Stingray 1.5

User's Guide

DAZDAQ LTD.

Stingray 1.5 User's Guide

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Getting Started

This section provides an introduction to Stingray and its features.

Introduction

Welcome

Welcome to Stingray, the ultimate protocol design, data acquisition, review and archiving tool for single and multiple microplate and tube reader technology. Stingray offers the easiest way to produce flexible and usable protocols for laboratory automation.

What is Stingray?

Stingray is a feature packed protocol design and execution tool. Stingray controls instrumentation, evaluates and re-assesses acquired data for research and diagnostic needs. Stingray provides unparalleled ease of use for specifying assay requirements and reviewing test results in a single Windows environment.

How is Stingray used?

Stingray has two levels of operation, which permit it to be both very powerful and very easy to use.

In the first level of operation, scientists, laboratory managers and those wishing to set up protocols and routine test parameters can define protocols and store them under named libraries. It is therefore possible to develop Stingray as a user defined protocol manager from which all named protocols can be recalled quickly and simply at any time in the future.

In the second level of operation, laboratory staff that need to run defined protocols set up by e.g. managers and commercial reagent suppliers can simply recall the parameters automatically by clicking on the appropriate test name.

This structure permits Stingray 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.

Stingray Features

32-bit Windows software
Familiar Windows 95 style user interface.

Context Sensitive Help
Press F1 or click a help button at any point whilst using Stingray for relevant Help information about the task at hand.

Multiple Document Interface
This means that any number of protocol, standards and readings files can be open at once. Windows can be arranged automatically using the standard tile and cascade commands.

Active Data Views
When data is changed in one view of a file, all other views of the data are modified instantly to reflect the changes made.

Drag And Drop Control Bars
Relevant control bars are displayed as and when required.

Short-Cut Keys
Experienced users can use key combinations to quickly perform common tasks.

Protocol/Results separation
Stingray splits assay use between two concepts, design and results. This approach allows an assay to be designed and re-used many times to run routine tests and provide a consistent reporting method. Isolating assay design from the end user also eliminates the possibility of accidental assay corruption. Stingray also provides a facility for changing the protocol file after readings have been made, if required.

Assay Master
Guides the user through the maze of protocol design.

Variable Microplate Dimensions
Stingray can be used with any microplate dimensions.

Virtual Microplate
Use a virtual microplate to design template layouts that are directly portable between microplate readers and single tube readings.

Flexible Template Configuration
Use the mouse to quickly design any possible template layout with replicates and in any fill direction. Undo command to recover from mistakes.

Template Layout Printing
Print the template layout out in colour to remind protocol users how to fill a microplate, or to insert tubes.

Good Laboratory Practice (GLP)
Easily create data entry screen to appear before a protocol run to gather assay specific information. This data can be included in the report.

Automatic Flagging
Stingray can automatically flag wells which satisfy expression based conditions or automatically flag the well that is the furthest from the mean for all groups with a percentage CV greater than a specified value. All flagged items are logged and can be included in the final report.

Powerful Expression Based Transformations
Create data transformations using standard mathematical grammar on raw or calculated matrices, using references to wells, defined groups (e.g. *sample1*) or groups of groups (e.g. *samplen*).

Transformation Wizards
Quickly create transformations for common tasks: blank correction, curve fit, matrix difference, kinetic difference and competitive bindings.

Curve Fits
- **Linear Regression on replicate points, with a specified validation correlation coefficient**
- **Point to Point**
- **Cubic Spline**
- **Polynomial Regression**
- **Four and Five Parameter Fit for sigmoidal plots**

Flexible Graph Axes and Titles
Logarithmic or Linear x and/or y axes.

Curve Fit data source from readings or archived standards file
Multiple standards on one plate
Competitive binding/inhibition concentrations
Twelve kinetic reduction methods available
The number of regression points can be specified where appropriate.

Positive or negative maximum slope
Calculate reductions using the most positive or most negative slope.

Kinetic Fail options
Specify what happens if a reduction method fails (where thresholds are involved).

Flexible Validation Specification
Specify validations for raw or calculated matrices using standard mathematical expressions on defined groups or wells, including Boolean operators and statistical operations.

Flexible Cut-Off Specification
Specify Cut-Offs as expressions for raw or calculated matrices using standard mathematical expressions on defined groups or wells, including Boolean operators and statistical operations.

Numerical Formatting
Specify how to format numerical data (for display/reporting purposes only) using decimal places or significant figures.

Flexible Reporting specification
Choose which data to include in the report and in which order. Edit report headers and footers, use any installed Windows fonts. The report can include a table of results (by group), a matrix of results (by well), fitted graphs, any fixed text, readings notes, validation results, calculation log, automatic flagging results, list of flagged wells and 3D graphs. Report layout can be previewed before readings are taken.

Editing of the protocol after readings have been taken
Make modifications to a protocol after readings have been made (user privilege dependent).

Stop kinetic test at any point preserving readings already made
View Cut-Offs
For individual wells or by defined groups.

3D Graphs
Stingray can plot 3D graphs of raw or calculated data. Views can be zoomed, rotated and shifted. 3D graphs can be used to easily identify and flag anomalies.

Manually Flagging Wells
Easily flag single wells or groups from various views, including standards graph. All flagged items are logged and can be included in the report.

Flag Individual Kinetic Points
Flag invalid kinetic points that will be ignored by kinetic reduction methods.

Sample Identifications
Specify sample identifications to be used in the report. Paste data from other applications with a single mouse click.

Flexible Standards Graph View
Zoom, flag points, flag defined groups, test the curve for unknown values and change the range to plot within.

Calculation Log
Details calculations, explains errors and tracks flagged items. The log can be included in the report and saved to a file.

Readings Notes
Specify notes regarding a specific protocol run - these notes are stored with the readings data and may be included in the report.

Data Exports
Export raw or calculated data to Excel or to any other application using Windows clipboard.

Automatic Exports
Automatically launch other applications with Stingray data after readings have been made.

Graphic Exports
Copy Stingray graphs into the Windows clipboard with one mouse click for immediate use in other applications.

Archived Standards Data
Extract standards data from protocol runs for use in transformations of future protocols.

Interactive standards editor
Create or edit archived standards data visually and test unknowns.

Locale format specifiers
Uses format specifiers of current locales, e.g. (1.23 UK, 1,23 German or French)

User names and passwords
Use in conjunction with LabLock to restrict user access and rights. LabLock can also be used to log user activity.

Supports Multiple Devices
Device drivers available for many readers. Dazdaq Ltd. are continually developing new device drivers for currently unsupported machines.

Multiple plate support
For use with assays that use multiple microplates

Scan/agglutination readings
View scan data in a many different projections, including bar, 3D and colour coded aerial views.

Import data from any text based source
Dazdaq Ltd. can develop import scripts for any currently unsupported file formats.

Off-line
Use Stingray without a connected instrument to analyse existing data.

Contacting DAZDAQ

Technical Queries

For technical questions about the software contact:

support@dazdaq.com

Sales Enquiries

For sales enquiries contact:

sales@dazdaq.com
Tel.: +44 (0) 1273 81 4414
Fax: +44 (0) 1273 81 4999

Further Information

Further and up to the minute information can be found on our web pages at

http://www.dazdaq.com

For information or problems regarding instrumentation contact your software supplier or hardware manufacturer.

Installation

Installation Requirements

Software Requirements

Windows 95/98 or Windows NT 4.0
Microsoft Excel 7 (95), Microsoft Excel 97 or Microsoft Excel 2000 is required for exporting data to Microsoft Excel.

Preferred Hardware

P90 Pentium Processor
16 MB RAM
4 MB free hard disk space
SVGA display running at 800x600
Mouse

Minimum Hardware

486 PC Compatible
4 MB RAM
4 MB free hard disk space
VGA display running at 640x480
Mouse

Previous Versions

If upgrading to a newer version of Stingray you must first uninstall any existing versions of Stingray or Dazdaq Stingray.

To uninstall Stingray go to the Add/Remove Programs option in the Windows Control Panel. Click Stingray and press the Remove button.

Installation Procedure

If Stingray has been provided with a CD launcher simply insert the CD into your drive and select the Install Now option.

If no CD launcher was provided install the program by running SETUP from the relevant drive using the Run option on the Start menu (e.g. D:\SETUP).

Note, administrative rights are required to install Stingray on an NT system.

Stingray Overview

Stingray separates design and set-up of assays from running the assay and analysing the results. This approach allows a diversity of assay protocols to be designed and stored by appropriate personnel with the option of password protection. This also allows operators to select and reuse routine tests very quickly and easily, providing a consistent reporting method and eliminating the possibilities of accidental assay corruption. Stingray also provides facilities for changing the protocol file after readings have been made.

Protocol Files

Protocol files (with a PRO file extension) specify the assay design. These files describe all the steps required to run an assay, including data acquisition methods, calculations, validations and report layout.

Data Files

Assays are contained within data files (with a DAT file extension for single plate data or an MPR extension for multiple plate data). These files contain the raw data from an assay and a reference to the protocol file that was used to generate the results. Data files also contain information specific to the assay; including the date and time the assay was executed, details of wells or kinetic points that were flagged and any notes made.

Standards Files

Stingray also uses standards files (with an STD file extension), which store archived standards data that can be used for calculations in other protocols.

Starting Stingray

Start Stingray by double clicking the Stingray icon or starting from Programs on the Windows Start Menu Bar.

If LabLock is in use with Stingray you will have to enter a user name and password before Stingray can be used. Depending on your user profile certain features may be disabled.

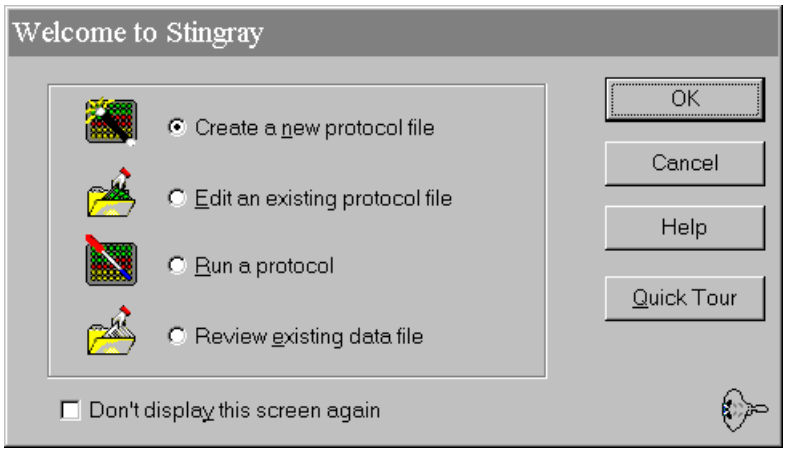


Figure 1 Stingray Welcome Dialogue Box

Four options are available upon start-up:

Create a new protocol
This automatically starts a blank file to set up a new protocol.

Edit an existing protocol file
This allows the editing of an existing protocol file. A standard Windows File dialogue box is opened and all protocol files are displayed. You can then edit the file using the options originally used to create the file.

Run a Protocol
This is a quick start option for running with an existing protocol. A standard Windows File Open dialogue box and all protocol files in the current directory are displayed. Stingray is now ready to run an assay.

Review existing data file
This option allows the re-evaluation of previously run assays. A standard Windows File Open dialogue box and all data files in the current directory are displayed. Select a data file for analysis.

Protocol Files

This section describes Stingray protocols in detail.

Overview

Protocol files (with a PRO file extension) specify the assay design. These files describe all the steps required to run an assay, including data acquisition methods, calculations, validations and report layout.

To create a new protocol file click **New Protocol**. This will create an empty protocol file ready for editing. Protocol control bars and an empty default sized virtual microplate will be displayed.

Assay Design should follow the general order defined below – which essentially involves following the protocol control bar buttons (on the left hand side of the Stingray display) in a downward order. Some steps will not be required depending on your assay needs.

Set-up the device(s) that will be used to acquire the raw data.
Configure the Assay Master.
Design the template layout.
Add GLP entries.
Configure automatic flagging settings.
Create required transformations.
Choose kinetic data reduction method.
Specify validation expressions.
Specify cut-off expressions.
Configure numerical formatting of results.
Design the report layout.
Result files management
Post read options.

Whilst designing and testing a protocol it is often the case that the ordering of these steps cannot be followed. It must be noted that changing the settings of certain items may affect the layout of the report and it is therefore recommended that designing the report layout should be left until last. If editing an item affects the layout of the report a message box will be displayed warning that the report layout should be modified or a default report layout should be used before saving can occur.

The steps above are now described in detail.

Device

The first step in creating any protocol file is specifying the data acquisition method. This simply means setting up which devices to use and how they should work. Figure 2 displays the Raw Data configuration box. Here raw data items can be added, removed and their order edited.

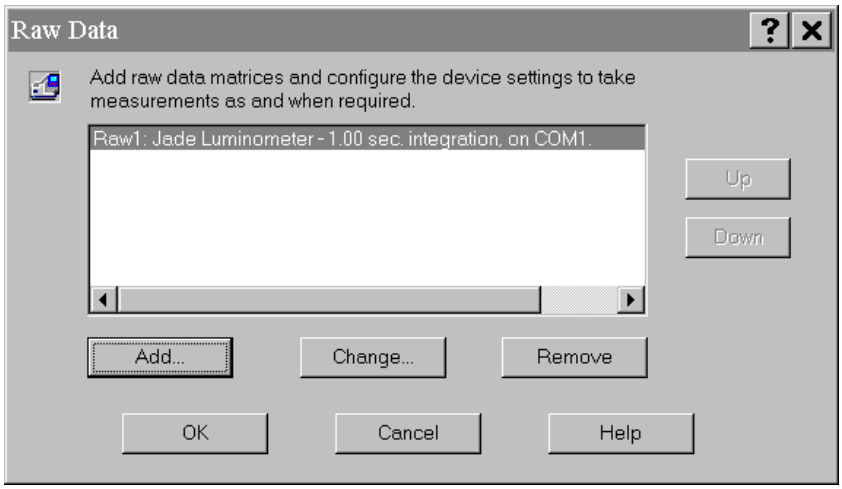


Figure 2 Raw Data Dialogue Box

Raw Data Items

Each raw data item represents a raw matrix of results acquired from an instrument.

In the simplest assay only one raw data item will be used. Click the **Add** button to add a new raw data item; this brings up the device select dialogue box. This is used to select a device to acquire the raw data from. Clicking the **Next** button displays the device configuration box that is specific to the selected device. Refer to your instrument manufacturer's manual for further information on device specific configuration.

Stingray allows multiple raw data items to be used. Each raw data item that is added will result in a separate matrix of raw data that will be available for inclusion in the final report and can be used as a basis for calculations.

For example, two raw data items could be set-up both using the same microplate reader but reading the plate with different labels (see Figure 3). These raw data items will be read in the order specified.

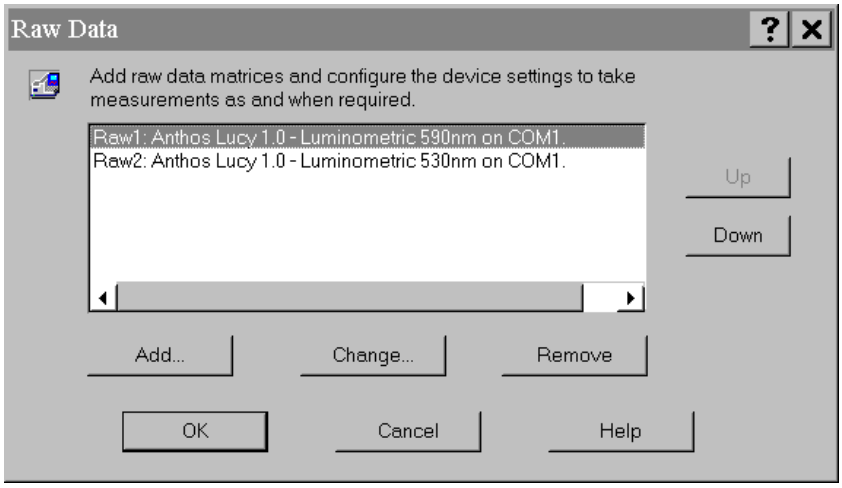


Figure 3 Dual Endpoint Raw Data Items

Multiple Labels/Matrices

Some configurations of devices, such as multiple labels, result in multiple matrices for a single raw data item. This is when a single readings command results in two or more matrices of raw data. Stingray extracts each matrix within the raw data item and makes them available for further analysis.

Limitations:

Only one raw data item can be used if multiple matrices are produced.

Stingray does not support scan mode with a plate repeat count.

Scan mode can be used with either multiple labels or multiple plates, not both.

Assay Master

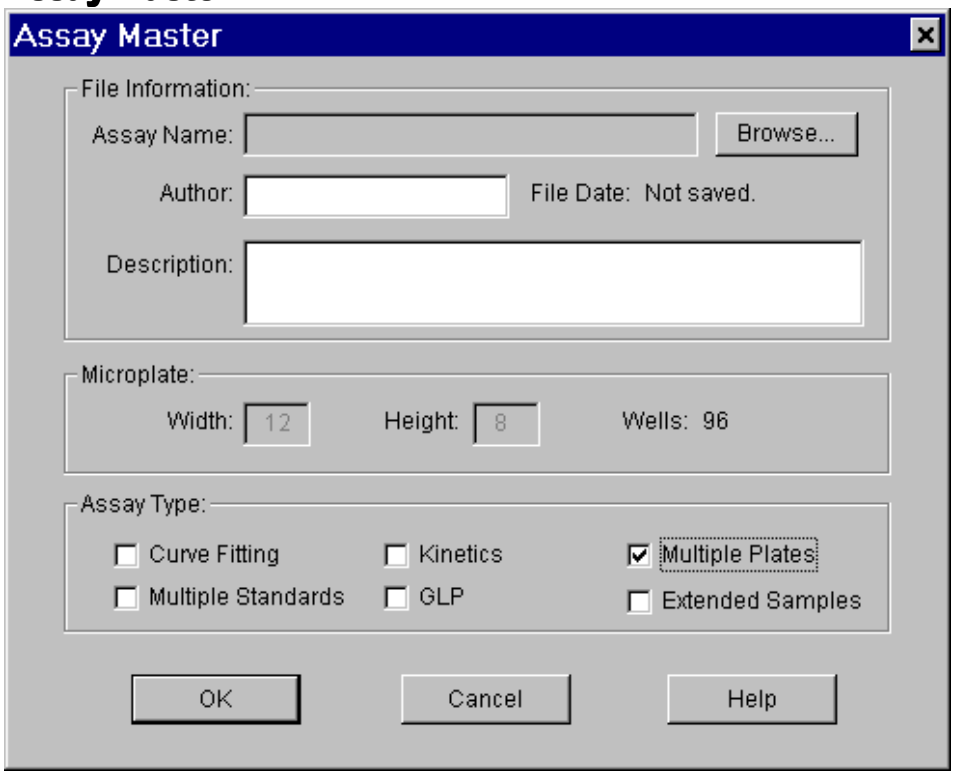


Figure 4 Assay Master Dialogue Box

File Information

Optionally enter information about the current protocol file.

Microplate

Stingray references wells on the template using letters and numbers. The letter indicates the template row and the number identifies the template column. For example, the well reference **C4** is the fourth well across and the third well down from the top left of the template. If the microplate has more than 26 rows then double letters must be used to refer to rows greater than row number 26. For example, **AA1** represents the first well on row 27, **AB3** represents the third well on row 28 and **BA14** represents the fourteenth well on row 53.

Assay Type

The Assay Type controls are used to add information about your assay and to set-up the protocol type. Simply check the required items to enable their use in the protocol. These options simplify navigation around the Stingray environment by hiding features that are not relevant for the protocol being created.

Curve Fitting

Tick this control if your protocol includes a curve fit.

Multiple Standards
Tick this control if you intend to use more than one set of standards in your protocol.

Kinetics
Tick this control if your protocol is kinetics protocol.

GLP
Tick this control if you wish to store user supplied text information with each run of the protocol.

Multiple Plates
Tick this control if this protocol will run across multiple microplates.

Extended Samples
For multiple plate protocols tick this control if all plates read after the first plate will contain samples only.

Template Configuration

The template layout must be configured to describe the group and type of each well on the *virtual* microplate. The well's group and type are used to identify which wells to use for calculations, automatic flagging, validations, cut-offs and reporting.

A well type describes how to fill each microplate well. Stingray well types include sample, standard, control, blank, spike or unused as required. Measurements are not taken or ignored in unused wells.

A well's number identifies which group it belongs to. Wells with the same type and number belong to the same group and each well is called a replicate. References in calculations to groups with replicates normally use the mean of the values of all the replicates.

Stingray 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.

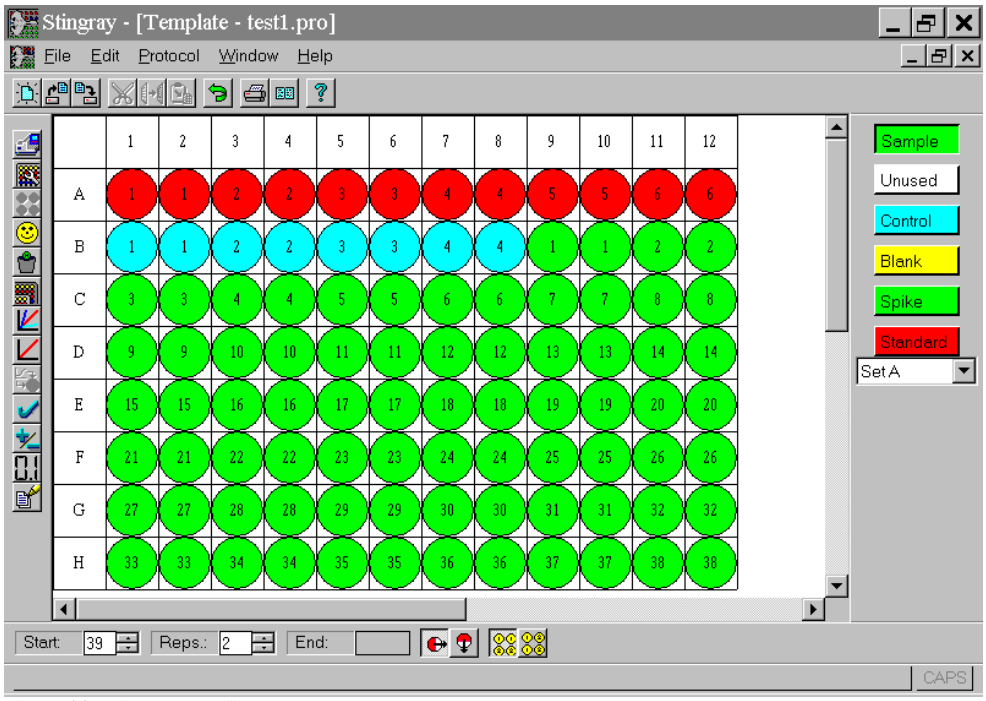


Figure 5 Template Layout Editor

To fill an area, specify the fill method and replicate direction using the number control bar located at the bottom of the screen.

Select a group type and select the wells to fill by clicking and dragging the mouse on the *virtual* microplate. Individual wells can be selected by a single click. The group start number increases as wells are set-up. To reset the group number to 1 double click on the text **Start:** in the numbering control bar.

If required double click on the group type button and the option to configure a user defined colour for that type is given.

To fill an entire plate with samples select the samples type button and single click in the space above letter **A** and on the left of **1** on the template. All wells will be filled with samples as specified by the number control bar.

To flag all wells select the unused type button and single click in the space above letter A and on the left of **1** on the template.

To undo the last change made click the undo button.

Printing Template Layout

The template layout can be printed using the **File | Print** command. A colour template printout is useful for reminding protocol users how to fill a microplate.

Multiple Plates

When creating a multiple plate protocol that does not use curve fitting the template layout specified is used for every plate read. Group numbering on subsequent plates is calculated from the previous plate. For example, if the first plate contains 10 samples then the first sample group on the second plate will be sample11. Thus, all of a group's members will be contained on the same physical microplate.

If curve fitting is used then there are two possible template layout situations depending on how the standards data is to be used:

LOCAL STANDARDS

In this situation all plates use the same template layout. Curve fitting transformations use standards data located on the same physical microplate.

Thus, any curve fit transformation for the first plate will generate a curve from standards data located on the first plate. Any curve fit transformation for the second plate will generate a curve from standards data located on the second plate, etc.

GLOBAL STANDARDS

In this situation standards data is only read on the first microplate. All curve fits in the protocol use the standards data from the first plate read.

On subsequent plates read Stingray converts the standard wells to sample wells - this avoids wasting microplate wells. All other well types behave as for local standards, i.e. blanks and controls will appear on every plate.

The numbering of the converted sample well groups starts from the highest sample number already on the plate. The standard ordering and number of members is preserved. Thus, the new sample groups will contain the same number of members and be in the same sequence and orientation as the standard groups.

Extended Samples

If using extended samples the template layout specified is used for the first plate read. All subsequent plates read will contain samples only. Use the Sample Fill Direction button (), located at the bottom of the display to specify how the samples will be filled on subsequent plates.

GLP

GLP is an acronym for Good Laboratory Practice.

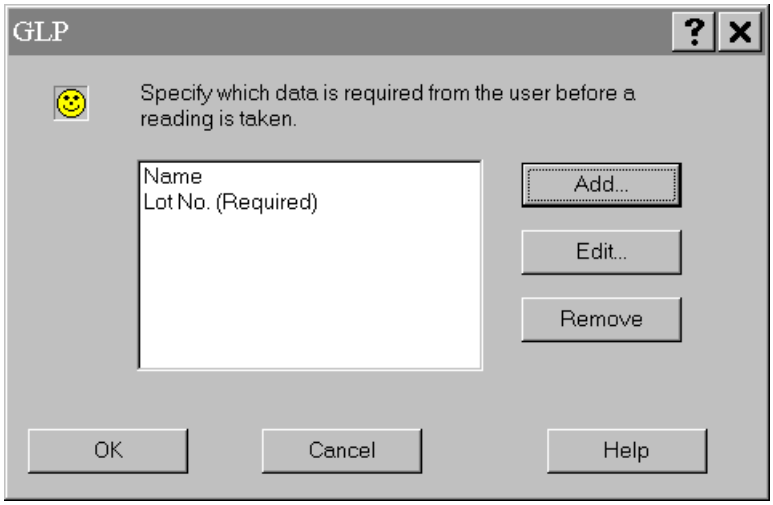


Figure 6 GLP Configuration Dialogue Box

This option allows you to define parameters that may be recorded by the user before the assay begins such as Name, Lot No., Reagent Lot, etc. The data entered by the user will be stored with the readings file and can be included in the report.

To add a new field click the Add button. Options may be either optional or required before an assay can be run. To require a user to enter data into the field before starting an assay tick the required box

Automatic Flagging

Automatic flagging automatically flags wells if the supplied conditions are satisfied. (If a well is flagged it will be ignored in any calculation and shown as flagged where relevant in the report.) Automatic Flagging is performed on raw data matrices or raw kinetic reductions only, before any other calculations are made.

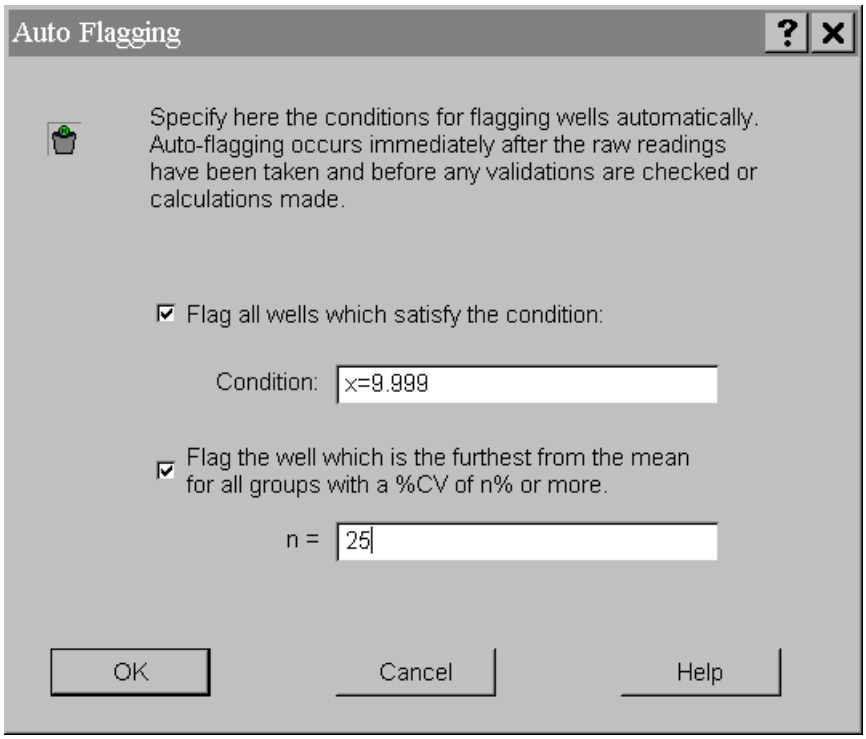


Figure 7 Automatic Flagging Dialogue Box

There are two methods of using Automatic Flagging:

1. Flag all wells that satisfy a given condition. Conditions are written using the standard expression grammar described further in Appendix A Expressions. All expressions must contain an *x* variable. This variable will be substituted by Stingray with a well's value. If the expression is evaluated true then the well will be flagged.

2. Flag the well that is the furthest from the mean for all groups with a percentage CV greater than a specified value.

Stingray performs auto-flagging from the top-left of the microplate, across and then down.

Table 1 below lists some examples.

Expression:	Effect:
`x = 9.999`	With this expression Stingray would flag all wells with a value of 9.999 in a raw matrix. This might be useful if the reader reports back a value of 9.999 to denote that a reading overflow occurred. Stingray would automatically flag all wells reported as overflow.
`x < mean (control1)`	With this expression Stingray would flag all wells that have a value that is less than the mean of all the replicates of the group control1. This would be useful for automatically spotting infected wells. In this example it is recommended that the control wells are placed at the bottom right of the microplate. Stingray performs auto-flagging from the top-left of the microplate, across and then down. If the control group members were at the top of the microplate members of the control group (when using replicates) would be flagged first. This would affect the mean value of the control group when the rest of the wells on the microplate are tested for auto-flagging.
`(x < standard1) or (x > standard9)`	With this expression Stingray would flag all wells that are outside of the range of the standards. Here it is assumed that each standard group has only 1 well. This expression would be useful if the curve fit being used is not valid for points outside the range of standards, i.e. if the fitted standards curve cannot be extrapolated.
`(x < (standard1 *0.9)) or (x > (standard9 * 1.1))`	With this expression Stingray would flag all wells that are 10% or more outside of the range of the standards. This expression would be useful if the curve fit method cannot be extrapolated by more than 10%.
`(x < mean (control1)) or (x > mean (control2))`	With this expression Stingray would flag all wells that are outside of the range of the control groups control1 and control2. Mean is used because each control group contains 3 replicates. However, caution must be made here since it is likely that some of the control wells will be flagged. For example, if control1 had 3 replicates and the values of each were 0.2, 0.3 and 0.4 then the mean of control1 is 0.3. By this expression Stingray would flag the first replicate of control1, 0.2 since it is less than 0.3.

Table 1 Auto Flagging Expression Examples

Method 2 might be used to remove anomalies automatically in replicate groups. It could be used in conjunction with a validation expression so that if the percentage CV were still over a specified percentage after 1 replicate was flagged then the assay would fail.

These options should be used with care to avoid removing too many results so as to invalidate the test. Details of Automatic Flagging calculations and flagged items are held in the Calculation Log and this can be included in the report to clarify why the wells are marked as flagged.

Transformations

Transformations are used to perform calculations on data matrices. A transformation takes an input matrix (such as a raw data matrix) and performs calculations to produce a new output matrix. Further transformations can be set-up using these new matrices. An unlimited number of transformations can be set-up.

Use the Transformation Manager (Figure 8) to create, edit and delete transformations. Use the Define Transformation Dialogue Box (Figure 9) to select the transform type and to set-up the names of the transform and the resulting matrix.

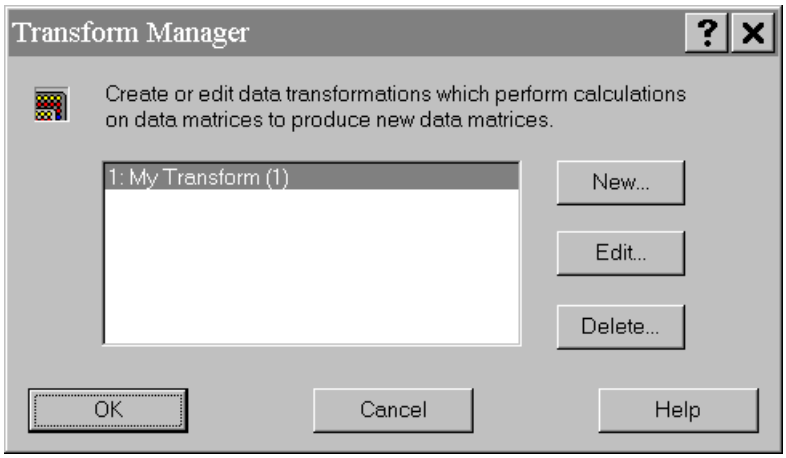


Figure 8 Transformation Manager Dialogue Box

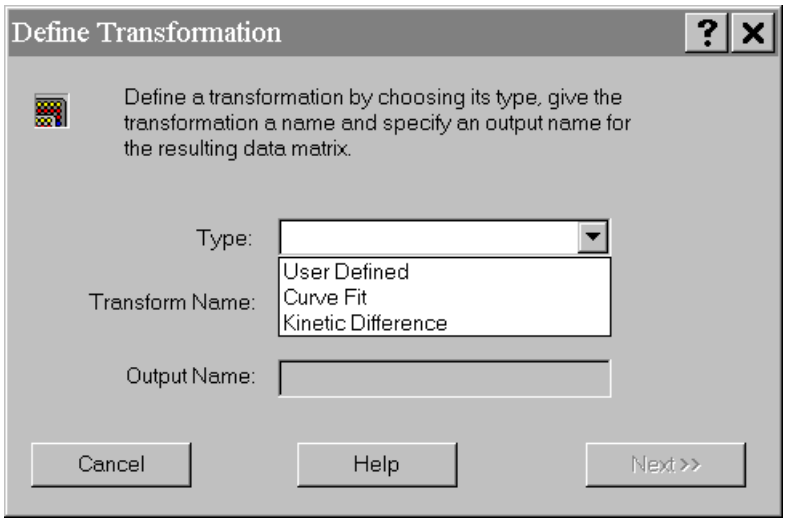


Figure 9 Define Transformation Dialogue Box

There are several different types of transformations available, depending on the protocol type.

User Defined Expressions
A user-defined expression transformation allows expressions composed of mathematical operators and references to wells on the input matrix to be entered to specify the transformation.

Curve Fit
A selected type of curve is fitted to the standards data and the resultant concentrations are calculated for all wells. This option is only available if Curve Fitting is enabled in the Assay Master.

Blank Correction
This transformation automatically sets up blank correction on a template by subtracting the mean value of the blanks from each sample raw data reading. This option is only available if there are blank wells defined on the template.

Matrix Difference
Use this transformation to quickly create a transformation which finds the difference between two matrices.

% Spike Recovery
This transformation is for the calculation of recovery of endotoxin spikes for LAL assays.

Competitive Bindings
This transformation is for the calculation of inhibition concentrations.

Kinetic Difference
This transformation is for the calculation of differences between two sets of kinetic data. Two sets of raw kinetic data are required.

Kinetic Mean
This transformation is for the meaning of replicated kinetic points.

User Defined Expressions

When defining a User Defined Expression Transformation a wizard step prompts whether to use auto-mean in the expressions. Use auto-mean if any reference to a group in your expressions refers to the mean value of all unflagged members of that group. Do not use auto-mean if you need to use other statistical operators on group data, such as *pcv, sd, var*.

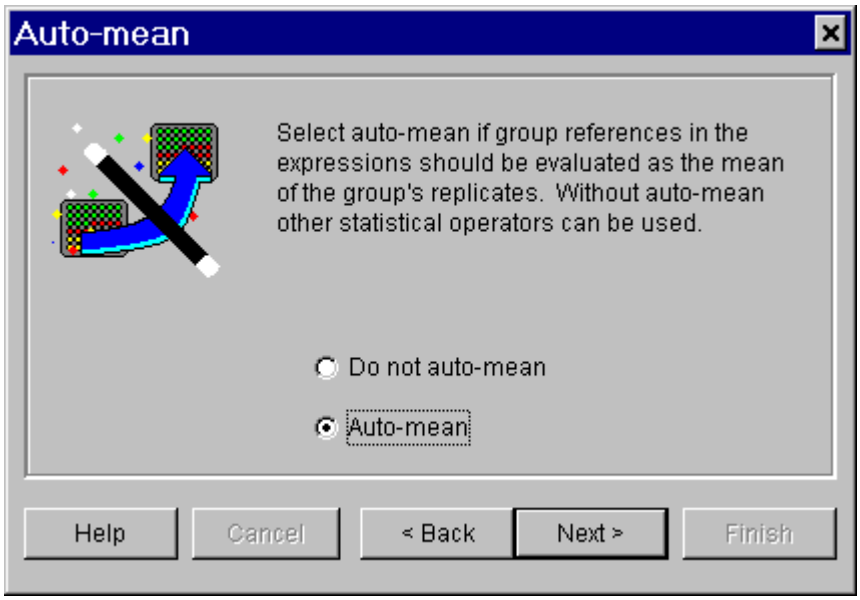


Figure 10 User Defined Expressions Auto-mean Dialogue Box

After defining a User Defined Expression Transformation the transformation expression editor will be displayed for the new transformation. To edit the expressions for a previously created user-defined transformation, right-click the mouse button on the template display to bring up the required transformation.

When editing an expression based transformation the transformation expression editor control bar will appear at the top part of the screen.

To specify an expression for a well you must first select the well or wells to store the expression in. This is done by either selecting them with the mouse from the transformation microplate view or by using the **Wells:** pull-down control.

The **x=** and **y=** pull-down controls are for specifying which matrix to use for variable reference. The **x=** pull-down control defaults to the transformation input matrix.

Once the target wells have been selected enter the expression into the **x'=** control and press return. If the expression is valid the expression editor view will be updated. If there is a syntax error in the expression a notification message will be displayed.

Figure 11 shows the editing of a transformation that finds the difference between **Raw1** and **Raw2**.

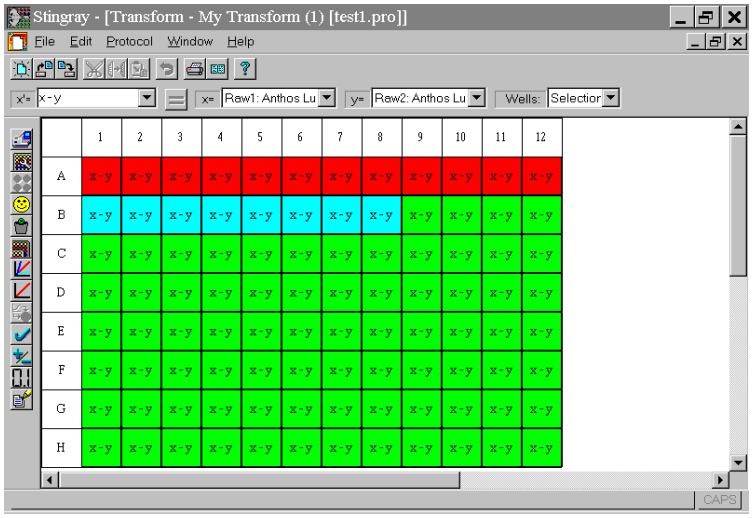


Figure 11 Transformation Expression Editor

Table 2 below lists some expression examples and explanations for a brief guide to user-defined expressions. Refer to Appendix A Expressions, for more information on expressions.

If a well does not have an expression associated with it then the value of that well in the transformed matrix will be the same as the value in the input matrix.

For clarity it is recommended that brackets are used in more complex expressions.

Transformation Expression:	Explanation:
`x*10`	If this expression is stored in well A1 then the calculation carried out will be the value of well A1 (in the matrix specified by the x= control) multiplied by 10. The result will be placed in the new matrix, the name of which was specified when the transformation was set-up.
`x – control1`	This expression contains a reference to control1. Before the result is calculated Stingray will parse this to an expression containing explicit well references. Thus, this expression depends on the well that it is in (the x value) and the location of the well or wells of control1. If the control1 contains more than 1 well then the mean of these wells will be used (assuming we are using auto-mean). The = button on the transformation expression editor control bar will display the entered expression with the explicit well references. This feature can be used to check that expressions are syntactically correct.
`sample1 - blank1`	This example simply subtracts the value of the group blank1 from the group sample1 and stores the result in the well it is stored in.
`samplen - blank1`	This example subtracts the value of group blank1 from the current sample group. If this expression was applied to all sample wells then when the expression is parsed the group number of the current well will replace samplen. For example if well A1 contained this expression and well A1 was a well of sample1 type then the expression would be sample1 - blank1. Similarly if the well at which the expression was stored was of sample2 type then the expression would be sample2 – blank1.
`gn - blank1`	This example behaves like the previous one except that the group and number of the well in which the expression was stored replace gn. This is useful for specifying an expression to apply to a whole plate.
`gn - blankn`	If this expression was stored in every well on a microplate and there are an equal number of sample and blank groups then the resulting transformation would be a background correction using separate blank wells for each sample group.
`sd(gn)`	This expression could be used to calculate the standard deviation of all groups on the template. This expression should be stored in every well on a microplate and we must specify not to use auto-mean. Each well in the resulting transformation will contain the calculated standard deviation of the unflagged members of its group.
`var(gn)`	Similar to the previous example, but calculates the variance of each group.

Table 2 Transform Expression Examples

Curve Fit

When setting up a curve fit transformation follow the transformation wizard to set-up the required parameters for the curve fit.

CURVE FIT METHOD

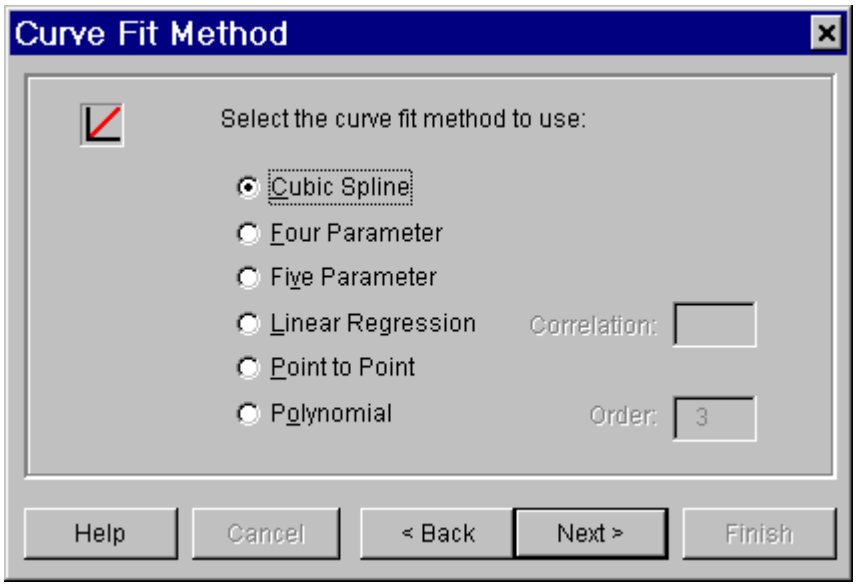


Figure 12 Curve Fit Method Dialogue Box

Linear Regression

This fit finds the line that best represents the linear fit of the data points. Linear regression is performed on all of the replicate points (not the means of each standard). Stingray uses the least squares method of linear regression.

Correlation Validation: Either a maximum or minimum figure can be selected for the correlation coefficient of the linear regression. If the validation fails then Stingray will display a warning message after the results have been calculated.

Point to Point

A line is fitted between each point for this method of curve fitting. Point-to-point uses the mean of each standard group.

Cubic Spline

This fits a cubic spline to the data. Cubic spline uses the mean of each standard group.

Polynomial Regression

A polynomial regression line is fitted to the data to the order specified, e.g. 2nd order (quadratic), 3rd order (cubic). Polynomial Regression uses the mean of each standard group.

For polynomial regression the mean squared error is calculated as follows:

$$mse = \sum_{i=0}^{n-1} \left| z_i - y_i \right|^2 / n$$

Four Parameter Fit

Stingray uses the Levenberg-Marquardt algorithm to determine the least squares set of coefficients that best fit the set of the mean of the standard points as expressed by the non-linear four parameter equation:

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

(A, B, C and D are the determined coefficients)

This fit method is suitable for sigmoidal plots that are symmetrical about C. The four-parameter fit uses the mean of each standard group.

Five Parameter Fit

Stingray uses the Levenberg-Marquardt algorithm to determine the least squares set of coefficients that best fit the set of the mean of the standard points as expressed by the non-linear five parameter equation:

$$y = D + \frac{A - D}{\left[1 + \left(\frac{x}{C}\right)^B\right]^M}$$

(A, B, C, D and M are the determined coefficients)

This fit method is suitable for sigmoidal plots that are not symmetrical about C.

The mean squared error for the four and five parameter fit methods is computed using the following formula:

$$mse = \frac{1}{n} \sum_{i=0}^{n-1} [y_i - f(x_i, a)]^2$$

EXTRAPOLATION

Extrapolation should be avoided in most applications. However, extrapolation is possible with any of Stingray's fit methods and should be used only with great caution.

Extrapolation with polynomial or cubic spline can cause serious problems because these curves may have one or more turning points outside the range of standards. This means that there may be multiple x values for a given y value. Stingray will use the first value x value it finds and this may lead to unexpected results.

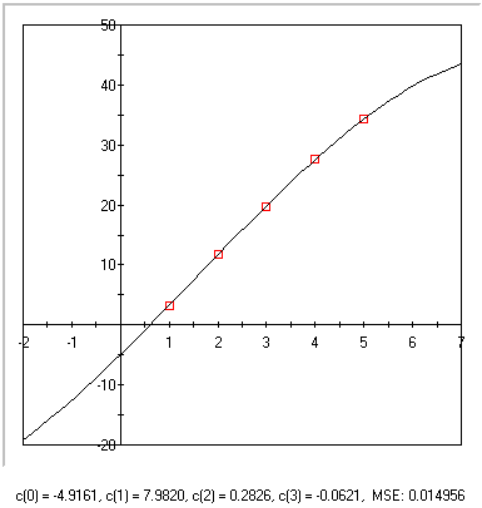


Figure 13 Polynomial Regression Example (1)

Figure 13 illustrates and example of this situation. Here polynomial regression using the 3rd order generates a curve through the standards points. Stingray will interpolate within the range of the standard x values (i.e. from x=1 to x=5) and determine the expected y value.

However, if we try to calculate the x value when y is 40 Stingray will unexpectedly report a negative number. To understand why this is happening we need to change the plotted x range of our standards graph.

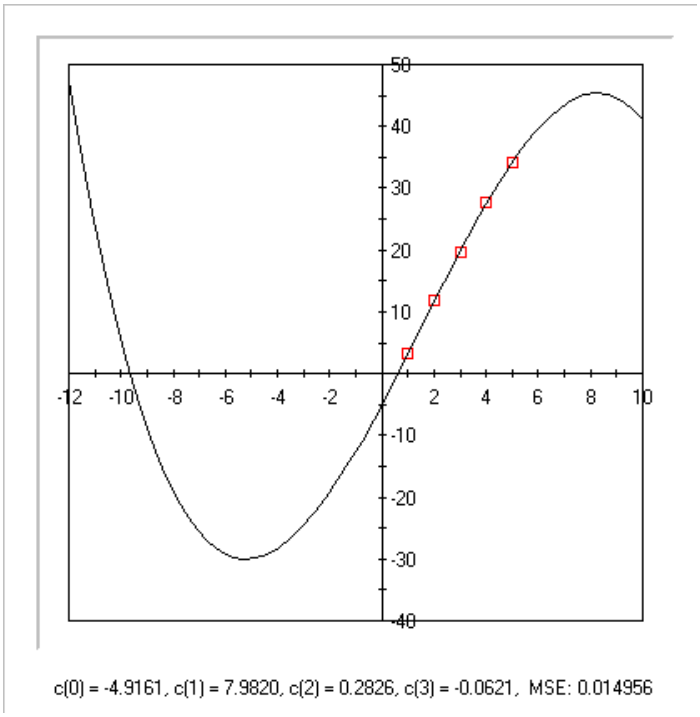


Figure 14 Polynomial Regression Example (2)

From Figure 14 we can see that there are 2 x values when y is 40. Stingray will report the first x value that it finds.

Remember, extrapolation should be used with extreme caution. To avoid this situation use an auto-flagging expression to mark all readings outside of the interpolation range.

For example, in the situation of 5 standards with ascending concentration values, the following auto-flagging expression:

```
(x < (standard1*0.95)) or (x > (standard5*1.05))
```

allows extrapolation but only within a 5% range of the curve. All points outside of this range would be marked as flagged.

Notes

There is a possibility with certain data sets that when calculating an x value from a y value using a cubic spline fit that Stingray will only give a lower bound on the number of solutions.

The four and five-parameter fit methods are not suitable for fitting straight-line data - the default linear regression will give an excellent fit in this case.

Due to the non-linear nature of the four and five parameter fit functions, for some data sets there may exist multiple fits with significantly different parameter values but similar MSE's.

It is recommended that users should seek advice from statistical or mathematical specialists when using the more complicated curve fitting routines (higher order polynomial and four and five parameter non-linear regression).

When comparing fits with other statistical packages consider Stingray's use of the mean of replicate points and method of MSE calculation.

LOG CURVE DATA POINTS

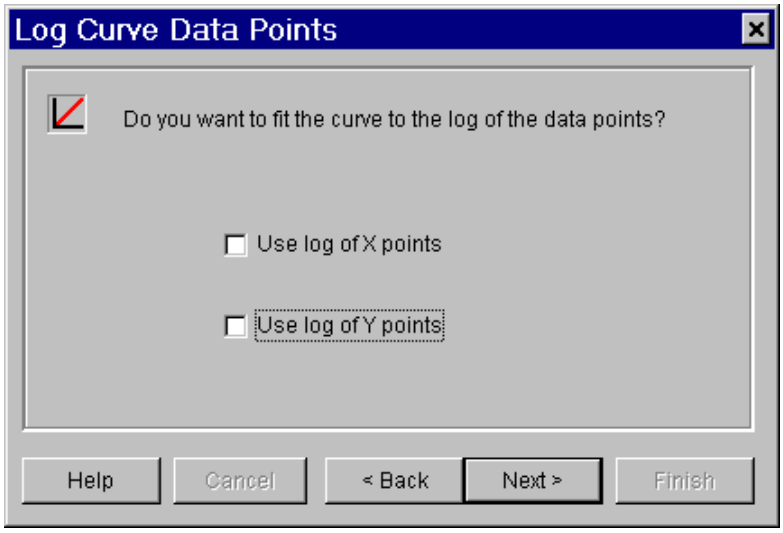


Figure 15 Log Curve Data Points Dialogue Box

This curve fit transformation wizard step asks whether the data to fit should be logged before fitting and anti-logged when calculated. See Appendix B Stingray Logarithms for more information.

GRAPH AXES AND TITLES

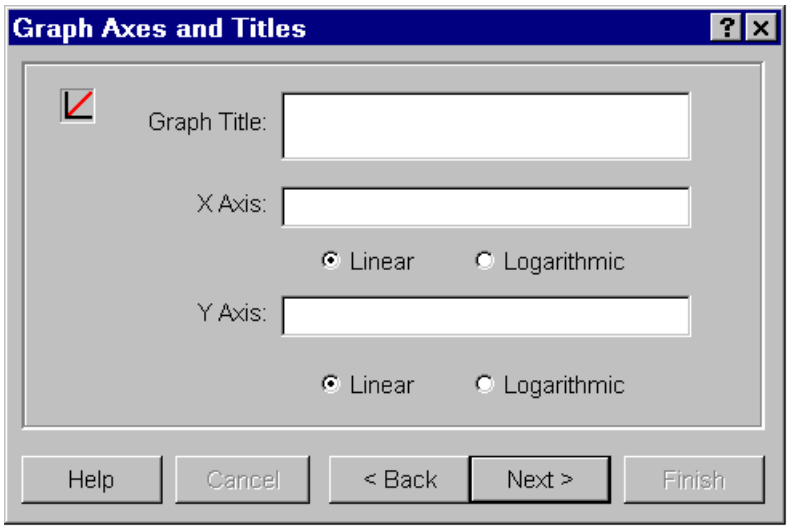


Figure 16 Graph Axes and Titles Dialogue Box

Choose the graphs axes types (linear or logarithmic) and enter titles for the axes and graph.

The axes type settings are for viewing purposes only. Settings here do not affect calculations made.

If data is spread over a wide range of numbers it is useful to project the graph using logarithmic axes.

See Appendix B Stingray Logarithms, for more information about logarithmic data and views with Stingray.

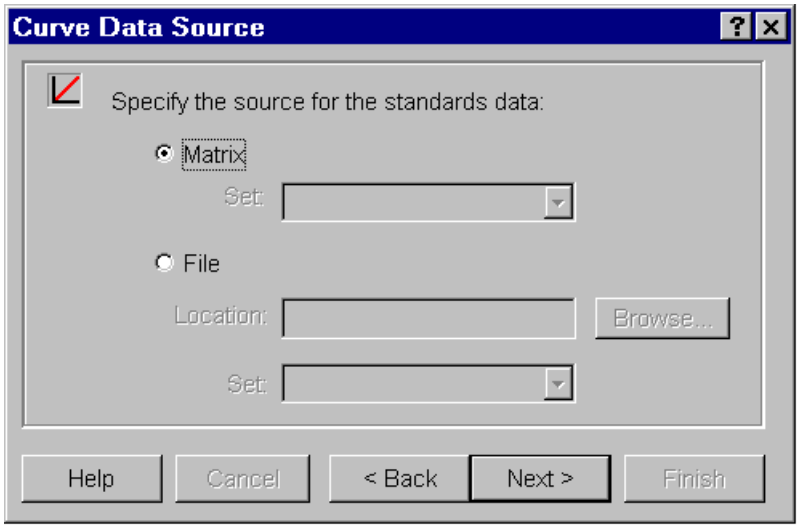


Figure 17 Curve Data Source Dialogue Box

Use this dialogue box to select the source of the data to be used to plot the curve. The standards data used for the curve fit can be on the plate or in a file.

If the data for the curve is read from standards on the microplate then the points to fit will be made up of the y values read for each defined standard and the x values specified in the protocol file.

If there are multiple standard sets on the plate then choose which standards set should be used for this transformation.

If the data is stored in an archived standards file then select the file and choose the standards set (standards files may contain more than one standards set).

Blank Correction

There are several different methods for performing blank correction. The required method will depend on the protocol being set-up. The Stingray blank correction wizard will guide you through setting up a blank correction transformation.

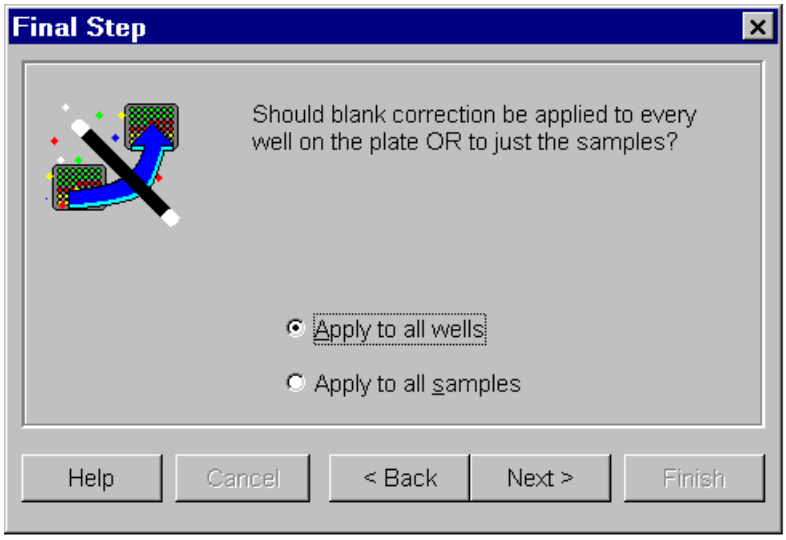


Figure 18 Blank Correction Wizard Dialogue Box

There are 2 main types of blank correction which depend upon the template layout:

The first type is where all groups or wells on the template will have the value of a single blank group removed.

The second type is where every sample group has a corresponding blank group. Each sample well will have the value of its corresponding blank group removed.

With the second type of blank correction if the plate layout contains samples with 2 or more replicates then there are 2 methods of blank correction. The Wizard will ask whether to remove the mean of the blank group from the **mean** of each group or from each **well**.

By choosing **mean** the value of each blank group is subtracted from the **mean** of each sample group. For example, if there are 2 replicates in sample1 then Stingray will calculate the mean of these samples and subtract the value of the blank group from this value. The result will be placed in both sample1 wells. This method is only useful if the blank correction is the last transformation and the mean results are required.

When **well** is selected the value of the blank group is subtracted from each and every well. As in the previous example, if there are 2 replicates in sample1 and each well has a different value, the blank correction will remove the value of blank1 from each well and the resulting sample1 wells will be different. This method is the more common method to use.

The wizard will look at the template layout and display the relevant options. Follow the blank correction wizard to set up the blank correction required for the protocol.

Depending on the blank correction method being used the wizard may ask whether to perform blank correction on all wells on the plate or to the samples only.

If blank correction is performed on all wells then the values of the blank group in the blank corrected matrix may be zero or negative. This is because if you remove the background count from the background count the result is zero. Also, if the blank group contains 2 or more replicates then when the mean of this group is removed from each member of the group then 1 or more blank wells will result in a negative value. Negative or zero wells may then be flagged if a curve fitting transformation is being used which cannot use negative or zero values (e.g. if a logarithmic axis is being used).

Matrix Difference

Follow the matrix difference wizard to create a transformation that finds a well-by-well difference between two matrices.

% Spike Recovery

This transformation is for the calculation of recovery of endotoxin spikes for LAL assays.

To set-up a % Spike Recovery transformation, create a template with an equal number of sample and spike groups. Add a % Spike Recovery transformation and enter the known endotoxin concentrations (NCS) for each defined spike group.

When the results are calculated, the following expression is evaluated on each sample well:

```
100 * (spiken - samplen) / ncs
```

This determines the % Spike recovery for each sample well.

Competitive Bindings

This transformation is for the calculation of inhibition concentrations. The transformation is available if the template layout has sample groups each containing 4 or more replicates. All sample groups must have the same number of replicates. Sample well replicates are adjacent and are filled horizontally or vertically.

For the transformation wizard set-up enter concentrations for each replicate member. These replicate members can themselves be treated as replicates by entering equal concentration values for different replicate members.

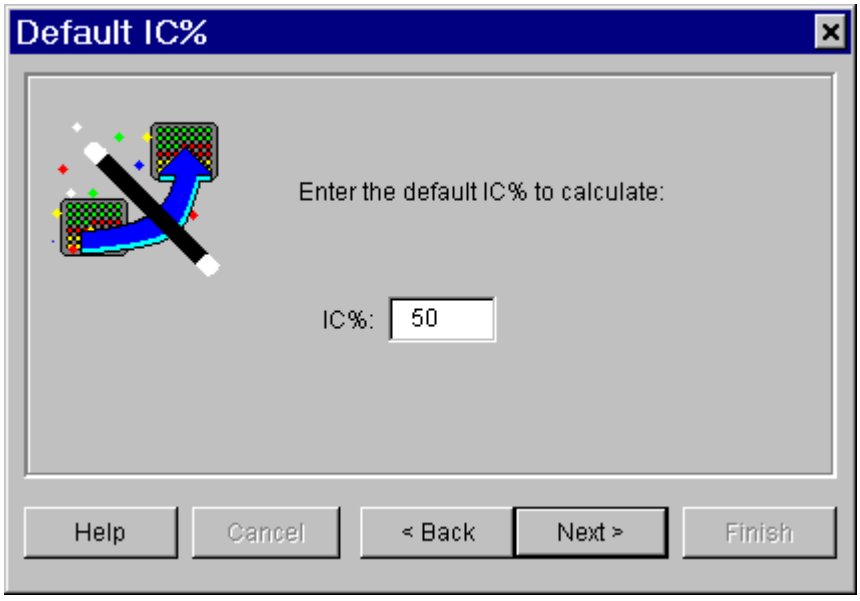


Figure 19 Default IC% Dialogue Box

Enter a default IC % value to be calculated for each sample group.

When readings have been made the four parameter fit method is used to fit a curve to each sample group using its unflagged replicates and the specified concentrations.

The Transformation calculates % bound for each sample well using the equation:

```
(Reading/(A-D)) * 100
```

Here, A and D are the results of the four parameter fit. For non-sample wells the result of the transformation is 0.

For competitive assays a competitive table is added to the report and each competitive graph read on the first plate in the assay run.

Kinetic Difference

Follow the matrix difference wizard to create a transformation that finds a point-by-point difference in all wells between two kinetic matrices. The result of this transformation is a further kinetic matrix.

Kinetic Mean

This transformation is for a kinetics protocol with replicates. The transformation finds all members of a group (its replicates) and creates a new kinetic graph that will be the same for each member. The new kinetic graph is made up of the points that are the mean of each reading for each cycle. If all points in the replicate groups are flagged the calculated point is flagged. Otherwise the unflagged points are used and the result is always unflagged.

This transformation method is made available when creating a kinetics protocol with replicated samples.

Standards

If standards are defined on a template a value must be assigned to the x value.

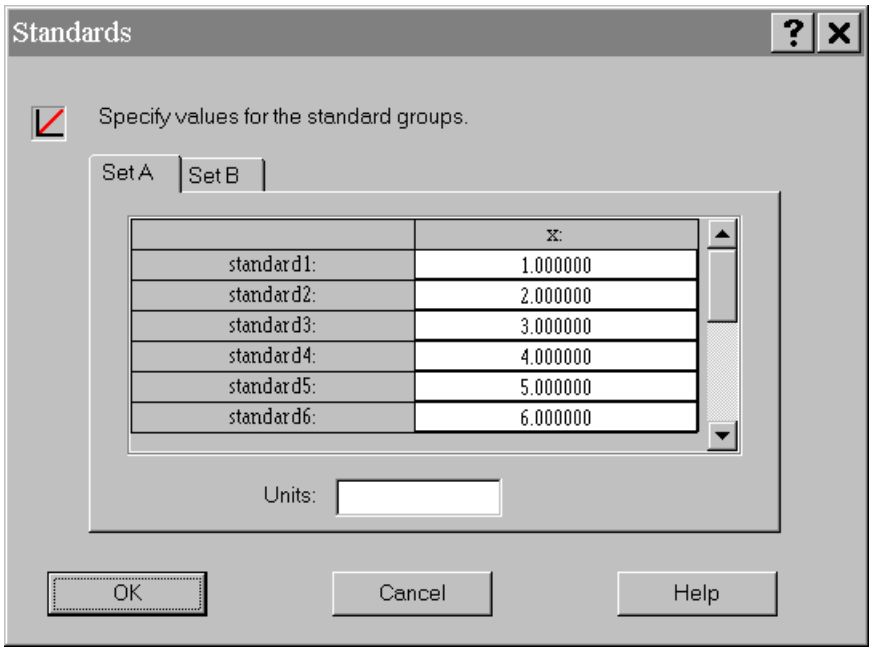


Figure 20 Standards Entry Dialogue Box

Double clicking a cell in the grid and typing a number into the dialogue box enters standard values. Press return to move to the next standard and press Esc to cancel editing.

Enter the units into the Units box. Any text can be used.

If multiple standards are in use then values need to be defined for each set. Select the appropriate set tab to change the displayed standards.

Multiple Standards

Multiple standards can be read from a single plate. If multiple standards are to be used on a single plate then you can specify names and colours for each standard set. Default names and colours of Set A and Set B are provided.

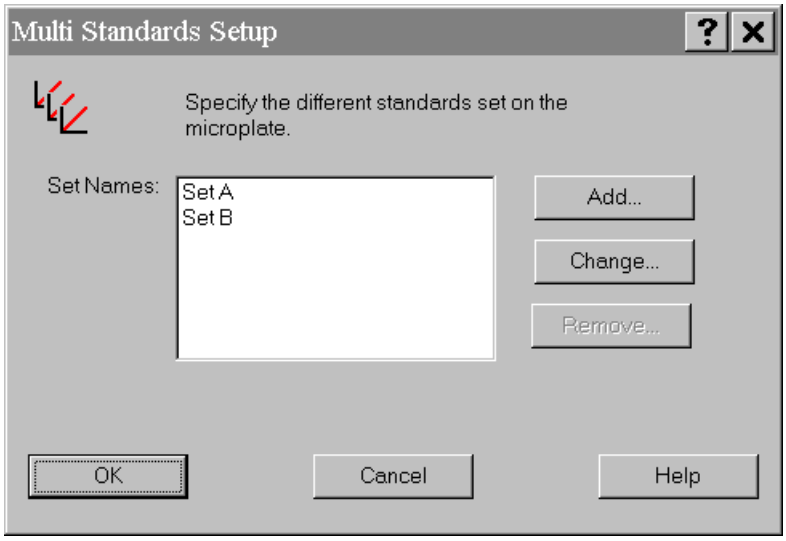


Figure 21 Multiple Standards Set-up Dialogue Box

Click on the Standards Sets control button and add the names of the sets. In the group control bar there will be a pull down control under the Standards group button. This will hold all of the names of the defined standards sets.

If there is a black button in the group control bar then you must specify the colour for this set of standards. Simply double click on the black button and choose an appropriate colour.

Reduction Methods

A reduction method performs some calculation on all readings within a well and results in a single number that is associated with the well. Reduction method is applied to all unflagged wells. The same reduction method is used throughout the protocol.

Reduction results can be used as input data for Stingray transformations.

The reduction methods available depend whether the protocol takes kinetics or scan readings:

Kinetic Reduction Methods

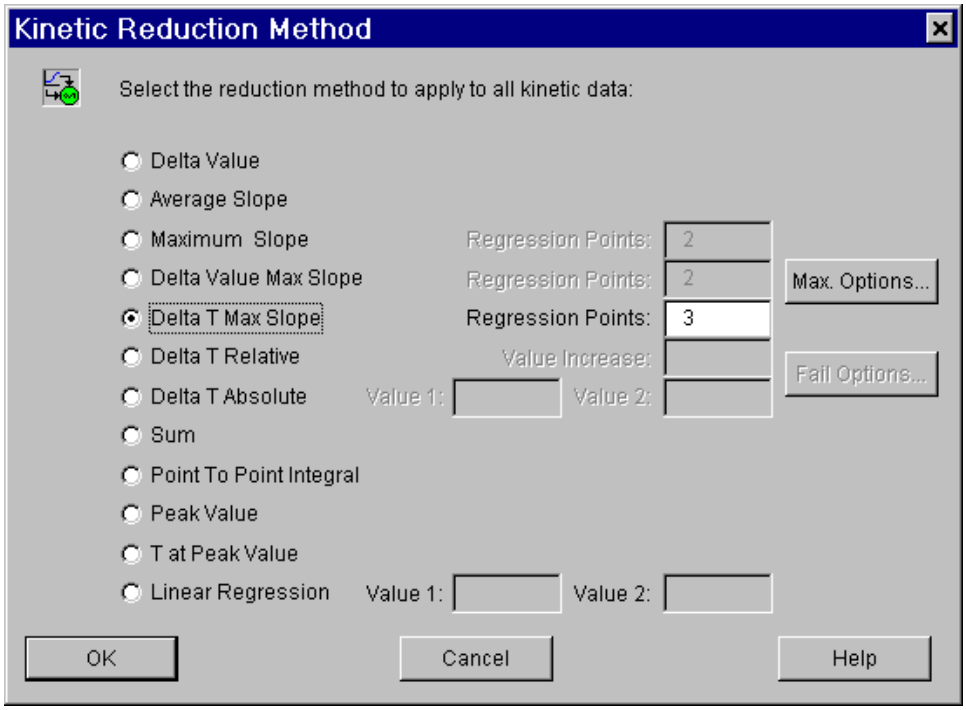


Figure 22 Kinetic Reduction Method Dialogue Box

All reduction methods ignore flagged kinetics points in all calculations. There are twelve kinetic reduction methods available:

DELTA VALUE

This reduction measures the difference between first and last kinetic reading.

AVERAGE SLOPE

Outputs the results in Value/sec. between first and last kinetic reading.

MAXIMUM SLOPE

Determines the maximum slope of the kinetic curve in Value/sec. This is the maximum rate of reaction. Maximum slope is found with linear regression. The number of points to be used for each linear regression can be specified.

E.g.: If you have 5 kinetic cycles and specify 3 regression points 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. Then the software finds which set of points produces the maximum slope and uses this to calculate the results.

DELTA VALUE - MAXIMUM SLOPE

Finds difference in value between the first kinetic measurement and the centre of the maximum slope. Maximum slope is calculated as before.

DELTA T - MAXIMUM SLOPE

Finds difference in seconds between the first kinetic measurement and the centre of the maximum slope. Maximum slope is calculated as before.

DELTA T - RELATIVE

This reduction determines the time in seconds taken for the kinetic reaction to reach a pre-set change in value. Value change is set in the Value increase box that appears when the reduction type is selected.

Fail options are available for this reduction method (see below).

DELTA T - ABSOLUTE

Determines the time taken for kinetic reaction to go from one pre-selected time (threshold 1) to another (threshold 2). When reduction type is selected enter the start and finish values in the respective boxes.

Fail options are available for this reduction method (see below).

SUM

This reduction is the sum of all readings taken on each well.

POINT-TO-POINT INTEGRAL

This reduction method finds the area under the kinetic chart using a point-to-point chart of the unflagged points.

PEAK VALUE

Measures the peak value of the reaction.

T AT PEAK

Measures the time taken to reach the peak value of the reaction.

LINEAR REGRESSION

This reduction method finds the linear regression using the unflagged points within the specified range. The time range is specified using parameters Value 1 and Value 2. These values are times and are relative from the first reading made for each well. The result of the reduction method is the slope of the linear regression.

For example, readings in well A1 may start at time 22. If value 1 = 10 and Value 2 = 20 then the linear regression will be found of points within the time range of 32 and 42 seconds.

Kinetic Reduction Maximum Slope Options

For reduction methods that use the calculated maximum slope click the **Max. Slope…** button to choose the way the maximum slope is determined.

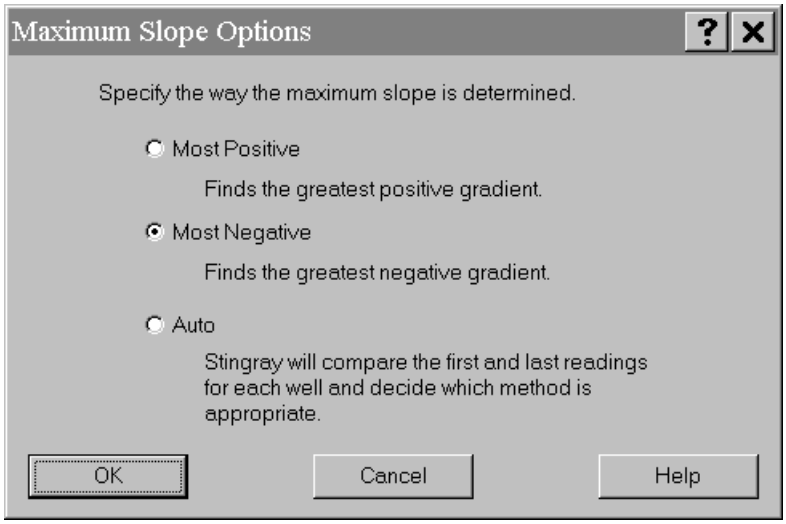


Figure 23 Kinetic Reduction Maximum Slope Options Dialogue Box

If the maximum slope required is the slope at the point where the kinetic readings increase the most over time, choose **Most Positive.** If the maximum slope required is the slope where the kinetic readings decrease the most over time, choose **Most Negative.**

Choosing **Auto** tells Stingray to look at the difference between the first and the last cycle read for each well to reduce. If the difference shows that the readings decrease over time the **Most Positive** method is used, otherwise the **Most Negative** method is used.

Kinetic Reduction Fail options

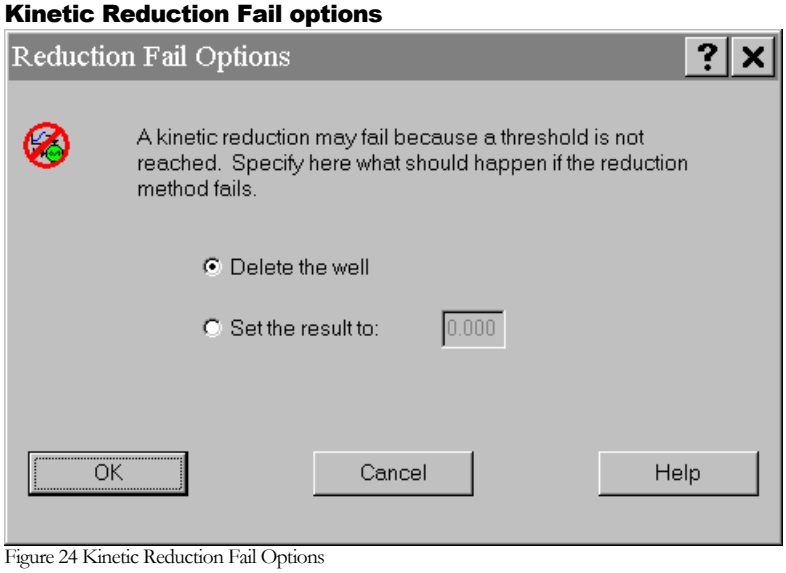


Figure 24 Kinetic Reduction Fail Options

The reduction methods Delta t relative and Delta t absolute may fail if the threshold(s) are not reached. In this case there are two options available.

The well can be flagged or the well can be assigned a specific value. If a specific value is assigned then a cut-off expression could be added which detects this case and labels the well in the report, if required.

Scan Reduction Methods

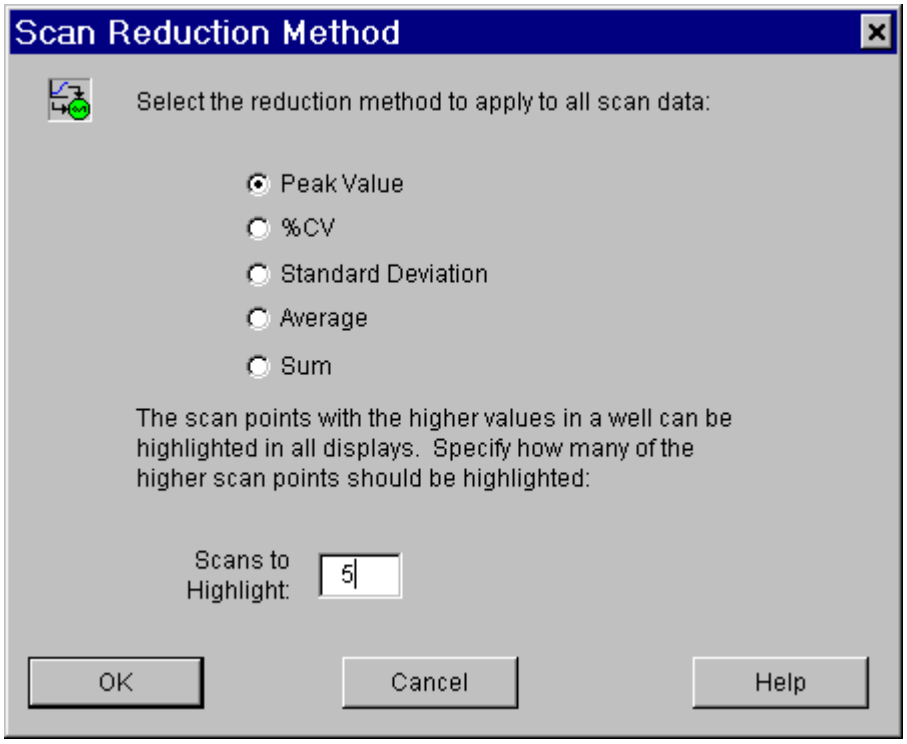


Figure 25 Scan Reduction Methods Dialogue Box

All reduction methods ignore flagged scan points in all calculations. There are five scan reduction methods available:

PEAK VALUE

The result of this reduction method is simply the highest scan point read.

%CV

Calculates the percentage coefficient of variance of the scan points.

STANDARD DEVIATION

Calculates the standard deviation of the scan points.

AVERAGE

Finds the average of the scan points.

SUM

Totals all scan points.

Scans To Highlight

Use the **Scans To Highlight** option to specify how many of the higher scan points to highlight in the scan bar view.

Validations

Use the validations control to specify validation expressions for each or any configured matrix. Simply enter expressions that must be true for the protocol to be valid. If the auto-mean check box is ticked then any references to groups (e.g. *sample1*) with more than one well will be 'mean'ed before the expression is evaluated. Disable auto-mean to use other types of evaluation, such as *pcv* (percentage CV), or *sd* (standard deviation) as part of the validation expression.

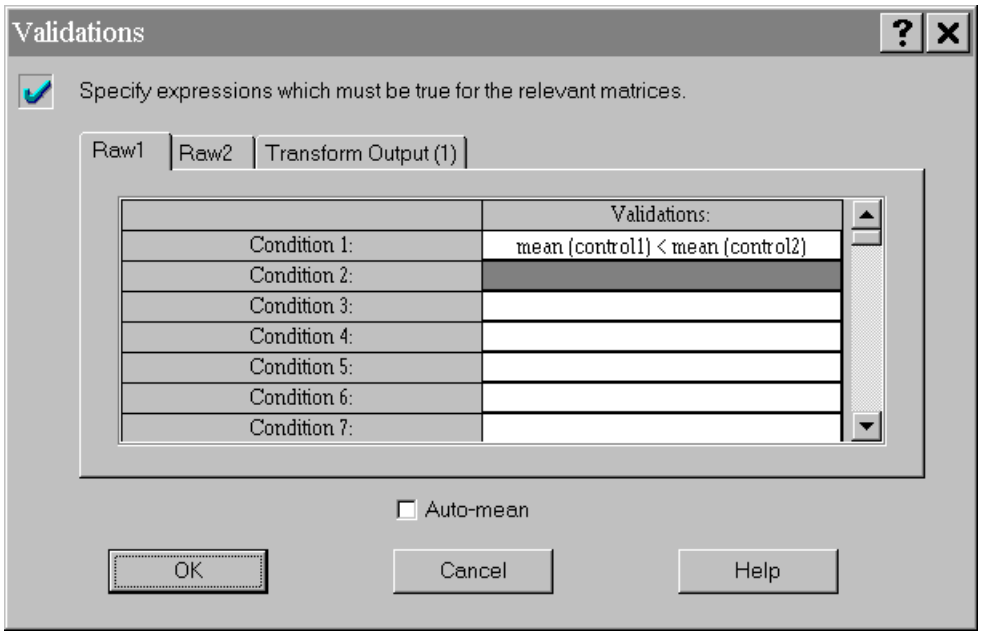


Figure 26 Validations

If any of the validations fail after (or during - for single tube readers) the reading process the user will be informed.

Table 3 below some examples of validation expressions, in all examples auto-mean is disabled.

Validation Expression:	Explanation:
`mean(control1)<mean(control2)`	The mean of control1 must be less than the mean of control2.
`pcv(control1)<30`	The percentage CV of control1 is less than 30%.
`mean(standard1) not mean(standard2)`	The mean of standard1 is not equal to the mean of standard2.
`mean(control1)<=mean(control2)`	The mean of control1 is less than or equal to the mean of control2.
`(mean(standard1)<mean(standard2)) and (mean(standard2)<mean(standard3))`	The mean standard1 is less than the mean of standard2 and the mean of standard2 are less than the mean of standard3. Note, it would be simpler to split this expression into two validations.
`sd(control1)<2`	The standard deviation of all members of control1 is less than 2.
`var(control1)<2`	The variance of all members of control1 is less than 2.

Table 3 Validations Expressions Examples

Cut-Offs

Specify expressions and labels to describe the results of data for each well or group. When Stingray generates cut-off labels it goes through all cut-off expressions testing a numeric value with the expression.

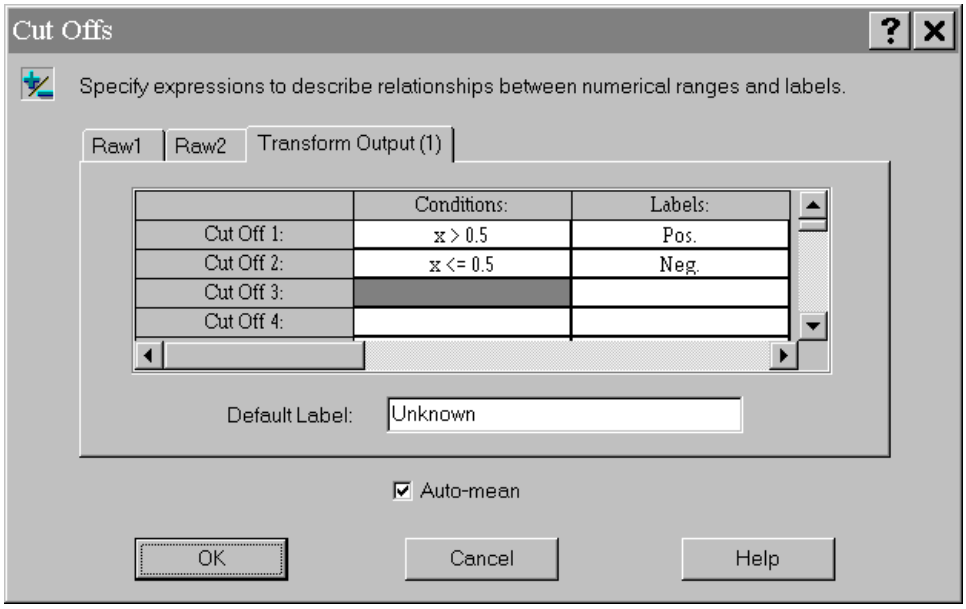


Figure 27 Cut-Offs Dialogue Box

Every cut-off expression must contain an *x* value that is replaced by the number to test. Stingray replaces the *x* in each cut-off expression with the number to test for. If the resulting expression is true then the well or group is associated with the matched expression's label.

For example, if the cut-off expression is *x < 0.5* and the label is *Neg* - then when the cut-off is determined for a well A1 who's value is 0.2 well A1 will be given the label *Neg*.

If there is more than one expression that is true then Stingray will include all of the labels of satisfied expressions. This is useful for defining grey areas.

Expression	Label
`x < mean (control1)`	Neg
`x > mean (control1)`	Pos
`(x < (mean (control1) * 1.1)) and (x > (mean (control1) * 0.9))`	Borderline

Table 4 Cut-Off Expression Examples

In this simplified example a well or group would be labelled either *Pos* or *Neg* depending on its value and the values of the replicates of control1. However, if the well or group were within a 10% range of control1 then it would ALSO be labelled *Borderline*.

If none of the cut-off expressions are true for a particular value, the default label is the resulting label.

As in the validation expressions, if auto-mean is enabled then the use of *sd,* or *pcv* is not available and all group references are mean by default.

See Appendix A Expressions, for more information on syntax and grammar.

Numerical Formatting

The resulting data from an assay run can be formatted for display purposes. Click the numerical formatting control button to specify how to format the numerical data for each matrix.

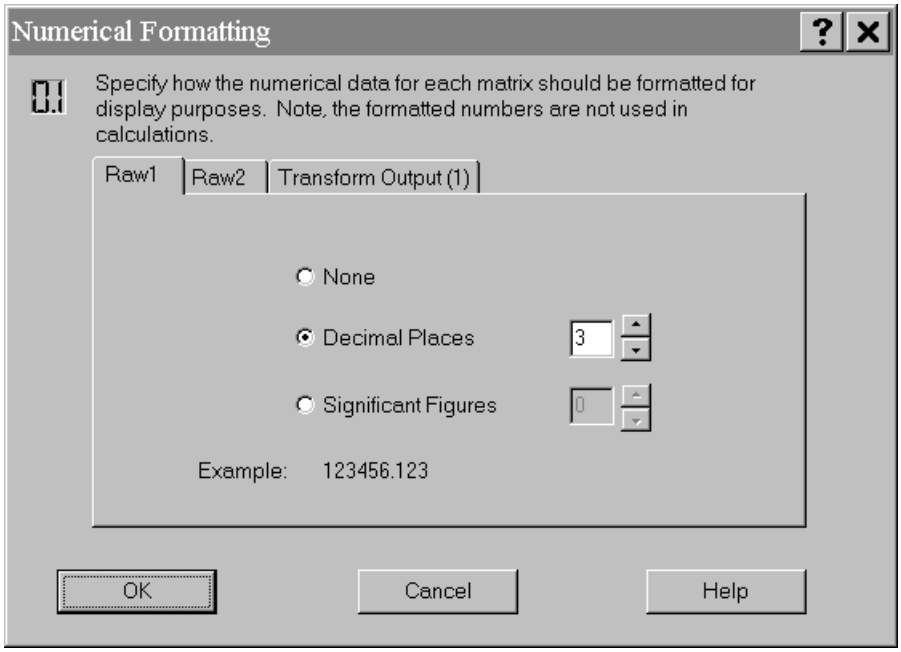


Figure 28 Numerical Formatting Dialogue Box

It must be noted that these settings are for display purposes only. No calculations are made using rounded data.

Report

The Report option is used to configure the contents of the final report. This report may be viewed on the screen or printed out after the readings have been taken.

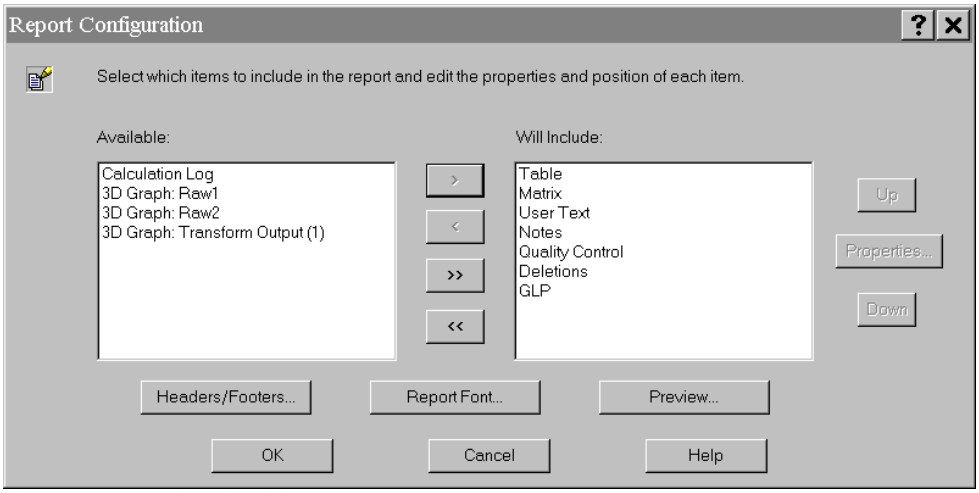


Figure 29 Report Configuration Dialogue Box

There are two list controls in the Report Configuration.

Available
Items shown here are those that can be included in the report but are not currently included.

Will Include
Items displayed in this category are currently selected to be included in the final report. Items will be included in the order displayed.

The items available for inclusion in the report will vary depending on the protocol. Table 5 below, describes each item that may be included the report. To edit the way an item is displayed in the report, select the included item and click the **Properties** button.

Report Item:	Explanation:
Table	A table of the raw and calculated data, including cut-offs, well references of group members and the group's percentage CV. Data in a table is displayed and calculated by group (e.g. sample1), specified in the leftmost column. The sample's ID replaces the group if ID's are specified after the protocol has been executed. Calculations are made using the mean of each group. Cut-offs are generated by using the mean of each group. Flagged wells are ignored in calculations and marked by a line through flagged wells. If a whole group is flagged its whole row will be marked as flagged. The items included in a table and in which order they appear can be edited through the table's properties. Select the table item and click the properties button to edit the table's contents and content's order.
Matrix	A matrix with the dimensions of the virtual microplate containing the raw and calculated data, including cut-offs, group names and percentage CV. The data in a matrix is displayed and calculated by well. Cut-offs are generated for each well. However, the percentage CV displayed for each well is the percentage CV of the group of which the well belongs to. Flagged wells will be displayed with a cross through them. The items included in a matrix and in which order they appear can be edited through the table's properties. Select the matrix item and click the properties button to edit the matrix's contents and content's order.
Standards Graph	This is a graphic of the standard graph used in a curve fit transformation. All standard graphs used by the protocol will be available for inclusion in the report. The width and height of the graphic image can be specified through the item's properties.
User Text	This is text that is specified by editing the item's properties. This text will be displayed in every run of the protocol file.
Validations	This is the result of the validations. A list of every specified validation expression for all matrices and whether it passed or failed.
Notes	These are the notes specified by the user of the protocol at run time and will therefore differ on every run of the protocol.
Calculation Log	This is a copy of the calculation log.
Automatic Flagging	This is a list of the automatic flagging details.
Flagged Wells	This lists all of the flagged wells.
3D Graph	This is a default 3D graph for each matrix. A 3D Graph report item will be available for all matrices in the protocol. The width and height of the graphic image can be specified through the item's properties.

Table 5 Report Items

Use the arrow icons to move a selected report item from one list to the other. Use the double arrow icons to move all the report items from one category to the other. Use the **Up** or **Down** buttons to move an included report item forwards or backwards in the report order.

Each data field has properties that may be changed. Click the Properties button to change. The properties depend upon the report item. All report items can be given a title and can be forced to start on a new page if required.

Use the **Headers/Footers…** button to control specify headers and footers for the report (see Figure 30). Any additional text can be included in the headers and footers.

Use the **Report Font…** to choose a font for the report. Be careful not to choose a font that is too large.

Click on the **Preview** button to view the Report layout. In the preview view data shown will be randomly generated and crossed boxes will represent graphs.

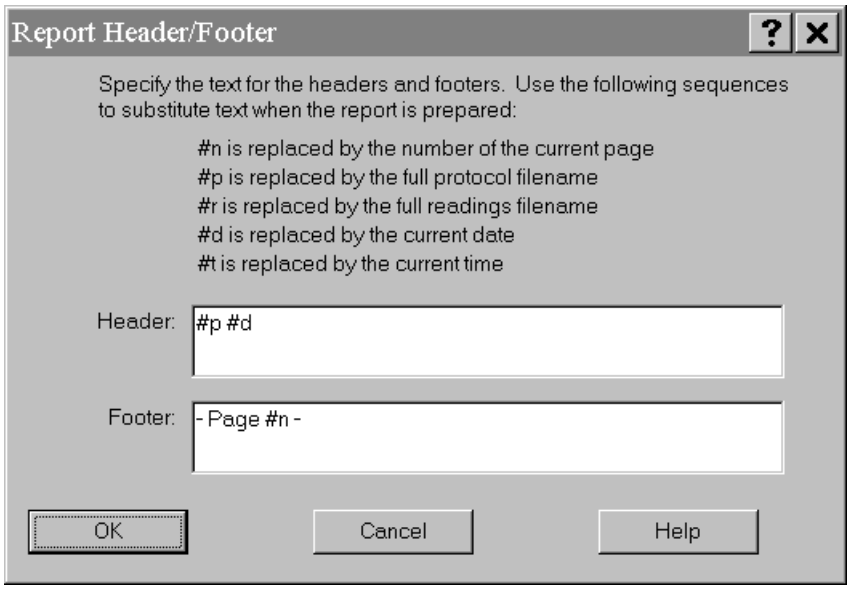


Figure 30 Report Headers and Footers Dialogue Box

Result files management

The Result files management options are used to specify the target directory and filename of results files.

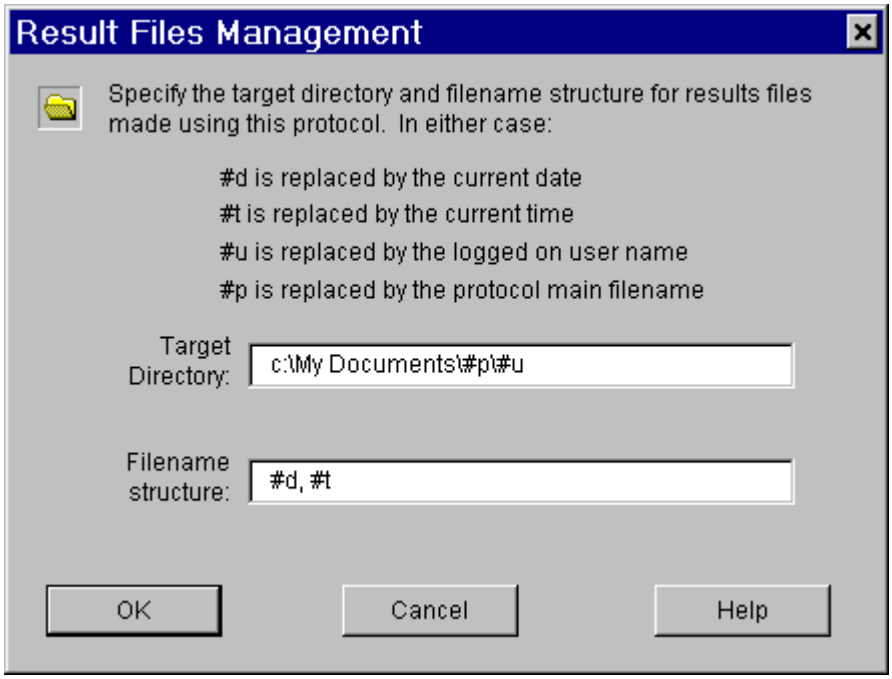


Figure 31 Result Files Management Dialogue Box

Specify a target directory for the results of this protocol to be stored in. If the specified directory does not exist then Stingray will create any necessary directory structures.

Specify a filename structure for the main part of the resulting data file's filename.

Results filenames will have either a DAT or MPR extension depending on whether the protocol is a single or multiple plates.

Use the detailed # macros to refer to Stingray variables. The format of the date used is specified in Window's Control Panel's regional settings.

Post read options

Use the post read options to automatically perform tasks after readings and calculations have been made.

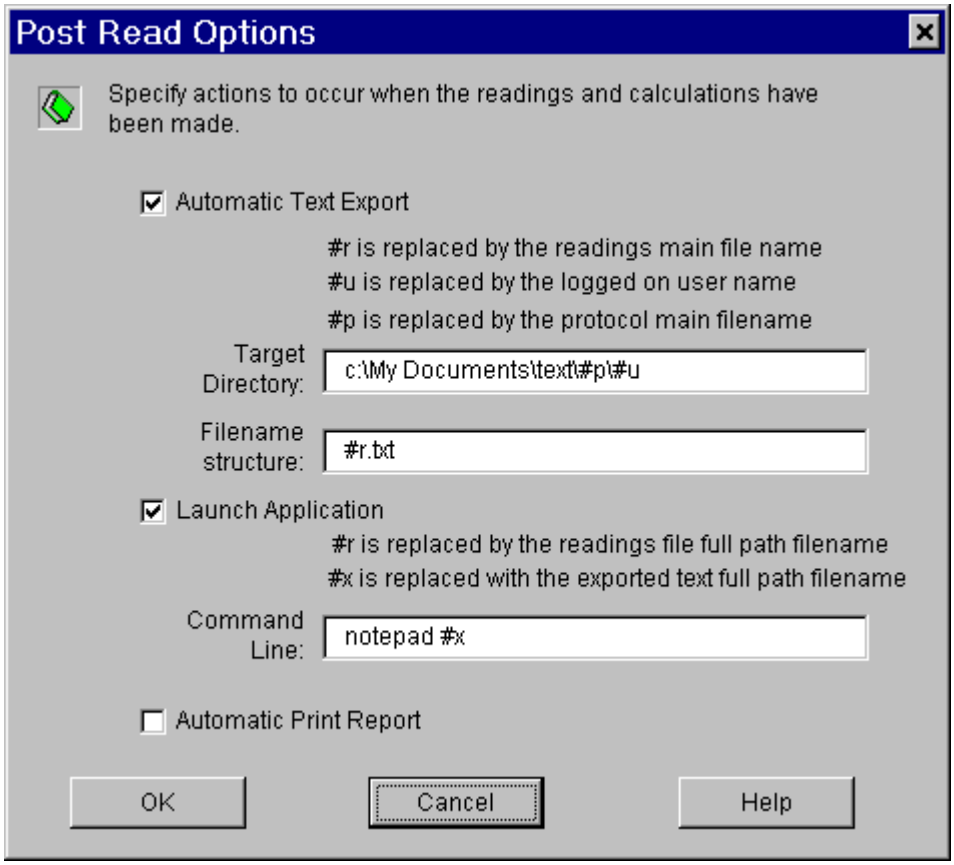


Figure 32 Post Read Options Dialogue Box

Automatic Text Export

Stingray can generate text based report files containing information on the report run. Use the options here to automatically create a text file after readings and calculations have been made. Specify a target directory and filename structure for the resulting text file. Use the detailed # macros to refer to Stingray variables.

Launch Application

Stingray can launch an external application after readings and calculations have been made. This is useful for processing Stingray data with other applications such as data-loggers or QC systems.

Specify a command line string for Stingray to execute. Use the # macros to refer to the exported text file or resulting Stingray data file.

Automatic Print Report

Automatically print report after results and calculations have been made. This is useful when running long protocols. The user could start a protocol and leave the instrument to continue. On return the user would have a hard copy of the results.

Readings Files

This section describes how to use Stingray results.

Overview

Readings files are used to store the raw data of an individual run of a protocol file. There are two types of readings files:

1. Single plate - containing raw data from a single plate run, denoted with a DAT extension.
2. Multiple plates - containing raw data from multiple plates, denoted with an MPR extension.

Running a protocol involves the following steps:

1. Select the protocol file.
2. Read the data.
3. Review the data.
4. Output the results.

Further steps may be required depending on the nature of the protocol. Readings files can be reviewed at a later date and all data specific to the reading will be available.

The following section covers these key steps.

Select the protocol file

When a protocol file is chosen, this automatically creates an empty reading file in which the data is then stored.

The easiest method for choosing which protocol to run is from the Stingray start-up screen (see Starting on page 15).

Alternatively, clicking File | New | Take new single or multiple plate readings, within Stingray will create a new readings file. If there is an active protocol document then you will be asked whether to use this protocol for the new readings file. If there is no active protocol document you must specify which protocol file will be used for the readings.

New readings files are given a default name generated by the current date and time. Data files can be renamed using the **Save As…** option.

Editing the protocol file of an open readings file

When developing a protocol file it is often the case that after a test reading has been made the protocol requires further modification. Stingray allows protocol processes to be changed after readings have been made so that the test data does not require rereading.

Right clicking on the readings matrix display after the readings have been made will display an option to **Open Protocol.** This feature can be used to quickly open the protocol file which the readings file uses. Figure 33 illustrates Stingray with an active readings file and its protocol file open after the menu option Windows | Tile Vertically has been executed.

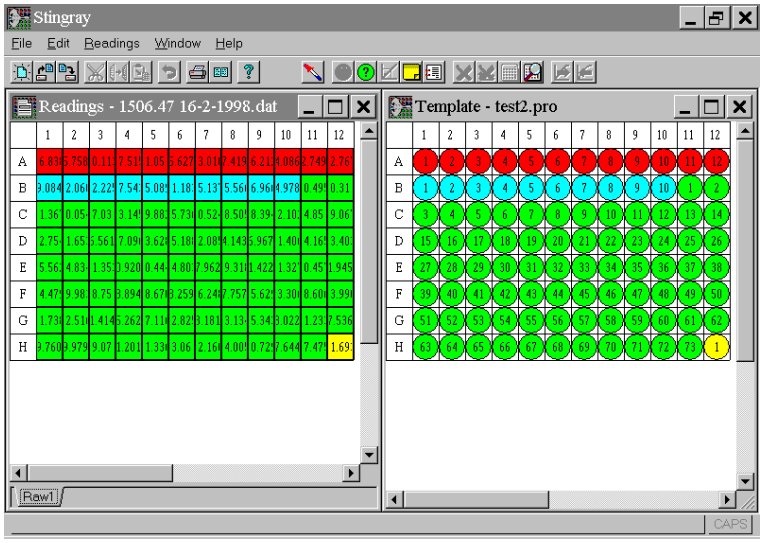


Figure 33 Editing the protocol of an active readings file

If changes are made to the protocol file and saved, then when the readings file becomes the active view, Stingray will warn that the protocol file has been modified and ask if results should be recalculated with the new protocol. The existing raw data will be used with the modified protocol file.

This facility is valuable for building and testing protocol files with real data without the need to repeat raw readings. Cut-offs, validations, automatic flagging and further transformations can be added, reduction methods, numerical formatting and existing transformations changed and the report layout modified all without rereading the raw data.

When using this feature it is important to remember that the raw data configuration should not be altered, as the raw readings are not modified.

Read the data

Reading data uses the settings in the selected protocol file to acquire data from connected readers. Simply click the measure button to begin the readings.

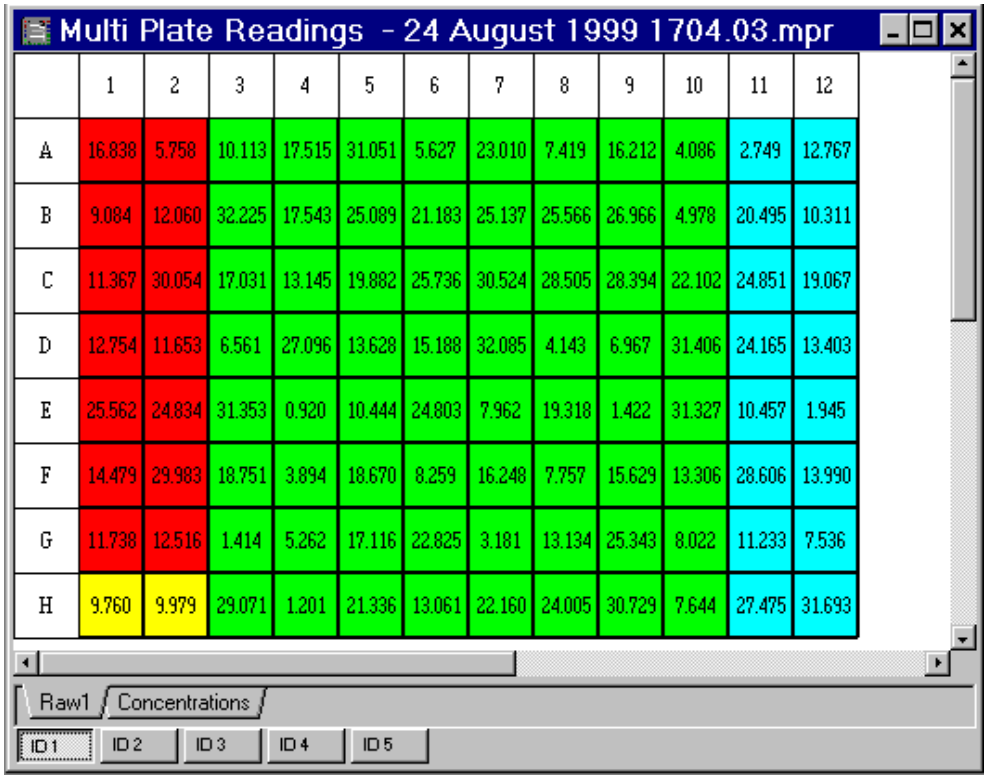
If the protocol uses GLP then data specific to this run of the protocol must be entered.

Review the data

After the raw readings have been made Stingray will perform the calculations and prepare the report as specified in the protocol file. There are two main ways to view the run's data output, by matrix or by report. If the protocol used a curve fit transformation a standards graph view will also be available. If the protocol used a competitive bindings transformation an IC graph will be available for each sample group. The calculation log is updated every time the calculations are made and holds details of calculations, validations, errors, warnings and flagged items made. Sample Identifications can be specified which will be used in the report. Notes can be made regarding the run of the protocol that will be saved with the data.

Multiple Plate Views

If the readings are read from multiple plates then buttons at the bottom of a readings window can be used to select specific plates for results data inspection. Any bar code information read with the microplate will also be displayed here.



	1	2	3	4	5	6	7	8	9	10	11	12
A	16.838	5.758	10.113	17.515	31.051	5.627	23.010	7.419	16.212	4.086	2.749	12.767
B	9.084	12.060	32.225	17.543	25.089	21.183	25.137	25.566	26.966	4.978	20.495	10.311
C	11.367	30.054	17.031	13.145	19.882	25.736	30.524	28.505	28.394	22.102	24.851	19.067
D	12.754	11.653	6.561	27.096	13.628	15.188	32.085	4.143	6.967	31.406	24.165	13.403
E	25.562	24.834	31.353	0.920	10.444	24.803	7.962	19.318	1.422	31.327	10.457	1.945
F	14.479	29.983	18.751	3.894	18.670	8.259	16.248	7.757	15.629	13.306	28.606	13.990
G	11.738	12.516	1.414	5.262	17.116	22.825	3.181	13.134	25.343	8.022	11.233	7.536
H	9.760	9.979	29.071	1.201	21.336	13.061	22.160	24.005	30.729	7.644	27.475	31.693

Figure 34 Multiple Plate Readings View

Endpoint Matrix Views

The reading matrix views are the first views displayed after readings are made. Each well in the virtual microplate represents readings or calculated data for a well. A tab control at the bottom of a reading matrix view allows switching between different matrices. The tab control contains labels for all raw matrices and the results of transformations as further matrices. Simply click on the tab of the matrix you are interested in.

Numerical data is displayed formatted by matrix as specified by the protocol file. This formatting is for display purposes only – if you leave the mouse over a particular well for one second the well's unformatted value (which is used for calculations) will be displayed.

If the numbers are too long to fit in each well, use the scroll bars and the **Zoom In** option available by right-clicking the mouse on a matrix view.

Any well that appears with a diagonal cross through it is marked as flagged. The well may have been flagged manually or will have been flagged automatically by the calculation process. Any automatic flagging made is detailed in the calculation log.

MATRIX CUT-OFFS

If cut-offs were defined for the selected matrix the option **Show with Cut-Offs** will be available on the right-click menu. This will display the cut-off labels evaluated for each well. It must be noted that these cut-off labels are determined individually for each separate well and may be different for different replicates of the same group.

3D GRAPHS

All readings matrices can be used to generate a 3D view. Simply right-click on the required matrix and select **New 3D View** to create a 3D representation of the matrix. The data used to generate the chart is the numbers in the *virtual* microplate's wells. 3D representations of a kinetic view use the calculated kinetic reduction.

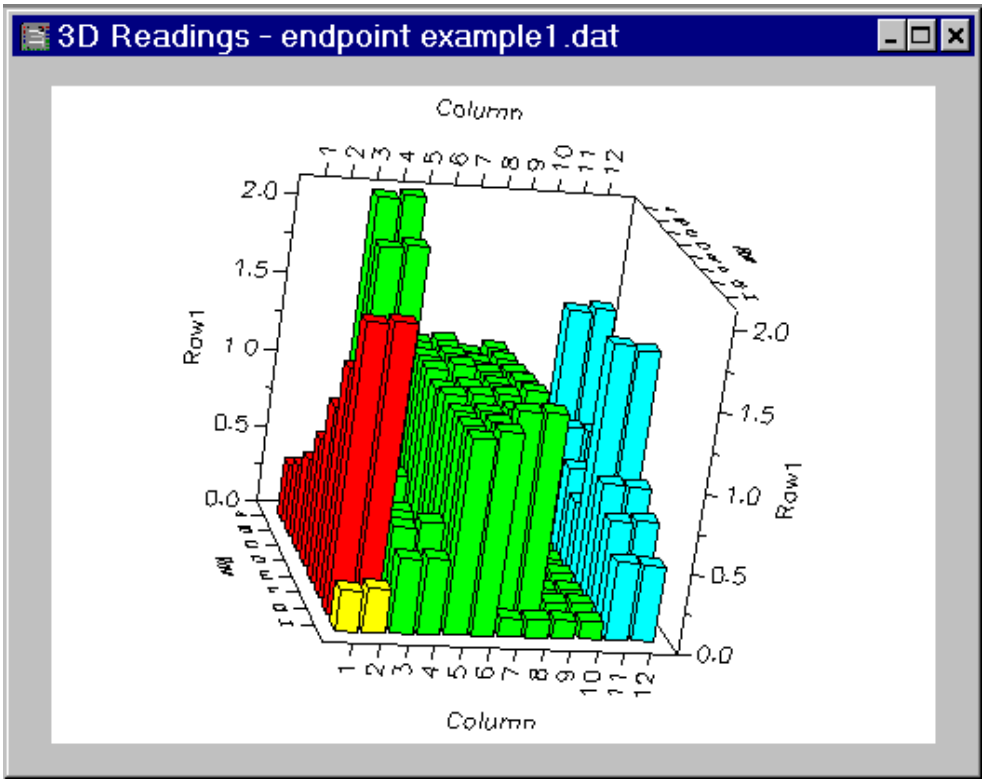


Figure 35 3D Microplate Projection

The 3D views are useful for quickly identifying anomalies that can then be easily flagged. To flag a well using the 3D view simply left click on the bar to remove. The bar's colour will change to the Windows selected colour. Next click the flag button. To unflag that well, click the flag button again.

A 3D view can be manipulated as follows:

Pressing and holding the right and left (or centre) mouse button(s) to rotate through the display as the mouse is moved. Press X, Y or Z (if required) to rotate through a particular plane.

Pressing shift whilst holding the right and left (or centre) will shift the graph in the mouse direction.

Pressing control whilst holding the right and left (or centre) will zoom in and out of the graph, as the mouse is move up and down.

Press the "r" key to reset zooming and shifting actions

Press Ctrl and hold down left mouse button then move the mouse to select a rectangle will zoom to the rectangle.

MANUALLY FLAGGING WELLS

The matrix view can be used for manually flagging individual or groups of wells. If a well is flagged it will be ignored in any calculation and marked as flagged where relevant in the report. A well can be marked as flagged from any of the matrices in a readings file. The date and time the flagging (or unflagging) is made will be stored in the calculation log and the flags list, either of which may be included in the report, depending on the protocol set-up.

To flag a well, select the well or wells to flag using the mouse and click the flag button. All views of the data will be updated to show the flagged wells. However, recalculation must occur now that the set of usable data has changed. Click the recalculation button to recalculate the data.

Kinetic Matrix Data Views

Kinetic data can be viewed in different ways. A small kinetic chart is displayed for each read well in the kinetic matrix display.

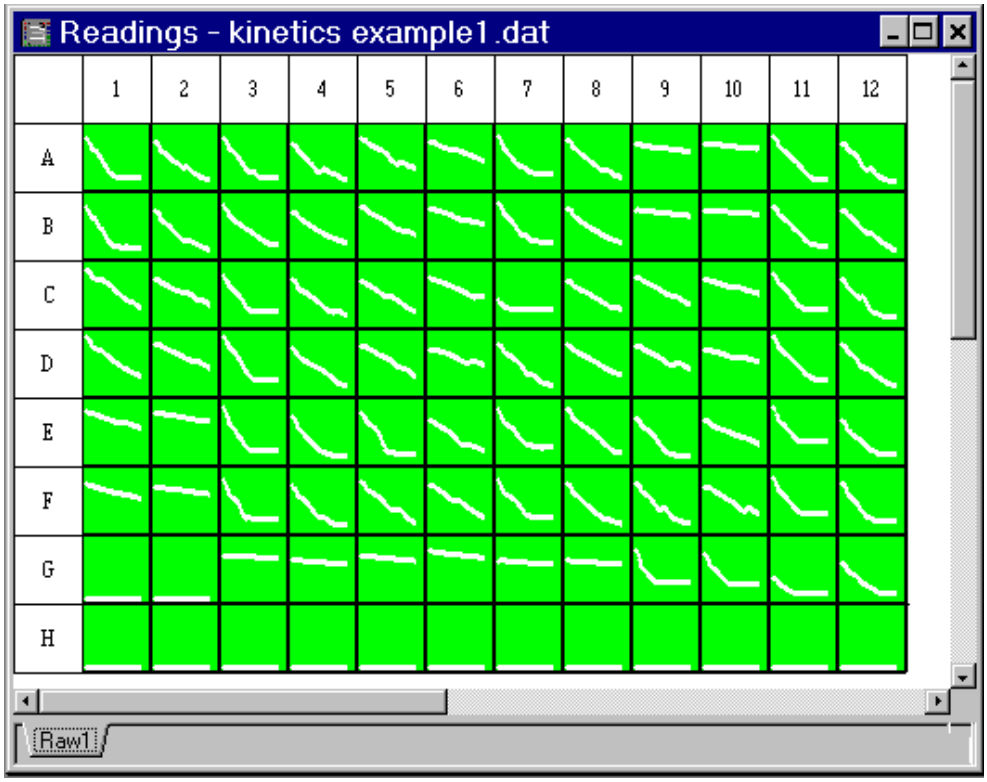


Figure 36 Kinetics Matrix View

To change the kinetic matrix data view, right-click on the required matrix and select **Data**. There are four data views available:

GRAPHS

This displays small kinetic data graphs within the matrix view. This is useful for comparing wells against other wells. To view zoomed kinetic graphs of a well simply double click on a small kinetic graph.

REDUCTION

This view displays the result of the kinetic reduction for each well. It is only available when the kinetic readings have finished.

CYCLE

This view displays the raw readings for each well of a particular kinetic cycle.

TIMES

This view displays the times each well was read for a particular cycle.

For the Cycle and Times view, specify a cycle to viewed and use the **Next Reading** and **Previous Reading** to step through the kinetic data. If a kinetic point has been flagged a vertical/horizontal cross will appear through the well

when the flagged cycle is view in Cycle or Times views. The only way to flag kinetic points is through the zoomed kinetic graphs.

The Reduction, Cycle and Times numerical data is displayed formatted according to the requirements for the matrix, given in the protocol file.

Use the **View Monochrome Graphs** option from the kinetics matrix right click data menu to display the kinetic matrix data in black and white. This is useful when printing the kinetic matrix data view on black and white printers.

Zoomed Kinetic Views

A zoomed kinetic view shows details of all kinetic readings for a particular well. Information of the kinetic reduction method results is also displayed.

Double click on a small kinetic chart in the kinetic data matrix view to display a zoomed kinetic graph.

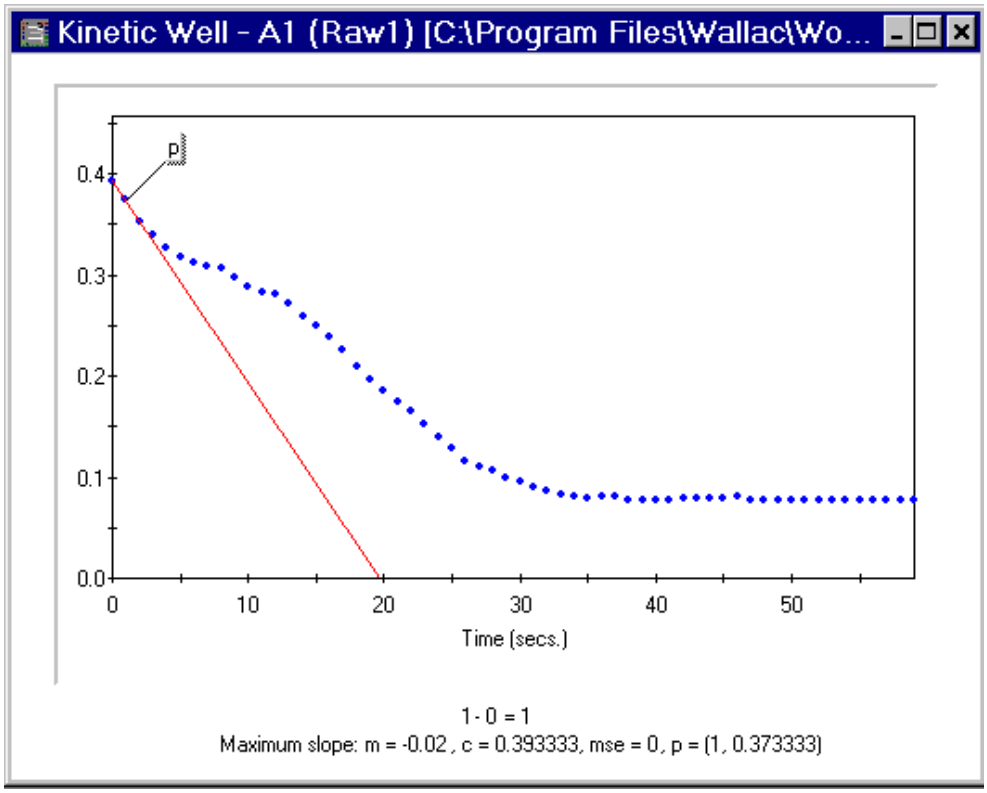


Figure 37 Zoomed Kinetics Graph

In a zoomed kinetic display individual kinetic points can be flagged by double clicking the point to remove. Flagged kinetic points will be removed from any reduction method. There must be at least 2 kinetic points for each kinetic well otherwise the reduction method will fail.

Right-clicking on a zoomed kinetic graph allows the kinetic reduction method to be changed and recalculations made.

By default the y-axis upper range of zoomed kinetic range is the maximum of all kinetic readings of the current matrix. This simplifies any visual comparison of kinetic charts from different wells of the same matrix.

Right click on a zoomed kinetic chart to change whether the y-axis range is based upon the matrix maximum or well maximum.

Scan Data Matrix View

Scan data can be viewed in different ways. A small scan chart is displayed for each read well in the scan matrix display.

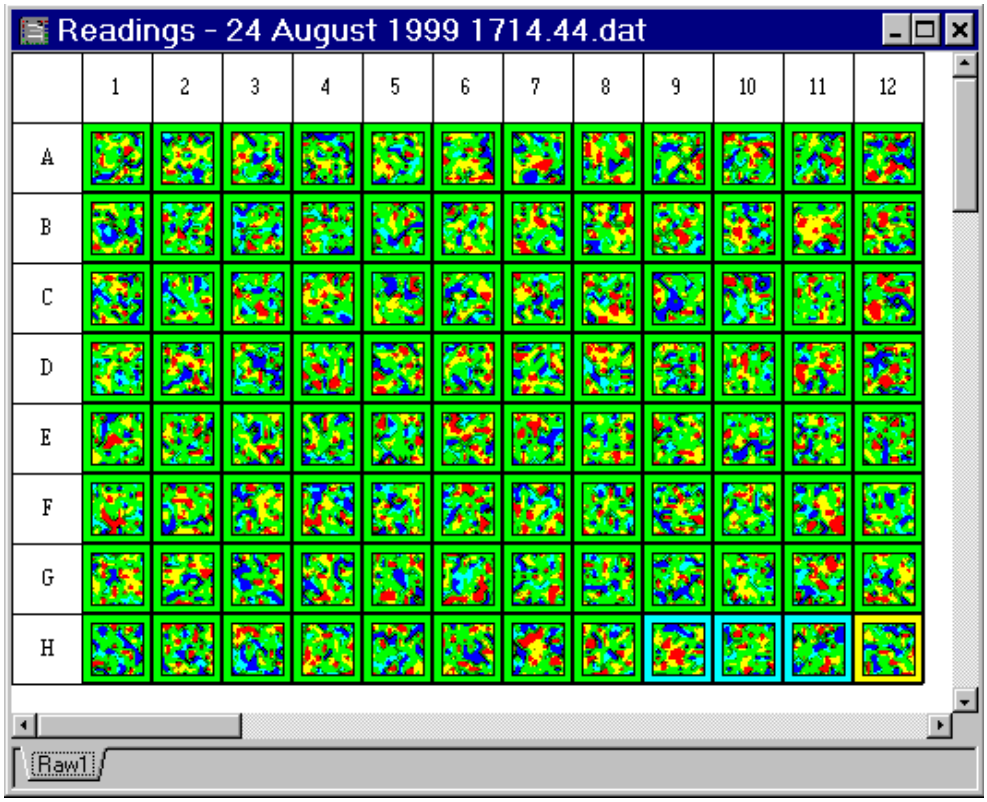


Figure 38 Scan Matrix View

To change the scan data view, right-click on the required matrix and select **Data**. There are three matrix data views available:

GRAPHS

This displays small scan data graphs within the matrix view. This is useful for comparing wells against other wells. To view a zoomed, more detailed scan graph simply double click on a small scan graph.

REDUCTION

This view displays the result of the scan reduction for each well.

SCAN READINGS

This view displays the raw readings for each well of a particular scan position. Scan readings are displayed in order from top-left to bottom-right. Use the **Next Reading** and **Previous Reading** to step through the scan data. If a scan point has been flagged a vertical/horizontal cross will appear through the well.

Zoomed Scan Views

A zoomed scan view shows details of all scan readings for a particular well. Information of the scan reduction method results is also displayed.

Double click on a small scan chart in the scan data matrix view to display a zoomed scan graph.

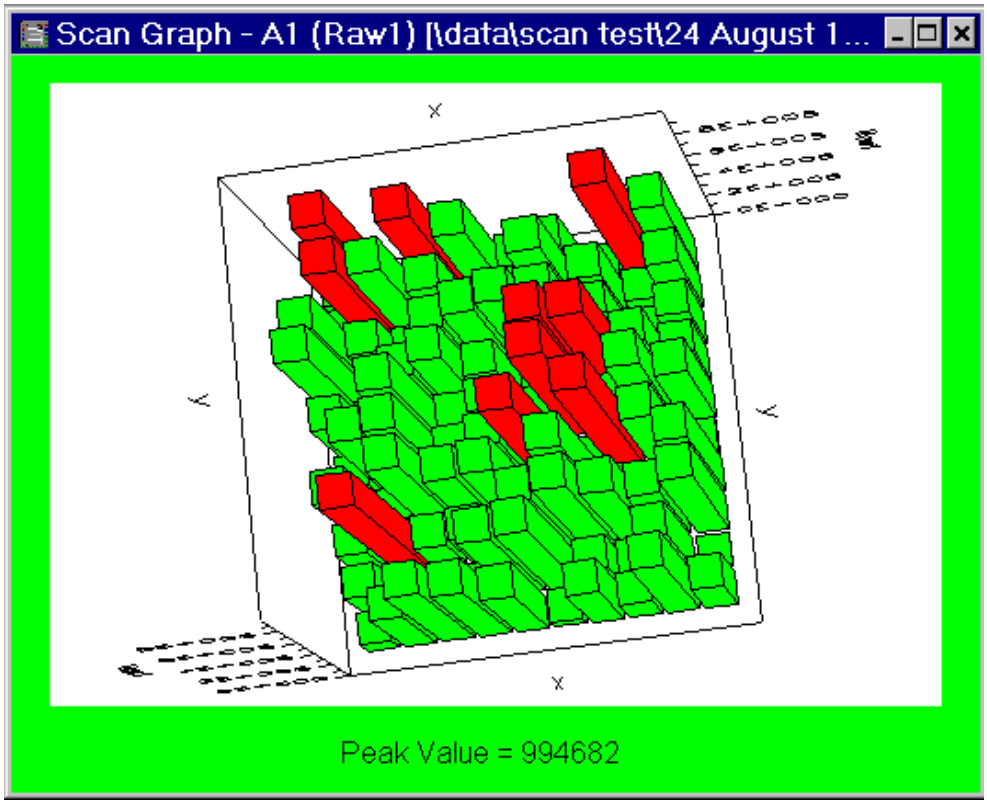


Figure 39 3D Scan Bar View

Zoomed scan graphs can be viewed in a variety of different ways.

There are 3 types of scan charts:

Bar

A 3D bar chart - each bar represents an unflagged read scan point. Bars with the higher readings can be highlighted.

Surface
A 3D surface projection of the unflagged scan points read.

Aerial
A 2D overhead view of the unflagged scan points read.

The bar scan chart type can be used to flag scan points that will be ignored from any scan reduction method. Clicking a scan bar and then click the flag cross to ignore the scan point. Click the flag button again to unflag a point. Click the Unflag All button to unflag all flagged points in all wells.

Further scan view options are available:

Show mesh
Displays an X-Y grid projected onto the 3D surface in a 3D view with a Z-axis.

Shade
Displays the data as a flat shaded surface in a 3D view with a Z-axis.

Show contours
Highlights the distribution of the data by displaying contour lines joining each of the distribution levels.

Colour zones
Highlights the distribution of the data by filling each level with a solid colour.

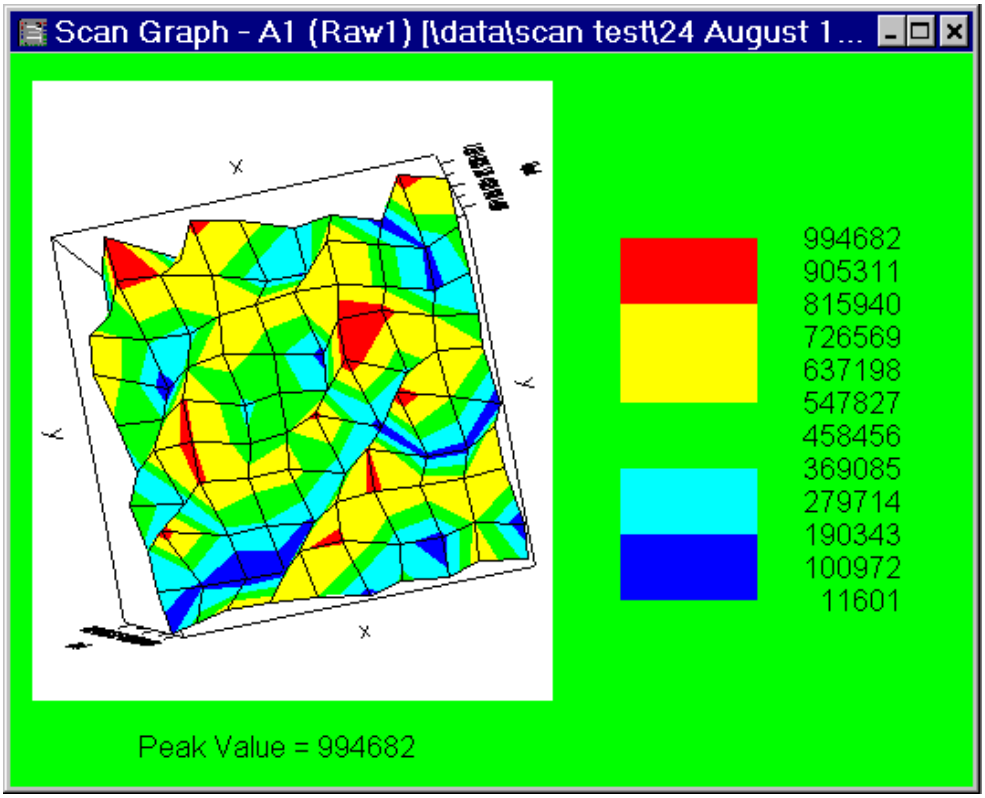


Figure 40 3D Scan Surface Projections With Shading, Mesh and Colour Zones

The 3D scan views can be manipulated as follows:

Pressing and holding the right and left (or centre) mouse button(s) to rotate through the display as the mouse is moved. Press X, Y or Z (if required) to rotate through a particular plane.

Pressing shift whilst holding the right and left (or centre) will shift the graph in the mouse direction.

Pressing control whilst holding the right and left (or centre) will zoom in and out of the graph, as the mouse is move up and down.

Press the "r" key to reset zooming and shifting actions

Press Ctrl and hold down left mouse button then move the mouse to select a rectangle will zoom to the rectangle.

Right-click on a zoomed scan graph to access the scan viewing options.

The scan reduction method can also be changed from the right-click menu.

Use the **Redraw All Scan Charts Like This…** option to view all scan wells in the scan matrix view with the settings of the current zoomed scan well.

Report View

The report view displays the data in report format as specified by the protocol. The report view can be zoomed in and out and can be printed out. If Sample Identifications are specified references to samples in the report (such as *sample1*) will be replaced with the specified Sample Identifications.

SAMPLE IDENTIFICATIONS

The Sample Identification control allows names to be given to samples. These names will be substituted in the report.

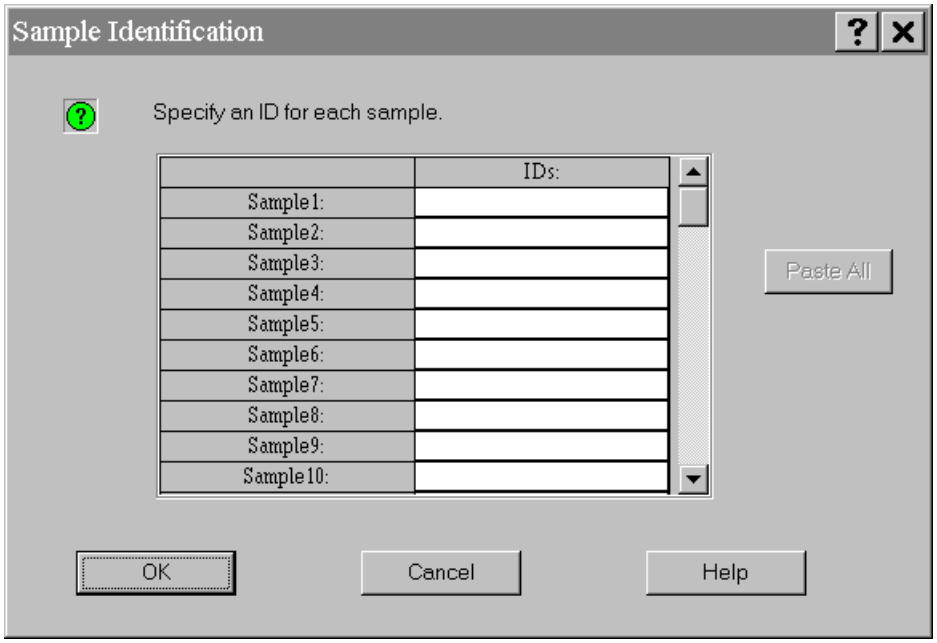


Figure 41 Sample Identification Entry Dialogue Box

To enter a name double click on a cell and enter its value. If data is contained in the Windows clipboard, use the **Paste All** button to copy textual data to the Sample Identification control.

Standards Graph View

DISPLAYING

If a protocol has transformations that use curve fitting, the fitted curve can be displayed. The first step is to select the matrix that is the output matrix of the transformation that uses a curve fitting transformation. Next click on the **Curve Fits** button to bring up a standards graph view.

STANDARDS POINTS

The standards graph view shows the points that the curve was fitted to as red squares. If the fit method used was linear regression then all points will be red squares, since the linear regression fit method uses all standard points.

For other fit methods blue triangles are also displayed, these represent the replicate points. The red squares are the mean of the replicate points for each standard group and the red squares are again the points that the curve was fitted to.

FLAGGING STANDARD POINTS

Points can be flagged in the standards graph view by double clicking on the point to remove. When a point is flagged on the graph the corresponding well or wells will be flagged and all other views updated. A red cross on the standards graph view denotes that a point has been flagged. The standards graph view will now be

invalid since the readings data needs recalculating with the new set of usable data. Click the **Recalculate** button to recalculate.

If a red square is flagged and the square represents the mean of the group, all of the group's replicates will be flagged.

To unflag a point simply double click on the flagged point on the standards graph.

CHANGING STANDARDS GRAPHS RANGE

The standards graph view's range to plot can be altered. This is useful to see how well the curve can be extrapolated beyond the range of the standards. Right click on a standards graph view and select **Change Range…**.

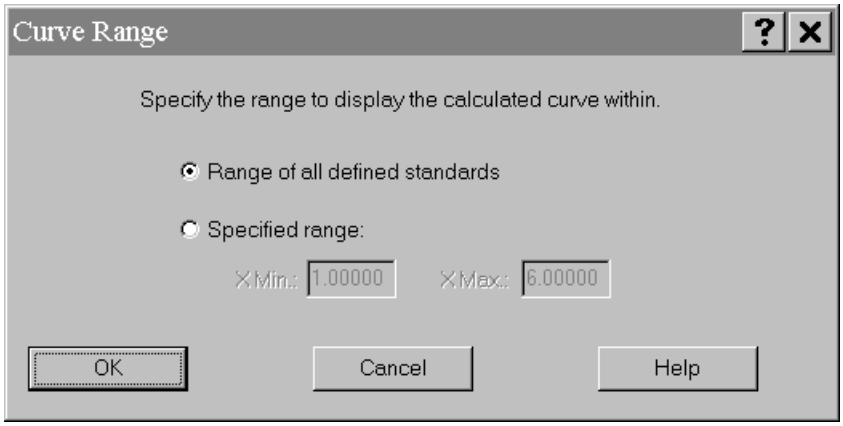


Figure 42 Standards Graph Curve Range Dialogue Box

TEST CURVE

The calculated curve can be tested. Known x or y values can be entered and Stingray will use the fitted curve to generate the corresponding y or x value. Right click on a standards graph view and select **Test Curve…**

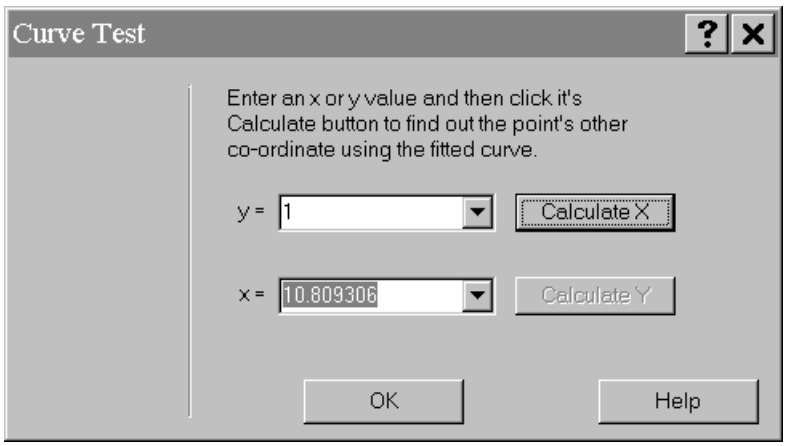


Figure 43 Standards Graph Curve Test Dialogue Box

Competitive Bindings Graph View

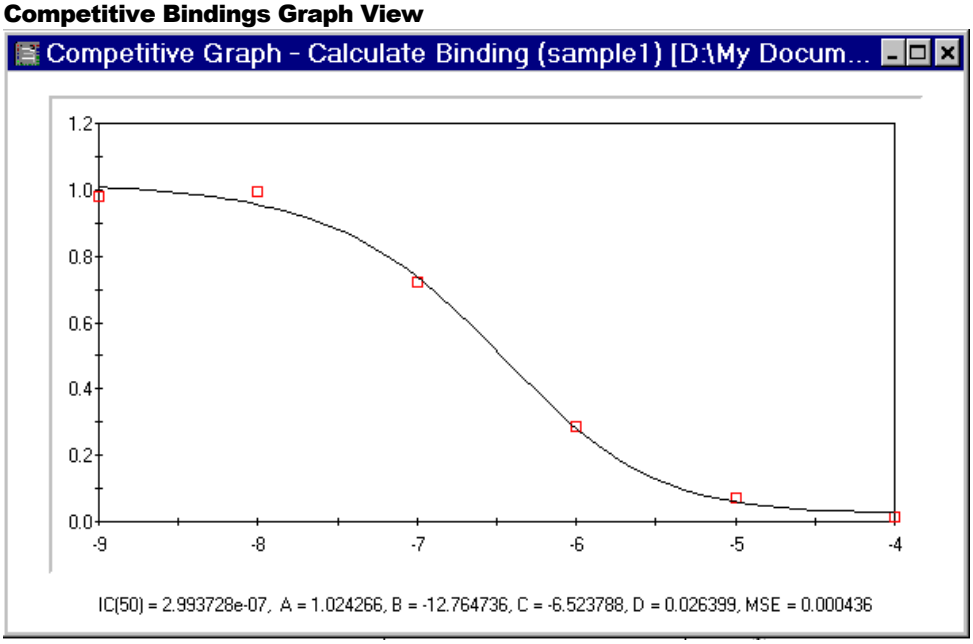


Figure 44 Competitive Graph View

DISPLAYING

If a protocol has a competitive bindings transformation, a fitted curve can be displayed for each sample group. Click the  button and choose which graph to view.

GRAPH POINTS

The graph shows the data points (concentration, absorbance) and the fitted four-parameter curve.

Competitive graph labels:

IC(n) = concentration of the substance resulting in the displacement of n% of the antibody. n is specified in the competitive bindings transformation in the protocol

A/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.

MSE = mean squared error of fit

FLAGGING SAMPLE POINTS

Flag a data point to ignore it in the curve fit by double clicking the point. When a point is flagged on the graph the corresponding well or wells will be flagged and all other views updated. A red cross on the competitive graph view denotes that a point has been flagged. Click the **Recalculate** button to recalculate. To unflag a point simply double click the flagged point on the standards graph.

CALCULATE IC(N)

Right click on the IC graph and use the **Calculate IC(n)** option to enter a % value and have Stingray calculate the concentration of the substance resulting in the displacement of n% of the antibody.

TEST CURVE

The calculated curve can be tested. Known x or y values can be entered and Stingray will use the fitted four parameter fit curve to generate the corresponding y or x value. Right click on a competitive graph view and select **Test Curve…**

Calculation Log

The calculation log holds details of calculations, validations, errors, warnings and flagged items made. The log is updated every time calculations are made.

The log time stamps entries and details the protocol file in use. If validations are used in a protocol the result of each validation is entered. If any errors occur during calculations details are entered into the log. If Stingray automatically flags wells because of mathematical error, automatic flagging or reduction error the details will be entered in the log. If the user manually flags wells (through any view) details will be entered in the log.

The contents of the calculation log can be saved to file. Right click on the log and select **Save Details…** This feature is useful for noting data for technical feedback and reporting any apparent faults, which may be included in a report

Notes

The user may enter notes regarding a specific run of the protocol at any time whilst the readings file is active. These notes are stored in the readings file and may be included in the report, depending on the protocol.

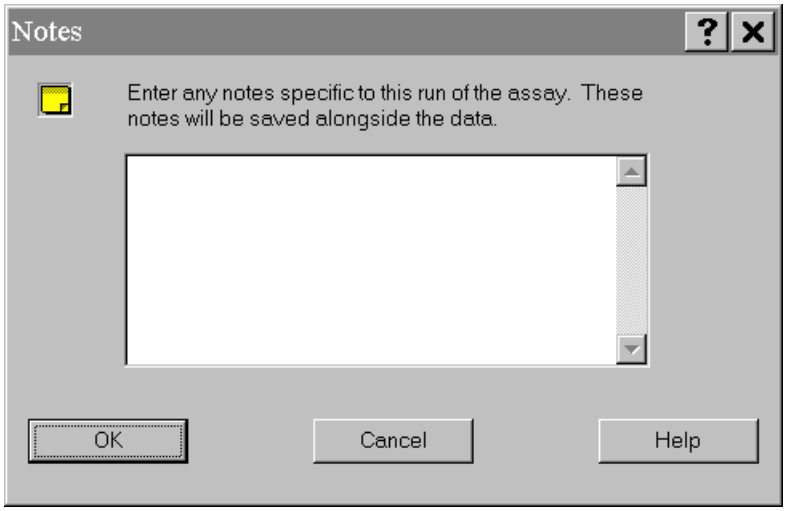


Figure 45 Notes Dialogue Box

Output the results

Data can be output from Stingray in 4 ways:

Output to the printer

Stingray data can be output through the report options. See page 55 for more information. Stingray views can also be printed using the **File | Print** option. This prints the active window and can be used to print any Stingray view, including data matrices, kinetic graphs (matrix or individual), standard graphs and 3D Graphs.

Export into an Excel document

Right click on a matrix view and select **Make Excel Document** to create an Excel document. Microsoft Excel 7.0 (95), Microsoft Excel 97 or Microsoft Excel 2000 must be installed on the system for this to work.

For kinetic data the resulting spreadsheet will contain all kinetic raw data for the selected matrix. For other data the selected matrix will appear in the resulting spreadsheet in the same orientation as it appears within Stingray.

Copy to the Windows clipboard

In any of the grid controls in Stingray, select the area to copy, right click the mouse and select **Copy** to copy the wells to the Windows clipboard. Images such as 3D graphs, scan charts, standard graphs, competitive charts, template layouts and kinetic graphs can also be copied by clicking the **Copy** button or selecting **Copy** from the **Edit** pull-down menu. With this method graphical and/or numerical data can easily be pasted into any other Windows application.

Text file export

A text file containing the contents of the report table can be generated and exported from Stingray. Right click on the readings matrix view and select Export Text File. Specify a target filename and a text file containing the report will be produced. Wells that are flagged are marked in brackets. If a whole group is flagged readings are not displayed - they are marked as flagged.

Alternatively use the Post Read Options in the protocol set-up to automatically export a text file after readings and calculations have been made.

Import Scripting

This section describes how to import text data from any source into Stingray.

Import scripts can be created which describe how to import any text based microplate data.

Import scripts can be launched from the command line – so Stingray operates in a stand-by mode, waiting for raw data.

Launching Stingray from the command line:

To launch Stingray from command line pass

Stingray %1 %2 %3

`%1` is the script import file

`%2` is the data file to import

`%3` is the protocol file to use

If Stingray is already running and a command line is executed then the currently open Stingray will execute the command line operation.

STIMP Overview

STIMP (The name given to Dazdaq Ltd.'s Import Scripting functionality) is a script based interpreted language that describes how to import microplate data from a text file of some arbitrary format. Currently STIMP can be used to import endpoint or kinetic data from text files containing single or multiple matrix data on single or multiple plate raw data into Stingray.

Stingray can be launched from the command line with a script filename, protocol filename and text filename to import. Stingray will create a new .dat or .mpr file containing the imported data. This can also be carried out if Stingray is already running.

The following section identifies the necessary steps to import a particular data file format by using an example situation. The end of this document contains a list of all STIMP instructions and the EBNF language definition.

Example

In this example we will show how to import data from the MicroLumat WinGlow .tkx file format version 1.24.

Gather example text files

We must first gather example text files that we want to import. The more text files we have to look at the easier it is to identify patterns in the file format structure. It is also useful if we know what the files to import contain. The following 2 Figures both show an example .TKX file that we would like to be able to import. (Note, in figures the ellipses denote continuation of data skipped here for conciseness.)

```
0.00  txk
1.00  1.60
2
100
TEST.KPT          16.04.1999  13:11:35
Simulated   1.24  3




Time  A1    A2
0.01  29700 36300
0.02  33000 39600
0.03  38500 45100
0.04  44000 49500
...
```

Figure 46 First portion of sample .TKX file containing 2 wells read with 100 cycles at a 0.01 interval.

```
0.00  txk
5.00  1.60
96
100
TEST2.KPT          16.04.1999  15:04:47
Simulated   1.24   3
```

Time	A1	A2	A3	A4	A5	A6	A7	A8	A9
	A10	A11	A12	B1	B2	B3	B4	B5	B6
	B7	B8	B9	B10	B11	B12	C1	C2	C3
	C4	C5	C6	C7	C8	C9	C10	C11	C12
	D1	D2	D3	D4	D5	D6	D7	D8	D9
	D10	D11	D12	E1	E2	E3	E4	E5	E6
	E7	E8	E9	E10	E11	E12	F1	F2	F3
	F4	F5	F6	F7	F8	F9	F10	F11	F12
	G1	G2	G3	G4	G5	G6	G7	G8	G9
	G10	G11	G12	H1	H2	H3	H4	H5	H6
	H7	H8	H9	H10	H11	H12			
0.05	29700	36300	37400	1000	3300	3300	14300	16500	13200
	4259	5206	5364	0	473	473	2051	2366	1893
	48	300	47	204	73	44	64	243	61
	264	114	158	125	237	99	38	120	271
	208	195	83	61	107	133	229	186	244
	297	14	152	40	121	37	43	30	83
	91	217	299	82	155	260	280	295	32
	209	56	82	118	247	228	47	87	278
	221	286	12	29	164	141	235	157	257
	277	115	243	253	216	96	278	120	156
	121	45	18	280	34	154			
0.10	33000	39600	40700	1100	3300	3300	15400	16500	14300
	4733	5679	5837	158	473	473	2209	2366	2051
	269	204	236	211	130	214	97	87	209
	121	93	37	151	208	28	185	251	113
	225	106	109	176	141	268	23	212	96
	248	188	201	137	29	49	267	271	293
	58	106	107	64	292	254	274	31	20
	268	146	46	149	272	173	129	270	300
	118	256	231	243	288	230	151	13	267
	169	223	256	168	179	166	35	237	239
	99	199	228	116	12	79			
0.15	38500	45100	46200	1100	3300	4400	16500	17600	15400
	5521	6468	6626	158	473	631	2366	2524	2209
	32	214	137	293	169	39	254	222	125
	76	102	209	240	44	277	215	39	156
	129	53	74	142	17	46	104	145	108
	231	86	268	156	179	143	49	154	126
	233	83	243	201	172	252	49	98	49
	261	241	205	193	30	107	134	158	84
	76	223	47	194	270	48	275	89	114

```
       86    186   105   150   34    206   243   156   79
       148   230   167   38    167   163
0.20   44000 49500 50600 2200  3300  4400  17600 19800 16500
       6310  7099  7257  316   473   631   2524  2840  2366
       263   156   133   269   145   279   154   13    213
       253   225   135   143   71    283   138   294   163
       147   210   148   254   116   85    60    190   216
       201   265   101   301   179   172   35    127   36
       202   189   253   173   87    10    126   103   222
       32    143   195   190   283   139   254   26    66
       147   123   100   59    216   280   123   78    277
       15    213   233   202   131   96    210   258   225
       144   154   261   109   287   248
0.25   50600 56100 56100 3300  4400  5500  19800 19800 17600
       7257  8045  8045  473   631   789   2840  2840  2524
       90    76    239   32    95    225   25    31    196
       97    43    56    123   43    75    166   269   50
       36    244   146   284   17    151   141   269   251
       259   94    214   87    232   243   82    74    106
       285   270   78    66    62    240   135   31    209
       198   286   218   63    209   161   143   194   22
       248   271   148   49    51    246   178   139   133
       264   65    259   245   25    94    254   53    225
       298   52    131   292   279   17
...
```

Figure 47 First portion of sample .TKX file containing 96 wells read with 100 cycles at 0.05 interval.

Identify file format structure and key variables

From Figures 1 and 2 and what we know about the data contained in these text files we can infer that the first number on the second line is the kinetic interval in ms. Also, the number on the 3rd line is the number of wells read and the number on the fourth line is the number of kinetic cycles read.

We can also see that the raw data is arranged as a table with a row for each cycle read and columns of each well read. The actual numerical raw data table starts on the line after the line beginning with Time.

Thus, there are variables at the top of the file to import, which describe the size and content of the raw data table.

Write script

Having identified the file format structure and key variables we can begin to write the script. Figure 3 contains the STIMP script that will import .TKX files.

```
/* Imports a WinGlow TKX file with v1 wells and v2 number of
cycles. */
set_nomatrices(1);
```

```
set_datatype(1);
/* Start going through the file. */
skip_nlines(2);
/* Read the number on the 3rd line down - this is the number
of wells, store it in v1.*/
read_integerintov(1);
set_nowells(v1);
/* The number on the next line is the number of cycles -
store in v2.*/
skip_line();
read_integerintov(2);
set_nocycles(v2);
/* Skip to readings. */
skip_untilafterstring("Time");
skip_line();
var_resetwell();
var_resetcycle();
%v2[
      read_timeintocurrentcycleandallwells();
      skip_allwhitespace();
      var_resetwell();
      %v1[  /* Read each well */
            read_readingintocurrentcycle();
            skip_allwhitespace();
            var_incrementwell();
      ]
      var_incrementcycle();
]
set_nocyclestocounter();
```

Figure 48 Import Script for .TKX file format.

We will save this script file as `wgtkx.imp`. All script files must be saved with an IMP extension.

Notice, that STIMP script files can contain C style comments and arbitrary tabs and spaces for formatting purposes.

We will now look more closely at this script file:

The first two instructions:

```
set_nomatrices(1);
set_datatype(1);
```

This tells Stingray that any file which we import using this script will contain 1 raw matrix of kinetic data.

The following 3 instructions then read in the number on the 3rd line into a STIMP variable - variable 1. The number of wells is then set to the value of variable 1.

Similarly the following 3 instructions then read in the number on the next line into variable 2. The number of cycles is then set to the value of variable 2.

We need to use these `set_` functions so that Stingray knows the size of the data we are importing with this script file and the text file.

The next instructions:

```
skip_untilafterstring("Time");
skip_line();
```

skips through the file to import until the start of the numerical raw data.

STIMP has well and cycle counters that are used to hold the position of where to store the next data item to import at. The instructions:

```
var_resetwell();
var_resetcycle();
```

Reset the well and cycle counter to the first well and the first cycle.

The next instruction contains a looping construct:

```
%v2[
…
]
```

All instructions inside the square bracket will be repeated the number of times specified by the value of variable 2. In this situation we are reading each row of the table and since each row represents a kinetic cycle - this will be repeated the number of times specified by the number of kinetic cycles now stored in variable 2.

For each kinetic cycle or table row the import script reads the first number as a time value into the current cycle (identified by the cycle counter) and stored at all wells using.

```
read_timeintocurrentcycleandallwells();
```

This is because in this situation it is assumed that all wells are read at the same time period. The import script then skips until the next number and the well cycle counter is reset. Remember that this data table contains columns of wells.

The next instruction is a nested loop which will repeat all instructions inside of the square brackets the number of times specified by variable 1 - i.e. the number of wells.

```
%v1[
…
]
```

Within this loop each number is read in from the .TKX file and stored in the current cycle. The well counter is incremented after each column is read.

At the end of the table row the cycle counter is incremented and the remaining rows are read - until all cycles/rows have been read in.

When all readings have been imported the last instruction

```
set_nocyclestocounter();
```

sets the number of cycles reported to Stingray as the current value of the cycle counter.

Create protocol file

Now that we have created our script we must create a Stingray protocol file. The protocol file must be compatible with the script file to import. When Stingray uses an import script it checks that the data type (kinetic or endpoint), the number of wells, the number of matrices, the number of cycles (if using kinetic) and multiple plates of the protocol file and file to import match. If they do not then the import procedure will not be allowed.

The easiest way to create a protocol is to use the NODEV - random number generator device driver to specify the data format expected. In this case we create a single NODEV raw data item reading 100 cycles.

We can then set-up the rest of the protocol as normal.

We will save our protocol file as "single kinetics 12x8.pro".

Test script

We can now test and run the import script with our text files to import. To do this run Stingray passing the command line arguments:

```
Stingray [Script Import file] [File to Import] [Protocol
File]
```

Note, do not forget to use quotes when specifying long file names.

In this example we would use:

```
Stingray wgtkx.imp kin1.txk "single kinetics 12x8.pro"
```

STIMP Data Types

There are 3 main data types.

Counter

STIMP provides four counters: well, cycle, matrix and plate. A counter can be reset, set to a particular value and incremented by one. Counters are useful when extracting repeated data - in a row or table using a loop. Some of the STIMP Read functions use the counter values to store imported data.

User Variable

STIMP gives the user 9 variables that can be used as temporary holders for numerical data. A variable is referred to by the letter v followed by the variable number 1-9.

STIMP Variable

There are several STIMP specific variables that must be set to tell Stingray about the data being imported. These STIMP variables: number of matrices, the number of wells, the data type (endpoint or kinetic) and the number of cycles (if data type is kinetic) should always be set by the end of the import script. The number of plates should be set if the script imports data from multiple plates. The plate width and height STIMP variables should be set if using the read_wellref instruction to parse well references in the script file (e.g. A1). The decimal separator should be set if the numerical data uses a decimal symbol other than a period.

STIMP Instructions

Some STIMP instructions require parameters. In this documentation:

n denotes a number or variable reference parameter. (A variable reference takes the form v followed by a number in the range 1-9)

c denotes a single character parameters (e.g. A)

s is a string parameter (e.g. "Enter the plate width")

There are four types of STIMP instructions:

Skip

Skip instructions are prefixed with skip_ . A skip instruction moves the file pointer throughout the text file to import.

Instruction:	Description:
`skip_char ()`	Skips a single char
`skip_nchars (n)`	Skips n chars
`skip_untilafterchar (c)`	Skips until after char c
`skip_untilbeforechar (c)`	Skips until before char c
`skip_untilafterstring (s)`	Skips until after string s
`skip_untilbeforestring (s)`	Skips until before string s
`skip_line ()`	Skips current line, positions at the start of next line. If mid way through current line then still skips to start of next line.
`skip_nlines (n)`	Skips n lines, positions at the start of next line
`skip_allspaces ()`	Skips all spaces
`skip_alltabs ()`	Skips all tabs
`skip_allwhitespace ()`	Skips all tabs, spaces and '\n' '\r'
`skip_untilbeforetext ()`	Skips until any letter
`skip_untilnumber ()`	Skips until a number, "-" or "." symbol
`skip_untildigit ()`	Skips until a 0123456789
`skip_untilwhitespace ()`	Skips all chars until the next whitespace
`skip_gobacktostart ()`	Resets pointer back to start of script - useful when information is at the end of the file

Table 6 Import Scripting Skip Instructions

Read

Read instructions are prefixed with read_ . A read instruction reads data from the current position of the file pointer into a STIMP variable or counter.

Instruction:	Description:
`read_wellref ()`	Reads well reference e.g. ("A1") from file pointer and sets the well counter to it. Requires set_platewidth and set_plateheight to have been used previously
`read_readingintocurrentwell ()`	Reads number from file pointer into current well. Use this instruction for reading endpoint data.
`read_readingintocurrentcycle ()`	Reads number from file pointer into current cycle. Use this instruction for readings kinetic data.
`read_timeintocurrentcycle ()`	Reads number from file pointer into current cycle time
`read_timeintocurrentcycleandallwells ()`	Reads number from file pointer into current cycle of all wells - must have set the number of wells using set_nowells before calling this
`read_hmstimeintocurrentcycle()`	Reads a time code of the format hh:mm:ss from file pointer - converts into seconds since 00:00:00 and stores into current cycle. Use this instruction for readings kinetic data. (See also, set_normalisetimes()) Also accepts hh:mm.
`read_hmsmtimeintocurrentcycle()`	Reads a time code of the format hh:mm:ss.ms from file pointer - converts into seconds since 00:00:00.00 and stores into current cycle. Use set_decimal separator to change the decimal separator between seconds and ms. Use this instruction for readings kinetic data. (See also, set_normalisetimes())
`read_integerintov(n)`	Reads number from file pointer and stores in a STIMP variable specified by n.

Table 7 Import Scripting Read Instructions

Set

Set instructions are prefixed with set_ . A set instruction sets the value of a STIMP variable.

Instruction:	Description:
`set_nomatrices (n)`	Sets the number of matrices being imported
`set_nocycles (n)`	Sets the number of cycles being imported
`set_nowells (n)`	Sets the number of wells being imported
`set_noplates (n)`	Sets the number of plates being imported. Only necessary if 2 or more plates, for multiple plates
`set_datatype (n)`	Sets the data type being imported where n is 0,1 or 2. 0 is endpoint, 1 kinetics, 2 scan
`set_decimalseparator (c)`	Sets the decimal separator character used for reading in numbers. Only need to use if character is other than period
`set_nocyclestocounter ()`	Sets the number of cycles to the number stored in the cycles counter
`set_nowellstocounter ()`	Sets the number of wells to the number stored in the wells counter
`set_nomatricestocounter ()`	Sets the number of matrices to the number stored in the matrices counter
`set_platewidth (n)`	Sets plate width to n - necessary if using read_wellref
`set_plateheight (n)`	Sets plate height to n - necessary if using read_wellref
`set_normalisetimes()`	Normalises all kinetic times in the current well of the current plate of the current matrix so that the first reading starts at time 0.

Table 8 Import Scripting Set Instructions

Variable

Variable instructions are prefixed with var_ . A variable instruction sets or increments a counter or sets a user variable.

Instruction:	Description:
`var_resetwell ()`	Sets the well counter to the first well
`var_resetmatrix ()`	Sets the matrix counter to the first matrix
`var_resetcycle ()`	Sets the cycle counter to the first cycle
`var_resetplate ()`	Sets the plate counter to the first plate
`var_incrementwell ()`	Increments the well counter
`var_incrementmatrix ()`	Increments the matrix counter
`var_incrementcycle ()`	Increments the cycle counter
`var_incrementplate ()`	Increments the plate counter
`var_incrementv(n)`	Increments variable n by 1
`var_setwell (n)`	Sets the well counter to the first well
`var_setmatrix (n)`	Sets the matrix counter to the first matrix
`var_setcycle (n)`	Sets the cycle counter to the first cycle
`var_setplate (n)`	Sets the plate counter to the first plate
`var_setv(n1, n2)`	Sets variable number n1 to the value of n2
`var_getfromuser(n, s1, s2)`	Gets a number from the user by displaying an input dialogue box where s1 is the window caption and s2 is the input prompt. The inputted number is stored in variable vn

Table 9 Import Scripting Variable Instructions

Looping Constructs

A loop repeats all items enclosed with square brackets. Loops may be nested.

Loop Construct:	Description:
`%n[ ]`	Loops n times
`%eof[ ]`	Loops until the end of the file is reached
`%vn[ ]`	Loops the number of times stored in variable n (where n is 1-9)
`%plate_width[ ]`	Loops width times where width was specified using set_platewidth
`%plate_height[ ]`	Loops height times where width was specified using set_plateheight
`%plate_nowells[ ]`	Loops the size of the plate, the size being either set by set_platewidth and set_plateheight or set_nowells

Table 10 Import Scripting Looping Constructs

EBNF Language Definition

```
digit = '1'|'2'|'3'|'4'|'5'|'6'|'7'|'8'|'9'|'0'
number = [-] {digit} [.] {digit}
char = 'a'|'b'|'c'|...|'z'|'A'|'B'|'C'|...|'Z'
word = char {char}
type = "skip" | "set" | "var" | "read"
commandname = type "_" action
command = commandname [argumentlist] ";"
argumentlist = "(" [argument { "," argument} ]")"
variablereference = v digit
argument = number | enclosed text | variablereference
enclosed text = "{ anything } "
condition = "eof" | "plate_width" | "plate_height" |
"plate_nowells"
loop_condidition = number | condition
loop = "%" loop_condidition "[" operation {operation} "]"
comment = "/*" { word | number} "*/"
```

`Multiple plate support`

This section contains further information about creating and using protocols that read data over multiple plates.

Creating a Multiple Plate Protocol

Create a new Stingray protocol as normal. The number of plates to read is not stored in the Stingray protocol it is specified when the protocol is run.

Enable the new Assay Master checkbox **Multiple Plates**. Unless you are using multiple plates and a concentration calculation this is the only difference between creating a single plate and multiple plate protocol.

If standards, control and blanks are only present on the first plate and all subsequent plates only contain samples, check the **Extended Sample** Assay master check box.

Multiple Plate Protocols with Concentration Calculation

When using multiple plates and a concentration calculation transformation you have to decide whether or not to read standards wells on every microplate. (This is when **Extended Samples** mode is not being used)

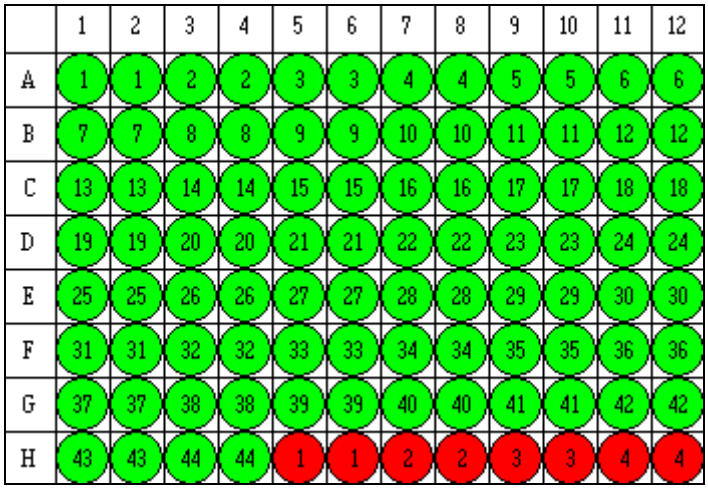
There are 2 options:

1. Standards are local to each microplate (each microplate template layout is the same). Data from each microplate is used to calculate the concentrations of the samples locally. Use this method if you need to calculate concentrations from standards on the same microplate.

2. Standards are global - the first microplate read contains standards wells, subsequent plates do not contain standards wells. Standards data from the first microplate is used to calculate concentrations for all samples on all plates. Use this method if you only want to read standards data on the first microplate.

With the **Multiple Plates** option checked Stingray adds a new step to the Curve Fit Transformation wizard. This new step asks whether standards should be local or global.

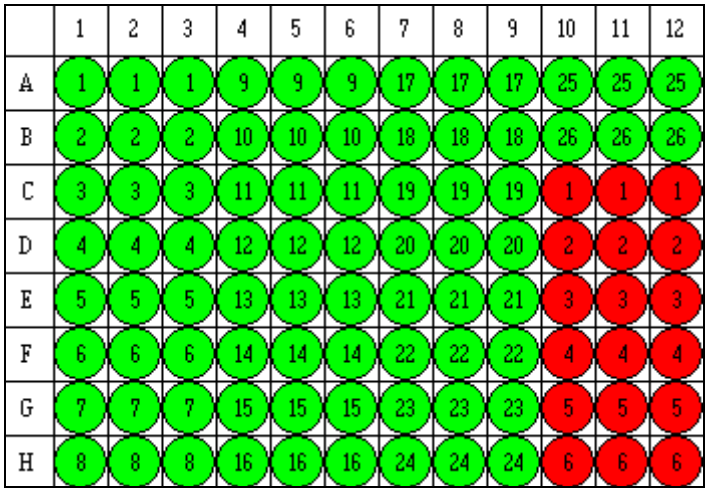
If standards are global then the standard wells on the first plate read will be replaced with sample wells in the same orientation and sequence on subsequent plates. Standards groups should be placed at the *last* part of the microplate. The *last* part of the microplate depends on the fill direction of the groups. Consider this when designing the template layout. Blank's, controls and spikes will be read on every plate read.



	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	2	2	3	3	4	4	5	5	6	6
B	7	7	8	8	9	9	10	10	11	11	12	12
C	13	13	14	14	15	15	16	16	17	17	18	18
D	19	19	20	20	21	21	22	22	23	23	24	24
E	25	25	26	26	27	27	28	28	29	29	30	30
F	31	31	32	32	33	33	34	34	35	35	36	36
G	37	37	38	38	39	39	40	40	41	41	42	42
H	43	43	44	44	1	1	2	2	3	3	4	4

Figure 49 Global standards template layout filled across

Figure 49 illustrates a typical situation where the standards have been placed at the *last* part of the microplate. The first plate read will contain 44 sample groups and 4 standards groups all in duplicate. With global standards the second plate will contain 48 sample groups and no standard wells. Wells A1 and A2 on the second plate read will contain sample group number 45. The standard groups in wells H5-H12 will be converted to sample groups. Thus, wells H5 and H6 on the second plate will contain sample group number 89.



	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	9	9	9	17	17	17	25	25	25
B	2	2	2	10	10	10	18	18	18	26	26	26
C	3	3	3	11	11	11	19	19	19	1	1	1
D	4	4	4	12	12	12	20	20	20	2	2	2
E	5	5	5	13	13	13	21	21	21	3	3	3
F	6	6	6	14	14	14	22	22	22	4	4	4
G	7	7	7	15	15	15	23	23	23	5	5	5
H	8	8	8	16	16	16	24	24	24	6	6	6

Figure 50 Global standards template layout filled downwards

Figure 50 illustrates another situation. In this case the wells are filled downwards rather than across. In this case, the *last* part of the microplate is the bottom right part of the screen. Here, first plate read will contain 26 sample groups and 6 standards groups all in triplicate. With global standards the second plate will contain 32 sample groups and no standard wells. Wells A1, A2 and A3 on the second plate read will contain sample group number 27. The standard groups in

wells C10-H12 will be converted to sample groups. Thus, wells C10, C11 and C12 on the second plate will contain sample group number 53.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	2	2	3	3	4	4	5	5	6	6
B	7	7	8	8	9	9	10	10	11	11	12	12
C	13	13	14	14	15	15	16	16	17	17	18	18
D	19	19	20	20	21	21	22	22	23	23	24	24
E	25	25	26	26	27	27	28	28	29	29	1	1
F	1	1	2	2	3	3	4	4	5	5	6	6
G	1	1	2	2	3	3	4	4	5	5	6	6
H	1	1	2	2	3	3	4	4	5	5	6	6

Figure 51 Global multiple set standards template layout with blank filled across

Figure 51 illustrates a further situation. In this case the wells are filled across, we have a blank group and 3 sets of standards groups (Sets A - red, B - pink, and C - dark green), all groups in duplicate. Note, the standards sets must be in order on the microplate - and the order is specified in the Multi Standards Set-up dialogue (Click Stingray's  button).

In this example the first plate read will contain 29 sample groups, a blank group and 3 sets of standards each with 6 standards groups. With global standards the second plate will contain 29+(3*6) = 47 sample groups. Every plate read will have blank wells at E11 and E12. Wells A1 and A2 on the second plate read will contain sample group number 30. All standard groups in wells F1-H12 will be converted to unique sample groups. This is why it is important that the standards sets appear in the correct order on the microplate (i.e. the first standards set nearest to the start of the microplate after all non-standard wells and the last standards set at the *last* part of the microplate).

Extended Samples

If using extended samples the template layout specified is used for the first plate read. All subsequent plates read will contain samples only. Use the Sample Fill Direction button (), located at the bottom of the display to specify how the samples will be filled on subsequent plates.

Multiple plate protocol expressions

An understanding of scope specification is required when using multiple plate protocols. References to groups or wells can be qualified with a scope identifier

that describes which plate to get data from. These scoped expressions are relevant throughout Stingray.

SCOPE IDENTIFIERS

Group or well references can optionally be proceeded by:

~L Denoting local scope

~F A reference on the first plate read

in any expression (user defined transformations, auto-flag expression, cut-off, validations).

Group references are global by default, namely any reference to a particular group is treated absolutely. Well references are local by default. All x or y references are treated as local by default also.

Example:	Description:
`A1~F`	Refers to the reading of well A1 on the first plate.
`A1~L`	Refers to the reading of well A1 on the current plate being evaluated.
`A1`	Refers to the reading of well A1 on the current plate being evaluated.
`blank1~F`	Refers to the group blank1 from the first plate.
`blank1~L`	Refers to the wells on the plate being evaluated that occupy the same position as group blank 1 on the first plate read.
`X`	The value being evaluated on the current plate.
`x~F`	The value being evaluated on the corresponding well from the first plate.
`x < control1`	Cut-off expression that compares each well with the value of control1 from the first plate.
`x < control1~L`	Cut-off expression that compares each well with the value of control1 and a control group on the same plate as the well.
`standard1&2:"raw3"~L` ***or*** `standard1&2#3~L`	The group's well positions would be found from set 1 of standard set 2 on the 3rd raw matrix local. This reference refers to those wells on the current plate being evaluated.
`x-blank1`	A simple blank correction that would use a blank group found on the first plate only.
`x-blank1~L`	A simple blank correction that would use a blank group local to each well.

Table 11 Scoped Expression Examples

GLOBAL SCOPE

By default group references are global by default. Thus, a simple group reference, such as **blank1** refers to the group blank1, which would always be on the first plate read. Group references are unique - all members of any given group will all be located on the same physical microplate.

LOCAL

If a group reference has local scope then Stingray looks at the template layout for the first plate to identify which wells belong to that group. Stingray then takes reading from those wells from within the CURRENT plate being evaluated.

For example, if we created a user defined transformation expression:

```
x+control1
```

Then the result of the transformation would be that all wells on all plates would be evaluated to the value of the well plus the value of control1 from the first microplate read.

However, we may want to refer to a local control group on each plate (i.e. we have a control group on every plate). In this situation the result for each well would be the value of each well plus the value of the control group on the plate the well was read from. Here we would use:

```
x+control1~L
```

to denote to use the local control group. Note, that the control1 group is only located on the 1st plate, but the ~L means use the group on the local plate equivalent of control1 on the first plate.

Thus, `control1~L` refers to the well positions of control1 on the first plate - but the readings in those well positions are on the current plate.

So if you use ~L the group refers to a group on the first plate read - but it will be interpreted as the wells of those group on the current plate.

~L should be used with care when the plate layouts of microplates read in a protocol differ. This happens when:

1. Reading standards on the first plate only. In this situation the standard wells are actually sample wells on plates after the first. Here, any reference to a standards group with the ~L when evaluated on plates other than the first will refer to a sample group.

2. Using extended samples. In this situation any reference to non-sample groups which use ~L will refer to sample groups on subsequent plates.

Running a Multiple Plate Protocol

The results of multiple plate protocols are stored in data files with an MPR extension.

To run a multiple plate protocol either click the Sample button from the template view of a protocol or go to the File | New menu option and select the **Take new multiple plate reading (.MPR)** option.

Confirm the protocol file to use and select the number of plates to read.

When the readings have been made use the buttons at the bottom of the MPR view to select a microplate to view. The Stingray report contains a table with combined results for all plates and matrices for each read microplate.

Standards Files

This section describes the creation and use of standards files for archiving standards data.

Standards File Data

Standards data can be stored in a file and used for future transformations eliminating the need for repeated standards readings. Standards files contain archived data only and do not store a fit method.

Creating Standards Files

Standards data can be made from scratch or extracted from readings files.

New Standards files can be set-up using the **New | Standards** option to create a new standards file from scratch.

Standards files can be created from data extracted from readings files. Simply click **New | Standards** when viewing results data. Standards data will be extracted from the current data file and stored in a new standards file. Flagged standards points will not be included in the resulting standards file.

Standards Editor

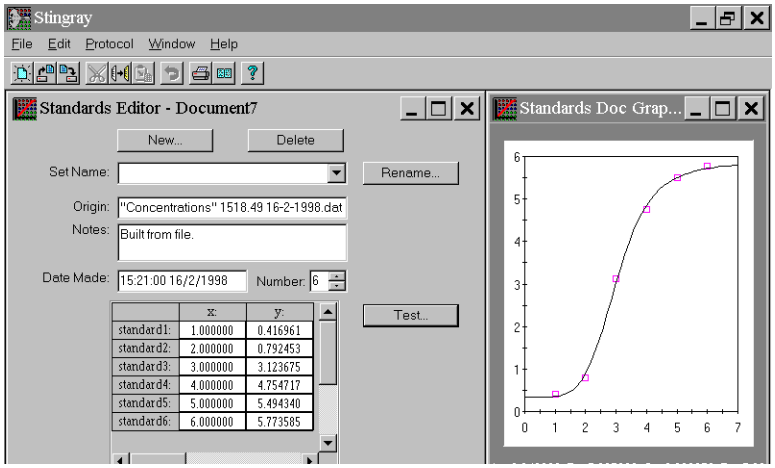


Figure 52 Standards Editor

Standards files can contain more than one set of standards data. A protocol file can be set-up that reads many different sets of standards on one plate. This data can be archived into a single standards file that can be used for transformations in other protocol files.

Use the **New…** and **Delete** buttons in the Standards Editor to add and remove standard sets to the standards file. The standards set can be renamed using the **Rename…** button.

The **Origin**, **Notes** and **Date Made** sections are useful for storing information about the standards file.

Select the number of standards points to use in each set and edit the standards values using the standards grid control.

Testing Standards

Click the **Test…** button to plot the standards points in an extended standards graph view window. The graph view will default to a linear regression fit method. Right clicking on the extended standards graph view allows curve-fitting parameters to be changed through the **Fit Method…** and **Axes/Titles…** options.

Editing Standards Data

Standards data can be edited through the standards editor grid control or by dragging the relevant points up and down.

The curve fit is automatically recalculated in the test window after a point has been dragged. To recalculate the curve after a point has been edited in the grid control, right click on the curve and select **Recalculate Fit…**.

LabLock

This section describes the functionality and use of LabLock.

Overview

LabLock is a companion product of Stingray. LabLock is used to create and edit user profiles that describe to Stingray what a user can and cannot do. Users logon to Stingray by entering their user name and password; Stingray allows each user to carry out the tasks that they have been granted. Insulating users against irrelevant Stingray functions can also simplify Stingray navigation.

For example, Jane a laboratory technician is granted permission to perform all Stingray operations, except the editing of readings files and raw data. Jane sets up and tests protocol files. Fred and other laboratory staff are only allowed to take readings by using Jane's protocols. Fred cannot accidentally (or intentionally) corrupt Stingray protocol files. Readings taken by any laboratory staff cannot be changed or modified after the readings have been made. The user name of the person carrying out the test is stored with the relevant readings files.

Stingray usage and activity can also be continually logged and monitored and logged on user names can be automatically included in reports.

User Profile Options

The following list describes Stingray operations that can be enabled or disabled for each user. (If LabLock is not in use Stingray enables all operations by default – unless otherwise stated.)

Create and edit protocol files.
Review protocol files.
Create and edit standards files.
Review standards files.
Create readings.
Review readings files.
Edit readings files.
Edit raw endpoint data (disabled by default).
Edit raw kinetic data (disabled by default).
Flag and unflag wells.
Flag and unflag kinetic cycles.

Editing Raw Data:

The editing of raw data is normally forbidden. However, the ability to quickly edit raw well readings or individual kinetic point readings (and their times) allows Stingray calculations to be easily tested and demonstrated.

Activity Log

LabLock can be used to log activity for any or all defined users. Logged activity includes:

When users log on and log off to Stingray.
When users load and save protocol/readings/standards files.
When users edit the value of a raw matrix well.
When users edit the value of a kinetic cycle in raw matrix well.
When users flag or unflag a well.
When users flag or unflag kinetic cycles.
When readings are started.
When readings are finished.
When readings are aborted.
When a report is printed.

Activity Log Location:

All activity is logged to a file named USAGE.LOG situated in the Stingray installation directory.

User Name Logging

Stingray can use the logged on user name:

as a default entry for GLP data.
as part of the report header and/or footers.
to associate well or kinetic cycle flagged items and unflagged items with a user.
as part of a target directory for results files

Editing Raw Data

If the LabLock options Edit raw endpoint data *or* Edit raw kinetic data are enabled for the logged on user then raw data can be changed manually. This is useful for testing Stingray calculations or for demonstration purposes.

To edit raw endpoint data simply double click on a well in a raw matrix to change and enter the new value.

Kinetic data can be edited in a similar way. To change kinetic reading values select the Data | Cycle view to view a matrix of kinetic readings for a particular cycle and double click on a well to edit it's value. To change kinetic reading times select Data | Times to view a matrix of the times a particular well was read at and double click on a well to edit it's value. Kinetic values can also be changed through the larger kinetic graphs. Double click on a kinetic graph in the Data | Graphs view to display a zoomed kinetic graph. Kinetic points can be dragged up and down; this is useful for quickly drawing test reduction slopes.

After changing raw data click the recalculate button to perform any necessary calculations.

Appendix A Expressions

Expressions are used to specify User Defined Expression Transformations, conditions for Automatic Flagging, Cut-Offs and Validation conditions. Stingray uses a simple but powerful mathematical grammar to parse and evaluate expressions. To understand how expressions work in Stingray it helps to learn the steps involved in the evaluation process, see Table 14 and Table 15 for details and examples.

Expression syntax and grammar is consistent throughout Stingray, however, the way which expressions are used depends on the feature being used:

Stingray Expressions

User Defined Expression Transformations

In User Defined Expression Transformations an expression is evaluated and the result is placed in an output matrix. The evaluation of User Defined Expressions depends upon the well which the expression is in and the matrices which the x and y variables refer to.

Auto-mean is always enabled for User Defined Expression Transformations (see below).

Validations

Validation expressions are Boolean expressions. If the expression results in a 1 the validation is true, if the result is 0 the validation is false.

Automatic Flagging

Automatic Flagging expressions are Boolean expressions. Automatic Flagging expressions are tested on every well in all raw matrices. An Automatic Flagging expression must contain an **x** variable. This **x** variable is replaced by the value of a well to test. If the expression is evaluated true, then the well will be flagged.

Auto-mean is always enabled for Automatic Flagging (see below).

(For kinetics the expression is tested on the result of the reduction).

Cut-Offs

Cut-Off expressions are Boolean expressions. Cut-Off expressions are tested on a well or a group. A Cut-Off expression must contain an **x** variable that is replaced by the value of a well or group to test. If a Cut-Off expression is evaluated true then the well or group tested will be given the label associated with the true expression.

List Operators

When expressions are evaluated the process depends whether auto-mean is enabled or disabled. If auto-mean is enabled then all references to groups in the expressions (such as *sample1*) will be replaced by the mean of the specified group.

If auto-mean is disabled an expression can use other list operators. The list operators available are described here.

List Operator:	Description:
`mean`	Mean – the average value.
`sd`	Standard Deviation – this is a measure of how widely values are dispersed from the average value (the mean). This is calculated using the "nonbiased" or "n-1" method.
`pcv`	Percentage CV – this is the Standard Deviation divided by the mean multiplied by 100.
`var`	Variance – this is simply the square of the Standard Deviation.
`max`	The maximum number in the list.
`min`	The minimum number in the list.
`log`	Calculates log10 of the mean value of the list (returns 0 on error).
`delog`	The inverse of log (i.e. 10 to the power of y).
`ln`	The natural log of the mean value of the list.
`exp`	The invert of ln of the mean value of the list (i.e. exponent to the power of y).

Table 12 List Operators

Binary Operators

Binary operators are operations performed on two values. Binary operators are used in the middle of two values to test.

Binary operator:	Description:
`and`	True if both operators are true (i.e. both values equal 1).
`or`	True if either or both operators are true.
`not`	True if both operators are not equal.
`=`	True if both operators are the same.

Table 13 Binary Operators

Step:	Description:	Example 1: (User Defined Expression Transformation)	Example 2: (User Defined Expression Transformation)	Example 3: (User Defined Expression Transformation)
Expression		sample1-blank1	x-blank1.	samplen-blankn
Situation		auto-mean is enabled, each group has two replicates and the expression is stored in well A1 of the first matrix.	auto-mean is enabled, each group has two replicates and the expression is evaluated for well A1 in the first matrix	auto-mean is disabled, each group has one member (no replicates) and the expression is evaluated for well A1 in the first matrix. Well A1 is of group type sample1.
1. Replace Macros	Replaces n in expressions with the group number. Replaces gn in expressions with the group type and group number.	sample1-blank1 No change.	x-blank1 No Change.	sample1-blank1 The n means the group number of the well that the expression is in. In this case the n is substituted for a 1 which is the group number of the well.
2. Expand	This step replaces variables in the expression with explicit well references. The explicit well references identify which well on which matrix to use. #1 means the first matrix defined, Raw1. #2, #3, etc. would identify subsequent matrices.	mean (a1#1, a2#1) – mean (h11#1, h12#1)	a1#1-mean (h11#1, h12#1) The x is replaced with a1#1	a1#1-b1#1
3. Replace with numbers	The values stored at each well replace the well references.	mean (0.023, 0.025) – mean (0.008, 0.007)	0.023-mean (0.008, 0.007)	0.023-0.003
4. Evaluate	The mathematical equation is evaluated.	0.00165	0.00165	0.020

Table 14 Expression Examples

Step:	Example 4: (User Defined Expression Transformation)	Example 5: (Cut-Off)	Example 6: (Automatic Flagging)	Example 7: (Validation)	Example 8: (Validation)
Expression	gn-blankn	x > control1	x=9.999	(standard1 < standard2) and (standard2 < standard3)	pcv (standard1) < 25
Situation	auto-mean is disabled, each group has one member (no replicates) and the expression is evaluated for well b3 in the second matrix. Well b3 is of group type standard2.	auto-mean is enabled, each group has three replicates and the expression is a cut-off expression, being evaluated for a well with value of 0.5, in the third matrix.	auto-mean is disabled, the automatic flagging is being tested for well A1 in Raw1.	auto-mean is enabled, this validation is being tested on Raw1.	auto-mean is disabled, this validation is being tested on Raw1.
1. Replace Macros	standard2 – blank2	x > control1	x=9.999	(standard1 < standard2) and (standard2 < standard3)	pcv (standard1) < 25
	The group and number of the well it belongs to (standard2) replace gn and blankn is replaced by the group number of the well, 2.	No change.	No change.	No change.	No change.
2. Expand	b3#2-c3#2	x > mean (c1#3, c2#3, c3#3)	a1#1=9.999	(mean (a1#1, a2#1) < mean (a3#1, a4#1)) and (mean (a3#1, a4#1) < mean (a5#1, a6#1))	pcv (a1#1, a2#1) < 25
3. Replace with numbers	0.405-0.005	0.5 > mean (0.023, 0.024, 0.022) The x is replaced by the value being tested, 0.5.|	0.023=9.999 The x is replaced by the value of the well being tested.	(mean (0.001, 0.002) < mean (0.010, 0.020)) and (0.010, 0.020)) < mean (0.060, 0.070)))	pcv (0.001, 0.002) < 25

4. Evaluate	0.400	1 Boolean expressions are evaluated to either 1 (true) or 0 false. For this Cut-Off expression, the result is 1, therefore the label associated with this expression will be stored for the well which had a value of 0.5.	0 This Boolean expression is evaluated to 0, false. This Automatic Flagging expression was evaluated to false. Therefore, the well passed this expression and will not be flagged.	1 This Boolean expression is evaluated to 1, true. This Validation succeeded.	1 This Boolean expression is evaluated to 1, true. This Validation succeeded.

Table 15 More Expression Examples

Appendix B Stingray Logarithms

Linear Regression Example

The example data set to fit a curve to is as follows:

X	Y:
1	0
2	1
4	2
8	3
16	4
32	5

Table 16 Example Data Set

If we plot this data on a graph with linear axes and the curve is fitted treating this data as non-logarithmic then we get the following curve if we use linear regression:

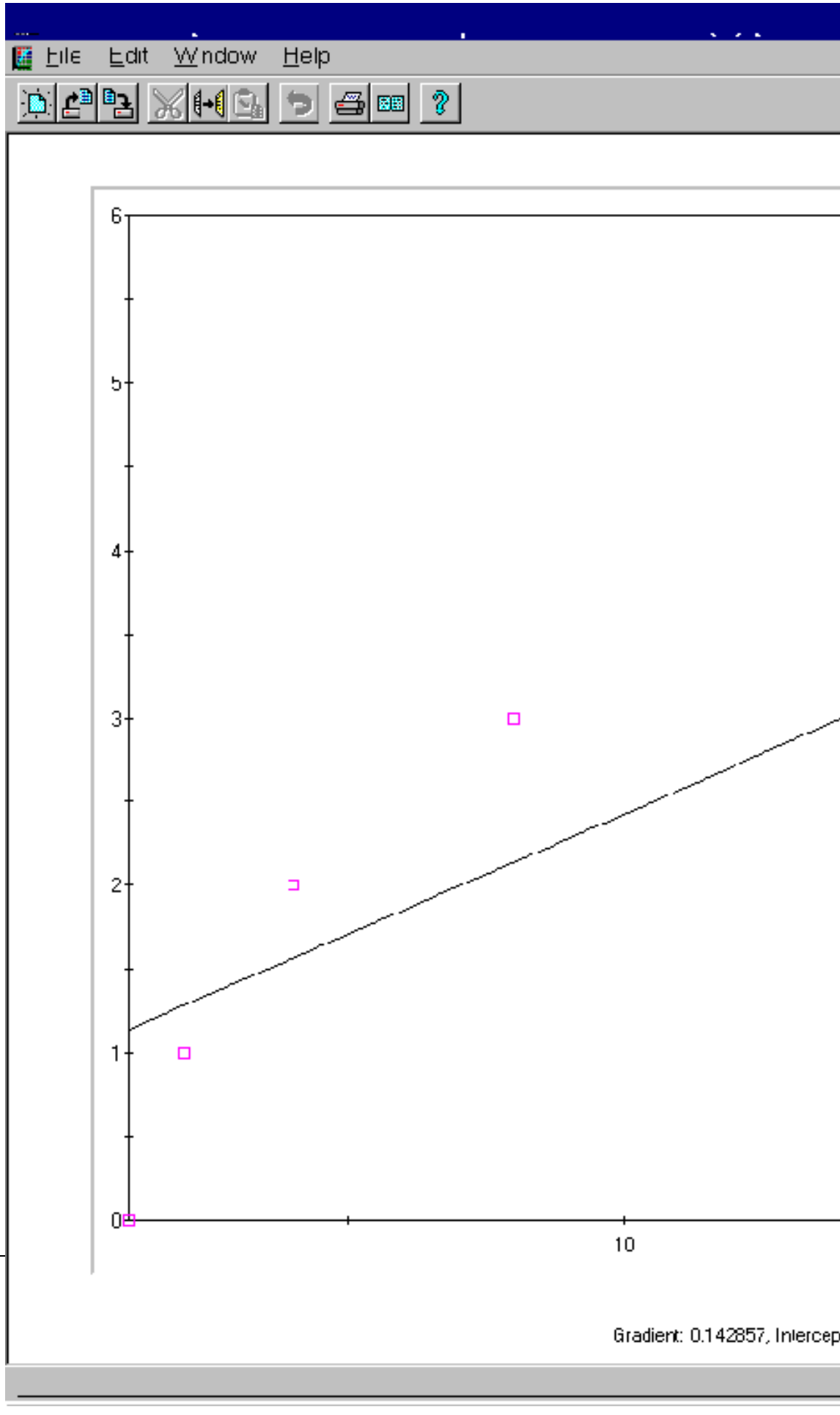

File Edit Window Help
6
5
4
3
2
1
0
10
Gradient: 0.142857, Intercep

Figure 53 Linear regression on the data set without data logging displayed on linear axes

The Stingray log curve data point wizard step allows curve data to be logged before the curve fit is made:

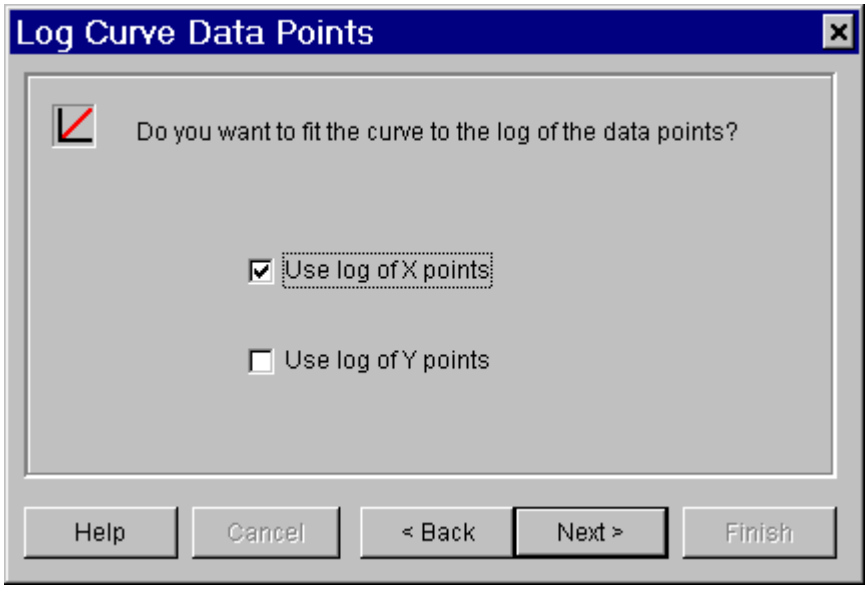


Figure 54 Stingray option to specify that the curve fit should be made on the log of the x data values

Now, the curve is fitted using the logged x data values. We get the resulting curve from a linear regression fit:

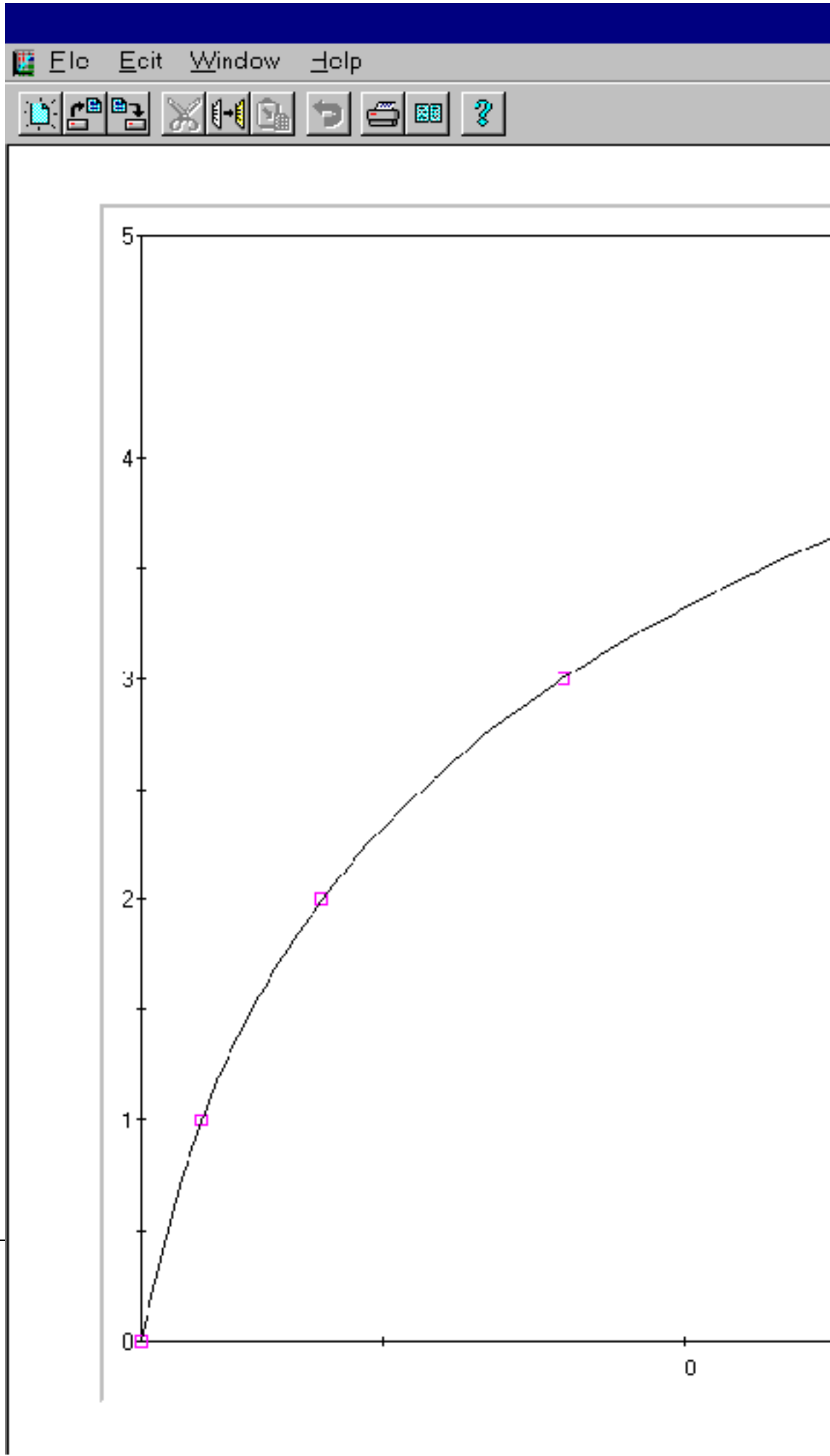

File
Edit
Window
Help
5
4
3
2
1
0
0

Figure 55 Linear regression on data set where x values are logged

These are the results we should expect - there is a straight line through all data points. However, since the x data is actually logarithmic the fitted straight line appears curved on a linear axis.

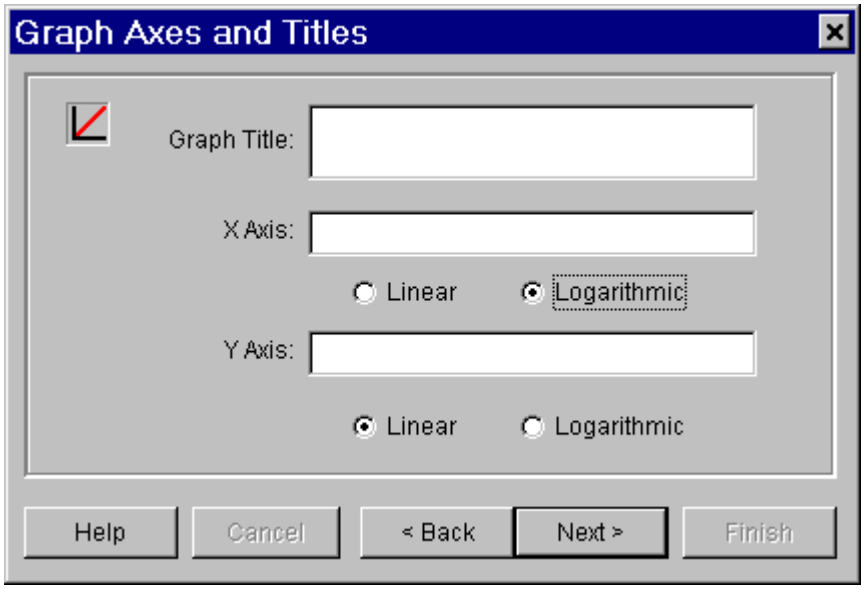


Figure 56 Changing axis type for projection.

By changing the axes type for viewing purpose we will get a visible straight line:

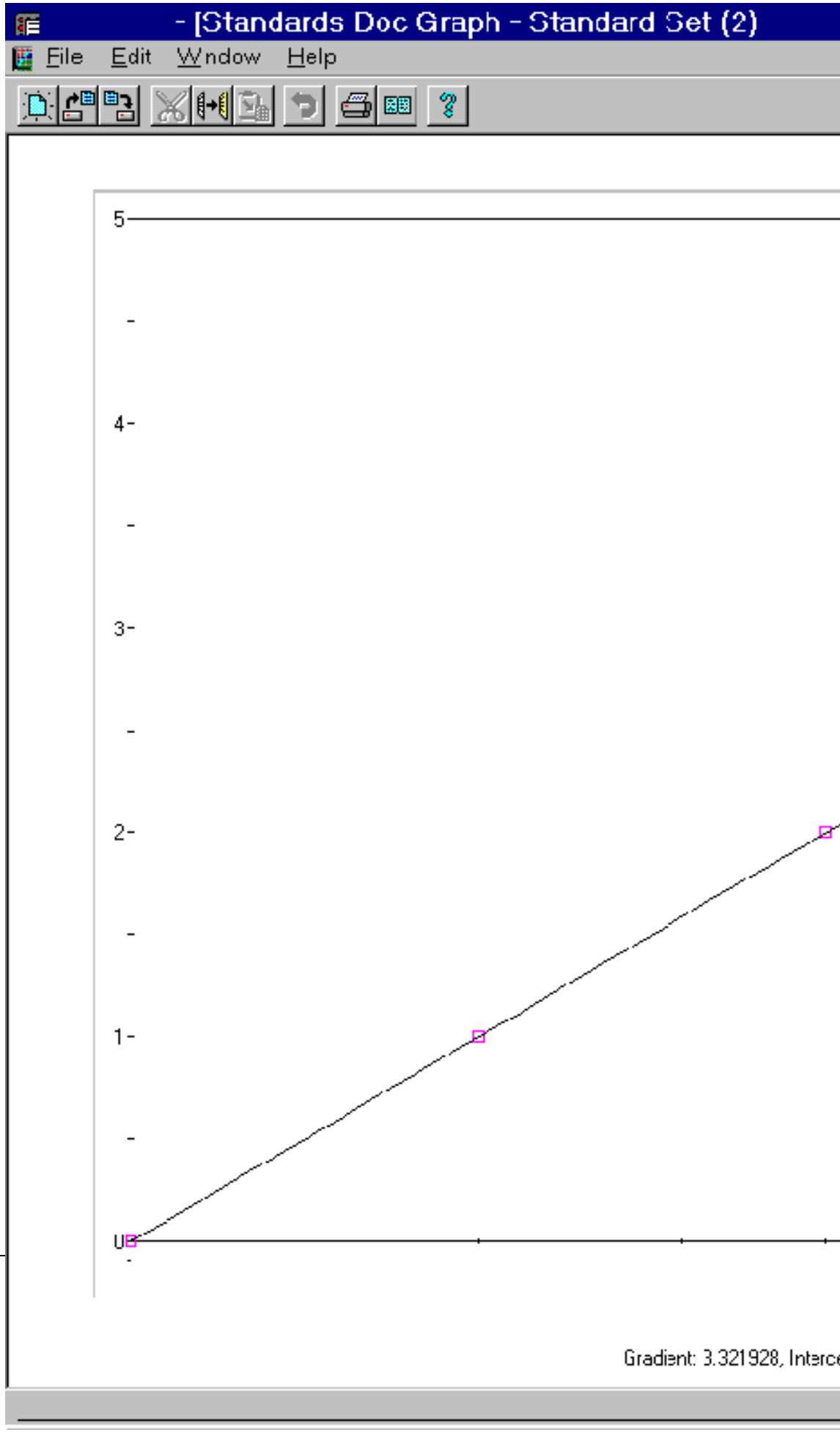

- [Standards Doc Graph - Standard Set (2)
File
Edit
Window
Help
5
4
3
2
1
0
Gradient: 3.321928, Interce

Figure 57 Linear Regression on data set with x values logged and projected on a log x axis

When to use logarithmic axes and log data values

If your data is spread over a wide range of numbers it may be useful to project the graph using logarithmic axes.

If you select to log your x values then you cannot use a standard with 0 concentration.

Appendix C Frequently Asked Questions

Why does Stingray always run in demo mode?

Stingray requires a dongle (software hard lock) for complete functionality. Contact your instrument supplier or Dazdaq Ltd for information about purchasing a dongle.

I have a dongle inserted into my PC but Stingray cannot detect it?

Ensure that the dongle is inserted into the printer port - it can be inserted into a COM port by mistake.

I have a dongle inserted into the COM port of my PC but Stingray cannot detect it? I am using an HP Laser Jet printer.

There are known problems with the HP-5L Laser Jet printer. To resolve the problems:

1. Set the LPT-1 communication mode of the PC to normal/compatible in the PC BIOS.

2. Restart Windows and run the HP-5L set-up for the drivers.

3. Select custom set-up and select PCL driver. Do not accept the Host driver. (When you install the software for the HP-5L you can choose two drivers Host based or PCL. The host-based driver uses the PC to process print data)

I have a dongle inserted into the COM port of my PC but Stingray cannot detect it? I am using Windows NT.

This problem occurs if a user without administrative rights installed Stingray on an NT system. There are 2 solutions to this problem:

1. Uninstall Stingray and log-on to Windows as the administrator. Repeat the installation process.

2. Log-on as administrator, check that the file softlok.sys exists in the system root's system32\drivers subdirectory. Run the following command from the Stingray sub-directory to add the necessary registry settings:

   ```
   regini softlok.ini
   ```

   (The file softlok.ini details the registry additions.)

In either case you should reboot the machine to start the new NT service. Stingray will then detect a correctly inserted dongle.

My reader is not listed as an available device driver...

Stingray can import data from text files generated by devices not currently supported by Stingray. See the section on import scripting for further information. Dazdaq Ltd. is continually developing new device drivers. Contact Dazdaq Ltd. for information about new device drivers or to request a new device driver to be implemented.

Why is the curve fitting transformation not available?

Ensure that the Assay Master Assay Type option Curve Fitting is checked and some standard wells on defined in the template layout.

Why are some of my standard points flagged when I use the cubic spline or point-to-point fit method with 2 or more replicates?

The lowest and highest standard points are flagged when the concentrations transformation is performed. Points are flagged during a cubic spline interpolation or point-to-point fit if they are outside of the range of the standard graph - these methods cannot be extrapolated. If you look at the standards graph the highest y point is the mean of the first standard points and the lowest y point is the mean of the last standard points. When using 2 or more replicates it is the mean of each standard group that is used, thus when interpolation is performed on each WELL one point from both the lowest and highest standard group will be out of range. Any point outside of this range has no solution.

The points are not ignored in the actual fit - they are simply marked as flagged because there is no result in the concentration transformation.

Stingray calculates the concentrations for all wells, not just the samples - this is why groups and wells outside of the range of data points will be flagged.

If required you could add a transformation step before the concentrations transformation that would mean each group first so the curve fit interpolates on each group. Simply create a user-defined transformation with auto-mean enabled and enter the expression gn in all wells.

Why does the four or five parameter fit method not find an optimal solution for my data set?

The four parameter and five parameter fit methods are only suitable for sigmoidal data sets. If the data set is not sigmoidal, it may be that there are no values for the coefficients A, B, C and D which would produce a feasible curve through all of these points using the constraints of the equation.

Why does Stingray keep asking me to use the default report - I loose my settings?

If small changes are made to the protocol they can affect the report content in a dramatic way. Stingray resets the report content when such changes are made to ensure that the report is valid. To avoid this situation leave the report set-up until you are happy with the rest of the protocol.

Why does my Auto-Flagging expression not work correctly for all wells?

Auto-Flagging expressions are evaluated well by well from the top left to bottom right of the microplate (e.g. A1, A2, A3, …, B1, B2, B3, …, H12). Stingray will flag each well as it is tested. This must be considered when designing the template layout. For example, the auto-flagging expression:

```
x < mean (control1)
```

used with a control group in replicate of 2 or more would flag members of the control group which are less than average. If members of control1 are flagged then the value of the mean of control1 changes - there are less members. Thus, the control1 group should be situated as the group closest to the bottom right of the microplate.

How can I overlay the kinetic curves?

Use the Export to Excel option from the right-click menu on the matrix view and create a combined graph using the Excel chart wizard.

The kinetics matrix printout is not very clear, what can I do?

Select the View Monochrome Graphs option from the Kinetics matrix, right click data menu prior to printing.

I am reading multiple plates, should I check the Multiple Plates and Extended Samples option in the Assay Master?

Check Multiple Plates and Extended Samples if blanks, controls and standards will only be read on the first plate. All readings on subsequent plates will be for samples.

If only the Multiple Plates option is ticked then the template layout will be the same for every plate read. Standards, blanks and controls will be read on every plate. However, when configuring a curve fit you can specify to read standards on the first plate only. In this situation the blanks and controls will be read on every plate but standards will be read on the first plate only. The wells occupied by standards on the first plate will be treated as samples in subsequent plates.

Why do I get strange concentration values calculated for my first and last standards when using polynomial regression and 2 or more replicates?

For all curve fit methods (except linear regression) Stingray calculates the curve from the mean of the unflagged standards points. When Stingray performs a curve fit transformation it calculates a concentration value for each well. Thus, Stingray will find the concentration for all wells including each standard well.

In the situation where we are using standards groups with 2 replicates, a replicate in the first and a replicate in the last standard group will be outside of the range of standards - this is because Stingray generated the curve from the mean of the replicates. When using more than 2 replicates there may be 2 or more such standards points out of range.

In these situations Stingray will have to extrapolate the curve to calculate a concentration. With a polynomial curve there will be turning points, this means that there is a possible ambiguity - it is possible that two or more points on the curve have identical y values but different x values. In this case Stingray may not choose the correct X value.

This situation does not occur if interpolating - as will be the case of all other standards groups and should be the case for samples. This is why extrapolation is not recommended with polynomial regression.

This situation will not affect the results but can be resolved when using blank correction or by adding a user-defined transformation. If using blank correction create a blank correction transformation that uses the mean of each group. If blank correction is not being used, create a transformation that calculates the mean of each well. To do this, simply create a user-defined transformation with auto-mean and enter the expression 'gn' into each relevant well. In either case the curve fit transformation uses the output of the first transformation that is the mean of each group.

How can I manually enter/adjust raw data in Stingray?

By default, Stingray raw data cannot be adjusted. However, it is often useful to be able to do this. This can be achieved by creating a Stingray user with editing rights. To do this:

1. Launch LabLock from the Stingray program group.
2. Click the New User… button to create a new user.
3. Enter a user name and password.
4. Tick the access rights for Edit Raw Endpoint Data and Edit Raw Kinetic Data as required.

5. Go to LabLock's File | Save As option - depending on whether LabLock has been used before.

6. Enter a filename for this user information file.

7. When prompted whether Stingray should use this profile click Yes.

8. Now launch Stingray as normal.

9. Stingray will prompt for a user name and password. Enter these details.

To edit endpoint raw data load any existing data file. Select the raw data matrix to edit and double click on the well to adjust. Enter the new value.

To edit kinetic raw data load any existing data file. Select the raw data matrix to edit and double click on a kinetic well to display a zoomed kinetic well view. Left click and hold the mouse button down on a kinetic point to adjust and drag the mouse up or down. Release the mouse button when dragging is complete.

When all adjustments have been made press the Recalculate button to recalculate the new results using the modified raw data.

To stop using LabLock use the Stop Client using LabLock command in LabLock's file menu.

How can I find out if I am using the latest version of Stingray?

Find the version number of Stingray from the application's Help | About menu option. Refer to Dazdaq Ltd.'s web page for information about the latest release of software.

Appendix D Conclusion of Independent Review of Curve Fitting Methods

Dr A.R. Humphries (Centre for Mathematical Analysis and its Applications) and Dr D.R. Robinson (Centre for Statistics & Stochastic Modelling) both of the University of Sussex have tested the curve-fitting module of the Stingray software version 1.5.

Tests were made on the linear regression, point-to-point, cubic spline, polynomial, four and five-parameter curve fitting functions. Results were compared against standard reference data sets and standard curve fitting routines.

We conclude from these independent tests that when used with appropriate data the curve fitting functions perform to a high degree of accuracy as detailed in the product's technical specification.

In particular the linear regression algorithm returned parameters to 6 decimal places of accuracy on all the data sets tested, and for low order (less than or equal to 5) polynomial fitting the routine returned parameters to 4 decimal places of accuracy on all but one data set tested. It is inherent in the non-linear nature of the four and five parameter fit functions that there need not be a unique best fit and thus the accuracy of the parameters found cannot be measured. The software has adequate safeguards in the sense that it did not return a nonsensical fit on any of the data sets tested.

Appendix E Glossary

Assay	A test to carry out.
Auto-mean	A mode of parsing expressions. If a Stingray expression references a group (such as sample1) that contains replicates the parser treats the group reference as a list of well references. If auto-mean is enabled Stingray will automatically find the mean of these replicates. If auto-mean is not enabled then another list operator can be used on the replicates.
Best fit	The value of a mathematical model's parameters when the difference between the data and the model is minimal. The accuracy of the result depends on the difference measure used and the initial parameter estimates.
Blank	A group type used for reading a background count.
Blank correction	A Stingray transformation for the compensation of a determined background count.
Boolean	A system of symbolic logic devised by George Boole to describe logical operations. Stingray allows the use of the Boolean operator and, or, and not in expressions. The results of Boolean expressions are either true or false.
Calculation log	Holds details of Stingray calculations, validations, errors, warnings and flagged items made. The log is updated every time calculations are made.
Control	A group type used for verifying an assay.
Correlation Validation	Either a maximum or minimum figure can be selected for the correlation coefficient of the linear regression. If the validation fails then Stingray will display a warning message after the results have been calculated.
Cut-off	A Boolean expression defining a range of values that can be labeled.
Cycle	A single read of a kinetic assay.
Data file	A Stingray file containing the raw data acquired in the assay, a reference to the protocol file, a list of any flagged items and any notes made during the assay.
Decay correction	A calculated compensation made to raw readings due to the gradual deterioration of readings over time. Stingray can automatically perform decay correction on tube reading assays.
Decay tube	A tube read repeatedly throughout the assay to determine the deterioration of readings over time.
Flag	A state of a well. If a well is flagged it will be ignored in any calculation and marked as flagged where relevant in the report.
Dispense	The preparation of wells prior to reading by administration of reagent.
Expression	A mathematical description of calculations or comparisons to be

	made.
Fast Kinetic	Repeated readings per well with a very small interval, typically < 1 second.
GLP	Acronym for Good Laboratory Practise. In Stingray this facility allows required data to be entered by the user and stored with an assay.
Group	A single well of a specified type or a collection of wells that are replicates of the same type.
Group reference	A specification of a well or collection of wells on the template layout. For example sample1 refers to the well defined in the template layout as sample1. If there is more than one well defined as sample1 the group reference refers to all of the members of the group – its replicates.
Incubation	A period of time where reactions occur. Stingray waits during these periods.
Initial Dispense	Any dispensing carried out before the readings are made.
Interval	The period of time between kinetic cycles.
Kinetics	An assay testing the rate of reactions.
Manual flagged items	Flaggings made by the user after the readings have been made to remove apparent assay anomalies.
Matrix	A set of numerical data corresponding to readings or calculations on the virtual microplate.
Multiple Standards	An assay with more than one set of standards on the virtual microplate.
Operand	A reference identifying a value which Stingray can perform mathematical operations or comparisons with in an expression.
Operator	A symbol (e.g. +,-,/,*,) or word (e.g. mean, sd, var, pcv, and, or, not) in an expression to identify mathematical functions or comparisons to be made.
Optimal solution	The value of a mathematical model's parameters when the difference between the data and the model is minimal. The accuracy of the result depends on the difference measure used and the initial parameter estimates.
Parser	The part of Stingray that interprets expressions.
Pre Read Options	Device procedures which occur before any readings are made but after any initial dispensing (such as incubation).
Protocol	The fixed set of parameters used to run an assay and present the data.
Automatic Flagging	Automatic flagging of wells that satisfy a condition.
Raw Data Item	A single matrix of data acquired from an external source (such as a microplate reader or tube reader).
Readings	The raw data used in an assay.

Recalculate	The method to repeat calculations for the new set of data after manually flagged items are made.
Reduction	The conversion of a kinetic graph for a well to a single number.
Replicate	A member of a group on the template layout.
Sample	A group type to test.
Slow Kinetic	Repeated readings per well with a very small interval, typically > 1 second.
Standard	A group type representing a known quantity which other wells are calculated by.
Standards File	An archived set of standards data.
Template layout	The arrangement of well groups and types on the virtual microplate.
Transformation	Calculations made on a matrix resulting in a new matrix.
Unused	A group type ignored or skipped throughout the assay.
Validation	A Boolean expression that ensures the assay is correct.
Virtual microplate	Stingray's representation of the actual microplate or assay tubes.
Well	A well on the virtual microplate or a well on an actual microplate.
Well group	The group of a particular well.
Well reference	A specification of a well on a microplate. Stingray references wells on the virtual microplate using letters and numbers. The letter indicates the microplate row and the number identifies the microplate column.
Well type	Describes how to fill each well in the virtual microplate.

Table 17 Glossary Of Terms

References

Windows, Windows NT, Excel are registered trademarks of Microsoft Corporation.

Hewlett-Packard and Laser Jet are registered trademarks of the Hewlett-Packard Company.

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