Manta
User's Guide

Table Of Contents

Getting started

System requirements

Minimum Requirements

The software will run on the following minimum system configuration:

CPU:	Pentium III or later
Memory:	128MB or greater
Available disk space:	50MB
Graphics:	800x600 resolution with 16 bit colour
Operating System:	Windows NT4(SP6), Windows 2000 or Windows XP
Other software:	Internet Explorer 5.0

Recommended

For optimum results with the software it is recommended you use a screen resolution of 1024x768.

It is also recommended that Internet Explorer 6.0 or later is used as some features are not available if using versions of Internet Explorer earlier than version 6.0. (The latest version can be downloaded from http://www.microsoft.com/windows/ie/default.asp)

Microsoft Word and Microsoft Excel versions 2000/XP or later are required for Word and Excel export option. (It is possible to Export numerical data to Excel 97, see page 133.)

Installing

Installing Manta

There are further requirements for running with Windows NT – see page 4 for more information.

Software Installation Procedure

1. Ensure you are logged on to Windows using an account with administrator privileges.
2. Close any open programs.
3. Insert the CD labeled **Manta** into your CD-ROM drive. If Auto-run is enabled on your system, the installation starts automatically and you can skip steps 4 and 5.
4. From the **Start** menu, select **Run**.
5. Type **D:\setup** (substitute the appropriate letter of your CD-ROM drive for D).
6. Follow the instructions on the screen.
7. When the installation has completed you may be prompted to restart the PC.
8. After installation is complete insert the program dongle and follow any onscreen messages.

After installation on Windows NT4, 2000 and XP it may be necessary to make changes as specified below.

System Administrator Information

User Files

By default, all user files are stored in the directory identified by the Windows CSIDL value:

CSIDL_PERSONAL

A typical path is **C:\Documents and Settings\username\My Documents**

This is typically the users My Documents folder.

Each user can override this by specifying their own setting from File | Options | Data in the main application window.

wtypes.dat

The file **wtypes.dat** in the installed Program Files directory contains settings which are common to all users of the software. It is therefore important that all users have Read and Write permissions on this file.

Windows NT compatibility issues

The software is compatible with Windows NT 4.0 Work Station; however there are some software requirements of the target system. These are:

Install as administrator

The software must be installed with a user account with administrator privileges. After installation, the software can be used by any user on the system (including users without administrator privileges).

User's data files are stored within the "My Assays" folder which is created within the "My Documents" sub-directory. In this way access to user data files can be restricted by the operating system on a per user basis.

Example data files are installed in the "My Assays" directory for the logged on user during installation. These example files can be manually copied to other users if required by the administrator.

NT Service Pack 6 or later

This can be downloaded from:

http://support.microsoft.com/default.aspx?scid=/support/servicepacks/WinNT/4.0/default.asp

Internet Explorer 5.x or later

Internet Explorer 5.x or later must be installed.

The latest version can be downloaded from
http://www.microsoft.com/windows/ie/default.asp

Tahoma font

The Tahoma font must be installed on your Windows 95 system. This is installed with:

Microsoft Office 97 for Windows

Microsoft Excel 97 for Windows

Microsoft Word 97 for Windows

Microsoft Access 97

Microsoft PowerPoint 97 for Windows

Microsoft Outlook 97

Microsoft Outlook 98

Microsoft Project 98 for Windows

If your system does not have the Tahoma font it can be downloaded from:

http://support.microsoft.com/default.aspx?scid=http://support.microsoft.com:80/support/kb/articles/Q188/0/81.ASP&NoWebContent=1

Also, it may be necessary to make changes as specified in System Administrator Information, page 3.

In accordance with Microsoft redistribution terms, Dazdaq cannot freely redistribute some of these components. Manta **can only be used if these components are installed.**

Windows 95 compatibility issues

The Manta software is compatible with Windows 95 (ensure you have the correct Installation version for Windows 95/98/ME), however there are some software requirements that must be installed on the target system. These are:

Internet Explorer 5.x

Microsoft no longer makes Internet Explorer 5.x available for download from their web site. To date Microsoft only has Internet Explorer 6.0 (and later) available for download and this is not compatible with Windows 95.

In accordance with Microsoft's Internet Explorer Life-Cycle requirements Dazdaq cannot redistribute Internet Explorer 5.0 or 5.5.

Thus, if you do not already have Internet Explorer 5.0 or 5.5 installed you must locate the installation files yourself. This can be achieved by searching the web. Dazdaq is not affiliated with any of these third parties.

Tahoma font

The Tahoma font must be installed on your Windows 95 system. This is installed with:

Microsoft Office 97 for Windows

Microsoft Excel 97 for Windows

Microsoft Word 97 for Windows

Microsoft Access 97

Microsoft PowerPoint 97 for Windows

Microsoft Outlook 97

Microsoft Outlook 98

Microsoft Project 98 for Windows

If your system does not have the Tahoma font it can be downloaded from:

http://download.microsoft.com/download/office97pro/fonts/1/W95/EN-US/tahoma32.exe

and

http://support.microsoft.com/default.aspx?scid=http://support.microsoft.com:80/support/kb/articles/Q188/0/81.ASP&NoWebContent=1

In accordance with Microsoft redistribution terms, Dazdaq cannot freely redistribute these components. Manta can only be used if these components are installed.

Upgrading

If you are upgrading from Stingray 1.x to Manta you will be supplied with an upgrade dongle. The first time you launch Manta the Upgrade Wizard will be launched. This wizard takes you through the upgrade procedure.

With an upgrade dongle, you can use the new software and the old software together on the same PC for a period of up to 30 days. After this period you must either permanently upgrade to the new version of the software or continue use with the old software.

Note: On systems which require a parallel dongle the following procedure must be observed: The new upgrade dongle must be inserted into the port when launching Manta. In the second step of the Upgrade Wizard, the new dongle must be taken out, the old one inserted (the old one is not detected if it is piggy-backing the new one) - the new one may be inserted into the back of the old one and be detected.

Starting Manta

After installation, you will see a Manta icon on your desktop.

To start Manta

- Click the Manta icon.

  OR

- From the **Start** menu, select **All Programs -** then click the Manta icon.

Basic features of Manta

Manta is the ultimate data acquisition, assay design and analysis software tool for microplate reader and automation technologies. Manta is Dazdaq's third generation Windows product totally redesigned from the ground up to simplify assay set-up and execution.

Key Features

Manta provides the easiest method of producing flexible and usable assays to maximise laboratory productivity, featuring:

NEW Stock of Wizards to minimise "clicks-to-results" for new and novice users.

NEW Transform model provides total flexibility in assay analysis design to cope with the most demanding requirements.

NEW Reusable components to get started quicker and eliminate work duplicity.

NEW Automatically recognises supported file formats to quickly import data from text files. Import scripting means new formats can be incorporated.

NEW Plug-in architecture to support new instrumentation, technologies, file formats, application methods and applications as they emerge.

Ready to Run

Straight away get to work with your instrument and application using pre-prepared application components.

Manta is shipped with predefined Assay Protocol components to greatly simplify the process of setting up and running an assay. Repositories of pre-defined Microplate Layouts, Data Acquisition settings, Transform settings, report styles and specific application Wizards are included.

For those in a hurry get your data into the software first, then layer your analysis and design your protocol with your real data. Once completed lock your protocol from further modification and share with colleagues.

What's New?

New Design

Manta 2.0 is a complete redesign over Manta 1.x. We have started again from the ground up to address user requirements and feedback gathered from over 8 years of Dazdaq software being used in hundreds of labs across the world.

By doing this we have taken advantage of significant changes in hardware and software technology to satisfy expectations of today's end-users.

Simple Design

During the development phase our main design focus was to simplify the end-user experience: rather than presenting a myriad of options we have tried to minimise the options displayed at each step, with a goal of "least mouse clicks to results". This does not mean we have sacrificed features; on the contrary Manta 2.0 now supports a much wider range of applications than its peers.

Get Started Quicker

As an example of this philosophy, the first thing a user may want to do with Manta 2.0 is create an Assay Protocol for their application requirements. After selecting this option from the main Organiser screen, the user selects an appropriate Wizard which creates a protocol for the specific type of application they are running (e.g, Quantitative, Qualitative, Enzyme Kinetics, etc).

The use of these Specific Application Wizards means that the user is only presented with options relevant to their application. Each wizard sets up the analysis behind the scenes and the novice user does not need a full understanding of how Manta 2.0 works to get started with typical assay requirements.

Transform Power

The secret to Manta's flexibility and power, which the more experienced user will soon become aware of, is the use of transforms.

The transforms are essentially the toolbox from which assay protocols are made. The protocol Wizards actually set-up these transforms for the user or the user can set-up their own transforms for more advanced applications. In either case after readings have been made or data imported, the transforms can be tweaked and further transforms added.

Thus, the Wizards take application requirements and set-up the transforms required behind the scenes. For example, the Quantitative Wizard may set-up a blank correction transform, a curve fit transform using the corrected data and a dilution factor transform depending on the user's requirements. Alternatively the generic Wizard can be used as a starting point to get the data in and then transforms can be manually added to support more complicated assay requirements.

More Power

The new Manta 2.0 architecture means that as Dazdaq develops new transforms and Wizards to support even more applications; as these become available they can be download to quickly address emerging technologies and new requirements without requiring a significant upgrade or change of working environment.

Stingray/Manta Comprehensive Version Comparison

Stingray 1.x	Manta
Back-fitting ambiguities with polynomial regression; Manta 1.x finds solutions outside the range of the standards (eg negative values), which are correct solutions to the back-fit of the polynomial but not the desired choice. See Manta 1.x manual for details.	NEW The user can specify a range to find a result in (e.g. the range of defined standards). If ambiguities occur, the user is warned.
Results files depend on protocol files. Any changes made to a protocol file affect all results files which depended on it.	NEW Results files contain their own copy of the protocol so any changes made to its protocol do not affect other results files.
No live readings – the user has to wait until readings are completed before seeing any results.	NEW Endpoint and kinetic readings are displayed during measurements.
Import and auto-launch cannot be used together.	NEW Data can be imported and automatically exported (as text, HTML, XLS, DOC, MHT).
Importing data from text files is difficult. The user has to manually select an appropriate import script to use to import a data file.	NEW Import script automatically detected for a selected file to import – the import script idea is transparent to the user.
Any changes to the protocol mean that the report configuration was reset to a default.	NEW Protocol changes do not affect the report configuration.
Poor quality report, images in the report sometimes appeared blocky.	NEW HTML generated report with high quality images.
No export to Word option	NEW Report can be exported to Word
Complicated to set up multiple plate protocols. The layouts which can be used for multiple plate microplates is limited.	NEW Support for multiple plates has been designed from the ground up. A multiple plate microplate layout editor has been included and plates can be set up in any way. Analysis can be performed across plates.
MDI window view means the user needs to spend time organising windows on the screen.	NEW Data views are automatically arranged.
No starting point for protocol setup. When creating a protocol the user is presented with many buttons and does not know where to start.	NEW Specific Application Wizards guide the user through the protocol setup for their particular application needs.
Export to Excel failed on versions of Excel > 97.	NEW Export to Excel supported on Office 2000 and XP.
Complicated Transform Setup. Only a handful of transforms are provided, most analysis needs to be setup using complicated expressions using "Matrix Transforms"	NEW 29 Transforms are included to support a wide range of requirements. The need for the novice user to learn expressions is limited. Many example files and descriptions are given.
No overlaid kinetic graphs.	NEW Kinetic graphs can be overlaid during and after readings.
Difficult to find protocol and results files.	NEW Outlook style file organiser.
No support for Manta 1.x files within Windows Explorer.	NEW Manta 2.0 files can be Opened and Edited from Windows Explorer (using double-click and right-click menu).
Competitive Bindings assay difficult to setup	NEW Competitive Bindings/IC(50) Wizard to

(confusion of well replicates and concentration replicates).	guide the user through configuration. This includes a clear representation of concentration replicates.
No support for Enzyme Kinetics assay.	NEW Enzyme Kinetics wizard setup.
No support for EC(20), EC(50), EC(80) calculation on standard curve fit.	NEW EC(20), EC(50), EC(80) and EC(n) calculations included for all fit methods and option B/B0 scaling of y axis.

Using the Help system

Accessing the Help

To find out more about how do something in Manta, access the online Help using any of the following methods.

To use the Contents, Index, or Search:

- From the **Help** menu, select **Contents & Index**. Use the buttons and links to navigate.

To get Help on a window or dialog:

- On any window, click the **Help** button.

  OR

- Press **F1** for help on any program window.

Finding information in the Help

You can find information in the Help in several ways.

To find information in the online Help:

1. From the **Help** menu, click **Contents & Index**.
2. If the left-hand window isn't visible, click the **Contents**, **Index**, or **Search** buttons.
3. In the Help window, do the following:

Click:	To:
Contents	View the table of contents for the online Help. Click each book to display pages that link to topics, and click each page to display the corresponding topic in the right window.
Index	Search for specific words or phrases or select from a list of index keywords. Click the keyword to display the corresponding topic in the right window.
Search	Locate words or phrases within the content of your topics. Type the word or phrase in the text field, press **ENTER**, and select the topic you want from the list of topics.
Glossary	Display a list of words, short phrases, and their definitions related to Manta. When you select a term from the **Term** list, its corresponding definition is displayed in **Definition**.

Moving around in the Help

Use the following types of navigation in the Help to move around and display information (click the hotspots to read about each):

- **Hyperlinks:** Hyperlinks are clickable items such as text (typically underlined and displayed in a different color) that perform an action, such as displaying another topic or a Web page.
- **Related Topics and See Also:** When you click a Related Topics or See Also button, a popup menu opens that displays a list of topics you can go to. These topics are relevant to what you are currently reading in the right window. Click a topic in the popup menu to open it in the right window.
- **Drop-down text:** When you click a drop-down hotspot, more information is displayed below the hotspot. You only need to click the hotspots you want to read. To hide the text, click the hotspot again.
- **Expanding text:** When you click an expanding hotspot, more information is displayed immediately to the right of the hotspot. You only need to click the hotspots you want to read. To hide the text, click the hotspot again.

- **Popups:** When you click a popup link, either a small window with text "pops up" or a topic opens "on top" of the currently open topic. Popups enable you to read additional information without leaving the current topic. When you finish reading the information in the popup, you can click any links it contains to jump to other information or close it by clicking again.
- **Browse sequences:** When you click the Previous or Next buttons, you can read through a series of topics that are arranged in a particular order. This allows you to learn about a subject in an easy-to-follow sequence.

Printing the Help

While using Manta's online Help, you can print topics and information right from the browser window.

To print a Help topic:

1. Right-click in the right pane and select **Print**. The Print dialog opens.
2. Click **Print**. The topic is printed to the specified printer.

Using the software

Overview

What is Manta?

Manta is a feature packed assay protocol design and execution tool for microplate based reader technologies.

Manta simplifies, coordinates and integrates:

- **Data acquisition** (from connected instrumentation with real-time results or through text file import)
- **Microplate layout specification**
- **Extensive analysis configuration**
- **Report generation**
- **Result archiving**

Why should I use Manta?

If you want to maximize laboratory productivity then Manta will help you by providing:

- **Guided assay protocol setup and use**
- **Real-time display of readings**
- **Total flexibility with analysis**
- **Consistent formatting of results and report generation**
- **One self-contained environment**

How do I work with Manta?

Manta has two levels of operation:

In the first level of operation, scientists, laboratory managers and those wishing to set-up routine test parameters can easily define named assay protocols which can be recalled and executed quickly and simply at any time in the future.

In the second level of operation, laboratory staff that need to run defined existing assay protocols can simply recall the parameters automatically by clicking on the appropriate protocol name to execute a routine test with no further configuration required.

This structure permits Manta to accommodate the power and versatility required by researchers setting up the most complex assays while at the same time being able to reset test parameters very quickly and simply by recalling the appropriate test protocol.

How can I start to learn about Manta?

The best place to start is to understand about the key concepts used in the software.

Concepts

This section describes the core concepts of Manta.

Data Acquisition

The Data Acquisition method describes how the raw data gets into Manta.

There are two different Data Acquisition methods:

Online

With Online Data Acquisition an instrument is physically connected to the PC. Manta is used to specify the measurement settings to use with the instrument. Manta starts and controls

the readings process and displays the data on the screen as the results arrive from the instrument.

Offline

With Offline Data Acquisition Manta imports data from existing text files. Manta can automatically recognise text formats containing microplate based data and import the data with little or no user input.

Whichever method of Data Acquisition is used the acquired data is treated in the same way after Data Acquisition is completed.

Microplate Layout

The Microplate Layout describes the positioning of the samples on the microplates associated with the test.

The layout corresponds to how the physical microplates were or will be pipetted when the data is acquired.

The microplate layout is specified using group types and group numbers. All wells with the same sample type and group number are replicates and are considered as belonging to the same group.

For example, a Quantitative application may require a microplate layout with Standard, Blank, Unknown, Pos and Neg Control group types. There may be 8 Standard groups, 1 blank group, 1 Pos and 1 Neg Control, with the rest of the microplate filled with Unknown groups. If the assay uses duplicates then each group will occur in two wells. In this example wells A1 and A2 may be occupied by replicates of the group "**Standard1**".

Within Manta each group type is represented by a particular colour and each group number by a number in the well.

Manta places no restriction on the positioning of wells within the layout – replicates do not need to be adjacent. However group numbering must start at 1 and ascend sequentially.

For Unknown group types Sample IDs can be specified.

For assays running across multiple microplates, microplate layouts can be defined for microplate sequences.

This microplate layout is setup in the Microplate Layout Editor (see page 58).

The microplate layout can be modified after readings have been made if pipetting errors have been made.

Transform

A Transform is a layer of analysis which performs an operation on input data resulting in output data. Transforms can be layered to define the analysis operations of the test.

Each transform has a number of settings used to specify the parameters of the analysis.

For example, the Factor transform takes an input matrix of endpoint data (this could be the raw measurements from the instrument) and then multiplies each endpoint value by a specified factor resulting in an output matrix of factored data.

Further transforms be created which take the output of one transform as their input.

Manta provides an extensive library of transforms to cater for even the most demanding assay requirements.

Manta includes a number of Wizards which automatically set up the required transforms for typical applications. For example, the Quantitative Wizard may setup a Blank Correction transform with Standard Curve Fit and Dilution Factor transforms to calculate concentrations of blank corrected data with an specified dilution factor for each Unknown group.

Assay Protocol

An Assay Protocol defines all of the settings and procedures required to perform a particular type of test.

An Assay Protocol is a self contained file which specifies the Data Acquisition settings, the Microplate Layout, the Transforms (which detail the analysis), the report options and result file management.

When an Assay Protocol is run its specified procedures for acquiring and processing the data are executed. After data acquisition the analyses are calculated, the report is automatically produced and all of the results are saved to a file. The result file contains its own copy of the Assay Protocol which was used to create the results (so any changes made to the original Assay Protocol do not affect the results).

Assay Results

Running an Assay Protocol produces the results of a test; these are the Assay Results.

Each run of an Assay Protocol is stored in its own self-contained Assay Results file.

The Assay Results file includes a copy of the Assay Protocol used to run the test, the raw and calculated data, any user interactions with the data (such as flagged wells or points), the audit and details of modifications made to the analyses.

Working with Manta

Manta allows you to work in different ways to speed up and automate your data acquisition and analysis processes. Here are some typical scenarios:

- I want to create a protocol defining measurements and analysis for a typical assay that will be used routinely – see page 21.
- I want to take some measurements with my instrument now and then maybe set up some analysis later – see page 19.
- I have some existing data in a text file, I want to import that now and analyse it – see page 19.
- I have imported my data and set up some analysis on it and now:
    - I want to perform the same analysis on other data files – see page 22.
    - I want to use the same analysis on new measurements controlled by Manta – see page 22.
- I want to create a protocol similar to one I have already created – see page 24.
- I want to change some analysis settings in results I have already made – see page 24.
- I want to re-evaluate my results using a different method – see page 25.
- I want to use Manta as part of an automated system (e.g. liquid handling) – see page 24.

How To

How to: Take measurements with an instrument now and setup some analysis afterwards

To setup and launch a basic Assay Protocol:

- From the Organiser Welcome Screen select **Create a new protocol**.
- The Select New Assay Protocol Wizard screen will be displayed, press **OK** to start the **Generic Wizard**.
- Press the **Next** button to step through the Wizard.
- On the **Data Acquisition Method** step press the **Create...** button to specify the measurements to perform in the instrument's own configuration window.
- On returning to the Wizard step press **Next**.
- On the **Microplate Layouts** step select an existing Microplate Layout or click **Create...** to specify the layout of your samples (you can change this later if required).
- Press **Next** to continue through the remaining steps of the Wizard. You can accept the default parameters or modify them as required.
- On the final Wizard step press the **Finish** button to launch the new Assay Protocol.

To take the measurements:

- Press the Start button on the horizontal toolbar in the main Run and Results window.
- After the readings have been completed you can Review the Results.

To setup analysis after the measurements have been taken:

- If you want to edit the microplate layout press the Edit Microplate Layout button.
- Press the Edit Transforms button in the main Run and Results window.
- Press the **Create...** button to create transforms as required.
- Press the **Recalculate** button in the main Run and Results window for the new results to be calculated.

How to: Import data from an existing text file and then analyse it

To setup and launch a basic Assay Protocol to import data:

- From the Organiser Welcome Screen select **Create a new protocol**.
- The Select New Assay Protocol Wizard screen will be displayed, press **OK** to start the **Generic Wizard**. (You can use a different Wizard if required, refer to the relevant section for further details of the other Wizards.)
- Press the **Next** button to step through the Wizard.
- On the **Data Acquisition Method** select **Import data to analyse from a text file...** and then press **Next**.
- Press the **Browse...** button and select the file to import and then press **OK**.
- Press the **Next** button and the Wizard will try to import your data.
- The next screen will show the results of the import attempt. If the Wizard was not successful follow the on screen instructions.

- If the data was imported successfully review the imported data and press the **Next** button to continue.
- On the **Microplate Layouts** step select an existing Microplate Layout or click **Create...** to specify the layout of your samples (you can change this later if required).
- Press **Next** to continue through the remaining steps of the Wizard. You can accept the default parameters or modify them as required.
- On the final Wizard step press the **Finish** button to launch the new Assay Protocol open it with the imported data.

To setup analysis after the data has been imported:

- If you want to edit the microplate layout press the Edit Microplate Layout button.
- Press the Edit Transforms button in the main Run and Results window.
- Press the **Create...** button to create transforms as required.

How to: Import text files as part of an automated process

Prepare your files

- Create one example text file that you would like to import; use your existing instrument and software to do this. (You only need one text file to import now, the others can be created later as part of your automated process.)

Create an Assay Protocol to import your text file

- From the Organiser Welcome Screen select **Create a new protocol**.
- The Select New Assay Protocol Wizard screen will be displayed, press **OK** to start the **Generic Wizard**. (You can use a different Wizard if required, refer to the relevant section for further details of the other Wizards.)
- Press the **Next** button to step through the Wizard.
- On the **Data Acquisition Method** select **Import data to analyse from a text file...** and then press **Next**.
- Press the **Browse...** button and select your text file (created above).
- Press the **Next** button and the Wizard will try to import your data.
- The next screen will show the results of the import attempt. If the Wizard was not successful follow the on screen instructions.
- If the data was imported successfully review the imported data and press the **Next** button to continue.
- On the **Microplate Layouts** step select an existing Microplate Layout or click **Create...** to specify the layout of your samples (you can change this later if required).
- Press **Next** to continue through the remaining steps of the Wizard. You can accept the default parameters or modify them as required.
- On the **Post Analysis** Wizard step, setup the options you require to output the data to other stages of your automated process. For example, you may want to export the resulting data as a text file and then launch another application with this text file. See page 51 for more details.

- On the final Wizard step press the **Finish** button to launch the new Assay Protocol and open it with the imported data. Your text data file will be imported, results calculated and any Post Analysis options performed.

Use the Assay Protocol as part of the automated process

- Within your automated process you can launch the Run and Results window to import, analyse and export the results. Use command line arguments, passing in the Assay Protocol filename and the name of the text file to import, for example:

  MRunRes <ProtocolFile> <DataFileToImport>

  (See Command Line Arguments, page 90, for more options.)

How to: Take measurements with an instrument for a typical assay that will be used routinely

To setup and launch a typical Assay Protocol:

- From the Organiser Welcome Screen select **Create a new protocol**.
- The Select New Assay Protocol Wizard screen will be displayed, select a Wizard which best describes the assay you are running (if you have further analysis requirements these can be added later).
- Press **OK** to start the Wizard.
- Press the **Next** button to step through the Wizard.
- On the **Data Acquisition Method** step press the **Create...** button to specify the measurements to perform in the instrument's own configuration window.
- On returning to the Wizard step press **Next**.
- On the **Microplate Layouts** step select an existing Microplate Layout or click **Create...** to specify the layout of your samples (you can change this later if required).
- Press **Next** to continue through the remaining steps of the Wizard specifying your assay parameters as you go.
- On the final Wizard step press the **Finish** button to launch the new Assay Protocol.

To take the measurements:

- Press the Start button on the horizontal toolbar in the main Run and Results window.
- After the readings have been completed you can Review the Results.

To run the assay again:

- Press the New button in the main Run and Results window.
- Click **OK** to use the current Assay Protocol (if multiple versions have been created select which version to use - typically you would use the latest)
- Start the assay as described above.

To run the assay again at a later date:

- From the Organiser Welcome Screen select **Run or open an existing protocol.**

If you have further analysis requirements

- Press the Edit Transforms button in the main Run and Results window.
- Press the **Create...** button to create transforms as required.

How to: Change an offline (importing) protocol to an online (takes measurements) protocol

- From the Organiser Welcome Screen select **Run or open an existing protocol.**
- In the Protocols View select the protocol you want to modify.
- Press the **Open** button in the Shortcuts Bar to display the Assay Protocol Editor for the selected file.
- In the top-left section titled **Readings** press the **Edit...** button.
- A message box will be displayed to confirm that you are changing the settings from importing to measurements, press **OK** to confirm this.
- The instrument's own configuration window will be displayed. Set up the measurements to take. (Note, the microplate dimensions must correspond to those already used in the assay protocol.)
- On returning to the Assay Protocol Editor press **OK** to save the changes.
- You can then press the **Run** button in the Shortcuts Bar to launch the modified Assay Protocol which will now take the measurements you have specified instead of importing data.

How to: Import other data files with an existing Assay Protocol

- From the Organiser Welcome Screen select **Run or open an existing protocol.**
- In the Protocols View select the protocol to use (this will be an assay protocol that you have already setup to import data).
- Press **Run** on the Shortcuts bar.
- A message box will be displayed confirming the type of file to import, press **OK**.
- A window box will be displayed to allow the selection of the file to import, select the file and press **OK**.

How to: Automatically start measurements, perform analysis and export results as part of an automated process

Create an Assay Protocol

- From the Organiser Welcome Screen select **Create a new protocol**.
- The Select New Assay Protocol Wizard screen will be displayed, select a Wizard which best describes the assay you are running (if you have further analysis requirements these can be added later).
- Press **OK** to start the Wizard.
- Press the **Next** button to step through the Wizard.
- On the **Data Acquisition Method** step press the **Create...** button to specify the measurements to perform in the instrument's own configuration window.
- On returning to the Wizard step press **Next**.
- On the **Microplate Layouts** step select an existing Microplate Layout or click **Create...** to specify the layout of your samples (you can change this later if required).

- Press **Next** to continue through the remaining steps of the Wizard specifying your assay parameters as you go.
- On the **Post Analysis** Wizard step, setup the options you require to output the data to other stages of your automated process. For example, you may want to export the resulting data as a text file and then launch another application with this text file. See page 51 for more details.
- On the final Wizard step press the **Finish** button to launch the new Assay Protocol.

Auto-start the Assay Protocol

- Within your automated process you can launch the Run and Results window, start the measurements automatically, analyse and export the results. Use command line arguments, passing in the Assay Protocol filename and using the **/run** option. Optionally use the **/exit** option to exit the Run and Results window after the Post Analysis options have been peformed, i.e.

  MRunRes /run <ProtocolFile>

  or

  MRunRes /run <ProtocolFile> /exit

  (See Command Line Arguments, page 90 for more details.)

How to: Use a compatible stacker system to run assays across multiple microplates

To setup and launch a typical Assay Protocol:

- From the Organiser Welcome Screen select **Create a new protocol**.
- The Select New Assay Protocol Wizard screen will be displayed, select a Wizard which best describes the assay you are running (if you have further analysis requirements these can be added later).
- Press **OK** to start the Wizard.
- Press the **Next** button to step through the Wizard.
- On the **Data Acquisition Method** step press the **Create...** button to specify the measurements to perform in the instrument's own configuration window.
- On returning to the Wizard step press **Next**.
- On the **Microplate Layouts**, press the **Advanced...** button and ensure the **Create Multiple Plate Microplate Layouts** option is ticked. Press **OK** to return to the wizard.
- Press the **Create...** button to launch the Microplate Layout Editor and use this to define the layout of each microplate to use. Refer to Multiple Plates section, page 61 for more information.
- Press the **Save and Close** button to return to the Wizard.
- Press **Next** to continue through the remaining steps of the Wizard specifying your assay parameters as you go.
- On the final Wizard step press the **Finish** button to launch the new Assay Protocol.

To take the measurements:

- Press the Start button on the horizontal toolbar in the main Run and Results window.
- As each new plate is automatically loaded and readings are taken they will appear on the screen.

- After the readings have been completed you can Review the Results.

To run the assay again:

- Press the New button in the main Run and Results window.
- Click **OK** to use the current Assay Protocol (if multiple versions have been created select which version to use - typically you would use the latest).
- Start the assay as described above.

To run the assay again at a later date:

- From the Organiser Welcome Screen select **Run or open an existing protocol**.

If you have further analysis requirements

- Press the Edit Transforms button in the main Run and Results window.
- Press the **Create...** button to create transforms as required.

How to: Create a protocol based on an existing protocol

- From the Organiser Welcome Screen select **Run or open an existing protocol.**
- In the Protocols View right-click on the protocol you want to use as a basis.
- Select **Copy**.
- Right-click again anywhere in the File List and select **Paste**.
- A copy of the original file will be created; you can rename it by right-clicking on it and select Rename.
- To modify the file, select it and press the **Open** button on the shortcuts bar.

How to: Use the software as part of an automated system (e.g. liquid handling)

Manta can work as part of an automated system in a variety of ways, see below for more information:

- My raw data is generated in text files, I want Manta to analyse it and then send the results to another application – see page 20.
- When the microplate is loaded in my instrument I want Manta to start the measurements, show the progress on screen, analyse the data and export the results – see page 22.
- I have a stacker system that works with Manta and I want to setup an assay which runs across multiple microplates – see page 23.

How to: Change the analysis settings in existing results

Open the existing results file:

- From the Organiser Welcome Screen select Open existing results.
- In the Results View select the results file to open and press the **Open** button on the Shortcuts bar.

Change the analysis settings:

- If you want to edit the microplate layout press the Edit Microplate Layout button.
- Press the Edit Transforms button in the main Run and Results window.
- Select the transform which you want change the settings of and press the **Edit...** button or press the **Create...** button to create further transforms as required.
- Press the **Recalculate** button in the main Run and Results window for the new results to be calculated.
- To switch between versions use the Versions control.

How to: Re-evaluate my results using a different method

Open the existing results file:

- From the Organiser Welcome Screen select **Open existing results**.
- In the Results View select the results file to open and press the **Open** button on the Shortcuts bar.

Specify new analysis settings:

- If you want to edit the microplate layout press the Edit Microplate Layout button.
- Press the Edit Transforms button in the main Run and Results window.
- If you want to remove all of the existing analysis: select the last transform in the list and press the **Remove** button, repeat this process until and transforms have been removed.
- Press the **Create...** button to create further transforms as required.
- Press the **Recalculate** button in the main Run and Results window for the new results to be calculated.
- To switch between versions use the Versions control.

Getting Around

The Manta user-interface consists of two main parts:

Organiser

The Organiser provides a convenient top-level view of your application data and simplifies navigation between the different folders of data. With the Organiser you can:

- Create, Edit and Run Assay Protocols
- Open existing Assay Results
- Manage Microplate Layouts, Data Acquisition and Exported Reports

Tip: Assay Protocols and Results can also be launched directly from Windows Explorer or from the command line.

Run and Results

When an Assay Protocol is run or Assay Results are reviewed they are displayed in a separate application window known as the Run and Results window.

Tip: Since the Run and Results window is separate to the Organiser you can open multiple Run and Results Windows at a time and use the Windows Task Bar to switch between them and return to the Organiser.

Organiser

Organiser Welcome Screen

This is the screen displayed when the Organiser is first launched. It contains the following options:

- **Create a new protocol**
- **Run or open an existing protocol**
- **Open existing results**
- **Create a new microplate layout**
- **Exit**

To return to the Welcome Screen at any time select the **Organiser** shortcut from the Shortcuts bar.

Windows

Organiser Window

The Organiser Window comprises of the following parts:

- Main Window - this contains either the File List or the Organiser Welcome Screen.
- Shortcuts Bar - optionally displayed to the left of the Main Window.
- Organiser Menu Bar - displayed across the top of the Main Window.
- Folder List - optionally displayed in between the Shortcuts Bar and Main Window.
- Caption Bar - the title of the selected view above the Main Window.

Folder List

The Folder List presents a hierarchical view of the organised data.

Showing the Folder List

The Folder List can be made visible by clicking on the Organiser caption:

If the revealed push-pin is then clicked, the Folder List will be permanently displayed.

Folder List Content

The Folder List has the following top-level branches:

Branch Name	Folder Contains files:
(Instrument Name)	Data Acquisition (*.DAQ)
Layouts	Microplate Layout (*.MLO)
Protocols	Assay Protocol (*.APR)
Results	Assay Results (*.ARS)
Reports	Exported Reports (*.DOC, *.XLS, *.MHT, *.HTM)

Selecting a branch displays the branch content in the main window.

These branches point to actual Windows folders. The path of these folders may be changed from Options.

Sub-branches

Branches may contain sub-branches (sub-directories). All sub-branches will be of the same type as their primary parent. To create a new sub-branch right-click in the File View and select **New** | **Folder**

The Folder List may be navigated using the relevant buttons on the Organiser Menu Bar.

File List

The File List is populated with the contents of the selected folder.

A Preview window is optionally displayed beneath the File List which contains more information about a file when a single file is selected.

Preview Pane Contents

The contents of the preview pane depends on the type of file selected as detailed here:

Folder View	Preview Pane Contents
Microplate Layout	A microplate control showing each plate layout in the microplate layout. If there are multiple plates in the plate layout then controls are available to step through each plate layout (the plate layouts appear in the order they are defined and note that this is not the same order they will be read if Advanced sequence steps have been defined).
Data Acquisition	A summary page detailing the readings to be made.
Assay Protocol	A summary of the Assay Protocol, including the name, the instrument settings (from the Data Acquisition), the well types used and microplate layout and also any configured transformations.
Assay Results	The latest report of the results. (If the results file has not yet been used on the PC then this may not exist and a Preview Not Available message will be displayed in the window.)
Reports	No preview window is displayed in Reports view.

Files

In the File List files can be selected using the normal Windows procedures. Right-click to display the default Windows Explorer context-sensitive pop-up menu. Relevant Organiser Menu Bar options are available when a single file is selected.

Sorting

Files are sorted by date by default, the most recent file is displayed and selected by default. The file sorting order can be change to sort by name and in reverse order: this is achieved by clicking on the column headings, **Name** and **Date Modified**.

Shortcuts Bar

The Shortcuts Bar is optionally displayed on the left of the main Organiser Window. It displays two different groups of icons:

Shortcuts

This group contains shortcuts to the most common folders.

Libraries

The group contains shortcuts to all folders.

Organiser Menu Bar

The Organiser Menu Bar extends across the top of the main area of the Organiser Window and contains the following buttons:

Back

Goes back to the previous view.

Forward

Moves forward to the next view that was already displayed.

Up

Moves up to the parent Folder. View the Folder List to see the underlying hierarchy.

New

Creates a New item for the current view (for example, when in Protocols View a new Assay Protocol is created).

Open

Opens the selected file.

Run

Runs the selected item.

(These buttons are disabled when inappropriate.)

Actions

Run Assay Protocol

To Run an Assay Protocol

First, navigate to the Protocols View. This can be done:

From the Organiser Welcome Screen: select the **Run or open an existing protocol** option**.**

or

Select **Protocols** in the Shortcuts bar.

or

Select the Protocols branch from the Folder List.

Next, select the Assay Protocol file to edit and select **Run** from the Organiser Menu bar. When this option is selected the Assay Protocol is Launched.

Edit Assay Protocol

To Edit an Assay Protocol

First, navigate to the Protocols View. This can be done:

From the Organiser Welcome Screen select the

Select **Protocols** in the Shortcuts bar. **Run or open an existing protocol** option**.**

or

Select the Protocols branch from the Folder List.

Next, select the Assay Protocol file to edit and select **Open** from the Organiser Menu bar. When this option is selected the Edit Assay Protocol window is displayed.

Open existing Assay Results

To Open an Assay Results File

First, navigate to the Results View. This can be done:

From the Organiser Welcome Screen select the **Open existing results** option**.**

or

Select **Results** in the Shortcuts bar.

or

Select the **Results** branch from the Folder List.

Next, select the Assay Results file to open and select **Open** from the Organiser Menu Bar**.** When this option is selected the Run and Results screen is displayed.

Create Microplate Layout

To Create a New Microplate Layout

From the Organiser Welcome Screen select the **Create a new microplate layout** option**.** When this option is selected the New Microplate Layout Wizard is launched.

Create Assay Protocol

To Create a New Assay Protocol

From the Organiser Welcome Screen select the **Create a new protocol** option**.**

Alternatively, within Organiser in the Shortcuts bar on the left of the screen select the **Protocols** shortcut and then click the **New** button in the Protocols View.

When this option is selected the New Assay Protocol Selection screen is then displayed.

Select New Assay Protocol Wizard

This screen allows you to select a Wizard to use to create a new Assay Protocol. The installed Wizards appear on the left as icons. Select one of these Wizard icons to find out more information about the Wizard and click **OK** to start the selected Wizard.

Tip: At Dazdaq Ltd. we are continuing to develop new Wizards to support new applications, visit our website at http://www.dazdaq.com/manta/plugins.htm to see which new Wizards are available.

Views

Data Acquisition View

To View the Data Acquisition folder

First, in Organiser click **Libraries** in the Shortcuts bar.

Next, press the shortcut with the name of your instrument.

The Data Acquisition View displays a File List of the Data Acquisition files.

A Preview window is optionally displayed which contains details of the operations performed by the selected Data Acquisition files.

The following Organiser Menu Bar buttons are available in this view:

New

Press **New** to create a new Data Acquisition file. The user interface for configuring the default instrument is displayed.

Open

Press **Open** to open an existing Data Acquisition file for review/edit.

Microplate Layouts View

To View the Microplate Layouts folder

First, in Organiser click **Libraries** in the Shortcuts bar.

Next, press the **Layouts** shortcut.

The Microplate Layouts View displays a File List of the Microplate Layouts.

A Preview window is optionally displayed which contains details of the selected Microplate Layout. The preview window contains a microplate control which can be used to view each plate layout in the microplate layout. If there are multiple plates in the microplate layout then you can step through each plate layout (the plate layouts appear in the order they are defined and note that this is not the same order they will be read if Advanced sequence steps have been defined).

The following Organiser Menu Bar buttons are available in this view:

New

Create a new Microplate Layout with the Microplate Layout Wizard

Open

Open the selected Microplate Layout for Review/Editing.

Protocol View

To View the Protocol folder

In the Organiser Welcome Screen, click **Run or open an existing protocol**

or:

In the Shortcuts Bar, select the **Protocols** shortcut.

The Protocols View displays a File List of the Assay Protocols.

A Preview Pane is optionally displayed which contains details of the selected Assay Protocol. The preview pane contains a summary of the Assay Protocol, including its name, the Data Acquisition settigns, the well types used, the microplate layout and any configured transforms.

The following Organiser Menu Bar buttons are available in this view:

New

Create a new Assay Protocol using a Wizard.

Open

Open the selected Assay Protocol for editing/review.

Run

Launch the selected Assay Protocol ready to run.

Results View

To View the Results folder

In the Organiser Welcome Screen, click **Open existing results**.

or:

In the Shortcuts Bar, select the **Results** shortcut.

The Results View displays a File List of the Assay Results.

A Preview window is optionally displayed which contains the report of the selected Assay Results file.

Open

The **Open** Organiser Menu Bar button opens the selected Assay Results file for review in the Run and Results window.

Reports View

To View the Reports folder

In the Shortcuts Bar, select the **Reports** shortcut.

The Report View displays a File List of reports which have been exported.

Open

The **Open** Organiser Menu Bar button is available in this view to open the selected file with its associated application.

Options

This window box displays various Manta settings; these are persisted across sessions of the program.

To access the Options window, in Organiser, select **File | Options**

The options are arranged in the following tabs:

View

Options to customise the view in the Organiser:

The **Show preview pane** option specifies whether a preview window is displayed in the various Folder Views. If this is ticked, when the main Organiser Window is displaying a File List an additional pane is displayed which displays a preview of an item selected in the File List.

The **Show shortcuts** option specifies whether the Shortcuts Bar is displayed on the left of the main Organiser Window.

Data

Option to change the location where all data files are stored:

The **Parent directory for data files** edit box details the location of the parent directory of all data files. All Manta data files will be located in a sub-directory of the parent directory.

This default parent directory is the user's My Documents directory.

The path can be changed to a new location (such as a network drive). The path entered must either be that of an existing directory, or else the child of an existing directory.

If a new Parent directory path is specified sub-directories will be created in the specified directory for the various folders.

Drivers

Option to specify which device driver to use:

This contains an list-box showing all installed device drivers with the default driver selected. (If there are no drivers installed then this list box is disabled and contains no entries.)

Application Wizards

Wizard Overviews

Generic Wizard

Description

Use this Wizard to create a basic Assay Protocol. It is the best place to start to get your data into the software quickly.

A basic Assay Protocol will take measurements with your connected instrumentation or import data from a file. When you run the Assay Protocol your data will be displayed in interactive views and within a typical report.

Basic operations such as blank correction, measurement-reference calculations and %CV calculation of replicates will also be automatically setup for you if they are relevant.

After you run the Assay Protocol created by the Wizard you can add your own specific analysis by creating transforms. An extensive library of transforms is provided to cope with even the most demanding application requirements.

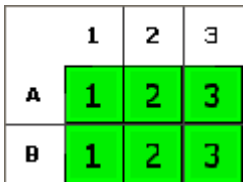
Measurements

Any type of measurements may be selected with any microplate layout.

Example 1

Setup: The Wizard is used to create an assay protocol that:

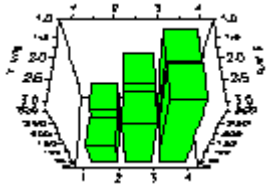
- Takes endpoint measurements from a 3x2 plate of 3 Unknowns in duplicate



Results: The readings are taken:

	1	2	3
A	98	219	298
B	102	217	312

Readings



3D Projection of readings

Group	Wells	Readings
Unknown1	A1,B1	100
Unknown2	A2,B2	218
Unknown3	A3,B3	305

Example 2

Setup: The Wizard is used to create an assay protocol that:

- Takes endpoint measurements from a 6x4 plate with 11 Unknowns in duplicate and 1 blank group.

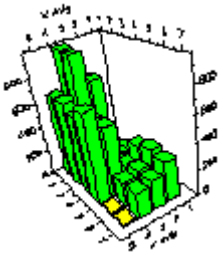
	1	2	3	4	5	6
A	1	1	2	2	3	3
B	4	4	5	5	6	6
C	7	7	8	8	9	9
D	10	10	11	11	1	1

- The Unknowns will be background correct by subtracting the mean of the Blank wells from each Unknown well.

Results: The readings are taken and the background correction performed.

	1	2	3	4	5	6
A	98	102	219	298	333	312
B	742	751	192	191	229	229
C	981	972	544	546	105	108
D	666	667	619	698	19	17

Raw Readings



3D Projection of Raw Readings

	1	2	3	4	5	6
A	80	84	201	280	315	294
B	724	733	174	173	211	210
C	963	954	526	528	172	180
D	648	649	601	680	1	-1

Blank Corrected Readings

Group	Wells	Raw Readings	Corrected
Unknown1	A1,A2	100	82
Unknown2	A3,A4	258.5	240.5
Unknown3	A5,A6	322.5	304.5
Unknown4	B1,B2	746.5	728.5
Unknown5	B3,B4	191.5	173.5
Unknown6	B5,B6	228.5	210.5
Unknown7	C1,C2	976.5	958.5
Unknown8	C3,C4	545	527
Unknown9	C5,C6	196.5	178.5
Unknown10	D1,D2	666.5	648.5
Unknown11	D3,D4	658.5	640.5
Blank1	D5,D6	18	0

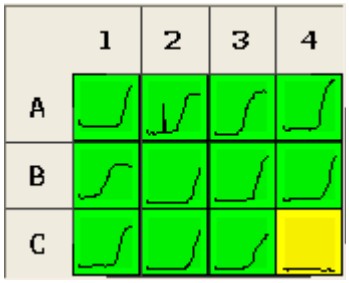
Example 3

Setup: The Wizard is used to create an assay protocol that:

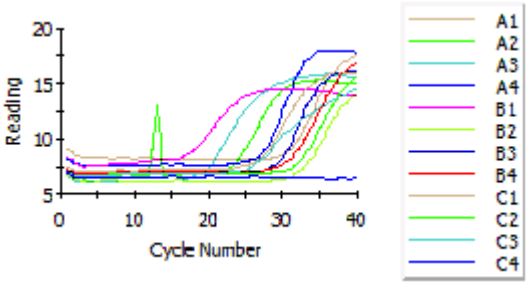
- Takes kinetic measurements (40 cycles) from a 4x3 plate with 11 Unknowns and 1 Blank well.

	1	2	3	4
A	1	4	7	10
B	2	5	8	11
C	3	6	9	1

Results: The kinetic readings are taken.



Raw Kinetic Readings



Overlaid Projection of Kinetic Readings

Group	Wells	Readings
Unknown1	A1	
Unknown2	B1	
Unknown3	C1	
Unknown4	A2	
Unknown5	B2	
Unknown6	C2	
Unknown7	A3	
Unknown8	B3	
Unknown9	C3	

Unknown10	A4	
Unknown11	B4	
Blank1	C4	

Ratio Wizard

Description

Use this Wizard to create a Ratio Assay Protocol.

With this type of protocol the ratio of two sets of measurements is calculated. The %CV of the replicates will also be determined.

Measurements

Two input matrices of endpoint measurement data are required.

Example

Setup: The Wizard is used to create an assay protocol that:

- Takes measurements with label A and label B
- Calculates the ratio of measurement A and B for each well

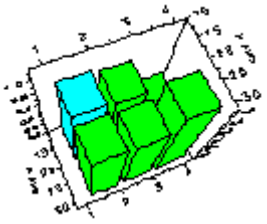
Results: The readings of each label are divided resulting in the calculate ratio.

	1	2	3
A	100	324	123
B	894	401	742

Label A

	1	2	3
A	102	351	224
B	911	421	642

Label B



3D Projection of Ratio

Group	Wells	Label A	Label B	Ratio
Control1	A1	100	102	0.980392
Unknown1	A2	324	351	0.923077

Unknown2	A3	123	224	0.549107
Unknown3	B1	894	911	0.981339
Unknown4	B2	401	421	0.952494
Unknown5	B3	742	642	1.15576

Qualitative Wizard

Description

Use this Wizard to create a basic Qualitative Assay Protocol.

With this type of protocol a cut-off point is used to label your Unknowns. Your Unknowns will be labeled depending on whether they are greater or less than your cut-off point. You can supply your own positive or negative label.

Your cut-off point is essentially a numeric value which can be made from readings from your plate (e.g. a Control group), an absolute numeric value, or a mathematical expression.

You can also optionally specify a grey-zone area. Any of your Unknowns that fall within this range are also labeled as Grey to denote that they are close to your cut-off point. The grey zone area is specified as a percentage, absolute numeric value or mathematical expression and is relative to your cut-off point.

The %CV of your replicates will also be calculated.

Measurements

A single matrix of endpoint measurements is required. If 2 matrices are selected a difference matrix will be calculated and used. If 3 matrices are selected then the 3rd matrix will be used.

Example

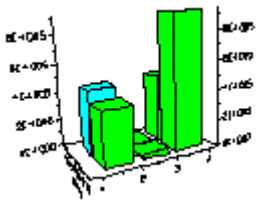
Setup: The Qualitative Wizard is used to create an assay protocol that:

- Makes measurements on a 3x2 plate with 1 control and 5 Unknowns.
- Uses a cut-off point that is the value of the measured Control well.
- Gives the label "Positive" for values greater than the cut-off point.
- Gives the label "Negative" for values less than the cut-off point.
- Uses a 10% grey zone

Results:

	1	2	3
A	449331	16717	471394
B	371955	31632	930599

Raw readings



3D Projection of raw readings

With these example results we can see from their 3D projection that:

- 3 of our Unknowns are less than our Control
- 2 of our Unknowns are greater than our Control
- 1 of our Unknowns is close to our Control

The table of the report looks like this:

Group	Wells	Readings	Cut-off
Control	A1	449331	(Cut-off Point)
Unknown1	A2	16797	Negative
Unknown2	A3	479394	Positive, Within Grey Zone
Unknown3	B1	371955	Negative
Unknown4	B2	31632	Negative
Unknown5	B3	930599	Positive

We can see that the Unknowns have been give labels based on their measurements relative to the Control group.

Quantitative Wizard

Description

Use this Wizard to create a Quantitative Assay Protocol, such as ELISA, IFMA, FIA.

With this type of protocol a concentrations curve is plotted from measurements of your Standards. Measurements of your Unknowns are then back fitted onto this curve to calculate their concentrations. An optional dilution factor can be applied to each Unknown group. The %CV of your replicates will also be calculated.

You can also select for **EC(20)**, **EC(50)**, **EC(80)** and **EC(n)** to be calculated from the curve and optionally plot the curve on a **B/B0** Y axis.

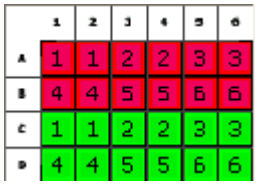
Measurements

A single matrix of endpoint measurements is required. If 2 matrices are selected a difference matrix will be calculated and used. If 3 matrices are selected then the 3rd matrix will be used.

Example

Setup: The Wizard is used to create an assay protocol that:

- Takes measurements on a 6x4 plate with 6 Standard and 6 Unknown groups in duplicate:



- Plots the measured Standard values against the specified concentrations:

Standard Group:	Concentration:
Standard1	1.0
Standard2	2.0
Standard3	3.0
Standard4	4.0
Standard5	5.0
Standard6	6.0

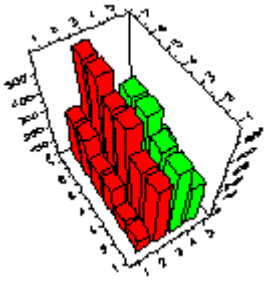
- Performs a linear regression on this data set and back-fits the Unknowns to calculate their concentrations.
- Applies a group specific dilution factor to the calculated concentration:

Unknown Group:	Dilution Factor
Unknown1	1
Unknown2	10
Unknown3	100
Unknown4	1
Unknown5	10
Unknown6	100

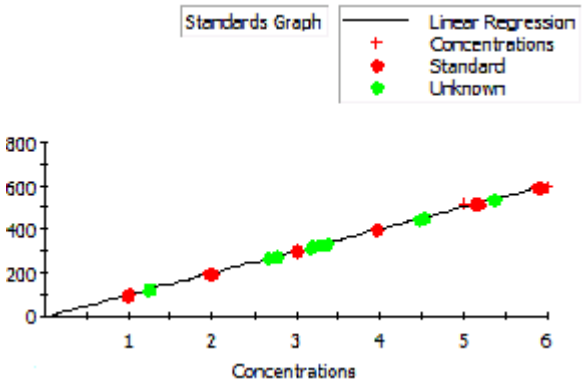
Results: The readings are plotted against the specified concentrations. The linear regression calculation results in a straight line which is used to calculate the concentrations of the samples. The dilution factors are then applied:

	1	2	3	4	5	6
A	101	99	198	201	303	304
B	399	400	521	518	594	598
C	450	457	542	541	321	319
D	279	269	333	340	123	125

Readings



3D Projection of readings



Linear Regression of readings with samples back fitted

Group	Wells	Readings	Calc. Concentrations	Dilutions
Standard1	A1,A2	100	0.995758	0.995758
Standard2	A3,A4	199.5	1.98063	1.98063
Standard3	A5,A6	303.5	3.01004	3.01004
Standard4	B1,B2	399.5	3.96027	3.96027
Standard5	B3,B4	519.5	5.14805	5.14805
Standard6	B5,B6	596	5.90526	5.90526
Unknown1	C1,C2	453.5	4.49477	4.49477
Unknown2	C3,C4	541.5	5.36581	53.6581
Unknown3	C5,C6	320	3.17336	317.336
Unknown4	D1,D2	274	2.71804	2.71804
Unknown5	D3,D4	336.5	3.33668	33.3668
Unknown6	D5,D6	124	1.23331	123.331

Enzyme Kinetics Wizard

Description

Use this wizard to create an Enzyme Kinetics Assay Protocol.

With this type of protocol the **Vmax** of samples at specified concentrations is calculated and can be compared. Kinetic measurements are taken and an exponential or linear fit is performed on each kinetic plot. The absolute slopes are then plotted against the specified concentrations. The Michaelis-Menten fit is applied to these concentration plots to determined each sample's **Vmax**. The Standard Deviation of each group can be optionally calculated.

Measurements

A single matrix of kinetic measurements is required.

Microplate Layout Requirements

The calculations are performed on a selected group type (e.g. Unknown or Standard). Each group of the selected type will contain the sample at specified concentrations..

In order to calculate **Vmax** there must be at least 2 concentrations used and each group must be diluted in the same way.

First, use the Wizard to create or select an existing microplate layout which specifies which wells belong to which group. For example, here we have 2 different samples we want to calculate the **Vmax** of; the top half of the plate is for the first sample and the bottom half for the second sample:

	1	2	3	4	5	6
A	1	1	1	1	1	1
B	1	1	1	1	1	1
C	2	2	2	2	2	2
D	2	2	2	2	2	2

Next, within the wizard specify the concentrations of each well. The large number displayed in each well of the microplate is the group number of the well and the subscript number identifies the concentration group. For example, wells **A1** and **C1** contain different samples but they are diluted in the same way:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	1_7	1_8	1_9	1_{10}	1_{11}	1_{12}
C	2_1	2_2	2_3	2_4	2_5	2_6
D	2_7	2_8	2_9	2_{10}	2_{11}	2_{12}

If required you can also specify if and how the concentration are replicated. For example, here each microplate column is diluted in the same way:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	1_1	1_2	1_3	1_4	1_5	1_6
C	2_1	2_2	2_3	2_4	2_5	2_6
D	2_1	2_2	2_3	2_4	2_5	2_6

Example

The wizard is used to create an assay protocol that:

- Takes kinetic measurements (over time) on a 6x4 plate with 4 unknown groups, each row contains a different sample and each column is serially diluted:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	2_1	2_2	2_3	2_4	2_5	2_6
C	3_1	3_2	3_3	3_4	3_5	3_6
D	4_1	4_2	4_3	4_4	4_5	4_6

- Determines the absolute linear slope of each kinetic plot (for each well). This is achieved by using Linear Regression on each well (readings against time) to calculate the absolute slope.

- The calculated absolute slopes are plotted against the specified concentrations for each group:

Dil.	**Conc.**
Conc. (1)	1
Conc. (2)	10
Conc. (3)	100
Conc. (4)	1000
Conc. (5)	10000
Conc. (6)	100000

- Calculates the **Vmax** of each group

IC50 Wizard

Description

Use this Wizard to create an IC50 Assay Protocol.

With this type of protocol the Inhibition Concentrations (typically IC50) for samples in serial dilution is calculated and can be compared. This is the concentration of the substance resulting in displacement of **n**% of the antibody. The % bound of each sample is also calculated.

Measurements

A single matrix of endpoint measurements is required. If 2 matrices are selected a difference matrix will be calculated and used. If 3 matrices are selected then the 3rd matrix will be used.

Microplate Layout Requirements

The calculations are performed on a selected group type (e.g. Unknown or Standard). Each group of the selected type will contain the sample at specified dilutions.

In order to calculate IC there must be at least 4 dilutions used and each group must be diluted in the same way.

First, use the Wizard to create or select an existing microplate layout which specifies which wells belong to which group. For example, here we have 2 different samples we want to calculate the IC(50) of; the top half of the plate is for the first sample and the bottom half for the second sample:

	1	2	3	4	5	6
A	1	1	1	1	1	1
B	1	1	1	1	1	1
C	2	2	2	2	2	2
D	2	2	2	2	2	2

Next, within the Wizard specify the dilutions/concentrations of each well. The large number displayed in each well of the microplate is the group number of the well and the subscript number identifies the concentration group. For example, wells **A1** and **C1** contain different samples but they are diluted in the same way:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	1_7	1_8	1_9	1_{10}	1_{11}	1_{12}
C	2_1	2_2	2_3	2_4	2_5	2_6
D	2_7	2_8	2_9	2_{10}	2_{11}	2_{12}

If required you can also specify if and how the concentration/dilutions are replicated. For example, here each microplate column is diluted in the same way:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	1_1	1_2	1_3	1_4	1_5	1_6
C	2_1	2_2	2_3	2_4	2_5	2_6
D	2_1	2_2	2_3	2_4	2_5	2_6

If blank wells are allocated then blank correction will be performed.

Example

Setup: The Wizard is used to create an assay protocol that:

- Takes measurements on a 6x4 plate with 4 Unknown groups, each row contains a different sample and each column is serially diluted:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	2_1	2_2	2_3	2_4	2_5	2_6
C	3_1	3_2	3_3	3_4	3_5	3_6
D	4_1	4_2	4_3	4_4	4_5	4_6

- Plots the measured values against the specified dilutions for each group:

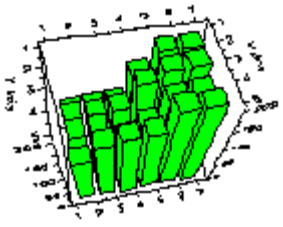
Dil.	**Conc.**
Conc. (1)	1
Conc. (2)	10
Conc. (3)	100
Conc. (4)	1000
Conc. (5)	10000
Conc. (6)	100000

- Calculates the IC50 and % bound of each group

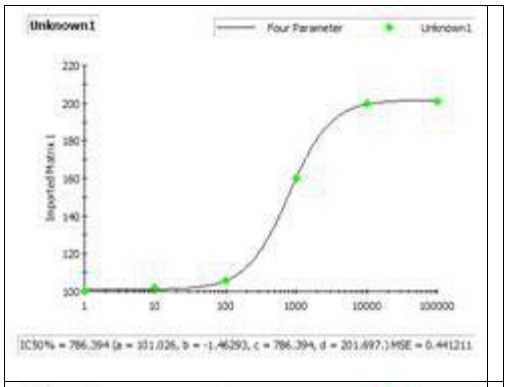
Results: The readings are plotted against the specified dilution for each group. The IC50 is determined from the fitted curve and the % bound calculated for each sample.

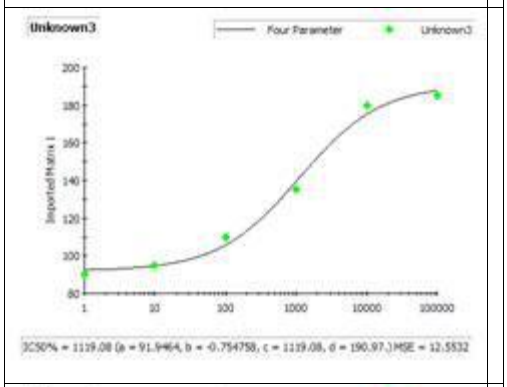
	1	2	3	4	5	6
A	100	102	106	160	200	201
B	130	132	135	180	199	202
C	90	95	110	135	180	185
D	120	152	160	161	205	203

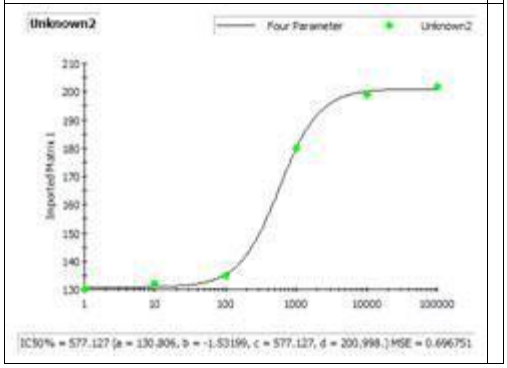
Readings

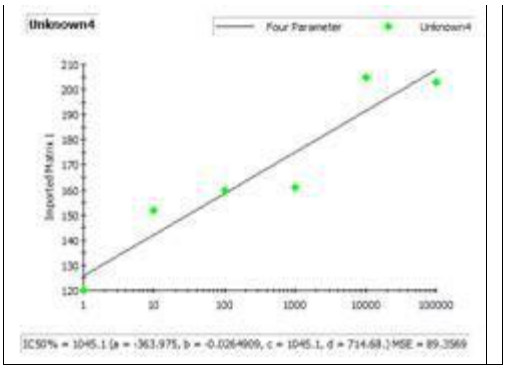


3D Projection of readings









Four Parameter Curve fit for each Group

Group	MSE	IC(50)
Unknown1	0.441211	786.394
Unknown2	0.696751	577.127
Unknown3	12.5532	1119.08
Unknown4	89.3569	1045.1

Calculated MSE and IC(50) for each group

	1	2	3	4	5	6
A	Unknown1 100 99.3338	Unknown1 102 101.32	Unknown1 106 105.294	Unknown1 160 158.934	Unknown1 200 198.668	Unknown1 201 199.661
B	Unknown2 130 185.207	Unknown2 132 188.056	Unknown2 135 192.33	Unknown2 180 256.44	Unknown2 199 283.509	Unknown2 202 287.783
C	Unknown3 90 90.8871	Unknown3 95 95.9364	Unknown3 110 111.084	Unknown3 135 136.331	Unknown3 180 181.774	Unknown3 185 186.823
D	Unknown4 120 11.125	Unknown4 152 14.0916	Unknown4 160 14.8333	Unknown4 161 14.926	Unknown4 205 19.0052	Unknown4 203 18.8197

Readings and calculated %bound by well

Wizard Steps

Generic

Welcome Wizard Step

Follow the instructions in the Wizard to setup a new Assay Protocol.

Use the **Next** and **Back** buttons to step through the Wizard or click **Cancel** to Exit and lose your changes.

Data Acquisition Method Wizard Step

Method

This Wizard step is for the selection, creation and editing of the Data Acquisition Method. This can be:

Online - take measurements with a connected instrument

The Data Acquisition Methods list box is populated with methods that have already been setup for the selected instrument and are applicable for the Wizard being used.

Each item in the list corresponds to a DAQ (Data Acquisition) file and displays the file's main name, followed by a dash, followed by the DAQ's description, including the microplate dimensions. If the DAQ file has its read-only attribute set then "(Locked)" is included at the end of the description.

Select an existing item to set-up an Assay Protocol which uses the specified measurement settings. New items can be created and existing item can be edited (unless they have been locked).

Create

Click the **Create...** button to launch the configuration screen of the instrument. After specifying the instrument measurement settings and returning to the Wizard the new item will be added to the end of the Data Acquisition Methods list and be selected by default (assuming it is applicable - see Tip below).

Edit

Double-click on an existing item to open up the selected item for editing – this displays the configuration screen of the instrument. (If the file is locked/read-only then a message box is displayed to denote that it is locked and cannot be edited.) After editing an item the new item will appear in the list and will be selected by default.

No instrument drivers installed

If there is no instrument driver installed (or if there is a problem with the instrument driver) then a warning will be given. In this situation an instrument driver cannot be used, however you can continue to use the wizard to import data from existing files. See **Advanced** option below to change driver.

Advanced

The **Advanced** button allows you to change the selected instrument driver. A selection of installed drivers is displayed. Making a change causes the DAQ list box to be repopulated.

Offline - import data from existing text files

The last item in the Data Acquisition Methods list box is the **Import data to analyse from a text file...** option. Select this option to set-up an Assay Protocol which will read in existing data from text files of a specific format.

Note: With this option you can create an Assay Protocol which imports a particular file format, the Assay Protocol can be re-used to import files of the specified format; you do not have to create a new Assay Protocol each time you want to import a file.

The selected Acquisition Method will be used each time the resulting Assay Protocol is run (but this can be edited later).

Applicability

You can only use Data Acquisition methods which are applicable for the Wizard being used, this is because specific applications use a specific type of measurement. For example, the Enzyme Kinetics wizard will only accept kinetic settings.

Tip: A description of the required applicability is displayed under the Data Acquisition list box and on the right of the **Create** button. New or edited items that are not applicable will not be included in the list box.

Import Wizard Steps

Select File to Import Wizard Step

This Wizard step is for the selection of a file to import. Use the **Browse...** button to locate an example file to import.

When the **Next** button is clicked the Wizard tries to determine how to import the selected file.

Tip: The resulting Assay Protocol will import ANY text file which uses the same format as the selected file. This means you do not have to re-run the Wizard to import more files of the same format; simply run the Assay Protocol created by the Wizard with a different file of the same format. On this Wizard step you specify one example of a file which you would like to import and the Wizard determines the file format from this example file; the resulting Assay Protocol will then be able to import this example file and any other file which follows the same file format (i.e. the data within the file will be different but will follow the same orientation).

Import Details Wizard Step

If this Wizard step is displayed it means that the Wizard is having difficulty determining how to import the data and that it needs more information to decide what to do. This could be because there are different ways it could import the data or that it cannot recognise the format.

The text file to import is displayed on the left and can be scrolled. Use the controls on the right to specify the type of data being import.

When the **Next** button is clicked the Wizard tries again to determine how to import the selected file using the information provided here.

Import Script Search Wizard Step

This Wizard step displays the results of the attempt to import the data. It will either inform that the text file cannot be imported or there has been a problem importing it. In these cases the **Next** button will be disabled and you cannot continue through the Wizard. If there was an ambiguity importing the text file then a list of possible Import Scripts are displayed, select one Import Script to use to continue.

If the text file could not be imported then you have the option to visit the Dazdaq Ltd. website to see if there are Import Scripts available for your file format. Alternatively, the Wizard can prepare an email to send your text file to Dazdaq Ltd. so that we can create a suitable Import Script for you (this feature requires compatible email software).

If there was a problem importing the text file then a message box is displayed warning of this and advising you to go **Back** and make changes.

Import Preview Wizard Step

This Wizard step displays a preview of the imported data in a microplate representation (of green wells) for your inspection. Continue through the Wizard if you are happy with the imported data.

The **Preview Properties...** button displays a window for changing the settings of the microplate preview – the available options depends on the type of data imported.

A **Parameter** control is displayed on the right if any additional parameters imported. The first column lists the parameter name and the second column the parameter value (imported from the file).

If the data being imported is not applicable for the application being set-up by the Wizard then a message box is displayed warning of this and the **Next** button is disabled. For example, the

Quantitative Wizard requires endpoint data, but if kinetic data has been imported then the Quantitative Wizard cannot be used. In this case go **Back** and select a different file to import or **Cancel** and use a different Wizard.

Microplate Layout Wizard Step

This Wizard step is for the selection, creation and editing of the Microplate Layout.

Microplate Layouts

This step displays thumbnail images of all existing and applicable Microplate Layouts. Included Microplate Layouts are compatible (match the microplate dimensions) with the selected Data Acquisition Method and are applicable for the Wizard in use. Create, edit or select a layout to use with the Assay Protocol.

Tip: A description of the Microplate Layout applicability is included under the Microplate Layouts and to the right of the Create button. (For example, the Quantitative Wizard requires Standard groups in the layout.) New or edited items that are not applicable will not be available.

Create

Click the **Create...** button to launch the Microplate Layout Editor with an empty Microplate Layout. The editor is set-up ready to use the group types relevant to the type of application being set-up.

Edit

Double clicking on a Microplate Layout opens up the selected item for review/editing in the Microplate Layout Editor.

Multiple Plate Microplate Layouts

A single Assay Protocol can run across 1 or more or microplates. To create a layout which runs across multiple microplates, press the **Advanced** button and tick the **Create Multiple Plate Microplate Layouts** option. With this option ticked any new Microplate Layouts created will be multiple plate.

Tip: Within each Microplate Layout thumbnail, if the Microplate Layout is locked then a padlock item appears on the top left of the thumbnail (it is locked if the file's read-only property is set). If the Microplate Layout comprises of multiple plates then a "dog-ear" appears in the right hand corner of the thumbnail.

Blank Correction Wizard Step

This Wizard step is used to specify how to perform Blank Correction. It works by allowing you to define associations between blank groups and other wells. Each well is corrected by its associated blank group.

Example:

With one particular application you may have 4 blank groups on your Microplate Layout, one in each corner of the microplate. With this situation you may want to subtract from the measurement of each well in each quarter of the microplate the measurement of its nearest blank group. Using this Wizard step you can define the associations between wells in each quarter of the microplate with its nearest blank group.

Select which blank group to define the associations from the combo-box or left click on a Blank group well. With the **Select** button pushed down (on the left) left-click on a well or drag over multiple wells on the microplate control. They will appear in the selected colour: this means they are associated with the selected Blank group. Press the **Erase** button down to erase the association in the same way with the mouse.

Wells which appear with a cross through cannot be associated with the selected Blank group; this is because they are already associated with another Blank group or are themselves Blank wells.

If you tick the check box titled "**Correct all wells with this group"** then all wells will be corrected by the selected Blank group. In this case you cannot select wells to associate in this mode (as all of the wells are selected).

If using a multiple Microplate Layout then associations can be made for each microplate and buttons are provided for movement through each microplate.

If you do not want to perform Blank Correction then go **Back** through the Wizard and either edit your Microplate Layout to remove the Blank groups or select a different Microplate Layout.

Post Analysis Wizard Step

This Wizard step provides options which can be performed after the Data Acquisition and analysis have been completed.

Automatically Print

If this option is ticked then the compiled report will be automatically printed with the default printer.

Export Report

If this option is ticked a report will automatically be exported. The following table identifies what each of the file extensions mean:

Report Type	Description
*.htm	A Web Page (complete with links to image files)
*.mht	A Web Archive file (a single self-contained file)
*.xls	Microsoft Excel (an Excel Workbook with Worksheets)
*.doc	Microsoft Word (a standard Word file with links to image files)

Some of these options may not be available if your PC does not have the required components: see page 134 for more information.

Export Results to Text File

If this option is ticked then each time the results are calculated a text file is created containing the exported raw and calculated results as described in the **Options...** button, page 88.

Launch another application

If this option is ticked you can specify an application to launch using the **Browse** button.

You can specify command line arguments to pass to the application. The **Add Macro** button appends the selected macro to the end of the **Command Line Arguments** text box. The **<ResultsFile>** macro is replaced with the full path of the Assay Results File.

Tip: The specific application is launched after the report and/or text file have been exported. This means you can launch another application passing the exported text file. This is useful to log the data with another system or as part of an automation process (e.g. liquid handling).

Assay Protocol File Details Wizard Step

This Wizard step allows the specification of the Assay Protocol filename being created by the Wizard and also the naming format of the Assay Results files which will be created each time the Assay Protocol is run. Notes can be entered relating to the Assay Protocol. You can also optionally specify the filename structure of the resulting Assay Results files.

Assay Protocol Filename

The default Assay Protocol filename given is unique and does not already exist. This can be edited as required. Note: all Assay Protocols are created in the Protocols directory displayed (this cannot be changed within the Wizard).

Notes

If required enter any notes regarding the Assay Protocol. These notes appear in the protocol description header at the top of each report produced by this protocol.

Assay Results Filename Structure

Push the **Advanced** button to display the options to specify the Results Directory and Filename. Here you can use text and macros to specify the name and location of the result files produced when running the Assay Protocol. Settings made here will be used for all Assay Protocols created in the future.

By default the Results Directory is specified as **<MyResultsFolder>**. This is the default Results directory and is a sub-directory called My Results in the specified Parent directory. (The Parent Directory can be specified in the Organiser Options).

The default Results Filename is **<ProtocolName>** (this is the name given to the Assay Protocol in the previous step)

These can be edited to specify valid directory and filename structures.

The **Add Macro:** combo box includes the macros:

<CurrentDate> <CurrentTime> <CurrentUser> <ProtocolName>

When the **Add Macro:** button is pressed the selected macro is appended to the end of the **Results Filename** text box. These macros can also be used in the Results directory (this is only available by manually typing them in).

The macros are expanded as suggested by their name.

When the **Results Directory** setting is expanded if the resulting directory does not exist then it will be created.

When the **Results Filename** setting is expanded if the resulting filename already exists in the specified **Results Directory** then a unique and sequential number is appended to ensure no existing file is overwritten.

Example:

If you specified the **Results Directory** as

<MyResultsFolder>\<ProtocolName>

and the **Results Filename** as:

<CurrentDate><CurrentTime>

Then each time the Assay Protocol is run, each results file will be stored in a sub-directory in <MyResultsFolder> named by the Protocol Name. The results filename will be made up of the date and time the result file is created.

Tip: You can specify any Results directory for the Protocol's result files to be located (including local or network directories). However, if the specified Results Directory is not a

sub-directory of **<MyResultsFolder>** then your files will not be accessible through the Organiser.

Thus, instead of specifying a fixed directory here it is recommended you either change the location of **<MyResultsFolder>** from the Organiser Options, or specify a sub-directory of **<MyResultsFolder>**, such as one made up of the protocol filename **<MyResultsFolder>\<ProtocolName>**. In this case Results will be located in a sub-folder identified by the protocol name within the Results folder which is easily accessible through the Organiser.

Final Wizard Step

This is the final Wizard step. On clicking **Finish** the Wizard creates the Assay Protocol using the specified settings. The resulting Assay Protocol is stored in the Protocols directory.

You can also optionally launch the new Assay Protocol after the Wizard has finished by ticking the **Launch the Assay Protocol Now** option.

If the Assay Protocol imports data then it can be launched with the text file used earlier in the Wizard. (If this option is not ticked then a different text file can be specified thought this must be of the same format as the original).

Click **Finish** to close the Wizard and launch the Assay Protocol as selected.

Ratio

Measurement, Reference Specification Wizard Step

This Wizard step allows you to specify the Ratio calculation.

Simply select the radio button corresponding to the division to perform.

Quantitative

Standard Set Selection Wizard Step

This Wizard step allows you to specify which set of standards to use in the Quantitative analysis. In a subsequent Wizard step you will specify their concentration values.

The wells belonging to the selected standards set flash. Use the controls to select which microplate (if multiple microplates are being used) and which set to use.

This Wizard step only appears when there are more than one standards set defined on the microplate layout.

Concentration Specification Wizard Step

This Wizard step is for the specification of the concentration values of each standard group.

Concentration values can be entered using the keyboard. As a group is selected its associated wells flash.

The up and down cursor keys can be used to change the standard group to edit.

Auto Series

If the **Auto Series** control is ticked, the **Delta** edit box can be used to enter a value to multiply or increase Group n's concentration by to get group n+1, starting with the first group. The auto-series operation is performed on pressing the **F** button or changing the operator (ie. + or *).

Paste

The **Paste** button pastes single or multi row data from the Windows clipboard into the Concentration table.

Fit Method Wizard Step

This Wizard step allows you to specify the fit method to use in the Quantitative analysis.

The raw or blank corrected measurements (Y) will be plotted against the specified concentration values (X) and then the selected fit method will be performed on (X,Y) data set. The resulting fit will be used to calculate concentration values for the unknowns.

The **Fit Method** drop-down list is populated with the installed curve fit methods. If the selected Fit Method has additional properties the **Edit** button is enabled and used to access these properties.

Optionally, the Y axis can scaled to B/B0 (the method used depends on the selected fit method)

Optionally, EC values can be calculated from the fit, default values are EC(20), EC(50) and EC(80). Enter comma separated values as required.

If the **Apply Dilution Factors** tick box is selected then Dilution Factors can be specified in the **Next** wizard step, otherwise Dilution Factors are not used.

Advanced

Push the **Advanced** button to reveal **Log/Anti-Log** options for X and Y data. If these are ticked then X and/or Y data is logged before fitting and anti-logged when values are read back from the fit. Use these options when fitting with linear values does not work, for example if you have a wide non-linear concentration sequence.

Dilution Factors Wizard Step

This Wizard step allows a dilution factor to be specified for each Unknown group. This means that the calculated concentration of each Unknown is multiplied by the specified factor.

The Group/Dilutions list on the right is populated with all Unknown groups on all the microplates which will be read. The up and down keys can be used to change the value to edit.

Auto Series

If **Auto Series** is ticked, the controls beneath it are enabled. The numeric edit box can be used to enter a value to multiply or increase Group n's concentration by to get group n+1, starting with the first group. (If the first group's value is 0 then the second is used rather than the first.) The auto-series operation is performed on pressing the **F** button or changing the operator (ie. + or *).

Qualitative

Cut-off Point Wizard Step

This Wizard step allows you to specify the cut-off point, how to label samples and details of any Grey Zone.

The **Cut-off point** is an expression describing the cut-off point. This is a number, expression or a group.

The **Negative Label** and **Positive Label** edit boxes allow labels to be specified. Any numerical value > the evaluated cut-off point will be labelled with the **Positive Label** and any point < will be given the **Negative Label**.

Ticking the **Grey Zone** box displays the Grey Zone edit control. Here an absolute number, expression or a percentage can be entered. If it is a percentage then this is an expression followed by a %. The percentage is found of the cut-off point. Any point which falls within this defined Grey Zone area is also marked as "Grey" as well as its determined label.

Example:

With a particular blood test application, it is necessary to label the Unknowns based on how they compare to the ratio of a Positive and Negative Control. The Microplate Layout includes 1 Pos Control group and 1 Neg Control group. Here you could enter a cut-off point expression: "Pos Control/Neg Control" which sets the cut-off point to this ratio. The Unknowns greater than this ratio will be labelled Positive and the Unknowns less than this ratio will be labelled Negative.

Tip: When using multiple plate Microplate Layouts any expressions used must be valid for all Microplate Layouts. Any references to groups must be localised – e.g. if the cut-off point was "**Control1**" then this would refer to the first Control group on all microplates.

Enzyme Kinetics

Kinetic Reduction Method Wizard Step

This Wizard step is used to specify the Kinetic Reduction method for the first step of the Enzyme Kinetics analysis.

With this type of application kinetic measurements are made resulting in a kinetic plot for each well. The first step of the Enzyme Kinetics analysis is to determine the absolute slope of each kinetic plot. This is determined from an exponential or linear fit performed on each kinetic plot.

Use the radio buttons to select which method to use to determine the slope of each kinetic plot.

Vmax Options

This Wizard step is used to specify Vmax options and the report content.

Options

If **Calculate Standard Deviation** is ticked the Standard Deviation of the Absolute Slopes is calculated.

If **Log X axis** it is ticked then the resulting graphs will be plotted with a logarithmic X axis.

Report Content

Tick boxes are provided to allow inclusion of items in the report:

Concentrations Table - list of the specified concentrations

Fit Results Table - the calculated MSE, R2, and Vmax of each group

Overlaid Graph – a single graph containing each plot from every plate overlaid in different colours

Individual Graphs - Graph of each group's fit

Concentrations Wizard Step

This Wizard step is used to specify the concentrations of the samples to calculate Vmax of.

Each sample group contains samples at specified concentrations; the measurements will be plotted against the concentrations specified here. From these plots Vmax is determined for each sample group.

Use the **Group Type** control to select which group to perform the analysis on.

Use the **Concentration Replicates** controls to specify if using concentration replicates, their number and the fill direction.

Concentrations

Each well on the microplate contains 2 numbers:

- The large number is the group which the well belongs to – this number identifies the sample; wells with the same number belong to the same group.
- The small number is the concentration group and represents the wells concentration; wells with the same small number are at the same concentration.

Wells which have the same large number and the same small number are replicates and contain the same sample at the same concentration.

Concentration values can be entered using the keyboard. The up and down keys can be used to change the value to edit. As a Concentration is selected, wells of that concentration will start to flash.

Auto Series

If **Auto Series** is selected the Delta edit box can be used to enter a value to multiply or increase Group n's concentration by to get group n+1, starting with the first group. (If the first Concentration value is 0 then the second is used rather than the first.) The auto-series operation is performed on pressing the **Fill** button or changing the operator (ie. + or *).

Paste

The **Paste** button pastes single or multi row data from the Windows clipboard into the Concentration table.

IC(50)

Concentrations/Dilutions Wizard Step

This Wizard step is used to specify how the samples to be compared are diluted.

Each sample group is diluted in the same way. The Inhibition Concentrations (IC) are determined for each sample group by fitting a plot of the measurements against the dilutions.

Use the **Group Type** control to select which group to perform the analysis on.

Use the **Concentration Replicates** controls to specify if using concentration replicates, their number and the fill direction.

Dilutions

Each well on the microplate contains 2 numbers:

- The large number is the group which the well belongs to – this number identifies the sample; wells with the same number belong to the same group.
- The small number is the concentration group and represents how the well is diluted; wells with the same small number are diluted in the same way.

Wells which have the same large number and the same small number are replicates and contain the same sample diluted in the same way.

Dilution values can be entered using the keyboard. The up and down keys can be used to change the value to edit. As a Dilution is selected, wells which will be diluted in that way start to flash.

Auto Series

If **Auto Series** is selected the edit box can be used to enter a value (Delta) to multiply or increase Group n's concentration by to get group n+1, starting with the first group. (If the first Concentration/Dilution value is 0 then the second is used rather than the first.) The auto-series operation is performed on pressing the **Fill** button or changing the operator (ie. + or *).

Paste

The **Paste** button pastes single or multi row data from the Windows clipboard into the Dilution table.

IC% and Report Content Wizard Step

This Wizard step is used to specify the IC% to calculate and the report content

IC%

An IC% value can be specified. The concentration of the substance resulting in the displacement of the specified % of the antibody will be calculated for each group.

Report Content

Under the Report Content section, tick boxes are provided to allow inclusion of items in the report.

Concentrations Table - list of the specified concentrations

Competitive Bindings Table - the calculated MSE and IC% of each group

Overlaid Competitive Graph – a single graph containing each plot from every plate overlaid in different colours

Competitive Graphs - Graph of each group's fit

Microplate Layout Editor

Microplate Layout Editor

The Microplate Layout Editor is used to specify how the physical microplates are pipetted; the analyses use the microplate layout to process the data accordingly.

Launching the Editor

To open the Microplate Layout Editor

- Create a new microplate layout with the Microplate Wizard
- Open an existing microplate layout from the microplate layouts view
- Edit the microplate layout of an assay protocol
- Edit the microplate layout before readings are started
- Edit the microplate layout after readings/import

Editor Views

The Microplate Layout Editor has two main views:

- Single Plate View - displayed when editing a single plate (see page 60).
- Multiple Plate View - displayed when editing a layout that runs over more than one plate (see page 61).

Microplate Wizard Overview

The Microplate Wizard is used to create a new microplate layout. You can launch the Microplate Wizard from the Organiser Welcome Screen.

You can follow the Microplate Wizard from start to finish to quickly create a typical microplate layout. You will need to specify the following information:

- Microplate Size - the width and height (and also optionally whether using multiple microplates)
- Categories/Sample Types - the type of application you are running and the sample types
- Groups - the number of groups of each sample type

Tip: You can launch the Microplate Layout Editor by pressing the **Finish** button when specifying the Size or Categories for more control over sample positioning and to create layouts for assays which run across multiple microplates.

Wizard Steps

Microplate Wizard Welcome Step

This is the first step of the Microplate Wizard. This wizard will help you to create a new single or multiple plate layout.

Click **Next** to continue with the Wizard, or **Cancel** to terminate the Wizard.

Microplate Wizard Size Step

This step allows you to specify the dimensions of the plate layout.

You can select a value from the drop-down list on the right, or enter your own values in the **Columns** and **Rows** boxes. *Note: the maximum plate dimension is **50x50**.*

If you wish to create a multiple plate layout, tick the **Multiple Plate** box.

Click the **Finish** button here to go straight to the microplate layout editor with the specified settings.

Click **Next** to continue with the Wizard, and specify the categories or sample types.

Click **Back** to go back to the Welcome step, or **Cancel** to terminate the Wizard.

Microplate Wizard Categories Step

This step allows you to specify the sample types that will be featured on the microplate.

You can do this by selecting a category or categories to which the microplate belongs, from a list on the left.

Alternatively, you can simply select the Sample Types you would like manually from the list on the right.

If you are creating a multiple plate layout, this is the last step of the Wizard. To continue with the creation of the layout, click **Finish** to be transferred to the microplate layout editor.

You can also click **Finish** if you're creating a single plate layout, or click **Next** to continue to the Groups step.

Click **Back** to go back to the previous step, or **Cancel** to terminate the Wizard.

Microplate Wizard Groups Step

This step allows you to allocate Groups to the Sample Types you selected in the previous step.

To do this, select a Sample Type from the list on the right, and enter the number of Groups to be allocated to it.

Click **Advanced** to display further options:

- **Fill Settings**: Click this button to alter the way the microplate is filled. See Fill Settings, page 63, for more information.
- **Vary samples**: This box is ticked when the Wizard is setting the Group allocation. If you alter any of the allocations manually, it will be unticked. To let the Wizard define the allocations again, tick the box.
- **First Well**: Click this button to display a pointer with which you can specify the first used well on the microplate. Alternatively, select the first used well from the drop-down list.

Click **Next** to continue with the Wizard.

Click **Back** to go back to the previous step, or **Cancel** to terminate the Wizard.

Tip: For more control over your sample positioning you can launch the Microplate Layout Editor by pressing the **Finish** button on the previous Size or Categories steps.

Microplate Wizard Summary Step

This is the final step of the Microplate Wizard. A display allows you to preview the microplate layout and the specified sample types.

Enter the name for the layout in the **Microplate Layout Name** box.

Click **Print...** to print a copy of the layout. Click **Print Preview** to view a preview of the printout.

Click **Finish** to accept the layout and complete the Wizard.

Click **Back** to go back to the previous step, or **Cancel** to terminate the Wizard.

Single Plate View

Overview

The Single Plate View of the microplate layout editor is used to specify the contents of each well in the microplate, this is each well's:

- Sample Type - identified by the colour of the well
- Group Number - identified by the number in the well

For instruments which support skipping wells, wells which are marked as unused will not be read by the instrument.

Window Controls

Microplate

The Microplate control is used to specify each well's sample type and group number. You can:

- **Set an individual well**

  Select the **Sample Type** and **Next group** in the Sample Types control, and then left click on a well. The **Next group** is automatically updated based on your **Replicates** setting so you can continue to set other single wells.

- **Set multiple wells**

  Left click the mouse on the first well and whilst holding down the mouse select the last well, as you move the mouse details of the groups which will be created are displayed near the mouse pointer. If the selected area does not contain enough wells to completely fill the specified replicates then a small exclamation icon is displayed to alert you of this. You can change the way multiple wells are filled from the Fill Settings.

Toolbar

- **Save and Close** – see page 67.
- **Microplate Preview** – see page 64.
- **Print Preview** – see page 65.
- **Print** – see page 65.
- **Sample IDs...** – see page 66.

Sample Types

This contains various controls for setting up the plate.

Multiple Plates

If editing a plate from a Multiple Microplate Layout the **< Previous** and **Next >** buttons can be used to edit the other defined plate layouts. The **Close Editor** returns to the Multiple Plate View.

Sample Type Controls

The Sample Type controls are located on the right side of the Single Plate View and are used to specify how to setup the microplate layout. They include:

- **Edit Types...** – see page 63.
- **Type Selector** - select the Sample Type to use to fill wells.
- **Fill Buttons**
    - Fill layout – see page 63.
    - Undo – see page 67.
    - Redo – see page 65.
    - Clear layout – see page 62.
- **Next group** - select the Group Number to use to start filling wells with. Note you can double click on the text **Next group** to reset it to 1.
- **Replicates(Members)** - select the number of replicates in each group
- **Fill Settings...** – see page 63.

Multiple Plate View

The Multiple Plate View in the Microplate Layout Editor allows you to define a sequence of plate layouts to read.

Defined Plate Layouts

The top **Defined Plate Layouts** control shows thumbnail images of your plate layouts. The following buttons allow you to create and edit these layouts.

- **Add Layout** – see page 64
- **Delete** – see page 64.
- **Edit...** – see page 64.
- **Repeat Last** – see page 65.

Pressing **Edit..** or double-click on a thumbnail to launch the Single Plate View to edit the layout.

(Note, changes to the number and ordering of the microplates cannot be made after the readings have been made/imported.)

Advanced:

Press the **Advanced** button to reveal the various Sequencecontrols. With these controls you can setup the order in which the **Defined Plate Layouts** (above) are used. This is useful if you intend to use the same layout on different microplates and saves you from having to recreate a layout for each microplate.

To achieve this you simply Add layouts from the top **Defined Plate Layouts** control to the bottom **Sequence** control.

You can also define repeated sections by selecting one or more thumbnails in the **Sequence** control (Select the first item, hold down CTRL key and select the last item; alternatively drag a rectangular area with the mouse) and then press the Repeat... button. The following buttons are available:

- **Add** – see page 64.
- **Remove** – see page 65.
- **Move** – see page 64.
- **Repeat...** – see page 65.

Tip: Press the Microplate Preview button to view the actual sequence of microplates that will be read.

Sample IDs (in Microplate Layout Editor)

To display the Sample IDs press the **Sample IDs...** button on the toolbar.

This window allows you to specify the Sample IDs of the Unknown groups on a microplate.

Sample IDs are used in place of the normal group name throughout the assay, including the report, the audit trail and the analysis windows. (They are *not* used in expressions.)

You can change the Sample IDs before, during and after readings. If they are changed after readings, it is necessary to click **Recalculate** (to regenerate the report).

Sample IDs can be entered by selecting the group and entering the new name. To number all groups consecutively, enter the desired ID, including a number, for the first group, then click **Auto Number**.

To copy the contents of the clipboard to the Sample IDs, click **Paste All**.

Click **Reset** to restore the Sample IDs to their original values.

Tip: Specifying Sample IDs within the Microplate Layout Editor means that each time a protocol which uses this layout is run, IDs specified with the layout are used. This is useful in a research scenario; for tests which you run repeatedly with the same samples. In this case specifying the ID's with the layout saves you from having to enter the Sample IDs each time you run the protocol. However, for scenarios such as a hospital situation where you may test different patient samples on each run of the protocol you would specify the Sample IDs with each run of the protocol.

Commands

Advanced Fill Settings

This window allows you to specify the fill settings for your microplate. There are two tabs, **Orientation** and **Order**.

Orientation

This tab allows you to specify the fill direction and number of replicates for your selected group. The following fill options are available:

- **By Row**
- **By Column**
- **Rectangle Mode**
- **Snake Mode**

If **Rectangle Mode** is selected, you can choose whether the replicates fill **By Row** or **By Column**.

You can specify the number of replicates using the entry box.

Order

Use this tab to change the order in which the Sample Types appear. Entries can be moved using the **Move Up** and **Move Down** buttons.

Clear Layout

You can clear the layout by selecting **Edit** | **Clear Layout** or by clicking the Clear Layout button from the Sample Types controls.

Edit Sample Type

This window is displayed when adding or editing a Sample Type. It allows you to specify the following Sample Type attributes:

- **Sample Group Name**
- **Sample Type**: select this from a drop-down list.
- **Population Number**: this is only editable when the Sample Type is first edited. It must be greater than any value previously allocated to a Sample of this type.
- **Sample Colour**: click **Change...** to open a colour selector.

A well in the bottom-left shows a preview of the Sample Type.

Edit Types

This window allows you to add, edit and remove Sample Types. Only non-system Sample Types can be edited or removed.

Click **Add...** to add a new Sample Type.

To edit an existing non-system Sample Type, select it from the list and click **Edit...** to edit the Sample Type

To remove a non-system Sample Type, select it from the list and click **Remove**. Note: if the removed Sample Type is used in another microplate layout, it will be possible to restore it when that microplate is opened.

Exit

To exit the editor, select **File** | **Exit**, or click the Close Window button in the top-right hand corner.

If the file needs to be saved, you will be prompted to do so.

Fill Layout

You can fill the unused wells of a layout by selecting **Edit** | **Fill Layout** or by clicking the Fill Layout button from the Sample Types controls.

The unused wells will be filled with the selected Sample Type according to the specified fill direction.

Fill Layout is not available when the Fill Direction is set to Rectangular Mode.

Fill Settings

This window can be reached by selecting **Edit** | **Fill Settings...**, or by clicking the **Fill Settings...** button. It allows you to specify the format in which the microplate is filled.

Four basic settings are available from this window. To select them simply click on the format you desire.

Click **Advanced** to display the Advanced Fill Settings window.

Hidden Sample Type

This message is displayed when opening a plate layout containing a Sample Type which has been removed during a previous session.

The options available depend on whether the current plate is locked or unlocked.

Clicking **Yes** (if it is unlocked) or **OK** (if it is locked) will recover the hidden type, and add the text "*Restored*" to its name.

If the plate is unlocked, clicking **No** will display the Hidden Sample Type Reallocation window, allowing you to allocate the hidden type to a different, unhidden type.

Clicking **Cancel** aborts the operation.

Hidden Sample Type Reallocation

This window allows you to select a Sample Type to be reallocated to a hidden Sample Type.

Click **OK** to perform the reallocation.

Microplate Preview

This is displayed by selecting **File** | **Microplate Preview**, or by clicking the Microplate Preview button on the toolbar.

Click **Print...** to print the layout.

Click **Print Preview** to preview the printout.

Multiple Plate Commands

Multiple Plate Add

Click the **Add** button in the Multiple Plate View to add any plate layout(s) highlighted in the upper **Defined Plate Layouts** control to the lower **Sequence** control.

Multiple Plate Add Layout

Click the **Add Layout** button in the Multiple Plate View to add a new empty layout to the end of the **Defined Plate Layouts**.

Multiple Plate Advanced

This button activates and deactivates the **Advanced** mode.

Refer to Multiple Plate View, page 61, for more information about Advanced mode.

Multiple Plate Close Editor

Click this button, when editing a single plate from a multiple plate layout, to close the editor and return to the multiple plate sequence editor.

Multiple Plate Delete

Click the **Delete** button in the Multiple Plate View to delete the highlighted plate from the **Defined Plate Layouts**.

If there is only one plate in the sequence, it cannot be deleted.

Multiple Plate Edit

Click the **Edit...** button in the Multiple Plate View to edit the highlighted plate layout in the Single Plate View.

Multiple Plate Move

Click the **<< Move** and **Move >>** buttons in the Multiple Plate View to move the highlighted plate layout(s) left or right one place in the **Sequence** control.

Multiple Plate Next

Click this button, when editing a single plate from a multiple plate layout, to edit the next plate in the sequence.

Multiple plate Previous

Click this button, when editing a single plate from a multiple plate layout, to edit the previous plate in the sequence.

Multiple Plate Remove

Click the **Remove** button in the Multiple Plate View to remove any highlighted plate layout(s) in the lower **Sequence** control.

Multiple Plate Repeat

Click the **Repeat...** button in the Multiple Plate View to display a window allowing you to set a repeat value to the highlighted plate layout(s) in the **Sequence** control. Enter a value in the **Repeat** box, or click **Read once** to disabled repeating. Click **OK** to keep the changes.

When a repeat value has been set, an arrow appears under the plate layout(s) which are to be repeated, with a number indicating the number of repeats. Click this number to edit the repeat value.

Multiple Plate Repeat Last

Click the **Repeat Last** button in the Multiple Plate View to copy the last defined plate layout to the end of the **Defined Plate Layouts**.

Next Group

The **Next group** control displays the value of the next group of the selected Sample Type.

You can specify the value of the next group by entering a value in the box, or using the up/down buttons.

Print

To print the layout, select **File** | **Print**, click the Print button on the toolbar, or press **CTRL+P**.

Tip: A colour microplate layout printout is useful for reminding protocol users how to pipette their microplates.

Print Preview

This displays a preview of the microplate printout. You can access it from the **File** | **Print Preview** pull-down, or by clicking the Print Preview button in the toolbar.

Click the **Print...** button to print the layout.

Redo

The last undone action can be redone by selecting **Edit** | **Redo**, by clicking the Redo button from the Sample Type controls, or by pressing **CTRL+Y**.

Replicates

This control displays the number of replicates in the current group of the selected Sample Type.

You can enter a different value in the box, or alter the value by clicking the up/down arrow buttons.

Sample IDs (in Microplate Layout Editor)

To display the Sample IDs press the **Sample IDs...** button on the toolbar.

This window allows you to specify the Sample IDs of the Unknown groups on a microplate.

Sample IDs are used in place of the normal group name throughout the assay, including the report, the audit trail and the analysis windows. (They are *not* used in expressions.)

You can change the Sample IDs before, during and after readings. If they are changed after readings, it is necessary to click **Recalculate** (to regenerate the report).

Sample IDs can be entered by selecting the group and entering the new name. To number all groups consecutively, enter the desired ID, including a number, for the first group, then click **Auto Number**.

To copy the contents of the clipboard to the Sample IDs, click **Paste All**.

Click **Reset** to restore the Sample IDs to their original values.

Tip: Specifying Sample IDs within the Microplate Layout Editor means that each time a protocol which uses this layout is run, IDs specified with the layout are used. This is useful in a research scenario; for tests which you run repeatedly with the same samples. In this case specifying the ID's with the layout saves you from having to enter the Sample IDs each time you run the protocol. However, for scenarios such as a hospital situation where you may test different patient samples on each run of the protocol you would specify the Sample IDs with each run of the protocol.

Sample Types

This window can be reached by selecting **Edit** | **Sample Types...** or by clicking the **Edit Types...** button. It displays a list of the available Sample Types, and allows you to select which types you would like to include on the plate.

Note: there must be at least one Sample Type ticked in the list.

Click **Edit...** to add further Sample Types to the list.

Click **OK** to add the ticked Sample Types to the microplate's available types.

Click **Cancel** to lose your changes.

Save

You can save the file in several ways:

- From the pull down menu option **File** | **Save**
- From the pull down menu option **File** | **Save As**
- Pressing the **Save** button on the toolbar
- Pressing the **Save and Close** button on the tool bar

The file can also be saved by:

- Clicking the Windows Close cross
- Pressing Alt+F4
- **File** | **Exit**

In these cases a message will be displayed asking whether you want to save the file.

Save and Close

This button saves the current microplate and closes the microplate editor without asking you for confirmation.

If the microplate has yet to be named, a window will appear asking you for a filename.

Save As

You can specify a filename to which to save the current microplate layout by selecting **File** | **Save As...**

Undo

The last action can be undone by selecting **Edit** | **Undo**, by clicking the Undo button from the Sample Types controls, or by pressing **CTRL+Z**.

Run and Results

Run and Results

Overview

This module is for running Assay Protocols and reviewing Results. Raw data can be viewed whilst readings are being made and the raw and calculated data can be inspected after the analyses are completed.

This module can also be used to adjust the various settings of the Assay Protocol before and after the measurements.

Running an Assay Protocol – page 85.

Reviewing Results – page 86.

Tip: This module can be launched from the command line to automatically process data as part of an automation process. See Command Line Arguments, page 133, for more information.

Getting Around

The main window consist of the following items:

Horizontal Toolbar

Vertical Toolbar

View Tabs

Horizontal Toolbar

The Horizontal Toolbar appears across the top of the main Run and Results window. Its content is different before readings have started and when the assay results are being displayed:

Before Readings

- **Open** – see page 83.
- **Scrubber** – see page 69.
- **Sample IDs** – see page 84.
- **Edit Protocol** – see page 92.
- **Start** - Starts the readings.
- **Stop** - Stops the readings in progress.

Displaying Assay Results

- **New** – see page 83.
- **Open** – see page 83.
- **Save** – see page 84.
- **Print Report** – see page 83.
- **Print Preview** – see page 83.
- **Recalculate** – see page 83.
- **Versions** – see page 85.

- **Sample IDs** – see page 84.
- **Edit/Review Transforms** – see page 82.
- **Edit Microplate Layout** – see page 86.

Vertical Toolbar

The Vertical Toolbar appears along the left hand side of the Run and Results window; it is comprised of the following buttons:

-  Microplate – see page 72.
-  3D Microplate – see page 71.
-  Zoomed Kinetic Chart – see page 72.
-  3D Kinetic Chart – see page 71.
-  Time/Temperature – see page 72.
-  Auto Arrange – see page 82.
-  Properties – see page 83.
-  Flag – see page 87.
-  Open in Word – see page 89.
-  Open in Excel – see page 89.

Scrubber Wizard

Scrubber Wizard

With this feature you can quickly and easily "scrub" and change the number of unknown wells to read. This makes applying small changes to the microplate prior to a run a very easy operation.

To launch the Scrubber Wizard press the Scrubber Wizard button on the Horizontal Toolbar in the Run and Results window prior to starting readings.

Scrubber Wizard Welcome Step

This is the first step of the scrubber Wizard. The Wizard allows you to change the number of Unknowns that will be read.

Click **Next** to continue the Wizard.

Click **Cancel** to terminate the Wizard.

Scrubber Wizard Allocation Step

This Wizard step allows you to define the number of Unknown (or Spike) wells to be read.

To make a well unused, left-click on it, or drag the mouse over a number of wells.

The window on the right shows the number of wells used. This can also be used to change the number of wells by selecting the group and entering a value.

If the layout contains multiple plates, use the navigation buttons to view each plate.

Click the **Reset** button to restore the original microplate layout.

Click **Next** to continue the Wizard.

Click **Back** to go to the previous Wizard step.

Click **Cancel** to terminate the Wizard.

Scrubber Wizard Completing Step

This is the final step of the Wizard.

If you tick the **Run assay protocol now** box, then readings will commence when you click **Finish**.

Click **Back** to go to the previous Wizard step.

Click **Cancel** to terminate the Wizard.

Tabs

View Tabs

The results are presented in a variety of views organised by the view tabs in the main Run and Results window:

- Data – see page 70.
- Analysis – see page 74.
- Audit Trail – see page 80.
- Report – see page 81.

The **Data** tab is the only tab displayed prior and during the measurement process. After readings the **Audit Trail** and **Report** tab are included and any further analysis information is provided in the **Analysis** tab.

Data

Data

The Data Tab in the Run and Results window is for viewing raw and calculated data. Before, during and after readings you can create and arrange different views of the data. Press a view button from the Vertical Toolbar to create a new view:

-  Microplate – see page 72.
-  3D Microplate – see page 71.
-  Zoomed Kinetic Chart – see page 72.

-  3D Kinetic Chart – see page 71.
-  Time/Temperature – see page 72.

Tip: Right-click on a view to display additional options in the Context Menu.

Data View Context Menu

Right-click on any of the views in the Data tab for more options:

Properties

Displays the property page for the selected view.

Remove View

Removes the selected view.

Copy as Image

Places an image copy of the current view on the Windows clipboard (using the current window size).

Views

3D Endpoint View

Description

This view displays a single matrix of endpoint readings projected in 3D.

Interaction

You can rotate the chart by holding down the middle mouse button (if there is no middle mouse button, hold down the right mouse button and the left mouse button at the same time), and moving the mouse. Pressing **X**, **Y** or **Z** during this operation will fix the rotation to the appropriate plane.

You can zoom in on the chart by holding down **CTRL** and dragging a rectangular area with the mouse. Alternatively, hold down **CTRL** and the middle mouse button (or the right and left mouse buttons at once) and move the mouse up or down to zoom in or out.

You can reset the zoom level by pressing the **R** key.

You can move the chart by holding down **SHIFT** and the middle mouse button (or the right and left mouse buttons at once) and moving the mouse.

Right-click on the view to display additional options in the Context Menu.

3D Kinetic Chart

Description

This view displays the kinetic charts of a well or series of wells in 3D. The X axis is Cycle Number, the Y axis is Reading and the wells appear in series on the Z axis.

Interaction

You can rotate the chart by holding down the middle mouse button (if there is no middle mouse button, hold down the right mouse button and the left mouse button at the same time), and moving the mouse. Pressing **X**, **Y** or **Z** during this operation will fix the rotation to the appropriate plane.

You can zoom in on the chart by holding down **CTRL** and dragging a rectangular area with the mouse. Alternatively, hold down **CTRL** and the middle mouse button (or the right and left mouse buttons at once) and move the mouse up or down to zoom in or out.

You can reset the zoom level by pressing the **R** key.

You can move the chart by holding down **SHIFT** and the middle mouse button (or the right and left mouse buttons at once) and moving the mouse.

Right-click on the view to display additional options in the Context Menu.

Microplate View

Description

This view displays a single matrix of results in a microplate format. The matrix may be endpoint or kinetic.

Interaction

Further information about a well can be displayed by hovering the mouse pointer over a well.

If you are in Flagging mode clicking on a well flags it. Flagged wells are marked with a diagonal cross. See Flagging, page 87, for more details.

Additionally if viewing a kinetic matrix: double-clicking on a well overlays it on a Zoomed Kinetic Chart view if one is already being displayed, if not a new Zoomed Kinetic Chart is created.

Right-click on the view to display additional options in the Context Menu.

Time Temperature View

Description

This view is available if temperature measurements were taken at regular intervals during readings. The chart displays temperatures over time.

If temperatures were taken with each and every reading over time then the temperatures can be viewed in the Microplate View and optionally plotted against readings.

Interaction

You can zoom in on the chart by holding down **CTRL** and dragging a rectangular area with the mouse. Alternatively, hold down **CTRL** and the middle mouse button (or the right and left mouse buttons at once) and move the mouse up or down to zoom in or out.

You can reset the zoom level by pressing the **R** key.

You can move the chart by holding down **SHIFT** and the middle mouse button (or the right and left mouse buttons at once) and moving the mouse.

Right-click on the view to display additional options in the Context Menu.

Zoomed Kinetic Chart

Description

This view displays the kinetic chart for a well or a series of wells.

Interaction

You can flag a kinetic point by selecting Flagging mode and clicking a point on a line on the chart. Flagged points appear as crosses in the same colour as the original line. You can unflag a flagged kinetic point by clicking on it again when in Flagging mode.

You can zoom in on the chart by holding down **CTRL** and dragging a rectangular area with the mouse. Alternatively, hold down **CTRL** and the middle mouse button (or the right and left mouse buttons at once) and move the mouse up or down to zoom in or out.

You can reset the zoom level by pressing the **R** key.

You can move the chart by holding down **SHIFT** and the middle mouse button (or the right and left mouse buttons at once) and moving the mouse.

Right-click on the view to display additional options in the Context Menu.

Properties

3D Endpoint Settings

If the **Rotate** check box is ticked then the view animates by rotating 90° about the Z axis.

3D Kinetic Settings

A drop-down list allows you to specify the **Chart Type**, Bar or Scatter.

If **Rotate** is checked then the chart rotates and animates 360° about the Y axis.

Data

This window allows you to specify the data to display.

Matrix

This **Matrix** combo is populated with the matrices which are applicable for the view type (i.e endpoint and or kinetic). Raw matrices are included prior and during reading and the calculated matrices are included after analysis is completed.

Plate

The Plate drop-down list displays the available plates (this is disabled if only using a single plate).

Drop-down lists allow you to specify the orientation of the X and Y axes.

Kinetic Controls

If a kinetic matrix is selected, additional controls are available:

The **Data** drop-down list allows you to select the type of data displayed in the kinetic view.

- Drop-down lists allow you to specify the data to be plotted on the **X Axis** and **Y axis**. These can include Cycle Number, Time, Reading and Temperature depending on the measured data.
- If using a microplate view, the **Chart** option displays the kinetic chart in each well of the microplate view. It also enables a **Relative Charts** option: if this is ticked all charts are scaled to the minimum and maximum readings on the matrix, otherwise each chart is scaled to the minimum and maximum reading of each well. The **Point** and **Time** options display the reading or time in each well at a specific cycle. The cycle can be selected from a drop-down list. (Any flagged points appear with a horizontal/vertical cross through them.) The matrix drop-down list allows the selection of endpoint or kinetic matrices to display.

Series

Here you can specify which well(s) to display. There are 3 options:

- **Wells:** allows you to select any wells from the list on the right. One or more items can be selected using the standard Windows procedure (hold down **SHIFT** and select the first and last items, or hold down **CTRL** and select separate items).
- **Well Groups:** allows you to select any groups on the selected plate from the list on the right. When a group is selected the wells belonging to that group are displayed.
- **All Wells:** automatically selects all used wells.

The **Show Series Labels** option allows you to display or hide the series labels on the chart.

Time Temp Settings

Select which temperature to view.

Analysis

The analysis tab appears in the Run and Results window if any of the configured transforms produce analysis control(s). An analysis control provides further information about the calculations performed and some allow you to use interactive controls to tweak parameters.

Analysis Controls

Analysis Control Context Menu

Right-click on any of the views in the Analysis tab for more options:

Properties

Displays the property page for the selected analysis control to allow further customisation of the view.

Edit Transformation

Displays the property page of the transform that the selected analysis control refers to. You can make changes to the analysis settings and then Recalculate the results.

Copy as Image

Places an image copy of the current view on the Windows clipboard (using the current window size).

Analysis Control Chart Interaction

Zooming and moving the chart

You can zoom in on the chart by holding down **CTRL** and dragging a rectangular area with the mouse. Alternatively, hold down **CTRL** and the middle mouse button (or the right and left mouse buttons at once) and move the mouse up or down to zoom in or out.

You can reset the zoom level by pressing the **R** key.

You can move the chart by holding down **SHIFT** and the middle mouse button (or the right and left mouse buttons at once) and moving the mouse.

Analysis Controls

Baseline Correction Analysis Control

This window displays the kinetic chart of the selected well in blue.

The baseline correction curve is displayed in red.

The details of the baseline correction are displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Competitive Binding Analysis Control

Normal Mode

This window displays the competitive graphs for each group.

The points in the group are plotted as crosses in the group's colour.

The footer displays the details of the fit method.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

The **Overlay** button activates Overlay mode.

Overlay Mode

In this mode a line is plotted to show the fit of each group on every plate. A key for the lines' colours is included at the top of the graph.

The **< Previous** and **Next >** buttons are disabled.

Flagging

Flagging is only available in Normal mode.

Any point on the graph can be flagged by selecting the **Flag** control and left-clicking on the point.

Chart labels are displayed on flagged points, detailing the point's well, group and value. When **Recalculate** is clicked the chart labels are removed, and the flagged point is displayed as an asterisk.

A point can be unflagged by clicking on it again with the **Flag** control selected.

Interaction

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Crossing Point Analysis Control

This window displays the kinetic chart of the selected well in blue.

The crossing point is marked with two red lines. The X value of the crossing point is displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Delta X for Delta Y Analysis Control

This window displays the kinetic chart of the selected well in blue.

The point at which the specified Delta Y is reached is marked with two dotted red lines, and an arrow with the point's X and Y values.

The point's X and Y values and the Delta X calculation are displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Delta Y Analysis Control

This window displays the kinetic chart of the selected well in blue.

The first and last points are marked with arrows on the graph.

The **Delta Y** calculation and its result are displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Fluorescent Background Analysis Control

This window displays the kinetic chart of the selected well in blue.

The fluorescent background corrected curve is displayed in red.

The details of the fluorescent background correction are displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Group Vmax Analysis Control

Normal Mode

This window displays the individual graphs for each group.

The points in the group are plotted as crosses in the group's colour.

The footer displays the details of the fit method.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

The **Overlay** button activates Overlay mode.

Overlay Mode

In this mode a line is plotted to show the fit of each group on every plate. A key for the lines' colours is included at the top of the graph.

The **< Previous** and **Next >** buttons are disabled.

Flagging

Flagging is only available in Normal mode.

Any point on the graph can be flagged by selecting the **Flag** control and left-clicking on the point.

Chart labels are displayed on flagged points, detailing the point's well, group and value. When **Recalculate** is clicked the chart labels are removed, and the flagged point is displayed as an asterisk.

A point can be unflagged by clicking on it again with the **Flag** control selected.

Interaction

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Integral Analysis Control

If the **Summation** method is used, the kinetic chart of the selected well is displayed in blue.

If the **Trapezium** method is used, the chart displays each trapezium in red, with the connecting points in blue.

In both cases, the result is shown in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Kinetic Fit Analysis Control

This window displays the kinetic chart of the selected well in blue.

The fitted line is displayed in red.

The output variables and coefficients are displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Kinetic Point Analysis Control

This window displays the kinetic chart of the selected well in blue.

The point at which the specified X value is reached is marked on the graph with an arrow displaying the X and Y values of the point.

The details of the interpolation are displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Linear Regression Analysis Control

This window displays the kinetic chart of the selected well in blue.

The linear fit is displayed as a red line.

The graph footer displays the fit details and result.

If you have selected to display the **Y at relative X'**, this point is marked on the fitted line with a dotted red line and an arrow detailing the X and Y values of the point.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Max Peaks Analysis Control

This window displays the kinetic chart of the selected well in blue.

The max peaks are with an arrow on the graph, and are displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Maximum Slope Analysis Control

This window displays the kinetic chart of the selected well in blue.

The maximum slope is marked as a red line, with an arrow pointing to its centre, displaying the X and Y values of the centre point.

If you have specified the result as the **Extrapolated Y of Max Slope**, a red dotted line displays the extrapolated line, and an arrow marks the extrapolated point.

The details of the calculations and the result are displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Peak Point Analysis Control

This window displays the kinetic chart of the selected well in blue.

The peak point is marked with two red lines, and is displayed in the graph footer.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Rate of Change Analysis Control

This window displays the kinetic chart of the selected well in blue.

The rate of change is displayed as a red line.

You can step through the available wells on the plate with the **< Previous** and **Next >** buttons.

Interaction

You can drag the vertical dashed lines to tweak the X range of points to include in the evaluation. Only those points which fall between the two vertical dashed lines will be included in the next Recalculation. These correspond to the X minimum and maximum range specified in the transform's Kinetic Options.

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Standard Curve Fit Analysis Control

This window displays the graph of the Standard Curve Fit.

Replicate points are plotted as red crosses. The fitted line is plotted through the X range of the Standards.

If the fit fails then the reason is displayed in the graph footer.

If EC values are calculated, these are displayed in the graph footer.

A key is displayed at the top-right of the graph detailing all symbols used, and any flagged Standard points.

Flagging

Any point on the graph can be flagged by selecting the **Flag** control and left-clicking on the point.

Chart labels are displayed on flagged points, detailing the point's well, group and value. When **Recalculate** is clicked the chart labels are removed.

If the flagged point is a Standard point, upon recalculation the point is shown with an asterisk through it.

If the flagged point is not a Standard point then upon recalculation the flagged point is not displayed.

A point can be unflagged by clicking on it again with the **Flag** control selected.

Interaction

You can interact with the analysis control chart – see page 74.

Right-click on an analysis control for further options in the Analysis Control Context Menu.

Properties

Selection

A drop-down list allows you to select which well is currently displayed.

If the view supports overlaying of well data the **Overlay** tick box can be selected to display the results of all wells together.

Plate

A drop-down list allows you to select which plate to display the data from.

Standard Curve Fit Analysis Properties

Show Calculated Points on Fit

The **Show Calculated Points on Fit** option, when ticked, displays all the backfitted points. The colour of each point corresponds to its group type.

Use Specified Range

When this option is ticked, you can specify the minimum and maximum X axis range values.

Audit Trail

Audit Trail

The audit trail tab in the Run and Results window contains details of operations performed on the data. Each entry is prefixed with the date and time.

Entries are created for the following operations:

- Calculations: all details of each transformation and its calculations are included.
- Accessing a file: when a file is opened or readings are completed.
- Creating Results: when a Results file is created after readings or the import of data.
- Versions Purged: when Purge Versions is performed.
- Saving the file.
- Flagging a well or a kinetic point.
- When the Microplate Layout is modified.

Report

Report

The report is displayed in the report tab of the Run and Results window. The report typically contains the following items:

Protocol Description

This table contains:

- **Assay Protocol Name**
- **Instrument** (for readings only)
- **Readings**
- **Well Types**
- **Microplate Layout**
- **Transforms**

Transform Items

Depending on the configured transforms, content may be added here. For example, a **Standard Curve Fit** transform adds a concentration table and a graph.

Table

The results for each group are listed in a table. Each row of the table contains the results from one group. The first column contains the group name; the second column, the wells which comprise that group.

For endpoint data either the mean of the unflagged wells, or the values of each replicate, are displayed, depending on the Report Options.

For kinetic data, graphs are shown for each well.

Additional columns may be added by any configured transforms.

Matrix

A matrix is included, in which each cell represents a well on the microplate. A key is included at the bottom of the matrix.

For endpoint data, flagged wells are marked with a strikethrough. Failed calculations are marked with '---'.

Flagged Table

This table lists the flagged points. There are columns for the flagged point, the flagging agent, and the flagging reason.

Tip: You can customise the report content using the Report Options...

Report Options

You can edit the report options by going to **File** | **Report Options...**

The following options are available:

- **Include Table**
- **Include Matrix**
- **Include Flag Details**
- **Include Transform Output**

See Report, page 81, for more information on these categories.

If you tick **Include Table**, you can specify whether the values of each raw replicate, or the mean values are displayed in the table.

If you tick **Include Matrix**, you can specify how many columns the matrix contains.

Click the **Font...** button to change the font used in the report.

Commands

Auto Arrange

Click this button to remove all the current results views in the Data Tab and set up the default views.

Edit/Review Transforms

You can edit transforms by selecting **Results** | **Edit Transforms**, clicking the **Edit Transforms** button on the horizontal toolbar, or by pressing **F9**. If you are not viewing the latest version of the results file, you will be able to review the transforms, but not edit them.

There are two tabs, **Transforms** and **Matrices**.

Transforms

A list of configured transforms is displayed in the main window.

To edit one of the transforms, select it and click **Edit...**, or double-click on it.

To remove a transform, select it and click **Remove**. You are not allowed to remove transforms whose output matrices are used as input matrices to another transform.

To create a new transform, click **Create...**

Matrices

This tab contains a list of the raw data matrices on the left and a list of the calculated matrices for the configured transformations on the right.

New

You can run new readings based on the protocol of the current results file, by selecting **File** | **New**, clicking the New button in the horizontal toolbar, or pressing **CTRL+N**.

If there is more than one version of the results file, you will be asked to select which version you wish to use. Clicking **Cancel** at this point aborts the New operation.

If the current results file needs saving, you will be asked whether you wish to do so before continuing with the New operation.

Open

You can open a results file by going to **File** | **Open**, clicking the Open button in the horizontal toolbar, or pressing **CTRL+O**.

If readings have been taken, and the current results file is unsaved, you will be prompted to save the file before opening a new one.

Post Analysis Options

These are the operations performed after the measurements/import and calculations have been performed as specified in the Post Analysis settings in the Assay Protocol.

The Post Analysis Options are also performed if the readings are stopped or stopped because the Run and Results window is exited during the read.

Print Preview

You can view a print preview of the report by selecting **File** | **Print Preview** or by clicking the Print Preview button in the horizontal toolbar.

You can print the report from the Print Preview window by clicking **Print...**

Print Report

You can print the report of a results file by selecting **File** | **Print Report**, clicking the Print button in the horizontal toolbar, or by pressing **CTRL+P**.

A Printer Selection window will be displayed allowing you to select which printer you wish to use.

Properties

Displays the property page of the current Data View or Analysis Control.

Purge Versions

This operation, accessible from the **Results | Purge Versions**, allows you to remove all versions of the results file except the last (the audit-trail data will be kept).

When you select this option you will be asked to confirm your decision. Click **OK** to go ahead and purge the versions, or **Cancel** to abort the operation.

Tip: Purge Versions can be used to reduce the size of the Assay Result file.

Recalculate

This button is enabled when changes have been made to the configured transforms, microplate layout or when a flagging operation has occurred.

The button will flash briefly to let you know that a recalculation is required.

Report Options

You can edit the report options by going to **File** | **Report Options...**

The following options are available:

- **Include Table**
- **Include Matrix**
- **Include Flag Details**
- **Include Transform Output**

See Report, page 81, for more information on these categories.

If you tick **Include Table**, you can specify whether the values of each raw replicate, or the mean values are displayed in the table.

If you tick **Include Matrix**, you can specify how many columns the matrix contains.

Click the **Font...** button to change the font used in the report.

Sample IDs

This window allows you to specify the Sample IDs of the Unknown groups on a microplate.

Sample IDs are used in place of the normal group name throughout the assay, including the report, the audit trail and the analysis windows. (They are *not* used in expressions.)

You can specify the Sample IDs in the Microplate Layout editor and before, during and after readings. If they are changed after readings, it is necessary to click **Recalculate** (to regenerate the report).

Sample IDs can be entered by selecting the group and entering the new name. To move between Sample IDs use the up and down cursor keys.

To number all groups consecutively, enter the desired ID, including a number, for the first group, then click **Auto Number**.

To copy the contents of the clipboard to the Sample IDs, click **Paste All**.

Click **Reset** to restore the Sample IDs to their original values.

Tip: Specifying Sample IDs within the Microplate Layout Editor means that each time a protocol which uses this layout is run, IDs specified with the layout are used. This is useful in a research scenario; for tests which you run repeatedly with the same samples. In this case specifying the ID's with the layout saves you from having to enter the Sample IDs each time you run the protocol. However, for scenarios such as a hospital situation where you may test different patient samples on each run of the protocol you would specify the Sample IDs with each run of the protocol.

Save

You can save the current results file by selecting **File** | **Save**, clicking the Save button in the horizontal toolbar or pressing **CTRL+S**.

If the results need recalculating you will be prompted to do so before saving.

Save As

You can save the results file to a specified filename by selecting **File** | **Save As**.

Save Protocol

You can save the protocol upon which a results file is based by selecting **File** | **Save Protocol**.

If there is more than one version of the results file, you will be prompted to select a version to save.

User Flag

This window appears when you are in flagging mode and have clicked on a well or a kinetic point. It allows you to enter a reason for flagging the well. This reason is included in the Audit Trail and in the flagging table in the report.

Tick the **Do not show this again** box if you don't wish this window to appear next time you flag a well or point.

Version Selector

This drop-down list is available on the horizontal toolbar and in the **Results** menu. It allows you to select which version of the results file to view.

Tip: You can only edit the analysis settings if you are reviewing the latest result version (when reviewing earlier results edit options are disabled).

Tasks

Launching and Running an Assay Protocol

The steps for running an Assay Protocol depend you are using an Online Protocol or Offline Protocol:

Online

On launching an Online Protocol the Run and Results window is displayed ready for the readings to be started. Initial results views are setup in the Data Tab, these views can be customised and interacted with before, during and after the readings are being made.

The Horizontal Toolbar includes the following buttons which can be used to modify the measurement and analysis settings before the readings are started:

Scrubber Wizard – see page 69.

Sample IDs – see page 84.

Edit Protocol – see page 92.

The **Start** and **Stop** buttons are used to control the measurement process.

If necessary you may want to edit the microplate layout before readings are started – see page 86.

Offline

When an Offline is launched the file to import can be specified with the command line arguments, see page 133.

If no command line arguments are specified a message box is displayed describing the file format which can be imported, the file to import can then be selected.

After the readings have been completed or data has been imported the calculations are made and then any configured Post Analysis options are performed.

You can then review the results, see page 86.

Edit the Microplate Layout before readings are started

You can modify the Microplate Layout before the readings are started. This is useful if:

- pipetting errors were made
- you use a different number of samples each time you run the protocol
- you want to change the analysis to be performed

There are two ways which you can do this:

- **Just change the number of unknowns to use** - use the Scrubber Wizard to increase or decrease the number of Unknown samples to use.
- **Freely edit the microplate layout** - select Edit Protocol and then press **Edit...** in the Microplate Layout section to edit the microplate layout. If your modifications affect the configured analysis then you will be advised whether to make further changes. (For example, if you remove the Blank group when using a Blank Correction transform.)

Modifying the Microplate Layout

You can modify the Microplate Layout after the readings have been made. This is useful if pipetting errors were made or if you want to change the analysis performed.

Select the Edit Microplate layout option from the horizontal toolbar. This will display the Microplate Layout editor.

If changes are made to the microplate layout which invalidate any of the transforms you will be alerted (for example if you remove a Blank group which is used in a Blank Correction transform)

After editing the Microplate Layout the Recalculate button flashes to signal that the changes made require a recalculation, you can continue to make further analysis settings or recalculate.

Reviewing Results

You can review results:

- by opening an existing result file (launch from the Organiser)
- after the measurements have been taken
- after the data has been imported

You can:

- View the raw and calculated data – see page 70.
- Flag data points – see page 87.
- View the report – see page 81.
- Print the report – see page 83.
- Export data – see page 88.
- View the audit-trail – see page 80.
- Modify the microplate layout – see page 86.
- View analysis controls – see page 74.

- Change the analysis settings – see page 87.
- Recalculate the results – see page 83.
- Run the protocol again – see page 87.
- Look at earlier versions – see page 87.

Flagging

You may flag a well or point which is not already flagged by selecting the Flag mode: press down the Flag button in the horizontal toolbar; then you can click on a well or kinetic point you wish to flag.

A flagged well or point can be unflagged by clicking on it again (whilst in Flag mode).

Unless you have specified otherwise, the User Flag window will appear, allowing you to enter a reason for the flagging operation (which will be included in the report).

In both cases, the Recalculate button will flash, indicating that you need to recalculate. The flagged points will not be included in the calculations.

Edit/Review Transforms

You can edit transforms by selecting **Results** | **Edit Transforms**, clicking the **Edit Transforms** button on the horizontal toolbar, or by pressing **F9**. If you are not viewing the latest version of the results file, you will be able to review the transforms, but not edit them.

There are two tabs, **Transforms** and **Matrices**.

Transforms

A list of configured transforms is displayed in the main window.

To edit one of the transforms, select it and click **Edit...**, or double-click on it.

To remove a transform, select it and click **Remove**. You are not allowed to remove transforms whose output matrices are used as input matrices to another transform.

To create a new transform, click **Create...**

Matrices

This tab contains a list of the raw data matrices on the left and a list of the calculated matrices for the configured transformations on the right.

Run the protocol again

You can run the protocol again by selecting the New command when reviewing the results.

Look at earlier versions of the results

Each time the Recalculate button is pressed a new set of results is created.

This means that all existing data and changes made are stored and can be tracked.

You can change the version of results being displayed using the Version selector.

Tip: To get rid of earlier versions select the Purge Versions option.

Exporting

Exporting Data

Data can be exported by Manta in the following file formats:

- Text
- HTML
- MHT
- Microsoft Excel
- Microsoft Word

You can:

- Export the data anytime after the results have been processed.
- Automatically Export the data as part of the Assay Protocol.
- Open the report in Word.
- Open the report in Excel.

Manual Data Export

You can export data from your Assay Results at any point using the following methods:

- Launch the Export Wizard
- Open in Excel
- Open in Word

Automatic Data Export

You can setup the Assay Protocol to automatically export the data so that the export operation occurs every time the protocol is run.

To set this up:

- When creating the protocol use the Post Analysis step of the Wizard

or if you have already created the protocol:

- Edit the protocol
- Specify the Export options as required

Export Results to Text File Options

This window is used to specify details about the text file to export.

You can enter a filename to create/use, or press the **Browse...** button to navigate to one.

If the **Append to existing file** option is ticked then the new export is appended to the specified file if it already exists (if not it is created).

Kinetic Matrices

If kinetic measurements are used then the controls within the **Kinetic Matrices** section can be used to customise the kinetic output for kinetic matrices.

Tick the **Include X values with each Y for Kinetic Matrices** to include X values of each kinetic point. If this is not ticked then only Y values will be exported. Use the drop-down lists to select the data to include.

Open in Word

Click this button, available in the vertical toolbar, to open a copy of the report in Word.

(Note, this function is only available if Microsoft Word/Office 2000 or later is installed.)

Open in Excel

Click this button, available in the vertical toolbar, to open a copy of the report in Excel.

(Note, this function is only available if Microsoft Excel/Office 2000 or later is installed.)

Export Wizard

Export Wizard

The Export Wizard can be used to export the report to a variety of file formats.

To launch the Export Wizard, select **File** | **Export Report**.

Report Export Wizard Welcome Step

This is the first step of the Report Export Wizard. This wizard will enable you to export the report content to a suitable format.

Click **Next** to continue the Wizard.

Click **Cancel** to terminate the Wizard.

Report Export Wizard Method Step

This Wizard step allows you to select which format you would like the report to be exported to. The following formats are available, depending on your computer's configuration:

Type	Description
.htm;.html	A Web Page (complete with links to image files)
*.mht	A Web Archive file (a single file)
*.xls	Microsoft Excel (an Excel Workbook with Worksheets)
*.doc	Microsoft Word (a standard Word file)

(Refer to Components Required For Export Report Options, *page 134, if some options are not available on your system.)*

Click **Next** to proceed to the next step, **Back** to go to the previous step.

Cancel will terminate the Wizard.

Report Export Wizard Filename Step

This Wizard step allows you to specify the a name and path for the file to be exported to.

Click the **Browse...** button to open a window allowing you to navigate to a suitable location.

A suggested file name and path is displayed. If this is not the first time this Results file has been exported, a number in brackets is appended to the filename, e.g. **(1)**.

Word documents and HTML documents of the same name cannot coexist in the same directory. A warning is displayed when you click **Next** if this is the case.

Click **Back** to go to the previous step, and **Cancel** to terminate the Wizard.

Report Export Wizard Completing Step

This is the final step of the Report Export Wizard. Upon clicking **Finish** or **Export Now**, the report will be exported to the specified location in the specified format.

If the **Open Report File once exported** tick box is ticked, the report file will be opened after exporting.

If **Export Now** is clicked, the Wizard will remain on screen after the export, enabling you to go back and create further report files.

If **Finish** is clicked, the Wizard will terminate after exporting the report.

Click **Back** to go to the previous step, and **Cancel** to terminate the Wizard.

Command Line Arguments

The Run and Results application can be launched from the command line. This means that Manta can be called from other applications.

Syntax

MRunRes [/run | /edit] {<ProtocolFile> [<DataFileToImport>]} | <ResultsFile> [/exit]

Where:

Item:	Meaning:
MRunRes	The application executable - this is located in the installation directory.
<ProtocolFile>	The full path to a protocol file (APR)
<DataFileToImport>	The full path to a text file to import (for a **<ProtocolFile>** which imports)
<ResultsFile>	The full path to an existing results file (ARS)
/run	The specified **<ProtocolFile>** file (which takes measurements) will start the readings once it has been loaded
/edit	Opens the specified **<ProtocolFile>** file for editing
/exit	The program exits after the post analysis options have been performed.

If the **<ProtocolFile>** imports data then a second parameter **<DataFileToImport>** can optionally be given which is the file to import, if this parameter is not given then a file open window is displayed to allow the manual selection of the file to import.

Examples

Example:	Action:
MRunRes <ResultsFile>	Opens the existing specified results file
MRunRes <ProtocolFile>	Launches the specified protocol file ready to start measurements.
MRunRes /run <ProtocolFile>	Launches the specified protocol file and starts the measurements straight away.
MRunRes /run <ProtocolFile> /exit	Launches the specified protocol file and starts the measurements straight away exits when finished.
MRunRes /edit <ProtocolFile>	Launches the specified protocol file for editing.
MRunRes <ProtocolFile> <DataFileToImport>	Launches the specified protocol which imports data and imports the specified file.
MRunRes <ProtocolFile> <DataFileToImport> /exit	Launches the specified protocol which imports data and imports the specified file then exits

Assay Protocol Editor

Edit Assay Protocol

You can edit an Assay Protocol by:

- Opening it from the Organiser
- Pressing the **Edit Protocol...** button in the Run and Results window prior to starting readings

Edit Assay Protocol Window

This window displays all the settings of your protocol, and allows you to edit/review them (if the protocol is read-only, you will not be allowed to edit any settings). It contains four subsections:

Readings

Click **Edit** to open the instrument editor. If you make any changes which alter the plate size or invalidate existing transforms, a warning will be displayed.

Microplate Layout

Click **Edit** to launch the microplate layout editor.

Transforms

Click **Edit** to change the transforms settings.

Advanced

This section contains five tabs. Each tab can be edited by selecting it and clicking **Edit**.

- Results Filename
- Print
- Report
- Export
- Launch Application

Advanced Options

Edit Assay Protocol Results Filename

This window allows you to specify the details for the results file that will be created when the protocol is run.

A **Target directory** box allows you to enter the path for the results file. Click the **Browse...** button to navigate to a suitable directory.

The **Filename** box allows you to enter the name of the results file. You can select macros from a drop-down list and add them to the Filename by clicking **Add Macro**.

The available macros are:

- **<CurrentDate>**
- **<CurrentTime>**
- **<CurrentUser>**
- **<ProtocolName>**

When the results file is created, these macros are expanded to the appropriate values.

Edit Assay Protocol Print

This window allows you to select whether the report is printed after the measurements are taken.

Edit Assay Protocol Report

This window allows you to specify which items will be included in the report. The following options are available:

- Include Table
- Include Matrix
- Include Flag Details
- Include Transform Output

See Report, page 81, for more information on these categories.

If you tick **Include Table**, you can specify whether the values of each raw replicate, or the mean values are displayed in the table.

If you tick **Include Matrix**, you can specify how many columns the matrix contains.

Click the **Font...** button to change the font used in the report.

Edit Assay Protocol Export

This window allows you to specify options for automatically exporting the data to a report and/or text file.

Report Export

If you tick the **Create and export a Report file option** the specified report file is automatically created after the readings have been made or text file imported. Further options are displayed allowing you to specify the format of the report. The following formats are available:

Type	Description
.htm;.html	A Web Page (complete with links to image files)
*.mht	A Web Archive file (a single file)
*.xls	Microsoft Excel (an Excel Workbook with Worksheets)
*.doc	Microsoft Word (a standard Word file)

(Refer to Components Required For Export Report Options, *page 134, if some options are not available on your system.)*

Text Export

If you tick the **Export Results to Text file** option then each time the results are calculated a text file is created containing the exported raw and calculated results as described in the **Options...** button, page 88.

Edit Assay Protocol Launch Application

This window allows you to specify whether an additional application is launched after readings have been taken.

An entry box allows you to enter the full path of the application you wish to launch. Click **Browse...** to navigate to the desired location.

There is an entry box for you to specify any command line arguments. A drop-down list allows you to select a macro. Click **Add Macro** to add it to the entry box.

The macro **<ResultsFile>** expands to the full filename of the results file.

Transforms

Auto Flag By Well Transform

Description

This transform is used to automatically flag each well which satisfies a specified expression.

Options

An expression is specified in terms of **x**.

The **x** value specified in the expression refers to the well. The following table lists some example uses:

Example Expression:	Action:
x=0	This is the default expression 0 which means that any well who's value is equal to 0 will be flagged.
x<Control1	Any well less than the mean of the replicates of **Control1** will be flagged.
pcv ([g]) > 25	All wells of any group which has a **%CV** > 25 will be flagged.
(pcv ([g]) > 25) and (x = furthest ([g]))	Flags the well furthest from the mean if the well belongs to a group who's **%CV** is > 25.

See **Expressions**, page 102, for more details on expressions.

Details

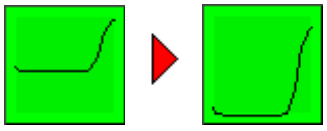
The **x** variable is evaluated as the value of the well on the input matrix. If the expression is evaluated to a non zero value then the well is auto-flagged.

Note, the wells are not actually flagged until all wells have been evaluated. This means that if the expression makes reference to a well which will be flagged by this transform when the expression is evaluated the well will be treated the same for wells. For example, if the expression was "**x < Control1**" and **Control1** was in triplicate then all wells < the mean of those **Control1** wells would be flagged. However, it is likely that at least 1 of **Control1**'s replicates will be < the mean of **Control1**, but since wells are not flagged until after all wells have been evaluated, the reference to **Control1** is consistent for all wells being evaluated.

Baseline Correction Transform

Description

This transform removes the inherent variable background, or baseline, from each kinetic well.



Options

There are 3 different Baseline Correction methods:

- Correct points by average **Y** between and including points **X1** and **X2**

- Correct points by average **Y** of **N** lowest points
- Proportional % between average of **N1** lowest and **N2** highest points

Details

For the first method, **X1** and **X2** must be >=1 and **X2** must be >= **X1**. If there are no included points between **X1** and **X2** then no correction will be made and the output well will be flagged.

For the second method, if the number of included points < **N** then the output well will be flagged. If the number of included points = **N** then the result is that all points are corrected by the average of all points.

For the third method, if there are equal low points or equal high points then these are used. For example, if specified to use the 2 lowest points and there are 2 points of 1.0 then the correction value is the mean of these, i.e. 1.0. **N1** and **N2** must be >= 1.

If the average of the lowest values is equal to the average of the highest values then this cannot be calculated and the output well will be flagged. If there are less than **N1** or **N2** valid readings then the output well will be flagged.

For the Proportional method the results are clipped - this means that all results will fall within the range 0 and 100, so values which are calculated to < 0 or > 100 will be set to 0 and 100 respectively.

Blank Correction Transform

Description

This transform performs Blank Correction on endpoint data by removing a background count. Wells marked as Blank wells are measured to determine the background count to use for correction.

Options

There are different types of Blank Correction which can be performed to accommodate any microplate layout:

- Single Blank Correction - where there is one Blank group on the plate
- One for One - each unknown is associated with its own Blank group
- Random Mapping - any association between Blanks and Unknowns can be specified. This allows for any type of Blank Correction configuration, such as microplate layouts with: Blanks in each corner of the plate, Blanks in each half of the plate, random Blanks, etc.

Details

The Blank Correction transform works by associating wells with a Blank group to correct. Each well can be associated with one Blank group. When the transform is evaluated each well has the mean of its associated Blank group subtracted from it.

When the Blank Correction transform is created default associations are created depending on the microplate layout:

- If there is one Blank group on the plate then all wells are associated with that Blank group.
- If there is an equal number of Blank groups and Unknown groups then each Unknown group is associated with the Blank group of the same number, e.g. **Unknown2** and **Blank1**, **Unknown2** and **Blank2**, etc.
- If the microplate layout does not fit into these two categories then no default associations are made; associations can be specified with the user interface as required.

Blank Correction is always performed plate by plate, that is a Blank group can only be associated with wells on the same plate it was read.

Competitive Binding Transform

Description

This transform calculates the Inhibition Concentrations (typically IC50) for samples in serial dilution; this is the concentration of the substance resulting in displacement of n% of the antibody. The transform also calculates the % bound of each sample.

Microplate Layout Requirements

This transform requires the samples to analyse to be laid out in a particular manner.

The calculations are performed on a selected group type (e.g. Unknown or Standard). Each group of the selected type will contain the sample at specified dilutions. In order to calculate IC there must be at least 4 dilutions used and each group must be diluted in the same way.

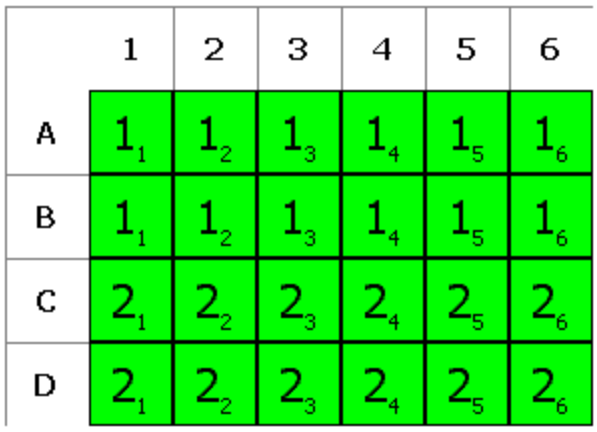
First, create or select an existing microplate layout which specifies which wells belong to which group. For example, here we have 2 different samples we want to calculate the IC(50) of; the top half of the plate is for the first sample and the bottom half for the second sample:

	1	2	3	4	5	6
A	1	1	1	1	1	1
B	1	1	1	1	1	1
C	2	2	2	2	2	2
D	2	2	2	2	2	2

Next, within the transform specify the dilutions/concentrations of each well. The large number displayed in each well of the microplate is the group number of the well and the subscript number identifies the concentration group. For example, wells **A1** and **C1** contain different samples but they are diluted in the same way:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	1_7	1_8	1_9	1_{10}	1_{11}	1_{12}
C	2_1	2_2	2_3	2_4	2_5	2_6
D	2_7	2_8	2_9	2_{10}	2_{11}	2_{12}

You can also specify if and how the concentration/dilutions are replicated. For example, here each microplate column is diluted in the same way:



Options

- **Conc.**

Select the groups to perform the Competitive Binding calculation on using the Competitive Group Type control. Specify if and how the concentrations are replicated. Enter the Concentrations for each dilution.

- **IC%**

Enter the Inhibition Concentrations % to calculate. The transform calculates the concentration of the substance resulting in the displacement of n% of the antibody.

- **Axes**

Specify the X and Y axes type (logarithmic or linear)

- **Report**

The following items can be optionally included in the report:

Concentrations Table - a list of the specified concentrations

Competitive Bindings Table - the calculated **MSE**, **R^2** and **IC%** of each group

Overlaid Competitive Graph – a single chart containing overlaid plots of each group

Competitive Graphs - a graph of each group's fit

Details

The Four Parameter Fit method is used to fit a curve to each group (of the selected Competitive Group Type). The data points of the plot are the specified concentrations (X) and the measurements of the wells (Y). (Flagged wells are not included.)

The calculated coefficients of the fit are as follows:

a and **d** = upper and lower asymptotes

b = slope (indicator of the sensitivity of the assay)

c = midpoint of the linear portion = **IC50** = **B50** = concentration of the substance resulting in the displacement of half of the antibody.

From these coefficients **y** is first calculated and from this **IC%(n)**, as follows:

y = d+ ((100-n) * ((a-d)/100))

IC%(n) = ((((a-d)/(y-d))-1)^(1/b))*c

The transform also calculates % bound for each well of each group using the absolute result of the equation:

(measurement/(a-d)) * 100

For each group, the Mean Squared Error **(MSE)** and **R²** of the fit is also calculated.

The transform produces the following group Transform Output Variables:

a: Calculated **a** coefficient (asymptote)

b: Calculated **b** coefficient (indicator of the sensitivity of the assay)

c: Calculated **c** coefficient (IC50)

d: Calculated **d** coefficient (asymptote)

MSE: Calculated **MSE**

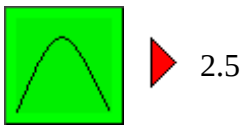
R²: Calculated **R²**

IC: Calculated **IC(n**)

Crossing Point Transform

Description

This kinetic reduction transform finds the X value where a specified crossing point value (Y) crosses the graph:



Options

- Crossing Point Value (Y) - this can be a number or an expression
- Number of fit points
- Slope direction
- Whether to flag the well if it is outside the X range
- What to do if there is more than one crossing point

Details

Finds the X value of the linearly-extrapolated crossing point of the specified Y value using the first specified number of fit points before and after a detected "bound" pair of points. For example, if cycles 3 and 4 bound the specified Y value, and the linear fit uses 3 fit points, then cycles 3,4 and 5 will be used.

If multiple bound conditions are detected then it uses the option of finding the first or last occurrence or flags the result.

Thus, the algorithm considers each adjacent point pair in the set and determines whether the pair bounds the specified Y value (by considering the specified slope direction). A linear fit is then performed on the successful bounding pair using the left and right points of the bounding pair plus the specified number of fit points -2 after the bound pair to determine X.

The well will be flagged if:

- Multiple crossing points are found and the option to flag is set on this condition.
- There are insufficient points to do linear fit over the specified number of fit points (this could occur if the crossing point is near the end of the data set and there are not the required number of fit points after the bound point)
- The linear fit does not match the specified slope direction
- A calculated slope is 0
- No bounding point pair is found
- The data set does not have more than 2 data points
- Any of the X values are descending or duplicated

A range of points to consider can be specified so that points that fall outside of the range are not considered.

%CV Transform

Description

This simple transform calculates the %CV of each group on the selected input matrix.

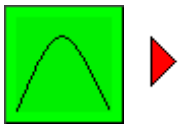
Details

Each group's %CV is calculated using all of the replicates of the group which are not flagged. The %CV is the Standard Deviation divided by the mean * 100. (If the mean or the Standard Deviation is 0 then the result is forced to 0.)

Delta X for Delta Y Transform

Description

This simple transform finds the change in X required for the specified change in Y to occur:



Options

The required change in Y is specified as Delta Y - this may be positive or negative.

Details

The X and Y value of the first included data point is determined. The data points are stepped through in the order they were read until a pair of points is found which bound the point where the

difference in Y between that point and the first point equals or exceeds the specified Delta Y. A simple linear interpolation is used between the two adjacent data points.

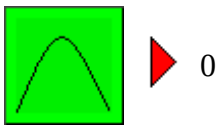
The result of the transform is the difference between the X value of this interpolated point and the first point.

A range of points to consider can be specified so that points that fall outside of the range are not stepped through.

Delta Y Transform

Description

This simple transform finds the change in Y from the first cycle and the last cycle e.g.:



Options

Depending on the measurements taken the X and Y axis type may be changed.

An optional subset of points to consider can be specified using an X range.

Details

The first cycle is the first cycle that is not flagged within the specified range of points to consider. Similarly the last cycle is the last cycle that is not flagged within the specified range of points to consider. If there are less than 2 included points the output well will be flagged.

A range of points to consider can be specified so that points that fall outside of the range are not stepped through.

Dilution Factors Transform

Description

This transform applies a specified dilution factor to selected sample groups, typically Unknowns.

Options

A factor can be specified for each group on any plate read.

Factors are typically specified for Unknown sample types only, however factors can optionally be specified for other sample types.

Details

Each well belonging to the list of groups with a factor specified is multiplied by the specified factor.

Groups not included in the list of factors assume a default factor of 1.

Dual Matrix Expression Transform

Description

This transform evaluates an expression in terms of **x** and **y** for two endpoint input matrices. The **x** variable refers to each well on the first specified input matrix and the **y** variable refers to each corresponding well on the second specified input matrix.

Options

The **x** and **y** variables refer to the first and second input matrices as specified on the Input Matrix tab.

The following table lists some example expressions:

Expression Example:	Effect:
x-y	Calculates the difference between each well on the first input matrix and its corresponding well on the second input matrix.
x/y	Calculates the ratio between each well on the first input matrix and its corresponding well on the second input matrix.
log2(x/y)	Calculates the logarithm of the ratio to base 2.

Refer to the **Expressions** section, page 102, for complete details of the expressions.

Details

The transform evaluates the specified expression for all included wells included in both input matrices, resulting in the output endpoint matrix.

Expressions entered will be general, and will be localised for each plate. For example, if you refer to the group **Blank1** in the expression then this refers to the first Blank group on each plate.

Expressions

Overview

Expressions can be used in many transforms to specify custom mathematical operations: this provides great flexibility in assay design.

Syntax

An expression is a sequence of mathematical operations and references to results from wells, groups, variables and other transform results.

The syntax of expressions is consistent throughout Manta, however the use of an expression depends on the context it is being used in.

Example Expressions:

Expression	Situation	Meaning
x*100	In a Matrix Expression transform	Multiplies each endpoint value by 100.
x-y	In a Dual Matrix Expression transform	Finds the difference between each well on the first input matrix and its corresponding well on the second input matrix.
(pcv ([g]) > 25) and (x = furthest ([g]))	In an Auto Flag By Well transform	Flags the well furthest from the mean if the well belongs to a group whose %CV is > 25.

Control1 * 0.9	In a Single Cut-Off transform	Here a cut-off point is specified as 90% of **Control1**, each well will be labelled in relation to this cut-off point.

An expression comprises of operations (such as **+**) and operands (such as numeric values).

Operands:

Operand	**Examples**
Number	1,1.23, -0.12, 1000
Well Reference	A1, A2, H12
Group Reference	**Standard1**, **Control1**, g, [g], [Control1]
Variable	w, x, y, z
Transform Output Variable	#1:X, #1.MSE, #1,**Unknown1**,IC

See page 114 for more information about Operands.

Operators:

Operator	**Examples**
Basic Mathematical	+,-,*,/
Comparison	and, or, not, <, >, =
List Operation	mean, sd, max, min

See page 118 for more information about Operators.

Using a combination of these operands and operators you can write versatile expressions that perform calculations with your results.

Expression Transform

Description

This transform allows you to enter an expression to evaluate and to name the result. The result will appear in a section in the report and can be referred to in other transforms.

Options

The following table lists some example expressions:

Expression Example:	**Effect:**
sd([Standard1])	Calculates the standard deviation of the wells in the group Standard1.
pcv([Standard1])	Calculates the %CV of the wells in the group Standard1.
max([Standard])	Finds the maximum reading in all Standard wells
1-(3*sd([Standard1])+3*sd([Standard6])/(Standard6-	Calculates z' as described by *J-H Zhang et al. (1999) J. Biomol.*

Standard1))	*Screen. 4:67-73*

You can enter a Result Name for the expression (this must be made up of alphabetical characters only).

Details

The result of the expression is made available as a transform matrix output variable using the specified name. This transform output variable can be referenced by other transforms. For example, you could use this transform to calculate the sensitivity of your assay and then use a Validation Expression transform to check the sensitivity is within a defined range.

Expressions entered will be general, and will be localised for each plate. For example, if you refer to the group Blank1 in the expression then this refers to the first blank group on each plate.

Factor Transform

Description

This simple transform multiplies each endpoint value by a specified factor.

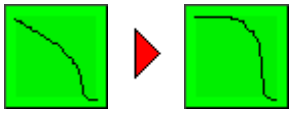
Options

The factor can be specified as an absolute value or as an expression.

Fluorescent Background Correction Transform

Description

This transform removes the effect of fluorescent background on temperature, typically required for melting curve analysis (using Temperature/Fluorescence plots), e.g.



Options

- Cursors **A1**, **A2**
- Cursors **B1**, **B2**
- Method

Details

Performs baseline correction and normalisation using two selected data regions. The respective data regions are defined as the data points within and including the positions of the two cursors in each pair. Baseline correction uses a mean Y value of data points within cursors **B1** and **B2**. Normalisation scaling uses a linear regression of data within cursors **A1** and **A2**. If cursor **A1** is below the X data range a value is assumed at the minimum X value. If cursor **B2** is above the X data range a value is assumed at the maximum X value.

Various correction modes (algorithms) may be applied by setting the Method as follows:

All modes apply a baseline subtraction -- the distinction is in the scaling method:

10: Applies a simple linearly-determined addition, it is not applied after cursor **B1**

11: As **10** but addition is used so that max Y approximately equals the first Y

20: The same addition as **10** though it is not applied after data falls to baseline + 3 SDs

21: As **11** but addition chosen so that max Y approximately equals the first raw Y

30: Applies a proportional correction, distributing Ys between 0 and cursor **A1**'s Y value

The well will be flagged if:

- Linear regression fails
- If any Xs are descending or duplicated
- If there are less than 2 points
- If cursor **A1** >= cursor **A2** or cursor **B1** > cursor **B2** (OK if equal)
- If cursors **A** contain < 2 points or if cursors **B** contain < 1 point

A warning is given

- If **Mode=3x** and cursor **A**'s defined top line crosses cursor **B**'s baseline within the data range - in which case the resulting Y contains the input Y minus the base Y value from cursors **B**

A range of points to consider can be specified so that points that fall outside of the range are not considered.

Group Vmax Transform

Description

This transform calculates the **Vmax** for each group of a specified group type.

Microplate Layout Requirements

This transform requires the samples to analyse to be laid out in a particular manner.

The calculations are performed on a selected group type (e.g. Unknown or Standard). Each group of the selected type will contain the sample at specified dilutions. In order to calculate **Vmax** there must be at least 2 concentrations used and each group must be diluted in the same way.

First, create or select an existing microplate layout which specifies which wells belong to which group. For example, here we have 2 different samples we want to calculate **Vmax** of; the top half of the plate is for the first sample and the bottom half for the second sample:

	1	2	3	4	5	6
A	1	1	1	1	1	1
B	1	1	1	1	1	1
C	2	2	2	2	2	2
D	2	2	2	2	2	2

Next, within the transform specify the dilutions/concentrations of each well. The large number displayed in each well of the microplate is the group number of the well and the subscript number identifies the concentration group. For example, wells **A1** and **C1** contain different samples but they are diluted in the same way:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	1_7	1_8	1_9	1_{10}	1_{11}	1_{12}
C	2_1	2_2	2_3	2_4	2_5	2_6
D	2_7	2_8	2_9	2_{10}	2_{11}	2_{12}

You can also specify if and how the concentration/dilutions are replicated. For example, here each microplate column is diluted in the same way:

	1	2	3	4	5	6
A	1_1	1_2	1_3	1_4	1_5	1_6
B	1_1	1_2	1_3	1_4	1_5	1_6
C	2_1	2_2	2_3	2_4	2_5	2_6
D	2_1	2_2	2_3	2_4	2_5	2_6

Options

- **Conc.**

Select the groups to perform the **Vmax** calculation on using the Group Type control. Specify if and how the concentrations are replicated. Enter each Concentration value.

- **Axes**

Specify the X and Y axes type (logarithmic or linear)

- **Report**

The following items can be optionally included in the report:

Concentrations Table - a list of the specified concentrations

Fit Results Table - the calculated **MSE**, **R²** and **Vmax** of each group

Overlaid Graph – a single chart containing overlaid plots of each group

Vmax Graphs - a graph of each individual group's fit

Details

The Michaelis-Menten method is used to fit a curve to each group (of the selected Group Type). The data points of the plot are the specified concentrations (X) and the measurements of the wells (Y). (Flagged wells are not included.)

The calculated coefficients of the fit are as follows:

Vmax = the determined maximum value of **y**

Km = The determined Michaelis-Menten coefficient.

For each group, the Mean Squared Error (**MSE**) and **R²** of the fit is also calculated.

The transform produces the following group Transform Output Variables:

Vmax: Calculated maximum value of **y**

Km: Calculated Michaelis-Menten coefficient.

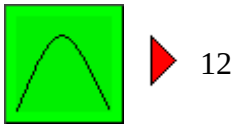
MSE: Calculated **MSE**

R²: Calculated **R²**

Integral Transform

Description

This simple transform finds the area underneath the chart:



Options

This can be calculated by simply summing the Y values or using the trapezium method.

Details

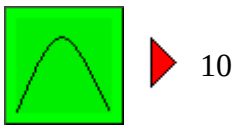
For the summation method the Y values of the included data points are simply totaled. There must be at least 1 data point.

For the trapezium method the area of the trapezium between **Y=0** and each adjacent point pair is determined (this area is always positive or 0). The point pairs are ordered by the order the order points were read. There must be at least 2 data points. If the data set is not in a continuous sequence about X then areas will be counted more than once. Also the area will be treated as contiguous where flagged points appear in between non flagged points. For example, if there is a flagged data point in between two non flagged data points then the area between the two non flagged data points will be calculated and included.

Kinetic Average Transform

Description

This kinetic reduction transform finds the average Y value of kinetic points in each well.



Options

The average of all points in each well can be found or you can specify a sub-range of points to consider.

Details

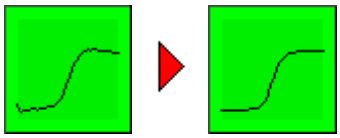
The sub-range is specified by defining minimum and maximum x values, all points within the range are consider. The range can be specified with numeric values, an expression or by dragging the vertical drag bars in the analysis control after the first evaluation. This is useful for calculating average baseline by eye.

If there are no points within the range in a well then the resulting well is flagged in the output matrix.

Kinetic Fit Transform

Description

Performs a curve fit on each kinetic well:



Options

Fit method

The available fit methods are determined by the modules installed on your system. See Fit Methods for further details.

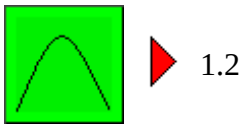
Details

Each resulting kinetic chart is built up by calculating Y for each X point using the fitted curve. Transform output well variables are generated for each well. In this way you can access any calculated coefficients for each well.

Kinetic Point Transform

Description

This kinetic reduction transform finds the Y value at a specified X value on a kinetic chart e.g.:



Options

An expression is specified to define the X value to find Y.

Details

An expression for determining an X cursor value is specified for all wells. This expression is evaluated on each well (this means there could be a different X cursor position in each well if the specified expression is related to the well.)

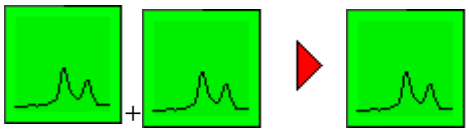
For each well, the transform inspects the data points in cycle order to find the first cycle pair which bound the specified X value, i.e. the two adjacent cycle points with X between them. The transform finds the Y value at the specified X value on the curve using ratios to determine the exact X, assuming a linear relationship between the two bounding points.

If a data point's X value exactly equals the X cursor, then the Y value of that point is used and the ratio calculation is not used.

Kinetic Ratio Transform

Description

This transform finds the ratio of two kinetic matrices:



Options

- The first input matrix is the numerator
- The second input matrix is the divisor

Details

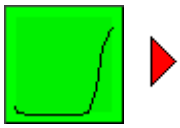
For each kinetic well, the transform determines which kinetic cycles are within the range and not flagged for both input matrices. For each kinetic point that has a corresponding flagged point within the range that is not flagged (identified by the same cycle number), the transform simply divides the point from the first matrix with its corresponding point of the second matrix. Thus, ratio points are only calculated if the cycle is not flagged and within the range in both input matrices.

If there are no points in the resulting output matrix, the well is flagged.

Linear Regression Transform

Description

This kinetic reduction transform finds the linear regression of each kinetic chart, e.g.:



Options

The result of the reduction can be the determined slope of the line through the kinetic points. Alternatively the transform can calculate the Y value on the slope at X' where X' is relative to the first point considered in the linear regression.

Details

The slope and intercept (**m** and **c**) are determined which minimise the sum of the squared errors – **sigma (m * x + c – y) ^ 2**.

There must be at least 2 data points.

A range of points to consider can be specified so that points that fall outside of the range are not considered. Flagged points are ignored.

Output Variables

For each calculated well the following well output variables are calculated:

Well Variable	Description
m	The determined slope
c	The determined intercept

List Operations

The following table details the various list operators that are available for use in expressions.

List Operator:	Description:	Parameters:	Example of use:
abs	Returns the absolute value of the list element.	1	**abs (-1.23)**
exp	Returns e raised to the power of a given number. Where e is the base of the natural logarithm (ie 2.71828182845904). Equivalent to Microsoft Excel's EXP command.	1	**exp(2)**
furthest	Finds the first value in the list of items that is furthest from the mean.	1 or more items	**furthest([Unknown1])**
log2	Calculates the logarithm of a number to base 2. The specified number must be positive. Equivalent to Excel's LOG	1	**log2(8)**

	command (with 2 as the second parameter).		
log10	Calculates the logarithm of a number to base 10. The specified number must be positive. Equivalent to Excel's LOG command.	1	**log10(2)**
logn	Calculates the natural logarithm of a number to the specified base. The specified number must be positive. Equivalent to Microsoft Excel's LN command.	1	**logn(2)**
matrixtotal	Calculates the total of all of the readings of the default matrix and plate. If all wells are flagged, returns 0.	0	**matrixtotal ()**
max	Returns the value of the item in the list with the greatest value.	1 or more items	**max (1,2,3)**
mean	Returns the mean of the items in the list	1 or more items	**mean ([Unknown1], [Unknown2])**
median	Returns the median of the items in the list. This is the number in the middle of the list when it is sorted into order. If the number of items in the list is odd this is the middle number, if it is even then it is the average of the two numbers in the middle.	1 or more items	**1 or more items**
min	Returns the item in the list with the least value.	1 or more items	**min (1,2,3)**
not	Performs the negation operation on the list element.	1	**not(1)** **not(x<10)**
numelements	Returns the number of items in the list. This can be used to find the number of unflagged wells references in a list if the list is made up entirely of group and/or well references.	0 or more items	**numelements ([Standard1, Standard2])**
pcv	Calculates the %CV of the elements in the list. (The %CV is the Standard Deviation divided by the mean * 100.)	1 or more items	**pcv([Unknown1])**
pow	Returns the result of a number raised to a power. Equivalent to Microsoft Excel's POWER command.	2 (number, power)	**pow (2,3)**
sd	Calculates the Standard Deviation of the elements in the list. The Standard Deviation is the square root of the Variance.	1 or more items	**sd([Unknown1])**
sum	Returns the total of all items in the list.	1 or more items	**sum (1,2,3)**
var	Calculates the Variance of the elements in the list. The Variance is the sum of the squares of the difference of each item and the mean divided by n-1 (where n is the number items in the list).	1 or more items	**var([Unknown1])**

Matrix Export Transform

Description

This transform exports raw and calculated data to a specified text file.

Options

- Filename to export to
- Append

For Kinetic Matrices, for each kinetic well:

- Y points can be output or both X and Y points , e.g. Readings only, or Readings with Time
- The output X and Y points can be specified, depending on the available raw data.

Details

The transform outputs all raw matrices and calculated matrices (which are evaluated before the Matrix Export transform) to the specified text file (for each plate read).

Max Peaks Transform

Description

This transform finds **N** peaks and optionally the area under each peak in each well. There is no output matrix produced, results are listed in an additional table added to the report.

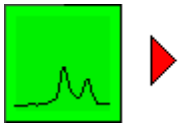


Table of peaks and optionally table of areas under each peak

Options

- Number of peaks to find (**N**)
- Whether to calculate the area under each peak
- Peak detection method - manual or automatic
- The result to show in the table (the X or Y value of each peak)

Details

The number of peaks to find can be set to values 1-4.

Automatic Peak Detection

This simple approach identifies possible peaks by identifying changes in gradient direction. This method is only suitable for smoothed data.

In the peak and peak area tables each column lists the peaks found, there is no association between the peaks on each row - each table column depends on the peaks found for that well.

Manual Peak Detection

A cursor is specified for each peak to find. Each cursor can be specified by positioning its drag bar in the analysis control or by specifying an expression (not well associated). A **+/-** range is also specified as an expression (also not well associated). If the evaluation of any of those expressions fails then the transformation will fail.

The first maxima within the range of each cursor is found.

In the peak and peak area tables each column corresponds to a specific cursor. If there is not a peak within the range for a specific cursor on a particular well this will be shown in the table.

Area under Peak Calculation

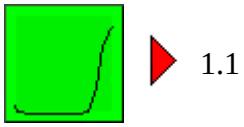
Each peak's valleys are determined by finding when the gradient between adjacent point's changes to above 0 on the right and left of the peak. The area between these valleys is determined using trapezium summation.

A range of points to consider can be specified so that points that fall outside of the range are not stepped through.

Maximum Slope Transform

Description

This kinetic reduction transform finds the maximum slope in each kinetic chart, this can be used to find the maximum rate of the reaction e.g.:



Options

The maximum slope to find can be specified as the:

- Most positive
- Most negative
- Auto - the general direction of each chart is determined and the appropriate maximum is found automatically

The maximum slope is determined using the specified number of linear regressions points.

The result of the transform can be the:

- Slope of maximum slope
- X at centre position of maximum slope (absolute or relative to first data point)
- Y at centre position of maximum slope (absolute or relative to first data point)
- The extrapolated Y value on the maximum slope at a specified X

Details

With Auto mode the general direction of each chart is determined by a linear regression calculation on all points in the chart. If the slope of this line is positive then the maximum positive slope is found, if negative then the maximum negative slope is found. This means that wells will be treated based on their general slope.

The maximum slope is determined performing linear regression on adjacent sets of **n** data points sliding by cycle through the sets (where **n** is the specified number of linear regression points). e.g.: If you have a kinetic chart with 5 data points and **n** = 3 the 1st slope is calculated on points 1, 2, 3, the 2nd slope on points 2, 3, 4 and the 3rd slope on points 3, 4, 5. The software finds which set of points has the maximum slope.

The X at the centre position is exactly half way between the first and last fit point in the set with the maximum slope. The Y at the centre position is caclulated using the X at the centre position and the determined maximum slope and intercept.

The extrapolated Y value on the maximum slope at a specified X is determined by calculation of the Y value on the equation of the maximum slope where X occurs.

A range of points to consider can be specified so that points that fall outside of the range are not considered. Flagged points are ignored.

Output Variables

For each calculated well the following well output variables are calculated:

Well Variable	Description
m	The slope of the maximum slope
c	The intercept of the maximum slope
X	X of centre point of the maximum slope
Y	Y of centre point of the maximum slope

Operands

This section describes the various operands which can be used with expressions.

Number

A number operand is simply a number, e.g. -1.23

Well Reference

A well reference operand is a reference to a specific well, such as A1. The available dimensions depend on the microplate layout read. For a microplate with more than 26 rows, 2 letters are used to identify rows 27+. A well on a particular matrix and plate can be specified by following with a period.

Examples:

Well Reference:	Evaluated To:
A1	The first well on the plate.
B1.2	Well B1 on the second matrix.
A1.2.3	Well B1 on the second matrix of the third plate.
AA1	The first column of row number 27
AB1	The first column of row number 28

Well Associations

In situations where an expression can be evaluated for any well or groups on a microplate, such as a user-defined expression, a "well associated" expression is used. This simply means that when the

expression is evaluated the evaluation considers it to make reference to a particular well. In this way more general expressions can be created which can be applied across microplates.

With a well associated expression the variable **w** can be used to refer to the associated well on a matrix and plate. If no matrix and plate are specified then the default matrix and plate are used. The default matrix is the input matrix and the default plate is the plate being evaluated.

Examples:

Example:	Refers to:
w	The associated well on the default matrix and plate
w.1	The associated well on the first matrix
w.2	The associated well on the second matrix
w.1.2	The associated well on the first matrix of the second plate

Variable

Prior to the evaluation of the expression some transforms assign values to the variables **x**, **y** and/or **z** variables. All references to these variables in expressions will be equated to the assigned value.

Examples:

In the **Matrix Expression** transform you can use variable **x** to refer to the well on the input matrix and enter an expression such as **x*100** to multiply all wells by 100.

In the **Dual Matrix Expression** transform the variables **x** and **y** are used to refer to the associated well on two different matrices so you could write an expression such as **x/y** to calculate the ratio of two sets of measurements.

Group Reference

A group operand is a reference to a specific group. A reference to a group is evaluated as the mean of the unflagged replicates of the group. So when the reference **Unknown1** is evaluated, the wells which contain replicates of **Unknown1** are identified and the mean of these wells which are not flagged is calculated.

For well-associated expressions **g** can be used to reference to group which the well belongs to.

If a group reference is enclosed in square brackets, e.g. **[Unknown1]**, the reference refers to the list of measurements of each unflagged replicate - this allows you to perform operations on the list of replicates other than mean. So **[Unknown1]** is actually expanded to the list of results for the unflagged members of **Unknown1**. This list can the be used with the List Operations.

When a well is associated – all references to **g** and **n** refer to the group of the associated well. The expressions can access the group type and group number from these variables. When a reference to a group is made it is evaluated to the mean of the unflagged replicates of the group. If you enclose a group reference in square brackets e.g. **[g]** the reference refers to the list of measurements of each unflagged replicate - this allows you to perform operations on the list of replicates other than mean. Also, when an expression is well associated you can omit the group number to refer to the group of the well which the expression is associated, for example **Unknown** would refer to the group **Unknown1** if its associated well's group number is 1.

If an expression is not associated with a particular well (such as a cut-off point) then the variables **g** and **n** cannot be used. In this situation if you omit the group number all wells in that group type are references, for example **Unknown** refers to all of the wells which are of **Unknown** type.

Examples:

Example:	Refers to:
g	The group that the associated well belongs to. This is evaluated as the mean of the unflagged replicates of the group.

[g]	The list of results made for each unflagged replicate. So for example if the associated well is **A1** which is a replicate of the group **Unknown1**, this reference refers to all wells in group **Unknown1**, including this well and other wells. A reference in square brackets cannot be used on its own because it is the list of results, a square bracket reference will be used as a parameter to a List Operation.
Control(n)	The Control group with the same group number as the group in the associated well. e.g. if the associated well contained **Unknown1** then this expression would refer to **Control1**
g.1	The group that the associated well belongs to on the first matrix.
g.1.2	The group that the associated well belongs to on the first matrix of the second plate.
Unknown1	The mean of the unflagged replicates of **Unknown1**.
Unknown1.2.3	**Unknown1** on the second matrix of the third plate.
sd([Unknown1])	The Standard Deviation of the included members of **Unknown1**.
sd([Unknown1,Unknown2])	The Standard Deviation of the included members of **Unknown1** and **Unknown2**.

Transform Output Variable

Some transforms produce output variables which contain further results of an evaluated transform. (The output variables produced by each transform are specified within the documentation of the transform.) These additional results are made available as Transform Output variables. There are 3 types or variables which depend on whether the calculations relate to the transform, specific groups or specific wells.

By Transform

These are variables that are associated with a transform as a whole.

For example, the **Standard Curve Fit** transform performs a curve fit, the calculated goodness of fit measures and any calculated coefficients of the fit are available as transform output variables. You may want to refer to these calculated variables for further analysis or validation.

To refer to a matrix variable use the syntax:

#<TransformNum>.<Variable>

Where:

<TransformNum> is the transform number (1 is the first)

<Variable> is the name of the variable

Examples:

Expression:	**Refers to:**
#1.MSE	The calculated MSE of the first transform (assuming it is a **Standard Curve Fit**).
(x-#2.c)/(#2.m)	The computed coefficients of the linear regression to calculate **y** from **x** (assuming the second transform is a **Standard Curve Fit** using

	Linear Regression).

By Group

These are variables calculated by the transform for specific groups.

For example, the **Competitive Binding** transform produces output variables for the calculated coefficients, **MSE** and **IC** value calculated for each group on the plate in dilutions. You may want to refer to these calculated variables for further analysis or validation of each group.

Use the syntax:

#<TransformNum>,<Group>,<Variable>

Where:

<TransformNum> is the transform number (1 is the first)

<Group> is the group. Note, the group references are "plate local" - this means that the numbering is relative to the plate rather than all plates in the assay. For example, **Unknown1**, refers to the first Unknown group on each plate.

<Variable> is the name of the variable

Examples:

Expression:	**Refers to:**
#1,Unknown1,IC	The IC value for the first Unknown group of the first transform (assuming it is a **Competitive Binding**)
#1,g,a	The calculated "a" coefficient for each group (assuming it is a transform which calculates coefficient a, e.g. **Competitive Binding**)

By well

These are variables calculated by the transform for specific wells.

For example, the **Kinetic Fit** transform produces output variables for the calculated goodness of fit measures and for any calculated coefficients for the fit made in each well. You may want to refer to these calculated variables for further analysis or validation of each well.

Use the syntax:

#<TransformNum>:<Variable>

Where:

<TransformNum> is the transform number (1 is the first)

<Variable> is the name of the variable

Examples:

Expression:	**Refers to:**
#2:c	The calculated **c** coefficient of the 2nd transform. This expression would be used in a

	Well Associated expression so the well being referred to is identified by the expression context.
#2:A1.c	The calculated **c** coefficient for well **A1**.

Operators

This section describes the various operators which can be used with expressions.

Basic Mathematical

The supported basic mathematical operators are **+**, **-**, ***** and **/**.

Comparison

A comparison can be mode using two operators. The comparison operators are:

>,**<**, **=**, **<=**, **>=**, **and**, **or**

The result of a comparison operator is either true or false, true is represented as non-zero, false as zero.

Examples

Comparison Operator:	**Description:**
x = y	True if x is equal to y.
x > y	True if x is greater than y.
x <= y	True if x is less than or equal to y.
(x > y) and (x > 100)	True if x is greater than y and x is greater than 100.

List Operations

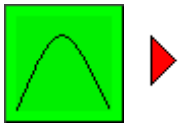
The list operators perform an operation on a list of zero or more comma separated operands. The evaluation of a list operator results in a single numeric value. Any flagged wells referred in the list will not be included in the calculations.

For the full list of List Operations, see page 110.

Peak Point Transform

Description

This kinetic reduction transform finds the maximum (peak) or minimum (dip) point in each kinetic chart. The result of the reduction can be the X or Y value of this point, e.g.:



Options

- The data point with minimum or maximum Y value can be found.
- The result can be the X or Y value of the determined point.

Details

The maximum or minimum point is found by stepping through the included points. If there are no included points the output well will be flagged.

A range of points to consider can be specified so that points that fall outside of the range are not stepped through.

If there are more than one points with the same peak/dip then the first (leftmost) peak/dip is used.

Output Variables

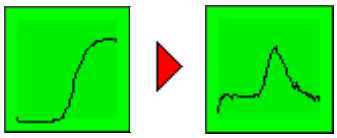
For each calculated well the following well transform output well variables are calculated:

Well Variable	Description
X	X of peak/dip
Y	Y of peak/dip (the peak/dip value)

Rate of Change Transform

Description

Calculates the rate of change (**dY/dX**) or (**-dY/dX**) for each plot, e.g:



Options

- Number of fit points (**N**)
- Invert Sign

Details

Performs linear regressions using groups of the specified number of fit points throughout the data set. The resulting data set is made up of the slopes of each group's regression and the average X values of each group. If Invert Sign is selected then each gradient is multiplied by -1.

The resulting data set is smaller by the **N-1** points.

The resulting well will be flagged if:

- It there are less than **N** points
- If any X points are descending or duplicated

Note, if the X axis is cycle number then the resulting data set will start at cycle 1 (to ensure cycle numbers remain as whole numbers starting at 1) this means the results are shifted left by the **(N-1)/2**. For other X axis types this shifting does not occur as whole numbers are not requried.

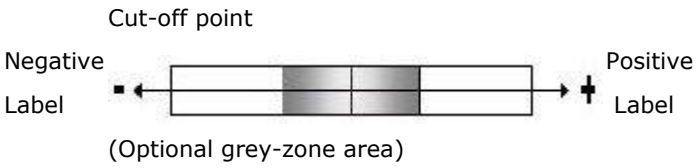
Single Cut-Off Transform

Description

This transform is for labelling samples based on a single cut-off point.

Options

The samples will be labeled depending on whether they are greater or less than a specified cut-off point. You can supply your own positive or negative label.



The cut-off point is essentially a numeric value which can be made from readings from your plate (e.g. a Control group), an absolute numeric value, or a mathematical expression.

For example, if you wanted to label your Unknowns based on how they compare to the ratio of a Pos Control and a Neg Control, you could enter a cut-off point expression: "**Pos Control/Neg Control**" which sets the cut-off point to this ratio. Refer to the **Expressions** section, page 102, for complete details of the expressions.

You can also optionally specify a grey-zone area. Any of your samples that fall within this range are also labeled as **Grey** to denote that they are close to your cut-off point. The grey zone area is specified as a percentage, absolute numeric value or mathematical expression and is relative to your cut-off point.

Details

If an expression is used for the cut-off point or grey zone then this expression must be valid on all microplate layouts - it must be a local plate expression (consider this when using multiple plates).

Standard Curve Fit Transform

Description

This transform performs a specified curve fit on Standards set values plotted against their specified concentrations. The resulting curve is then used to read off interpolated (and optionally extrapolated) concentrations for samples. You can also select for **EC(20)**, **EC(50**), **EC(80)** and **EC(n)** to be calculated from the curve and optionally plot the curve on a **B/B0** Y axis.

Options

- **Fit method**

The available fit methods are determined by the modules installed on your system.

The Y axis can be optionally scaled to **B/B0** (the method used depends on the selected fit method)

EC values can be calculated from the fit, default values are **EC(20)**, **EC(50)** and **EC(80)**

See Fit Methods for further details.

- **Standards Set**

Which Standards set to use

- **Concentrations**

The specified concentrations

- **Axes**

The axes type used to plot the data (linear or logarithmic)

- **Range**

A range to calculate concentrations can be specified, this can be used to specify whether or not and to what degree extrapolation occurs. Note, extrapolation depends on the fit method.

Details

Transform Output variables are calculated for Goodness of Fit Measures and for any calculated coefficients, see Fit Methods for further details.

The specified concentration values must be >= 0

Report Content

A graph of the curve fit is included in the report showing the Standard values with the calculated curve.

A table listing the details of the fit is provided, this includes the specified **EC(n)** results, the Goodness of Fit Measures and any calculated coefficients.

A table of the specified concentrations and readings (the X and Y data set) is also included in the report. Any flagged data points are marked with a strikethrough.

Standard Deviation Transform

Description

This simple transform calculates the Standard Deviation of each group on the selected input matrix.

Details

Each group's Standard Deviation is calculated using all of the replicates of the group which are not flagged. The Standard Deviation is the square root of the Variance.

Matrix Expression Transform

Description

This transform evaluates an expression in terms of x for each well on the specified input endpoint matrix resulting in an endpoint output matrix.

This can also be used to access the well output variables of another transform.

Options

The **x** variable refers to the wells on the input matrix specified on the Input Matrix tab. (You can also use the **g** variable to refer to the group that the well belongs to)

The following table lists some example expressions:

Expression Example:	**Effect:**
x*100	Multiplies each endpoint value by 100.
x-Blank1	Removes the value of the group **Blank1** from each well. If **Blank1** consists of replicates the mean of the unflagged replicates is used.
log10(x)	Calculates the logarithm of each well to base 10.
sd([g])	Calculates the Standard Deviation of the group that the well belongs to. (By definition, all wells in the same group will have the same result)
pcv([g])	Calculates the %CV of the group that the well belongs to.
#1:c	Sets each well to contain the value of the first calculated transform's output well variable that is named **c**. This could be the calculated IC50 of a four parameter fit through a well.
(#1:a - #1:d)/2	Sets each well to contain the value of the first calculated transform's output well variable that is named **a** divided by the 1st calculated transform's output well variable that is named **d**.

Refer to the **Expressions** section, page 102, for complete details of the expressions.

Details

The transform evaluates the specified expression for all included wells on the input endpoint matrix, resulting in the output endpoint matrix. The **x** variable is evaluated as value of the well on the input matrix.

Expressions entered will be general, and will be localised for each plate. For example, if you refer to the group **Blank1** in the expression then this refers to the first blank group on each plate.

Validation Transform

Description

This transform tests that statistical operations on groups fall within defined limits. For example, a validation condition can be created which tests that the **%CV** of the replicates of a Control is < 15.

A table is added to the report detailing whether each validation passed or failed. If any validations fail then a message box is displayed during the calculation phase warning the user and listing the failed results.

Details

Validation conditions can be performed on any endpoint matrix. Specify which matrix of data to use under the Input Matrix tab (you may want to use a Raw or Calculated matrix).

Under the Validation tab you can **Add**, **Edit** and **Remove** Validation Conditions. A Validation Condition consists of a statistical operator to perform, a group or range of groups to consider, a comparison operator and a value to compare against.

The statistical operators available are: **Standard Deviation**, **%CV**, **Variance** and **Mean**.

The validation condition can be applied on any specific group, group type or all samples. If a group type is specified (e.g. All Controls) then the validation condition is applied to every group of that type. Similarly if All Samples is selected then all groups on all plates are tested.

When the validation condition is evaluated, the statistical operator is performed on all unflagged replicates of each group. For groups with less than 2 unflagged replicates the validation is not performed when using Standard Deviation, %CV and Variance (as these operators require 2 or more values to make sense).

If using multiple-plates the group references are defined using "plate local" reference but the condition is evaluated on every plate. This means that the group numbering is relative to the plate rather than all plates in the assay. For example, if the validation condition refers to the group **Control1**, this is the first Control group on each plate: if every plate contains 1 Control group, then the "plate local" reference "**Control1**" refers to "**Control1**" on the first plate and "**Control2**" on the second plate. In the report a validation table is produced for each plate: here the expressions refer to the "assay global" group number rather than the "plate local".

The value to compare against can be a numerical value or an expression.

Validation Expression Transform

Description

This transform allows you to enter an expression which is validated. If the expression fails then a warning is displayed to alert the user. The result of the validation is included in the report, marked with pass or fail.

Options

The following table lists some example expressions:

Expression Example:	Effect:
Standard1 < Standard2	The mean of Standard1 wells must be less than the mean of Standard2 wells.
pcv([Standard1]) < 25	The %CV of Standard1 must be < 25.
#1.ZPrime > 0.5	This assumes that a z' transform has been setup as the first transform and checks that the calculate z' is > 0.5

Variance Transform

Description

This simple transform calculates the variance of each group on the selected input matrix.

Details

Each group's Variance is calculated using all of the replicates of the group which are not flagged. The Variance is the sum of the squares of the difference of each replicate and the mean divided by n-1 (where **n** is the number replicates which are not flagged).

Z' Transform

Description

This transform calculates z' as described by *J-H Zhang et al. (1999) J. Biomol. Screen. 4:67-73*. z' satisfies the requirement for assessing both assay window and precision for accurate assay performance evaluation.

According to the z' value model, computed values less than 0 indicate implausible assays; those greater than 0 indicate do-able assays, and values of 0.5 and above indicate excellent assays, which are readily transferable from assay development to an HTS screen.

Options

There are two different methods by which z' can be calculated.

If you are using at least 24 replicates you can use:

3*sd(High) + 3*sd(Low)

If you are using less than 24 replicates you can use:

6*sd(All)

Where sd is the standard deviation. (Here the sd of each group is calculated and then average sd is then calculated)

You must also specify the groups:

High - this is the group measuring the absence of the displacing substance

Low - this is the group measuring the completely displaced tracer

All - this is the group type measuring all of the concentrations (typically Standard)

Details

The transform produces the transform output matrix variable **ZPrime**

This can be referred to in other transforms, for example you could write a Validation Expression transform which ensures the calculated z' is > 0.5:

#1.ZPrime > 0.5

(Where the first transform created is Z')

Details

If the expression is evaluated to 0 then the validation fails, otherwise it passes.

Properties

Axes

This step allows you to specify the details of each axis.

If **Logarithmic** is ticked then the axis is logarithmic, otherwise it is linear.

If **Always Positive** is ticked then the Axis always starts at 0 (and no negative part is displayed).

If **Specify Title** is ticked you can enter a title to be used in place of the default axis title.

Baseline Correction Method

This window allows you to specify the method used to perform the baseline correction.

There are three methods available:

- Correct points by average of points between points **X1** and **X2**
- Correct points by average of **N** lowest points
- Proportional % between average of **N1** lowest and **N2** highest points

In each case you can specify the relevant parameters by entering their values in a text box.

See the Baseline Correction Transform help page for more information on the correction methods.

Blank Correction

This allows you to specify associations between Blank groups and wells to be corrected.

You can select a Blank group from a drop-down list. All included inherently Blank sample types are included (eg **Blank**, **BlankX**, **BlankY**, etc.).

You can make associations between a Blank group and a well, when the **Select** button is pressed, by left clicking on the well or by dragging a rectangular area. When the **Erase** button is pressed down wells can be selected in the same way for disassociation with the selected Blank group.

Wells of a Blank group cannot be associated with a different Blank group than itself.

When in erase mode and a Blank well is selected the association between the Blank well and itself is removed, resulting in the Blank well not being corrected by itself.

It is not allowed to have no wells associated with any Blank groups.

If there are some wells on the plate that are not of the Unused type and are not associated with any Blank wells then, when **OK** is clicked or the tab is changed, a warning is given. This situation *is* allowed.

If the check box titled **Correct all wells with this group** is ticked then all wells on the display are selected, the **Select** and **Erase** buttons are disabled and all wells will be corrected by the selected group (the user cannot select wells to associate in this mode).

Conc.

The term "replicates" is used here in two different ways, in both cases "replicates" still means the wells which belong to a group. The term "group replicates" are the members of the group as is used elsewhere throughout the software. The term "concentration replicates" are the members of the group which share the same concentration.

This allows you to specify the concentrations for each dilution, and how the concentrations are replicated.

The **Group Type** list allows you to select a group type to perform the calculations on.

The **Concentration Replicates** section allows you to specify the number of concentration replicates (using the **Number** drop-down list) and their fill **Direction**, which can be Across or Down. With an Across fill direction, for example, if wells **A1**,**A2**, **B1** and **B2** were **Unknown1** wells, and 2 concentration replicates were used, **A1** and **A2** would be subscript 1 and **B1** and **B2** would be subscript 2. With a Down fill direction, **A1** and **B1** would be subscript 1 and **A2** and **B2** would be subscript 2.

The large number displayed in each well of the microplate is the group number of the well. The concentration replicate number is shown as a subscript numeral.

Concentration/Dilution values can be entered using the keyboard. Use the up and down keys change the value to edit.

Tick **Auto Series** to enter a value by which to multiply or increase Group n's concentration by to get group n+1, starting with the first group. (If the first Concentration/Dilution value is 0 then the second is used rather than the first.) The auto-series operation is performed on pressing the **Fill** button or changing the operator (i.e. + or *).

Use the **Paste** button to paste single or multi row data from the Windows clipboard into the Concentration table.

Concentrations

Concentration values can be entered using the keyboard. The up and down keys can be used to change the group to edit.

If Auto Series is selected the numeric edit box can be used to enter a value to multiply or increase Group n's concentration by to get group n+1, starting with the first group. (If the first Standard value is 0 then the second is used rather than the first.) The auto-series operation is performed on pressing the **Fill** button or changing the operator (i.e. **+** or *****).The **Paste** button allows you to paste single or multi row data from the Windows clipboard into the Concentration table.

Crossing Point

This window allows you to specify the **Crossing Point**, number of **Fit Points** and **Slope Direction** for the Crossing Point transform.

The **Advanced** button displays a drop-down list which allows you to select the transform's behaviour when more than one crossing point is found.

Cut-Off

This window allows you to specify the Cut-Off Point to which all wells will be compared. You can enter a group reference, an absolute numerical value, or a mathematical expression.

Text boxes allow you to enter text for the Negative and Positive labels.

If you tick the **Grey Zone** box, a further text box appears allowing you to enter a grey zone. This may be expressed as a percentage, absolute numerical value or a mathematical expression.

Difference

This window allows you to specify the **Delta Y** parameter for the Delta X for Delta Y transform.

Dual Input Matrix

This allows you to specify the input matrices for transforms that require two input matrices. You can select any Raw matrix or Calculated matrices that occur before the transform being edited. If the transform expects endpoint matrices as input, then only endpoint matrices are available. If the transform expects kinetic matrices as input, then only kinetic matrices are available.

Click the **Advanced** button to display a box listing the sample types of the microplate layout. For each selected sample type no calculations will be made for wells of those sample type. In these cases items will be added to the Audit Trail detailing each excluded well under Group Calculations.

Expression

This allows you to enter an expression to be evaluated.

See the **Expressions** section, page 102, for more information.

Factor

This window allows you to specify the factor by which all points on a plate will be multiplied.

You may specify the factor as an absolute numerical value or a mathematical expression.

Factors

This allows you to specify a dilution factor for each group.

The **Advanced** button displays the **Calculate Factors** list, which can be used to specify which groups factors can be applied to.

Auto Series allows you to enter a value to multiply or increase Group n's concentration by to get group n+1, starting with the first group. (If the first group's value is 0 then the second is used rather than the first.) The auto-series operation is performed on pressing the **Fill** button or changing the operator (i.e. + or *).

File

This window is used to specify details about the text file to export.

You can enter a filename to create/use, or press the **Browse...** button to navigate to one.

If the **Append to existing file** option is ticked then the new export is appended to the specified file if it already exists (if not it is created).

Kinetic Matrices

If kinetic measurements are used then the controls within the **Kinetic Matrices** section can be used to customise the kinetic output for kinetic matrices.

Tick the **Include X values with each Y for Kinetic Matrices** to include X values of each kinetic point. If this is not ticked then only Y values will be exported. Use the drop-down lists to select the data to include.

IC%

This step allows you to enter a value for the **IC%** to be calculated. The value must be in the range 0 to 100.

Input Matrix

This allows you to specify the input matrix for transforms that require one input matrix. You can select any Raw matrix or Calculated matrices that occur before the transform being edited. If the transform expects one single endpoint matrix as input, then only endpoint matrices are available. If the transform expects one single kinetic matrix as input, then only kinetic matrices are available.

Click the **Advanced** button to display a listbox listing the sample types of the microplate layout. 0 or more items can be selected. For each selected sample type no calculations will be made for wells of those sample type. In these cases items will be added to the Audit Trail detailing each excluded well under Group Calculations.

Integral

This window allows you to specify which method to use when calculating the Integral of each kinetic chart.

Kinetic Options

This window allows you to specify settings relating to the input data for a kinetic transform.

You can specify the data for the X and Y axes using the drop-down lists.

A range of input data points may be specified by entering minimum and maximum X values.

Tip: The minimum and maximum X values can also be selected using the vertical dashed lines in the transform's analysis control - in this way you can see line up to the raw data.

Linear Regression

This window allows you to specify whether the result of the Linear Regression transform is the **Slope** of the line through the points of each kinetic chart, or the **Y at relative X'**.

Max Peaks

This window allows you to specify how many peaks are to be found in each kinetic chart. This can be a value from 1 to 4. A tickbox allows you to select whether to calculate the area under each peak.

The Detection Method can be specified as **Automatic** or **Manual**.

A drop-down list allows you to select whether X or Y values are displayed in the report table.

Max Slope

This window allows you to specify the details for the Maximum Slope transform.

A text box allows you to enter the number of Linear Regression points.

Three radio buttons allow you to specify the maximum slope as **Most Positive**, **Most Negative** or **Auto**. If **Auto** is specified the maximum slope is determined from the general direction of each chart.

Further radio buttons allow you to specify the Result. If you select **X** or **Y at centre of Max Slope**, a tickbox allows you to specify **Relative from first point**. If you select **Extrapolated Y of Max Slope**, a text box allows you to enter an X value to extrapolate.

Method

This step allows you to select a curve fit method. You can click the **Edit** button to access the curve fit's properties.

You can optionally calculate EC Values from the results of the fit. If selected you can enter of a list of comma separated numbers (n) to calculate EC(n).

Some methods display a further tick box to select to scale the Y axis based on **B/B0**. If this is ticked then the resulting fit line will go through (0,0). (Note, this option does not appear for the Kinetic Fit transform).

Clicking the **Advanced** button displays two Log/Anti-log tick boxes:

If **Log/Anti Log X** or **Y** is ticked then all input X or Y data points are logged as specified.

When Y is calculated from X:

If **Log/Anti Log X** is ticked then the X is Logged

Then Y is Calculated from X as normal

If **Log/Anti Log Y** is ticked then the calculated Y is Anti-Logged

When Y is calculated from X:

If **Log/Anti Log Y** is ticked then the Y is Logged

Then X is Calculated from Y as normal

If **Log/Anti Log X** is ticked then the calculated X is Anti-Logged

Log means log base 10

Anti-log means 10 to the power of the number

Output Matrix

This allows you to specify the name of a Transform output matrix.

Any value can be given for each field.

Peak

This window allows you to specify whether the maximum (peak) or minimum (dip) point in each kinetic chart is found.

Two radio buttons allow you to specify whether the X or Y value in each case is given as the result.

Point

This window allows you to specify an X value at which to find the Y value.

Range

This step allows you to select the range the calculated concentrations must fall in. If a calculated concentration falls outside of the specified range then the result will be flagged.

There are three options: the Range of Standards, a numerical minimum and maximum value, or to Accept all calculated concentrations.

If the resulting fit is such that the specified range is outside of the range of points that can be calculated from the fit then the specified range is ignored and the range is determined by the limitations of the fit. For example, if you specify the range as 0 – 100 but the fit is only valid between 20 and 100 then the range will be 20-100.

Rate of Change Method

This window allows you to specify the number of fit points used in calculating the rate of change for each kinetic chart. The number of fit points is the number of points used to calculated the slope of each segment

A tickbox allows you to specify whether to invert the sign of the result, this can be used to avoid negative results.

Report

This allows you to specify which items are included in the Report:

- **Concentrations Table** - a list of the specified concentrations.
- **Fit Results Table** - the calculated **MSE** and **IC%** of each group.
- **Overlaid Graph** – a single graph containing each plot from every plate overlaid in different colours.
- **Individual Graphs** - a graph of each group's fit.

Source

This allows you to specify the source Standards data set. If multiple plates are used then you can select any plate with inherently Standard groups. If there is more than one Standard type on a plate, you can choose between types with the Group drop-down list. The selected standard group will flash. You can select the source matrix from the Matrix drop-down list.

Transform Select

Validations

This allows you to edit the validation condition. You can edit an item by selecting it and clicking **Edit**, or by double-clicking on it.

The **Add** and **Edit** buttons display a Validation window that allows you to edit the condition.

The first drop-down list allows the selection of a statistical operator.

The second drop-down list allows you to select any group, All Samples, or an All option for each group type.

The third drop-down list displays the comparison operators.

The fourth drop-down list can be used to select or enter a new value or expression.

Advanced tips and tricks

Backing up application data

As part of normal PC administration it is usual to periodically copy files to a different location.

All user data files are stored within the **My Assays** sub-directory of the **My Documents** folder.

Command Line Arguments

The Run and Results application can be launched from the command line. This means that Manta can be called from other applications.

Syntax

MRunRes [/run | /edit] {<ProtocolFile> [<DataFileToImport>]} | <ResultsFile> [/exit]

Where:

Item:	Meaning:
MRunRes	The application executable - this is located in the installation directory.
<ProtocolFile>	The full path to a protocol file (APR)
<DataFileToImport>	The full path to a text file to import (for a **<ProtocolFile>** which imports)
<ResultsFile>	The full path to an existing results file (ARS)
/run	The specified **<ProtocolFile>** file (which takes measurements) will start the readings once it has been loaded
/edit	Opens the specified **<ProtocolFile>** file for editing
/exit	The program exits after the post analysis options have been performed.

If the **<ProtocolFile>** imports data then a second parameter **<DataFileToImport>** can optionally be given which is the file to import, if this parameter is not given then a file open window is displayed to allow the manual selection of the file to import.

Examples

Example:	Action:
MRunRes <ResultsFile>	Opens the existing specified results file
MRunRes <ProtocolFile>	Launches the specified protocol file ready to start measurements.
MRunRes /run <ProtocolFile>	Launches the specified protocol file and starts the measurements straight away.
MRunRes /run <ProtocolFile> /exit	Launches the specified protocol file and starts the measurements straight away exits when finished.
MRunRes /edit <ProtocolFile>	Launches the specified protocol file for editing.
MRunRes <ProtocolFile> <DataFileToImport>	Launches the specified protocol which imports data and imports the specified file.
MRunRes <ProtocolFile> <DataFileToImport> /exit	Launches the specified protocol which imports data and imports the specified file then exits

Export numerical data to Excel 97

The **Export to Excel** option is only enabled if Excel 2000/XP or later is installed.

However, a spreadsheet can be created when using Excel 97 by exporting the Report as HTML, then in Excel go to File | Open and select "All files" then got to reports directory (or where ever it was created) then open the file. The text and numeric data will be imported. In this way the data in the tables and matrices can be referenced from cells, however the images will not be imported (as these are not compatible with Excel 97).

Components Required For Export Report Options

The following table lists the components that are required for each Export Report type to be available. If the specified components are not available on your system then the options will be disabled.

Export Report Type	File Extension	Components Required
A Web Page (complete with links to image files)	*.htm;*.html	None (this option is always available)
A Web Archive file (a single file)	*.mht	None (this option is always available)
Microsoft Excel (an Excel Workbook with Worksheets)	*.xls	If Microsoft Excel 2000/XP or later are installed. (For details of how to export data with Microsoft Excel 97, see page 133.)
Microsoft Word (a standard Word file)	*.doc	If Word 2000/XP or later are installed. (It is not available with Word 97, the file format used is not compatible)

Contacting Dazdaq

Contact Details

Address:	**Dazdaq Ltd.** 7 Queen Square, Brighton BN1 3FD England
Telephone:	+44 (0) 1273 777648
Fax:	+44 (0) 1273 777174
World Wide Web:	www.dazdaq.com

Sales

Contact your account instrument sales representative for information about the latest products and services or contact Dazdaq Ltd. for further details about our products:

Phone:	+44 (0) 1273 777648
Fax:	+44 (0) 1273 777174
Email:	sales@dazdaq.com
World Wide Web:	www.dazdaq.com

Technical Support

Contact your account sales representative for information about Technical Support for your instrumentation.

Before contacting Dazdaq Ltd. technical support for help with the Manta software prepare the following information:

- Your Operating System version (e.g. Windows XP, Windows 2000, etc.).
- Find the version of Manta in the **Help** | **About** box in the Organiser.

Phone:	+44 (0) 1273 777648
Fax:	+44 (0) 1273 777174
Email:	support@dazdaq.com
World Wide Web support:	www.dazdaq.com/support

End-user License Agreement

End-user License Agreement

BY CLICKING ON THE INSTALLATION "YES" BUTTON OR OPENING THE PACKAGE, YOU ARE CONSENTING TO BE BOUND BY AND ARE BECOMING A PARTY TO THIS AGREEMENT. IF YOU DO NOT AGREE TO ALL OF THE TERMS OF THIS AGREEMENT, CLICK THE "NO" BUTTON. (IF APPLICABLE, YOU MAY RETURN THE PRODUCT TO THE PLACE OF PURCHASE FOR A FULL REFUND.) IF THE COPY OF THE SOFTWARE YOU RECEIVED WAS ACCOMPANIED BY A PRINTED OR OTHER FORM OF "HARD-COPY" END USER LICENSE AGREEMENT WHOSE TERMS VARY FROM THIS AGREEMENT, THEN THE HARD-COPY END USER LICENSE AGREEMENT GOVERNS YOUR USE OF THE SOFTWARE.

DAZDAQ LTD. END USER LICENSE AGREEMENT

REDISTRIBUTION OR RENTAL NOT PERMITTED

This Agreement has 3 parts. Part I applies if you have a free of charge license to the accompanying software (the"Software"). Part II applies if you have purchased a license to the Software. Part III applies to all license grants. If you initially acquired a copy of the Software free of charge and you wish to purchase a license, contact DAZDAQ Ltd. ("DAZDAQ Ltd.") on the Internet at dazdaq@dazdaq.com. For purposes of this Agreement, "Licensor" shall be defined as follows: If you have acquired a third party product or service and such product or service included the Software, then such third party shall be the Licensor. Otherwise, DAZDAQ Ltd. shall be the Licensor.

PART I - TERMS APPLICABLE WHEN LICENSE FEES NOT (YET) PAID (LIMITED TO EVALUATION USE)

LICENSE GRANT. Licensor grants you a non-exclusive license to use the Software free of charge if (a) your use of the Software is for the purpose of evaluating whether to purchase an ongoing license to the Software. The evaluation period for use by or on behalf of a commercial entity is limited to ninety (90) days; Government agencies (other than public libraries) are not considered educational, religious or charitable non-profit organizations for purposes of this Agreement. If you are using the Software free of charge, you are not entitled to hard-copy documentation, support or telephone assistance. If you fit within the description above, you may use the Software in the manner described in Part III below under "Scope of Grant."

DISCLAIMER OF WARRANTY. Free of charge Software is provided on an "AS IS" basis, without warranty of any kind, including without limitation the warranties that the Software is free of defects, merchantable, fit for a particular purpose or non-infringing. The entire risk as to the quality and performance of the Software is borne by you. Should the Software prove defective in any respect, you and not Licensor or its suppliers assume the entire cost of any service and repair. In addition, the security mechanisms implemented by the Software have inherent limitations, and you must

determine that the Software sufficiently meets your requirements. This disclaimer of warranty constitutes an essential part of this Agreement. No use of the Software without payment of license fees to Licensor is authorized hereunder except under this Disclaimer.

PART II - TERMS APPLICABLE WHEN LICENSE FEES PAID

LICENSE GRANT. Subject to payment of applicable license fees, Licensor grants to you a non-exclusive license to use the Software and accompanying documentation ("Documentation") in the manner described in Part III below under "Scope of Grant."

LIMITED WARRANTY. Licensor warrants that for a period of ninety (90) days from the date of acquisition, the Software, if operated as directed, will substantially achieve the functionality described in the Documentation. Licensor does not warrant, however, that your use of the Software will be uninterrupted or that the operation of the Software will be error-free or secure. In addition, the security mechanisms implemented by the Software have inherent limitations, and you must determine that the Software sufficiently meets your requirements. Licensor also warrants that the media containing the Software, if provided by Licensor, is free from defects in material and workmanship and will so remain for ninety (90) days from the date you acquired the Software. Licensor's sole liability for any breach of this warranty shall be, in Licensor's sole discretion: (i) to replace your defective media or Software; or (ii) to advise you how to achieve substantially the same functionality with the Software as described in the Documentation through a procedure different from that set forth in the Documentation; or (iii) if the above remedies are impracticable, to refund the license fee you paid for the Software. Repaired, corrected, or replaced Software and Documentation shall be covered by this limited warranty for the period remaining under the

warranty that covered the original Software, or if longer, for thirty (30) days after the date (a) of delivery to you of the repaired or replaced Software, or (b) Licensor advised you how to operate the Software so as to achieve substantially the same functionality described in the Documentation.

Only if you inform Licensor of your problem with the Software during the applicable warranty period and provide evidence of the date you purchased a license to the Software will Licensor be obligated to honour this warranty. Licensor will use reasonable commercial efforts to repair, replace, advise or, for individual consumers, refund pursuant to the foregoing warranty within thirty (30) days of being so notified.

If any modifications are made to the Software by you during the warranty period; if the media is subjected to accident, abuse, or improper use; or if you violate the terms of this Agreement, then this warranty shall immediately terminate. Moreover, this warranty shall not apply if the Software is used on or in conjunction with hardware or software other than the unmodified version of hardware and software with which the Software was designed to be used as described in the Documentation.

THIS IS A LIMITED WARRANTY, AND IT IS THE ONLY WARRANTY MADE BY LICENSOR OR ITS SUPPLIERS. LICENSOR MAKES NO OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NONINFRINGEMENT OF THIRD PARTIES' RIGHTS. YOU MAY HAVE OTHER STATUTORY RIGHTS. HOWEVER, TO THE FULL EXTENT PERMITTED BY LAW, THE DURATION OF STATUTORILY REQUIRED WARRANTIES, IF ANY, SHALL BE LIMITED TO THE ABOVE LIMITED WARRANTY PERIOD. MOREOVER, IN NO EVENT WILL WARRANTIES PROVIDED BY LAW, IF ANY, APPLY UNLESS THEY ARE REQUIRED TO APPLY BY STATUTE NOTWITHSTANDING THEIR EXCLUSION BY CONTRACT. NO DEALER, AGENT, OR EMPLOYEE OF LICENSOR IS AUTHORIZED TO MAKE ANY MODIFICATIONS, EXTENSIONS, OR ADDITIONS TO THIS LIMITED WARRANTY.

PART III - TERMS APPLICABLE TO ALL LICENSE GRANTS

SCOPE OF LICENSE GRANT.

You may:

• use the Software on any single computer;

• use the Software on a second computer so long as only one (1) copy is used at a time;

• use the Software on a network, provided that a licensed copy of the Software has been acquired for each person permitted to access the Software through the network;

• copy the Software for archival purposes, provided any copy must contain all of the original Software's proprietary notices; or

• if you have purchased a Right to Copy license for multiple copies of the Software, make the number of copies of Software (but not the Documentation) stated on the packing slip or invoice, provided any copy must contain all of the original Software's proprietary notices. The number of copies on the invoice is the total number of copies that may be made for all platforms. Additional copies of Documentation may be purchased from Licensor.

You may not:

• permit other individuals to use the Software except under the terms listed above;

• permit concurrent use of the Software;

• modify, translate, reverse engineer, decompile, disassemble (except and solely to the extent an applicable statute expressly and specifically prohibits such restrictions), or create derivative works based on the Software;

• copy the Software other than as specified above;

• rent, lease, grant a security interest in, or otherwise transfer rights to the Software; or remove any proprietary notices or labels on the Software.

TITLE. Title, ownership rights, and intellectual property rights in the Software shall remain in DAZDAQ Ltd. and/or its suppliers. The Software is protected by copyright

and other intellectual property laws and by international treaties. Title and related rights in the content accessed through the Software is the property of the applicable content owner and is

protected by applicable law. The license granted under this Agreement gives you no rights to such content.

TERMINATION. This Agreement and the license granted hereunder will terminate automatically if you fail to comply with the limitations described herein. Upon termination, you must destroy all copies of the Software and Documentation.

EXPORT CONTROLS. None of the Software or underlying information or technology may be downloaded or otherwise exported or reexported (i) into (or to a national or resident of) Cuba, Iraq, Libya, Sudan, North Korea, Iran, Syria or any other country to which the U.S. has embargoed goods; or (ii) to anyone on the U.S. Treasury Department's list of Specially Designated Nationals or the U.S. Commerce Department's Table of Denial Orders. By downloading or using the Software, you are agreeing to the foregoing and you are representing and warranting that you are not located in, under the control of, or a national or resident of any such country or on any such list.

In addition, if the licensed Software is identified as a not-for-export product (for example, on the box, media or in the installation process), then, unless you have an exemption from the United States Department of State, the following applies: EXCEPT FOR EXPORT TO CANADA FOR USE IN CANADA BY CANADIAN CITIZENS, THE SOFTWARE AND ANY UNDERLYING TECHNOLOGY MAY NOT BE EXPORTED OUTSIDE THE UNITED STATES OR TO ANY FOREIGN ENTITY OR "FOREIGN PERSON" AS DEFINED BY U.S. GOVERNMENT REGULATIONS, INCLUDING WITHOUT LIMITATION, ANYONE WHO IS NOT A CITIZEN, NATIONAL OR LAWFUL PERMANENT RESIDENT OF THE UNITED STATES. BY DOWNLOADING OR USING THE SOFTWARE, YOU ARE AGREEING TO THE FOREGOING AND YOU ARE WARRANTING THAT YOU ARE NOT A "FOREIGN PERSON" OR UNDER THE CONTROL OF A "FOREIGN PERSON."

LIMITATION OF LIABILITY. UNDER NO CIRCUMSTANCES AND UNDER NO LEGAL THEORY, TORT, CONTRACT, OR OTHERWISE, SHALL LICENSOR OR ITS SUPPLIERS OR RESELLERS BE LIABLE TO YOU OR ANY OTHER PERSON FOR ANY INDIRECT, SPECIAL, INCIDENTAL, OR CONSEQUENTIAL DAMAGES OF ANY CHARACTER INCLUDING, WITHOUT LIMITATION, DAMAGES FOR LOSS OF GOODWILL, WORK STOPPAGE, COMPUTER FAILURE OR MALFUNCTION, OR ANY AND ALL OTHER COMMERCIAL DAMAGES OR LOSSES. IN NO EVENT WILL LICENSOR BE LIABLE FOR ANY DAMAGES IN EXCESS OF THE AMOUNT LICENSOR RECEIVED FROM YOU FOR A LICENSE TO THE SOFTWARE, EVEN IF LICENSOR SHALL HAVE BEEN INFORMED OF THE POSSIBILITY OF SUCH DAMAGES, OR FOR ANY CLAIM BY ANY THIRD PARTY. THIS LIMITATION OF LIABILITY SHALL NOT APPLY TO LIABILITY FOR DEATH OR PERSONAL INJURY RESULTING FROM LICENSOR'S NEGLIGENCE TO THE EXTENT APPLICABLE LAW PROHIBITS SUCH LIMITATION. SOME JURISDICTIONS DO NOT ALLOW THE EXCLUSION OR LIMITATION OF INCIDENTAL OR CONSEQUENTIAL DAMAGES, SO THIS EXCLUSION AND LIMITATION MAY NOT APPLY TO YOU.

HIGH RISK ACTIVITIES. The Software is not fault-tolerant and is not designed, manufactured or intended for use or resale as on-line control equipment in hazardous environments requiring fail-safe performance, such as in the operation of nuclear facilities, aircraft navigation or communication systems, air traffic control, direct life support machines, or weapons systems, in which the failure of the Software could lead directly to death, personal injury, or severe physical or environmental damage ("High Risk Activities"). Accordingly, Licensor and its suppliers specifically disclaim any express or implied warranty of fitness for High Risk Activities.

MISCELLANEOUS. This Agreement represents the complete agreement concerning the license granted hereunder and may be amended only by a writing executed by both parties. THE ACCEPTANCE OF ANY PURCHASE ORDER PLACED BY YOU IS EXPRESSLY MADE CONDITIONAL ON YOUR ASSENT TO THE TERMS SET FORTH HEREIN, AND NOT THOSE IN YOUR PURCHASE ORDER. If any provision of this Agreement is held to be unenforceable, such provision shall be reformed only to the extent necessary to make it enforceable. This Agreement shall be governed by California law, excluding conflict of law provisions (except to the extent applicable law, if any, provides otherwise). The application of the United Nations Convention of Contracts for the International Sale of Goods is expressly excluded.

U.S. GOVERNMENT END USERS. The Software is a "commercial item," as that term is defined in 48 C.F.R. 2.101 (Oct. 1995), consisting of "commercial computer software" and "commercial computer software documentation," as such terms are used in 48 C.F.R. 12.212 (Sept. 1995). Consistent with 48 C.F.R. 12.212 and 48 C.F.R. 227.7202-1 through 227.7202-4 (June 1995), all U.S. Government End Users acquire the Software with only those rights set forth herein.

Glossary

A

Accuracy: A measure of the degree of agreement between a measured value and the standard or accepted value for that sample.

Assay Protocol: The specification of all steps to run an assay. This includes the Data Acquisition parameters, the Microplate Layout, the analysis settings (transforms), file management and reporting information.

B

Blank: This is a Sample Type which is measured as a background count which can be used in a blank correction calculation.

D

Data Acquisition: This describes how the raw data gets into the software. This can be either offline via importable text files or online through control of a connected instrument.

G

Goodness of fit measure: A value calculated to quantify how well the curve fit models the data set, examples include MSE, R^2, SS, SYX.

Group: A well or wells of the same group number and sample type.

Group Number: This is a number used to identify which group a sample belongs to. Wells which have the same sample type and group number contain the same sample and are replicates. For example, if wells A1 and A2 are Unknown1 then both wells contain the same sample,Unkown1.

I

IC50: Inhibition Concentration; this isthe concentration of the substance resulting in displacement of 50% of the antibody.

Import Scripting: A technique used to automatically recognise and import data from text files.

in vitro: In an artificial environment outside of a living organism

Included Well: Wells are only included in a calculation if they have not been flagged for exclusion. A well is not included if there was a problem reading the well, the user has flagged the well, there was a problem with a previous calculation on the well or the well was auto-flagged. A well can also be excluded because the transformation is configured to only use certain group types.

M

Microplate: This term is used loosely to describe any sized micro titre plate, including a petri dish.

Microplate Layout: This describes the positioning of the samples on the microplate(s) associated with the test, i.e. the way in which the microplate(s) used by the assay protocol are pipetted.

O

Offline Protocol: Readings are imported from an existing text file of a particular file format.

Online Protocol: Measurements are taken with a connected instrument controlled by the software.

Output Variables: Additional results of a transform or curve fit which can be referenced in expressions.

P

Plate Layout: A description of how a particular microplate is filled.

Precision: A measure of the degree of agreement between repeated measurements of the same sample.

Protocols Directory: This is the location where all your Assay Protocols are stored. It is a sub-directory of My Assays called My Protocols, ie <Parent Directory>\My Assays\My Protocols. (The <Parent Directory> is normally your My Documents folder, but this can be changed under Organiser Options | Data.)

R

Raw Data: The readings taken with an instrument or the data imported from a text file.

Replicate: A sample of the same Group Number and Sample Type that is measured in more than one well.

S

Sample: Item being measured by a reader.

Sample IDs: This is a name or number given to an Unknown group to identify it (for example, a patient ID). Each specified Sample ID will be used instead of the group type and number making the association between the sample and the microplate layout clearer.

Sample Type: This is the nature of the substance to be measured, for example Unknown, Pos Control,Standard. Each sample type is represented by a particular colour.

Standard: A sample of which some property (typically its concentration) is known that is used in the quantitative analysis of unknowns.

T

Transform: A Transform is a layer of analysis which performs an operation on input data resulting in output data. Transforms can be layered to define the analysis operations of the test.

U

Unflagged: A well or data point that is not marked as flagged

Unknown: A sample that you want to calculate results for (typically by comparing against Standards and/or Controls).

Unused well: A well that is not used in a template layout - it has no well group and type. Some instruments will not read unused wells and normally the well will be empty.

W

Well: A position on the microplate. The letter identifies the row and the number identifies the column. A1 Top left H12 Bottom right (for 12x8) AA1 Left well row 27 AB1 Left well row 28 AZ1 Left well row 52 BA1 Left well row 53