adjusted using either the ND tab in the Setup Options dialog box or the **File / Move ND Wheel...** command. An icon in the Toolbar is available as a shortcut to the **File / Move ND Wheel...** command.

When the ND wheel is adjusted using the Setup dialog box, the programmed position will take effect after the dialog box is closed by selecting the OK button. When the ND wheel is adjusted using the **File / Move ND Wheel...** command, however, the changes occur immediately and the dialog box remains open to accept further user input. This feature is useful to adjust the ND wheel while data is being monitored in the Fluorescence and Fluorescence Ratio data windows.

In the Mixed-Mixed mode of data acquisition, each of the programmed filter wheel positions can have a unique ND filter wheel position. In all other modes, the first ND wheel position is used for all filter wheel positions.

Adjustment of the ND wheel during data acquisition is disabled. That is, during data collection (i.e., after either Start or Timed has been selected), the Setup Dialog box cannot be opened and the **File / Move ND Wheel...** command is disabled.

Avoiding Peak Pile Up

The PMT in the Dye Fluorometer is linear to a count rate of 10×10^6 counts/sec. When this count rate is reached, exposure of the PMT to increasing amounts of light may cause the observed count rate to *decrease*. This is normal behavior, which is caused by *Peak Pile Up*.

When the count rate becomes excessive, the time between pulses becomes very short and the detection circuitry cannot distinguish between the end of one pulse and the beginning of the next. The result is that pairs of pulses in close sequence get counted as one pulse and the overall count rate begins to decline. Peak Pile Up can be detected by using the neutral density wheel to attenuate the excitation light intensity. If the count rate is observed to *increase* after the aperture setting of the neutral density wheel is *decreased*, then peak pile up is probably occurring.

If Peak Pile Up occurs, the user can decrease the count rate in one of several ways to put the PMT in the linear range.

1. Decrease the exposure of the sample to illumination light by decreasing the aperture of the neutral density wheel.
2. Decrease either the sample concentration or the concentration of fluorescent species in the sample.
3. Place an additional neutral density filter in the detector assembly. Refer to the user manual for the Model D46 Detector for instructions on how to add an optional neutral density filter to this detector.

Using the Shutters and Avoiding Shoot Through

The Excitation and Emission Shutters

The number of shutters available in the Dye Fluorometer depends on your hardware setup. Versions of the Dye Fluorometer that have separate illumination and detection modules contain two shutters, one in each module. The excitation shutter in the illumination module and the emission shutter in the detection module are controlled independently by the Data Acquisition software. The three operation modes for the shutters are selected using the Mode tab of the Setup dialog box. See *Chapter 5: Command Reference* for an explanation of these settings.

The shutters are used for two purposes:

1. The excitation shutter is used to block illumination of the sample.
2. The emission shutter is used to block detection of the excitation light by the PMT (photomultiplier tube) and to prevent exposure of the PMT to room light.

Blocking excess illumination light with the excitation shutter is commonly used to limit photobleaching of the sample. Photobleaching is generally observed as a steady decline in the fluorescence of a sample that can be stopped by blocking the excitation light. The susceptibility of a particular fluorescent probe to photobleaching is dependent on the particular probe and its environment.

When the Dye Fluorometer software is started after the illuminator and detectors are turned on, the shutters will initially be set to the closed position. If the user exits the Data Acquisition software with the shutters in the open position, it is possible that the shutters will initially be in the open position the next time the Data Acquisition software is started if the computer has not been shut off in the interim. It is always good practice to close the excitation and emission shutters prior to quitting the Data Acquisition program.

Avoiding Shoot Through

BE CAREFUL NOT TO EXPOSE THE PMT TO EXCESSIVE LIGHT.

The emission shutter can and should be used to limit the exposure of the PMT to excess light. Excessive light can be caused by exposure of the PMT to room light or the excitation light. Exposure of the PMT to excitation light is commonly caused by *shoot through*.

Shoot through is when the excitation and emission wavelengths are set to similar values so that excitation light from the illuminator reaches the detector. This is avoided by careful selection of interference filters and by not allowing interference filters with overlapping passbands to be positioned in the excitation and emission filter wheels at the same time. For answers to questions about the selection of interference filters, the user should refer to the C&L Instruments web site or contact C&L Instruments directly. The URL for the C&L web site is listed on the cover of this manual.

The proper:

- selection of interference filters,
- placement of filters in the filter wheels, and
- operation of the Dye Fluorometer

provides the best insurance to avoid shoot through.

The Multi mode.

When a filter wheel is operated in the Multi mode, the sequence of filters will be in the order in which they were installed in the filter wheel. Avoiding shoot through should be a primary consideration when deciding on which position to install particular filters in a filter wheel.

The Mixed Mode

When the Dye Fluorometer is operated in the Mixed-Mixed mode, the likelihood that shoot through can occur is increased because the excitation and emission filters can be positioned in any pair combination as programmed in the Mixed tab of the Setup Options dialog box. Moreover, intermediate filter positions can be transiently placed in the light path during repositioning of the filter wheels in this mode.

When the filter wheel repositions while in the Mixed-Mixed mode, the sequence of intermediate positions will be between the start and end positions. That is, if a filter wheel is programmed to go from position 1 to position 6, then it will briefly pass through the intermediate positions 2, 3, 4, and 5 on its way from position 1 to 6. The reverse sequence will occur if it is programmed to go from position 6 to 1. This should be borne in mind when positioning filters in the filter wheels when the Dye Fluorometer is used in this mode.

It is important to note that, in this acquisition mode, the display of data is disabled during the time it takes for the filter wheel(s) to reposition. Thus, the presence of shoot through is not readily apparent in the display.

To avoid shoot through during repositioning of the filter wheel in the Mixed mode, it is recommended that the user follow one or more of the following practices:

1. Carefully select the filter position when installing filters in the filter wheel(s). If the excitation or emission is to alternate between two positions, then shoot through can be avoided by placing the filters in adjacent positions.
2. Use the shutters in the Auto Always mode. In this mode, the shutter will close prior to repositioning the filter wheel(s) and will reopen once the filter wheel(s) are stationary. See *Chapter 5: Command Reference* for more details about the Auto Always mode.

Acquiring Analog Data

The C&L Dye Fluorometer can record analog data along with the recording of fluorescence data. Eight analog inputs are provided that can be used to acquire data in the 0 to +5 Volt range. The user should avoid applying voltages outside this range to these

inputs. If the user requires collecting data over a wider range (e.g., 0 to +10 Volts), a voltage divider should be inserted between the signal source and the analog input of the Dye Fluorometer. Contact C&L instruments if assistance is required. The acquired data is viewed in the Analog window. The Dye Fluorometer acquires analog data in synchrony with the fluorescence data. Details of the timing of analog and fluorescence data acquisition are discussed in *Chapter 9: Timing Considerations*.

In order to display analog data in the Analog window and to record analog data from a particular channel, the analog channel must be first enabled. Analog channels are enabled and disabled through software using the Analog tab in the Setup dialog box. See *Chapter 5: Command Reference* for details of this feature.

Enabling an analog channel connects the input signal to the Analog to Digital Converter (ADC) used by the Data Acquisition system. When an analog channel is disabled, the input signal is disconnected from the ADC and the input to the ADC for that channel is grounded (the signal is not grounded). This prevents a “floating signal” from being presented to the ADC.

Recording and Saving Data

Recording or acquiring data is initiated with the **Record / Start** or **Record / Timed** command. The **Record** command will begin data acquisition in a manner that requires the user to select the **Record / Stop** command to stop the recording of data. Selecting **Record / Timed** will begin data acquisition that will last for the duration of time specified in the Record tab of the Setup Options dialog box or until the **Record / Stop** command is selected, whichever occurs first.

In the normal mode of operation, starting the recording of data using either the **Record / Start** or **Record / Timed** commands will start data recording immediately and the recording of data will be continuous until it is stopped. This is the preferred mode for continuous and uninterrupted data recording. The Single Sample Mode is used for non-continuous recording of data.

Using the Single Sample Mode

The Single Sample Mode is used when the user wants the collection of data to be interrupted one or more times, but yet have the data recorded in one file. It is also useful for data averaging. With this mode, numerous measurements of the fluorescence and analog signal can be made and averaged together as one data point in an automated fashion.

Operation of the Dye Fluorometer in the Single Sample Mode is selected by enabling this feature using the check box in the Record tab of the Setup Options dialog box. (See *Chapter 5: Command Reference*). When enabled, selecting the **Record / Start** command will open a dialog box, as shown in the following figure.

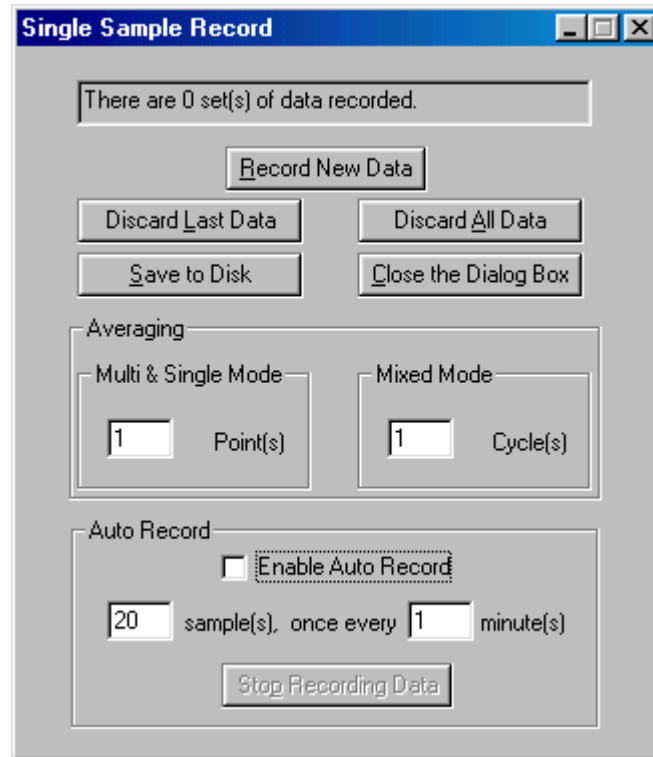


Figure 17. The Single Sample Record dialog box.

The Single Sample Mode dialog box contains 5 selection buttons, 4 places for data entry and a message box. The Single Sample Mode is setup by entering values in the data entry boxes labeled **Multi & Single Mode**, and **Mixed Mode**. A check box enables the **Auto Record** feature.

In the Single Sample Mode, data is recorded to a data buffer. The user has the option of saving the data to disk at any time, or deleting the last or all of the data points in the buffer. The 5 selection buttons controls recording of data into this buffer, deleting data from the buffer and saving the data to disk. This is discussed more fully below.

Multi & Single Mode

This dialog box is used for signal averaging. The entry in this dialog box indicates how many data points will be averaged together to generate one data point in the data acquisition file. This dialog box is used when the data acquisition mode is any combination of Multi and Single (i.e., Single-Single, Single-Multi, Multi-Single, Multi-Multi). An entry of 1 indicates that no signal averaging will occur. Valid entries are between 1 and 500. For instance, when the value in this dialog box is set to 50, 50 points will be collected and the average value of the 50 points will be placed in the data buffer. If the sampling mode is set to Multi, then the average of the 50 points at each wavelength combination will placed in the buffer.

Mixed Mode

This dialog box is used for signal averaging. This dialog box is used when the Dye Fluorometer is operated in the Mixed-Mixed mode of data acquisition. The entry in this dialog box indicates how many cycles of the filter wheel are used to average data. One cycle is considered one complete sequence of filter positions, as programmed in the table shown in the Mixed tab of the Setup dialog box. An entry of 1 indicates that no signal averaging will occur. Valid entries are between 1 and 500. For instance, when the value in this dialog box is set to 50, 50 points for each wavelength combination in **Mixed Mode** will be collected and the average value of the 50 points will be placed in the data buffer.

Auto Record

Auto record is used to automate the collection of data in the Single Sample Mode. When the Auto Record feature *is not* used, single data points or averaged data points will be collected whenever the **Record New Data** button is selected. With Auto Record *is* used, a data set is recorded once every interval that is indicated in the Auto Record dialog box. Data will be recorded in this manner for the total number of samples that has been specified in this dialog box. For instance, a setting of “20 sample(s), once every 1 minute” will record one data sample every minute for a total of 20 samples on a total of 20 minutes when the **Record New Data** button is selected.

A check in the **Enable Auto Record** check box enables this feature. If you wish to interrupt the automatic recording of data, select the **Stop Recording Data** button.

Data averaging can be used together with the auto record feature. If the time needed to acquire data is longer than the duration of time between data acquisition periods entered in the Auto Record dialog box, the user will be prompted to lengthen the auto record time. This may occur if:

1. The duration of the Dwell Time specified in the Mixed tab of the Setup Options dialog box is too long.
2. Too many data points are averaged to complete the sequence in the allotted time.
3. The Sampling time is too long.

Record New Data

Record New Data begins a data acquisition sequence. When this button is selected, a data set is recorded and stored in a data buffer. These data can be a single set of points or an averaged set of points, as specified by the values entered in the **Multi & Single** or **Mixed** data entry fields.

Discard Last Data

Discard Last Data will delete the last set of data that was collected the last time that **Record New Data** was selected. The number of data sets collected is indicated in the message box in the top of the **Single Sample Mode** dialog box. **Discard Last Data** can be selected repeatedly to remove more than one data set.

Discard All Data

Discard All Data will clear the data buffer and delete all previously collected data using the **Single Sample Mode**. All data will be deleted from the data buffer that was acquired since the last time that **Discard All Data** was selected.

Close the Dialog Box

Close the Dialog Box will close the **Single Sample Mode** dialog box. It is important to note that selecting **Close the Dialog Box** will not clear the data buffer of previously acquired data. To clear the data buffer, the user must select **Discard All Data**. Once the **Single Sample Mode** dialog box is closed, it can be reopened using the **Record / Start** command. Closing and reopening this dialog box does not affect the data in the data buffer.

Save to Disk

Save to Disk permanently saves the data that has been stored in the data buffer to disk using the file name and location specified in the Record tab of the Setup Options dialog box. The Auto Increment File feature is also supported. When Auto Increment File is enabled, selecting **Save to Disk** will save the file to the next numbered file name.

It is important to note that selecting **Save File to Disk** does not clear the data buffer. If **Record New Data** is selected after selecting **Save File to Disk**, the next file saved to disk will contain the original data plus the newly added data. Usually, **Discard All Data** is selected after selecting **Save to Disk**, however, this not need be the case. In some instances, it may be useful to save a second file with added data.

Tips for Using Single Sample Mode

The Auto Record feature is particularly useful for collecting data under four types of conditions.

1. If a sample is especially vulnerable to photobleaching, **Auto Record** can be used with the **Auto Always** shutter mode to block illumination of the sample between periods of data acquisition.
2. If the user wants to record data over a long period of time, but continual data acquisition is not needed. Recording data continuously over a long period of time generates large data files that are more cumbersome to analyze. This is generally not required if the signal is changing slowly.
3. If the fluorescence signal is noisy, the signal-averaging feature of **Auto Record** can be used to eliminate random noise.
4. If several different samples are measured and the user wishes to record the data in one file. This is especially useful with the Model CV1 Cuvette Accessory. A data set can be acquired for one sample and the sample changed prior to collection of the next data set.

Resizing Windows during the Recording of Data

The Windows[®] operating system functions in a multitasking and object-oriented environment. Because of this, some program functions are curtailed when others are permitted to take precedence. As a result, some features in the Windows[®] operating system can cause loss of data during periods of rapid data acquisition. This is not a deficiency in the design of the Data Acquisition software, but rather a consequence that exists in all software written to operate in a multitasking environment.

During data acquisition and at all other times, the operating system must monitor the users' input via the keyboard and the mouse. Certain input operations can significantly decrease the processing time available to other operations. The primary Windows[®] operation that can affect data collection by the Data Acquisition software is the positioning and resizing of program windows. When the user clicks on the title bar of an active window to reposition it on the screen, execution of the underlying program is curtailed for the duration of time in which the mouse button is depressed. For this reason, repositioning and resizing windows during data acquisition is discouraged since it may lead to loss of data. Whether or not data is lost depends on several factors, but the primary factor is the rate of data acquisition. It is recommended that the user size and position the data windows prior to starting data windows acquisition.

Chapter 8: Viewing Data

Overview

The C&L Instruments Data Acquisition software allows the user to monitor Fluorescence, Fluorescence Ratios and Analog data simultaneously in separate data windows. Moreover, data can be monitored in real time between and during periods of active data collection. This chapter is intended to provide the user with a practical guide for viewing data with the Data Acquisition software. A detailed discussion of all the features of the software are provided in *Chapter 5: Command Reference*.

Use of the Right Mouse Button

The Data Acquisition software has been written to take advantage of the right mouse button. Clicking the right mouse button in various areas of the data windows will open an option selection box, which is appropriate for that area of the data window, to allow the user to change display options. This can be used as a short cut to more quickly change display options, rather than using menu items in the main Menu Bar. The options available with the right mouse button are discussed in this chapter in relation to the particular data window.

Data Windows

Data obtained by the Dye Fluorometer is viewed in three types of data windows. These are the Fluorescence, Fluorescence Ratio, and Analog windows. In addition, a Timer window is displayed to show the progress of time as the data is updated in the data windows. Details of these windows are discussed below.

Timer Window

The Timer window shows either a blank or static graph when the filter wheels are not in the Run mode. After selecting **File / Run**, a horizontal bar is displayed on this graph. The movement of horizontal bar indicates the progress of time and the position of the new data being updated in the data windows. The Timer windows cannot be resized or disabled.

During the recording of data, a digital clock is displayed under the X axis of this graph to indicate the amount of time that has elapsed since the recording of data was started.

Fluorescence Window

The Fluorescence window is used to display the intensity of the fluorescence measured by the Dye Fluorometer. The **Display** option in the main Menu Bar can be used to enable the display of one or more fluorescence channels. Many of the display features can also be obtained using the right mouse button. Clicking the right mouse button in the center of the Fluorescence window will open the **Display** selections.

Clicking the right mouse button in the top center portion of the graph will open the selection of fluorescence channels that can be enabled or disabled for display. If only one channel is available for display, as with the Single-Single data acquisition mode, this

dialog box will not appear. This feature is useful to limit the display in a particular Fluorescence window to the data channel(s) of interest.

Several Fluorescence windows can be opened using the **V**iew / **N**ew **F**luorescence menu item and each can be set to display any available fluorescence channels.

Data Overflow

The Dye Fluorometer can accumulate 65,536 counts (i.e., 2^{16}) within one sampling period. If more counts are detected within the sampling period, a data overflow condition will exist. If this occurs in any fluorescence channel, a warning message (DATA OVERFLOW) is displayed along the X Axis of the Fluorescence window. In addition, the fluorescence channel in which the overflow occurred will be underlined in the top of the Fluorescence window.

To correct a data overflow condition, the user has several options.

- Decrease the intensity of the illumination using the Neutral Density wheel.
- Decrease the concentration of the sample or the concentration of the fluorescent species in the sample.
- Decrease the sampling time so that fewer counts will be detected within the sampling time period.
- Insert an additional neutral density filter in the detector (see the Model D48 Detector User Manual).

It is important to note that this data overflow condition is different from the problem caused by exceeding the count rate of the PMT. In a data overflow condition, the limitation is not the PMT, but rather the amount of counts that can be detected within the sampling period. This limitation is not necessarily caused by excessive emission intensity. Selecting a long sampling time that is not appropriate for the intensity of the fluorescence emission can also cause data overflow. See also *Avoiding Peak Pile Up* in *Chapter 7: Acquiring Data*.

Fluorescence Ratio Window

The fluorescence ratio window is used for monitoring the ratio of fluorescence intensities between any two fluorescence channels. As with the other data windows, many of the display options are available using the right mouse button. The numerator and denominator of the ratio can be selected by clicking in the top center position of the graph with the right mouse button or by selecting **D**isplay in the main Menu Bar.

Clicking in the center of the graph with the right mouse button will open options for scaling the graph. These options are also available by selecting **V**iew in the main Menu Bar.

Several Fluorescence Ratio windows can be opened using the **V**iew / **N**ew **F**luorescence **R**atio menu item and each can be set to display the fluorescence intensity ratio between any two fluorescence channels.

The Fluorescence Ratio window is not available in the Mixed-Mixed Mode of data acquisition.

Analog Window

The analog window is used to display the acquired analog voltages. The default scaling of the Y Axis is the 0 to +5 Volt limit of the Analog to Digital Converter. As with the other data windows, many of the display options are available using the right mouse button.

The displayed analog channels can be selected by clicking in the top center position of the graph with the right mouse button or by selecting **D**isplay in the main Menu Bar. The analog channels that are available for display are those channels that have been enabled in the Analog tab of the Setup Options dialog box. See *Chapter 5: Command Reference* and *Acquiring Analog Data* in *Chapter 7: Acquiring Data*.

Clicking in the center of the graph with the right mouse button will open options for scaling the graph. These options are also available by selecting **V**iew in the main Menu Bar.

Several Analog windows can be opened using the **V**iew / **N**ew **A**nalog menu item and each can be set to display one or more analog channels.

Chapter 9: Timing Considerations

Overview

As explained briefly in *Chapter 3: Fundamentals of Data Acquisition*, the C&L Dye Fluorometer can be considered a time sharing device. This means that the measurement of fluorescence at various wavelengths, which are selected using filters in the filter wheels, is performed in a sequential fashion. In addition, the Data Acquisition software permits acquisition of analog data that is acquired along with fluorescence data in a synchronous fashion. The user should be familiar with these timing considerations for proper interpretation of the data collected by the C&L Dye Fluorometer.

Timing and Data Acquisition Modes

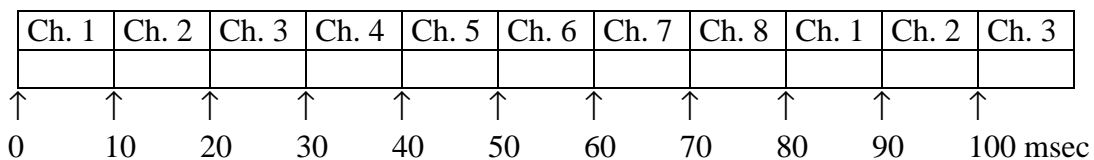
The excitation and emission filter wheels can each operate in three modes: Multi, Single and Mixed. Up to five combinations of these modes can be used to acquire data in one of five separate data acquisition modes: Multi-Multi, Single-Single, Multi-Single, Single-Multi, and Mixed-Mixed.

In all data acquisition modes other than the Mixed-Mixed mode, fluorescence data is acquired for all filter positions, whether or not the display of these data is enabled in a Fluorescence window. Furthermore, these data are acquired in a continuous fashion without interruption. For instance, in the Multi-Multi mode, fluorescence counts from position 2 are acquired immediately after collection from position 1 has terminated. Thus, the timing relationship between data points can be easily determined from the setting of the sampling time in the **Setup Options**.

Timing of Fluorescence Data Acquisition in the Multi Modes

The timing relationship between data points can be viewed graphically as a time line. The following graph would indicate the timing relation if either the excitation and/or emission filter wheel were operated in the Multi mode with a sampling time of 10 milliseconds. This would be the case if the data acquisition mode were set to Multi-Multi, Multi-Single, or Single-Multi. The upward arrow indicates the beginning of a data sampling period.

Timeline 1

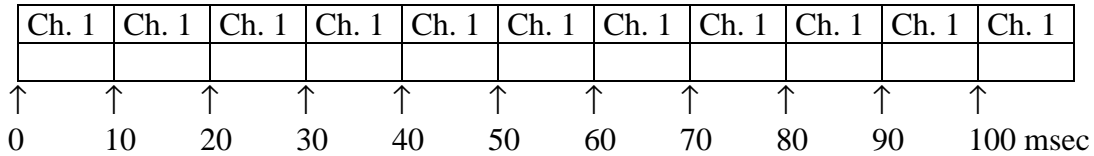


Using this time line, it can be seen that the data points for a particular channel are separated by the time required for a full revolution of the filter wheel. In this instance, with an 8-position filter wheel and a sampling time of 10 milliseconds, the 10-millisecond periods for the repetitive collection of data from channel 1 are spaced by 80 milliseconds. The rate of data collection for any one channel is dependent on the sampling time, whether a 4- or 8-position filter wheel is used in the C&L Dye Fluorometer.

Timing of Fluorescence Data Acquisition in the Single-Single Mode

In the Single-Single mode, the timing considerations are similar, except that data is collected from only one channel. The time line in this case would look as follows.

Timeline 2



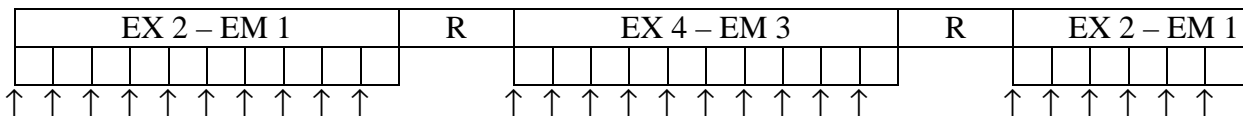
Timing of Fluorescence Data Acquisition in the Mixed-Mixed Mode

The timing considerations when the C&L Dye Fluorometer is operated in the Mixed-Mixed mode are slightly more complicated. The main difference lies in the fact that data acquisition is temporarily disabled during the time in which the filter wheels are repositioning and the shutter(s) are closed and opened (if the shutters are in the Auto Always mode). A time of 300 mseconds is allotted for this repositioning operation.

When the filter wheels are stationary at a programmed position, data is collected at the specified sampling rate as if the fluorometer was operating in the Single-Single mode. Upon completion of the dwell period, data collection is interrupted for 300 mseconds, the filter wheels are repositioned, and the cycle continues.

The following timeline would indicate the timing relation in the Mixed-Mixed mode, with the assumption that the sampling time is set to 100 milliseconds with a constant dwell time of 1 second. It is also assumed that the filter wheels cycle between two position settings, composed of filters 2 and 4 in the excitation filter wheel and filters 1 and 3 in the emission wheel. "R" refers to the time period in which the filter wheels are repositioning. The upward arrow indicates the beginning of a data sampling period, with the box above the arrow indicating its duration.

Timeline 3



Timing of Analog Data Acquisition

The C&L Dye Fluorometer can acquire Analog data in addition to fluorescence data. The acquisition of analog and fluorescence data occurs in a synchronous fashion. As a result, the rate of analog data acquisition is dependent on the sampling time, as specified in the Multi, Single or Mixed tabs of **Setup Options** dialog box.

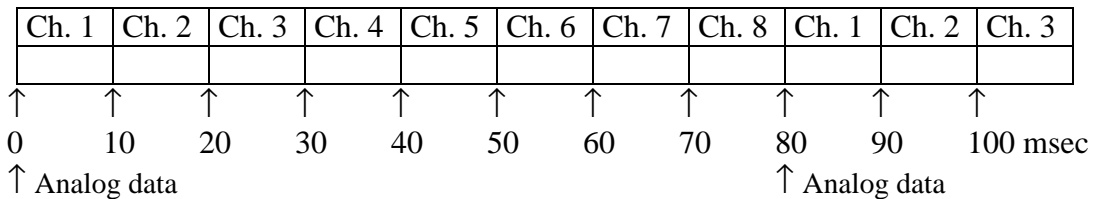
Up to eight analog data channels can be acquired. Specific channels are enabled using the Analog tab in the **Setup Options** dialog box. In all data acquisition modes, analog data is acquired for the analog channels that are enabled, whether or not the display of these data is enabled in an Analog window.

Unlike fluorescence data, the analog data from all enabled channels is captured simultaneously. The data captured in this way can be considered a “snap shot” of the voltages that are present on the analog channel inputs at that moment in time. The time at which this “snap shot” is taken is dependent on the data acquisition mode.

Timing of Analog Data in the Multi Modes

When the C&L Dye Fluorometer is operated in the Multi-Multi, Single-Multi or Multi-Single modes, the analog data is captured at the beginning of the sampling time period for Filter 1. The time line below illustrates when the analog channels are read, in relation to the acquisition of fluorescence data described above.

Timeline #



Timing of Analog Data in the Single-Single and Mixed-Mixed Modes

When the C&L Dye Fluorometer is operated in either Single-Single or Mixed-Mixed mode, analog data is acquired at the same rate as the fluorescence data. In these modes, the capture of analog data occurs once during each sampling period and at the same time as the fluorescence data.

Synchronizing External Events to the Acquisition of Fluorescence and Analog Data

Features in the Dye Fluorometer make it possible to synchronize external events to the acquisition of data. The timing information from the operation of the filter wheel(s) is available as TTL pulses from the PC-DAQ Controller card or the RS-DAQ Controller. This feature can be used to trigger stimulators or other laboratory devices so that these events can occur in synchrony with the collection of data.

The user is referred to the hardware manuals for the PC-DAQ Controller card or the RS-DAQ Controller for a further discussion of this topic.

Chapter 10: Troubleshooting

If you have trouble operating the C&L Instruments Dye Fluorometer, please follow these steps in the indicated order.

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

<i>Symptom</i>	<i>Possible Problem</i>	<i>Possible Solution</i>
Main program window opens without any child windows and most menu items are disabled.	A configuration file has not been loaded.	Use the <u>F</u> ile, <u>O</u> pen command to either open or create a new configuration file.
Fluorescence data is not visible in the Fluorescence window.	Run command is not enabled.	Enable the Run command using Run menu item under <u>F</u> ile menu or the Run icon.
	Viewing of Fluorescence data is not enabled	Make sure the Fluorescence window is active, then enable viewing of data using the View menu.
	Data is off scale	Use the <u>U</u> nzoom feature in a Fluorescence window.
	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 <u>O</u> ptions.
Analog data is not visible in the Analog window.	Run command is not enabled.	Enable the Run command using Run menu item under <u>F</u> ile menu or the Run icon.
	Acquisition of Analog data is not enabled.	Enable one or more analog data channels using the Analog tab in the Setup Options dialog box.
	Viewing of Analog data is not enabled.	Make sure the Analog window is active, then enable viewing of data using the View menu .
	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 <u>O</u> ptions.
Selecting Record / Timed or pressing the Timed Recording icon does not appear to initiate data recording.	Zero time has been entered as Recording Time duration in the Record tab of the Setup Options dialog box.	Enter a number in the Recording Time section in the Record tab of the Setup Options dialog box.
	The Run command has not been select to place the filter wheels in a data acquisition mode.	Enable the Run command using Run menu item under <u>F</u> ile menu or the Run icon.

When the Run command is selected, I get a message box indicating that the excitation module (or detection module or neutral density wheel) is not detected.	The illumination source or the detector is not turned on.	Turn on the illuminator and the detector before starting the Data Acquisition program.
	The cables connecting the illumination source or the detector to the breakout box of the PC-DAQ Controller card is disconnected.	Properly connect the cables from the illumination source and the detector to the breakout box of the PC-DAQ Controller card.

Chapter 11: Agreement, License and Warranty

Agreement and License

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Should you have any questions concerning this agreement, you may contact C&L at 314 Scout Lane, Hummelstown, PA, USA, 717-566-3642.

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