

USER  
MANUAL

For Microsoft® Windows

**MasterPlex®**

**ReaderFit**

*Quantitative Analysis Module*



**For Research Use Only**

601 Gateway Blvd.  
Suite 100  
South San Francisco, CA 94080

**TELEPHONE**

1.888.615.9600 (toll free)  
1.650.615.7600

**FACSIMILE**

1.650.615.7639

Part no. P-33340-10201

**TRADEMARKS**

Microsoft® is a registered trademark of Microsoft Corporation.

**COPYRIGHT**

© 2009-2010 Hitachi Software Engineering America, Ltd. All Rights Reserved.

Ver P072010-22

## **LICENSE AGREEMENT**

BEFORE OPENING THIS PACKAGE, YOU SHOULD CAREFULLY READ THE FOLLOWING TERMS AND CONDITIONS. BY OPENING THIS PACKAGE YOU AGREE TO BECOME BOUND BY THE TERMS AND CONDITIONS OF THIS AGREEMENT, WHICH INCLUDES THE SOFTWARE LICENSE AND LIMITED WARRANTY. IF YOU DO NOT AGREE WITH THESE TERMS AND CONDITIONS, YOU SHOULD PROMPTLY RETURN THE PACKAGE UNOPENED TO HITACHI SOFTWARE ENGINEERING AMERICA, LTD. ("HISAL") or HISAL Distributor AND YOUR MONEY WILL BE REFUNDED.

The enclosed software is licensed, not sold, to you for use only upon the terms of this Agreement, and HISAL reserves any rights not expressly granted to you. You are responsible for the selection of the Software to achieve your intended results, and for the installation, use and results obtained from the Software. You own the media on which the Software is originally or subsequently recorded or fixed, but HISAL retains ownership of all copies of the Software itself.

### **LICENSE**

You may:

- a. Use the Software on a single machine at any given time.
- b. Obtain limited numbers of Copy Protection Devices. Additional, Copy Protection Devices are provided only as a convenience of running the software.
- c. In no manner engineer or reverse-engineer the copy protection hardware, or whole or part of the software.
- d. Copy the software only for backup provided that you reproduce all copyright and other proprietary notices that are on the original copy of the Software provided to you. Certain Software, however, may include mechanisms to limit or inhibit copying. Such Software is marked copy protected.
- e. Transfer of the Software and all rights under this Agreement to another party together with a copy of this Agreement if the other party agrees to accept the terms and conditions of this Agreement. If you transfer the Software, you must at the same time either transfer all

## **LICENSE AGREEMENT**

---

copies whether in printed or machine-readable form, to the same party or destroy and copies not transferred.

### **RESTRICTIONS**

You may not use, copy, modify, or transfer the Software, or any copy, in whole or in part, except as expressly provided for in this Agreement. Any attempt to transfer any of the rights, duties or obligations hereunder except as expressly provided for in this Agreement is void.

**YOU MAY NOT RENT, LEASE, LOAN, RESELL FOR PROFIT, OR DISTRIBUTE.**

### **TERM**

This Agreement is effective until terminated. You may terminate it at any time by destroying the Software together with all copies in any form. This Agreement will immediately and automatically terminate without notice if you fail to comply with any term or condition of this Agreement. You agree upon termination to promptly destroy the Software together with all copies in any form.

### **LIMITED WARRANTY**

HISAL warrants, for the period of ninety (90) days from the date of delivery of the Software to you as evidenced by a copy of your receipt, that:

(1) The Software, unless modified by you, will perform the function described in the documentation provided by HISAL. Your sole remedy under the warranty is that HISAL will undertake to correct within a reasonable period of time any marked Software Error (failure of the Software to perform the functions described in the documentation).

HISAL does not warrant that the Software will meet your requirements, that operation of the Software will be uninterrupted or error-free, or that all Software Errors will be corrected.

(2) The media on which the Software is furnished will be free from defects in materials and workmanship under normal use. HISAL will, at its option, replace or refund the purchase price of the media at no charge

to you, provided you return the faulty media with proof of purchase to HISAL. HISAL will not have any responsibility to replace or refund the purchase price of the media damaged by accident, abuse or misapplication.

**THE ABOVE WARRANTIES ARE EXCLUSIVE AND IN LIEU OF ALL OTHER**

## **LICENSE AGREEMENT**

---

WARRANTIES, WHETHER EXPRESS OR IMPLIED, INCLUDING THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. NO ORAL OR WRITTEN INFORMATION OR ADVICE GIVEN BY HISAL, ITS EMPLOYEES, DISTRIBUTORS, OR AGENTS SHALL INCREASE THE SCOPE OF THE ABOVE WARRANTIES OR CREATE ANY NEW WARRANTIES. SOME STATES DO NOT ALLOW THE EXCLUSION OF IMPLIED WARRANTIES, SO THE ABOVE EXCLUSION MAY NOT APPLY TO YOU. IN THAT EVENT, ANY IMPLIED WARRANTIES ARE LIMITED IN DURATION TO NINETY (90) DAYS FROM THE DATE OF DELIVERY OF THE SOFTWARE. THIS WARRANTY GIVES YOU SPECIFIC LEGAL RIGHTS. YOU MAY HAVE OTHER RIGHTS, WHICH VARY FROM STATE TO STATE.

### **LIMITATIONS OF REMEDIES**

HISAL's entire liability to you and your exclusive remedy shall be the replacement of the Software media or the refund of your purchase price as set forth above. If HISAL or the HISAL's distributors are unable to deliver replacement media which is free of defects in materials and workmanship, you may terminate this Agreement by returning the Software and your money will be refunded.

REGARDLESS OF WHETHER ANY REMEDY SET FORTH HEREIN FAILS ITS ESSENTIAL PURPOSE, IN NO EVENT WILL HISAL BE LIABLE TO YOU FOR ANY DAMAGES, INCLUDING ANY LOST PROFITS, LOST DATA OR OTHER INCIDENTAL OR CONSEQUENTIAL DAMAGES ARISING OUT OF THE USE OR INABILITY OF SUCH DAMAGES, OR FOR ANY CLAIM BY ANY OTHER PARTY. SOME STATES DO NOT ALLOW THE LIMITATION OR EXCLUSION OR LIABILITY FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES TO THE ABOVE LIMITATION OR EXCLUSION MAY NOT APPLY TO YOU.

### **GOVERNMENT LICENSEE**

If you are acquiring the Software on behalf of any unit or agency of the United States Government, the following provisions apply:

The Government acknowledges HISAL's representation that the Software and its documentation were developed at private expense and no part of them is in the public domain.

## **LICENSE AGREEMENT**

---

The Government acknowledges HISAL's representation that the Software is Restricted Computer Software as that term is defined in Clause 52.227-19 of the Federal Acquisition Regulations (FAR) and is commercial Computer Software as that term is defined in Subpart 227.401 of the Department of Defense Federal Acquisition Regulations supplement (DFARS) The Government agrees that:

If the Software is supplied to the Department of Defense (DOD), the Software is classified as Commercial Computer Software and the Government is acquiring only restricted rights in the Software and its documentation will be as defined in Clause 52.227-19 (c) (2) of the FAR.

If the Software is supplied to any unit or agency of the United States Government other than DOD, the Governments rights in Software and its documentation

### **RESTRICTED RIGHTS LEGEND**

Use, duplication, or disclosure by the Government is subject to restrictions as set forth in subparagraph.

(c) (1) (11) of the rights in Technical Data and computer software clause of DFARS 52.227-7013.

Hitachi Software Engineering America, Ltd.  
601 Gateway Boulevard, Suite 100  
South San Francisco, CA 94080

### **EXPORT LAW ASSURANCES**

You acknowledge and agree that the Software is subject to restrictions and controls imposed by the United States Export Administration Act ("The Act") and the regulations thereunder. You agree and certify that neither the Software nor any direct product thereof is being or will be acquired, shipped, transferred or reexported, directly or indirectly, into any country prohibited by the Act and the regulations thereunder or will be used for any purpose prohibited by the same.

### **GENERAL**

This agreement will be governed by the laws of the State of California, except for that body of law dealing with conflicts of law.

Future updates of the Software will be available for purchase by licensees for a fee provided a registration card has been received by Hitachi Software Engineering America, Ltd.

## **LICENSE AGREEMENT**

---

Should you have any questions concerning this Agreement, you may contact HISAL at <http://www.miraibio.com>.

You acknowledge that you have read this Agreement, understand it and agree to be bound by its terms and conditions. You further agree that it is the complete and exclusive statement of the agreement between us which supersedes any proposal or prior agreement, oral or written, and any other communications between us in relation to the subject matter of this Agreement.

## CONTENTS

| CHAPTER 1                                             | PAGE |
|-------------------------------------------------------|------|
| <b>Welcome</b>                                        |      |
| About This Manual .....                               | 1    |
| Technical Support .....                               | 2    |
| <br>CHAPTER 2                                         |      |
| <b>Installing MasterPlex®</b>                         |      |
| Requirements .....                                    | 3    |
| Installing MasterPlex® ReaderFit .....                | 4    |
| Installing a License .....                            | 10   |
| <br>CHAPTER 3                                         |      |
| <b>Getting Started</b>                                |      |
| Overview of MasterPlex® ReaderFit Analysis ....       | 11   |
| Starting MasterPlex® ReaderFit .....                  | 12   |
| Paste the raw data from plate reader result file .... | 13   |
| Importing Measurement Results                         |      |
| And Analyte Assign .....                              | 15   |
| Import .csv, .txt, .xls or Open .mlx* files           |      |
| by drag and drop .....                                | 23   |
| Tab categorized work flow .....                       | 24   |
| Viewing Data in the Input Data Tab .....              | 26   |
| Saving Plate Data .....                               | 31   |
| <br>CHAPTER 4                                         |      |
| <b>Defining a Plate – Input Data tab</b>              |      |
| Designating Well Type and Group .....                 | 33   |

|                                               |    |
|-----------------------------------------------|----|
| Setting Standard Concentration.....           | 42 |
| Linking a Standard Data Set .....             | 48 |
| Working with Diluted Unknowns .....           | 49 |
| Working With Templates .....                  | 52 |
| Preferences .....                             | 57 |
| Creating a Virtual Plate .....                | 62 |
| Working with the Virtual Analyte Filter ..... | 67 |
| Quality Control Manager.....                  | 70 |

## CHAPTER 5

### **Standard Curve & Concentration -**

#### ***Fit Curves tab***

|                                                                       |    |
|-----------------------------------------------------------------------|----|
| Go to Fit Curves tab .....                                            | 75 |
| Generating Standard Curve & Computing Analyte<br>Concentrations ..... | 78 |
| Reviewing Calculated Standard Data .....                              | 80 |
| Best Fit Calculation Option .....                                     | 86 |
| Statistics Toolbox .....                                              | 88 |
| Printing and Exporting the Standard Data .....                        | 89 |

## CHAPTER 6

### **Reviewing Data - View Results tab**

|                                     |    |
|-------------------------------------|----|
| Add or Delete a Column .....        | 92 |
| Sort or Filter the Column Data..... | 94 |
| Exporting a Data.....               | 96 |
| Printing a Data.....                | 98 |

## CHAPTER 7

### **Data Charts – Create Graphs tab**

|                                |     |
|--------------------------------|-----|
| Viewing a Data Chart .....     | 99  |
| Chart format .....             | 104 |
| Analyte Selector .....         | 105 |
| Changing Color Palette.....    | 107 |
| Changing Chart Properties..... | 108 |
| Printing a Chart .....         | 109 |

|                                     |     |
|-------------------------------------|-----|
| Copying or Saving Chart Image ..... | 111 |
|-------------------------------------|-----|

## CHAPTER 8

### **Export Data – Customized Report Manager tab**

|                                                        |     |
|--------------------------------------------------------|-----|
| Importing a User Defined Stylesheet .....              | 113 |
| Exporting a User Defined Stylesheet .....              | 114 |
| Delete Stylesheet File from Style Sheet List .....     | 115 |
| Including standard curve images.....                   | 116 |
| Transform Original Data into Your Customized Data..... | 116 |

## APPENDIX A

### **Preferences**

|                           |     |
|---------------------------|-----|
| Grid Customize Menu ..... | 118 |
| Print Preview Menu .....  | 125 |

## APPENDIX B

### **MasterPlex® ReaderFit Toolbars**

|                                             |     |
|---------------------------------------------|-----|
| Main File Menu and Toolbar .....            | 132 |
| Input Data Tab Toolbar .....                | 134 |
| View Results Tab Toolbar .....              | 135 |
| Create Graphs Tab Toolbar .....             | 136 |
| Customized Report Manager Tab Toolbar ..... | 137 |

## APPENDIX C

### **Model Equations**

|                                       |     |
|---------------------------------------|-----|
| Four Parameter Logistic Curve .....   | 138 |
| Five Parameter Logistic Curve .....   | 139 |
| Heteroscedasticity .....              | 143 |
| Weighted Nonlinear Least Square ..... | 149 |
| Results of Weighting .....            | 150 |

## CHAPTER 1

*Welcome to the MiraiBio MasterPlex® ReaderFit User Manual. MasterPlex® ReaderFit software analyzes results files (\*.csv, \*.txt or \*.xls) from the plate reader instruments.*

## 1.1

**About This Manual**

This manual explains how to use the MasterPlex® ReaderFit application module to:

- Open blank plate and then paste the raw value from result files
- Import results files (\*.csv, \*.txt or \*.xls) from the plate reader instruments
- Designate standard, unknown, control, and background wells
- Generate standard curves
- Compute analyte concentrations
- Generate data charts and reports

**What's New in MasterPlex® ReaderFit**

MasterPlex® ReaderFit offers new features, including the ability to:

- Merge plates using virtual plate feature so that it can analyze beyond 100 panels at one time
- Make a sample marking and groups easily and quickly using Auto-grouping feature or dragging grouping feature
- Calculate a fold change especially for being used relative gene analysis
- Normalize the data so that it can analyze between difference plates
- Generate a custom reports using style sheet

**Conventions Used in This Manual**

This manual describes the steps required to perform the various tasks associated with the MasterPlex® ReaderFit software. The manual uses a step format to explain the various tasks associated with MasterPlex® ReaderFit. A symbol may follow a step instruction. It indicates the software response to the action performed by the user.

## **Screen Captures**

Screen captures may accompany the step instructions for further illustration. The screen captures in this manual may not exactly match those displayed on your screen.

### **1.2**

## **Technical Support**

You can contact MiraiBio Technical support at:

Hitachi Software Engineering America, Ltd.

601 Gateway Boulevard, Suite 100

South San Francisco, CA 94080

USA

Tel: +1 (650) 615-7600

Toll Free: +1 (888) 615-9600

Fax: +1 (650) 615-7639

E-mail: [support@miraibio.com](mailto:support@miraibio.com)

[www.miraibio.com](http://www.miraibio.com)

---

## CHAPTER 2 | Installing MasterPlex® ReaderFit

*This chapter explains the minimum hardware and software requirements needed to install and use MasterPlex® ReaderFit. It provides installation instructions for a computer for your analysis.*

### 2.1

#### Requirements

For optimum performance, MasterPlex® ReaderFit requires hardware and software that meet or exceed the following specifications.

##### Minimum Hardware Requirements

| Platform            | PC                                                                                    |
|---------------------|---------------------------------------------------------------------------------------|
| CPU                 | Intel Pentium 4 2 GHz or equivalent,<br>Intel Pentium 4 2 GHz or better (recommended) |
| Memory (RAM)        | 512MB or higher for Windows XP/Vista/7                                                |
| Storage space (HDD) | 120 MB available hard drive space for the installation                                |
| Input devices       | Keyboard and mouse or other pointing device                                           |
| Video RAM           | 32MB or higher                                                                        |
| Monitor resolution  | XGA (1024x768 pixels or higher; 1280 x1024 recommended)                               |
| Monitor color       | 16-bit color (high color) or higher                                                   |
| CD-ROM drive        | Required for CD media version. Not applicable for download version.                   |

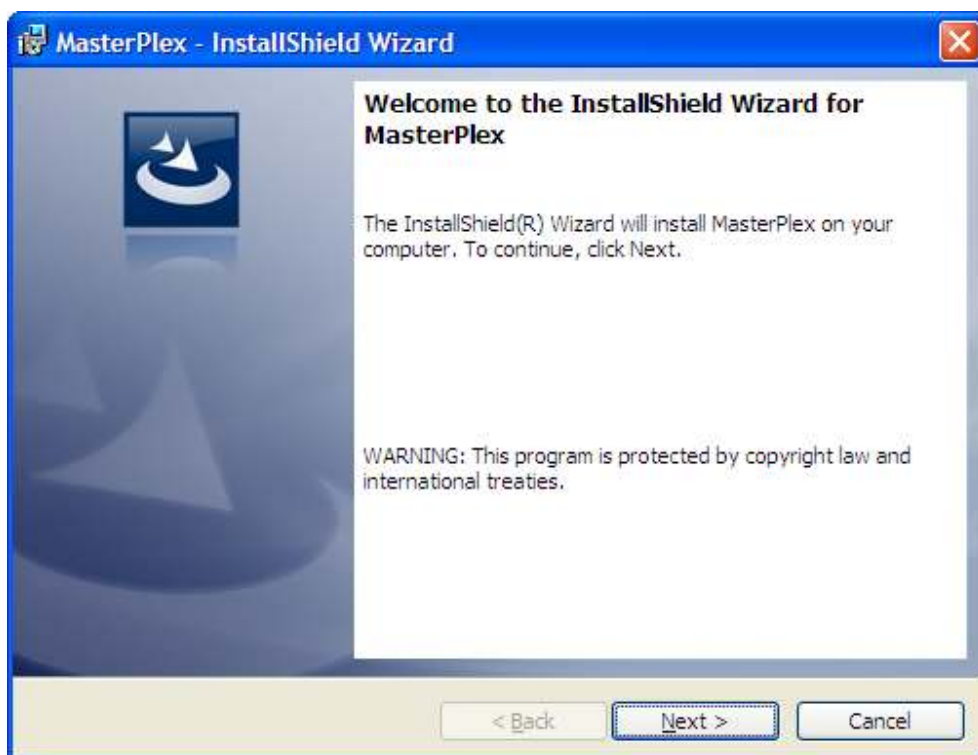
##### Software Requirements

|                  |                                                                        |
|------------------|------------------------------------------------------------------------|
| Operating system | Microsoft Windows XP/Vista/7,<br>Microsoft .NET3.5 framework required. |
|------------------|------------------------------------------------------------------------|

## 2.2

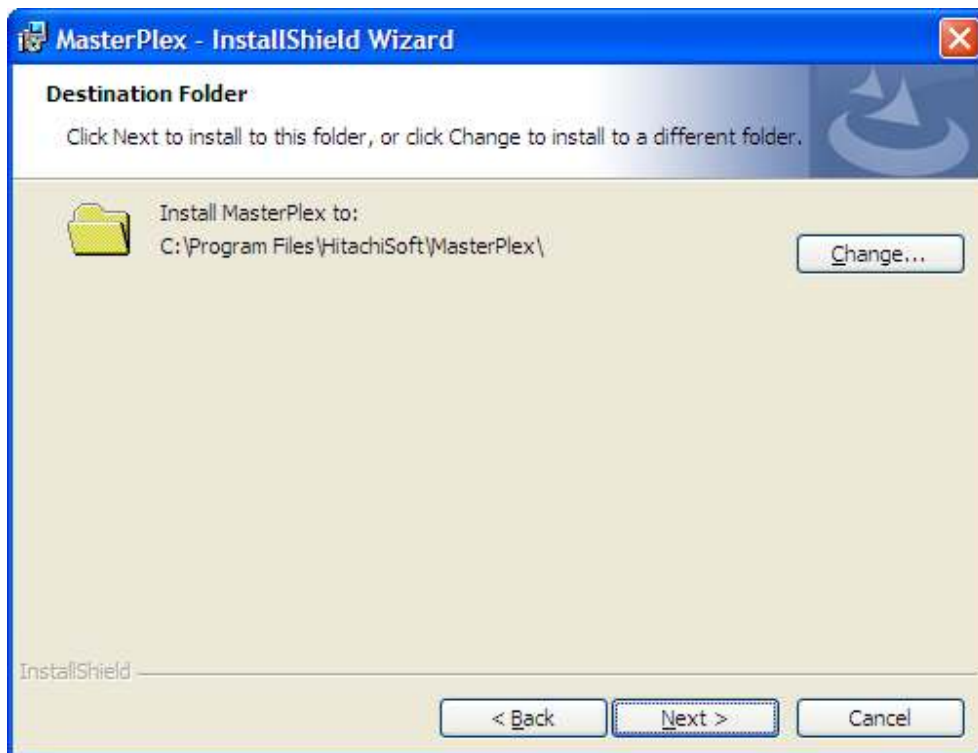
### Installing MasterPlex® ReaderFit

1. Insert the MasterPlex® CD-ROM in the workstation computer and double-click setup.exe.  
⇒ The installation begins and the InstallShield Wizard appears (Figure 2.1).



**Figure 2.1 InstallShield Wizard, Welcome screen**

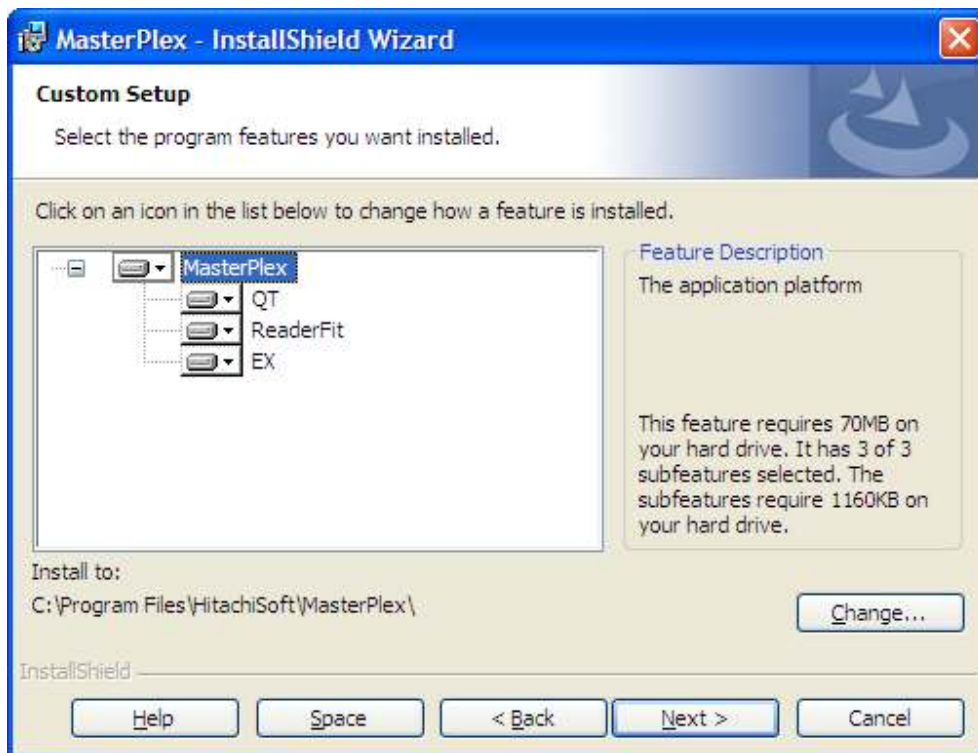
2. To continue the installation, click **Next**.  
⇒ The Customer Information window appears (Figure 2.2).



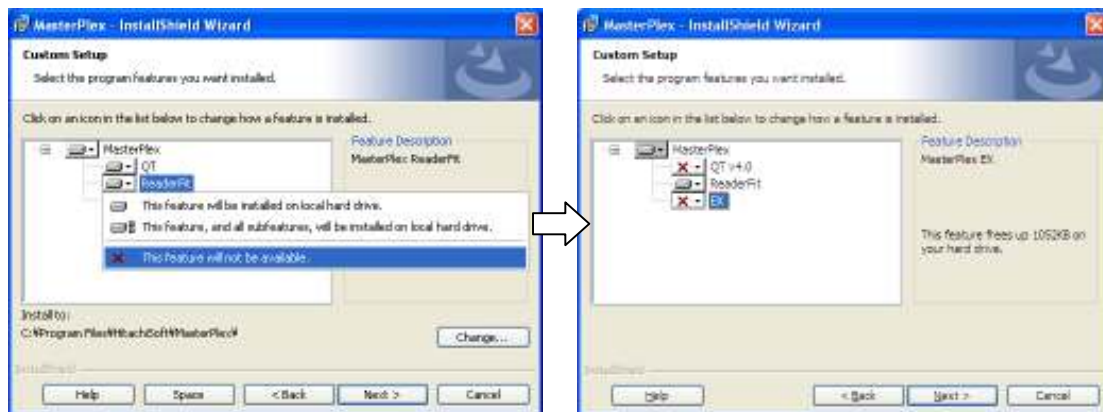
**Figure 2.2 Customer Information screen**

3. Input both User Name and Company Name, the click **Next**.  
⇒ The Set Type window appears (Figure 2.3).

## CHAPTER 2 INSTALLING MASTERPLEX® READERFIT



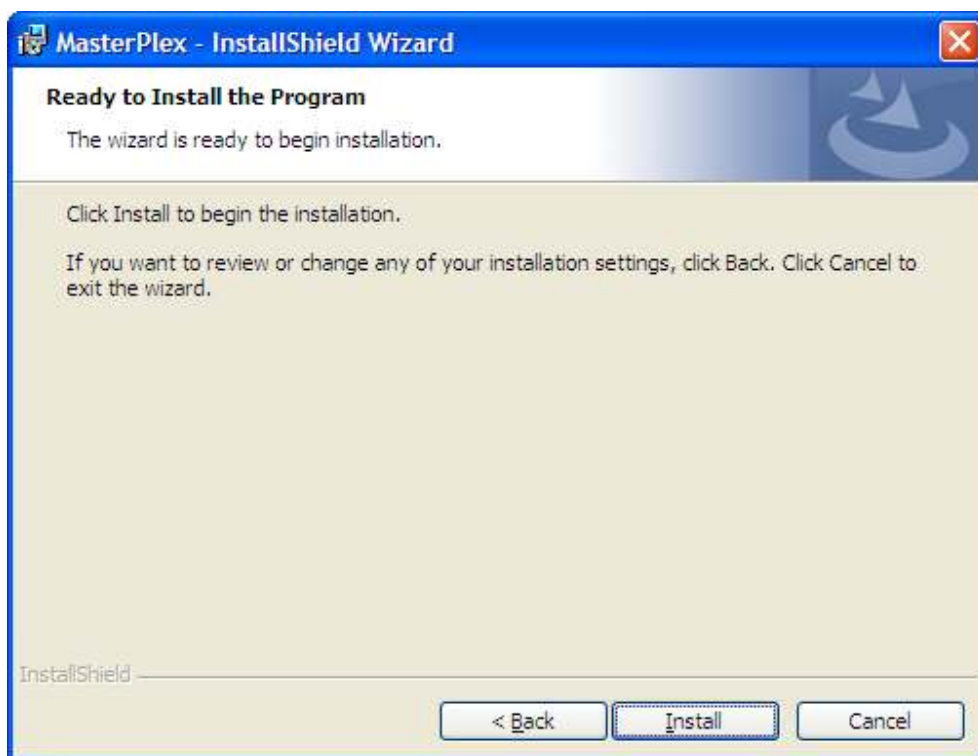
4. Make sure the module name you purchased and click  icon you don't want to install.



**Figure 2.4 Install module selection window**

5. To continue, click **Next**.

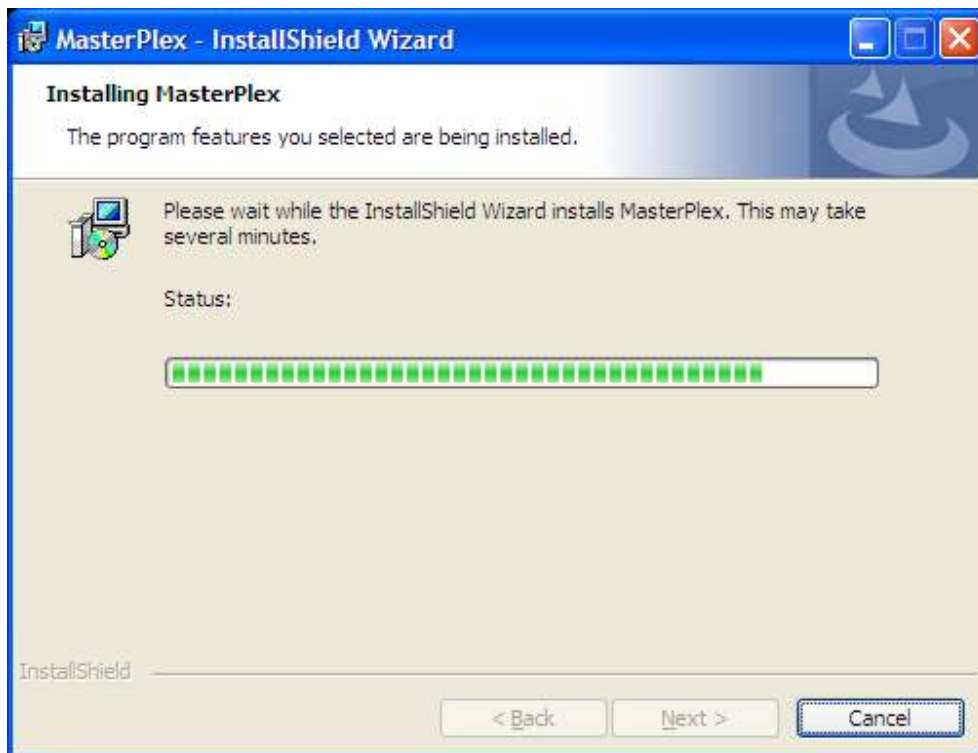
⇒ The Ready to Install the Program window appears (Figure 2.5).



**Figure 2.5 Ready to Install the Program window**

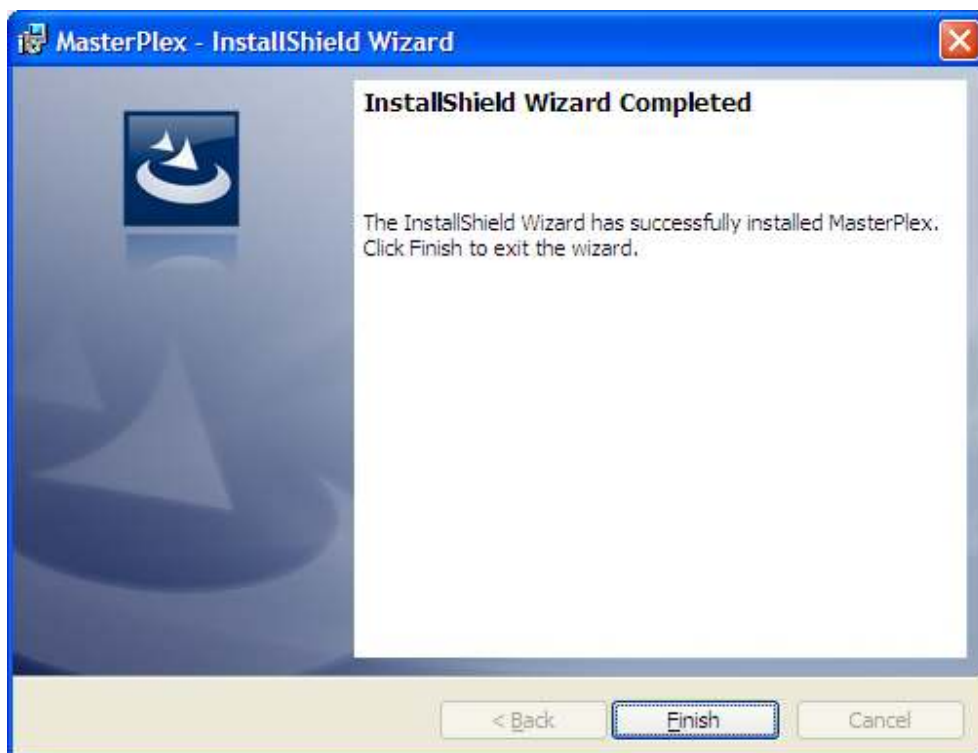
6. Click **Install**.

⇒ The Start Copying Files window appears (Figure 2.6).



**Figure 2.6 InstallShield Wizard, Start Copying Files window**

7. After the installation is completed, the InstallShield Wizard Complete window appears (Figure 2.7).



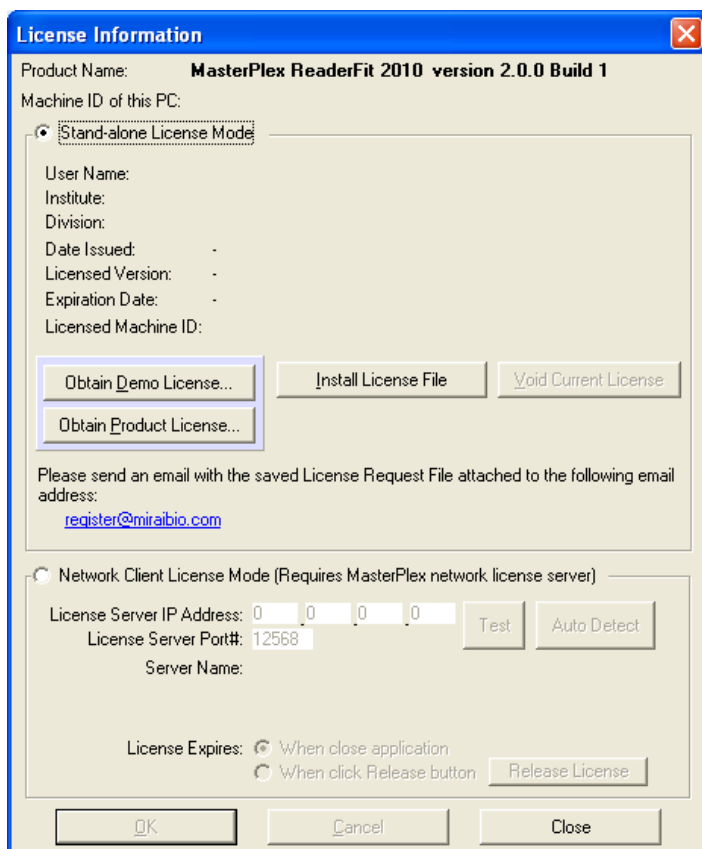
**Figure 2.7 InstallShield Wizard Complete window**

8. Click **Finish** to finish the installation and close the window.

## 2.3

### Installing a License

1. Double-click the MasterPlex® icon  on the workstation desktop.  
⇒ The License Information dialog box appears (Figure 2.5).



**Figure 2.5 License Information dialog box**

2. To view instructions on how to obtain a license (\*.lic), click **Obtain Product Licenses**.
3. After you have obtained a license, click **Install New License**.  
⇒ The Open dialog box appears.
4. Use the Open dialog box to locate the license (\*.lic) and double-click the file.  
⇒ The license is installed.

## CHAPTER 3 | Getting Started

*This chapter provides a brief overview of data analysis using MasterPlex® ReaderFit. It also explains how to start the software, import a result file (.csv, .txt or .xls) from plate reader instruments, and the user interface components.*

### 3.1

#### Overview of MasterPlex® ReaderFit Analysis

MasterPlex® ReaderFit software analyzes results files (.csv, .txt or .xls) from the major plate reader instruments. The analysis steps include:

- Import a results file (.csv, .txt or .xls)
- Designate well types (standard, unknown, background, or control) and well groups (identify members of a standard data set or replicate unknowns)
- Define the standard data set (enter standard concentrations and select a model equation for the standard curve)
- Associate or *link* a standard data set to an unknown group(s)
- Compute the analyte concentrations
- Save the results file in MasterPlex® ReaderFit file format (.mxqs).

The .mxqs file includes information associated with the file (for example, well definitions and interpolated concentrations)

After the concentrations are calculated, you can:

- View the results in graphs or several different report formats
- Create a *virtual plate* (a simulated microtiter plate) that contains data from user-selected actual plates (.csv, .txt, or .xls)

## 3.2

### Starting MasterPlex® ReaderFit

On the desk top, double-click the MasterPlex® icon . Alternatively, you can click the Windows start menu button  and select **Programs > MasterPlex > MasterPlex**.

⇒ The MasterPlex® user interface appears and lists up all detected applications in the application pane (Figure 3.1).

You can import a results file (.csv, .txt or .xls) or paste the raw data in the blank plate from this interface.

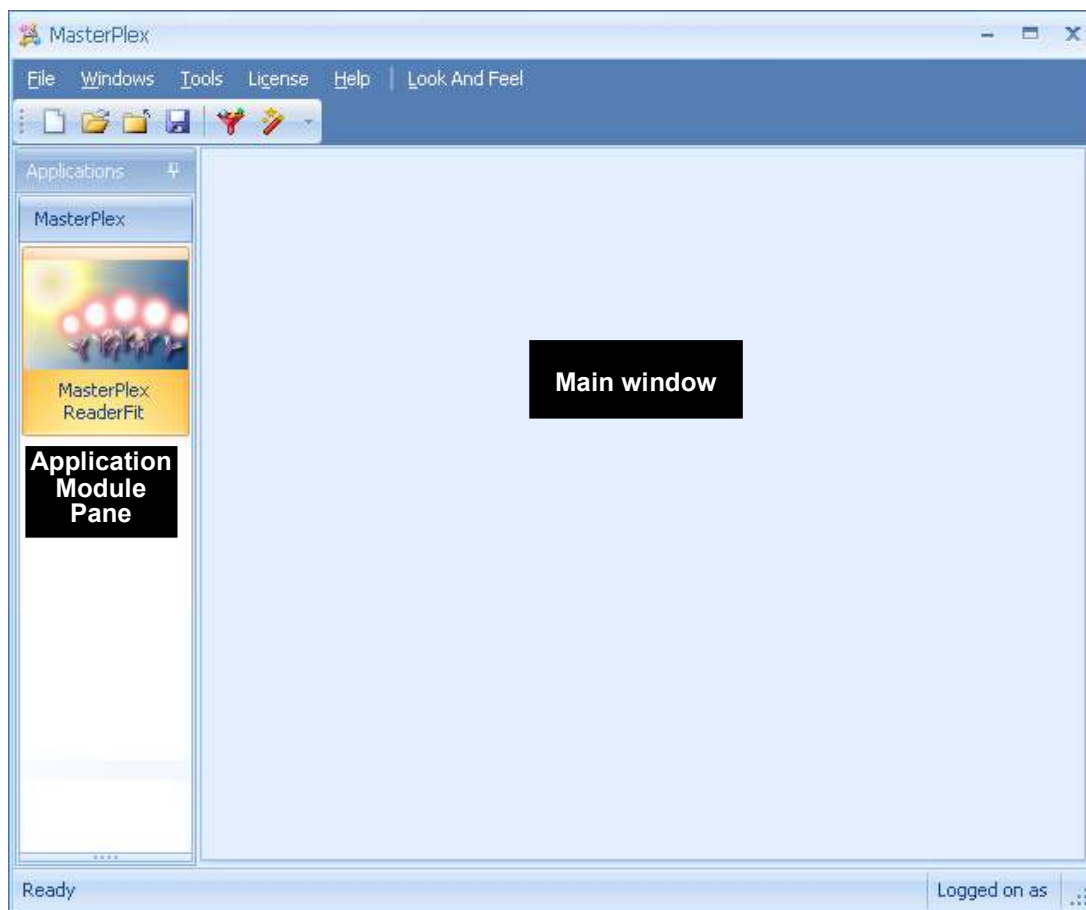
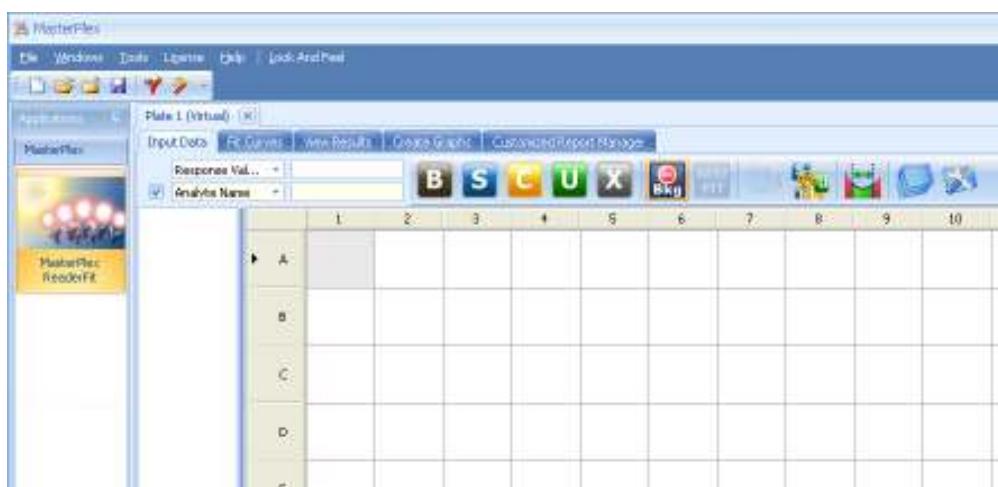


Figure 3.1 MasterPlex® user interface

### 3.3

#### Paste the raw data from plate reader's result file

To begin a MasterPlex® ReaderFit analysis, open blank plate and simply paste the copied data from the plate reader result file. MasterPlex ReaderFit opens 100 x 100 size blank plate automatically when the program is launched (Figure 3.2).



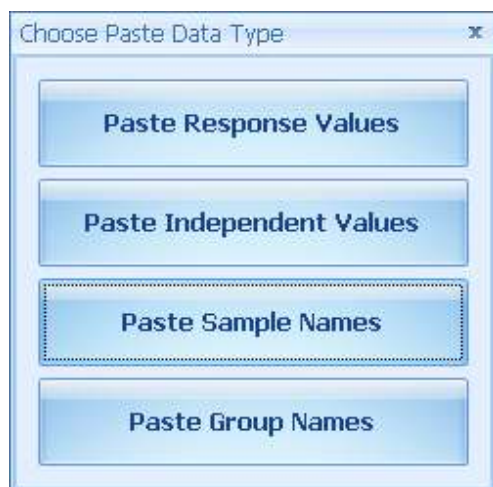
**Figure 3.2 Default blank plate**

#### Paste copied data in the blank plate

1. Copy the result data from plate reader.
2. Select the top left cell you want to paste the data
3. Press **Ctrl + V** or right click and select **Paste Data** command (Figure 3.3).  
⇒ Data type selection window appears (Figure 3.4).



**Figure 3.3 Paste Data command from right click menu**



**Figure 3.4 Data type selection window**

4. Choose appropriate data type you want to paste in.
5. Copied data is pasted in the blank plate (Figure 3.5).

|     | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        | 9        | 10       | 11 |
|-----|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----|
| ▶ A | 41.00000 | 39.00000 | 37.00000 | 112.0000 | 92.00000 | 2426.500 | 43.00000 | 36.00000 | 48.00000 | 80.00000 |    |
| B   | 10516.00 | 10034.50 | 66.00000 | 4911.000 | 106.5000 | 186.0000 | 41.00000 | 38.00000 | 45.50000 | 55.00000 |    |
| C   | 6152.000 | 6299.000 | 39.00000 | 5011.000 | 65.00000 | 43.00000 | 38.00000 | 52.00000 | 40.00000 | 45.00000 |    |
| D   | 3104.000 | 2990.500 | 41.00000 | 5591.000 | 3989.500 | 113.0000 | 38.00000 | 44.00000 | 40.00000 | 42.00000 |    |
| E   | 1040.000 | 1094.000 | 45.00000 | 3070.000 | 1808.000 | 270.0000 | 49.00000 | 40.00000 | 36.00000 | 47.00000 |    |
| F   | 292.0000 | 300.0000 | 39.00000 | 1129.000 | 119.0000 | 41.00000 | 42.00000 | 40.00000 | 37.00000 | 49.00000 |    |
| G   | 108.5000 | 104.0000 | 45.00000 | 107.5000 | 111.0000 | 70.00000 | 41.00000 | 39.00000 | 326.0000 | 41.00000 |    |
| H   | 56.00000 | 55.00000 | 51.00000 | 5578.000 | 59.00000 | 50.00000 | 38.00000 | 37.00000 | 128.5000 | 39.00000 |    |
| I   |          |          |          |          |          |          |          |          |          |          |    |

**Figure 3.5 Pasted data in the blank plate**

6. To delete the copied data in the plate, press **DELETE** key or choose **Delete Selected Wells** command from right click menu.

## 3.4

### Importing Measurement Results and Analyte Assign

To begin a MasterPlex® ReaderFit analysis, import a .csv, .txt or .xls file using toolbar, menu bar or application icon.

### Importing Scanning Results Using the File Open Menu, File Open Icon or Application Icon

1. Choose **File > Open**, click the **File Open** icon  or click the application icon .

⇒ The Open dialog box appears (Figure 3.6).

2. Enter the file path for the .csv, .txt or .xls that you want to import.

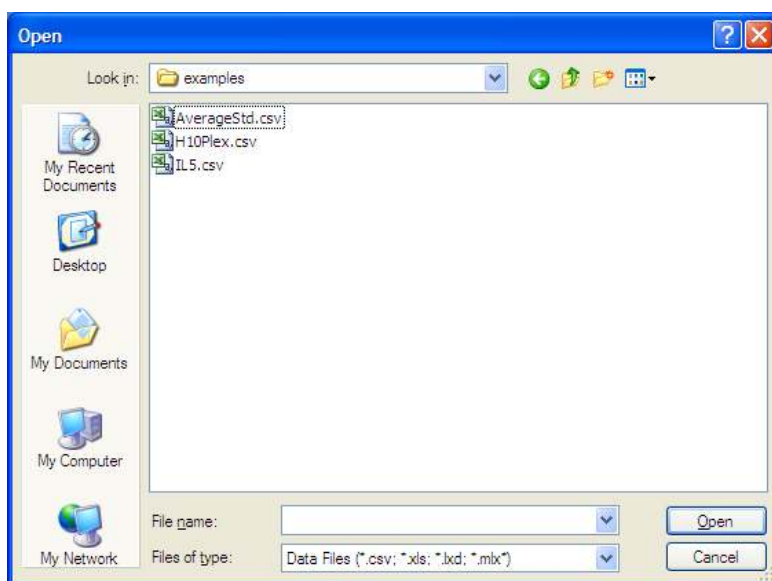
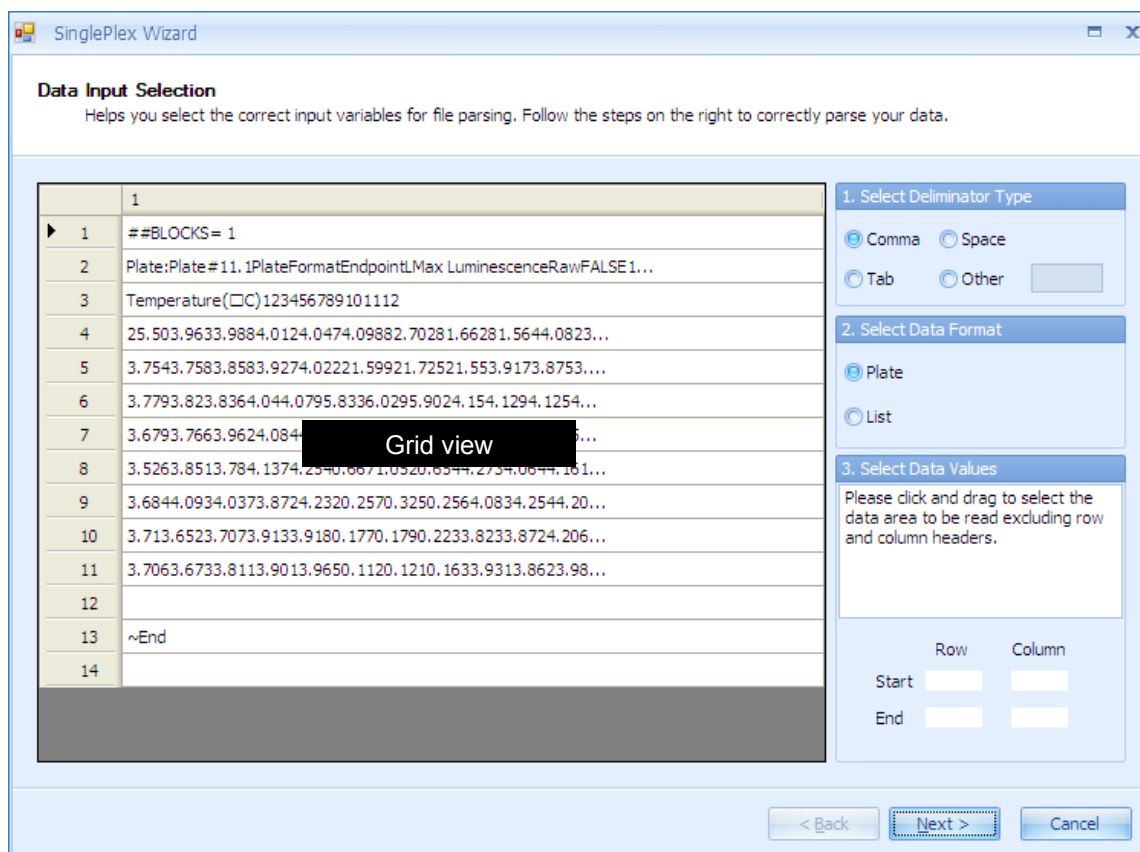


Figure 3.6 Open dialog box

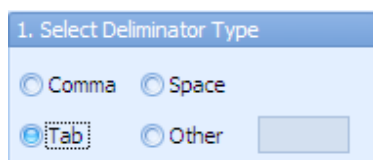
3. Navigate to the directory of the .csv, .txt or .xls that you want to import.
4. Select one or more .csv, .txt or .xls files and click **Open**.  
⇒ File import wizard appears (Figure 3.7).

## Data Input Selection Page



**Figure 3.7 Data Input Selection Page**

1. Select one of the delimiter type from select delimiter type box (Figure 3.8) so that your result data is correctly delimited in the file display grid.  
⇒ Data delimitation in the grid view will be changed (Figure 3.9).



**Figure 3.8 Select Delimiter Type Box**

### Figure 3.9 Data Delimitation Change

2. Select Data Format

☒ Plate

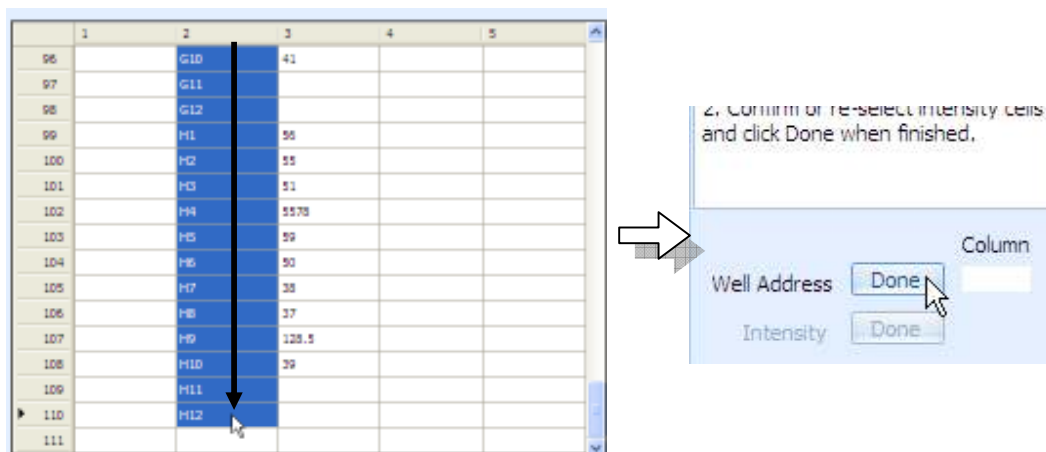
☐ List

3. (In case of Plate) Select data area you want to import by mouse dragging (Figure 3.11).

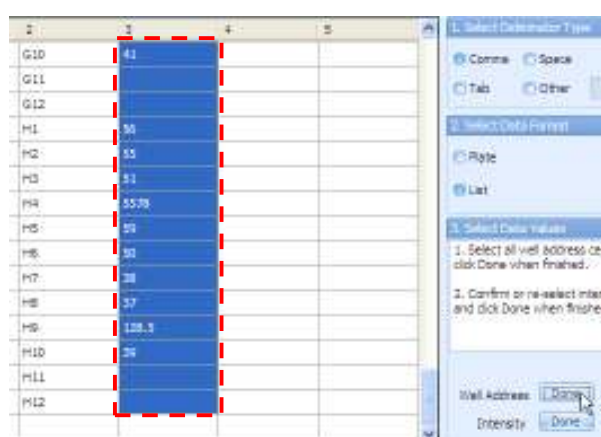
**Figure 3.11 Data selection by mouse dragging**

4-1. (In case of List) First select well address by mouse dragging, then click Done button for well address column (Figure 3.12).

⇒ Column next to the well address is automatically selected(Figure 3.13).



**Figure 3.12 Well Address Selection**



**Figure 3.13 Auto Intensity Data Selection**

4-2. (In case of List) Next, select intensity data by mouse dragging, then click done button for intensity column. If auto selected column is the intensity data column, just click done button for intensity column (Figure 3.14).



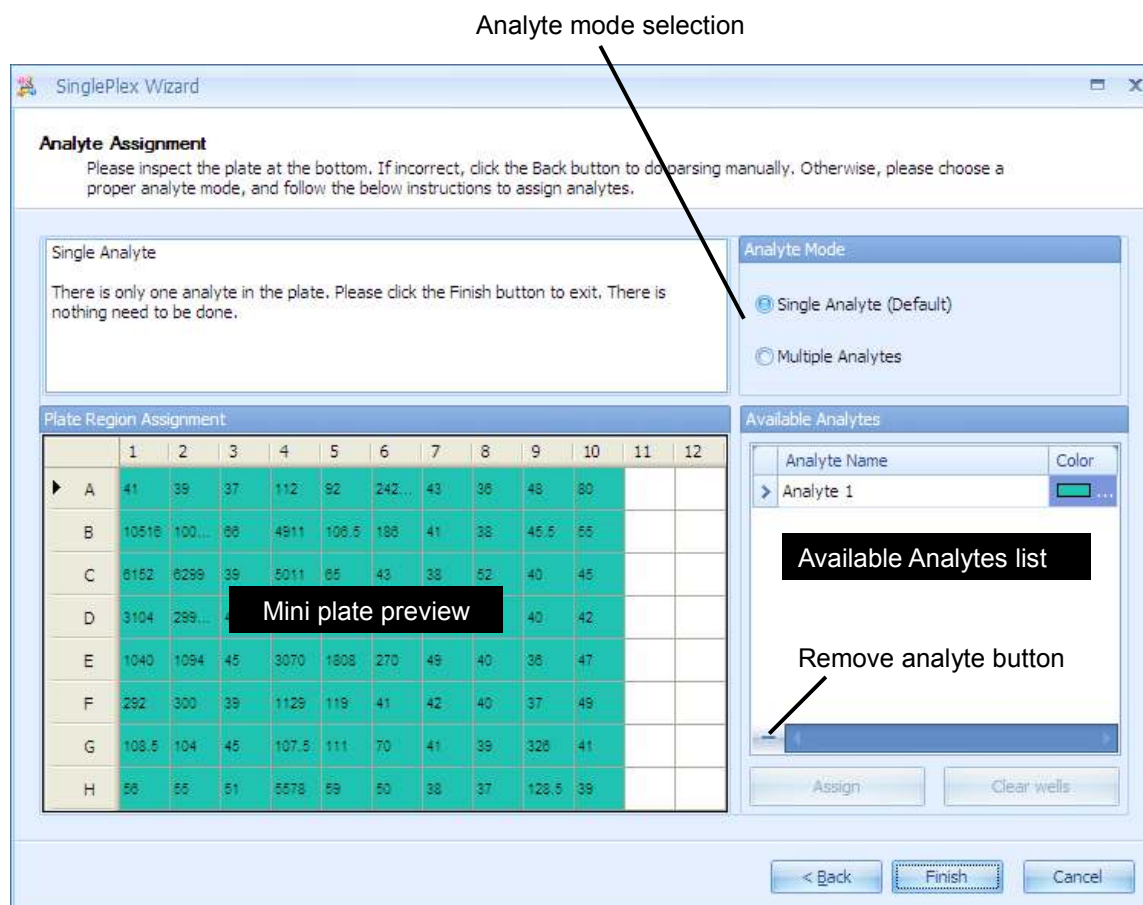
**Figure 3.14 Click Done Button for Intensity Column**

5. Click Next to proceed (Figure 3.15).



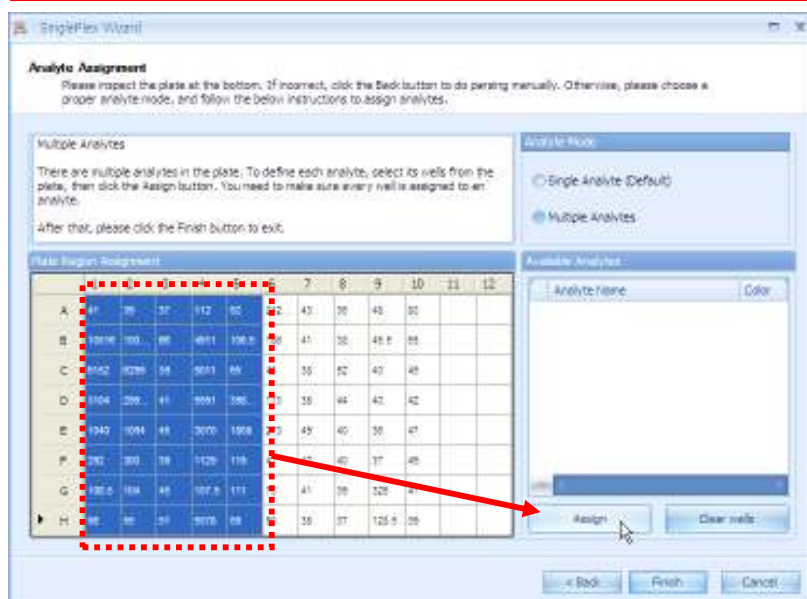
**Figure 3.15 Next Button**

## Analyte Assignment Page

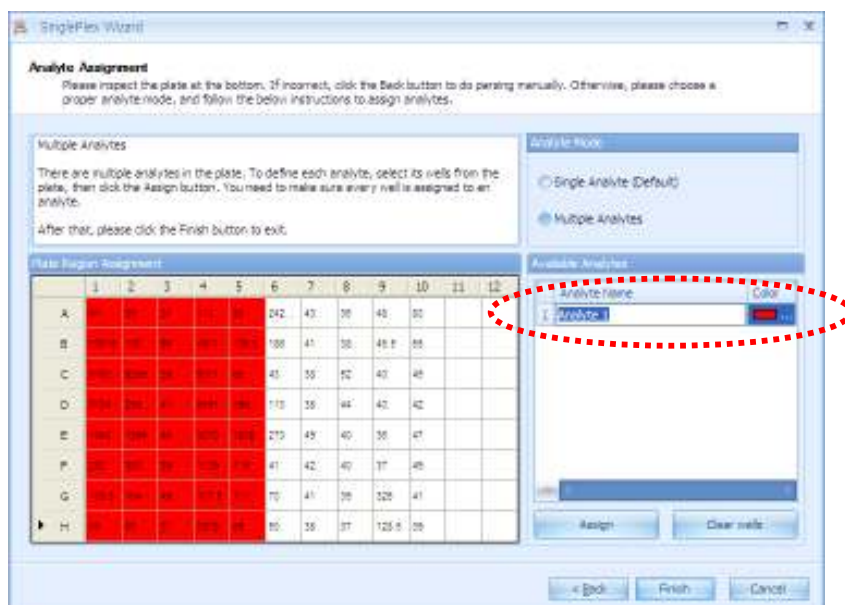


**Figure 3.16 Analyte Assignment Page**

1. Select wells for the analyte your are trying to specify.
2. Click Assign button
  - ⇒ Selected wells are registered as an analyte, and listed in the Available Analytes list. (Figure 3.17)
3. Rename the analyte name or the color if you want. (default analyte name is 'Analyte n', color is random) (Figure 3.18)
4. Repeat analyte assignment. (Figure 3.19)
5. When all wells were assigned, click OK.
  - ⇒ Analyte assignment dialog closes and plate is build on plate view. (Figure 3.20)



**Figure 3.17 Data Selection**



**Figure 3.18 Data Assignment**

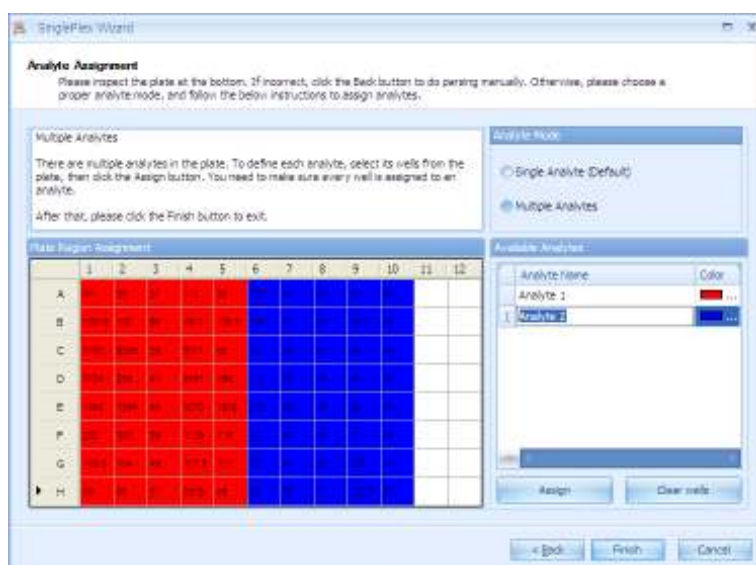


Figure 3.19 Naming and Color Change

**Input Data** | **Fit Curves** | **View Results** | **Create Graphs** | **Customized Report Manager**

Response Val... = 58.00  
Analyte Name = IL-10

**B S C U X** **Bkg** **111**

|   | 1                 | 2                 | 3              | 4                | 5                | 6               | 7             | 8             | 9              | 10            | 11 | 12 |
|---|-------------------|-------------------|----------------|------------------|------------------|-----------------|---------------|---------------|----------------|---------------|----|----|
| A | 41.88<br>IL-10    | 38.00<br>IL-10    | 37.88<br>IL-10 | 112.00<br>IL-10  | 92.88<br>IL-10   | 2426.50<br>IL-2 | 43.88<br>IL-2 | 36.00<br>IL-2 | 46.88<br>IL-2  | 88.00<br>IL-2 |    |    |
| B | 10516.88<br>IL-10 | 18834.50<br>IL-10 | 96.88<br>IL-10 | 4911.00<br>IL-10 | 106.58<br>IL-10  | 186.00<br>IL-2  | 41.88<br>IL-2 | 38.00<br>IL-2 | 45.88<br>IL-2  | 55.00<br>IL-2 |    |    |
| C | 6152.88<br>IL-10  | 6289.00<br>IL-10  | 39.88<br>IL-10 | 5811.00<br>IL-10 | 65.88<br>IL-10   | 43.00<br>IL-2   | 38.88<br>IL-2 | 52.00<br>IL-2 | 40.88<br>IL-2  | 45.00<br>IL-2 |    |    |
| D | 3104.88<br>IL-10  | 2998.50<br>IL-10  | 41.88<br>IL-10 | 5591.00<br>IL-10 | 3999.58<br>IL-10 | 113.00<br>IL-2  | 38.88<br>IL-2 | 44.00<br>IL-2 | 40.88<br>IL-2  | 42.00<br>IL-2 |    |    |
| E | 1040.88<br>IL-10  | 1894.00<br>IL-10  | 46.88<br>IL-10 | 3878.00<br>IL-10 | 1608.88<br>IL-10 | 278.00<br>IL-2  | 49.88<br>IL-2 | 46.00<br>IL-2 | 36.88<br>IL-2  | 47.00<br>IL-2 |    |    |
| F | 250.88<br>IL-10   | 388.00<br>IL-10   | 39.88<br>IL-10 | 1129.00<br>IL-10 | 119.88<br>IL-10  | 41.00<br>IL-2   | 42.88<br>IL-2 | 48.00<br>IL-2 | 37.88<br>IL-2  | 49.00<br>IL-2 |    |    |
| G | 108.58<br>IL-10   | 104.00<br>IL-10   | 46.88<br>IL-10 | 107.50<br>IL-10  | 111.88<br>IL-10  | 78.00<br>IL-2   | 41.88<br>IL-2 | 38.00<br>IL-2 | 326.88<br>IL-2 | 41.00<br>IL-2 |    |    |
| H | 56.88<br>IL-10    | 53.00<br>IL-10    | 51.88<br>IL-10 | 5578.00<br>IL-10 | 59.88<br>IL-10   | 58.00<br>IL-2   | 38.88<br>IL-2 | 37.00<br>IL-2 | 128.58<br>IL-2 | 39.00<br>IL-2 |    |    |

Figure 3.20 Analyte Assign dialog

### 3.5

#### Import .csv, .txt, .xls or Open .mlx\* Files by drag and drop

1. Open Windows Explorer and adjust the window size so that you can view both the MasterPlex® ReaderFit and Windows® Explorer application windows.
2. Use Windows Explorer to navigate to the .csv, .txt, .xls or .mxqs file(s) that you want to open.
3. Select the file(s) of interest, then click and hold the mouse button while you drag the selected file(s) to the MasterPlex® application menu bar area (Figure 3.21).

To select adjacent files, press and hold the **Shift** key while you click the first and last file in the selection. To select nonadjacent files, press and hold the **Ctrl** key while you click the files of interest.

4. Release the mouse button.

⇒ The file(s) open in MasterPlex® ReaderFit.



**Figure 3.21 MasterPlex® ReaderFit and Windows® Explorer application windows**

*Use a drag-and-drop operation to open a .csv, .txt, .xls or .mxqs file(s) in the MasterPlex® application menu bar area*

## 3.6

### Tab categorized work flow

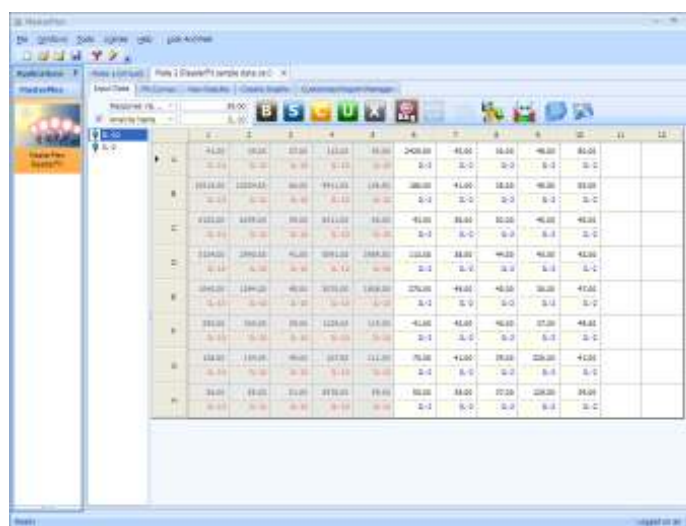
ReaderFit application module consists of five tab pages, **Input Data**, **Fit Curves**, **View Results**, **Create Graphs** and **Customized Report Manager** (Figure 3.22), designed to match the work flow in a typical multiplex data analysis session.



**Figure 3.22 ReaderFit application module tabs**

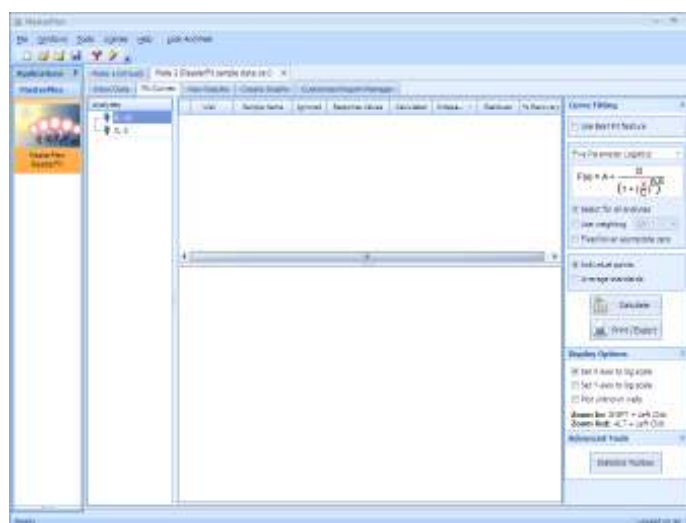
#### Define a Plate

- Mark sample as Background, Standard, Unknown or Control
- Fill out standard value



#### Draw Standard Curve

- Choose a model equation, and set options
- Review regression curve and data
- Make some outliers and re-calculate the curve



### Review Data

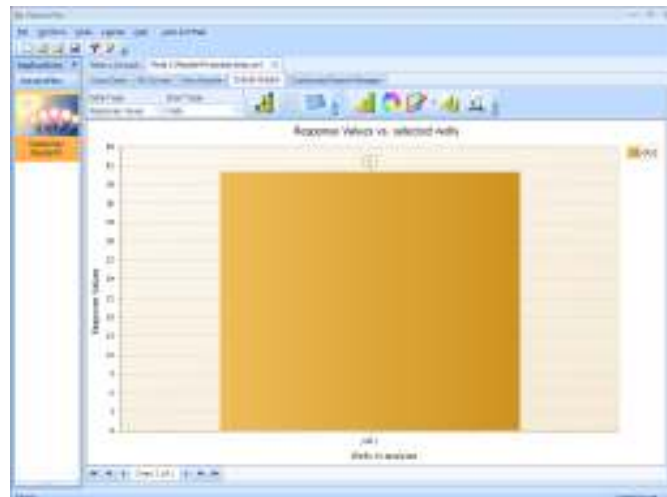
- Review all data
- Print or export the data



| Wavelength | Absorbance | ... |
|------------|------------|-----|
| 215        | 0.00       | ... |
| 225        | 0.00       | ... |
| 235        | 0.00       | ... |
| 245        | 0.00       | ... |
| 255        | 0.00       | ... |
| 265        | 0.00       | ... |
| 275        | 0.00       | ... |
| 285        | 0.00       | ... |
| 295        | 0.00       | ... |
| 305        | 0.00       | ... |
| 315        | 0.00       | ... |
| 325        | 0.00       | ... |
| 335        | 0.00       | ... |
| 345        | 0.00       | ... |
| 355        | 0.00       | ... |
| 365        | 0.00       | ... |
| 375        | 0.00       | ... |
| 385        | 0.00       | ... |
| 395        | 0.00       | ... |
| 405        | 0.00       | ... |
| 415        | 0.00       | ... |
| 425        | 0.00       | ... |
| 435        | 0.00       | ... |
| 445        | 0.00       | ... |
| 455        | 0.00       | ... |
| 465        | 0.00       | ... |
| 475        | 0.00       | ... |
| 485        | 0.00       | ... |
| 495        | 0.00       | ... |
| 505        | 0.00       | ... |
| 515        | 0.00       | ... |
| 525        | 0.00       | ... |
| 535        | 0.00       | ... |
| 545        | 0.00       | ... |
| 555        | 0.00       | ... |
| 565        | 0.00       | ... |
| 575        | 0.00       | ... |
| 585        | 0.00       | ... |
| 595        | 0.00       | ... |
| 605        | 0.00       | ... |
| 615        | 0.00       | ... |
| 625        | 0.00       | ... |
| 635        | 0.00       | ... |
| 645        | 0.00       | ... |
| 655        | 0.00       | ... |
| 665        | 0.00       | ... |
| 675        | 0.00       | ... |
| 685        | 0.00       | ... |
| 695        | 0.00       | ... |
| 705        | 0.00       | ... |
| 715        | 0.00       | ... |
| 725        | 0.00       | ... |
| 735        | 0.00       | ... |
| 745        | 0.00       | ... |
| 755        | 0.00       | ... |
| 765        | 0.00       | ... |
| 775        | 0.00       | ... |
| 785        | 0.00       | ... |
| 795        | 0.00       | ... |
| 805        | 0.00       | ... |
| 815        | 0.00       | ... |
| 825        | 0.00       | ... |
| 835        | 0.00       | ... |
| 845        | 0.00       | ... |
| 855        | 0.00       | ... |
| 865        | 0.00       | ... |
| 875        | 0.00       | ... |
| 885        | 0.00       | ... |
| 895        | 0.00       | ... |
| 905        | 0.00       | ... |
| 915        | 0.00       | ... |
| 925        | 0.00       | ... |
| 935        | 0.00       | ... |
| 945        | 0.00       | ... |
| 955        | 0.00       | ... |
| 965        | 0.00       | ... |
| 975        | 0.00       | ... |
| 985        | 0.00       | ... |
| 995        | 0.00       | ... |

### Review Data by Chart

- Review all data on the chart
- Customize chart properties
- Print or export the chart



### Export Customized Data

- Transform the MasterPlexReaderFit xml data to original data format
- Import or export the style sheet data

### 3.7

## Viewing Data in the Input Data Tab

The ReaderFit application module starts in the Input Data tab. If any other tab page is displayed, click the **Input Data** tab to display the Input Data tab as shown below (Figure 3.23).



Figure 3.23 Input Data tab page

1. If more than one application window is open, select the **Cascade**, **Tile Horizontal**, or **Tile Vertical** menu from the window menu bar to arrange the application windows for easier viewing.
2. To change the data displayed in the well grid:
  - a. Click an analyte in the Analyte pane.
  - b. Make a selection from the data type upper drop-down list.
 

⇒ The well grid displays the data for the selected analyte.

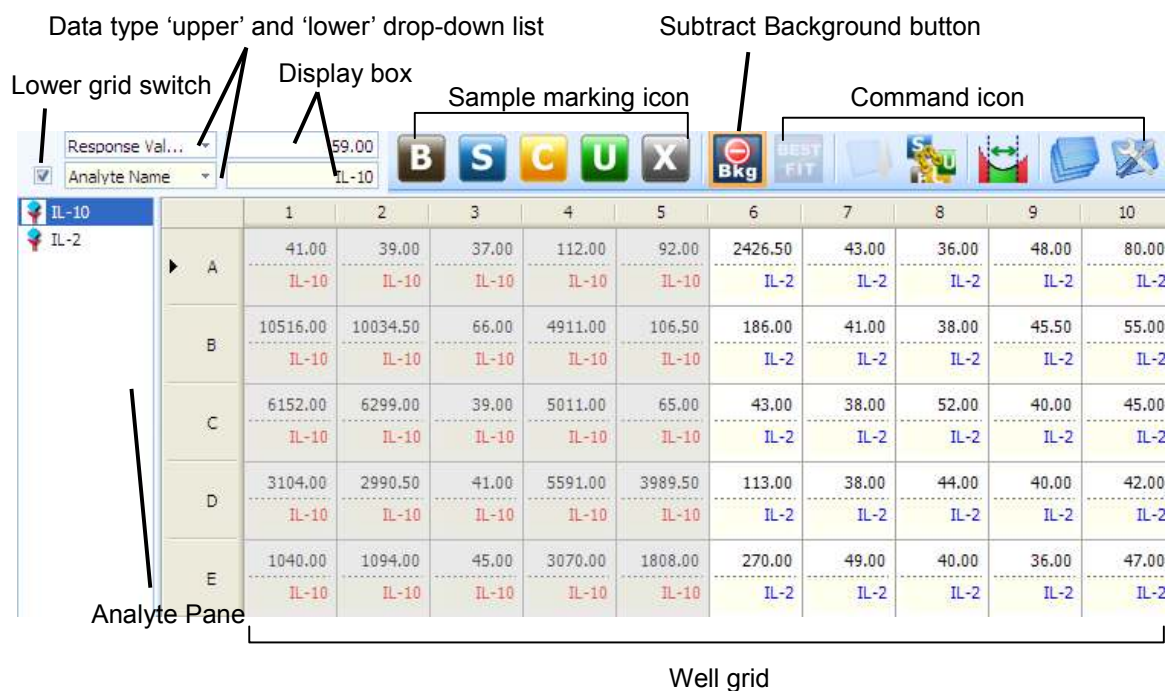
Figure 3.24 shows the components of the Input Data tab. Table 3.1 lists the

types of data available for display in the plate view.

3. To view background-subtracted data, click the **Subtract background** button .

⇒ The Input Data tab displays background-subtracted data.

For more information on background calculation options, see *Background Type* on section 4.6.



**Figure 3.24 Input Data tab and Analyte pane**

## **Plate View Components**

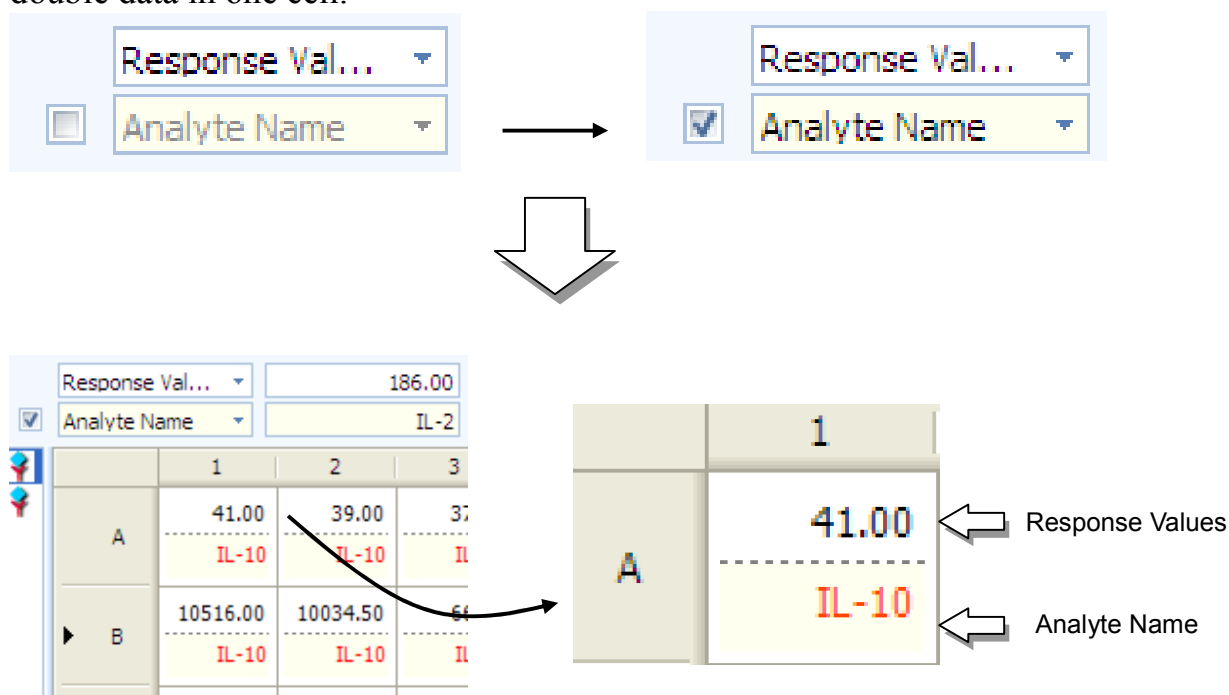
|                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Well Grid</b>                                    | A representation of a microtiter plate that displays the well contents for the analyte selected from the analyte panel and data type selected from the data drop-down list. Some data types can be edited (see Table 3.1). Select one of the wells (The wells turn gray), then click the same well once again to edit mode.                                                                                              |
| <b>Data type ‘upper’ and ‘lower’ drop-down list</b> | Shows the types of data available for display in the well grid. Make a selection from this drop-down list to choose the data type displayed in the well grid. Click the drop-down arrow to view the list and select a data type. (See Table 3.1 for a description of the data types.) The well grid can be separated into upper and lower grids by clicking lower grid switch. (See Figure 3.8 for more further details) |
| <b>Lower grid switch</b>                            | Enables the lower grid data selection and display.                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Display box</b>                                  | Displays the selected data type value for the active (selected) well.                                                                                                                                                                                                                                                                                                                                                    |
| <b>Analyte pane</b>                                 | Displays a list of the analytes in an assay.                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Sample marking icon</b>                          | Icons for sample marking.                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Subtract background</b>                          | Displays the background-subtracted value.                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Command icon</b>                                 | Icons for operating input data tab.                                                                                                                                                                                                                                                                                                                                                                                      |

**Table 3.1 Data Types in the well grid**

| <b>Data Type</b>     | <b>Description</b>                                                                                                  | <b>Edit Data</b> |
|----------------------|---------------------------------------------------------------------------------------------------------------------|------------------|
| Response Values      | The luminescence intensity measured by the plate reader instruments.                                                | No               |
| Calculated Values    | The analyte concentration that is computed (interpolated or extrapolated) from the user-selected standard curve.    | No               |
| Independent Values   | The dilution factor for the well.                                                                                   | Yes              |
| Standard Links       | Shows the standard number that is linked to each well or well group.                                                | Yes              |
| Outlier Status       | A check mark indicates the well is outlier and the well data are not included in the calculation of concentrations. | Yes              |
| Sample Name          | User-specified name for the well.                                                                                   | Yes              |
| Replicate Group Name | The group name of the well. Wells that belong to the same group have the same group number.                         | Yes              |
| Analyte Name         | The analyte name assigned to the well. Wells that belong to the same group have the same group number.              | Yes              |
| Dilution Factor      | The dilution factor for the well.                                                                                   | Yes              |
| Response Mean        | Shows the Response Value average within the group.                                                                  | No               |
| Response SD          | Shows the Response Value standard deviation within the group.                                                       | No               |
| Response %CV         | Shows the Response Value %CV within the group.                                                                      | No               |
| Calculated Mean      | Shows the concentration average within the group.                                                                   | No               |
| Calculated SD        | Shows the concentration standard deviation within the group.                                                        | No               |
| Calculated %CV       | Shows the concentration %CV within the group.                                                                       | No               |
| Intensity            | Shows the Normalized data within the group. (This data type is available in the lower drop-down list only.)         | No               |

### Display Double Data Information in one Cell

ReaderFit has an unique feature for data viewing on the well grid. You can select two data type from various kind of data, and it is displayed in the one cell separated into upper and lower. Figure 3.8 shows how to display the double data in one cell.



**Figure 3.25 Upper and lower grid display**

Well grid can be separated into upper and lower grid. Each grid displays separate data type.

## 3.8

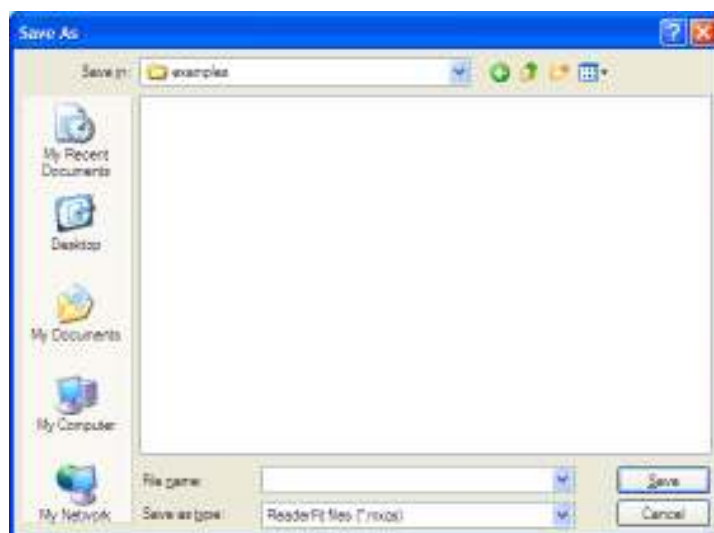
### Saving Plate Data

After you import a scanning results file (.csv, .txt or .xls), the data can be saved to a MasterPlex® ReaderFit file format (.mxqs). The .mxqs file includes all data associated with a plate such as well definitions and computed (interpolated or extrapolated) concentrations.

To save results data (.csv, .txt or .xls) to a MasterPlex® file (.mxqs):

1. Click the **Save** button . Alternatively, select **File > Save** from the main menu.

⇒ The Save As dialog box appears (Figure 3.26).



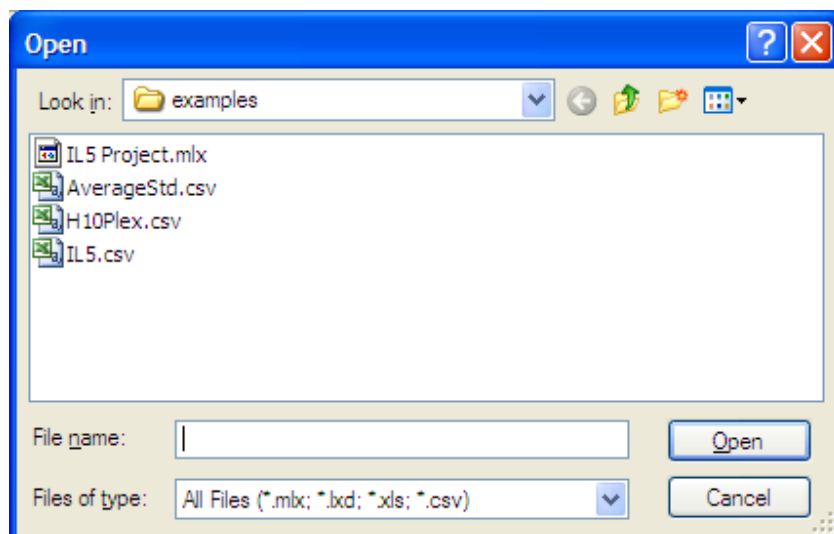
**Figure 3.26 Save As dialog box**

2. Confirm the default directory where the file will be saved or choose another directory.
3. Enter a file name and click **Save**.

### Opening a MasterPlex® File (.mxqs)

1. Click the **Open** button . Alternatively, select **File > Open Plate** from the main menu.

⇒ The Open dialog box appears (Figure 3.27).



**Figure 3.27 Open dialog box**

2. Confirm the default directory or choose another directory.
3. Select a file name (.mxqs) and click **Open**.  
 ⇒ An application module window opens and displays the results data (Figure 3.28).

|                       |          |                                                                        |       |         |         |         |       |       |       |       |    |
|-----------------------|----------|------------------------------------------------------------------------|-------|---------|---------|---------|-------|-------|-------|-------|----|
| Response Val... 59.00 |          | <div> <div>B S C U X</div> <div>Bkg BEST FIT</div> <div> </div> </div> |       |         |         |         |       |       |       |       |    |
| Analyte Name IL-10    |          |                                                                        |       |         |         |         |       |       |       |       |    |
| IL-10                 |          | 1                                                                      | 2     | 3       | 4       | 5       | 6     | 7     | 8     | 9     | 10 |
| IL-2                  |          |                                                                        |       |         |         |         |       |       |       |       |    |
| A                     | 41.00    | 39.00                                                                  | 37.00 | 112.00  | 92.00   | 2426.50 | 43.00 | 36.00 | 48.00 | 80.00 |    |
|                       | IL-10    | IL-10                                                                  | IL-10 | IL-10   | IL-10   | IL-2    | IL-2  | IL-2  | IL-2  | IL-2  |    |
| B                     | 10516.00 | 10034.50                                                               | 66.00 | 4911.00 | 106.50  | 186.00  | 41.00 | 38.00 | 45.50 | 55.00 |    |
|                       | IL-10    | IL-10                                                                  | IL-10 | IL-10   | IL-10   | IL-2    | IL-2  | IL-2  | IL-2  | IL-2  |    |
| C                     | 6152.00  | 6299.00                                                                | 39.00 | 5011.00 | 65.00   | 43.00   | 38.00 | 52.00 | 40.00 | 45.00 |    |
|                       | IL-10    | IL-10                                                                  | IL-10 | IL-10   | IL-10   | IL-2    | IL-2  | IL-2  | IL-2  | IL-2  |    |
| D                     | 3104.00  | 2990.50                                                                | 41.00 | 5591.00 | 3989.50 | 113.00  | 38.00 | 44.00 | 40.00 | 42.00 |    |
|                       | IL-10    | IL-10                                                                  | IL-10 | IL-10   | IL-10   | IL-2    | IL-2  | IL-2  | IL-2  | IL-2  |    |
| E                     | 1040.00  | 1094.00                                                                | 45.00 | 3070.00 | 1808.00 | 270.00  | 49.00 | 40.00 | 36.00 | 47.00 |    |
|                       | IL-10    | IL-10                                                                  | IL-10 | IL-10   | IL-10   | IL-2    | IL-2  | IL-2  | IL-2  | IL-2  |    |

**Figure 3.28 Input Data tab**

## 4 | Defining a Plate – *Input Data tab*

*After you import a scanning results file (.csv, .txt or .xls), your analysis begins by defining a plate. This chapter explains how to define and save a plate. The steps to define a plate include:*

- **Designate well type** to identify the standard, unknown, background, and control wells.
- **Create a standard data set(s)** by entering the concentration for each well in the standard data set. A plate can have more than one standard data set.
- **Link each well group to a standard data set** to specify the standard that is used to compute (interpolate or extrapolate) the analyte concentrations.

The plate definition can be saved as a template that can be applied to other plates. The Template Manager helps you manage your templates. For more information on templates, see *Working With Templates* (on section 4.5).

### 4.1

## Designating Well Type and Group

### Selecting Wells

To select a well in the Input Data tab, click the well in the well grid. There are three ways to select multiple wells:

- To select adjacent wells (Figure 4.1), press and hold the mouse button while you drag the pointer over the wells that you want to select. Click and release the mouse button to select the highlighted wells.
- To select adjacent wells, press and hold the **Shift** key while you click the first and last well in the selection.
- To select nonadjacent wells (Figure 4.2), press and hold the **Ctrl** while you click the wells.

|   | 1       | 2      | 3      | 4      |
|---|---------|--------|--------|--------|
| A | 20.00   | 367.00 | 506.50 | 341.00 |
|   | 63      | 67     | 76     | 80     |
| B | 1308.00 | 429.00 | 480.50 | 392.00 |
|   | 95      | 66     | 78     | 79     |
| C | 1084.50 | 82.00  | 88.00  | 78.00  |
|   | 72      | 79     | 75     | 70     |
| D | 525.00  | 88.50  | 82.00  | 96.50  |
|   | 79      | 66     | 76     | 74     |
| E | 201.00  | 37.50  | 38.00  | 37.00  |
|   | 81      | 82     | 85     | 96     |
| F | 66.50   | 32.00  | 32.00  | 34.00  |
|   | 80      | 79     | 66     | 74     |
| G | 39.00   | 18.00  | 18.00  | 23.00  |
|   | 78      | 81     | 68     | 69     |

**Figure 4.1 Well grid selection**

To select adjacent wells, press and hold the **Shift** key while you click the first and last well in the selection. Alternatively, press and hold the mouse button while you drag the mouse over the wells of interest.

|   | 1       | 2      | 3      | 4      |
|---|---------|--------|--------|--------|
| A | 20.00   | 367.00 | 506.50 | 341.00 |
|   | 63      | 67     | 76     | 80     |
| B | 1308.00 | 429.00 | 480.50 | 392.00 |
|   | 95      | 66     | 78     | 79     |
| C | 1084.50 | 82.00  | 88.00  | 78.00  |
|   | 72      | 79     | 75     | 70     |
| D | 525.00  | 88.50  | 82.00  | 96.50  |
|   | 79      | 66     | 76     | 74     |
| E | 201.00  | 37.50  | 38.00  | 37.00  |
|   | 81      | 82     | 85     | 96     |
| F | 66.50   | 32.00  | 32.00  | 34.00  |
|   | 80      | 79     | 66     | 74     |
| G | 39.00   | 18.00  | 18.00  | 23.00  |
|   | 78      | 81     | 68     | 69     |

**Figure 4.2 Well grid random selection**

To select nonadjacent wells, press and hold the **Ctrl** key while you click the wells of interest.

## Designating Well Type

Table 4.1 shows the types of wells that are available.

1. Select the well(s) that you want to define.
  2. To define (or *mark*) the well(s), click one of the icons located on the upper well grid (Figure 4.3). You can also right-click the selection and choose a well type from the pop-up menu that appears Figure 4.4. (Table 4.1).
- ⇒ The well type is applied to the selected well(s).



**Figure 4.3 Sample mark icons**



**Figure 4.4 Well grid pop-up menu**

*Right click a well to display the pop-up menu*

**Table 4.1 Sample mark icon and context menu to define wells**

| Well Type                                                                                              | Button                                                                              | Context menu on the well grid |
|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------|
| <b>Background</b><br>Wells that contain no analytes.                                                   |    | <b>Background</b>             |
| <b>Standard</b><br>Wells that contains analyte of known concentration.                                 |    | <b>Standard</b>               |
| <b>Unknown</b><br>Wells that contains analytes of unknown concentration.                               |    | <b>Unknown</b>                |
| <b>Control</b><br>Wells that contain analytes that function as controls for a particular assay design. |   | <b>Control</b>                |
| <b>Unmark</b><br>Clear the current marking.                                                            |  | <b>Unmark</b>                 |



If a well belongs to a group, unmarking the well also removes the well from the group.

3. Repeat step 1 and step 2 to mark and group other well(s).

### Designating Well Groups

After you have defined the wells, the wells are organized into *groups* automatically so that the software can identify:

- Replicate unknowns
- A standard data set

MasterPlex® ReaderFit automatically places all background wells into one group. You can define one or more groups of control wells per plate.



**NOTE:** A group can include nonadjacent wells. A plate can have more than one group of standards or unknowns.

### Grouping Wells by Pattern

The purpose of pattern grouping is to provide users another way to easily and quickly make replicate groups. Pattern here means two things: the group type (e.g., standard, unknown...) and the dimensions of the group (i.e., rows and columns). This function acts similarly to the Resizing feature of Microsoft Excel. It is especially useful when the plate has many groups/replicates that follow similar group patterns.

1. Define the group pattern by selecting a group of wells, and marking and grouping them together. We will group other wells into this pattern.
2. Select all wells of the pattern group (Figure 4.5).

|   | 1             | 2            | 3            | 4            | 5 | 6 | 7 |
|---|---------------|--------------|--------------|--------------|---|---|---|
| A | 20.00<br>63   | 367.00<br>67 | 506.50<br>76 | 341.00<br>80 |   |   |   |
| B | 1308.00<br>95 | 429.00<br>66 | 480.50<br>78 | 392.00<br>79 |   |   |   |
| C | 1084.50<br>72 | 82.00<br>79  | 88.00<br>75  | 78.00<br>70  |   |   |   |
| D | 525.00<br>79  | 88.50<br>66  | 82.00<br>76  | 96.50<br>74  |   |   |   |
| E | 201.00<br>81  | 37.50<br>82  | 38.00<br>85  | 37.00<br>96  |   |   |   |
| F | 66.50<br>80   | 32.00<br>79  | 32.00<br>66  | 34.00<br>74  |   |   |   |
| G | 39.00<br>78   | 18.00<br>81  | 18.00<br>68  | 23.00<br>69  |   |   |   |
| H | 26.50<br>68   | 19.00<br>69  | 17.50<br>80  | 27.00<br>42  |   |   |   |

**Figure 4.5 Well groups**

3. Move the pointer to the bottom-right corner of the selection. When you see the pointer turn into a black cross, hold down the left mouse button and drag the pointer over the selection. During dragging, you will see in real-time that new wells are selected and grouped into the pattern, as indicated by a red-line border (Figure 4.6).

|   | 1             | 2            | 3            | 4            |
|---|---------------|--------------|--------------|--------------|
| A | 20.00<br>63   | 367.00<br>67 | 506.50<br>76 | 341.00<br>80 |
| B | 1308.00<br>95 | 429.00<br>66 | 480.50<br>78 | 392.00<br>79 |
| C | 1084.50<br>72 | 82.00<br>79  |              |              |
| D | 525.00<br>79  | 88.50<br>66  |              |              |
| E | 201.00<br>81  | 37.50<br>82  |              |              |
| F | 66.50<br>80   | 32.00<br>79  |              |              |
| G | 39.00<br>78   | 18.00<br>81  |              |              |
| H | 26.50<br>68   | 19.00<br>69  |              |              |

|   | 1             | 2            | 3            | 4            |
|---|---------------|--------------|--------------|--------------|
| A | 20.00<br>63   | 367.00<br>67 | 506.50<br>76 | 341.00<br>80 |
| B | 1308.00<br>95 | 429.00<br>66 | 480.50<br>78 | 392.00<br>79 |
| C | 1084.50<br>72 | 82.00<br>79  | 88.00<br>75  | 78.00<br>70  |
| D | 525.00<br>79  | 88.50<br>66  | 82.00<br>76  | 96.50<br>74  |
| E | 201.00<br>81  | 37.50<br>82  | 38.00<br>85  | 37.00<br>96  |
| F | 66.50<br>80   | 32.00<br>79  | 32.00<br>66  | 34.00<br>74  |
| G | 39.00<br>78   | 18.00<br>81  | 18.00<br>68  | 23.00<br>69  |
| H | 26.50<br>68   | 19.00<br>69  | 17.50<br>80  | 27.00<br>42  |

**Figure 4.6 Making Well groups by mouse Dragging**

4. Once you are satisfied with the selection, just release the mouse button. The software will automatically finish the grouping(Figure 4.7).

|     | 1             | 2            | 3            | 4            |
|-----|---------------|--------------|--------------|--------------|
| ▶ A | 20.00<br>63   | 367.00<br>67 | 506.50<br>76 | 341.00<br>80 |
| B   | 1308.00<br>95 | 429.00<br>66 | 480.50<br>78 | 392.00<br>79 |
| C   | 1084.50<br>72 | 82.00<br>79  | 88.00<br>75  | 78.00<br>70  |
| D   | 525.00<br>79  | 88.50<br>66  | 82.00<br>76  | 96.50<br>74  |
| E   | 201.00<br>81  | 37.50<br>82  | 38.00<br>85  | 37.00<br>96  |
| F   | 66.50<br>80   | 32.00<br>79  | 32.00<br>66  | 34.00<br>74  |
| G   | 39.00<br>78   | 18.00<br>81  | 18.00<br>68  | 23.00<br>69  |
| H   | 26.50<br>68   | 19.00<br>69  | 17.50<br>80  | 27.00<br>42  |

**Figure 4.7 Well groups**



**NOTE:** When starting drag, you can move the pointer, you can move it either downwards or rightwards, which results in different ways to select wells. To switch between the two modes, just drag the pointer back into the pattern group, and then drag it out in either direction. So, it is determined by your first move direction when you are dragging the pointer out of the pattern group.

|   | 1             | 2            | 3            | 4            |
|---|---------------|--------------|--------------|--------------|
| A | 20.00<br>63   | 367.00<br>67 | 506.50<br>76 | 341.00<br>80 |
| B | 1308.00<br>95 | 429.00<br>66 | 480.50<br>78 | 392.00<br>79 |
| C | 1084.50<br>72 | 82.00<br>79  | 88.00<br>75  | 78.00<br>70  |
| D | 525.00<br>7   | 88.50<br>66  | 82.00<br>76  | 96.50<br>74  |
| E | 201.00<br>81  | 37.50<br>82  | 38.00<br>85  | 37.00<br>96  |
| F | 66.50<br>80   | 32.00<br>79  | 32.00<br>66  | 34.00<br>74  |
| G | 39.00<br>78   | 18.00<br>81  | 18.00<br>68  | 23.00<br>69  |
| H | 26.50<br>68   | 19.00<br>69  | 17.50<br>80  | 27.00<br>42  |

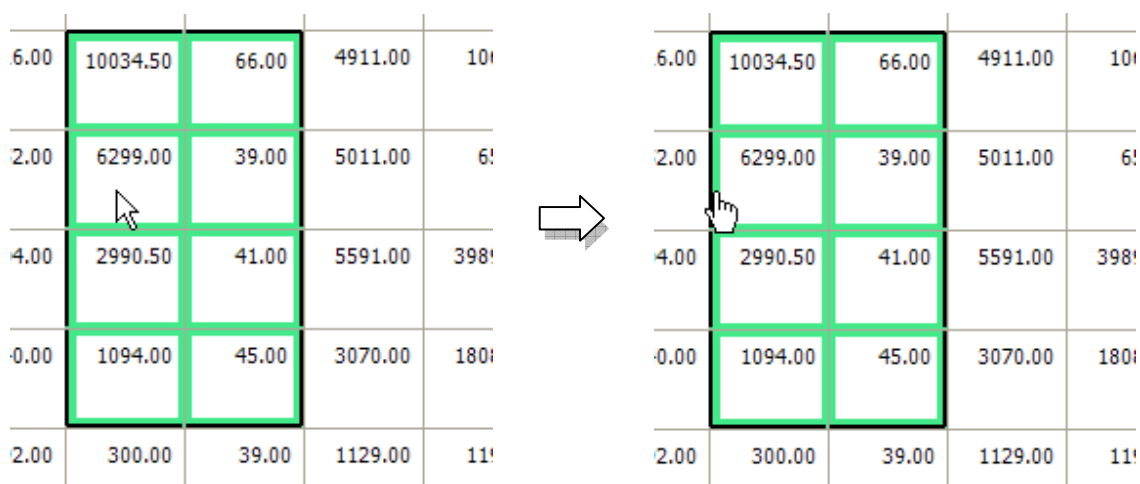
|   | 1             | 2            | 3            | 4            |
|---|---------------|--------------|--------------|--------------|
| A | 20.00<br>63   | 367.00<br>67 | 506.50<br>76 | 341.00<br>80 |
| B | 1308.00<br>95 | 429.00<br>66 | 480.50<br>78 | 392.00<br>79 |
| C | 1084.50<br>72 | 82.00<br>79  | 88.00<br>75  | 78.00<br>70  |
| D | 525.00<br>79  | 88.50<br>66  | 82.00<br>76  | 96.50<br>74  |
| E | 201.00<br>81  | 37.50<br>82  | 38.00<br>85  | 37.00<br>96  |
| F | 66.50<br>80   | 32.00<br>79  | 32.00<br>66  | 34.00<br>74  |
| G | 39.00<br>78   | 18.00<br>81  | 18.00<br>68  | 23.00<br>69  |

**Figure 4.8 Making Well groups by mouse Dragging**

*Dragging downwards as the first move (above) vs. dragging rightwards as the first move (below)*

### Select all wells within the group at one time

1. While hovering over a replicate group border, the mouse pointer changes to a 'hand' icon (Figure 4.9).



**Figure 4.9 Mouse pointer changes to 'hand' icon**

2. Click the border while mouse pointer is hand icon.  
⇒ Entire wells within the group are selected (Figure 4.10).



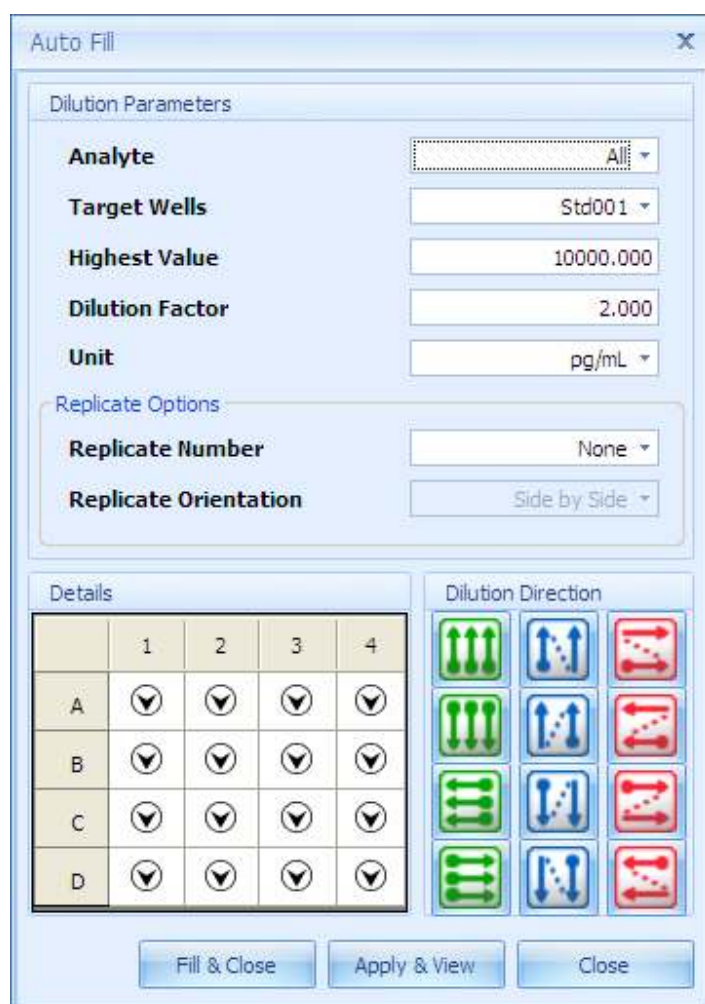
**Figure 4.10 Selected wells**

## 4.2

### Setting Standard Concentrations

After you define and group the standard wells, use the auto fill feature to help you automatically enter the standard concentrations. **Auto-Fill** icon is enabled when one or more standard groups are in the plate.

1. Click the **Auto-Fill** button  located above the well grid. Alternatively, Right-click the standard data set and select **Auto-Fill** from the popup menu.  
⇒ The Auto Fill dialog box appears (Figure 4.11).



The Auto Fill dialog box is titled "Auto Fill" and contains the following sections:

- Dilution Parameters:**
  - Analyte:** A dropdown menu with "All" selected.
  - Target Wells:** A dropdown menu with "Std001" selected.
  - Highest Value:** A text input field containing "10000.000".
  - Dilution Factor:** A text input field containing "2.000".
  - Unit:** A dropdown menu with "pg/mL" selected.
- Replicate Options:**
  - Replicate Number:** A dropdown menu with "None" selected.
  - Replicate Orientation:** A dropdown menu with "Side by Side" selected.
- Details:** A 4x4 grid of wells (A-D rows, 1-4 columns). Each cell contains a downward arrow icon.
- Dilution Direction:** A 3x3 grid of icons representing different dilution patterns (e.g., vertical, horizontal, diagonal).

At the bottom of the dialog box are three buttons: "Fill & Close", "Apply & View", and "Close".

Figure 4.11 Auto Fill dialog box

2. Make a selection from the Analyte drop-down list.
3. Select target standard group you want to fill out.
4. Enter the starting concentration for the standard data set.
5. Enter the dilution factor.
6. Make a selection from the concentration unit drop-down list
7. To select a dilution direction for the standard data set, click a dilution direction arrow.  
 ⇒ The gradient map shows the location and direction of the dilution gradient(s) (Figure 4.12).



This gradient map specifies a separate dilution gradient in each column of the standard data set. The starting concentration is at the top of a column.



This gradient map specifies one dilution gradient per standard data set. The starting concentration is at the upper left well and the end concentration is at the lower right well. Click an arrow to choose a dilution direction.

**Figure 4.12 Example dilution gradient maps**

*Click a dilution direction arrow to choose the dilution gradient configuration for the standard data set.*

7. To specify the same starting concentration, dilution factor, and concentration units for all analytes in the standard data set, choose the **Fill for all analytes** option. To specify a different starting concentration, dilution factor, or concentration unit for a different analyte, repeat step 2 through step 4.
8. Click **Apply** button when finished entering the concentration, the dilution, and the dilution direction for all analytes in the standard data set. If you want to close the dialog box at the same time, click **Fill & Close** button.

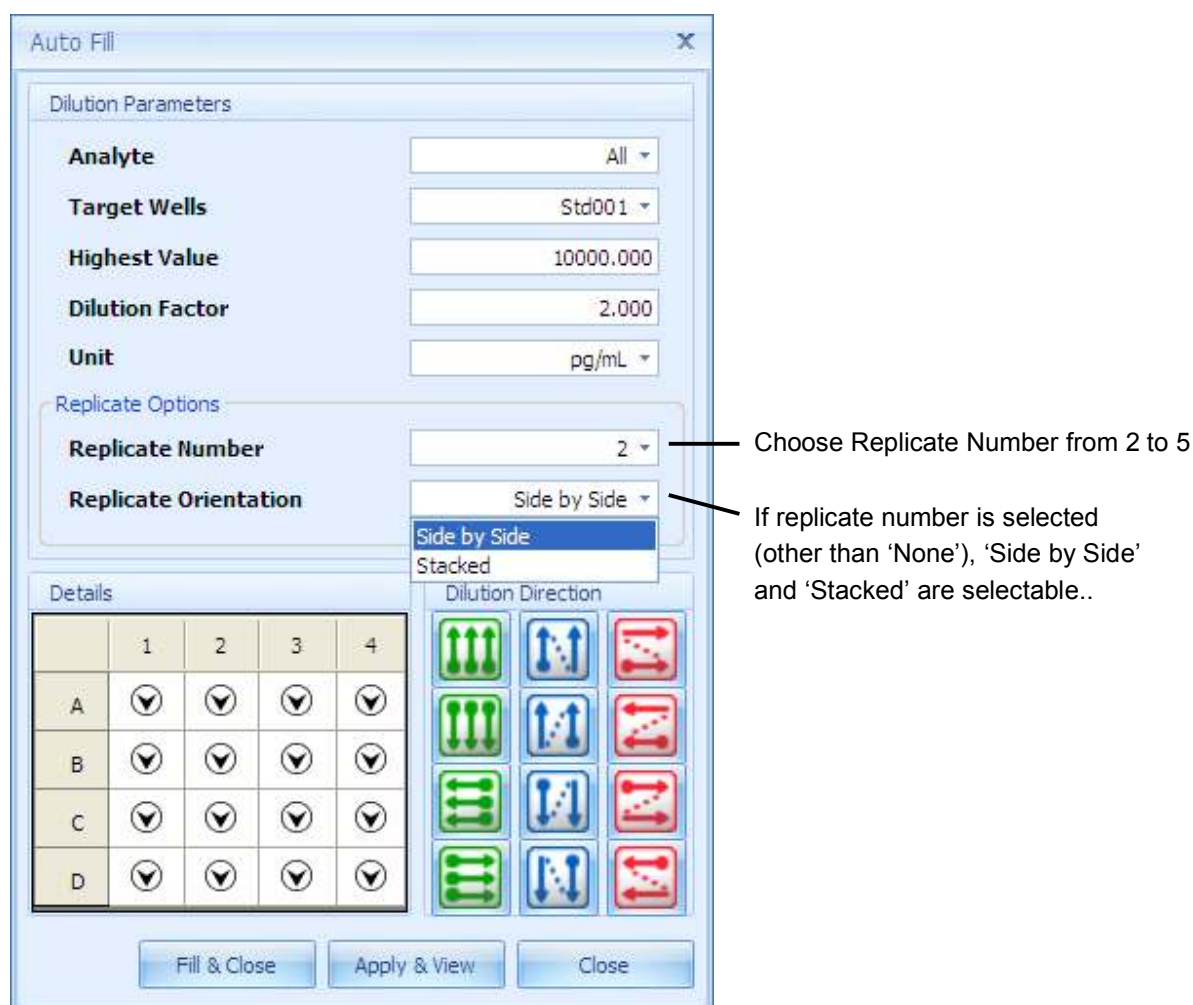


**NOTE:** If you want to fill the standard value for your desired wells, select wells on the well grid, and choose 'Selected Wells' at the target well selection in the Auto-Fill dialog. Auto-Fill process is applied for only the wells you selected.

---

### Fill in for replicate standard samples

If you have replicate standard samples in your plate, and if you want to fill the same diluted standard concentration value for each replicates, use replicate filling option (Figure 4.13). Figure 4.14 and 4.15 shows each 'Side by Side' and 'Stacked' replicate example.



**Figure 4.13 Replicate Options**

|   | 1        | 2        | 3     | 4     |
|---|----------|----------|-------|-------|
| A | 10000.00 | 10000.00 | 39.06 | 39.06 |
| B | 5000.00  | 5000.00  | 19.53 | 19.53 |
| C | 2500.00  | 2500.00  | 9.77  | 9.77  |
| D | 1250.00  | 1250.00  | 4.88  | 4.88  |
| E | 625.00   | 625.00   | 2.44  | 2.44  |
| F | 312.50   | 312.50   | 1.22  | 1.22  |
| G | 156.25   | 156.25   | 0.61  | 0.61  |
| H | 78.13    | 78.13    | 0.31  | 0.31  |

Replicate Number : 2  
Replicate Orientation: Side by Side  
Dilution Direction:

| Details |   |   |   |   | Dilution Direction |  |  |
|---------|---|---|---|---|--------------------|--|--|
|         | 1 | 2 | 3 | 4 |                    |  |  |
| A       | ▼ | ▼ | ▼ | ▼ |                    |  |  |
| B       | ▼ | ▼ | ▼ | ▼ |                    |  |  |
| C       | ▼ | ▼ | ▼ | ▼ |                    |  |  |
| D       | ▼ | ▼ | ▼ | ▼ |                    |  |  |

**Figure 4.14 Side by Side Replicate Options**

|   | 1        | 2        | 3        | 4       | 5       | 6       | 7       | 8       |
|---|----------|----------|----------|---------|---------|---------|---------|---------|
| A | 10000.00 | 10000.00 | 10000.00 | 5000.00 | 5000.00 | 5000.00 | 2500.00 | 2500.00 |
| B | 2500.00  | 1250.00  | 1250.00  | 1250.00 | 1250.00 | 625.00  | 625.00  | 312.50  |

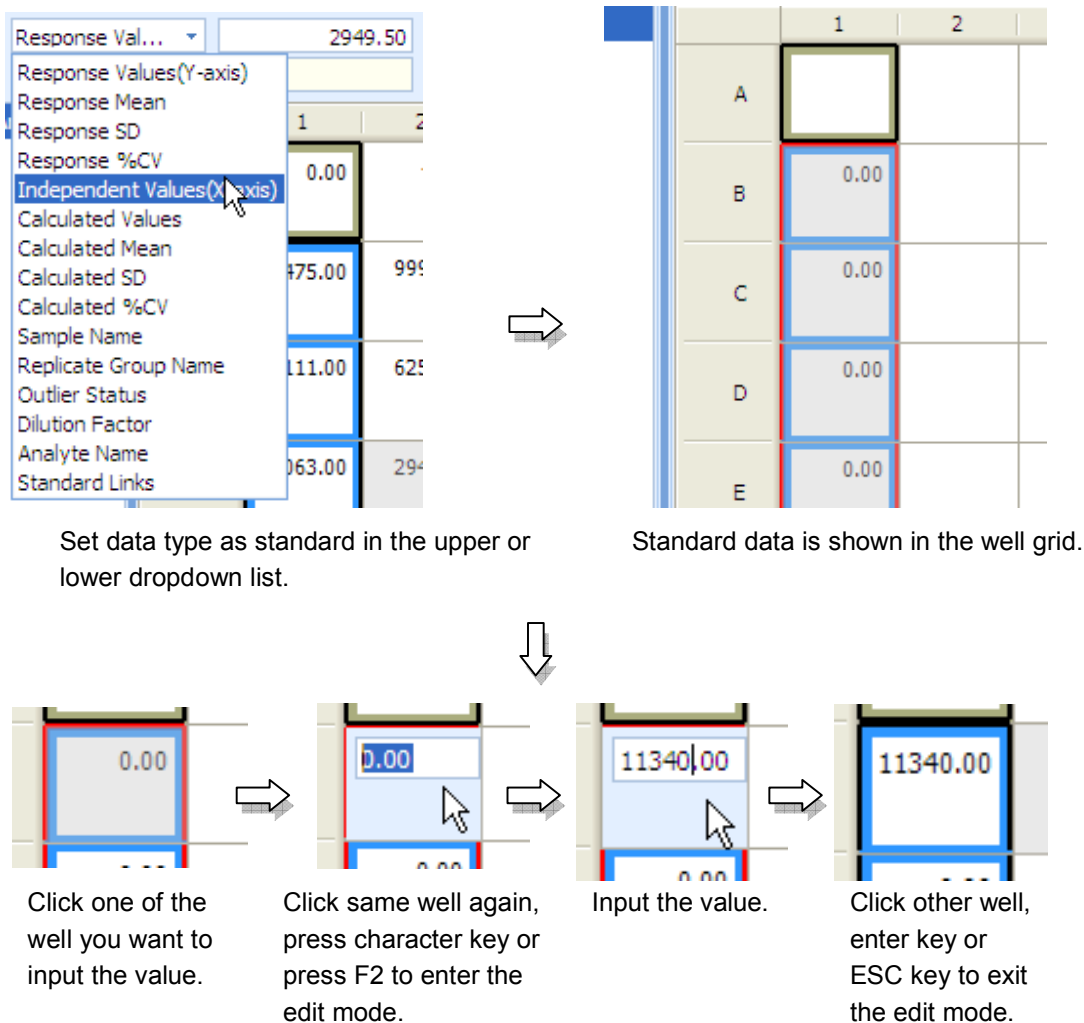
Replicate Number : 3  
Replicate Orientation: Stacked  
Dilution Direction:

| Details |   |   |   |   | Dilution Direction |  |  |
|---------|---|---|---|---|--------------------|--|--|
|         | 1 | 2 | 3 | 4 |                    |  |  |
| A       | ▶ | ▶ | ▶ | ▶ |                    |  |  |
| B       | ▶ | ▶ | ▶ | ▶ |                    |  |  |
| C       | ▶ | ▶ | ▶ | ▶ |                    |  |  |
| D       | ▶ | ▶ | ▶ | ▶ |                    |  |  |

**Figure 4.15 Stacked Replicate Options**

### Input Standard Data Manually

If your standard data series does not have sequential diluted values, use direct edit mode on the well grid(Figure 4.16) .



**Figure 4.16 Input manually using edit mode**

## 4.3

### Linking a Standard Data Set

Background, control, and unknown wells must be associated with or *linked* to the standard data set that will be used to calculate concentrations. By default, the first standard that you define will be linked to the background, control, and unknown well groups.

If there is more than one standard on the plate, you can link a user-selected standard to a user-selected well group(s).

1. To link a well group to a standard data set, press and hold the **Ctrl** key while you click the group and the standard data set that you want to link.



**NOTE:** A standard data set can be linked to multiple groups of the same well type, but each group can have only one standard.

2. Click the **Link Standard** button .

3. To check the status, select Standard Links data type from upper or lower drop-down(Figure 4.17).



|   | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        | 9        |     |
|---|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----|
| A | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Sta |
| B |          |          | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Sta |
| C |          |          | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Sta |
| D |          |          | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Sta |
| E |          |          | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Sta |
| F |          |          | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Sta |
| G |          |          | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Sta |
|   | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Standard | Sta |

**Figure 4.17 Checking Linking Status**

## 4.4

### Working With Diluted Unknowns

If you need to dilute a sample prior to an assay, you can specify a dilution factor in the well grid. MasterPlex® ReaderFit can compute the diluted analyte concentration.

For a diluted unknown:

$$\text{Original concentration} = \text{Dilution factor} * \text{Calculated concentration.}$$

### Editing a Dilution Factor

1. Select 'Dilution Factor' data type from upper drop-down list or lower drop-down list (Figure 4.18).

⇒ Current dilution factor settings are shown in the plate well grid.

|   | 1    | 2    | 3    | 4    |
|---|------|------|------|------|
| A | 1.00 | 1.00 | 1.00 | 1.00 |
| B | 1.00 | 1.00 | 1.00 | 1.00 |

**Figure 4.18 Display Dilution Factor**

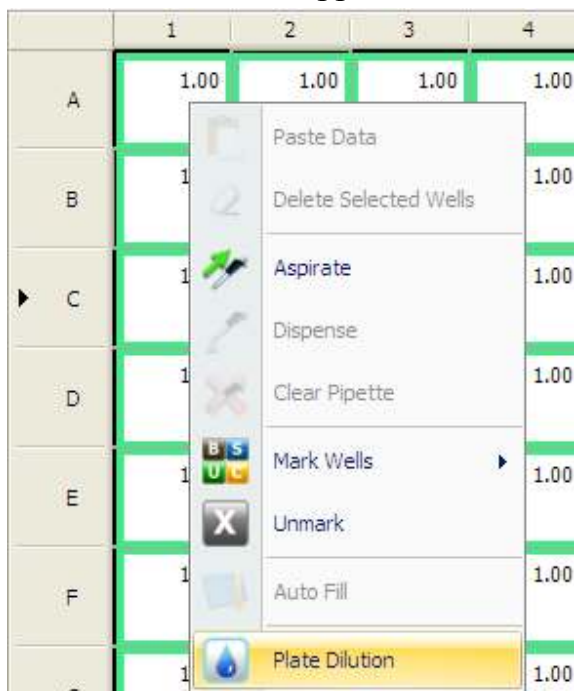
2. Click one of the desired well your want to set the dilution.
3. Click the same well again to enter the edit mode(Figure 4.19).

|   | 1        | 2    | 3    | 4    |
|---|----------|------|------|------|
| A | <i>i</i> | 1.00 | 1.00 | 1.00 |
| B | 1.00     | 1.00 | 1.00 | 1.00 |

**Figure 4.19 Dilution Factor Edit Mode**

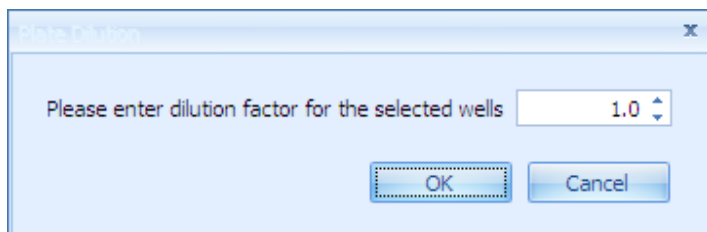
### Editing a Dilution Factor using batch input feature

1. Select multiple wells you want to set the dilution at one time.
2. Right click on the well grid.  
⇒ Context menu appears (Figure 4.20).



**Figure 4.20 Dilution Factor Menu**

3. Select **Plate Dilution** menu. Plate Dilution dialog appears (Figure 4.21).



**Figure 4.21 Dilution Factor Input Dialog**

4. Edit value directly or change the value using spin button.
5. Click OK when finish.  
⇒ Dilution factor is updated on the well grid (Figure 4.22).

|   | 1    | 2    | 3    | 4    |
|---|------|------|------|------|
| A | 2.00 | 2.00 | 2.00 | 1.00 |
| B | 2.00 | 2.00 | 2.00 | 1.00 |
| C | 1.00 | 1.00 | 1.00 | 1.00 |

**Figure 4.22 Inputted Dilution Factor**

### **Dilution for Unknowns**

Samples can be diluted prior to the assay and analysis. After MasterPlex® ReaderFit interpolates the diluted unknown analyte concentrations from the standard curve, it can compute and display the original, undiluted concentration in the well grid.

$$\text{Original concentration} = \text{Diluted concentration} * \text{Dilution Factor}$$

## 4.5

### Working With Templates

A plate definition includes:

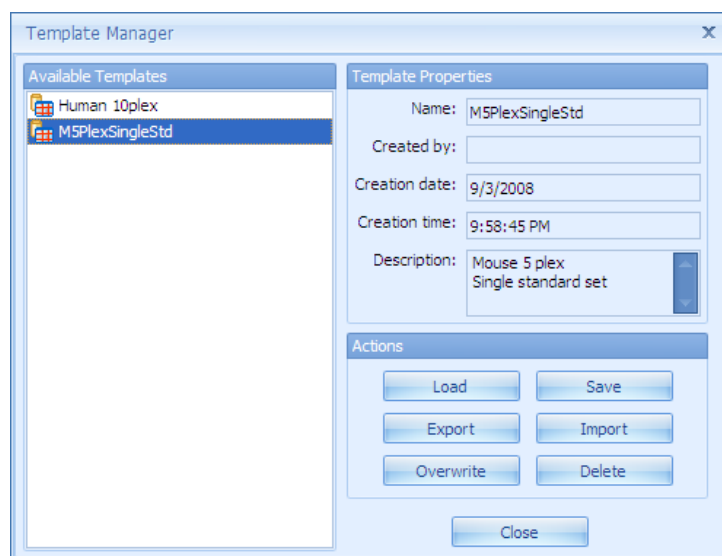
- Well types and well groups
- Standards (including standard concentrations, associated model equation, and concentration units)
- Links between the standard(s) and well groups
- Data calculated for the plate (for example, analyte concentrations or standard data curves)
- Data manually entered in the plate (for example, sample names or dilution factors)

You can save the plate definition as a template. You can apply a template to an active plate. Templates may also be exported, imported, or deleted.

### Opening the Template Manager

The Template Manager is a tool that helps you manage your templates.

1. Click the **Template Manager** button  .  
⇒ The Template Manager appears (Figure 4.23).
2. Click a template in the Available Templates list to view information about the template.



**Figure 4.23** Template Manager shows available templates

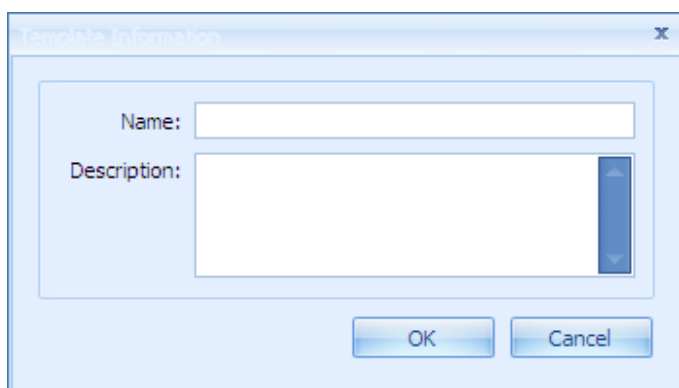
*Click a template to view information about the template.*

### Saving a Template

You can save the current plate definition to a template.

1. After you have finished defining a plate, open the Template Manager and click the **Save** button.

⇒ The Template Name and Description box appears (Figure 4.24).



**Figure 4.24 Template Name and Description box**

2. Enter a name and descriptions for the template and click **OK**.

⇒ The new template is added to the Available Template list.

### Loading a Template

You can apply or *load* a saved template to the current plate.

1. In the Template Manager, select the template that you want to apply to the plate.
2. Click the **Load** button.

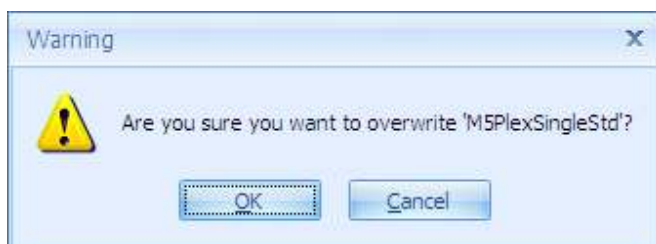
⇒ The template is applied and the well grid shows the new well attributes (well type, well group, and links to standard data sets).

### Overwriting a Template

You can overwrite an existing template with the current plate definition.

1. In the Template Manager, select the template that you want to overwrite
2. Click the **Overwrite** button.

⇒ A confirmation box appears (Figure 4.25).



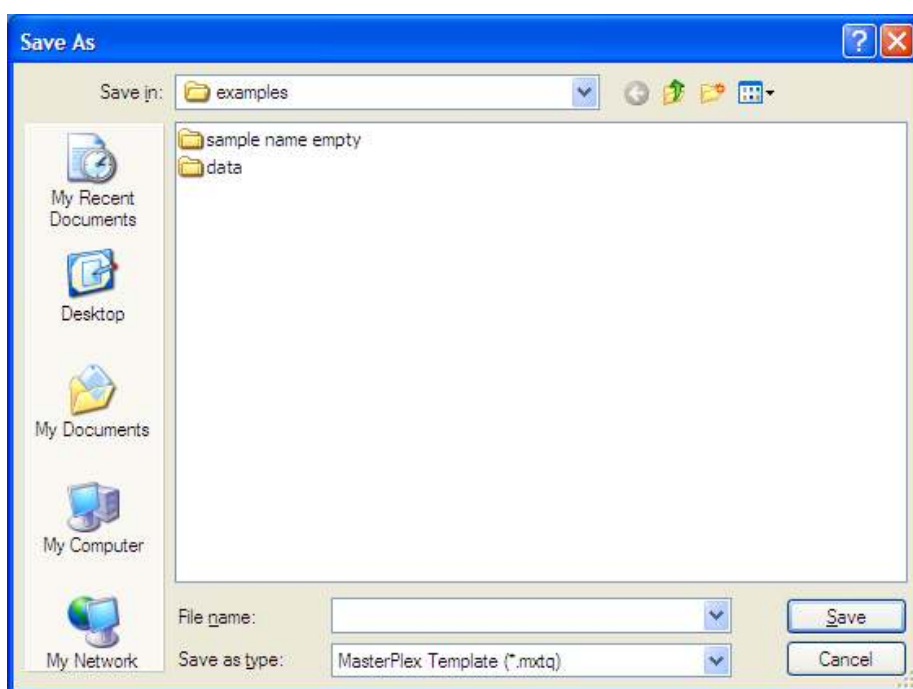
**Figure 4.25 Confirmation box**

1. Click **OK** to overwrite the selected template with the current plate definition.

### Exporting a Template

You can export a template to a user-specified location.

1. In the Template Manager, click the template you want to export.
2. Click the **Export** button.
  - ⇒ The Save As dialog box appears (Figure 4.26).



**Figure 4.26 Save As dialog box**

3. Choose the directory for the template that you want to export.

4. Enter a name for the template (\*.mxtq).



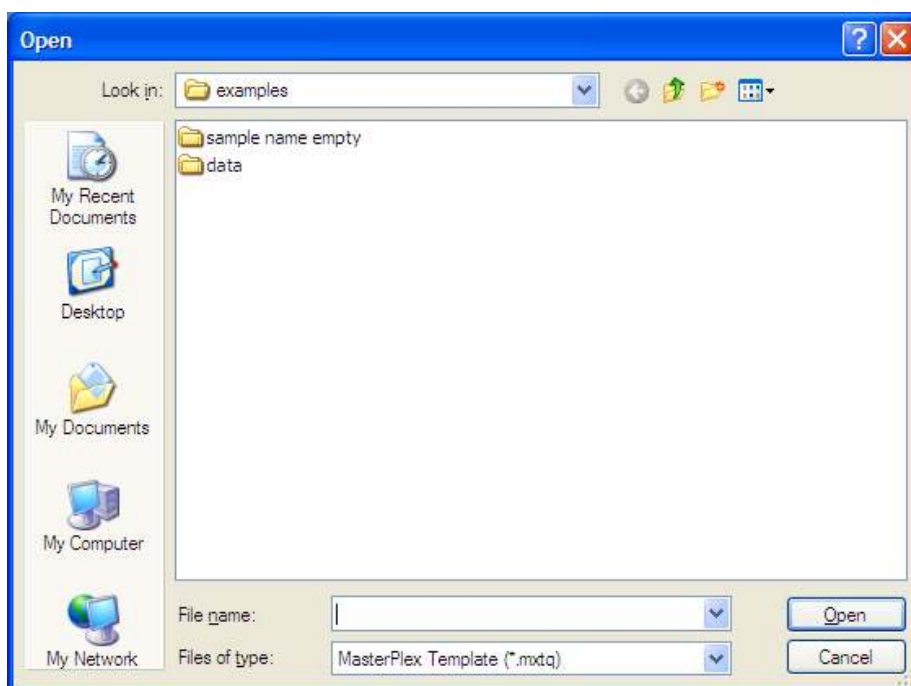
**NOTE:** A template must have a .mxtq file extension. Changing the extension will render the exported template unusable.

### Importing a Template

You can import a template (.mxtq) from a user-specified location.

1. In the Template Manager, click **Import** button.

⇒ The Open dialog box appears (Figure 4.27).



**Figure 4.27 Open dialog box**

2. Choose the directory with the template that you want to import.

3. Select the template and click **Open**.

⇒ The template name is added to the Template Manager.

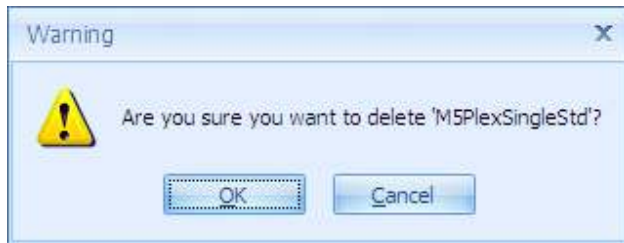
### Deleting a Template

You can delete a template (.mxtq) from the system.

1. In the Template Manager, click the template that you want to delete.

2. Click **Delete** button.

⇒ A confirmation box appears (Figure 4.28).



**Figure 4.28 Confirmation box**

3. Click **OK** to delete the template.

⇒ The template is removed from the Template Manager.



**WARNING:** This permanently removes the template from the system.

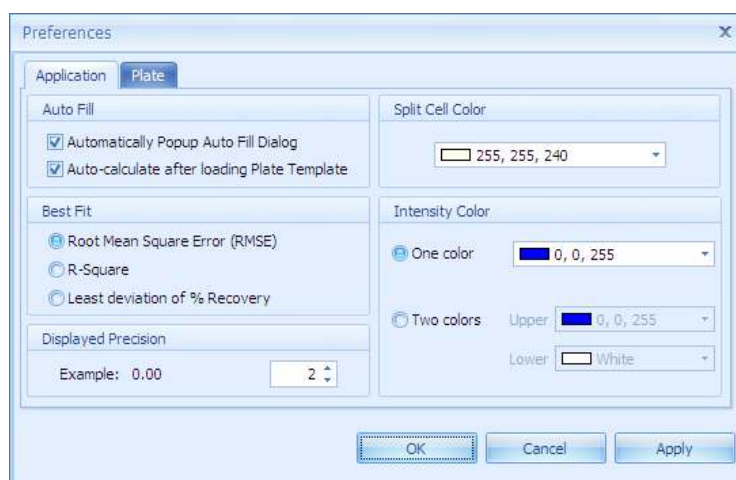
---

## 4.6

### Preferences

Preferences are user-modifiable software settings. They are displayed in the Preferences dialog box.

- To open the Preferences dialog box (Figure 4.29), click the **Preferences** button .



**Figure 4.29 Preferences dialog box**

### Application Preferences

#### Auto Fill

**Automatically Popup  
Auto Fill Dialog**

Check this option if you want to open autofill dialog automatically when you mark the standard sample.

**Auto-calculate after  
loading Plate Template**

Check this option if you want to calculate automatically right after the template loading.

#### Best Fit

**Root Mean Square  
Error (RMSE)**

Use RMSE index to choose the best curve fit combination.

**R-Square**

Use R-square to choose the best.

**Least deviation of %  
Recovery**

Use LD of % Recover to choose the best.

## Split Cell Color

Color lower grid by specified color.

|   | 1               | 2               | 3            | 4              |
|---|-----------------|-----------------|--------------|----------------|
| A | 41.00<br>189    | 39.00<br>194    | 37.00<br>193 | 112.00<br>226  |
| B | 10516.00<br>208 | 10034.50<br>152 | 66.00<br>199 | 4911.00<br>203 |
| C | 6152.00<br>225  | 6299.00<br>246  | 39.00<br>247 | 5011.00<br>220 |

**Figure 4.30 Colored lower well grid**

## Intensity Color

### One Color

Use one color for representing the value shading.

### Two Color

Use two colors for representing the value shading.

Intensity Color

☒ One color LimeGreen

☐ Two colors Upper 255, 0, 0

Lower 0, 0, 192



|   | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9      | 10     |
|---|---------|---------|---------|---------|---------|---------|---------|---------|--------|--------|
| A | 10.00   | 9.00    | 19.00   | 6903.00 | 48.50   | 1162.00 | 5573.00 | 2145.00 | 443.00 | 187.00 |
| B | 9929.00 | 8989.50 | 6994.00 | 4487.00 | 64.00   | 179.00  | 4746.00 | 1005.00 | 209.50 | 95.00  |
| C | 5850.50 | 5807.00 | 1297.00 | 4276.00 | 37.50   | 18.00   | 3271.00 | 7722.50 | 104.00 | 45.00  |
| D | 2656.00 | 3049.00 | 63.50   | 3277.00 | 1846.00 | 109.00  | 1957.00 | 6467.00 | 43.00  | 32.00  |
| E | 1104.50 | 914.00  | 57.50   | 1501.00 | 1842.00 | 138.00  | 7233.00 | 5535.50 | 27.50  | 185.00 |
| F | 300.00  | 404.00  | 1396.00 | 433.00  | 133.50  | 15.00   | 6247.50 | 3727.50 | 15.00  | 99.00  |
| G | 143.50  | 116.00  | 7453.00 | 61.00   | 58.00   | 7190.00 | 4878.00 | 2840.50 | 779.00 | 70.00  |
| H | 42.50   | 40.00   | 7817.00 | 3178.00 | 54.50   | 7301.00 | 3625.50 | 1662.50 | 343.00 | 31.00  |

Click 'one color' and select desired color for the maximum value. The color density decreases directly with the value.

Intensity Color

☐ One color LimeGreen

☒ Two colors Upper 255, 0, 0

Lower 0, 0, 192

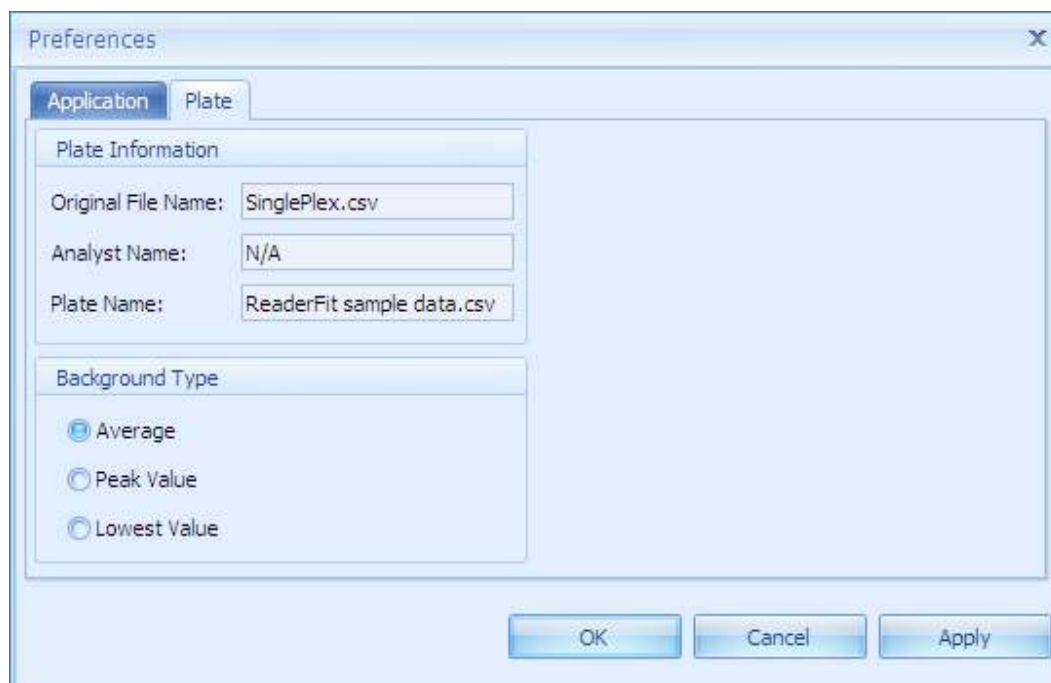


|   | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9      | 10     |
|---|---------|---------|---------|---------|---------|---------|---------|---------|--------|--------|
| A | 10.00   | 9.00    | 19.00   | 6903.00 | 48.50   | 1162.00 | 5573.00 | 2145.00 | 443.00 | 187.00 |
| B | 9929.00 | 8989.50 | 6994.00 | 4487.00 | 64.00   | 179.00  | 4746.00 | 1005.00 | 209.50 | 95.00  |
| C | 5850.50 | 5807.00 | 1297.00 | 4276.00 | 37.50   | 18.00   | 3271.00 | 7722.50 | 104.00 | 45.00  |
| D | 2656.00 | 3049.00 | 63.50   | 3277.00 | 1846.00 | 109.00  | 1957.00 | 6467.00 | 43.00  | 32.00  |
| E | 1104.50 | 914.00  | 57.50   | 1501.00 | 1842.00 | 138.00  | 7233.00 | 5535.50 | 27.50  | 185.00 |
| F | 300.00  | 404.00  | 1396.00 | 433.00  | 133.50  | 15.00   | 6247.50 | 3727.50 | 15.00  | 99.00  |
| G | 143.50  | 116.00  | 7453.00 | 61.00   | 58.00   | 7190.00 | 4878.00 | 2840.50 | 779.00 | 70.00  |
| H | 42.50   | 40.00   | 7817.00 | 3178.00 | 54.50   | 7301.00 | 3625.50 | 1662.50 | 343.00 | 31.00  |

Click 'two colors' and select desired color for the maximum and minimum value. The color shifts upper to lower directly with the value.

**Figure 4.32 Example of Intensity color**

## Plate Preferences



### Plate Information

|                           |                                                                                                                       |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------|
| <b>Original File Name</b> | Displays the name assigned to the result file in the plate reader software. To edit the plate name, enter a new name. |
| <b>Analyst Name</b>       | Displays the analyst name entered in the plate reader.                                                                |
| <b>Plate Name</b>         | Shows plate name of this file.                                                                                        |

### Background type

|                     |                                                                                                                                                                                                                                                             |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Average</b>      | Calculate average value in the background group.<br>Background (Bkg) Response Value = (Bkg Response Value <sub>1</sub> + Bkg Response Value <sub>2</sub> + ... Bkg Response Value <sub>n</sub> )/n<br>where n = the number of background wells in the plate |
| <b>Peak Value</b>   | Take highest value in the background group.                                                                                                                                                                                                                 |
| <b>Lowest Value</b> | Take lowest value in the background group.                                                                                                                                                                                                                  |

### Threshold

You can select one of the criteria for threshold marker from Response Value, Concentration and Error range. Select one of them and enter a Response Value, concentration or error range threshold for a plate. The software automatically marks wells that contain data less than the user specified threshold with a red border (Figure 4.18).

To set a threshold(s):

1. Check '**Show threshold marker**' box
  2. Check one of the radio button in front of the data type you want to use as a threshold marker.
  3. Select equity equal symbol and input the value in the box.
  4. Click **Apply** to reflect current setting to the plate, or click **OK** to reflect and close the dialog box.
- ⇒ A red border marks wells that contain data less than or greater than threshold for all analyte (Figure 4.28).

|   | 1        | 2        | 3       | 4       | 5       | 6       | 7       | 8       | 9      | 10     |
|---|----------|----------|---------|---------|---------|---------|---------|---------|--------|--------|
| A | 28.00    | 29.00    | 40.50   | 1022.00 | 126.00  | 1932.50 | 1964.00 | 787.00  | 271.00 | 105.00 |
| B | 11083.00 | 10831.00 | 587.00  | 5457.00 | 133.50  | 621.50  | 1465.00 | 472.50  | 128.00 | 66.00  |
| C | 9072.00  | 8899.00  | 58.00   | 5459.00 | 201.00  | 62.00   | 866.50  | 5011.50 | 80.00  | 48.00  |
| D | 7044.00  | 7087.00  | 63.00   | 4589.50 | 481.00  | 50.00   | 511.00  | 3186.00 | 47.00  | 38.00  |
| E | 4987.00  | 4440.00  | 95.50   | 575.00  | 2722.00 | 415.00  | 4701.00 | 2289.50 | 41.00  | 154.00 |
| F | 2824.00  | 2861.00  | 280.00  | 218.50  | 72.00   | 49.00   | 3372.00 | 1472.00 | 30.00  | 89.00  |
|   | 1484.00  | 1451.00  | 2190.00 | 157.50  | 63.00   | 4727.00 | 2160.50 | 831.00  | 310.50 | 59.00  |

**Figure 4.31 Well grid**

### Outlier Options

**Show threshold  
Marker**

Show red rectangle indicator inside the grid if the threshold conditions meet the criteria.

**Response Value**

Use Response Value for threshold conditions.

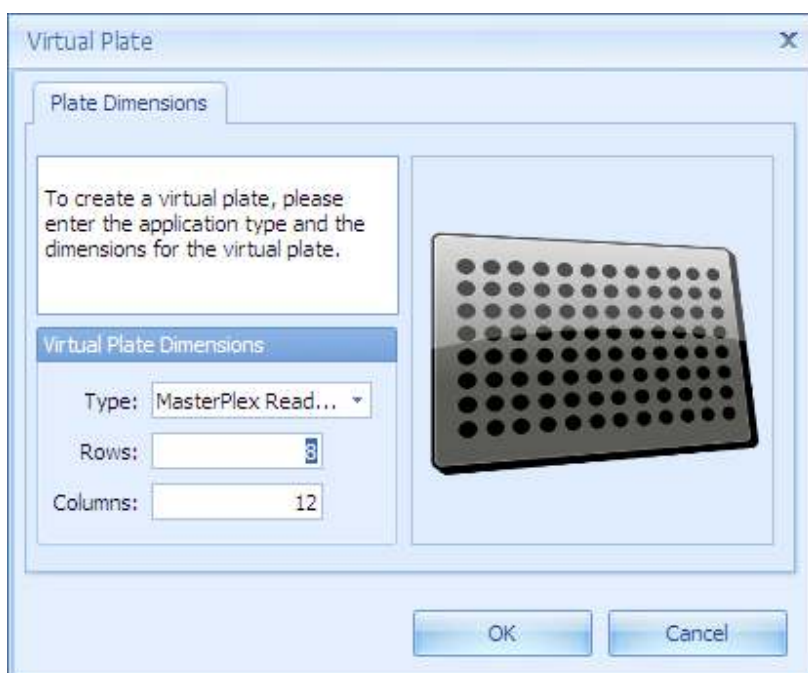
---

|                           |                                                                                                                                                |
|---------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Concentration</b>      | Use Concentration value for threshold conditions.                                                                                              |
| <b>Error Range</b>        | Use Error Range for threshold conditions.                                                                                                      |
| <b>Automatic outliers</b> | Automatically check on/off the outlier check box for the wells. To check on, click <b>Set</b> button. To check off, click <b>Clear</b> button. |

## 4.7

### Creating a Virtual Plate

1. Open the measured results files (.csv, .txt or .xls) or MasterPlex® ReaderFit files (.mlx\*) that are the data sources for the virtual plate.
2. Click the **Virtual Plate** button   
⇒ The Virtual Plate dialog appears (Figure 4.33).



**Figure 4.33 Plate Wizard, Plate Dimensions tab**

3. Enter the number of rows and columns for the virtual plate. Click **OK**.  
⇒ A module window opens and displays the empty well grid of the virtual plate (Figure 4.34).

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |
| G |   |   |   |   |   |   |   |   |   |    |    |    |
| H |   |   |   |   |   |   |   |   |   |    |    |    |

**Figure 4.34 Virtual plate**

### Selecting Data from a Source Plate

The virtual pipette copies (*aspirates*) data from user-selected wells in a source plate and pastes (*dispenses*) the data into a virtual plate. The virtual pipette copies all of the analyte data in a well, including the computed analyte concentrations. It remains loaded until you dispense or clear the pipette.



**NOTE:** The data source plates must contain the same type and number of analytes, otherwise concentrations cannot be calculated. If the source plates contain the same number of analytes, but they are named differently, use the virtual analyte filter to rename analytes so that the nomenclature is consistent. (See *Working with the Virtual Analyte Filter* on section 4.8.)

1. In the source plate, select the wells of interest.

To select adjacent wells, press and hold the mouse button while you drag the mouse pointer to select the wells of interest.



**NOTE:** Selecting non-adjacent wells is not recommended.

2. Right-click the selected wells and select **Aspirate** from the pop-up menu that appears (Figure 4.35).
  - ⇒ The data for the analytes in the selected wells are added to the virtual pipette and is ready to dispense into a virtual plate.

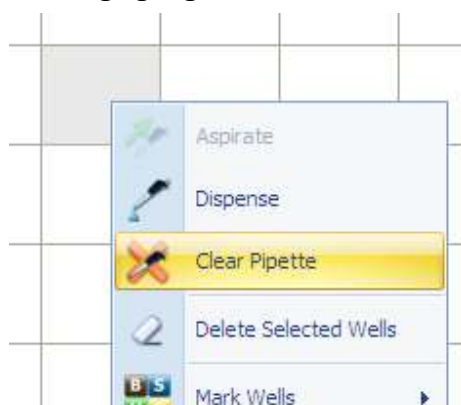
 **NOTE:** If the background is subtracted in the source plate, the virtual pipette aspirates and transfers background-subtracted values. If you do not want to aspirate background-subtracted values, make sure the background subtraction is turned off before you aspirate data into the virtual pipette. (Click the  button to turn background subtraction on or off.)

|   | 1        | 2        | 3        | 4      |
|---|----------|----------|----------|--------|
| A | 41.00000 | 39.00000 | 37.00000 | 112.00 |
| B | 10516.00 | 10034.50 | 66.00000 | 4911.0 |
| C | 6152     |          |          | 11.0   |
| D | 3104     |          |          | 91.0   |
| E | 1040     |          |          | 70.0   |
|   | 292.0    |          |          | 29.0   |

**Figure 4.35 Aspirating Data**

*Right-click selected wells to display the pop-up menu.*

- To clear the data from the virtual pipette, right-click and select **Clear** from the pop-up menu (Figure 4.36).



**Figure 4.36 Clear Aspirated Data**

### Adding Data to a Virtual Plate

After the virtual pipette aspirates data from the source plate, it is ready to dispense the data into the virtual plate.

1. Position the mouse pointer over the virtual plate.
2. Click the first well to which the data will be added.
3. Right-click the well and select **Dispense** from the pop-up menu that appears.
  - ⇒ The data are added to the virtual plate (in the same configuration as in the source plate) (Figure 4.37).

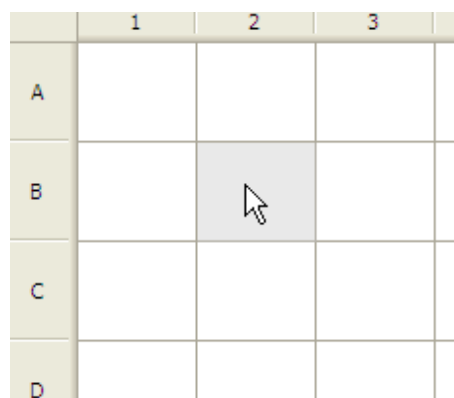
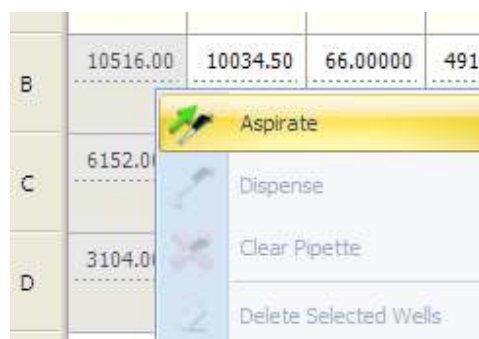


**NOTE:** If the number or names of the analytes in the virtual pipette is different from that in the virtual plate, the virtual analyte filter automatically appears. For more information on using the filter, see *Working With the Virtual Analyte Filter* on section 4.9.

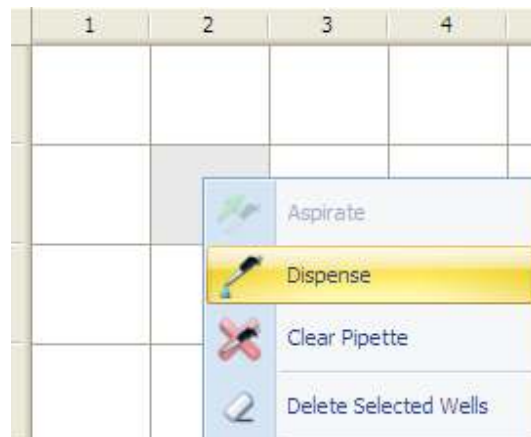


**NOTE:** Data in a virtual plate cannot be removed, but can be overwritten.

1. Open a .mlx or .csv.
2. Select the wells of interest in the source plate (.csv or .mlx). Right-click the selected wells and choose aspirate from the pop-up menu.
3. In the virtual plate, select the first well where you want to dispense the data.



4. Right-click the well and select **Dispense** from the pop-up menu.



5. The data are added to the virtual plate (starting at the selected well) in the same configuration as in the source plate.

|  | 1 | 2        | 3 | 4 |
|--|---|----------|---|---|
|  |   |          |   |   |
|  |   | 10516.00 |   |   |
|  |   | 6152.00  |   |   |
|  |   | 3104.00  |   |   |
|  |   | 1040.00  |   |   |

**Figure 4.37 Adding data to a virtual plate**

Open a source plate (.mlx or .csv, .txt or .xls) and create a virtual plate (click the  button to generate the blank virtual plate).

## 4.8

### Working With the Virtual Analyte Filter

In a multiplex assay, all of the plate wells must contain:

- The same types of analytes with the same nomenclature
- The same number of analytes

This is true for virtual plates as well. When you add data to a virtual plate, MasterPlex® ReaderFit compares the name and number of the analytes in the virtual pipette to those in the virtual plate. The virtual pipette will not dispense if there are discrepancies between the number or names of analytes in the pipette and the virtual plate. If the number of analytes in the pipette is greater than that of the destination plate, the virtual analyte filter automatically appears (Figure 4.38).

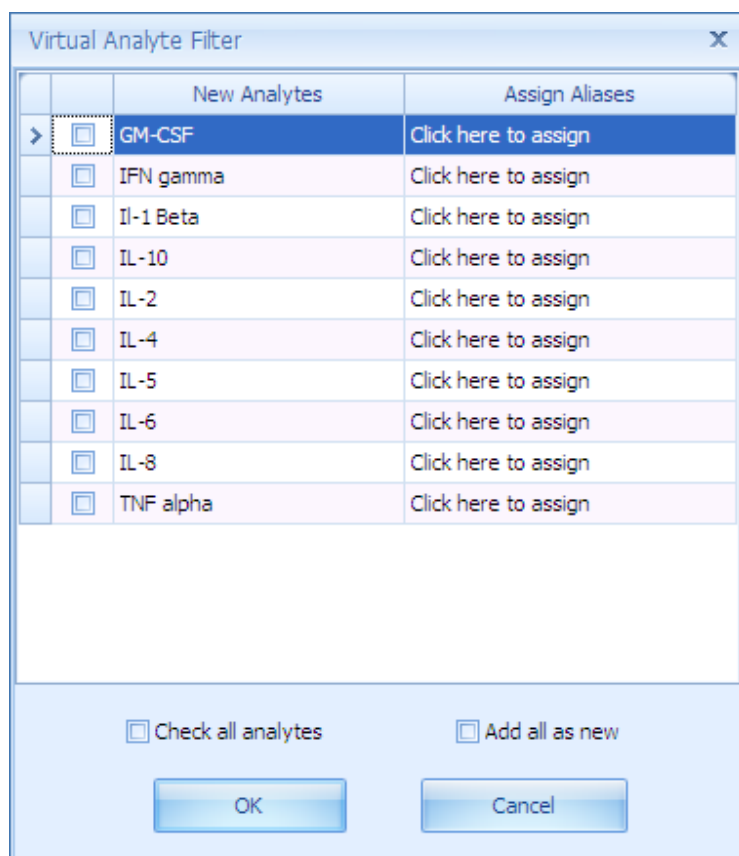
The virtual analyte filter displays a list of the analytes that are present in the virtual pipette. It enables you to choose the analytes that you want to add to the virtual plate and, if necessary, rename them to be consistent with the number and name of analytes in the virtual plate.

If you add data to a virtual plate from source wells that contain different analyte names or a different number of analytes, data holes are created. As a result, a well in the virtual plate appears blank if the analyte selected in the analyte panel is not present in the well. If a plate file (.csv, .txt, xls, .mlx, or virtual) contains data holes, the concentrations cannot be calculated.



**NOTE:** In order to prevent data holes, if the number of analytes in the virtual pipette is less than the number of analytes in the destination plate, the data cannot be added to the virtual plate.

---

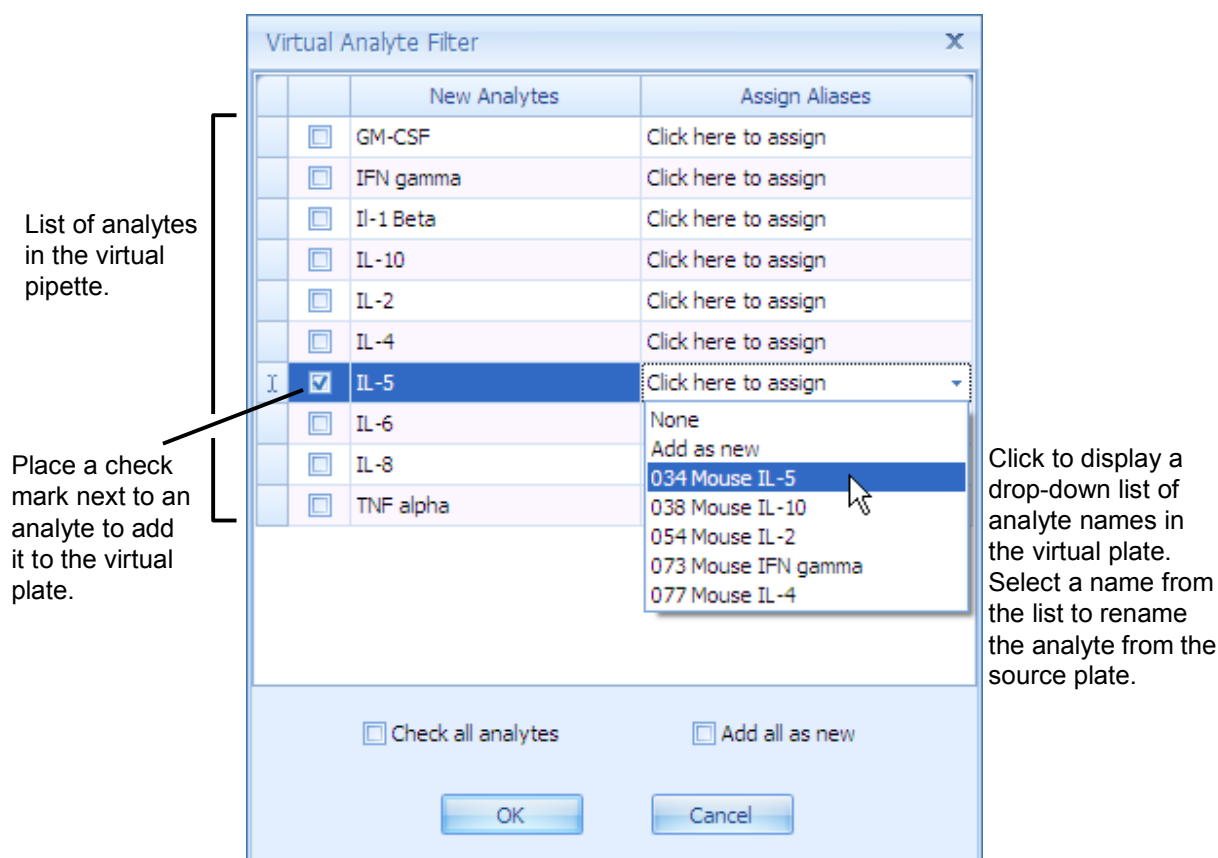


**Figure 4.38 Virtual analyte filter shows the analytes in the virtual pipette**

### Selecting and Renaming Analytes

If the virtual analyte filter appears, you must select and, if necessary, rename the analytes to match the number and names of the analytes in the virtual plate.

1. In the virtual analyte filter (Figure 4.38), place a check mark next to each analyte that you want to add to the virtual plate. To select all analytes for the virtual plate, click **Check All**.
2. To rename an analyte so that it is consistent with the nomenclature in the virtual plate:
  - a. Click **here to assign** next to the analyte that you want to rename.
    - ⇒ A drop-down list shows the names of the analytes in the virtual plate (Figure 4.39).
  - b. Select a name from the drop-down list.
    - ⇒ The virtual analyte filter displays the new name for the analyte.



**Figure 4.39 Virtual analyte filter**

3. To save the renaming assignments for use again with the same source plate (.csv, .txt or .xls or .mxqs) during the current session, choose the **Save this assignment** option.

If you want to aspirate other data from the same source plate, choose the **Use last saved assignments** option in the virtual analyte filter to automatically rename all of the analytes in the filter.

4. Click **OK**.

⇒ The data are added to the virtual plate and the virtual analyte filter closes.

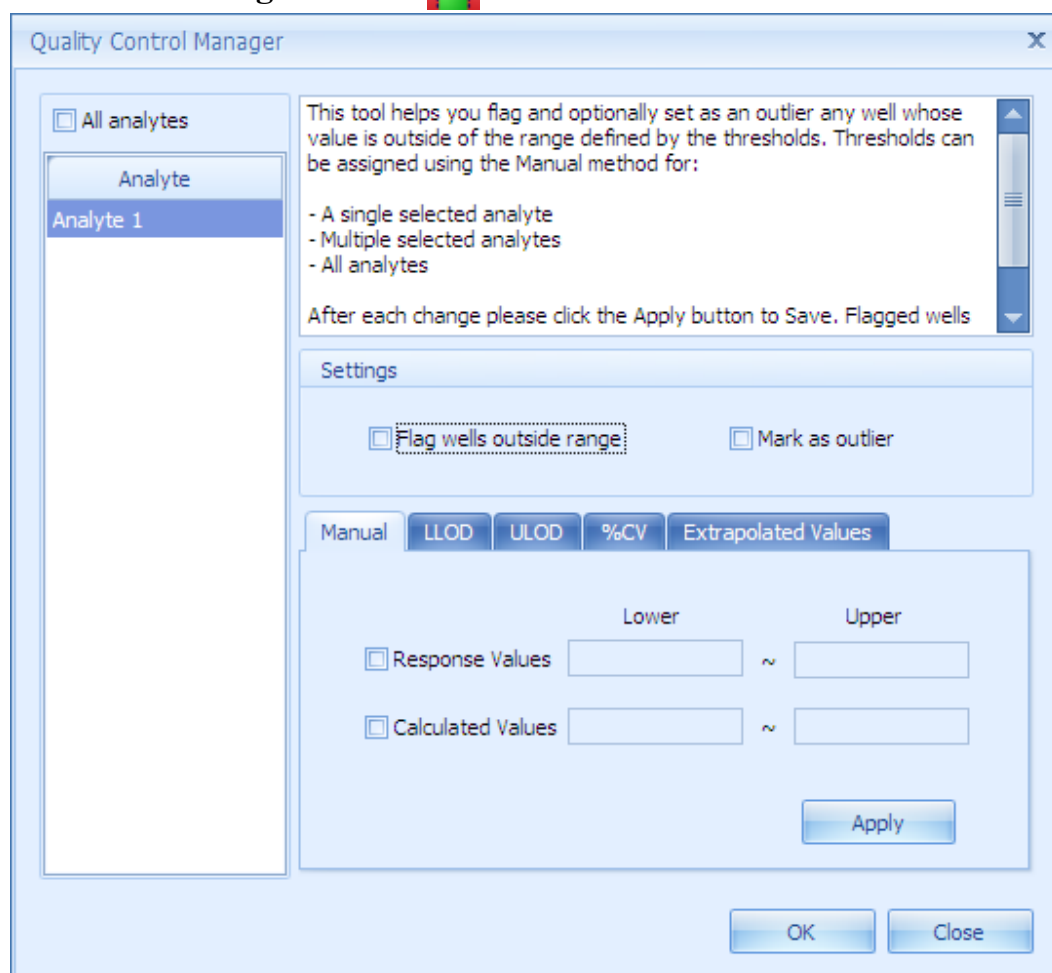
## 4. 9

### Quality Control Manager

Quality Control Manager helps you flag and optionally set as an outlier any wells whose value is outside of the range defined by the thresholds.

Thresholds can be assigned using the manual method for

- A single selected analyte
  - Multiple selected analytes
  - All analytes
- To open the Quality Control Manager (Figure 4.40), click the **Quality Control Manager** button .



**Figure 4.40 Quality Control Manager dialog**

The software automatically marks wells that contain data less than the user

specified threshold with a red border (Figure 4.41).

To set a threshold(s):

1. Select analytes you want to attach the threshold criteria from the analyte pane.
  - ⇒ Use All analytes check box or Ctrl key for multiple selection.
2. Check ‘**Show threshold marker**’ box and/or ‘**Mark as outlier**’ box.
3. Select the threshold criterion from the threshold tab.
4. Set threshold conditions and click apply button. Close the dialog box.
  - ⇒ A red border marks wells that contain data meet the threshold criteria.
  - ⇒ If you choose ‘**Mark as outlier**’ at the same time, the data are marked as outlier and outlier check boxes are checked (Figure 4.42).

|   | 1        | 2        | 3     | 4       | 5       | 6       | 7     | 8     | 9     | 10    |
|---|----------|----------|-------|---------|---------|---------|-------|-------|-------|-------|
| A | 41.00    | 39.00    | 37.00 | 112.00  | 92.00   | 2426.50 | 43.00 | 36.00 | 48.00 | 80.00 |
| B | 10516.00 | 10034.50 | 66.00 | 4911.00 | 106.50  | 186.00  | 41.00 | 38.00 | 45.50 | 55.00 |
| C | 6152.00  | 6299.00  | 39.00 | 5011.00 | 65.00   | 43.00   | 38.00 | 52.00 | 40.00 | 45.00 |
| D | 3104.00  | 2990.50  | 41.00 | 5591.00 | 3989.50 | 113.00  | 38.00 | 44.00 | 40.00 | 42.00 |
| E | 1040.00  | 1094.00  | 45.00 | 3070.00 | 1808.00 | 270.00  | 49.00 | 40.00 | 36.00 | 47.00 |
| F | 292.00   | 300.00   | 39.00 | 1129.00 | 119.00  | 41.00   | 42.00 | 40.00 | 37.00 | 49.00 |
|   | 100.00   | 104.00   | 45.00 | 107.00  | 111.00  | 70.00   | 41.00 | 38.00 | 36.00 | 41.00 |

**Figure 4.41 Well grid**

|   | 1                                   | 2                                   | 3                                   | 4                                   | 5                                   | 6                                   | 7                                   | 8                                   | 9                                   | 10                                  |
|---|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| A | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            |
| B | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| C | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            |
| D | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> |
| E | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            |
| F | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            | <input type="checkbox"/>            |
|   | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/>            |

**Figure 4.42 Outlier check boxes**

## Settings

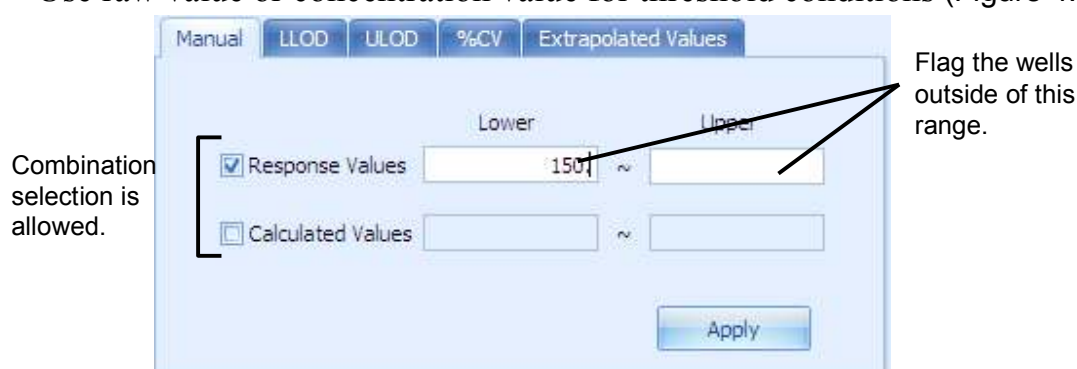
**Flag wells outside range** Show red rectangle indicator inside the grid if the threshold conditions meet the criteria.

**Mark as outlier** Mark flagged data as outlier

## Threshold Options

### Manual

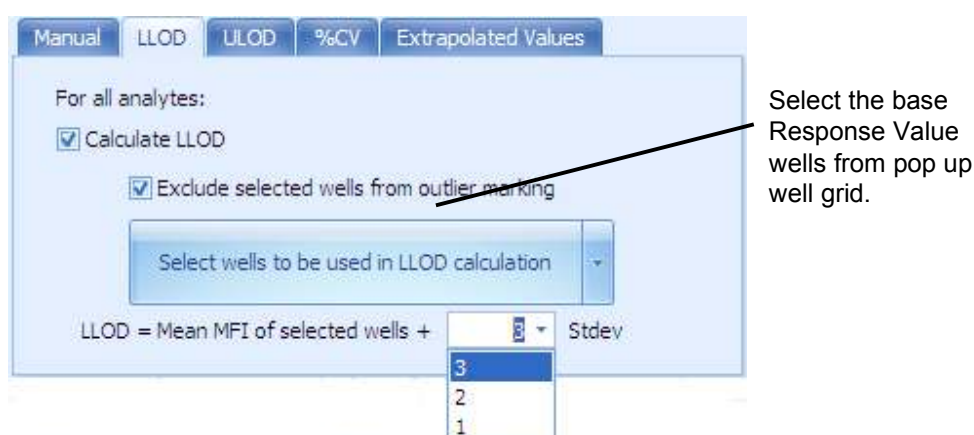
Use raw value or concentration value for threshold conditions (Figure 4.43).



**Figure 4.43 Manual threshold tab**

### LLOD(Lower Limit of Detection)

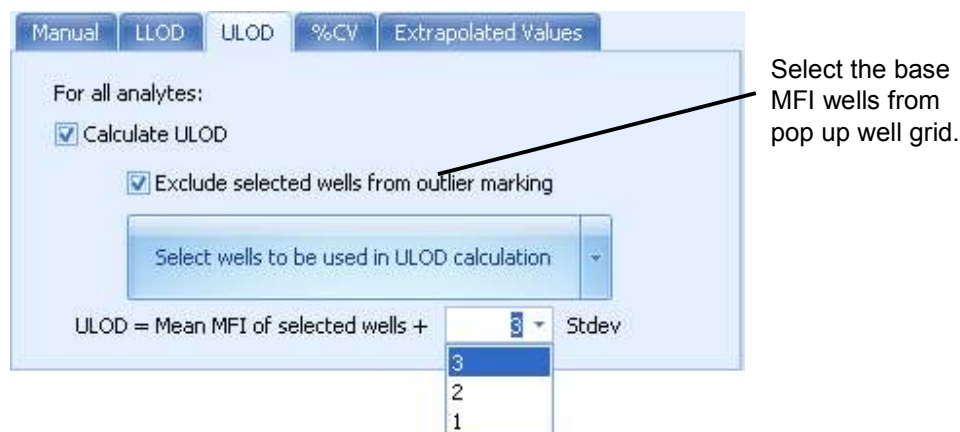
Flag the lower values than the LLOD calculation value (Figure 4.44). LLOD is based on the Response Value mean value of the selected wells plus the standard deviation multiplied by the user selected number.



**Figure 4.44 LLOD tab**

### ULOD(Upper Limit of Detection)

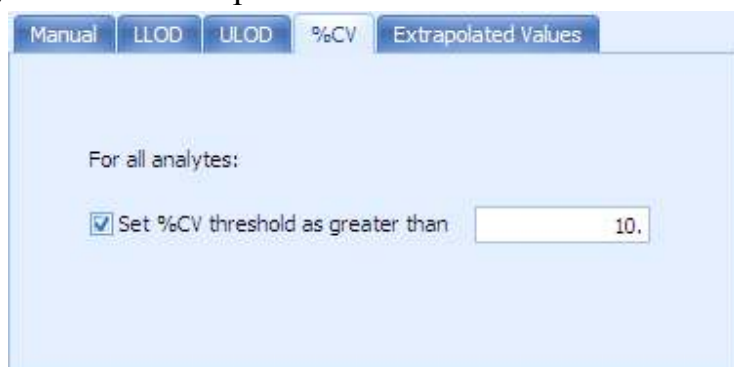
Flag the upper values than the ULOD calculation value (Figure 4.45). ULOD is based on the MFI mean value of the selected wells plus the standard deviation multiplied by the user selected number.



**Figure 4.45 ULOD tab**

### CV

Use %CV value of the group as a threshold criterion (Figure 4.46). Flag the values greater than the specified %CV value.

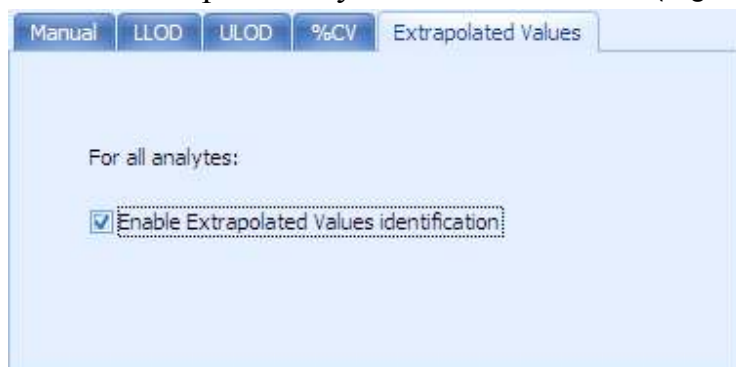


**Figure 4.46 %CV tab**

---

### **Extrapolated Values**

Flag the values extrapolated by the standard curves (Figure 4.47).



**Figure 4.47 Extrapolated values tab**

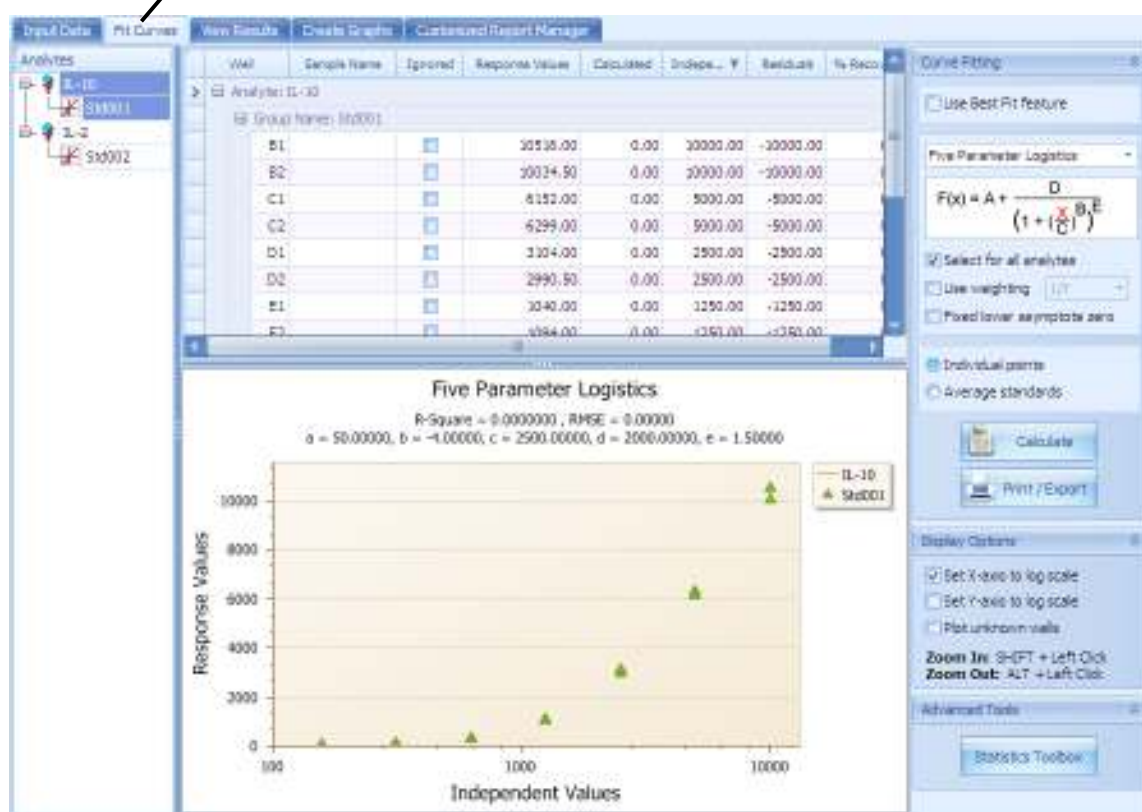
**- Fit Curve tab**

*This chapter explains how to generate standard curves and compute (interpolate or extrapolate) analyte concentrations from the standard curves.*

**5.1****Go to Fit Curves Tab**

Click **Fit Curves** tab then application window displays the Fit Curves tab page (Figure 5.1).

Fit Curves tab



Standard Data  
Group pane

Standard Curve Data Grid and Chart

Commands and  
Display Options

**Figure 5.1 Fit Curves page**

Each well in a standard data set represents an x,y data point. The Response value is plotted on the y-axis and the concentration is plotted on the x-axis. MasterPlex® ReaderFit uses nonlinear regression (curve fitting) analysis to fit a user-specified model equation to the standard data set and generate a standard curve.



---

**NOTE:** The standard curve may not pass through each point in the standard data set.

---

The software computes the  $R^2$  value ( $0 \leq R^2 \leq 1$ ) for the model equation.  $R^2$  measures the goodness of fit of the model equation to the standard data set (where  $R^2 = 1$  is the probability that the model predicts the data perfectly). The steps to create a standard curve include:

1. Mark the standard wells.
2. Link the standard data set to the unknown well group(s) of interest.  
(The analyte concentrations are interpolated from the standard curve that is linked to the unknown well group.)
4. Enter the standard concentrations.
5. Select a model equation for the standard data set.
6. Calculate the standard curves.



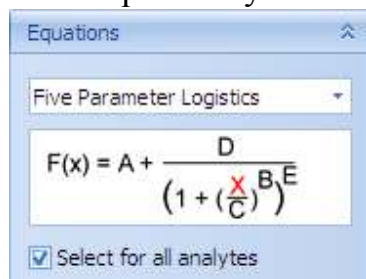
---

**NOTE:** A plate can have more than one standard data set. The standard data sets may have different concentrations or model equations.

---

### Selecting a Model Equation for the Standard Data Set

1. Select an analyte from the left analyte pane.
2. In the right pane, select one equation from the drop-down list.  
⇒ Equation symbol is shown under the drop-down list (Figure 5.2).



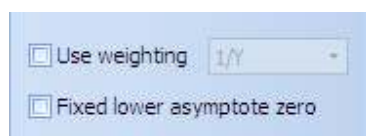
**Figure 5.2 Model Equations drop-down list**

*Model equations available for regression analysis of a standard data set*

3. Select a model equation.
4. To apply the selected model to all analytes, choose the **Select for all analytes** option.
5. To apply weighting during curve fitting, choose the **Use Weighting** option and select a weighting method from the drop-down list.
6. To fix the lower asymptote to zero (sets A = 0), select the **Fixed lower asymptote zero** option (Figure 5.3). This is an option of the Five Parameter Logistics and Four Parameter Logistics equations.



**NOTE:** This feature is reasonable to use if enough background was subtracted and the data has little user error, but for most data sets the  $R^2$  and concentration values will not be improved with this feature on.



**Figure 5.3 Weighting and Fixed asymptote option**

*Model equations available for regression analysis of a standard data set*



**NOTE:** For more information about model equations and weighting methods, see Appendix C.

## 5.2

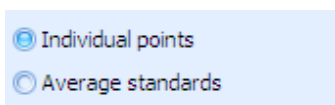
**Generating Standard Curves & Computing****Analyte Concentrations**

MasterPlex® ReaderFit carries out a two step calculation sequence when it fits the standard curves. The software:

- Fits a standard curve for all defined standard data sets
- Interpolates or extrapolates analyte concentrations for the unknown groups that are linked to the standard data set

**Standard Points Options** (Figure 5.4)

|                                       |                                                                                        |
|---------------------------------------|----------------------------------------------------------------------------------------|
| <b>Individual points</b><br>(default) | Displays each point in the standard data set individually on the standard curve chart. |
| <b>Average standards</b>              | Displays averaged data points within the same standard values, with an error bar.      |

**Figure 5.4 Standard Points option****Standard Points Options** (Figure 5.5)

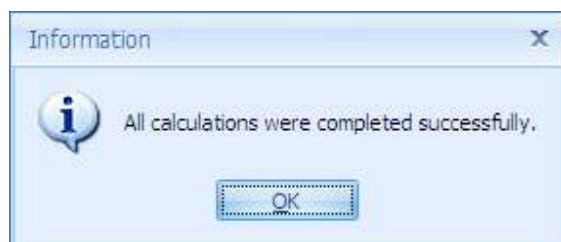
|                                |                                            |
|--------------------------------|--------------------------------------------|
| <b>Set X-axis to log scale</b> | Plot X-axis data on the chart by log scale |
| <b>Set Y-axis to log scale</b> | Plot X-axis data on the chart by log scale |
| <b>Plot unknown wells</b>      | Plot unknown wells on the curve            |

**Figure 5.5 Chart Scale option**

### Generating Standard Curves

1. To generate the standard curves and compute (interpolate or extrapolate) the analyte concentrations, click the **Calculate** button  **Calculate**

⇒ A message box confirms the calculations are completed (Figure 5.6).

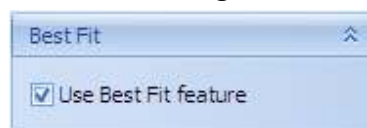


**Figure 5.6 Message box**

### Generating Standard Curves by using Best Fit

1. To generate the standard curves and compute the analyte concentrations by using Best Fit feature, check 'Use Best Fit feature' on (Figure 5.7), then click the **Calculate** button  **Calculate**.

⇒ A message box confirms the calculations are completed (Figure 5.6).

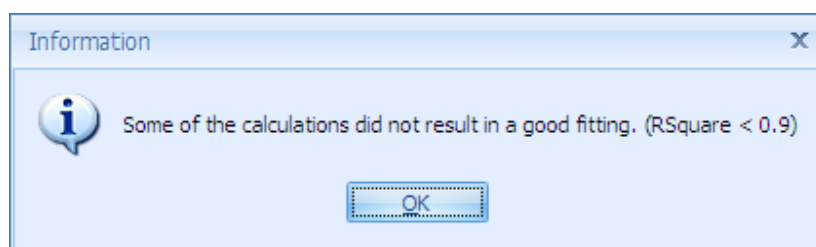


**Figure 5.7 Best Fit feature check box**

---

 **NOTE:** If overall R-square value is less than 0.9, a warning message box appears (Figure 5.8).

---



**Figure 5.8 Warning Message box**

## 5.3

## Reviewing Calculated Standard Data

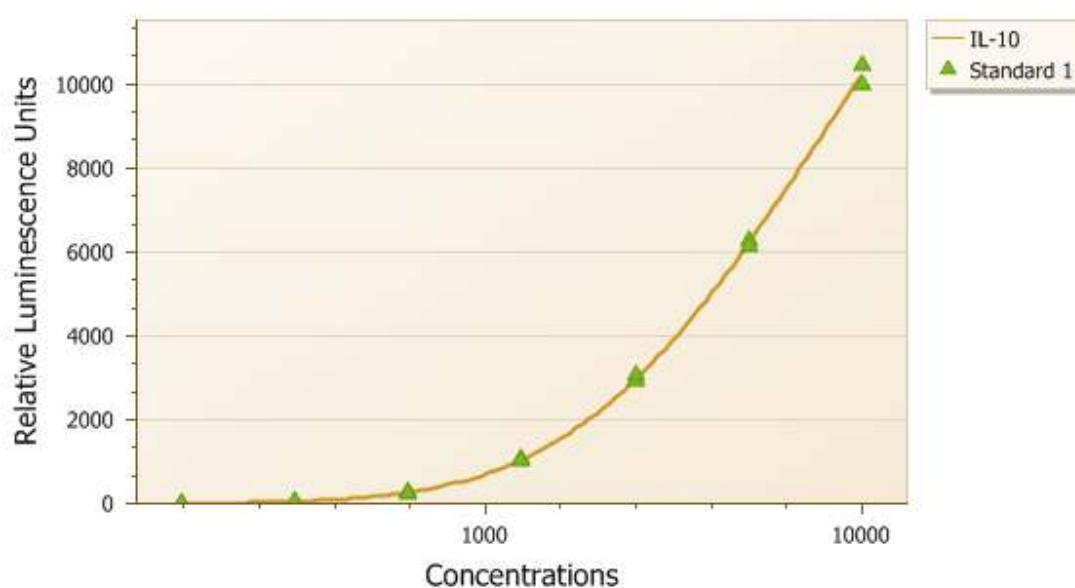
1. Standard data grid and Standard chart view are updated (Figure 5.7).

| Well                   | Sample Name | Ignored                  | RLU      | Calculated | Expected ▼ | Residuals | % Recovery |
|------------------------|-------------|--------------------------|----------|------------|------------|-----------|------------|
| Analyte: IL-10         |             |                          |          |            |            |           |            |
| Group Name: Standard 1 |             |                          |          |            |            |           |            |
| B1                     |             | <input type="checkbox"/> | 10516.00 | 10429.40   | 10000.00   | 429.40    | 104.29     |
| B2                     |             | <input type="checkbox"/> | 10034.50 | 9609.67    | 10000.00   | -390.33   | 96.10      |
| C1                     |             | <input type="checkbox"/> | 6152.00  | 4908.06    | 5000.00    | -91.94    | 98.16      |
| C2                     |             | <input type="checkbox"/> | 6299.00  | 5042.17    | 5000.00    | 42.17     | 100.84     |
| D1                     |             | <input type="checkbox"/> | 3104.00  | 2567.05    | 2500.00    | 67.05     | 102.68     |
| D2                     |             | <input type="checkbox"/> | 2990.50  | 2491.46    | 2500.00    | -8.54     | 99.66      |
| E1                     |             | <input type="checkbox"/> | 1040.00  | 1211.88    | 1250.00    | -38.12    | 96.95      |
| E2                     |             | <input type="checkbox"/> | 1094.00  | 1249.38    | 1250.00    | -0.62     | 99.95      |

## Five Parameter Logistics

R-Square = 0.9999550

a = -1.632, b = -0.780, c = 1333.200, d = 22988.260, e = 4.286,



**Figure 5.9 Standard Data Table and Chart**

2. To view multiple standard data, select the analyte from the analyte pane during [CTRL] key pressing down.

⇒ Data table and chart are updated by the multiple standard (Figure 5.8).

## STANDARD CURVES &amp; CONCENTRATION

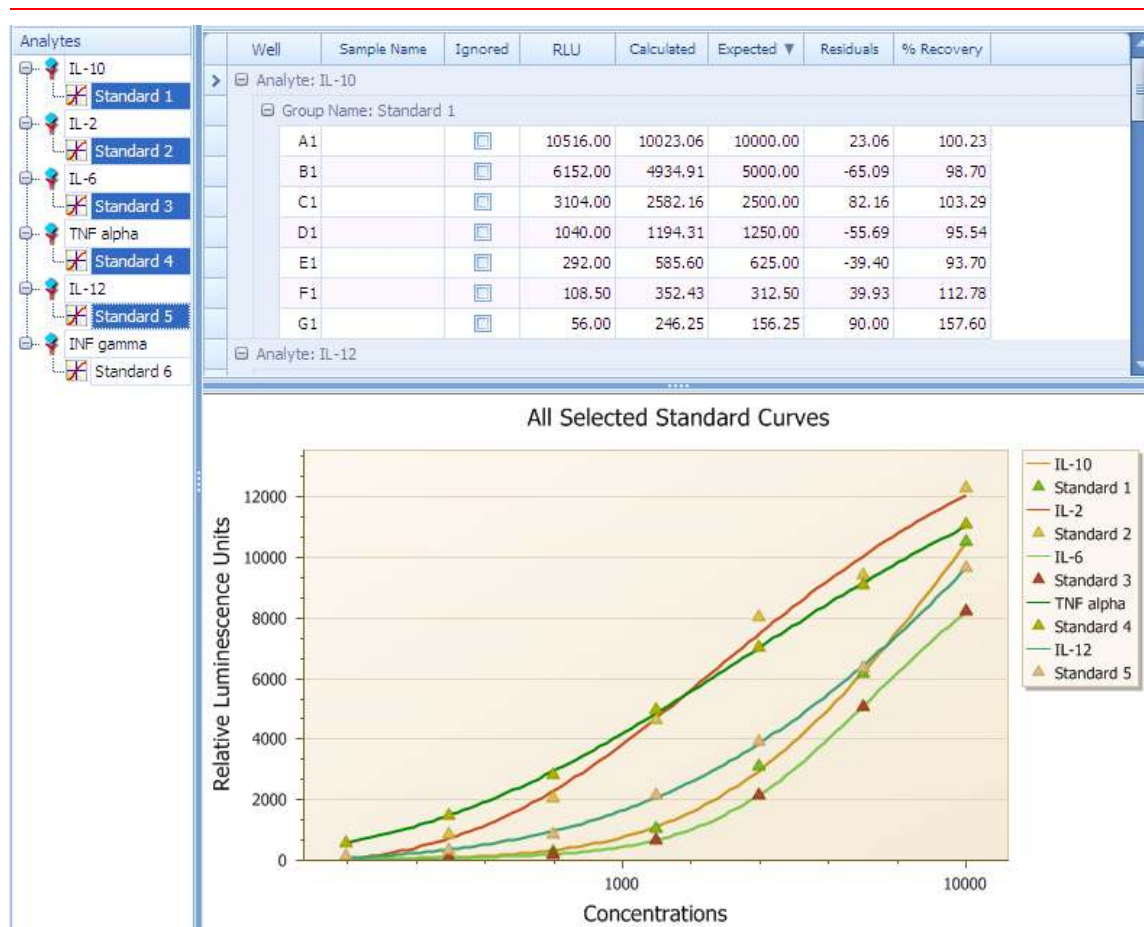
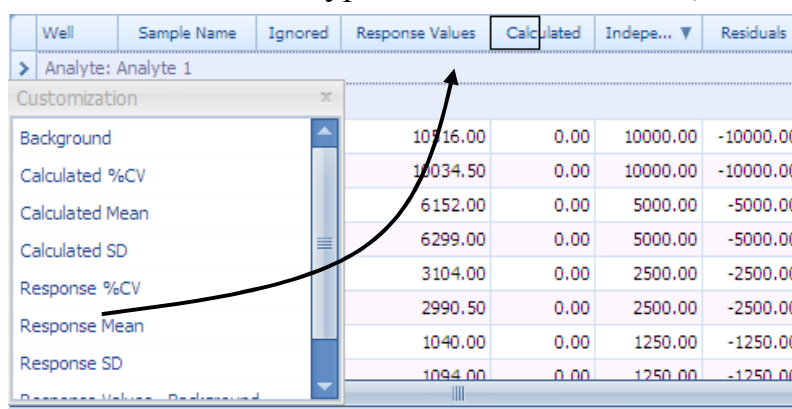


Figure 5.10 Multiple Standard Data

2. In the standard data grid, you can add, delete, sort, interchange the column position and set filtering for customized data viewing (Figure 5.9, 10, 11).

⇒ There are 15 data types available to review (Table 5.1).



Select one of the data types from the pop-up menu and drag&drop it onto the data grid where you would like to add the column.

Figure 5.11 Add column from column chooser box

|   | Sample Name     | Ignored                  | Response Values | Calculated | Independ... | Residuals |
|---|-----------------|--------------------------|-----------------|------------|-------------|-----------|
|   | Up Name: Std001 |                          |                 |            |             |           |
| 2 |                 | <input type="checkbox"/> | 55.00           | 199.04     | 156.25      | 42.79     |
| 1 |                 | <input type="checkbox"/> | 56.00           | 203.36     | 156.25      | 47.11     |
| 2 |                 | <input type="checkbox"/> | 104.00          | 336.75     | 312.50      | 24.25     |
| 1 |                 | <input type="checkbox"/> | 108.50          | 345.86     | 312.50      | 33.36     |
| 2 |                 | <input type="checkbox"/> | 300.00          | 609.27     | 625.00      | -15.73    |
| 1 |                 | <input type="checkbox"/> | 292.00          | 600.61     | 625.00      | -24.39    |

Select the data type you want to delete in the data grid column and drag&drop it away from the column.

Figure 5.12 Delete columns

|  | Sample Name  | Ignored                  | Response Values | Calculated | Independ... | Residuals |
|--|--------------|--------------------------|-----------------|------------|-------------|-----------|
|  | Name: Std001 |                          |                 |            |             |           |
|  |              | <input type="checkbox"/> | 55.00           | 199.04     | 156.25      | 42.79     |
|  |              | <input type="checkbox"/> | 56.00           | 203.36     | 156.25      | 47.11     |
|  |              | <input type="checkbox"/> | 104.00          | 336.75     | 312.50      | 24.25     |
|  |              | <input type="checkbox"/> | 108.50          | 345.86     | 312.50      | 33.36     |
|  |              | <input type="checkbox"/> | 300.00          | 609.27     | 625.00      | -15.73    |
|  |              | <input type="checkbox"/> | 292.00          | 600.61     | 625.00      | -24.39    |

Select the data type you want to move in the data grid and drag&drop it to the new position.

Figure 5.13 Interchange the column position



**NOTE:** There are some other features for the data grid to customize the grid view. See appendix A section A.1 *Grid Customize Menu* for further details.

**Table 5.1 Data Types in the well grid**

| Data Type                  | Description                                                                                                |
|----------------------------|------------------------------------------------------------------------------------------------------------|
| Well                       | Well name                                                                                                  |
| Analyte                    | Analyte name                                                                                               |
| Sample Name                | User-specified name for the well.                                                                          |
| Replicate Group Name       | The group number of the well. Wells that belong to the same group have the same group number.              |
| Ignored                    | If this is checked, this data is not included for the regression analysis.                                 |
| Response Values            | The light intensity measured by the plate reader instrument.                                               |
| Response Values-Background | Background subtracted value from Response Value.                                                           |
| Response Mean              | Shows the Response Value average within the group.                                                         |
| Response SD                | Shows the Response Value standard deviation within the group.                                              |
| Response %CV               | Shows the Response Value %CV within the group.                                                             |
| Calculated                 | The concentration that is calculated (interpolated or extrapolated) from the user-selected standard curve. |
| Independent Values         | Standard data user inputted.                                                                               |
| Calculated Mean            | Shows the concentration average within the group.                                                          |
| Calculated SD              | Shows the concentration standard deviation within the group.                                               |
| Calculated %CV             | Shows the concentration %CV within the group.                                                              |
| Residuals                  | Residual = Observed (or calculated) concentration – Expected concentration                                 |
| %Recovery                  | %Recovery = (Calculated/Expected) x 100.                                                                   |
| Background                 | Response Value background value for the plate.                                                             |

4. Check the fitted curve on the standard curve chart (Figure 5.12).

If the standard curve uses a sigmoidal model (for example, the Four Parameter Logistics equation, Figure 5.12), the software interpolates the analyte concentration when:

Highest standard Response Value  $\leq$  Response Value  $\leq$  Lowest standard data point

The software extrapolates the analyte concentration when:

$A < \text{Response Value} < \text{Lowest standard Response Value}$

or

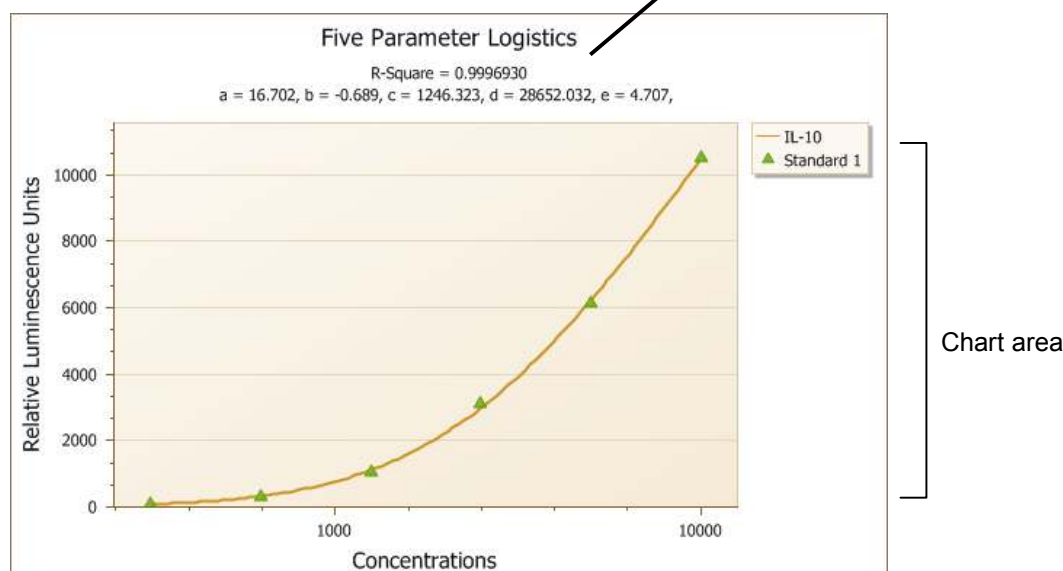
Highest standard Response Value < Response Value < D

where A is the lower asymptote and D is the upper asymptote of the sigmoidal curve (Figure 5.12).

A Response Value less than A or greater than D is beyond the range of the standard curve model and the concentration value cannot be extrapolated.

If Response Value < A, the well grid displays the lowest standard Response Value preceded by < (Figure 5.12). If Response Value > D, the well grid displays the highest standard Response Value preceded by >.

Equation parameters



**Figure 5.14 Five Parameter Logistics model equation, x-axis log scale**

$A = 38.367$  (bottom asymptote),  $D = 22988.119$  (top asymptote). Response Value values less than A or greater than D are beyond the range of the model equation.

### Specifying Outliers for Standard Data Point

1. Click one of the data points on the chart you want to eliminate from the calculation (Figure 5.13).

⇒ Small data window pops up under the cursor and the corresponding data is highlighted in the data table (Figure 5.16).



**Figure 5.15 Click one of the data points on the chart**

|   | Well           | Sample Name | Group... | Ignored                  | Response Values | Calculated | Indepe... ▼ |
|---|----------------|-------------|----------|--------------------------|-----------------|------------|-------------|
|   | Analyte: IL-10 |             |          |                          |                 |            |             |
| > | B1             |             | Std001   | <input type="checkbox"/> | 10516.00        | 10023.06   | 10000.00    |
|   | C1             |             | Std001   | <input type="checkbox"/> | 6152.00         | 4934.91    | 5000.00     |
|   | D1             |             | Std001   | <input type="checkbox"/> | 3104.00         | 2582.16    | 2500.00     |
|   | E1             |             | Std001   | <input type="checkbox"/> | 1040.00         | 1194.31    | 1250.00     |
|   | F1             |             | Std001   | <input type="checkbox"/> | 292.00          | 585.60     | 625.00      |

**Figure 5.16 Corresponding Data is highlighted**

2. Check the corresponding box in the ignored column by clicking on it (Figure 5.17).

|   | Well           | Sample Name | Group... | Ignored                             | Response Values | Calculated | Indepe... ▼ |
|---|----------------|-------------|----------|-------------------------------------|-----------------|------------|-------------|
|   | Analyte: IL-10 |             |          |                                     |                 |            |             |
| I | B1             |             | Std001   | <input checked="" type="checkbox"/> | 10516.00        | 10023.06   | 10000.00    |
|   | C1             |             | Std001   | <input type="checkbox"/>            | 6152.00         | 4934.91    | 5000.00     |
|   | D1             |             | Std001   | <input type="checkbox"/>            | 3104.00         | 2582.16    | 2500.00     |
|   | E1             |             | Std001   | <input type="checkbox"/>            | 1040.00         | 1194.31    | 1250.00     |
|   | F1             |             | Std001   | <input type="checkbox"/>            | 292.00          | 585.60     | 625.00      |

**Figure 5.17 Check on the box by clicking**

## 5.4

**Best Fit Calculation Option**

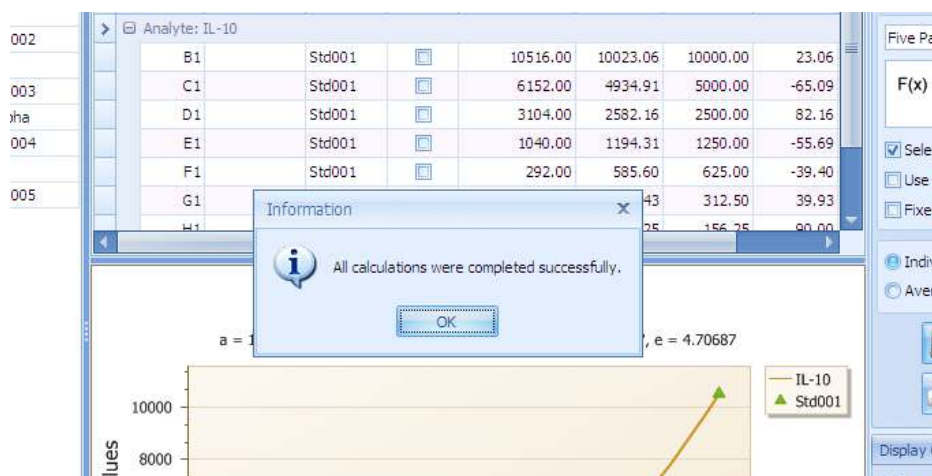
ReaderFit has an advanced feature called **BEST FIT**. This feature finds the best equation model and weighting combination for your standard data automatically, and does this for all analytes.

1. Go to Input Data tab.
2. Click the **BEST FIT**  button.  
 ⇒ Progress window appears and MasterPlex® begins searching for the best combination (Figure 5.16).



**Figure 5.18 Progress Window for BEST FIT Calculation**

3. Once the best fit has been found for all analytes and all calculations have been done, the Fit Curves tab is displayed. (Figure 5.17).



**Figure 5.19 Notification Dialog of BEST FIT calculation**

---

**Best Fit method options**

Best Fit search is based on the index value selected in the preference dialog.

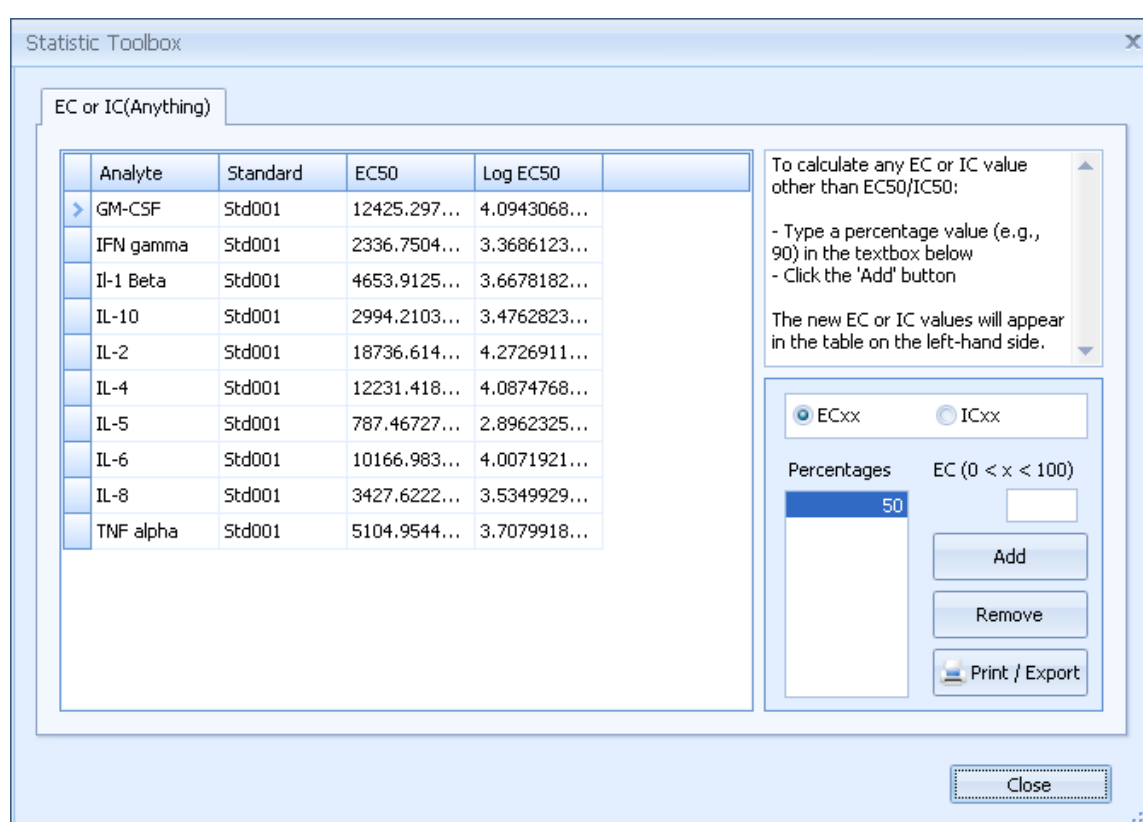
There are three searching index available in the preference dialog.

|                                      |                                                                                                           |
|--------------------------------------|-----------------------------------------------------------------------------------------------------------|
| <b>Root Mean Square Error (RMSE)</b> | Use RMSE index to choose the best curve fit combination.                                                  |
| <b>R-Square</b>                      | Use R-square to choose the best combination.                                                              |
| <b>Least deviation of % Recovery</b> | Use LD of % Recover to choose the best. This index is good for the needs of lower concentration accuracy. |

## 5.5

## Statistics Toolbox

The Statistic Toolbox has a EC(effective concentration) or IC(inhibition concentration) value list (Figure 5.20). MasterPlex® ReaderFit calculates the EC or IC value whenever the fitting curve is drawn. You can check the default EC<sub>50</sub> or IC<sub>50</sub> value from here or you can add any percentage value you want to check.



**Figure 5.20 EC or IC<sub>anything</sub> list**

### Adding a percentage

1. Choose EC or IC you want to display by the radio button.
2. Click the text box above Add button, and then input desired value.
3. Click Add button
  - ⇒ MasterPlex® QT calculates the EC or IC value based on the inputted value and add the result in the list.

## 5.6

## Printing and Exporting the Standard Data

You can print or export the chart and data table of the standard data.

1. Click the Print  button.  
⇒ Print preview window appears (Figure 5.21).

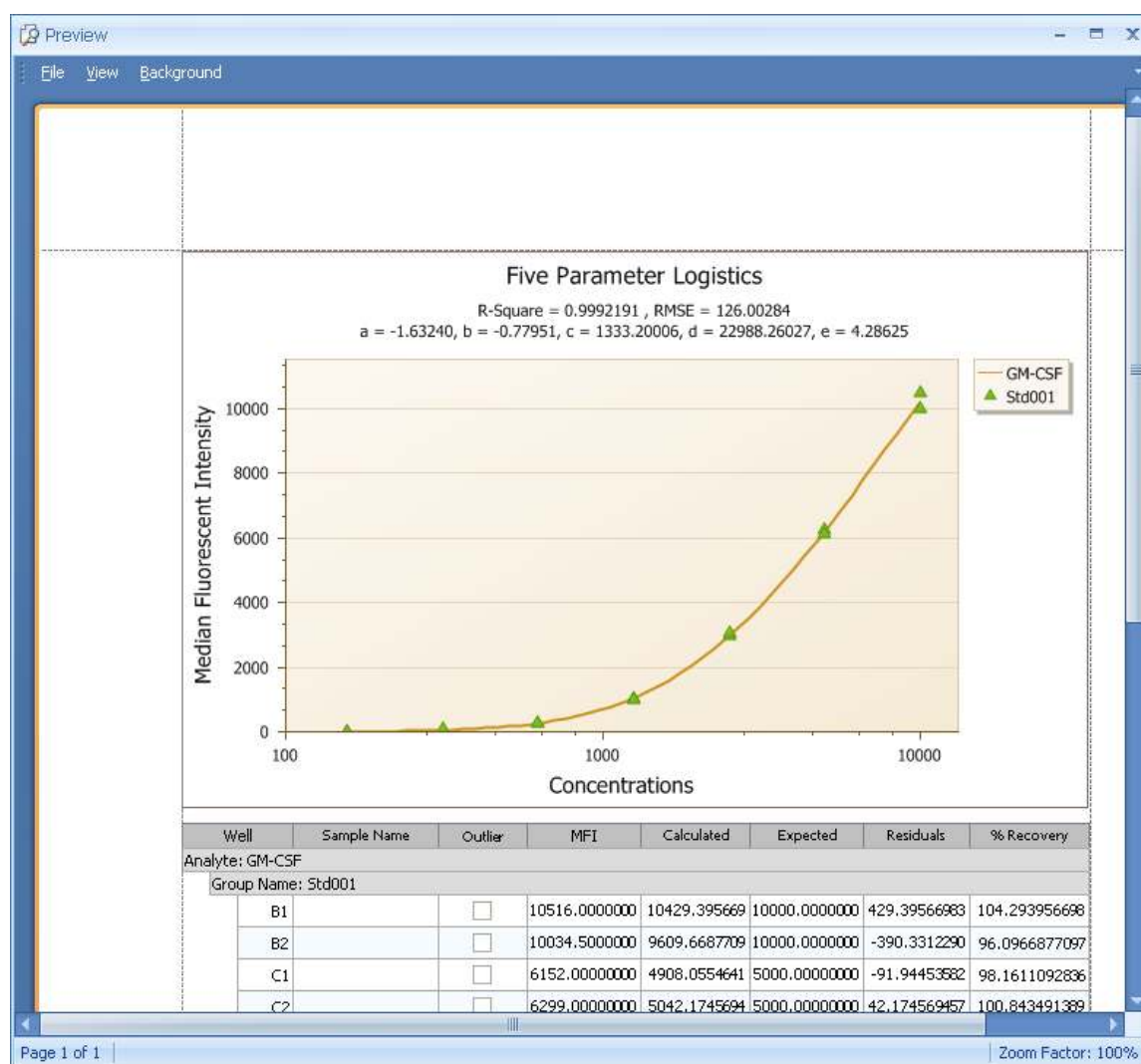


Figure 5.21 Print Preview window

2. To print this, click **Print**  icon from the menu bar.  
⇒ Print setting dialog appears.

3. To export this, click Export Document  drop-down icon.
- ⇒ There are 8 file types and 7 image types to export the document (See appendix A section A.2 *Print Preview Menu* for further details and task options).



**NOTE:** You can also copy or export the Standard Curve chart by right clicking the mouse button on the chart. It will show 'Copy' and 'Export Image' in the pop-up menu. The 'Copy' menu enables you to copy the chart image in bitmap format.

---

## CHAPTER

**6 Reviewing Data – View Results tab**

*View Results tab is to review the data across all analytes. In this tab, you can:*

- Add or delete the data column via column selector box
- Sort or filter the column data
- Change the column layout
- Make groups to categorize the data
- Print or export the data

View Results tab  
/

| Well | Sample Name | Replicate Group... | Response values | Response Mean | Calculated values | Calculated Mean |
|------|-------------|--------------------|-----------------|---------------|-------------------|-----------------|
| B1   | S10001      |                    | 10516.00        | 30275.25      | 10429.40          | 10019.53        |
| C1   | S10001      |                    | 6152.00         | 6225.50       | 4908.06           | 4975.11         |
| D1   | S10001      |                    | 3104.00         | 3047.25       | 2567.05           | 2529.28         |
| E1   | S10001      |                    | 1040.00         | 3057.00       | 1211.88           | 1230.63         |
| F1   | S10001      |                    | 292.00          | 296.00        | 600.61            | 604.94          |
| G1   | S10001      |                    | 106.50          | 306.25        | 345.86            | 341.30          |
| H1   | S10001      |                    | 56.00           | 55.50         | 203.36            | 201.20          |
| A2   |             |                    | 39.00           | 0.00          | 0.00              | 0.00            |
| B2   | S10001      |                    | 10034.50        | 30275.25      | 9809.67           | 10019.53        |
| C2   | S10001      |                    | 6259.00         | 6225.50       | 5042.17           | 4975.11         |
| D2   | S10001      |                    | 2990.30         | 3047.25       | 2491.48           | 2529.28         |
| E2   | S10001      |                    | 1094.00         | 3057.00       | 1249.38           | 1230.63         |
| F2   | S10001      |                    | 300.00          | 296.00        | 606.27            | 604.94          |
| G2   | S10001      |                    | 104.00          | 306.25        | 336.75            | 341.30          |
| H2   | S10001      |                    | 55.00           | 55.50         | 196.04            | 201.20          |
| A3   |             |                    | 37.00           | 0.00          | 0.00              | 0.00            |
| B3   |             |                    | 66.00           | 0.00          | 0.00              | 0.00            |
| C3   |             |                    | 39.00           | 0.00          | 0.00              | 0.00            |
| D3   |             |                    | 41.00           | 0.00          | 0.00              | 0.00            |
| E3   |             |                    | 45.00           | 0.00          | 0.00              | 0.00            |
| F3   |             |                    | 39.00           | 0.00          | 0.00              | 0.00            |
| G3   |             |                    | 45.00           | 0.00          | 0.00              | 0.00            |
| H3   |             |                    | 51.00           | 0.00          | 0.00              | 0.00            |
| A4   |             |                    | 112.00          | 0.00          | 0.00              | 0.00            |

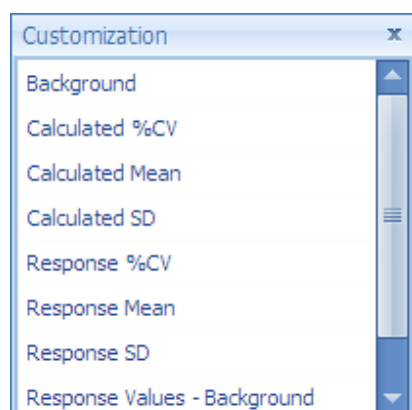
**Figure 6.1 View Results Table**

## 6.1

**Add or Delete a column**

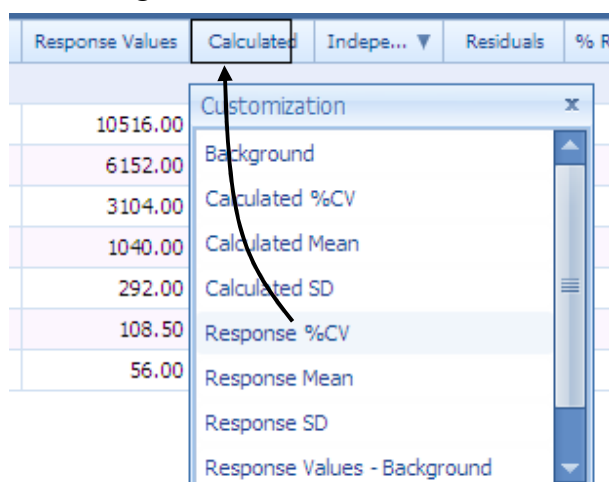
1. Click **Show Columns Selector**  **Show Columns Selector** button.

⇒ Column selector appears (Figure 6.2).



**Figure 6.2 Column Selector box**

2. To add a data column, select one of the data types and drag & drop it onto the data grid (Figure 6.3)



**Figure 6.3 Add data type to the data grid**

3. To delete the column from the data grid, select desired column and drag & drop it away from the column (Figure 6.4).

| Response Values | Response Mean | Response SD | Independ... | Calculated... |
|-----------------|---------------|-------------|-------------|---------------|
| 55.00           | 55.50         | 0.71        | 156.25      | 3.06          |
| 56.00           | 55.50         | 0.71        | 156.25      | 3.06          |
| 104.00          | 106.25        | 3.18        | 312.50      | 6.45          |

Figure 6.4 Delete data type from the data grid

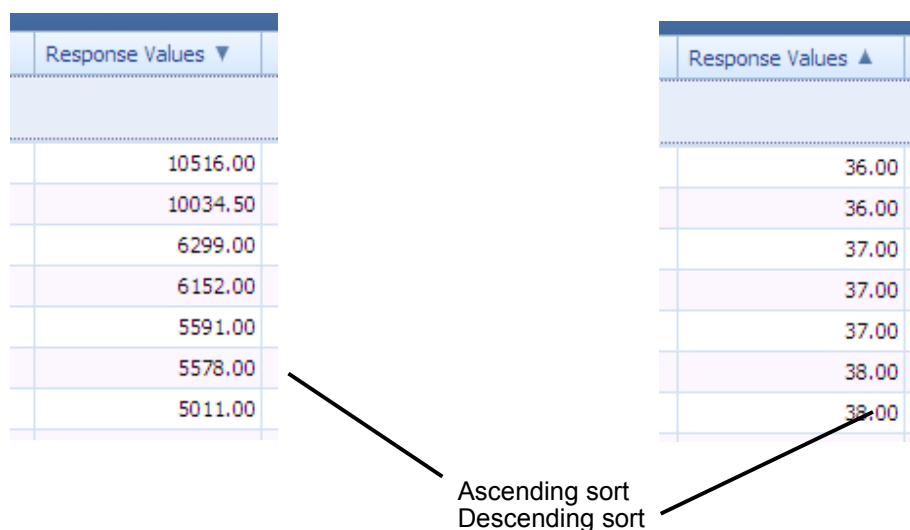
Table 6.1 Data Types in the Data Table

| Data Type                    | Description                                                                                                         |
|------------------------------|---------------------------------------------------------------------------------------------------------------------|
| Well                         | Well name                                                                                                           |
| Analyte Name                 | Analyte name                                                                                                        |
| Sample Name                  | User-specified name for the well.                                                                                   |
| Replicate Group Name         | The group number of the well. Wells that belong to the same group have the same group number.                       |
| Outlier                      | Well data are not included in the calculation.                                                                      |
| Response Values              | The light intensity measured by the plate reader instrument.                                                        |
| Response Values - Background | Background subtracted value from Response Value.                                                                    |
| Response Mean                | Shows the Response Value average within the group.                                                                  |
| Response SD                  | Shows the Response Value standard deviation within the group.                                                       |
| Response %CV                 | Shows the Response Value %CV within the group.                                                                      |
| Calculated                   | The concentration that is calculated (interpolated or extrapolated) from the user-selected standard curve.          |
| Independent Values           | Standard data user inputted.                                                                                        |
| Calculated Mean              | Shows the concentration average within the group.                                                                   |
| Calculated SD                | Shows the concentration standard deviation within the group.                                                        |
| Calculated %CV               | Shows the concentration %CV within the group.                                                                       |
| Residuals                    | Residual = Observed (or calculated) concentration – Expected concentration                                          |
| %Recovery                    | %Recovery = (Calculated/Expected) x 100.                                                                            |
| EC50 / IC50                  | Half maximal effective concentration (EC <sub>50</sub> ), half maximal inhibitory concentration (IC <sub>50</sub> ) |
| Dilution Factor              | Dilution factor for Background, Unknown and Control.                                                                |

## 6.2

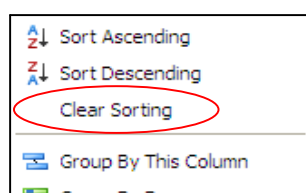
### Sort or Filter the Column Data

To sort by specific column, click the column title. Ascending and descending are changed alternatively (Figure 6.5).



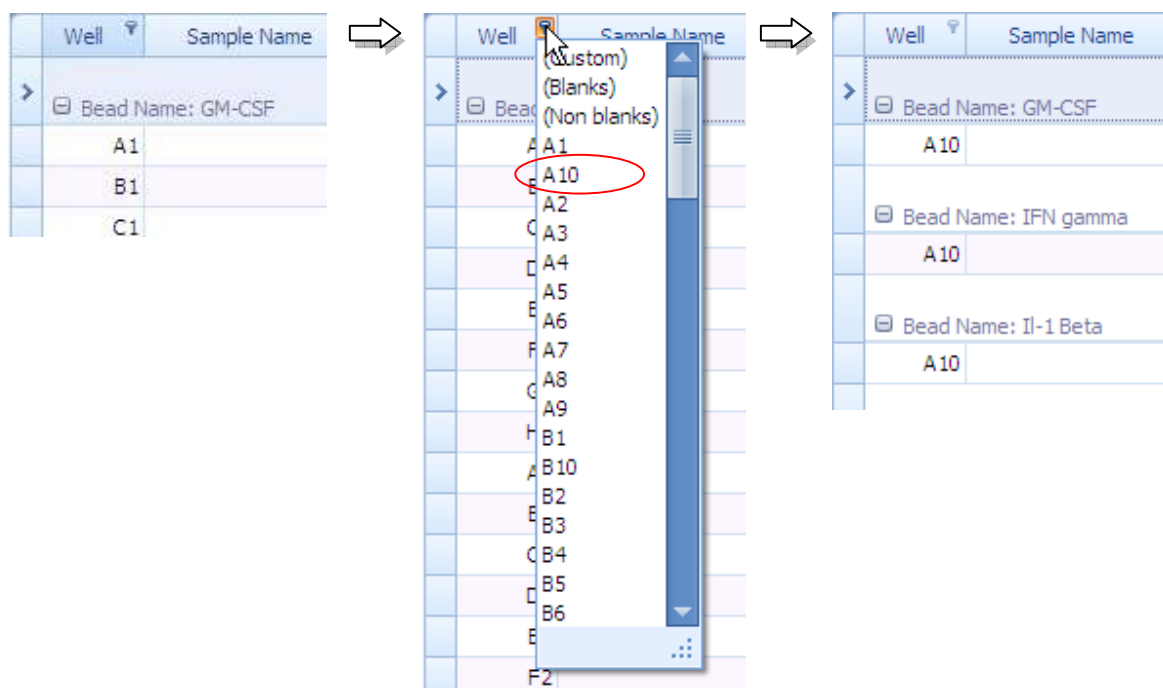
**Figure 6.5 Sort Column Data**

To clear the sort, right click on the column you want to clear the sort, select 'Clear Sorting' from the menu (Figure 6.6).



**Figure 6.6 Clear Sorting**

To filter by specific data in the column, click upper right side of the column you want to use it as filter base (Figure 6.7).



**Figure 6.7** Filter by the data in the column



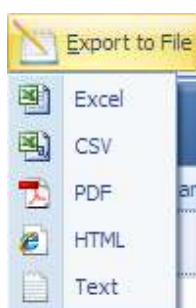
**NOTE:** There is a way to construct more complex filter conditions using **Filter Builder**. See appendix A section A.1 'Create Complex Filter Criteria' paragraph.

## 6.3

### Exporting a Data

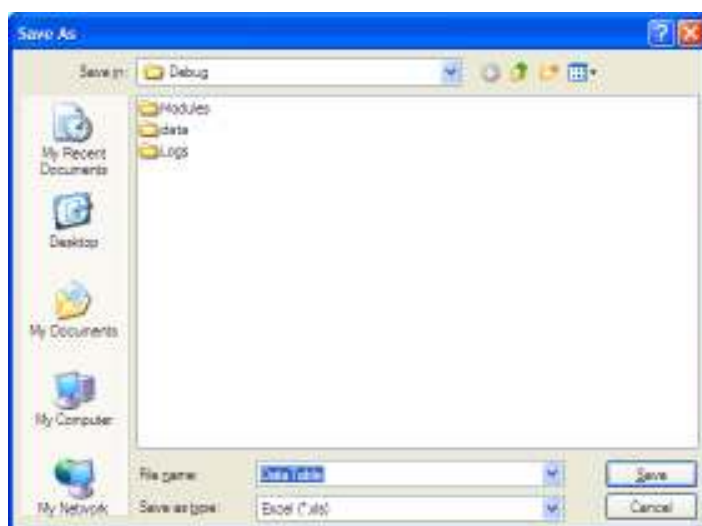
You can export your data table data from **Export to File** button .

1. Click Export to File drop-down button.
2. Select the file format you want to export
  - ⇒ There are five file formats available: Excel, CSV, PDF, HTML and Text (Figure 6.8).



**Figure 6.8 Export to File menu**

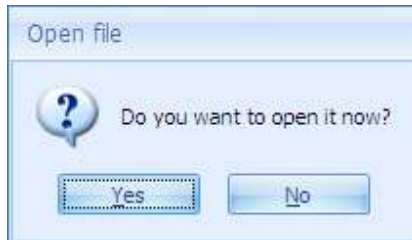
3. File save dialog appears. Set file path and input file name, then click OK (Figure 6.9).



**Figure 6.9 File save dialog**

4. After saving the file, an Open file prompt appears (Figure 6.10). If you want to open the saved file immediately using the program the files extension is associated with, click Yes.

⇒ The saved file is opened on the program. (Figure 6.11).



**Figure 6.10 Confirmation dialog**

The image shows a Microsoft Excel spreadsheet titled 'DataTable.xls [Compatibility Mode] - Microsoft Excel'. The spreadsheet has a table with the following columns: Sample Name, Replicate Group, Response Values, Response Mean, Calculated Values, and Calculated Mean. The data is organized into groups (A1, B1, C1, D1, E1, F1, G1, H1, A2, B2, C2, D2, E2, F2, G2, H2, A3, B3, C3, D3) and replicates (Rep1, Rep2, Rep3, Rep4, Rep5, Rep6, Rep7, Rep8, Rep9, Rep10, Rep11, Rep12, Rep13, Rep14, Rep15, Rep16, Rep17, Rep18, Rep19, Rep20, Rep21, Rep22). The 'Response Values' column contains numerical data, and the 'Response Mean' column contains the mean of the response values for each group. The 'Calculated Values' and 'Calculated Mean' columns are currently empty.

|    | Sample Name | Replicate Group | Response Values | Response Mean | Calculated Values | Calculated Mean |
|----|-------------|-----------------|-----------------|---------------|-------------------|-----------------|
| A1 |             |                 | 41.00           | 0.00          | 0.00              | 0.00            |
| B1 |             | Std001          | 30536.00        | 10518.00      | 0.00              | 0.00            |
| C1 |             | Std001          | 6152.00         | 6152.00       | 0.00              | 0.00            |
| D1 |             | Std001          | 3104.00         | 3104.00       | 0.00              | 0.00            |
| E1 |             | Std001          | 1040.00         | 1040.00       | 0.00              | 0.00            |
| F1 |             | Std001          | 292.00          | 292.00        | 0.00              | 0.00            |
| G1 |             | Std001          | 108.50          | 108.50        | 0.00              | 0.00            |
| H1 |             | Std001          | 58.00           | 58.00         | 0.00              | 0.00            |
| A2 |             |                 | 39.00           | 0.00          | 0.00              | 0.00            |
| B2 |             | Std002          | 10034.50        | 10034.50      | 0.00              | 0.00            |
| C2 |             | Std002          | 6299.00         | 6299.00       | 0.00              | 0.00            |
| D2 |             | Std002          | 2990.50         | 2990.50       | 0.00              | 0.00            |
| E2 |             | Std002          | 1094.00         | 1094.00       | 0.00              | 0.00            |
| F2 |             | Std002          | 300.00          | 300.00        | 0.00              | 0.00            |
| G2 |             | Std002          | 104.00          | 104.00        | 0.00              | 0.00            |
| H2 |             | Std002          | 55.00           | 55.00         | 0.00              | 0.00            |
| A3 |             |                 | 37.00           | 0.00          | 0.00              | 0.00            |
| B3 |             |                 | 88.00           | 0.00          | 0.00              | 0.00            |
| C3 |             |                 | 39.00           | 0.00          | 0.00              | 0.00            |
| D3 |             |                 | 41.00           | 0.00          | 0.00              | 0.00            |

**Figure 6.11 Opening in Excel**

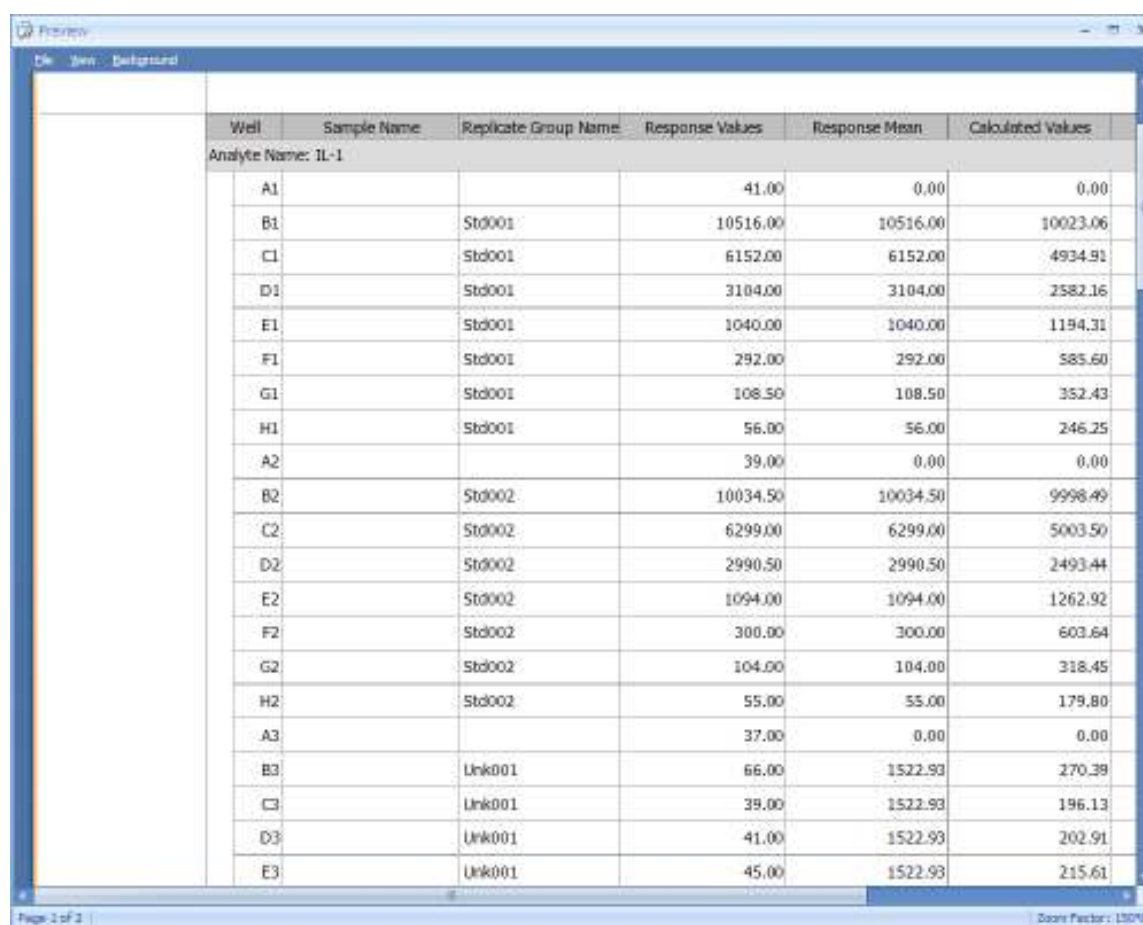
## 6.4

### Printing a Data

You can preview your data with the **Print Preview** button  .

1. Click the **Print Preview** button.

⇒ Print preview window appears (Figure 6.12).



| Well               | Sample Name | Replicate Group Name | Response Values | Response Mean | Calculated Values |
|--------------------|-------------|----------------------|-----------------|---------------|-------------------|
| Analyte Name: IL-1 |             |                      |                 |               |                   |
| A1                 |             |                      | 41.00           | 0.00          | 0.00              |
| B1                 |             | Std001               | 10516.00        | 10516.00      | 10023.06          |
| C1                 |             | Std001               | 6152.00         | 6152.00       | 4934.91           |
| D1                 |             | Std001               | 3104.00         | 3104.00       | 2582.16           |
| E1                 |             | Std001               | 1040.00         | 1040.00       | 1194.31           |
| F1                 |             | Std001               | 292.00          | 292.00        | 585.60            |
| G1                 |             | Std001               | 108.50          | 108.50        | 352.43            |
| H1                 |             | Std001               | 56.00           | 56.00         | 246.25            |
| A2                 |             |                      | 39.00           | 0.00          | 0.00              |
| B2                 |             | Std002               | 10034.50        | 10034.50      | 9998.49           |
| C2                 |             | Std002               | 6299.00         | 6299.00       | 5003.50           |
| D2                 |             | Std002               | 2990.50         | 2990.50       | 2493.44           |
| E2                 |             | Std002               | 1094.00         | 1094.00       | 1262.92           |
| F2                 |             | Std002               | 300.00          | 300.00        | 603.64            |
| G2                 |             | Std002               | 104.00          | 104.00        | 318.45            |
| H2                 |             | Std002               | 55.00           | 55.00         | 179.80            |
| A3                 |             |                      | 37.00           | 0.00          | 0.00              |
| B3                 |             | Unk001               | 66.00           | 1522.93       | 270.39            |
| C3                 |             | Unk001               | 39.00           | 1522.93       | 196.13            |
| D3                 |             | Unk001               | 41.00           | 1522.93       | 202.91            |
| E3                 |             | Unk001               | 45.00           | 1522.93       | 215.61            |

**Figure 6.12 Print Preview**

2. To print, click the **Print**  icon from the menu bar.

⇒ Print setting dialog appears.



**NOTE:** For more printing options, see appendix A section A.2 'Print Preview' menu.

## CHAPTER 7 | Data Charts – Create Graphs tab

MasterPlex® ReaderFit can display Response Value, concentration or standard data in many graph formats in the create graphs tab.

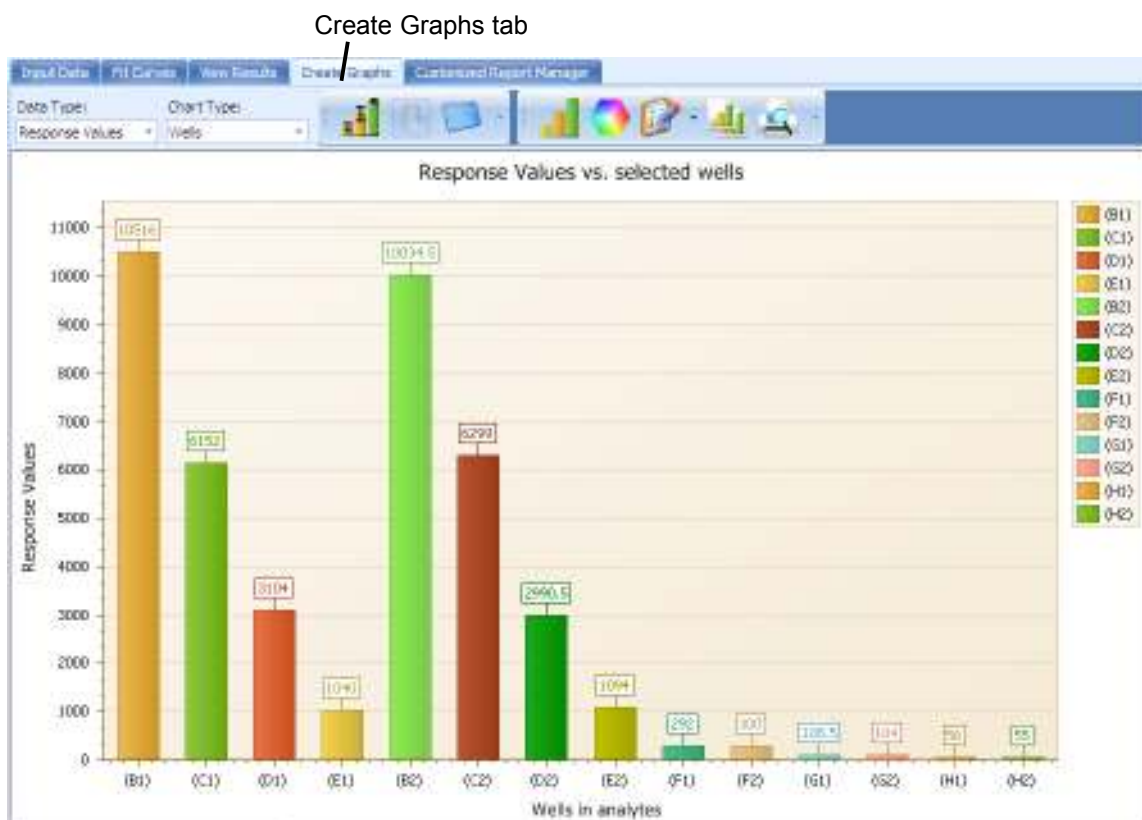
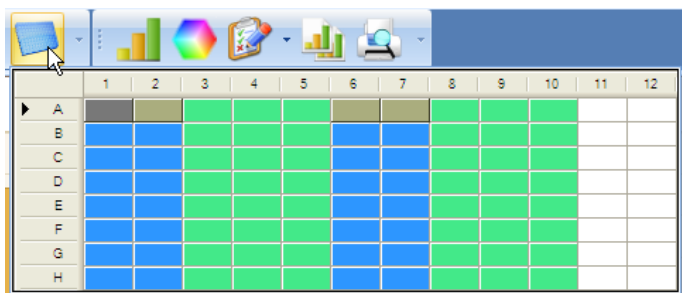


Figure 7.1 Create Graphs tab

### 7.1

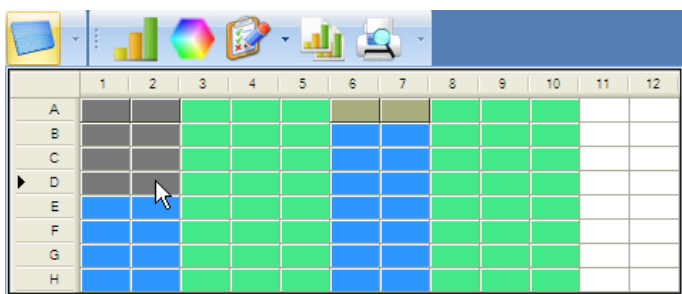
#### Viewing a Data Chart

1. Click the Well Selector  button.  
⇒ A mini sized well plate is displayed under the button (Figure 7.2).

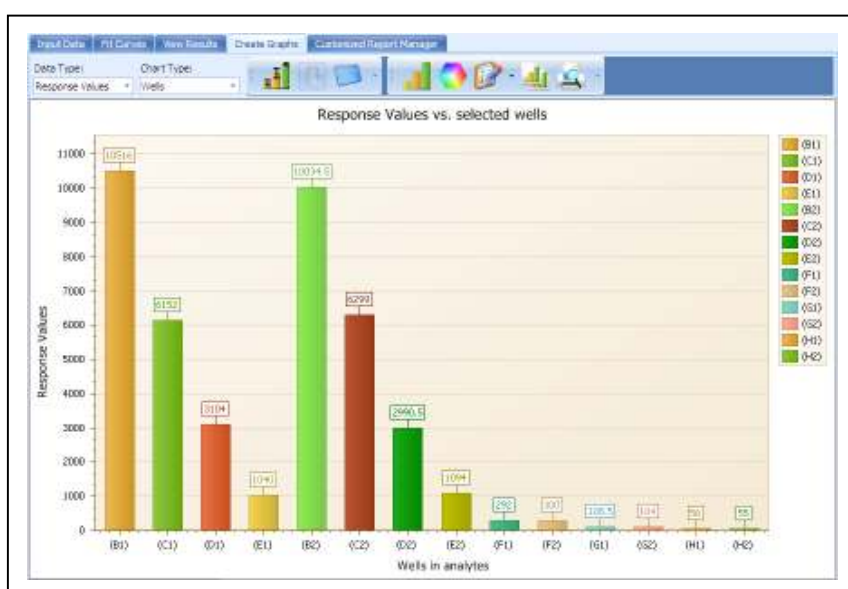


**Figure 7.2 Well Selector**

2. Select the wells you want to display on the chart. You can select multiple wells by pressing [CTRL] key (Figure 7.3).



**Figure 7.3 Multiple Well Selection**



**Figure 7.4 Bar Chart for Selected Wells**

3. To display another data type for the selected wells, click the **Data Type** drop-down list and select one of the data type.

**Table 7.1 Data Types**

| <b>Data Type</b> | <b>Displays...</b>       |
|------------------|--------------------------|
| Response Value   | Response Value           |
| Concentration    | Calculated concentration |

4. To change the data type, click the Chart Type drop-down list and select one of two chart types.

**Table 7.2 Chart Types**

| <b>Chart Type</b>    | <b>Displays...</b>                                                                      |
|----------------------|-----------------------------------------------------------------------------------------|
| Well Group           | Analyte data for each user selected well.                                               |
| Wells by Analyte     | Analyte data for each user selected well by analyte.                                    |
| Wells per Analyte    | Analyte data for each user selected well per analyte.                                   |
| Group by Analyte     | Plot the selected data type which has sample name, across the analytes. (Figure 7.5)    |
| Group by Sample Name | Plot the selected data type which has sample name, across the sample name. (Figure 7.6) |

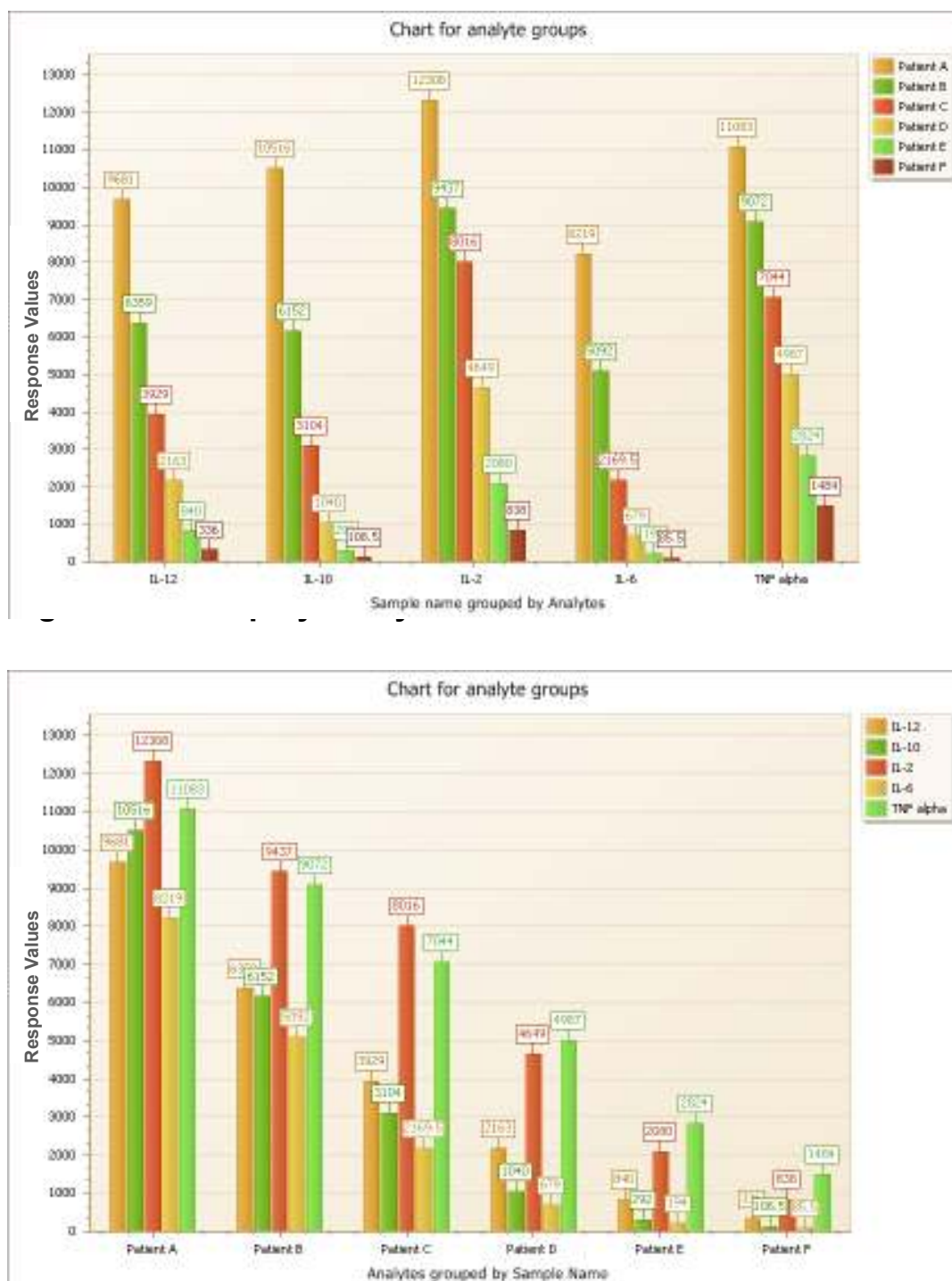


Figure 7.6 Group by Sample name chart

## Replicate View

Plotting group values is available by clicking the **Replicate View** button . Replicate View is available only on Bar style charts. If you are on another type of chart when you click the Replicate View button, it automatically re-selects the Bar chart and displays the data with error bars. Figure 7.7 shows an example of the replicate view chart.

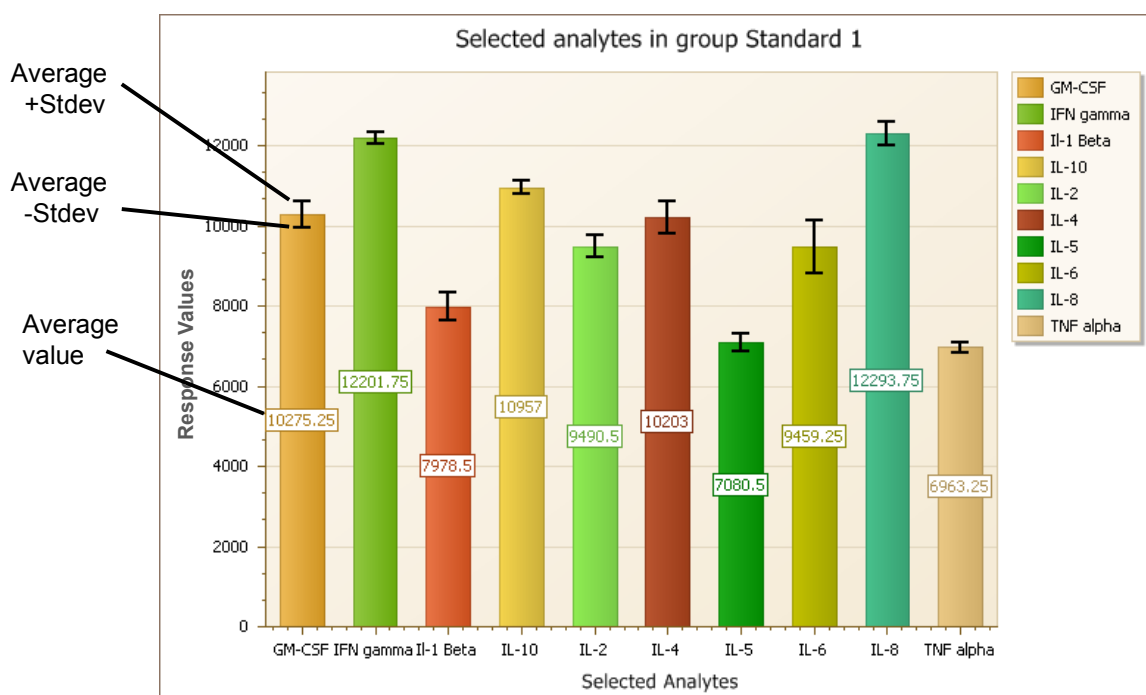


Figure 7.7 Replicate View chart

## 7.2

### Chart Format

MasterPlex® ReaderFit provides various chart formats (Table 7.3). To change a chart format for the selected well data, click the **Chart Gallery** button , and make a selection from the drop-down list of **Chart Gallery**.

**Table 7.3 Available chart format**

| Icon                                                                                | Chart Name     | Features                                  |
|-------------------------------------------------------------------------------------|----------------|-------------------------------------------|
|    | Bar            | Bar style x-y chart                       |
|    | Bar 3D         | 3D bar style x-y chart                    |
|    | Manhattan Bar  | 3D bar style x-y-z chart                  |
|    | Point          | Point style x-y chart                     |
|    | Line           | Line interpolated style x-y chart         |
|   | Step Line      | Step line interpolated style x-y chart    |
|  | Spline         | Spline interpolated style x-y chart       |
|  | Line 3D        | 3D line interpolated style x-y chart      |
|  | Step Line 3D   | 3D step line interpolated style x-y chart |
|  | Spline 3D      | 3D spline interpolated style x-y chart    |
|  | Area           | Area painted style x-y chart              |
|  | Spline Area    | Spline area painted style x-y chart       |
|  | Area 3D        | 3D area painted style x-y chart           |
|  | Spline Area 3D | 3D spline area style x-y chart            |
|  | Pie            | Pie style circular chart                  |
|  | Pie 3D         | 3D pie style circular chart               |
|  | Doughnut       | Doughnut style circular chart             |
|  | Doughnut 3D    | 3D doughnut style circular chart          |
|  | Radar Line     | Line interpolated style radar chart       |
|  | Radar Point    | Point plotted style radar chart           |

## 7.3

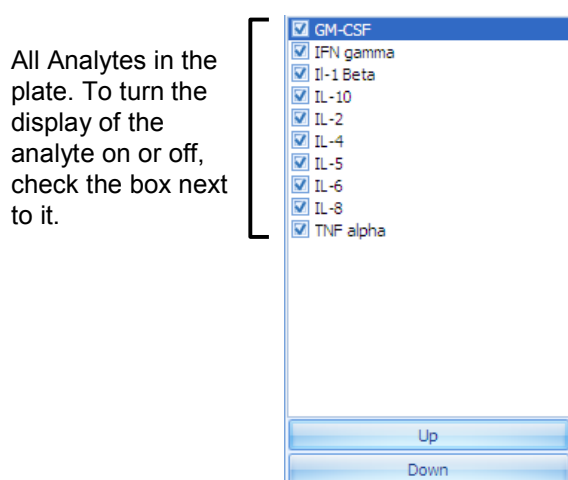
### Analyte Selector

In the Well Group chart, **Analyte Selector** allows you to change the analytes position in the chart and allows you to display on/off setting for each analyte. To move the analyte position,

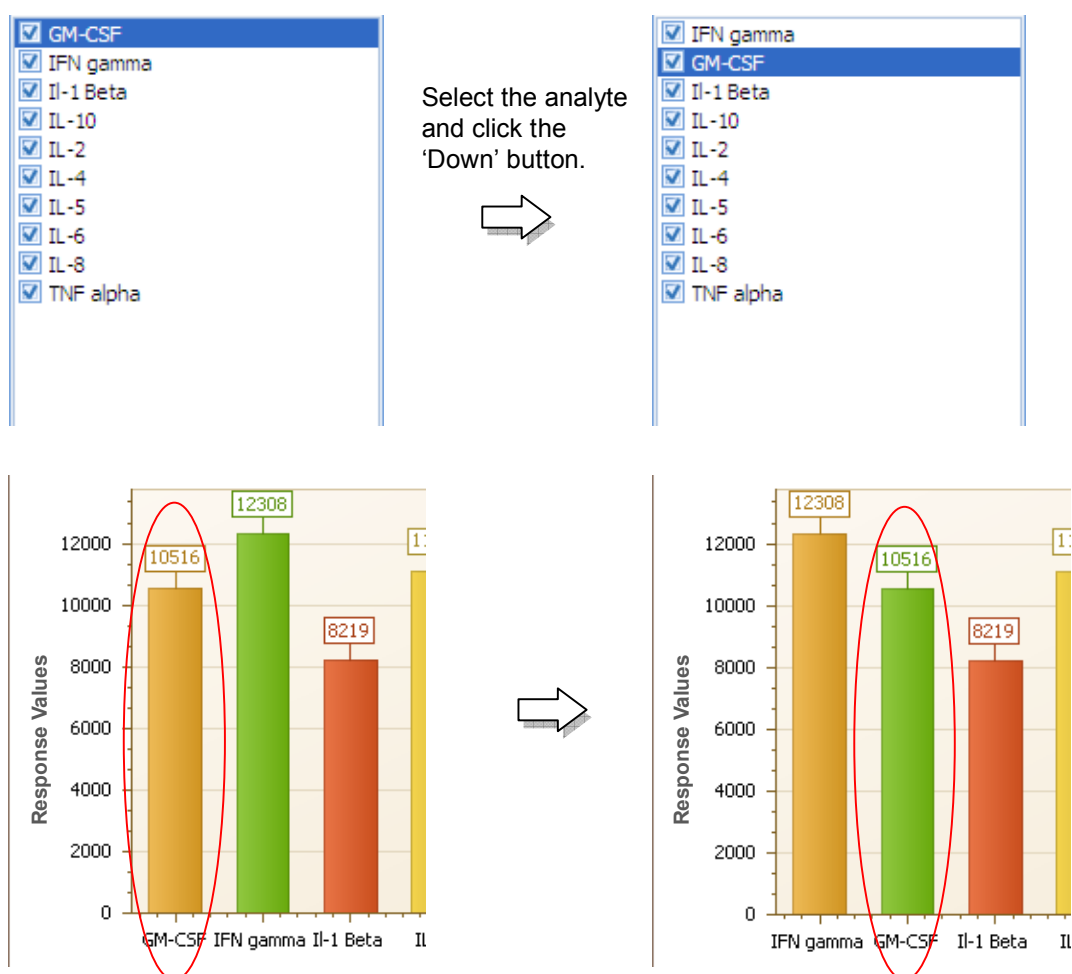
1. Click **Analyte Selector** drop-down list (Figure 7.8).
2. Select the analyte you want to move.
3. To move the analyte to the left, click **Up** button. To move the analyte to right, click **Down** button.

⇒ The analyte moves one position to the left (or right) from its current position (Figure 7.9).

In the Single Analyte chart, the Analyte Selector allows you to select the analyte you want to display in the chart (Figure 7.10).



**Figure 7.8 Analyte Selector drop-down list**



**Figure 7.9 Change the analyte position**

All Analytes in the plate. To select the analyte, click the radio button next to the analyte name.

The figure shows a list of analytes with radio buttons for selection. The selected analyte is GM-CSF. Below the list are 'Up' and 'Down' buttons.

- ☒ GM-CSF
- ☐ IFN gamma
- ☐ IL-1 Beta
- ☐ IL-10
- ☐ IL-2
- ☐ IL-4
- ☐ IL-5
- ☐ IL-6
- ☐ IL-8
- ☐ TNF alpha

Up  
Down

**Figure 7.10 Analyte Selector drop-down list**

## 7.4

### Changing the Color Palette

There are 30 color palettes available from **Color Palette** button . Figure 7.11 shows the palette names and their corresponding color patterns.

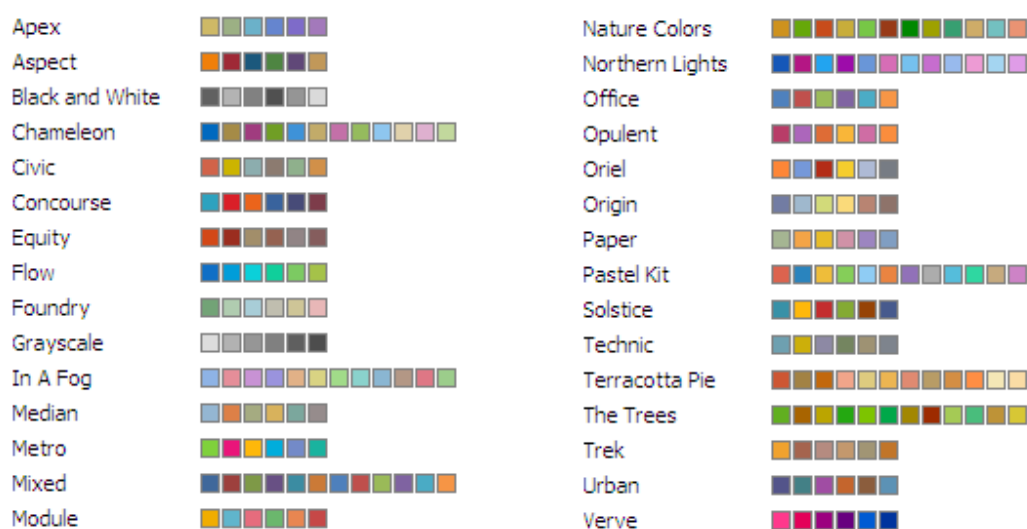


Figure 7.11 Color Palettes

## 7.5

### Changing Chart Properties

The Create Graphs tab has great flexibility in customizing the chart. To enter the chart properties dialog, click **Chart Properties** button . You can choose to change the entire chart's properties or just one of them. To change the entire chart's properties, click button  of the  button. To change one of the chart's properties, click small drop-down arrow right side of the button (Figure 7.12). Figure 7.13 shows Chart Properties dialog, and Table 7.4 explains the property categories.

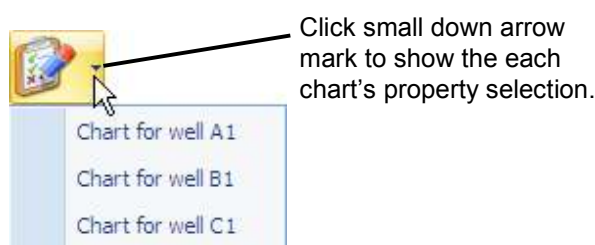


Figure 7.12 Chart Property for Individual Chart



Figure 7.13 Multiple Well Selection

**Table 7.4 Chart Properties**

| Properties   | Icon                                                                                | Features                                                                                                                                               |
|--------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| Legends      |    | Customize the legend's properties.                                                                                                                     |
| Diagram      |    | Customize the diagram's properties.                                                                                                                    |
| Axes         |    | Customize X and Y axes of the diagram. Note that you may select an axis to be modified on the chart preview.                                           |
| Chart Titles |    | Add chart titles to be displayed within a chart.                                                                                                       |
| Point Labels |    | Customize point label properties of the selected series. Note that you may select a series to be modified on the chart preview.                        |
| Series Views |    | Customize series view properties of the selected series. Note that you may select a series to be modified no the chart preview.                        |
| Appearance   |  | Choose a palette to color series or their data points. Also choose the style, which specifies the chart's appearance depending on the current palette. |

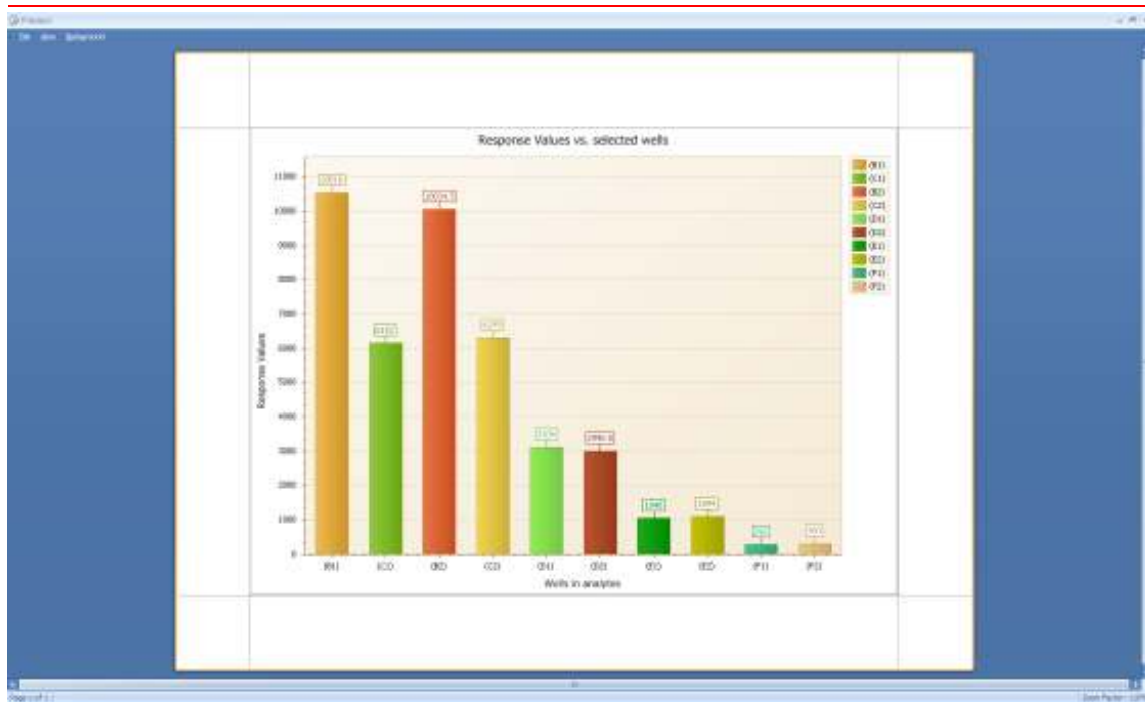
## 7.6

### Printing a Chart

You can print your data with the **Print** button .

1. Click the **Print Preview** button.

⇒ Print preview window appears (Figure 7.14).



**Figure 7.14 Print Preview**

- To print, click the **Print**  icon from the menu bar.  
⇒ Print setting dialog appears.



**NOTE:** For more print options, see appendix A section A.2 '*Print Preview*' menu.

## 7.7

### Copying or Saving Chart Image

The software can export the chart image to other applications. The data may be copied to the system clipboard or saved in different file formats.

1. Right click on the chart you want to copy or save.
2. To copy the chart image in bitmap format, click '**Copy**'.
  - ⇒ The image is sent to the clipboard and you can paste the image data on other applications.
3. To save the chart image in other formats, click '**Export Image**'.
  - ⇒ The File Save dialog is opened (Figure 7.15).
4. Input the file name and select one of the file formats from the 'Save as type' drop-down list. There are five formats available: bitmap, png, html, pdf and Excel.

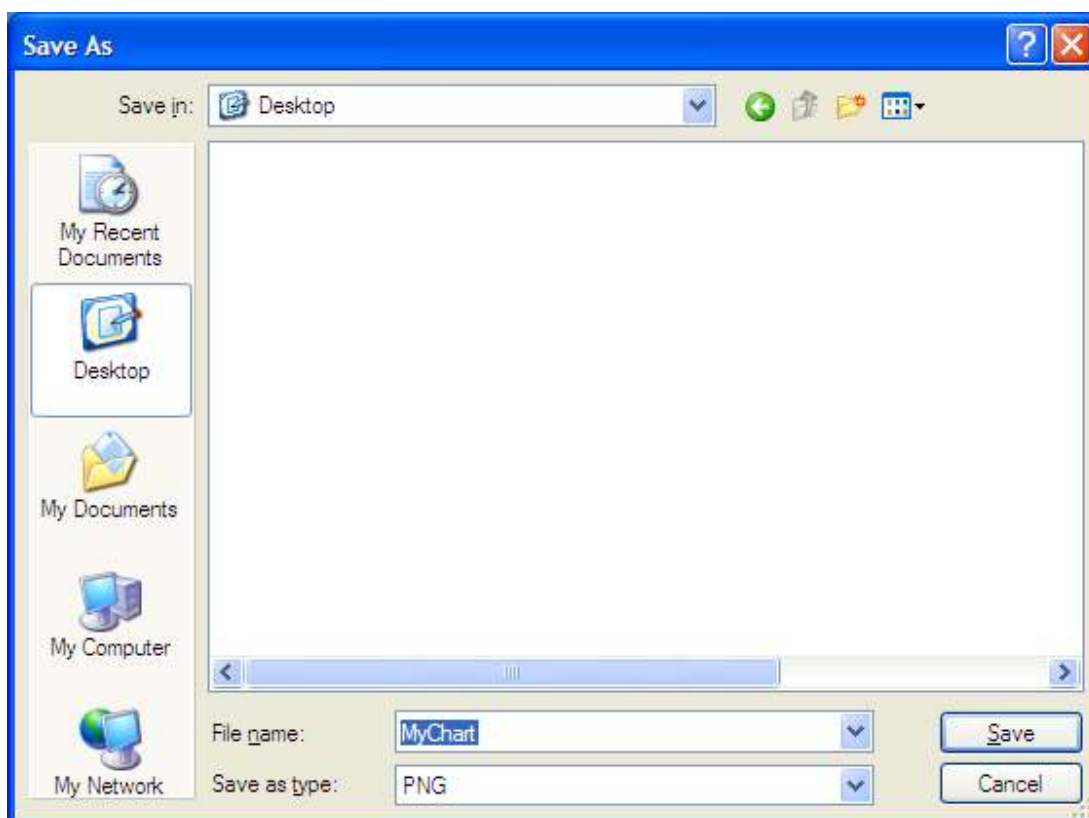


Figure 7.15 Chart Image Export dialog

## CHAPTER 8 **Export Data – Customized Report Manager tab**

*This chapter explains how to export MasterPlex® ReaderFit data transformed by the user. Custom report is a powerful and flexible tool for presenting and exporting your data. Compared to regular report, Custom report has greater flexibility on what and how to present data. While ReaderFit stores its analysis results in an XML format document, it is possible for users to present their data in whatever format they want. The only thing users need to do is to define their presenting formats in XSL files (Extensive Stylesheet Language).*



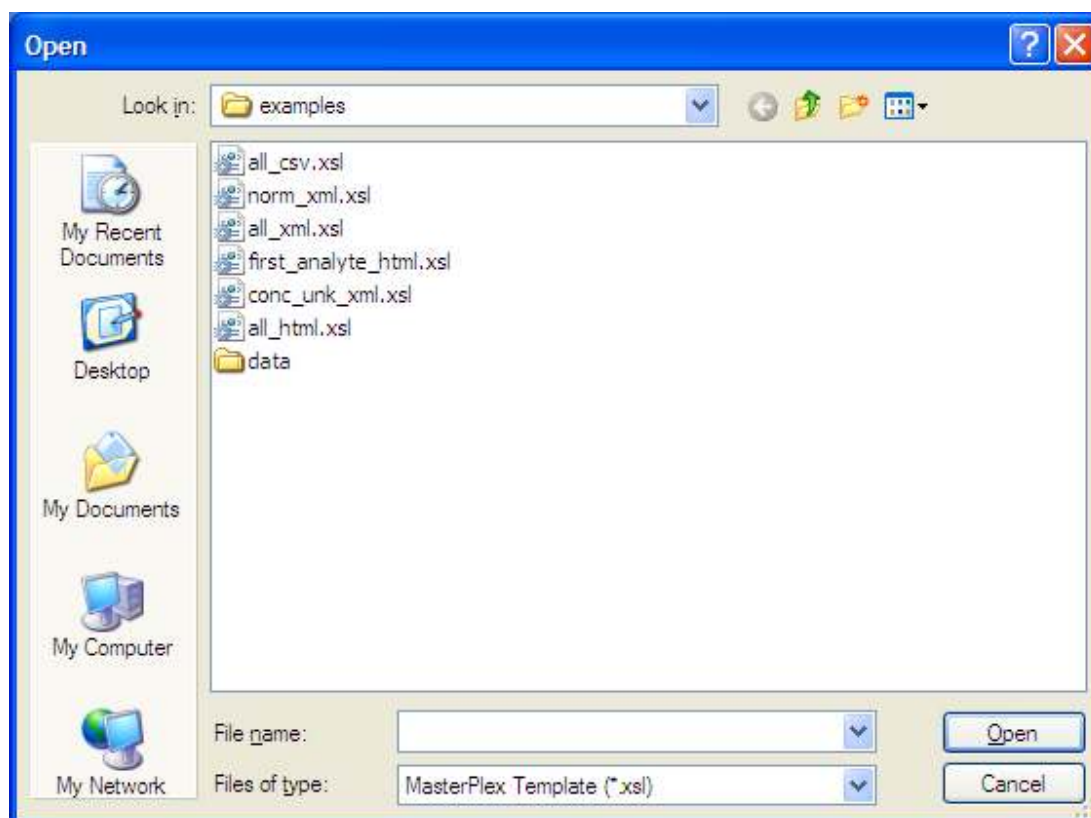
**Figure 8.1 Customized Report Manager tab**

## 8.1

### Importing a User Defined Stylesheet

1. Click **Import** button .

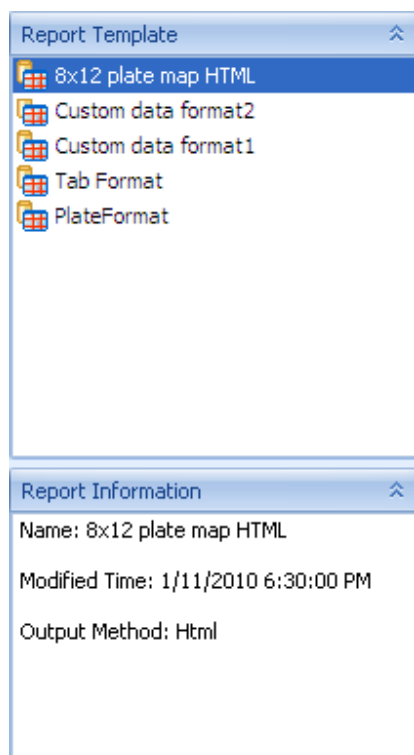
⇒ The file open dialog appears (Figure 8.2).



**Figure 8.2 Open File Dialog**

2. Select the file you want to import to the Customized Report Manager.

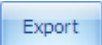
⇒ Style sheet's name is displayed in the Style Sheet list, and XSL Information window shows style sheet's information (Figure 8.3).

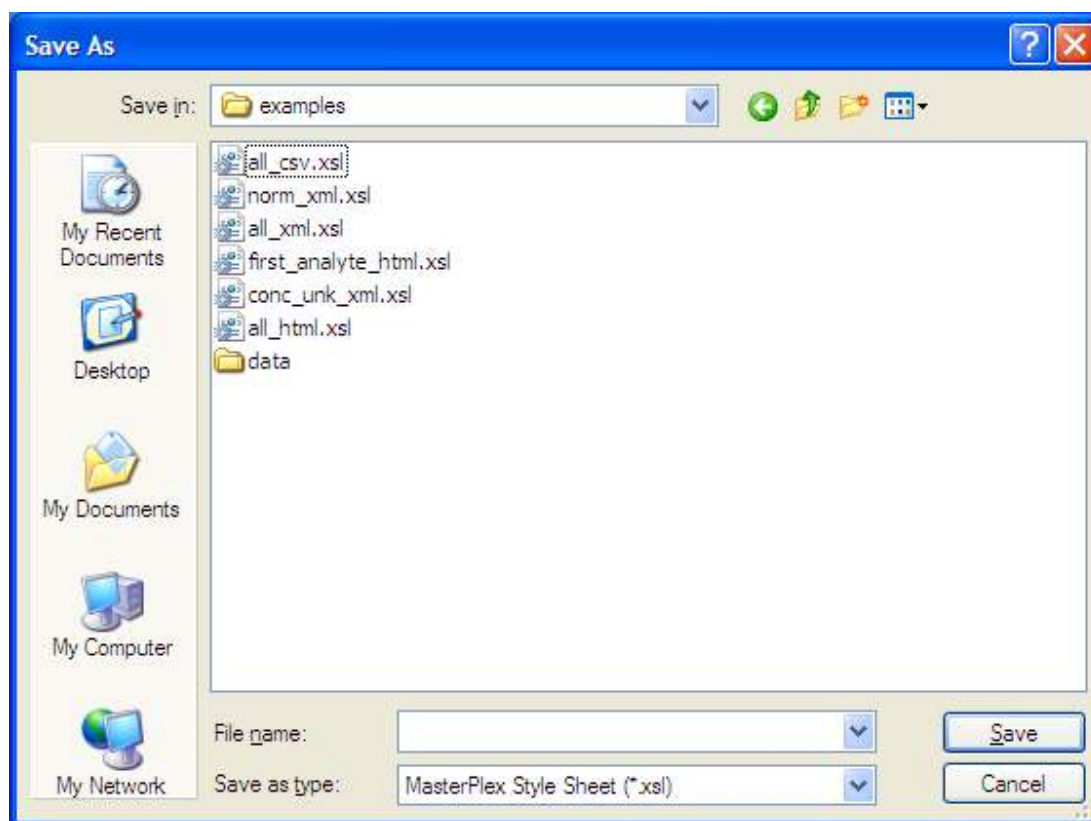


**Figure 8.3 Style Sheet List and Information**

## 8.2

### Exporting a User Defined Stylesheet

1. Click **Export** button  .  
⇒ The file save dialog appears (Figure 8.4).



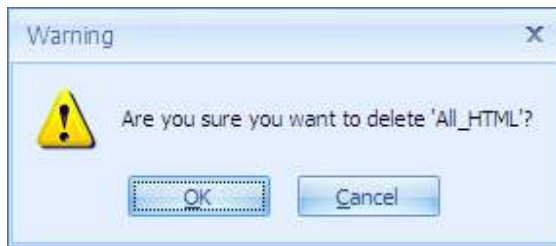
**Figure 8.4 Save File Dialog**

2. Set the destination and input file name, then click the Save button.  
⇒ Style sheet's name is displayed in the Style Sheet window, and XSL Information window shows style sheet's information (Figure 8.3).

## 8.3

### Delete Style Sheet File from Style Sheet List

1. Select the style sheet you want to delete from the style sheet list.
2. Click the **Delete** button .  
⇒ The confirmation dialog appears (Figure 8.5).
3. Click OK to delete.

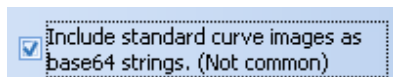


**Figure 8.5 Confirmation Dialog**

## 8.4

### Including standard curve images

1. Check Include standard curve images.



⇒ The transformed data includes standard curve image data as base64 encoding.

## 8.5

### Transform Original Data into Your Customized Data

1. Select the style sheet you want to apply from the style sheet list.
2. Click the Apply button .

⇒ The transformed data is shown in the preview window (Figure 8.6).

Save Print

**Flat File by Determination**

| FileName                                                        | RunDate   | RunTime | SampleWell | AnalyteName | WellType | SampleName |   |
|-----------------------------------------------------------------|-----------|---------|------------|-------------|----------|------------|---|
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | GM-CSF      | Other    |            | 4 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | IFN gamma   | Other    |            | 1 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | IL-1 Beta   | Other    |            | 4 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | IL-10       | Other    |            | 2 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | IL-2        | Other    |            | 1 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | IL-4        | Other    |            | 3 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | IL-5        | Other    |            | 8 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | IL-6        | Other    |            | 1 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | IL-8        | Other    |            | 3 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | A1         | TNF alpha   | Other    |            | 2 |
| C:\Documents and Settings\tshimizu\Desktop/examples/H10Plex.csv | 9/12/2008 | 8:10 PM | B1         | GM-CSF      | Other    |            | 1 |

**Figure 8.6 Transformed Data (HTML format)**

3. Save to file or print from the menu button.

**APPENDIX**

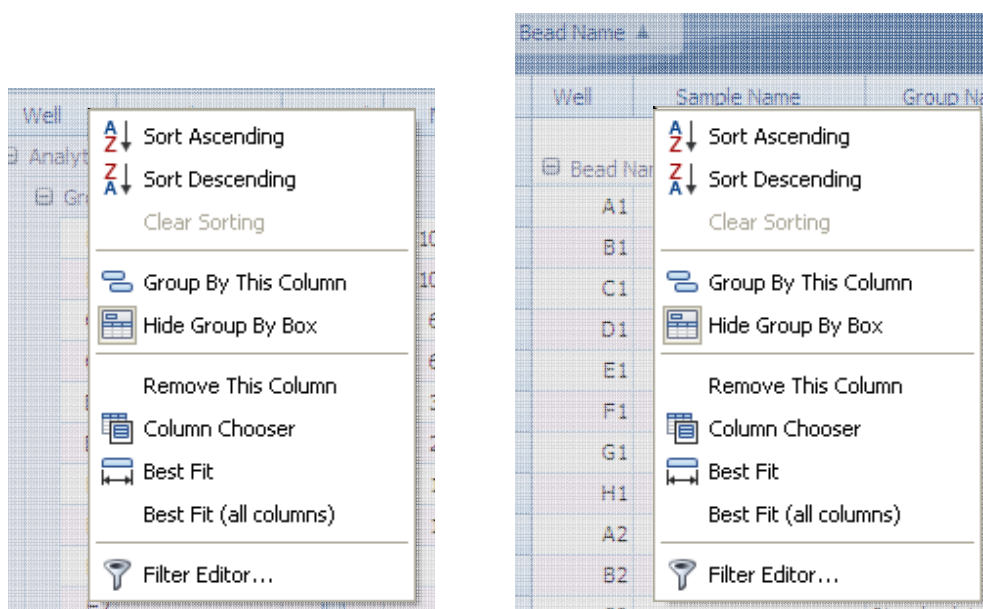
**A**

*This appendix explains more details about Data Grid (for Fit Curves tab and View Results tab) and Print Preview.*

**A.1**

**Grid Customize Menu**

Grid customize menu allows you to customize your grid data viewing more flexibly and efficiently. Grid Customize Menu is invoked by right clicking on the both grid column, standard curve data grid and data table grid (Figure A.1).



**Figure A.1 Grid Menu in the Fit Curves tab and View Results tab**

**Table A.1 Main toolbar buttons and functions**

| Icon | Command                | Function                                      |
|------|------------------------|-----------------------------------------------|
|      | <b>Sort Ascending</b>  | Sort right clicked column by ascending.       |
|      | <b>Sort Descending</b> | Sort right clicked column by descending.      |
|      | <b>Clear Sorting</b>   | Clear sorting of right clicked column (if the |

|                                                                                    |                               |                                                                                                                                          |
|------------------------------------------------------------------------------------|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                    |                               | columns is sorted).                                                                                                                      |
|   | <b>Group By This Column</b>   | Make a group by right clicked column. The group is located under the last position of current group hierarchy.                           |
|   | <b>Hide Group By Box</b>      | Display/Hide <b>Group Box</b> (Figure A.2) on the upper side of the columns. It enables you to make group(s) by drag and drop operation. |
|                                                                                    | <b>Remove This Column</b>     | Remove right clicked column.                                                                                                             |
|   | <b>Column Chooser</b>         | Show Column Chooser box.                                                                                                                 |
|   | <b>Best Fit</b>               | Adjust the width of right clicked column.                                                                                                |
|   | <b>Clear Filter</b>           | Clear filter condition of right clicked column (if column is filtered).                                                                  |
|  | <b>Filter Editor</b>          | Open <b>Filter Builder</b> window (Figure A.6).                                                                                          |
|                                                                                    | <b>Best Fit (all columns)</b> | Adjust the width of all columns.                                                                                                         |

## Group Box

The Group Box appears on the upper side of the column (Figure A.2) by choosing the **Group By Box** menu from the right click menu on the grid column. You can drag one of the column you want to group to this box (Figure A.3). Also you can multi-group by repeating same way (Figure A.4).

|                      |             |                    |                 |               |
|----------------------|-------------|--------------------|-----------------|---------------|
| Analyte Name ▲       |             |                    |                 |               |
| Well                 | Sample Name | Replicate Group... | Response Values | Response Mean |
| > Analyte Name: IL-1 |             |                    |                 |               |
| A1                   |             |                    | 41.00           | 0.00          |
| B1                   |             | Std001             | 10516.00        | 10516.00      |
| C1                   |             | Std001             | 6152.00         | 6152.00       |

**Figure A.2 Group Box**

APPENDIX A  
PREFERENCES

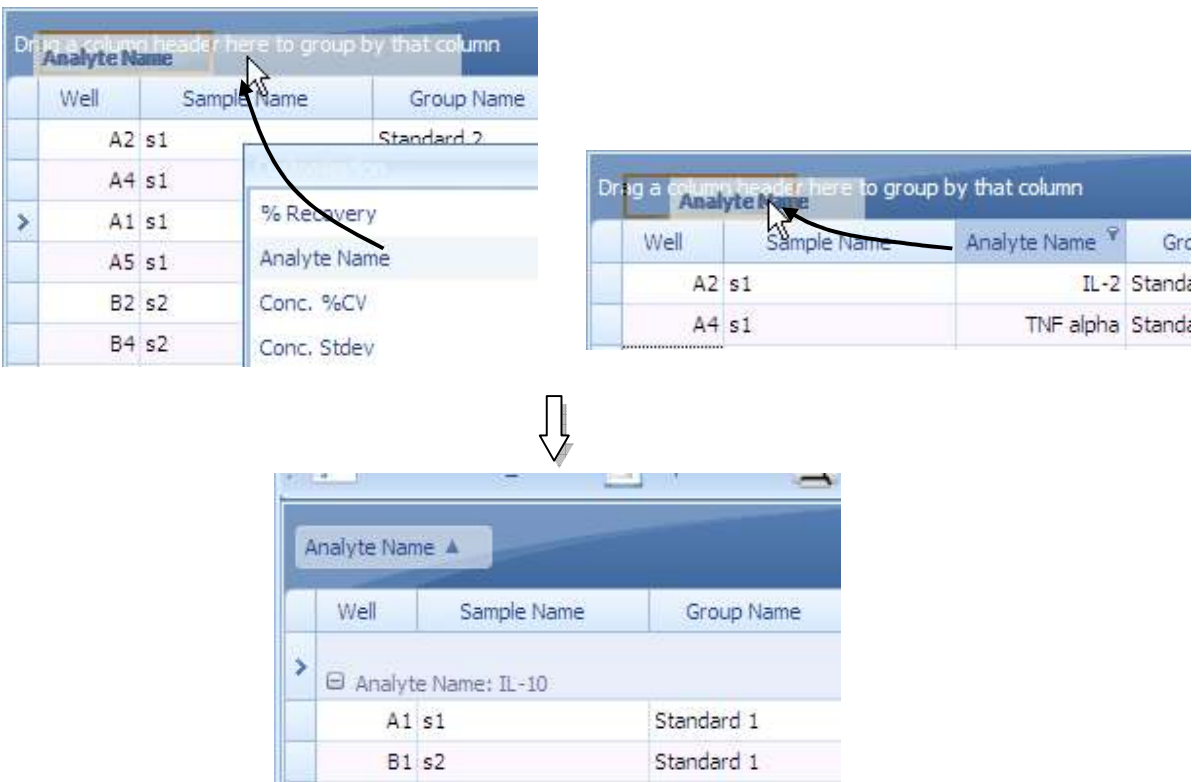


Figure A.3 Group by Drag & Drop

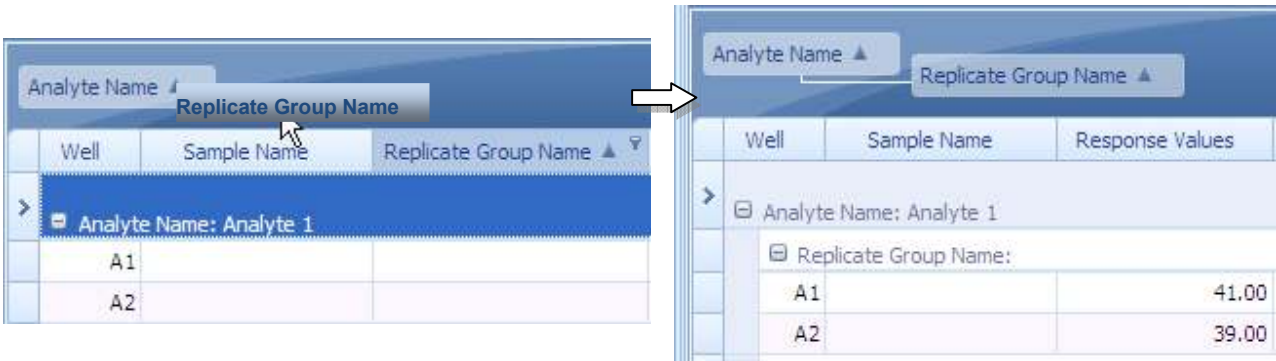


Figure A.4 Multi-Group by Drag & Drop

## Group Box Menu

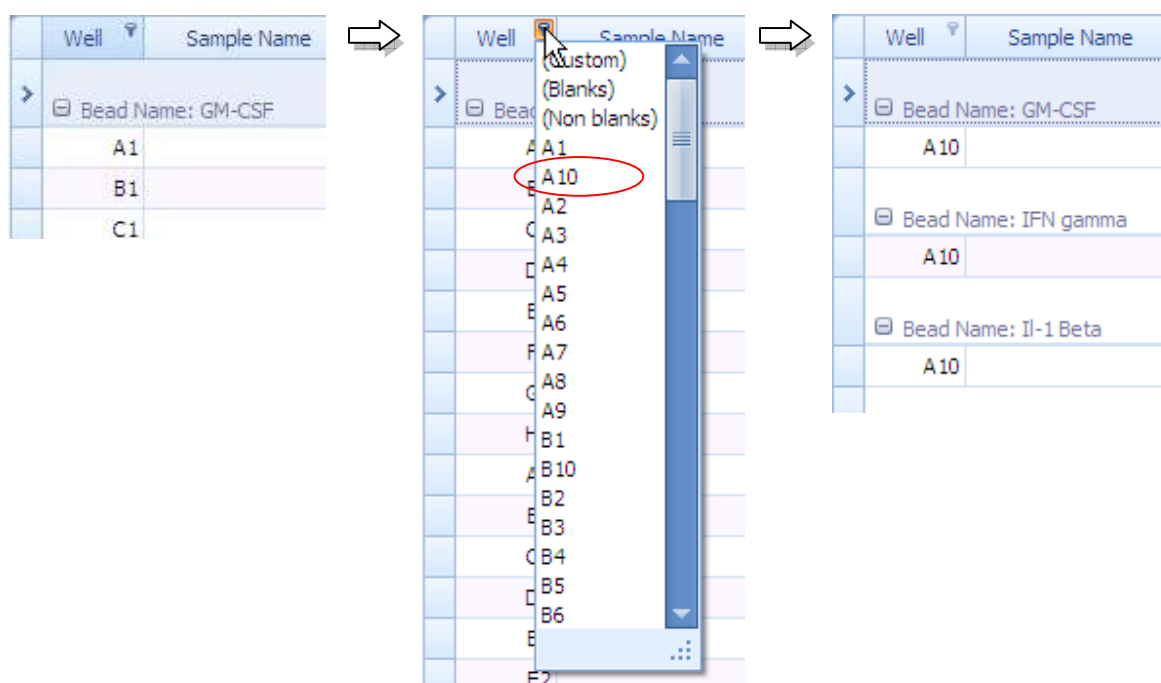
Group Box has context menu. Table A.2 shows the menus.

**Table A.2 Main toolbar buttons and functions**

| Icon                                                                              | Command               | Function                                                      |
|-----------------------------------------------------------------------------------|-----------------------|---------------------------------------------------------------|
|  | <b>Full Expand</b>    | Expand all group trees.                                       |
|  | <b>Full Collapse</b>  | Collapse all group trees.                                     |
|  | <b>Clear Grouping</b> | Clear group and return the group columns to the grid column.. |

## Working with Column Filter

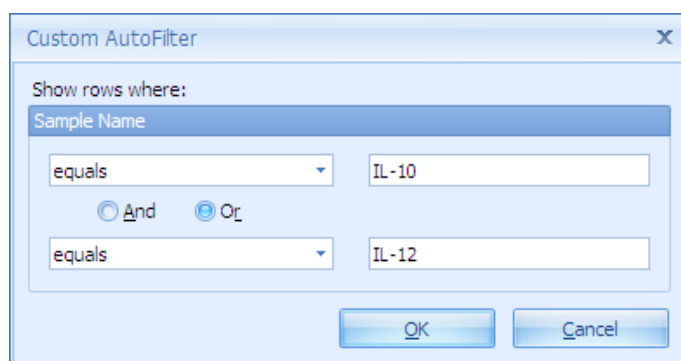
There are two ways to set a filter. The first way is by using the filter drop-down menu from the column (Figure A.5). You can filter the column by selecting the specific data in the column. The second way is by using the **Filter Builder** window (Figure A.6).



**Figure A.5 Direct Filtering from the column**

## Create Complex Filter Criteria

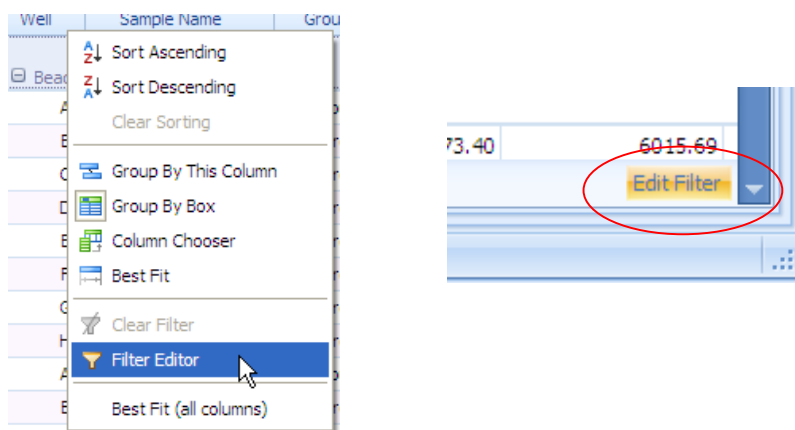
To construct filter criteria involving multiple columns and various comparison operators, use the filter drop-down list and click **Custom**. This invokes the **Custom AutoFilter** dialog (Figure A.6) which allows you to compare a column with one or two values. To construct using more various operators and multiple conditions, use the filter builder. See ‘**Basic step for constructing Filter Criteria by using Filter Builder**’ paragraph in this section.



**Figure A.6 Custom AutoFilter dialog**

## Filter Builder Window

By using the filter builder, you can filter the data more specifically. To open the filter builder window, choose **Filter Editor** from the right click menu on the column or click **Edit Filter** button on the bottom of the data grid (Figure A.7).

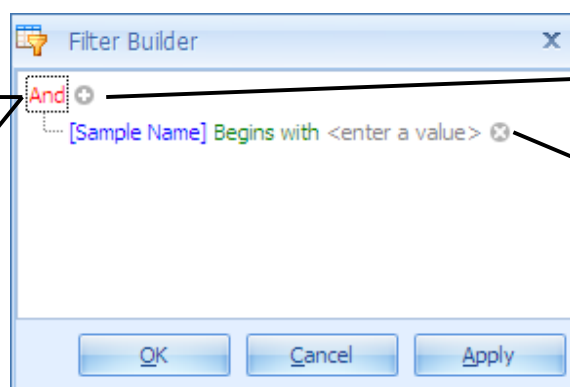
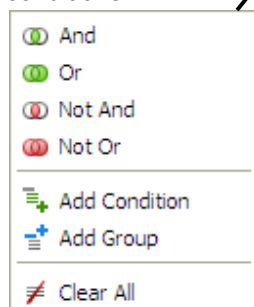


**Figure A.7 Opening the Filter Builder**

## Basic steps for constructing filter criteria with the Filter Builder

1. Right click on the grid column.
  2. Choose Filter Editor.
- ⇒ Filter Builder window appears (Figure A.8).

Click to change the current operator, add/remove conditions and groups of conditions.



To add filter condition, click [+]

To remove filter condition, click [x]

Figure A.8 Filter Builder Window

3. Click **[Sample Name]** and choose one of the column item.
4. Click **Equals** and choose one of the operator. Table A.3 shows the conditions you can choose from.
5. Click **<Enter a value>** and enter the operand value.
6. Click the **Apply** button to apply the filter setting to the grid. If you want to apply and close the Filter Builder window, click the **OK** button.

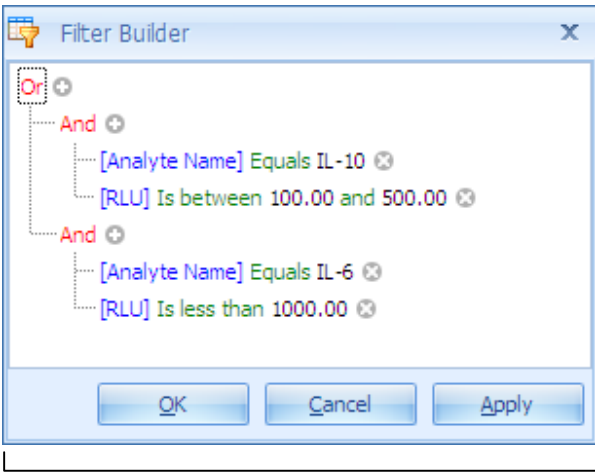
Table A.3 Available Commands for Filter

| Icon | Operator                        | Function                                                  |
|------|---------------------------------|-----------------------------------------------------------|
| =    | <b>Equals</b>                   | Shows only [Sample Name] = <value>.                       |
| ≠    | <b>Does not equal</b>           | Shows only [Sample Name] ≠ <value>                        |
| >    | <b>Is greater than</b>          | Shows only [Sample Name] > <value>.                       |
| ≥    | <b>Is greater than or equal</b> | Shows [Sample Name] = <value> and [Sample Name] > <value> |
| <    | <b>Is less than</b>             | Shows only [Sample Name] < <value>                        |
| ≤    | <b>Is less than or equal</b>    | Shows [Sample Name] = <value> and [Sample Name] < <value> |

## APPENDIX A

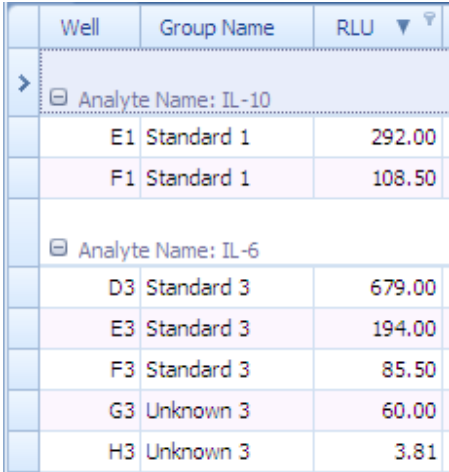
### PREFERENCES

|                                                                                   |                         |                                                                                 |
|-----------------------------------------------------------------------------------|-------------------------|---------------------------------------------------------------------------------|
|  | <b>Is between</b>       | Shows only the value which has between first <value> and second <value>         |
|  | <b>Is not between</b>   | Shows only the outside value which has between first <value> and second <value> |
|  | <b>Contains</b>         | Shows only the value contains <value>                                           |
|  | <b>Does not contain</b> | Shows only the value does not contains <value>                                  |
|  | <b>Begins with</b>      | Shows only the value starts with <value>                                        |
|  | <b>Ends with</b>        | Shows only the value ends with <value>                                          |
|  | <b>Is like</b>          | Shows only [Sample Name] > <value>                                              |
|  | <b>Is not like</b>      | Shows only [Sample Name] > <value>                                              |
|  | <b>Is blank</b>         | Shows only value are blank.                                                     |
|  | <b>Is not blank</b>     | Shows only value are not blank.                                                 |
|  | <b>Is any of</b>        | Shows only value which has <value>s.                                            |
|  | <b>Is none of</b>       | Shows only value does not have <value>s.                                        |



Shows only,  
Analyte name is 'IL-10' and Response Value is between 100 and 500,  
or  
Analyte name is 'IL-6' and Response Value is less than 1000





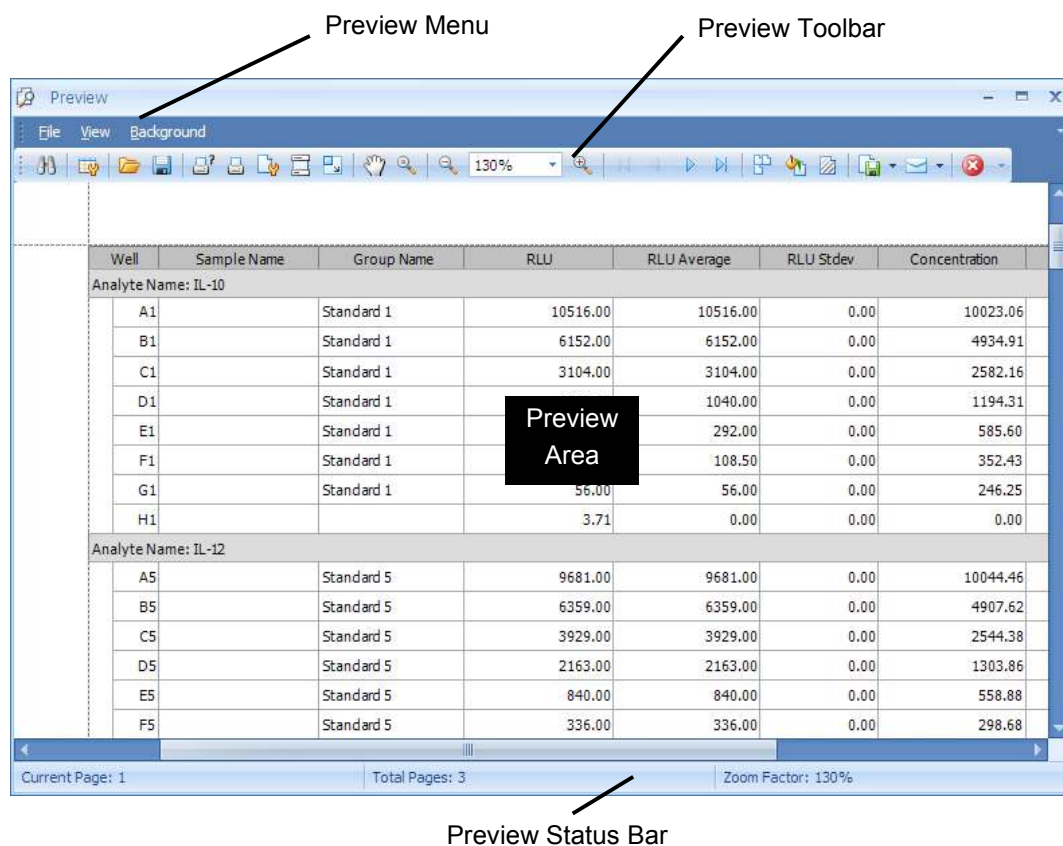
| Well                       | Group Name | RLU    |
|----------------------------|------------|--------|
| <b>Analyte Name: IL-10</b> |            |        |
| E1                         | Standard 1 | 292.00 |
| F1                         | Standard 1 | 108.50 |
| <b>Analyte Name: IL-6</b>  |            |        |
| D3                         | Standard 3 | 679.00 |
| E3                         | Standard 3 | 194.00 |
| F3                         | Standard 3 | 85.50  |
| G3                         | Unknown 3  | 60.00  |
| H3                         | Unknown 3  | 3.81   |

**Figure A.9 Example of the complex filter criteria**

## A.2

### Print Preview Menu

Print preview allows you to zoom, navigate, print out, set printing options, export and other useful tasks (Figure A.10).



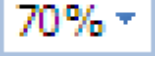
**Figure A.10 Print Preview Window**

**Table A.4 Preview Toolbar buttons and functions**

| Icon                                                                                | Command          | Function                                                 |
|-------------------------------------------------------------------------------------|------------------|----------------------------------------------------------|
|  | <b>Search</b>    | Search specific word or value from the preview document. |
|  | <b>Customize</b> | Customize the printing items.                            |
|  | <b>Open</b>      | Open preview document files(*.prnx).                     |

## APPENDIX A

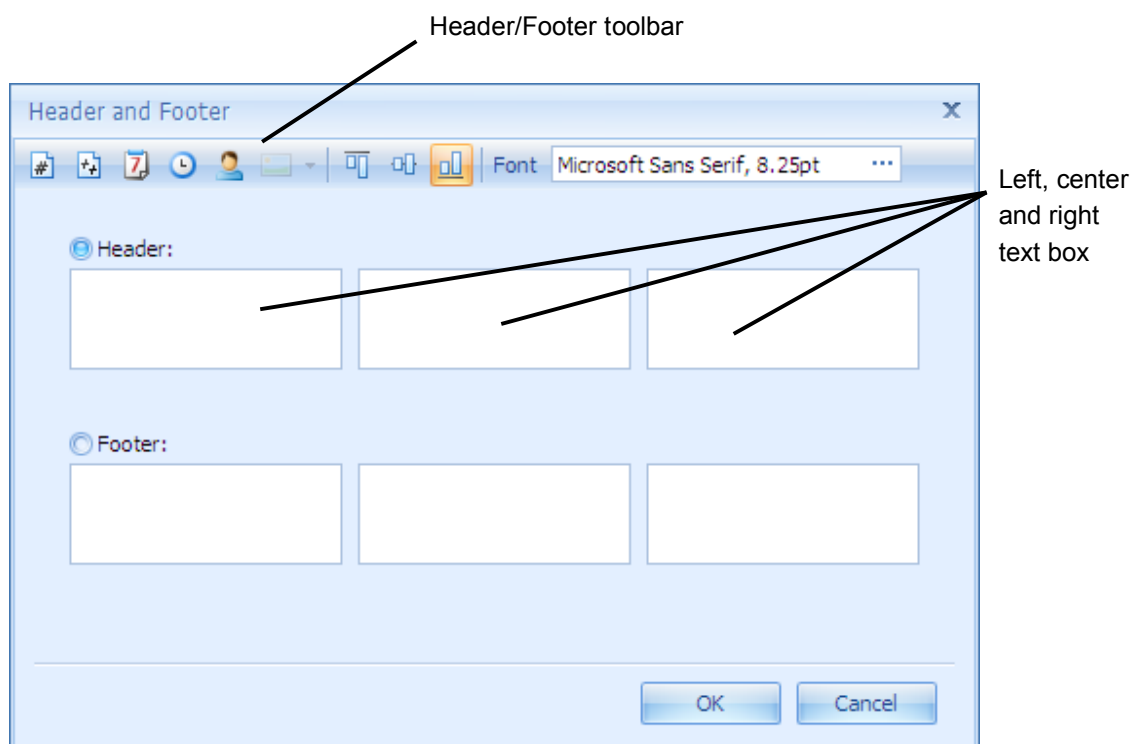
### PREFERENCES

|                                                                                     |                          |                                                          |
|-------------------------------------------------------------------------------------|--------------------------|----------------------------------------------------------|
|    | <b>Save</b>              | Save preview document by *.prnx format.                  |
|    | <b>Print...</b>          | Open print dialog.                                       |
|    | <b>Print</b>             | Print the preview document.                              |
|    | <b>Print Setup...</b>    | Open print setup dialog.                                 |
|    | <b>Header and Footer</b> | Set up header and footer options (Figure A.10).          |
|    | <b>Scale</b>             | Set page scale.                                          |
|    | <b>Hand Tool</b>         | User hand icon                                           |
|   | <b>Magnifier</b>         | User magnifier                                           |
|  | <b>Zoom Out</b>          | Zoom out the preview document.                           |
|  | <b>Zoom</b>              | Set zoom size from the drop-down list.                   |
|  | <b>Zoom In</b>           | Zoom in the preview document.                            |
|  | <b>First Page</b>        | Show first page.                                         |
|  | <b>Previous Page</b>     | Show previous page.                                      |
|  | <b>Next Page</b>         | Show next page                                           |
|  | <b>Last Page</b>         | Show last page.                                          |
|  | <b>Multiple Pages</b>    | Select multi pages to preview.                           |
|  | <b>Color...</b>          | Open color picker and set the document background color. |

|                                                                                   |                           |                                                                                                           |
|-----------------------------------------------------------------------------------|---------------------------|-----------------------------------------------------------------------------------------------------------|
|  | <b>Water Mark...</b>      | Open water mark setting dialog (Figure A.10).                                                             |
|  | <b>Export Document...</b> | Export the document by selected format.<br>PDF, HTML, MHT, RTF, Excel, CSV, Text, Image                   |
|  | <b>Send via E-Mail...</b> | Export the document by selected format, and send it via e-mail.<br>PDF, MHT, RTF, Excel, CSV, Text, Image |
|  | <b>Exit</b>               | Close preview window.                                                                                     |

### Insert Header/Footer

To insert header/footer into your printing document, open **Header and Footer** dialog (Figure A.11). Select header or footer radio button, then input text or items from the header/footer toolbar (Table A.5) in the left, center or right text box.



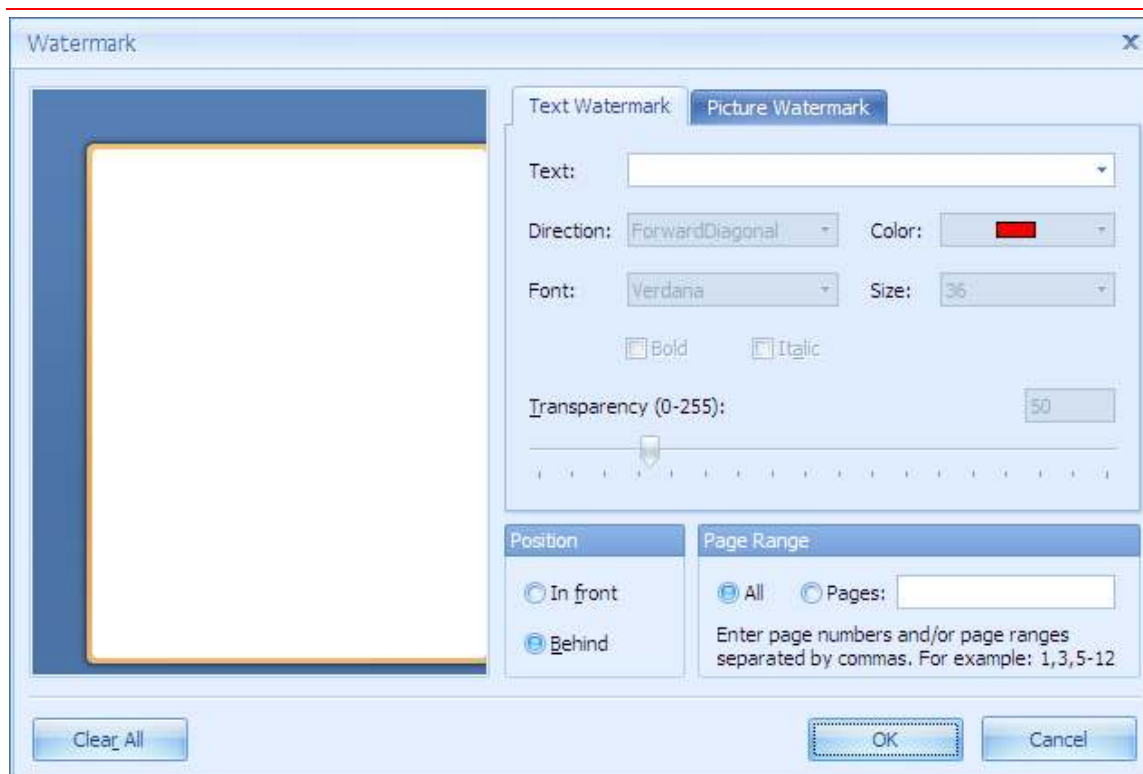
**Figure A.11 Header and Footer dialog**

**Table A.5 Header and Footer Toolbar buttons and functions**

| Icon        | Command                  | Function                                                   |
|-------------|--------------------------|------------------------------------------------------------|
|             | <b>Page Number</b>       | Insert page number.                                        |
|             | <b>Page # of Pages #</b> | Insert '(current page) of (total pages)' type page number. |
|             | <b>Date Printed</b>      | Insert date printed.                                       |
|             | <b>Time Printed</b>      | Insert time printed.                                       |
|             | <b>User Name</b>         | Insert user name who login the windows.                    |
|             | <b>Image</b>             | Insert the image.                                          |
|             | <b>Align to Top</b>      | Align all headers/footers to the top level.                |
|             | <b>Align to Center</b>   | Align all headers/footers to the center level.             |
|             | <b>Align to Bottom</b>   | Align all headers/footers to the bottom level.             |
| <b>Font</b> | <b>Font</b>              | Open font dialog for the header and footer font.           |

### Insert Water Mark

To insert water marks into your printing document, open **Water Mark** dialog (Figure A.12). There are two types of watermarks you can insert in your document, one is 'Text' and another is 'Picture'. They are separated into two tabs. To insert a text watermark, use 'Text Watermark' tab. To insert a picture watermark, use the 'Picture Watermark' tab.



**Figure A.12 Watermark dialog**

**Table A.6 Text Watermark properties**

| Property            | Function                                                                                                                                                                                                         |
|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Text</b>         | Input text word in the text box or select predefined words from the drop-down list<br>Predefined words: ASAP, CONFIDENTIAL, COPY, DO NOT COPY, DRAFT, EVALUATION, ORIGINAL, PERSONAL, SAMPLE, TOP SECRET, URGENT |
| <b>Direction</b>    | Select text direction from one of the followings:<br>Horizontal, Vertical, BackwardDiagonal, ForwardDiagonal                                                                                                     |
| <b>Color</b>        | Open color picker and choose text font color.                                                                                                                                                                    |
| <b>Font</b>         | Open font dialog and choose text font.                                                                                                                                                                           |
| <b>Size</b>         | Specify text font size.                                                                                                                                                                                          |
| <b>Transparency</b> | Set transparency for the insert text.                                                                                                                                                                            |
| <b>Position</b>     | Select text position, In front or Behind.                                                                                                                                                                        |
| <b>Page Range</b>   | Set page range for the text water mark.                                                                                                                                                                          |

**Table A.7 Picture Watermark properties**

| <b>Property</b>             | <b>Function</b>                                                                                                                                                                                    |
|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Load image...</b>        | Open file open dialog and specify the image file to be inserted.                                                                                                                                   |
| <b>Size mode</b>            | Select one of the size mode from followings.<br>Clip: Insert the image as same size as original image.<br>Stretch: Stretch the image to horizontal direction.<br>Zoom: Zoom the image to the page. |
| <b>Horizontal alignment</b> | Set horizontal alignment, left, center or right.                                                                                                                                                   |
| <b>Vertical alignment</b>   | Set vertical alignment, left, center or right.                                                                                                                                                     |
| <b>Tiling</b>               | Fill up the page by the image.                                                                                                                                                                     |
| <b>Transparency</b>         | Set transparency for the insert image.                                                                                                                                                             |
| <b>Position</b>             | Select image position, In front or Behind.                                                                                                                                                         |
| <b>Page Range</b>           | Set page range for the image water mark.                                                                                                                                                           |

## APPENDIX A PREFERENCES

| Bead Name: GM-CSF |              |          |         |     |          |         |
|-------------------|--------------|----------|---------|-----|----------|---------|
| A1                | Background 1 | 41.00    | 0.00    | 189 | 103.94   | 84.27   |
| B1                | Standard 1   | 10516.00 | 2970.39 | 208 | 10429.40 | 2843.14 |
| C1                | Standard 1   | 6152.00  | 2970.39 | 225 | 4908.06  | 2843.14 |
| D1                | Standard 1   | 3104.00  | 2970.39 | 213 | 2567.05  | 2843.14 |
| E1                | Standard 1   | 1040.00  | 2970.39 | 181 | 1211.88  | 2843.14 |
| F1                | Standard 1   | 292.00   | 2970.39 | 165 | 600.61   | 2843.14 |
| G1                | Standard 1   | 108.50   | 2970.39 | 190 | 345.86   | 2843.14 |
| H1                | Standard 1   | 56.00    | 2970.39 | 183 | 203.36   | 2843.14 |
| A2                | Background 1 | 39.00    | 0.00    | 194 | 64.60    | 84.27   |
| B2                | Standard 1   | 10034.50 | 2970.39 | 152 | 9609.67  | 2843.14 |
| C2                | Standard 1   | 6299.00  | 2970.39 | 246 | 5042.17  | 2843.14 |
| D2                | Standard 1   | 2990.50  | 2970.39 | 252 | 2491.46  | 2843.14 |
| E2                | Standard 1   | 1094.00  | 2970.39 | 223 | 1249.38  | 2843.14 |
| F2                | Standard 1   | 300.00   | 2970.39 | 217 | 609.27   | 2843.14 |
| G2                | Standard 1   | 104.00   | 2970.39 | 259 | 336.75   | 2843.14 |
| H2                | Standard 1   | 55.00    | 2970.39 | 202 | 199.04   | 2843.14 |
| A3                | Unknown 1    | 37.00    | 540.89  | 193 | <64.60   | 671.22  |
| B3                | Unknown 1    | 66.00    | 540.89  | 199 | 240.47   | 671.22  |
| C3                | Unknown 1    | 39.00    | 540.89  | 247 | 64.60    | 671.22  |
| D3                | Unknown 1    | 41.00    | 540.89  | 183 | 103.94   | 671.22  |
| E3                | Unknown 1    | 45.00    | 540.89  | 201 | 143.07   | 671.22  |
| F3                | Unknown 1    | 39.00    | 540.89  | 213 | 64.60    | 671.22  |
| G3                | Unknown 1    | 45.00    | 540.89  | 207 | 143.07   | 671.22  |
| H3                | Unknown 1    | 51.00    | 540.89  | 177 | 180.04   | 671.22  |
| A4                | Unknown 1    | 112.00   | 540.89  | 226 | 352.75   | 671.22  |
| B4                | Unknown 1    | 4911.00  | 540.89  | 203 | 3865.87  | 671.22  |
| C4                | Unknown 1    | 5011.00  | 540.89  | 220 | 3944.48  | 671.22  |
| D4                | Unknown 1    | 5591.00  | 540.89  | 262 | 4418.11  | 671.22  |
| E4                | Unknown 1    | 3070.00  | 540.89  | 165 | 2544.35  | 671.22  |
| F4                | Unknown 1    | 1129.00  | 540.89  | 238 | 1273.49  | 671.22  |
| G4                | Unknown 1    | 107.50   | 540.89  | 182 | 343.86   | 671.22  |
| H4                | Unknown 1    | 5578.00  | 540.89  | 241 | 4407.15  | 671.22  |
| A5                | Unknown 1    | 92.00    | 540.89  | 204 | 310.71   | 671.22  |
| B5                | Unknown 1    | 106.50   | 540.89  | 200 | 341.85   | 671.22  |
| C5                | Unknown 1    | 65.00    | 540.89  | 172 | 237.15   | 671.22  |
| D5                | Unknown 1    | 3989.50  | 540.89  | 232 | 3177.84  | 671.22  |
| E5                | Unknown 1    | 1808.00  | 540.89  | 218 | 1722.64  | 671.22  |
| F5                | Unknown 1    | 119.00   | 540.89  | 179 | 366.03   | 671.22  |
| G5                | Unknown 1    | 111.00   | 540.89  | 211 | 350.80   | 671.22  |
| H5                | Unknown 1    | 59.00    | 540.89  | 201 | 215.54   | 671.22  |
| A6                | Unknown 1    | 2426.50  | 540.89  | 218 | 2122.04  | 671.22  |
| B6                | Unknown 1    | 186.00   | 540.89  | 219 | 471.89   | 671.22  |
| C6                | Unknown 1    | 43.00    | 540.89  | 202 | 126.21   | 671.22  |
| D6                | Unknown 1    | 113.00   | 540.89  | 218 | 354.68   | 671.22  |

Figure A.13 Example of the text and image watermark

**APPENDIX B**

*The toolbars that are available depend on the types of windows that are open in the main display area.*

**B.1**

**Main File Menu and Toolbar**

**Table B.1 Main File menu and toolbar**

| Menu Bar Command                | Main Toolbar Button                                                                 | Function                                                                                                                    |
|---------------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| <b>File → New</b>               |    | Open blank plate for ReaderFit application.                                                                                 |
| <b>File → Open</b>              |  | Displays the Open dialog box so that a results file (.csv, .txt or .xls), MasterPlex® ReaderFit file (.mxqs) may be opened. |
| <b>File → Close</b>             |  | Close currently opened plate data file.                                                                                     |
| <b>File → Save</b>              |  | Save currently opened plate data.                                                                                           |
| <b>File → Save as</b>           | -                                                                                   | Save currently opened plate data as different file name.                                                                    |
| <b>File → Recent Files</b>      | -                                                                                   | List up the files recently opened.                                                                                          |
| <b>File → Exit</b>              | -                                                                                   | Close MasterPlex® application.                                                                                              |
| <b>Analyte Filter</b>           |  | Valid analyte filter feature.                                                                                               |
| <b>Virtual Plate</b>            |  | Generate virtual plate. It opens plate dimension input dialog.                                                              |
| <b>Windows → Show Tab style</b> | -                                                                                   | Set tab style window display                                                                                                |
| <b>Windows → Cascade</b>        | -                                                                                   | Arrange the window by cascade style.                                                                                        |
| <b>Windows → Tile</b>           | -                                                                                   | Arrange the window by horizontal style.                                                                                     |

**APPENDIX B**  
**MASTERPLEX® ReaderFit TOOLBARS**

|                                |   |                                                                       |
|--------------------------------|---|-----------------------------------------------------------------------|
| <b>Horizontal</b>              |   |                                                                       |
| <b>Windows → Tile Vertical</b> | - | Arrange the window by vertical style.                                 |
| <b>Tools → User Management</b> | - | Open user management dialog.<br>(For 21 CFR Part 11 module only)      |
| <b>Tools → Log Viewer</b>      | - | Open log viewer.<br>(For 21 CFR Part 11 module only)                  |
| <b>Tools → Verify files</b>    | - | Open verification checker dialog.<br>(For 21 CFR Part 11 module only) |
| <b>License → Applications</b>  | - | Open application license information dialog.                          |
| <b>License → Plugins</b>       | - | Open plugin license information dialog.                               |
| <b>Help → Manuals</b>          | - | Open manuals folder.                                                  |
| <b>Help → Tutorial</b>         | - | Open video tutorial.                                                  |
| <b>Help → Online Support</b>   | - | Open support URL by the default browser.                              |
| <b>Help → About</b>            | - | Display splash screen with application version information.           |
| <b>Look and Feel</b>           | - | Change application skins.                                             |

## B.2

### Input Data Tab Toolbar

The Input Data tab toolbar is available on the top of the well grid in the plate tab.



**Figure B.2 Plate toolbar**

**Table B.2 Plate toolbar buttons and functions**

| Menu Bar Command               | Toolbar Icon                                                                        | Function                                       |
|--------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------|
| <b>Background</b>              |    | Mark selected wells as Background.             |
| <b>Standard</b>                |  | Mark selected wells as Standard.               |
| <b>Unknown</b>                 |  | Mark selected wells as Unknown.                |
| <b>Control</b>                 |  | Mark selected wells as Control.                |
| <b>Unmark</b>                  |  | Unmark selected wells.                         |
| <b>Subtract Background</b>     |  | Toggle subtract background function.           |
| <b>Best Fit</b>                |  | Start best fit mode.                           |
| <b>Auto Fill</b>               |  | Open Auto Fill dialog.                         |
| <b>Link Standard</b>           |  | Make link(s) between standard data and others. |
| <b>Quality Control Manager</b> |  | Open Quality Control Manager.                  |

|                          |                                                                                   |                               |
|--------------------------|-----------------------------------------------------------------------------------|-------------------------------|
| <b>Template Manager</b>  |  | Open template manager.        |
| <b>Plate Preferences</b> |  | Open plate preference dialog. |

## B.3

### View Results Tab Toolbar

The View Results toolbar is available on the top of the data grid in the View Results tab.



**Figure B.4 Calculation toolbar**

**Table B.4 Calculation toolbar buttons and functions**

| Menu Bar Command                  | Toolbar Button                                                                      | Function                                                                     |
|-----------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| <b>Show/Hide Columns Selector</b> |  | Show or hide columns selector.                                               |
| <b>Export to File</b>             |  | Export data table by selected format.<br>Format: Excel, CSV, PDF, HTML, Text |
| <b>Print Preview</b>              |  | Open print preview window                                                    |
| <b>Table Template</b>             |  | Open table template manager.                                                 |
| <b>Merge Cells By Group</b>       |  | Toggle the cell merge mode.                                                  |

## B.4

### Create Graphs Tab Toolbar

The chart toolbar is available on the top of the chart view area in the Create Graphs tab.



**Figure B.5 Chart toolbar**

**Table B.5 Chart toolbar buttons and functions**

| Menu Bar Command        | Toolbar Button                                                                      | Function                                        |
|-------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------|
| <b>Replicate View</b>   |    | Toggle replicate view mode.                     |
| <b>Analyte Selector</b> |  | Shows drop-down list for all analytes.          |
| <b>Well Selector</b>    |  | Shows mini-sized plate view.                    |
| <b>Chart Gallery</b>    |  | Shows drop-down list of available chart format. |
| <b>Color Palette</b>    |  | Shows drop-down list of available palette.      |
| <b>Chart Properties</b> |  | Open chart properties dialog.                   |
| <b>Chart Template</b>   |  | Open chart template manager.                    |
| <b>Print Preview</b>    |  | Open print preview.                             |

## **B.5**

### **Customized Report Manager Tab Toolbar**

The Customized Report Manager toolbar is available on the top of the preview area in the Customized Report Manager tab.



**Figure B.5 Chart toolbar**

**Table B.6 Chart toolbar buttons and functions**

| <b>Menu Bar Command</b> | <b>Toolbar Button</b>                                                             | <b>Function</b>                   |
|-------------------------|-----------------------------------------------------------------------------------|-----------------------------------|
| <b>Print</b>            |  | Print current displayed document. |

APPENDIX C

*This appendix provides background on the Four and Five Parameter Logistic curves. It also explains how weighting methods can improve the fit of nonlinear models to data with non-constant variability (heteroscedasticity).*

C.1

Four Parameter Logistic Curve

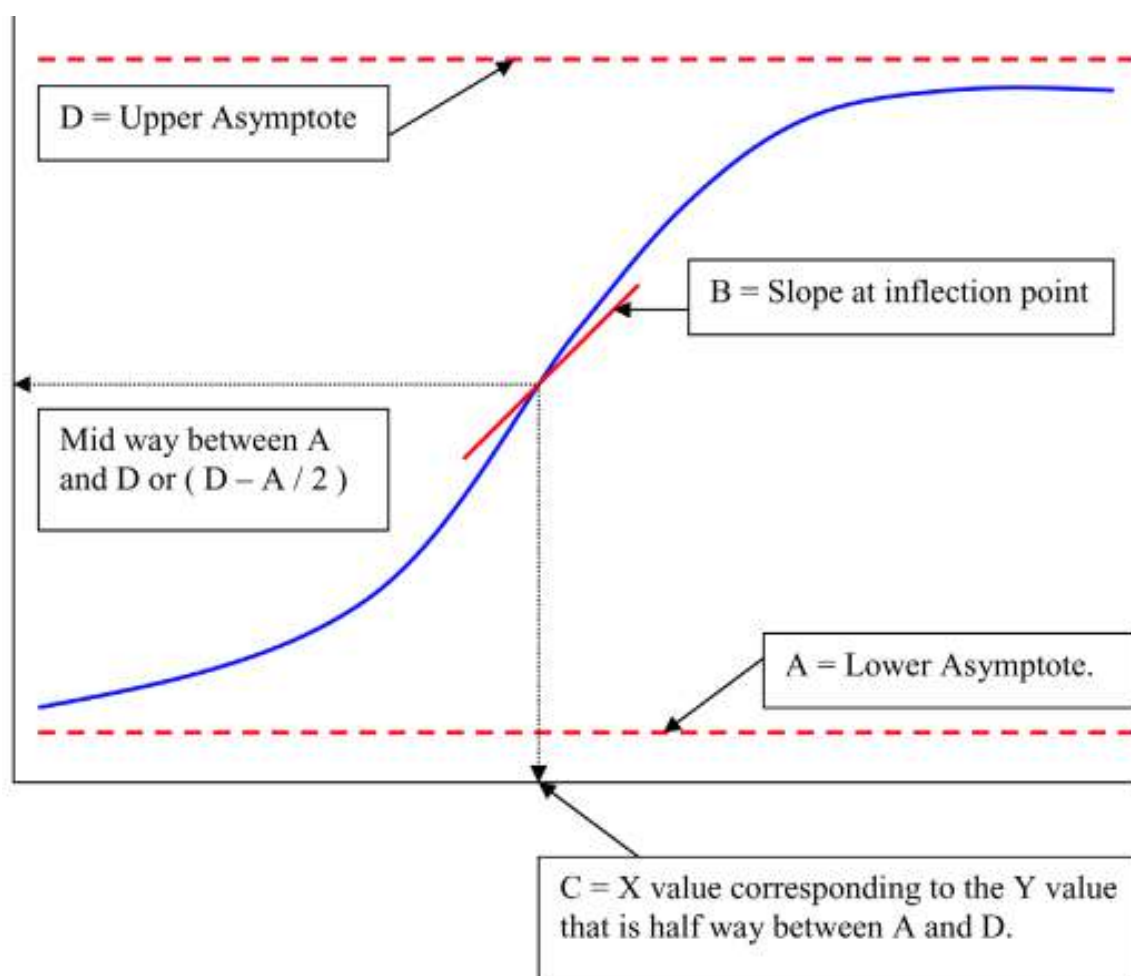


Figure C.1 Four parameter logistics curve

In Figure C.1, the asymptotes D and A are the upper and lower limits of the model. An asymptote is a value that the function never reaches. Therefore, the value of a function at or beyond an asymptote cannot be

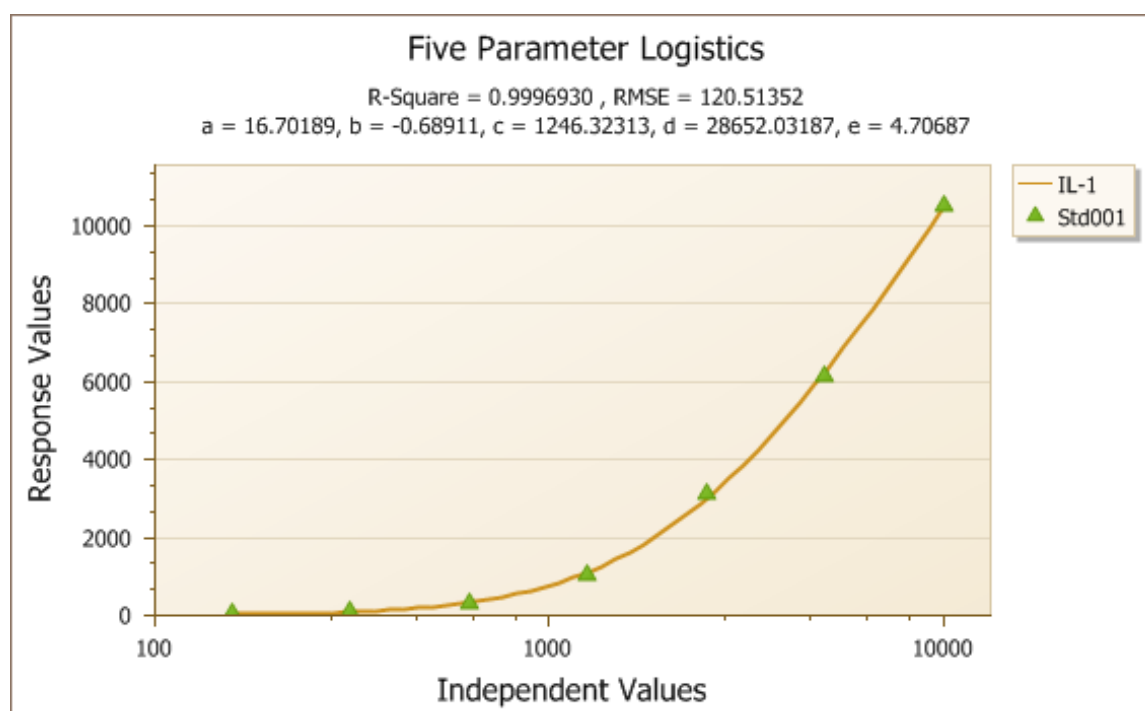
predicted. If Response Value  $\leq A$  or Response Value  $\geq D$ , the Response Value is out of the calculable range of the Four Parameter Logistics model. It is not possible to mathematically extrapolate values that are equal to or beyond the asymptote values.

For example, imagine the function  $F(x) = \text{Log}(x)$ . The vertical asymptote for this function is the line  $x = 0$ . This means the function can never reach the line where  $x = 0$ . For example,  $\log(0)$  cannot be calculated.

In Figure C.1, B is the slope at the inflection point. It is the speed of the function as it moves away from the inflection point. C is the most interesting parameter. The C parameter corresponds to the x value that is associated with the y value at the midpoint between the minimum and maximum limits of the function. In a biochemical assay, C corresponds to the concentration (since it is on the x-axis) that produces a 50% response.

## C.2

### Five Parameter Logistic Curve



**Figure C.2 Five parameter logistic curve**

A Five Parameter Logistic curve introduces an additional parameter to the Four Parameter Logistic model to compensate for asymmetric data. In Figure C.2, we can see that the curve is not symmetrical because the lower part of the curve behaves quite differently from the upper portion. The additional parameter E compensates for this asymmetric behavior and adjusts the curve.

### **Fixed Lower Asymptote Zero**

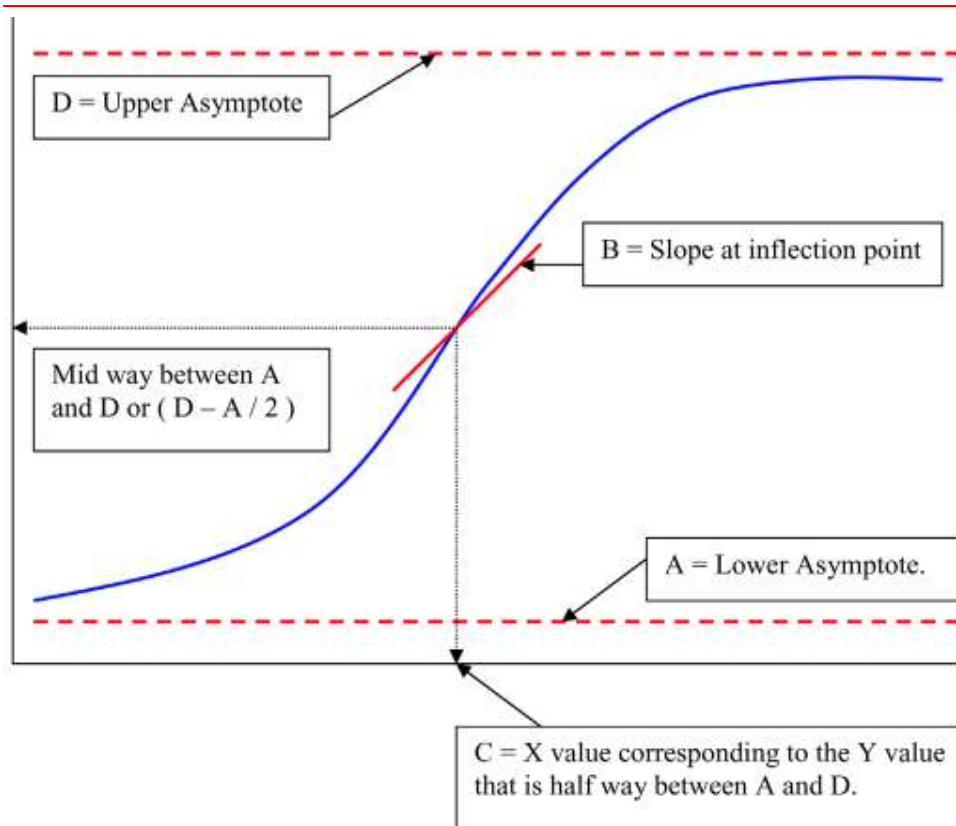
Usually an asymptote is estimated where a function is tending toward a constant value. A fixed asymptote is a restriction that tells the function to never go beyond that value. For example, setting  $A = 0$ , as in the 'Fixed lower asymptote zero' option in the Fit Curves tab. It is used with logistic functions, like the Four Parameter Logistics (4PL) equation shown below.

$$F(x) = \frac{A - D}{\left(1 + \left(\frac{x}{C}\right)^B\right)} + D$$

#### **Four Parameter Logistics Model**

The assumption that the lower asymptote is zero is probably incorrect in some cases, but it allows the user to reduce the number of parameters in the equation.

The asymptote sets a bound for the parameter. In the 4PL model, there are lower and upper asymptotes, defined as the A and D parameters respectively (See diagram below).



**Figure C.1 Four parameter logistics curve**

MasterPlex® ReaderFit extrapolates the analyte concentration when:

$A < \text{Response Value} < \text{Lowest standard Response Value}$

or

$\text{Highest standard Response Value} < \text{Response Value} < D$

where A is the bottom asymptote and D is the top asymptote of the sigmoidal curve. An Response Value less than A or greater than D is beyond the range of the standard curve model and the concentration value cannot be extrapolated.

In the 2-dimensional representation of the curve shown above, Response Value is the Y-axis value. Theoretically, Response Value cannot be less than zero. So, setting the lower asymptote to zero is reasonable. But there can be measured data that actually has some negative Response Values, which can be

caused by user error.

MasterPlex® ReaderFit provides a function to subtract the background noise from the Response Values to obtain more accurate responses. If the user subtracts a reasonable amount of background, it could be assumed that the lowest detectable Response Value is zero. Note that  $\log(0)$  is undefined. So, in some cases, fixing the lower asymptote to zero is theoretically correct.

However, in reality, most datasets contain some slight amount of error, so fixing the lower asymptote to zero can actually make it more difficult to achieve a good curve fit. For this reason, a slightly better R2 value is usually reported when the curve fitting is done with this feature turned off (unchecked), which is the default, but the difference in the R2 value would be very small – usually less than 1/100,000th or 0.00001. Any change in the graph would be unnoticeable. The difference in the interpolated concentrations would also be very small – usually less than 2% if any.

Asymptotes can be used to gauge the similarity of functions. For example, if two curves calculated with the Four Parameter Logistics equation end up having very close to the same A and D parameters, their interpolated concentrations would be in the same range.

The lower and upper asymptotes (A and D) of the 4PL and 5PL models usually calculated automatically during the curve fitting process and are derived from the characteristics of the data. The “Fixed lower asymptote zero” feature makes it possible to fix the lower asymptote to a hardcoded value of zero, which is useful in some experiments. This feature is reasonable to use if enough background was subtracted and the data has little user error. But for most data sets, the R2 and concentration values will not be improved with this feature on.

### **Log-Log Model**

The Log-Log model transforms the data to log scale for the x and y values. It applies linear regression to fit a straight line through these points. This model is appropriate for data that are intrinsically linear.

## C.3

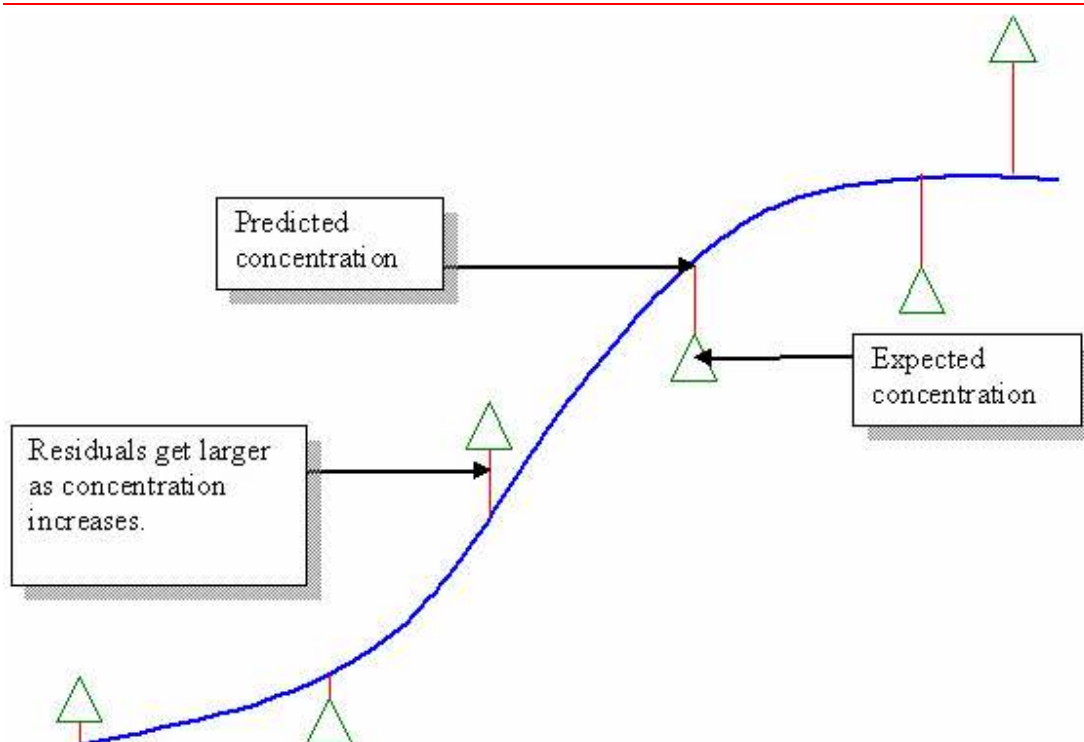
### Heteroscedasticity

Fitting nonlinear models to observed data is often complicated by non-constant or heterogeneous variability. Heterogeneous variability or *heteroscedasticity* occurs in most types of observed data. This is especially true for biochemical assays where concentration or dose is the predictor. Therefore, we can expect that measurement error varies with respect to the mean. In the plate reader system, Response Values are based on vary with the concentration. In this case, we expect the error in detecting Response Value to increase as concentration increases. This is best seen in Figure C.3, a residual plot from a plate reader cytokines assay.

A residual plot is a graphical representation of how far away an observed concentration is from its expected value. It plots residuals against observed concentrations. In Figure C.3, we can see that the deviation of the observed concentration from the expected value increases as concentration increases. This means the variability is not constant.

Residual plots help you detect non-constant variability as well as outliers. If a residual plot exhibits data points in a wedge or funnel-shaped pattern, then we can expect the underlying data to have non-constant variability.

Non-constant variability complicates curve fitting because the regression process assumes the errors are constant across all data points. When the data violate this assumption, the resulting curve fit is less than optimal. This is illustrated in Figure C.4.



**Figure C.4 Data with non-constant variability**

*The residual increases as concentration increases.*

When we fit a model to the data, the curve is applied to all of the data points as closely as possible so that the distances between the predicted and expected concentrations are minimized. In Figure C.4 we can think of the lines that represent the residuals as ropes and each data point as a wrench. Curve fitting can be imagined as pulling the curve line so that it is as close to each point as possible without snapping the actual curve.

The best curve fit is reached when the curve is pulled as close as possible to each data point without breaking the actual curve model.

The nonlinear least square algorithm accomplishes this task. The nonlinear (or linear) least square algorithm assumes that all points have the same variability, so all points influence the curve fit equally.

However, data that exhibit non-constant variability violate this assumption. As a result, data points with greater variability assert more ‘pull’ on the curve.

Data points at higher concentrations have more variability than those at lower concentrations, and have greater influence on the curve fit than the points at a lower concentration. As a result, accuracy or sensitivity at the lower

concentrations decreases.

### **Levenberg-Marquardt algorithm (LMA)**

The Levenberg–Marquardt algorithm (LMA) used by MasterPlex® ReaderFit minimizes a function over a space of parameters of the function. It is commonly used in least-square-fitting and nonlinear regression (curve fitting). LMA interpolates between the Gauss-Newton algorithm (GNA) and gradient descent. LMA is more robust than GNA, but can be slower for very large datasets. Robustness is a measure of how well the algorithm can do the curve fitting with fewer data.

Given two points on a data grid, LMA iteratively takes steps from point 1 towards point 2, adjusting its slope each time. This is harder to visualize in a multidimensional space, but LMA adjusts in all directions simultaneously as it fits a multivariate function.

LMA adjusts the parameters of the equation (i.e. the Five Parameter Logistics or Four Parameter Logistics equations) at each iteration of the algorithm until there is no improvement in the curve fit, or until it reaches a maximum threshold (100 iterations in this case). To accomplish the curve fitting, it combines two common statistical math techniques: Gradient Descent and Gauss-Newton (GNA).

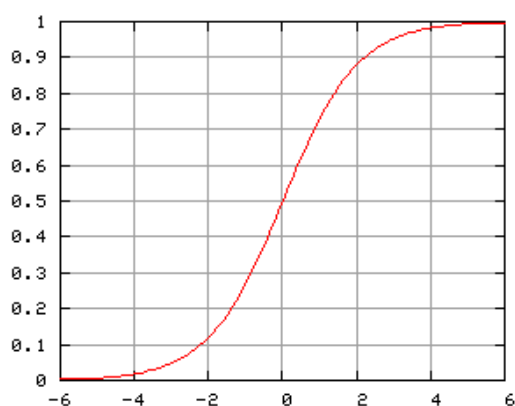
**Gradient Descent:** Take steps proportional to the negative of the gradient of the function at the current point. Each step reduces the sum of squares.

**Gauss-Newton (GNA):** Determine the amount of change in sum of squares when each parameter changes. This determines the slope at that point.

Gradient descent usually works well in early iterations, while Gauss-Newton usually works well in later iterations. LMA automatically switches between these two methods. The result is a curve fit with a high degree of precision over a wide variety of datasets. No algorithm can do curve fitting for all

datasets since some datasets are too sparse or have faulty data. But a good algorithm can find a curve fit for many datasets, and have a degree of precision in its parameter calculations.

To track the data points, MasterPlex® ReaderFit uses a class of a nonlinear transfer function, called a sigmoid function, for example the Five Parameter Logistics model. This typically produces a sigmoidal, or S-shaped curve (Figure C.5).



**Figure C.5 A sigmoidal or S-shaped curve**

MasterPlex® ReaderFit uses 4 or 5 parameters models to help in the curve fitting for the nonlinear model. The Five Parameter Logistics model compensates for asymmetry in the curve. To a certain extent, it is useful to have more parameters to tweak during the curve fitting process, thus producing a more precise curve to fit the data. This is why the Five Parameter Logistics model usually outperforms the Four Parameter model for most datasets.

MasterPlex® ReaderFit includes a Best Fit feature to automatically choose a model and weighting method that produces the best curve fit. However, choosing a model can be a scientific decision, based on chemistry or biology. MasterPlex® ReaderFit still provides the flexibility for the scientist to choose the model and weighting method themselves, which may be desirable in some cases, depending on the assay.

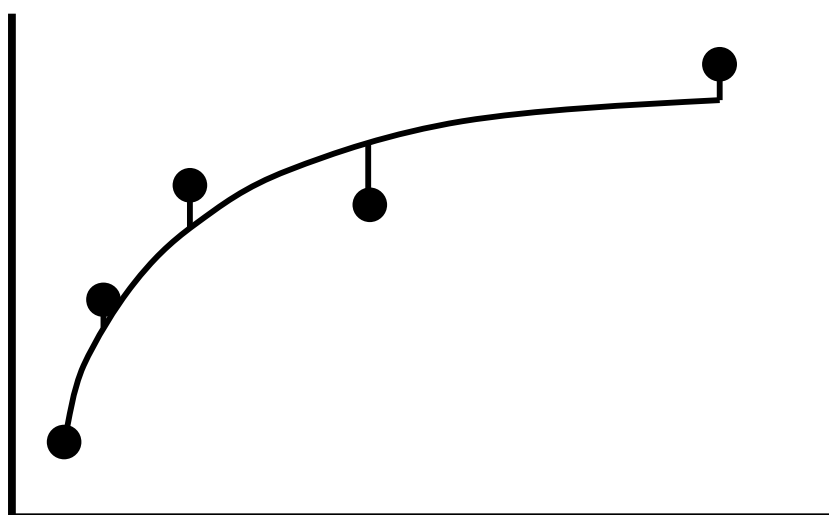
---

## Interpreting the Result

One measure of goodness of the curve fit is the  $R^2$  value.  $R^2$  is a fraction between 0.0 and 1.0. A high  $R^2$  value means the curve came close to the points. When all calculations have been completed,  $R^2$  is reported on the curve chart of the Standard tab.

## Calculating $R^2$

$SS_{\text{reg}}$  is the sum of the squares of the distances of the points from the best-fit curve determined by nonlinear regression.



**Figure C.6 A sigmoidal or S-shaped curve**

$SS_{\text{tot}}$  is the sum of the square of the distances of the points from a horizontal line through the mean of all Y values.

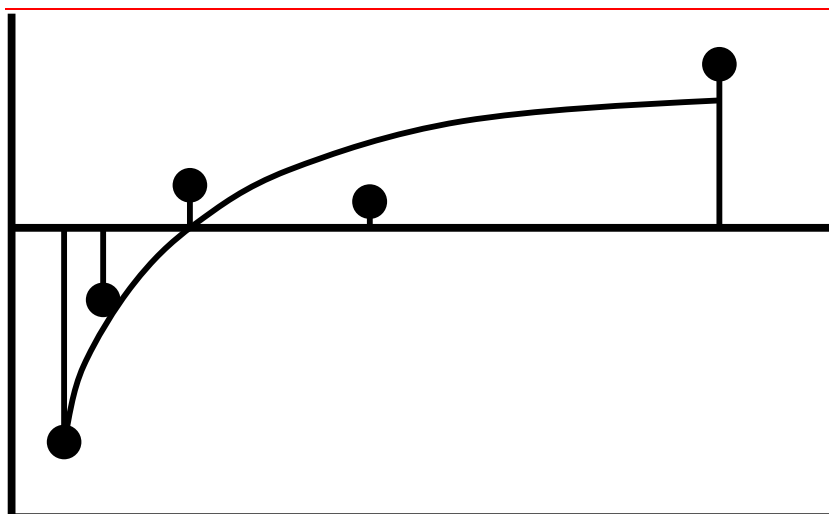


Figure C.8 A sigmoidal or S-shaped curve

$R^2$  is calculated using this equation:

$$R^2 = 1.0 - \frac{SS_{reg}}{SS_{tot}}$$

Note that  $R^2$  is not really the square of anything. Also,  $R^2$  could be negative.  $R^2$  will be negative when the best-fit curve fits the data worse than a horizontal line at the mean Y value. This could happen if you pick an inappropriate model, or fix a parameter to an inappropriate constant value.

### Weighting Methods

- $1/Y^2$  : for when you expect the average distance of the points from the curve to be higher when Y is higher, but the relative distance to be a constant.
- $1/Y$  : for when it follows a Poisson distribution or there's a counting error

- $1/X$  or  $1/X^2$  : for weighting the left part of the graph more than the right

The only problem with weighting methods is that the user cannot always predict the weight values, because of unknown error distribution. But for many measured datasets, it is often the case that the Response Values are more accurate at their lower levels, so it may be reasonable to use the  $1/Y^2$  weighting method to put more weight on the lower part of the curve.

## C.4

### Weighted Nonlinear Least Square

The weighted nonlinear least square method of curve fitting is one way to correct for non-constant variability. In this method, weights are assigned to each point so that all points have equal influence on the curve. Instead of minimizing the residuals, the method minimizes residuals based on the weight at each point. In mathematical terms, the non-constant variability is made constant again by these additional weighting factors. If the weight for a point is higher, it will influence the curve fit more. The weight is the inverse of the variance, so points with low variability have more influence during curve fitting, which seems logical. As a result, the curve fit represents the data better and the sensitivity often increases at lower concentrations.

- $1/Y^2$  : for when you expect the average distance of the points from the curve to be higher when Y is higher, but the relative distance to be a constant.
- $1/Y$  : for when it follows a Poisson distribution or there's a counting error
- $1/X$  or  $1/X^2$  : for weighting the left part of the graph more than the right

The only problem with weighting methods is that the user cannot always

---

predict the weight values, because of unknown error distribution. But for many measured datasets, it is often the case that the Response Values are more accurate at their lower levels, so it may be reasonable to use the  $1/Y^2$  weighting method to put more weight on the lower part of the curve.

## **C.5**

### **Results of Weighting**

In addition to the R-Square value, MasterPlex® ReaderFit computes % Recovery values that measure how well the calibration curve (standard curve) fits the observed data.

$\% \text{ Recovery} = \text{Observed Concentration} / \text{Expected Concentration} * 100$

The closer % Recovery is to 100, the better the curve fit at that point. If % Recovery is less than 100, the point is below the curve. If it is greater than 100, the point is above the curve.