Data Acquisition Software User Manual for the Digital Photon Counting System
Version 1.0

C&L Instruments, Inc.
314 Scout Lane
Hummelstown, PA 17036

Phone: 717-566-3642
Fax: 717-566-3643
E-mail: support@fluorescence.com
Web site: http://www.fluorescence.com

Table of Contents

List of Figures

Chapter 1: About the DPC System Data Acquisition Software

Program Description

The DPC (Digital Photon Counting) System Data Acquisition Software is an easy-to-use 32-bit software application. This version of the software applies to the DPC data acquisition software that uses the Model PC-DAQ controller card. The DPC Data Acquisition offers powerful data collection features using a standard Windows® interface. The software allows the user to:

- Acquire photon counting data from any TTL source. Counting rates are software-selectable and can be set from 2 to 10,000 data points per second.
- Make analog measurements in synchrony with photon counting data (DPC-2 only). Up to eight channels of analog data (0 to 5 Volt) can be collected.
- View photon counting data and analog channels on the computer monitor, in real time, as it is acquired.

This manual is applicable for both the DPC-1 and DPC-2. The main difference between these two software packages is that the DPC-2 includes the capabilities for analog data acquisition. Throughout this manual, it will be noted which software features only apply to the DPC-2.

System Requirements

The computer used for the DPC-1 and DPC-2 data acquisition and analysis 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 the DPC Data Acquisition software, 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 DPC Data Acquisition software. It is assumed that the user is already familiar with the following:

- Photon counting and/or fluorescence spectroscopy concepts and procedures
- How to use Windows® 95 or Windows® 98

Obtaining Current Documentation

The most current version of this 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 DPC-1 or DPC-2 system, 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 (I/O 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.

Note that a similar PC card (PC-DAQ Controller) manufactured by C&L Instruments is used by the C&L Instruments Dye Fluorometer. If you wish to upgrade your DPC system to the multiwavelength capabilities of the photon counting system available in the Dye Fluorometer, please contact C&L Instruments to determine if your version of the PC card supplied with your DPC system is capable of operating the Dye Fluorometer

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 DPC-1 and DPC-2 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 software files and the default configuration file will be placed in subdirectory structure under either the C:\Program Files\CandL\DPC 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 DPC hardware and 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***.

Data Acquisition Mode

Data is acquired by specifying the acquisition aperture time and by turning data monitoring on. All data is recorded as a function of elapsed time. If analog data is acquired together with photon counts, these data are acquired at the same rate and in synchrony. Further details of data acquisition are a topic of ***Chapter 7: Acquiring Data***.

Shutters (DPC-2 Only)

The DPC-2 system is equipped with a Breakout box. This accessory connects to the PC-DAQ card in the PC with a SCSI cable. The Breakout box contains the analog input BNC connectors. It also has TTL output connectors for the control of external shutters (shutters are not provided). The shutter outputs 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 control shutters to limit either the exposure of the sample or the collection of light by the photomultiplier.

Fluorescence and Photon Counting

Together with a TTL-output photomultiplier, the DPC-1 and DPC-2 can be used as a very sensitive photometer. A typical applications is for fluorescence measurements, in which the intensity of light emitted from a fluorescent sample is measured when excited by excitation energy. C&L Instrument can provide photomultipliers for these applications. Photon-counting PMT's capture 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 DPC data acquisition system. The photon-counting method of light detection is unparalleled for high sensitivity and accurate detection of low light levels. The DPC System counts these pulses in discrete time intervals. The count rate is proportional to the intensity of the light impinging on the photomultiplier.

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 PMT Counts window. One or more PMT Counts widow(s) 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**. The sampling time is determined by a value keyed into the Setup Options dialog box.

Data acquisition sequences can be acquired continuously or as single data points, depending on the mode of data acquisition. The DPC 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 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.

Measuring Fluorescence by Balancing Factors

Since the DPC System is often used for the measurement of fluorescence emission, this section can be used as a guide to facilitate these types of measurements. Several factors influence the intensity of the acquired fluorescence signal by the DPC System. Obviously, the concentration of the fluorescent dye and its quantum yield affect the observed intensity. But there are additional factors that can be controlled that affect the number of fluorescence counts detected within the sampling time. Two primary factors are the excitation lamp intensity and setting of the sampling time.

Excitation Lamp Intensity

The PMT used in photon-counting applications will respond to light intensity in a linear fashion up to a maximum count rate that is specified by the manufacturer of the PMT. The user should adjust the excitation intensity in order to maintain the PMT in the linear range. Adjusting either the characteristics of the sample or the excitation energy or emission collection efficiency can be used to alter the count rate.

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 light from a weakly fluorescing sample. A long sampling time, however, may cause saturation of the counter in the DPC system, especially 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 DPC system uses a 16 bit counter and can count up to 2^{16} (i.e., 65,536) counts within a sampling period before saturation of the counter occurs. A feature in the software will indicate 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 is discussed in more detail in ***Chapter 7: Acquiring Data***.

The DPC system can display the observed light intensity 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 (DPC-2 Only)

Along with the acquisition of PMT Counts data, the DPC-2 system 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 DPC-2 is equipped with a 12 bit analog-to-digital converter, providing a resolution of 1.2 mVolt within the 0 to 5 Volt range. This feature gives the user the ability to record events that may be occurring simultaneously with the recording of photon counts.

Chapter 4: Getting Started

Overview

This chapter will quickly familiarize the user with the basic operating steps required for collecting data with the DPC system.

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 all the features of the instrument 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. The fluorescence and analog signals can be monitored between periods of data acquisition.

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.
2. Launch the DPC Data Acquisition software.
3. Specify and/or load previously specified **Setup Options** for data acquisition.
4. Enable the **Run** feature to begin monitoring PMT Counts.
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 DPC system 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***.

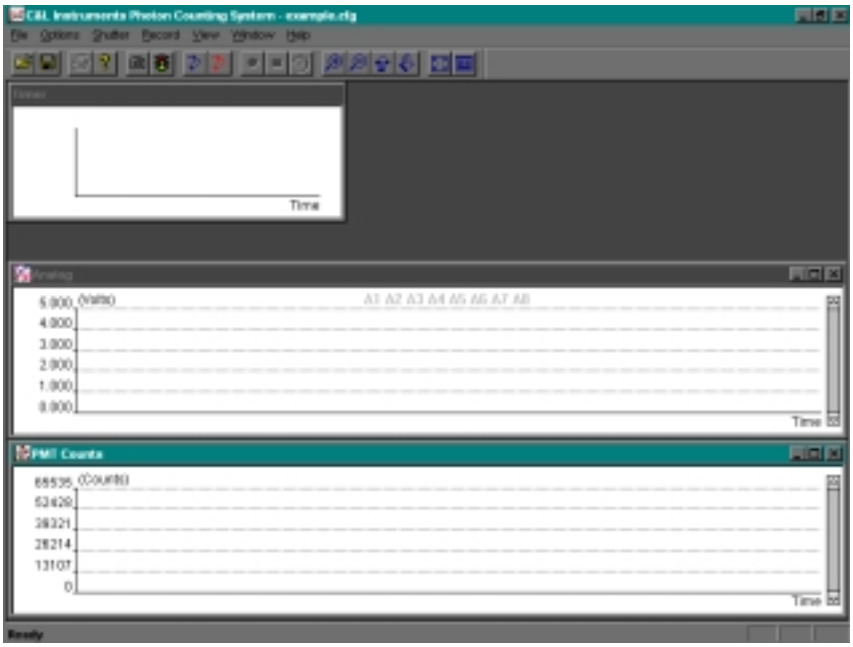


Figure 1. The main program window.

When the Data Acquisition program is first started, the main program window contains either two or three “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 **File** / **Open** command. Data Acquisition will automatically open the last configuration file used. If no configuration files are shown in the Open Configuration File dialog box, type a name and select Open. The acquisition program will open a new dialog box asking you if you want to create this file.

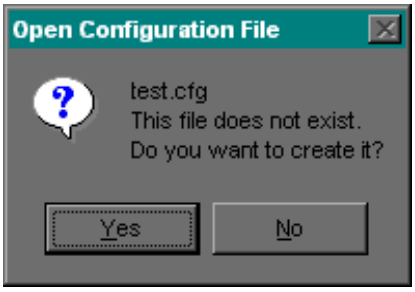


Figure 2. The Open Configuration File dialog box.

The child windows—Timer, PMT Counts and Analog—are used to view the data as monitored by the Digital Photon Counting system. 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: **File**, **Options**, **Shutter**, **Record**, **Display**, **View**, **Window** and **Help**. Through these drop-down menus, all features of the Digital Photon Counting system 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.

- **File** - Allows the user to **Open** and **Save** configuration files, specify **Setup Options**, control the fluorometer hardware components, print the contents of data windows, and exit the program.
- **Options** - Allows the user to specify the **Color** of various channels and text and check the interface settings for the DPC controller PC **Board**.
- **Shutter** - Allows the user to open and close the excitation and emission shutters manually.
- **Record** - Allows the user to **Start** and **Stop** the data acquisition process or used the **Timed** feature for fixed duration data acquisition.
- **Display** - Allows the user to specify the data channels to be displayed in the Analog windows (DPC-2 only).

- **View** - Allows the user to toggle the main program window's **Toolbar** and **Status Bar** on and off. Also allows the user to perform various actions to manipulate the presentation of data in the active PMT Counts or Analog (DPC-2 only) windows. These actions include **Zoom In**, **Zoom Out**, **Unzoom** or **Move Up** or **Move Down** the scale of the Y-axis, and change the **Settings…** of the zoom function.
- **Window** - Allows the user to open **New PMT Counts**, or **New Analog** data windows. Also allows the user to select the active window and to arrange the child windows within the main program widow using the **Cascade, Tile** and **Arrange Icons** options.
- **Help** - Allows the user to access on-line information **About C&L** Instruments.

File Menu

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

- **Open...**,
- **Save**,
- **Save As...**,
- **Setup Options...**,
- **Run**,
- **Print...**,
- **Print Preview**,
- **Print Setup...**,
- **1 [filename] (etc.)**
- **Exit**

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 As…

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 Options…

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 DPC to begin monitoring photon counts in the PMT Counts window and analog data in the Analog window (DPC-2 only). After the Run command has been enabled, data is displayed in the Analog window provided that data channels have been enabled for viewing using the **Display** menu item.

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 DPC 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 **Shutter Mode, Sampling Time**, **Record**, and **Analog**. 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.

Shutter Mode

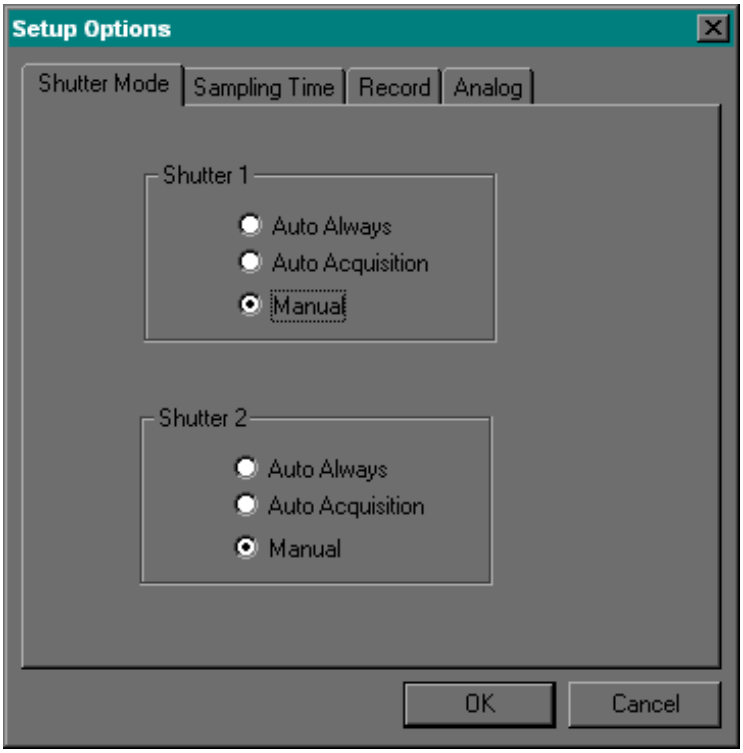


Figure 3. The Shutter Mode tab in the Setup Options dialog box.

Specifying Shutter Control Modes

The **Shutter Mode** tab displays the choices for control of two shutters by the DPC system. The settings in the **Shutter 1** and **Shutter 2** sections are used to indicate the shutter control mode for the excitation and emission shutters. These shutters can each be controlled in three ways: **Auto Always**, **Auto Acquisition**, and **Manual,** which are described below. Contact C&L Instruments for instructions on how to connect shutters to the DPC system

- In the **Auto Always** mode, the shutter(s) will always remain in an automatic control mode. Manual control of the shutter using the **Shutter** menu is disabled in this shutter mode. **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 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 mode allows shutters to be used to insure that the sample and/or the detector are exposed to light only during data acquisition.
- In the **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 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. In between periods of data acquisition, the shutters can be controlled manually.

- In the **Manual** mode, the shutter(s) are controlled only by the options in the Shutter menu. Selecting **Start**, **Stop** or **Timed** does not change the state of the shutter(s) while in the manual mode.

Sampling Time Tab

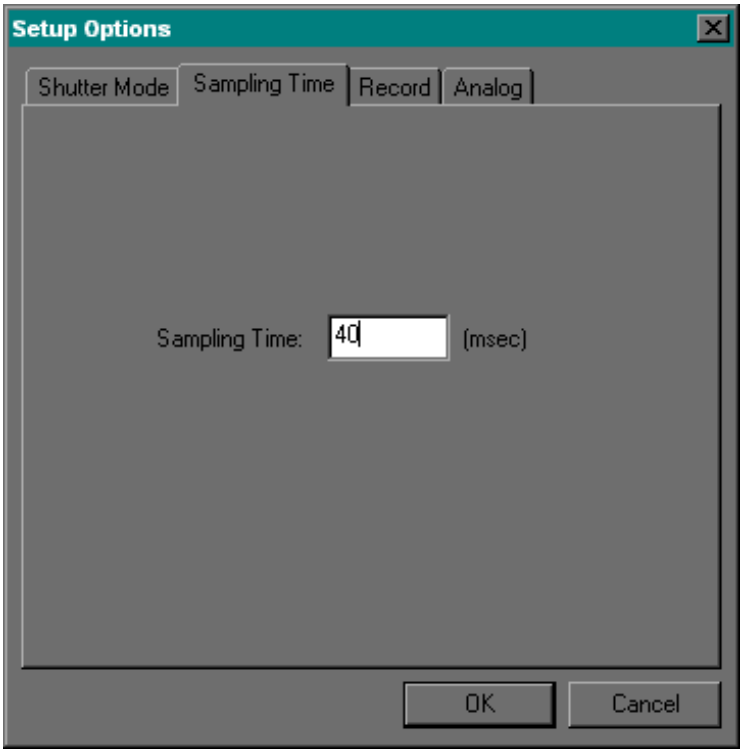


Figure 4. The Sampling Time tab in the Setup Options dialog box.

The setting of the sampling time determines the time interval over which photons are collected. The minimum valid entry is 0.1 milliseconds. The DPC uses a 16-bit counter, so the sampling time must be set so that less than 65,536 photons are counted in one sampling period. Further details about the setting of the sampling time can be found in ***Chapter 7: Acquiring Data***.

Record Tab

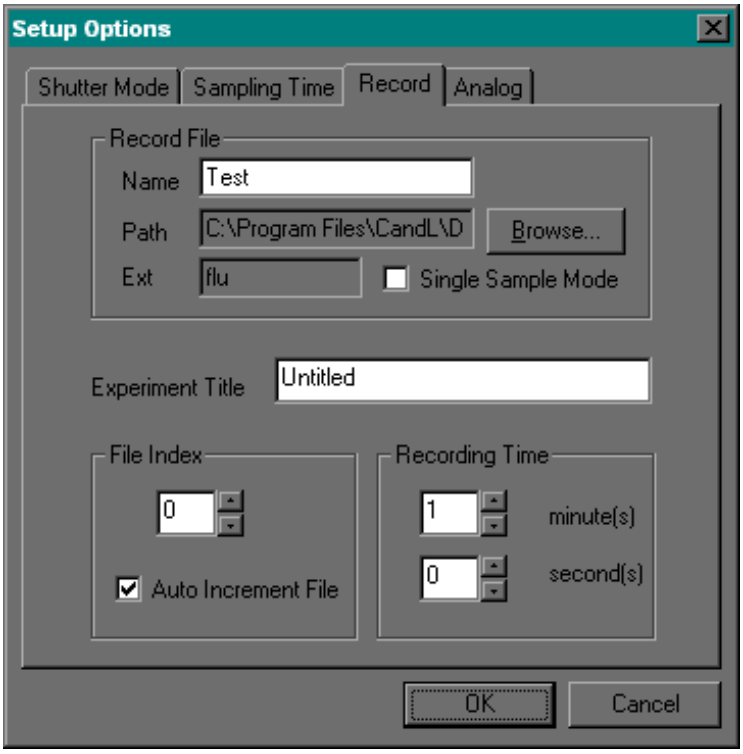


Figure 5. The Record Tab in the Setup Options dialog box.

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 will be 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 sampling rate specified in the **Sampling Time** dialog box. 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 C&L Instruments 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, 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. Recording of analog data is only available in DPC-2 version of the Digital Photon Counting system.

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***.

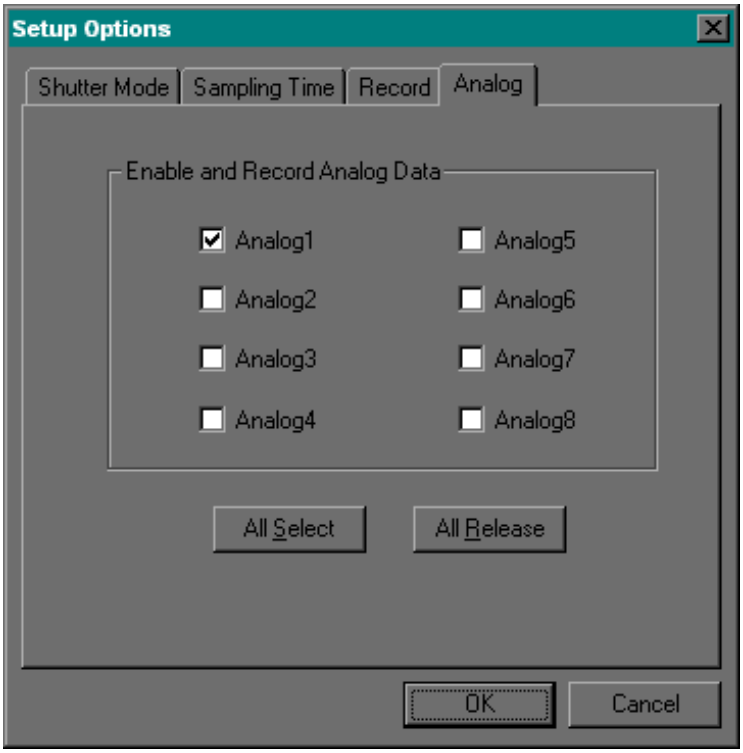


Figure 6. The Analog tab in the Setup Options dialog box.

Options Menu

The Options menu contains the following menu items:

- **Color…**
- **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 PMT Counts and Analog data windows. Up to eight channels of data can be displayed

in the Analog data window (DPC-2 only). 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.

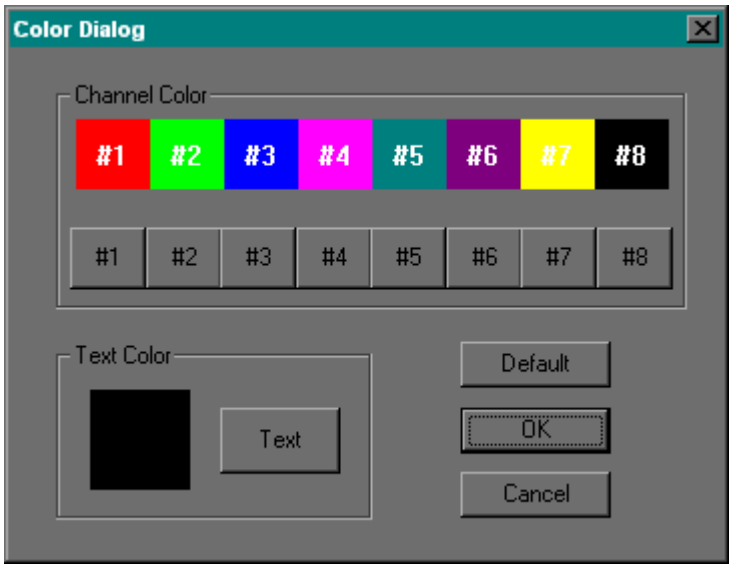


Figure 7. The Color Dialog Box.

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 / Board

Selecting Board will open a dialog box that indicates the hardware interrupt and the base address used by the PC card. This dialog box is only used for diagnostic purposes should the PC 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 Shutter 1** - Opens the shutter 1 (if closed) or closes it (if open).
- **Open Shutter 2** - Opens the shutter 2 (if closed) or closes it (if open).

When a shutter is open, a check is placed next to the entry in this menu.

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 monitoring of the data in the data windows (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 (see **File** / **Run**, above).

Display Menu

The **Display** menu allows the user to specify the data channels that will be displayed in the Analog data window. The **Display** menu is available on the menu bar *only* when the Analog window is active.

Analog Window Display Options

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

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

Number of Channels Enabled

The number of analog channels that are enabled on this menu depends on the setting in the **Analog** tab of the **Setup Options** dialog box. Only the analog channels that have been enabled in the **Analog** tab are available for selection in the **Display** menu.

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.

View Menu

The **View** 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 **View** 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 **View** menu.

- **Toolbar**
- **Status Bar**

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

- **Toolbar**
- **Status Bar**
- **Lines**
- **Zoom In**
- **Zoom Out**
- **Move Up**
- **Move Down**
- **Unzoom**
- **Settings…**

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

- **Toolbar**
- **Status Bar**
- **Counts**
- **Counts/sec**
- **Zoom In**
- **Zoom Out**
- **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 **PMT Counts** 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 PMT Counts and Analog (DPC-2 only) 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 PMT Counts or Analog 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 PMT Counts or Analog. 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 PMT Counts or Analog.
- 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 PMT Counts or Analog 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 PMT Counts or Analog 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 PMT Counts 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 intensity data in counts, then the maximum value will be 65,535 counts (e.g., 2^{16}-1). If the scale is set to display the photon count 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 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.

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.

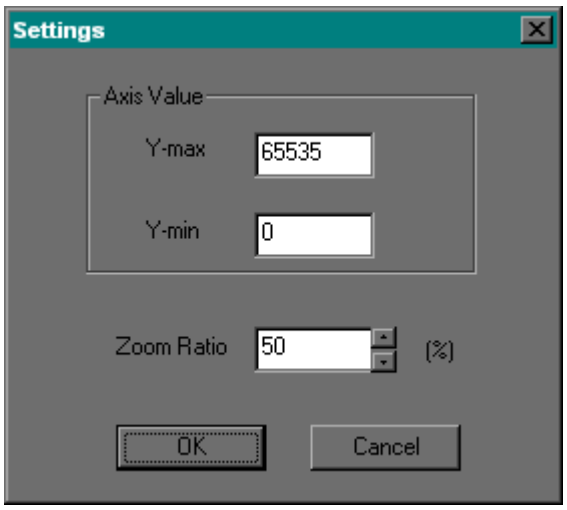


Figure 8. The Settings dialog box.

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 PMT Counts**

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

Window / New PMT Counts and / New Analog

Selecting **New PMT Counts** or **New Analog** opens an additional PMT Counts or Analog 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 PMT Counts windows can be used to monitor the photon count intensity 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 software 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 may 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 / Open**

 **File / Print…**

 **File / Setup Option…**

 **Shutter / Open Shutter 1**

 **Record / Start**

 **Record / Timed**

 **View / Zoom Out**

 **View / Move Down**

 **View / Setting…**

 **File / Save**

 **Help / About C&L…**

 **File / Run**

 **Shutter / Open Shutter 2**

 **Record / Stop**

 **View / Zoom In**

 **View / Move Up**

 **View / Unzoom**

Chapter 7: Acquiring Data

Overview

This chapter describes how to use the C&L Digital Photon Counting system to acquire photon-counting 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 DPC.

Data and Configuration Files

The C&L DPC 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 Digital Photon Counting system Data Acquisition software 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 DPC system. 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 Data Acquisition software of the C&L DPC system. One or more configuration files can be stored for recalling previously saved setting. 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 photon counts at a particular sampling time and with specific analog channels, a new configuration file can be saved for later recall after the DPC system 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 DPC system can be set for the measurement of photon counts and analog data using the tabbed **Setup Options** dialog box.

Using Run

The **Run** command is used to initiate the monitoring of PMT Counts and Analog data (DPC-2 only). **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 (PMT Counts, Analog) 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 data monitoring activity. This feature can be used prior to setting the DPC system 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.

Avoiding Peak Pile Up

PMT's used with the C&L Instruments Digital Photon Counting system will count photons in a linear fashion up to a specified count rate. 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 attenuating the light intensity impinging on the PMT. If the count rate is observed to *increase* after the light intensity is *decreased*, then peak pile up is probably occurring.

If Peak Pile Up occurs, the user can decrease the count rate by lowering the exposure of PMT to the measured light by using a neutral density filter.

Using the Shutters in the Measurement of Fluorescence

Shutters

The C&L Digital Photon Counting system can control two independent shutters. If the DPC system is used for measurement of fluorescence intensity, these shutters can be configured to function as excitation and emission shutters. An excitation shutter in the illumination optical path and an emission shutter in the detection path can be controlled independently by the Data Acquisition software. The three operation modes for the shutters are selected using the Shutter Mode tab of the Setup dialog box. See ***Chapter 5: Command Reference*** for an explanation of these settings.

For fluorescence measurements, the shutters are used for two purposes:

1. The excitation shutter (Shutter 1) is used to block illumination of the sample.
2. The emission shutter (Shutter 2) is used to block detection of the excitation light by the PMT 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 Data Acquisition software is started, 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. If you use the shutter control features of the DPC system, it is always good practice to close the shutters prior to quitting the Data Acquisition program.

Acquiring Analog Data

The C&L DPC system can record analog data along with the recording of photon counts (DPC-2 only). 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 Digital Photon Counting system. 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 PMT Counting data. Details of the timing of analog and PMT Counts 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 DPC 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 **Recond / 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, which ever 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 photon counts and analog signal can be made and averaged together as one data point in an automated fashion.

Operation of the DPC system 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.

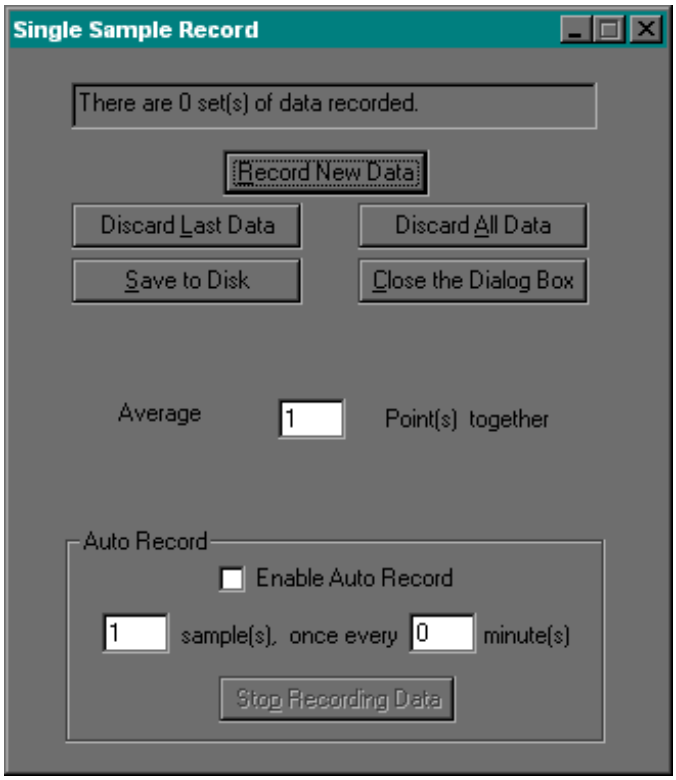


Figure 9. The Single Sample Record dialog box.

The Single Sample Mode dialog box contains 5 main selection buttons, 3 places for data entry and a message box. The Single Sample Mode is setup by entering values in the data entry box labeled "Average" and the entry boxes labeled **Auto Record**.

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 6 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.

Data Averaging

The entry on the Data Averaging data box indicates how many data points will be averaged together for signal averaging purposes. An entry of 1 indicates that no signal averaging will occur. Valid entries are between 1 and 500. A value greater than 1 will initiate on-line data averaging. When this feature is enabled, the signal-averaged data is displayed on the data widows.

Data averaging affects the PMT Counts and Analog data.

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, either 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 indicted in the Auto Record dialog box. Data will be recorded in this manner for the total number of points that has been specified in this dialog box.

For instance, if Auto record is set to record *20 sample(s), every 2 minute(s),* then one data set will be recorded when the **Record New Data** button is selected and additional data sets will be recorded every 2 minutes until of total of 20 sets are recorded.

The Auto Record feature is enabled with the check box.

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. Too many data points are averaged to complete the sequence in the allotted time.
2. 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 either a single data point or a data point acquired by signal averaging.

Discard Last Data

Discard Last Data will delete the last data point 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 Record** 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 that was collected 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.

Stop Recording Data

Stop Recording Data aborts a data acquisition sequence that had been initiated enabling the Auto Record feature. When this button is selected, a data acquisition sequence that had been programmed to occur in an automated fashion using Auto Record can be aborted.

Tips for Using Single Sample Mode

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

1. In the measurement of fluorescence, some samples are 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 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 C&L Instruments 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 Digital Photon Counting system allows the user to monitor PMT Counts and Analog data (DPC-2) 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 DPC system 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 DPC system are viewed in two types of data windows. These are the PMT Counts 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 Data Acquisition software is 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.

PMT Counts Window

The PMT Counts window is used to display the intensity of the light measured by the PMT attached to the DPC system. Many of the display features can be obtained using the right mouse button. Clicking the right mouse button in the center of the PMT Counts window will open the **Display** selections.

Several PMT Counts windows can be opened using the **View / New PMT Counts** menu item and each can be set to display the data with different scaling.

Data Overflow

The Digital Photon Counting system 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, a warning message (DATA OVERFLOW) is displayed along the X Axis of the PMT Counts window.

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

- Decrease the intensity of the light impinging on the PMT.
- If measuring fluorescence, 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. If decreasing the sampling time will generate too much data for a given experiment, the signal averaging feature can be used to limit the amount of data recorded.

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 light impinging on the PMT. Selecting a long sampling time that is not appropriate for the intensity of the measured light can also case a data overflow condition. See also ***Avoiding Peak Pile Up*** in ***Chapter 7: Acquiring Data***.

Analog Window

The analog window is used to display the acquired analog voltages (DPC-2 only). 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 **Display** 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 Analog widow graph with the right mouse button will open options for scaling the graph. These options are also available by selecting **View** in the main Menu Bar.

Several Analog windows can be opened using the **View / New Analog** menu item and each can be set to display one or more analog channels.

Chapter 9: Timing Considerations

Overview

The Data Acquisition software acquires analog data (DPC-2 only) in synchrony with the PMT Counts data. The user should be familiar with these timing considerations for proper interpretation of the data collected by the DPC system.

Synchronous Data Acquisition Timing

The C&L DPC system can acquire Analog data in addition to PMT Counts data (DPC-2 only). The acquisition of analog and PMT Counts data occurs in a synchronous fashion. As a result, the rate of analog data acquisition is dependent on the sampling time, as specified in Sampling Time tab of **Setup Options** dialog box.

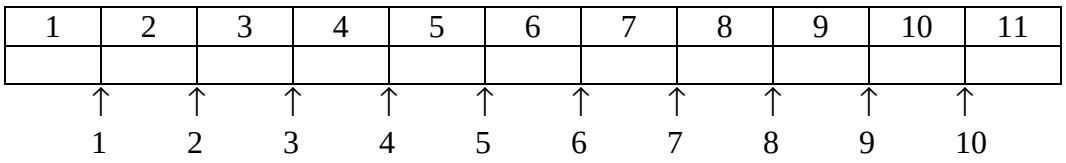
Up to eight analog data channels can be acquired. Specific channels are enabled using the Analog tab in the **Setup Options** dialog box. Analog data is acquired for the analog channels that are enabled, whether or not the display of these data is enabled in an Analog window.

In the DPC system, photons are counted over the period of time designated as the sampling time. At the end of this sampling time, data is acquired simultaneously from all the analog channels. 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 synchronous with the end point of each sampling time window.

This is illustrated in the following Timeline diagram. The first row of numbers indicates the data number of the PMT Counts data. The width of the box represents the duration of the sampling time. The second row of arrows indicates the timing of the analog data acquisition. Note that a given data point of the Analog data is acquired at the end of the corresponding sampling widow in which PMT Counts were accumulated.

Timeline

PMT point number



Analog point number

Chapter 10: Troubleshooting

If you have trouble operating the C&L DPC system, please follow these steps in the indicated order.

1. Consult the manuals for the hardware components of the C&L DPC system.
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.

Symptom	*Possible Problem*	*Possible Solution*
Main program window opens without any child windows and most menu items are disabled.	A configuration file has not been loaded.	Use the File, Open command to either open or create a new configuration file.
PMT Counts data is not visible in the PMT Counts window.	Run command is not enabled.	Enable the Run command using Run menu item under File menu or the Run icon.
	Viewing of PMT Counts data is not enabled	Make sure the PMT Counts window is active, then enable viewing of data using the View menu.
	Data is off scale	Use the Unzoom feature in a PMT Counts 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 Options.
Analog data is not visible in the Analog window.	Run command is not enabled.	Enable the Run command using Run menu item under File 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 .
	You have the DPC-1, not DPC-2	Analog data acquisition is a feature of the DPC-2, not DPC-1. To acquire analog data, you must upgrade to the DPC-2.
	Colors of data point(s) are the same as the graph background color.	Change the color of the data points using the Color… menu item under Options.
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 is not enabled.	Enable the Run command using Run menu item under File menu or the Run icon.

Chapter 11: Agreement, License and Warranty

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