

# OPTIMA

**Software Manual – Part IIIb:  
Excel based Evaluation Software**

**Version 2.10**

This manual was designed to guide OPTIMA users through the software features.

Although these instructions were carefully written and checked, we cannot accept responsibility for problems encountered when using this manual. Suggestions for improving this manual will be gratefully accepted.

BMG LABTECH reserves the right to change or update this manual at any time. The Revision-Number is stated at the bottom of every page.

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# 1 Evaluation Software - Data Reduction

The data reduction package provides powerful Excel macros for easy data calculations as well as all the functions possible with the Excel software. Once a measurement is performed the data is automatically saved as a database file in Excel. The test run will be present in a list of all saved test runs on the first worksheet in Excel.

You can access the data by clicking on the Excel  icon in the tool bar or selecting 'Results | Excel' from the main menu bar. Or you can go directly to Excel from the Windows Start menu: 'Programs | BMG LABTECH | OPTIMA | OPTIMA – Evaluation'. If the evaluation software is opened from the start menu then a login screen will appear as with the control software. The same user path and password applies.

At the top of every worksheet is the normal Excel menu with all the functions from the Excel program. In addition there is a OPTIMA menu on the far right with some special functions specific to the data reduction package. A toolbar box  also appears there (see chapter 1.2.2). The functionality is shortly explained with tooltip texts appearing when you move the cursor over the buttons.

*Note:* The worksheets are designed for a screen resolution of 800 x 600 pixels or higher.

## 1.1 The Worksheets

The OPTIMA evaluation software will be opened with up to 8 worksheets:

### Test Runs

This worksheet is displayed when Excel is opened. It lists all the test runs that have been performed along with the 3 identifiers, the layout, microplate, date, time and database file number. Select the test run you want by double clicking on the test run.

### Raw Data

This worksheet gives the raw results from the measurement. You can select the data you want to include in the signal curve sheet and define the ranges for kinetic calculations.

### Signal Curve

You can view the curve formed by the kinetic points for a single well or a group of wells. If you only have one kinetic point (only one column of measurements), the signal curve sheet will not appear.

### Evaluation

View the results on three tables; you can select how the data is presented on the table, i.e. averages, raw data, blank subtraction, standard concentrations, etc.

### Sample IDs

Contains a list of all sample IDs. This sheet is only available if sample IDs are defined.

### Standard Curve

Plot of the curve from the standards and the results of the unknowns according to the curve. This worksheet is only available if standards are defined.

### Result List

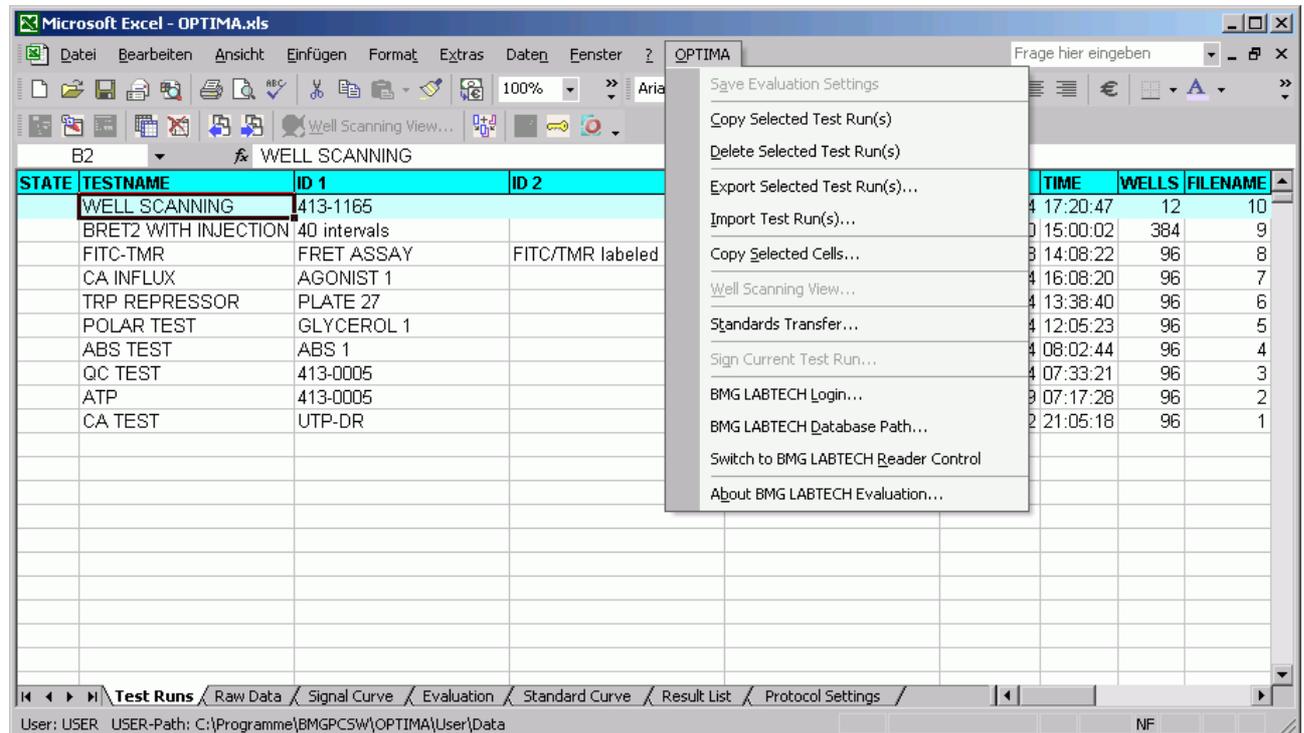
Shows the raw data, the average, the standard concentration, SD, %CV values of replicates and the recalculated sample concentrations in list form. This worksheet is only available if standards are defined.

### Protocol Settings

In this sheet you can see the used protocol settings for your test run, also two fields with the audit trail and signature entries.

## 1.2 Test Runs Worksheet

The Test Runs worksheet is automatically displayed when Excel is opened. It lists all the test runs that have been saved or imported. At the bottom of the worksheet, the user name and the user path are shown; the test runs for that user only are displayed on the worksheet.



### State

The state field appears on the very left and describes the history of a test run. The following states are defined:

- O:** test run has been imported from an older version of the database; this means, in its history no validation checks have been made.
- C:** test run has been copied.
- M:** modified test run (e.g. wells have been taken out (see Save under 1.3) or Sample IDs (see chapter 1.6) have been changed).
- X:** manipulations have been detected since the generation of the test run (manipulations done outside the evaluation software).
- S:** signed test run (it is not possible to save further changes to this test run). It can also appear combined with other markers, e.g. 'MS'.

If the state field is empty, the test run is still in its original state.

### Testname

The testname is listed first and appears as it is defined in the protocol definition.

### ID1 / ID2 / ID3

These are the plate identifiers that were created before the measurement (see chapter *Plate Identification* of software manual part II: Control Part).

### Date and Time

The date and time that the measurement took place.

### Wells

Plate format (number of wells) of the microplate.

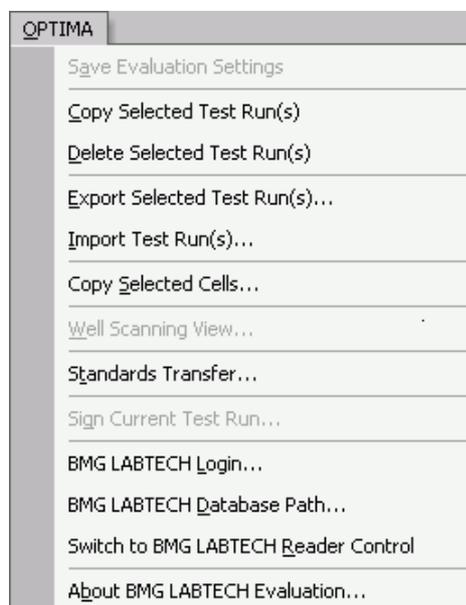
## Filename

The number of the database file assigned to the test run.

Select the test run which results you want to view and double click it. You can also select the test run and select one of the options from the OPTIMA pull-down menu located in the toolbar.

### 1.2.1 OPTIMA Pull-Down Menu

The following options are located under the OPTIMA menu entry at the top of the Excel worksheets.



#### Save Evaluation Settings

Here you can save the changes you have made during an evaluation session. Your program settings (evaluation methods, control states etc.) and the changes you made to values (changed sample Ids, wells marked as 'deleted', changed comments) will be saved and used again for this test run when you reopen it.

This option is disabled, until you have made changes to your session or if you have signed the test run. When you make changes during a session, you have also the possibility to save and store them before choosing another test run or before signing a test run.

Each time you save your changes, an audit trail entry is made. The audit trail entries can be seen on the Protocol Settings sheet.

#### Copy Selected Test Run(s)

This feature allows you to copy an entire test run - a reproduction of all the raw data, layout, etc. It is then possible to modify the data and save the changes without losing the original data. Highlight the test run you wish to copy by clicking the test name, then choose 'Copy Selected Test Run(s)'. The copied test run is listed on the test run list with an 'C' to the left of the name, if the test has no other marker.

For example, if you want to remove raw data from the calculations, you can copy the original test run and then remove the data you do not want to use and save these modifications.

#### Delete Selected Test Run(s)

If you want to delete a test run, select it and choose 'Delete Selected Test Run(s)'. You can select and delete several test runs at a time.

#### Export Selected Test Run(s)...

Using the export function, you can export the test run(s) onto a diskette or a hard drive folder of your choice. Highlight one or more test runs at a time and select 'Export Selected Test Run(s)...'. A dialogue box will ask for the destination drive and folder as well as the file name. The extension for exported test run files is '.RUC'.

**Import Test Run(s)...**

With the import function, you can import test runs. Click on 'Import Test Run(s)...', and the next window will ask for the file name, the folder and drive where it is located. The imported test runs will be added to the list on the Test Runs worksheet. To be compatible to previous versions of the BMG software, also files with the extension '.RUN' resp. '.RUM' will be accepted for import.

**Well Scanning View...**

If the opened test run contains well scanning data you can open the window to view the data. For more details about the presentation of the well scanning data see chapter 1.10.

**Copy Selected Cells...**

Select the data you want to copy in a worksheet and choose 'Copy Selected Cells...'. A new workbook is created and the selected data will be copied into a sheet within the new workbook. This workbook can be saved under a new name and can be used like any Excel workbook.

**Standards Transfer...**

This opens the Standards Transfer Wizard, see chapter 1.11.

**Sign Current Test Run...**

You can sign a test run, if you have opened it. You can make more than one signature for each test run. An audit trail entry is made for each signature. The signatures can be seen on the Protocol Settings sheet.

If you have signed a test run, it is not possible to save further changes. If you would like to make changes to a signed test run, you must make a copy of it with the 'Copy Selected Test Run(s)' menu point. The signature will be removed in the copied version.

For more details about signatures see chapter *Digital Sign Function* of software manual part IV: FDA 21 CFR part 11.

**BMG LABTECH Login...**

This allows you to change the logged in user. The functions are the same as in the control part (see chapter *Login Screen* of software manual part II: Control Part).

**BMG LABTECH Database Path...**

A destination directory for the test runs can be specified. The change is valid only for the user who is logged in. The directory must be a subdirectory of the user directory. Select the drive and directory in the user path window (see chapter *User Directories* of software manual part II: Control Part).

**Switch to BMG LABTECH Reader Control**

With this function you can switch back to the OPTIMA control software or use the  button in the toolbar. The evaluation software will stay open in the background.

**About BMG LABTECH Evaluation...**

This opens an information window about the evaluation software.

To access the Raw Data, Signal Curve and Standard Curve worksheets for a test run, double click on the desired test run.

## 1.2.2 OPTIMA Toolbar

The OPTIMA toolbar contains twelve buttons.



### Save Evaluation Settings

Same functionality as the correspondent menu option, see chapter 1.2.1



### Copy settings from Test Run / Paste settings to Test Run

When you leave a test run, it is possible to save the settings you made during your session, e.g. evaluation methods, sorting options etc. When the test run is opened again, the evaluation software will be restored with the same adjustments.

If you would like to copy this settings to a series of test runs, first select this test run on the test run sheet and press the 'Copy settings from Test Run' button. The settings are now stored in the memory. If you now select a series of test runs and press the 'Paste settings to Test Run' button, your settings will be copied to these test runs.



### Copy Selected Test Run(s)

Same functionality as the correspondent menu option, see chapter 1.2.1



### Delete Selected Test Run(s)

Same functionality as the correspondent menu option, see chapter 1.2.1



### Export Selected Test Run(s)

Same functionality as the correspondent menu option, see chapter 1.2.1



### Import Test Run(s)

Same functionality as the correspondent menu option, see chapter 1.2.1



### Well Scanning View... Well scanning view

Same functionality as the correspondent menu option, see chapter 1.10 *Display Well Scanning Data*.



### Standards Transfer

If you would like to apply a standard measurement to another test run with samples, you can use this feature. A detailed description can be found in chapter 1.11.



### Sign Current Test Run

Same functionality as the correspondent menu option, see chapter 1.2.1



### BMG LABTECH Login

Same functionality as the correspondent menu option, see chapter 1.2.1



### Switch to BMG LABTECH Reader Control

This button enables you to directly switch from the evaluation software to the control part.

### 1.3 Raw Data Worksheet

This worksheet displays all the raw data for each interval or cycle from the measurement. Each row represents the data from a specific well. Each column represents the data at a particular interval (well mode) or cycle (plate mode).

Test Name **GROUP A STANDARD** ID 1: **Rhodamin** ID 2: ID 3: 2001.09.12 11:42:17

Order by rows  
 Order by columns

Calc. Range Start 1  Stop 1 
 Calc. Range Start 2  Stop 2

All

Well	Cont.	Cycles	2	3
	t	0	143	286
	C	0,0	0,0	0,0
A01	SA1	1	56668	57495
A01	SA1	2	249	248
A01	SA1	3	1565	1516
A01	SA1	4	62	59
A02	SA2	1	19777	19642
A02	SA2	2	136	134
A02	SA2	3	894	902
A02	SA2	4	42	42
A03	SA3	1	8902	8630
A03	SA3	2	69	72
A03	SA3	3	532	564
A03	SA3	4	35	42
A04	SA4	1	4762	4514
A04	SA4	2	34	40
A04	SA4	3	354	376
A04	SA4	4	36	32
A05	SA5	1	2947	2845
A05	SA5	2	21	23
A05	SA5	3	258	290
A05	SA5	4	37	35

#### Description of the chart:

Order by rows	The order of the wells appears sorted by rows on the microplate (default).																								
Order by columns	The order of the wells appears sorted by columns on the microplate.																								
Well	The coordinates of the well in the microplate (A01= row A, column 1)																								
Cont.	<p>Content of the well as labeled in the layout definition.</p> <p>Here you can select a specific well or a group of wells to be displayed in the Signal Curve sheet by highlighting the well name(s) in the Cont. Column. If you do not select anything here the signal curve for all wells will be displayed, although with a maximum of 253 wells.</p> <table border="1" style="display: inline-table;"> <thead> <tr> <th>Well</th> <th>Cont.</th> </tr> </thead> <tbody> <tr><td>A01</td><td>S1</td></tr> <tr><td>A02</td><td>S2</td></tr> <tr><td>B03</td><td>S3</td></tr> <tr><td>B04</td><td>S4</td></tr> <tr><td>B05</td><td>S5</td></tr> <tr><td>B06</td><td>S6</td></tr> <tr><td>B07</td><td>S7</td></tr> <tr><td>B08</td><td>S8</td></tr> <tr><td>B09</td><td>S9</td></tr> <tr><td>B10</td><td>S10</td></tr> <tr><td>B11</td><td>S11</td></tr> </tbody> </table>	Well	Cont.	A01	S1	A02	S2	B03	S3	B04	S4	B05	S5	B06	S6	B07	S7	B08	S8	B09	S9	B10	S10	B11	S11
Well	Cont.																								
A01	S1																								
A02	S2																								
B03	S3																								
B04	S4																								
B05	S5																								
B06	S6																								
B07	S7																								
B08	S8																								
B09	S9																								
B10	S10																								
B11	S11																								
CH	<p>Chromatic/Channel: Number of the used filter setting. The measurement channels are marked with 'A' resp. 'B', the chromatic numbering will always start with 1, which corresponds to the first defined filter combination in the multichromatic sheet (see chapter <i>Multichromatics</i> of software manual part II: Control Part).</p> <p><b>t:</b> In the channel column, the "t" stands for time. For more than one cycle or interval the "t" row shows the time in seconds in which the cycle / interval occurred.</p> <p><b>C:</b> In the channel column, the "C" stands for Celsius. If the incubator was used during the measurement or if the Temperature Monitoring Feature is switched on (see chapter <i>Temperature Monitoring Feature</i> of software manual part II: Control Part), this row displays the temperature of the instrument at the particular kinetic cycle (only plate mode). In well mode, an additional column "Temp" appears, which gives the starting temperature at each measurement of a well.</p>																								
Cycles / Intervals	Lists the number of the particular interval (well mode) or cycle (plate mode).																								

### Calc. Range Start 1 / Stop 1

Calc. Range Start 1    Stop 1

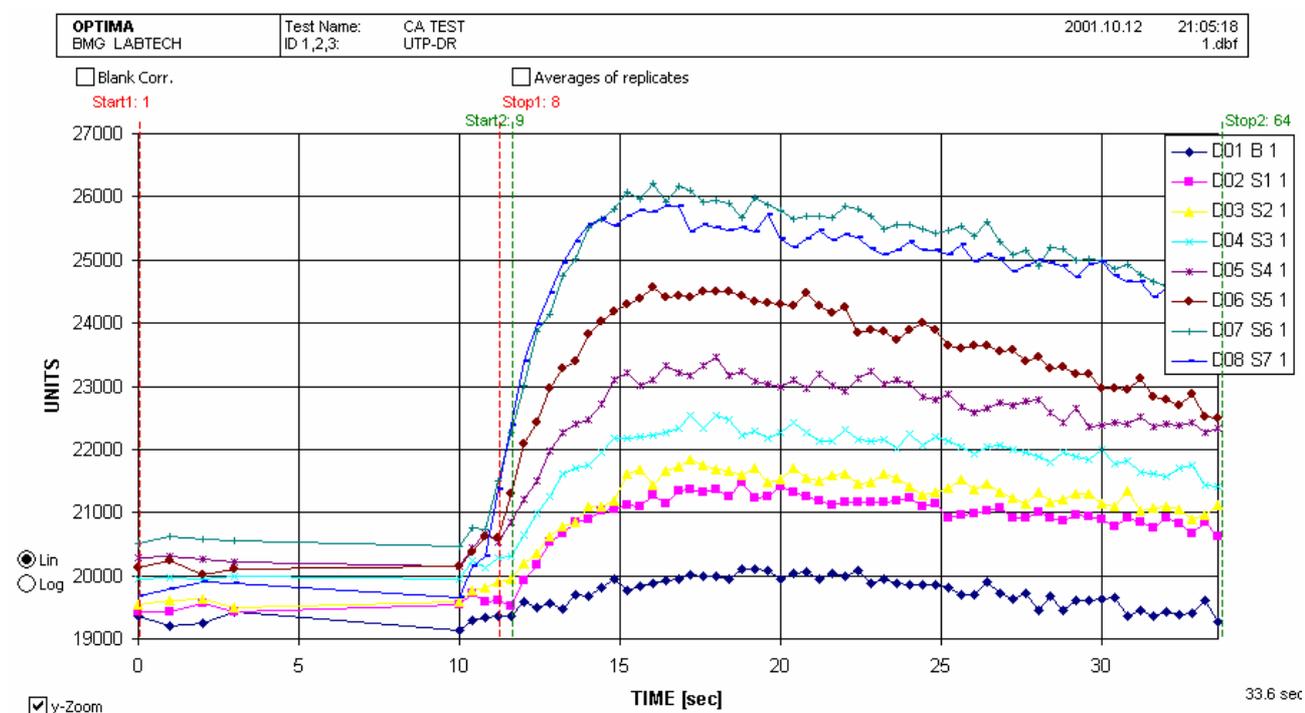
You can select the kinetic intervals / cycles that will be included in the data calculations by entering the interval that you want to start with and the interval you want to stop at. The cycles / intervals you select will be highlighted in red.

### Calc. Range Start 2 / Stop 2

Here you can select a second range of kinetic intervals / cycles. With this function it is possible to calculate the difference or the quotient of range 1 and range 2. The cycles / intervals selected for range 2 will be highlighted in green.

## 1.4 Signal Curve Worksheet

The signal curve worksheet graphically plots the data points for one well or a group of wells selected in the Raw Data Worksheet, see chapter 1.3 (Cont. description). This sheet only appears if you have more than one kinetic point.



In plate mode, you can also include the temperature line. The temperature will be displayed on the right axis of the chart.

You can change the scale for the units by clicking on the scale in the lower left corner.

If threshold is the evaluation type (selected in the Evaluation Worksheet, see chapter 1.5), then the legend will show a threshold line (as 'The') and the desired threshold number.

**Blank Corr.:** Appears only if blanks are defined. If checked, the average of the blanks will be subtracted from each data point of the corresponding signal curves. The blank curve itself will not be shown.

**Averages of replicates:** If checked, the averages of each selected content and channel will be calculated and displayed as signal curve. In the legend the names of the contents appear instead of the names of the wells.

**Y-Zoom:** A checkbox to zoom the signal curve in y-direction. The lowest and highest measurement data will be used as lower/upper limits.

**Lin / Log:** Buttons to switch between linear and logarithmic scaling of the y-axis.

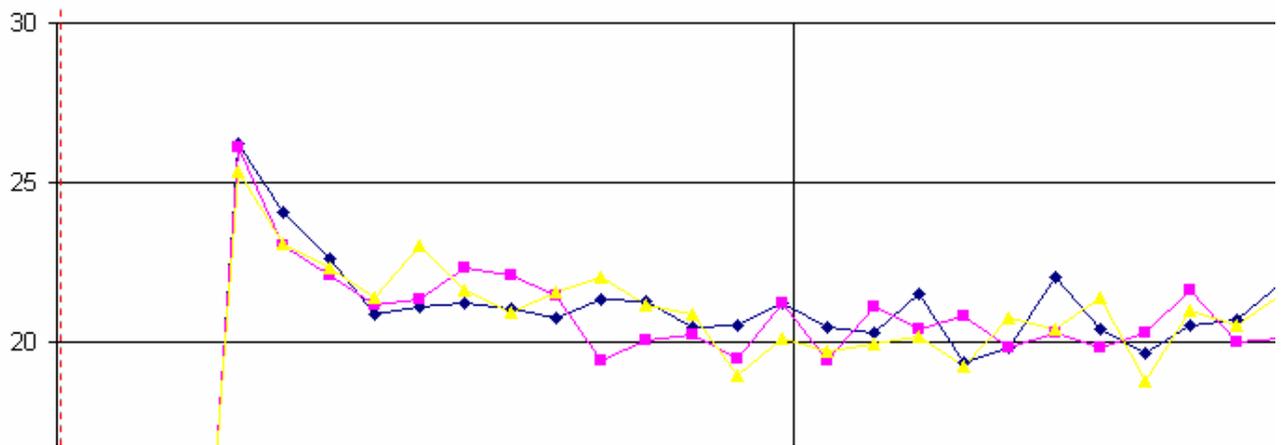
**Curve calculation:**

This feature is useful for calculations between different chromatic datasets, e.g. FURA-2 applications. It is available for all measurements with more than one chromatic or more than one channel. The wavelength calculation is disabled if polarization or anisotropy values are selected on the raw data sheet.

If an appropriate test run is chosen, a checkbox 'Activate curve calculation' appears. If it is checked, three dropdown boxes appear. In the first and third you can select the wavelength as operand, in the second you can choose the operator. You can calculate the ratio, the product, the difference or the sum between the values of two chromatic datasets.

Blank Corr.   
  Averages of replicates   
  Activate curve calculation  
Em 410 / Em 515

Start1: 1



### 1.5 Evaluation Worksheet

You can specify the evaluation method in the evaluation worksheet. It contains tables where you can see calculated data.

You can see the most important settings from the used protocol definition on the top portion of the evaluation sheet. You can see all settings in the Protocol Settings sheet (see chapter 1.8).

**Note:** The header section of 6-, 12-, 24-, 48- and 96-evaluation sheets is fixed for better general view of the results. If you use the scrollbar at the left of the window, only the tables move.

<b>OPTIMA</b>		Testname: GROUP A+B+C STANDARD	2001.09.12 12:09:07	<input type="checkbox"/> Hide protocol settings		
BMG LABTECH		ID 1,2,3: Rhodamin+4 Methylumb +Coumarin	66.dbf, imported			
Fluorescence, plate mode equidistant		Kinetic window	1	2	3	4
Microplate: BMG LABTECHNOLOGIES		No. of cycles	3	-	-	-
		Cycle time [s]	143	-	-	-
		Meas. start time [s]	0,0	-	-	-
		No. of flashes	10	-	-	-
No.	Excitation	Emission	Gain			
1	544	590	118			
2	355	460	048			
3	340	440	116			
4	485	520	093			
		Volume group	1	2	3	4
		Volume [µl]	20	-	-	-
		Injection cycle	1	-	-	-
		Shaking after inject. [s]	-	-	-	-
Pos. delay [s]: 0,2		Calculation Start1: 1 Stop1: 2		Start2: 3 Stop2: 3		
Reading direct. 1						
Comment: 110 µl color reagent per well						

Calculation: Sum  Use average of blanks of all groups Chromatic 1 Table content Layout

Table 1		Range1		1		Layout							
A	SA1	SA4	SA5	SA6	SA7	SA8	SA9	SA10	SA11	BA			
B					XA2		XA1						
C	SB1	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	BB			
D													
E	SC1	SC4	SC5	SC6	SC7	SC8	SC9	SC10	SC11	BC			
F													
G	XD1	XD4	XD5	XD6	XD7	XD8	XD9	XD10	XD11	BD			
H													
		1	2	3	4	5	6	7	8	9	10	11	12

#### Hide protocol settings

It is possible to hide the header area and the comment to give better overview for the data by checking this checkbox.

#### Reading Direction Icon Legend

		bidirectional reading		unidirectional reading	
		start left	start right	start left	start right
horizontal reading	start top	1	2	3	4
	start bottom	5	6	7	8
vertical reading	start top	9	10	11	12
	start bottom	13	14	15	16

**Comment**

It is possible to insert a comment on the worksheet by typing the comment into the comment field between header and table 1. You can save the comment permanently if you use the ‘Save Evaluation Settings’ option of the OPTIMA menu (see chapter 1.2.1). If you leave the test run without saving, you are prompted for to save.

**1.5.1 Calculations on the Three Tables in the Evaluation Sheet**

The data is presented on three different tables. Each table can have different information based on what you choose from the table content menu on the right side above each table.

A table represents the wells of the microplate and data is presented on the table according to the layout defined in the test protocol.

<b>OPTIMA</b>	Testname: GROUP A+B+C STANDARD	2001.09.12 12:09:07	<input checked="" type="checkbox"/> Hide protocol settings
BMG LABTECH	ID 1,2,3: Rhodamin+4 Methylumb +Coumarin		66.dbf, imported

Calculation: Sum  Use average of blanks of all groups Chromatic: 1 Table content: Layout

Table 1 Range1: 1

A	SA1	SA2	SA3	SA4	SA5	SA6	SA7	SA8	SA9	SA10	SA11	BA
B							XA2		XA1			
C	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	BB
D							XB13		XB12			
E	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9	SC10	SC11	BC
F							XC13		XC12			
G	XD1	XD2	xd3	xd4	XD5	XD6	XD7	XD8	XD9	XD10	XD11	BD
H							XD13		XD12			
	1	2	3	4	5	6	7	8	9	10	11	12

Table 2 Range1: 1 Standard concentration:

A	10	5	2,500	1,250	0,625	0,313	0,156	78,13E-3	39,06E-3	19,53E-3	9,77E-3	
B												
C	1000	500	250	125	62,500	31,250	15,625	7,813	3,906	1,953	0,977	
D												
E	10000	5000	2500	1250	625	312,500	156,250	78,125	39,063	19,531	9,766	
F												
G												
H												
	1	2	3	4	5	6	7	8	9	10	11	12

Responsible for standard curve

Table calculation: None Range1: 1 Raw data - blank:

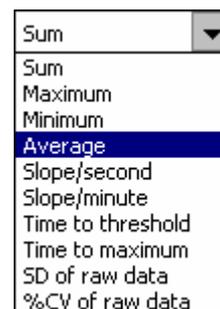
A	112705	35996	14515	6802	4006	2345	1494	1298	920	766	907	
B							424		6006			
C	117	94	65	70	250	91	-24	138	94	20	5	
D							-89		-91			
E	8	-28	59	27	-28	19	-5	73	48	41	44	
F							-90		-15			
G	977	566			68	91	174	47	48	12	38	
H							-97		41			
	1	2	3	4	5	6	7	8	9	10	11	12

Gray fields contain deleted values

## Calculation Pull-Down Menu for Kinetic Evaluations

These calculations are for kinetic assays where there are more than one cycle/ interval. They are related to the selected intervals / cycles defined by the calculation start 1/stop 1 resp. start 2/stop 2 controls on the Raw Data sheet. The selection in this menu is valid for all three tables.

You can add all the kinetic points of the selected range of cycles / intervals together for each well. The sum will be used for the tables in the evaluation sheet. Sum is the default method of calculation for both well mode and plate mode, and is selected the first time the evaluation software is entered. After that, the evaluation program will remember the last settings used and automatically re-use these settings the next time the worksheet is entered.



### **Maximum**

Finds the maximum value for each well (for the selected range of intervals / cycles). The maximum of the selected range will be listed in the tables of the evaluation sheet.

### **Minimum**

Finds the minimum value for each well (for the selected range of intervals / cycles). The minimum of the selected range will be listed in the tables of the evaluation sheet.

### **Average**

Calculates the average of all readings for each standard, sample and/or blank replicate in the selected range of intervals / cycles. The average of the selected range will be listed in the tables of the evaluation sheet.

### **Slope/second**

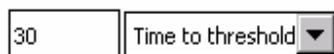
Calculates the linear regression curve and gives the corresponding slope per second value for each well (for the selected range of intervals / cycles). The slope of the selected range will be displayed in the tables of the evaluation sheet.

### **Slope/minute**

Calculates the linear regression curve and gives the corresponding slope per minute value for each well (for the selected range of intervals / cycles). The slope of the selected range will be displayed in the tables of the evaluation sheet.

### **Time to threshold**

When you select threshold over time, an additional box appears next to the pull-down menu.



You must enter the threshold that you are interested in in this field. The time it takes for the threshold to be reached is then indicated in the evaluation sheet tables.

### **Time to maximum**

Gives the time until the maximum value in the selected range of cycles / intervals is reached.

### **SD of raw data**

Calculates the standard deviation of the raw data for each well for the selected range of cycles / intervals.

$$SD = \sqrt{\frac{n \sum x^2 - (\sum x)^2}{n^2}}$$

**%CV of raw data**

Calculates the standard deviation of the raw data for each well over the selected range of cycles / intervals divided by the average of the raw data for this well / range, expressed in percent.

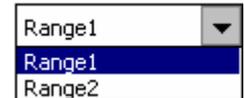
**Use average of blanks of all groups**

Use average of blanks of all groups

Here you can collect the blank values from different groups as average instead of using an individual blank value for each group. This box appears only if the test protocol contains blanks and more than one group is defined. If it is checked, then the average of all blanks is used for calculation.

**Range Pull-Down Menu**

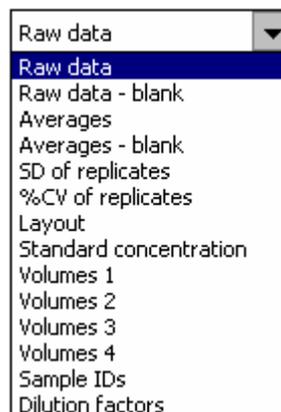
You can select the range you are interested in for each table.

**Range 1**

The measurement values of range 1 (Calc. Range start 1 ... Stop 1 on the raw data worksheet) will be used in the corresponding table.

**Range 2**

The measurement values of range 2 (Calc. Range start 2 ... Stop 2 on the raw data worksheet) will be used in the corresponding table.

**1.5.2 Table Content Pull-Down Menu****Raw Data**

The raw data with no calculations is displayed.

**Raw Data - blank**

The average of the blanks (background) subtracted from the raw data is displayed.

**Averages**

The mean of the replicates is displayed.

**Averages - blank**

The average of the blanks subtracted from the average of replicates is displayed.

**SD of replicates**

The standard deviation of the replicates and the blanks is displayed. This is important for determining limit of sensitivity.

$$SD = \sqrt{\frac{n \sum x^2 - (\sum x)^2}{n^2}}$$

**%CV of replicates**

Calculates the standard deviation of the raw data for replicates and blanks divided by the average of the raw data for replicates and blanks, expressed in percent.

**Regression coeff. (r)**

The regression coefficient as calculated from the linear regression equation is displayed. Appears only if 'Slope/second(minute)' is chosen in the calculation pull down menu.

**Layout**

The contents (standards, samples, blank) as defined in the layout section of the test protocol, are displayed.

**Standard concentration**

The concentrations of the standards that were defined the test protocol (see chapter *Concentrations / Volumes / Shaking* of software manual part II: Control Part) are displayed.

**Volumes 1, 2, 3, 4**

The injection volumes for volume group 1 ... 4 as defined in the test protocol (see chapter *Concentrations / Volumes / Shaking* of software manual part II: Control Part) are displayed.

**Sample IDs**

Shows the Sample ID for each well (as defined before test start, see chapter *Plate Identification* of software manual part II: Control Part)

*Note:* If you use very long sample IDs you will only see a part here (approximately up to 10 characters). To see the full sample ID, use the Sample IDs worksheet (see chapter 1.6).

**Dilution factors**

Shows the dilution factor for each well (as defined before test start, see chapter *Plate Identification* of software manual part II: Control Part).

**Multichromatic Data**

If more than one filter setting was used in the test protocol (multichromatic), it is possible to view the data for each setting by using the channel drop-down box. The box contains numbers corresponding to the order of the filter combinations. The number is in the order, in which the filters were defined in the protocol definition (see chapter *Defining Protocols* of software manual part II: Control Part). If you use a polarization test, the channels are displayed as 'A' and 'B'. If you use a dual luminescence test, the chromatic settings are displayed as 1A, 1B, 2A, 2B etc. (see chapter *Multichromatics* of software manual part II: Control Part).

Choose the number in the box that corresponds to the raw data you want to view. You can choose different numbers for each of the three tables to make comparisons of the data.

Which filter setting corresponds to which position can be seen in the header of the evaluation sheet. The pull-down box will be empty if only one filter pair has been used.

1	▼
1	
2	
3	
4	
5	
6	
7	
8	

**1.5.3 Data for Standard Curve**

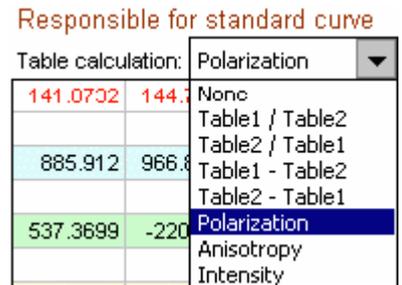
Table 3 is used for defining the data used for the Standard Curve. If you choose ‘Raw data – blank’ then the results, if valid, will be plotted on the Standard Curve worksheet. This standard curve will be the basis for calculating the unknowns.

The data selection that is valid for plotting the standard curve includes raw data (minus blank) and averages (minus blank).

A standard curve cannot be formed from volumes or layout information. If the data from table three cannot form a graph, the standard curve worksheet will be blank. If a value in table 3 is negative, the logarithmic scale for the standard curve cannot be selected.

**Calculations in the three tables**

There is an additional pull-down menu on the left, between the second and third table. You can select optional calculations between the tables here. When a calculation is chosen then all three tables will convert to the same type of data (i.e., ‘Raw Data’, ‘Averages’, etc). If no calculation is possible the third table will be gray.



Description	Explanation
None	All 3 tables are independent
Table1 / Table2	The content of table 1 is divided by the content of table 2, results are shown in table 3.
Table2 / Table1	The content of table 2 is divided by the content of table 1, results are shown in table 3.
Table1 - Table2	Values from table 2 are subtracted from the values from table 1 and the results are shown in table 3
Table2 - Table1	Values from table 1 are subtracted from the values from table 2 and the results are shown in table 3
*Polarization	The polarization values in mP units are calculated using channel A and channel B, results are shown in table 3.
*Anisotropy	The anisotropy values are calculated using channel A and channel B, results are shown in table 3.
*Intensity	The intensity values are calculated using the values from both channels, results are shown in table 3.

**Notes:** The items marked with an asterisk appear only in polarization mode.

If there is a division by zero, the respective value will be marked with ‘DivByZero’ in the evaluation tables.

**Removing data:** If you want to eliminate the results of a well from the data reduction, highlight them in one of the three tables and press ‘Delete’. The content name of this well will now appear in lower case letters. Its value will not be used in calculations. Pressing ‘Delete’ again will restore the data value.

**Save changes:** If you remove data in the described way, you can permanently save the changes by using the ‘Save Evaluation Settings’ option from the OPTIMA menu. If you leave the test run without saving, the software will ask you if you want to save your changes or not.

*Note:* Saving changes is only possible if the test run has no signature (see chapter *Digital Sign Function* of software manual part IV: FDA 21 CFR part 11). If you want to make changes to a signed test run, you must make a copy of it.

## 1.6 Sample IDs Worksheet

This worksheet is only available if you have defined Sample IDs before starting the test run (see chapter *Plate Identification* of software manual part II: Control Part).

It contains a list with all sample IDs. You can choose between sorting for rows, columns, well content or sample IDs.

<input type="radio"/> Sort column up				
<input type="radio"/> Sort column down				
<input checked="" type="radio"/> Sort row up				
<input type="radio"/> Sort row down	<input type="radio"/> Sort up	<input type="radio"/> Sort down	<input type="radio"/> Sort up	<input type="radio"/> Sort down
<b>Well</b>	<b>Contents</b>	<b>Sample IDs</b>		
A12	BA	Blank A		
C12	BB	Blank B		
E12	BC	Blank C		
G12	BD	Blank D		
A01	SA1	RG 100%		
A02	SA2	RG 80%		
A03	SA3	RG 60%		
A04	SA4	RG 40%		
A05	SA5	RG 20%		

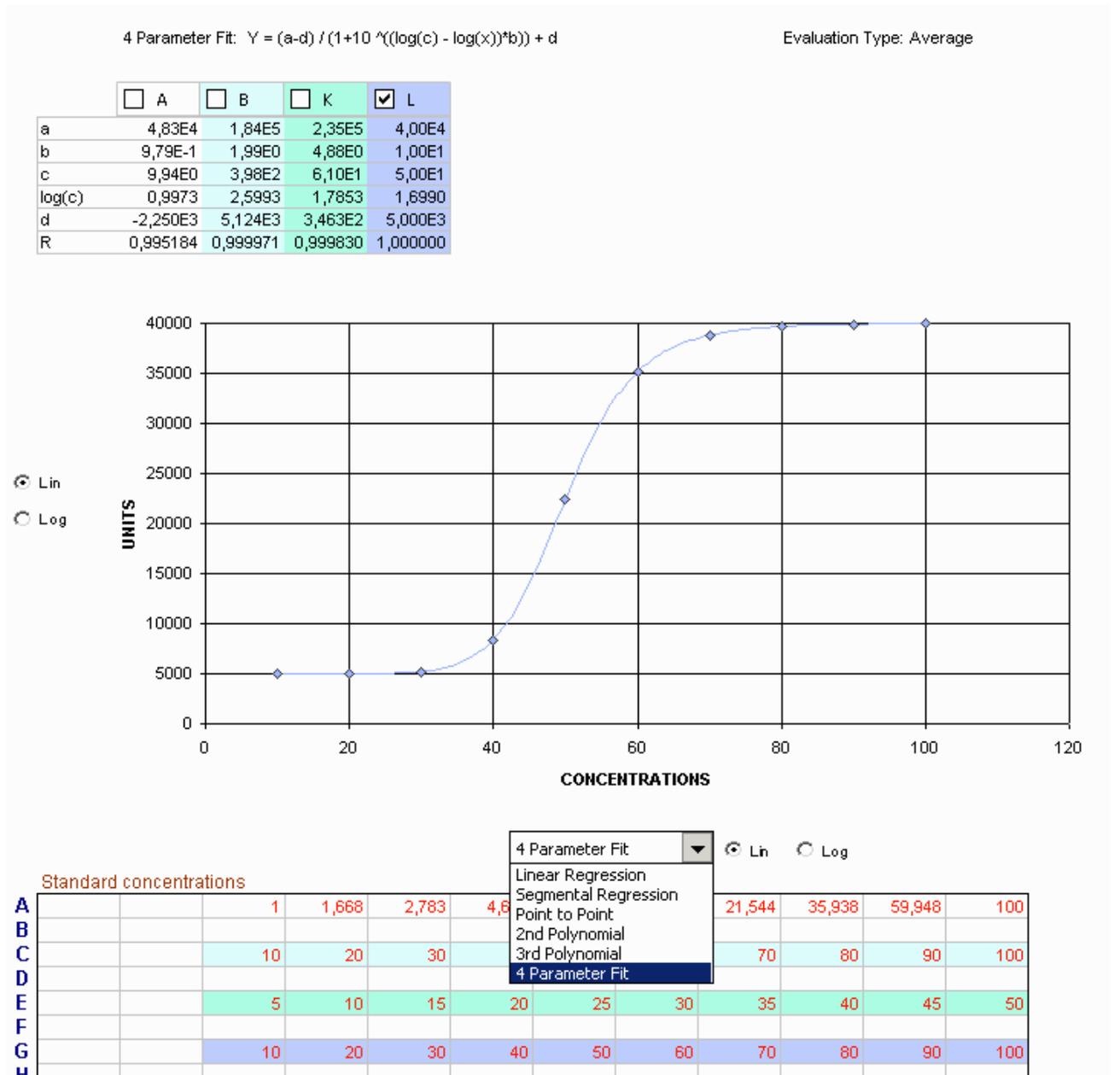
It is possible to change the sample IDs here by editing the respective fields in the Sample ID column. You can save your changes permanently by choosing the 'Save Evaluation Settings' option from the OPTIMA menu. If you leave the test run without saving, you are prompted for to save.

*Note:* Saving changes is only possible if the test run has no signature.

### 1.7 Standard Curve Worksheet

After the information in table three of the Evaluation Worksheet is selected, click on the tab for standard curve at the bottom of the screen. At the top of the sheet you will see the parameter table where the parameters for each group are listed. You can select the groups you wish to see by clicking the corresponding checkbox above the parameter table. Below, you find the standard curve graph, which displays the concentration and measurement units of your standard replicates. The graph can be plotted in linear or logarithmic scale by selecting the button below the graph.

*Note:* If one of the values equals zero or lower, logarithmic scaling is not possible.



The calculated unit values for the standard concentrations defined in the test setup are plotted in a 'Standard curve'. The graph can be plotted on linear or logarithmic scale in x or y direction by clicking the 'Lin' or 'Log' button of the correspondent axis. You can select the groups to be plotted with the checkboxes above the graph.

#### Curve Fits

There is a pull-down menu for selecting one of six curve fits below the graph. You can choose the one that best fits the data and provides the best results for calculating the unknowns.

Notes: a) The Segmental Regression curve fit is useful especially for solubility applications. It tries to split the data range into two regions with optimal regression fit, displaying also the coordinates of the intersection point. If the parameter r for the whole data range is bigger than 0.98, only one regression line will be drawn.

b) The 4 Parameter Fit option is not available if the data is not suitable for this type of curve fit. Values which are greater than the maximum or lower than the minimum of the 4 Parameter Fit asymptote are marked with an asterisk.

**Calculation of the Unknowns**

Below the graph there are two tables:

Standard concentrations

A				0.000001	5E-07	2.5E-07	1.25E-07	6.25E-08	3.13E-08			
B				0.000001	5E-07	2.5E-07	1.25E-07	6.25E-08	3.13E-08			
C				0.000001	5E-07	2.5E-07	1.25E-07	6.25E-08	3.13E-08			
D				0.000001	5E-07	2.5E-07	1.25E-07	6.25E-08	3.13E-08			
E				1E-07	5E-08	2.5E-08	1.25E-08	6.25E-09	3.13E-09			
F				1E-07	5E-08	2.5E-08	1.25E-08	6.25E-09	3.13E-09			
G				1E-08	5E-09	2.5E-09	1.25E-09	6.25E-10	3.13E-10			
H				1E-08	5E-09	2.5E-09	1.25E-09	6.25E-10	3.13E-10			
	1	2	3	4	5	6	7	8	9	10	11	12

Calculated concentrations

Table calculation: None      Range1      Channel 1      Raw data - blank

A		*	*	9.5E-07	2.89E-07	2.04E-07	1.42E-07	9.97E-08	*			
B		*	*	*	2.86E-07	1.89E-07	1.26E-07	9.67E-08	3.5E-08			
C												
D												
E												
F												
G												
H												
	1	2	3	4	5	6	7	8	9	10	11	12

\*: The signal of the respective sample is lower or higher than the asymptote min or max values!

Used for calculation:

Group    A    B    C    D

**First Table: Standard Concentrations**

This table contains the standard concentrations as defined in the layout (see chapter Concentration / Volumes / Shaking of software manual part II: Control Part).

**Second Table: Calculated Concentrations**

This table contains the calculated concentrations based on the curve fitting method chosen for the standard concentrations. They are calculated by using the formula for linear regression, segmental regression, point to point, 2<sup>nd</sup> and 3<sup>rd</sup> polynomial or the 4-parameter fit.

**Used for Calculation**

You can select the standard of the group, which is used for calculating the sample values with these buttons. With the button 'Group' selected, which is activated by default, every group uses its own standard for calculation.

Note: In the case of 2<sup>nd</sup> and 3<sup>rd</sup> degree polynomial equations, it is possible that there are problems calculating the unknowns. More than one concentration could be possible or the point could be out of the range of the curve. In these cases, the table will contain asterisks in place of the data.

*	The signal of the respective sample is out of the signal range of the standards. No extrapolation is possible.
*	The signal of the respective sample is lower or higher than the asymptote min or max values (can only appear if 4-parameter-fit is chosen).
**	There is more than one concentration possible for the signal (units). No clear relationship between signal and concentration.

### 1.8 Result List Worksheet

This worksheet is only available if the standard curve worksheet is visible. This means that standards must be defined in the layout, and in the third table of the Evaluation Worksheet one of the following selections must be used: 'Raw data' (minus blank) and 'Averages' (minus blank).

<b>OPTIMA</b> BMG LABTECH	Testname: DNA1 ID 1,2,3:	2002.07.26 10:51:24 1.dbf, imported	<input type="checkbox"/> Hide parameter settings <input type="checkbox"/> Hide standard curve						
Fluorescence, plate mode equidistant									
Evaluation type: Sum									
Calculation: Start1: 2 Stop1: 2 Start2: 0 Stop2: 0									
Selected: Range1									
Table calculation: None									
Curve fitting: Linear Regression; X-Axis lin, Y-Axis lin									
<table border="1" style="width:100%; border-collapse: collapse;"> <tr><td>m</td><td>3,319E3</td></tr> <tr><td>b</td><td>-1,403E2</td></tr> <tr><td>r</td><td>0,980503</td></tr> </table>				m	3,319E3	b	-1,403E2	r	0,980503
m	3,319E3								
b	-1,403E2								
r	0,980503								
Comment: DNA Test									
Sort contents		Sort sample IDs		Sort rows		Sort columns		<input checked="" type="checkbox"/> Avg of replicates	<input checked="" type="checkbox"/> Use dilution factor
<input checked="" type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down		
<b>Content</b>	<b>Sample ID</b>	<b>Well</b>	<b>Dilution factor</b>	<b>Raw data - blank</b>	<b>Avg of replicates</b>	<b>SD of replicates</b>	<b>%CV</b>	<b>Calculated concentr.</b>	
S1		A01	1,000	70	65	5	7,7	61,94E-3	
S1		A02		60					

The worksheet contains a list of all raw data based on the selection you made for the third table on the evaluation sheet. The data values are grouped by replicates. The calculated concentrations are also shown using the curve fitting method of the current standard curve worksheet. The most important measurement parameters, the parameter table for the standard curve and the graph of the standard curve appear in the header. Both can be hidden by checking the checkboxes 'Hide parameter settings' and 'Hide standard curve' to get a better overview of the data.

The data can be sorted by plate rows, plate columns, well contents or sample IDs in upward or downward direction using the corresponding checkboxes.

If you check the box 'Use dilution factor' the calculated concentrations are multiplied with the corresponding dilution factor.

OPTIMA BMG LABTECH		Testname: DNA1 ID 1,2,3:		2002.07.26 10:51:24 1.dbf, imported		<input checked="" type="checkbox"/> Hide parameter settings <input checked="" type="checkbox"/> Hide standard curve			
Sort contents		Sort sample IDs		Sort rows		Sort columns		<input checked="" type="checkbox"/> Avg of replicates	<input type="checkbox"/> Use dilution factor
<input checked="" type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down		
Content	Sample ID	Well	Dilution factor	Raw data - blank	Avg of replicates	SD of replicates	%CV	Calculated concentr.	
S1		A01	1,000	70	65	5	7,7	61,94E-3	
S1		A02		60					
S2		B01	1,000	1315	1330	15	1,1	0,443	
S2		B02		1345					
S3		C01	1,000	2630	3125	495	15,8	0,984	
S3		C02		3620					
S4		D01	1,000	3277	3247	30	0,9	1,021	
S4		D02		3217					
S5		E01	1,000	5046	6720	1674	24,9	2,067	
S5		E02		8394					
S6		F01	1,000	7973	8149	176	2,2	2,498	
S6		F02		8324					
S7		G01	1,000	11068	15460	4392	28,4	4,700	
S7		G02		19851					
S8		H01	1,000	15318	15542	224	1,4	4,725	
S8		H02		15765					
X1		A03	1,000	5033	5002	31	0,6	1,550	
X1		A04		4971					
X2		B03	1,000	8965	9069	104	1,1	2,775	
X2		B04		9173					
X3		C03	1,000	1941	1964	23	1,1	0,634	
X3		C04		1986					
X4		D03	1,000	2140	2142	2	0,1	0,688	
X4		D04		2144					
X5		E03	1,000	2763	2805	42	1,5	0,887	
X5		E04		2846					
X6		F03	1,000	28519	28726	207	0,7	8,698	
X6		F04		28932					

### 1.9 Protocol Settings Worksheet

Here you can see all settings defined in the protocol used for the current test run. You can also see the history of changes for a test run in the audit trail text box. If the test run has been signed, the signatures will appear in the signature text box.

<b>OPTIMA</b>	Testname: MULTI DEL PT INJ	2005.12.15 10:24:45
BMG LABTECH	ID 1,2,3:	3.dbf

Simultaneous dual luminescence, plate mode equidistant	Kinetic window	1	2	3	4
Plate type: BMG LABTECH 96	No. of cycles	1	-	-	-
Top optic used	Meas. start time [s]	0,0	-	-	-
	Meas. interval time [s]	0,10	-	-	-

Chromatic No.	1A	2A	3A	4A	1B	2B	3B	4B
Gain	2351	4095	2679	4095	2447	4095	2427	1758
Emission filter	520	590	544	612	544	lens	520	empty

Required value A[%]: 40	Volume group	1	2	3	4
Required value B[%]: 40	Volume [µl]	indiv.	-	indiv.	-
	Used pump	1	-	1	-
	Pump speed [µl/s]	150	-	230	-
Positioning delay [s]: 0,2	Smart dispensing used	-	-	-	-
	Pump syringe vol. [ml]	2.5	-	2.5	-
Shaking width [mm]: 1	Injection cycle	1	-	1	-
Shaking mode: double orbital	Injection start time [s]	0.0	-	15.2	-
Additional shaking: 1s before each cycle	Shaking after inject. [s]	5	-	123	-

Target temperature [°C]: 28,9

Reading direction: 1 

Calculation Start1: 1 Stop1: 1 Start2: 0 Stop2: 0

Well scanning: None

Comment:

Software version control: 2.00 P1  
 Software version evaluation: 2.00 P1 B:0008  
 Serial number: 413-0445  
 User: USERRC16

Audit trail  
 Donnerstag, 15. Dezember 2005 - 10:26:21, User 'USERRC16': Data record created by performing test protocol 'MULTI DEL PT INJ' (started: Donnerstag, 15. Dezember 2005 - 10:24:45) using reader 413-0445.  
 Donnerstag, 15. Dezember 2005 - 10:30:25, User 'USERRC16': Automatic initialization of evaluation settings done during first opening of test run.

Signatures

All settings are described in chapter *Defining Protocols* of software manual part II: Control Part.

**Notes:** The 'Last required value [%]' is the last required value which has been used to perform an automatic gain adjustment using this test protocol. A gain can also be typed in manually and hence the required value [%] is not taken into account.

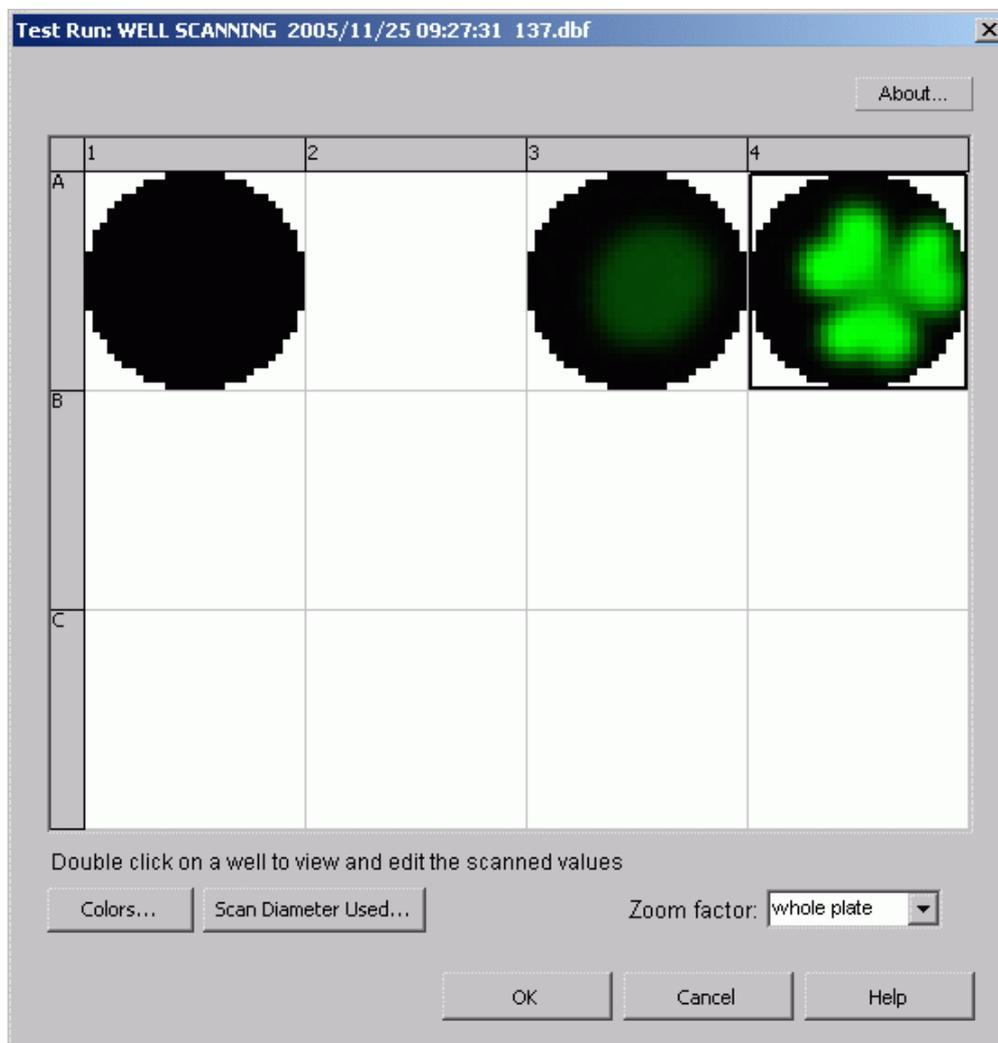
Date and time information for audit trail and signatures is displayed using the format as defined in the Windows Control Panel (Regional Settings) under long date and time format.

## 1.10 Display Well Scanning Data

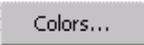
If the opened test run contains well scanning data, (see chapter *Defining Protocols* of software manual part II: Control Part) you can display thus data in the evaluation software.

### 1.10.1 Well Scan Plate View

After you've selected the OPTIMA Pulldown Menu 'Well Scanning View...' or you've pressed the Toolbar Button , a new window will be opened.



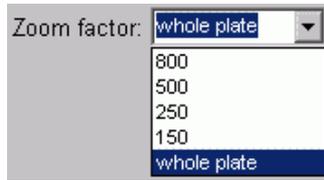
The Well Scanning View window displays the measured well scanning data graphically in a grid according to the microplate format. The header of the window contains the test run name, the date and time that the measurement took place and the filename assigned to the test run.

You can choose between three different display modes. To change the mode and the color settings press  (see chapter 1.10.2 *Color Settings*).

If you are using layout groups, the layout grid will be displayed using the background colors belonging to the layout groups used.

You can double click on a well to get a zoomed view of the measurement values with additional information and to edit the measurement values (see chapter 1.10.4 *Detailed View of Well Scanning Data for a Selected Well*).

Press  to change the diameter of the circle/rectangle which defines the valid data points (see chapter 1.10.3 *Change Scan Diameter Used*).



Use this control to zoom to the selected value in percent (whole plate is equal to 100%).

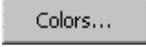


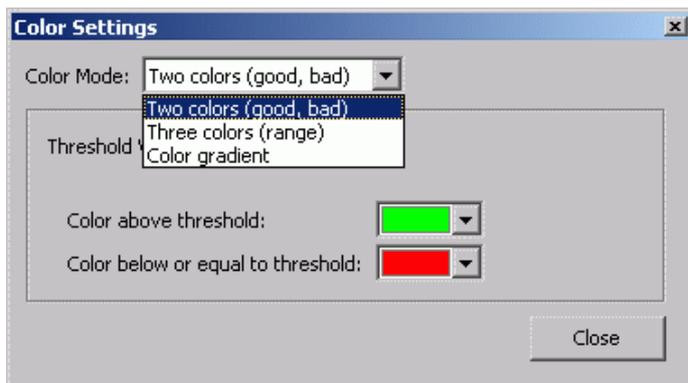
If the measurement was performed with two channels or more than one chromatic, an additional control appears to select the channel/chromatic to be displayed:

If you press the OK button, the changes performed will be assumed and saved. To revoke the changes, press the Cancel button. In both cases the window will be closed.

Only the average value of all used data points in the well (see also chapter 1.10.3 *Change Scan Diameter Used* and 1.10.4 *Detailed View of Well Scanning Data for a Selected Well*) will be displayed in the 1.3 *Raw Data Worksheet* and used for further calculation.

### 1.10.2 Color Settings

After pressing  in the Well Scanning View window or the Detailed View window, the following dialog box will appear:



The selected settings affect the way the well scanning data will be displayed in both the Well Scanning View window and the Detailed View window. You can change the display mode by selecting one of the three color modes available:

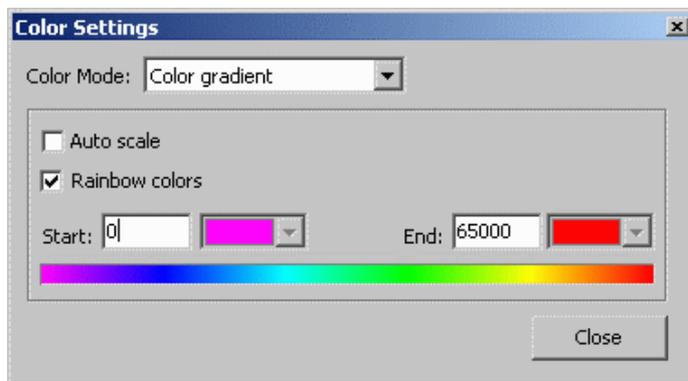
#### **Two colors (good, bad)**

If you are only interested in a good / bad decision, you should choose the option to display different colors for all values under a certain threshold and for all values above the threshold. You can select the two colors and change the threshold value.

#### **Three colors (range)**

Same concept as 'Two colors', but here you can also define a range 'in-between' to be displayed in a third color.

#### **Color gradient**



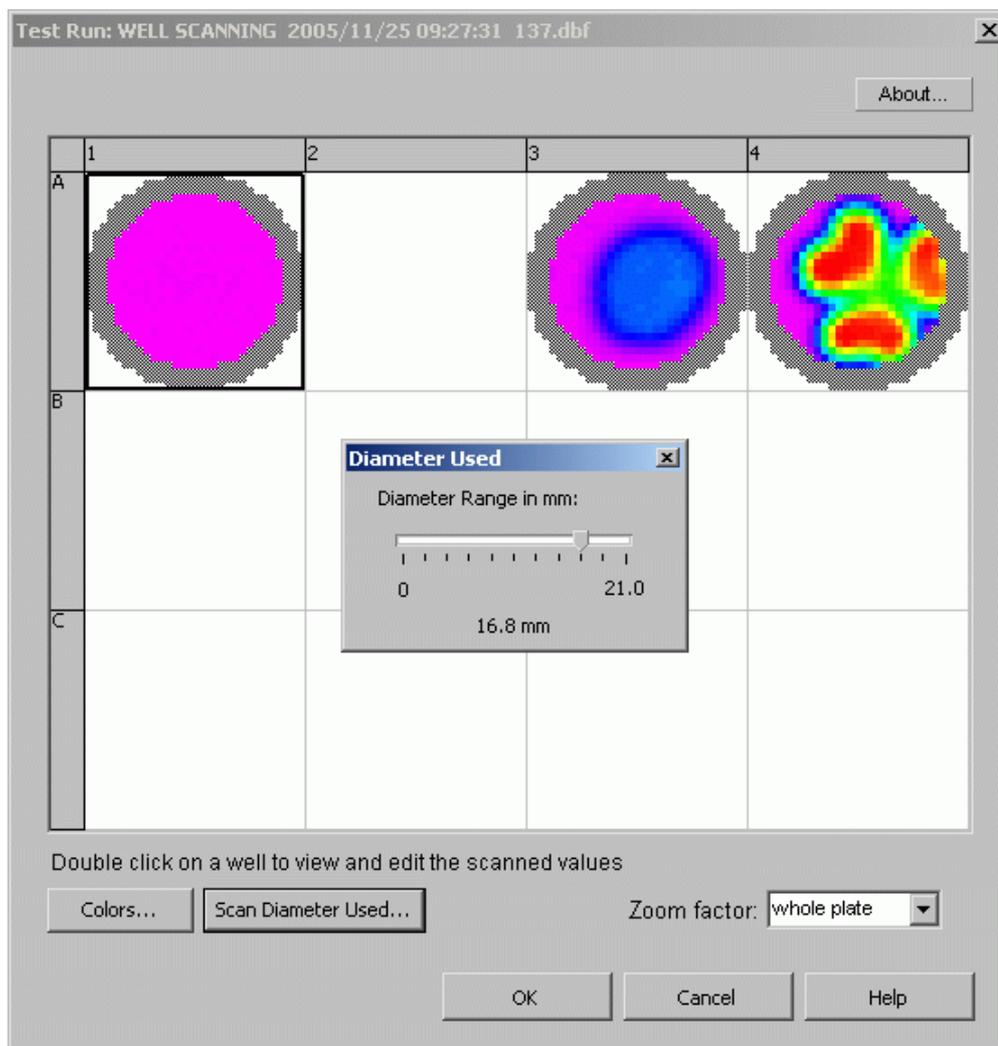
The measurement values will be displayed in different shades of colors or gray levels. You can select a start and an end color. It is also possible to use colors from the rainbow spectrum. You can define the start and the end values to enlarge the range of the color gradient used.

Use the auto scaling function to set the start and the end values automatically to the minimum and maximum measurement value of the selected Channel/Chromatic for the whole plate.

**Note:** For this option, it is recommended to use a graphic mode with more than 256 colors (windows control panel)

### 1.10.3 Change Scan Diameter Used

After pressing **Scan Diameter Used...** in the Well Scan Plate View or the Detailed View window, the Diameter Used window opens.



The Scan Diameter Used describes the diameter of a circle (for a round well shape) or of a rectangle (for a rectangular well shape) that defines the area within which the measured data points are used for further calculation.

Changing the diameter size allows you to reduce this area (i.e. if you find out that the results measured near the border of the well are not certain).

The used diameter window contains a slider control to change the diameter used.

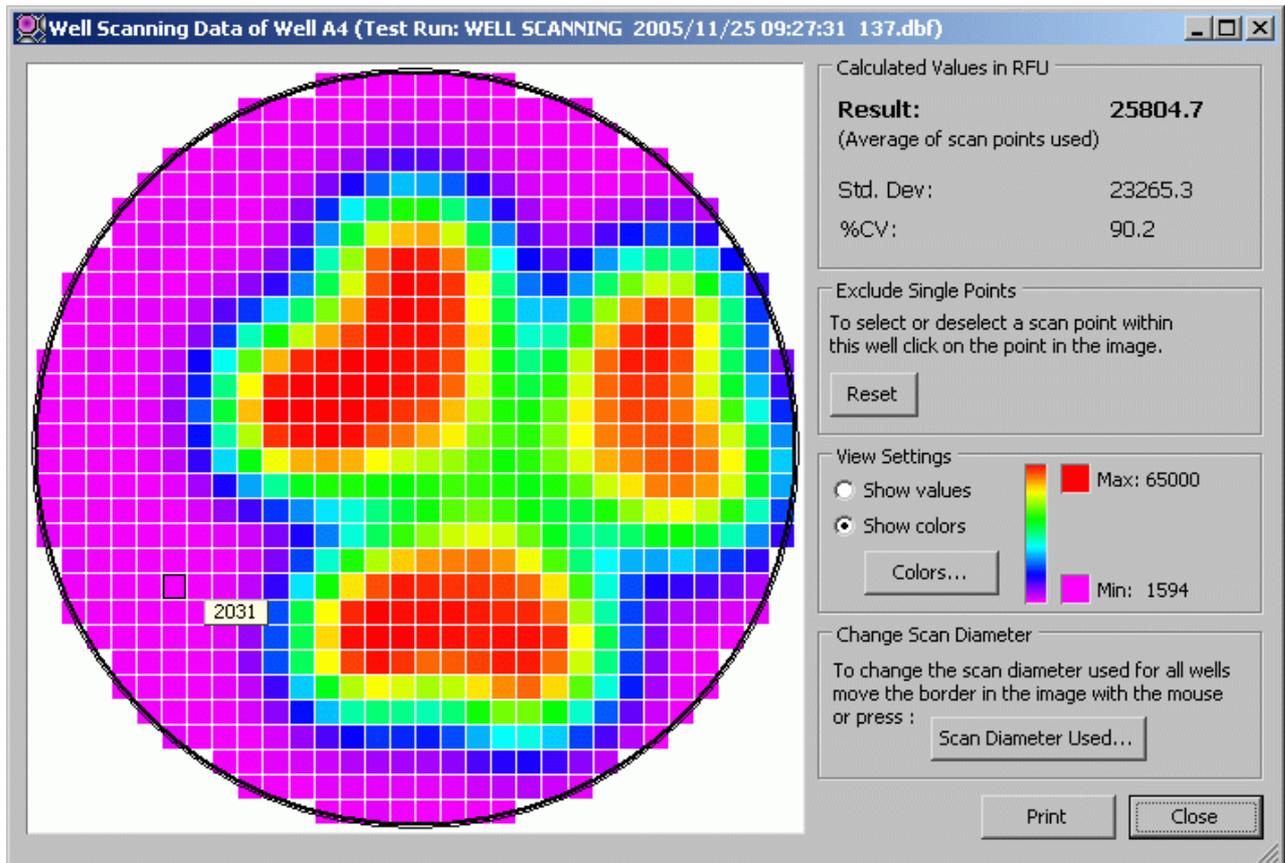
Move the slider with the mouse to change the diameter. The data points outside the area will be displayed in a gray pattern that indicates, that these points will not be used for calculating the average value of the well scanned.

You can also change the diameter in the Detailed View of a well.

**Note:** Changing the diameter always effects all wells, even if you change it in the Detailed View of a well.

### 1.10.4 Detailed View of Well Scanning Data for a Selected Well

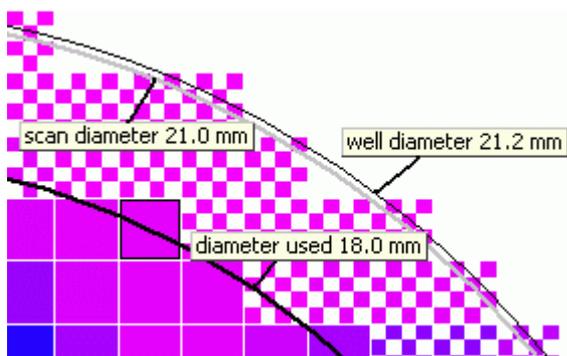
The window with a detailed view of the measured data for one well can be opened if you double click on the well in the Well Scanning View window of the whole microplate.



In this window you can see the selected well in a zoomed view with additional information.

If you move the mouse over a scan point, a hint with the measured value will be displayed.

The picture contains three circles (for a round well shape) or rectangles (for a rectangular well shape) with the following meaning:



**Fat line, black:** Shows the scan diameter used. All scan points outside are marked as not used and therefore displayed in a grid pattern.

**Fat line, gray:** Shows the physical scan diameter. This is the diameter used by the reader as limit when the well is scanned. Only scan points of the defined matrix whose center lies inside the area defined by this diameter are measured. You can define the scan diameter in the protocol settings of a test run in the reader control software.

**Thin line, black:** Shows the border of the well as it is defined in the microplate database.

If you move the mouse over one of these border lines, a hint with the identifier and the size of the border will be displayed.

## Description of the Dialog

### Calculated Values

#### Result

This value is calculated as average of all scan points used. It will be displayed in the Raw Data Worksheet and used as base value for further calculations.

#### Std. Dev

The calculated standard deviation of the scan points used.

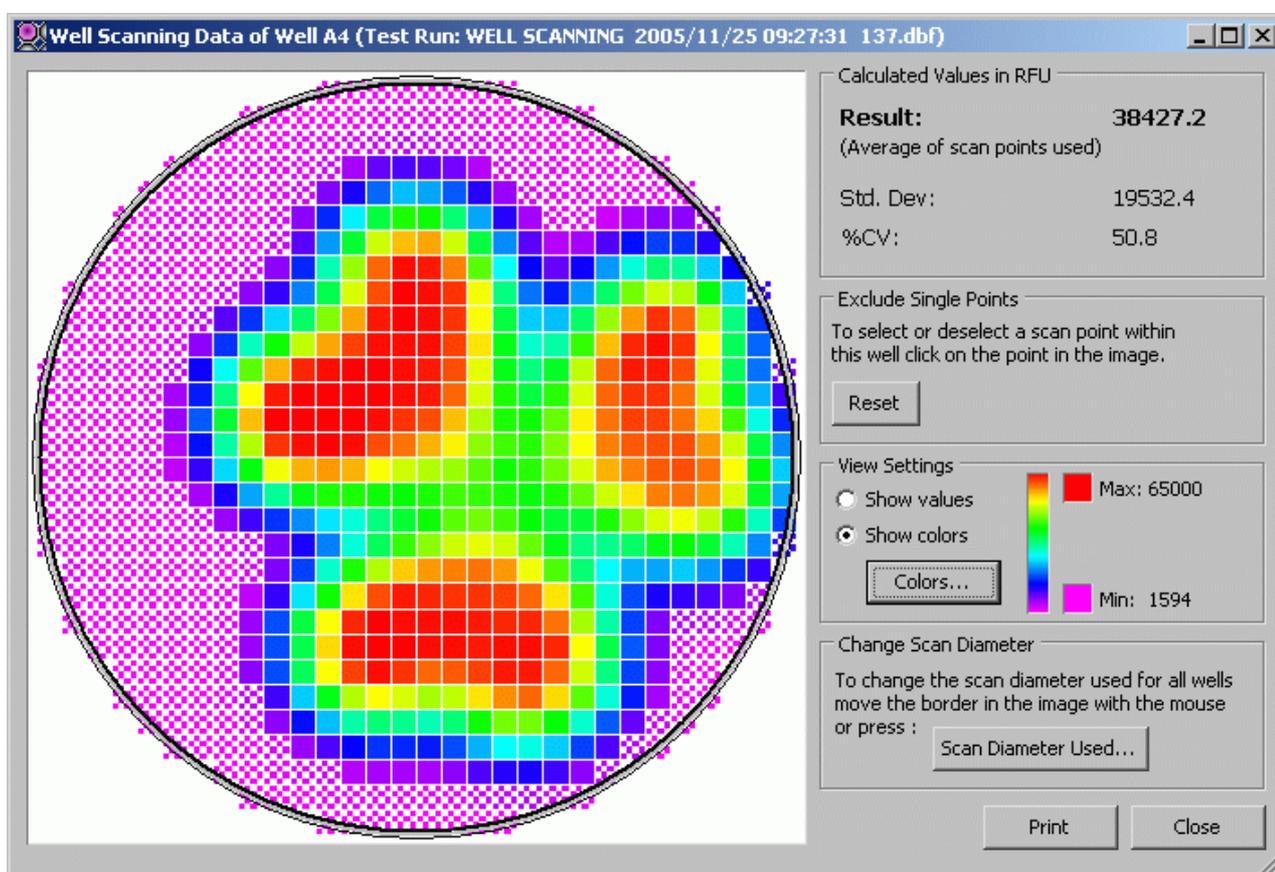
#### %CV

The calculated standard deviation, divided by the average of the scan points used, expressed in percent.

### Exclude Single Points

You can exclude single scan points of the selected well, by clicking on it in the image. If you click on an excluded scan point, the exclusion will be revoked.

Excluded (unused) scan points are drawn in a grid pattern.



**Note:** If a scan point is excluded by the *Scan Diameter Used*, it is not possible to revoke this exclusion by clicking on it. Therefore you have to increase the *Scan Diameter Used*.

Pressing the **Reset** button will change back the state of each scan point to 'used', if its center lies inside the area defined by the *Scan Diameter Used*.

### View Settings

To display the values of each measured scan point, select **Show values**. The image changes and the value is shown instead of a colored rectangle. It is recommended to maximize the window if you use this function, so that the font can be displayed in a readable size.

To change back to the selected color mode, select **Show colors**.

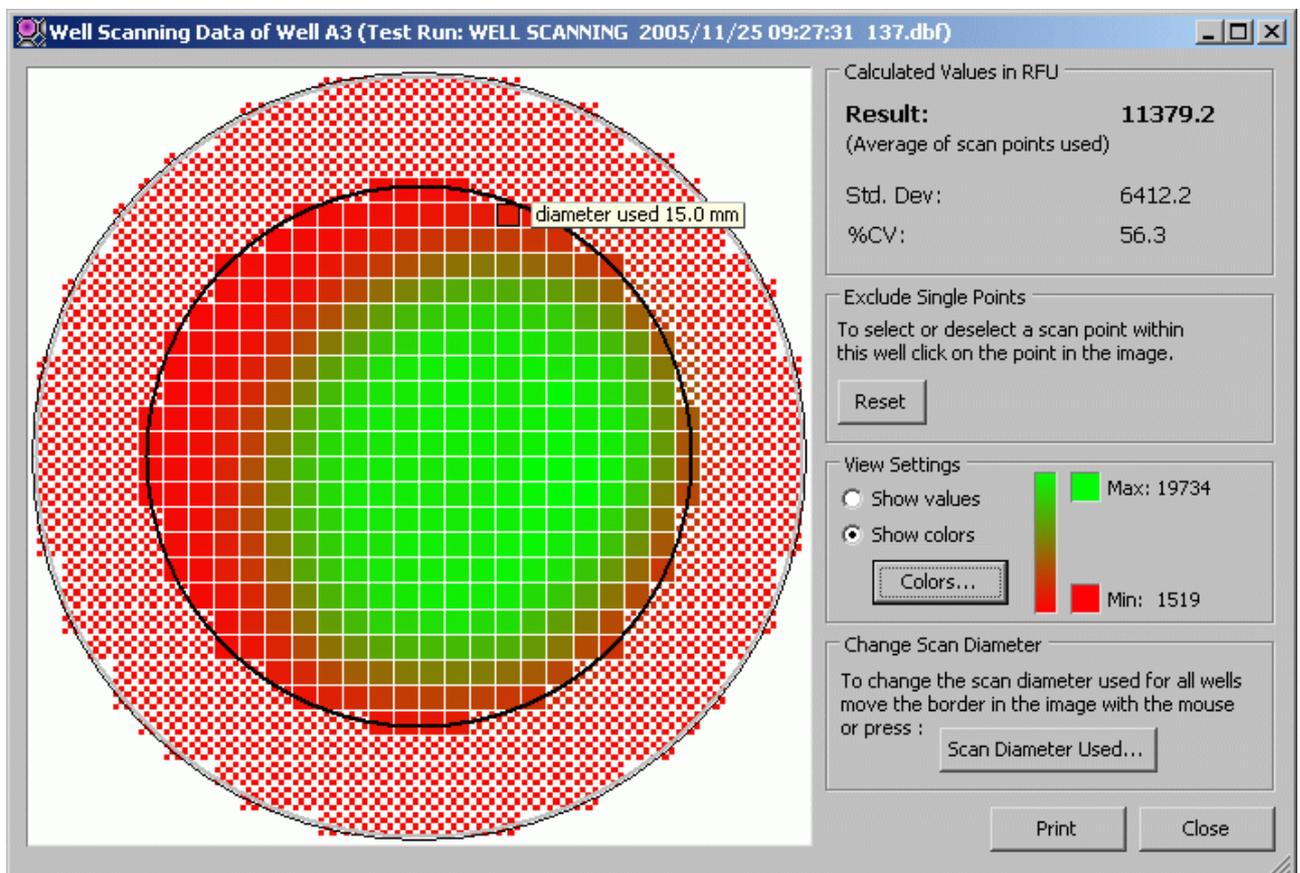
Press  to change the selected color mode and its settings (see Color Settings)

The color legend shows the color gradient between the minimum value (**Min:**) and the maximum value (**Max:**) of the selected well.

### Change Scan Diameter

Press  to change the diameter of the circle/rectangle which defines the valid data points (see 1.10.3 *Change Scan Diameter Used*).

Alternatively you can move the border in the image with the mouse to change the diameter. Move the mouse over the fat black line in the image until the hint shows *diameter used xxx mm* and the mouse cursor changes to two arrows. Then press the mouse button (the color of the border changes to blue), move it to the desired size and release the mouse button. Note, that the new diameter concerns all wells!



**Print:** Prints the screen on any available printer.

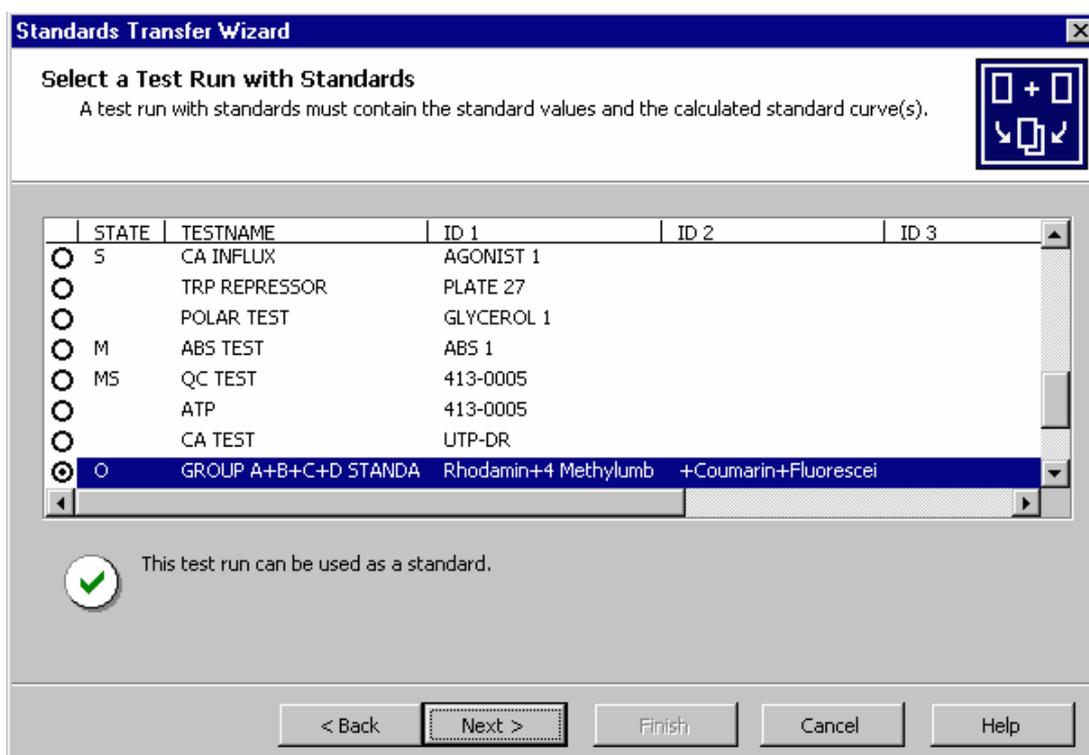
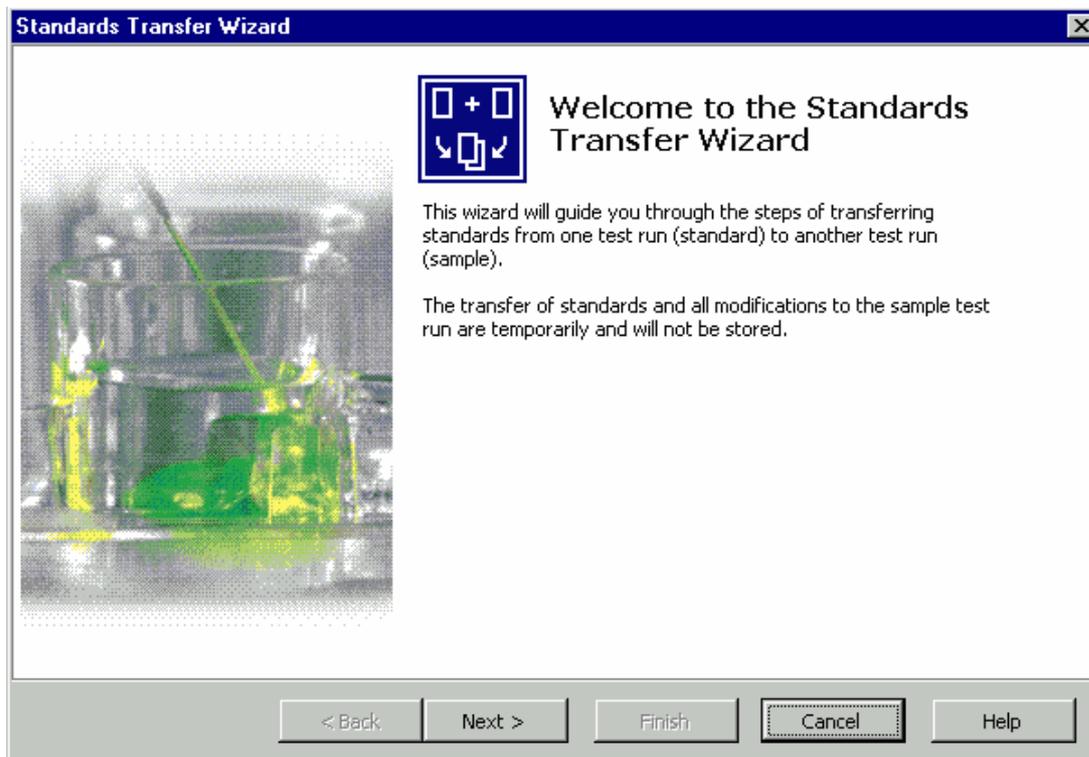
**Close:** Closes the detailed view window.

## 1.11 Standards Transfer Wizard

This feature is made for applying standard parameters gained from a plate with standards (called standard model in the following text) for a following series of plates with samples.

**Note:** The measurement parameters relevant for the calculation (e.g., gain value, cycle time, etc.) of your standard model must match the ones from your sample test. If they don't match, the software will not allow you to use the respective sample test.

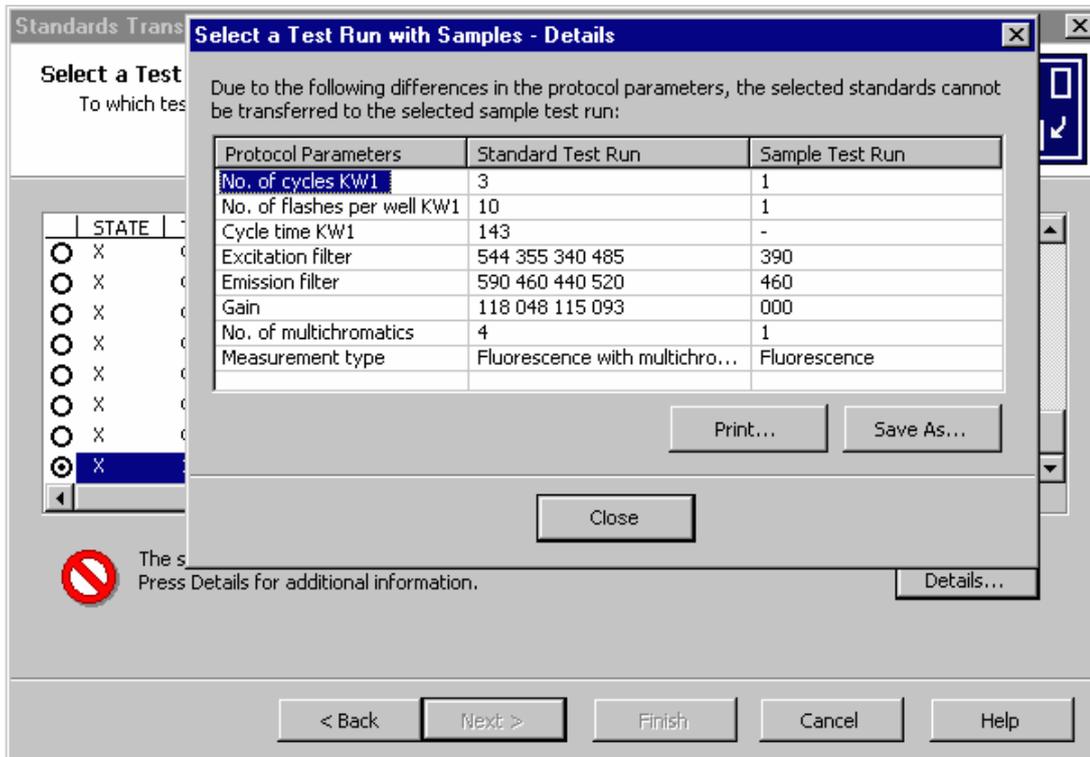
To use this feature, you should first edit your standard test run and choose the calculation methods you are interested in. Save the changes you made and press the button 'Standards Transfer'. The following user dialogs will open:



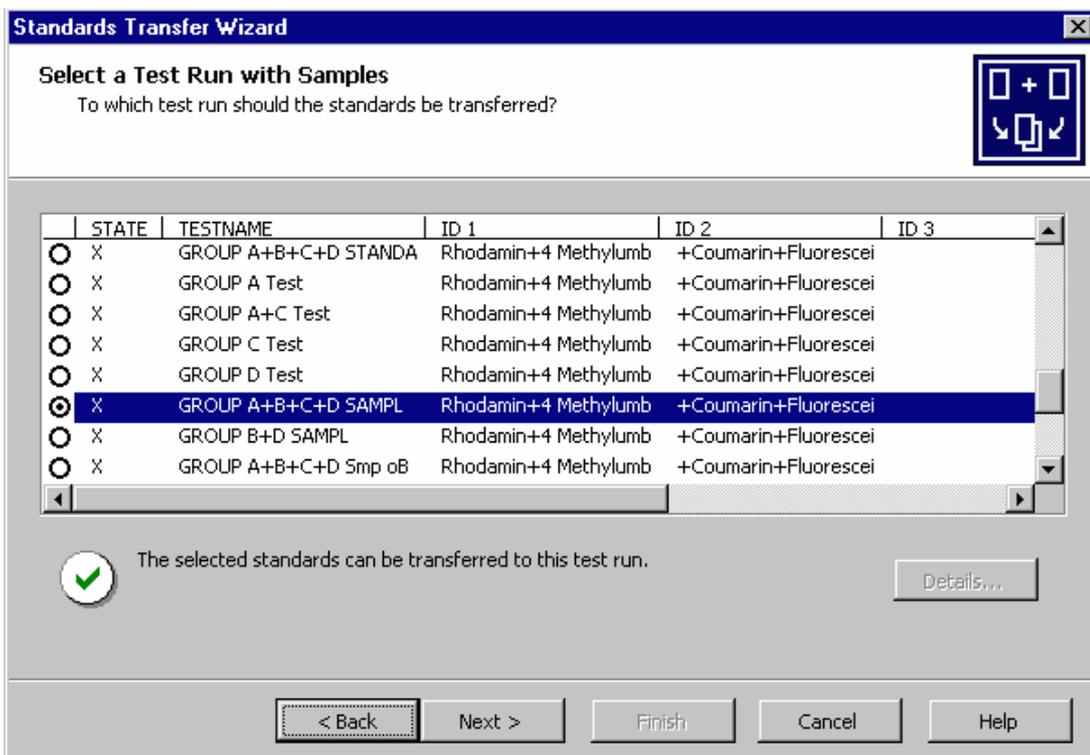
The test runs of your user directory appear in the list box. You can select them with cursor or mouse. Below the list box, a field with information whether or not the current test run can be used as standard appears. Choose the test run with the standards you wish to apply and press the 'Next' button. The test run will be loaded as standard model and the parameters for the curve fitting method will be calculated.

On the next screen, you can choose the sample test you like to combine with the loaded standard model.

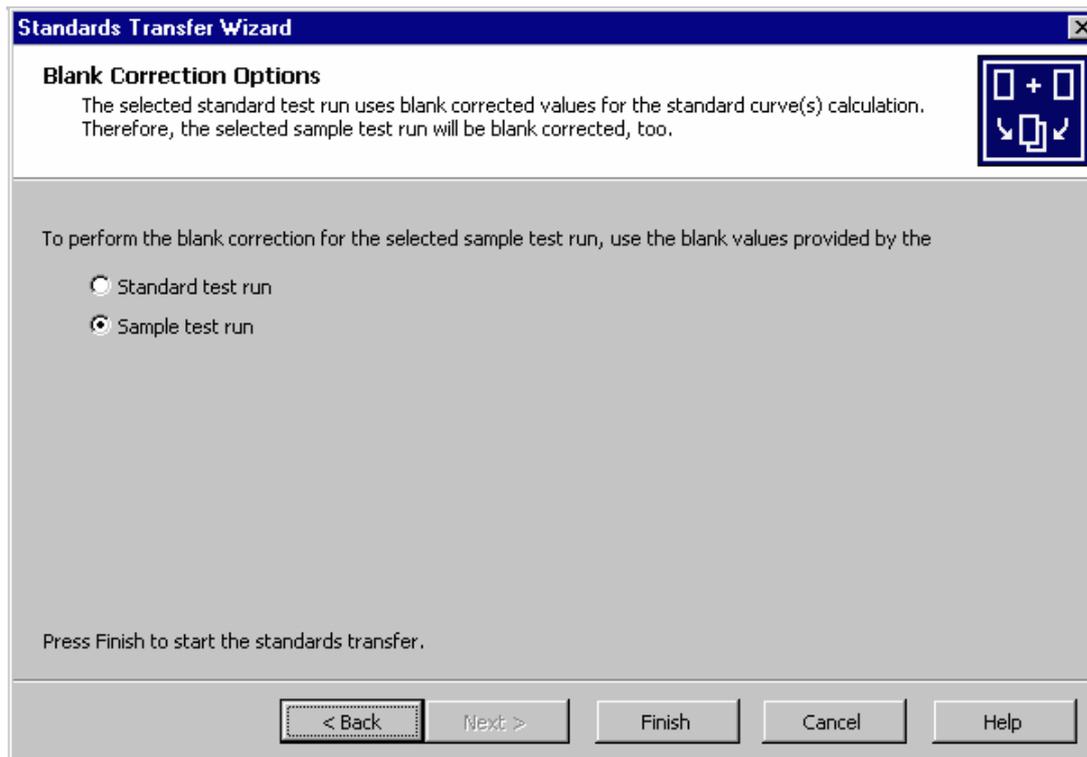
If the sample test run you have chosen is not applicable for your standard model, the 'Details' button is enabled. By pressing it, you can see a list of the measurement parameters not matching to the ones of your standard model. If you need it, you can print the list or store it in an ASCII file.



If you have chosen a test run which is applicable to your standard model, the wizard will appear like this:



If both the standard plate and your sample test run contain blanks, the following screen will appear where you can choose if to use the blanks of the standard plate or the blanks of your test run for the calculation:



By default, the blanks on the sample plate will be used. If you press apply, the software will switch to the standard curve sheet.

The design of the 'Standard concentration' table has now changed compared to the normal view. A table with the list of standard concentrations from the standard plate and their corresponding measurement units will appear. The second table contains the calculated concentrations of your samples. The header areas of all sheets appear now with light green background color. Below the measurement data tables appear a green text box with the information about your standard and sample plate.

Blank corrected standard concentrations of standard plate GROUP A+B+C+D STANDA (11.dbf) - 2001/09/12, 13:01:20

Content	Standard	Units	Content	Standard	Units	Content	Standard	Units
SA11	9.77E-3	403	SB11	0.977	13	SC11	9.766	16
SA10	19.53E-3	383	SB10	1.953	19	SC10	19.531	11
SA9	39.06E-3	423	SB9	3.906	66	SC9	39.063	24
SA8	78.13E-3	616	SB8	7.813	30	SC8	78.125	46
SA7	0.156	710	SB7	15.625	-2	SC7	156.250	-2
SA6	0.313	1103	SB6	31.250	59	SC6	312.500	14
SA5	0.625	1855	SB5	62.500	170	SC5	625	0
SA4	1.250	3189	SB4	125	34	SC4	1250	-4
SA3	2.500	6893	SB3	250	37	SC3	2500	30
SA2	5	17356	BB		389	SC2	5000	-33
SA1	10	54709				SC1	10000	14
BA		383				BC		402

Calculated concentrations using GROUP A+B+C+D STANDA (11.dbf) as standard plate

Table calculation: None

Range1

Channel 1

Raw data - blank

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B							0.369		0.862			
C												
D							0.288		0.297			
E												
F							0.296		0.300			
G												
H							0.292		0.308			

Used for calculation:

Group 
  A 
  B 
  C 
  D 
  G 
  H

You are currently using standards from another test run for calculation.

Standard test: GROUP A+B+C+D STANDA (11.dbf) 2001/09/12 13:01:20  
 Sample test: GROUP A+B+C+D SAMPL (25.dbf) 2001/09/12 13:01:20

Blank correction options:  
 For the blank correction of the samples the blank(s) of the sample plate are used.

**Note:** The controls on the Raw Data - and the Evaluation – sheet responsible for the standard calculation are now disabled.

## 2 Microsoft Office Macro Security

Many features in Microsoft Office are created in or depend on the Office integrated **Visual Basic for Application (VBA)** programming language. Since VBA is a potential mean to create and deploy macro viruses, Microsoft Office programs allow the usage of VBA features only in one of three **Security Levels**. These three Security Levels provide an adjustable protection against macro viruses.

For the highest level of protection, Microsoft recommends setting the macro Security Level to High or Medium and using digital signatures.

A digital signature on a Microsoft Office VBA macro is like a wax seal on an envelope: it confirms that the macro originated from the signer and that the macro has not been altered since it was signed.

When you open an Excel workbook or load an add-in that contains a digitally signed VBA macro, the digital signature appears on your computer as a certificate. The certificate names the macro's source, plus additional information about the identity and integrity of that source.

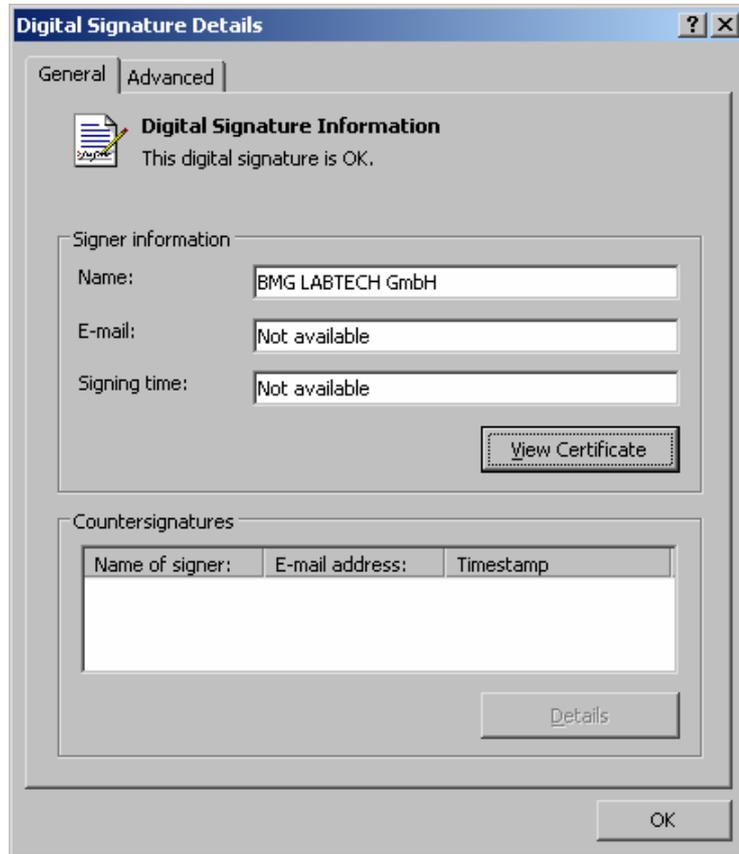
The BMG LABTECH Evaluation Excel workbook uses VBA macros as well to implement its features. In order to assure the highest security, all BMG LABTECH VBA macro codes are digitally signed.

If you have set the Excel **Security Level** to **High** or **Medium** and open the BMG LABTECH Evaluation Excel workbook for the first time, you will see a **Security Warning** dialog box regarding the digitally signed macro with BMG LABTECH GmbH as author specified:



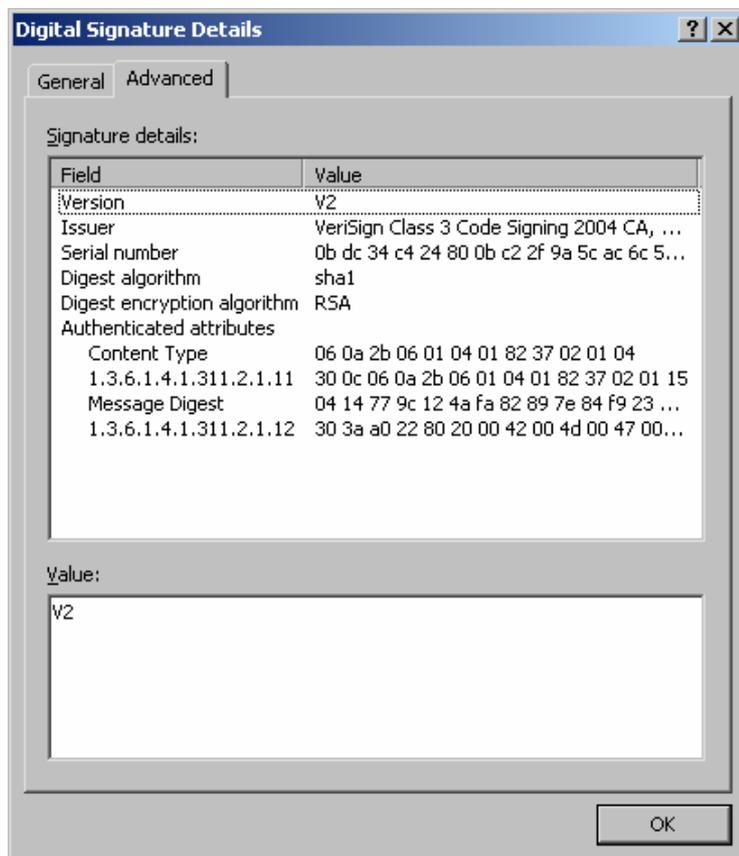
*Security Warning dialog box for macros by BMG LABTECH.*

To view details about the digital signature, click Details.... This displays the Digital Signature Details dialog box as shown in the next figure.



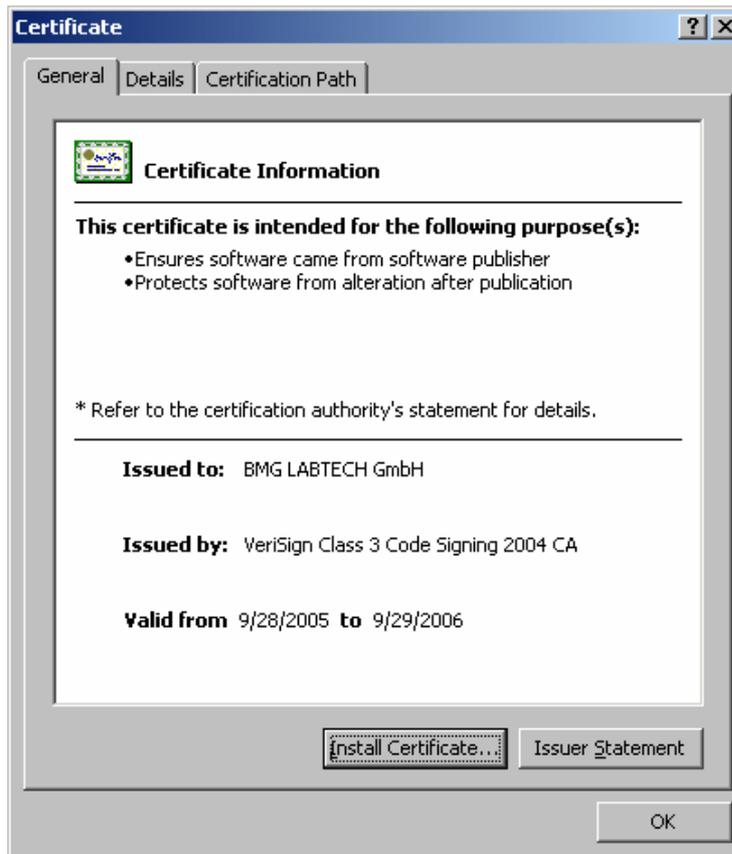
Digital Signature Details dialog box.

If you click on the **Advanced** tab, you will be able to view the **Signature** details as shown below.



Digital signature details.

To view the certificate, click **View Certificate** on the **General** tab.



*Digital certificate issued to BMG LABTECH.*

Back in the **Security Warning** dialog box, note that the **Enable Macros** button is disabled since BMG LABTECH is at this very moment an unknown, and therefore, not trusted source, which means you cannot enable VBA macros from sources that you do not trust. To enable it, you first have to trust the source of the VBA macro. To trust a source, select the **Always trust macros from this source** check box. This makes the Enable Macros selection available.

Click **Enable Macros**. This will load the signed BMG LABTECH VBA macro. In addition, BMG LABTECH will be added to your **Trusted Sources**. You can verify this in the **Trusted Sources** list (to get there, click **Tools**, point to **Macro**, and then click **Security**. In the **Security** dialog box, click the **Trusted Sources** tab).

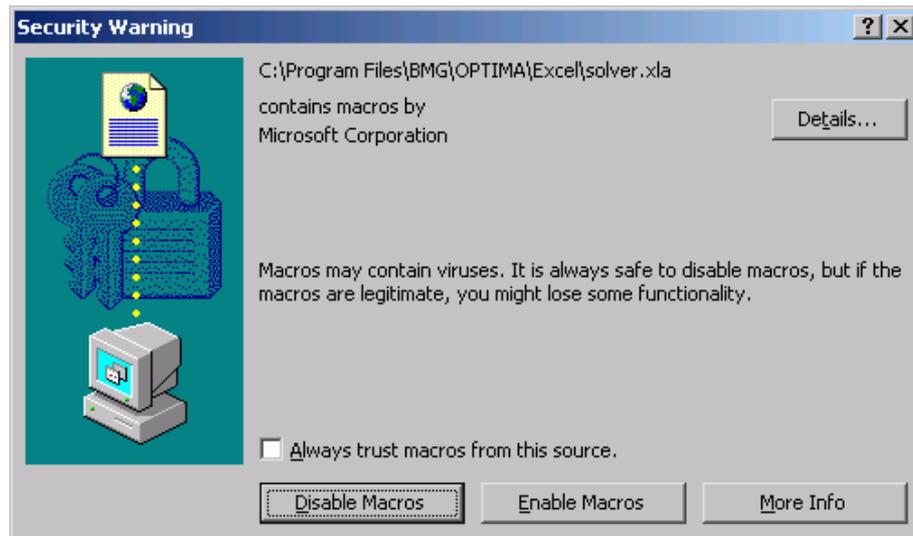
When you open the BMG LABTECH Evaluation workbook the next time, you will find that you won't be prompted and asked whether or not you want to enable the BMG LABTECH VBA macro. Instead, the VBA macro will be loaded without any user intervention. This is because the BMG LABTECH VBA macro was digitally signed using a certificate that corresponds with one that is now in the list of **Trusted Sources** certificates.

If you click **Disable Macros**, the signed BMG LABTECH VBA macro is disabled and won't be loaded.

The BMG LABTECH Evaluation Excel workbook uses VBA macros from two sources:

- BMG LABTECH GmbH and
- Microsoft® Corporation.

Therefore, you will be prompted two times: once for BMG LABTECH, as shown above, and once for the referenced VBA macros from Microsoft:



*Security Warning dialog box for macros by Microsoft.*

For further details on security and virus protection please see the Office help or [www.microsoft.com/security](http://www.microsoft.com/security).

## 3 Known Problems and Solutions

There are some possible errors due to operating system configuration:

1. Error 429 'ActiveX component can't create object or return reference to this object'
2. Error 1004 'VBA initialization failed'
3. Error 40009 'No current row'

For solutions see the following chapters.

### 3.1 Error 429 'ActiveX component can't create object or return reference to this object'

If this message appears when you open the Excel evaluation sheet, you should use the Fix429 program from the installation CD-ROM (under ~\OPTIMA V1.xx\Evaluation\).

This program checks a registry key responsible for ActiveX data access objects delivered with Microsoft programs (Office (Excel), Visual Basic, Internet Explorer...) regarding presence and correct value.

```
Key:      HKEY_CLASSES_ROOT\LICENSES\F4FC596D-DFFE-11CF-9551-00AA00A3DC45
Value:    mbmabptebkjcldgtjmskjwtsdhjbmkmwtrak
```

A wrong value of this key will cause the error 429 ('ActiveX component can't create object or return reference to this object') at startup of the evaluation software.

- If the key does not exist, it will be created (after pressing the 'Correct Error' button).
- If the key exists but has a wrong value, the old value will be saved under the name 'backup' in the same key and the key value will be changed to the correct value. Should you encounter problems with other programs, you can restore the old key value using the 'Restore' button of the Fix429 program.

*Note:* The patch program 'Fix429' will be called automatically during the installation of the OPTIMA evaluation part, so this error should occur (after installation) only if you install a new Microsoft program, which may have deleted or changed this registry key.

### 3.2 Error 1004 'VBA initialization failed'

When you get this error message after starting the evaluation part, some Excel add-ins are missing or are not enabled, e.g. the VBA macro language.

Please start the evaluation part directly from the windows start menu (and not using the Excel button in the control part). There will be a message telling you what parts are missing and asking you whether you want to install these Excel parts. Please answer 'Yes'.

Using Excel XP it is also necessary to set the security level to medium or low as the high level will not allow execution of our set of macros. To do so, please use the Excel menu command 'Extras | Security'.

### 3.3 Error 40009 'No current row'

If this message appears when you open the evaluation worksheet, you must update the Dao350.dll file installed on your computer.

- Close Excel and open a Windows Explorer.
- Open ~:\Program Files\Common Files\Microsoft Shared\Dao\.
- Rename the existing Dao350.dll file in the folder to Daoold.dll.
- From the OPTIMA installation CD-ROM go to the directory ~:\OPTIMA Vx.xx-x\Evaluation\ Excel97\_RunTimeError40009\. Copy the file Dao350.dll from the CD and paste it in the ~\Microsoft Shared\Dao\ folder.
- Reopen the software. There should be no error messages.

## 4 Support

If you have any problems / questions regarding the software / the instruments, you should visit our web page (<http://www.bmglabtech.com>) and read the 'Frequently Asked Questions' (FAQ) on the Support page. If you can not find an answer there, please contact BMG LABTECH using the following email addresses:

- Problems / questions regarding software:  
[support@bmglabtech.com](mailto:support@bmglabtech.com)
- Problems / questions regarding the instruments:  
[tech.service@bmglabtech.com](mailto:tech.service@bmglabtech.com)

You can also use our on-line bug report form:

<http://www.bmglabtech.com/support>