CytoCalc™ Data Analysis Software

User Guide

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How to Use This Guide

Purpose of this guide

Applied Biosystems *CytoCalc™ Data Analysis Software User's Guide* details the procedures for installing and using version 3.00 or later of the CytoCalc™ Data Analysis software.

Audience

This guide is intended for novice and experienced CytoCalc Data Analysis software users who are analyzing data obtained with the CytoFluor® II or CytoFluor Series 4000 Fluorescence Multi-Well Plate Readers. Although the software can used with models 2300 and 2350, a different User's Guide is required.

Structure of this guide

Applied Biosystems *CytoCalc™ Data Analysis Software User's Guide* is divided into chapters. Each chapter page is marked with a tab and a header to help you locate information within the chapter.

The table below describes each chapter.

Chapter 1, Installing the CytoCalc Data Analysis Software	Contains introductory information and software installation and startup instructions.
Chapter 2, Using the Plate Layout Screen	Describes how to use the plate layout screen to assign wells, groups, and standards groupings.
Chapter 3, Displaying, Graphing, and Printing Data	Describes how to analyze, graph, display, and print data from the CytoFluor II Fluorescence Multi-Well Plate Reader.
Appendix A, Example Files	Describes the different example files included with the software.
Appendix B, Hints for Using Excel	Contains hints for using Microsoft Excel.

Appendix C, Helpful Hints	Describes helpful hints on most frequently asked questions.
Appendix D, Technical Support and Training	Describes how to contact Technical Support, obtain technical documents, and obtain customer training information.

Conventions

This guide uses the following conventions to make text easier to understand.

General conventions

- **Bold** indicates user action:

    "Click **OK** when the installation is complete."

- *Italic* text denotes new or important words, and is also used for emphasis:

    "A standards *group* consists of replicate wells for a single point on the curve."

Notes

A note calling out important information to the operator appears as:

Note: *Subsequent changes to a work file can be saved using Save in the File menu (Alt, F, S).*

CytoCalc is a Microsoft® Windows®-based application for 100% IBM®-PC/AT-compatible computers. This manual assumes that you have a basic understanding of MS-DOS® conventions for naming files and directories, and familiarity with Windows.

Menu commands

You can select menu commands in two ways:

- Click with the mouse
- Use keyboard shortcuts

The procedures in this manual use the mouse instruction followed by the keyboard shortcut in parentheses.

For example:

    Select **Exit** from the File menu (Alt, F, X).

Tasks are performed in the following sequence:

1. Select the screen items you wish to operate on.

2. Select the command from the menu bar or ribbon.

3. Execute the command (double-click the command, click **OK**, or press **Enter**).

Installing the CytoCalc Data Analysis Software

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This chapter includes the following sections:

1

1.1 Introduction

The CytoCalc™ Data Analysis Software is a graphical, mouse-driven, user interface used to automatically analyze fluorescence data obtained from the CytoFluor® II Multi-Well Fluorescence Reader or the CytoFluor Series 4000 Multi-Well Fluorescence Reader.

You use the CytoCalc Plate Layout screen to assign your wells to groups and groupings instead of using the CytoFluor Plate Layout screen.

You use two types of files:

- Work files (*.CFL) that you create to define the multi-well plate layout, calculation conventions, and display/printout format.

- Data files (*.MFR) that contain raw fluorescence data from the CytoFluor II or CytoFluor Series 4000, or fluorescence data collected with controlled temperature from the CytoFluor Series 4000.

You open a CytoFluor II data file onto an open CytoCalc work file. You can use the same *.CFL work file to process any number of *.MFR data files, or you can process a *.MFR data file using more than one *.CFL work file.

The CytoCalc software uses the editing and graphics display features of Microsoft® Excel to display charts, graphs, and tables of calculated data.

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Requirements The CytoCalc software requires the following:

- 100% IBM® PC/AT compatible 386 computer (486 DX recommended)

- MS-DOS version 3.3 or later (6.2 recommended)

- Windows version 3.1 or later

- Microsoft Excel version 4.0 or later

- 4 MB RAM

- VGA graphics adapter and monitor

- 3.5-inch high-density floppy disk drive

- 40 MB hard disk drive (>200 MB recommended)

- Microsoft-compatible mouse

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1.2 Installing the Software

What you receive with CytoCalc

Your CytoCalc package contains the following:

- One program disk (3.5-inch high density floppy disk) for software version 3.00

- One User's Guide

- One Applied Biosystems Software License Agreement

Please read the license agreement before opening the floppy diskette package.

Installing Microsoft Excel

You must purchase and install Microsoft Excel separately. When you install Excel, allow the installation program to modify the path statement in your AUTOEXEC.BAT file to include the DOS Path to Excel. Otherwise, CytoCalc cannot access Excel.

Setting Excel Version 5.0 file location

If you are using version 5.0 or higher of Excel, do the following:

1. Start Excel from the Program Manager by double-clicking the Excel icon.

2. Select **Options** from the Tools menu.

3. Select the **General** tab.

4. Delete the text from the **Default File Location** field in the lower part of the screen. This field must be blank.

5. Click **OK**.

To check the Default File Location setting (you must install Cytocalc before checking):

1. From the File menu in the Plate Layout screen, open **EX1SF8X2.CFL** (work file) and **BTS5009.MFR** (data file). See Section 3.1.1, Viewing Data on the Plate Layout Screen, for help on opening files.

2. Select **Format** from the Data menu. The Data Format dialog box is displayed.

1

3. Select **Calc Data** and **Scan 1**. Other settings are not important at this time.

4. Click **Calculate**. A Microsoft Excel spreadsheet should be displayed, showing data and grid lines. If the screen is blank (no grid lines), the Default File Location parameter is not blank. Repeat the procedure to set this field blank

Installing CytoCalc

To install the CytoCalc software:

1. Power up your computer and start Windows.

2. Activate the Windows Program Manager.

3. Insert the CytoCalc diskette into the floppy disk drive.

4. Select **Run** from the File menu.

5. Type **a:install** (type b: if the floppy drive is drive b:)*.*

6. Click **OK**.

   The default destination directory is C:\CYTO2. You can change the destination directory by typing a directory name when prompted.

7. Click **Continue**.

8. Click **OK** when the installation is complete.

   The installation program completes the setup by creating a CytoCalc group and icon in the Program Manager.

1

1.3 Starting the Software

Starting the CytoCalc Software

To start the CytoCalc software:

1. Power up your computer.

    The Windows Program Manager appears.

    Note*: If your computer is not set to automatically start Windows, the DOS prompt appears instead of the Program Manager. At the C:\ prompt type **WIN** and press **Enter**.*

    ---

2. Double-click the **CytoCalc** icon.

    The Plate Layout screen appears.

Using CytoCalc with CytoFluor II software

To use the Cytocalc software simultaneously with the Cytofluor II software:

1. Hold down the **Alt** key and press the **Tab** key repeatedly until the Program Manager appears. Release the keys.

2. Double-click the **CytoFluor II** icon to start the Cytofluor II program.

Use the Alt and Tab keys to toggle between the CytoCalc software, the CytoFluor II software, and the Program Manager. You can collect data with the CytoFluor II software, and immediately open the data file (*.MFR) into the CytoCalc software for processing.

Creating a new directory

The C:\CYTO2 directory is the default destination directory for data. We recommend that you create additional directories for storage of your data files. Use the File Manager to create these directories. Refer to your Windows manual for more information on creating directories.

To create additional directories using the File Manager:

1. Toggle to the Program Manager.

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2. Double-click the File Manager icon.

3. Select **Create Directories** from the File menu and create your data directories.

1.4 Exiting the Software

To exit the CytoCalc software:

1. Select **Exit** from the File menu (ALT, F, X).

   The Exit CytoCalc dialog box appears (Figure 1-1).

Select to save your plate layout

```
┌─────────────────────────────────────┐
│ ━    Exit CytoCalc                   │
├─────────────────────────────────────┤
│ This will end your CytoCalc session. │
│          ⊠ Save Changes              │
│  ┌──────────────┐  ┌──────────────┐  │
│  │     OK       │  │    Cancel    │  │
│  └──────────────┘  └──────────────┘  │
└─────────────────────────────────────┘
```
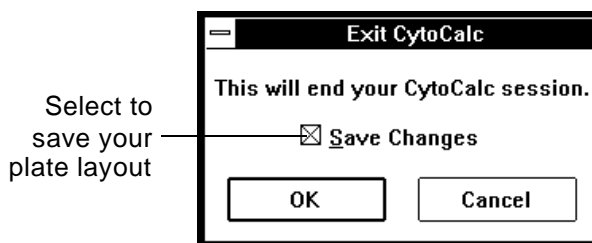
Figure 1-1 Exit CytoCalc Dialog Box

2. Click the **Save Changes** check box, if necessary.

   Note: When you select Save Changes, the plate layout currently on your screen appears as the default plate layout the next time you start up the CytoCalc software. If you do not select save changes, the last saved plate layout appears the next time you start up.
   ---

3. Click **OK**.

   Click **Cancel** if you do not want to exit the CytoCalc software.

Using the Plate Layout Screen

2

This chapter includes the following sections:

2.1 Overview of the Plate Layout Screen

2

Introduction   When you open the CytoCalc software, the Plate Layout screen appears. The Plate Layout screen displays a graphical representation of a multi-well plate. You use the CytoCalc Plate Layout screen instead of the CytoFluor II Plate Layout screen. You use the Plate Layout screen to:

- Select the plate type you are using
- Assign wells to reflect the way your plate is organized
- Assign standards groupings and a curve fit
- Save your layout as a work file

The CytoCalc software uses this information to determine how the data of specific cells are used to perform calculations.

Default Plate Layout screen   The first time you start the CytoCalc software, the default Plate Layout screen appears (Figure 2-1). This layout is preset to a 96-well plate with all wells identified as unknowns. You can change from the default by selecting a different plate type from the Plate Type menu. Select New from the File menu to display the original default layout.

You can customize the default Plate Layout screen to your most frequently used plate setup by saving your well assignments as a work file when you exit from the CytoCalc software. The next time you start the CytoCalc software, the layout from the last work file you saved is displayed.
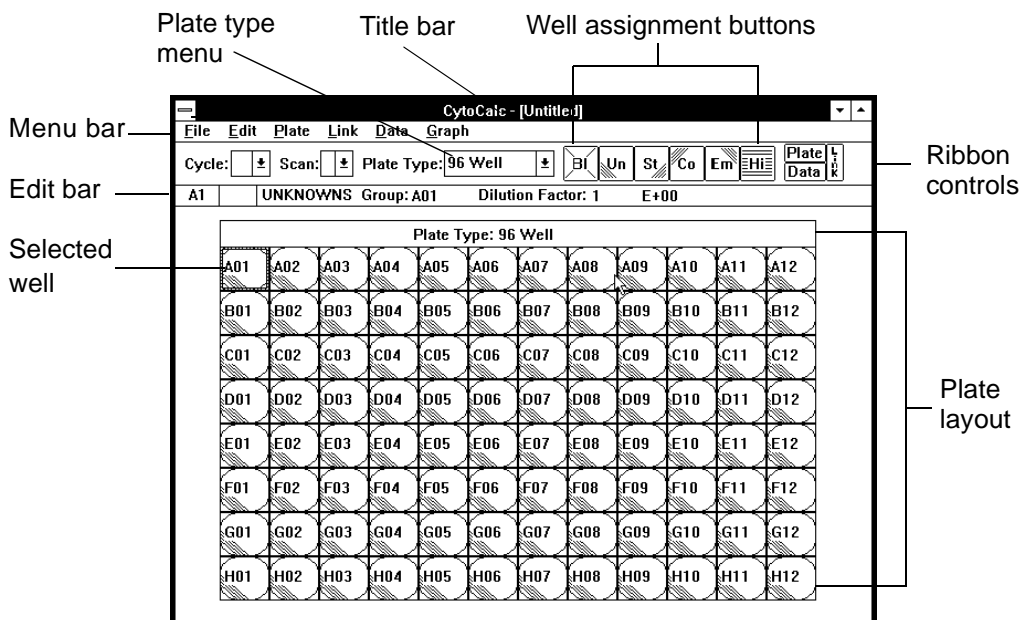
Figure 2-1 Default Plate Layout Screen

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Parts of the screen

The Plate Layout screen includes the following features:

- **Selected well**—Appears with a box around it. The box appears around a single selected well or the first well of a group.The first well position is the default selection.

- **Edit bar**—Contains information specific to the well type you selected. You use the Edit bar to enter the Group Name, concentration, dilution factor, and so on when you are assigning wells. The information that you need to enter depends on the well type that you are assigning. You activate the edit bar by clicking on any field in the edit bar.

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To change any of the information in a field in the edit bar, select the information by click-dragging across it (F2 and TAB). The Cancel (✗) and Accept (✓) buttons appear, and you can retype the information.

Click the Cancel (✗) button to cancel your changes. Click the Accept (✓)button to store the changes.

- **Menu bar**—Lists available menus. Each menu contains a list of commands for working with the plate layout, and managing data and files.

- **Title bar**—Contains the work file name associated with the displayed plate layout.

- **Ribbon controls**—Provide shortcuts for selecting the most frequently used commands listed in the Menu bar. Until you scan a plate or load data from a previous scan, only the Plate Type menu, buttons for well assignments (except the Hi button), and Link are active.

  You can disable the ribbon display by selecting **Ribbon** from the Plate pull-down menu.

  The Plate Type menu contains a list of available plate types. You use the list to change from the default plate type to a different plate type.

  The well assignment buttons correspond to commands in the Plate menu. They allow you to assign wells directly without using the menu.

- **Plate layout**—graphically represents of the selected plate type. The default wells appear yellow, are numbered with well position numbers, and are defined as unknowns.

2.2 Assigning Wells and Groups

This section describes:

- Well types
- Groups and groupings
- Assigning wells

2.2.1 Well Types

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The choices for well types and their appearance on the Plate Layout screen are defined in Table 2-1.

Table 2-1   Well Types

Well Types	Appearance	Color	Description
Blank Well		Gray	Wells that are used for calculating a background value for subtraction. You can subtract a blank value from all of the wells in your assay. You cannot subtract more than one blank group at a time from any well.
Unknown Well		Yellow	Wells containing samples of unknown value.

2

Table 2-1   Well Types   (continued)

Well Types	Appearance	Color	Description
Standards Well		Green	Wells that are used to obtain values for plotting standards curves. You need a minimum of three points to plot a curve, and you must have at least two replicate wells for each point. A standards *group* consists of the replicate wells for a single point on the curve. A standards *grouping* consists of all standards groups (points) in a single curve. You can have more than one standards grouping (curve) on your plate layout, but you can assign only one standards grouping at a time to an unknown. Refer to "Assigning standards and controls to unknowns" on page -20 for more information.
Control Well		Red	Wells that are used to obtain values against which Unknown fluorescence values are compared. You need a minimum of three wells in a control group. You may have more than one control group on a plate.
Empty Well		White	Wells that have been left unfilled or are not to be included in the assay. Empty wells can be scanned, but their values are not used in calculations.

Table 2-1   Well Types (continued)

Well Types	Appearance	Color	Description
Hidden Well		Black	Wells containing data that you want to omit from calculations. For example, values that are obvious outliers, or values that exceed the system limits (99999).

2.2.2 Groups and Groupings

Understanding groups and groupings

When you assign wells, you also assign them to groups to identify replicates of the same material. All the wells of the same group are treated the same way in calculations.

When you assign standards wells, you:

- Assign replicates wells to groups
- Assign groups to standards groupings
- Assign a curve fit to a standards grouping

When you assign all other types of wells, you assign replicate wells to groups.

Group names

Assigned wells are labeled with the name entered in the Group field in the Edit bar. Default group names are listed in Table 2-2.

Table 2-2   Default Group Names

Well Type	Group Name
Blank	Bl01
Unknown	Name of first well in group
Standards	St01

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Table 2-2   Default Group Names (continued)

Well Type	Group Name
Control	Co01
Empty	Em
Hidden	Hi

You can change the group name to customize it to your plate layout. Group names:

- Can contain up to four letters or numbers
- Must be the same length to ensure that the groups are sorted properly

Refer to Appendix C, Helpful Hints, for suggestions on naming groups.

2.2.3 Assigning Wells

To assign wells:

1. Select the wells you want to assign:

    - Click on a single well to select it
    - Click and drag to select a group of wells

2. Make the appropriate well assignment.

    Type the appropriate information in the fields in the edit bar that appears for each selected well type.

If you want to assign …	Then …
Blank wells	• Click the **Blank** (Bl) button (Alt, P, B). • Change the group name, if necessary.
Unknown wells	• Click the **Unknown** (Un) button (Alt, P, U). Press **F2** if the well type is already Unknown. • Change the group name, if necessary. • Enter a dilution factor, if necessary. Highlight the default value of +00 and enter your value with the appropriate sign. You can enter up to 5 digits, including decimals. The exponent range is ± 99.
Standards wells	• Click the **Standards** (St) button (Alt, P, S). • Change the group name, if necessary. • Enter a name for the standards grouping. You can enter up to 4 characters. See "Multiple experiments on a single plate" on page C-1 for hints on naming. • Enter the concentration value. You can enter up to 5 digits, including decimals. The exponent range is ± 99. You must enter at least 3 concentration values (data points) in duplicate (minimum of 6 wells) for a valid standard curve. See Section 2.3.1, Curve Fits, for data point requirements for each curve fit. If you do not have the minimum number of data points required for the selected curve fit, an "Invalid Standard Curve" message is generated. ***Note:*** *You must enter a concentration value in the standards Value field.* • Enter the concentration Units. You can enter up to 5 characters, for example, mg/ml.

2

2

If you want to assign …	Then …
Control wells	• Click the **Control** (Co) button (Alt, P, C). • Change the group name, if necessary. • Enter the concentration value. You can enter up to 5 digits, including decimals. The exponent range is ± 99. You must include 3 wells (enter the concentration in triplicate) in the control group. If you do not have 3 data points in the control group, an "Invalid Control Group" message is displayed.
Empty wells	Click the Empty (Em) button (Alt, P, E).

3. When you are finished, click the ✓ button (accept) to accept the definition. Click the ✗ button (cancel) to cancel the setting.

   All of the wells assigned to the same group are labeled with the group name.

4. To assign additional wells, repeat step 1 through step 3.

Copying well assignments

You can copy a defined well assignment and paste it to an unassigned well.

1. Select a defined well or group of wells.

2. Select **Copy** from the Edit menu (Alt, E, C or Ctrl+ Ins).

3. Select the destination well (or the first well of a group).

4. Select **Paste** from the Edit menu (Alt, E, P or Shift+ Ins).

   Note: You can undo the Paste command immediately by selecting Undo from the Edit menu. (Alt, E, U or Alt+Backspace).

   The copied well or wells appear in the new location.

5. Repeat step 3 and step 4 to assign remaining wells.

2.3 Assigning Groups to Standards Groupings

This section describes:

- Using the Link editor
- Curve fits
- Using the Format command

2

Introduction Group assignments direct the software to subtract the blank from the raw data, and calculate unknowns using the appropriate standards curve.

A standards grouping consists of groups containing replicates of standards that span the range of interest. The CytoCalc software can support more than one standards grouping (experiment) on a plate.

Each standards grouping can have more than one unknown group assigned to it. The software does not calculate an unknown value from more than one standards curve at a time.

Each unknown group can have only one control group assigned to it. A single control can be assigned to more than one unknown.

You can assign groups to groupings in two ways:

- Graphically, using the Link feature
- Manually, using the Format command on the Data menu

Changes made with Link are mirrored in the manual displays, and changes made using the Format command are mirrored in the Link display.

After you collect data, you can graph each unknown group with a standards curve or with a control group. See Chapter 3, Displaying, Graphing, and Printing Data, for more information.

2

Example   For example, if an assay plate contains:

- Replicate groups for two unknowns: Un01 and Un02.

- A single standards grouping named RNA, which is made up of four standards groups: RNA1, RNA2, RNA3, and RNA4. (Each of these groups contains three replicate wells)

- Two Control groups: Co01 and Co02

Both Un01 and Un02 can be assigned to the RNA standards grouping. Either Co01 or Co02 can be assigned to both Un01 and Un02. But Co01 and Co02 cannot both be assigned to a single unknown.

2.3.1   Curve Fits

The CytoCalc software uses 10 different curve fits that you can assign to your standards groupings. Once you assign a curve fit, it is used in subsequent data calculations.

You can use the Hide button to hide all out-of-range (99999) values so that they are not included in the calculation of the best-fit curve.

You can wait until after you have collected data to assign a curve fit. You can then test different fits by assigning them one at a time and using the built-in graphing capability to find the curve that best fits the data.

The CytoCalc software generates standards curve fits using the following functions and number of standards points:

Curve Fit	Function	Standards Points Required
LIN-LIN	$[C] = a + b(fl)$	3
LOG-LOG	$\log[C] = a + b(\log(fl))$	3
LIN-LOG	$[C] = a + b(\log(fl))$	3
LOG-LIN	$\log[C] = a + b(fl)$	3
LOGIT-LOG	$[C] = \dfrac{(a-d)}{\left(1 + \left(\frac{(fl)}{c}\right)^{b}\right)} + d$	5
2nd ORDER POLYNOMIAL	$[C] = a + b(fl) + c(fl)^{2}$	3
LIN-RECIPROCAL	$[C] = a + b\left(\dfrac{1}{(fl)}\right)$	3
LIN-SQUARE ROOT	$[C] = a + b\sqrt{(fl)}$	3
RECIPROCAL SQUARE ROOT	$[C] = a + b\left(\dfrac{1}{\sqrt{(fl)}}\right)$	3
4-PARAMETER FIT*	$[C] = \dfrac{(a-d)}{\left(1 + \left(\frac{(fl)}{c}\right)^{b}\right)} + d$	4

* Calculated using Levinburg-Marquardt algorithm

Where:
[C] = concentration
[fl] = fluorescence

2

2

Note: *When you use the logit-log curve type, the highest and lowest of the standards values are interpreted as the high and low asymptotes of the sigmoid curve. If more than one standard yields the same maximum or minimum fluorescence value, hide all but the one closest to the "knee" in the curve. The other intermediate values should span the midpoint between the high and low values for best fit.*

2.3.2 Using the Link Editor

This section describes:

- Assigning groups to groupings
- Reassigning groups to groupings
- Assigning a different curve fit

Assigning groups to groupings

To use the Link editor:

1. Click **Link** or select **Link** from the Link menu.

    If there is only one standards grouping, it is selected by default. If there are more than one, one of the groupings is selected.

2. Double-click on all groups you want to assign to the selected standards grouping. Groups assigned to the standards grouping are highlighted.

3. Select a curve fit from the Curves menu.

4. Double-click on the next standards grouping.

    Previously assigned groups contain grayed text and are not available.

5. Repeat step 2 and step 3 as necessary to link additional groups on the plate with the appropriate standards grouping.

6. Click **Data** or **Plate** to exit the Link editor and save the assignments. The Accept Current Links dialog box appears:

    •   Click **Yes** to save your changes and return to the Plate Layout screen.

    •   Click **No** to cancel your changes and return to the Plate Layout screen.

    •   Click **Cancel** to cancel your changes and return to the Link editor.

2

Reassigning groups to groupings

You can reassign groups from one standards grouping to another.

To reassign a group:

1. Select the standards grouping from which you want to reassign a group.

2. Double-click on a well to deselect the group.

    A deselected group appears as shown in the example in Figure 2-2.

Deselected unknowns group
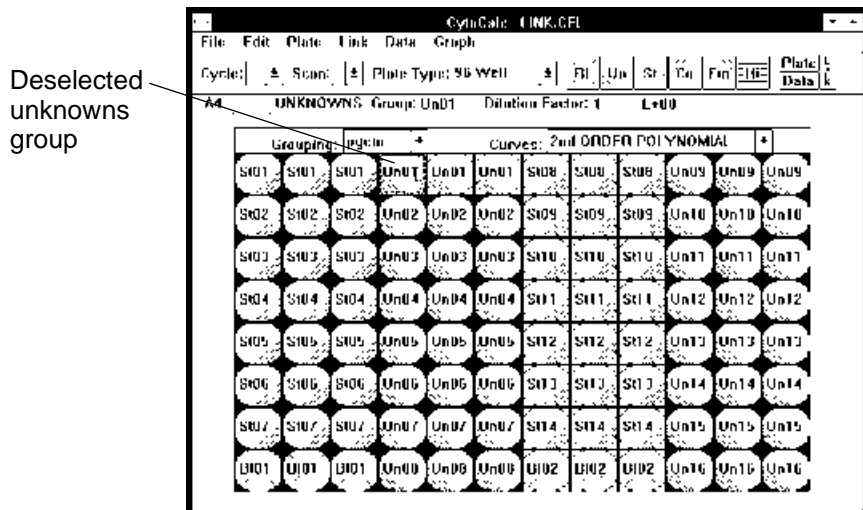
Figure 2-2 Deselected Unknown Group

3. Select the standards grouping to which you want to
    reassign the group.

    The example in Figure 2-3 shows the Un01 group
    unassigned and the cfth standards grouping selected.

Un01 available
for linking

cfth standards grouping
selected
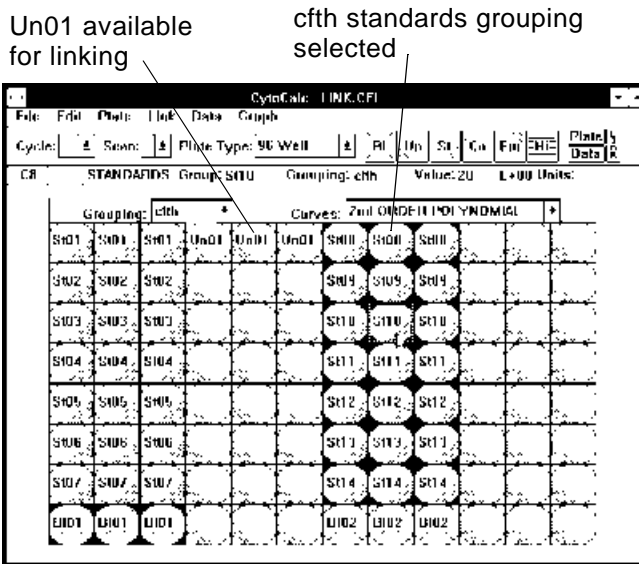
Figure 2-3 cfth Standards Grouping

4. Select the group you want to assign to the selected
    standards grouping.

    The example in Figure 2-4 shows the UN01 group
    assigned to the cfth standards grouping.
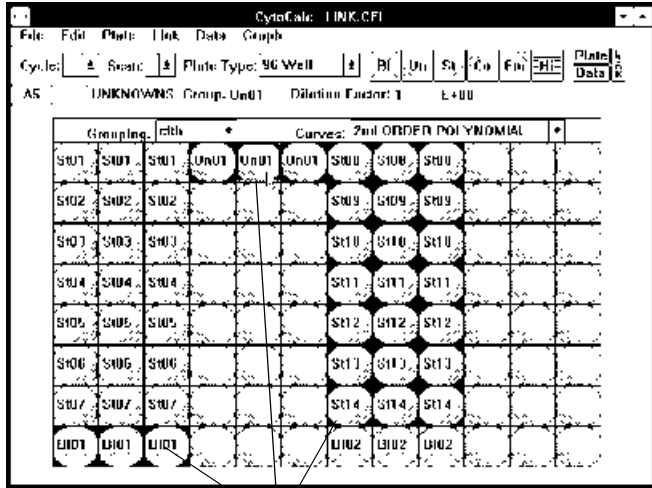
Groups are assigned only to the active Standards Grouping.

Figure 2-4 Groups Assigned to the cfth Standards Grouping

Assigning a different curve fit

You can assign a different curve fit to the selected standards grouping by selecting the curve you want from the Curves pull-down menu.

The example in Figure 2-5 shows the Curves pull-down menu. The selected curve fit, 2nd Order Polynomial, will be applied to the cfth standards grouping.

Curve selection
for the cfth
grouping
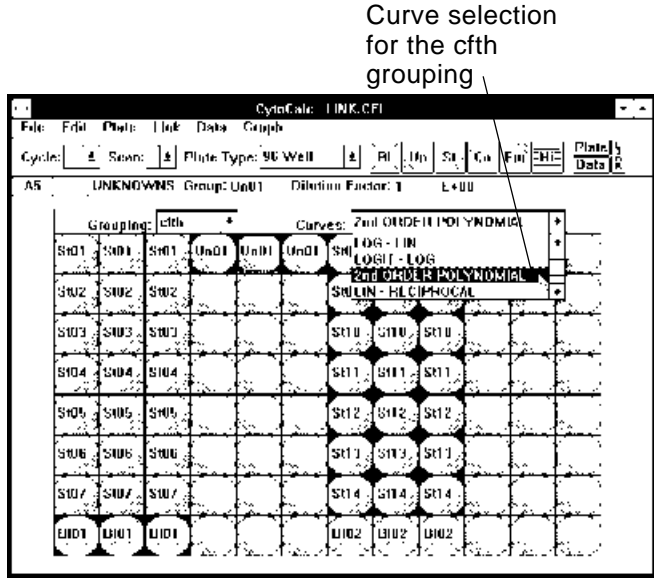
Figure 2-5 Curves Pull-Down Menu

2.3.3 Using the Format Command

This section describes:

- Assigning a curve fit to a standards grouping
- Assigning standards and controls to unknowns
- Assigning groups to blanks

The assignments you made with the Link editor can also be made manually using the Format command on the Data menu.

Assigning a curve fit to a standards grouping

To assign a curve to a standards grouping:

1. Select **Format** from the Data menu (ALT, D, F). The Data Format dialog box appears (Figure 2-6).

2
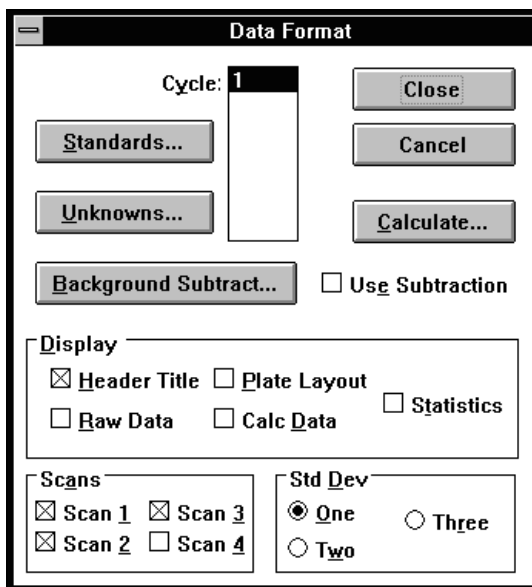
Figure 2-6 Data Format Dialog Box

2. Click the **Standards** button. The Standards dialog box appears (Figure 2-7).

2

Grouping names

Display-only list of groups belonging to the selected grouping

Selected curve fitting
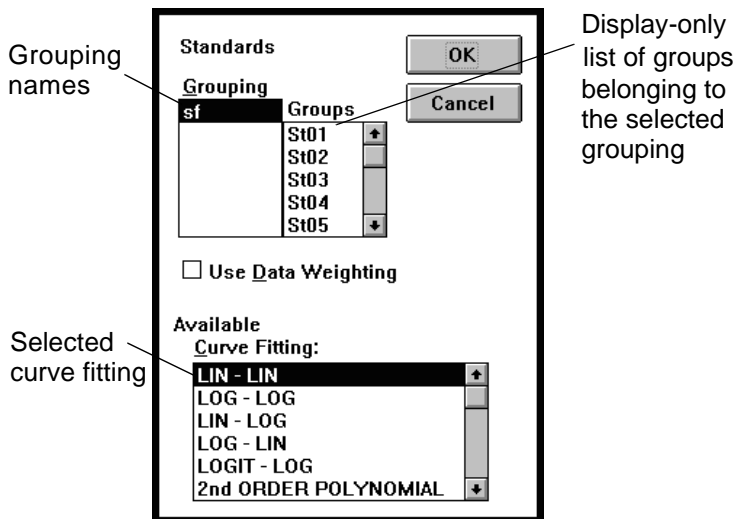
Figure 2-7 Standards Dialog Box

3. Select the name of the grouping to which you want to assign a curve.

    The groups of replicate wells that are assigned to the selected grouping are displayed in the Groups box. You cannot make selections from this list.

4. Click the curve fitting formula you want to assign to the selected standards grouping. Use the scroll bar to view all of available formulas, if necessary.

5. Repeat step 3 and step 4 if you have more than one standards grouping to assign.

6. Click **OK** to save the changes. Click **Cancel** to close the box without updating assignments.

Assigning standards and controls to unknowns

To assign standards and controls to unknowns:

1. Select **Format** from the **Data** menu (ALT, D, F).

    The Data Format dialog box appears (Figure 2-6).

2. Click **Unknowns**. The Unknowns dialog box appears (Figure 2-8).
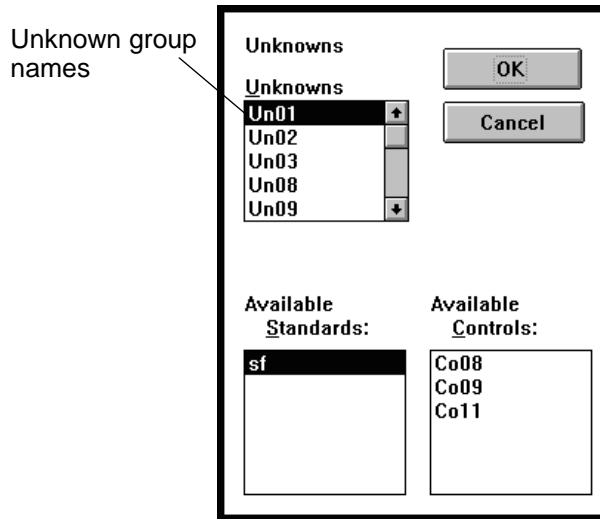
Figure 2-8 Unknowns Dialog Box

3. Select the unknown group to which you want to make assignments.

4. Click the standards grouping to which you want the unknown to be assigned. You can assign an unknown to only one Standards grouping at a time.

5. Click the Control group that you want to assign to the unknown. You can assign only one Control to an unknown at a time.

6. Repeat step 3 through step 5 if you want to make additional assignments.

7. Click **OK** when you are finished. Click **Cancel** to close the box without updating the plate layout.

When you select an Unknown group, the Standards Grouping to which it is assigned is highlighted. To cancel the assignment, click on the Standards Grouping name and the highlight disappears.

Assigning groups to blank wells

To assign groups to blank wells:

1. Select **Format** from the **Data** menu (ALT, D, F). The Data Format dialog box appears (Figure 2-6).

2. Click **Background**. The Background Subtract dialog box appears (Figure 2-9).
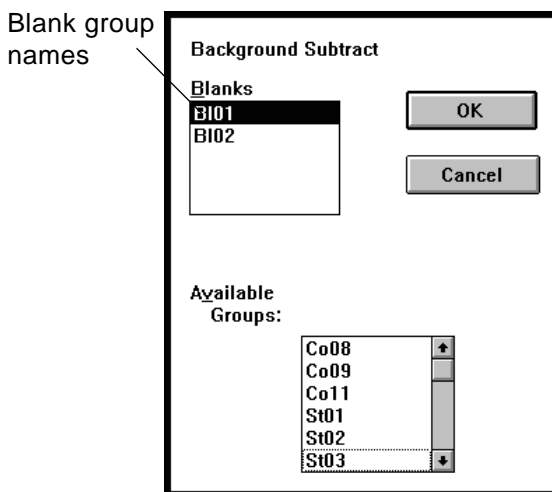
Blank group names

Figure 2-9 Background Subtract Dialog Box

3. Click the name of the blank group that you want to use to obtain a background value.

4. Click the groups from which you want to subtract the background value. You may select as many as are applicable. You can subtract only one background value at a time from any well.

> **Note:** *Standards are listed as individual groups, not as standards groupings. Make sure that you select all standards groups belonging to a single grouping.*

5. Repeat step 3 and step 4 if you want to assign groups to another blank group. Once you click the name of another blank group, only the remaining available groups (the ones you did not assign earlier) appear in the list box.

6. Click **OK** when you are finished. Click **Cancel** to close the box without updating the plate layout.

2

2.4 Saving the Plate Layout as a Work File (*.CFL)

2

The plate layout information is saved as a work file with a *.CFL extension. A *work file* contains well assignments and group assignments for data calculation. It does not contain data.

Saving a work file

To save a plate layout as a work file:

1. Select **Save As** from the **File** menu (ALT, F, A). The File Save As dialog box appears.

2. Type a file name in the File Name box.

3. Change directories, if necessary.

4. Click **Save**.

    Click **Cancel** if you do not want to save the file.

Note: *Subsequent changes to a work file can be saved using Save in the File menu (ALT, F, S).*

Viewing sample work files

To view a sample work file:

1. Select **Open** from the File menu and use the directory dialog box to find and open one of the sample work files: EX1SF8X2.CFL, EX2SF8X2.CFL, EX3SF8X2.CFL, or EX4SF8X2.CFL.

2. Select **Format** from the Data menu and use the Standards, Unknowns, and Background Subtract buttons to view the group assignments of the sample file.

3. Use the Link button to view previously made assignments.

4. Close the file without saving changes when you are finished.

Displaying, Graphing, and Printing Data

3

This chapter includes the following sections:

3.1 Displaying Data on the Plate Layout Screen

This section describes:

- Viewing data on the Plate Layout screen
- Ribbon controls

3.1.1 Viewing Data on the Plate Layout Screen

3

When you scan a plate with the CytoFluor II Fluorescence Reader, the result is a data file with a *.MFR extension. The file is created by the CytoFluor II software and contains the raw data (fluorescence) values and the scanner parameters selected to perform the scan.

To view data, open a *.CFL (work) file and an *.MFR (data) file:

1. Select **Open** from the **File** menu (Alt, F, O). The File Open dialog box appears (Figure 3-1).
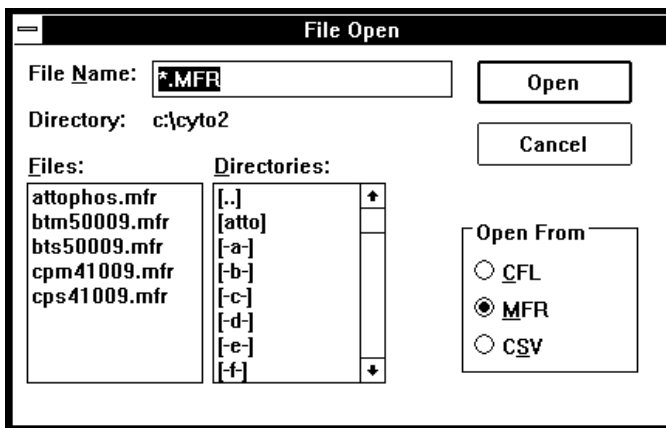
Figure 3-1 File Open Dialog Box

2. Click **CFL** to see the available work files. Change directories or scroll through the list, if necessary.

3. Select the name of the file.

4. Click **Open**.

   The file you selected appears on the plate layout screen.

5. Select **Open** from the File menu (Alt, F, O). The File Open dialog box appears.

   Note: *The CSV option allows you to import data that was exported from older CytoFluor 2300 series readers.*

   ---

6. Click **MFR** to see the available data files. Change directories or scroll through the list, if necessary.

7. Select the name of the file.

8. Click **Open**. The data from the file you selected appears on the Plate Layout screen. An example of the Plate Layout screen with data displayed is shown in Figure 3-2.

9. From the Cycle pull-down list, select the cycle of data to display. If kinetic data has been collected, you can select **Kin**. to display kinetic data.

   Note: *The Kin. option is available only if the current .MFR file contains kinetic data.*

3

Active Boxes   Active Buttons
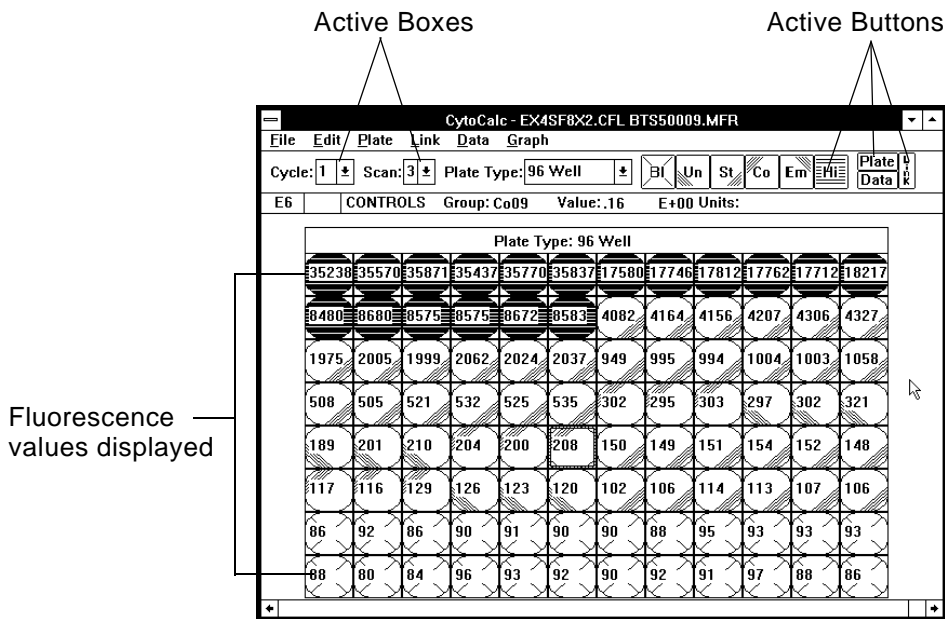
Figure 3-2 Plate Layout Screen with Data Displayed

3.1.2 Ribbon Controls

When data is displayed on the Plate Layout screen, the Cycle, Scan, and Data menus and the Hidden button on the ribbon are active.

Data and Plate buttons

When you click the Data button, the wells display the fluorescence values obtained during the scan. When you click the Plate button, the wells display the group names. You can switch between Data and Plate without losing data.

Cycle and Scan boxes

The Cycle box and the Scan box display values appropriate for the displayed data file. If you have more than one cycle for a plate or more than one scan per cycle, select the appropriate number from the pull-down list. Scroll bars appear if there are multiple cycles or scans. You use the vertical scroll bar to change cycles. You use the horizontal scroll bar to change scans. The data obtained for the selected cycle or scan is then displayed.

If you have more than one cycle in the data file, you can perform data calculation and graphing for only one cycle at a time. If you have more than one scan per cycle, you can include any or all of them in data calculation and graphing.

Hide (Hi) Button

The Hide (Hi) button enables you to hide obvious out-of-range data before calculating the results. Use this button to hide wells that you do not want to include in the analysis.You can hide wells that are obvious outliers or wells that have a value beyond the system limits (99999).

You can perform data calculation before hiding wells. Unknown wells that have values out of range when a control is applied are identified with asterisks in the Excel spreadsheet after statistical calculation. You can use this information to decide which wells to hide. All out of range or blank-equivalent standards values must be hidden so that they will not be included in the best-fit standards curve calculation.

Hiding Wells

To hide wells:

1. Select the well or wells that you want to hide.

2. Click the **Hi** button (Alt, P, H).

    The selected wells change to hidden wells. The type is listed as Hidden. It is not necessary to enter any other information.

When data is displayed, the value of the hidden well can be viewed on the plate layout screen, but the data will not be included in calculations.

3

3.2 Customizing the Excel Data Display

This section describes:

- Using the Data Format dialog box
- Display parameters
- Scans options
- Std Dev options

3.2.1 Using the Data Format Dialog Box

3

The Data Format dialog box (Figure 3-3) allows you to customize how data is displayed in the Excel spreadsheet. The following parameters and options determine how the data is displayed in Excel:

- Header title
- Plate layout
- Raw data
- Calculated data
- Statistics
- Number of scans
- Standard deviation range

Data Format

Cycle of data displayed ———— Cycle: 1

Standards...

Unknowns...

Background Subtract... ☐ Use Subtraction

Close

Cancel

Calculate...

Display parameters ————

Display
☒ Header Title ☐ Plate Layout
☐ Raw Data ☐ Calc Data
☐ Statistics

Scan options ————

Scans
☒ Scan 1 ☒ Scan 3
☒ Scan 2 ☐ Scan 4

Standard deviation options ————

Std Dev
⦿ One ○ Three
○ Two

3
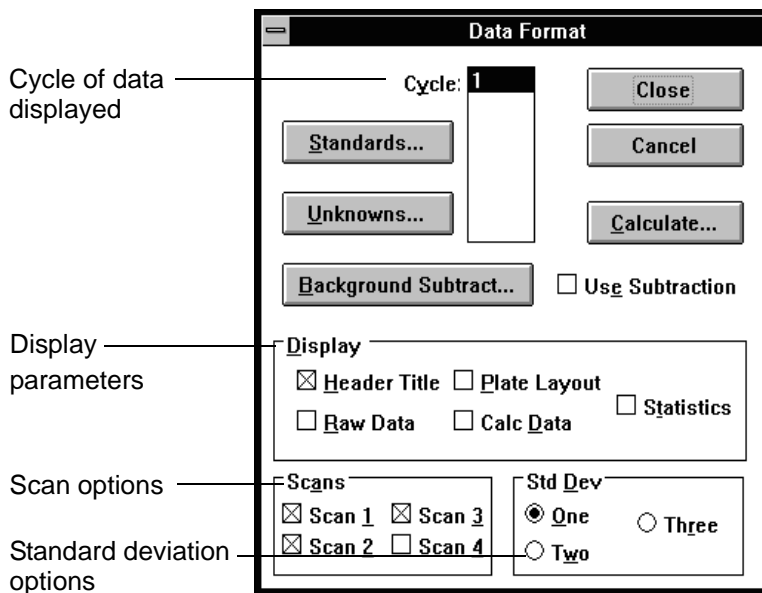
Figure 3-3 Data Format Dialog Box

3.2.2 Display Parameters

The Display box in the Data Format dialog box contains options that you can include on your spreadsheet. Select an option by clicking the check box. You can choose as many of these options as you want, but the fewer you choose, the faster the calculation will run.

Header Title   Select this option to display the scanner setup parameters for the data file.

An example header title display is shown in Figure 3-4. Use the scroll bars to view all of the information.

	1	2	3	4	5	6	7	8	
1	BTS50009.MFR								
2	Sat Aug 06 22:33:09 1994								
3	Standard Deviation is 1x								
4									
5	Background Subtract is On			Data Weighting is Off					
6									
7	COMMENT	009-sf-black titertek-top-8/8/94-fgb							
8									
9	MODE:	AUTO	MIX TIME:	0		RDS/WELL	3		
10	CYC-CYC:	0:00:00							
11	SCAN	1	1	2	2	3	3	4	
12	FILTER	EX	EM	EX	EM	EX	EM	EX	
13	CENTER	485	530	485	530	485	530	485	
14	WIDTH	20	25	20	25	20	25	20	
15	POSN	1	1	1	1	1	1	1	
16	GAIN	70		80		90		55	
17	AUTOSCALE OFF								
18	PLATE:	CytoPlate 96 well			ROWS:	8	COLS:	12	
19	CYCLES:	1							
20									

Sheet1

Figure 3-4 Header Title Display in Excel

Plate Layout   Select this option to display your data in a row by column array corresponding to the wells in your microplate.

An example is shown in Figure 3-5.

	1	2	3	4	5	6	7	8	
131	PLATE LAYOUT FORMAT DISPLAY				Background Subtract is On			Data Weightin	
132									
133	CYCLE	SCAN		ELAPSED TIME					
134	1		1	0:00:00					
135									
136		1	2	3	4	5	6	7	
137	A	5071	5160	5182	5141	5153	5175	2555	
138	B	1266	1276	1281	1254	1294	1254	618	
139	C	308	312	312	314	315	318	152	
140	D	75	76	79	79	80	78	40	
141	E	21	21	21	21	21	21	11	

Sheet1

Figure 3-5 Plate Layout in Excel

Raw Data   Select this option to view the raw data for each well displayed in column format.

An example is shown in Figure 3-6.

	1	2	3	4	5	6	7	8	
31	RAW DATA DISPLAY				Background Subtract is On			Data Weighting	
32	CYCLE 1	SCAN 1	SCAN 2	SCAN 3					
33	WELL	DATA	DATA	DATA				⊹	
34	A01	5071	14381	35148					
35	A02	5160	14616	35570					
36	A03	5182	14695	35781					
37	A04	5141	14589	35437					

◄ ◄ ► ►\ Sheet1 /

Figure 3-6 Raw Data Display in Excel

Calc Data   Select this option to view the Value Format display, which is a table sorted by group name that displays all assignments, setup parameters, and data for each well position on the plate.

An example is shown in Figure 3-7.

	1	2	3	4	5	6	7	8	
173	VALUE FORMAT DISPLAY				Background Subtract is On			Data Weighting	
174	CYCLE	SCAN	ELAPSED TIME						
175	1	1		0:00:00			⊹		
176									
177	Standard Equation(s)								
178	sf is [c] = 0.0290576 + 0.00779095(fl) r=0.999904								
179									
180	Group	Group	RC	Grouping	Fluores	Value	Units	Dilution	
181	Type	Name							
182									
183	Standard	St01	A01	sf	5071	40		N/A	
184			A03	sf	5182	40		N/A	
185			A05	sf	5153	40		N/A	
186		St02	A07	sf	2555	20		N/A	
187			A09	sf	2591	20		N/A	
188			A11	sf	2579	20		N/A	
189		St03	B02	sf	1276	10		N/A	
190			B04	sf	1254	10		N/A	
191			B06	sf	1254	10		N/A	
192		St04	B07	sf	618	5		N/A	

◄ ◄ ► ►\ Sheet1 /

Figure 3-7 Calc Data Display in Excel

Statistics Use this option to calculate and display the concentrations, average values of replicates, the standard deviation, variance, CV, and other statistical parameters of the data. This table is sorted by group and grouping. Actual values for standards and controls are listed beside their average fluorescence values. The average value of unknown replicates, calculated from the assigned standards curve, is listed in the column to the left of the average fluorescence value. An example is shown in Figure 3-8.

	1	2	3	4	5	6	7	8	
496	STATISTICS RESULTS				Background Subtract is On			Data Weighting	
497									
498	CYCLE	SCAN	ELAPSED TIME						
499	1	1	0:00:00						
500								✛	
501	Standard Equation(s)								
502	sf is [c] = 0.0290576 + 0.00779095(fl)	r=0.999904							
503									
504	Group	Group	Value	Average	SD (n)	V (n)	SD (n-1)	V (n-1)	
505		Name							
506									
507	Standard	St01	40	0	0	0	0	0	
508		St02	20	2574.5	14.9805	224.417	18.3427	336.455	0
509		St03	10	1260.83	10.391	107.972	12.7196	161.788	
510		St04	5	634.5	11.3615	129.083	12.4441	154.855	
511		St05	2.5	312.667	3.14466	9.88889	3.4382	11.8212	
512		St06	1.25	156.333	2.98142	8.88889	3.25902	10.6212	
513		St07	0.63	77.3333	1.88562	3.55556	2.05456	4.22121	
514		St10	0.08	11	0.816497	0.666667	0.868646	0.754545	
515		St12	0.02	3	0.816497	0.666667	0.868646	0.754545	

Sheet1

Figure 3-8 Statistics Display in Excel

3.2.3 Scans Options

A CytoFluor II cycle can consist of up to four scans of one microplate. Each scan can have a different excitation/emission filter and PMT gain combination. Select any combination of scan check boxes to choose which scan data to display. You can view the results from one up to all four scans.

3

3.2.4 Standard Deviation (Std Dev) Options

The CytoCalc software can calculate standard deviation ranges of one, two, and three. You can choose only one at a time. The chosen standard deviation appears with the header information if Header Title has been chosen. The calculated value appears with other statistics if Statistics has been chosen.

3

3.3 Displaying and Printing the Data in Excel

This section describes:

- Displaying the data in Excel
- Displaying kinetic data in Excel
- Printing the data from Excel
- Exiting from Excel

3.3.1 Displaying the Data in Excel

3

To display data in Excel:

1. Select **Format** from the Data menu (Alt, D, F). The Data Format dialog box appears (Figure 3-3).

2. Select the appropriate parameters and options.

3. Click **Calculate** in the Data Format dialog box. A spreadsheet with your data is displayed.

Note: If the screen is blank (no grid lines), the Microsoft Excel Default File Location parameter is not set correctly. See "Setting Excel Version 5.0 file location" on page 1-4.

---

4. Use the scroll bar to view more of the spreadsheet.

5. Use **Save As** from the File menu to save the file under a new name.

Graphing concentration versus average fluorescence

If you selected the Statistics option, you can make a graph of concentration versus fluorescence without leaving the Excel data display spreadsheet.

1. Use the scroll bar to access the Statistics Results area of the spreadsheet.

2. Select the data for the x-axis (concentration) by highlighting the concentration values of the groups you want to include.

3. Select the data for the y-axis (average fluorescence) by holding down the CNTRL key and click-dragging down the Average column until the fluorescence values of the same groups are highlighted. (Both columns must be highlighted identically.)

4. Click the **Chart Wizard** button, click and drag the outline of the chart size you want.

   The chart wizard displays a series of dialog boxes.

5. Select **Next** in the first dialog box.

6. Select **XY** (Scatter) and then Next in the second dialog box.

7. Select the grid pattern and then **Next** in the third dialog box.

8. Select **Next** in the fourth dialog box.

9. Add a legend if you want in the fifth dialog box.

10. Select **OK** or **Finish**.

Excel plots and displays the results.

3

3.3.2 Displaying Kinetic Data In Excel

To display maximum slope data collected using the CytoFluor Kinetic option in Microsoft® Excel software:

1. Select **Format** from the Data menu (Alt, D, F). The Data Format dialog box appears (Figure 3-9).
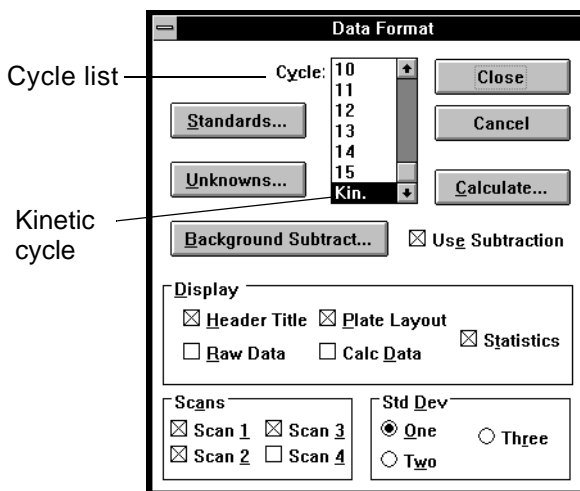
Figure 3-9 Data Format Dialog Box

2. Select **Kin**. from the Cycle list.

3. Select Statistics and any other appropriate display options.

4. Click **Calculate.**

5. A spreadsheet containing the maximum slope values (change in fluorescence units) is displayed (Figure 3-10).

KIN2.MFB								
Standard Deviation is 1x								
Background Subtract is Off			Data Weighting is Off					
STATISTICS RESULTS					Background Subtract is Off		Data Weighting is Off	
KIN.	SCAN		CYCLE TIME					
	1		0:02:00					
Standard Equation(s)								
1 is [c] = -26.7055 + 0.0564388(fl) r=0.993797								
Group	Group Name	Value	Average	SD (n)	V (n)	SD (n-1)	V (n-1)	CV
Standard	St01	200	3926.25	32.9574	1086.19	38.0553	1448.25	0.96927
	St02	100	2426.75	41.3665	1711.19	47.7653	2281.58	1.96831
	St03	50	1409.5	32.3303	1045.25	37.3318	1393.67	2.64859
	St04	25	765.5	14.3265	205.25	16.5423	273.667	2.16105
Unknown	E01	0	3905	0	0	0	0	0
	E02	0	2336	0	0	0	0	0
	E03	0	1372	0	0	0	0	0

Maximum slope values (Δ Fluorescence)

Figure 3-10 Maximum Slope Data Display

3.3.3 Printing the Data from Excel

3

Printing an entire spreadsheet

To print your entire spreadsheet, select **Print** from the File menu (Alt, F, P). Follow the instructions presented in the dialog boxes.

Printing an area of a spreadsheet

To print a particular area of the spreadsheet:

1. Highlight the area you want to print.

2. Select **Print** from the File menu.

3. Select **Selection** from the Print What dialog box to print the selected area.

4. Select **OK**.

Refer to the Excel user's manual for details on using more of the Excel printing features or Excel version 4.0.

3.3.4 Exiting Excel

To exit Excel and return to the Plate Layout screen:

Select **End Data Display** from the File menu
(Alt, F, D, Enter).

The Excel software closes, and the CytoCalc Data
Format dialog box appears.

*Note: Always use the End Data Display command to return
to CytoCalc to avoid opening additional Excel windows. Do
not switch from Excel to the Plate Layout screen by using
the Close command or by clicking in the Plate Layout
screen. Do not minimize the Excel window.*

3

3.4 Using the Graph Menu

This section describes:

- Overview of the Graph menu
- Graphing standards curves
- Graphing unknowns
- Graphing unknowns using kinetic data

3.4.1 Overview of the Graph Menu

Use the Graph menu to define the mathematical curve that best fits the data points within a standards grouping. The CytoCalc software uses the selected curve fit to calculate unknowns assigned to the standards grouping. In addition, a least-squares best-fit curve is calculated for the selected curve fit and is displayed on a graph with the necessary coefficients and correlation parameters.

You can create a bar chart that displays the raw fluorescence values for each unknown well or group, along with ±1, 2, or 3 standard deviation error bars. If you have a control group assigned to an unknown group, the error bars will be those of the control. You can display the bar chart of unknowns in concentration units as calculated from the assigned standards curve.

Many of the choices in the Graph dialog boxes are the same as the choices in the Data Format dialog boxes because the two features mirror each other. If you make changes in the Graph dialog boxes, the changes also appear in the Data Format dialog box.

3

3.4.2 Graphing Standards Curves

To create a graph of a standards curve:

1. Select **Standards** from the Graph menu (Alt, G, S). The Graph Standards dialog box appears (Figure 3-11).

3
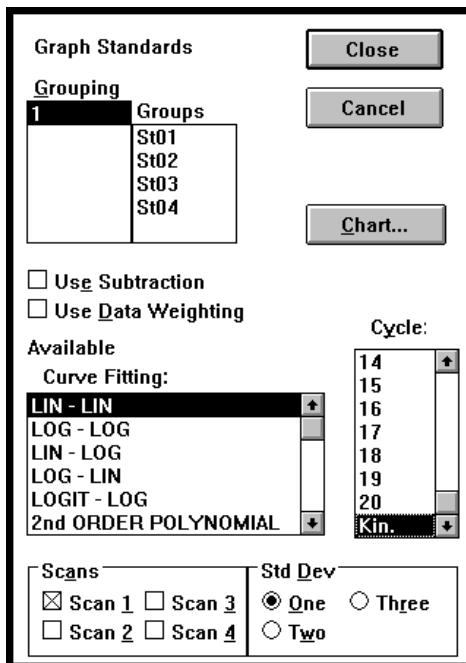
Figure 3-11 Graph Standards Dialog Box

2. Select a Grouping.

3. Select the following parameters as needed.

Parameter	Description
Use Subtraction	Subtracts the average value of the assigned Blank group from the other groups on your plate.
Use Data Weighting	Applies data weighting during curve fitting.
Cycle	Displays the cycle to be graphed, or selects kinetic data for graphing. You can select only one cycle or kinetic data at a time.
Available Curve Fitting	Applies a curve fit to use for graphing standards groupings. The default curve fit is a linear transformation. You can make or change a curve fit assignment at this screen.
Scans	Selects the scans to be graphed and displayed. You can select multiple scans in any combination.
Std Dev	Calculates the selected standard deviation range. You can choose only one at a time.

3

4. Click **Chart** when you are finished.

    The CytoCalc software accesses Microsoft Excel and creates graphs of your data. When calculations are finished, a display similar to the examples in Figure 3-12 or Figure 3-13 appears.

Raw fluorescence data

When graphing raw fluorescence data, each chart displays the least-squares best-fit curve for the formula you chose, the raw data points, and the standard deviation limits. The legend shows the symbols for data points and the standard deviation values.

You can save and print your charts using the commands on the Excel File menu. You can also change scaling and add text to the charts. Refer to the Excel manual for instructions.
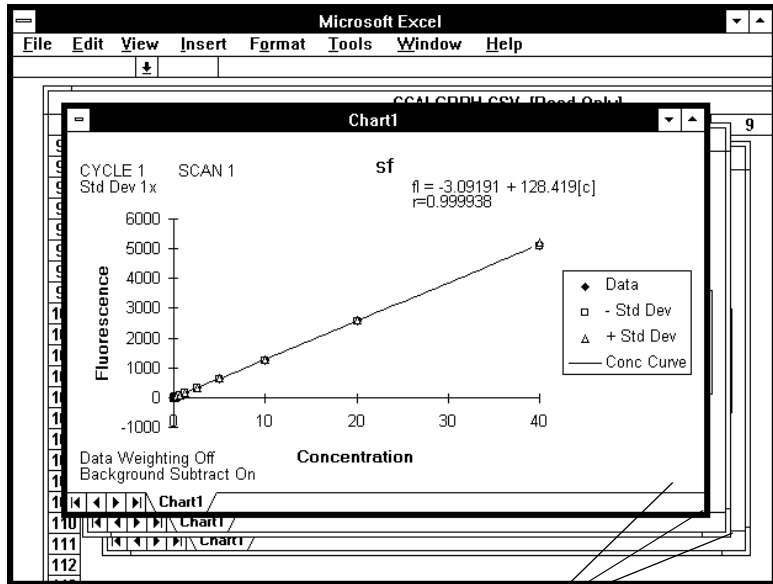
One chart for each scan of each cycle is displayed

Figure 3-12 Standards Chart

Kinetic data

When graphing kinetic data, the CytoCalc software creates a graph showing the maximum change (initial rate) in fluorescence for each concentration level.

NOTE: The Y-axis label in Figure 3-13 refers to change in fluorescence or Δ Fluorescence.

Change in fluorescence ────
(Δ Fluorescence)

KIN.   **SCAN 1**   **1**   fl = 472.674 + 17.6995[c]
Std Dev 1x   r=0.993797

Data Weighting Off   **Concentration**
Background Subtract Off
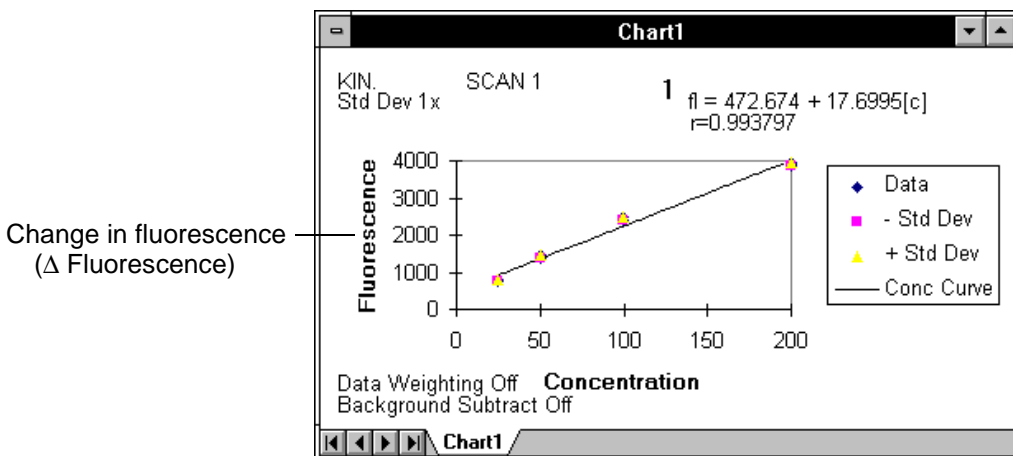
Figure 3-13 Standards Chart (Initial Rate) for Kinetic Data

3

3.4.3 Graphing Unknowns

To create a bar chart of unknowns:

1. Select **Unknowns** from the Graph menu (Alt, G, U). The Graph Unknowns dialog box appears (Figure 3-14).
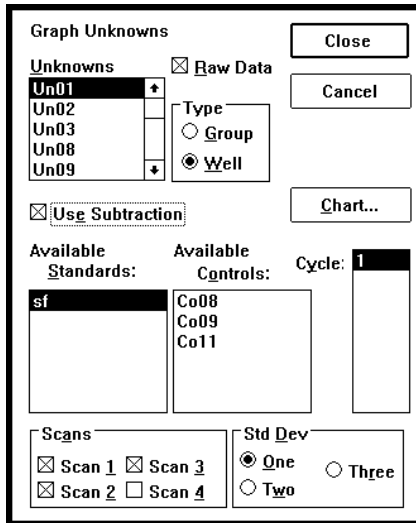
Figure 3-14 Graph Unknowns Dialog Box

2. Select an unknown. Any previously assigned standards grouping and control group are highlighted.

3. Select the following parameters as needed.

Parameter	Description
Raw Data	Displays raw fluorescence values when selected. Displays concentration values when deselected and the unknowns are assigned to a standards curve.

Parameter	Description
Type	Determines type of plot: • **Group**—Plots the average value for each group. • **Well**—Plots data for each individual well.
Use Subtraction	Subtracts the average value of the assigned blank group from the other groups on your plate.
Available Standards	Displays available standards groupings. You make or change assignments.
Available Controls	Controls against which the unknowns are compared. You can make or change assignments.
Cycle	Determines cycle to be graphed. You can select only one cycle at a time.
Scans	Selects the scans to be graphed and displayed. You can select multiple scans in any combination.
Std Dev	Calculates the selected standard deviation range. You can select only one at a time.

4. Click **Chart** when you are finished.

The CytoCalc software accesses Microsoft Excel and creates a chart with your data. When calculations are finished, a display similar the example in Figure 3-15 appears.

3
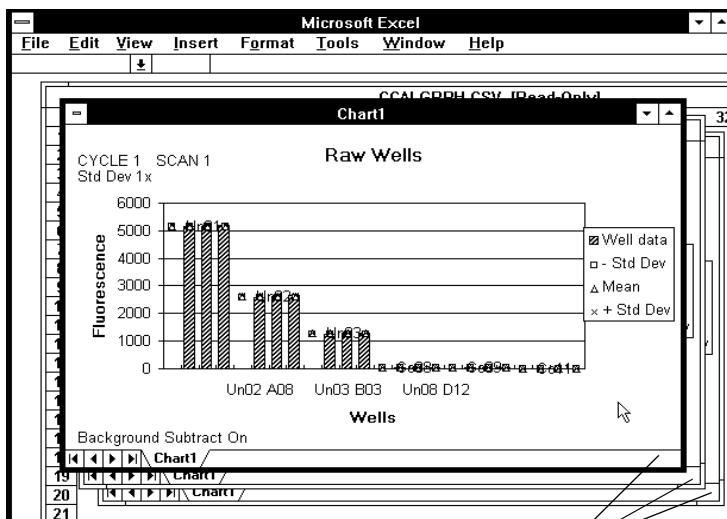
One chart for each scan of each cycle is displayed

Figure 3-15 Unknowns Chart

The chart shows a bar graph using either controls and unknowns (raw data) or standards and unknowns (calculated data). You see a separate chart for each scan of each cycle.

You can save and print your charts using the commands on the Excel File menu. You can also change scaling and add text to the charts. Refer to the Excel manual for instructions.

5. Select **End Data Display** from the File menu. (Alt, F, D, Enter)

   The Excel software closes, and the CytoCalc Data Format dialog box appears.

3.4.4 Graphing Unknowns Using Kinetic Data

To create a bar chart of the maximum change (initial rate) in fluorescence of unknowns using kinetic data:

1. Select **Unknowns** from the Graph menu.

   The Graph Unknowns dialog box appears (Figure 3-16).
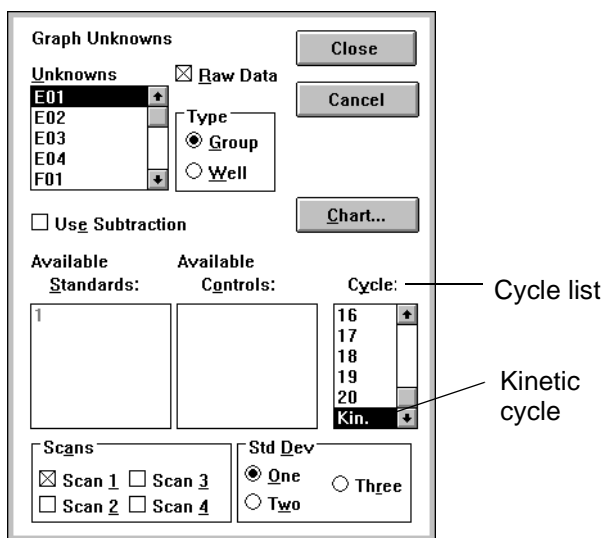
Figure 3-16 Graph Unknowns Dialog Box

2. Select an unknown.

3. Select **Raw Data** to display the change in fluorescence.

4. Select **Group**.

5. Select **Kin.** from the Cycle list (Figure 3-16).

6. Select the appropriate parameters.

7. Click **Chart**.

   A chart using data from the Kinetics cycle is created
   and displayed (Figure 3-17).
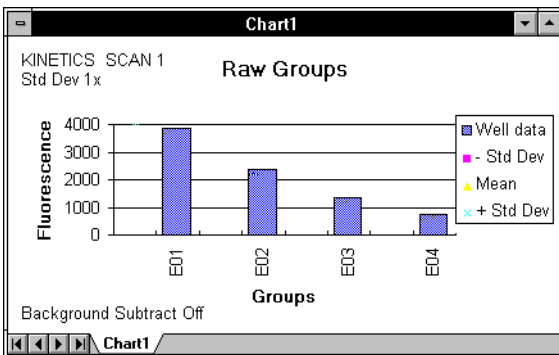
Figure 3-17 Unknowns Chart for Kinetic Data

3

3.5 Printing from the Plate Layout Screen

The CytoCalc software enables you to print the results of data calculation from the Plate Layout screen. The printout can contain all or part of the information included on the Excel spreadsheet.

To print the results from a data file:

1. Select **Print** from the File menu (Alt, F, P). The File Print dialog box appears (Figure 3-18).
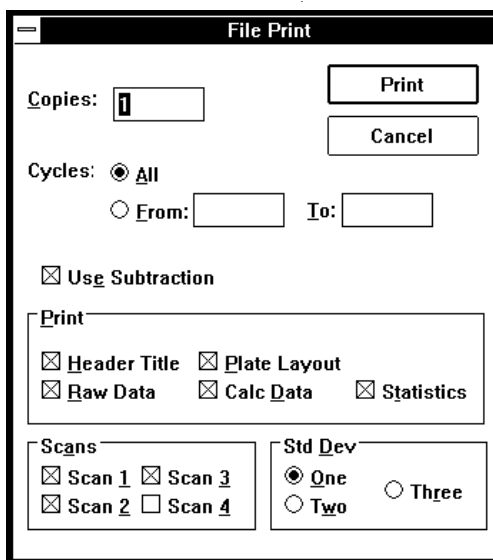
Figure 3-18 File Print Dialog Box

2. Select the number of copies to print. The default is 1.

3. Select the following parameters as needed:

Parameter	Description
Cycle	Specifies the number of cycles to print.
Use Subtraction	Subtracts the average value of the assigned blank group from the other groups on your plate.
Print	Allows selected parameters appear on the printed report.
Scans	Allows selected parameters to appear on the printed report.
Std Dev	Allows selected parameters to appear on the printed report.

4. Click **Print** when you are finished. Click **Cancel** to close the box without printing.

The files are printed in an Excel spreadsheet layout.

3

3.6 Converting Files

The CytoCalc software allows you to convert your data files (*.MFR) to a format that can be read by the TiterCalc or Delta-Soft software packages.

Converting files

To convert files:

1. Select **Convert** from the File menu (Alt, F, C). The File Convert dialog box appears Figure 3-19.
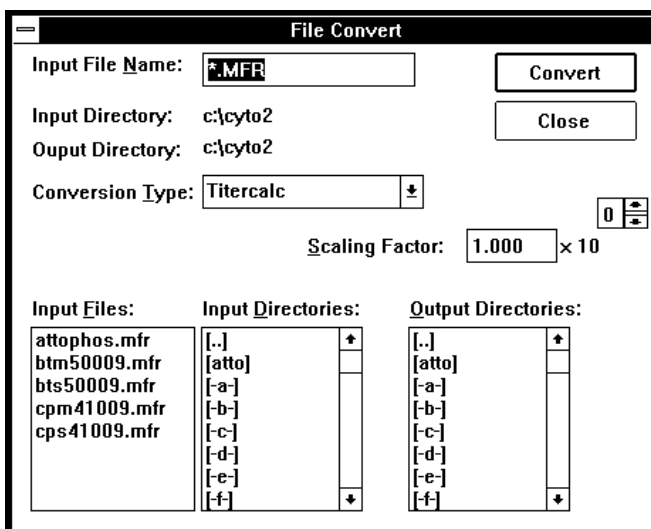
Figure 3-19 File Convert Dialog Box

2. Change the input and output directories, if necessary.

3. Select the file that is to be converted from the Input Files list box.

4. Select the Conversion Type, if necessary. TiterCalc is the default.

5. Enter the scaling factor. Titercalc and Delta-Soft require a range of 0 to 9.999. Use the box above the number 10 to enter an exponent value. The default exponent is 0. You can enter: 1, 0, -1, -2, -3, -4, or -5.

6. Click **Convert** to convert the files. You can use the converted files in the selected calculation software.

    Click **Close** to close the box without converting files.

Converted file name format

Files are saved in the chosen conversion type. The converted file name consists of the eight characters (maximum) of the *.MFR file name, with a new appended extension identifying the cycle and scan.

For example, the conversion file containing the data from cycle 1 and scan 4 of data file CAPRINE.MFR would have the name CAPRINE.014.

3.7 Deleting Files

You can delete CytoCalc files from your hard disk to recover working space. You can make copies of the files on a different disk for backup or print out the data files.

To delete files:

1.   Select **Delete** from the File menu (Alt, F, D).The File Delete dialog box appears.

2.   Click the file type to delete.

3.   Use the scroll bar to change directories if necessary.

4.   Select the file to delete.

5.   Click **Delete** to delete the file. An alert box appears asking you to confirm your choice.

    •   Click **Yes** to confirm deletion.
    •   Click **No** to cancel the deletion and return to the Delete dialog box.

6.   Click **Close** to return to the Plate Layout screen.

3

3

Example Files

A

This appendix contains:

- Overview
- File description

Overview A group of pre-configured work files (*.CFL) and data files (*.MFR) are loaded on your computer when you install the CytoCalc software. These template files contain example plate layout and data handling information.

You can use the example files to:

- Click on different wells to see the information displayed in the edit bar for each well type.

- Use the Data and Graph pull-down menus to see how the calculation and graphing options are configured.

- Change any of the calculation and graphing options to see the effect of your changes on the data display and printout.

- Save changed files to a different name using the Save As command on the File menu without changing the template file.

Opening the template files
1. Select **Open** from the File menu to open a *.CFL template work file.

2. Select **Open** from the File menu to open a *.MFR data file onto the template.

File description   The first four work files listed are increasingly complex
configurations for use with the either the BTS*.MFR or
CPS*.MFR data files. These files illustrate four different ways
of treating data from a single scan of a 96-well plate. Use the
same data file as you step through each work file.

The following template files are included:

- **EXAMPLE1.CFL**—Defines a single standard curve
  made up of 12 dilutions with six replicates of each
  concentration, and 2 rows of blanks.

- **EXAMPLE2.CFL**—Redefines some of the standards
  wells from the EX1SF8X2.CFL file as unknowns.
  Calculated values of these unknowns should
  correspond to the standards values from which they
  were converted.

- **EXAMPLE3.CFL**—Introduces the hide feature. Use this
  feature when the data contains obvious outliers,
  especially if standards are out of range of the
  CytoFluor II Fluorescence Plate Reader (99999 for
  CytoFluor II, 9999 for CytoFluor 2300/2350).

- **EXAMPLE4.CFL**—Introduces control wells to the
  template. This example is helpful in illustrating how to
  use the Unknowns command on the Graph menu. Note
  the differences when the Raw Data check box is
  selected or unselected and when the control wells are
  either assigned or not assigned to an unknown.

- **CFATTO.CFL**—Used with the ATTOPHOS.MFR data
  file as an example of a ligand-binding (ELISA) assay
  requiring use of the 4-parameter fit to the logistic
  equation.

- **KINETICS.CFL**—Used with the KIN2.MFR data file as
  an example of a kinetic curve of Attophos dilutions.
  Uses a 4-parameter fit.

- **MA*.CFL work files**—Used with the MA147S.CSV
  data file as examples of CytoFluor 2300 series data
  analyses.

A

Hints for Using Excel

B

This appendix contains:

- Setting page orientation
- Using Print Preview and setting page breaks
- Displaying the regression equation

Setting page orientation

The CytoCalc software uses Microsoft Excel to display all data and graphics. When Excel displays CytoCalc data, all Excel features are available. The spreadsheet printout is best formatted when printed in the landscape mode on an 8.5-inch X 11-inch page using an HP LaserJet printer. You can check the orientation of the page in Excel by using Print Preview in the File menu.

To set page orientation:

1. Select **Page Setup** from the File menu.

2. Click **Landscape**.

3. Click **OK** to exit the setup box.

You can also change the printer setup through the Windows Control Panel.

Using Print Preview and setting page breaks

The CytoCalc Data Format dialog box allows you to select any combination of five display options (Header, Raw Data, Plate Layout, Value Format, and Statistics Format) for Excel. A default page break is inserted after each of the five display options. You can preview your Excel spreadsheet before printing it, and customize it by adding page breaks if necessary.

To preview your printout:

1. Select **Print Preview** from the File menu.

2. Step through each page using the Next/Previous buttons at the top of the screen.

3. If satisfactory, click **Print**.

4. Select **Close** to return to the spreadsheet.

To insert additional page breaks:

1. Go to column A and click on the row where you want the page break to occur.

2. Select **Page Break** from the Insert menu.

3. Repeat this process until you have formatted your printout.

Displaying the regression equation

When you use the Graph Standards commands to find a best-fit curve for your standards, the graph and associated information are displayed as an Excel Chart. If Excel displays any extra toolbars at the bottom of the screen, the regression equation and some of the statistics parameters will not be displayed on the chart.

To hide extra toolbars:

1. Select **Toolbars** from the View menu.

2. Click on the selection boxes of the toolbars you want to hide.

3. Click **OK**.

   When you close Excel in this configuration, the setup will be retained the next time Excel is opened.

B

Helpful Hints

C

Multiple experiments on a single plate

Use unique names that have the same number of characters when you define Standards Grouping names for each experiment on the plate. This correctly displays group assignments made with the Link feature when you review them with the Data Format feature, and when you review assignments made with the Data Format feature in the Link editor. All names are case sensitive — abc ≠ Abc ≠ aBc, etc.

Examples of acceptable Standards Grouping name combinations on a plate:

- ab1, ab2, ab3 (each name unique and of the same length).

- a, b, cde, de, df, da (unique leading characters with different lengths and all names with the same leading character are of equal length).

If you use Standards Grouping names with different lengths and identical leading characters, then the assignments you make are displayed incorrectly in when you switch between the Link feature and the Data Format feature. Only the display is affected. Calculations are not affected.

Assigning group names

When you assign group names sequentially by number, use leading zeroes for all single digit values. Group lists are sorted alphanumerically for display. Only the display order is affected by the sorting. Calculations are not affected.

C

For example:

- Use: (Un01, Un02,... Un09, Un10, ...Un20).

- Do not use: (Un1, Un2,...Un9, Un10, ...Un20).

  This may be sorted as: Un1, Un10, Un11,....Un19, Un2, Un20, and so on.

Modifying group parameters

To change group parameters except the group name:

1. Click on one well of the group.

2. Make the desired change in the Edit bar.

3. Click the Accept button (Enter). The change is applied to all other group members without affecting any other group parameter or assignment.

To change the group name:

1. Click on one well of the group.

2. Enter the new group name in the edit bar.

3. Click **Yes** on the Change all occurrences? dialog box.

   Click **No** to apply the new name to only the highlighted wells.

4. Reassign the group to the appropriate standards grouping.

Standards curves and calculating unknowns

When you select Standards from the Graph menu, and choose one of equations in the list box, the curve relating fluorescence (y-axis) to concentration of standard (x-axis) is displayed. The calculated regression equation is displayed on the graph as:

fl [fluorescence] = f(c) [concentration of standard]

When you click Standards from the Data Format dialog box, and then Calculate, the CytoCalc software calculates unknown concentration values from their measured fluorescence using the transpose of the above equation. In this case, fluorescence becomes the independent (x-axis) variable. The regression equation is displayed in the Value Format Display and the Statistics Results sections of the spreadsheet as:

> c [Unknown concentration] = f(fl) [measured Unknown fluorescence]

Background Subtract

When background (blank)-subtracted data are displayed, the blanks display their raw fluorescence values. Blank values are not subtracted from themselves.

Hide button

Out-of-range standards values distort the calculated standards curve. Use the Hide button to mask any raw data that is out of range (99999).

An apparently normal data value can appear when you use Background Subtract with an out-of-range value (for example, 99999 - 23520 = 76479). This can be indicated by replicate values that are exactly the same.

Older Cytofluor data

Earlier versions of the CytoCalc software (2.00.06 and earlier) were used to analyze data from the older CytoFluor 2300/2350 model scanners. Data was stored in*.CSV data files. You can still work with these files by selecting the **CSV** radio button on the File Open dialog box.

C

Technical Support and Training

D

This appendix contains the following sections:

D.1 Contacting Technical Support

Overview You can contact Applied Biosystems for technical support:

- By e-mail
- By telephone or fax
- Through the Applied Biosystems web site

NOTE: *For information on obtaining technical documents such as Applied Biosystems user documents, MSDSs, and certificates of analysis, see "Obtaining Technical Documents" on page D-8.*

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By E-mail You can contact technical support by e-mail for help in the product areas listed below.

Product/Product Area	E-Mail Address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com
• Biochromatography • Expedite™ (8900) DNA Synthesis System • PNA • Pioneer™ Peptide Synthesis System • Proteomics Solution 1™ (PS1) System • ICAT™ reagent • FMAT™ 8100 HTS System • Mariner™ Mass Spectrometers • Voyager™ Mass Spectrometers • CytoFluor® 4000 Fluorescence Plate Reader	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS SCIEX)	support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

By telephone or fax (North America)

To contact Applied Biosystems Technical Support in North America, use the telephone or fax numbers in the table below.

*NOTE: To schedule a service call for other support needs, or in case of an emergency, dial **1.800.831.6844, then press 1.***

Product/Product Area	Telephone	Fax
ABI PRISM® 3700 DNA Analyzer	**1.800.831.6844**, then press **8**[a]	1.650.638.5981
DNA Synthesis	**1.800.831.6844**, press **2**, then press **1**[a]	1.650.638.5981
Fluorescent DNA Sequencing	**1.800.831.6844**, press **2**, then press **2**[a]	1.650.638.5981
Fluorescent Fragment Analysis (including GeneScan® applications)	**1.800.831.6844**, press **2**, then press **3**[a]	1.650.638.5981
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1.800.831.6844**, press **2**, then press **4**[a]	1.650.638.5981
ABI PRISM® 3100 Genetic Analyzer	**1.800.831.6844**, press **2**, then press **6**[a]	1.650.638.5981
Peptide Synthesis (433 and 43*x* Systems)	**1.800.831.6844**, press **3**, then press **1**[a]	1.650.638.5981
Protein Sequencing (Procise® Protein Sequencing Systems)	**1.800.831.6844**, press **3**, then press **2**[a]	1.650.638.5981

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Product/Product Area	Telephone	Fax
PCR and Sequence Detection	**1**.**800**.**762**.**4001**, then press: **1** for PCR[a] **2** for TaqMan® applications and Sequence Detection Systems including ABI PRISM® 7700, 7900, and 5700[a] **6** for the 6700 Automated Sample Prep System[a] or **1**.**800**.**831**.**6844**, then press **5**[a]	1.240.453.4613
• Voyager™ MALDI-TOF Biospectrometry Workstations • Mariner™ ESI-TOF Mass Spectrometry Workstations • Proteomics Solution 1™ (PS1) System • ICAT™ reagent	**1**.**800**.**899**.**5858**, press **1**, then press **3**[b]	1.508.383.7855
Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products)	**1**.**800**.**899**.**5858**, press **1**, then press **4**[b]	1.508.383.7855
Expedite™ (8900) Nucleic Acid Synthesis Systems	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855
PNA Custom and Synthesis	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855

Product/Product Area	Telephone	Fax
FMAT™ 8100 HTS System CytoFluor® 4000 Fluorescence Plate Reader	**1.800.899.5858**, press **1**, then press **6**[b]	1.508.383.7855
Chemiluminescence (Tropix)	**1.800.542.2369** (U.S. only), or **1.781.271.0045**[c]	1.781.275.8581
LC/MS (Applied Biosystems/MDS Sciex)	**1.800.952.4716**	1.508.383.7899

a. 5:30 A.M. to 5:00 P.M. Pacific time.

b. 8:00 A.M. to 6:00 P.M. Eastern time.

c. 9:00 A.M. to 5:00 P.M. Eastern time.

By telephone or fax (outside North America) To contact Applied Biosystems Technical Support or Field Service outside North America, use the telephone or fax numbers below.

Region	Telephone	Fax
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	61 3 9730 8600	61 3 9730 8799
China (Beijing)	86 10 64106608 or 86 800 8100497	86 10 64106617
Hong Kong	852 2756 6928	852 2756 6968
Korea (Seoul)	82 2 5936470/6471	82 2 5936472
Malaysia (Petaling Jaya)	60 3 79588268	603 79549043
Singapore	65 896 2168	65 896 2147
Taiwan (Taipei Hsien)	886 2 2358 2838	886 2 2358 2839
Thailand (Bangkok)	66 2 719 6405	662 319 9788

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Region	Telephone	Fax
Europe		
Austria (Wien)	43 (0)1 867 35 75 00	43 (0)1 867 35 75 11
Belgium	32 (0)2 532 4484	32 (0)2 582 1886
Denmark (Naerum)	45 45 58 60 00	45 45 58 60 01
Finland (Espoo)	358 (0)9 251 24 250	358 (0)9 251 24 243
France (Paris)	33 (0)1 69 59 85 85	33 (0)1 69 59 85 00
Germany (Weiterstadt)	49 (0) 6150 101 0	49 (0) 6150 101 101
Italy (Milano)	39 (0)39 83891	39 (0)39 838 9492
Norway (Oslo)	47 23 12 06 05	47 23 12 05 75
Portugal (Lisboa)	351.(0)22.605.33.14	351.(0)22.605.33.15
Spain (Tres Cantos)	34.(0)91.806.1210	34.(0)91.806.12.06
Sweden (Stockholm)	46 (0)8 619 4400	46 (0)8 619 4401
Switzerland (Rotkreuz)	41 (0)41 799 7777	41 (0)41 790 0676
The Netherlands (Nieuwerkerk a/d IJssel)	31 (0)180 392400	31 (0)180 392409 or 31 (0)180 392499
United Kingdom (Warrington, Cheshire)	44 (0)1925 825650	44 (0)1925 282502
European Managed Territories (EMT)		
Africa, English speaking (Johannesburg, South Africa)	27 11 478 0411	27 11 478 0349
Africa, French speaking (Paris, France)	33 1 69 59 85 11	33 1 69 59 85 00
India (New Delhi)	91 11 653 3743 91 11 653 3744	91 11 653 3138
Poland, Lithuania, Latvia, and Estonia (Warszawa)	48 22 866 4010	48 22 866 4020

Region	Telephone	Fax
For all other EMT countries not listed (Central and southeast Europe, CIS, Middle East, and West Asia	44 1925 282481	44 1925 282509
Japan		
Japan (Hacchobori, ChuoKu, Tokyo)	81 3 5566 6230	81 3 5566 6507
Latin America		
Caribbean countries, Mexico, and Central America	52 55 35 3610	52 55 66 2308
Brazil	0 800 704 9004 or 55 11 5070 9654	55 11 5070 9694/95
Argentina	800 666 0096	55 11 5070 9694/95
Chile	1230 020 9102	55 11 5070 9694/95
Uruguay	0004 055 654	55 11 5070 9694/95

Through the Applied Biosystems web site

To contact Technical Support through the Applied Biosystems web site:

1. Go to **www.appliedbiosystems.com**

2. Click **Services & Support** at the top of the page, then click **Frequently Asked Questions**.

3. Click **Contact Support** in the contents list at the left of the screen.

4. Click your geographic region for the product area of interest.

5. In the Personal Assistance form, enter the requested information and your question, then click **Ask Us RIGHT NOW**.

6. In the Customer Information form, enter the requested information, then click **Ask Us RIGHT NOW**.

Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert.

D.2 Obtaining Technical Documents

D

Overview   You can obtain technical documents, such as Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents for free, 24 hours a day. You can obtain documents:

- By telephone
- Through the Applied Biosystems web site

Ordering documents by telephone   To order documents by telephone:

1. From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.

2. Follow the voice instructions to order documents (for delivery by fax).

NOTE: *There is a limit of five documents per fax request.*

Obtaining documents through the web site   To view, download, or order documents through the Applied Biosystems web site:

1. Go to **www.appliedbiosystems.com/**

2. At the top of the page, click **Services & Support** at the top of the page, then click **Documents on Demand**.

3. In the search form, enter and select search criteria, then click **Search at the bottom of the page**.

4. In the results screen, do any of the following:

    - Click 🔲 to view a PDF version of the document.

    - Right-click 🔲, then select **Save Target As** to download a copy of the PDF file.

    - Select the **Fax** check box, then click **Deliver Selected Documents Now** to have the document faxed to you.

    - Select the **Email** check box, then click **Deliver Selected Documents Now** to have the document (PDF format) e-mailed to you.

NOTE: *There is a limit of five documents per fax request, but no limit on the number of documents per e-mail request.*

D

D.3 Obtaining Customer Training Information

D

To obtain Applied Biosystems training information, go to **www.appliedbiosystems.com**, click **Services & Support** at the top of the screen, then click **Training**.

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