

OPTIMA

Software Manual – Part II: Control Software

Version 2.20

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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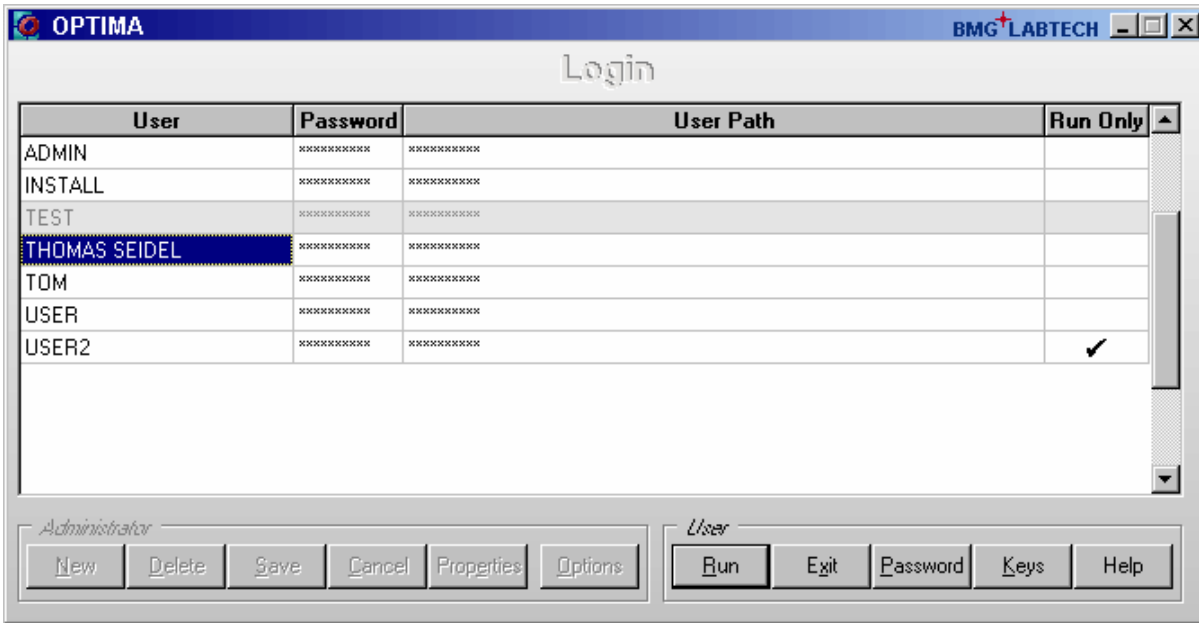
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1 Login Screen

When the control or data analysis part of the software is opened, a login screen appears (The Login Screen function can be switched off, see chapter 3.3 *Program Configuration*). This feature allows more than one user to perform test runs on one PC. The data and test protocols are stored in an individual user folder. The user can also edit test parameters and some evaluation features without the changes applying to all users.



For logging in as the standard user 'USER', by default no password is necessary.

The initial administrator password is 'bmg'. When the password is entered, the **Administrator** buttons will become available and new users can be created or changes for existing user entries can be made (see chapter 1.2 *Administrator Functions*).

Run Only

If this field is checked, the respective user is only able to start pre-defined test protocols. The user can perform a gain adjustment and enter plate / sample IDs, but can not change or delete an existing test protocol or copy / import protocols or create new protocols. Changing offset or filter values or microplate definitions is also not possible for this type of user.

The Run Only property can only be set / unset by the administrator. When using this option, it is strongly recommended to select Run Only also for the default user 'USER' (or to define a password for this account) and to change the administrator password to something other than 'bmg'.

Note: Instead of setting / unsetting this option every time a protocol change is necessary, you can also create two user entries using the same directory, one of these entries with Run Only option and the other one without this option.

1.1 User Functions

Run

After clicking 'Run', the OPTIMA software will start using the test protocol and measurement results paths of the selected user. For all users other than the default user 'USER', you need to enter the password first.

Exit

Use this button to close the login screen without starting the program itself / without logging on.

Password

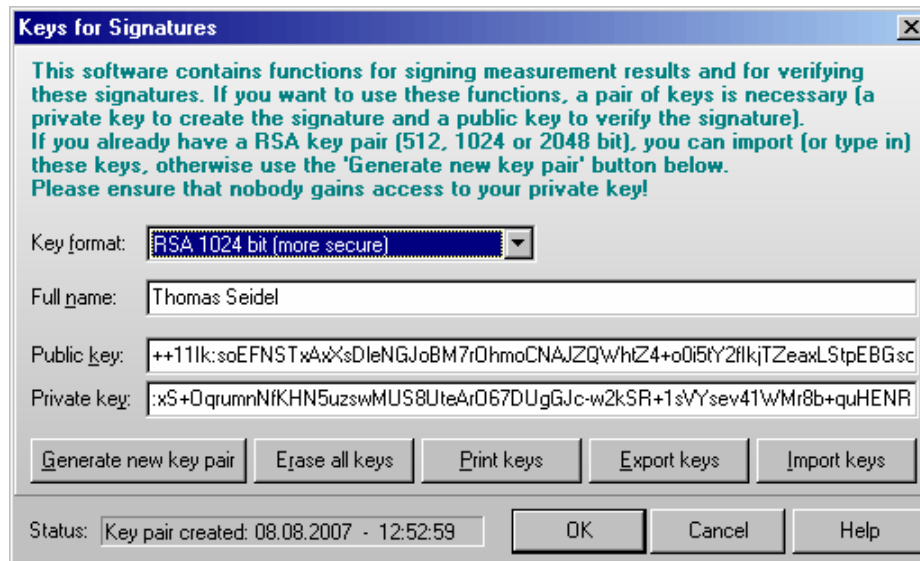
Use this button to change your password. Password restrictions might apply (see chapter 1.3.1 *Password Policies and Password Aging*).

Keys

Opens the 'Keys for Signatures' dialogue box (see below).

1.1.1 Signature Keys

If you want to digitally sign data records you need to have a pair of RSA keys: a private key for creating a digital signature and a public key for verifying this signature. A key pair can be generated or imported using the 'Keys' dialogue.



To **generate** a new key pair please select between 512, 1024 and 2048 bit key length and enter the **full name** which should appear as part of the signature. The longer the key, the more secure it will be, but creation of the key pairs and signing data records / verifying signatures will last longer. The largest amount of time will be necessary for generating the key pair, signing and verifying will only last a few seconds on a modern computer when using 2048 bit keys and less than a second when using 512 or 1024 bit keys. The keys will be stored inside the user data base. The private key will be encrypted, as everybody who has access to this key could sign in your name! Therefore, **please ensure that nobody gains access to your private key**.

If you already have a RSA key pair, you can **import** or type in these keys. An example of the expected file format is shown below.

When creating or importing a new key pair, existing public keys will not be overwritten. Old public keys will still be stored inside the user data base to be able to verify older signatures. Use the '**Erase all keys**' button if you want to remove keys.

It is possible to **print** out or **export** keys (you can decide whether you want to print/export your private or public key(s) or both).

Example of an exported key file:

```
[CurrentKeys]
Name=Thomas Seidel
Created=2003-07-22 - 16:17:14
Key Format=RSA, 1024 bit
PrivateKey=:QKUrnlp3zpjFky+30-M0QssGnRYRRJ9Hb8wEdDyYO8RDlw5PtWpJnzc ...
PublicKey=++11Ik:cDGk7Ran3sVSxNkhjirqMNLKXK-7qHoal-8T-vLo5xMYTe2SaRI ...

NumberOfOldPublicKeys=2

[OldKey1]
Name= Thomas Seidel
Created=2003-07-22 - 16:16:54
Key Format=RSA, 512 bit
PublicKey=++11Ik:h+BJtQm6NxBMDbIh4fpGZEs4ujF1GgOrCq+RWjaCygf3GcCee+6S ...

[OldKey2]
Name= Thomas Seidel
Created=2003-07-22 - 16:12:38
Key Format=RSA, 512 bit
PublicKey=++11Ik:p4SIFP6ngf1vxM9v+CB50F00zb3dNeOxcdOwG90GmDybeUf01xX6 ...
```

Note: Date and time information in this dialogue is displayed using the short date and long time format, which has been defined using the Windows Control Panel (regional settings).

1.2 Administrator Functions

The buttons of the 'Administrator' group will become available after entering the administrator password.

New

Click on 'New' to create a new user account. Enter the user name, the initial password (you can also leave this field empty and let the user define the password later). Select the directory for the user's data and test protocols. For directory options, click on the far right side under 'Directory' and a button appears [...]. After clicking this button, a user path dialog box appears where you can select the directory for storing test protocols and measurement data (see chapter 1.4 *User Directories*).

Delete

When an existing user entry needs to be deleted, highlight this entry and click 'Delete'.

Save

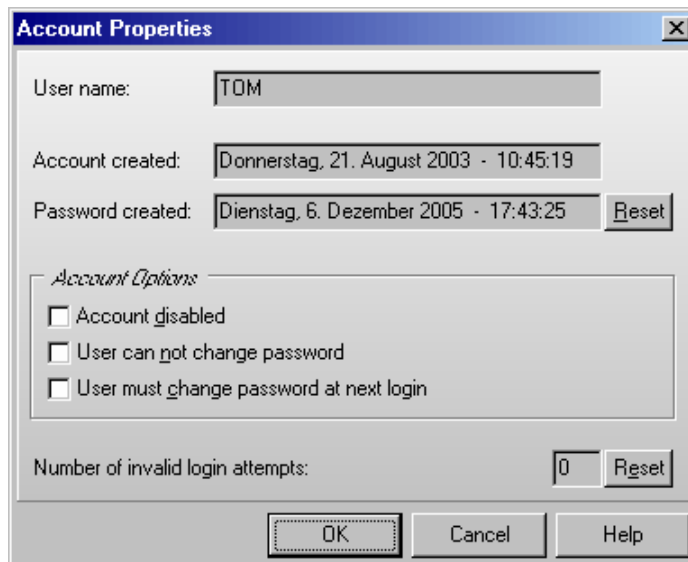
After clicking the 'Save' button all changes made will be saved in the user data base.

Cancel

If you click 'Cancel', all changes made after the last save will be discarded.

Properties

After clicking this button, a dialogue showing the properties of the selected user account will appear.



Here you can disable / enable the user account, you can force the user to change his password at the next login or you select an option to not allow the user to change his password. In addition, you can reset the counter for invalid login attempts.

Note: Date and time information in this dialogue window is displayed using the long date and long time format, which has been defined using the Windows Control Panel (regional settings).

Options

After clicking the 'Options' button the 'Administrator Options' dialogue will appear (see chapter 1.3 *Administrator Options*).

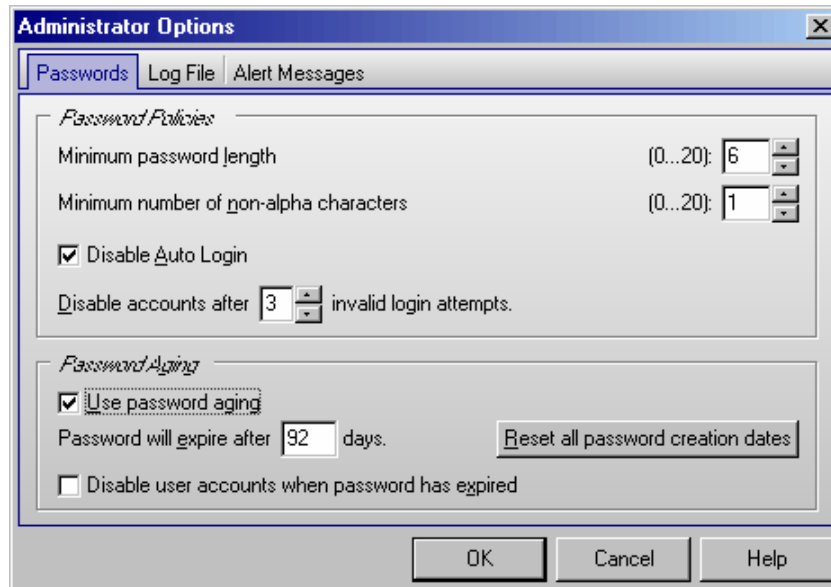
Note: Exporting the user table into a XLS (Excel), text or HTML file is possible after right clicking on the table.

1.3 Administrator Options

The Administrator Options dialogue box can be reached from the login screen.

1.3.1 Password Policies and Password Aging

In the first sheet the Administrator can define the policies for password. In addition it is possible to use password aging (passwords will be valid only for a defined time to force the users to change their passwords from time to time).



Password Policies

Here the required minimum length of passwords and the minimum number of non-alpha characters (numbers, special characters) can be specified. If you use 0 in both fields user accounts without passwords are possible.

The administrator can disable the 'Auto Login' function (see chapter 3.3.1 *Program Configuration*) here.

If you want an account to be disabled after a certain number of invalid login attempts (login attempts using a wrong password), select the number in the last line of the Password Policies box. If 0 is specified here, accounts will never be disabled.

When this software is used in an FDA 21 CFR part 11 compliant environment, BMG LABTECH recommends using a minimum password length of 6 characters including at least one non-alpha character. In this case, you should disable the auto login function and choose to disable account after 3 invalid login attempts. In addition, you should change the initial administrator password to something other than 'bmg' and you should also define a password for the default user 'USER'. See also Software manual part IV: FDA 21 CFR part.

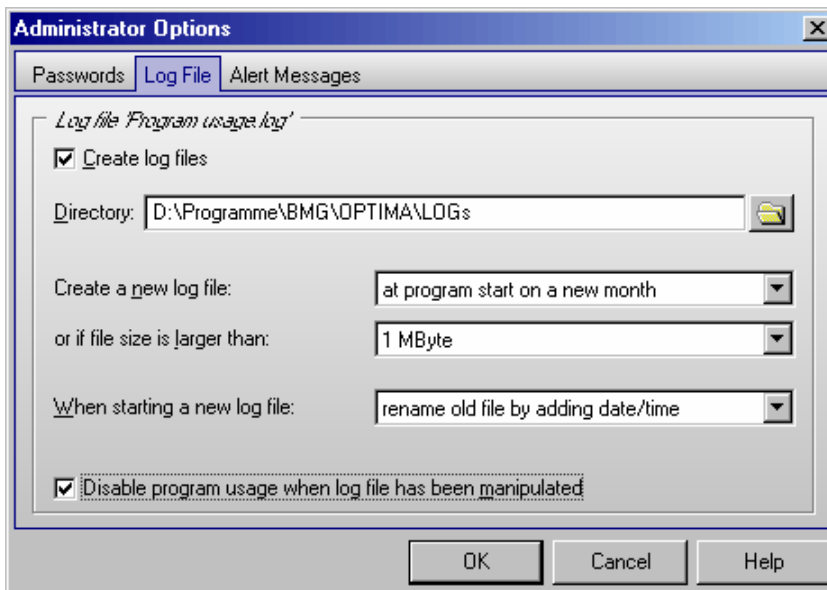
Password Aging

If passwords should be valid for only a certain amount of time, you can use the password aging function. The user will be prompted to change his password several days before the defined time is over. After the defined time, logging on is no longer possible. If the option '**Disable user accounts when password has expired**' has been selected only the administrator can reactivate the account (by deselecting the 'Account disabled' check box in the 'Account Properties' dialogue (see chapter 1.2 *Administrator Functions*), otherwise the user can reactive his account by changing the password (see chapter 1.1 *User Functions*). When changing the password aging settings you can use the '**Reset all password creation dates**' button to avoid that some existing passwords are already expired.

When this software is used in an FDA 21 CFR part 11 compliant environment, BMG LABTECH recommends using the password aging function with a setting of 92 days (3 months) or 183 days (half a year). See also Software manual part IV: FDA 21 CFR part.

1.3.2 Program Usage Log File

The BMG LABTECH software can create a log file (named 'Program usage.log') containing information about all important program actions, such as logging on, defining a test protocol, changing offset or filter values, performing a measurement and so on. This function can be switched on in the second sheet of the 'Administrator Options' dialogue box.



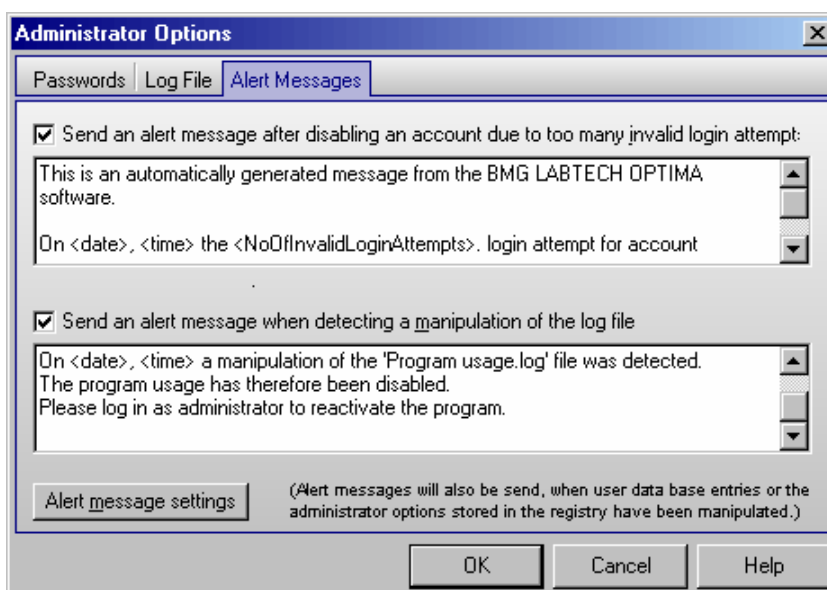
Here you can also specify the directory where this file is to be stored and under which conditions a new file should be started. When starting a new log file, the old file might be renamed or erased. The log file will be protected against manipulation by calculating a cryptographically secure hash value. The integrity of the log file will be checked at every program start. If the log file has been manipulated, the program usage can be disabled. To re-enable program usage the administrator needs to log in. You can also use the 'Check Data Integrity' tool (see also Software manual part IV: FDA 21 CFR part) to check the integrity of the log file.

When this software is used in an FDA 21 CFR part 11 compliant environment BMG LABTECH recommends using the log file function.

Note: There will be an additional run log file (see chapter 9.3 *Run Log Window*) which contains the communication between reader and computer. This file is intended to deliver background information in case of problems.

1.3.3 Alert Messages

Using the third sheet of the administrator options dialogue, it is possible to define alert messages, which can be sent under certain circumstances via email (e.g. to the administrator) or saved into an alert message file.



You can define a message, which will be sent after disabling an account due to too many invalid login trials and you define a message, which will be sent after detecting a manipulation of the log file.

In addition, alert messages will be sent when user data base entries or the administrator options stored in the registry have been manipulated.

After clicking the 'Alert message settings' button a dialogue will appear, where you can define the way in which to send these messages.

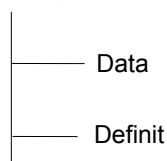
To be able to send emails, the computer needs to be connected to the internet. For the email options, you can use the same settings you are using in your normal email program. If you are not sure about these settings ask your system administrator.

It is also possible to add all alert messages to a text file. You can specify the directory and the file name in the lower part of this dialogue box.

1.4 User Directories

After the first login for a new user, two subdirectories will be automatically created under the defined user path (see chapter 1.2):

<user path>



Definit: In this subdirectory test protocols will be stored.

Data: The measurement data is stored in this subdirectory. Here you will find '<number>.dbf' (raw data) files and the 'Measure.db*' files (test runs overview data base table).

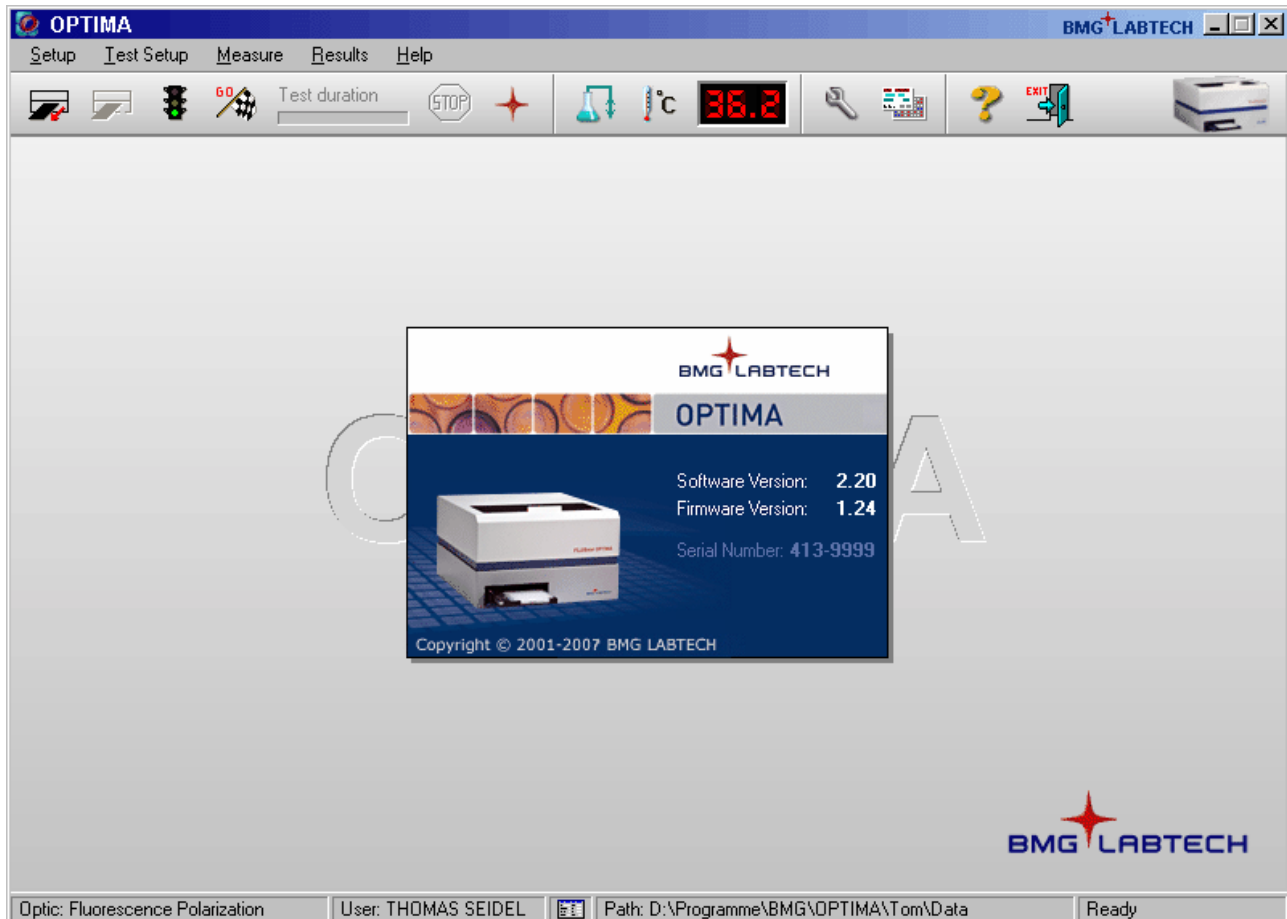
It is possible, at any time, to redirect the path of the data and to store the information in another directory. You can do this in the OPTIMA control software by selecting '**Setup**' and then '**Path**' or '**Login**' (see also chapter 3.5 Login and 3.6 Data Path).

2 Control Software Overview



2.1 Main Screen

After logging on, the main screen for the control software opens. An information screen appears in the middle containing details of the software and firmware versions.

Note: Write down the software version and firmware version so it is easily accessible if you need technical support. You can also retrieve this information by selecting **'Help'** and **'About OPTIMA'** in the main menu.



The status bar at the bottom of the main screen shows the currently installed optic (readers with auto optic switching) or the selected reading mode (other readers), the user logged in and the path used for storing the measurement results. You can change these settings by clicking the respective field of the status bar.

The little icon right of the user field shows whether the user is allowed to change test protocols  or if he is only allowed to run pre defined protocols  ('Run Only' mode).












In the last part of the status bar the current reader status will be shown.





Note: The first section does not exist when using a LUMIstar OPTIMA reader, as this reader can only measure in luminescence mode.

2.2 Icons and Menu Commands

The main menu is displayed across the top of the screen. Click the item to display the pull-down menu for additional options. You can also use the corresponding buttons of the tool bar that perform the same functions as the main menu items. The instrument must be turned on and connected for certain functions to be available.



<i>Menu command</i>	<i>Icon</i>	<i>Function</i>
Setup ...		Menu for configuring the instrument and program settings. Select the reader configuration (installed optic or measurement method), filters, change offsets values and select a directory for measurement data.
Setup Program Configuration	"	Changes program behavior, define options for ASCII export.
Setup Exit		Exits OPTIMA control software.
Test Setup Test Protocol		Defines the test parameters – e.g. number of flashes, intervals / cycles, content of the wells in the microplate, standard concentrations, injection volumes and times, etc..
Measure Plate Out		Moves microplate carrier out of the instrument.
Measure Plate In		Moves microplate carrier into the instrument.
Measure Measure		Performs a measurement using a pre-defined test protocol. Before the measurement starts, you can enter plate IDs and perform an automatic gain adjustment.
Measure Quick Start		Performs a simple end point measurement using the full plate without the need to define a test protocol before.
Measure Pause After Current Cycle		Just select method, filters and plate type.
Measure Stop Now		Stops the active test run after finishing the current cycle (only available for plate mode tests). *
Measure Prime		Stops the active test run or the execution of a script.
Measure Temperature		Prepares the pumps for injection, if pumps are installed. Define pump speed.
Measure Script Mode	-	Defines the temperature of the incubator.
		Opens the script mode window. **

Menu command	Icon	Function
Results Open Evaluation Software		Opens the Excel based BMG LABTECH evaluation software for data reduction. ***
Open MARS Data Analysis Softw.		Opens BMG LABTECH's new generation Data Analysis Software MARS. ***
Open Last Test Run	[Ctrl]+ 	This command will start MARS and will open the last test run performed. If MARS has already been started it will be activated (the input focus will be transferred to this software part) and the last test run will be opened.
Help Contents		Opens the online help.
Help Contact Addresses	-	Shows BMG LABTECH's contact addresses.
Help BMG Web Page	-	Starts an internet browser and makes a connection to the BMG LABTECH web page.
Help About OPTIMA	-	Shows the software and firmware version.
Help System Information	-	Shows information about the operating system, the CPU speed, the current Excel version and the amount of memory.

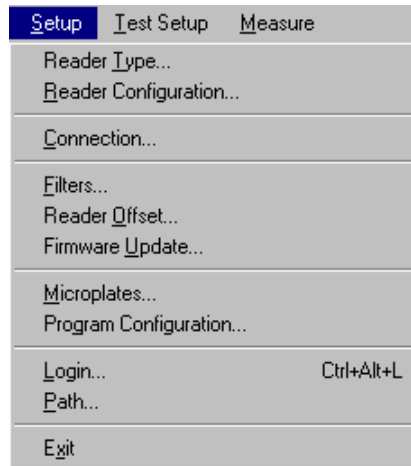
- * The 'Pause After Current Cycle' function is not available when using the script mode.
- ** The script mode is not available when the program is used in ActiveX or DDE mode, e.g. as part of a robotic system or in combination with Stacker Control.
- *** The function to open BMG LABTECH's new generation Data Analysis software MARS or to open the Excel based Evaluation software is only available if the respective software part has been installed. To change between an icon for MARS and an icon for the Excel based Evaluation software use the popup menu which appears after right clicking the icon.

3 Setup

3.1 Instrument Setup

The configuration settings for the instrument include the installed optic (reader equipped with auto optic switching) or the measurement method, the communication port, filter definitions, instrument offsets, definition of microplate dimensions and the path for measurement data.

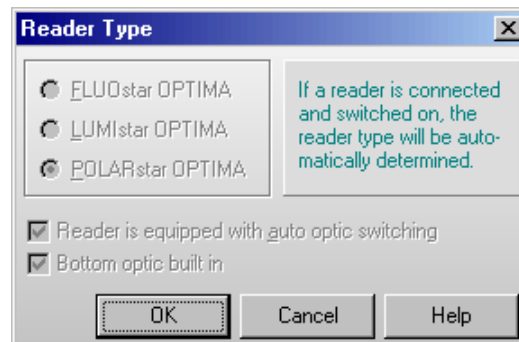
You can use the **'Setup'** pull-down menu



or the  button.

3.1.1 Reader Type

If a reader is connected to the computer and switched on, this window (accessible via **'Setup | Reader Type'**) will show the type of reader which was automatically recognized. Depending on the reader type different measurement methods can be used.



The software remembers the last reader type used and will use this type if no reader is connected, but in this case you can change the reader type using this dialogue. This might be useful if you want to define a test protocol for a reader currently not available.

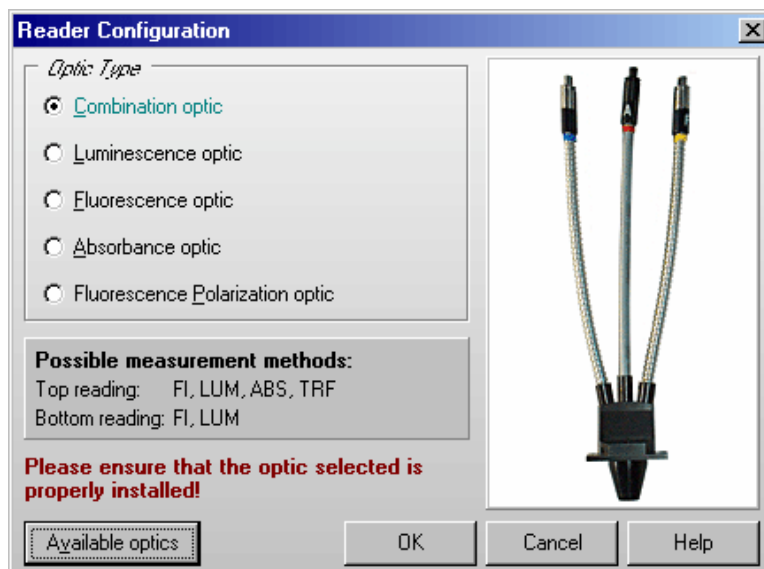
3.1.2 Reader Configuration

The LUMIstar OPTIMA can only measure using the luminescence method, therefore, the reader configuration dialogue is not available for this reader.

The FLUOstar OPTIMA can measure fluorescence intensity and time-resolved fluorescence and (optional) absorbance and luminescence. In addition, the POLARstar OPTIMA can read using the fluorescence polarization method. If the reader is equipped with auto optic switching, the reading methods possible depend on the installed optic. You can use the **'Setup | Reader Configuration'** function to define the optic type. If the reader is not equipped with auto optic switching, the reader configuration dialogue is used to select the reading method (see below).

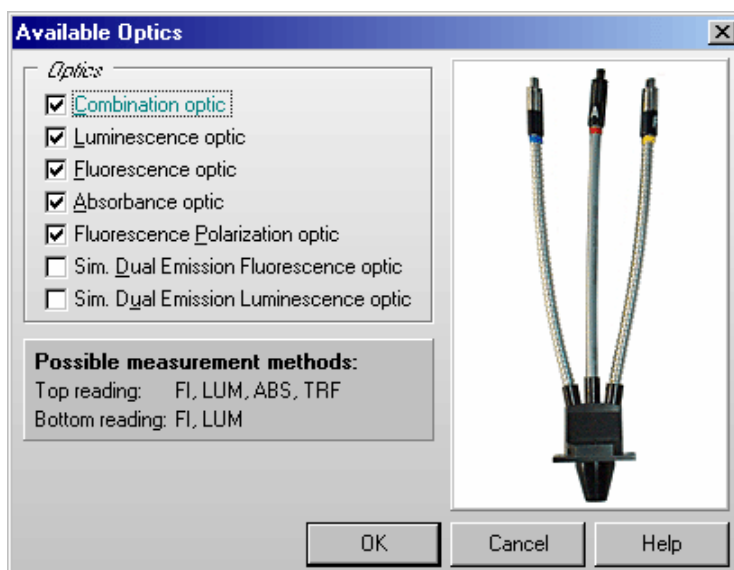
FLUOstar or POLARstar OPTIMA with auto optic switching

Using the 'Setup | Reader Configuration' function, you can define which optic has been installed. You will see a picture of the optic selected on the right. For some measurement methods there are different optimized optics available, e.g. for absorbance measurement. If these optics look very different, the picture will show more than one optic.



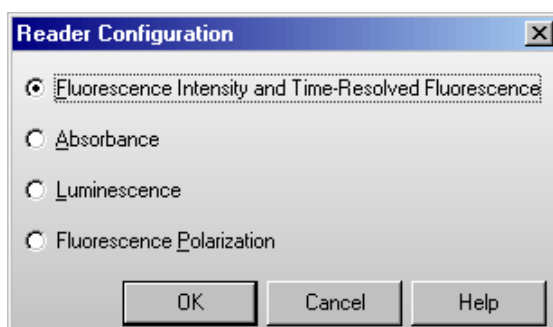
After selecting an optic, the reading modes possible with the reader connected (or selected using 'Setup | Reader Type') are listed on the left.

Use the 'Available Optics' dialogue to define which optics you have available.



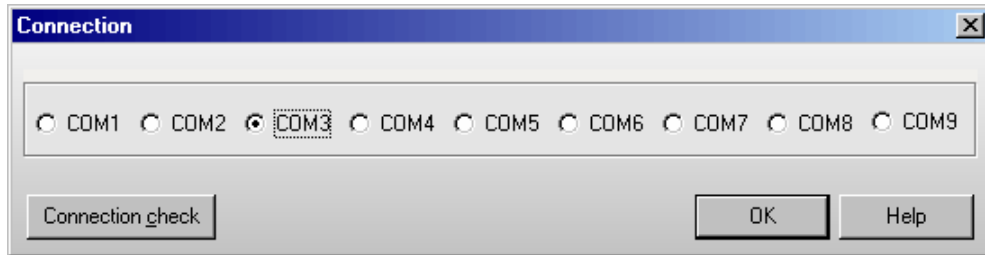
FLUOstar or POLARstar OPTIMA without auto optic switching

Using the 'Setup | Reader Configuration' function, you can designate the measurement method. Please ensure that an optic suitable for the measurement method selected is installed and that you have switched to top or bottom reading, according to your needs.

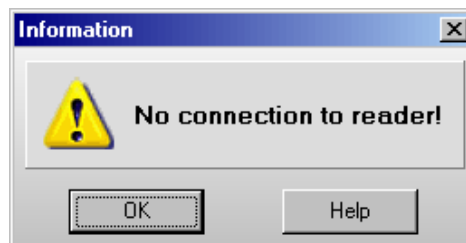


3.1.3 Connection

You must select the corresponding communication port on the PC and check the communication status between the instrument and the PC. You can only select com ports which are available on the PC. If there is no communication between the PC and the instrument, check that the power to the instrument is switched on. If there is still no communication, try a different com port.

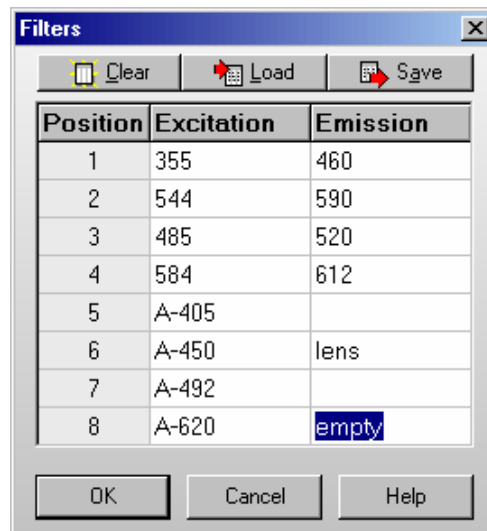


Connection check allows you to validate communication between the PC and the instrument. If there is no communication, a message box will appear:



3.1.4 Filters

The readers of the OPTIMA family can be configured with up to 8 excitation filters and up to 8 emission filters. Click on 'Setup | Filters' to open the filter dialogue.



The position in the table corresponds to the filter position in the filter wheel (see picture below). Enter the filter wavelength values in the corresponding fields. For absorbance mode an empty position should be named on the emission side.



- Clear** After clicking this button, the table contents will be removed.
- Load** Loads filter information from an ASCII or Excel (.xls) file into the table. This might be useful if you are using more than one set of filter wheels.
- Save** Saves filter information from the table into an ASCII or Excel (.xls) file.
- OK** Saves the filter positions and returns to the main menu.
- Cancel** Closes this window without saving changes.

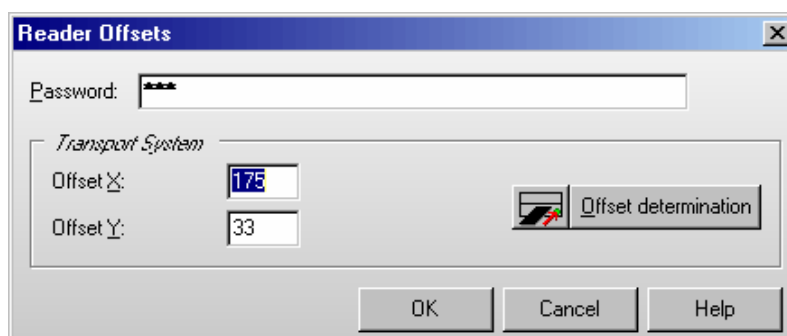
Notes: The table is common to all users.

Users with activated 'Run Only' option (see 1 Login Screen) are not allowed to change the filter settings.


3.1.5 Reader Offsets

Every OPTIMA reader is accurately calibrated at the factory and has individually defined offset values. The offset values correspond to the home position of the microplate carrier. Correct offset values are important for optimal measurement results. The offset values are stored in the reader EEPROM. The determination of the offset values should be carried out only by a qualified service technician. If the plate carrier is removed or replaced, the offset values should be re-calibrated.

The Reader Offsets screen can be reached by selecting '**Reader Offset**' in the '**Setup**' menu.



You can now enter new values. Do not perform an 'Offset determination' unless you are qualified to do so!

The  button will move the plate carrier to the defined offset position.

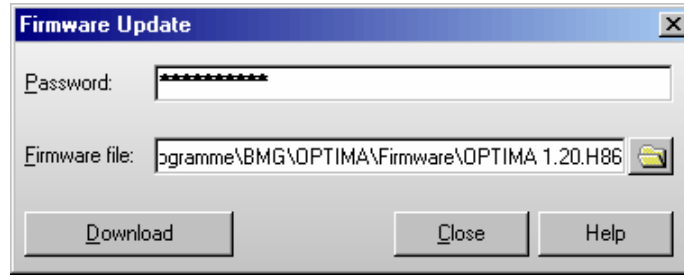
The '**Offset determination**' button is for service personnel only.

Click on '**OK**' to save the offset values into the reader EEPROM.


Note: Users with activated 'Run Only' option (see chapter 1 *Login Screen*) are not allowed to change offset values.

3.1.6 Firmware Update

This function allows you to update the reader firmware (content of the built in Flash-EEPROM).



If you received a new firmware file from BMG LABTECH, you should copy this file into the folder ~:\Program Files\BMG\OPTIMA\Firmware.

Make sure that the reader is connected to the computer and switched on. After typing in the correct password (you will get the password with the new firmware) select the firmware file using the  button, then press 'Download'. The OPTIMA program will bring the reader into the download mode and then start the download process. Downloading the new firmware is an automatic process, which will last a few minutes. Do not interrupt this process by switching the reader or the computer off or by closing the download program!

After the download is completed, the reader needs to be switched off and on again to activate the new firmware.

If the download process is interrupted, the reader will not work, but even in this state it is usually possible to repeat the download process (you need to restart the OPTIMA software after an interrupted download process).

3.2 Microplate Database

The OPTIMA software contains a data base with dimensions of microplates from most microplate manufacturers. While most plates have the standard spacing and footprint, some plates have slightly different dimensions, and must be positioned accordingly for optimal results.

In addition, new plates that are not on the current list can easily be defined by selecting '**Setup | Microplates**'. If microplate dimensions are edited, the changes will apply to all users.

Microplate ▾	Length	Width	X (1)	Y (1)	X (n)	Y (n)	Plate Format ▲
▶ BMG LABTECH 96	127.76	85.48	14.38	11.24	113.38	74.24	96
CORNING 384 LOW FLANGE	127.80	85.50	12.12	8.99	115.68	76.51	384
CORNING 384 LOW VOL FL. BOTTO	127.76	85.47	12.13	8.99	115.63	76.48	384
CORNING 384 LOW VOL ROUND BI	127.74	85.50	12.12	8.99	115.62	76.51	384
COSTAR 12	127.76	85.48	24.88	16.74	102.88	68.74	12
COSTAR 24	127.76	85.48	15.13	13.49	112.63	71.99	24
COSTAR 384	127.80	85.50	12.12	8.99	115.68	76.51	384
COSTAR 48	127.76	85.48	18.38	10.24	109.38	75.24	48
COSTAR 6	127.76	85.48	24.88	23.24	102.88	62.24	6
COSTAR 96	127.80	85.50	14.30	11.20	113.50	74.30	96
COSTAR 96 HALF AREA	127.80	85.50	14.30	11.20	113.50	74.30	96
DYNEX 96	128.00	85.80	14.40	11.20	113.60	74.10	96
FALCON 384	127.60	85.60	12.10	9.10	115.50	76.50	384
FALCON 96	127.70	86.00	14.40	11.50	113.30	74.50	96

Buttons: New, Edit, Copy, Export, Import, Delete, Close, Help

To select the first microplate, beginning with e.g. 'B', simply press the key [B]. To select more than one microplate, press [Shift] together with [↑] or [↓] or press [Ctrl] and click on the desired microplate names with the left mouse button. The microplates in this list can be sorted by name or by plate format. To change the sort order, click the 'Microplate' or 'Plate Format' section of the table headline.

New

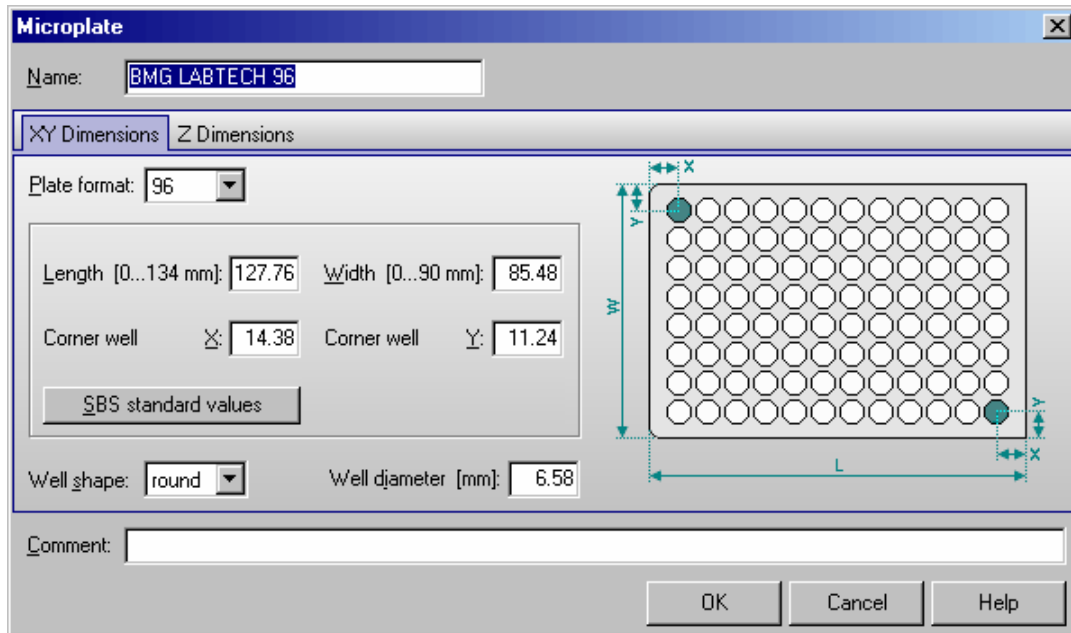
Click on 'New' to enter the plate dimensions for a new plate. A new window will appear that allows you to enter the necessary coordinates. Enter the plate format (well number) and the mechanical dimensions of the microplate (see Edit below).

Note: The dimensions should be measured from the center of the wells and need to be very accurate in order for the wells to be positioned exactly during measurements.

Edit

Select a microplate entry that you want to modify. A second window appears that displays the dimensions and plate format. All dimensions are in mm.

XY Dimensions



Name	Name of the new microplate
Plate Format	Select the total number of wells in the plate – 1536, 384, 96, 48, 24, 12 or 6 wells.
Length	The outer length of the entire microplate, from border to border.
Width	Outer width of entire microplate, from top to bottom.
Corner well X	Distance from the left border of the microplate to the center of a well in the first column = distance from the center of a well in the last column to the right border of the microplate. *
Corner well Y	Distance from the top border of the microplate to the center of a well in the first row = distance from the center of a well in the last row to the bottom border of the microplate. *
Well shape	Select between round and square. This information is necessary for well scanning.
Well diameter	Diameter of a well. This information is necessary for well scanning.

* *The software shows by default only one X and Y distance for the corner wells, based on the assumption that the plate is symmetrical.*

If you want to define asymmetrical plates, use the key combination [Ctrl]+[U] or click the plate drawing. The dialogue will then show input boxes for the distance to the top left well (X(1), Y(1)) and to the bottom right well (X(n), Y(n)). When opening an existing asymmetrical plate definition, the dialogue will of course also be opened in the "asymmetrical" version. When you define a new microplate using this mode, the values for X(n) and Y(n) will be pre-calculated as soon as the length and X(1) or the width and Y(1) are entered. It is possible to override the pre-calculated values. Please note: this calculation will only take place as long as there are 0 values inserted for X(n) and Y(n).

SBS standard values

After clicking this button, the SBS standard X and Y dimensions for the selected plate format will be entered. Please select the well shape and define the well diameter / width yourself.

You can use the **comment** field to store additional information about the microplate, for example order numbers or the microplate material.

Z Dimensions

Use the second sheet to define the Z dimensions. All dimensions need to be entered in mm. If the plate manufacturer does not provide these dimensions, you can use an accurately calibrated caliper to obtain the following dimensions. Please see the diagrams for the correct measurement of the dimensions.

Plate height	Total height of the microplate.
Well depth	Depth of a well, measured in the middle of the well.
Border height	Height of the border of the microplate.
Distance between plate and well bottom	Distance between the bottom of the microplate and the outer bottom of the wells.
Stack height	Vertical distance between two microplates, if the plates are stacked. As the well bottom is higher than the plate bottom, this stack height is lower than the plate height.

Copy

Select a microplate that you want to copy. A window will appear allowing you to rename the microplate. The dimensions are copied exactly. This option allows you to edit a microplate's dimensions without changing the original.

Export

Select a microplate definition that you want to export to a disk or to a different directory. A new window will ask for the destination drive and directory. The file name will get an extension '.MPC'.

Import

Imports microplate definition from a disk or another directory and adds it to the existing data base. The extension for the microplate file must be '.MPC' or '.MPL' (microplate definitions exported from other / older BMG LABTECH programs).

Delete

Deletes an existing microplate and its dimensions from the data base.

Close

Returns to main menu.

Notes: Exporting the microplate table into a XLS (Excel), text or HTML file is possible after right clicking the table.

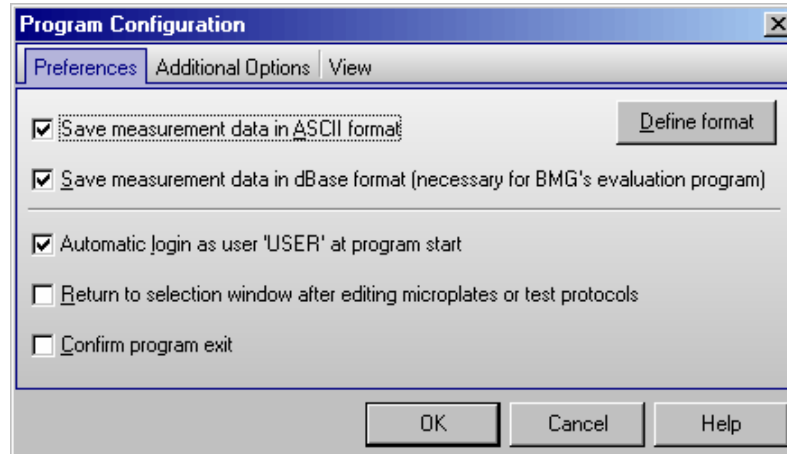
Users with activated 'Run Only' option (see chapter 1 *Login Screen*) are not allowed to edit, copy or import microplate definitions.

3.3 Program Configuration

Program Configuration allows the user to modify the appearance of the software. You can activate a feature by ticking the box next to the statement.

3.3.1 Preferences Sheet

In the first sheet you can select how the measurement data should be stored. Here you can also define some general program behavior settings.



Save measurement data in ASCII format

By default all measurement data is saved in dBase format in the specified user directory (see below). This type of data can be used with the BMG LABTECH Data Analysis software. If you prefer to use a different data analysis software package, which will not work with dBase formats, then it is also possible to store the data in ASCII format. If you select this option then the **'Define Format for ASCII Export'** window becomes available (see chapter 3.4).

Save measurement data in dBase format

If this option is selected, all measurement data is stored in a set of dbase files in the data directory of the user logged in, e.g. 'C:\Program Files\BMG\OPTIMA\

Note: It is possible to use both formats simultaneously.

Return to selection window after editing microplates or test protocols

The selection window for tests and microplates contains previously defined setups and options to edit, export, import, etc.. By default, you can return to the main control software after creating or editing a test or microplate definition without returning to the selection window. If you wish to see the selection window for the microplates or test protocol definitions again, check the box in the 'Program Configuration' window.

Auto login

If you choose the option 'Automatic login as user "USER" at program start' there will be no login screen at program start, instead you will be logged in as the default user 'USER'. It is still possible to use the login function later ('Setup | Login').

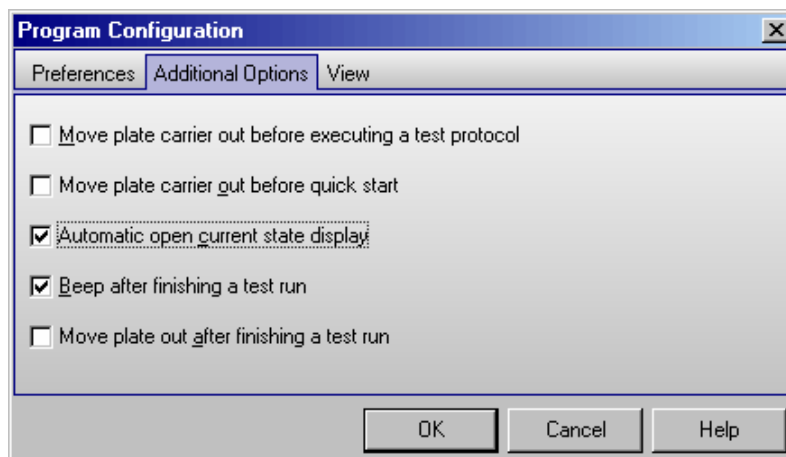
Note: Setting this option is only possible, if you are logged in as "USER" and if the administrator has not disabled this function (see chapter 1 *Login Screen*).

Confirm program exit

If you deselect this option there will be no confirmation window when exiting the program.

3.3.2 Additional Options Sheet

In the second sheet you can change the program behavior around measurements.



Move plate carrier out before executing a test protocol

Select this option if you want the plate carrier to be moved out when starting the execution of a test protocol. The plate carrier will be moved out after selecting the test protocol and opening the 'Start Measurement' dialogue.

Move plate carrier out before quick start

Select this option if you want the plate carrier to be moved out when opening the 'Quick Start' dialogue.

Automatic open current state display

If this option is selected the Current State display will be automatically opened after starting a measurement.

Beep after finishing a test run

If you select this option there will be a short acoustic signal after a test run is finished.

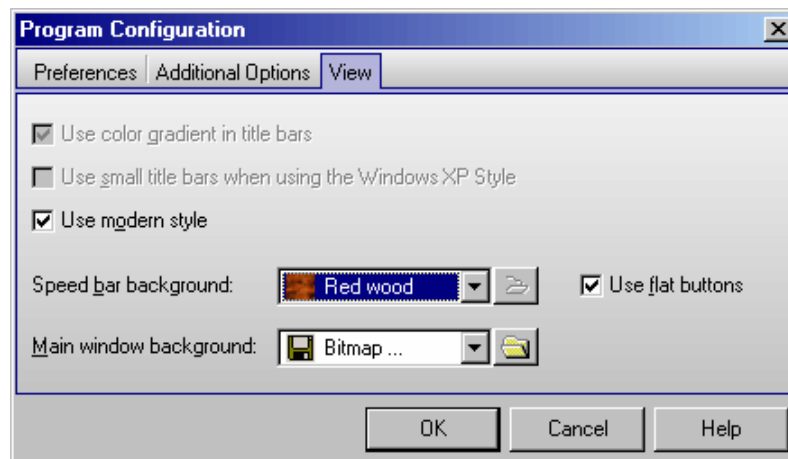
Move plate out after finishing a test run

If you select this option the plate will be automatically moved out of the reader as soon as the test run is finished, the current state window has been closed and the measurement data is saved.

Note: This option is not active if the software is used in ActiveX or DDE mode (e.g. in combination with Stacker Control) or in script mode (see chapter 7).

3.3.3 View Sheet

Using this sheet you can change the program look.



Use color gradient in title bar

The border or title bar at the top of the software can be either a solid color or a gradient from dark to light. This is only for appearance and does not affect any other feature of the software.

Note: In Windows 98, 2000, XP or Vista you define the title bar style using the windows control panel. Therefore, this option will be grayed out.

Use small title bars when using the Windows XP Style

Using this option you can decide whether you want to get small or large title bars for all program sub windows. The title bar of the main program window and of the login screen will not be changed. The style of Windows standard dialog boxes, e.g. for file name selection or for printing options, will also not be changed.

Note: This option is only available when Windows XP is used and the Windows XP style has been selected. It is not available when using the classic style.

Use modern style

You can choose between a classic and a more modern appearance of the software. The look of the software is also influenced by the display style selected using Window's control panel (under 'Display Properties').

Note: The screen shots used in the online help have been created using Windows XP's classic style in combination with the 'Use modern style' option of the BMG LABTECH software.

Speed bar background

It is possible to define the style of the speed bar. Choose any of the pre defined background styles or use one of your own bitmap files (BMP-, GIF-, JPEG format).

Use flat buttons

If this option is selected the buttons of the speed bar will appear flat (they will look like icons). In this case, the border of a button will only be visible when the mouse cursor is moved over the button.

Main window background

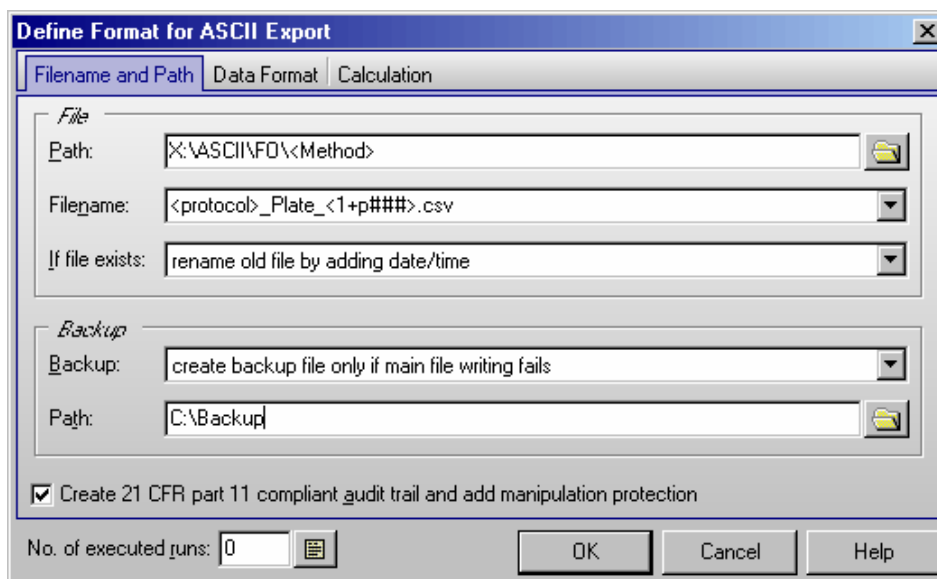
Similar to the speed bar it is also possible to define the background of the main program window. Choose any of the pre defined background styles or use one of your own bitmap files.

Note: All settings from the program configuration window besides the Auto Login option are user specific, therefore, each user can select his preferences independently.

3.4 ASCII Export Function

If you click the 'Define format' button in the 'Program Configuration' window (see chapter 3.3.1) the '**Define Format for ASCII Export**' window will appear. In this window, you can choose the name and format of the file and where the data is saved.

3.4.1 Filename and Output Path



File

Path

The data path where the ASCII files will be stored. This can be a network directory, too. To create a new directory simply type the path you wish in. You can use the special functions <protocol> and <method> as part of the directory name to get your protocols sorted (explanation see below under Filename).

Filename


The file name can be a constant name (something you type in) or you can use one of the following options given in the drop down menu:

- <protocol> Name of the used test protocol.
- <method> Name of the used method, e.g. 'Fluorescence Intensity' or 'Luminescence'.
- <ID1>...<ID3> The information written in the plate identification window before the measurement begins.
- <1+#> Tests will be assigned consecutive numbers. When the OPTIMA software is restarted the numbers begin again with '1'. You can change the start number; for example, enter the number 5 and the test numbers will increase consecutively starting at 5.

You can add a '#' character in order to increase the number of digits used. (<1+###> will produce file names 001, 002, 003, etc.)

If you want a number to be changed only after e.g. every tenths plate you should add this value in parenthesis before the > character, e.g. <1+###(10)>.

To use the 'Total no. of executed runs' instead of the number of executed runs after program start add a 'T' before the '#', e.g. <1+T#####>.

To use the 'No. of executed runs for the used protocol (used test definition)' add a 'P' before the '#', e.g. <1+P###>. You can see a Run Statistics after pressing the  button.

In script mode (see chapter 7) you can add a 'B' before the '#' to get the plate numbers of the current batch run.

Counting down is also possible, use a '-' instead of the '+'.

- `<A+#>` Identical to consecutive numbers except now the file name will be consecutive alphabetical letters. The number of letters used can be modified (i.e. `<A+###>` will result in AAA, AAB, AAC, etc).
- `<date>` insert current date
 You can specify the date format if you add a format description after “`<date:.`” using `yy` or `yyyy` for the year, `m` or `mm` for the month and `d` or `dd` for the day:

<code>yy</code>	year with two digits (1999 => 99, 2000 => 00)
<code>yyyy</code>	year with four digits
<code>m</code>	one or two digits for the month (January => 1, December => 12)
<code>mm</code>	month with two digits (January => 01)
<code>mmm</code>	abbreviated name of the month (January => Jan.)
<code>mmmm</code>	full name of the month
<code>d</code>	day with one or two digits
<code>dd</code>	day always with two digits
<code>ddd</code>	abbreviated name of the day (Monday => Mo.)
<code>dddd</code>	full name of the day
<code>ddddd</code>	date in the format defined as “Short Date Format” under windows (‘Settings Control Panel Regional Settings’)
<code>dddddd</code>	date in the format defined as “Long Date Format” under windows

Example: `<date:yyyy_mm_dd>`
 If you do not specify the format “`yyyymmdd`” will be used.
- `<time>` insert current time
 You can specify the time format if you add a format description after “`<time:.`”:

<code>h</code> or <code>hh</code>	for the hour (one or always two digits)
<code>m</code> or <code>mm</code>	for the minute
<code>s</code> or <code>ss</code>	for the second
<code>t</code>	time in the format defined as “Short Time Format” under windows (‘Settings Control Panel Regional Settings’)
<code>tt</code>	time in the format defined as “Long Time Format” under windows
<code>am/pm</code> or <code>a/p</code> or <code>AM/PM</code> or <code>Am/Pm</code>	use 12 hours format and show am or pm (a or p...)

Example: `<time:hh.mm.ss>`
 If you do not specify the format “`hhmmss`” will be used.
- `<ser_number>` Serial number of the reader used

You can use more than one of these parameters at one time in the filename (example: `<protocol> plate <1+###>.dat`). If a file extension is not specified then ‘.DAT’ will be added automatically.

If file exists

If a file with the same name already exists, then there are several options:

- **Rename the old file by adding date and time** to distinguish it from the more recent file.
- **Overwrite old file.**
- **Append** the new **data** to the existing file (it will list separate tests in the same file; each test can be separated by a dashed line. See also the ‘Separate data blocks with an empty line’ option described below).

Backup

Here you can choose what to do if the data storage to the above defined directory fails:

- ***no backup file***
Show an error message when creation / writing into the defined ASCII file failed. The measurement results are still available in the database files (dBase format, for usage with e.g. Excel).
- ***create backup file only if main file writing fails***
If creation / writing into the defined ASCII file failed the ASCII file will be stored in the specified backup directory (same filename and behavior if file already exists as defined above). If writing into the backup file fails too, then there will be an error message. This setting is recommended if the main directory is on a network drive.
- ***always create backup file***
The ASCII file will be stored in the specified main directory. A second copy will be stored in the backup directory (same filename and behavior if file already exists as defined above). There will be no error message when writing into the main file failed. But there will be an error message if writing into the backup file fails.
- ***always create backup file (no error message when this fails)***
The ASCII file will be stored in the specified main directory. A second copy will be stored in the backup directory (same filename and behavior if file already exists as defined above). There will be no error message when writing into the main and/or backup file failed.

Path

Specify the path for storing the backup copy of the ASCII file.

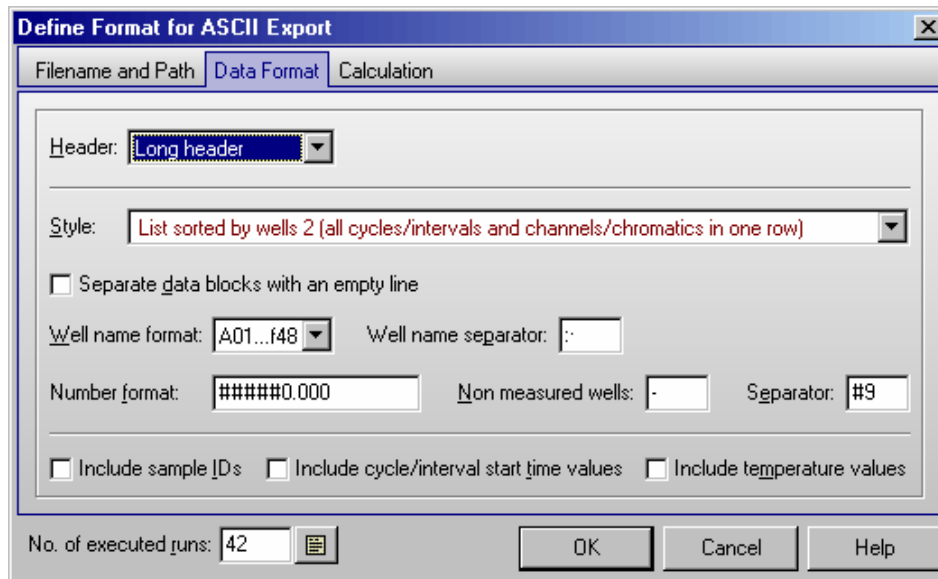
Create 21 CFR part 11 compliant audit trail and add manipulation protection

If you use this option, there will be an additional file created for each ASCII file (same file name, but with additional file extension '.at'). This file will contain an anti manipulation hash value and the audit trail for the data stored in the connected ASCII file. The anti manipulation hash value will protect the whole ASCII file and the audit trail entries (see software manual part IV: FDA 21 CFR part 11).

The hash value will be created when the control part of the BMG LABTECH software creates the ASCII data file. It can be checked using the "Check Data Integrity" tool (see software manual part IV: FDA 21 CFR part 11).

3.4.2 Data Format

On the second sheet ('Format'), you can specify the data format:



Header

It is possible to include a description of the test run.

- **Short header**
Describes the test run in 5 lines (test protocol name, date, plate IDs, number of cycles, and number of channels / chromatics).
- **Long header**
Contains the information of the short header but also includes the measurement mode, filters and the gain settings.
- **Full header**
Using the full header option all protocol settings including things like the layout, the used target temperature, the firm- and software versions and the reader serial number will be included in the ASCII file. BMG LABTECH recommends this option if you want to use ASCII files as data records according to the 21 CFR part 11 rule (see software manual part IV).
- **Danish style**
If you use this option there will be no header, but in the first column of every data line the plate ID1 will be listed. This may be useful, if you combine data from more than one test run in one ASCII file and search, for example, for the highest result in any well of all plates. When you have found this result, you not only see the well name, you also see immediately the plate identification.

If you use the option 'Short', 'Long' or 'Full header' there will also be a line with the 'Channel / Multichromatic' number and a line with the 'Cycle / Interval' number before each data block.

Style

The data can appear as a **'Table'** (raw data side by side in a matrix) or a column (**'List'**). It is possible to include the well numbers and to include or exclude non measured wells (wells which are not used in the layout of the protocol executed).

If you choose the option **'Table with well numbers in plate layout style'** you will get the data represented in a table, where the well names are shown similar to what is printed on a microplate:

	1	2	3	4	...
A	28672	22726	24340	25977	
B	22908	23479	25483	27633	
C	23722	23839	23020	23503	
...					

Tests that have more than one cycle or interval will have data blocks representing the results from each cycle / interval. Multichromatic measurements will appear with the data from the optic module first used, followed by the data from the second module, etc.

If you choose the option '**List sorted by wells**', you will get the measurement results for all cycles in one line per well; but if your test uses more than one optic module, you will still get a separate data block for each chromatic (for each optic module used). Depending on the calculation method selected (see chapter 3.4.3), you will also get separate data blocks for each channel when using dual emission or fluorescence polarization methods and / or a data block for the ratio or the polarization values.

If you choose the option '**List sorted by wells 2**', you will get all measurement results for one well in one line per well. Depending on the protocol executed and on the selection in the Calculation sheet (see below), the line will first contain the values of the first cycle/interval for the first chromatic (for a dual emission protocol this might be one value for channel A, one value for channel B and a ratio), then the values for the second chromatic and so on. After this, the values of the second cycle/interval will follow and so on. When you perform a dual chromatic dual emission protocol and would like to store raw data and ratio, you will get the data in the following order:

First cycle 1A, 1B, Ratio-1, 2A, 2B, Ratio-2, second cycle 1A, 1B, Ratio-1, 2A, 2B, Ratio-2, third cycle ...

To make the usage easier, the selections are grouped and each group will be shown in a different color. In addition, a hint (which appears when moving the mouse cursor to the style selection pull down box) will show a short example of the file format to be expected.

Separate data blocks with an empty line

If you use multichromatics or test protocols using two channels or if you use test protocols with more than one cycle / interval you will get more than one block of data in your ASCII file. By checking this box these blocks are separated by an empty line. There will also be an empty line separating the data from different test runs if you use the 'Append data' option (see above).

Well name format

Select whether or not you want the well name to always contain a two digit number (e.g. A09 instead of A9). If you prefer the well name with a one digit number, you might want to choose a trailing blank in front of the well names for column 1 to 9 to achieve an equally aligned layout.

Note: When using the 'Table with well numbers in plate layout style' it is not possible to define a well name format. Here the well identification is done in a way similar to what is printed on a microplate.

Well name separator

Select how the well name should be separated from the following measurement values: for example use a comma, a semi colon or back slash. By selecting '#9', a tab will be inserted.

Number format

Select the number of digits before and after the decimal point. A position described with a # symbol will be a number or a space; a position described with a zero will be a number or a zero.

For absorbance tests, you should specify a format of 0.000 because the OD values can range from 0.000 to 4.500. By adding an 'm' to the format string (e.g. '###0m') the numbers will be expressed in milliOD (0 to 4500 mOD).

Non-measured wells

Select the appearance of a non-measured well, for example: hyphen (-), backslash (/), n.a., or a zero (0).

Separator

Select how the individual raw data number will be separated: use for example a comma, a semi colon or back slash. By selecting '#9', a tab will be used.

Include sample IDs

Store the sample IDs (see chapter 6.5) in the ASCII file (after the measurement value).

Include cycle/interval start time values

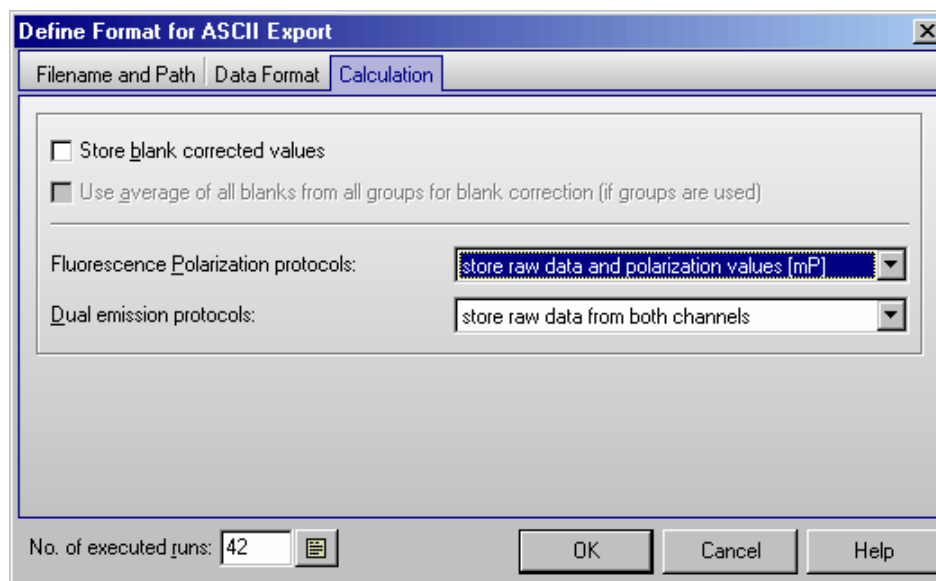
If this option is selected, the cycle start times (plate mode) or the interval start times (well mode) will be included. The time values will be stored in a separate line in front of each measurement values block or in one line before the data when using a 'List sorted by wells' style. If you have decided to include a header, the time information is preceded by 'Time [s]: '; otherwise only the numbers will be stored.

Include temperature values

Store the temperature which was measured during the measurement of each cycle (plate mode) or during the measurement of each well (well mode).

Note: For this function it is necessary, that the built in incubator is switched on or that the temperature monitoring function is used (see chapter 5.2 *Incubation*).

3.4.3 Data Calculation during ASCII Export



Store blank corrected values

If this option is selected, the measurement values stored in the ASCII file will be blank corrected.

Use average of all blanks from all groups for blank correction

This option is only important when different layout groups (see chapter 4.3.9 *Using Layout Groups*) are used. If this option is not selected, the measurement values of a group will be corrected using only blanks from the same group. If you select this option, the correction will be done using the average of all blanks from all groups.

Fluorescence Polarization protocols

For fluorescence polarization tests, you can decide whether you want the raw data for both channels to be stored, or the polarization or anisotropy values, or a combination of raw data and polarization or anisotropy values. The polarization values will be calculated and stored in mP, the anisotropy values in mA. For these values you should use a number format with enough space for decimal numbers, e.g. '####0.000'.

$$\text{Polarization values: } P = \frac{\text{Ch.A} - (\text{Ch.B})}{\text{Ch.A} + (\text{Ch.B})}$$

$$\text{Anisotropy values: } A = \frac{\text{Ch.A} - (\text{Ch.B})}{\text{Ch.A} + 2(\text{Ch.B})}$$

Dual emission protocols

For dual emission tests, you can decide whether you want the raw data for the two channels or the ratio of channel A / channel B to be stored. It is also possible to get both the raw data and the ratio.

Number of executed runs

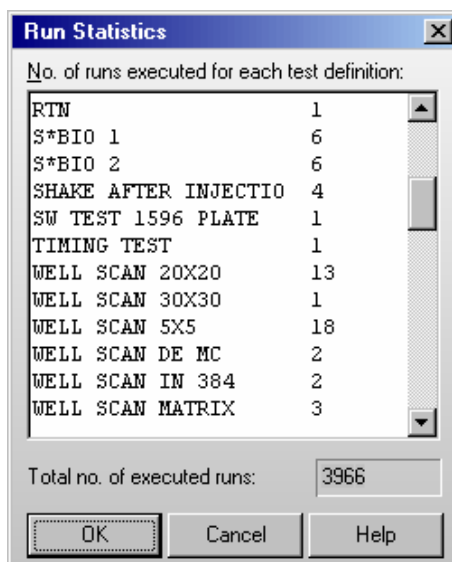
The 'counter' tracks the number of measurements that were performed after the program was started. You can manually type in a starting number. This number will be used in the filename options <1+#> and <A+#>.

To see a **Run Statistics** press the  button.

Note: All settings from the 'Define Format for ASCII Export' window are user specific; therefore, each user can select his preferences independently.

3.4.4 Run Statistics

In this window, you see a list of all used test protocols together with a number showing you how often a protocol was executed. It is possible to edit these numbers or to delete a protocol from the list.



You can use this number together with the <1+P#> or <A+P#> option for defining the ASCII export filename or as part of a plate ID.

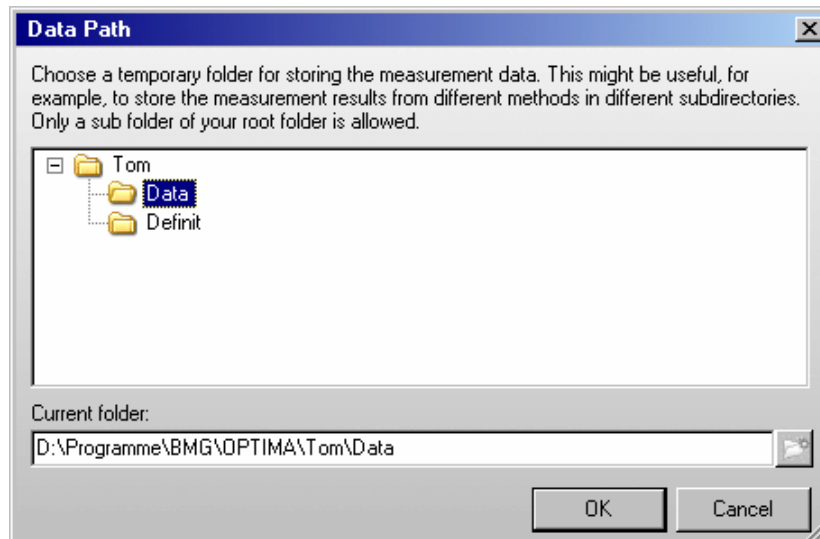
In this window, you also see the '**Total no. of executed runs**'. This is the total number of all tests executed using this computer after installing the OPTIMA software.

3.5 Login

The login function is described in chapter 1 Login Screen.

3.6 Data Path

This window allows you, to temporarily change the directory for your measurement results to a directory different than the one specified using the Login screen (see chapter 1). This might be useful, for example, to store the measurement results from different methods in different subdirectories.



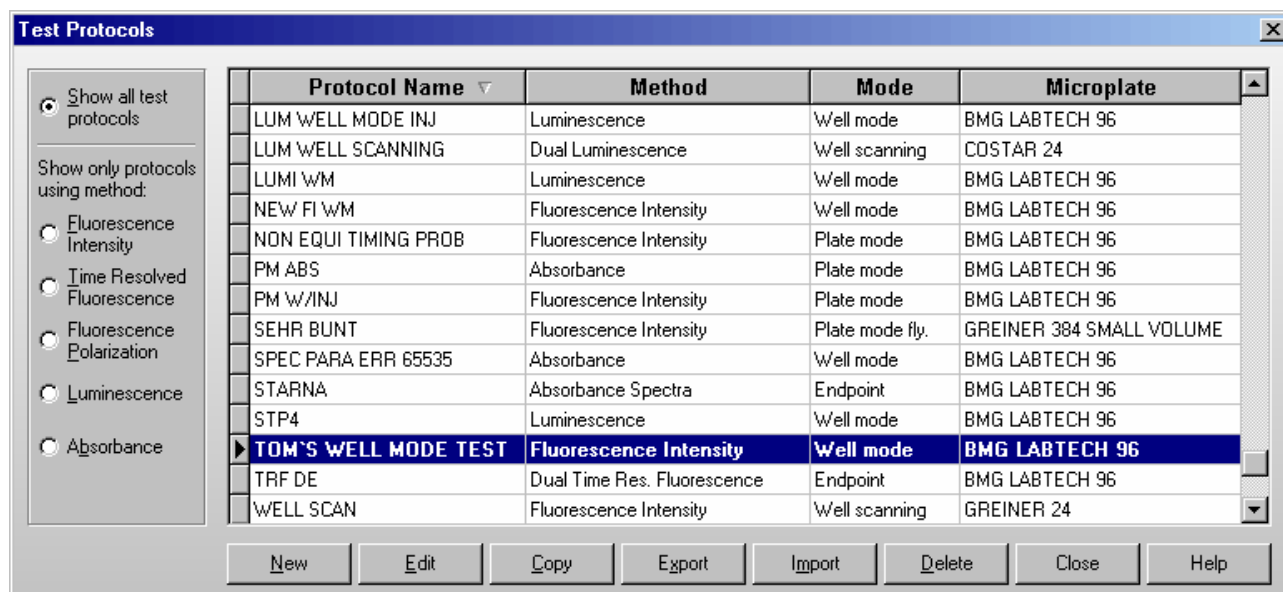
4 Defining Protocols

4.1 Protocol Selection Window

To create or edit a protocol click on  or select 'Test Protocol' from the 'Test Setup' menu.

The FLUOstar OPTIMA can measure fluorescence intensity and time-resolved fluorescence and (optional) luminescence and absorbance. In addition, the POLARstar OPTIMA can read in fluorescence polarization mode. The LUMIstar OPTIMA can only measure in luminescence mode.

The 'Test Protocols' window lists all previously defined tests and gives you options for creating new test protocols or modifying existing protocols. The window will show only protocols using a method which can be performed with the reader currently connected. If no reader is connected, the selection depends on the reader type used last or the reader type manually selected in the 'Reader Type' dialogue (see chapter 3.1.1).



By default the protocols are sorted by method first and then alphabetically by name. If you prefer sorting by name, only click the 'Protocol Name' part of the table headline. To switch back to the original sorting, click 'Method'. It is also possible to get the list sorted by the mode or the microplate used. Simply click on the 'Mode' or 'Microplate' part of the table headline. Besides displaying all available protocols, you might select to see only protocols using a certain measurement method by clicking one of the radio buttons on the left side of this dialogue box.

Note: As the LUMIstar OPTIMA can only measure using the luminescence method, the method selection box will not appear when using this type of reader.

To select the first test protocol, beginning with e.g. 'T', simply press the key [T]. To select more than one protocol (e.g. for export) use [Shift] together with [↑] or [↓] or press [Ctrl] and click on the desired protocol names with the left mouse button.

New

Define a new test protocol. See chapter 4.2.1.

Edit

Modify an existing test protocol. Select the protocol and click on 'Edit' or double click on the test name.

Copy

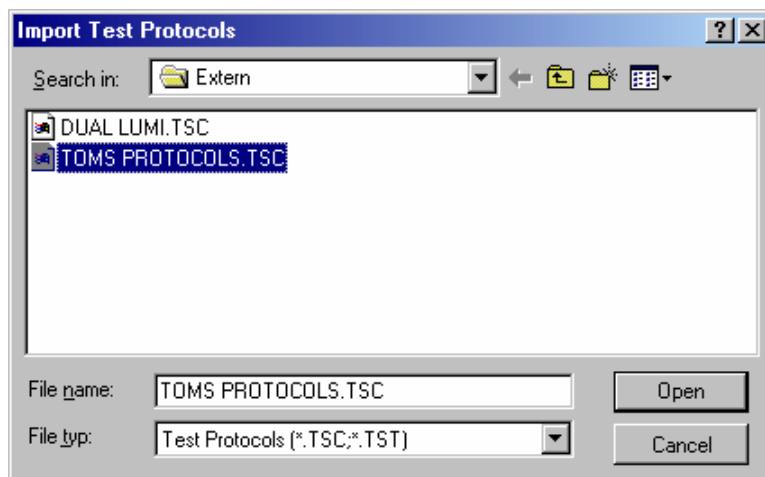
Select the protocol you want to duplicate. A new dialogue appears and asks for a name for the copy. The duplicate will appear on the test protocol list under the new name. Making a duplicate protocol means that you can modify a protocol without changing the original.

Export

Using the export function, you can export test protocols onto a diskette or a hard drive folder of your choice. Select the protocol(s) you want to export then click on 'Export'. A dialogue box will ask for the destination drive and folder as well as a name for the file. The exported file will get the file name extension '.TSC'.

Import

To import a test protocol click on 'Import'. Choose the folder. A list of files with extensions '.TSC' and '.TST' will appear:



Select the desired file and click on 'Open'. The imported test protocols will be added to the list of protocols.

Notes: It is possible to import test protocols from the NOVOstar or from the FLUOstar OPTIMA software.

It is also possible to import test protocols created using FLUOstar (old 403 series), FLUOstar Galaxy and LUMIstar Galaxy. If you import a test protocol from one of these programs, the OPTIMA import function will ask you to import the layout definitions used by these tests immediately after importing the test definitions (In OPTIMA, the layout information is stored with the other test parameters, the above mentioned programs use different data bases for layout and test definitions.).

Delete

Remove a test protocol from the list by selecting the protocol and clicking on 'Delete'.

Close

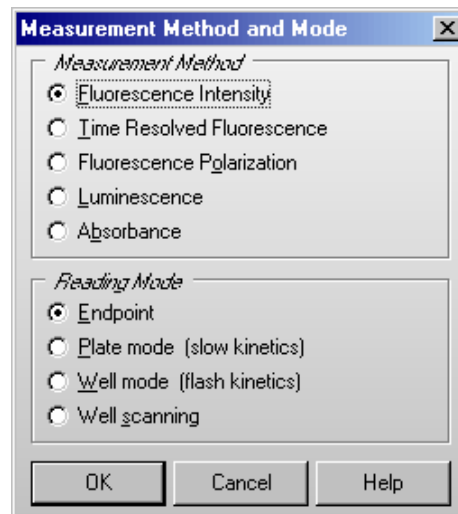
Closes the test protocol selection window.

Notes: Exporting the test protocols table into a XLS (Excel), text or HTML file is possible after right clicking the table.

Users with activated 'Run Only' option (see chapter 1 *Login Screen*) are not allowed to edit, copy or import test protocols.

4.2 Creating a New Test Protocol

When creating a new test protocol a dialog box will ask for the measurement method and which mode you want to use to perform the test run. Select the method and mode that is appropriate for the assay. The following chapters will explain the different reading modes and measurement methods.



Note: Depending on the reader type, not all measurement methods might be possible. As the LUMIstar OPTIMA can only measure using the luminescence method, this dialogue will only provide a selection of the different reading modes when using this type of reader.

After the method and mode is selected, the next window allows you to enter the test protocol parameters.

4.3 Fluorescence Intensity Protocols

4.3.1 Basic Parameters – Endpoint

If you want to measure each well only once (if you do not expect a kinetic reaction) and if you do not want to perform well scanning (see chapter 4.3.4), you should define the test protocol in endpoint mode.

Test Name

Assign a test name, as you would like it to appear on the list of test protocols.

Microplate

Use the pull-down menu to select the microplate used in the assay. All microplates defined under 'Setup | Microplates' will be listed. To select the first microplate, beginning with e.g. 'B', simply press the key [B] after opening the pull-down box (using e.g. [↓]).

General Settings

Positioning delay

You can define a waiting period after a well of the microplate moves to the measurement position and before the measurement begins. The delay time allows the liquid to settle and the surface to become stable so that the measurement is more accurate. For homogeneous fluorescence assays BMG LABTECH recommends a delay time of 0.2 s. For fluorescence measurements of cell assays, a delay time of 0.5 s is adequate. Liquid movements and hence delay time are influenced by viscosity.

Note: If you enter 0 here, the firmware will use 20 ms as smallest possible delay time. See also flying mode.

Flying mode

This is a time optimized parameter for endpoint / plate mode. When this function is selected, the measurement, using 1 or 3 flashes only, will occur at the exact moment that the center of the well is under the measurement head. The plate carrier does not stop as the well passes the measurement position. If you have defined injections, this setting will be ignored.

Measurement start time

Defines the time when the measurement will start, relative to the time when the measurement position is reached plus positioning delay. Defining a measurement start time larger than 0 makes sense when i.e. injection or shaking is performed before the measurement.

Number of flashes per well

You can define up to 200 flashes per well. All the measurement values obtained for all flashes are averaged for one intensity value per well. Therefore, the greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the reading time.

Optic

If your reader is equipped with auto optic switching you can select the **top** or **bottom optic** for fluorescence and luminescence mode protocols. If your reader is not equipped with auto optic switching you can select the top or bottom optic manually (see Instrument Manual for details).

Note: Bottom measurement is not possible in simultaneous dual emission mode.

Filter Settings

Number of multichromatics

There is the possibility to analyze 8 fluorophores per well. Enter the number of fluorophores to be analyzed, then click the arrow button or on the 'Multichromatic' tab to define the filter combination for each fluorophore to be analyzed.

Excitation and Emission filter

If you only want to analyze one fluorophore, you can select the filter combinations directly in this window. When you use the pull-down menu, the list of filters, as defined under 'Setup | Filters' (see chapter 3.1.4 *Filters*), is displayed.

Gain

This value will be used to adjust the sensitivity of the photo multiplier tube (PMT). A higher gain factor will increase the signal. Choose a gain value which will keep the measurement result of higher concentrations within the range of the instrument (i.e. 0 to 65000 relative fluorescence units). You can enter the gain settings here or perform an automatic gain adjustment before the measurement.

Simultaneous dual emission

If your reader has two fluorescence measurement channels (using two PMTs) built in (POLARstar OPTIMA), you can use this option to measure light of two different wave lengths simultaneously. When switching on this option, a second emission filter box and a second gain box will appear.

Filter Settings

No. of multichromatics (1...4): 1 →

Simultaneous dual emission

Excitation filter: 485 / Emission filter (ch. A/B): 520 / Gain (ch. A/B): 646 / 1023

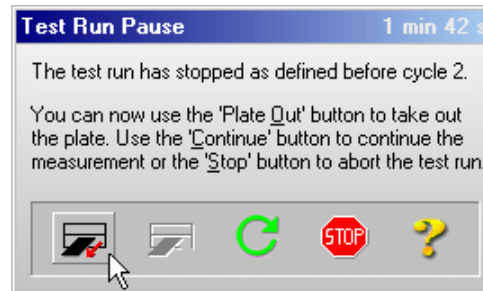
By selecting the emission filter for channel A, the filter for channel B is selected automatically. Due to mechanical constraints of the reader hardware for channel B the filter 180° opposite to the A channel filter will always be used. This means, if you are using the filter installed in filter wheel position 1 for channel A, the filter installed in position 5 will be used for channel B.

Orbital Averaging

If you use microplates with up to 96 wells, instead of measuring one point in the middle of the well you might use the orbital averaging function (see chapter 4.3.5). This is useful if you use large wells and if the probe is not equally distributed, e.g. when using cell assays.

Pause before plate reading

You can define a pause before the plate reading itself starts. This function might be useful to incubate the plate for a certain time. You can define a pause time of up to 3600 s (one hour). If you define 0 s as pause time, the Test Run Pause window will appear, otherwise the measurement will automatically be continued after the defined pause time.



In the caption bar of the window you can see the time elapsed since the beginning of the pause.

The pause window provides the possibility of bringing the plate out (to make a manual injection, to incubate or to change the plate).

Note: The pause function is not available in script mode (see chapter 7).

Comment

Here you can enter a short description of the test protocol (up to 255 characters). This text will also be visible later in the Data Analysis software (see software manual part III).

Check timing

After defining all necessary settings (do not forget the filters), click the button '**Check timing**'. At the bottom of the test protocol definition window, the '**Total measurement time**' for the entire plate calculated by the reader will be displayed. After pressing the 'Check timing' button the 'Timing Overview' sheet will become available (see chapter 4.3.14).

Note: The 'Check timing' function is only available if the reader is switched on.

Limitations for 1536 well plates

Your reader has to be specially prepared for measuring 1536 well plates. Measuring 1536 well plates is only possible in endpoint or plate mode. The following limitations exist for the test protocol:

- only horizontal bidirectional reading, starting top left
- no multichromatics
- no injection
- no shaking

Printing the Protocol

To print the protocol use the key combination [Shift]+[Ctrl]+[P]. A standard print dialog box will appear. Here you can decide, whether you only want to print the current sheet ('Selection'), all sheets ('All') or a selection of sheets ('Pages'). When using portrait format two sheets will be printed on one page, using landscape format only one.

Click on '**OK**' to save the protocol settings and to add the test protocol to the list.

4.3.2 Basic Parameters – Plate Mode

Plate mode can be used for slow kinetics, i.e. the reaction lasts for an extended period of time, even after injection. All wells defined in the layout are read once during a plate cycle; it is possible to read up to 250 cycles. You can split the measurement into up to 4 kinetic windows. You can define the cycle time independently for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve. Injections can be defined for any cycle.

Key words:

Cycles	The number of times the entire plate will be measured. Each well is read only once per cycle if no multichromatics are used, otherwise it will be measured as many times as chromatics are used within each cycle.
Cycle time	The amount of time it takes to measure the plate during one cycle. You can use the Minimum cycle time as calculated by the instrument (fastest possible time) or enter a higher time if you want a delay between the cycles.

Test Name

Assign a test name, as you would like it to appear on the list of test protocols.

Microplate

Use the pull-down menu to select the microplate used in the assay. All microplates defined under 'Setup | Microplates' will be listed. To select the first microplate, beginning with e.g. 'B', simply press the key [B] after opening the pull-down box (using e.g. [↓]).

General Settings

Positioning delay

You can define a waiting period after a well of the microplate moves to the measurement position and before the measurement begins. The delay time allows the liquid to settle and the surface to become stable so that the measurement is more accurate. For homogeneous fluorescence assays BMG LABTECH recommends a delay time of 0.2 s. For fluorescence measurements of cell assays, a delay time of 0.5 s is adequate. Liquid movements and hence delay time are influenced by viscosity.

Note: If you enter 0 here, the firmware will use 20 ms as smallest possible delay time. See also flying mode.

Flying mode

This is a time optimized parameter for endpoint / plate mode. When this function is selected the measurement, using 1 or 3 flashes only, will occur at the exact moment that the center of the well is under the measurement head. The plate carrier does not stop as the well passes the measurement position. If you have defined injections, the injection cycles will be performed in non flying mode.

Number of kinetic windows

You can split the measurement into up to 4 kinetic windows. You can define the number of cycles, the measurement start time, the number of flashes and the cycle time independently for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve. If you choose to use more than one kinetic window, a new sheet 'Kinetic Windows' (see chapter 4.3.6) will become available, where you can define the parameters for all kinetic windows. To switch to this sheet, click the arrow button or on the 'Kinetic Windows' tab. If you use only one kinetic window, you can define all necessary parameters on the 'Basic Parameters' sheet.

Kinetic Window 1

Number of cycles

This is the amount of times the entire plate will be measured for kinetic window 1. Each well defined in the layout will be measured once per cycle if no multichromatics are used, otherwise it will be measured as many times as chromatics are used within each cycle. You can define up to 250 cycles (250 is the total number for all kinetic windows).

Measurement start time

Defines the time when the measurement will start for all cycles belonging to kinetic window 1, relative to the time when the measurement position is reached plus positioning delay. Defining a measurement start time larger than 0 makes sense when i.e. injection or shaking is performed before the measurement.

Number of flashes per well and cycle

You can define up to 200 flashes per well and cycle. All the measurement values obtained for all flashes for a cycle are averaged for one intensity value per well. Therefore, the greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the minimum cycle time and, therefore, the reading time.

Cycle time

You can define the duration of each cycle from 1 to 10000 seconds. When the instrument is switched on, you can click the '**Check timing**' button and the cycle time is automatically validated by the instrument (You will see the minimum cycle time below this group box. If the cycle time you have defined is smaller than this minimum cycle time, it will be automatically corrected to the minimum time.). If you want to increase the cycle time (if you want a delay between cycles), you can manually enter a time greater than the minimum cycle time calculated by the 'Check timing' function.

For example, the instrument gives a time of 25 seconds per cycle, but you can change this to 85 seconds so that there will be a delay of 60 seconds between cycles.

Note: For protocols using only one measurement = one cycle (endpoint tests), this time value does not matter, therefore, this input box will be disabled. For defining endpoint test protocols you can also use the special endpoint mode, see chapter 4.3.1.

Optic

If your reader is equipped with auto optic switching you can select the **top** or **bottom optic** for fluorescence and luminescence mode protocols. If your reader is not equipped with auto optic switching you can select the top or bottom optic manually (see Instrument Manual for details).

Note: Bottom measurement is not possible in simultaneous dual emission mode.

Filter Settings

Number of multichromatics

There is the possibility to analyze 8 fluorophores per well. Enter the number of fluorophores to be analyzed, then click the arrow button or on the 'Multichromatic' tab to define the filter combination for each fluorophore to be analyzed.

Excitation and Emission filter

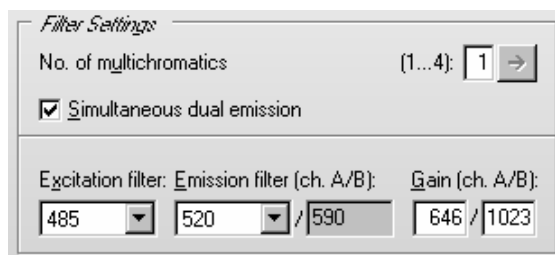
If you only want to analyze one fluorophore, you can select the filter combinations directly in this window. When you use the pull-down menu, the list of filters, as defined under 'Setup | Filters' (see chapter 3.1.4 Filters), is displayed.

Gain

This value will be used to adjust the sensitivity of the photo multiplier tube (PMT). A higher gain factor will increase the signal. Choose a gain value which will keep the measurement result of higher concentrations within the range of the instrument (i.e. 0 to 65000 relative fluorescence units). You can enter the gain settings here or perform an automatic gain adjustment before the measurement.

Simultaneous dual emission

If your reader has two fluorescence measurement channels (using two PMTs) built in (POLARstar OPTIMA), you can use this option to measure light of two different wave lengths simultaneously. When switching on this option, a second emission filter box and a second gain box will appear.



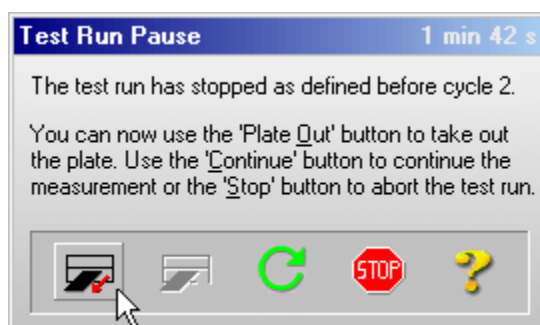
By selecting the emission filter for channel A, the filter for channel B is selected automatically. Due to mechanical constraints of the reader hardware for channel B the filter 180° opposite to the A channel filter will always be used. This means, if you are using the filter installed in filter wheel position 1 for channel A, the filter installed in position 5 will be used for channel B.

Orbital Averaging

If you use microplates with up to 96 wells, instead of measuring one point in the middle of the well you might use the orbital averaging function (see chapter 4.3.5). This is useful if you use large wells and if the probe is not equally distributed, e.g. when using cell assays.


Pause before cycle

You can define a pause for plate mode tests. The default setting is 0, which results in an uninterrupted measurement. If you want a pause, enter the cycle number before which the reader should pause. The reader will pause the measurement before the defined cycle. You can define a pause time of up to 3600 s (one hour). If you have defined 0s as pause time, the Test Run Pause window will appear, otherwise the measurement will automatically be continued after the defined pause time.



In the caption bar of the window you can see the time elapsed since the beginning of the pause.

The pause window provides the possibility of bringing the plate out (to make a manual injection, to incubate or to change the plate) or stopping the test run.

You can also pause after a measurement has started (plate mode only with more than one cycle). Go to 'Measure' and select 'Pause After Current Cycle' or use the  button.

Notes: It is possible to define a pause before the first cycle, e.g. to incubate the plate for a certain time.

The pause function is not available in script mode (see chapter 7).

Comment

Here you can enter a short description of the test protocol (up to 255 characters). This text will also be visible later in the Data Analysis software (see software manual part III).

Check timing

Minimum cycle time 1

The minimum cycle time can only be calculated by the reader. After defining all necessary settings (do not forget the filters), click the button '**Check timing**'. The shortest possible cycle time will be displayed. If you have defined a cycle time, which is shorter than this minimum time, it will automatically be corrected. At the bottom of the test protocol definition window, the calculated '**Total measurement time**' for the entire plate will be displayed.

Note: The 'Check timing' function is only available if the reader is switched on.

After pressing the 'Check timing' button the 'Timing Overview' sheet will become available (see chapter 4.3.14).

Limitations for 1536 well plates

Your reader has to be specially prepared for measuring 1536 well plates. Measuring 1536 well plates is only possible in endpoint or plate mode. The following limitations exist for the test protocol:

- only one kinetic window
- only horizontal bidirectional reading, starting top left
- no multichromatics
- no injection
- no shaking

Printing the Protocol

To print the protocol use the key combination [Shift]+[Ctrl]+[P]. A standard print dialog box will appear. Here you can decide, whether you only want to print the current sheet ('Selection'), all sheets ('All') or a selection of sheets ('Pages'). When using portrait format two sheets will be printed on one page, using landscape format only one.

Click on '**OK**' to save the protocol settings and to add the test protocol to the list.

4.3.3 Basic Parameters – Well Mode

Well mode protocols should be used for fast kinetics, i.e. if the reaction, usually initiated by an injection, occurs over a very finite amount of time. Measurements can then be performed in small intervals when the reaction occurs. In well mode, each well, as defined in the layout, will be measured individually for the defined number of intervals.

For example, a test can be defined with 3 intervals and an injection. Therefore, well A1 will be moved to the measurement position, a measurement is performed, then an injection, followed by two more measurements. When all defined measurement intervals have been performed on well A1 the microplate carrier moves to the next defined well for the same protocol. Once all the defined wells have been measured, the assay is finished.

It is not possible to measure 1536 well plates in well mode. Use plate mode (see chapter 4.3.2) for such plates.

Key words:

Intervals	Number of times the well is measured. Up to 250 intervals per well can be defined.
Interval time	The length of time, in seconds, for each measurement interval. This includes the flashes and the time for measuring the emission light.
Total measurement time per well	The amount time it takes to perform all the measurement intervals, including any injections, on one well. It is calculated by multiplying the number of intervals by the interval time (and taking into account all used kinetic windows and the measurement start times).

Test name

Assign a test name, as you would like it to appear on the list of test protocols.

Microplate

Use the pull-down menu to select the microplate used in the assay. All microplates defined under 'Setup | Microplates' will be listed. To select the first microplate, beginning with e.g. 'B', simply press the key [B] after opening the pull-down box (using e.g. [↓]).

General Settings

Positioning delay

You can define a waiting period after the microplate moves a well to the measurement position and before the measurement begins. The delay time allows the liquid to settle and the surface to become stable so that the measurement is more accurate. For homogeneous fluorescence assays BMG LABTECH recommends a delay time of 0.2 s. For fluorescence measurements of cell assays, a delay time of 0.5 s is adequate. Liquid movements and hence delay time are influenced by viscosity.

Number of kinetic windows

You can split the measurement into up to 4 kinetic windows. You can define the number of intervals, the interval time and the start time independently for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve. If you choose to use more than one kinetic window, a new sheet 'Kinetic Windows' (see chapter 4.3.7) will become available, where you can define the parameters for all kinetic windows. To switch to this sheet, click the arrow button or on the 'Kinetic Windows' tab. If you use only one kinetic window, you can define all necessary parameters on the 'Basic Parameters' sheet.

Kinetic Window 1

Measurement start time

= Start time for kinetic window 1. Defines the time when the measurement will start, relative to the time when the measurement position is reached plus position delay.

Example - measurement start time 5 seconds: After the reader plate carrier reaches the measurement position, the defined positioning delay time starts. After this, there will be an additional delay of 5 seconds before the measurement itself starts.

Using this measurement start time, it is possible to start the measurement after an injection.

Number of intervals

This is the amount of times a measurement will be successively taken on each well (equals the number of readings on each well). You can define up to 250 intervals (250 is the total number for all kinetic windows). This is typically used for kinetic assays, in which the dynamics of a reaction changes very quickly over time. Each interval is plotted as a kinetic point displaying the change over time.

Number of flashes per well and interval

You can define up to 200 flashes per measurement interval. All the measurement values from the flashes defined for one interval are averaged to produce one intensity value per well. The greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the reading time.

Interval time

You can define the duration of each interval from 0.02 to 100 seconds. When the instrument is active, you can click the 'Check timing' button (see below) and the interval time is automatically validated by the instrument. If you want to increase the interval time (if you want a delay between the intervals), you can manually enter a time. If you have defined an interval time in any of the kinetic windows which is shorter than the respective minimum interval time, it will automatically be corrected to the shortest possible value. If necessary, the start times for following kinetic windows will also be corrected.

The minimum interval time depends on the number of flashes (1 flash equals 0.02 seconds, 10 flashes equals 0.2 seconds, etc.) and on the usage of multichromatics.

For example, the instrument gives a minimum interval time of 0.4 seconds, but you can change this to 5 seconds so that there will be a delay of 4.6 seconds between intervals.

Note: For endpoint tests (tests using only one measurement = one interval), this time value does not matter, therefore, this input box will be disabled. In these cases the measurement of the one interval might also last longer than 100 seconds.

End time of kinetic window 1

This value is automatically calculated (Measurement start time plus Number of intervals multiplied by Interval time). The start time for kinetic window 2 needs to be higher than or equal this end time.

Optic

If your reader is equipped with auto optic switching you can select the **top** or **bottom optic** for fluorescence and luminescence mode protocols. If your reader is not equipped with auto optic switching you can select the top or bottom optic manually (see Instrument Manual for details).

Note: Bottom measurement is not possible in simultaneous dual emission mode.

Filter Settings

No. of multichromatics

There is the possibility to analyze up to 8 fluorophores per well. In well mode, all fluorophore settings will be measured in one cycle (one well at a time). Enter the number of fluorophores to be analyzed, then click the arrow button or on the 'Multichromatic' tab to define the filter combination for each fluorophore to be analyzed.

Excitation and Emission filter

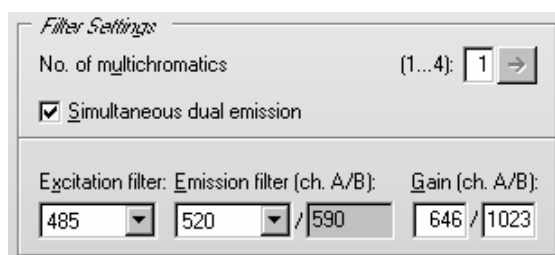
If you only want to analyze one fluorophore, you can select the filter combinations directly in this window. When you use the pull-down menu, the list of filters, as defined under 'Setup | Filters', is displayed.

Gain

This value will be used to adjust the sensitivity of the photo multiplier tube (PMT). A higher gain factor will increase the signal. Choose a gain value which will keep the measurement result of higher concentrations within the range of the instrument (i.e. 0 to 65000 relative fluorescence units). You can enter a gain manually or perform an automatic gain adjustment before the measurement.

Simultaneous dual emission

If your reader has two fluorescence measurement channels (using two PMTs) built in (POLARstar OPTIMA), you can use this option to measure light of two different wave lengths simultaneously. When switching on this option, a second emission filter box and a second gain box will appear.



Filter Settings

No. of multichromatics (1...4): 1 →

Simultaneous dual emission

Excitation filter: Emission filter (ch. A/B): Gain (ch. A/B):

485 520 / 590 646 / 1023

By selecting the emission filter for channel A, the filter for channel B is selected automatically. Due to mechanical constraints of the reader hardware for channel B the filter 180° opposite to the A channel filter will always be used. This means, if you are using the filter installed in filter wheel position 1 for channel A, the filter installed in position 5 will be used for channel B.

Orbital Averaging

If you use microplates with up to 96 wells, instead of measuring one point in the middle of the well you might use the orbital averaging function (see chapter 4.3.5). This is useful if you use large wells and if the probe is not equally distributed, e.g. when using cell assays.

Comment

Here you can enter a short description of the test protocol (up to 255 characters). This text will also be visible later in the Data Analysis software (see software manual part III).

Check timing

Minimum interval time 1

The minimum interval times (this time might be different for different kinetic windows) can only be calculated by the reader. After defining all necessary settings (do not forget the filters), click the button '**Check timing**'. The shortest possible interval time will be displayed. If you have defined an interval time in any of the kinetic windows which is shorter than the respective minimum time, it will automatically be corrected. If necessary, the start times for all following kinetic windows will also be corrected. At the bottom of the test protocol definition window, the calculated '**Total measurement time**' for the entire plate will be displayed.

Note: The 'Check timing' function is only available if the reader is switched on.

Total measurement time per well

This time will be calculated using the settings in the protocol, i.e. the interval time, the number of intervals and the start time for all kinetic windows. If the defined interval time for the last interval (in the last kinetic window), is higher than the minimum interval time, the calculated total measurement time per well will show a value slightly too high, as the reader, when the last interval is finished will not wait for the defined interval time before it goes on to the next well. If you have defined an injection which will last longer than the last measurement (which usually makes no sense), this time will be too short. (The total measurement time calculated by the reader will always be correct.)

After pressing the '**Check timing**' button, the 'Timing Overview' sheet will become available (see chapter 4.3.14).

Printing the Protocol

To print the protocol use the key combination [Shift]+[Ctrl]+[P]. A standard print dialog box will appear. Here you can decide, whether you only want to print the current sheet ('Selection'), all sheets ('All') or a selection of sheets ('Pages'). When using portrait format two sheets will be printed on one page, using landscape format only one.

Click on '**OK**' to save the protocol settings and to add the test protocol to the list.

4.3.4 Basic Parameters – Well Scanning

If you want to measure more than one point of a well, you can use the orbital averaging option (see chapter 4.3.5) or you can define a well scanning protocol. Well scanning means measuring different points of a well in a matrix style. Besides getting an average measurement value of all scan points, using this mode you will also be able to see measurement values from the single scan points in the Current State Display (see chapter 6.7.4 *Display of Well Scanning Data*) and in the Data Analysis software (see software manual part III).

Well scanning can only be used for microplates up to 96 wells.

Most parameters are similar or identical to well mode protocols (see chapter 4.3.3), but it is not possible to measure more than one interval in this mode.

Test name

Assign a test name, as you would like it to appear on the list of test protocols.

Microplate

Use the pull-down menu to select the microplate used in the assay. All microplates defined under 'Setup | Microplates' will be listed. To select the first microplate, beginning with e.g. 'B', simply press the key [B] after opening the pull-down box (using e.g. [↓]).

General Settings

Positioning delay

You can define a waiting period after the microplate moves a well to the measurement position and before the measurement begins. The delay time allows the liquid to settle and the surface to become stable so that the measurement is more accurate. For homogeneous fluorescence assays BMG LABTECH recommends a delay time of 0.2 s. For fluorescence measurements of cell assays, a delay time of 0.5 s is adequate. Liquid movements and hence delay time are influenced by viscosity.

Notes: This positioning delay time does not only occur once per well, it occurs for every scan point and has therefore a dramatic impact on the total measurement time, especially when using a high number of scan points, e.g. 30x30.

If you enter 0 here, the firmware will use 20 ms as smallest possible delay time.

Measurement start time

Defines the time when the measurement will start, relative to the time when the measurement position is reached plus position delay.

Example - measurement start time 5 seconds: After the reader plate carrier reaches the measurement position, the defined positioning delay time starts. After this, there will be an additional delay of 5 seconds before the measurement itself starts.

Using this measurement start time, it is possible to start the measurement after an injection.

Number of flashes per scan point

You can define up to 200 flashes scan point. All the measurement values from the flashes defined for one scan point are averaged to produce one intensity value. The greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the reading time.

Optic

If your reader is equipped with auto optic switching you can select the **top** or **bottom optic** for fluorescence and luminescence mode protocols. If your reader is not equipped with auto optic switching you can select the top or bottom optic manually (see Instrument Manual for details).

Note: Bottom measurement is not possible in simultaneous dual emission mode.

Filter Settings

No. of multichromatics

There is the possibility to analyze up to 8 fluorophores per well. In well mode, all fluorophore settings will be measured in one cycle (one well at a time). Enter the number of fluorophores to be analyzed, then click the arrow button or on the 'Multichromatic' tab to define the filter combination for each fluorophore to be analyzed.

Excitation and Emission filter

If you only want to analyze one fluorophore, you can select the filter combinations directly in this window. When you use the pull-down menu, the list of filters, as defined under 'Setup | Filters', is displayed.

Gain

This value will be used to adjust the sensitivity of the photo multiplier tube (PMT). A higher gain factor will increase the signal. Choose a gain value which will keep the measurement result of higher concentrations within the range of the instrument (i.e. 0 to 65000 relative fluorescence units). You can enter a gain manually or perform an automatic gain adjustment before the measurement.

Simultaneous dual emission

If your reader has two fluorescence measurement channels (using two PMTs) built in (POLARstar OPTIMA), you can use this option to measure light of two different wave lengths simultaneously. When switching on this option, a second emission filter box and a second gain box will appear.

Filter Settings

No. of multichromatics (1...4): 1 →

Simultaneous dual emission

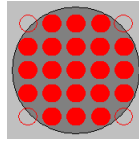
Excitation filter: Emission filter (ch. A/B): Gain (ch. A/B):

485 520 / 590 646 / 1023

By selecting the emission filter for channel A, the filter for channel B is selected automatically. Due to mechanical constraints of the reader hardware for channel B the filter 180° opposite to the A channel filter will always be used. This means, if you are using the filter installed in filter wheel position 1 for channel A, the filter installed in position 5 will be used for channel B.

Well Scanning

Here you can define the **matrix size** for the scanning procedure and therefore the number of measurement (scan) points. When using a plate with round wells (see well shape in chapter 3.2 Microplate Database), only measurement points which are inside the circle with the defined **diameter** will be measured (in the example below 21 out of 25 possible points):



You can define the scan diameter (or the scan width for microplates with square wells) between 1 mm and the diameter / width of the well.

Notes: Well scanning is slower than orbital averaging (see chapter 4.3.5).

The defined number of flashes (or the defined measurement interval time in luminescence mode) and the defined positioning delay time will be used for every single scan point!

Comment

Here you can enter a short description of the test protocol (up to 255 characters). This text will also be visible later in the Data Analysis software (see software manual part III).

Check timing

After defining all necessary settings (do not forget the filters), click the button '**Check timing**'. At the bottom of the test protocol definition window, the calculated '**Total measurement time**' for the entire plate will be displayed.

After pressing the '**Check timing**' button, the 'Timing Overview' sheet will become available (see chapter 4.3.14).

Note: The 'Check timing' function is only available if the reader is switched on.

Printing the Protocol

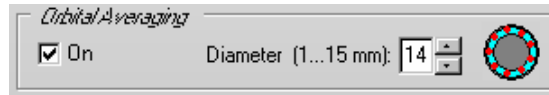
To print the protocol use the key combination [Shift]+[Ctrl]+[P]. A standard print dialog box will appear. Here you can decide, whether you only want to print the current sheet ('Selection'), all sheets ('All') or a selection of sheets ('Pages'). When using portrait format two sheets will be printed on one page, using landscape format only one.

Click on '**OK**' to save the protocol settings and to add the test protocol to the list.

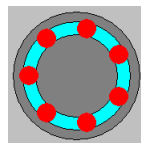
4.3.5 Orbital Averaging

If you use microplates with up to 96 wells, instead of measuring one point in the middle of the well you might use the orbital averaging function. This is useful if you use large wells and if the probe is not equally distributed, e.g. when using cell assays.

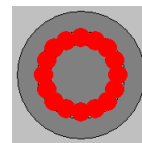
Using this mode, the measurement takes place on an orbit with definable diameter. In **absorbance and fluorescence modes** the defined number of flashes will be equally distributed over the orbit. For luminescence mode, see chapter 4.6.1. For each well there will be one orbital movement per cycle/interval (in multichromatic mode one movement per chromatic per cycle/interval). The maximum number of flashes possible depends on the diameter defined. In orbital averaging mode only the average measurement value of all scan points will be displayed (opposite to well scanning, see chapter 4.3.4).



A little icon inside the orbital averaging box will illustrate how the measurement points are distributed over the well:



7 flashes



16 flashes, smaller diameter

Notes: As it is possible to use different measurement interval times in different kinetic windows (see chapters 4.3.6 and 4.3.7), you will also see these icons in the Kinetic Windows sheet.

The defined positioning delay time occurs only once for each well in orbital averaging mode (opposite to well scanning, see chapter 4.3.4). There will be no positioning delay between the measurements of single scan points.

4.3.6 Kinetic Windows – Plate Mode

It is possible to split the measurement into up to 4 kinetic windows. You can define the cycle time individually for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve.

Fluorescence Intensity - Plate Mode

Basic Parameters | **Kinetic Windows** | Layout | Concentrations / Volumes / Shaking | Timing Overview (One cycle)

Number	No. of cycles (1...250)	Meas. start time (0...1200 s)	No. of flashes (0...200)	Cycle time (1...10000 s)	Minimum cycle time
1	3	0.0	10	42	42 s
2	10	0.0	20	52	52 s
3	2	0.0	20	52	52 s
4	0	0.0	10	1	

Equidistant kinetic cycles (all cycles are as long as necessary for the longest/latest injection or measurement to take place)

Total measurement time: 12m30s

Number of cycles

This is the amount of times the entire plate will be measured. Each well defined in the layout will be measured once per cycle if no multichromatics are used, otherwise it will be measured as many times as chromatics are used within each cycle. You can define up to a total of 250 cycles divided into up to 4 kinetic windows.

Measurement start time

Defines the time when the measurement will start for all cycles belonging to the respective kinetic window, relative to the time when the measurement position is reached plus positioning delay. Defining a measurement start time larger than 0 makes sense when i.e. injection or shaking is performed before the measurement.

Number of flashes (all modes except luminescence)

You can define up to 200 flashes per well and cycle. All the measurement values obtained for all flashes of a cycle sent to one well are averaged for one intensity. Therefore, the greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the minimum cycle time and, therefore, the reading time.

Measurement interval time (only luminescence mode)

Defines the measurement time (defines how long the light is measured). By increasing this time, you will get higher (and usually more accurate) measurement values.

Cycle time

You can define the duration of each cycle from 1 to 10000 seconds (=2hr46min40s). When the instrument is switched on, you can click the '**Check timing**' button and the cycle time is automatically validated by the instrument. If you want to increase the cycle time (if you want a delay between cycles), you can enter a time greater than the minimum cycle time calculated by the 'Check timing' function.

For example, if the instrument gives a minimum cycle time of 25 seconds and you change this to 85 seconds, there will be a delay of 60 seconds between cycles.

Equidistant kinetic cycles

If you use this option, the minimum cycle time for all cycles is as long as the cycle with the latest / longest injection or measurement requires.

If you do not use this option, the time for cycles with injection can be different from cycles without injection and the minimum cycle time for each kinetic window can be different. This allows very short sampling rates in kinetic areas with a fast change in signal.

Note: Even if you choose this option, it is possible to define different kinetic cycle times for different kinetic windows, but the software will ensure that the timing of all wells is equal by adding waiting times after processing each well.

Minimum cycle time

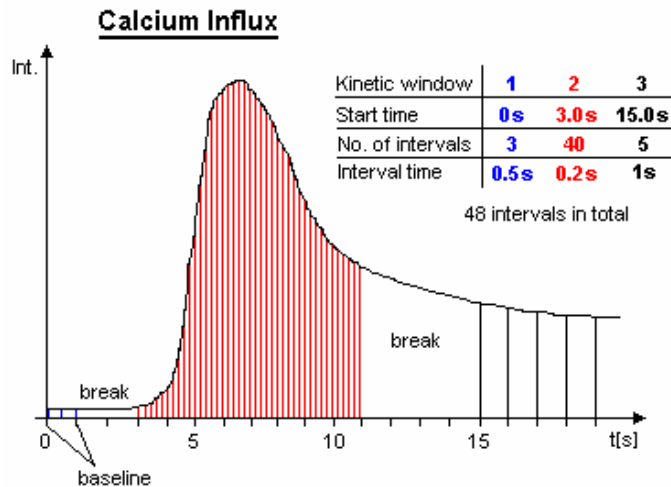
The minimum cycle times can only be calculated by the reader. If you do not use the Equidistant kinetic cycles option, the Minimum cycle time can be different in different kinetic windows (depending on the Measurement start time and the Number of flashes). After defining all necessary settings (do not forget the filters), click the button '**Check timing**'. The shortest possible cycle times will be displayed. If you have defined a cycle time in any of the kinetic windows which is shorter than the corresponding minimum time, it will automatically be corrected. At the bottom of the test protocol definition window, the calculated '**Total measurement time**' for the entire plate will be displayed.

Note: The 'Check timing' function is only available if the reader is switched on.

4.3.7 Kinetic Windows – Well Mode

It is possible to split the measurement into up to 4 kinetic windows. You can define the interval time independently for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve.

Example:



Fluorescence Intensity - Well Mode

Basic Parameters | **Kinetic Windows** | Layout | Concentrations / Volumes / Shaking | Injection Timing | Timing Overview

Number	Start time (0...1200 s)	No. of intervals (0...250)	No. of flashes (0...200)	Interval time (0.02...100 s)	End time (s)	Minimum interval time
1	0.0	3	5	1.00	3	0.06 s
2	3.0	40	10	0.20	11	0.10 s
3	12.0	5	10	2.00	22	0.10 s
4	25.0	0	10	0.10	0	

Total meas. time/well: 22 s

Check timing Total measurement time: 32m50s

OK Cancel Help

Start time

Defines the time when the measurement for the selected kinetic window will start, relative to the time when the measurement position is reached plus position delay. The start time for a kinetic window needs to be higher than the end time of the kinetic window before.

Number of intervals

This is the amount of times a measurement will be successively taken on each well. You can define up to a total of 250 intervals.

Number of flashes (all modes except luminescence)

You can define up to 200 flashes per measurement interval. All measurement values obtained for all flashes defined for one interval are averaged to produce one intensity value per well. The greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the reading time.

Measurement interval time (only luminescence mode)

Defines the measurement time (defines how long the light is measured). By increasing this time, you will get higher (and usually more accurate) measurement values.

Interval time

You can define the duration of each interval from 0.02 to 100.0 seconds. When the instrument is switched on, you can click the **‘Check timing’** button and the interval times are automatically validated by the instrument. If you want to increase an interval time (if you want a delay between the intervals), you can manually enter a time.

For example, the instrument gives a time of 0.4 seconds but you can change this to 5 seconds so that there will be a delay of 4.6 seconds between intervals.

End time

This value is automatically calculated (Start time plus Number of intervals multiplied by Interval time). The start time for the next kinetic window needs to be higher than this end time.


Minimum interval time

The minimum interval times (this time value might be different for different kinetic windows) can only be calculated by the reader. After defining all necessary settings press the button **‘Check timing’**. The shortest possible interval times will be displayed. If you have defined an interval time in any of the kinetic windows which is shorter than the respective minimum time, it will automatically be corrected. If necessary, the start times for all following kinetic windows will also be corrected. At the bottom of the test protocol definition window, the calculated **‘Total measurement time’** for the entire plate will be displayed.

Note: The ‘Check timing’ function is only available if the reader is switched on.

4.3.8 Layout Definition

This sheet contains a grid representing the wells of the microplate that you selected. You can define wells containing samples, blanks, standards and controls.

If you edit a 384 or 1536 well layout, you can use the zoom buttons  to enlarge or reduce the layout display.

Content

To define the content of a well, select the content type first by clicking the appropriate button (e.g. sample, standard, blank, ...) and then double click one well in the layout grid or select a group of wells (see below).

Sample	X	The well's content has unknown concentration.
Blank	B	The well contains water or buffer for measuring background.
Standard	S	The well's content has a known concentration and is used to formulate a standard curve in the data reduction
Negative Control, Positive Control, Control	N P C	The well's content has known concentrations, but will not be used for the standard curve calculation. It can be used for comparisons or for special evaluation calculations.

Index

The **Index** is the reference number of the sample or standard. The index box displays the number that will be used for the next sample or a standard. If **Increase** is selected, each sample or standard will be labeled with consecutive numbers. With **Constant** the number will remain the same, in the case of continuous replicates. In the example picture above, the index box shows that the next standard well number is 6.

Increase	For each well the next consecutive number will be used. The number shown in the index box is the number of the next well.
Constant	The number remains fixed; use this if the samples are identical.

Replicates

Replicates are the number of repeated samples. If you have duplicates of a sample on the microplate then you select '2' and whether they are labeled in the horizontal or vertical direction.


Methods of Labeling (Auto Fill-Out Function)

1. Select the appropriate content type and then double click on each well of that type.
2. If the samples are in successive rows or columns, select increase if the samples should be labeled with consecutive numbers, or choose constant if they are continuous replicates. Click on the first well with the left mouse key and drag across the wells containing the samples, standards, controls or blanks.
3. If a row or column contains the same contents (samples, standards, ...) click the row letter or the column number and all wells of that row / column will be labeled.
4. To fill the entire microplate select the appropriate content (samples, standards, ...) and click on the format number (e.g. '96') in the top left corner.

Notes: You can **export** and **re-import** a complete layout (containing also the concentration and volume values, see chapter 4.3.10) using the context menu of the layout grid (right click on the grid). Exporting only the layout grid into a XLS (Excel), text or HTML file is also possible.

You can **undo** the last 5 layout changes using the context popup menu or using the key combination [Ctrl]+[Z].

Reading direction

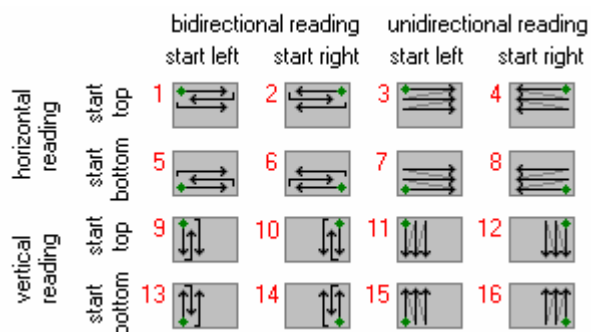
After pressing the reading direction button (), you can choose between **horizontal** or **vertical** reading (horizontal: the plate carrier will move from left to right reading across successive columns, vertical: the plate carrier will move up and down reading in successive rows).

It is also possible to select a **bidirectional** or **unidirectional** reading mode. Example: Using a bidirectional horizontal reading of a full 96 well plate, the reading will continue after reading A1 ... A12 with B12 and then go back via B11, B10, ... to B1. If you choose unidirectional reading, the plate carrier would move to B1 after A12 and continue reading with B2, B3,

It is possible to **start** the reading **from any of the four corners**.

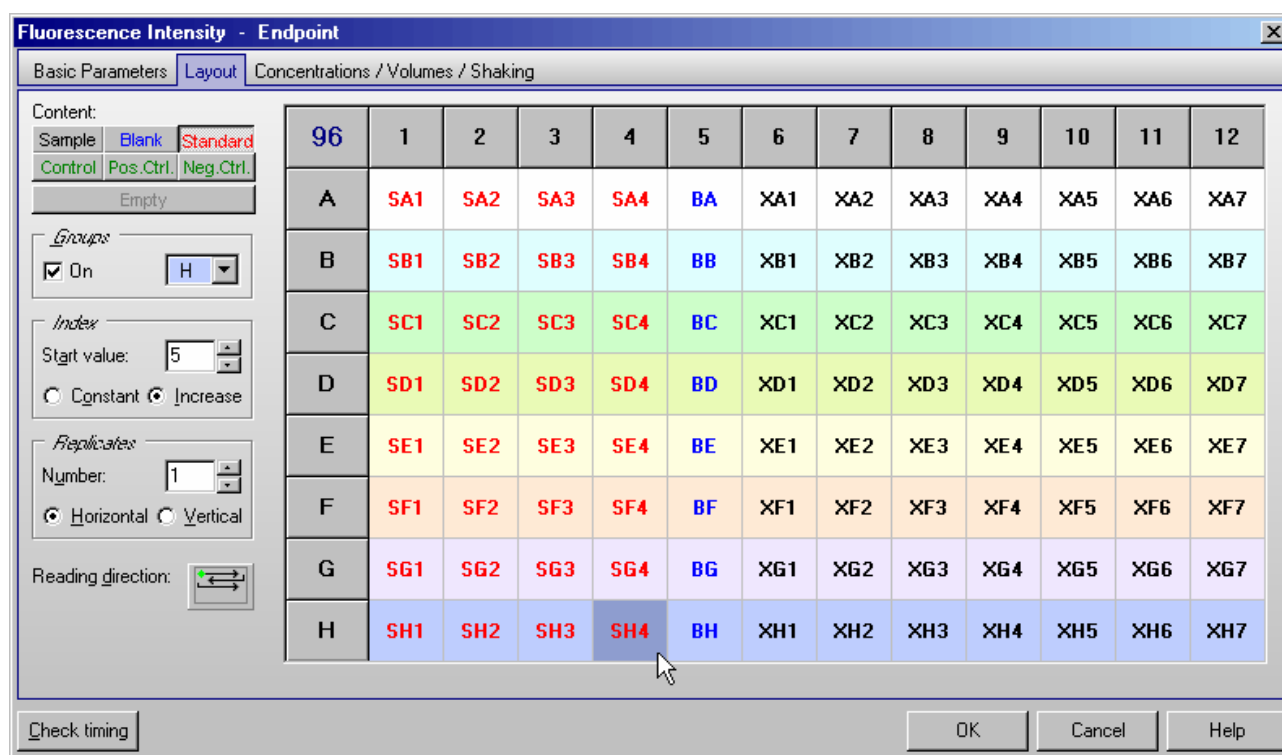
Note: The reading direction has no bearing on the measurements; it is intended to optimize the amount of time it takes to read the plate.

Possible reading modes:



4.3.9 Using Layout Groups

It is possible to use up to 12 independent layout groups (sets of samples, blanks, controls and standards). Each group can be individually blank corrected and it is possible to calculate a separate standard curve for each group (see software manual part III: Data Analysis).



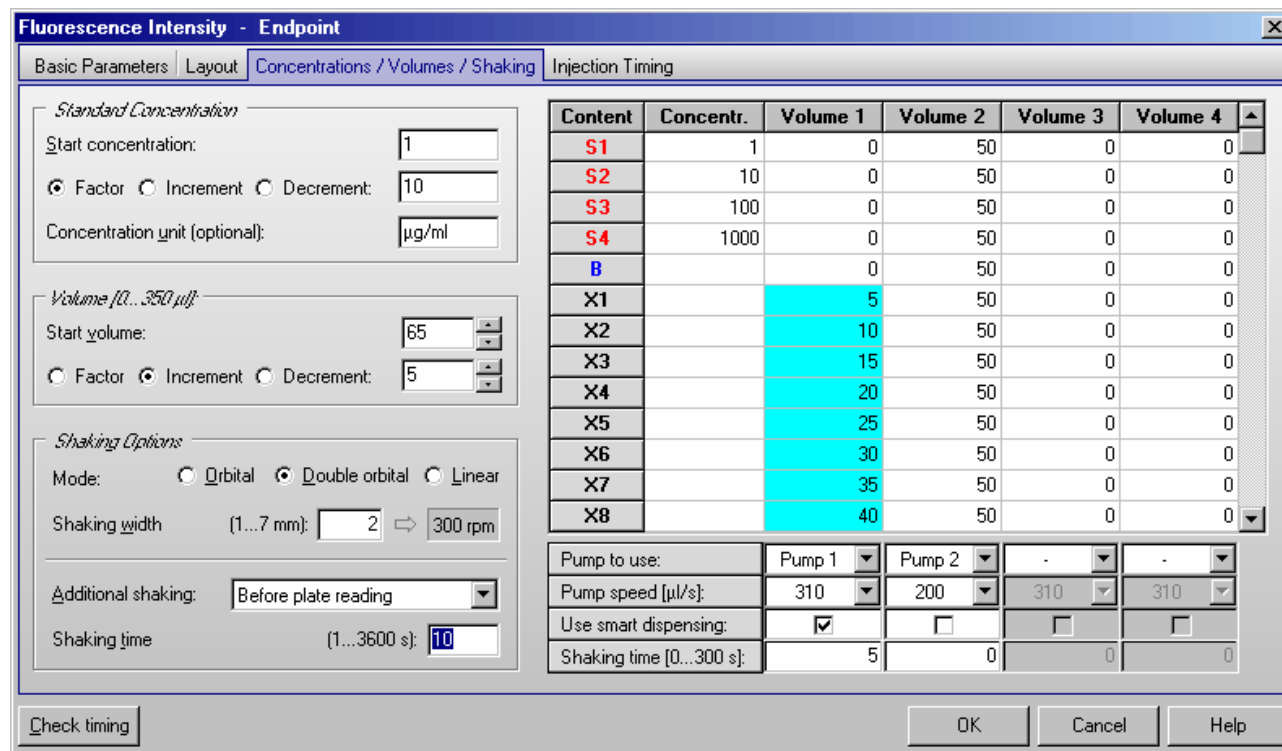
To activate the usage of groups activate the 'On' check box in the groups box. Use the pull down box next to this check box to select one of the 12 possible groups (A...L). Now enter samples, blanks and standards as described above. The group will be shown in the layout grid using different background colors and by inserting the group letter between content type identifier (e.g. B for blank, S for standard and X for sample) and index.

Notes: When switching on the group mode, all existing layout entries will be converted to group A.

When switching off the group mode, all layout entries not belonging to group A will be erased.

4.3.10 Concentrations / Volumes / Shaking

To define the standard concentrations and the injection volumes, go to the **‘Concentrations / Volumes / Shaking’** sheet. In this sheet you will see a table that lists contents and their reference numbers. Concentration refers to the known concentration of the standards. Volume 1 ... 4 refers to the injection volumes of volume groups 1 ... 4, respectively. The concentrations (of standards) and volumes for injection can be entered manually into each space or by using the auto fill-out function.



The **Auto fill-out function** can be used to define the concentrations and volumes without entering them manually. This function works very similar to the layout definition auto fill-out function. The concentrations or volumes will automatically be calculated using the given **Start** value and a number to be multiplied (**Factor**), added (**Increment**) or subtracted (**Decrement**).

Click with the left mouse button on the table headline **‘Concentration’** or **‘Volume 1’** ... **‘Volume 4’**. You can also select a specific set of wells by finding the first well and clicking and scrolling down with the left mouse button or using the cursor keys [↓] and [↑] together with [Shift]. The calculated values will automatically be entered into the table.

Standard Concentration

If you have defined standards in you plate layout it is necessary that you enter the **standard concentrations** here. These concentrations will later be used to calculate a standard curve and to calculate the concentrations of the samples (unknowns) in the Data Analysis software (see software manual part III).

Optionally you can define the **unit** for the concentration values. This unit will then be shown in the Data Analysis software.

Volume

You can use up to 4 injection or volume groups in one test protocol. Please enter the injection volumes you want to use into the table. Besides entering the volumes manually you can also use the auto fill-out function (see above). It is not necessary to use all volume groups consecutive (you can for example use only volume 2 and 4).

You can enter the volumes in steps of 0.5 µl. If you prefer steps of 0.166 µl (1/6 µl = minimum step of the injectors) add the following line to the [Configuration] section of the ‘FLUOstar OPTIMA.ini’ file: MinVolumeStep=6, for minimum steps of 1 µl use MinVolumeStep=1. You will find this configuration file in the OPTIMA main installation directory, usually ‘~\Program Files\BMG\OPTIMA\’.

Notes: You can **export** and **re-import** a complete layout (including the concentration and volume values) using the context menu of the table (right click on the table). Exporting only this table into a XLS (Excel), text or HTML file is also possible.

You can **undo** the last 5 changes of the concentrations and volumes table using the context pop up menu or using the key combination [Ctrl]+[Z], as long as there have been no changes to the layout since then.

Pump to use

Select which pump should be used to inject the defined volume(s). It is possible to use the same pump in different volume groups.

Pump speed

The pump can dispense at different speeds. The injection speed is defined in $\mu\text{l} / \text{second}$. The default speed is $310 \mu\text{l} / \text{second}$ which should be appropriate for most assays. There are pre-defined speeds available by using the pull-down menu.

Factors determining pump speed:

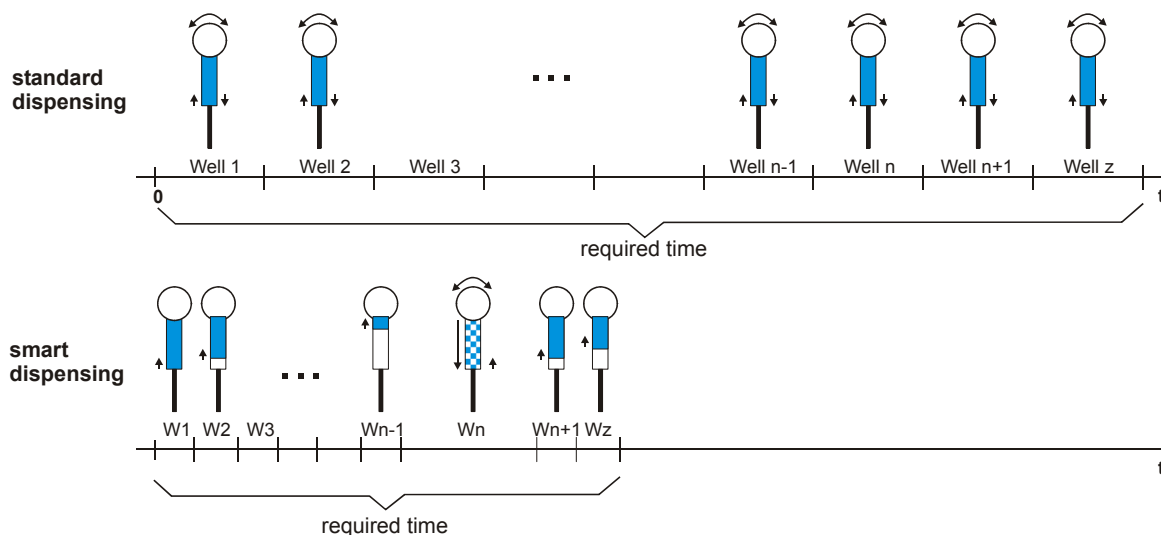
Viscosity: Liquids, such as water and buffer, can be dispensed at higher speeds. For highly viscous solutions you should use a slower speed to ensure higher precision and lower the risk of air bubbles.

Cells: Solutions containing cells should have a lower speed because it causes less stress on the cells.

Volume: Higher speeds are necessary for small volumes (below $3\text{--}5 \mu\text{l}$) to ensure the best performance.

Use smart dispensing

When using this option, the assigned pump will work in smart dispensing mode, which means that at the beginning of the test run the syringe will be filled completely, and then the defined volume will be injected in each well. Therefore, there is no aspiration of liquid for each well. Only when the remaining volume in the syringe is smaller than the required injection volume, another full stroke will be aspirated before the next injection. Dependent upon the size of the volumes, it might happen that an asynchronous pickup of reagent takes place on some wells. The processing of these wells will then take more time than the processing of the other wells (see picture).



The reader will calculate on which wells an asynchronous pickup of liquid is necessary for all volume groups. When equidistant cycles are selected, the reader will calculate the required time for the injections for all wells and will ensure an equidistant timing for all wells by inserting additional waiting time for the wells / cycles where no injection takes place or where the injection is shorter.

The required time to dispense a complete plate will be much less when using the smart dispensing feature since for the majority of the wells usually only an injection without valve movement takes place.

Notes: Due to technical reasons, it is not possible to use the same pump in different volume groups where the smart dispensing feature is activated in one volume group but deactivated in another group. The selected pump will then always operate in smart dispensing mode.

The smart dispensing feature is not available for well mode protocols.

The smart dispensing feature is only available for readers with serial numbers beginning with 413-2400.

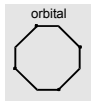
Shaking time

If you want shaking after an injection, add the desired shaking time here. It is possible to define different shaking times for different volume groups. The shaking width and shaking style (orbital or linear) will be defined for all volume groups (and for possible additional shaking) in the 'Shaking Options' group box on the left side of this sheet.

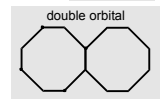
Shaking Options

Shaking mode

Orbital: Shaking mode is circular; mixing is more complete, especially around the edges of the microplate.



Double orbital: The shaking function is performed as orbital movement. The plate carrier makes a figure eight movement.



Linear: Shaking mode is from right to left.

Shaking width

The width (linear) or diameter (orbital) of the shaking motion. The range is 1 mm to 7 mm. The width depends on several conditions:

Plate format: For plates with larger wells, such as 6 or 24 well plates, you can use a larger diameter (slower speed) while you should use a smaller diameter (faster speed) for plates with smaller wells.

Samples: If the wells contain cells, the diameter should be larger so the force of the shaking does not stress the cells. For viscous solutions, you should also use a larger shaking diameter.

Volume: A smaller diameter is suggested for smaller volumes.

Additional shaking

If you want additional shaking (other than shaking after injection), you can choose in plate mode between shaking before or after each cycle or before / after the first cycle. In addition, it is possible to select shaking after inject cycle(s). Opposite to the shaking definable for every volume group (see above), this shaking does not occur immediately after each injection. It will occur after all injections of a cycle have been completed (When using a full 96 well plate this shaking does occur only once and not 96 times.).

When using another reading mode (endpoint, well mode, well scanning), additional shaking is only possible before plate reading.

Shaking time

The duration of time for additional shaking is defined in seconds. The maximum shaking time is 300 seconds (5 minutes).

Shaking after Injection without Injection

If you need shaking at a certain time, but no injection at this time, you can use any of the four volume groups. Please set all injection volumes in the table column belonging to this group to 0, but select a pump (which will not be used). You can then specify the shaking duration at the bottom of the table. The start time of this shaking procedure can be defined under 'Volume group X injection start time' in the 'Injection Timing' sheet (see chapter 4.3.11 / 4.3.12).

Limitations for Injection

The maximum amount per injection is 500 μl (6, 12, 24 and 48 well plates). The maximum amount per well (Volume 1 + Volume 2 + Volume 3 + Volume 4) is 350 μl for 96 well plates and 100 μl for 384 well plates respectively. Injection is not possible in 1536 well plates.

BMG LABTECH does not recommend using volumes below 2.5 μl for a 500 μl syringe. If a 1 ml syringe is installed do not use volumes below 5 μl and for a 2.5 ml syringe use a minimum of 15 μl .

Note: It is necessary to prime all used pumps before performing a test with injections (see chapter 5.1 *Priming*).

4.3.11 Injection Timing – Plate Mode

Fluorescence Intensity - Plate Mode

Basic Parameters | Layout | Concentrations / Volumes / Shaking | **Injection Timing** | Timing Overview (One cycle)

Injection Cycles

Volume group 1 injection cycle [Slider] (1...20):

Volume group 2 injection cycle [Slider] (1...20):

Volume group 3 injection cycle [Slider] (1...20):

Volume group 4 injection cycle [Slider] (1...20):

Equidistant kinetic cycles (all cycles are as long as necessary for the longest/latest injection or measurement to take place)

Start Times

Volume group 1 injection start time (0...1200.0 s):

Volume group 2 injection start time (0...1200.0 s):

Volume group 3 injection start time (0...1200.0 s):

Volume group 4 injection start time (0...1200.0 s):

Timing Example for one Cycle:

Meas. start time = 0.0 s
 Vol.gr.1 inj. time = 2.0 s
 Vol.gr.2 inj. time = 7.0 s

Posit. delay | Start inj. 1 | Start measurement | Start injection 2

Total measurement time:

Note: Only the input elements for volume groups, where a pump is defined in the corresponding layout definition, are available (see chapter 4.3.10).

Injection Cycles

Volume group 1 injection cycle

The cycle in which the injection of volume group 1 will be performed. The defined cycle number cannot be greater than the number of cycles defined.

Volume group 2 injection cycle ... Volume group 4 injection cycle

Same for volume group 2 ... 4.

Equidistant kinetic cycles

If you use this option, the cycle time for all cycles is as long as the cycle with the latest / longest injection or measurement requires – otherwise the time for all cycles without injection can be smaller.

Start Times

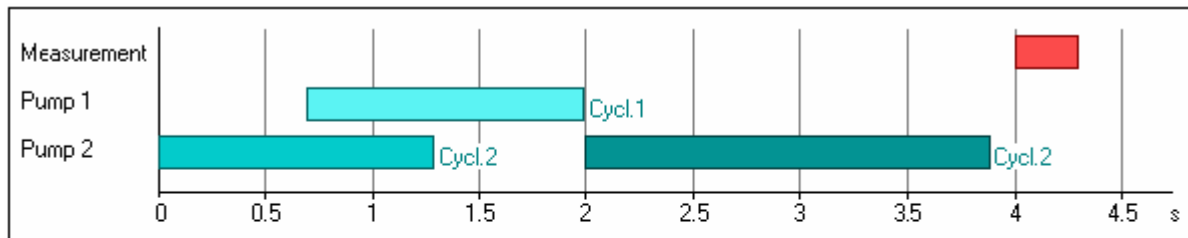
Using the Start Times box, it is also possible to meet special requirements regarding timing on one well in a slow kinetic. The start of the injection(s) or of the measurement (in Basic Parameters or Kinetic Windows sheet) can be delayed up to 1200 seconds on each well after the time when the well reaches the measurement position and the positioning delay is over.

Volume group X injection start time

Defines the time when the injection of volume group 1, 2, 3 or 4 is initiated, relative to the time when the measurement position is reached, plus positioning delay.

Example: The following scenario would be possible with this method:

- Inject the volumes defined in volume group 1 in the first cycle at 0.7 s
- An incubation time of 10 minutes could be realized by setting the cycle time of the first cycle (kinetic window 1) for 600 s.
- Inject the volumes from group 2 in the second cycle (10 minutes later) at 0 s and the volumes from group 3 in the same cycle at 2 s (which then will take place just after the volume group 2 injection).
- The measurement could be started at 4 s. This will then be directly after injection 3 (the measurement will start in all kinetic cycles at the same time).



Legend

- Measurement
- Volume group 1
- Volume group 2
- Volume group 3

4.3.12 Injection Timing – Endpoint, Well Mode and Well Scanning Test Protocols

Click on the 'Injection Timing' tab in order to define the injection times:

The screenshot shows the 'Fluorescence Intensity - Well Mode' window with the 'Injection Timing' tab selected. The 'Injection' section contains the following settings:

Volume group 1 injection start time	(0...1200.0 s):	7.0
Volume group 2 injection start time	(0...1200.0 s):	2.0
Volume group 3 injection start time	(0...1200.0 s):	0.0
Volume group 4 injection start time	(0...1200.0 s):	0.0

The 'Time-optimized measurement' checkbox is unchecked. The 'Timing Example' graph shows a fluorescence intensity curve with markers for 'Start measurement 1' at 0s, 'Start inj. 2' at 2s, and 'Start injection 1' at 7s. The legend for the graph indicates: Meas. start time 1 = 0.0 s, Vol. group 1 inj. time = 7.0 s, and Vol. group 2 inj. time = 2.0 s.

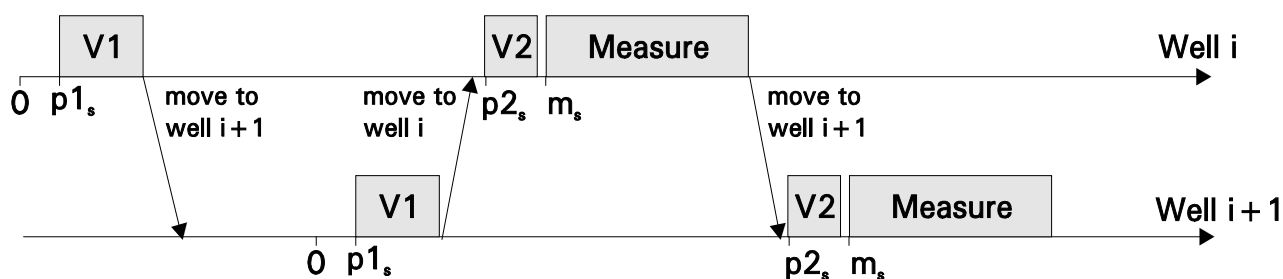
At the bottom, the 'Check timing' button is active, and the 'Total measurement time' is displayed as 32m50s. The 'OK', 'Cancel', and 'Help' buttons are also visible.

Volume group X injection start time

Defines the time the injection for volume group 1, 2, 3 or 4 is initiated, relative to the time when the measurement position is reached, plus positioning delay. The time can be before the beginning of the measurement (Measurement start time > Volume group X injection time), it can be during or after the measurement. You can only define the injection time for those volume groups, where volumes are defined and a pump is selected in the 'Concentration / Volumes / Shaking' sheet.

Time-optimized measurement

This check box does not exist for endpoint protocols. If a kinetic test run with time optimization is defined, then two consecutive wells are processed at the same time. This mode is especially useful when there is a long incubation time between e.g. an injection and the measurement. An injection into a well is, in this case, immediately followed by an injection into the next well. Then the first well is measured followed by the second well. By injecting into the next well in the sequence, the incubation time of that well is underway during the measurement of the first well. The ability to use Time-optimized measuring is contingent on the injection and measurement start times, as well as on the duration of each action. This mode is only possible when there is a time lag between different actions; this delay time must be longer than the time necessary to perform all actions.



Example for well mode test with time optimization

V1	Injection of volume 1
V2	Injection of volume 2
$p1_s$	Volume group 1 injection start time
$p2_s$	Volume group 2 injection start time
m_s	Measurement start time

4.3.13 Multichromatics

When you choose to analyze more than one fluorophore per well (well mode or plate mode), you must define the filter combinations for each fluorophore. First enter the number of fluorophores to be analyzed ('Basic Parameters' sheet) and then click the arrow key or the 'Multichromatic' tab. A new sheet will appear:

Number	Excitation filter	Emission filter	Gain (0...4095)
1	355	460	1042
2	485	520	606
3	544	612	1989
4			0
5			0
6			0
7			0
8			0

Check timing Total measurement time: 12m30s OK Cancel Help

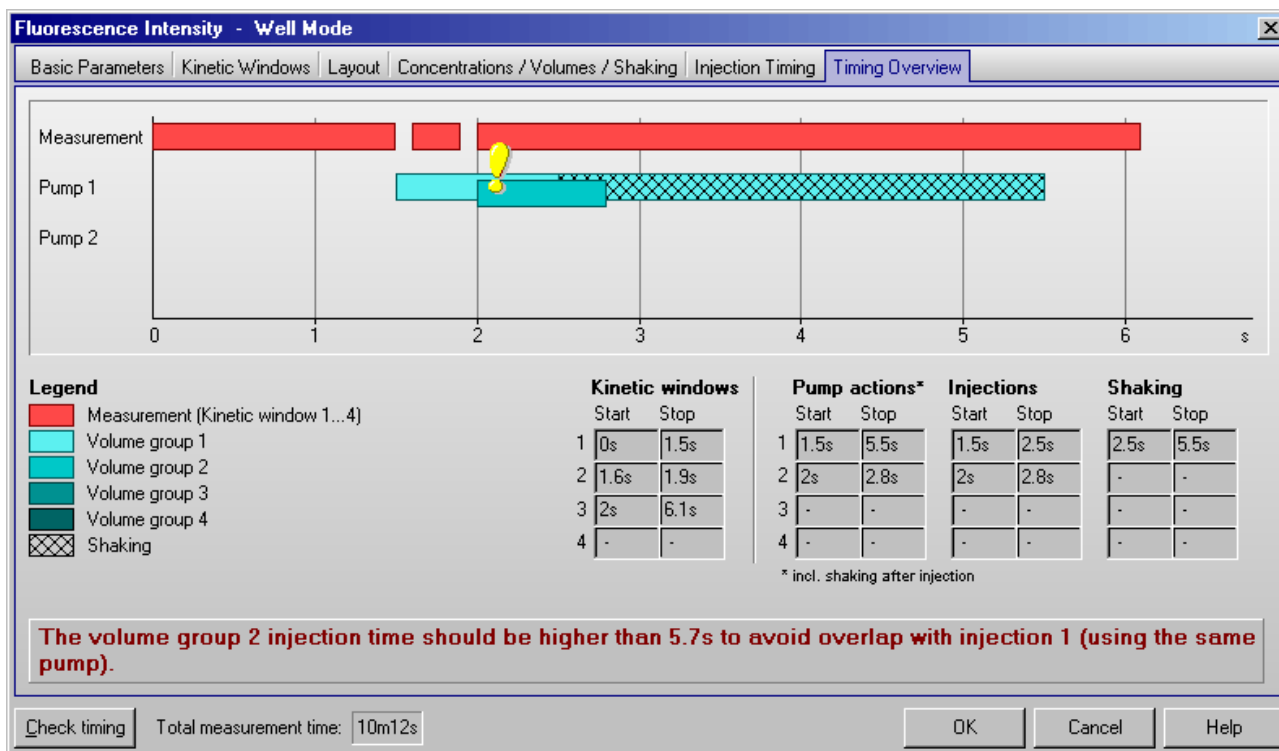
Now you can use the pull-down menu in each row to define the filters. The filters shown in the pull-down menu are listed as they are defined in the 'Setup | Filter' section (see chapter 3.1.4 *Filters*).

It is possible to define the gain for each filter combination. The gain is the amplification of the signal in the PMT. This function allows you to optimize the sensitivity for each assay. Enter the gain manually (from 0 to 4095) or perform an automatic gain adjustment for each filter combination before the measurement (see chapter 6.4 *Gain Adjustment*).

Note: For luminescence method protocols the excitation filter column does not exist, for absorbance method protocols the gain column do not exist when using a firmware version ≥ 1.20 (auto gain).

4.3.14 Timing Overview

After clicking the 'Check timing' button (lower left corner of the test protocol definition window), the following sheet will become available:



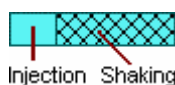
In this sheet, you can see a graphic overview of the measurement and all injections for one well. (For plate mode tests, all actions are displayed in the timing overview of one cycle. The displayed injections may occur in different cycles, as defined under 'Injection / Timing'. The cycle used will be listed behind each injection bar.)

If there is a timing problem, e.g. overlapping injections using the same pump this will be marked by an exclamation mark and a description will inform you about the origin of the timing problem.

In addition, you will see tables containing the timing for measurements and the injection times. If you use the 'Shaking after injection' option (definable in the 'Concentrations / Volumes / Shaking' sheet), you will see these times as well.

Note: The pump action times listed include time for shaking.

An injection action can contain different steps:



Injections are not possible during shaking.

4.4 Time-Resolved Fluorescence Protocols

Time-resolved fluorescence can be measured in endpoint, plate or well mode or in well scanning mode. Most parameters are similar to fluorescence intensity protocols (see chapters 4.3). Multichromatic measurements are possible. If the reader is equipped with two fluorescence measurement channels simultaneous dual emission measurements are also possible.

In the 'Filters and Integration' box you can define the optimal integration time for a time-resolved assay.

Integration Start and Time

For integration start, enter the time in which the measurement should begin after the flash. This time should provide the maximum signal from the fluorophore after the flash has dissipated. The integration time is the length of the measurement (i.e. how long the PMT reads the emission light). To find the optimal times for the fluorophore, see chapter 4.4.1.

If you have one time-resolved fluorophore you can define the same filter setting for up to 8 (multichromatic) tests and try different integration times (see chapter 4.4.1). You can then compare the measurement data to find the optimal time for sensitivity.

4.4.1 Multichromatics for Time-Resolved Fluorescence Protocols

Using the Multichromatics function, you can determine the optimal integration time for a time-resolved assay. Using one time-resolved fluorophore, you can define the same filter combinations for all 8-filter settings and define 8 different integration times. You can then compare the data in the data analysis part to find the optimal time for the fluorophore.

For example, if you were using Eu^{3+} , you would define all 8-filter settings equal. You can keep the integration time constant and vary the integration start time in order to find the optimal start time. You can then keep the integration start time constant and vary the integration time to find the optimal measurement time.

Time Resolved Fluorescence - Endpoint

Basic Parameters | Layout | Concentrations / Volumes / Shaking | **Multichromatic** | Timing Overview

Number	Excitation filter	Emission filter	Gain (0...4095)	Integr. start (12...1510 μs)	Integr. time (10...1510 μs)
1	Ex	Em	700	400	100
2	Ex	Em	700	400	200
3	Ex	Em	700	400	300
4	Ex	Em	700	400	400
5	Ex	Em	700	400	500
6	Ex	Em	700	400	600
7	Ex	Em	700	400	700
8	Ex	Em	700	400	800

Check timing Total measurement time: 42 s OK Cancel Help

4.5 Fluorescence Polarization Protocols

In fluorescence polarization mode, 2 PMTs (photo multiplier tubes) are used for measuring two different channels. Channel A measures vertically polarized light and channel B measures horizontally polarized light.

Fluorescence polarization can be measured in endpoint, plate or well mode. Most parameters are similar to fluorescence intensity protocols (see chapter 4.3). Multichromatic measurements and injections are not possible.

Positioning delay

The positioning delay in fluorescence polarization tests is recommended to be 1 second, so that the surface of the liquid is stable before measurement.

No. of flashes

BMG LABTECH recommends 50 for the number of flashes for the most accurate results. All flashes are averaged together for one intensity value.

Filters

For fluorescence polarization measurement, two identical emission filters should be installed in the filter wheel positioned 180° from each other. When you select one filter, the other filter is automatically selected.

Gain

You can enter the gain value for each channel here. The gain will differ slightly for each channel. It is recommended to perform an automatic gain adjustment right before test start (see chapter 6.4.2 *Gain Adjustment – Fluorescence Polarization*).

4.6 Luminescence Protocols

Luminescence can be measured in all reading modes (endpoint, plate or well mode or well scanning mode). Most parameters are similar to fluorescence intensity protocols (see chapter 4.3). Multichromatic measurements are possible. If the reader is equipped with two luminescence measurement channels simultaneous dual emission measurements are also possible.

Positioning delay

At least 0.2 seconds are recommended for luminescence measurements.

Measurement interval time

Defines the measurement time (defines how long the light is measured). By increasing this time, you will get higher (and usually more accurate) measurement values.

Interval time (only in well mode)

You can define the duration of each interval from 20 ms to 100.0 seconds. When the reader is switched on, you can click the 'Check timing' button and the interval time is automatically validated by the reader. If you do not use multichromatics, the minimum interval time is equal to the defined measurement interval time. If you use multichromatics, the minimum interval time is equal to the defined measurement interval time multiplied by the number of multichromatics plus the time necessary to switch the filters.

If you want to increase the interval time (if you want a delay between the intervals), you can enter a time manually. If you have defined an interval time in any of the kinetic windows which is shorter than the respective minimum interval time, it will automatically be corrected to the shortest possible value. If necessary, the start times for following kinetic windows will also be corrected.

For example, the instrument gives a minimum interval time of 0.1 seconds, but you can change this to 0.2 seconds so that there will be a delay of 0.1 seconds between intervals.

Note: For endpoint tests (tests using only one measurement = one interval), the time value defined here does not matter.

Optic

If your reader is equipped with auto optic switching you can select for luminescence (and fluorescence) mode protocols the top or bottom optic. If your reader is not equipped with auto optic switching you can select the top or bottom optic manually (see Instrument Manual for details).

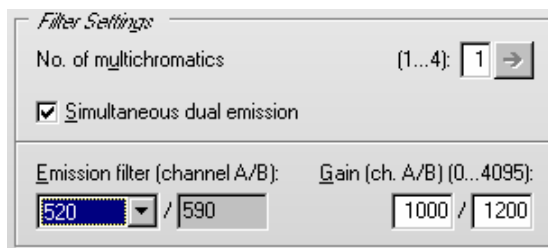
Note: Bottom measurement is not possible in simultaneous dual emission mode.

Emission filter

It is possible to choose an emission filter or use an 'empty' position to measure without filter. If a lens is installed in the emission filter wheel use this.

Simultaneous dual emission

If your reader has two luminescence measurement channels (using two PMTs) built in, you can use this option to measure light of two different wave lengths simultaneously. When switching on this option, a second filter box and a second gain box will appear.



By selecting the filter for channel A, the filter for channel B is selected automatically. Due to mechanical constraints of the reader hardware for channel B the filter 180° opposite to the A channel filter will always be used. This means, if you are using the filter installed in filter wheel position 1 for channel A, the filter installed in position 5 will be used for channel B.

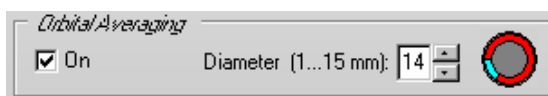
Time to normalize the results

As the measurement values depend on the measurement interval time, it is usually a good idea to normalize the measurement values. This will allow you to compare the measurement values of test runs using different measurement interval times. This will also allow you to compare the results of different kinetic windows using different measurement times. To switch off the normalization enter 0 here.

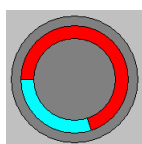
Example: When you set the *Time to normalize the results* to 1.0s (to get the results in counts per second) and use a *Measurement interval time* of 0.1s the raw measurement values will be multiplied by 10.

4.6.1 Orbital Averaging for Luminescence Protocols

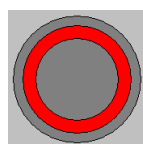
Using this mode, the measurement takes place on an orbit with definable diameter. In **luminescence mode** the measurement is done by continuously measuring the emitted light during the defined measurement interval time (see chapter 4.6). For each well there will be one orbital movement per cycle/interval (in multichromatic mode one movement per chromatic per cycle/interval). In orbital averaging mode only the average measurement value of all scan points will be displayed (opposite to well scanning, see chapter 4.3.4).



As the time necessary for one orbital movement only depends on the selected diameter, it might happen, that the measurement occurs only during a part of the orbital movement. The little icon in the orbital averaging box will show you which part of the orbit is used for the measurement.



70 % coverage



100 % coverage

It is recommended to define the measurement interval as long as the orbital movement lasts (this time is shown as maximum possible time besides the measurement interval time input box). There will be a warning message if the defined measurement time covers less than 50% of the orbital movement.

Notes: As it is possible to use different measurement interval times in different kinetic windows (see chapters 4.3.6 and 4.3.7), you will also see these icons in the Kinetic Windows sheet.

The defined positioning delay time occurs only once for each well in orbital averaging mode (opposite to well scanning, see chapter 4.3.4).

4.7 Absorbance Protocols

Absorbance can be measured in all reading modes. Most parameters are similar to fluorescence intensity protocols (see chapter 5.3). Simultaneous dual emission measurements are not possible.

Positioning delay

The positioning delay should be defined as at least 0.5 seconds.

No. of flashes

In absorbance mode the number of flashes should be defined as at least 20 to minimize the deviation from flash to flash.

Multichromatics

Define up to 8 filter settings for multiple absorbance analysis. Select 280 and 260 excitation (absorbance) filters for DNA absorbance.

Filter settings

Important parameter for absorbance mode is the filter setting. The absorbance filter should be on the excitation side. The emission side should have a position defined as 'empty'.


Path Length Correction

If you use this function, the measurement values will be normalized to show the absorption for 1 cm path length. Please enter the liquid volume per well. Based on the microplate well dimensions (see chapter 3.2) the current path length will be calculated. Based on this length the correction will be performed. Please make sure, that all wells contain the same amount of liquid when using this function.

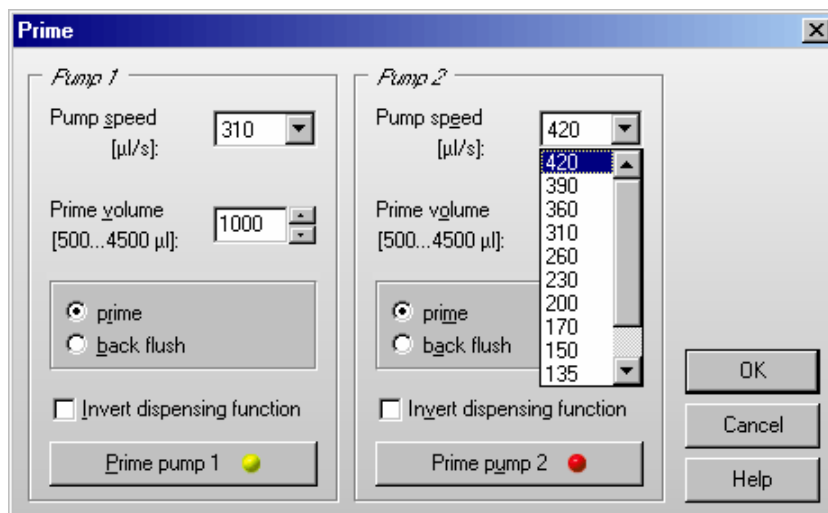
5 Priming / Incubation

5.1 Priming

The reader may have up to 2 pumps. Before starting a test run it is necessary that all used injector pumps are initialized. This can be achieved by using the prime function. The priming procedure needs to be repeated once after each program start. Solutions for injections can be placed inside the reagent box.

To open the prime window, use the  button or choose the menu command '**Measure | Prime**'.

Note: Before priming a pump, the injection needle must be removed from the measurement head to prevent contamination of the instrument.



Pump speed

The pump speed selected here will only be used for priming. The injection speed for test protocols is defined in the 'Concentrations / Volumes / Shaking' sheet of the test protocol definition window (see chapter 4.3.10).

Priming

It is necessary to fill the tubing with the dispensing solution prior to starting a measurement. Place the tubing in the solution to be dispensed and place the injection needle in a waste container. Do not leave the needle in the measurement head or you will risk contamination of the instrument. The volume of the syringe can be 500 µl, 1 ml or 2.5 ml. A prime volume of at least twice the syringe volume is recommended for priming the tubings and the syringe. A higher volume can be used for washing the tubing after the measurements are complete. The prime volume can be selected in steps of the syringe size, e.g. in steps of 500 µl if the pump is equipped with a 500 µl syringe.

After clicking on a 'Prime pump' button, a message will appear to remind you to remove the injection needle from the measurement head. Repeat the procedure for all pumps you want to use.

Back flush

If the pumps have been in use, it is important to flush out any solutions that could be considered a contamination reagent (non contaminating reagent could be i.e. distilled water or a water / alcohol solution). The back flush feature also allows you to conserve expensive reagents, since the entire syringe can be emptied after use.

Invert dispensing function


The default position of the plunger is at the bottom of the syringe barrel. In this position, the injection starts with the liquid in the syringe barrel being pumped through the tubing and then the syringe is refilled as the plunger comes back down.

It is possible to change the order of the plunger's movements by clicking on '**Invert dispensing function**'. The plunger will begin at the top of the syringe barrel and the syringe barrel will, in this case, first fill and then dispense through the tubing.

The invert function is helpful in cases where the solutions have particles or cells that may settle at the bottom of the syringe barrel, and, therefore, will not be dispensed uniformly.

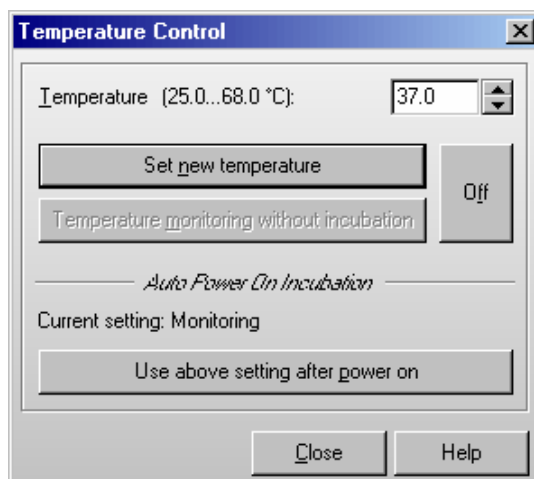
5.2 Incubation

5.2.1 Temperature Control

The incubation can be activated through the temperature button  or by selecting 'Temperature' under the 'Measure' menu.

The temperature range of the incubator is 25 °C to 45 °C (optional up to 60 °C). The chosen temperature must be higher than the ambient temperature. The temperature can be set in 0.1 °C increments. The temperature can also be monitored without activating the incubator (see chapter 5.2.2 *Temperature Monitoring Feature* below).

You can enter the temperature manually or toggle to the desired temperature using the arrow buttons.



Click on 'Incubator on'. The temperature indicator in the toolbar of the control software will be activated. The indicator will be red until the selected temperature is reached, then the indicator turns green.

It is possible to perform a measurement before the target temperature is reached. In the Excel data reduction, the current temperature for each kinetic point during the measurement is displayed in the raw data worksheet.

The 'Incubator on' button changes to 'Set new temperature' if you select a new target temperature during incubation.

5.2.2 Temperature Monitoring Feature

It is possible to monitor the instrument's temperature without using the incubator. The temperature sensor of the incubator will be activated and update the temperature display.

Click the 'Temperature monitoring without incubation' button. The temperature will appear on the temperature display in the control software. When using this function, the indicator color will be cyan. The temperature will also be stored with the measurement data and displayed in the Raw Data Worksheet Data Analysis software (see software manual part III).

5.2.3 Auto Power On Incubation

When you click the 'Use above setting after auto power on', the currently selected temperature value will be used as the default target temperature for the incubator.

If you have defined a value within the allowed temperature range, the built-in incubator will be automatically switched on the next time the reader is switched on. Using this function, the incubator will be turned on even without starting the OPTIMA software.

By using a target temperature of 00.1 °C (selected e.g. by clicking the 'Temperature monitoring without incubation' button), only the temperature monitoring function is switched on by default, the heating plates will not be used.

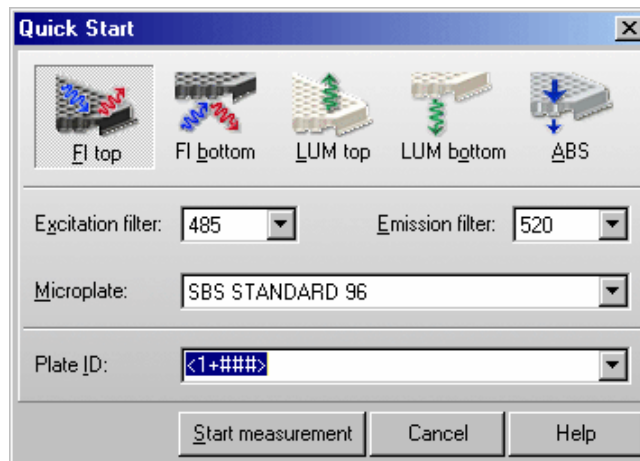
To switch off the auto power on incubation function, select a target temperature of 0 °C (either by clicking the 'Off' button or by manually entering this value), and click the 'Use above setting after auto power on' button.

6 Performing a Measurement

6.1 Quick Start

The Quick Start function can be used to measure a plate without defining a test protocol using the fluorescence intensity, luminescence or absorbance method. Using this function the full plate will always be measured as an endpoint test. Please use the standard measurement method based on pre-defined protocols (see chapter 4) if you want to measure only a part of the microplate, if you want to perform a kinetic measurement, if you want to use dual emission / multichromatics, if you need injections or shaking ... or if you want to measure using fluorescence polarization method. The measurement of 1536 well plates is also not possible using the quick start function.

After using the menu command **Measure | Quick Start** or after clicking the  button the following dialogue will appear:



Please select the **measurement method** first by clicking the appropriate button.

Note: If your reader is not equipped with auto optic switching, the method selection is done using the 'Reader Configuration' dialogue (see chapter 3.1.2 *Reader Configuration*).

The **filter** and **microplate** selection is specific for the method and top/bottom selection done on top of the dialogue.

You have the option to add one **plate identifier**. Here you can use the same special functions as when executing a pre-defined protocol (see chapter 6.3 *Plate Identification*).

After clicking '**Start measurement**' an automatic gain adjustment will be performed in fluorescence intensity and absorbance modes. In luminescence mode, a fixed gain of 3400 will be used.

As soon as the measurement itself has been started, you can open the Current State display (see chapter 6.7) to have a look onto the measurement values. You can also setup the program to automatically open the Current State display (see chapter 3.3.2 *Additional Options Sheet*).

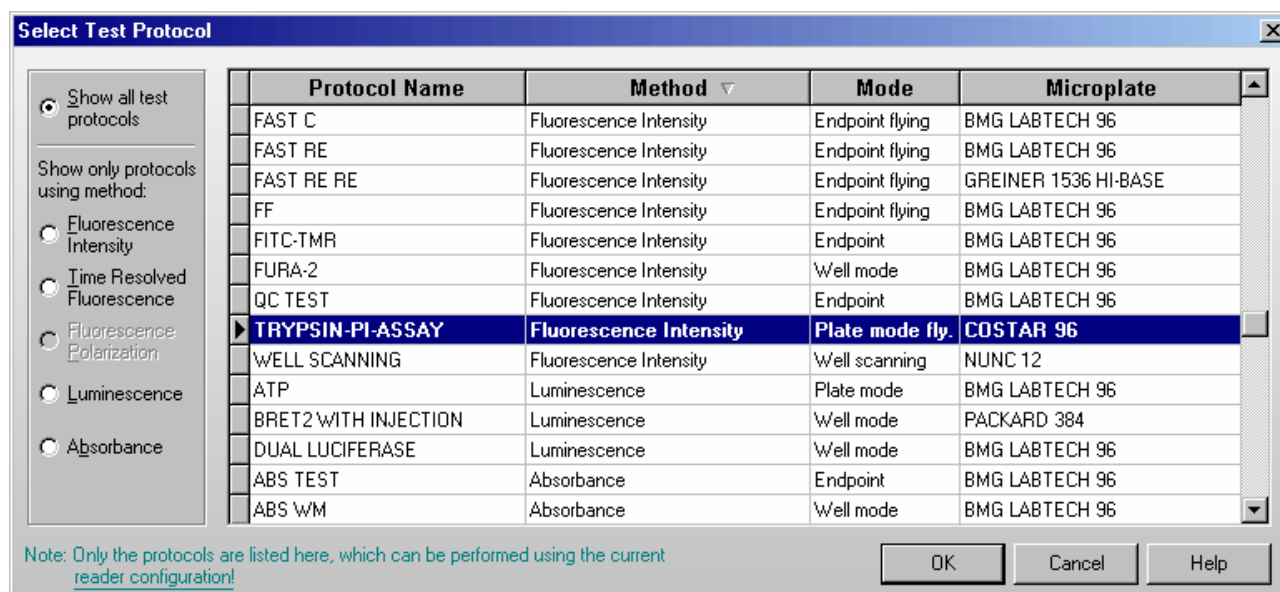
Notes: For absorbance measurements a positioning delay of 0.5 s will be used, for the other methods 0.2 s. Fluorescence measurements will be performed using 10 flashes per well, absorbance measurements will use 20 flashes per well. For luminescence measurements a measurement interval time of 1 s will be used.

To use the Quick Start function firmware version 1.20 (or newer) needs to be installed.

6.2 Executing Pre-Defined Test Protocols

A selection window displays all the defined protocols which can be performed using the installed optic, if the reader is equipped with auto optic switching.

If the reader is not equipped with auto optic switching you will see here only the protocols using the measurement method currently selected, i.e. fluorescence method. Use the 'Reader Configuration' dialogue to change the measurement method.



By default the protocols are sorted by method first and then alphabetically by name. If you prefer a sorting by name only click the 'Protocol Name' part of the table headline. To switch back to the original sorting click 'Method'. It is also possible to get the list sorted by the mode or the used microplate. Simply click on the 'Mode' or 'Microplate' part of the table headline.

Besides displaying all available protocols you might select to see only protocols using a certain measurement method by clicking one of the radio buttons on the left side of this dialogue box.

Note: As the LUMIstar OPTIMA can only measure using the luminescence method the method selection box will not appear when using this type of reader. This box will also not appear if the reader is not equipped with auto optic switching.

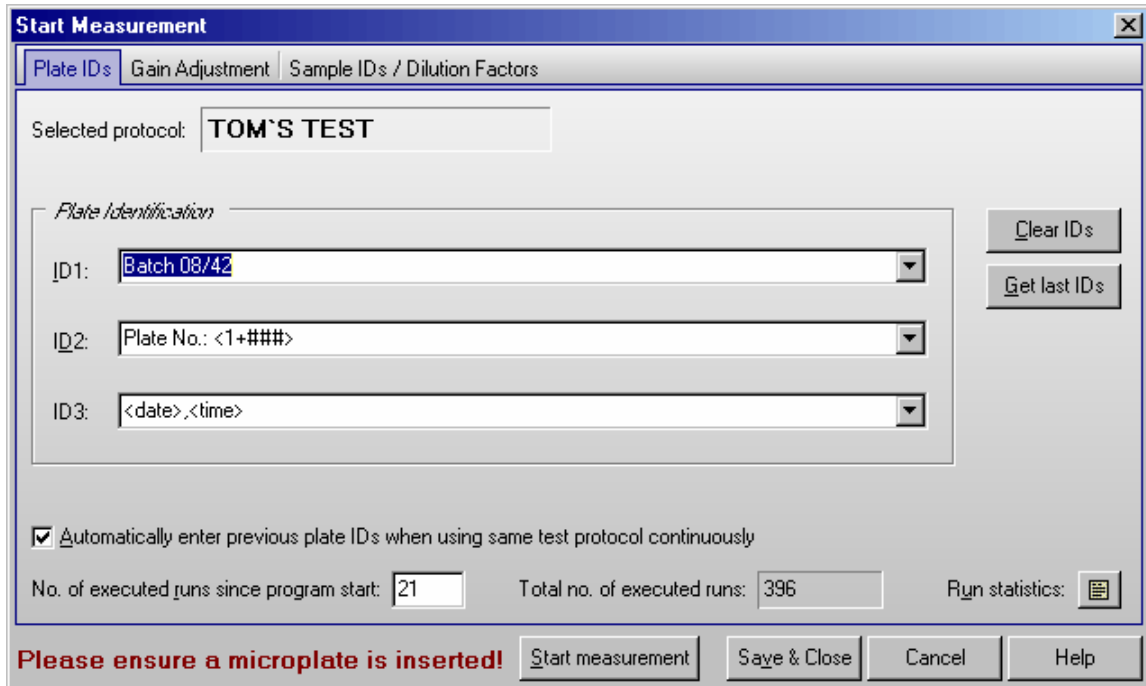
To select the first protocol, beginning with e.g. 'T', simply press the key [T].

Double click on the desired protocol or select the protocol and click '**OK**'. Click '**Cancel**' to return to the main menu.

After selecting the protocol the next window gives you the option to add identifiers to the test, to define dilution factors, to perform a gain adjustment and to start the measurement.

6.3 Plate Identification

After selecting the test protocol a window appears, where you can add identifiers to the test. This is an optional feature that allows you to give the plate specific identifying features such as a title, a description, or a number. Three identifiers with up to 100 alphanumeric characters are possible.



The drop down menu for each identifier allows you to choose several options for the IDs or you can manually type in a description.

ID Options


<protocol> The name of the used test protocol will be entered.

<method> Name of the used method, e.g. 'Fluorescence Intensity' or 'AlphaScreen'.

<1+#> Consecutive numbers for each test run (with the same test name) will be automatically entered. You can manually change the start number in the space 'No. of executed runs since program start.' The number of digits to be used can be changed by adding '#' characters, i.e. using <1+####> 001, 002, 003, etc. will be generated.

To use the number of loop executions in a script mode run instead of the number of executed runs after program start, add a 'B' before the '#', e.g. <1+B####>.

To use the 'Total no. of executed runs' instead of the number of executed runs after program start, add a 'T' before the '#', e.g. <1+T####>.

To use the 'No. of executed runs for the used test protocol', add a 'P' before the '#', e.g. <1+P##>. You can see a Run Statistics after pressing the  button (see chapter 3.4.4).

Counting down is also possible, use a '-' instead of the '+'.</p>
</div>

<A+#> Same concept as consecutive numbers using the alphabet.

Example: <A+####> → AAA, AAB, AAC ...

<date> The date of the test run.

You can specify the date format if you add a format description after "<date:" using yy or yyyy for the year, m or mm for the month and d or dd for the day:

yy year with two digits (1999 → 99, 2000 → 00)

yyyy year with four digits

m one or two digits for the month (January → 1, December → 12)

mm month with two digits (January → 01)

2007-11-12

0413F0011A

75/115

mmm	abbreviated name of the month (January → Jan.)
mmmm	full name of the month
d	day with one or two digits
dd	day with two digits
ddd	abbreviated name of the day (Monday → Mo.)
dddd	full name of the day
dddddd	date in the format defined as “Short Date Format” under windows (‘Settings Control Panel Regional Settings’)
dddddd	date in the format defined as “Long Date Format” under windows
Example:	<date:yyyy_mm_dd>

If you do not specify the format, the date format defined as “Short Date Format” under windows will be used.

<time>

Time of the test run.

You can specify the time format if you add a format description after “<time:”:

h or hh	for the hour
m or mm	for the minute
s or ss	for the second
t	time in the format defined as “Short Time Format” under windows (‘Settings Control Panel Regional Settings’)
tt	time in the format defined as “Long Time Format” under windows
am/pm or a/p or AM/PM or Am/Pm	use 12 hours format and show am or pm (a or p...)

Example: <time:hh.mm.ss>

If you do not specify the format, the time format defined as “Long Time Format” under windows will be used.

<ser_number> Serial number of the reader used.

Clear IDs

Delete the plate IDs that are entered.

Get last IDs

Recall the ID settings used for the last test run performed by the user currently logged in.

Automatically enter the previous plate IDs when using same test protocol


If this box is checked, then the same plate identifiers will be entered automatically when using the same protocol. You can still edit the identifiers or use the ‘Clear IDs’ button.

6.4 Gain Adjustment

By clicking the tab for Gain Adjustment, the sheet with the gain settings and the layout defined will appear.

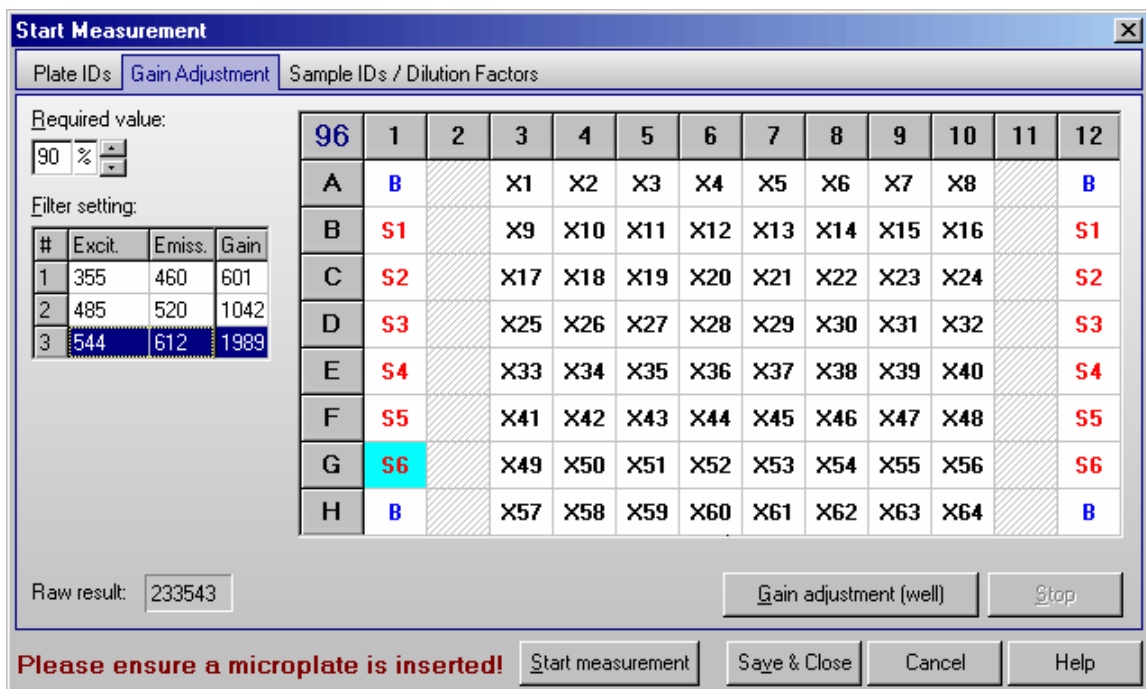
The purpose of a gain adjustment is to optimize the signal amplification so that the results have the maximum sensitivity and dynamic range. The gain is usually performed on the well containing the standard with the highest concentration of fluorophore (highest intensity). This sets the gain so there is no overflow in the higher intensity wells (an overflow means the relative fluorescent units of a well exceeds the maximum range, i.e. 65,000 rfu).

Zoom Feature

For 384 and 1536 well formats, the gain adjustment screen has a zoom function, allowing you to zoom in a certain section of the layout. To zoom in and out of the screen use the  icons.

The gain adjustment window is specific for each measurement method. The parameters for different methods are described on the following pages.

6.4.1 Gain Adjustment – Fluorescence and Luminescence Method



The filter settings are displayed on the left (in luminescence mode there are no excitation filters, only emission filters). If the protocol is multichromatic, then all filter combinations used will be displayed (you can perform a gain adjustment for each filter set).

Automatic Gain Adjustment for one Well

Select a well for gain adjustment, then click the 'Gain adjustment (well)' button. The instrument samples the well eleven times in 3 seconds to find the optimal gain. The optimal gain value appears in the box next to the filter settings.

'Stop' will terminate the gain adjustment process.

Automatic Gain Adjustment for the entire Plate

There are occasions when a well gain adjustment is not possible, for example, when the well with the highest intensity is not known. You can then do a gain adjustment on the entire plate. The instrument finds the well with the highest intensity and determines the gain. Click on the blue microplate format number in the top left corner of the layout (i.e. 96 or 384). All wells are selected and the caption of the gain adjustment button changes to 'Gain adjustment (plate)'. Click this button and the instrument will return a gain value in the box next to the filter settings.

Note: An automatic gain adjustment for the entire plate is not possible for 1536 well plates.

Gain Adjustment for Simultaneous Dual Emission Protocols

When using a simultaneous dual emission protocol the gain adjustment is always done simultaneously for the two channels (A and B). Nevertheless it is possible to define different required (target) values and, therefore, to define a target ratio.

In addition, it is still possible to type in values manually for each channel.

Gain Adjustment Plate is not possible in simultaneous dual emission mode.

Required value (Ch.A/B):
 60 % 40 %

Filter setting / channel:

#	Emiss.	Gain
1A	460	657
1B	510	785
2A	520	742
2B	540	921

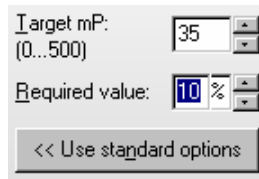
Channel A Channel B
 Raw results: 137042 98472

6.4.2 Gain Adjustment – Fluorescence Polarization Method

The gain adjustment window for fluorescence polarization protocols looks a little bit different compared with the other methods.

There are two PMTs (photo multiplier tubes) involved in measuring fluorescence polarization, one for channel A (vertical light) and one for channel B (horizontal light). As with fluorescence intensity protocols, you want to perform the gain adjustment on the well with the highest concentration of free fluorophore. Due to the fact that the relationship between channel A and B determine the fluorescence polarization value, it is important that the two channels are optimized for the best results. Select the desired well and click on 'Gain adjustment'. In this mode it is not possible to perform a gain adjustment on the entire plate.

The gain settings for both channels needed to reach the defined Target mP value (see below) are determined during the automatic gain adjustment. For the adjustment procedure a required value (target raw measurement value) of 10 percent of the measurement range is used by default. This value should work fine for most assays. If you want to use a different required value click on the 'Use advanced options' button. An input field for this value will appear:



The screenshot shows a dialog box with two input fields and a button. The first field is labeled 'Target mP: (0...500)' and contains the number '35'. The second field is labeled 'Required value:' and contains '10%' with a percentage sign. Below these fields is a button with the text '<< Use standard options'.

The value you enter in this window is used as a target value for channel A. An optimal value for channel B is calculated automatically, based on the entered Target mP (see below).

Target mP

It is recommended to use free fluorescein for gain adjustment, but it is possible to use other fluorophores or labeled biomolecules. The theoretical fluorescence polarization value for free fluorescein is 35 mP. Enter this value in the field 'Target mP'. In case you use a different fluorophore or a labeled biomolecule, you need to use the theoretical mP value of this molecule as 'Target mP'.

Now, when you perform a gain adjustment on the well with the highest concentration of free fluorophore, the result of that well will become equal to the target value entered (so for fluorescein the polarization value of the well is equal to 35 mP).

Custom Target mP value

If you are using a different fluorophore or a fluorescein-labeled peptide (bound fluorescein), you must change the 'Target mP' value. If you do not know what this value should be, you can start with, for example, 35.

Perform a gain adjustment as described above.

Start the measurement. Use the current state display (see chapter 6.7) or the Data Analysis software (see software manual part III) to check the measurement values. If the results are negative, then you need to increase the Target mP value.

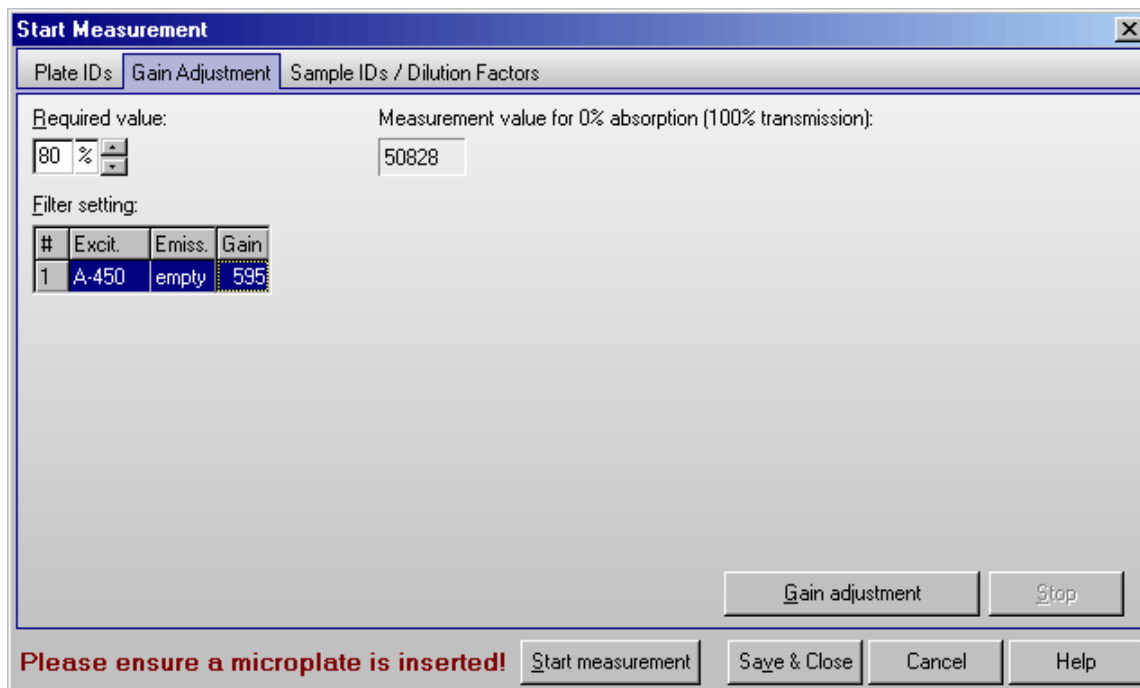
The results using a randomly selected target value are not absolute numbers, but it is still possible to see the binding curve and the inflection point from the polarization data.

6.4.3 Gain Adjustment – Absorbance Method

In absorbance mode, the gain is determined when the instrument measures the full transmission of light (0% absorbance). The gain is dependent on the filter wavelength used.

During the gain adjustment procedure, the plate carrier moves out of the way of the optics and the instrument samples the full transmission of light.

Beginning with firmware version 1.20 the gain adjustment for absorbance method protocols is done automatically as part of the measurement, therefore, you will not see this sheet if your reader uses firmware 1.20 or newer.



The filter setting is displayed in the gain adjustment window; there should be a filter for excitation and the emission side should usually say 'empty'. If you use a multichromatic protocol perform a gain adjustment for all filter sets used. Please select the desired filter pair, before clicking the 'Gain adjustment' button.

Required value for absorbance is recommended at 80%.

'Stop' will terminate the gain adjustment process.

6.4.4 Required Value

The measurement range for fluorescence is 0 to 65,000. If an overflow occurs, the measurement value will be 65,000. The measurement range for luminescence depends on the measurement interval time (max. 200,000,000 for 100 s). To avoid an overflow, it is necessary to set that the gain so the values stay within the range of the instrument.

In the top left corner of the gain adjustment screen is a '**Required Value**' number. This value refers to the percentage of the maximum value of the dynamic range. The default value is 90%. Therefore, when you perform a gain adjustment on the well with the highest concentration of fluorophore, the result will be 90% of the maximum fluorescence value, i.e. $65,000 \times 0.90$. This prevents an overflow in case of deviation of the intensities over the plate.



If the measurement is an endpoint test, then the required value can remain at the default value of 90%.

For a kinetic assay, an injection can increase the intensity values over the entire plate. If you do not know the final results you should lower the required value to 50% (or less) of maximum value in order to have enough space for higher intensity values and to prevent an overflow.

6.5 Sample IDs / Dilution Factors

You may enter identification values for each well in this sheet. These values will be shown in the data analysis software (see software manual part III). It is also possible to store these IDs together with the measurement results in an ASCII file (see chapter 3.4 *ASCII Export Function*).

In addition, it is possible to define dilution factors for all wells which do not contain blanks or standards. The dilution factor will be taken into account when calculating the unknown concentrations (see software manual part III: Data Analysis software).

Note: If you are using replicates, the sample ID and the dilution factor you entered for one well will be used for all replicates.

Well	Content	Sample ID	Dilution
A1	XA1	Sample 008/1	1
A2	XA2	Sample 008/2	1
A3	XA3	Sample 008/3	1
B1	XB1	Sample 009/1	2
B2	XB2	Sample 009/2	2
B3	XB3	Sample 009/3	2
C1	XC1	Sample 010/1	2.5
C2	XC2	Sample 010/2	2.5
C3	XC3	Sample 010/3	2.5
D1	XD1	Sample 011/1	5
D2	XD2	Sample 011/2	5

Options

- Automatically enter sample IDs previously used with this protocol
- Export / Print also lines without sample ID entry
- Include dilution factors when exporting / printing

Dilution Factor Auto Fill Function

Start dilution:

Factor Increment Decrement

Use the left mouse button to select the cells to be filled out in the dilution factor column of the table or click the caption for all cells.

Please ensure a microplate is inserted!

Start measurement Save & Close Cancel Help

Clear IDs

Pressing this button will clear all ID fields.

Get last IDs

By clicking this button you can recall the last IDs used for the selected test even if you did not use the option 'Automatically enter sample IDs previously used with this test protocol' (see below).

Table sort order

You can choose between sorting for rows, columns or well content. The selected sort order will also be used for printing or exporting the sample ID / dilution factor list.

Print ID list

You can print out the ID list on any available printer.

Import IDs

Import sample IDs / dilution factors from a text file (created using the export function or using a text editor like Notepad) or from a XLS (Excel format) file.

Format for Sample ID text files:

In the first column of such a file, there is the well number (e.g. 'A1'), the sample ID for this well should be in the same line beginning with position 12. If you want to include dilution factors, these values should be entered beginning at position 44.

Format for Sample ID Excel files:

	A	B	C	D
1	Well	Content	Sample ID	Dilution
2	B2	X1	Probe 007.1	2
3	B3	X2	Probe 007.2	2
4	B4	X3	Probe 007.3	0.5
5	B5	X4	Probe 007.4	1
6	C2	S3	Standard 50%	1
7	C3	S4		1
8	C4	B	Blank	1
9	C5	B	Blank	1

The well order in the files does not matter. If there are no dilution factors included, the dilution factors entered so far will not be changed.

Export IDs

Export current sample IDs (and if the appropriate option described below is selected also the dilution factors) into a text or XLS (Excel format) file.

Options

Automatically enter sample IDs previously used with this test protocol

If this option is checked, then the last used sample IDs for the selected test automatically will be reentered as default values.

Export / Print also lines without sample ID entry

If this option is checked all lines will be exported or printed, otherwise only the lines where a sample ID was entered would be exported or printed. When exporting into an Excel file, all lines will always be exported.

Include dilution factors when exporting / printing

If this option is checked the dilution factors will also be exported or printed.

Dilution Factor Auto Fill Function

If the dilution factor is equal for a high number of wells, you can use the auto fill out function. Enter the desired dilution factor inside the group box 'Dilution Factor Auto Fill Function'. Then select the corresponding cells in the table by clicking a cell or by selecting a range of cells using the left mouse button. To use this factor for all wells (besides standards and blanks) click the caption of the dilution column of the table. You might also specify a factor or increment / decrement value to automatically increase / decrease the dilution values.

Note: Exporting the table into a HTML file (in addition to the XLS or ASCII format) is possible after right clicking the table.

The settings from this window are user specific, therefore each user can select his preferences independently.

6.6 Measurement

After entering IDs / performing the gain adjustment, you have the following options:

Start measurement: Begins the measurement using the defined gain.

Save & Close: Saves the results of the gain adjustment, the ID settings and the dilution factors and return to the main menu without performing a measurement.

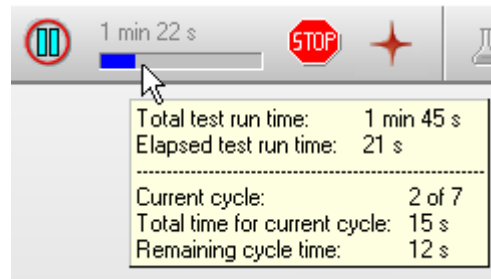
Cancel: Closes this window without saving the gain adjustment results and ID settings and without starting the test.



6.6.1 Test Run Information

On top of the main program window, there is a time gauge indicating the elapsed time of the test. The remaining time will be displayed above the time gauge. It is updated dynamically during the measurement.




If you move the mouse cursor to the time gauge, a small window with additional timing information will appear.



If you perform a plate mode kinetic measurement the quick start button () will change into a pause button (). This button has the same function as the menu entry 'Measure | Pause After Current Cycle': the active test run will pause after finishing the current cycle and a pause / continue window will appear (see chapter 4.3.2).


6.6.2 Stopping the Test Run

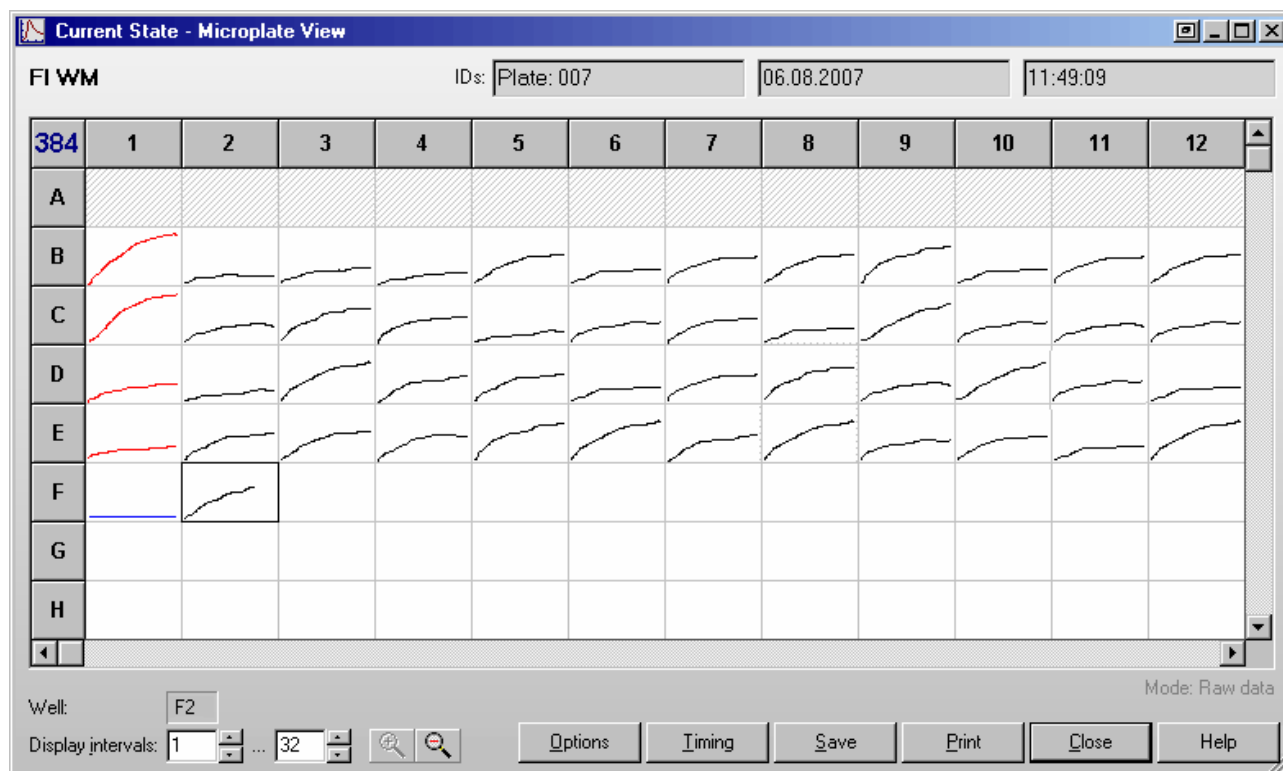
It is possible to terminate a measurement procedure after it has started by pressing the stop button () or by using the menu function 'Measure | Stop Now'. If there is already measurement data available, a dialog box will ask if you want to save the incomplete data.

In script mode (see chapter 7), you can use this function to immediately stop any activity.

6.7 Current State Graphics

6.7.1 Current State – Microplate View

When the measurement has started, the 'Start Measurement' button will change to the 'Current State Graphics'  button. This function allows you to view raw data graphically as the measurement occurs.



The Current State window displays a grid of the microplate format. The measurement results can be displayed as curves: Each measurement value is represented by a dot and you can see the relative position of the values. You can choose between a curve of points or lines. It is also possible to display the measurement values of the last measured cycle / interval numeric or use colors for a fast overview (see chapter 6.7.2 *Current State - Options*).

It is possible to change the size of this window. The software will remember the last used size and position user specific.

When using 384 or 1536 well plates it is possible to zoom in and out using the zoom buttons .

Current cycle	Shows the number of the cycle currently being measured (only for plate mode tests).
Well	Shows the name of the current well (only for well mode tests).
Display cycles / intervals	Kinetic points that will be displayed. This can be changed manually, otherwise the default number of cycles / intervals as defined in the test protocol will be used.
Options	Opens the 'Current State Options' dialog box.
Timing	Opens the 'Timing Overview' window.
Save	Saves the data as bitmap (windows BMP format or JPEG format).
Print	Prints the screen on any available printer.
Close	Closes the current state window.

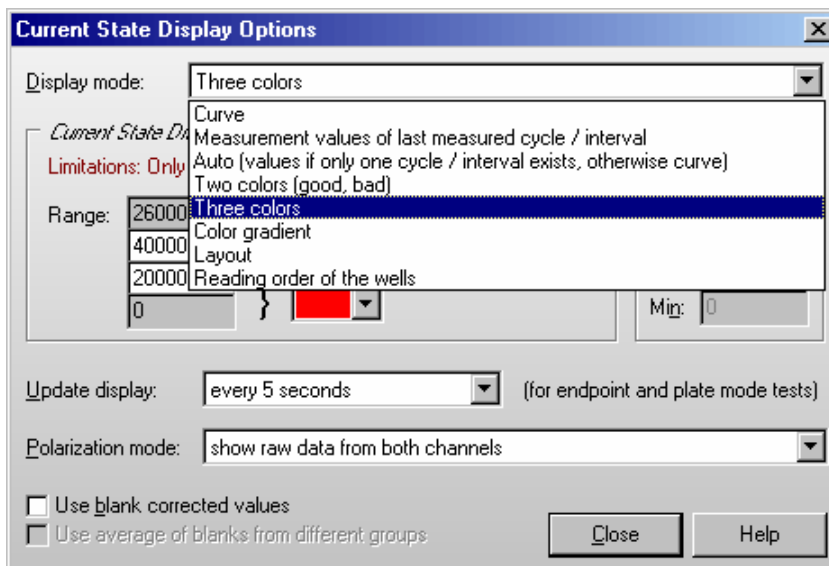
If you are using layout groups (see chapter 4.3.9), the layout grid will be displayed using the background colors belonging to the layout groups used. When you move the mouse pointer over a well, the well content (e.g. 'SA1') will be shown for a few seconds.

For test protocols with well scanning (see chapter 4.3.4 *Basic Parameters – Well Scanning*) the single scan points will be displayed, if you use the two or three colors display mode or when using the color gradient display mode (see chapter 6.7.4 *Display of Well Scanning Data*).

You can double click on a square to get a zoomed view of the measurement values (see chapter 6.7.3 *Current State – Well View*).

6.7.2 Current State – Options

After clicking the 'Options' button in the Current State window, the following dialog box will appear:



Display Mode

The measurement results can be displayed as:

Curve

The measurement results can be displayed as a curve of points or lines. If there is more than one chromatic used (multichromatic, more than one filter pair), you will see the results of all chromatrics displayed together.

Measurement values of last measured cycle / interval

You will see the last measurement values in numeric style. Due to space limitations, only the results for up to two chromatrics (filter pairs) will be shown (the first two). To see values from an earlier cycle / interval, you can change the number in the Cycles / Intervals input box of the Current State window.

Note: When you use this option in plate mode with an update display setting (see below) of more often than 'only after a cycle is completed', for all wells, which are not yet measured in the current cycle, '...' will be displayed to avoid that measurement values of different cycles can be mixed up.

If you want to see the last available measurement value for each well, regardless whether this measurement value is from the current cycle or still from the previous cycle, add the following setting to the [Configuration] section of the 'FLUOstar OPTIMA.ini' file: `ClearBeforeUpdateInMValuesMode=False`. You will find this configuration file in the OPTIMA main installation directory, usually '~\Program Files\BMG\OPTIMA\'.

Auto

If you use this option, you will get numeric values for all tests with only one cycle / interval (endpoint tests) and a curve display for all other tests.

Two colors

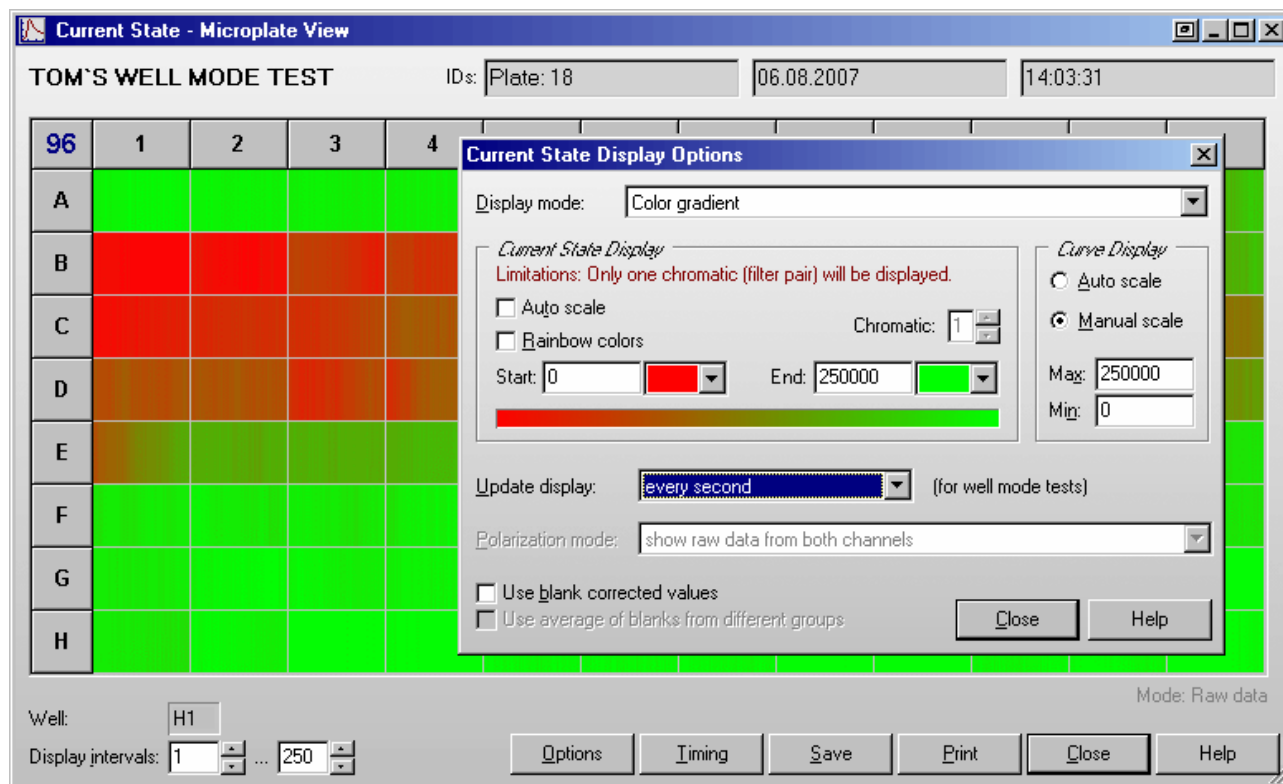
If you are only interested in a good / bad decision, you should choose the option to display different colors for all values under a certain limit and for all values above the limit. You can select the two colors and change the limit value. Only the results from one chromatic / channel will be displayed, but if your test uses multichromatics you can decide which chromatic should be used for the current state display.

Three colors

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. If your measurement values use only a part of the total measurement range (i.e. 0...30000), it might be useful to change the start and/or end value to enlarge the used range of the color gradient. Alternatively use the auto scale option. Similar to the Two and Three colors option, it is only possible to display the results from one chromatic / channel.

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

Layout

Shows which wells are used for samples, standards and blanks.

Curve Display

The settings in this group will be used for the Curve display in the Current State window and for the Current State Zooming window.

Auto scale: The limits for the graph will be selected automatically according to the measurement results.

Manual scale: This options allows you to personally specify the graph limits. You can use this function to enlarge parts of the graph of special interest.

Max: Maximum value displayed

Min: Minimum value displayed

Update display

Here you can specify how often the display is updated. In well mode the display is updated at least after finishing the measurement of a well, in plate mode it is updated at least after each cycle. If you select any of the 'Update every X seconds' options, there will be updates even for unfinished cycles / wells showing the already available measurement values.

Notes: For updating the Current State Overview and the Current State Zooming window, a large amount of computing power is necessary, especially for tests with a large number of cycles / intervals or multichromatics. If the computer is too slow to redraw the current state display between two measurement values, the update sequence will be lowered automatically.

The update display modes for well mode tests and plate mode tests are independent.

This option is not available for well mode endpoint test runs without multichromatics, as here only one value per well (two for simultaneous dual emission protocols) will be transmitted. When using the well scanning feature all scan points will also be transmitted at once, therefore, the update function does not make sense in these cases.

Absorbance mode

The measurement results from absorbance mode test runs can be displayed in

- **OD units**
- **mOD units**

Polarization mode

The measurement results from polarization tests can be displayed as

- **Raw data from both channels**
- **Polarization values in mP units**

These values are calculated from the results of channel A and channel B:

$$P = \frac{\text{Ch.A} - (\text{Ch.B})}{\text{Ch.A} + (\text{Ch.B})}$$

- **Anisotropy values in mA units**

$$A = \frac{\text{Ch.A} - (\text{Ch.B})}{\text{Ch.A} + 2(\text{Ch.B})}$$

Use blank corrected values

If you use this option, all results will be blank corrected before display.

Notes: If you use this option during well mode tests, measurement results cannot be displayed before the first "Blank" well is measured. Therefore, it might be a good idea to place at least one blank at the beginning of reading (depending on the selected reading direction, see chapter 4.3.9 *Using Layout Groups*) if you are interested in the current state display. As soon as the measurement values of additional blanks become available, the entire current state display will be recalculated and updated.

If you use this option during plate mode tests, the current state display will only be updated when a cycle is finished (regardless of the selection under 'Update display').

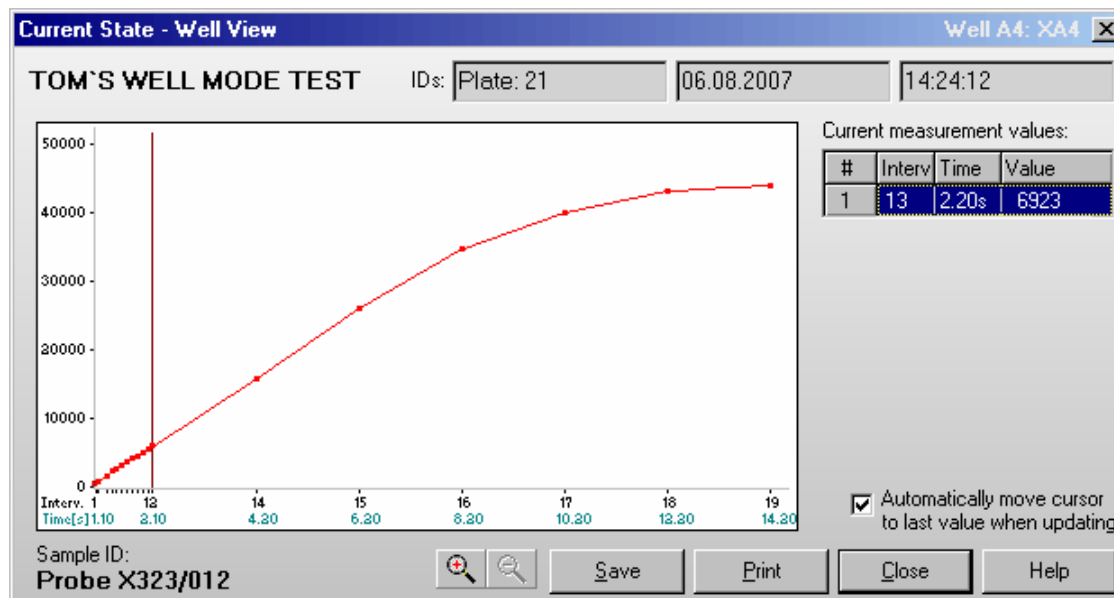
Use average of all blanks from all groups for blank correction

This option is only available when different layout groups (see chapter 4.3.9 *Using Layout Groups*) are used. If this option is not selected, the measurement values of a group will be corrected using only blanks from the same group. If you select this option, the correction will be done using the average of all blanks from all groups.

Note: All settings from this window are user specific, therefore each user can select his preferences independently.


6.7.3 Current State – Well View

After double clicking on a well in the Current State window, you get a zoomed view of the measurement values. Here the measurement values are always displayed as a curve (when using well scanning the display might look different, depending on the selected display mode, see chapter 6.7.4). There is also a table on the right side of the window where you can see measurement results for all used filter pairs in numeric style. When you move the mouse pointer to the filter setting number column of the table a little hint box, containing the filter setting details, will appear.



A little red arrow will show the end of the measurement range (only if the displayed range includes this value).

You can click and drag the cursor line to each kinetic point to see the measurement value(s) of this point. It is also possible to move the cursor by using the following keys: [←], [→], [PgUp], [PgDn], [Pos1] and [End].

Using the  keys you can zoom into the curve and out again.

When the option '**Automatically move cursor to last value when updating**' is selected, the cursor will automatically move to the newest value when the measurement result of a new cycle becomes available. The table on the right side will show the new value(s) numerically.

If you have defined a Sample ID for the selected well, this ID will be displayed below the graph.

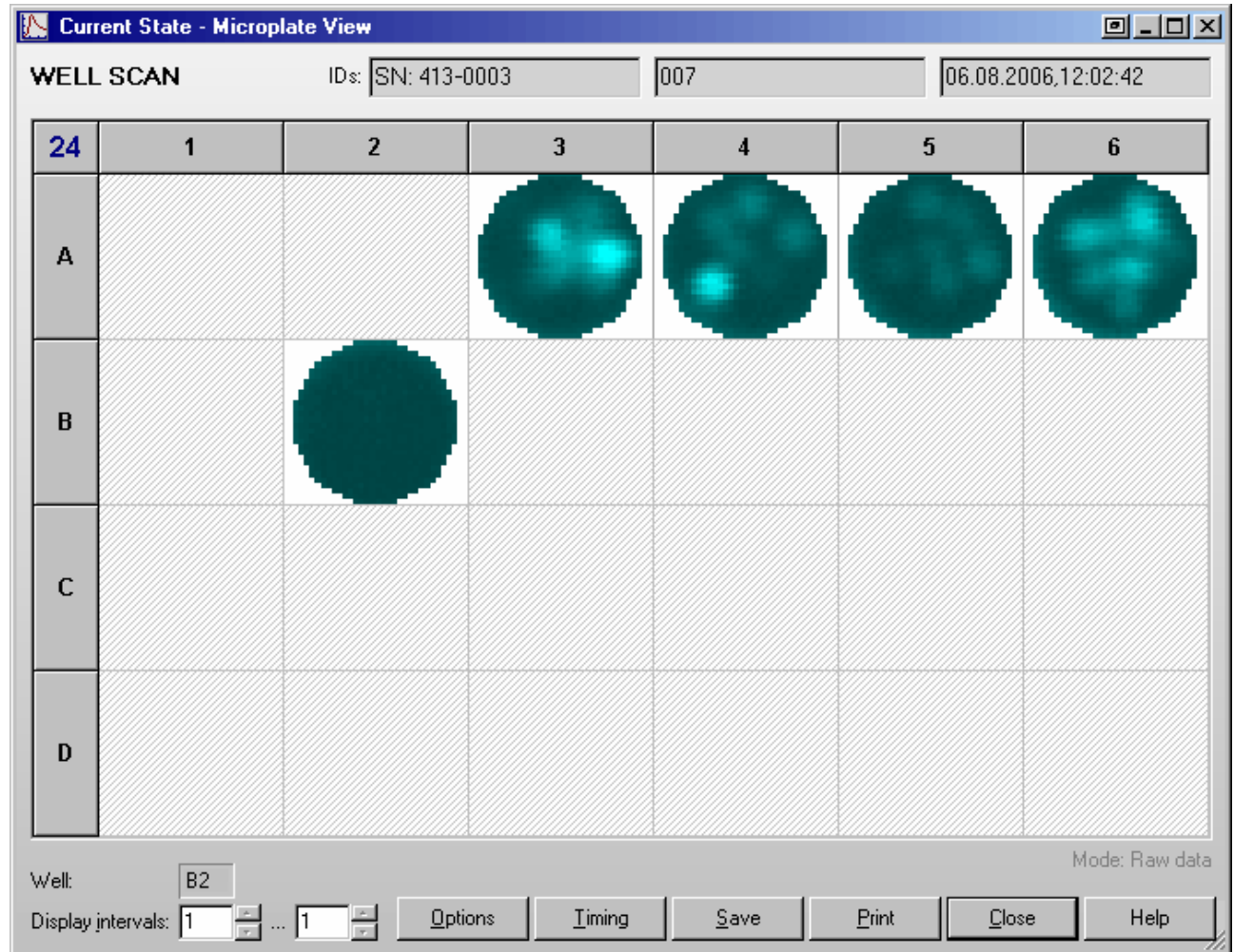
Save Saves the data as bitmap (windows BMP or JPEG format) on the hard drive.

Print Creates a print out of the screen on any available printer.

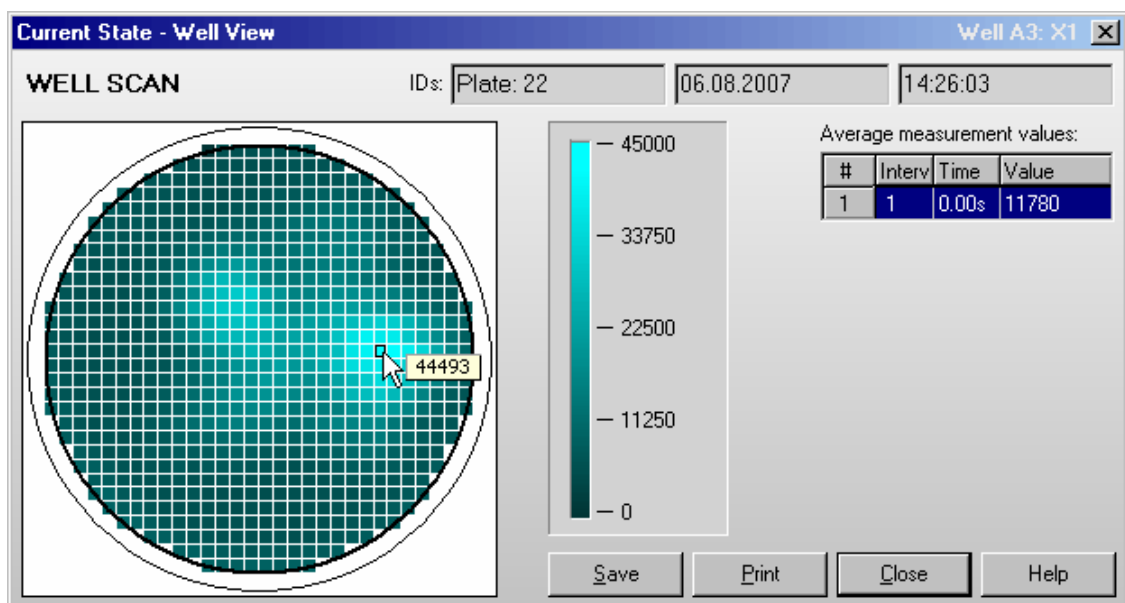
Close Closes this window.

6.7.4 Display of Well Scanning Data

When performing a protocol using well scanning, you can see the single scan values in two and three colors and color gradient display mode (see chapter 6.7.2 *Current State – Options*). Example using the color gradient display mode:



Clicking one well will open the Current State – Well View window:

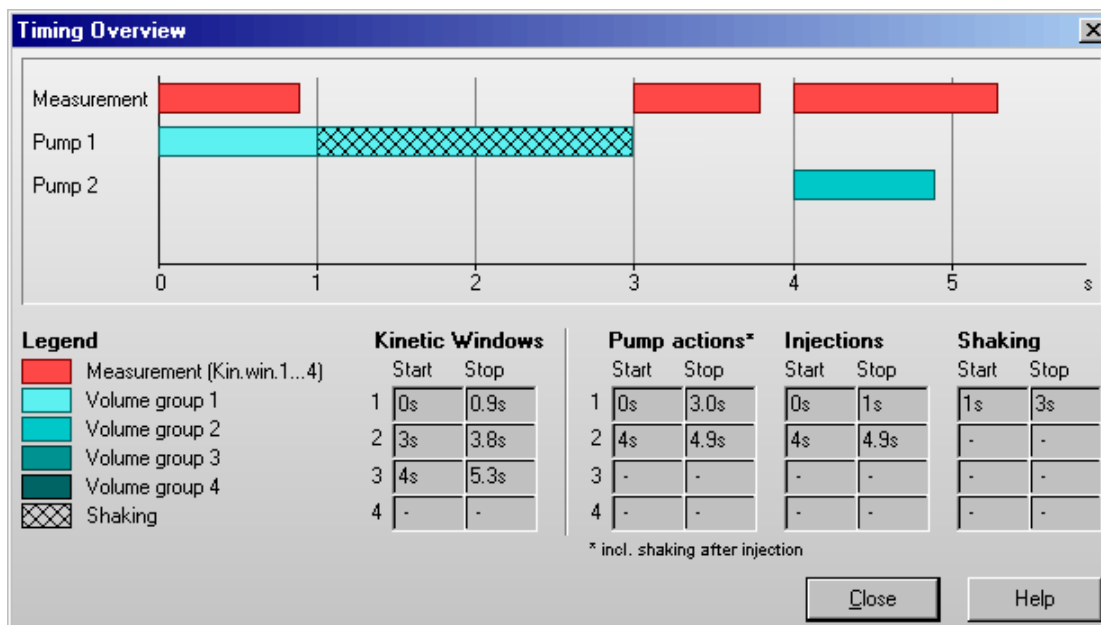


If you move the mouse cursor over the well display, the measurement value of the selected scan point will be displayed. The table on the right shows the average measurement value of the well.

Note: If you use a multichromatic or dual emission protocol, the values of the chromatic / channel selected in the Current State Options dialogue will be displayed. The table on the right of the zooming display will show the average measurement values of all chromatims / channels. The selected chromatic / channel will be marked by a blue bar.

6.7.5 Timing Overview

After clicking the 'Timing' button in the Current State window, you will get a window showing an overview of the timing of the test currently running.

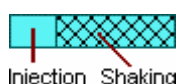


In this window, you can see a graphic overview of the measurement and all injections for one well. (For plate mode tests all actions are displayed in the timing overview of one cycle. The displayed injections may occur in different cycles. The used cycle will be listed behind each injection bar.)

In addition, you will see tables containing the measurement and the injection times.

Note: The listed pump action times include the time for shaking after injection.

An injection action can contain different steps:



7 Script Mode

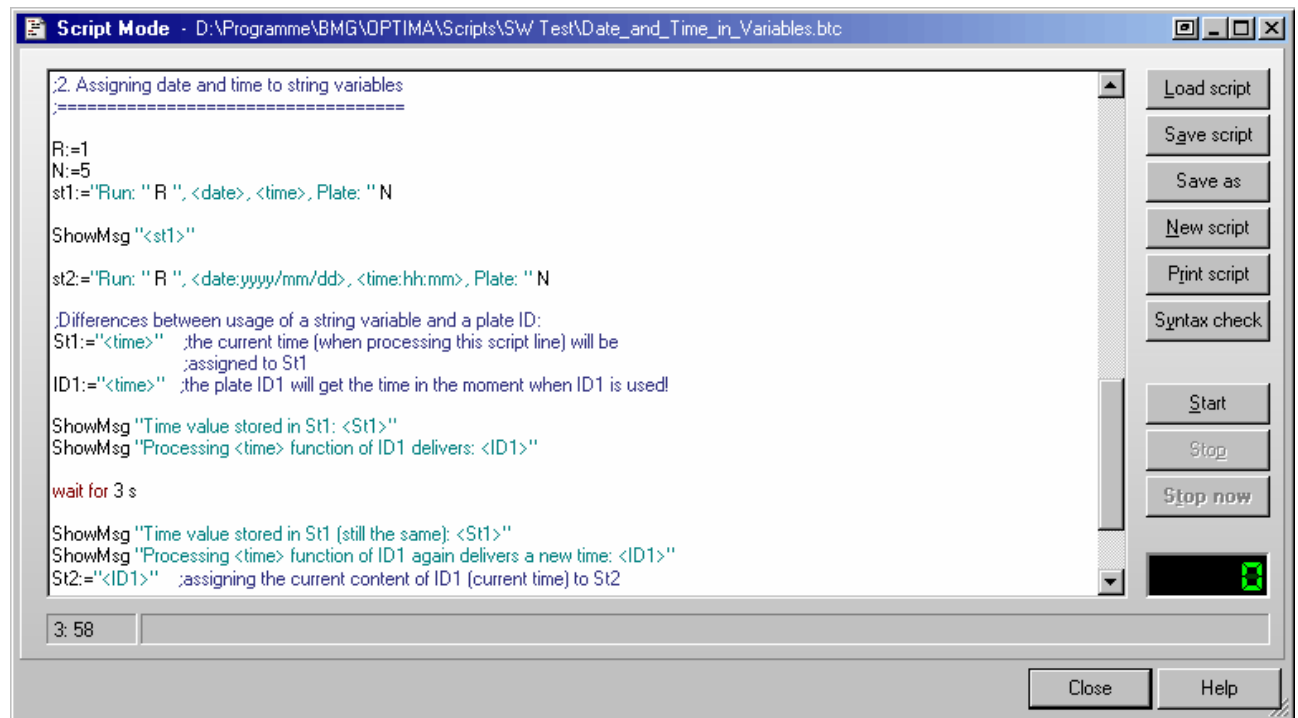
7.1 Script Mode Window

Using this window you can define your own run scripts, which gives you **unlimited flexibility**. You can use this function, for instance, to use different test protocol definitions in one batch run or to perform more than one measurement on a plate.


All available script language commands are described in chapter 8 The Script Language.

Note: The script mode is not available when the program is used in ActiveX or DDE mode, e.g. as part of a robotic system or in combination with Stacker Control.

7.1.1 Control Elements



- Load script:** Loads a script file. Using the mouse you can also move a script file directly from an explorer window into the script window (drag and drop).
- Save script:** Saves all changes.
- Save as:** Saves the current script under a new file name.
- New script:** Clears the script editor window to allow entering a new script.
- Print script:** Prints the script on any available printer.
- Syntax check:** Performs a syntax check. If there is a syntax error in the script you will get a message. It will also be checked, whether the protocols specified as parameter for e.g. the R_Run command exists and whether optional path parameters are correct. There will be a range check performed for all numeric parameters. *Hotkey:* [Ctrl]+[F9]
- Start:** Starts the execution of the script. *Hotkey:* [F9]
- Stop:** Stops the execution of the script after finishing the current command.
- Stop now:** Stops the execution of the script immediately (the command currently processed will be terminated). *Hotkey:* [Ctrl]+[F2]
- Close:** Closes this window. You can close (and reopen) this window even when the script is currently executed.

Clicking the  button beside the 'Minimize' and 'Maximize' button or using the system menu function 'Default position and size' will bring the script mode window back to its default size and position. To open the system menu, use [Alt]+[], click on the form's icon or right click on the form's caption. You can also double click the form's background to activate this function.

In the left part of the script mode window status section you will see the current cursor position (line: column) while editing the script.

The software will automatically remember the last script used (user specific) and will reopen the script the next time you open the script mode window.

The script mode window is resizable. The size and position used will be stored user specifically.

7.1.2 Editor Functions

To **undo** the last change of the script use the key combination [Ctrl]+[P] or [Alt]+[Back].

Block **indent / outdent function**: After selecting one or more lines and using the key combination [Ctrl]+[K] or [Ctrl]+[Tab], two spaces will be inserted on the beginning of the selected lines. (The selected block will be indented by 2 characters.) To outdent a block, use [Ctrl]+[U] or [Ctrl]+[Shift]+[Tab].

It is possible to **search text** forward or backward beginning from the cursor position or from the top / end of the editor content. The following key combinations can be used to activate the search function:

1. Borland Style:

To open the search parameters window use [Ctrl]+[Q], [F], to repeat the last search (in the defined search direction) use [Ctrl]+[L]. Use [Shift]+[Ctrl]+[L] to search against the defined direction, e.g. to go back to the previous occurrence.

2. Microsoft Style:

To open the search parameters window use [Ctrl]+[F], to search the next occurrence in forward direction use [F3], for backward direction use [Shift]+[F3].

If you select something before using the [Ctrl]+[Q], [F] / [Ctrl]+[F] key combination, this selection will be used as default search string, otherwise if the cursor is positioned onto a word this word will be taken as default value.

Note: Searching something, which includes line breaks, is not possible. You might change the width of the window to remove soft line breaks.

All above mentioned functions are also available using the **pull-up menu** (right click into the editor window).

[Ctrl]+[Y] will **delete** the cursor line, [Ctrl]+[Q],[Y] will delete everything in the current line after the cursor position.

[Ctrl]+[F1] will provide **context sensitive help**, as this key combination will open the Script Language chapter of the online help. If the word at the cursor position is a known command / language element, the page of the online help containing this element will be opened (it might be necessary to scroll down some lines to see the description).

7.1.3 Syntax Highlight Function

To ease the reading of script code, a syntax highlight function has been implemented. Reserved words (as for example for, to, do, begin, end) are displayed in **red**. String constants are shown using **cyan**. **Blue** will be used for comments.

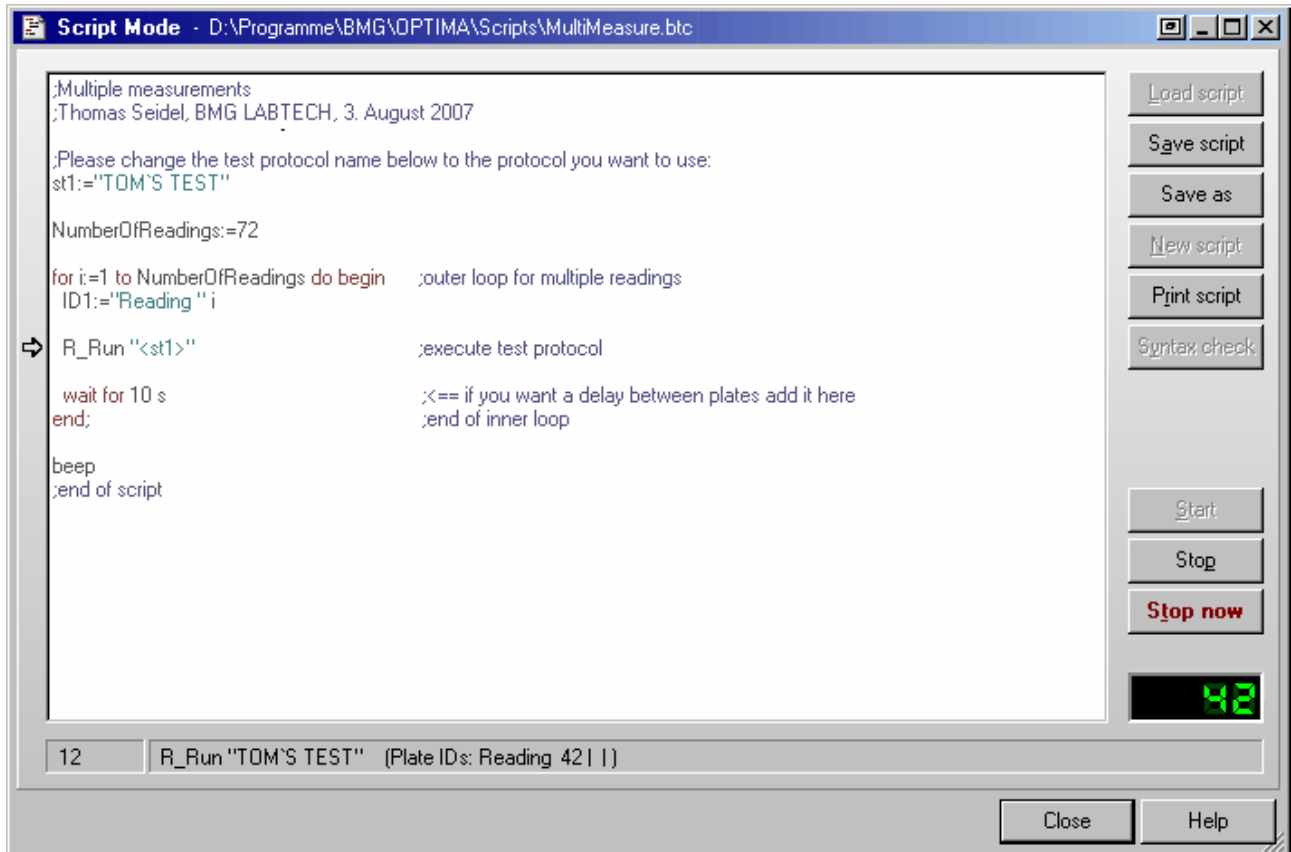
Example:

```
for i:=1 to NumberOfReadings do begin           ;loop for multiple readings
  ID1:="Reading " i                             ;define plate ID
  R_Run "TOM'S PROTOCOL"                       ;execute test protocol
end;                                             ;end of loop
```

If you use a color printer for printing a script, the colors of the syntax highlight function will also be used for the print out. When using other printers the colors will be replaced by different levels of gray and bold or non-bold fonts.

7.1.4 Executing Scripts

When executing a script a pointer (⇒) on the left side of the script mode window will indicate the line currently processed.



In the left part of the status section you will see the number of the script line currently processed. In the remaining part of the status section, you will see the currently processed command and, for many commands, some additional information.

You will also find all script commands listed in the run log file / run log window (see chapter 9.3).

The **loop** display on the lower right side of the window shows the value of the loop variable if the script execution is currently inside a loop (see 'for ... to' loop in chapter 8.2.2). If you use nested loops, this loop counter will show the value of the inner most loop.

7.2 Script Auto Run

It is possible to optionally specify a script, which will automatically be executed after starting the program (immediately after initialization and reading the EEPROM content). To use this feature, specify the name of the script in the command line after the program name preceded by */s*, e.g.

```
FLUOstar.exe /s"C:\Program Files\BMG\OPTIMA\Scripts\Autorun.btc" /v.
```

If you also add */v* to the command line, the script editor will be opened and the script specified will be loaded into this window and executed there. Without the */v* parameter the script will be executed in the background. When specifying only */v*, the script mode window will be automatically opened and the last used script will be loaded, but the script execution will not be started.

Notes: If no path is specified, the script will be searched inside the '\ExeDll' subfolder of the OPTIMA main directory. It is possible to omit the file extension ".btc", but it is not possible to use files with extensions different than ".btc". If the path or file name contains spaces, you need to put it into quotation marks (").

8 The Script Language

8.1 Introduction

Scripts can either be automatically generated or written by the user using the ‘Script Mode Window’ (see chapter 7.1).

You will find some example scripts in the subfolder `Scripts` of the OPTIMA installation directory (usually `c:\Program Files\BMG\OPTIMA\`).

The script language is case insensitive. Nevertheless, it is recommended to follow the writing style from this manual to ease the reading of script code.

All **string** values / string parameters need to be enclosed in double quotes ". Everything behind a semicolon ; is considered to be a **comment**. During execution of the script these comments will be ignored.

Note: If all parameters of a command do not fit in one line, you should enter a / at the end of the line and continue in the next line, e.g.:

```
R_GainWell "DUAL EMISSION FLUO" /  
           "D:\Program Files\BMG\OPTIMA\Tom\Definit" /  
           2 5 80 40 1
```

8.1.1 Typographical Conventions

{ } Curly brackets are used to characterize a place holder for a command parameter. Replace the expression with an appropriate numeric or string value (depending on the parameter type) or with a variable.

() Round brackets enclose optional items.

Boldface Boldfaced words represent elements of the script language (commands or reserved words).

Monospace type Example code is displayed using a monospace font type (fixed pitch font).

Note: When items are shown enclosed by < >, these signs need also to be entered - opposite to the signs { } and (). Items enclosed by < > will be replaced by the item values when the script line containing the item is processed. These signs are used to mark system constants or variables and for conversion functions, like <IntToStr(n)>.

8.2 Batch Control Commands

8.2.1 Wait Commands

wait for {n} s

Wait for {n} seconds before proceeding with the next line. You can specify the time in 0.1 s steps. Instead s for seconds you can also use m for minutes or h for hours.

Example:

```
wait for 3.5 s
```

wait for temp >= {n}

Wait for the built in incubator of the reader to reach the specified temperature before proceeding with the next line (you can specify the temperature in 0.1 °C steps).

You should use this command after switching on the incubator using the R_Temp command.

Example:

```
R_Temp 37  
wait for temp >= 36.9
```

wait for temp <= {n}

Wait for the temperature of the reader to reach or fall below the specified temperature before proceeding with the next line.

You can use this command after setting the incubator's target temperature to a lower value using the R_Temp command or after switching off the incubation. To get temperature values transmitted even when the incubator is switched off, use the temperature monitoring function (see chapter 5.2.2) by sending a 'R_Temp 0.1' command.

8.2.2 Loop Commands

```
for i:={n} to {m} do begin  
  {commands}
```

```
  ...
```

```
end;
```

for ... to - loop: All commands between the for...to- and the end-line will be repeated {m-n+1} times. A maximum of 100 nested loops ('begin ... end' blocks used in combination with 'for ... to' or 'if ... then') is possible.

Example:

```
for i:=1 to 5 do begin  
  R_Run "MY TEST"  
end;
```

break

Leave a loop and continue operation with the first command after the loop end. (When used outside a loop, this command will end the script execution.)

8.2.3 Comparisons

if ID1="value" **then** ...

if ID2="value" **then** ...

if ID3="value" **then** ...

Perform the command after 'then' only if the ID1 (2, 3) string is equal the declared value. *

if st{n}="value" **then** ...

Perform the command after 'then' only if the value of the string variable st{n} (st0 ... st9) is equal the declared value. *

if st{n}=st{m} **then** ...

Perform the command after 'then' only if the values of both string variables are equal. * Instead of a string variable you can also use one of the ID values.

if {variable}=value **then** ...

Perform the command after 'then' only if the (numeric) value of the variable is equal the declared value. *

if {variable1}={variable2} **then** ...

Perform the command after 'then' only if the (numeric) value of the variable1 is equal the value of variable2. *

* Instead of '=' you can also use '<>' for not equal, '>' for greater than, '>=' for greater than or equal, '<' for smaller than and '<=' for smaller than or equal.

If you want to perform more than one command after the comparison, you can use '**begin**' and '**end;**' to define a block of commands.

Example:

```
if A>5 then begin
  beep
  ShowMsg "Information: A is larger than 5"
end;
```

A maximum of 100 nested 'begin ... end' blocks (used in combination with 'if ... then' or 'for ... to') is possible.

Note: The string comparisons work case insensitive, e.g. "ABC"="abc".

8.2.4 Other Batch Control Commands

beep

This command produces a short acoustic signal (you can use this for example, to announce the end of a batch operation).

restart

Restart operation with first script line (useful e.g. for endless demos).

halt

End the execution of the script.

terminate

End the execution of the script and terminate the software. This might be especially useful in combination with the script auto run function (see chapter 7.2).

8.3 Dialogue Commands

8.3.1 Message Boxes

ShowMsg {Message} {Type} ({Title})

Show the defined message in a small window. Depending on the {Type} (Info, Warning, Error), the window will contain an appropriate icon. Optional: you can specify the window title (caption). If you do not specify a title, 'Information', 'Warning' or 'Error' will be used (depending on the specified {Type}).

Use '&' inside the message text to specify the start of a new line.

The processing of the script will be continued when the user clicks the 'OK' button (or uses the enter key).

Example:

```
ShowMsg "This is an example message.&&Press enter to continue." Info
```

Ask {Question} ({Title}) (Yes: {cmd}) (No: {cmd})

Open a question window which displays the {Question} text and contains a 'Yes', a 'No' and a 'Cancel' button.

Optional: You might enter a title; otherwise 'Question' will be used.

When the user clicks the 'Cancel' button, the script operation will be terminated.

After *Yes*: you can enter a command to be performed when the user clicks the 'Yes' button.

Similarly, you can enter a command to be performed after clicking the 'No' button after *No*:. .

Example:

```
Ask "Continue operation?" No: Halt
```

8.3.2 Input Commands

{Variable}:=**MenuSelection** {Label|Item 1|Item 2 ...} ({Title}) ({Default selection})

This command will show a selection window containing a list of items that are selectable by radio buttons.

The first part of the first parameter (everything until the | character) will be used as a label (or caption) of the radio group box. All following parts of this string (separated by a | character) will be used as items (selectable entries) of the radio group box. If you use '&' inside the item text, the following letter will be used as a hot key.

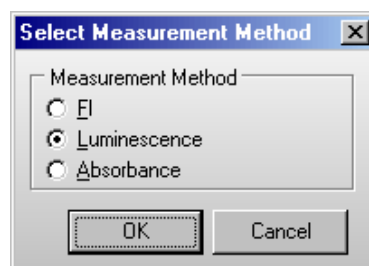
Specifying a title or defining a default selection is optional. If you do not specify a title, 'Please select' will be used. If you do not define a default selection, the first item will be preselected.

The return value is the index of the selected item (counting starts with 1 for the first item).

The size of the dialogue box will automatically be calculated based on the length of the title, the label and the longest item.

Example:

```
n:=MenuSelection "Measurement Method|&FI|&Luminescence|&Absorbance" /
"Select Measurement Method" 2
```



{Variable}:=**InputInteger** {Label} ({Title}) ({Min}) ({Max}) ({Default value})

This command will show an input box for integer values. Using the first parameter you specify the text in front of the input field. Similar to the Ask and ShowMsg command you can use '&' inside the text for a line break.

If you do not specify a title, 'Input' will be used.

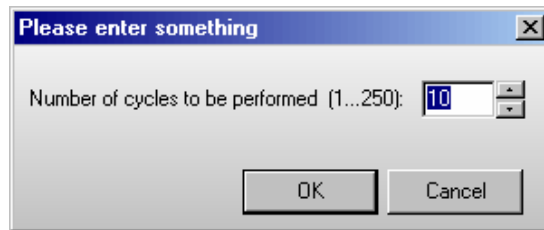
If you specify a minimum and maximum value, only input values in this range will be accepted. If the range between minimum and maximum value is not larger than 250, up/down scroll buttons will appear beside the input field.

Optionally, you can define a default value (a value, which will be pre-entered into the input field).

The value entered will be assigned to the target variable (variable specified left of :=).

Example:

```
i:=InputInteger "Number of cycles to be performed (1...250):" /
  "Please enter something" 1 250 10
```

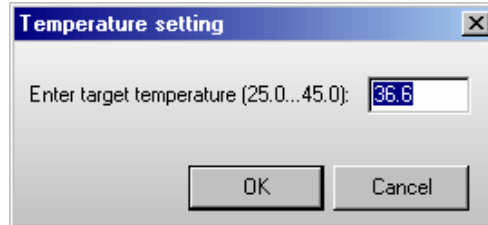


{Variable}:=**InputFloat** {Label} ({Title}) ({Min}) ({Max}) ({Default value})

This command is similar to the IntegerInput command, but will be used for floating point values.

Example:

```
r:=InputFloat "Enter target temperature (25.0...44.5):" /
  "Temperature setting" 25 45.0 36.7
```



{Stringvariable}:=**InputString** {Label} ({Title}) ({Default value})

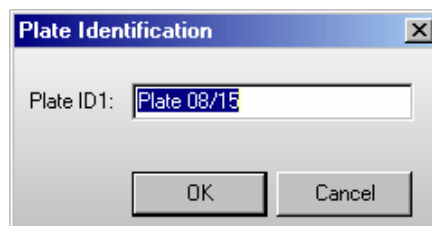
This command is similar to the IntegerInput command, but will be used for string values. As target variable, only a string variable (st0 ... st9) is allowed.

Examples:

```
st1:=InputString "Plate ID1:"
```

or

```
st1:=InputString "Plate ID1:" "Plate Identification" "Plate 08/15"
```



8.3.3 Script Memo

AddToMemo {Text} ({Title})

This command will add the text to a memo / log window. If this window is not yet open, it will be opened.

During the first usage of this command, you might optionally specify a window title, otherwise "Script Memo" will be used. To format the text the following options can be used:

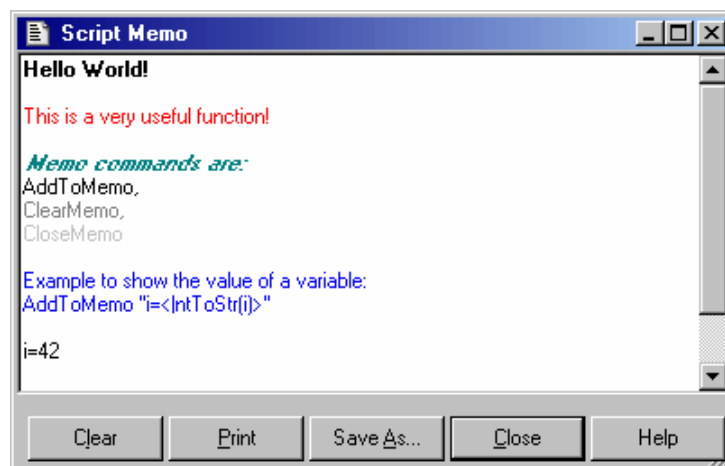
	use a bold font style
<i>	use an italic font style
<bi>	use bold italic
 or </i> or </bi>	use normal font style
<c:{ColorName}>	use the defined color, possible values are: Black, Red, Maroon, Lime, Green, Yellow, Olive, Blue, Navy, Fuchsia, Purple, Aqua, Teal, Silver, Gray

Note: These format options will be used for the whole text added with the AddToMemo command. It is not possible to format only a part of the text added by one AddToMemo command. These format options will also be used for the text added by subsequent AddToMemo commands until the options are overwritten by new format options.

Similar to the Ask and ShowMsg command you can use "&" inside the text for a line break.

Example:

```
AddToMemo "<b><c:red>Hello World!" "NEW !!!"
```



ClearMemo

This command will clear the memo window.

CloseMemo

This command will close the memo window without clearing its content. It will automatically be reopened with the next AddToMemo command. It is also possible to close the window manually.

Note: The title and text of the memo window will be reset at every script start.

8.4 Reader Commands

8.4.1 Init Command

R_Init ({InitMode})

This command will initialize the reader.

Using the optional {InitMode} parameter you can change the behavior. This parameter is binary coded (see table for the high byte). To the low byte the TestMode from the configuration file will be added.

Bit 0	move plate carrier to transport lock position
-------	---

Examples:

```
R_Init 0           normal initialization
R_Init $0100      initialization including a movement of the plate carrier to the transport lock position
```

Note: The high byte of the InitMode parameter will only be used if a firmware version 1.20 or higher is installed.

8.4.2 Plate Carrier Movement

R_PlateOut

Move plate carrier out of the reader to stack position 1.

R_PlateOutR

Move plate carrier out and to the right side (stack position 2).

R_PlateIn

Move plate carrier into the reader.

R_PlateUser {x} {y}

Move plate carrier to position x, y.

8.4.3 Incubator Control

R_Temp {n}

Switch the built in incubator of the reader on and set {n} as target temperature. The allowed temperature range for the standard incubator is 25.0 ... 45.0. If your reader is equipped with an extended incubator you can use the range 10.0 ... 60.0.

Use R_Temp 0 to switch the incubator off.

Use R_Temp 0.1 to switch the **temperature monitoring function** on without using the incubator.

Notes: The target temperature should be at least 5 degrees above the ambient temperature.

You can use this command together with a **wait for temp** command.

When the incubator is switched on or the temperature monitoring function is used, the current temperature will be displayed inside the icon bar of the main program window.

8.4.4 Gain Adjustment

Notes: Gain adjustment is not necessary for absorbance protocols when using a firmware version 1.20 or higher. Gain adjustment over the whole plate (R_GainPlate) is not available for fluorescence polarization or dual emission protocols.

R_GainPlate {Protocol name} ({Path to protocol definition}) {Desired raw result for channel 1} {Desired raw result for channel 2} {Chromatic number}

Perform a gain adjustment for all measurement points of the plate using the declared parameters.

The parameter {Path to protocol definition} is optional. Use this, if you want to use a test protocol belonging to a different user account.

Please specify the desired raw result (required value) in % of the measurement range.

Example:

```
R_GainPlate "QC Test" 80 0 1
```

R_GainWell {Protocol name} ({Path to protocol definition}) {Well column} {Well row} {Desired raw result for channel 1} {Desired raw result for channel 2} {Chromatic number} ({Target mP value for fluorescence polarization protocols})

Perform a gain adjustment on the specified well using the declared parameters.

The parameter {Path to protocol definition} is optional.

Example:

```
R_GainWell "FAST TEST" 5 2 80 0 1
```

(Perform gain adjustment for channel A (req. value 80%) with the first filter setting used in the protocol (chromatic 1) on well B5.)

R_GainWell in Fluorescence Polarization Mode

In fluorescence polarization mode you can either define the required values for both channels or you can define the required value for channel A and a target mP value (to use this mode set the required value for channel B to 0). When using the second option, the software will calculate the necessary required value for channel B itself.

Example:

```
R_GainWell "FP PLATE" 2 2 80 0 1 35
```

Note: In fluorescence polarization mode a fine adjustment between the two channels will be performed automatically.

R_GetKFactor {Protocol name} ({Path to protocol definition}) {Well column} {Well row} {Chromatic No.} {Target polarization value}

Calibrate the ratio between the two measurement channels (fine adjustment for fluorescence polarization tests) using the declared parameters.

Note: This fine adjustment between the two channels will also be performed automatically during the R_GainWell command.

R_SetGain {Protocol Name} ({Path to protocol data base}) {Chromatic} {channel} {gain value}

Change the gain value defined in a test protocol to the specified value (range 0...4095).

Example:

```
R_SetGain "FP PLATE" 1 "B" 222
```

(Sets the gain value for chromatic 1 (=filter setting 1), channel B to 222.)

8.4.5 Run Commands

n:=R_CalculateTestDuration {Protocol name} ({Path to protocol definition})

Calculate the duration of executing a test protocol and stores this value (in seconds) in variable n.

ID1:=, ID2:=, ID3:=

Change the ID values used by the R_Run command. You can enter <Loop> to use the current value of the loop variable (For ...To command), e.g.: ID1:="Batch: <Loop>". You can also combine numerical variables with strings, e.g.: ID1:="Temperature=" Temp1. Here you can use all special functions that are described in the chapter 6.3 *Plate Identification*.

R_SetConfig {Measurement method}

This command is only necessary if the reader is not equipped with auto optic switching. It is used to change the reader configuration (measurement method). You can only start a test run using a test protocol matching the selected measurement mode, e.g. you can only start a luminescence test when the reader configuration is set to luminescence.

Parameter	Meaning
Measurement method	'F': fluorescence method
	'A': absorbance method
	'L': luminescence method
	'P': fluorescence polarization method

Note: You need to ensure that appropriate measurement heads are installed.

If you try to switch to an optional measurement method which is not available (e.g. luminescence on a reader without the luminescence option) you will get an error message.

R_SetSampleIDs {Protocol name} ({Path to protocol definition}) {Sample IDs filename}

This command can be used to load a set of sample IDs from a file (ASCII or Excel (XLS)). The file content should use the same syntax as for the 'Import IDs' function of the Sample IDs sheet of the Start Measurement dialogue. You can use this dialogue to create the sample IDs file.

Examples:

```
R_SetSampleIDs "FI TEST" "E:\SIDs.txt"
```

```
R_SetSampleIDs "TOM'S TEST" "c:\BMG\OPTIMA\Tom\Definit" "E:\SIDs.xls"
```

If the sample IDs file contains dilution factors, these factors will also be imported. If the file contains entries for wells which are not used in the selected test protocol, these entries will be ignored (e.g. well A13 for a protocol using a 96 well plate). If the Sample IDs file contains invalid entries, e.g. an impossible well name like A49 or XYZ, there will be an error message.

R_ClearSampleIDs {Protocol name} ({Path to protocol definition})

The command R_ClearSampleIDs can be used to clear the sample IDs associated with the specified protocol.

R_ClearDilutionFactors {Protocol name} ({Path to protocol definition})

This command will reset all dilution factors associated with the specified protocol to 1.

R_Run {Protocol name} ({Path to protocol definition}) ({Path for measurement data})

Start a measurement using the declared parameters.

Specifying the path to the protocol definition and specifying the path for measurement data is optional (useful, if you want to perform a protocol belonging to a different user), but it is not possible to only specify the path for measurement data. If you omit these parameters, the path belonging to the user currently logged in will be used.

8.5 System Variables

There are a few predefined variables you can use in scripts.

8.5.1 Numerical System Variables

<Temp> current temperature of the incubator built into the reader (floating point value with 0.1 °C resolution)

Note: To use this variable it is necessary to switch the incubator on (R_Temp {n}) or to use the temperature monitoring function (R_Temp 0.1).

After Gain Adjustment:

<GainA> gain value for channel A obtained during gain adjustment

<GainB> gain value for channel A obtained during gain adjustment

<RawResultA> row result obtained during gain adjustment for channel A

<RawResultB> row result obtained during gain adjustment for channel A

<GainAdjWellColA> column of well with highest signal found during R_GainPlate (gain adjustment over the whole plate) for channel A

<GainAdjWellRowA> row of well with highest signal found during R_GainPlate for channel A

<KFactor> K factor (fine adjustment between the two channels) obtained during R_GainWell or R_GetKFactor in fluorescence polarization mode (floating point value)

Note: To convert a column and row value into a well name (e.g. column 5, row 3 => C5), use the function **<WellName(Column, Row)>**.

8.5.2 String Type System Variables

<User> This system variable contains the name of the user currently logged in (see chapter 1).

<ProtocolPath> and

<DataPath>

These variables contain the path to the test protocol definition data base and the path for storing the measurement data in dBase format (measurement data base). Before using the R_Run command (see chapter 8.4.5) these paths are the default paths belonging to the user currently logged in (as defined in the login screen or as selected using the 'Setup | Path' command, see chapter 1 and 3.6). If you use a R_Run command inside the script in combination with the optional path parameters (e.g. R_Run "FAST" "D:\My Files\OPTIMA\Definit" "D:\My Files\OPTIMA\Data") afterwards these system variables will show the paths used for the last run command. These variables are especially useful in combination with the Call and Execute commands (see chapter 8.10).

<ASCIIPath>

This variable contains the current path for storing the measurement data in ASCII format (as defined using 'Setup | Program Configuration | Save measurement data in ASCII format | Define format', see chapter 3.4).

Example:

Execute "Merge.exe <DataPath> <User> H ID1"

8.5.3 Boolean System Variable

<PlateCarrierOut> true, if the reader plate carrier is outside the reader

You can use this boolean variable in combination with the 'if ... then' command.

Examples:

```
if <PlateCarrierOut> then ...
```

(perform the command after 'then' only if the reader plate carrier is outside the reader)

```
if Not(<PlateCarrierOut>) then ...
```

(perform the command after 'then' only if the reader plate carrier is inside the reader)

Note: It is not possible to use this boolean variable anywhere else than in comparisons; it is not possible to use this variable in calculations or as part of strings.

In addition, it is currently not possible to define your own boolean variables and use these for comparisons in the above style (use numeric variables and comparisons in the style 'if MyVar<>0 then ...' or 'if MyVar=0 then ...' instead of boolean variables).

8.6 Numerical Variables

You can use up to 100 numerical variables (type double float, 8 bytes precision) in your scripts. In addition, up to 10 string variables can be used (see chapter 8.7). You do not need to declare the variables. A variable will be automatically initialized with a value of 0.0 when it is used first time.

Variable Names

A variable name can be up to 50 characters long. You can use letters and numbers for the name, but it must start with a letter. Example for correct variable names: i, Temp, Temp2.

Variable names are case insensitive.

Do not use any of the reserved words: begin, break, do, empty, end, flying, for, halt, ID1, ID2, ID3, if, next, no:, NotLastPlate, plate, restart, StopperZPosIsDifferent, TestPlateOnReaderCarrier, TestPlateOn-StackerTable, then, to, wait, yes:.

Please also do not use any of the command names for variables, which means anything beginning with R_ and S_ or AddToMemo, Ask, Beep, Call, ClearMemo, CloseMemo, Execute, ShowMsg / ShowMessage and Terminate or any of the names of system variables: Barcode, LastPlateDetected, Magazine1In, Magazine2In, PlateCarrierOut, PlatesIn1 or PlatesIn2. In addition, do not use st0 ... st9, as these are the predefined names for the string variables.

Mathematical Operations

Available operations: addition (+), subtraction (-), multiplication (*) and division (/). Only one operation per line is allowed.

Limitations

Use only one command / mathematical operation per line.

It is not possible to combine calculations with other commands.

Wrong:

```
R_Temp Temp + 1
```

Correct:

```
Temp := Temp + 1
R_Temp Temp
```

One operation between 'for' and 'to' is allowed, but not after 'to'.

Wrong:

```
for i:=1 to 3*a do begin
```

Correct:

```
b := 3*a
for i:=1 to b do begin
```

Also allowed:

```
for i:=4*c to d do begin
    ...
    if <Barcode_String>="abc" then break ;continue with first command after end;
end;
```

8.6.1 Converting Numerical Values into String Values

To use a numerical value as part of a string value, e.g. for the test name parameter of the R_Run command, you need to use the `<IntToStr(n)>` or `<FloatToStr(n)>` conversion function.

Examples:

```
for i:=1 to NoOfRuns do begin
  R_Run "Test <IntToStr(i)>"
end;
```

```
Temp:=36.5
st1:="<FloatToStr(Temp)>"
```

When the variable `n` contains a real number, `<IntToStr(n)>` will return the integer part of `n`; that is, `n` rounded toward zero, and then converted into a string.

`<FloatToStr(n)>` will return the real number converted into a string.

For example

```
X:=2.5
ID1:="Plate <IntToStr(X)>"
ID2:="Plate <FloatToStr(X)>"
```

will result in `ID1=Plate 2` and `ID2=Plate 2.5`.

You can use the `<IntToStr(n)>` and `<FloatToStr(n)>` functions in your scripts on all places where a string is used. These functions need to be inserted inside the quotation marks.

8.7 String Variables

You can use up to 10 string variables in your scripts. The name of these variables need to start with 'st' followed by a number '0'... '9' (st0, st1, ... st9). You do not need to declare the variables. The string variables will be initialized with an empty string.

To assign a value to a string variable you can use string constants, string variables, numerical variables or any combination of these.

Examples:

```
st1:="This is a string."  
st2:="Current temperature: <Temp>°C"  
st3:="<st1> <st2>"  
st4:="The value of n is " n "." or  
st4:="The value of n is <FloatToStr(n)>."
```

You can use a string variable for all commands where a string parameter is expected. You need to enter the name of the string variable in <brackets> inside the quotation marks.

Examples:

```
ShowMsg "<st3>!" info  
R_Run "<st4>"
```

It is also possible, to compare strings.

Examples:

```
if st1="value" then halt  
if st2<>st3 then R_Run "TOMS TEST"
```

Note: Everything, which can be done with string variables, can also be done with the three plate identifiers ID1 ... ID3. The only difference is, that you can use some special functions for the plate IDs, as for example ID1:="Date: <date>" (see chapter 6.3).

8.8 Measurement Data

8.8.1 R_GetData

To get access to the measurement data you can use the **R_GetData** command. This is useful, if you want to perform different actions in your script depending on the measurement values.

Syntax:

Variable := **R_GetData** {well name} {cycle/interval} {chromatic} {channel}

or

Variable := **R_GetData** {well column} {well row} {cycle/interval} {chromatic} {channel}

When the well name is computed (e.g. inside a loop) the numeric version is easier to use, but the alphanumeric version is easier to read. When using the alphanumeric version, the well name is case sensitive (the rows in 1536 well plates are named A...Z and a...f).

The cycle/interval, the chromatic and the channel parameters are optional. If you do not specify these parameters, cycle/interval 1, chromatic 1 and channel A will be used.

Examples:

Value:=R_GetData "A1" 1 2 "B" *Get the measurement value for well A1, cycle 1, chromatic 2, channel B.*
Value:=R_GetData "B09" 3 *Get the measurement value for well B9, cycle 3, chromatic 1, channel A.*
Value:=R_GetData 4 5 3 *Get the measurement value for well E4, cycle 3, chromatic 1, channel A.*
Value:=R_GetData "a12" *Get the measurement value for well a12 (1536 well plate), cycle 1, chromatic 1, channel A.*

Note: The R_GetData command uses the dBase format measurement files and will therefore only work if the 'Save measurement data in dBase format' option has not been switched off (menu command 'Setup | Program Configuration')!

8.8.2 R_GetRawDataFileNumber

To get the file number (= name) of the latest raw data file (<Number>.dbf), you can use the **R_GetRawDataFileNumber** command. This might be useful in combination with the call of an external program (see chapter 8.10), which performs some operations with the measurement data, e.g. a merge of different measurement runs into a single run. Optionally it is possible to specify a data path for this command as parameter. If you do not specify the path, this command will return the last raw data file number from the Measure.dbf which was used during the last R_Run command or (if there was no R_Run command inside the script before using the R_GetRawDataFileNumber command) from the Measure.dbf inside the current measurement data path (see <DataPath> in chapter 8.5.2).

Syntax:

Variable := **R_GetRawDataFileNumber** {(Path to measurement data)}

8.9 Date and Time Functions

8.9.1 Using Date and Time in Numerical Variables

It is possible to assign the current date and time to numeric variables by using the following functions:

- N:=<now>** The integral part of the delivered value is the number of days that have passed since 12/30/1899. The fractional part is the fraction of a 24 hour day that has elapsed since midnight.
- T:=<time>** delivers the fraction of the current day that has elapsed since midnight
- D:=<date>** delivers the number of days that have passed since 12/30/1899

Using these functions, it is possible to measure run times and to write scripts where certain actions need to take place at a defined time after other actions.

Example:

```
Tstart:=<now>
R_Run "WHATEVER"
Tend:=<now>
DeltaT:=Tend-Tstart
DeltaT:=DeltaT * 86400 ;convert fractions of a day into seconds
WaitTime:=600 - DeltaT
wait for WaitTime s
;Next action ...
```

This example script will perform one measurement and will then wait the remaining time to 600 seconds before continuing with the following commands. This way you can achieve a distance of 10 minutes between the start of the first measurement and the following action.

Note: It is not allowed to combine the <now>, <date> and <time> commands with calculations.

Wrong:

```
DeltaT:=<now>-Tstart
```

Correct:

```
Tend:=<now>
DeltaT:=Tend-Tstart
```

or

```
DeltaT:=<now>
DeltaT:=DeltaT-Tstart
```

8.9.2 Using Date and Time in String Variables

It is also possible to assign the current date and time to string variables by using the <date> and <time> function.

<date> will be replaced by the date and **<time>** will be replaced by the time in the moment when this script line is processed. The date and time format can be specified in the same way as you can do when using <date> and <time> for the plate IDs. If you do not specify the format the “Long Date Format” / “Long Time Format” as defined in the windows control panel will be used.

Example:

```
R:=1
N:=5
st1:="Run: " R ", <date>, <time>, Plate: " N
st2:="Run: " R ", <date:yyyy/mm/dd>, <time:hh:mm>, Plate: " N
```

Note: If you assign <date> or <time> to any of the plate IDs, the date and time value will be assign in the moment, when this ID is used, e.g. when performing a R_Run command.

Assigning a time value to a string variable allows to use this value several times (it will not change unlike the time in ID1:="<time>").

8.10 Calling External Applications

Call {Command line} ({Execution style})

The command Call will start the specified external program and wait until this program has been finished (has been closed). First parameter for both commands is the command line of the program to be started (program name and optional command line parameters). The program name can contain a complete path. If no path information is provided, the sub directory \ExeDll of the OPTIMA installation directory is used (usually c:\Program Files\BMG\OPTIMA\ExeDll).

Example:

```
Call "C:\Program Files\Norton Commander\NC.exe"
```

The program will be started in its default size. By default it will not be activated (the input focus remains on the OPTIMA program).

Optionally you can define the execution style by adding the word Activated, Minimized or Hidden as a second parameter.

When using **Activated**, the program will be started and the input focus will be shifted to this program.

Example:

```
Call "D:\Programme\Norton Commander\NC.exe" Activated
```

When using **Minimized**, the program will be started in a minimized version (iconized). When using **Hidden**, the external program will be started invisibly (as long as the program does not open a window, e.g. an error message, by itself).

Example:

```
Call "Merge.exe <DataPath> <User> H ID1" Hidden
```

Note: It does not make sense to combine the options Activated, Minimized and Hidden, therefore, it is only allowed to use one of these options per Call command.

Execute {Command line} ({Execution style})

The command Execute will also start the specified program, but will not wait until the external program has been closed. You can use the same parameters as described above for the Call command.

Example:

```
Execute "Merge.exe <DataPath> <User> H ID1" Hidden
```

The command Execute can also be used to open files as long as Windows can recognize the file type.

Examples:

```
Execute "D:\Picture.bmp" ;opens the specified picture using the program  
;associated with *.bmp files
```



```
Execute "OPTIMA.hlp" ;opens the OPTIMA online help
```

9 Known Problems and Solutions

1. Disabled Plate In / Out Buttons
 2. Access Rights Problems under Windows NT / 2000 / XP
- For solutions see the following chapters.

9.1 Disabled Plate In / Out Buttons



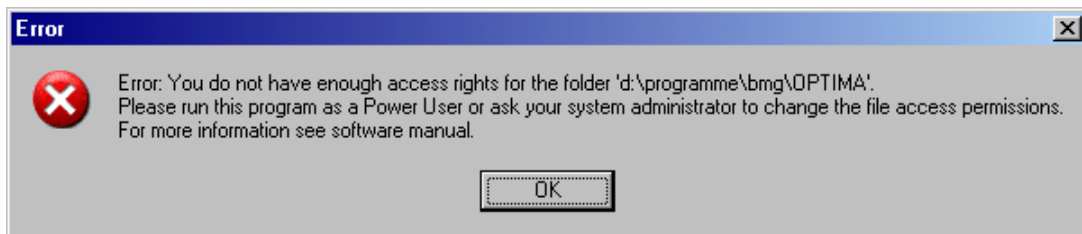
If both plate buttons ( , ) are disabled and there is no test run active and the communication to the reader is OK, then it is most likely that there is a wrong setting in the configuration file '`~\Program Files\BMG\OPTIMA\FLUOstar OPTIMA.ini`'. The value behind '`DisablePlateCmds=`' should be '`False`'. This parameter will be set to '`True`' if the reader is connected to a robotic/stacker system and the plate in/out movement will be controlled only using the robotic software.

9.2 Access Rights under Windows NT / 2000 / XP

9.2.1 File Access Permissions

Microsoft changed the default access permissions for all program files newly installed. In all Windows versions before Windows 2000, any standard user has access to the files installed using a standard installation program. Beginning with Windows 2000, a normal user (non-power user) has only read access by default. As we store important information in data base files, all users of the BMG LABTECH software need to have write access to certain files in the program directory (usually `~\Program Files\BMG\OPTIMA`).

If you have used the option '**Program should be usable also for non power users**' during installation (see Software manual part I), the write access rights to the necessary files will be set during the installation. If you did not use this option or if you have added new users / user groups after installation, the following error might appear:



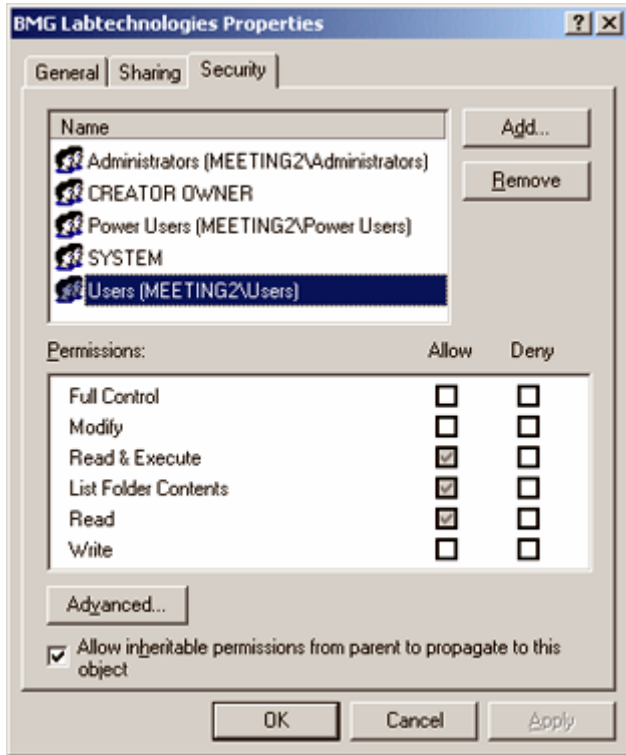
Solution

Logon as administrator (or user with administrative rights).

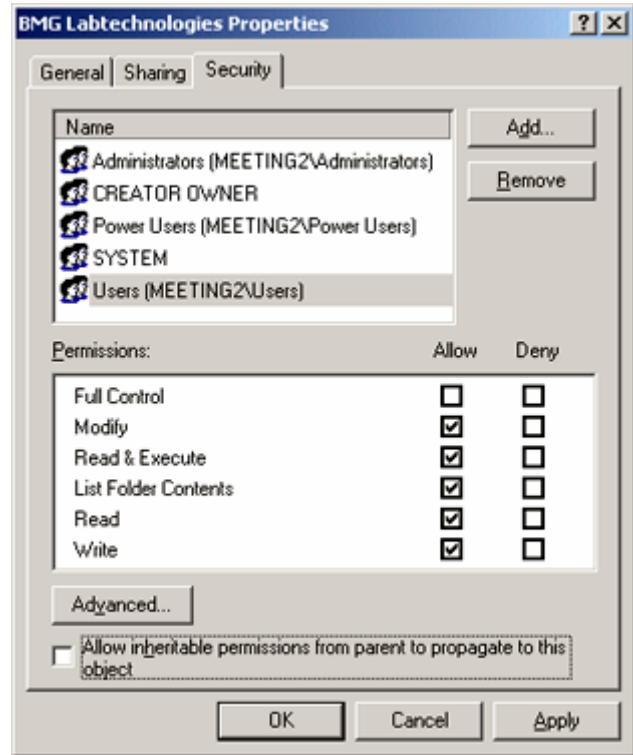
Run the program '**SetPermission**' from the OPTIMA CD-ROM (folder `~:\OPTIMA Vx.xx\`).

Alternatively, you can also manually change the permissions: Open the Windows Explorer and right-click the folder where the BMG LABTECH software is installed (usually `~\Program Files\BMG\OPTIMA`). Choose 'Properties', then 'Security'. Check the 'Modify' permission, 'Write' permission will be checked automatically. Uncheck the box 'Allow inheritable permissions from parent to propagate to this object'. Now all users should be able to write to the BMG LABTECH directory and work with the software.

Default settings:



Customized settings:



Listing of files where write access is necessary

The following files / directories need to have full access to write (temporary) data.

BMG LABTECH Software		
Directory	File	Usage
~:\Program Files\BMG\OPTIMA\	FLUOstar OPTIMA.ini * OPTIMA.rns OPTIMA.log	Configuration file Run Statistik File Log file
~:\Program Files\BMG\OPTIMA\Temp		Directory for temporary files
~:\Program Files\BMG\OPTIMA\User		Subdirectories for test protocol definitions and measurement data of standard user
~:\Program Files\BMG\OPTIMA\Admin		Subdirectories for test protocol definitions and measurement data of administrator
~:\Program Files\BMG\OPTIMA\ <UserName>		Subdirectories for test protocol definitions and measurement data of defined users
~:\Program Files\BMG_Flashtools		Used by Flash-EPROM Update Program

* This file is named 'FLUOstar OPTIMA.ini' instead of 'OPTIMA.ini' for compatibility reasons.

Borland Database Engine		
Directory	File	Usage
~:\Program Files\Borland\Common Files\BDE\	idapi32.cfg	BDE properties
	idapi32.bak	Backup of idapi32.cfg
~:\ You can use the BDE Administrator to change the location of the pdoxusr.net file (Configuration Drivers Native Paradox Net Dir).	pdoxusr.net	Paradox network control file

9.2.2 Registry Access

There are two places in the registry where the BMG LABTECH software stores information:

All user specific things, like program settings (e.g. the program window position or current state display options), are stored in the registry part `HKEY_CURRENT_USER`. There are no access problems to this part.

All settings which are important for all users, like the selected communication port or reading mode, will be stored in the `HKEY_LOCAL_MACHINE` part of the registry (as this part of the registry is intended for non-user specific information). For unintelligible reasons, Microsoft changed the default access permissions for this part of the registry beginning with Windows 2000. On all Windows versions before Windows 2000, every user has read and write access to this general information part of the local registry, beginning with version 2000 a standard user (non-power user), by default, only gets read access to newly generated keys in this part of the Windows registry.

This access limitation can cause the Error: '0509: The program has not been correctly installed or you are using W2000/XP without appropriate registry access rights.' at program start if you did not use the option '**Program should be usable also for non power users**' during installation (see Software manual part I).

Solution

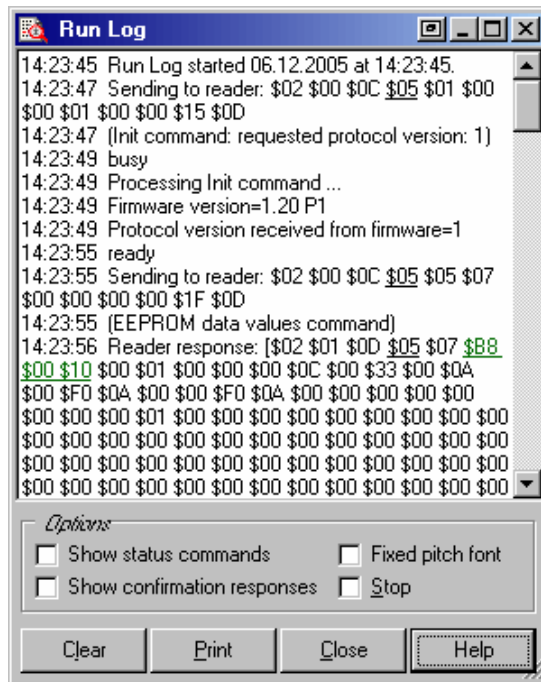
- Logon as Administrator (or a user with administrative rights).
- Run the program '**SetPermission**' from the OPTIMA CD-ROM (folder ~:\OPTIMA Vx.xx\).

or

- Start the program 'Regedt32' (Windows XP: 'Regedit', use the 'Run' from windows start menu).
- Go to 'HKEY_LOCAL_MACHINE\Software\BMG Labtechnologies'.
- Select 'Permissions'.
- Add read and write rights for everyone to this key and all sub keys.

9.3 Run Log Window

The Run Log window (control part) shows all commands sent to the reader and all responses. You can open the Run Log window using the key combination [Shift]+[Ctrl]+[L] from the program main window.



It is possible to stop the process of adding new entries to the run log by checking the 'Stop' checkbox.

You can **print** the entire run log or a marked part of the run log.

9.3.1 Search Function

It is possible to search the log window forward or backward beginning from the cursor position or from the top / end of the text.

The following key combinations can be used to activate the search function:

1. Borland Style:

To open the search parameters window use [Ctrl]+[Q][F], to repeat the last search (in the defined search direction) use [Ctrl]+[L]. Use [Shift]+[Ctrl]+[L] to search against the defined direction, e.g. to go back to the previous occurrence.

2. Microsoft Style:

To open the search parameters window use [Ctrl]+[F], to search the next occurrence in forward direction use [F3], for backward direction use [Shift]+[F3].

If you select something before using the [Ctrl]+[Q][F] / [Ctrl]+[F] key combination this selection will be used as default search string, otherwise if the cursor is positioned onto a word (something containing letters, not just hexadecimal numbers) this word will be taken as default value.

Note: Searching something, which includes line breaks, is not possible. You might change the width of the Run Log window to remove soft line breaks.

9.3.2 Run Log File

The run log will automatically be saved into the file 'OPTIMA.log' in the OPTIMA main directory (usually '~\Program Files\BMG\OPTIMA\'). You can switch off the generation of the run log file by changing the line 'RunLog=true' in the configuration file '~\Program Files\BMG\OPTIMA\FLUOstar OPTIMA.ini' (section [Debug]) into 'RunLog=false'.

Note: At the next program start the existing content of this file will be erased. Whenever you need support from BMG LABTECH due to a software / firmware malfunction you should send us the log file together with a description of the nature of the problem.

10 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
- Problems / questions regarding the instruments:
tech.service@bmglabtech.com

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

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