BMG LABTECH

Omega

Software Manual – Part II: Control Software

Version 5.10

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

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

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

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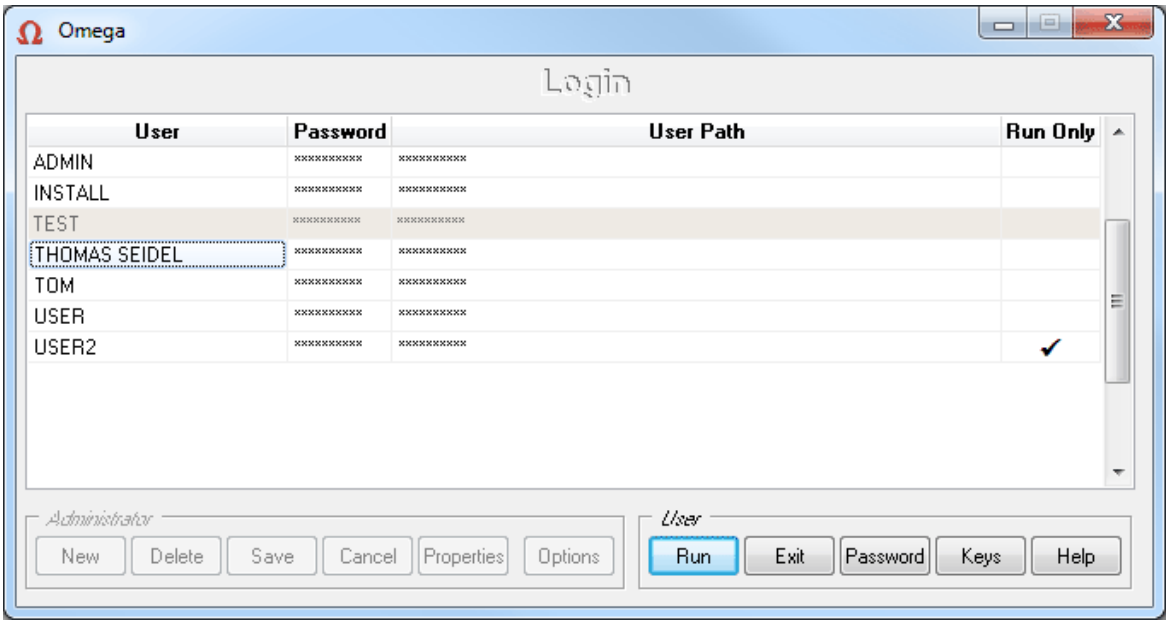
1 Login Screen

A login screen appears when the control or data analysis part of the software is opened. 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.

The Login Screen function can be switched off, see chapter 3.3.1 *Program Configuration*.



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 can only start pre-defined test protocols. The user can perform a gain adjustment and enter plate / sample IDs. This type of user cannot create, change, delete, copy, import or export new or existing test protocols, microplate definitions, or offset / filter values.

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’.

Hint: Instead of setting or unsetting this option every time a protocol change is necessary, two user entries can use the same User Path, one of these entries with the Run Only option and one without.

1.1 User Functions

Run

After clicking ‘Run’, the Omega 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

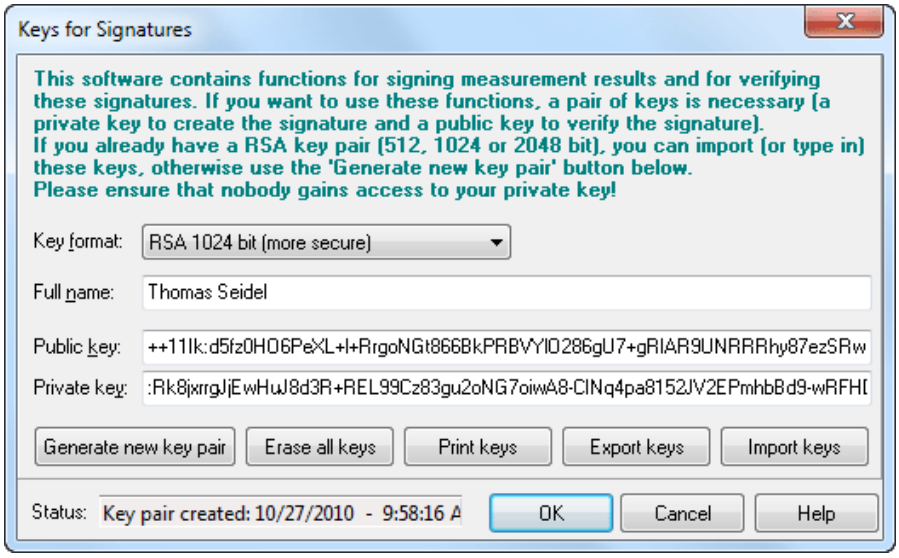
Use this button to change your password. Password restrictions might apply (see chapter 1.3.1 *Password Policies and Password Aging*).

Keys

This button opens the ‘Keys for Signatures’ dialogue box (see below).

1.1.1 Signature Keys

If you want to digitally sign data records (see also Software manual part IV: FDA 21 CFR part 11) 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 take longer. Generating the key pair requires the largest amount of time; 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, to avoid that anybody else could sign a test run under your name! **Please ensure that nobody gains access to your private key**.

If you already have an 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 and can be used 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=2007-07-22  -  16:17:14
Key Format=RSA, 1024 bit
PrivateKey=:QKUrnq1p3zpjFky+3O-M0QssGnRYRRJ9Hb8wEdDyYO8RDlwi5PtWpJnzc …
PublicKey=++11Ik:cDGk7Ran3sVSxNkhjirqMNLDKXK-7qHoa1-8T-vLo5xMYTe2SaRI …

NumberOfOldPublicKeys=2

[OldKey1]
Name= Thomas Seidel
Created=2007-07-22  -  16:16:54
Key Format=RSA, 512 bit
PublicKey=++11Ik:h+BJtQm6NxBMDbIh4fpGZEs4ujF1GgOrCq+RWjaCygf3GcCee+6S …

[OldKey2]
Name= Thomas Seidel
Created=2007-07-22  -  16:12:38
Key Format=RSA, 512 bit
PublicKey=++11Ik:p4SIFP6ngf1vxM9v+CB5OFO0zb3dNeOxcdOwG9OGmDybeUf0lxX6 …
```

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; this can be used to 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

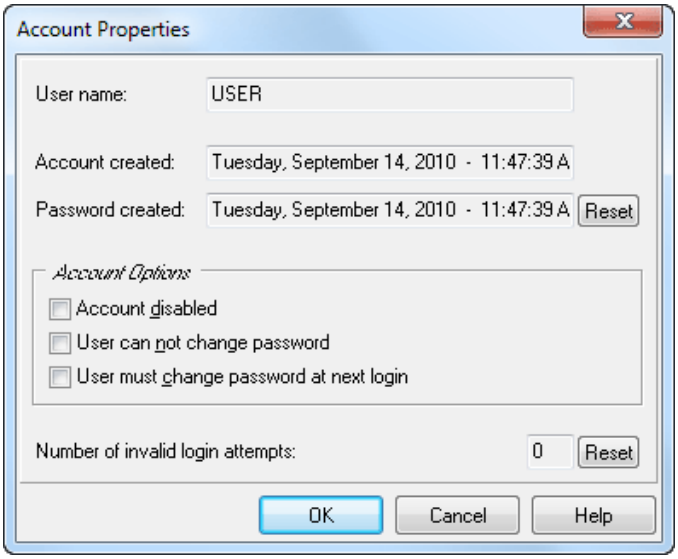
After clicking the ‘Save’ button all changes made will be saved in the user data base.

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 can 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 box will appear (see chapter 1.3 *Administrator Options*).

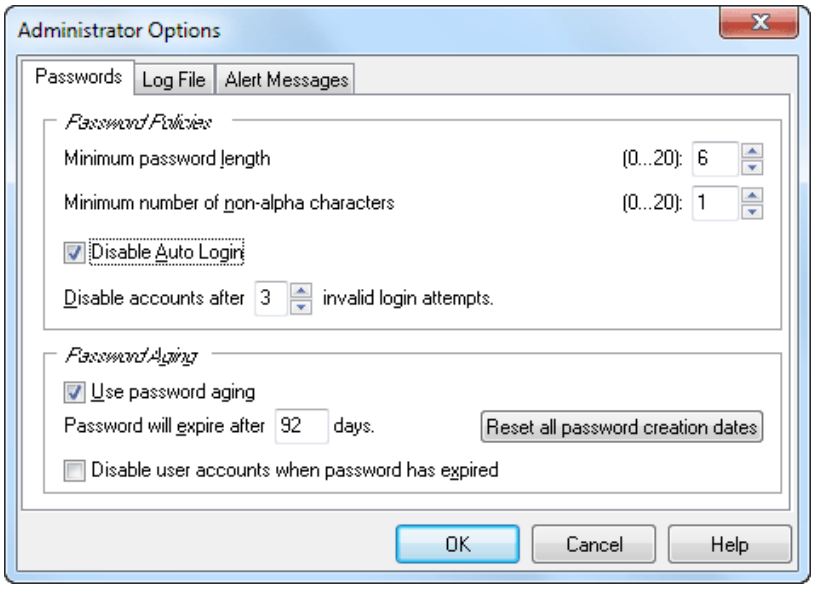
Note: Exporting the user table into a XLS (Excel), text or HTML file is possible after right clicking on the table.

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 here (see chapter 3.3.1 *Program Configuration*).

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 11.

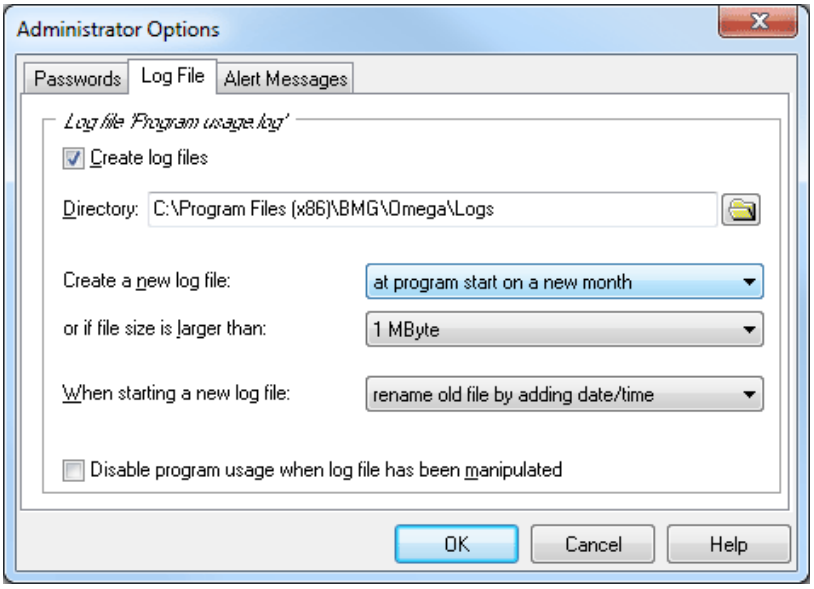
Password Aging

If you want to make passwords 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. This can be done 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 prevent existing passwords from expiring.

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 11.

1.3.2 Program Usage Log File

The BMG LABTECH software can create a log file (named 'Program usage.log') that contains 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.



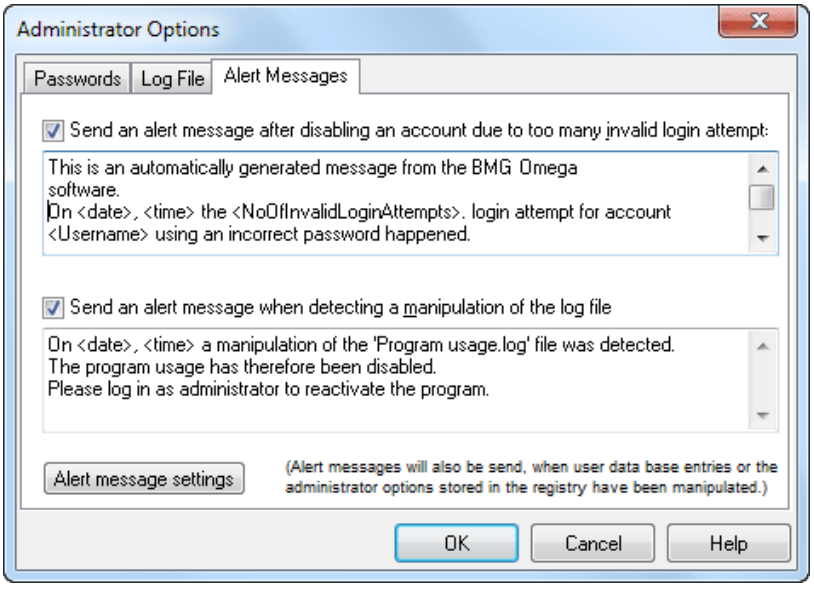
Here you can also specify the directory where this file is to be stored and under which conditions a new file should be started. When starting a new log file, the old file might be renamed or erased. The log file will be protected against manipulation by calculating a cryptographically secure hash value. 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 Software manual part IV: FDA 21 CFR part 11) 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 which contains the communication between reader and computer (see chapter 10.3 *Run Log Window*). This file is intended to deliver background information in case of problems.

1.3.3 Alert Messages

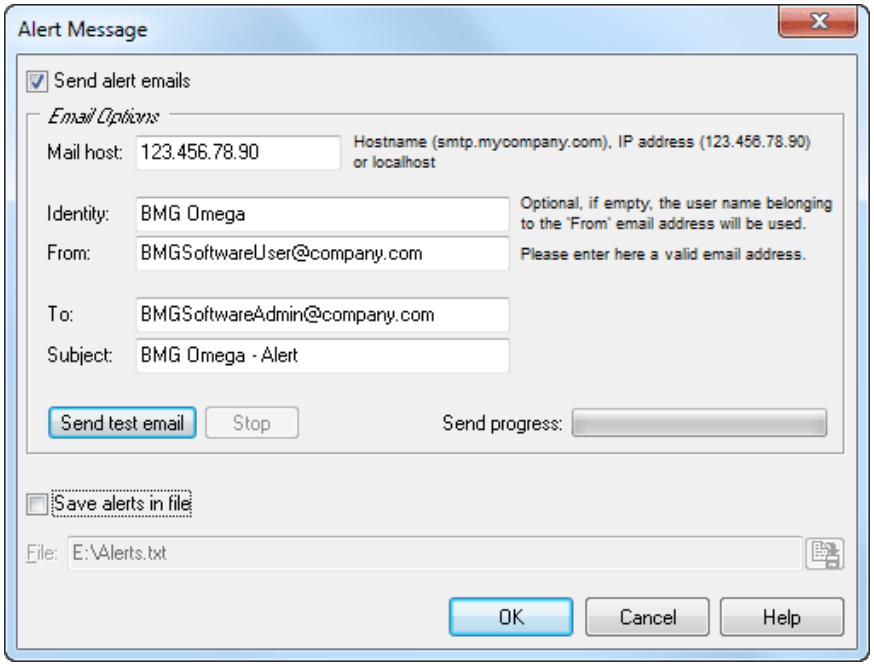
By using the third sheet of the administrator options dialogue, it is possible to define alert messages, which can be sent under certain circumstances via email (e.g. to the administrator) or saved into an alert message file.



You can define a message, which will be sent after disabling an account due to too many invalid login trials and you can 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>

- Data
- Definit

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.*' files (test runs overview data base table).

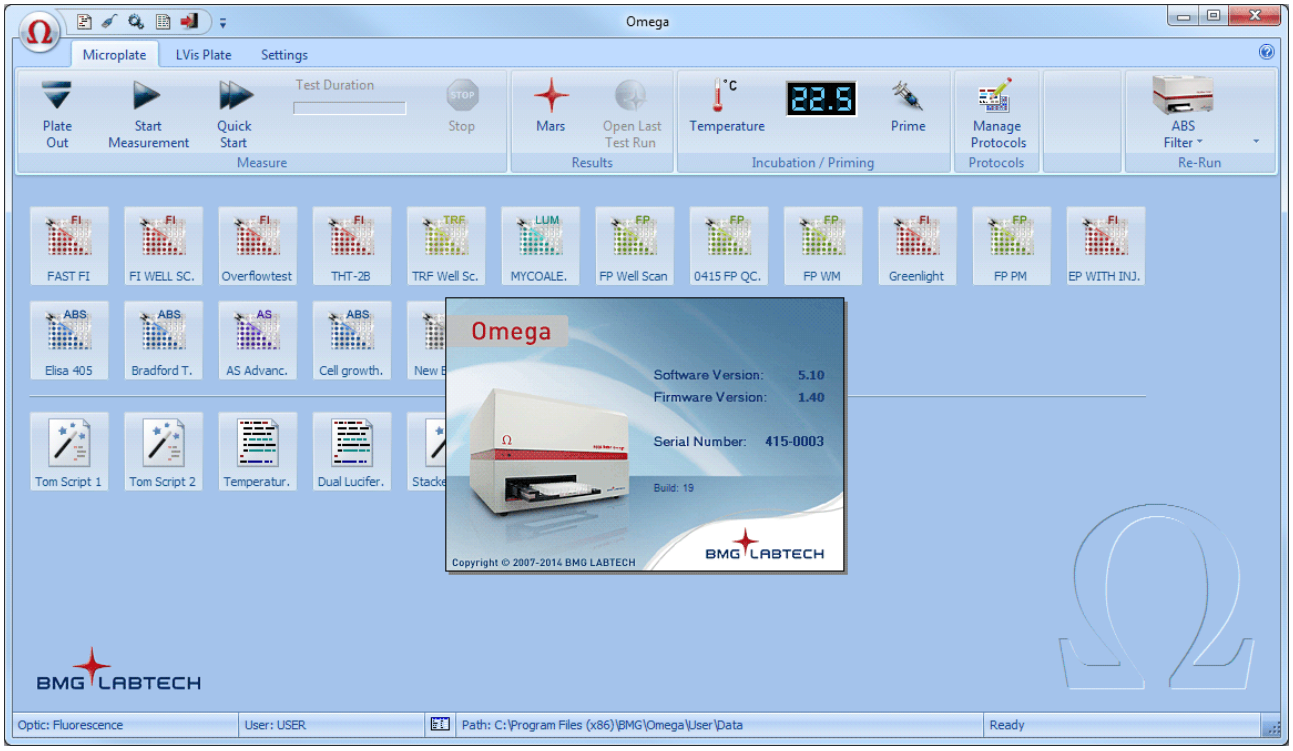
It is possible, to redirect the path of the data and to store the information in another directory at any time. Do this in the Omega control software by selecting '**Login**' under the Application Button in the top left corner (see also chapter 2.2.1: *Application Button Menu*)

Hint: The '**Login**' dialogue can also be opened by double clicking on the '**User:**' section of the status bar at the bottom of the Omega software.

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. This information is also accessible by clicking on the Application Button in the top left corner and then selecting '**About Omega**'.

The status bar at the bottom of the main screen shows the optic currently installed, the user logged in and the path used for storing the measurement results. These settings can be changed by clicking that field of the status bar (when clicking the optic field only an information window will appear).

The small icon to the 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).

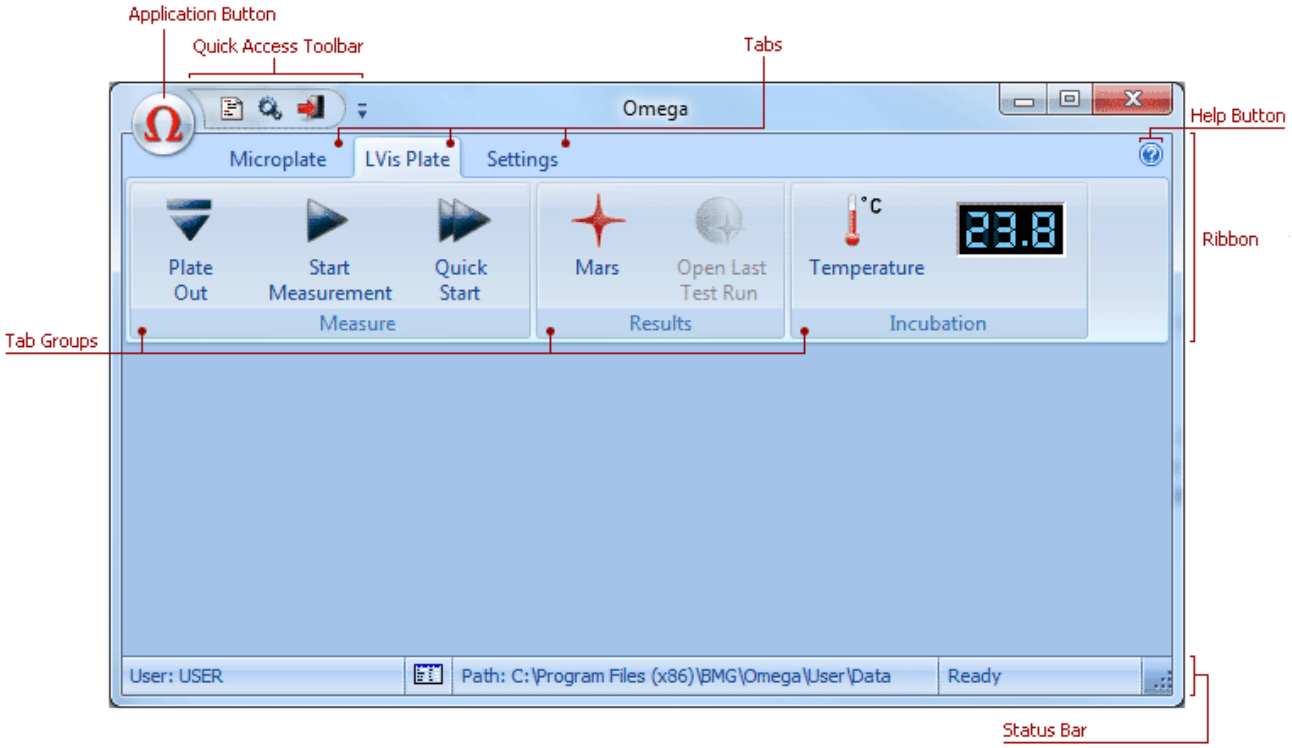
In the last part of the status bar, the current reader status will be shown.

Notes: To change the style of the program window use 'View' under 'Settings Tab' and 'Program Configuration' (see chapter 3.3.3).

The first section of the status bar does not exist when using a LUMIstar or SPECTROstar Omega or NEPHELOstar Plus reader, as these readers can only measure using one method (luminescence, absorbance or nephelometry).

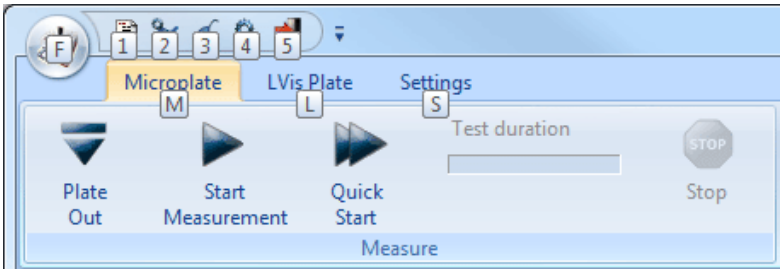
2.2 Menu Commands

The Omega software uses a ribbon style menu, seen in the newer Windows software:



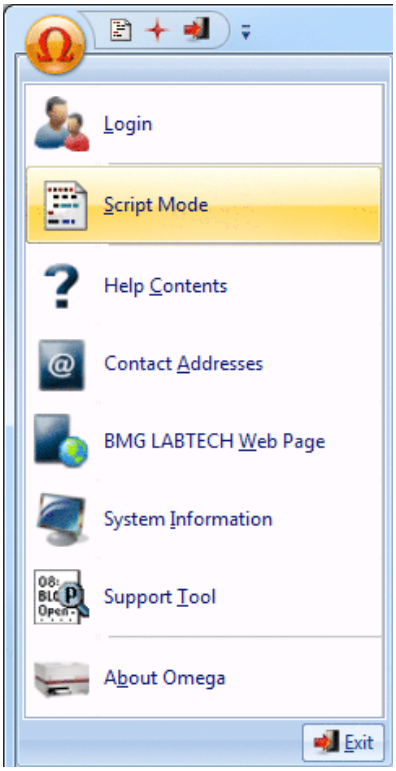
You can minimize the ribbon using the popup menu (right click onto the ribbon) or by double clicking a tab header or by using the key combination [Ctrl]+[F1].

If you want to use the keyboard to access the menu please press the [Alt] key. The key strokes necessary to activate a function will be shown:



2.2.1 Application Button Menu

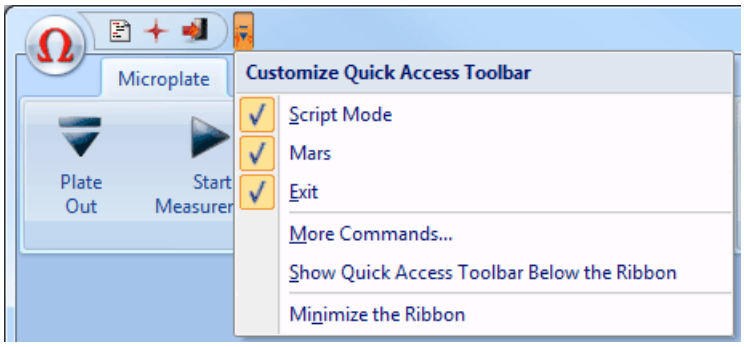
The Application Button, located in the top left corner, can be used to access certain software features.



Application Button Commands	Icon	Function
Login		Opens the login window.
Script Mode		Opens the script mode window.[1]
Help Contents		Opens the online help. *Hint:* **Hit F1** and a page specific Help menu pops up.
Contact Addresses		Shows BMG LABTECH's contact addresses.
BMG LABTECH Web Page		Starts an internet browser and makes a connection to the BMG LABTECH web page.
System Information		Shows information about the operating system, the CPU speed and the memory available.
Support Tool		Starts the BMG LABTECH Support Tool.
About Omega		Shows the software and firmware version.

[1] Script mode is not available when in ActiveX or DDE mode, e.g. as part of a robotic system.b

2.2.2 Quick Access Toolbar



There are three predefined Quick Access Toolbar buttons – Script Mode, Mars and Exit. The Quick Access Toolbar can be modified by the right drop down menu.

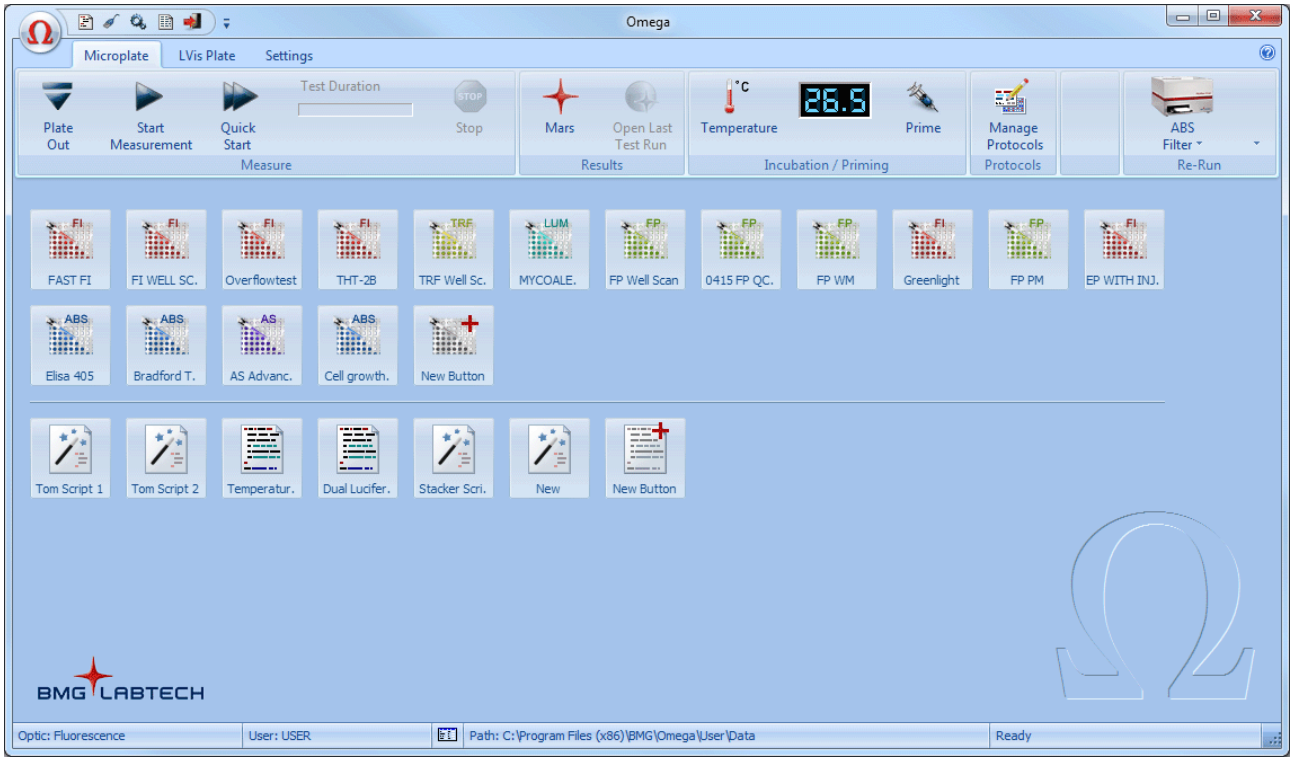
Quick Access Toolbar Commands	Icon	Function
Script Mode		Opens the script mode window.[1]
MARS		Opens BMG LABTECH's MARS Data Analysis software.
Exit		Exits Omega control software.

[1]Script mode is not available when in ActiveX or DDE mode, e.g. as part of a robotic system.

Hint: Right click on most icons will give the option to 'Add to Quick Access Toolbar'.

2.2.3 Microplate Tab

From this tab, microplate measurements are started; results can be opened; incubation is controlled; protocols can be managed; and previous test protocols can be re-run.



Microplate Tab Commands		Icon	Function
Measure tab group	Plate Out		Moves microplate out of the instrument.[1]
	Plate In		Moves microplate into the instrument.[1]
	Start Measurement		Starts a measurement using a pre-defined test protocol. Before the measurement starts, plate and sample IDs can be entered.
	Quick Start		Performs an end point measurement using the full plate without defining a test protocol. Select wavelength, plate type and IDs.
	Current State Graphics		Opens the current state display.
	Pause		Interrupts the active test run after finishing the current cycle (only available for kinetic endpoint measurements).[2]
	Stop		Stops the active test run or the execution of a script.
Results tab group	MARS		Opens BMG LABTECH's MARS Data Analysis software.
	Open Last Test Run		Opens the last test run performed using MARS.
Incubation / Priming	Temperature Control		Opens the temperature dialogue box to set the incubator.
	Priming		Prepares the pumps for injection, only available if pumps are installed.
Protocols	Manage Protocols		Opens the manage test protocol dialogue box, where all test protocol parameters can be edited or created anew.

Microplate Tab Commands		Icon	Function
Re-Run tab group	Re-Run <Protocol Name>		Top part of this button repeats the execution of the last used test protocol. The bottom arrow lets users edit this protocol, change IDs or delete the protocol from the re-run list.
	Re-Run List (Right down arrow)		Shows a list of up to the 10 last used test protocols. Clicking one of the entries will repeat the execution of this protocol.

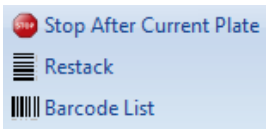
[1] This option is not available when the reader is connect to a stacker and magazine 1 is inserted, as here the plate movement from the source magazine into the reader and out of the reader to the destination magazine after the measurement, is part of the batch or single plate measurement routine.

If the plate button is disabled and the reader is not attached to a stacker and there is no measurement or other action active and the communication to the reader is OK, then it is most likely that there is an incorrect setting in the configuration file '`~\Program Files\BMG\Omega\Omega.ini`'. The value behind '`DisablePlateCmds=`' should be '`False`'. This parameter will be set to '`True`' if the reader is used in a robotic system, as here the plate in/out movement will be only controlled using the robotic software.

[2] The 'Pause After Current Cycle' function is not available when using a stacker or in script mode (see chapter 6.8). Due to technical reasons this pause function is also not available when performing absorbance spectra plate mode measurements.

Stacker Functions

When a stacker is connected to the reader the following three additional functions will be offered inside the Measure tab group:



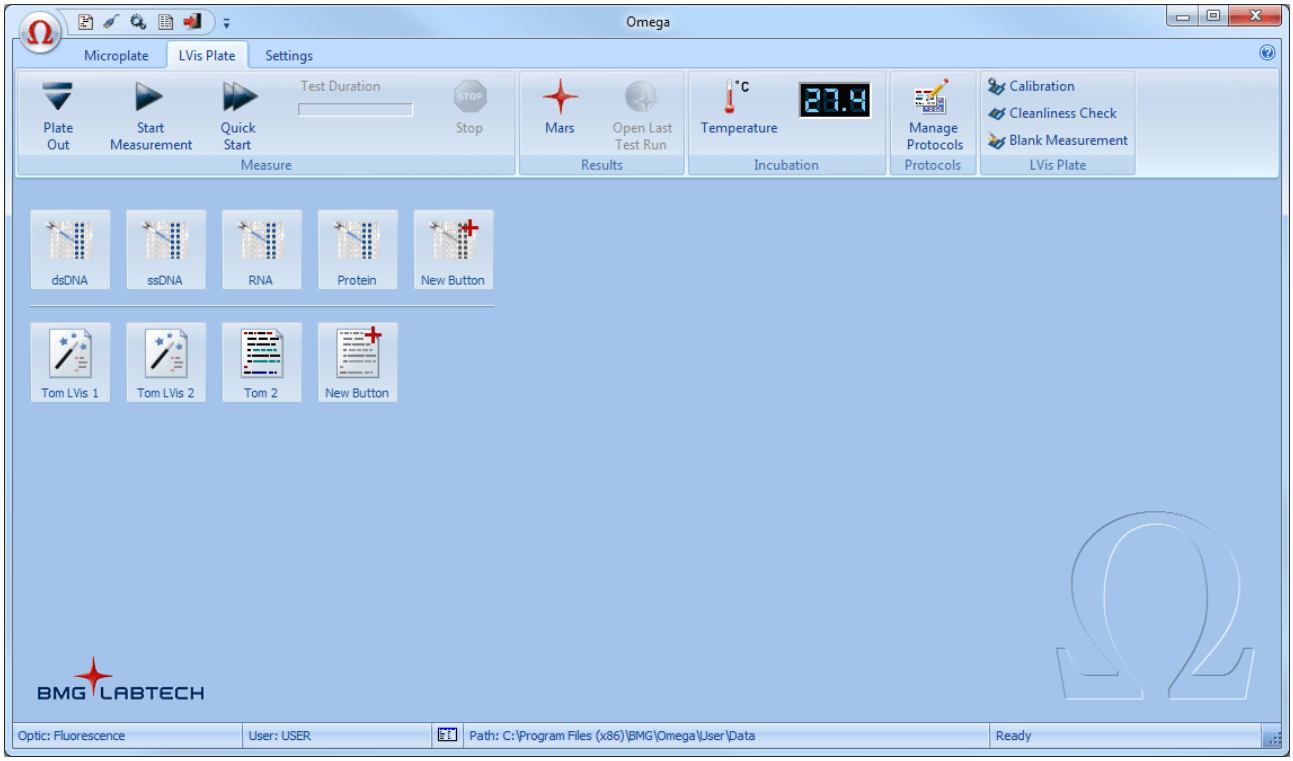
Microplate Tab Commands	Icon	Function
Stop After Current Plate		This function is only available in batch mode (reader with a stacker attached) or in script mode when a script containing batch measurements is used. After selecting this function the measurement of the current plate will be finished and this plate will be transferred to the destination stack. Then the batch operation will be stopped.
Restack		Restacks all plates from magazine 2 to magazine 1.
Barcode List		Opens the Barcode List. [1]

[1] This function is only available if the stacker is equipped with a barcode reader and barcodes have already be read.

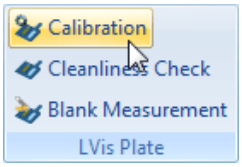
Please see chapter 6 for a description of microplate measurements.

2.2.4 LVis Plate Tab

This tab provides the same functions as the Microplate Tab (see chapter 2.2.3) for the BMG LABTECH LVis Plate (micro drop section).



The LVis Plate Tab does not contain the Prima and the Re-Run functions.



There is a special menu group 'LVis Plate' containing functions to calibrate the LVis Plate, to perform a cleanliness check and to determine the blank values for sequential blanking.

Please see chapter 7 for a description of LVis Plate measurements.

Hints: This tab only exists if the reader is equipped with a spectrometer for absorbance measurements, e.g. it does not exist when using a NEPHELOstar Plus.

If you do not own an LVis Plate you can switch off the LVis Plate Tab using the 'Settings | Program Configuration' function.

You can also use the Microplate Tab to perform measurements using the LVis Plate. This also allows you to use the re-run function. (When the LVis Plate Tab has been switched off you will find the LVis Plate menu group inside the Settings Tab.)

It is possible to export a protocol from one tab and to import it into the data base of the other tab, but the LVis Plate Tab protocol data base will only accept protocols using the BMG LVis Micro Drop plate.

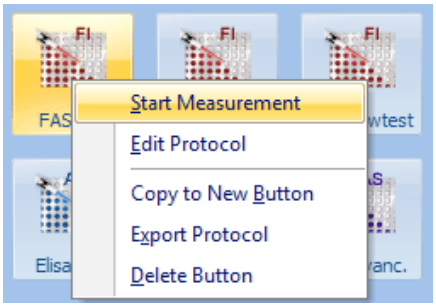
Using other parts of the LVis Plate than the micro drop section is only possible via the Microplate Tab. To measure the quality check filters (holmium and neutral density filters) please define a protocol using the standard BMG LABTECH 96 plate.

2.2.5 User-Definable Buttons

You can create up to 36 user-definable buttons in the Microplate and LVis Plate tab of the main program window for faster access to frequently used protocols.

Creating a new button is possible by clicking the 'New Button', copying an assay button to a user button (using the assay button popup menu or simply by dragging the assay button to the user button region) or by copying an existing user button.

You can also drag a Microplate or LVis Plate protocol from the protocol selection window directly to the user button region.



Right clicking a user button opens a menu with allows you to start the measurement immediately (without opening the Start Measurement dialogue) and to edit the test protocol.

You can change the position of a user button by simply dragging it to its new position (drag-and-drop).

Notes: The left click function is configurable (see chapter 3.3.1).

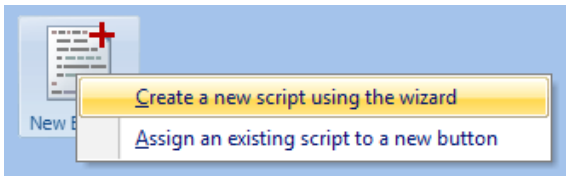
If you do not want to use user-definable buttons you can switch this feature off using the program configuration dialogue.

The user-definable buttons use the user-specific protocol data bases. Therefore, each user can create his own set of buttons and can define the protocols independently.

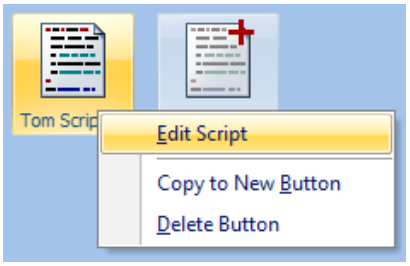
Run Only users (see chapter 1 *Login Screen*) are not allowed to create or delete user-definable buttons or to edit the associated test protocols.

2.2.6 Script Buttons

You can create up to 12 script (see chapter 8 *Script Mode*) buttons in the Microplate and LVis Plate tab of the main program window.



There are two options: You can assign an existing script to a button for faster access or you can use the script wizard (see chapter 8.3) to get a script generated automatically. The script wizard is intended for commonly performed tasks like measuring a plate (or a batch of plates) using different measurement methods.



Right clicking a script button opens a menu with allows you to edit a script or to copy it to a new button. When right clicking a wizard script button there is the additional option to export this script to a file. You can use this script file then as a starting point for more individual tasks.

Notes: Script buttons are not available when the program is used in ActiveX or DDE mode, e.g. as part of a robotic system.

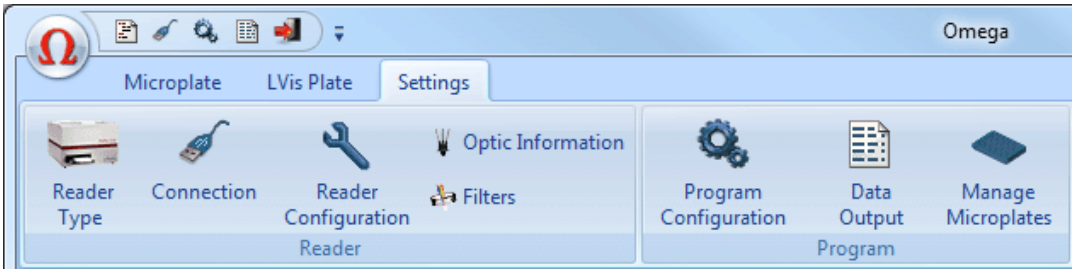
If you do not want to use script buttons you can switch this feature off using the program configuration dialogue.

The script buttons are user-specific. Therefore, each user can create his own independent set of buttons.

Run Only users (see chapter 1 *Login Screen*) are not allowed to create or delete script buttons.

3 Settings

To change the settings of the instrument or the software use the '**Settings**' Tab:

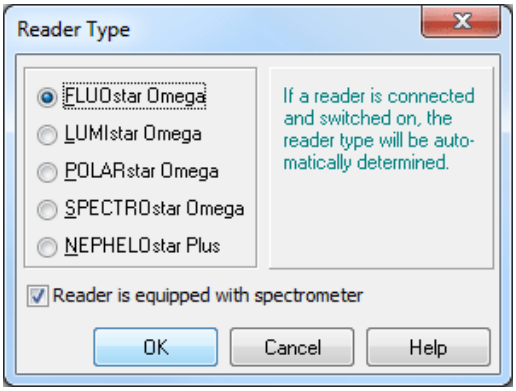


3.1 Reader Configuration

3.1.1 Reader Type

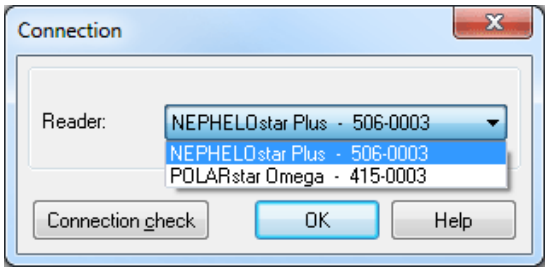
If a reader is connected to the computer and switched on, this window (accessible via '**Settings | Reader Type**') will show the type of reader which was automatically recognized. Depending on the reader type different measurement methods can be used.

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 box. This might be useful if you want to define a test protocol for a reader currently not available.

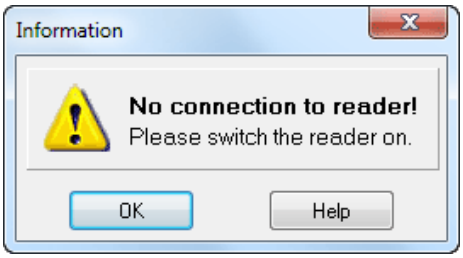


3.1.2 Connection

After installing the software, connect the reader to the computer using a USB cable. Select the reader using the '**Connection**' function which is located under the '**Settings**' Tab in the Reader menu group.



Check the connection by clicking the '**Connection check**' button. If there is no communication between the computer and the instrument, a message box will appear:



In this case, check that the power to the instrument is switched on and the cables are plugged in.

3.1.3 Fan Control

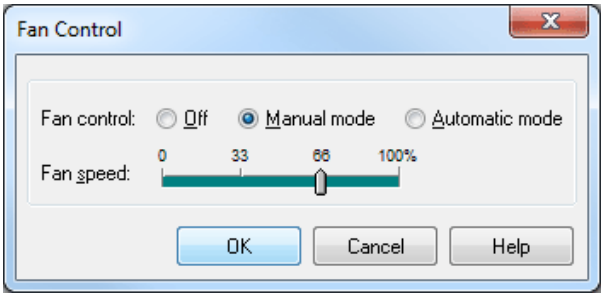
The fan ventilating capability in the Omega readers has the following advantages:

- to ventilate the reader after long test runs at higher temperatures to remove excessive humidity in the reader and avoid corrosion
- to more quickly reach desired temperatures for long-term incubation experiments
- to stabilize the environmental conditions during a measurement run more efficiently.

The 'Fan Control' mode in the Omega readers is only accessible if firmware version 1.30 or higher is installed and the reader is equipped with a fan.

To access the Fan Control settings, click on 'Reader Configuration' in the 'Settings' tab and choose 'Fan Control' in the 'Reader Configuration' window:

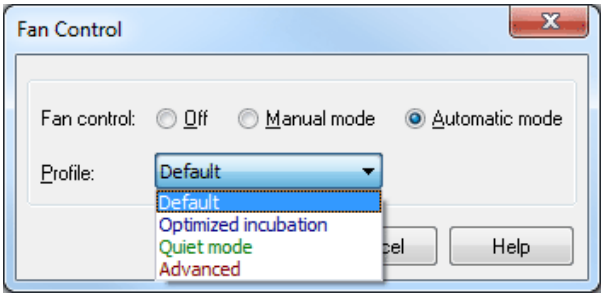
The Fan Control setting offers 3 modes: Off, Manual mode, Automatic mode.



Off: the fan is permanently switched off.

Manual mode: the fan speed is set manually and only one specific setting from 0-100% with an increment of 33% is applied permanently, independent of the reader's condition. Setting the value to 0% is the same as having the fan set to the off position.

Automatic mode: the fan is controlled by pre-defined settings in the Default, Optimized incubation, and Quiet mode profile. When selecting Advanced individual settings can be applied.

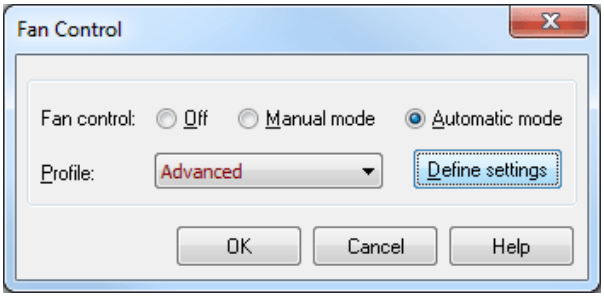


Default profile: Standard setting for the majority of applications when no incubation experiments are planned or evaporation is of no concern.

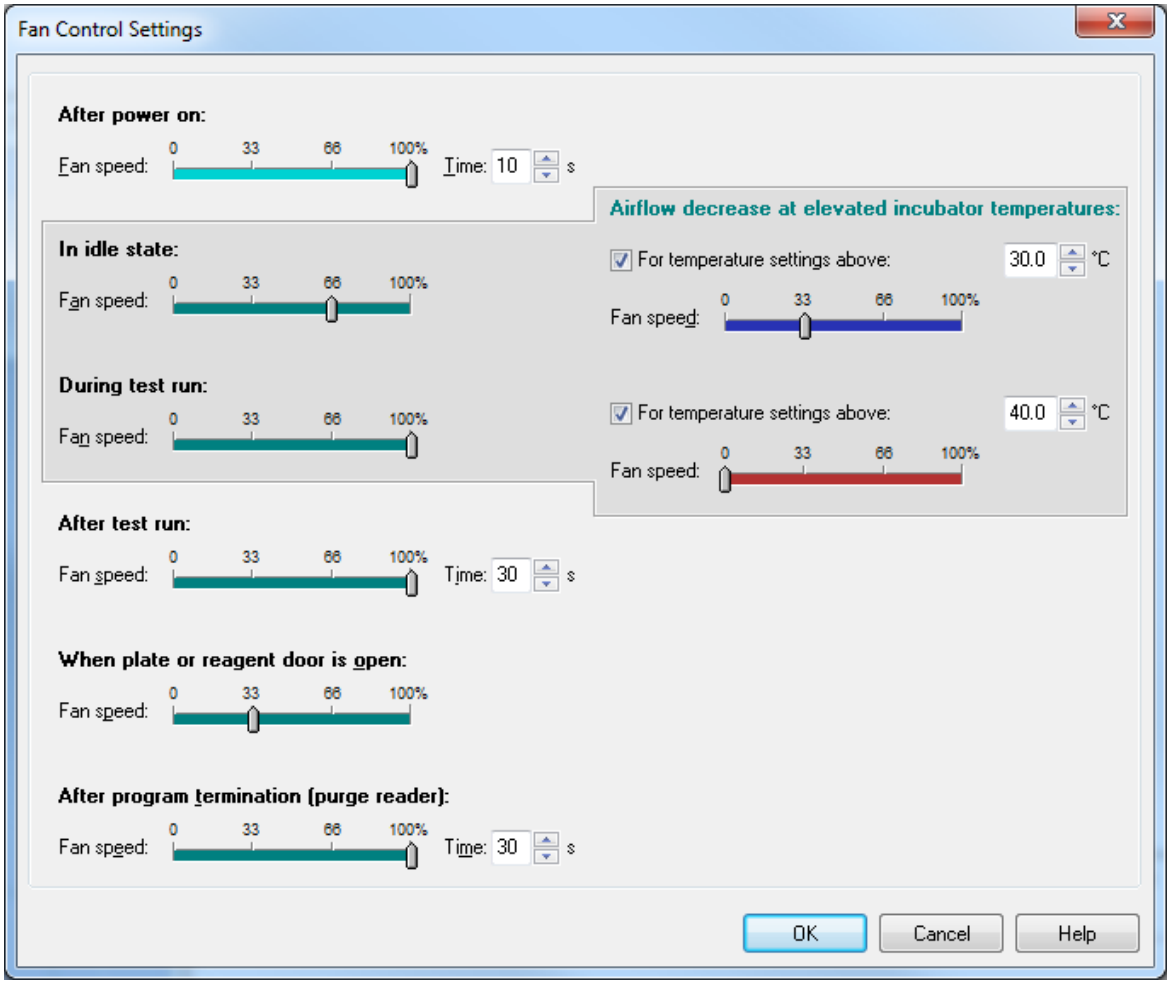
Optimized Incubation profile: Specific setting with a reduced fan speed in the idle state to stabilize the environmental conditions for incubation experiments more effectively.

Quiet mode profile: Specific setting with the fan speed reduced to 0% in the idle state or when the reagent/microplate door is open. This setting is recommended when working with low volumes to prevent evaporation.

Advanced profile: Define the fan speed individually for several reader conditions.



When clicking on 'Define settings' a new dialog appears to set the fan speed by dragging the highlighted slider to a position between 0-100% at a 33% increment. After setting the fan speed for all conditions, click 'OK' to save these settings. Shown below are the settings for the 'Default' profile and are described in detail.



Fan speed after power on: this speed is set to 100% for 10 sec per default. This serves to ventilate the reader initially after a long no-use period in order to get rid of any accumulated humidity inside the reader.

Fan speed in idle state: The reader is switched on and no test run is active.

Fan speed during test run: The reader is switched on and a test run is active.

Airflow during incubation: The two settings for temperature and fan speed control the airflow in order to reach and maintain a stable environment. If the temperature is below the upper or lower limit of the set target temperatures the fan speed defined for the idle state or during test run will be used.

Fan speed after test run: The reader has finished a test run and subsequently the reader is ventilated to remove excessive humidity. In particular, this is useful after long-term incubation experiments and when a new test run with other environmental settings will follow.

Fan speed when plate or reagent door is open: When this reader state is active the air exchange between inside the reader and the ambient environment is at greatest. Thus be cautious when deciding the settings for the fan speed. It is recommended to set the speed at a lower level than for the other reader state conditions.

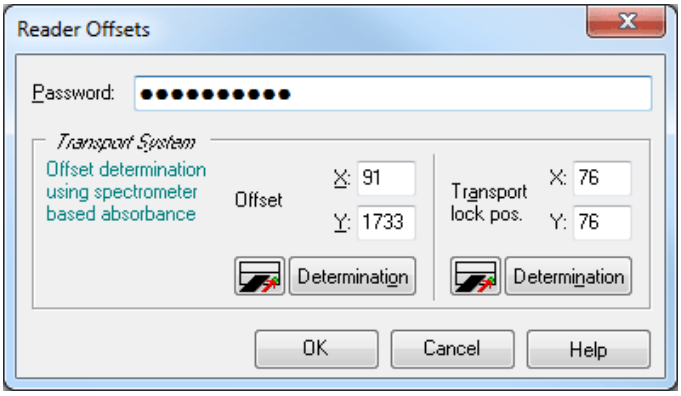
Fan speed after program termination: When the software is closed, it is assumed that no further experiment will be executed. Thus the reader will be excessively purged for 30 sec (default) to remove any humidity. It is recommended to always close the Control Software first and wait until the fan operation is finished before turning off the reader.

The individual reader states have different executive priorities for fan operation and are lined out below:

1	Microplate or reagent door Open
2	Incubator On with nominal temperature > Lower Limit
3	Incubator On with nominal temperature > Upper Limit
4	Active test run (reader busy)
5	Reader standby (idle mode)

The reader states are evaluated internally and the respective fan speed is used according to the priority level. For instance, if the microplate door is open this setting applies and lower priority states are not considered. If the door is closed again, the fan speed will be set to the corresponding event state.

3.1.4 Reader Offset



Every Omega reader is accurately calibrated at the factory and each has specific offset values, which 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. If the plate carrier is removed or replaced, the offset values should be re-calibrated. **The determination of the offset values should be carried out only by a qualified service technician**.

The Reader Offsets dialogue can be reached by selecting '**Reader Offset**' in the '**Reader Configuration**' dialogue, which is under the 'Settings' tab.

Note of warning: **Do not perform an 'Offset determination' unless you are qualified to do so!**

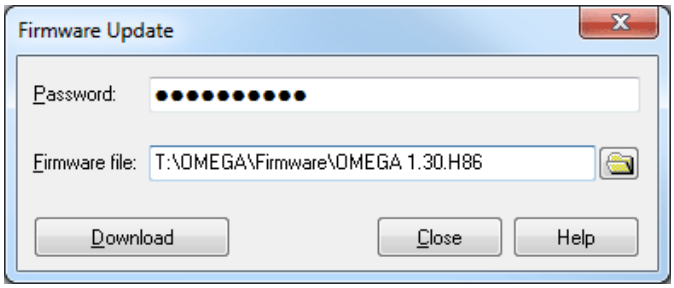
The buttons will move the plate carrier to the defined positions.

The '**Determination**' buttons will start the determination procedure for the corresponding offset values.

Click '**OK**' to save all changed 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.

3.1.5 Firmware Update



This function updates the reader firmware (content of the built in Flash-EPROM) and updates the firmware of the spectrometer built-in into the reader.

New firmware files from BMG LABTECH should be copied into the folder ~:\Program Files\BMG\Omega\Firmware.

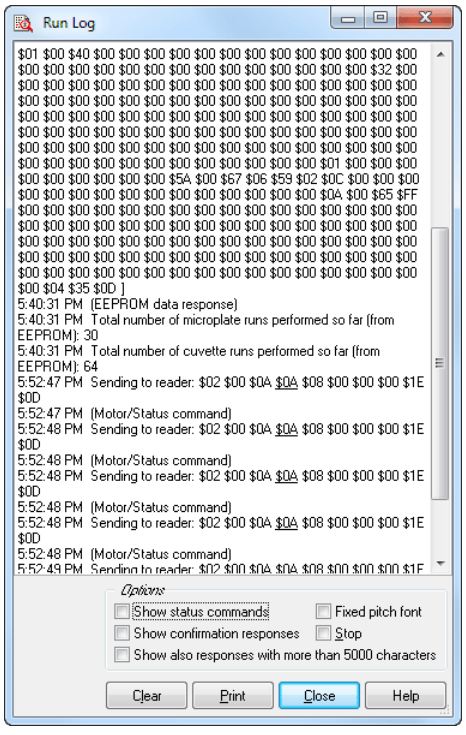
Make sure the reader is connected to the computer and turned on. After typing in the correct password (password will come with new firmware), select the newest firmware file using the button and then press '**Download**'. The Omega program will bring the reader into the download mode and will start the firmware download program 'FlashTools'. Downloading the new firmware is an automatic process, which will last a few minutes.

Note about Download Progress: If the green light on the instrument is slowly blinking, the firmware download is still taking place.

After the download is complete, the firmware download program will close automatically. The reader will reset automatically to activate the new firmware.

Note: Do **not interrupt** this process by turning off the reader or computer or by closing the software! 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 (just restart the software after an interrupted download process and repeat).

3.1.6 Show Run Log Window



The '**Run Log**' window shows all commands sent to the reader and all responses. The Run Log window can be opened using the key combination [Shift]+[Ctrl]+[L] from the main window of the control program.

Hint: If support is needed due to a malfunction, please send BMG LABTECH this log file together with a description of the problem before restarting the program or this will be erased.

It is possible to stop the process of adding new entries to the run log by checking the '**Stop**' checkbox.

By default only the first bytes of responses are shown as listing very long responses (e.g. the measurement data responses of absorbance, spectra plate mode test runs) will slow down the data transfer process. Check the last check box of this window to see complete responses.

'**Print**' the entire run log or a marked part of the run log.

3.1.7 Run Log 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:

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].

Selecting something before using [Ctrl]+[Q][F] or [Ctrl]+[F] will be used as the default search string, otherwise the word the cursor is positioned onto (something containing letters, not hexadecimal numbers) will be used.

Note: Searching something, which includes line breaks, is not possible. Change the width of the Run Log window to remove soft line breaks.

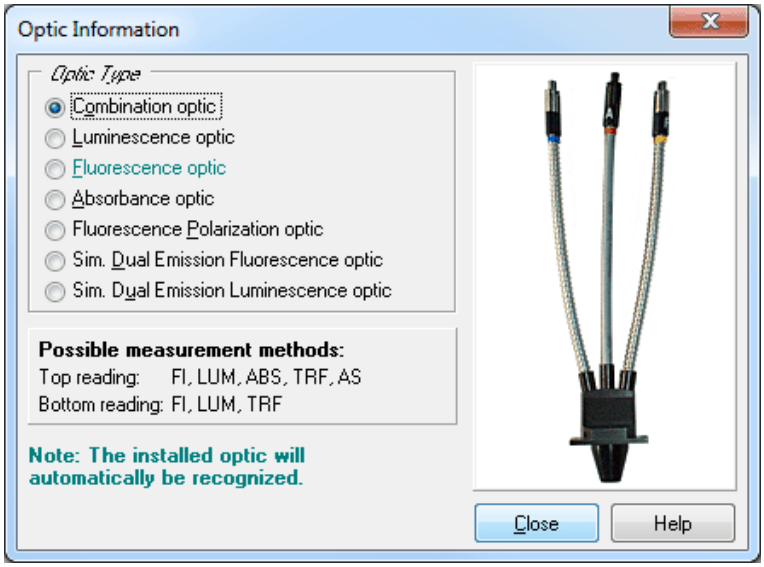
3.1.8 Run Log File

The run log will automatically be saved into the file '`Omega.log`' in the Omega main directory (usually '`~\Program Files\BMG\Omega\`'). The generation of the run log file can be stopped by changing the line '`RunLog=true`' in the configuration file '`~\Program Files\BMG\Omega\Omega.ini`' (section `[Debug]`) into '`RunLog=false`'.

Note: At the next program start the existing log file will be renamed into '`Omega.bak`' and a new log file will be started.

3.1.9 Optic Information

The FLUOstar Omega can measure fluorescence intensity and time-resolved fluorescence as well as (optional) absorbance, luminescence and AlphaScreen. In addition, the POLARstar Omega can read in fluorescence polarization mode. The measurement methods which can be used also depend on the optic installed. You can use the '**Setup** | **Optic Information**' function to get information about which measurement methods can be performed with which optic.



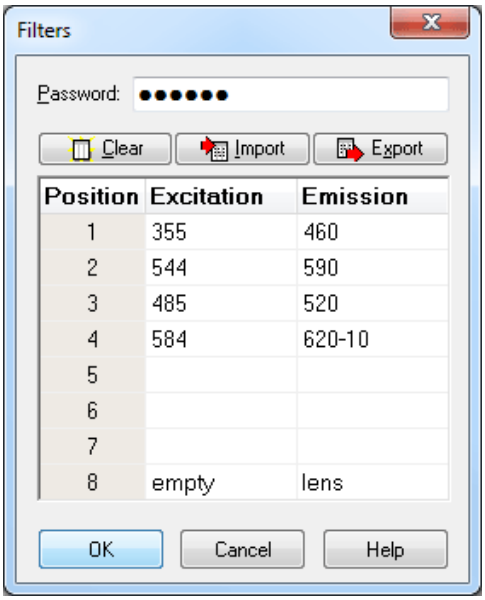
The optic type currently installed will be marked using a cyan text color.

After selecting an optic type, you will see a picture of the optic on the right. For some measurement methods, there are different optimized optics available, e.g. fluorescence measurement. If these optics look significantly different, the picture will show more than one optic. The measurement methods which can be performed using the optic selected with the reader connected (or selected using 'Setup | Reader Type') are listed on the left.

The LUMIstar Omega can only measure in luminescence mode, the SPECTROstar Omega can only measure using the absorbance method and the NEPHELOstar Plus can only measure using the nephelometry method. Therefore, the optic information dialogue is not available for these readers.

3.1.10 Filters

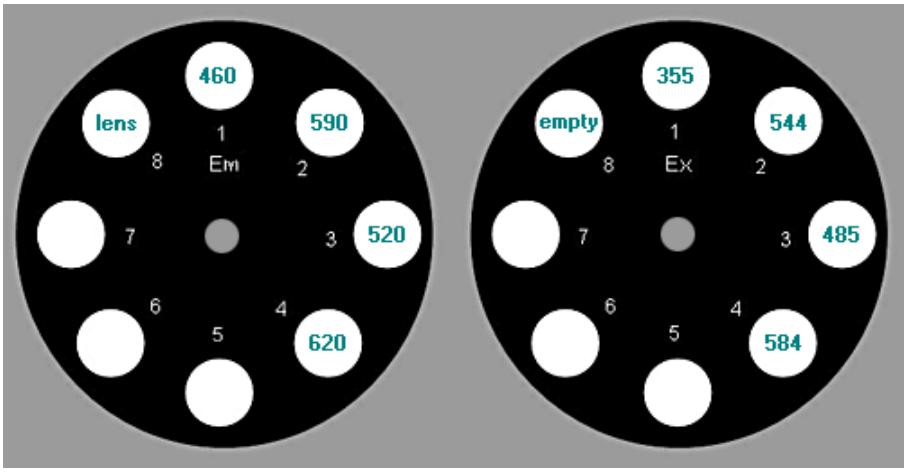
The Omega family of readers can be configured with up to 8 excitation filters and up to 8 emission filters. Click on '**Settings** | **Filters**' to open the filter dialogue.



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.

To be able to change the filter table, you need to enter the password 'bmg'. This password protection is intended only to prevent changes by mistake.

For absorbance method measurements, position 8 on the emission side should contain a lens or should be empty. For absorbance method measurements using a spectrometer, position 8 on the excitation side should be empty (or should contain a UV filter).



Clear After clicking this button, the table contents will be removed.

Import Imports 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.

Export Exports filter information from the table into an ASCII or Excel (.xls) file.

OK Saves the filter positions and returns to the main program window.

Cancel Closes this window without saving changes.

Notes: The table is common to all users.

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

This dialogue is not available when using a NEPHELOstar Plus reader.

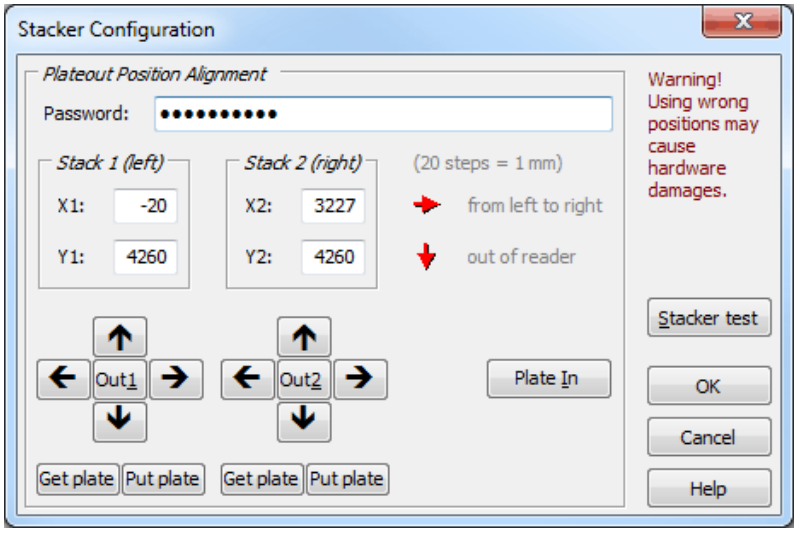
3.2 Stacker Configuration

To open the Stacker Configuration dialogue use the menu command ‘Settings | Stacker Configuration’.

This function is only available, if a stacker is attached to the reader.

3.2.1 Position Alignment

For precise operation of the stacker / reader system, it is necessary to adjust the positions of the reader plate carrier relative to the stacker. Use the ‘Setup | Stacker Configuration’ command to open the following window.

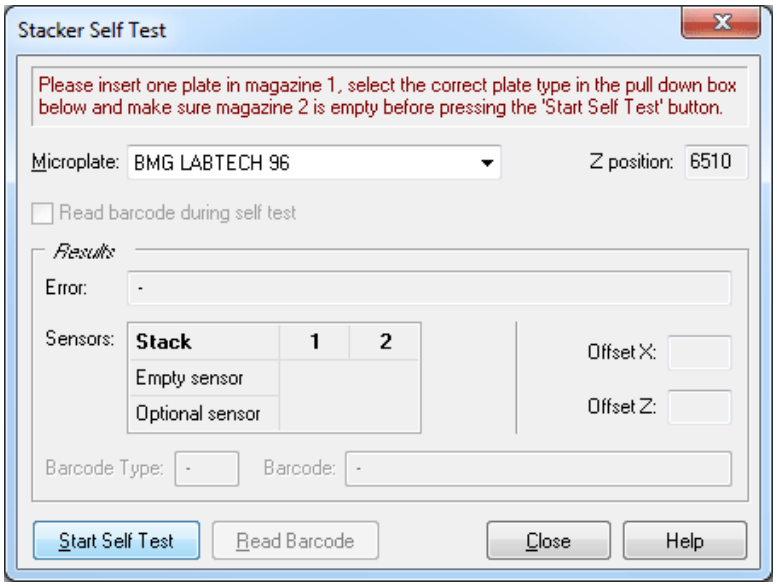


Make sure, that both stacker magazines are inserted. Use the ‘**Out1**’ button to move the reader plate carrier to the position under stack 1. Check the position by inserting the position alignment pin through the little hole in the right corner of the magazine. If the position is not absolutely accurate, change the value X1 / Y1 and press ‘Out1’ again or use the arrow buttons to change the position step by step. By pressing an arrow button, the corresponding value will increase or decrease by one step (0.05 mm) and the plate carrier will be moved to this new position. If you press and hold the [Ctrl] key while using an arrow button, the value will change by 10 steps (0.5 mm). Using [Shift] it will change by 20 steps (1 mm), using [Alt] it will change by 100 steps (5 mm). After determining the correct position insert a microplate and use the ‘Get plate’ and ‘Put plate’ buttons to perform a final check.

Repeat this procedure for the stack 2 position.

3.2.2 Stacker Self Test

The Stacker Self Test dialogue is accessible from the Stacker Configuration dialogue (see previous chapter) or using the ‘Settings | Stacker Self Test’ menu command.



Please insert one microplate in magazine 1 and make sure that magazine 2 is empty. Make sure that both magazines are locked. Select the correct microplate type.

After pressing the '**Start Self Test**' button, the Stacker will perform a complete self test procedure (including checking the barcode reader, if installed, and if the '**Read barcode during self test**' option is selected). If an error occurs, a message will be displayed.

The table below the error message box shows the status of built-in sensors. The empty sensor detects if there are plates in a stack (1 = no plates in). The optional sensor is used only for stack 1 in this version. It works as a sensor to check whether or not the reader plate carrier is empty.

During the self-test procedure, the x and z transport systems will be calibrated. The measured offset values will be shown.

If a barcode reader is built in, it will be used to try to read the barcode of the inserted plate.

When you press the '**Read Barcode**' button, the barcode reader will be triggered. You can use this function to align the barcode reader or test its function (you will see the red laser beam). As there will be no plate movement to the barcode read position, a built in barcode reader will not read a barcode value using this function.

3.3 Program Configuration

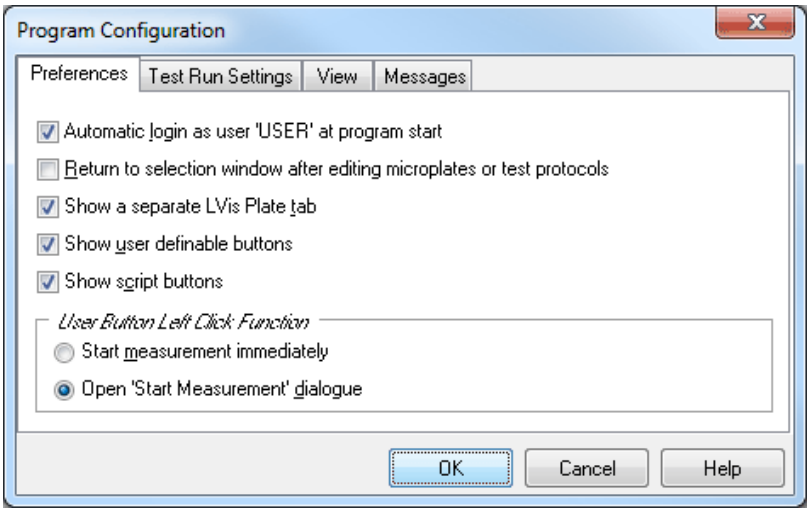
To open the Program Configuration dialogue use the menu command ‘Settings | Program Configuration’.

The Program Configuration dialogue box allows users to modify the appearance and behavior of the software. Activate a feature by ticking the box next to the statement.

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

3.3.1 Preferences Tab

Change different program features using this tab.



Automatic login as user ‘USER’ at program start

If this option is chosen there will be no login screen at program start, instead the default user ‘USER’ will be automatically logged in. It is still possible to use the login function later (‘Application Button Menu | Login’ or click onto the user name inside the status bar).

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

Return to selection window after editing microplates or test protocols

By default, returning to the main control software happens after creating or editing a test or microplate definition without returning to the selection window. To see the selection window for the microplates or test protocol definitions again, check this box.

Show a separate LVis Plate tab

The LVis Plate tab provides the same functions as the Microplate Tab for the BMG LABTECH LVis Plate (micro drop section). If you do not own an LVis Plate you can switch off the LVis Plate Tab using this function.

You can also use the Microplate Tab to perform measurements using the LVis Plate.

Show user definable buttons

You can define up to 36 user buttons in the Microplate and LVis Plate tab of the main program window for faster access to frequently used protocols. If you do not want to use this feature you can switch it off here.

Show script buttons

You can define up to 12 script buttons in the Microplate and LVis Plate tab of the main program window for faster access to frequently used scripts. If you do not want to use this feature you can switch it off here.

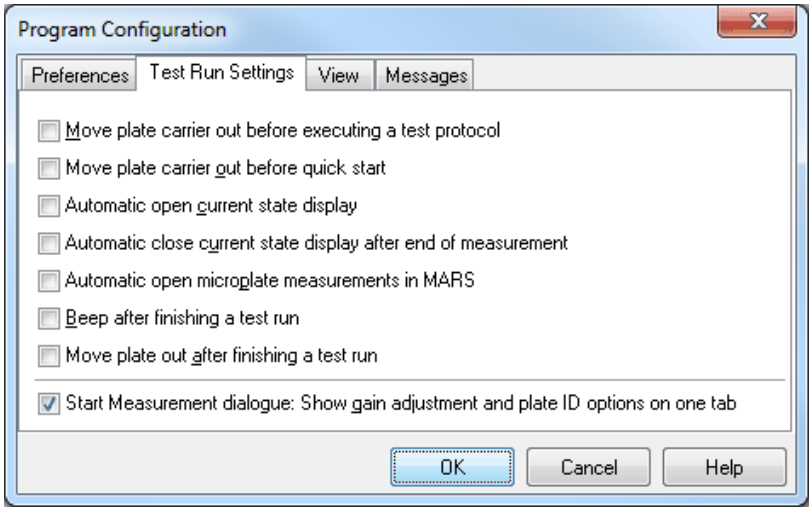
User Button Left Click Function

If the first option is selected a left click onto a user-definable button will start the measurement immediately using the pre-defined (last used) Plate IDs and Sample IDs. Changing these IDs is possible by using the first menu entry of the popup menu which appears after right clicking the button ('Change IDs').

If you prefer that the dialogue appears, where you can change the IDs, after a left click instead of an immediate measurement start select the second option. In this case the first menu entry in the popup menu will be changed to 'Start Measurement'.

3.3.2 Test Run Settings Tab

Change the program behavior for measurement runs using this tab.



Move plate carrier out before executing a test protocol

Selecting this option moves the plate carrier out when starting 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

Selected by default, this option moves the plate carrier out when opening the 'Quick Start' dialogue box.

Automatic open current state display for microplate measurements

Select this option to open the Current State display after starting a measurement.

Automatic close current state display after end of measurement

Select this option to close the Current State display after the measurement has ended.

Hint: As long as the current display window is open no new measurement can be started.

Automatic open microplate measurements in MARS

Select this option to automatically open MARS after measuring a microplate.

Beep after finishing a test run

Select this option to hear a short acoustic signal after a test run is finished.

Move plate out after finishing a test run

Select this option to have the plate automatically move 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.

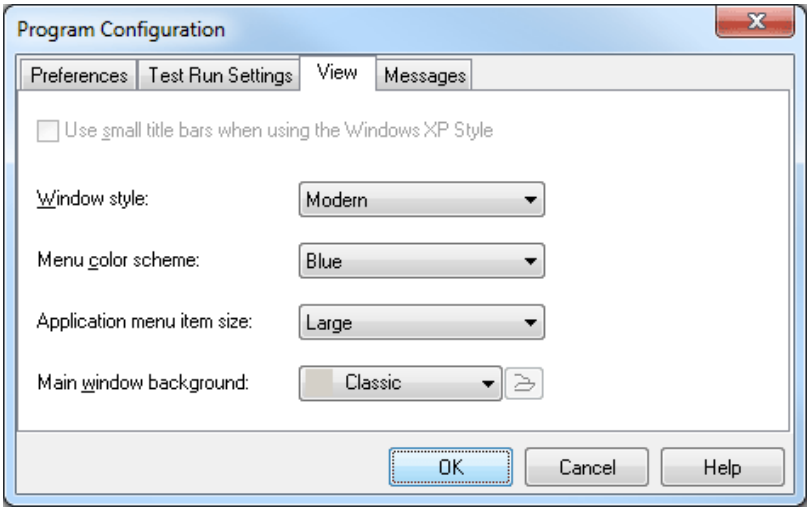
Note: This option is not active if the software is in ActiveX, DDE or script mode (see chapter 6.8).

Start Measurement dialogue: Show gain adjustment and plate ID options on one tab

Beginning with version 2.10 the gain adjustment and plate ID options will be shown on a single tab, which, therefore, is very full. If you prefer the old style (separate tabs for gain adjustment and plate IDs) unselect this check box.

3.3.3 View Tab

Change the look of the program using this tab.



Use small title bars when using the Windows XP Style

Select this option to use small title bars for all program sub windows. The title bar of the main program window and of the login screen will not be changed. Also, the style of Windows standard dialog boxes will not be changed, e.g. for file name selection or for printing options.

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.

Windows style

Select one of three window styles: Classic, Modern or Modern Blue. 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 this manual have been created using Windows 7 in combination with the 'Modern style' option of the BMG LABTECH software.

Menu color scheme

Select blue, silver or black as the menu color scheme.

Application menu item size

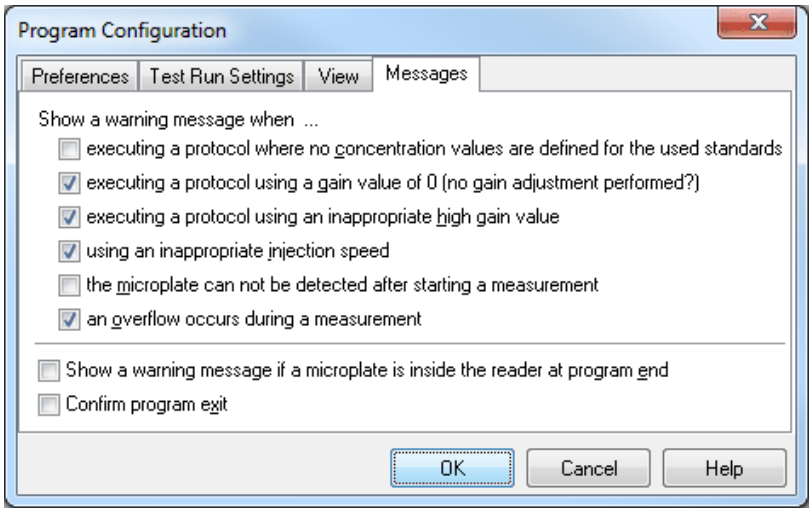
Select either normal or large for the application menu size.

Main window background

Select the background for the main window by choosing a predefined background styles or an original bitmap file. When using a bitmap file the dimensions will be scaled to fit.

3.3.4 Messages Tab

Change the configuration of messages using this tab.



You can for example define to get a warning message displayed when trying to execute a test protocol with an inappropriate gain value or injection speed. Not all options are available for all type of readers.

Perform an additional microplate search at program end

If a stacker is attached to the reader there will be a plate search performed at program start. Using this option you can activate an additional search at program end. If a plate is found inside the reader plate carrier or on the stacker table, it will be moved to magazine 2.

Show a warning message if a microplate is inside the reader at program end

If no stacker is attached to the reader, you can get an optional warning message if a microplate is inside the reader at program end. You will then be asked whether you want the plate been moved out or not.

Note: This feature will only work if the plate has been moved in after initializing the reader or if a measurement has been performed.

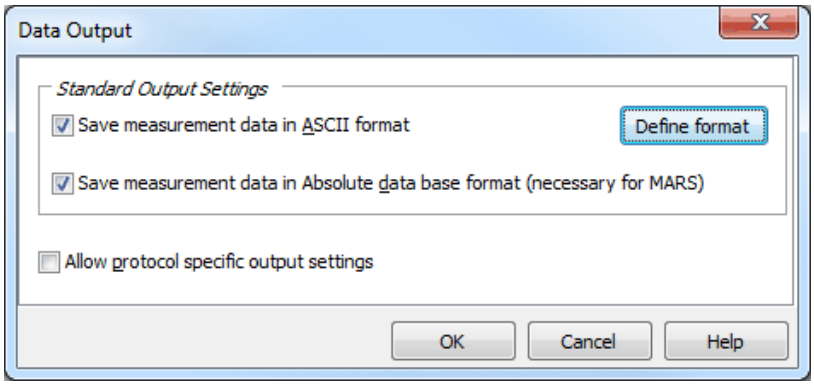
Confirm program exit

Deselect this option to avoid the confirmation window when exiting the program.

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 Data Output

To open the Output Settings dialogue use the menu command ‘Settings | Data Output’.



Standard Output Settings

Save measurement data in ASCII format

By default all measurement data is saved in Absolute Data Base format in the specified user directory (see chapter 1 *Login Screen*). BMG LABTECH’s Data Analysis software, MARS, uses this file format.

To use a different evaluation software package, store data in ASCII format. Select this option and the ‘**Define Format for ASCII Export**’ window becomes available (see chapter 3.4.1: *Filename and Output Path*).

Hint: This function allows for the data to be saved in **ASCII format**, which can be used in third party LIMS or data analysis programs.

Save measurement data in Absolute Data Base format (necessary for BMG’s data analysis program)

This option is selected by default. All measurement data is stored in a set of Absolute Data Base files under the logged in user’s data directory. BMG LABTECH’s Data Analysis software, MARS, uses this file format. It is possible to export data using both ASCII and Absolute Data Base formats.

Hint: The Absolute Data Base file has information that can aid in trouble shooting, therefore it is recommended to keep this option checked when saving data in ASCII format in case it needs to be referenced later by a BMG LABTECH technical specialist.

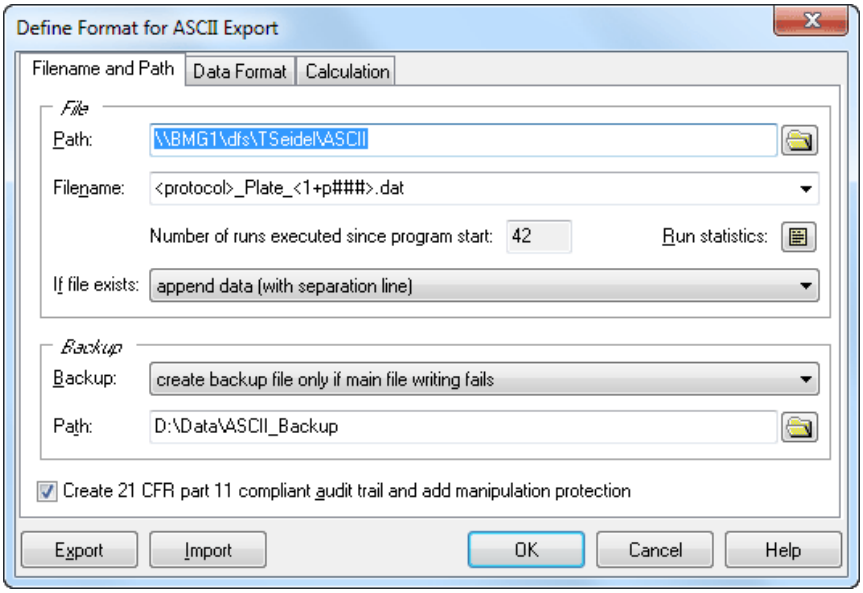
Allow protocol specific output settings

This option allows for protocol specific ASCII export of data with a new ‘**Output**’ tab on the advanced ‘**Test Protocols**’ setup page (see chapter 4.3.15).

Note: When this is used, all protocols where no specific settings have been defined will use the ***Standard Output Settings*** defined above.

3.4.1 Filename and Output Path for ASCII files

The '**Define Format for ASCII Export**' window sets up how, where and what measurement data to export.



File

Path

Specify the data path where the ASCII files will be stored, even a network directory can be used. To create a new directory, simply click on the folder to the right or type in the path. Use the special functions <protocol>, <method>, <date> and <time> as part of the directory name to sort protocols (see the description of these options under Filename).

Filename

The filename can be a constant typed in name or the following options in the drop down menu can be used:

<protocol> Name of the test protocol used.

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

<ID1>…<ID3> The information written in the plate identification window before the measurement begins.

<1+#> Consecutive numbers will be assigned to the test runs. When the software is restarted the numbers begin again with '1', but the start number can be changed. For example, enter the number 5 and the test numbers will increase consecutively starting at 5.

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

To change a number only after e.g. every tenth plate 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##>. Run Statistics can be seen after pressing the button.

In batch mode (using a stacker) or in script mode 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, but the file name will be consecutive alphabetical letters. The number of letters used can be modified (e.g. <A+###> will result in AAA, AAB, etc).

<date> Insert current date

Specify the date format by adding 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)

mmm – abbreviated name of the month (January => Jan.)

mmmm – full name of the month

d – day with one or two digits

dd – day always with two digits

ddd – abbreviated name of the day (Monday => Mo.)

dddd – full name of the day

ddddd – 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>

The default format "yyyymmdd" will be used.

<time> Insert current time

Specify the time format by adding a format description after "<time:":

h or hh – for the hour (one or always two digits)

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>

The default format "hhmmss" will be used.

<ser_number> Serial number of the reader used

<barcode> Barcode of the current plate (only available if the reader is attached to a stacker equipped with a barcode reader and only available for measurements using the stacker, not for single plate measurements without stacker magazine 1 inserted).

More than one of these parameters can be used in the filename (example: <protocol> plate <1+###>.dat).

Notes: If a file extension is not specified then '.CSV' will be added automatically.

Characters that are not allowed to be used in file names (e.g. ":") will be removed automatically.

Number of executed runs

This 'counter' tracks the number of measurements that were performed after the program was started. This is the number that will be used in the filename options <1+#> and <A+#> and it can be manually changed to begin with any number.

To see the **Run Statistics** press the button. This window lists all test protocols used together with a number showing how often. It is possible to edit these numbers or to delete a protocol from the list.

Hint: 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.

This window also shows the 'Total no. of executed runs' which is the total number of all test runs executed using the reader connected.

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 data with separation line*** adds the newly measured data to the existing file and it separates each test with a dashed line.
- ***append data without separation line*** adds the newly measured data to the existing file but it does not include a dashed line.

Backup

If the above defined directory fails, this function allows for an alternate plan.

Backup options:

- ***no backup file*** shows an error message when it fails to create or write the defined ASCII file.
- ***create backup file only if main file writing fails.*** This option allows for the ASCII file to be stored in the specified backup directory when creating or writing to the above defined ASCII file failed (the same filename and behavior if file already exists will be used as defined above). If writing into the backup file fails also, then there will be an error message.

  Hint for networked data: This setting is recommended if the main File Path defined above is on a network drive, then the Backup Path can be on the local drive.
- ***always create backup file.*** This option allows the ASCII file to be stored using the main File Path and a second copy will be stored in the backup directory (the same filename and behavior if file already exists will be used as defined above). There will be no error message if writing to the main file failed, but there will be if writing into the backup file fails.
- ***always create backup file (no error message when this fails).*** This option allows for the ASCII file to be stored in the specified main directory and a second copy in the backup directory (the same filename and behavior if file already exists will be used as defined above). There will be no error message when writing to the main and/or backup file fails.

Path

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

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

Hint for FDA 21 CFR part 11 compliancy: BMG LABTECH recommends using this option if the ASCII files are to be used in accordance with the 21 CFR part 11 rule (see software manual part IV).

This option creates a second file to the ASCII file with anti manipulation hash values, as well as an audit trail (the file will have the same name and will include the file extension '.at'). The anti manipulation hash value will protect the whole ASCII file and the audit trail (see software manual part IV: FDA 21 CFR part 11).

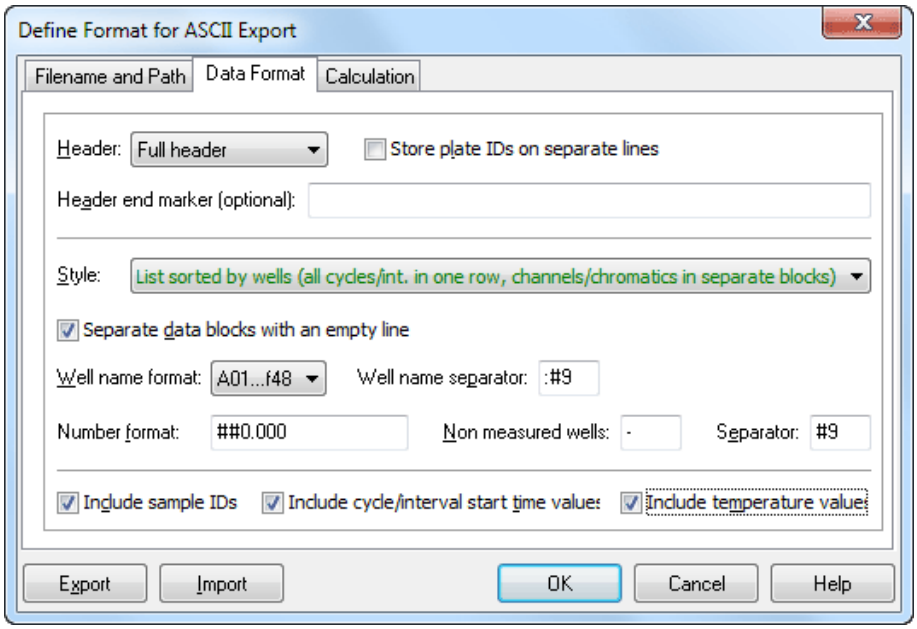
The hash value will be created when the control part of the BMG LABTECH software creates the ASCII data file. It can be checked using the "Check Data Integrity" tool (see software manual part IV).

Export / Import

It is possible to export and re-import all of the ASCII settings. This might be especially useful when using **protocol specific output settings** (see chapter 4.3.15) to transfer the settings from one protocol to another.

3.4.2 Data Format

Specify the format of the exported ASCII data



Header

To include a description of the test run, the following options can be chosen:

- **No Header** does exactly what it says.
- **Only ID1** uses ID1 as the header (see chapter 6.3).
- **Short header** uses 5 lines to describe the test run (test protocol name, date, plate IDs, number of cycles, and number of wavelengths).
- **Long header** contains the information of the short header but also the measurement mode.
- **Full header** contains all protocol settings including layout, target temperature, the firmware and software versions, and the reader serial number.

Hint for FDA 21 CFR part 11 compliancy: BMG LABTECH recommends this option if the ASCII files are to be used in accordance with the 21 CFR part 11 rule (see software manual part IV).

When using the 'Short', 'Long' or 'Full header' there will also be a line with the 'Channel or Wavelength' number and a line with the 'Cycle or Interval' number before each data block.

- **Danish style** has no header, but in the first column of every data line the plate ID1 will be listed.

Hint: This may be useful, if data from more than one test run is combined into one ASCII file. The file can be searched for example the highest result in any well of all plates. This allows for the plate identification to be easily and immediately seen.

Store plate IDs on separate lines

Check this box to store the IDs on separate lines, by default the three plate IDs will be stored on one line:

```
i.e. ID1: FAST TEST  ID2: 07.08.2009,21:17:19  ID3: 415-0002
```

Header end marker

When importing the ASCII file, certain data analysis software or LIMS can use markers to detect the end of the file header. Use this optional feature to enter text after the file header to mark the end of the header.

Use '&' inside the text to specify the start of a new line and '&' can be used more than once. This might be useful to achieve a certain number of header lines or to enter several lines with additional information.

Style

The exported ASCII data can appear as a '**Table**' (raw data side by side in a matrix) or a column ('**List**'). It is possible to include **well numbers** and to include **only measured wells**.

Choose the option '**Table with well numbers in plate layout style**' to export the data 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 or interval. Multichromatic measurements will appear with the data from the first chromatic first, followed by the data from the second chromatic, etc.

Hint: By default, the **well name separator** will not be added in front of the line with the well column numbers (as it would look strange if there were say a comma before the 1). However, if a comma or tab needs to be added at that spot (i.e. for importing a comma separated files into Excel), add the following line under [Configuration] in the configuration file Omega.ini:

`IncludeWellNameSeparatorInColumnNumbersRowForASCIIFiles=True`

To open the configuration file, use the key combination [Shift]+[Ctrl]+[I] from the main screen of the Omega Control software. If the section [Configuration] does not yet exist, add it to the end of the file. Save the file. It is not necessary to restart the software for this setting to become active.

The option '**List sorted by wells**' will export the measurement results for all cycles in one line per well; if the test uses more than one wavelength, a separate data block for each wavelength will be given.

The option '**List sorted by wells 2**' will export the measurement results for all cycles in one line per well; if the test uses more than one wavelength, it will be added to the same line.

Hint: 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 the test protocol has more than one cycle, interval or wavelength more than one block of data will be in the exported 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 when using the 'Append data' option (see above).

Well name format

The well name can contain a two digit number or a one digit number (e.g. A09 instead of A9). A trailing blank in front of the well names for column 1 to 9 can be used 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.

It is recommended to specify a format of 0.000 because the OD values can range from 0.000 to 3.500 (without path length correction). By adding an 'm' to the format string (e.g. '###0m') the numbers will be expressed in milliOD (0 to 3500 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 a comma, a semi colon or back slash. By selecting '#9', a tab will be used.

Note: A small dot will be shown when entering a space into the separator input box instead of the space to make it easier to check the input.

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

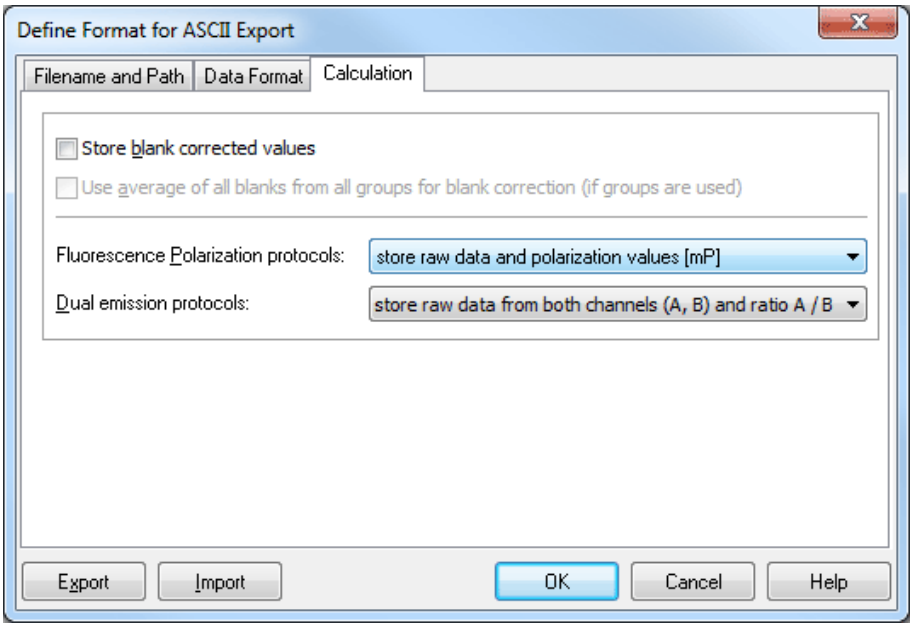
If this option is selected, the cycle start times (plate mode) or the interval start times (well mode) will be included. The time values will be stored in a separate line in front of each measurement values block or in one line before the data when using a 'List sorted by wells' style. If a header is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

Include temperature values

Store the measured temperature taken at the measurement of each cycle (kinetic measurement).

Note: The built in incubator needs to be switched on or the temperature monitoring function needs to be 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. Select this option to blank correct 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 and 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'.

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

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

Dual emission protocols

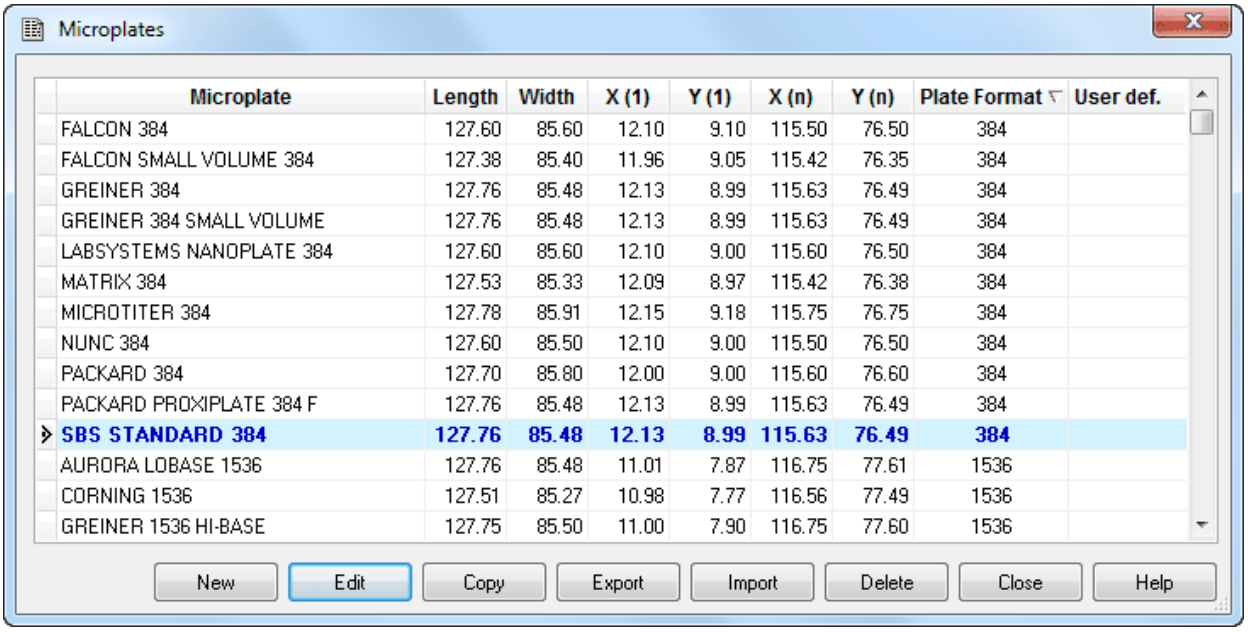
For dual emission tests, you can decide whether you want the raw data for the two channels (order A, B or B, A) or the ratio of the two channels (A / B or B / A) to be stored. It is also possible to get both the raw data and the ratio.

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

3.5 Manage Microplates

The Omega software contains a data base with microplate dimensions from most microplate manufacturers. Most plates have the SBS standard spacing and footprint; some plates will have slightly different dimensions and must be positioned accordingly for optimal results.

To open this dialog box select '**Manage Microplates**' in the 'Settings' tab.



Note: To export the microplate definitions into a XLS (Excel), text or HTML file right click on the table.

By default the microplate definitions are sorted by plate format first and then alphabetically by name. Click on the 'Microplate' part of the table header to sort by name and click 'Plate Format' to switch back.

Hint: To select the first microplate that begins with e.g. 'B', press the [B] key. 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.

Creating User Defined Microplates

The following options allow new microplates not in the current list to be easily added to the database. When a new microplate is added a check mark will appear in the 'User def.' column to the far right.

Hint: After clicking the 'User def.' part of the table header all User defined microplates will be listed first. It is useful to export these definitions before performing an upgrade because these are not transferred (see Software Manual Part 1: Installing software, section 1.5.3).

Note: Users with activated 'Run Only' option (see chapter 1 *Login Screen*) cannot edit, copy or import microplate definitions.

New button pulls up a dialogue box new plate dimensions can be entered (see next chapter).

Edit button modifies a selected microplate through a new dialogue box (see next chapter).

Copy button copies the dimensions of a selected microplate after a new name dialogue box appears.

Hint: Use this option to edit a microplate's dimensions without changing the original definition.

Export button exports a selected microplate entry to a disk or to a different directory. A new window will ask for the destination drive and directory and the file name will have the extension '**.MPC**'.

Import button imports a microplate definition from a disk or another directory. The file extension must be '.MPC' or '.MPL' (microplate definitions exported from older BMG LABTECH programs). Or just drag and drop a *.MPC (*.MPL) file directly into the microplate selection window.

Delete button removes a selected microplate entry from the data base.

Close button exits this window.

3.5.1 Define a New Microplate or Edit an Existing One

This dialogue box allows for existing microplate definitions to be changed or for new plates to be added to the database. Enter a '**Name**' for the plate and specify the mechanical dimensions of the microplate in mm.

Hint: Using the wrong microplate or having the wrong dimensions to a microplate can cause the data to 'trend' across a plate and to increase the %CV of replicates, especially for 384, 1536, and 3456 well plates. Always check the dimensions when using a new microplate type or manufacturer.

Warning: If a microplate's dimensions are edited, the changes will apply to all users.

XY Dimensions Tab

The first tab defines the X and Y dimensions of a microplate. Measurements are taken from the center of the well and need to be accurate so that the wells are exactly positioned underneath the measurement optics.

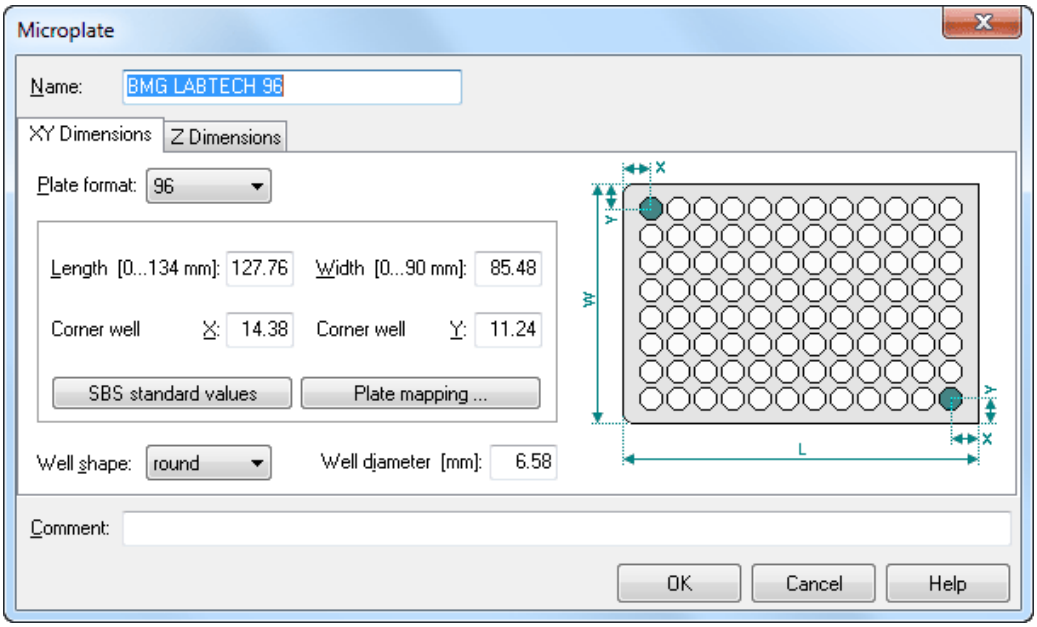


Plate format selects the total number of wells in the plate – 1536, 384, 96, 48, 24, 12 or 6 wells.

Length is the outer length of the entire microplate, from left border to right border.

Width is the outer width of entire microplate, from top to bottom.

Corner well X is the distance from the left border of the microplate to the center of a well in the first column; this should equal the distance from the center of a well in the last column to the right border of the microplate.*

Corner well Y is the distance from the top border of the microplate to the center of a well in the first row; this should equal the distance from the center of a well in the last row to the bottom border of the microplate.*

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

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 defining 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).

Well shape is either round or square; this information is necessary for well scanning. Here you can also define the shape of the special measurement positions of the BMG LVis plate (cuvette or micro drop).

Well diameter is the diameter of a well; this information is necessary for well scanning.

SBS standard values button will insert the SBS standard X and Y dimensions for the selected plate format. Select the well shape and define the well diameter or width.

Plate mapping will automatically determine the positions of the corner wells for unknown microplates, 96 to 1536 wells (see next chapter).

Z Dimensions Tab

The second tab defines the Z dimensions of the microplate, which may be necessary when the Omega is automated. If these dimensions are not provided, an accurately calibrated caliper can be used to obtain the dimensions. See the diagrams for the correct measurement dimensions.

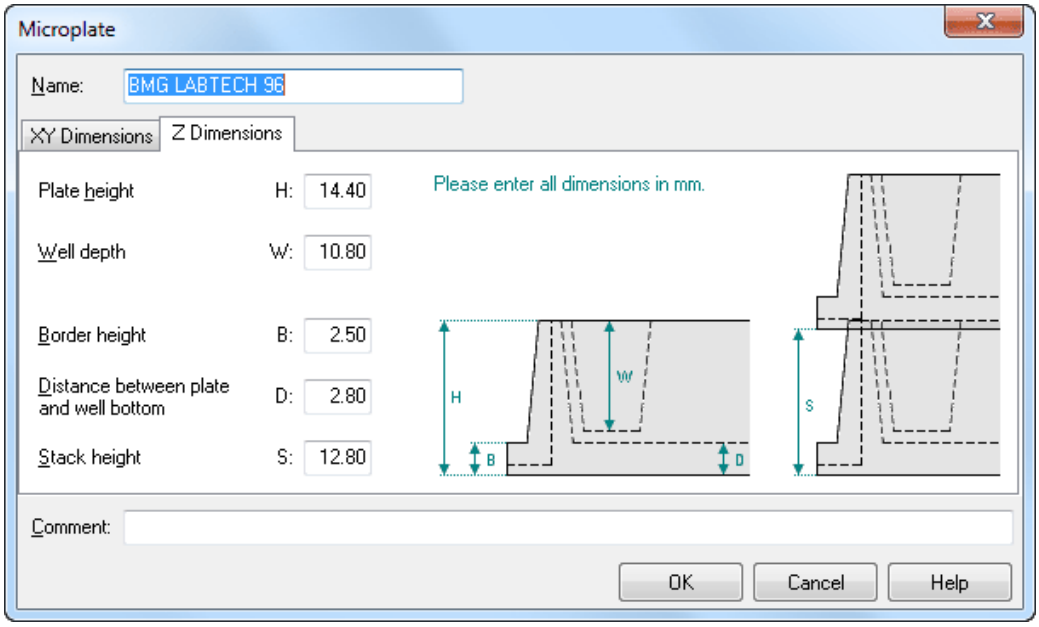


Plate height is the total height of the microplate.

Well depth is the depth of a well, measured in the middle of the well.

Border height is the height of the border of the microplate.

Distance between plate and well bottom is the distance between the bottom of the microplate and the outer bottom of the wells.

Stack height uses the 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.

Comment field can store additional information, for example order numbers or the microplate material.

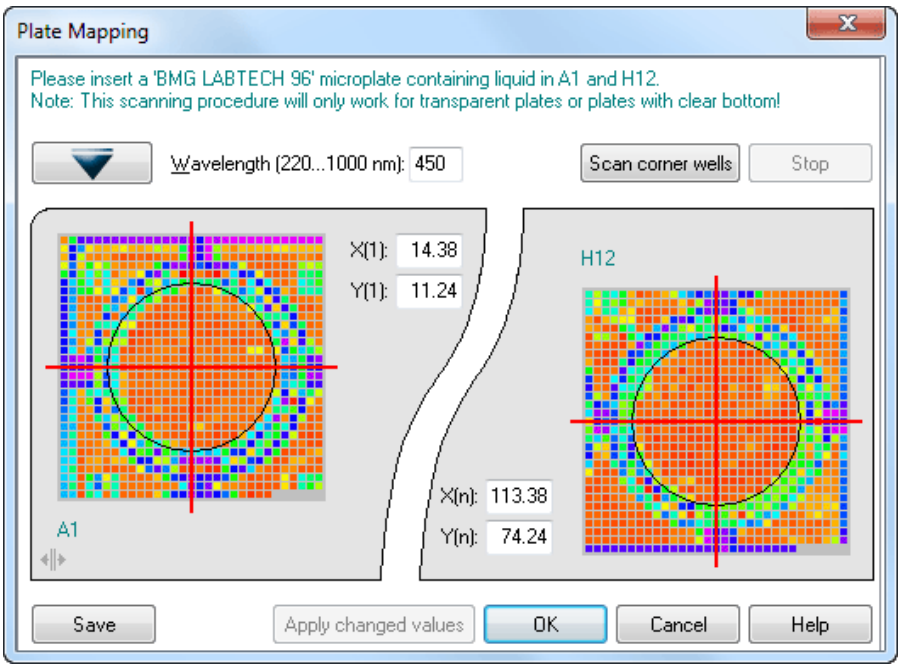
Notes: Users with activated 'Run Only' option (see chapter 1 *Login Screen*) are not allowed to edit microplate definitions.

If an original BMG LABTECH plate definition is changed, there will be a backup created ('Original Name_ORG'). To retrieve the original definitions, delete the changed definition; then either rename the _ORG definition the original name, or copy the _ORG definition and name the copy the original name.

3.5.2 Plate Mapping

The plate mapping function will determine the exact well positions of an unknown, transparent or clear bottom, microplate that has 96 to 1536 wells.

First, fill the top left and bottom right wells of the microplate with liquid – water or any buffer will work. Next, put the microplate into the reader (or insert the plate into magazine 1 if your reader is attached to a stacker). Define an appropriate **wavelength** (Omega readers) – the default is 450 nm and is appropriate for water or define the **laser beam focus** and **intensity** (NEPHELOstar Plus). Click the '**Scan corner wells**' button. The instrument will scan the top left and then bottom right corner wells.



After the scanning procedure has been completed, a picture of the top left and the bottom right wells will be shown. **Drag the red bars** to optimum X and Y positions. It is also possible to change these values by typing numbers into the appropriate input boxes.

The original (start) positions will be shown using black markers.

Hints: Right or left click on the well display or use the [+] and [-] keys to **change the color range**. This may make finding the best position easier. Press the [0] key to use the initial color selection.

Hold the [Ctrl] key while moving the mouse cursor over the well display, the measurement values of the single scan points will be shown.

By clicking the '**Apply changed values**' button, the currently specified X and Y values will be stored without closing the dialogue box. A new scan can be started based on these values. If the 'Apply changed values' function is not clicked before starting a new scan, the original X and Y coordinates will be used.

Notes: The scanning procedure will only work for transparent plates or plates with a clear bottom!

Omega readers: As this scanning is performed using the absorbance spectrometer, this function is only available if the reader is equipped with a spectrometer.

It is possible to '**Save**' the display in bitmap (*.BMP) or JPEG format. Use the key combination [Shift]+[Ctrl]+[P] to **print out** the dialogue box. A standard print dialog box will appear.

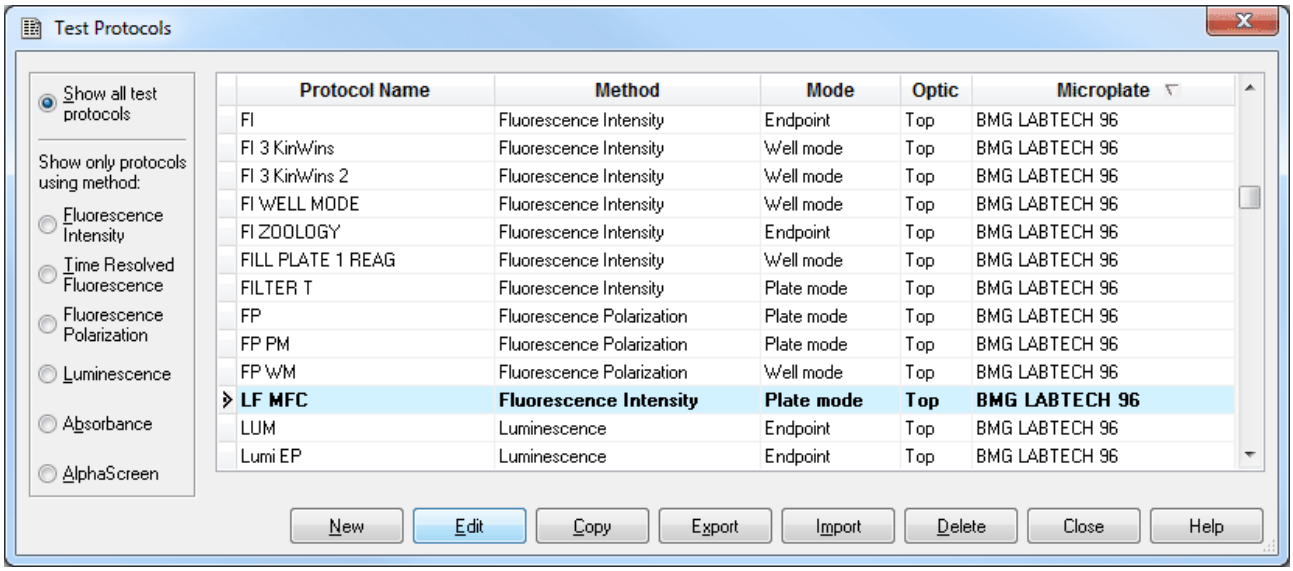
4 Manage Protocols

4.1 Protocol Selection Window

To create or edit a predefined protocol, click on the '**Manage Protocols**' button ( ) in the Protocols tab group. The '**Test Protocols**' dialogue window will appear. It lists all previously defined protocols and gives options for creating new test protocols or modifying existing ones.

The FLUOstar Omega can measure fluorescence intensity and time-resolved fluorescence, as well as (optional) luminescence, absorbance and AlphaScreen. In addition, the POLARstar Omega can measure fluorescence polarization. The LUMIstar Omega can only measure using the luminescence method and the SPECTROstar Omega can only measure absorbance. The NEPHELOstar Plus can only measure using the nephelometry method.

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 Omega, the SPECTROstar Omega and the NEPHELOstar Plus can only measure using one method, the method selection box will not appear when using these types of reader.

To select the first test protocol that begins 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 a previous test protocol. Select the test and click on 'Edit' or double click on the test protocol name.

Copy

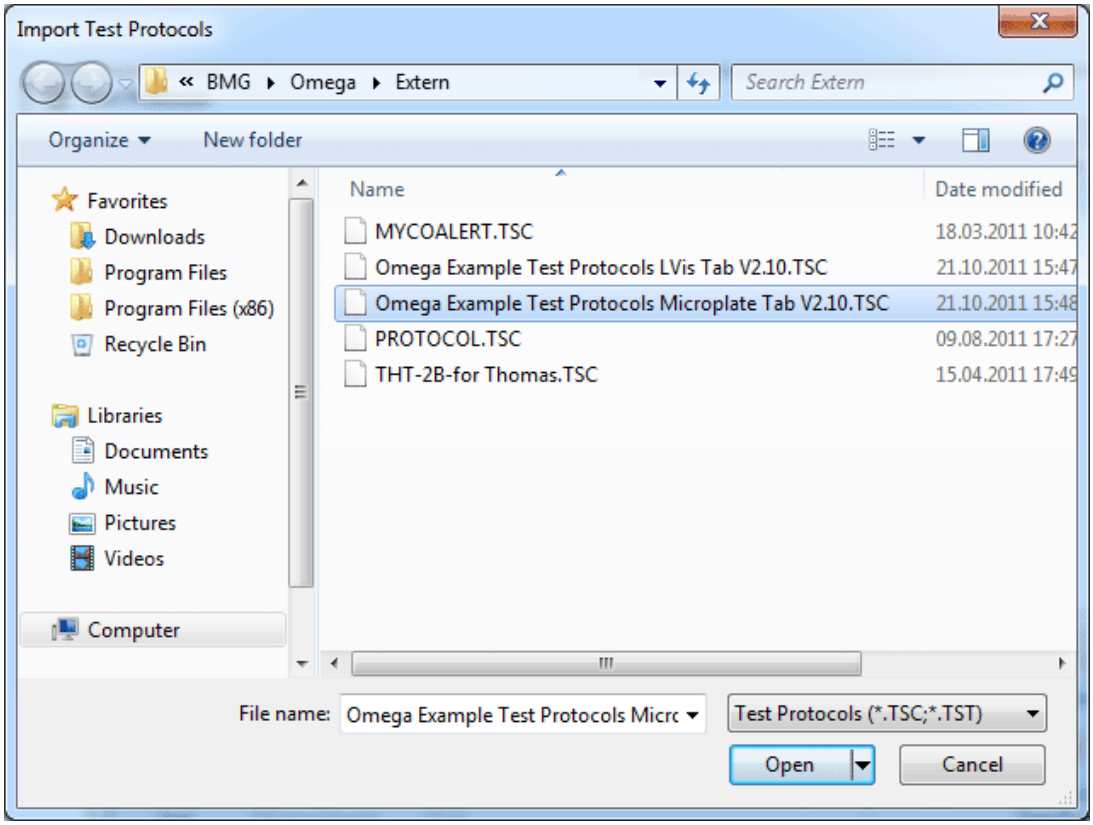
Select the protocol you want to duplicate. A new screen appears and asks for a name for the copy. The duplicate will appear on the test protocol list under the new name. Making a duplicate protocol means that you can modify a protocol without changing the original.

Export

By 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 extension for exported test protocol files is ‘.TSC’.

Import

To import a test protocol click on ‘Import’. Choose the folder.



Select the desired file and click on ‘Open’. The imported test protocols will be added to the list of protocols. By using the mouse, you can also move a *.TSC file directly from an explorer window into the protocol selection window (drag and drop).

Notes: It is possible to import test protocols created using the FLUOstar OPTIMA, OPTIMA or NOVOstar program (file extension ‘.TST’ or ‘.TSC’).

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 Omega import function will ask you to import the layout definitions used by these tests immediately after importing the test definitions (In Omega, 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.

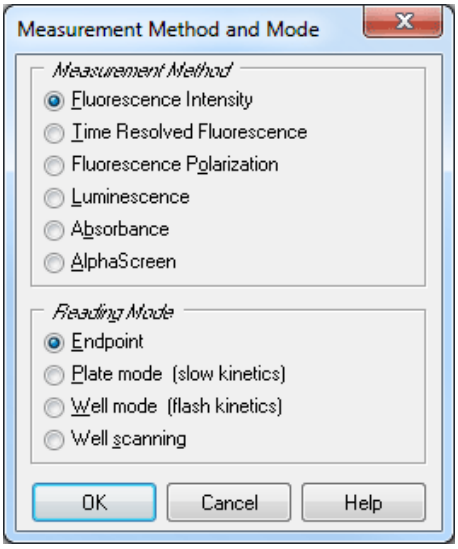
Notes: Exporting the test protocols table into a XLS (Excel), text or HTML file is possible after right clicking the table.

The context menu (right click a protocol entry) also offers functions to convert endpoint protocols into plate mode protocols and vice versa and to convert well mode protocols into well scan protocols and vice versa.

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

Click on '**NEW**'; the next 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.



Notes: Depending on the reader type, all measurement methods might not be available. As the LUMIstar Omega, the SPECTROstar Omega and the NEPHELOstar Plus can only measure using one measurement method, this dialogue will only provide a selection of the different reading modes when using these types of reader.

For AlphaScreen only endpoint can be used.

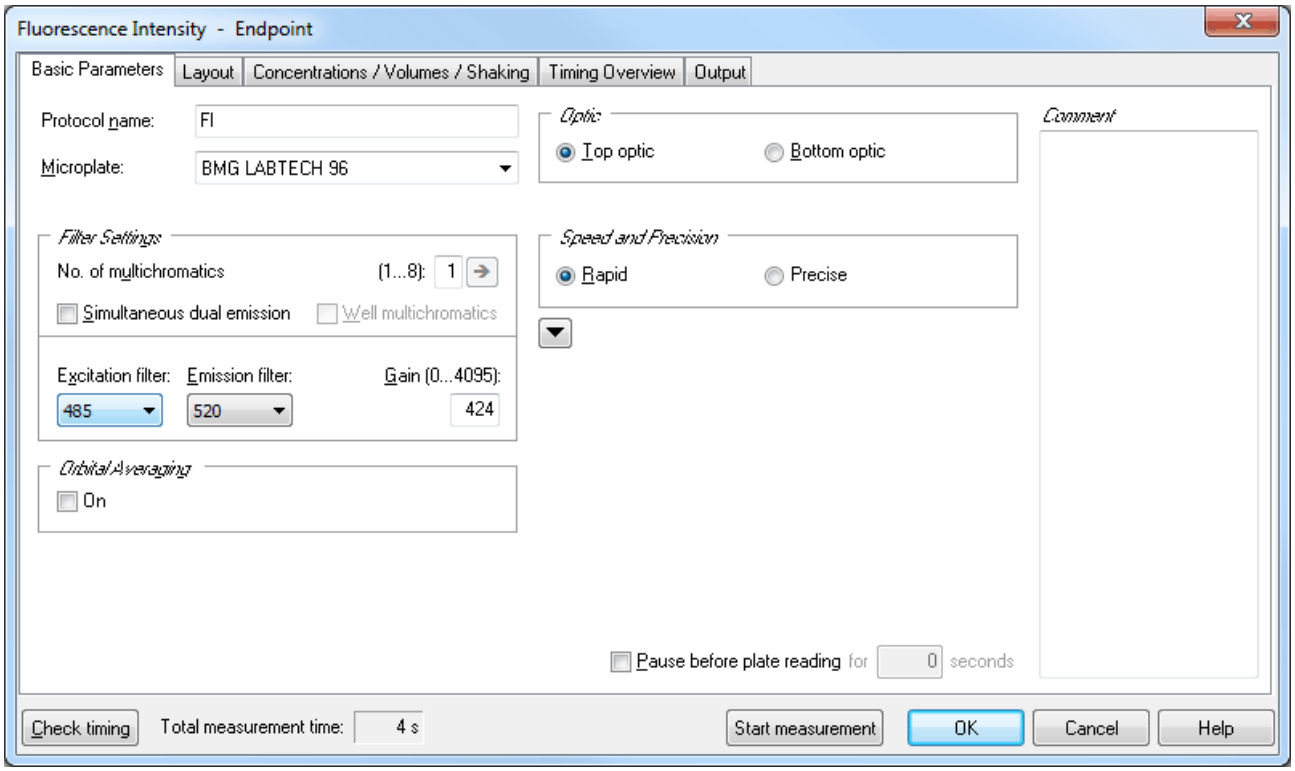
After the method and mode is selected, the next window allows you to enter the test protocol parameters.

4.3 Fluorescence Intensity Protocols

Fluorescence intensity can be measured in endpoint, plate or well mode or in well scan mode. Multichromatic measurements are possible. If the reader is equipped with two fluorescence measurement channels (POLARstar Omega) simultaneous dual emission measurements are also possible.

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 protocol 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 that begins with e.g. ‘B’, simply press the key [B] after opening the pull-down box.

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 click on the ‘Multichromatic’ tab to define the filter combination for each fluorophore to be analyzed.

Excitation and **Emission filter**

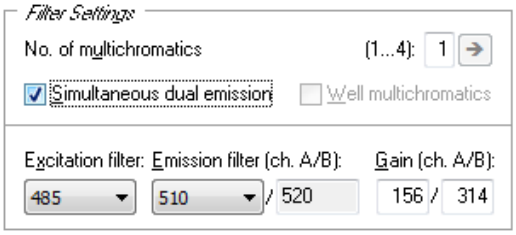
If you only want to analyze one fluorophore, you can select the filter combinations directly in this window. When you use the pull-down menu, the list of filters, as defined under ‘Setup | Filters’ (see chapter 3.1.10 Filters), is displayed.

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 (e.g. 0 to 260 000 relative fluorescence units). You can enter the gain settings here or perform an automatic gain adjustment before the measurement (see chapter 6.4).

Simultaneous dual emission

If your reader has two fluorescence measurement channels (using two PMTs) built in (POLARstar Omega), 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.

Well multichromatics

If you use more than one chromatic and if this box is not checked, in endpoint and plate mode all wells will be measured using the first chromatics first and then all well will be measured using the second chromatics. If this box is checked the measurement of the multichromatics will be done per well. All chromatics will be measured consecutively in the first well (changing the filter and gain setting in between) before moving to the next well.

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.

Note: Orbital Averaging can not be combined with Flying mode (see below).

Optic

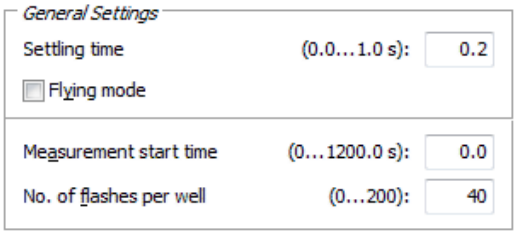
You can select the **top** or **bottom optic** for fluorescence and luminescence mode protocols.

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

Speed and Precision

The two choices in this box, depending on microplate format, balance speed and precision through adjustments to settling time and the number of flashes per well.

Hint: To manually input the **Settling time** or **No. of flashes per well (cycle or scan point)** or to use **Flying mode** or to define a **Measurement start time**, click the arrow button , the *Speed and Precision* box turns into a ***General Settings*** box:



General Settings

Settling time

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 settling time. Defining a measurement start time larger than 0 makes sense when e.g. injection or shaking is performed before the measurement.

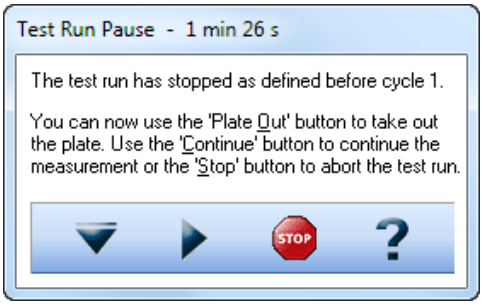
Number of flashes per well

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.

To switch back to the Speed and Precision box use the arrow button .

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. 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 when using a stacker or in script mode (see chapter 6.8).

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.

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.

Start Measurement

You can click this button to immediately start the measurement after defining all necessary settings. This will open the 'Start Measurement' window (see chapter 6.3).

Note: The 'Start Measurement' function is only available if the reader is switched on.

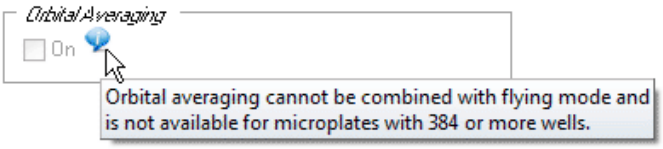
Click on '**OK**' to save the protocol settings and to add the test protocol to the list.

Limitations for 1536 well plates

Your reader has to be specially prepared for measuring 1536 well plates. Injections are not possible.

Disabled Options

Some options are mutual exclusive. If an option can currently not be used, there will be a small info symbol. When moving the mouse cursor to this symbol an explanation will appear:



4.3.2 Basic Parameters – Plate Mode

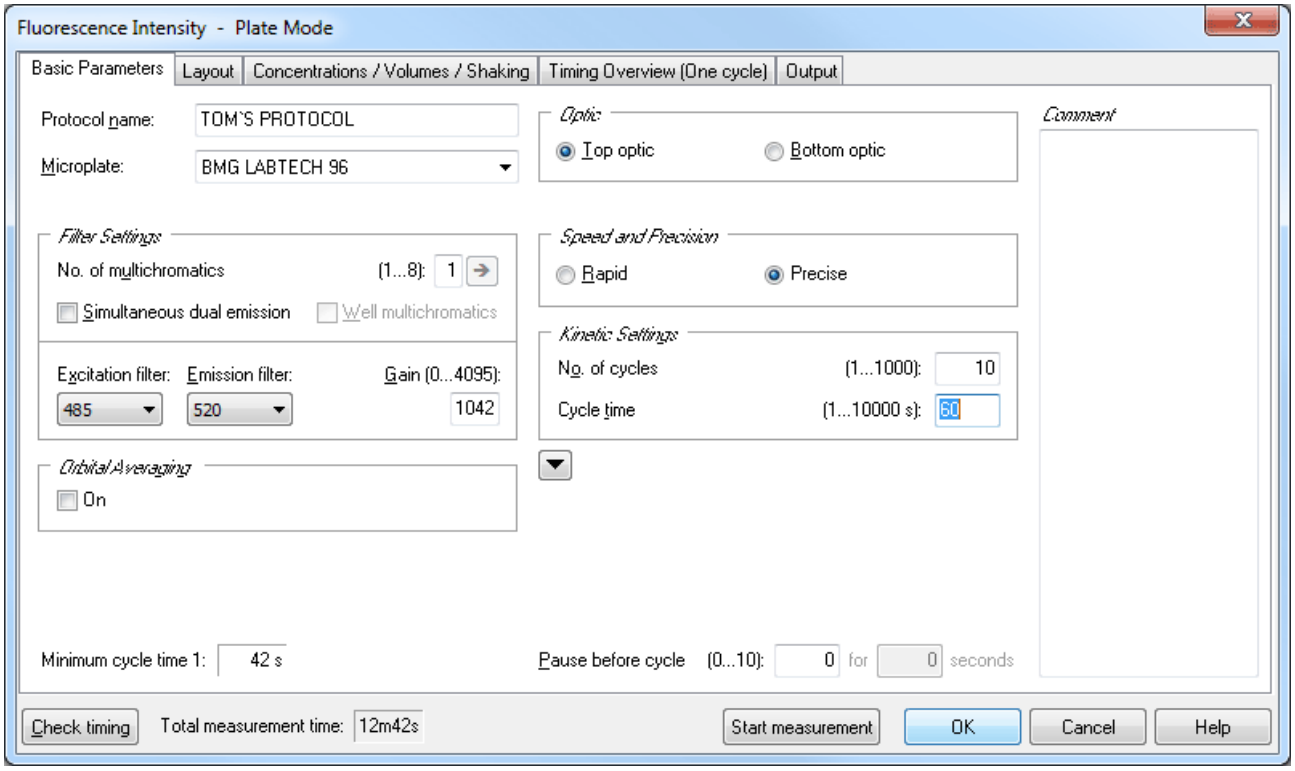
The Basic Parameters tab for Endpoint and Plate Mode are similar, except Plate Mode has two additional *Kinetic Settings,* **No. of cycles** and **Cycle time**.

Plate mode can be used for slow kinetics, e.g. 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 1000 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.

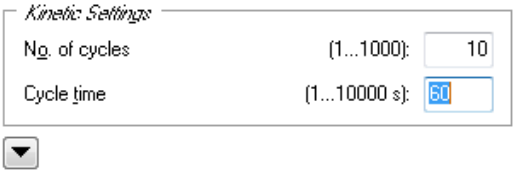
* To use more than 250 cycles a firmware version 1.30 or newer is necessary.

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.



Kinetic Settings



To measure the plate more than one time, use the Kinetic Settings box:

No. of cycles allows for the entire plate to be measured up to 1000 times with each well in the layout measured once per cycle.

Cycle time allows for the duration of each cycle to be defined from 1 to 10,000 seconds.

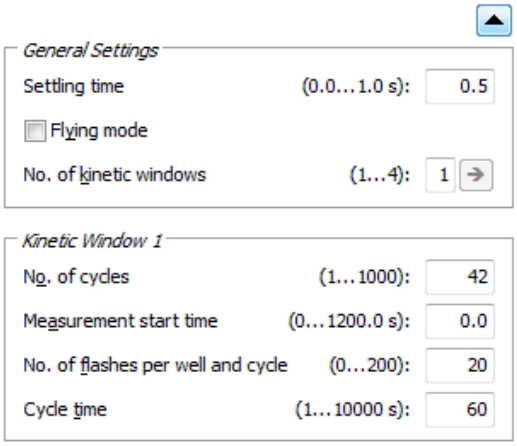
Example settings: A user wants to take 1 reading every 15 minutes for 6 hours for cell growth measurements. Kinetic settings are: No. of cycles = 24 (4 readings for 6 hours) and Cycle time = 900s.

Hint: Click the '**Check timing**' button (see below) when the instrument is switched on and the **Minimum cycle time** will be calculated. If the defined cycle time is smaller than the minimum cycle time, it will be automatically corrected up to the minimum cycle time. If the minimum cycle time is lower, it will do nothing.

After clicking the button the group boxes General Settings and Kinetic Window 1 become available.

General Settings

Settling time



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 1000 cycles (1000 is the total number for all kinetic windows). The number of cycles for the other kinetic windows can be defined in a separate sheet (see chapter 4.3.6).

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 settling time. Defining a measurement start time larger than 0 makes sense when e.g. 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.

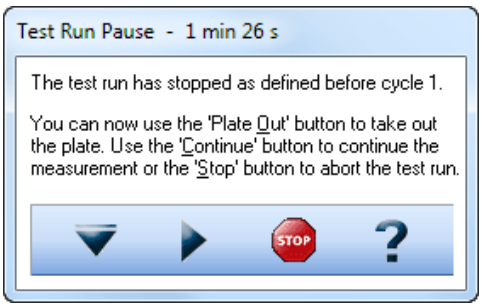
Cycle time

You can define the duration of each cycle from 1 to 10 000 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: A protocol using only one cycle is the same as an endpoint test, thus the cycle time input box is disabled. For defining endpoint test protocols you can also use the special endpoint mode, see chapter 4.3.1.

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. If you have defined 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) 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 the 'Pause' 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 when using a stacker or in script mode (see chapter 8).

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.

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.

4.3.3 Basic Parameters – Well Mode

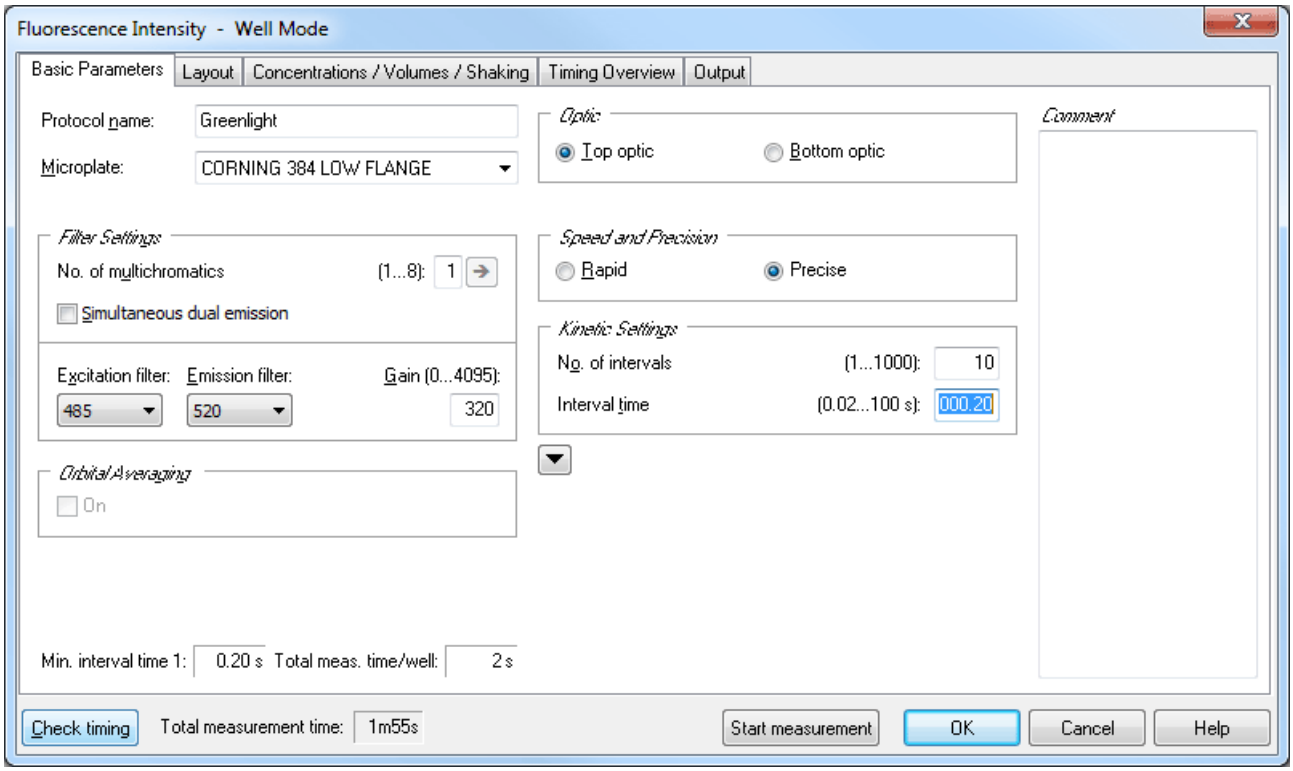
Well mode tests should be selected for fast kinetics, e.g. if the reaction, usually initiated by an injection, occurs over a very finite amount of time. Measurements can then be performed in small intervals when the reaction occurs. In well mode, each well, as defined in the layout, will be measured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

Key words:

Intervals	Number of times the well is measured. Up to 1000 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 measure-ment 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).

* To use more than 250 intervals a firmware version 1.30 or newer is necessary.



Kinetic Settings

To measure each well more than one time, use the Kinetic Settings box:

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 1000 intervals. 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.

Interval time

You can define the duration of each interval from 0.02 to 100.0 seconds. When the instrument is active, you can click the 'Check timing' button 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 whether the rapid or precise mode is used and on the usage of multichromatics.

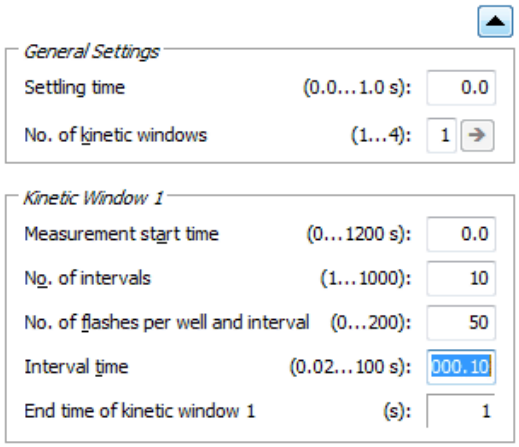
For example, the instrument gives a minimum interval time of 0.4 seconds, but you can change this to 5 seconds so that there will be a delay of 4.6 seconds between intervals.

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. For defining endpoint test protocols you can also use the special endpoint mode, see chapter 4.3.1.

Hint: Click the '**Check timing**' button (see below) when the instrument is switched on and the **Minimum interval time** will be calculated. If the defined interval time is smaller than the minimum interval time, it will be automatically corrected up to the minimum interval time. If the minimum interval time is lower, it will do nothing.

After clicking the button the group boxes General Settings and Kinetic Window 1 become available.

General Settings



Settling time

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 4.3.6) 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

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 settling 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 1000 intervals (1000 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 100.0 seconds. When the instrument is active, you can click the 'Check timing' button 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 0.01 seconds, 10 flashes equals 0.1 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. For defining endpoint test protocols you can also use the special endpoint mode, see chapter 4.3.1.

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.

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.

Total measurement time per well

This time will be calculated using the settings in the protocol, e.g. 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.)

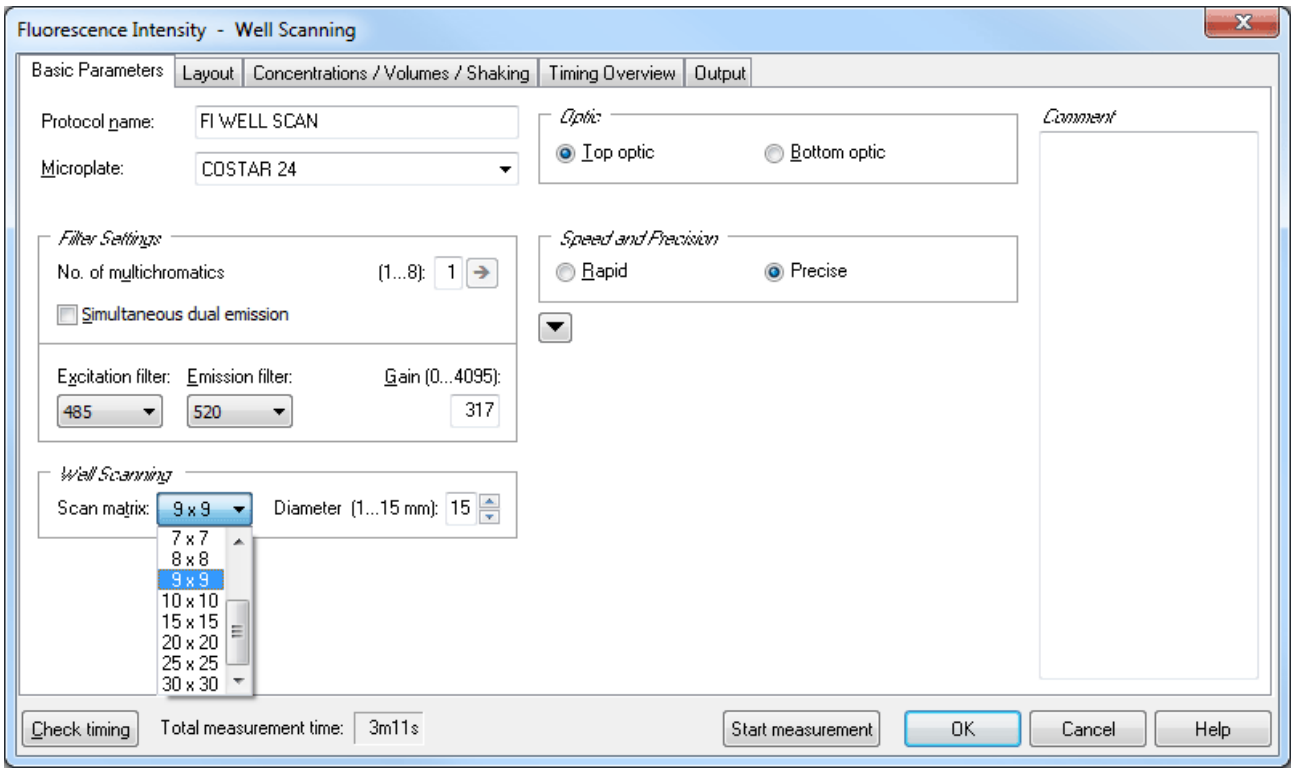
After pressing the '**Check timing**' button, the 'Timing Overview' sheet will become available (see chapter 4.3.14).

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

4.3.4 Basic Parameters – Well Scan

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 scan protocol. Well scan 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) and in the Omega Data Analysis software (see software manual part III).

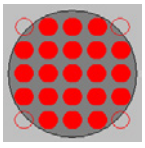
Well scan can only be used for microplates up to 96 wells. Well scan is not available for fluorescence polarization measurements.



Most parameters are similar or identical to endpoint protocols (see chapter 4.3.1). It is not possible to measure more than one cycle or interval in this mode.

Well Scan

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 3.5.1), 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.

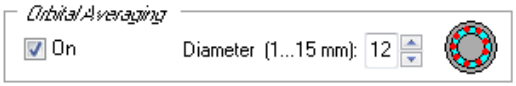
Notes: Well scanning is slower than orbital averaging (see chapter 4.3.5).

The defined number of flashes (or the defined measurement interval time in luminescence mode) and the defined settling time will be used for every single scan point, and have therefore a dramatic impact on the total measurement time, especially when using a high number of scan points, e.g. 30x30.

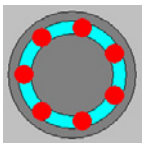
4.3.5 Orbital Averaging

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.

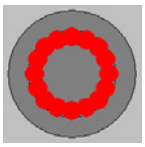
Using this mode, the measurement takes place on an orbit with definable diameter. In **absorbance and fluorescence modes** the defined number of flashes will be equally distributed over the orbit. For luminescence mode, see chapter 4.6.1. For each well, there will be one orbital movement per cycle/interval (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 small icon inside the orbital averaging box will illustrate how the measurement points are distributed over the well:



7 flashes



16 flashes, smaller diameter

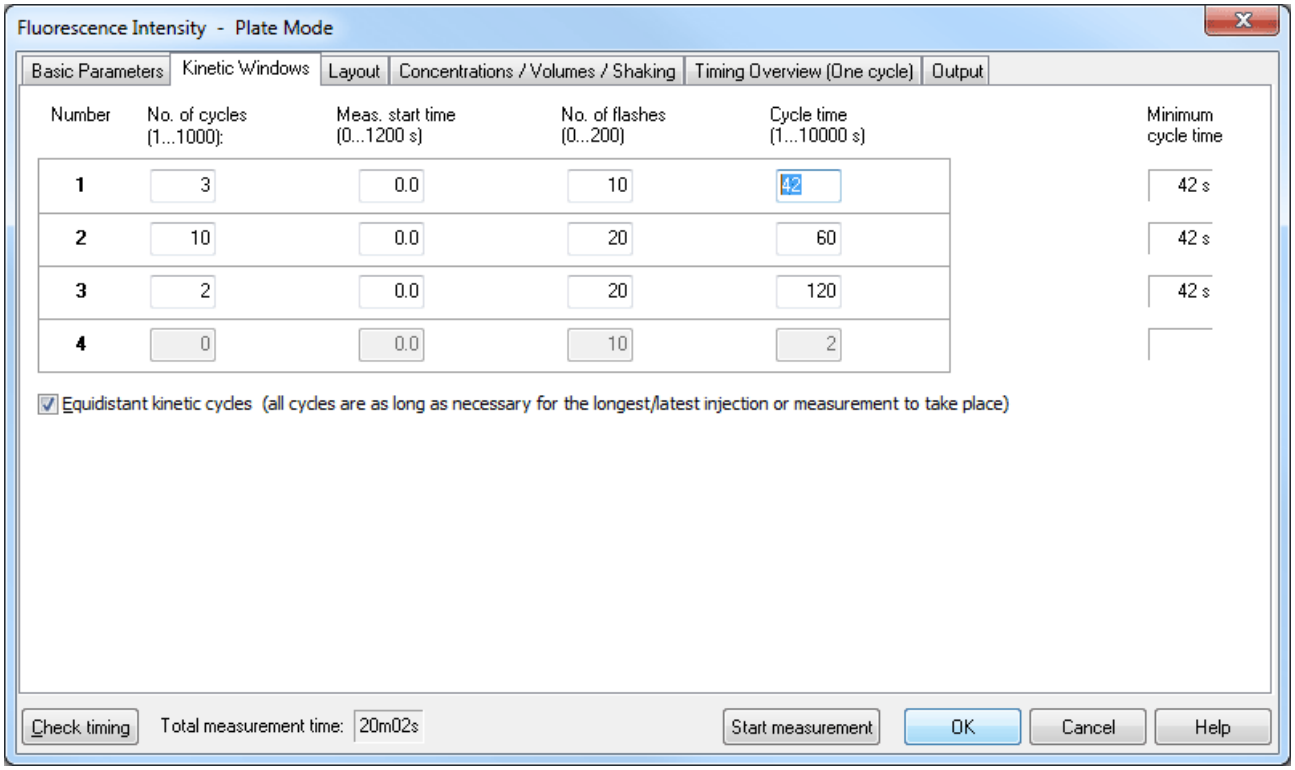
Notes: As it is possible to use different measurement interval times in different kinetic windows (see chapters 4.3.6 and 4.3.7), you will also see these icons in the Kinetic Windows sheet.

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

Due to technical reasons orbital averaging is not available for spectrometer based absorbance measurements.

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.



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 1000 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 settling time. Defining a measurement start time larger than 0 makes sense when e.g. injection or shaking is performed before the measurement.

Number of flashes (all modes except luminescence and nephelometry)

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 and nephelometry method)

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

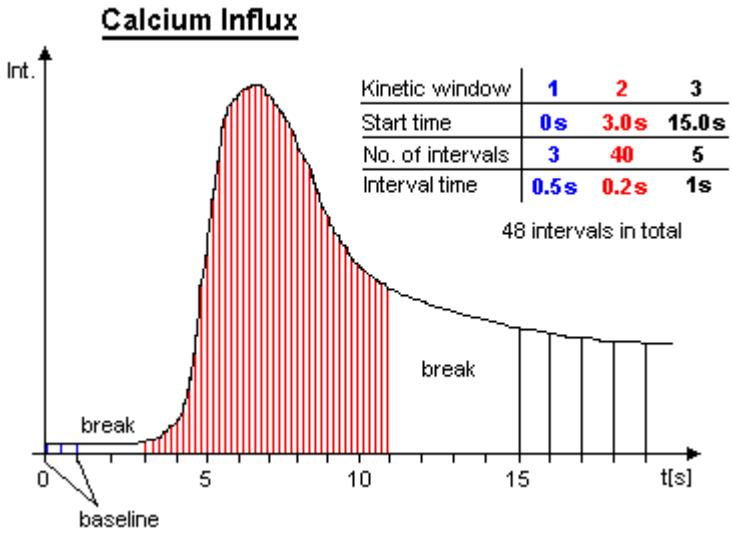
The minimum cycle times can only be calculated by the reader. If you do not use the Equidistant kinetic cycles option, the Minimum cycle time can be different in different kinetic windows (depending on the Measurement start time and the Number of flashes). After defining all necessary settings (do not 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.

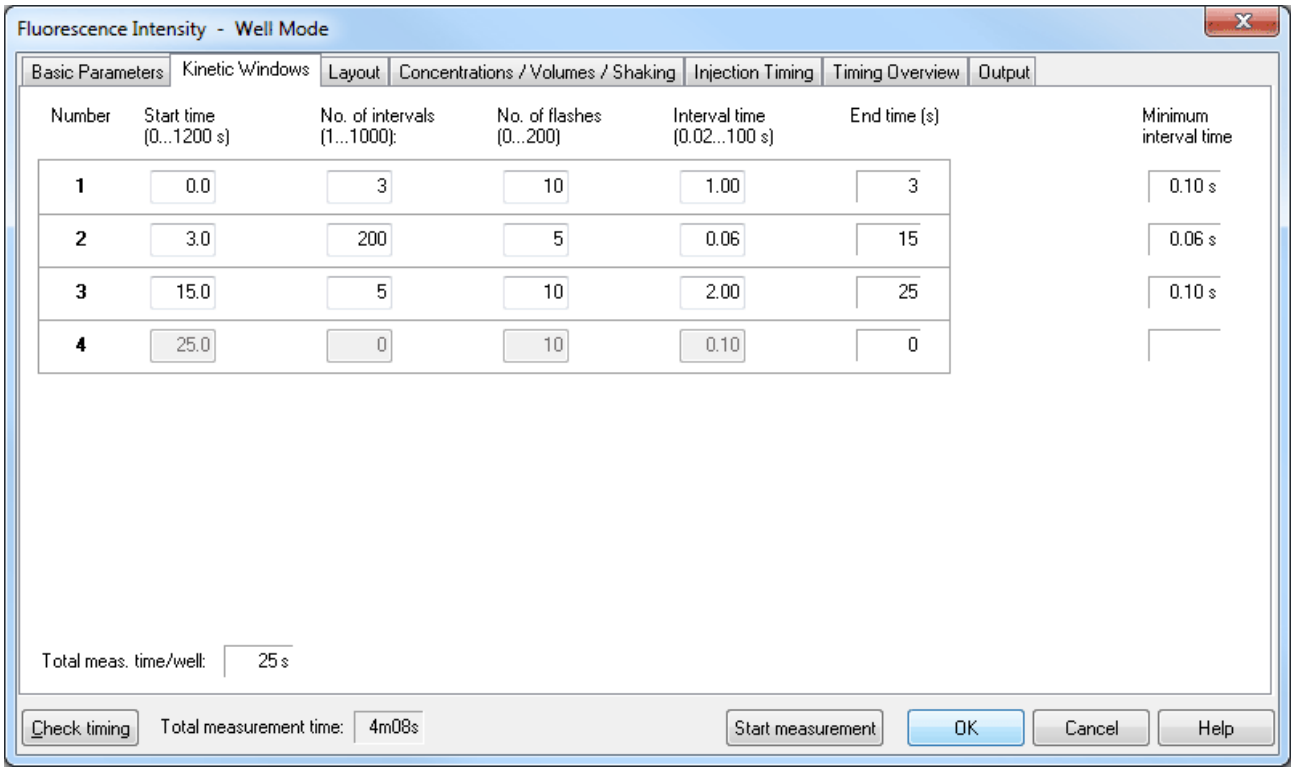
Note: The 'Check timing' function is only available if the reader is switched on.

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:





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 1000 intervals.

Number of flashes (all modes except luminescence and nephelometry)

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 and nephelometry method)

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.

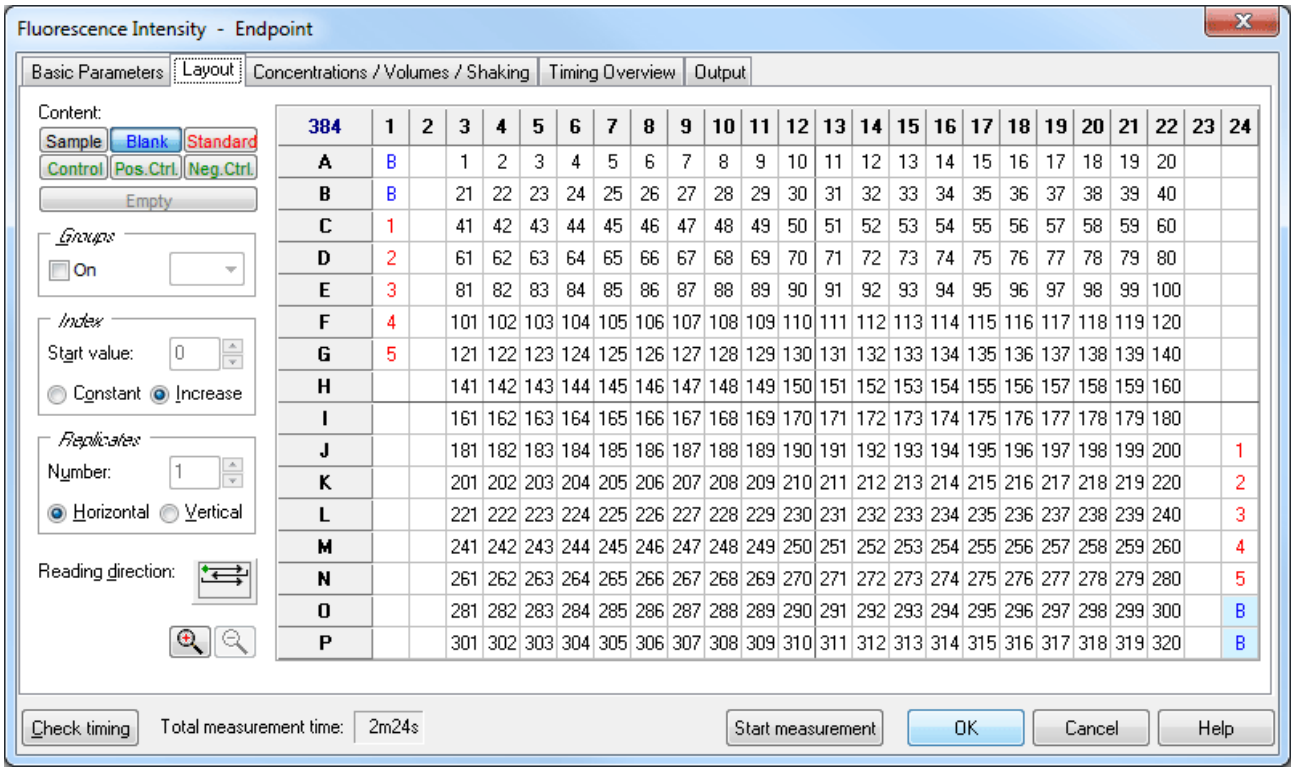
Minimum interval time

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

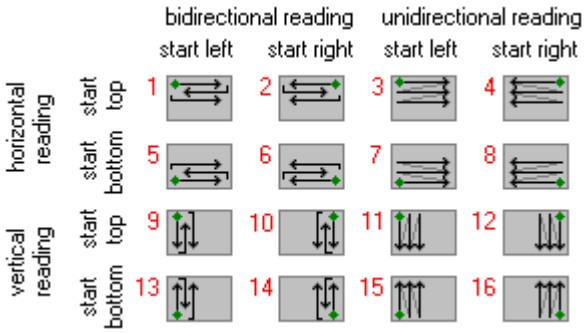
After pressing the reading direction button ( ), you can choose between **horizontal** or **vertical** reading (horizontal: the plate carrier will move from left to right reading across successive columns, vertical: the plate carrier will move up and down reading in successive rows).

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**.

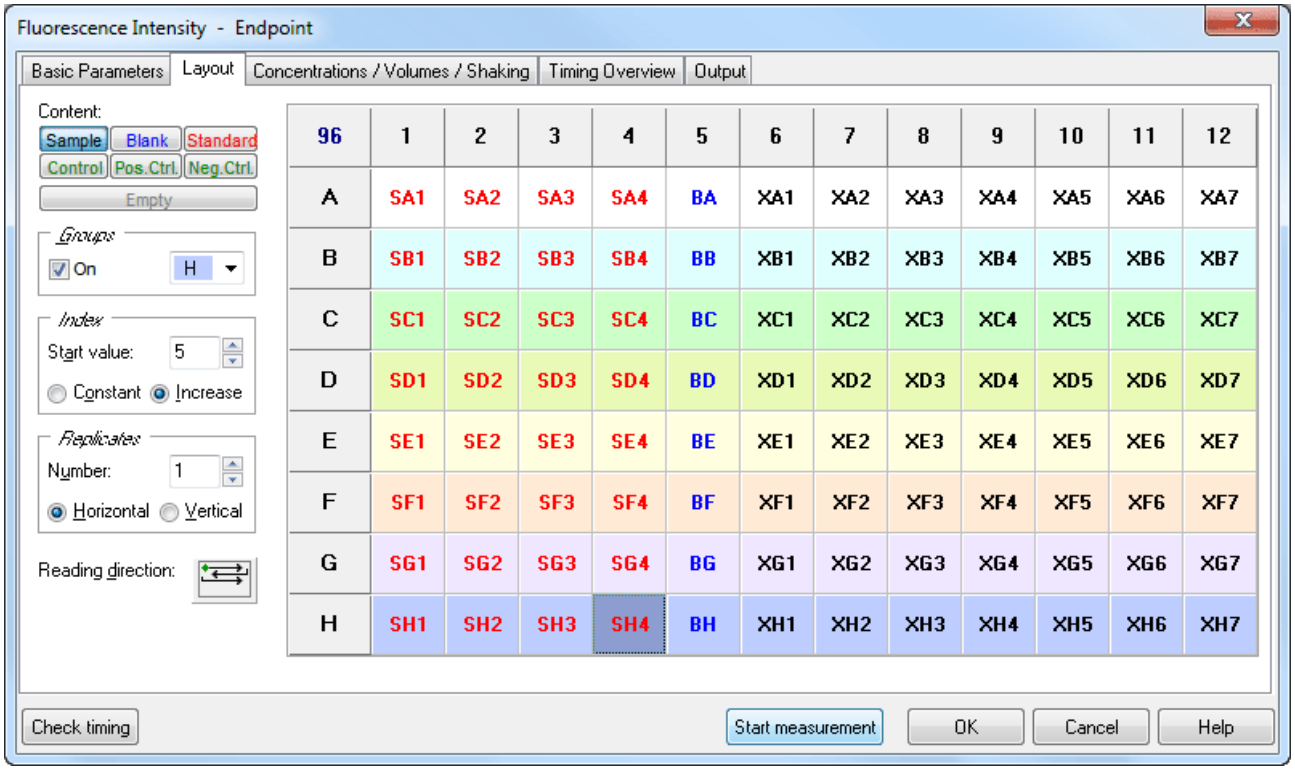
Note: The reading direction has no bearing on the measurements; it is intended to optimize the amount of time it takes to read the plate.

Possible reading modes:



4.3.9 Using Layout Groups

It is possible to use up to 26 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).



96	1	2	3	4	5	6	7	8	9	10	11	12
A	SA1	SA2	SA3	SA4	BA	XA1	XA2	XA3	XA4	XA5	XA6	XA7
B	SB1	SB2	SB3	SB4	BB	XB1	XB2	XB3	XB4	XB5	XB6	XB7
C	SC1	SC2	SC3	SC4	BC	XC1	XC2	XC3	XC4	XC5	XC6	XC7
D	SD1	SD2	SD3	SD4	BD	XD1	XD2	XD3	XD4	XD5	XD6	XD7
E	SE1	SE2	SE3	SE4	BE	XE1	XE2	XE3	XE4	XE5	XE6	XE7
F	SF1	SF2	SF3	SF4	BF	XF1	XF2	XF3	XF4	XF5	XF6	XF7
G	SG1	SG2	SG3	SG4	BG	XG1	XG2	XG3	XG4	XG5	XG6	XG7
H	SH1	SH2	SH3	SH4	BH	XH1	XH2	XH3	XH4	XH5	XH6	XH7

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 26 possible groups (A...Z). 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.

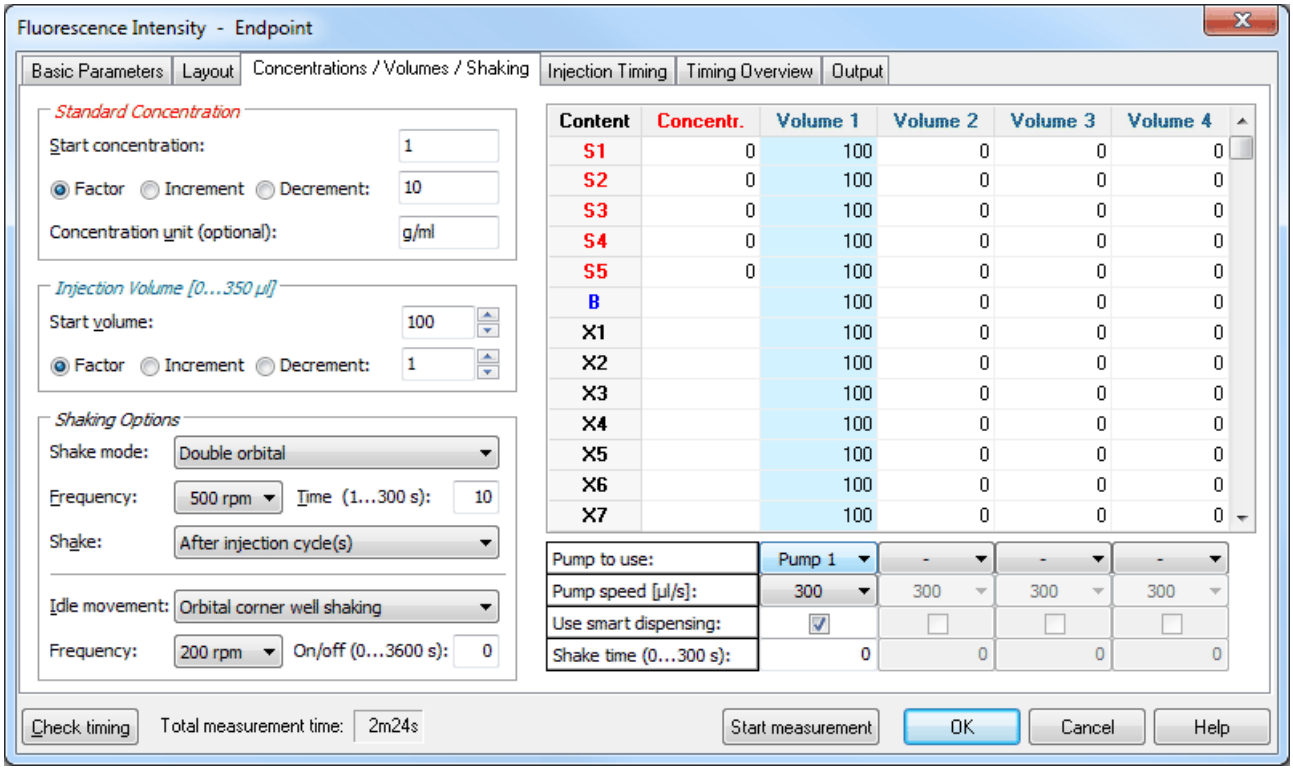
Notes: When switching on the group mode, all existing layout entries will be converted to group A.

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 '`Omega.ini`' file: `MinVolumeStep=6`, for minimum steps of 1 µl use `MinVolumeStep=1`. You will find this configuration file in the Omega main installation directory, usually '`~\Program Files\BMG\Omega\`'.

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 popup 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 µl / second. The default speed is 310 µl / second which should be appropriate for most assays. There are pre-defined speeds available by using the pull-down menu.

Factors determining pump speed:

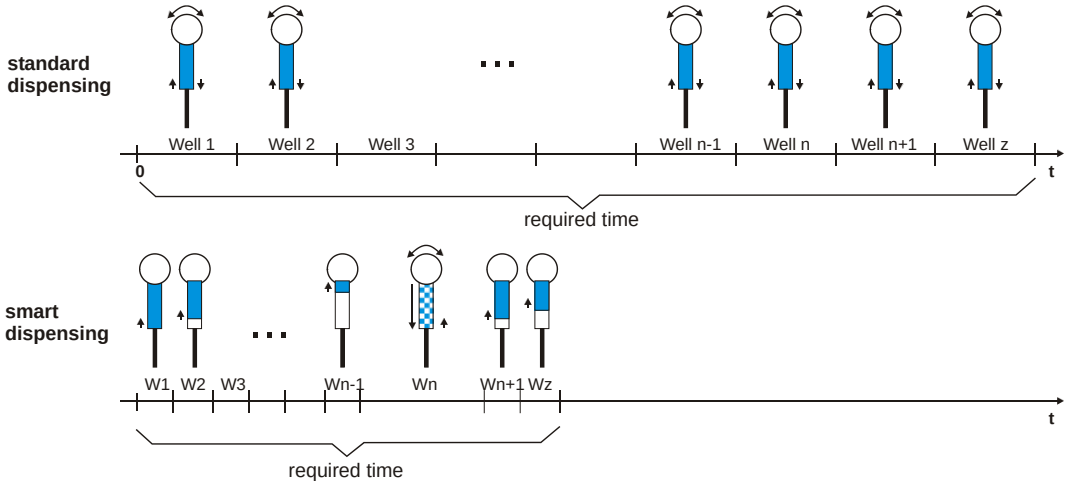
Viscosity: Liquids, such as water and buffer, can be dispensed at higher speeds. For highly viscous solutions you should use a slower speed to ensure higher precision and lower the risk of air bubbles.

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...5 µl) 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.

Shake time

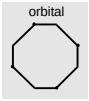
If you want shaking after an injection, add the desired shaking time here. It is possible to define different shaking times for different volume groups. The shake mode and frequency will be defined for all volume groups (and for possible additional shaking) in the 'Shaking Options' group box on the left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

Shaking Options

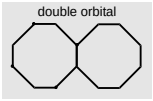
Shake mode

Linear: Shaking mode is from right to left.

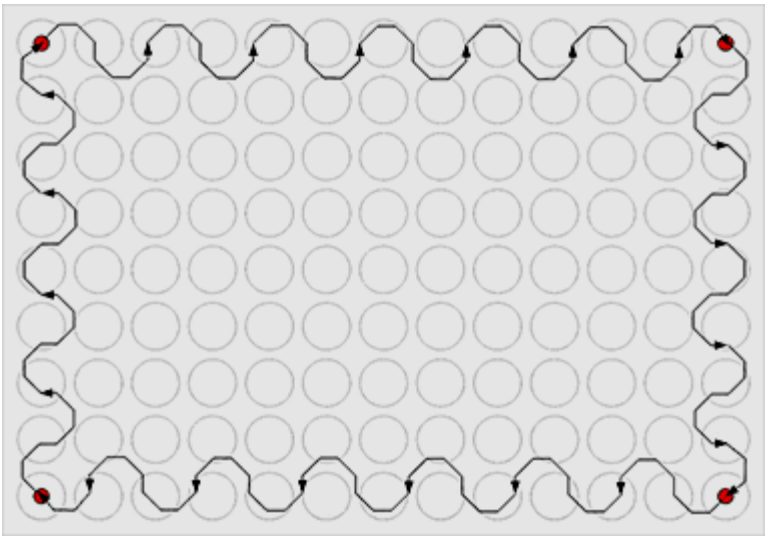
Orbital: Shaking mode is circular; mixing is more complete, especially around the edges of the microplate.



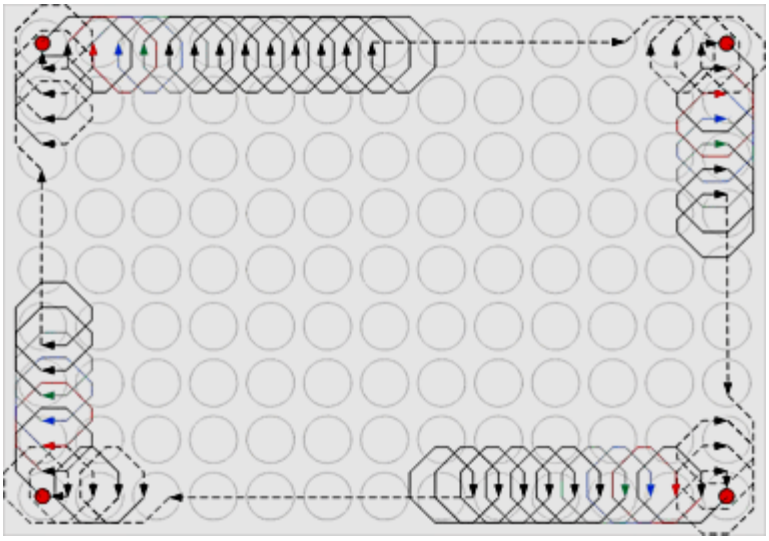
Double orbital: The shaking function is performed as orbital movement. The plate carrier makes a figure eight movement.



Meander corner well shaking: Meander shaking movement overlaid with a movement between the 4 corner wells of the plate.



Orbital corner well shaking: Orbital shaking movement overlaid with a movement between the 4 corner wells of the plate.



Frequency

Defines the speed of the shaking motion. The frequency to be used depends on several conditions:

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

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

Volume: A higher frequency is suggested for smaller volumes.

Time

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

Shake

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.).

In endpoint, well or well scan mode, additional shaking is only possible before plate reading.

Idle movement

For plate mode protocols an additional plate carrier movement during the idle time can be defined. The idle time is the waiting time between the end of all kinetic cycle i actions (measurements, injections, …) and the start of kinetic cycle i+1. If **none** has been selected, the plate carrier will stay in the start position (first well or shaking position) during the idle time. It is also possible to **wait at the incubation position** (a dedicated waiting position where the microplate is fully covered by the incubator heating plates), or to select a (linear) **movement among corner wells**. When **meander corner well shaking** or **orbital corner well shaking** has been selected, the plate carrier will move among the 4 corner wells. This movement will be overlaid with a meander or orbital shaking movement (see pictures above).

Notes: The idle movement feature is only available for plate mode protocols.

To use this function a firmware version 1.40 or newer is necessary.

Frequency

For the last two idle movement options different frequencies can be used. This parameter defines the repetition frequency of one single shaking polygon. Since the number of motor movements is the same as for orbital shaking, it has the same shaking effect as orbital shaking.

On/off

If a value larger than 0 is entered here, the idle movement will be interrupted periodically. This value is used for both, the active time and the pause time.

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 select the entry “Shake only” in the ‘Pump to use’ pull down box. 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).

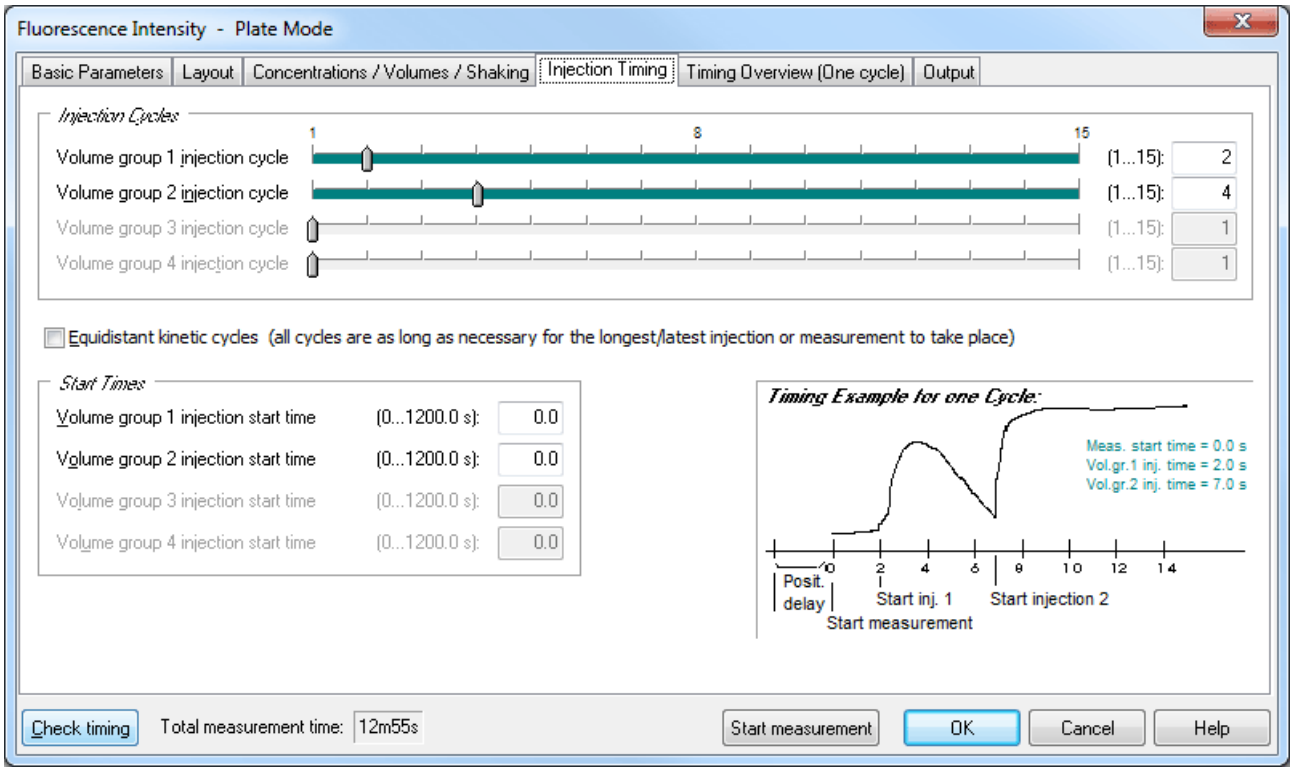
Limitations for Injection

The maximum amount per injection is 500 µl (6, 12, 24 and 48 well plates), when using a standard 500 µl syringe or a larger syringe. When using a 250 µl syringe or a 100 µl syringe the volume per injection must not exceed the syringe size. 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



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

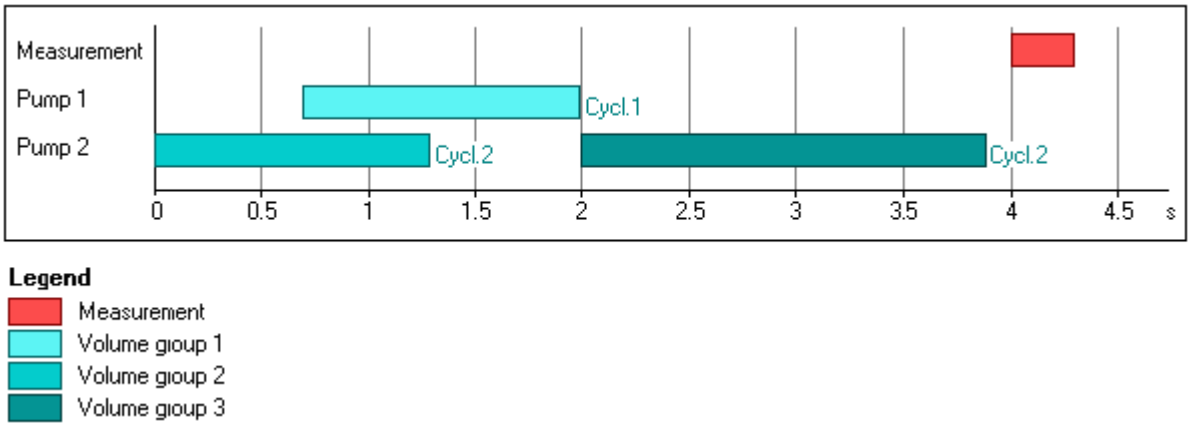
By using the Start Times box, it is also possible to meet special requirements regarding timing on one well in a slow kinetic. The start of the injection(s) or of the measurement (in Basic Parameters or Kinetic Windows sheet) can be delayed up to 1200 seconds on each well after the time when the well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

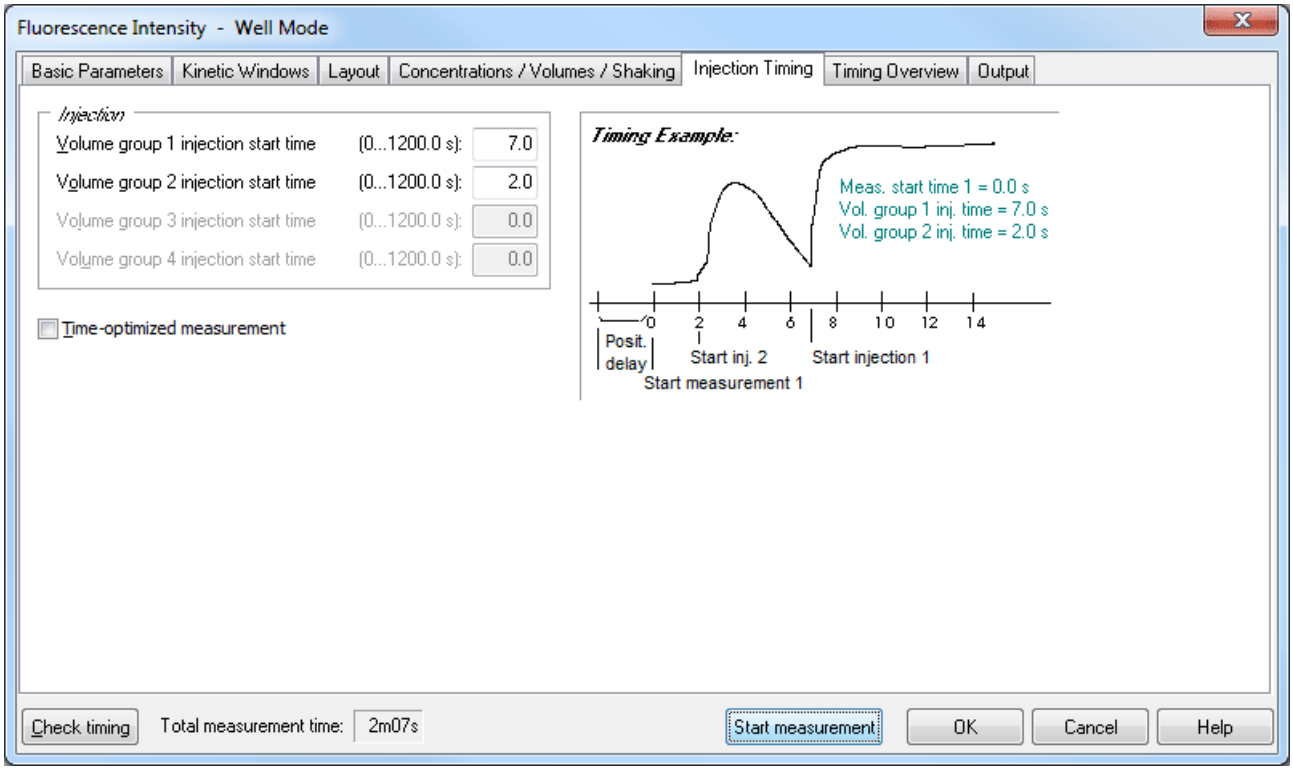
Example: The following scenario would be possible with this method:

- 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).



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

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

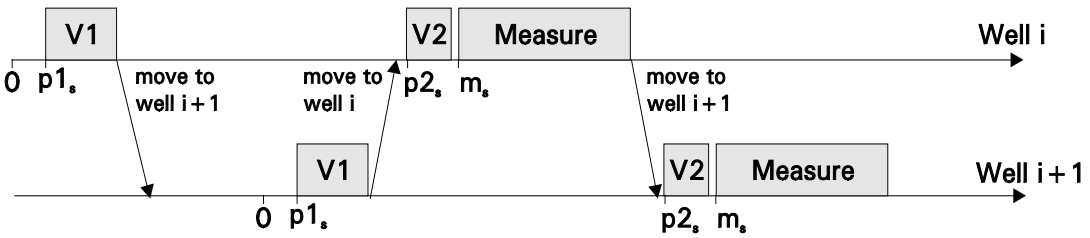


Volume group X injection start time

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 settling time. 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.

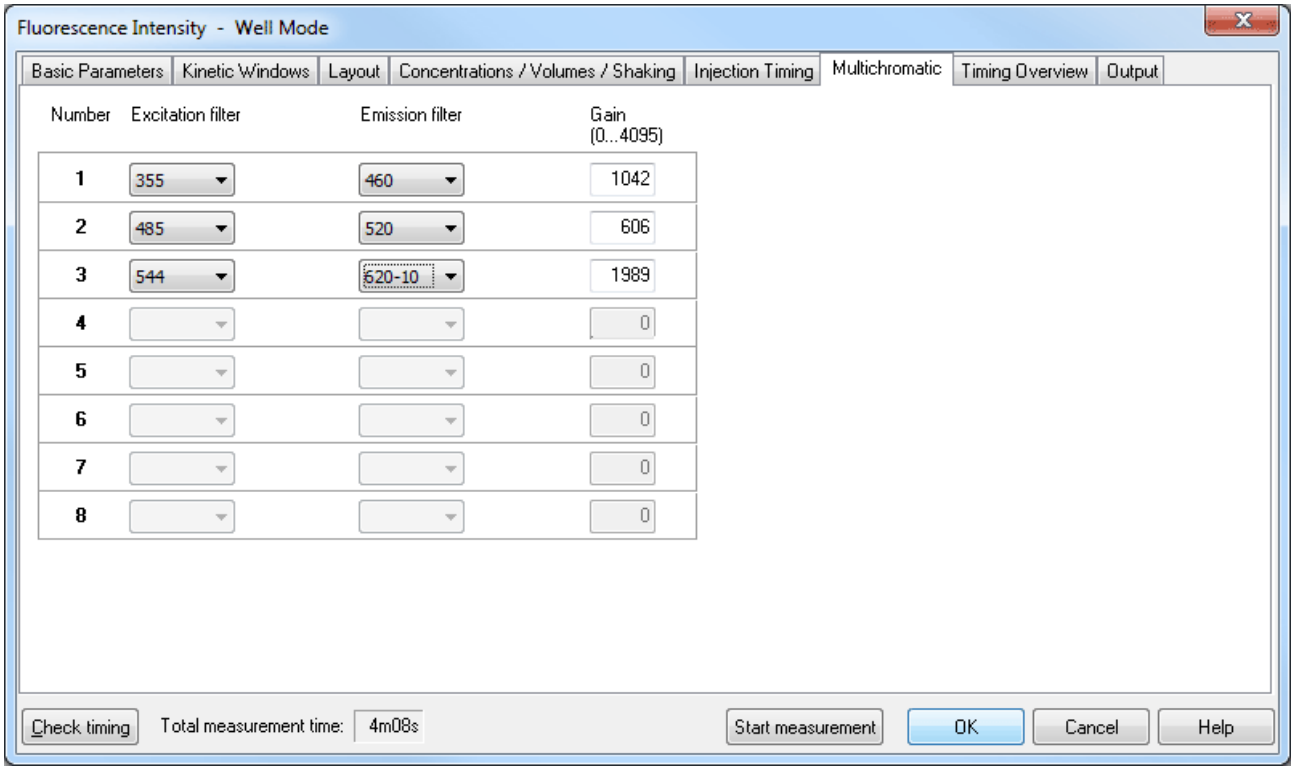


Example for well mode test with time optimization

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, 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 button or click on the ‘Multichromatic’ tab. A new sheet will appear:



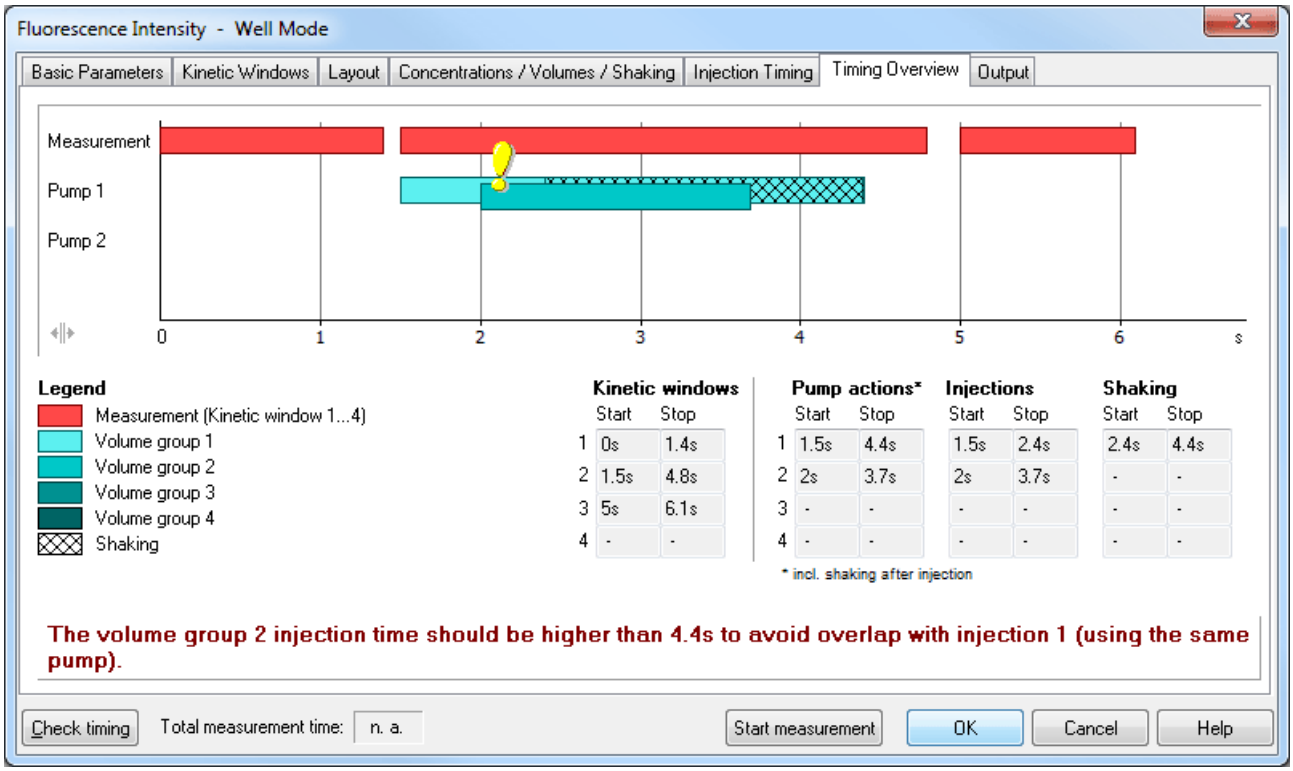
Now you can use the pull-down menu in each row to define the filters. The filters shown in the pull-down menu are listed as they are defined in the ‘Setup | Filter’ section (see chapter 3.1.10 Filters).

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 exists, for absorbance method protocols the emission filter and the gain columns do not exist.

4.3.14 Timing Overview

The timing overview offers a graphic display of the measurement and injection timing. After clicking the 'Check timing' button (lower left corner of the test protocol definition window), the following sheet will become available:



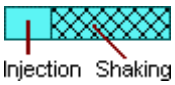
In this sheet, you can see a graphic overview of the measurement and all injections for one well. (For plate mode tests, all actions are displayed in the timing overview of one cycle. The displayed injections may occur in different cycles, as defined under 'Injection / Timing'. The cycle used will be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

You can change the timing by moving the timing bars using the mouse (left click a bar and drag it to its new position). Time values changed by moving a bar will be displayed in cyan. You can also change a time value by clicking the value inside the table. This will bring you to the protocol definition sheet where this time value can either be directly entered or influenced (e.g. the kinetic window stop time can be change by changing the number of intervals).

An injection action can contain different steps:

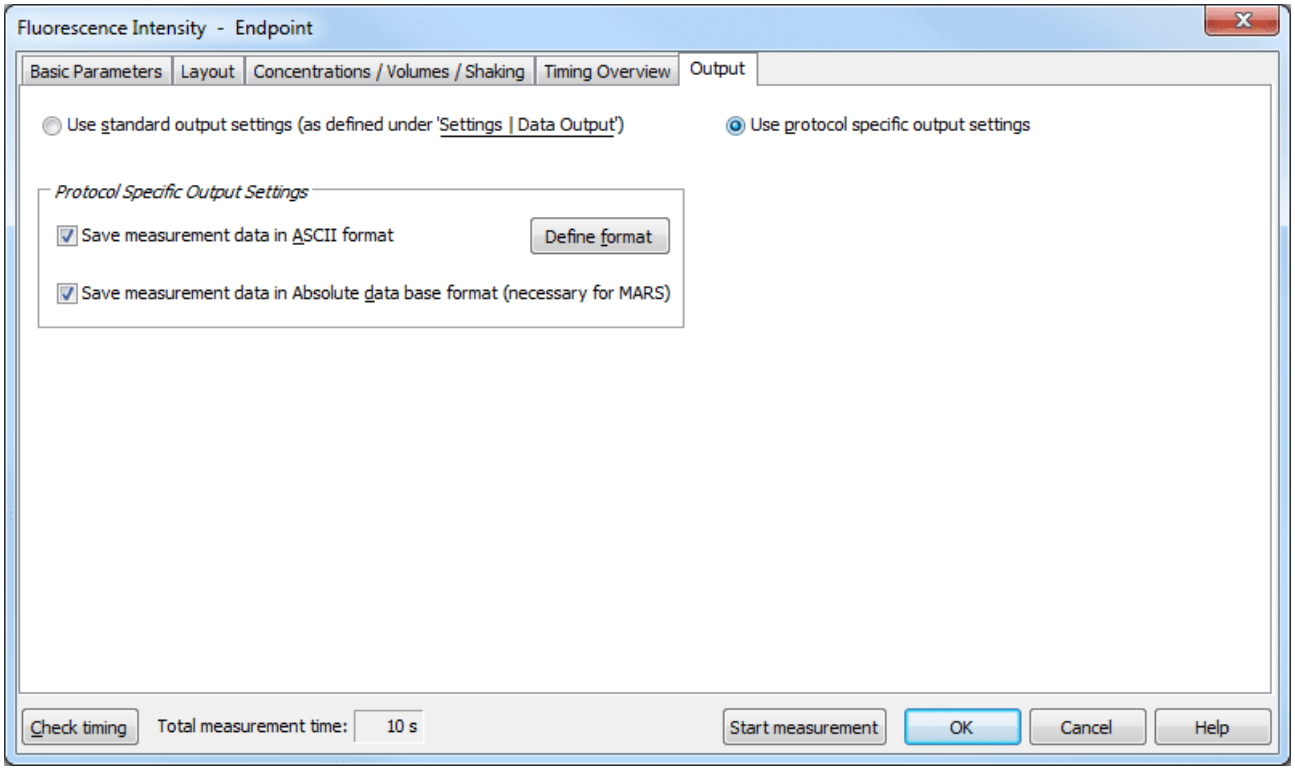


Note: The pump action times listed include the time for shaking.

Injections are not possible during shaking.

4.3.15 Output Sheet

If you have activated the option 'Allow protocol specific output settings' in the 'Data Output' dialogue (see chapter 3.4), an additional sheet 'Output' will be available as part of the protocol editors:

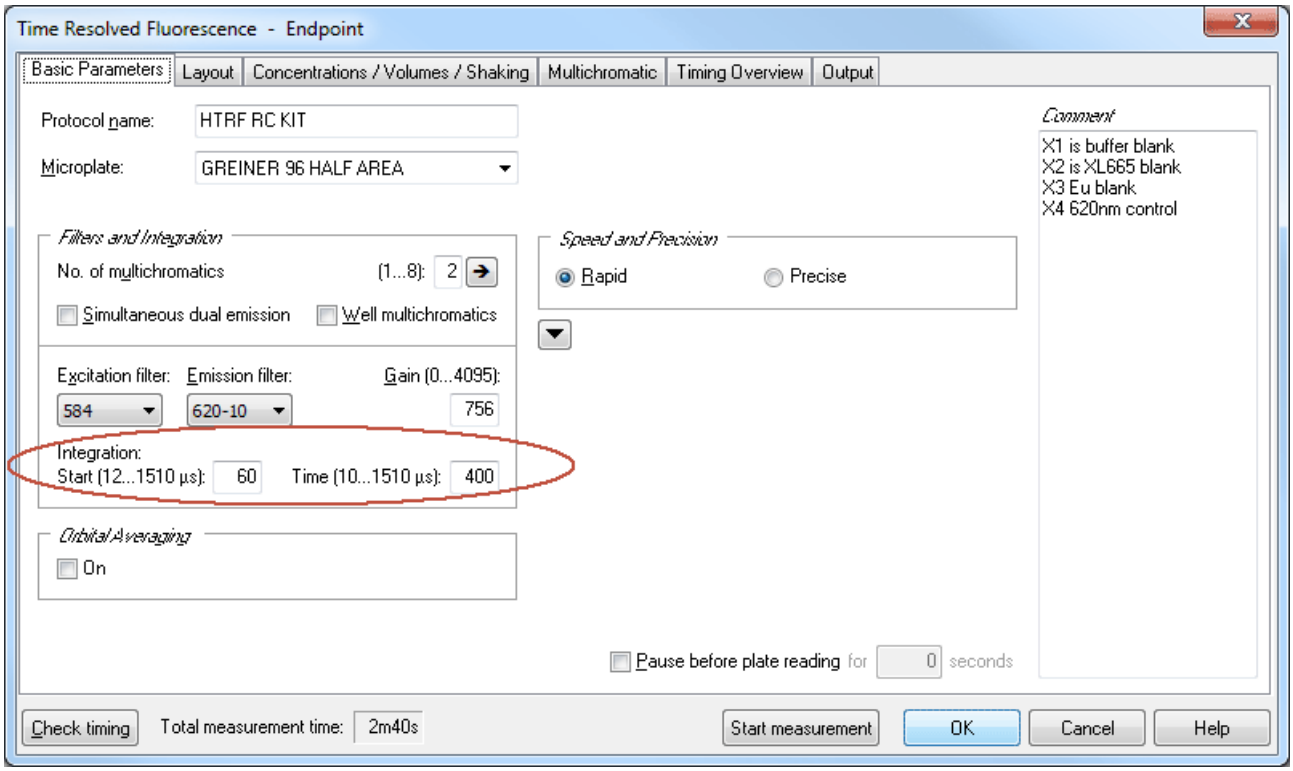


Here you can choose between **standard output settings** and **protocol specific output settings**.

If you have selected to use protocol specific output settings you can decide for the protocol currently edited whether the measurement data should be stored in **ASCII** and / or **Absolute Data Base format**. After clicking the '**Define format**' button you can define the ASCII export format specific for each protocol (see chapter 3.4.1).

4.4 Time-Resolved Fluorescence Protocols

Time-resolved fluorescence can be measured in endpoint, plate or well mode or in well scan mode. Most parameters are similar to fluorescence intensity protocols (see chapter 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

Enter the time after the flash when the measurement should begin. This time should provide the maximum signal from the fluorophore after the flash has dissipated.

Integration time

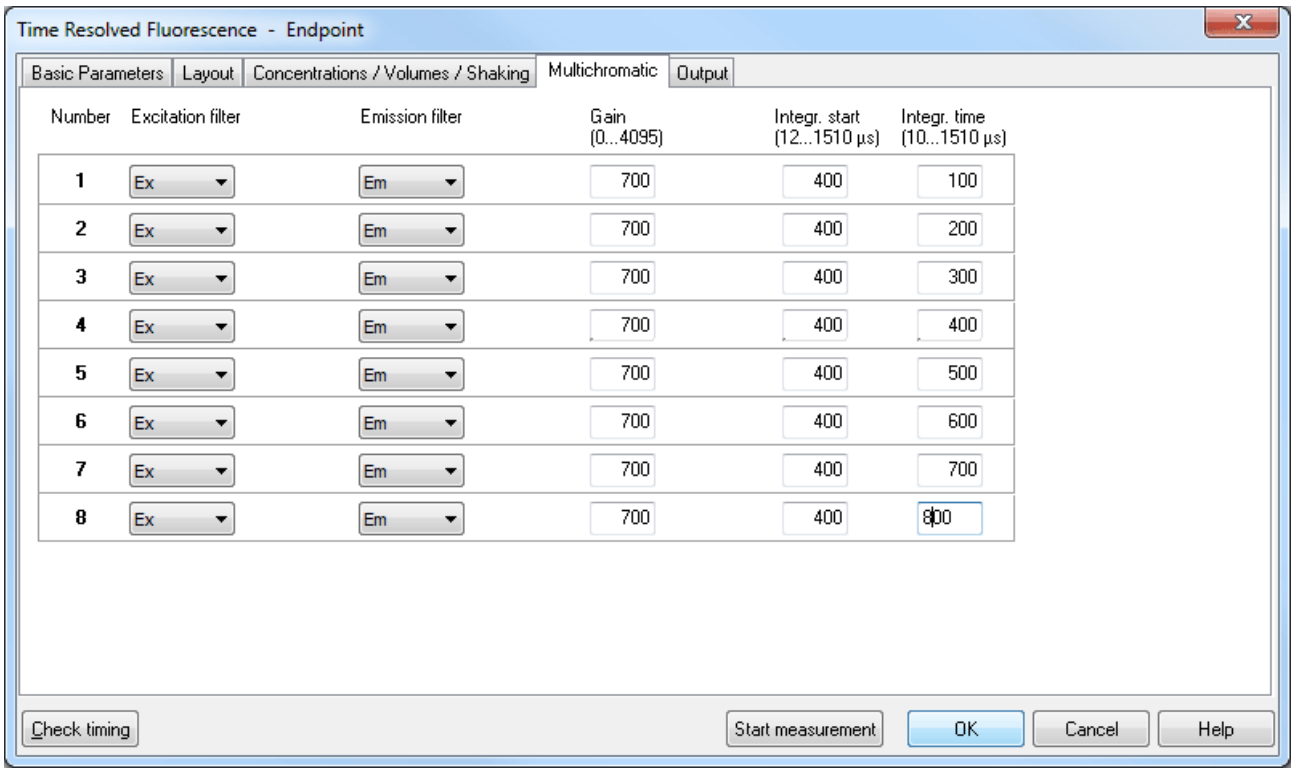
The integration time is the length of the measurement (e.g. how long the PMT reads the emission light).

To find the optimal time for maximum sensitivity you can define 8 as no. of multichromatics and try different integration times using the same filter setting (see chapter 4.4.1). You can then compare the measurement data.

4.4.1 Multichromatics in Time-Resolved Fluorescence Mode

By 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.

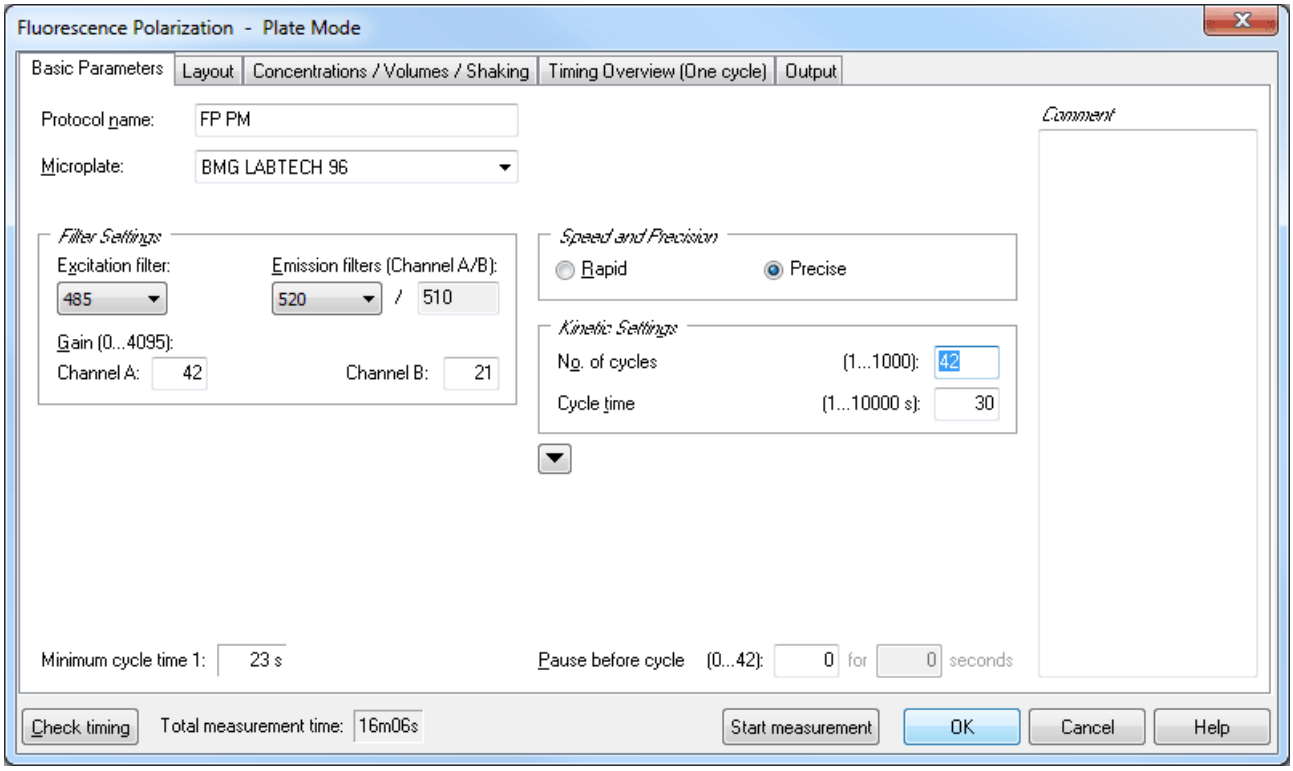
For example, if you were using Eu^{3+}, you would define all 8-filter settings equal. You can keep the integration time constant and vary the integration start time in order to find the optimal start time. You can then keep the integration start time constant and vary the integration time to find the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



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.



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*).

Settling time

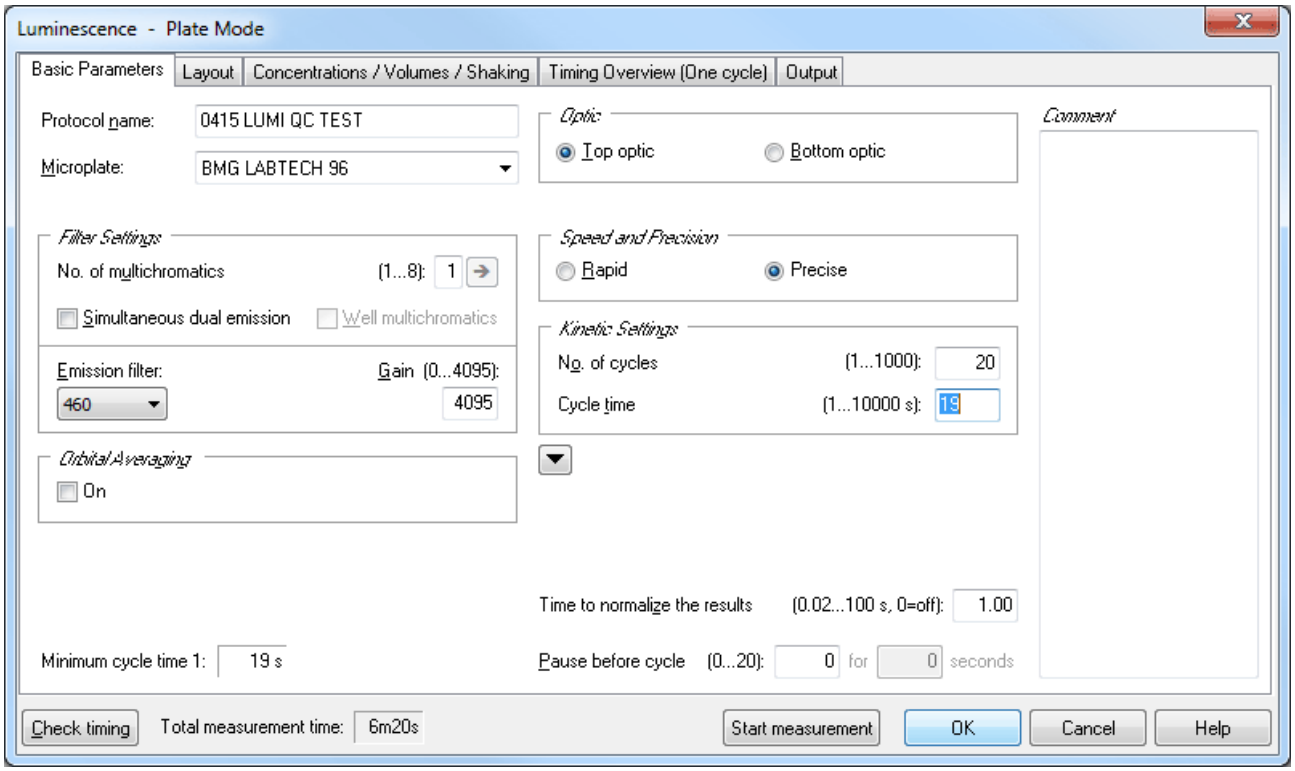
The settling time in fluorescence polarization tests is recommended to be 1 second, so that the surface of the liquid is stable before measurement.

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.

4.6 Luminescence Protocols

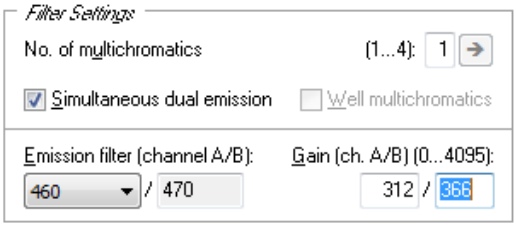
Luminescence can be measured in endpoint, plate or well mode or in well scan mode. Most parameters are similar to fluorescence intensity protocols (see chapter 4.3). Multichromatic measurements are possible. If the reader is equipped with two luminescence measurement channels simultaneous dual emission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



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.

Optic

You can select for luminescence (and fluorescence) mode protocols to use the top or bottom optic.

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

Settling time

At least 0.2 seconds are recommended for luminescence tests.

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.

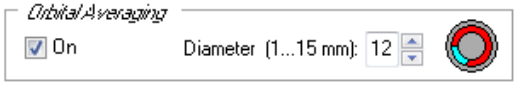
Time to normalize the results

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.

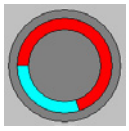
Example: When you set the *Time to normalize the results* to 1s (to get the results in counts per second) and use a *Measurement interval time* of 0.1s the raw measurement values will be multiplied by 10.

4.6.1 Orbital Averaging in Luminescence Mode

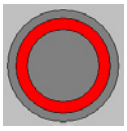
Using this mode, the measurement takes place on an orbit with definable diameter. In **luminescence mode** the measurement is done by continuously measuring the emitted light during the defined measurement interval time (see chapter 4.6). For each well, there will be one orbital movement per cycle/interval (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 small icon in the orbital averaging box will show you which part of the orbit is used for the measurement.



70 % coverage



100 % coverage

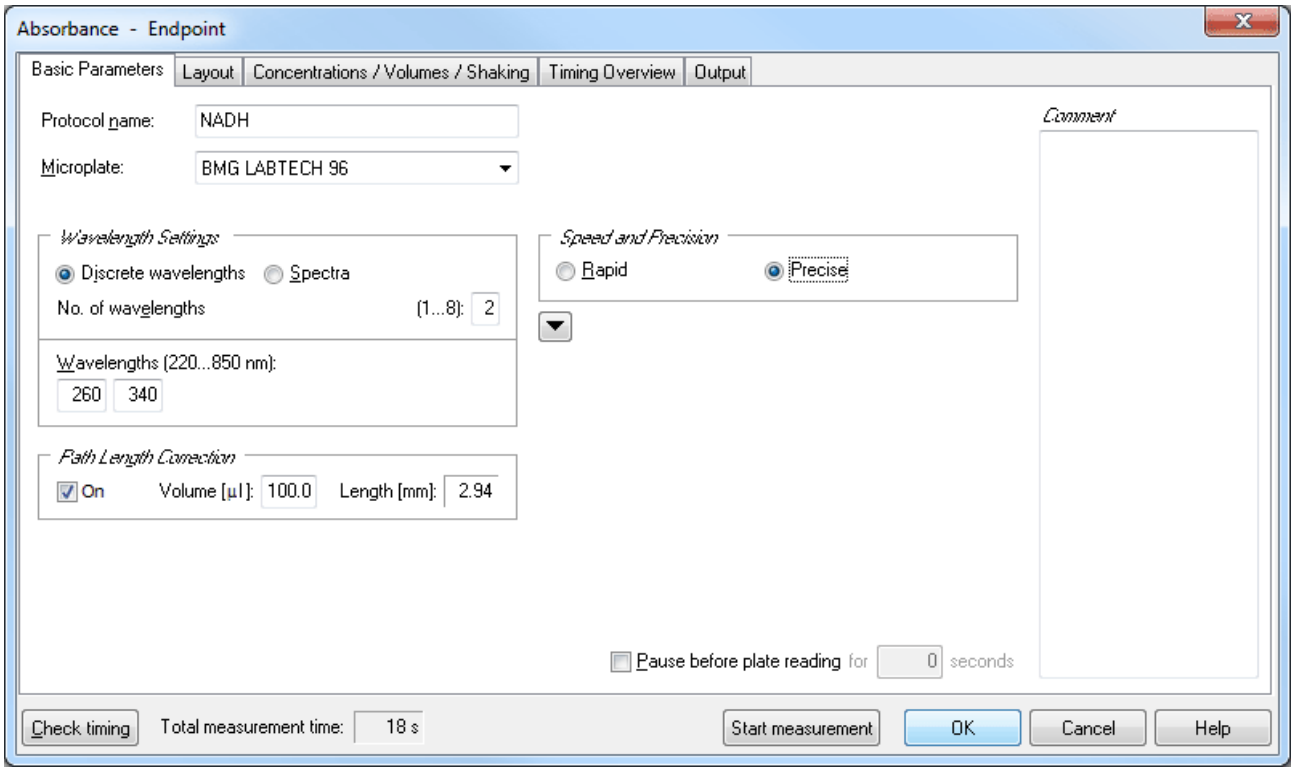
It is recommended to define the measurement interval as long as the orbital movement lasts (this time is shown as maximum possible time besides the measurement interval time input box). There will be a warning message if the defined measurement time covers less than 50% of the orbital movement.

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 settling 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 4.3). If the reader is equipped with a spectrometer, besides measuring discrete wavelengths, you can also measure whole spectra (however, this is only in endpoint and plate mode possible), see chapter 6.7.4 *Display of Spectra Data*).



Wavelength Settings

If the reader is equipped with a spectrometer, you can specify here up to 8 discrete wavelengths to be measured.

For endpoint or plate mode protocols, you can alternatively also measure whole spectra (you need a firmware version 1.13 (or newer) to measure whole spectra in plate mode). For spectra measurements, you specify the start and end wavelengths, and the resolution (1, 2, 5 or 10 nm).

Please note: measuring over a wide wavelength range with a high resolution (1 or 2 nm) produces a huge amount of data. Therefore, you should only measure the wavelength range you are really interested in.

If the reader is not equipped with a spectrometer, the absorbance measurement will be performed using a PMT (photo multiplier tube). In this case, you can select which excitation filter(s) should be used. If you want to measure up to three wavelengths, you can select the necessary filter inside this sheet; if you want to measure more than three wavelengths (maximum 8), you can select the filters using the multichromatics sheet (see chapter 4.3.13).

Position 8 from the emission filter wheel will be used. This position should contain a lens or should be empty. For absorbance method measurements using a spectrometer, position 8 on the excitation side should be empty (or should contain a UV filter).

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 3.5.1) the current path length will be calculated. Based on this length the correction will be performed.

Notes: Please make sure, that all wells contain the same amount of liquid when using this function.

The path length correction box is not available when using the BMG LABTECH LVis plate. Here the path length values determined using the LVis Path Length Determination Wizard (see chapter 7.2.1) will always be used.

Settling time

The settling time should be defined as at least 0.5 seconds.

Note: Flying mode is not available when using a spectrometer.

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.

Injections

For injections in combination with spectrometer based absorbance measurements a firmware version 1.20 or newer is necessary. For plate mode protocols also firmware versions 1.11 – 1.13 can be used.

4.7.1 Sequential Blanking

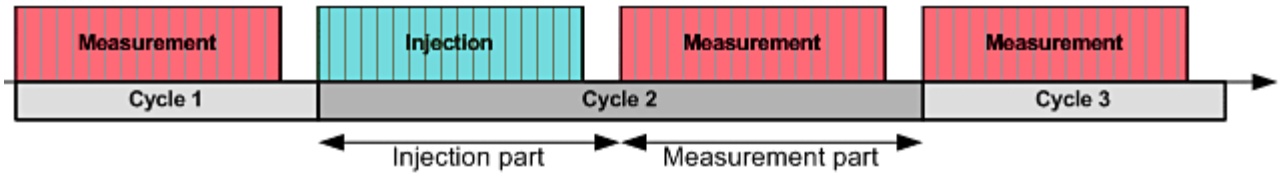
When you define a test protocol using the BMG LABTECH LVis Micro Drop plate you can use sequential blanking. In this mode the individual blank measurement value of each well will be subtracted from the measurement values. To use this function the blank values need to be measured before starting the measurement. This can be done using the blank measurement function of the LVis Plate menu group (see chapter 7.2.3 *LVis Plate Blank Measurement*).

4.7.2 Injections in Combination with Spectrometer Measurements

Plate Mode / Endpoint Measurements of Discrete Wavelengths

When not using the spectrometer (e.g. when performing a fluorescence or luminescence measurement or a pmt based absorbance measurement), it is possible to measure and inject at the same time:

Due to the fact, that the measurement position of the spectrometer is not located at the same place as the injection position, injection and measurement can not take place at the same time when performing a spectrometer based measurement. Therefore, a cycle containing an injection is divided into two parts – first part is the injection and second part is the measurement (cycle 2 of the following example).

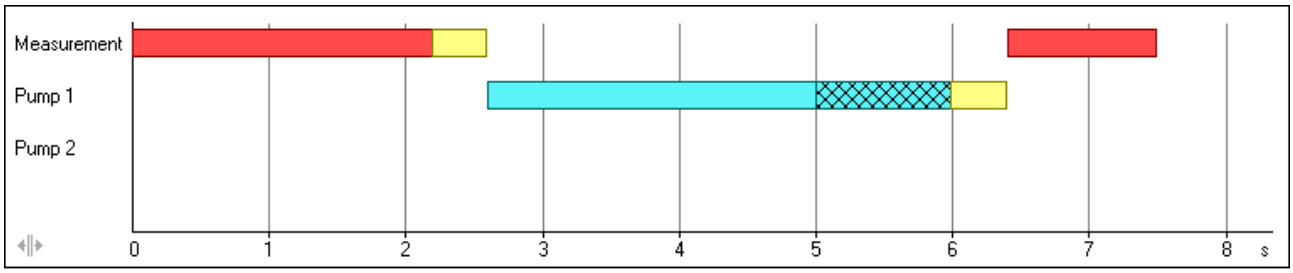


Please note that in injection cycles the injection part will be finished for ALL selected wells first before the measurement part for ALL selected wells will start. This is important when you expect a fast reaction because measurements can only start after the whole injection part has been completed.

When using the ‘Equidistant kinetic cycles’ feature, the software will ensure that timing of all wells is exactly the same.

Well Mode / Endpoint Spectra Measurements

When using **well mode** (or when using the endpoint mode in combination with spectra measurement) the microplate needs to be moved to the injection position before the injection takes place and will be moved back to the measurement position afterwards. This takes place for each well. During this movement times (yellow bars in the picture below) no other actions can take place.



Legend

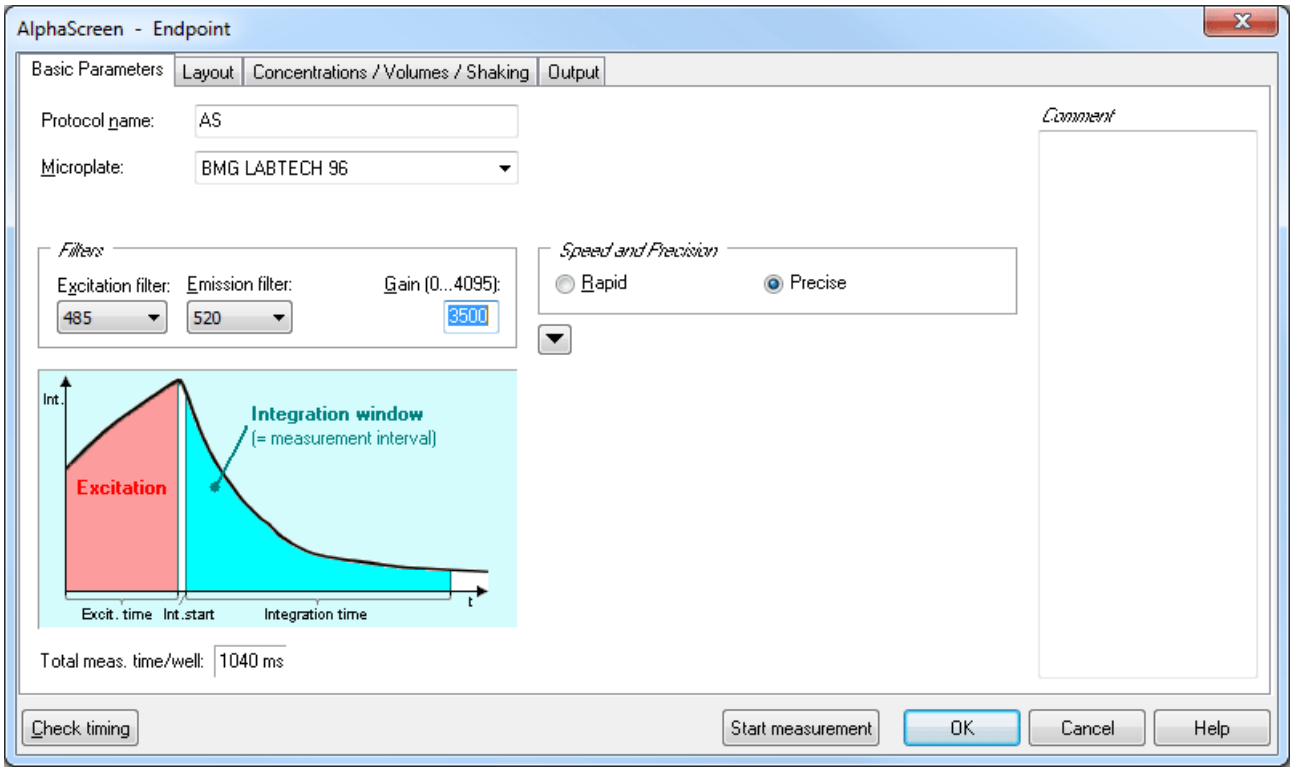
- Measurement (Kinetic window 1...4)
- Volume group 1
- Volume group 2
- Volume group 3
- Volume group 4
- Shaking
- Plate carrier movement

4.8 AlphaScreen Protocols

In AlphaScreen™ (Amplified Luminescence Proximity Homogeneous Assay) mode the samples are excited at a wave length of 680 nm for a defined time (excitation time) and the emission in the range of 520 nm to 620 nm is measured.

Most parameters are similar to fluorescence intensity protocols (see chapter 4.3). Well scan and multichromatic or kinetic measurements are not possible in AlphaScreen mode.

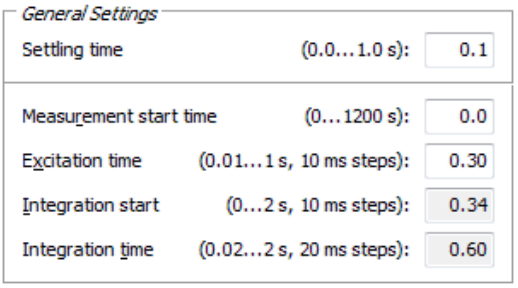
An automatic gain adjustment is not possible, but the gain setting can be changed manually. A gain value of about 3600 is recommended.



Speed and Precision

The two choices in this box, depending on microplate format, balance speed and precision through adjustments to settling time, excitation and integration time.

After clicking the button the group box General Settings becomes available, where you can define these parameters individually.



Excitation time

You can define how long there should be excitation light.

Integration start

Defines the time when the measurement (integration) of the emission light should begin. As the best results are achieved if the integration of the emission light starts approximately 40 ms after the end of the excitation, this time value will always be used.

Integration time

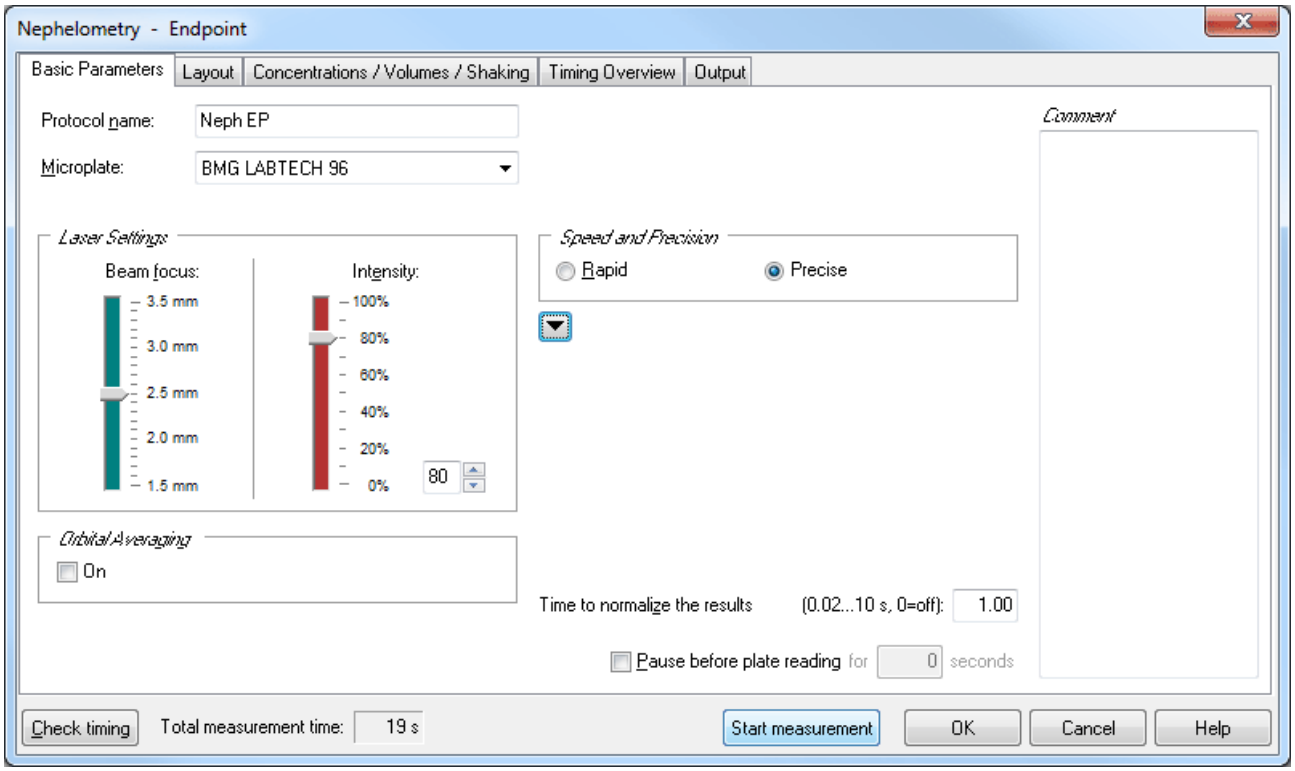
The integration time is the length of the measurement (i.e. how long the PMT reads the emission light). The integration time will always be twice as long as the excitation time.

Notes: Longer excitation and longer emission (integration) times result in higher measurement values, but also increase the time necessary for reading the plate.

For AlphaScreen measurements a firmware version 1.12 or newer is necessary.

4.9 Nephelometry Protocols

Nephelometry can only be measured using a NEPHELOstar Plus reader. It can be measured in all reading modes. Most parameters are similar to luminescence protocols (see chapter 4.6). Nephelometry will be measured using a laser. You can change the laser beam focus and the laser intensity. There are no filters involved.



Changing the **laser beam focus** is important when measuring wells with smaller diameters, such as 384 vs. 96-well plates. However the overall signal will decrease as the beam focus decreases. In addition, a narrower beam can also help to achieve better results with solutions that have a larger meniscus, such as solutions with higher percentage DMSO and solutions with detergents (i.e. Tween-20).

The **laser intensity** should be lowered when the signal is near the maximum RNU. The intensity can be adjusted higher when lowering the beam focus to allow higher overall signal units.

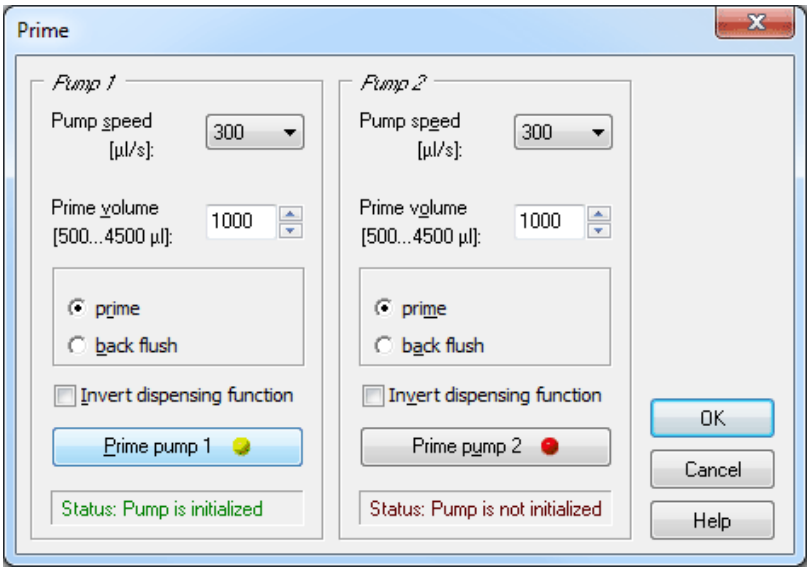
5 Priming / Incubation

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 menu command '**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 100 µl, 250 µl, 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 (default size).

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 e.g. 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.

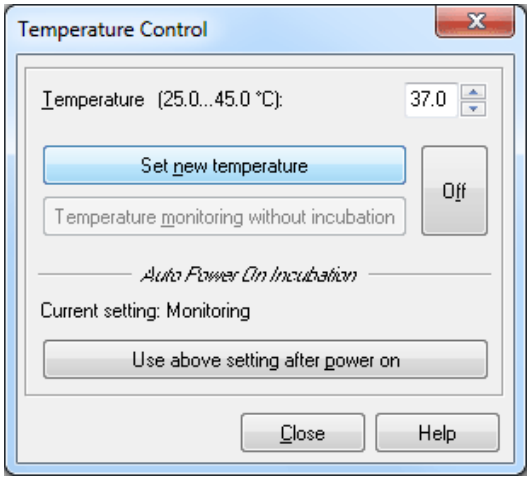
The invert function is helpful in cases where the solutions have particles or cells that may settle at the bottom of the syringe barrel, and, therefore, will not be dispensed uniformly.

Note: You can also prime the pumps without using the software. When you open the reagent door, you will see two buttons on the left side. To prime a pump double click the respective button (see Operating Manual for details).

5.2 Incubation

5.2.1 Temperature Control

The incubation can be activated through the '**Temperature**' menu command.



The temperature range of the incubator is 25 °C to 45 °C (optional up to 65 °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 of the 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 Omega 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.

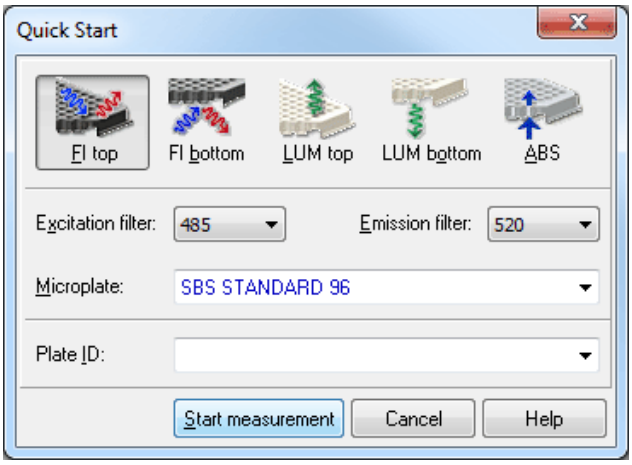
To switch off the auto power on incubation function, select a target temperature of 0 °C (either by clicking the 'Off' button or by manually entering this value), and click the 'Use above setting after auto power on' button.

6 Microplate Measurements

6.1 Quick Start

The 'Quick Start' function can be used to measure a plate without defining a test protocol using fluorescence intensity, luminescence, absorbance and nephelometry 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 in fluorescence polarization mode.

After clicking the '**Quick Start**' button the following dialogue box will appear:



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

Notes: Only the measurement methods are selectable, which can be performed with the reader connected and using the optic currently installed.

The measurement method selection is not available when using the Quick Start function of the LVis tab, as here always absorbance will be used.

When using a NEPHELOstar Plus reader instead of the method and filter selection you can define here the laser beam focus and the laser intensity.

The **filter** and **microplate** selection will be stored independently for each 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 I*).

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).

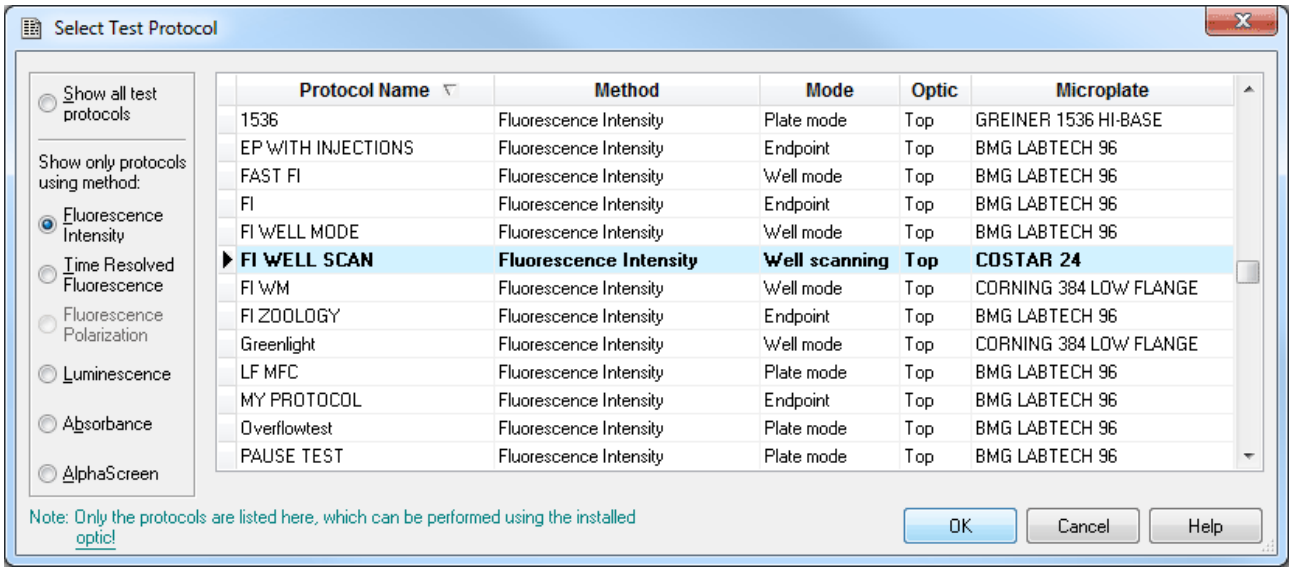
Note: For absorbance measurements a settling time of 0.5 s will be used, for the other methods 0.2 s. Fluorescence measurements will be performed using 10 flashes per well, absorbance measurements will use 20 flashes per well. For luminescence and nephelometry measurements a measurement interval time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

6.2 Executing Pre-Defined Test Protocols

The ‘Start Measurement’ button allows for pre-defined protocols to be used (see chapter 4).

6.2.1 Select Test Protocol

After pressing the ‘Start Measurement’ button, a selection window displays all the defined protocols which can be performed using the installed optic (see chapter 3.1.9).



By default the protocols are sorted by method, then alphabetically by name. Change the way test protocols are sorted by clicking other table column headers (Protocol Name, Mode or Microplate).

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.

To select the first protocol that begins with e.g. ‘T’, simply press the key [T].

OK will start the selected protocol, as will double clicking on it.

Cancel will return to the main window.

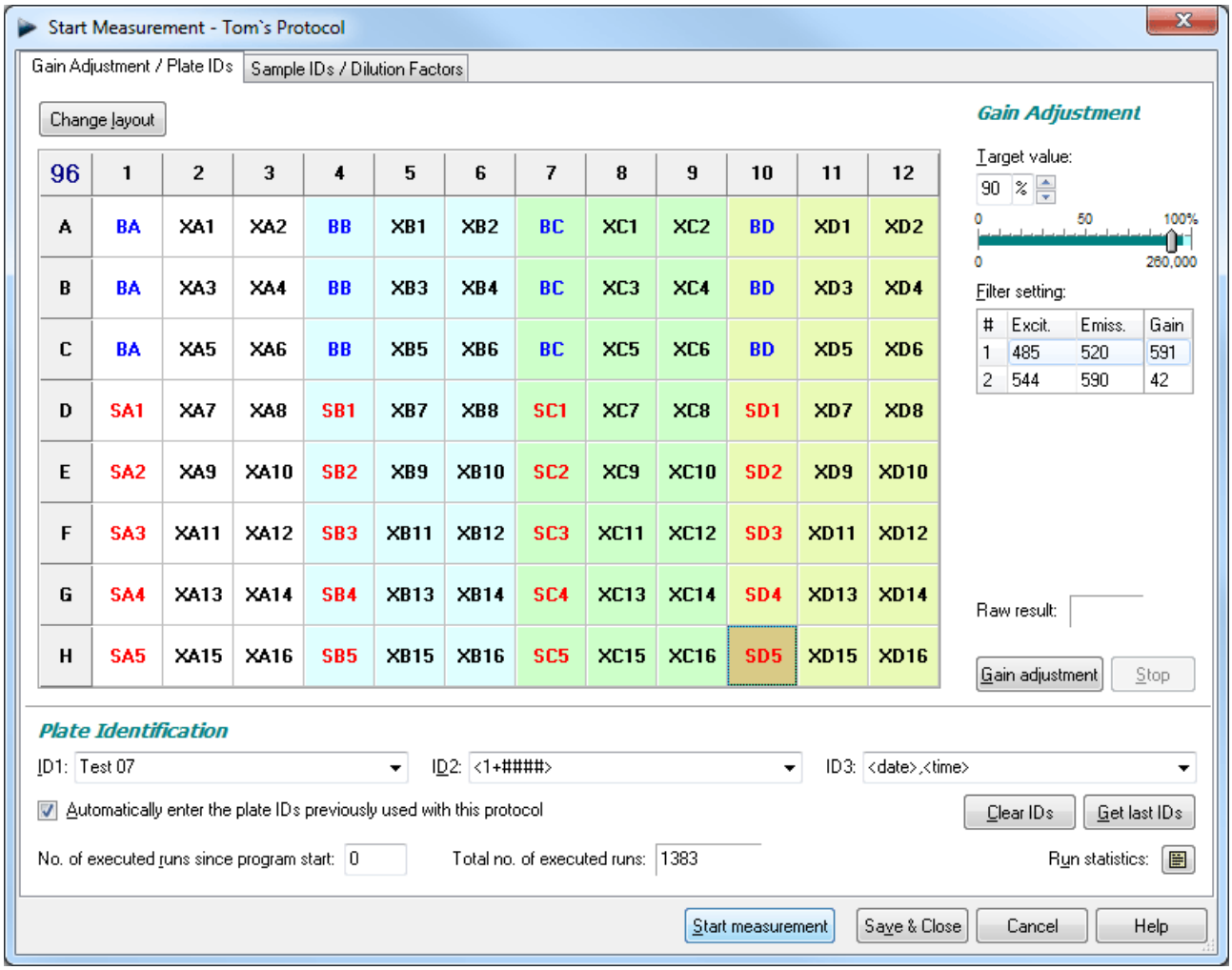
After selecting the protocol the next dialogue gives you the option to add identifiers to the test run, to define dilution factors, to perform a gain adjustment and to start the measurement.

Notes: As the LUMIstar Omega, the SPECTROstar Omega and the NEPHELOstar Plus can only measure using one method, the method selection box will not appear when using these types of reader.

Exporting the test protocols table into a Excel (.xls / .xlsx), text or HTML file is possible after right clicking the table.

6.3 Plate IDs

After selecting the test protocol, a new window appears where plate specific identifying features (IDs) can be added, i.e. title, description, or a number. Three IDs with up to 100 alphanumeric characters are possible. Each identifier has a drop down menu with several options or a unique description can be manually typed in.



96	1	2	3	4	5	6	7	8	9	10	11	12
A	BA	XA1	XA2	BB	XB1	XB2	BC	XC1	XC2	BD	XD1	XD2
B	BA	XA3	XA4	BB	XB3	XB4	BC	XC3	XC4	BD	XD3	XD4
C	BA	XA5	XA6	BB	XB5	XB6	BC	XC5	XC6	BD	XD5	XD6
D	SA1	XA7	XA8	SB1	XB7	XB8	SC1	XC7	XC8	SD1	XD7	XD8
E	SA2	XA9	XA10	SB2	XB9	XB10	SC2	XC9	XC10	SD2	XD9	XD10
F	SA3	XA11	XA12	SB3	XB11	XB12	SC3	XC11	XC12	SD3	XD11	XD12
G	SA4	XA13	XA14	SB4	XB13	XB14	SC4	XC13	XC14	SD4	XD13	XD14
H	SA5	XA15	XA16	SB5	XB15	XB16	SC5	XC15	XC16	SD5	XD15	XD16

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, e.g. using <1+###> 001, 002, 003, etc. will be generated.

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)>.

In batch mode (using a stacker) or in script mode (see chapter 6.8) you can add a 'B' before the '#' to get the plate numbers of the current batch run, 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.

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

<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)
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
ddddd	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...)

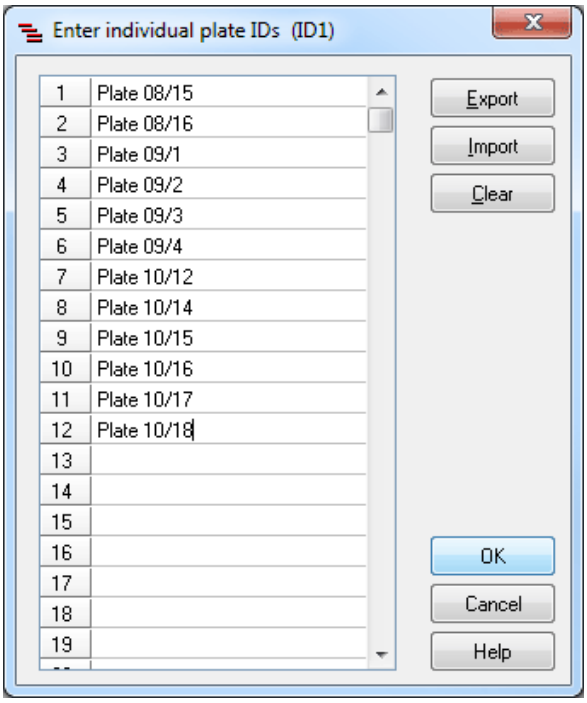
Example: <time:hh.mm.ss>

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.

<barcode> Barcode of the current plate (only available if the reader is attached to a stacker equipped with a barcode reader and only available for measurements using the stacker, not for single plate measurements without stacker magazine 1 inserted).

- <table> Only for Stacker batch measurements: use an individual identifier for each plate: After selecting <table> you can open a dialog box for entering the IDs for each plate by pressing the button right to the Plate ID input box .



The ID values entered here are user specific. You can import the table from a pure text (ASCII) file or from an Excel file. Exporting is possible using these two formats and in addition using the *.html format.

You can combine all these things. If the resulting ID is longer than 100 chars it will be truncated in the Absolute Data Base files but not in ASCII data files.

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.

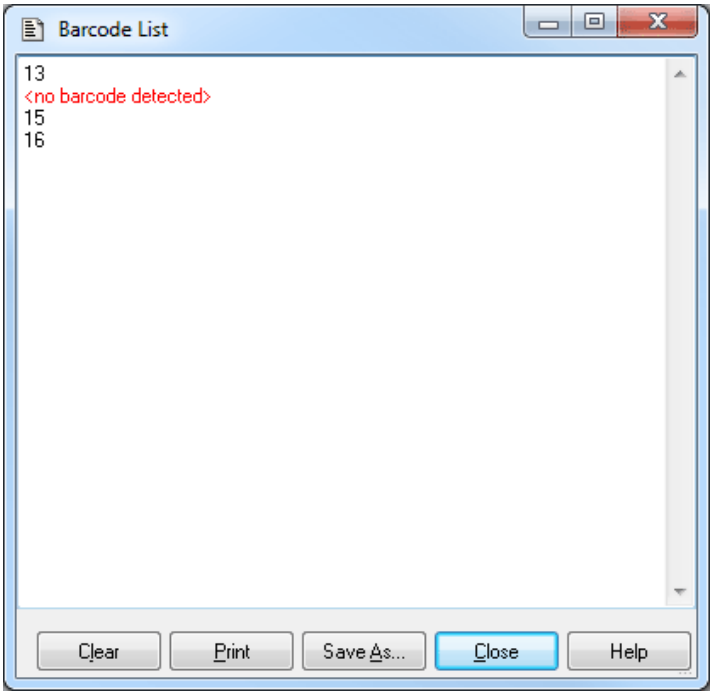
Automatically enter the plate IDs previously used with this protocol

If this box is checked, then the plate identifiers which have been used during the last execution of the selected protocol will be entered automatically. You can still edit the identifiers or use the ‘Clear IDs’ button.

6.3.1 Barcode List

The barcode list window shows all the barcode values read so far during batch runs. To open the barcode list window, use the command ‘Barcode List’ inside the ‘Measure’ menu tab group. This function is only available if the reader is attached to a stacker equipped with a barcode reader and is only enabled when at least one barcode has been read.

You can edit the barcode list. It is possible to save the barcode list as a pure text file or to print out the list.



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, e.g. 260,000 rfu).

Notes: The gain adjustment for absorbance method protocols is done automatically as part of the measurement, therefore, the gain adjustment option does not exist when using the absorbance method. It is also not available in AlphaScreen mode, but using this mode, you can manually change the gain value inside the test protocol editor.

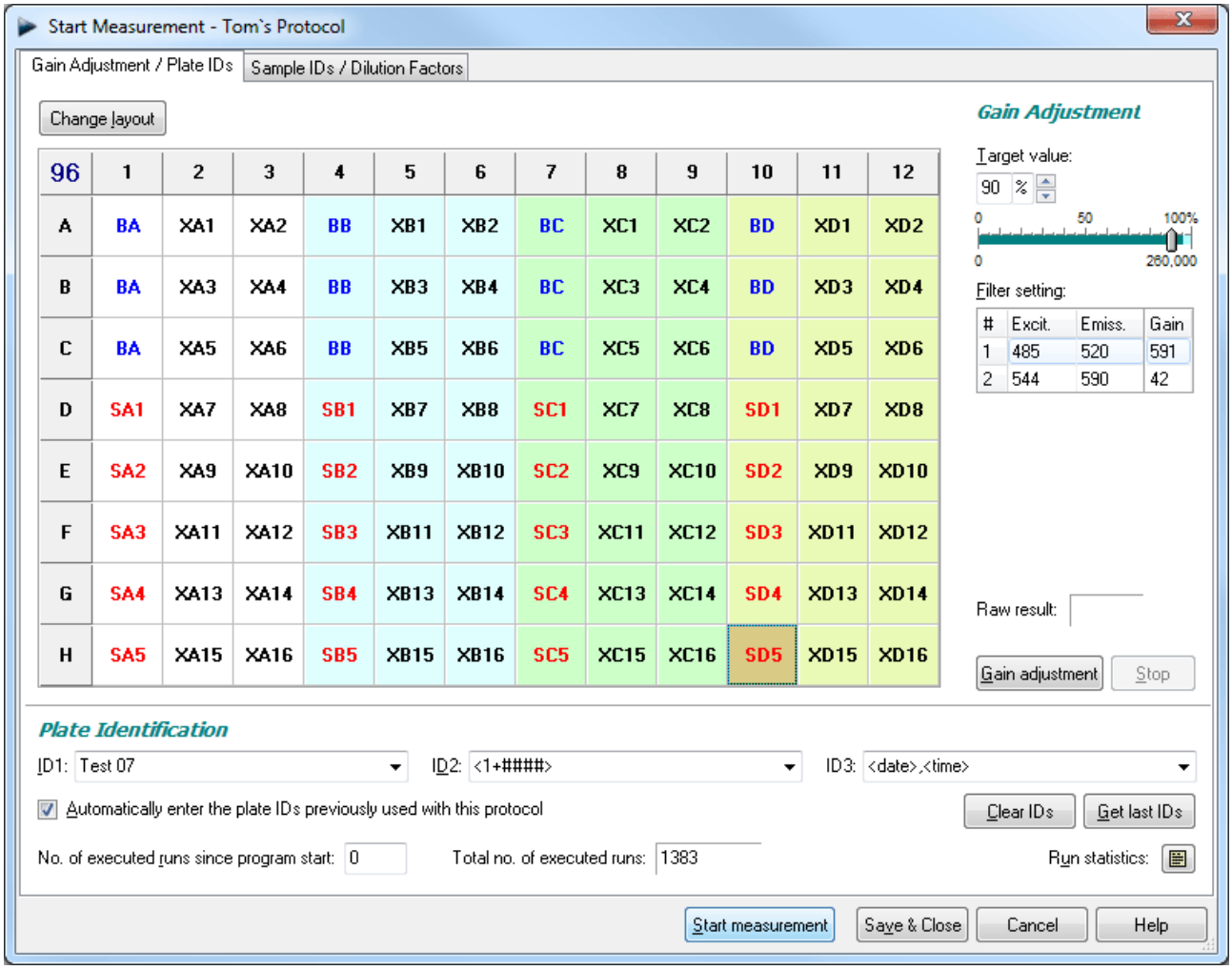
If you prefer that the plate ID options and the gain adjustment functions are shown on separate tab you can select this using the Program Configuration dialogue (see chapter 3.3.2).

Zoom Feature

For 384 and 1536 well formats, the layout grid 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 buttons.

The gain adjustment dialogue will look slightly different when using different measurement methods. The parameters are described on the following pages.

6.4.1 Gain Adjustment – Fluorescence and Luminescence Method



96	1	2	3	4	5	6	7	8	9	10	11	12
A	BA	XA1	XA2	BB	XB1	XB2	BC	XC1	XC2	BD	XD1	XD2
B	BA	XA3	XA4	BB	XB3	XB4	BC	XC3	XC4	BD	XD3	XD4
C	BA	XA5	XA6	BB	XB5	XB6	BC	XC5	XC6	BD	XD5	XD6
D	SA1	XA7	XA8	SB1	XB7	XB8	SC1	XC7	XC8	SD1	XD7	XD8
E	SA2	XA9	XA10	SB2	XB9	XB10	SC2	XC9	XC10	SD2	XD9	XD10
F	SA3	XA11	XA12	SB3	XB11	XB12	SC3	XC11	XC12	SD3	XD11	XD12
G	SA4	XA13	XA14	SB4	XB13	XB14	SC4	XC13	XC14	SD4	XD13	XD14
H	SA5	XA15	XA16	SB5	XB15	XB16	SC5	XC15	XC16	SD5	XD15	XD16

Filter and Gain Table

The filter settings and the gain values are displayed on the right (in luminescence mode there are no excitation filters, only emission filters). If the protocol is multichromatic, then all filter combinations used will be displayed. In this case you can perform a gain adjustment for each chromatics (for each filter set). Please select the appropriate table row before clicking the Gain adjustment button.

Automatic Gain Adjustment for one Well

Select the well for gain adjustment. Start the automatic gain adjustment by clicking the button '**Gain adjustment**' button. The instrument measures the well eleven times to find the optimal gain. The optimal gain value then appears in the table next to the chromatic number. It will also be stored in the test protocol. If you are using a dual channel protocol, the gain adjustment will be performed simultaneously for both channels. The optimal gain value(s) will be shown in the filter and gain table.

'**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 (e.g. 96 or 384). All wells are selected. Click the '**Gain adjustment**' button and the instrument will return a gain value in the box next to the filter settings.

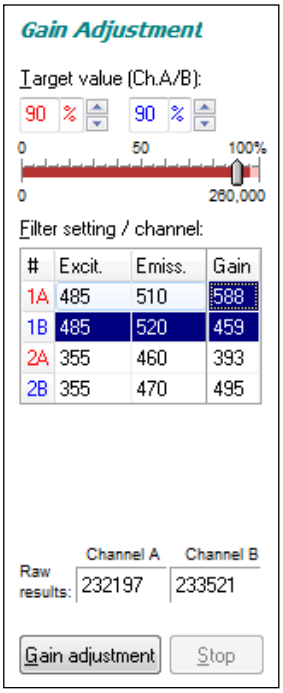
Note: An automatic gain adjustment for the entire plate is not possible for 1536 well plates.

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 target values and, therefore, to define a target ratio.

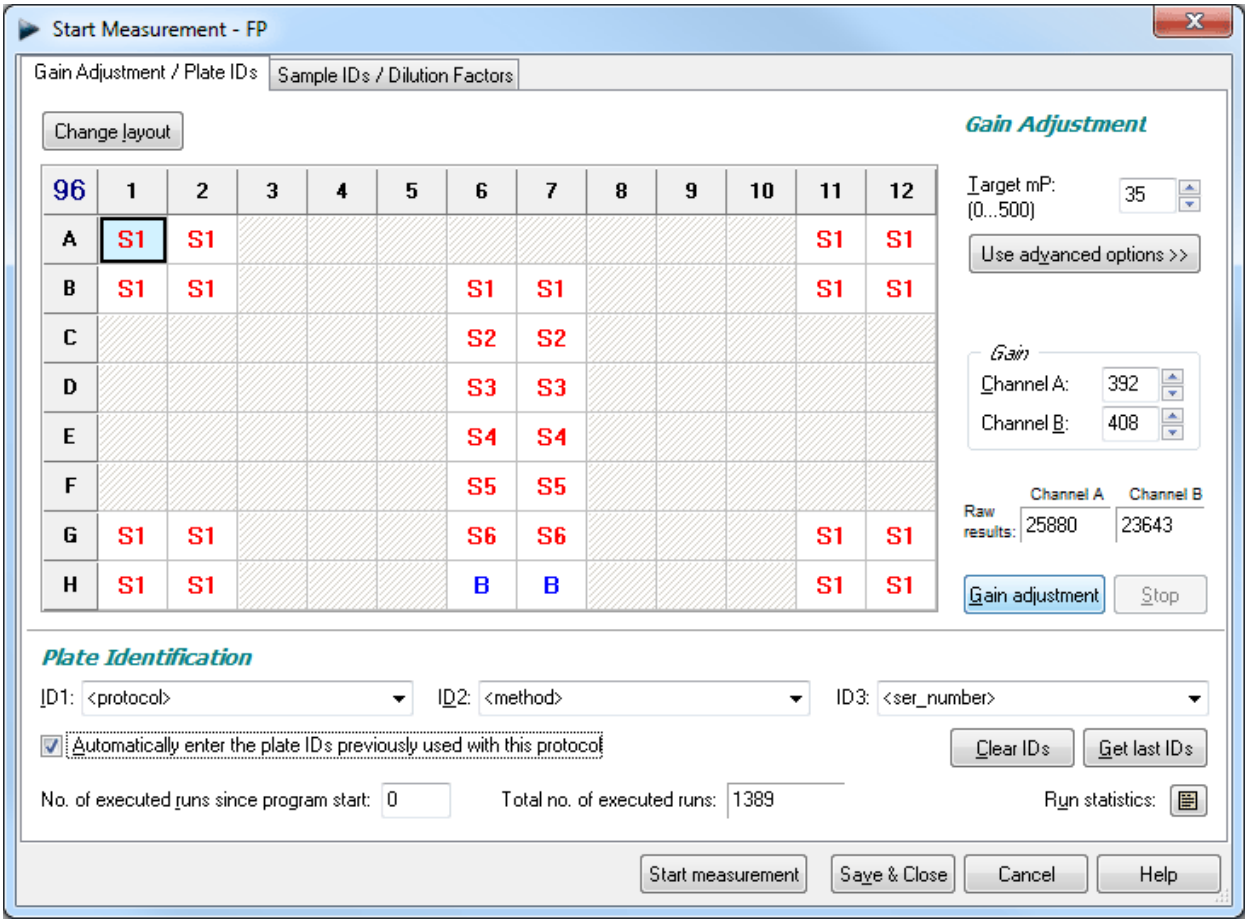
In addition, it is still possible to type in values manually for each channel.

If two different wells are found showing the highest signal for channel A and B during a gain adjustment plate procedure, the A channel well will be marked with a red frame and the B channel well with a blue frame.



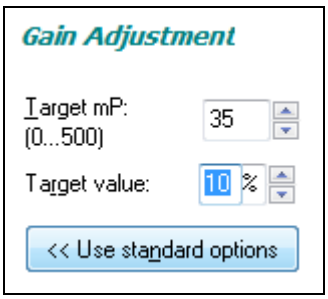
6.4.2 Gain Adjustment – Fluorescence Polarization Method

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 target value click on the '**Use advanced options**' button. An input field for this value will appear:



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.

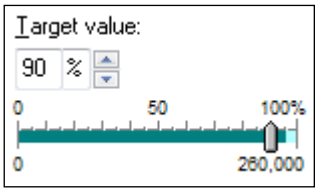
Start the measurement. Use the current state display (see chapter 6.7) or the Omega Data Analysis software (see software manual part III) to check the measurement values. If the results are negative, then you need to increase the Target mP value.

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 Target Value

The measurement range for fluorescence is 0 to 260 000. If an overflow occurs, the measurement value will be 260 000. 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 '**Target 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, e.g. 260 000 x 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 target 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 target value to 50% (or less) of maximum value in order to have enough space for higher intensity values and to prevent an overflow.

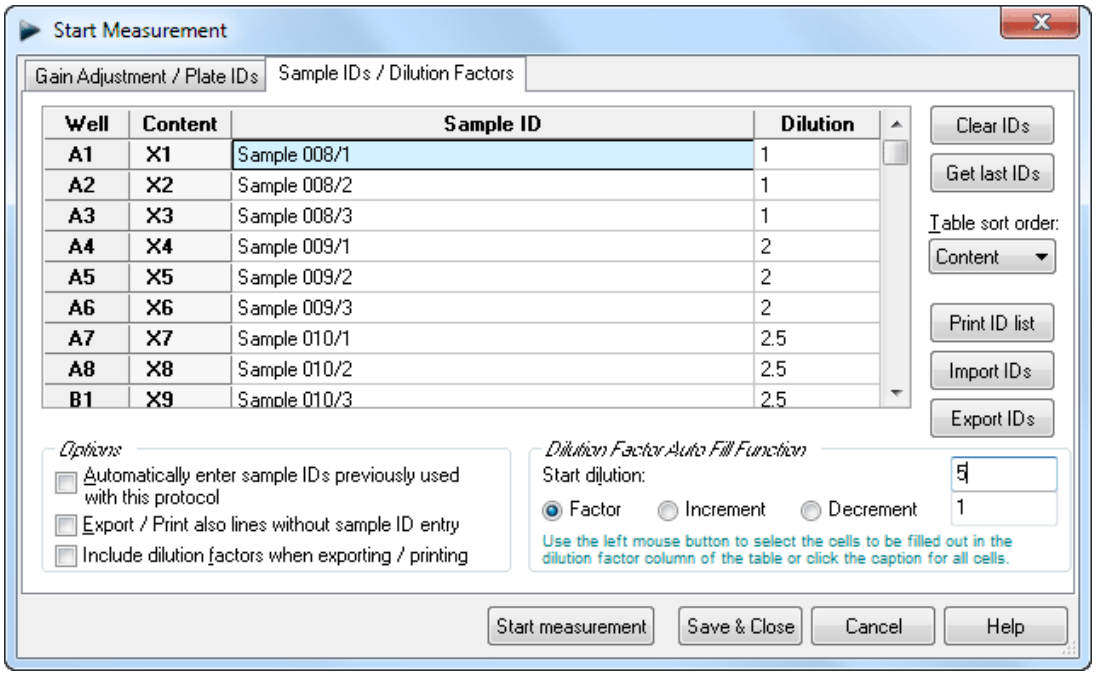
You can also use the slider to change the target value. Here you can also see the selected target raw value (depending on the measurement method used).

6.5 Sample IDs / Dilution Factors

You may enter identification values for each well in this sheet. These values will be shown in the Sample IDs sheet and in the Omega 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 3.4.2).

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 (software manual part III: Data Analysis).

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



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 Excel format (.xls / .xlsx) 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.

Notes: Please enter the header column using upper and lower case letters exactly as shown, as the import function works case sensitive.

If the file to be imported contains wells, which are not used in the selected test protocol, these entries will not be imported. If there are well used in the test protocol, but not in the import file, the sample IDs of these wells 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 (see chapters 6.3 and 6.5) and performing the gain adjustment (see chapter 6.4), 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 Stacker Operation

If the reader is connected to a stacker, instead of the 'Start measurement' button, there will be a '**Measure one plate**' button.

If stacker magazine 1 is installed, the plate will automatically be fetched from this magazine before the measurement (or before a gain or focus adjustment procedure). After the measurement, the plate will be moved to magazine 2. If magazine 1 is not installed (or not locked), everything works as if there would be no stacker, which means, you are responsible for moving the microplate into the reader using using the reader plate button or the menu function 'Measure | Plate In' / 'Measure | Plate Out'.

When a stacker is attached to the reader, there is an additional button '**Measure all plates**'. This button will start a batch measurement, where all plates available in stacker magazine 1 will be measured and finally transferred to magazine 2. It is possible to add more plates during the operation. If the box '**Don't measure the last plate**' is checked, the last plate will not be measured. This is useful, when the last plate is used as cover.

Notes: Please ensure that you use microplates of the same type as defined in the selected test protocol, as there are huge differences in the Z dimensions of different plate types.

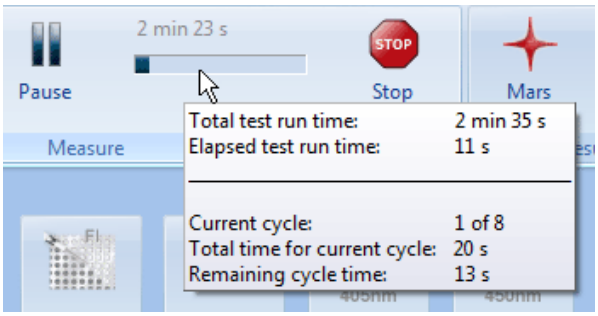
Make sure, that the plateout positions are aligned before using the stacker, see chapter 3.2 Stacker Configuration.

If you want to get the plates back in the same order as they were before starting the measurement, use the menu command 'Measure | **Restack**'. This command will transfer all plates from magazine 2 back to magazine 1.

6.6.2 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.

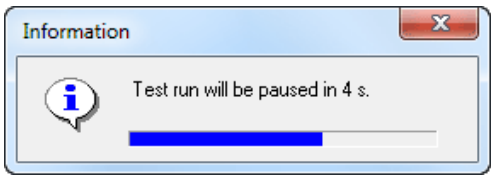
If you move the mouse cursor to the time gauge, a small window with additional timing information will appear.



If you perform a **plate mode kinetic measurement** the quick start button ( ) will change into a pause button ( ). Click this button to pause the active test run after finishing the current cycle. A pause / continue window will appear (see chapter 4.3.2).

Notes: The pause function is not available when using a stacker or in script mode. Due to technical reasons this pause function is also not available when performing absorbance spectra plate mode measurements.

A count down window will appear after using the 'Pause' command, showing the remaining time until the current cycle is finished.

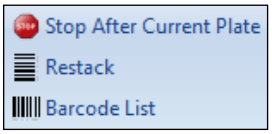


6.6.3 Stopping the Measurement

It is possible to stop a measurement procedure after it has started by clicking the stop button . If there is already measurement data available, a dialog box will ask if you want to save the incomplete data.

In batch mode (using a stacker) or in script mode (see chapter 8), you can use this function to immediately stop any activity. Depending on the state of the system in the monitor moment of using the stop function, it might be necessary to reinitialize the stacker and the reader (the software will tell you this). If during this moment there is a plate inside the reader, a dialogue will appear asking you whether this plate should be moved out to stacker magazine 1 or 2.

In batch mode, there is also an additional menu command 'Measure | **Stop After Current Plate**' available. When you use this command, the measurement of the current plate will be finished and the plate will be moved out to magazine 2 before the batch processing is terminated.

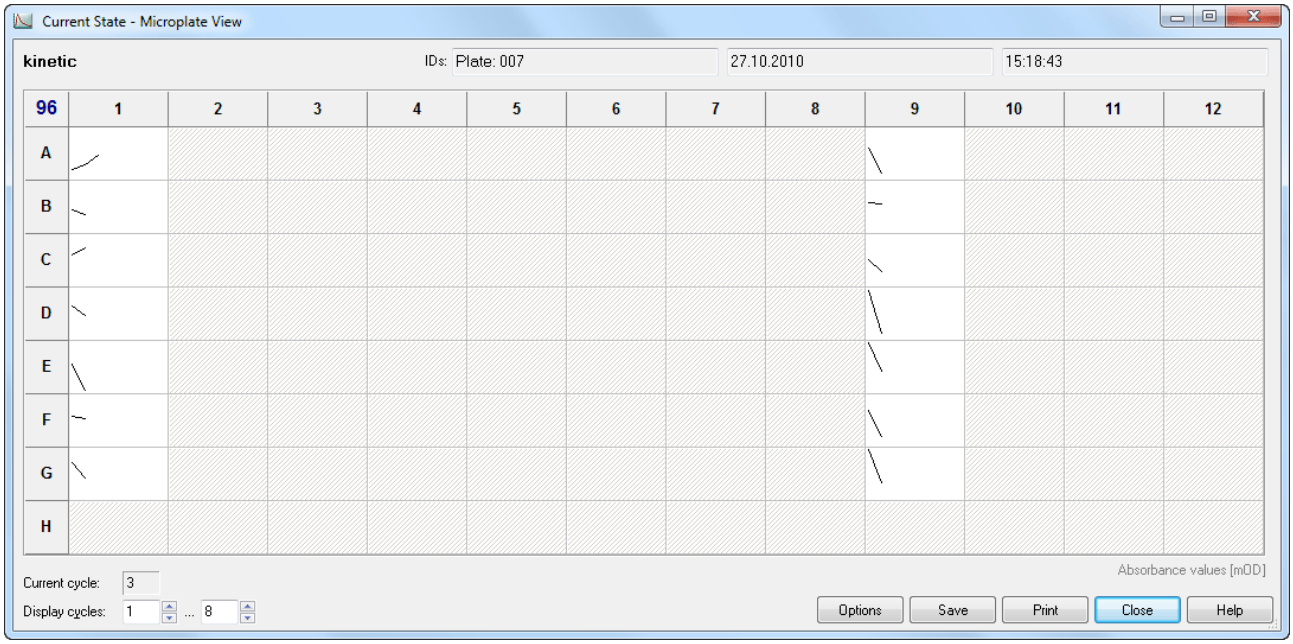


Note: If you use a stacker and want to get the plates back in the same order as they were before starting the measurement, use the menu command 'Measure | **Restack**'. This command will transfer all plates from magazine 2 back to magazine 1.

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 – Microplate View 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.

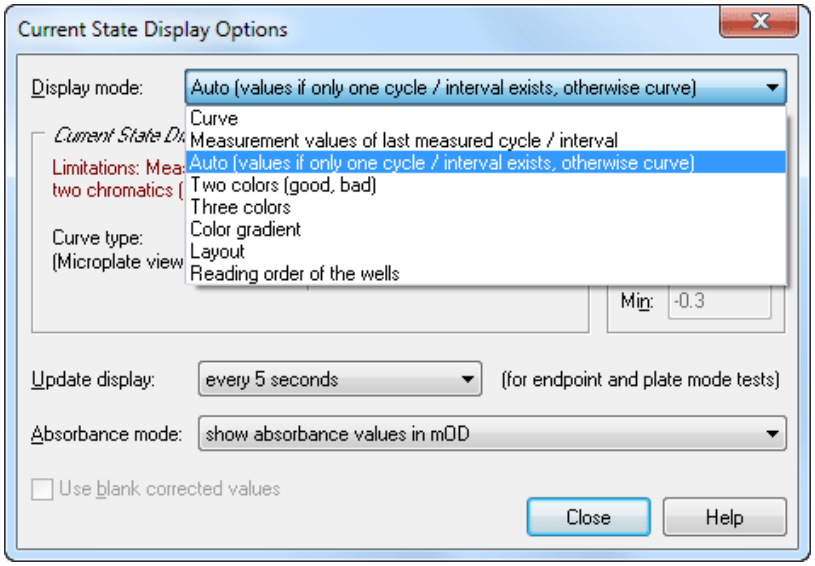
When performing an absorbance spectra measurement, you can see the spectrum of each well (see chapter 6.7.4 *Display of Spectra Data*).

For test protocols with well scanning (see chapter 4.3.4) 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 6.7.5 *Display of Well Scan 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 – Microplate View 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 chromatics 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 chromatics (filter pairs / wavelengths) 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 – Microplate View 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 '`Omega.ini`' file: `ClearBeforeUpdateInMValuesMode=False`. You will find this configuration file in the Omega main installation directory, usually '`~\Program Files\BMG\Omega\`'.

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

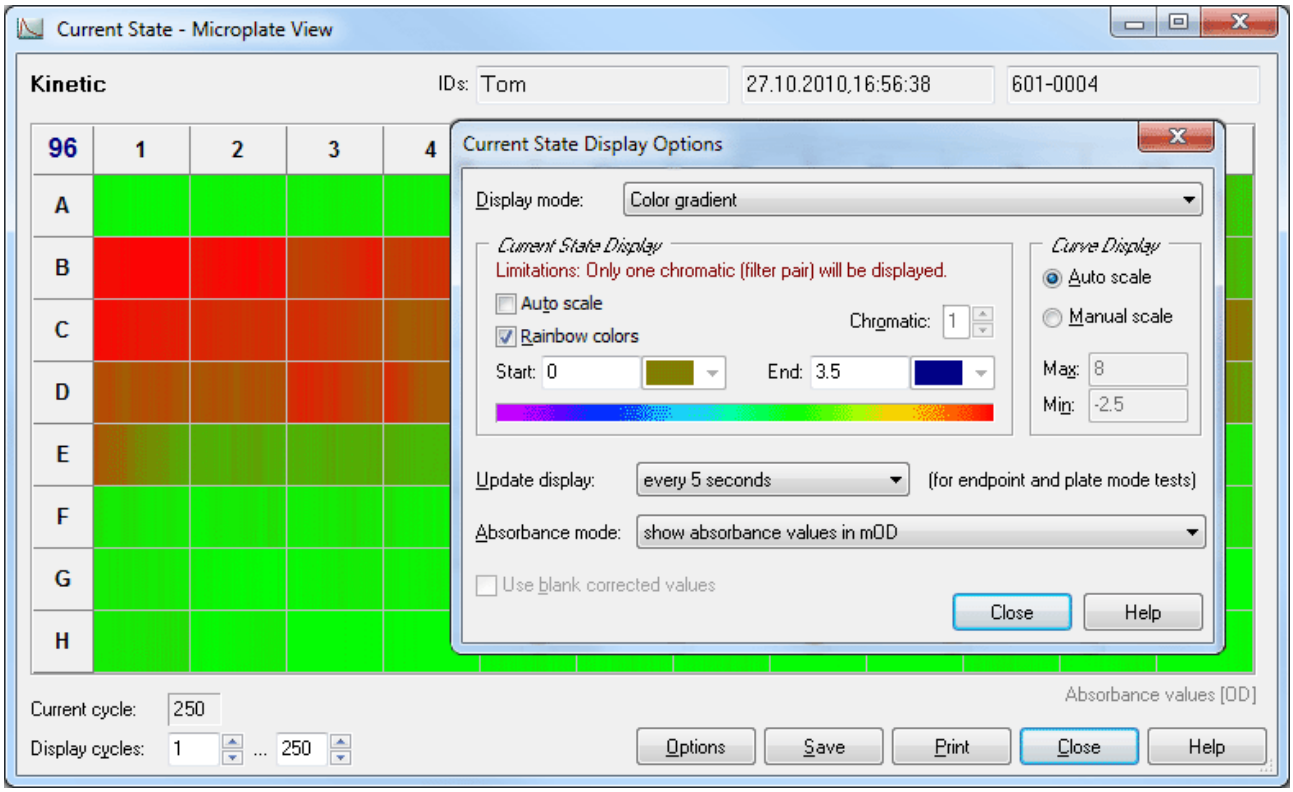
If you are only interested in a good / bad decision, you should choose the option to display different colors for all values under a certain limit and for all values above the limit. You can select the two colors and change the limit value. Only the results from one chromatic / channel will be displayed, 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 (e.g. 0...30 000), 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.

Reading order of the wells

Shows in which order the wells are measured.

Curve Display

The settings in this group will be used for the Curve display in the Current State – Microplate View window and for the Current State – Well View 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 – Microplate View and the Current State – Well View 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 scan feature all scan points will also be transmitted at once, therefore, the update function does not make sense in these cases. Due to technical reasons the display will only be updated after a cycle is completed when performing absorbance spectra plate mode measurements.

Absorbance mode

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

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

Polarization mode

The measurement results from fluorescence 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:

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

- ***Anisotropy values in mA units***

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

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 4.3.8 Layout Definition) 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').

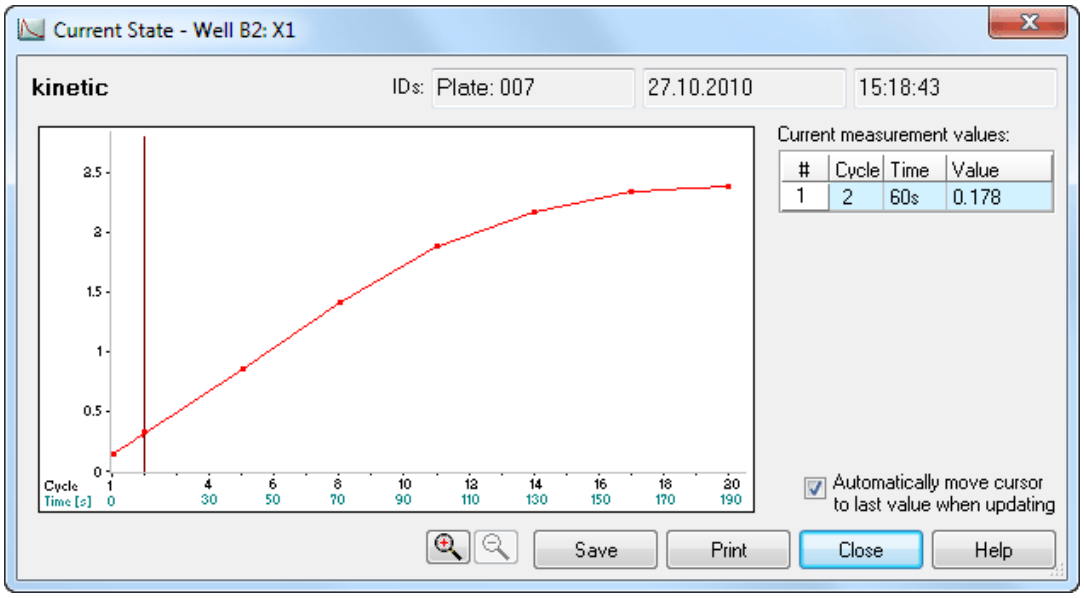
Use average of all blanks from all groups for blank correction

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 – Microplate View window, you get a zoomed view of the selected well. Here the measurement values are always displayed as a curve (when performing a spectra measurement or when using well scanning the display might look different, depending on the selected display mode, see chapters 6.7.4 and 6.7.5). 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 small hint box, containing the filter setting details, will appear.



A small 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].

By using the buttons or using the [+] and [-] 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 use a multichromatic or dual emission protocol, the table on the right of the zooming display will show the measurement values of all chromatics / channels. If you use a color display mode (two colors, three colors, color gradient), only one channel / chromatic will be displayed in the Current State – Microplate View display (see chapter 6.7.1). The selected chromatic / channel will be marked inside the table by a blue bar. Besides using the Current State Options dialogue (see chapter 6.7.2), you can change the chromatic / channel selection also by moving the blue bar to a different row.

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

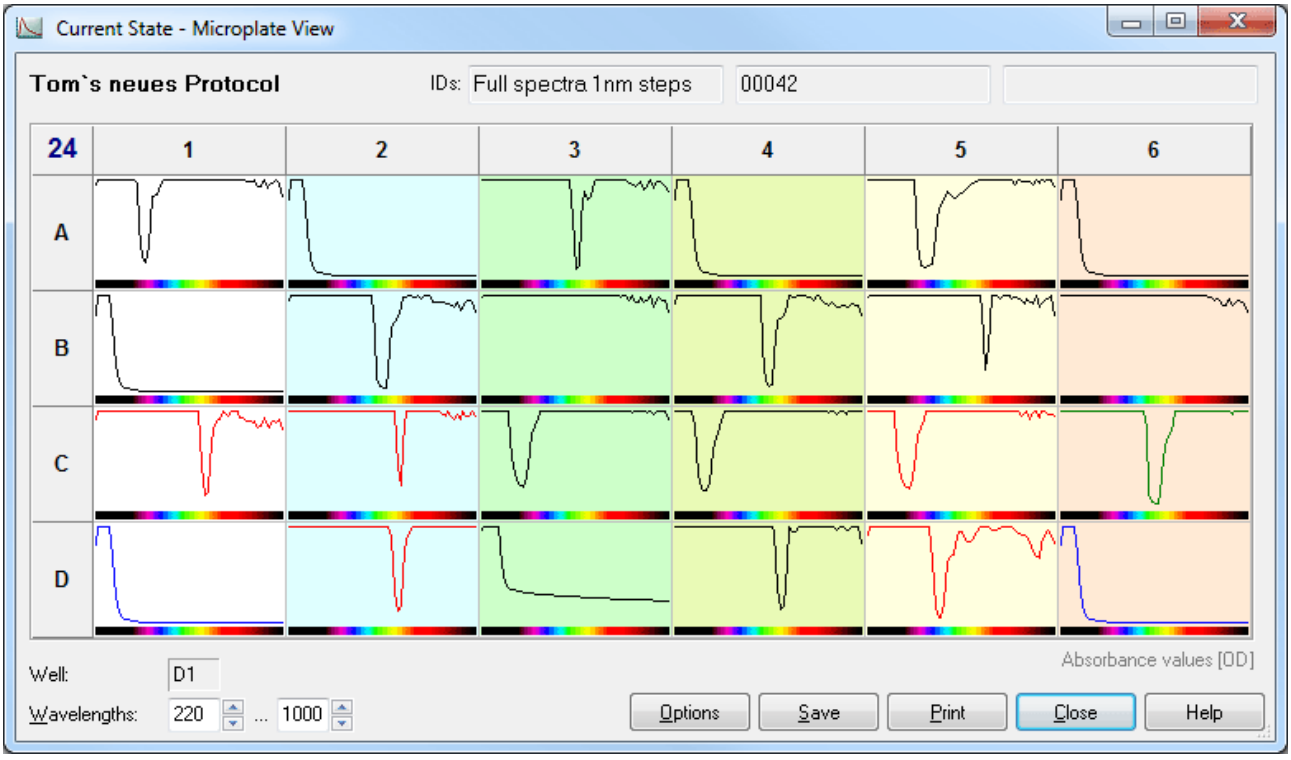
Save Saves the data as bitmap (windows BMP or JPEG format) on the hard drive.

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

Close Closes this window.

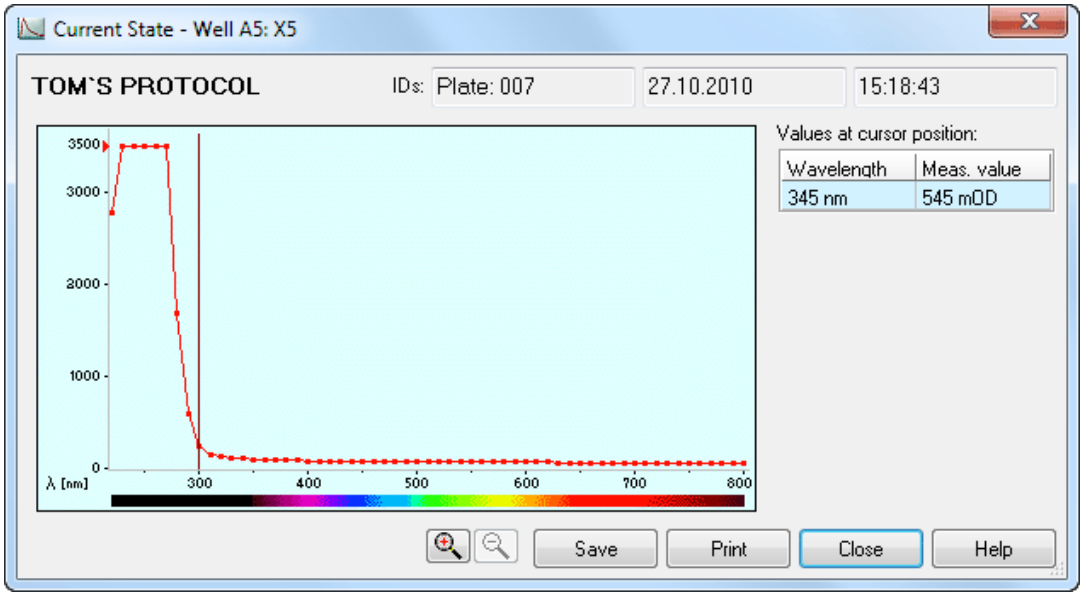
6.7.4 Display of Spectra Data

When performing a protocol in absorbance spectra mode, you can see the spectra of all wells used:



When performing plate mode measurements, you can select the cycle of which the data should be displayed.

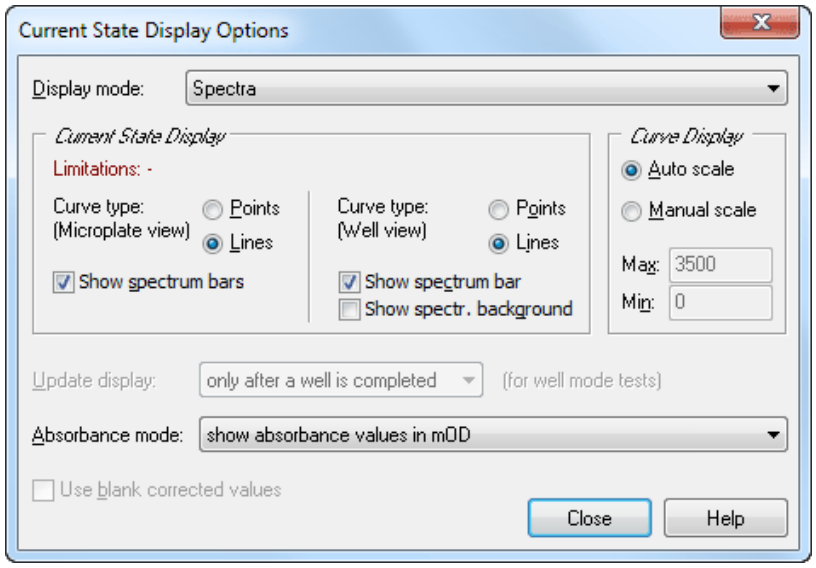
Clicking on one well will open the Current State – Well View window:



If you move the mouse cursor over the display, the measurement value of the wavelength selected will be displayed inside the table. It is also possible to move the cursor by using the following keys: [←], [→], [PgUp], [PgDn], [Pos1] and [End].

By using the buttons or using the [+] and [-] keys, you can zoom into the spectrum curve and out again.

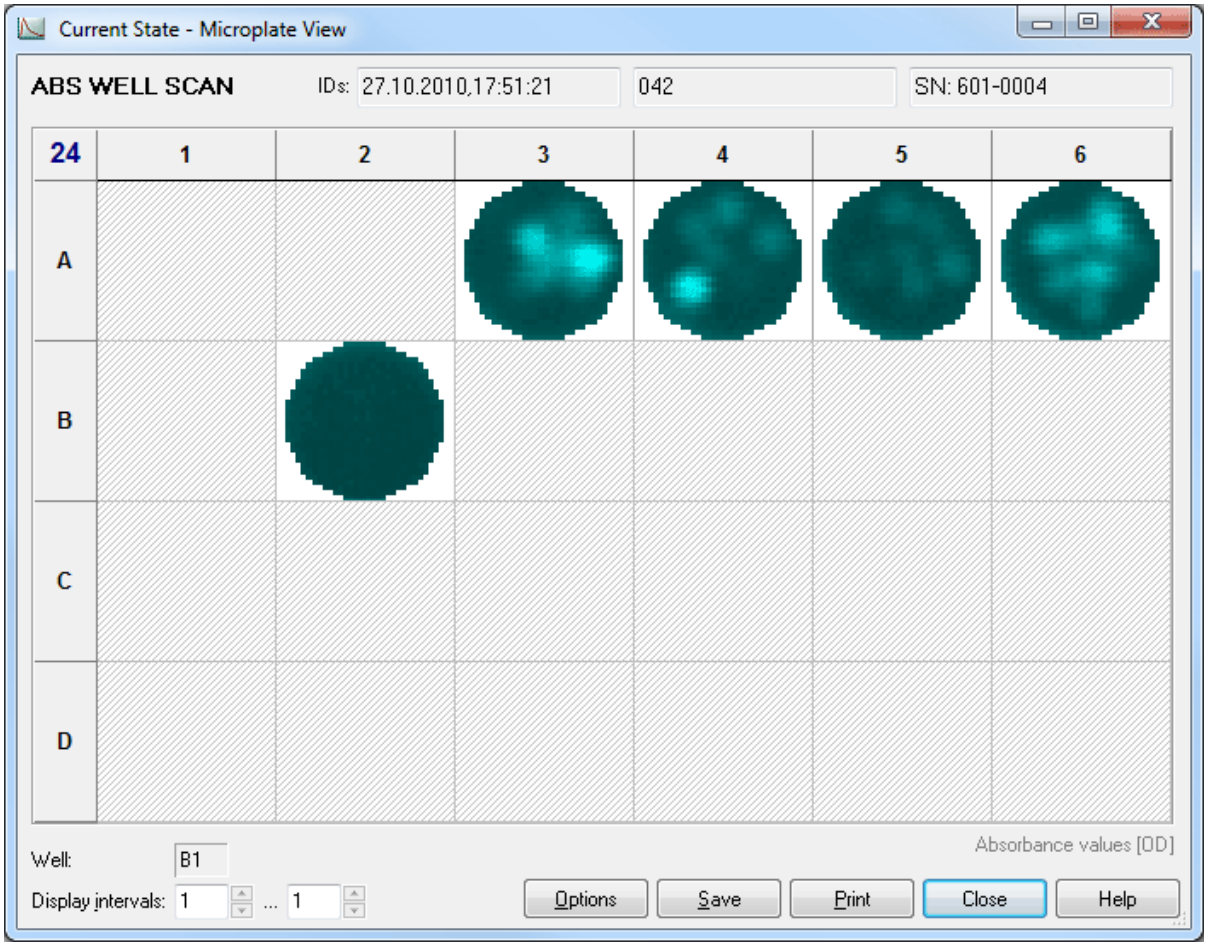
Inside the Options dialogue, you can decide whether you want to see a spectrum bar below each spectrum or not. This bar might help you with the orientation of the spectrum.



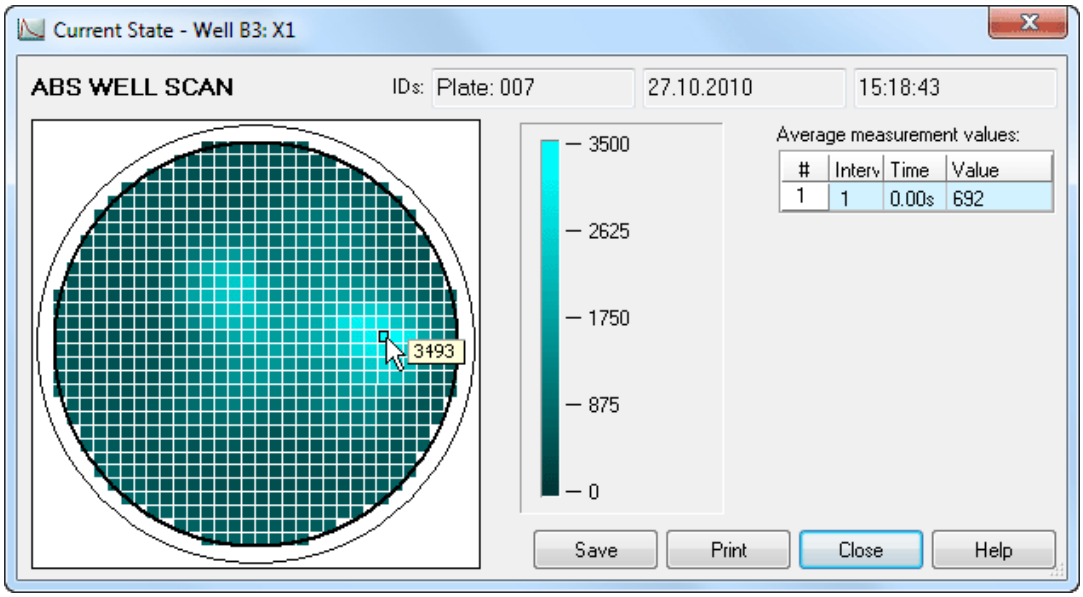
For the well view display, it is also possible to display the background in colors according to the measured wavelengths.

6.7.5 Display of Well Scan Data

When performing a protocol using matrix 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 on one well will open the Current State – Well View window:

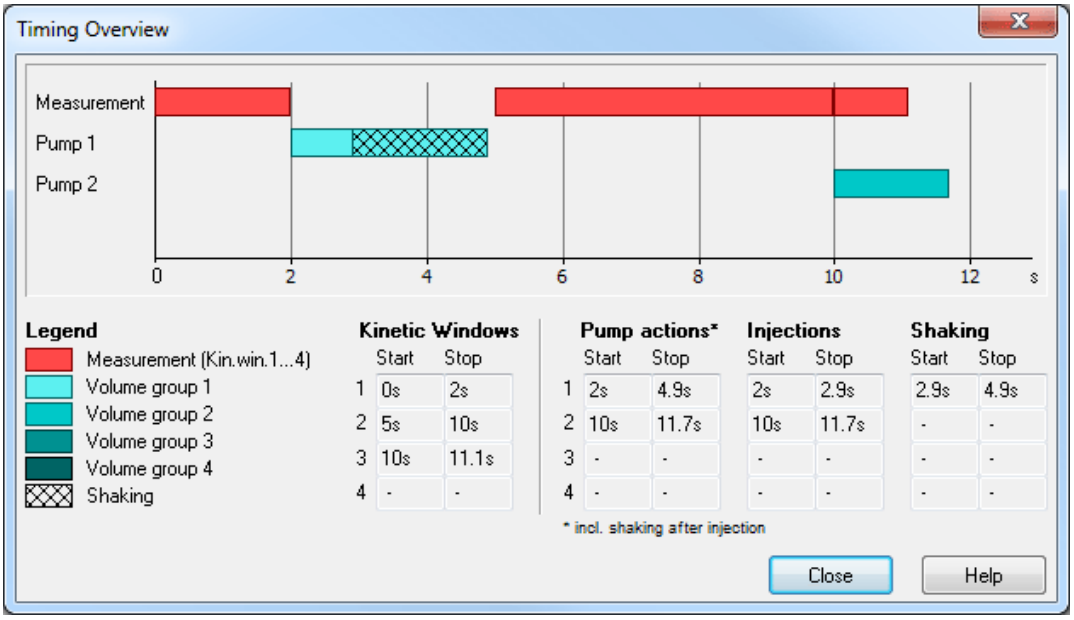


If you move the mouse cursor over the well display, the measurement value of the selected scan point will be displayed. The table on the right shows the average measurement value of the well.

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 chromatics / channels. The selected chromatic / channel will be marked by a blue bar.

6.7.6 Timing Overview

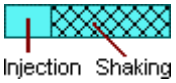
After clicking the 'Timing' button in the Current State – Microplate View window, you will get a window showing an overview of the timing of the test currently running.



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: 

6.8 Repeating the Execution of a Test Protocol (“Re-run”)

At the right side of the Microplate Tab menu is the “Re-run” button. Repeat the execution of the last used test protocol by clicking the top section of this button.

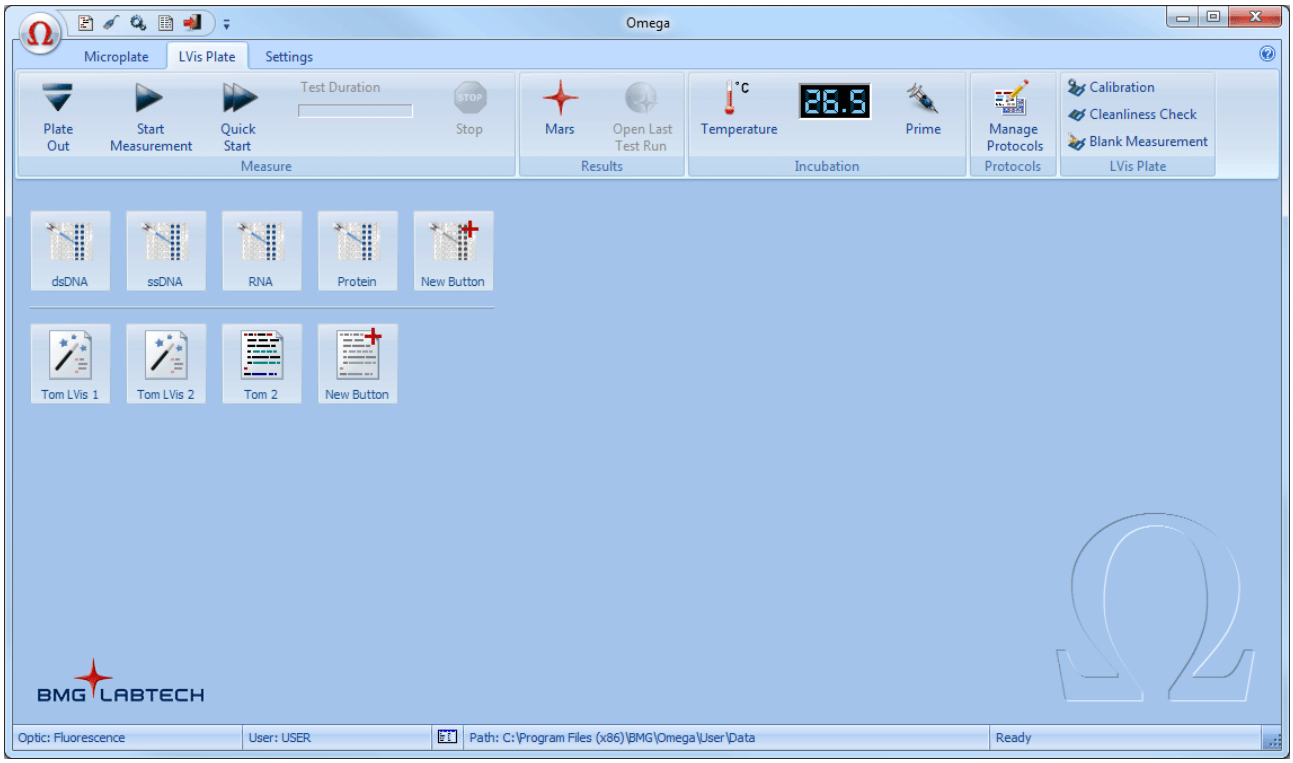
Click the button at the bottom and a context menu will appear. Users have functions to edit the protocol, to open the normal start measurement window (to change IDs or gain values), and to remove the protocol from the re-run list. There is also a function to assign the protocol to a user-definable button (see chapter 2.2.5).

The button at the right end of the icon bar ( ) provides a list containing up to ten last used test protocols. Use this list to repeat the execution of one of these protocols.

7 LVis Plate Measurements

BMG LABTECH's Low Volume (LVis) Microplate is available to perform low volume measurements like DNA.

This LVis Plate tab provides the same functions as the Microplate Tab (see description in chapter 6) for the BMG LABTECH LVis Plate (micro drop section measurements only).



The LVis Plate Tab uses a separate protocol data base. This allows you to define protocols with the same names as protocols from the Microplate Tab. Using the LVis Plate Tab you can only define protocols using the BMG LVis Micro Drop plate.

Hints: This tab only exists if the reader is equipped with a spectrometer for absorbance measurements, e.g. it does not exist when using a NEPHELOstar Plus.

If you do not own an LVis Plate you can switch off the LVis Plate Tab using the 'Settings | Program Configuration' function (see chapter 3.3.1).

Well scanning is not possible when using the BMG LVis Micro Drop plate.

The LVis Plate Tab does not contain the Re-Run function (see chapter 6.8).

You can also use the Microplate Tab to perform measurements using the LVis Plate. This also allows you to use the re-run function. (When the LVis Plate Tab has been switched off you will find the LVis Plate menu group inside the Settings Tab.)

It is possible to export a protocol from one tab and to import it into the data base of the other tab, but the LVis Plate Tab protocol data base will only accept protocols using the BMG LVis Micro Drop plate.

Using other parts of the LVis Plate than the micro drop section is only possible via the Microplate Tab. To measure the quality check filters (holmium and neutral density filters) please define a protocol using the standard BMG LABTECH 96 plate.

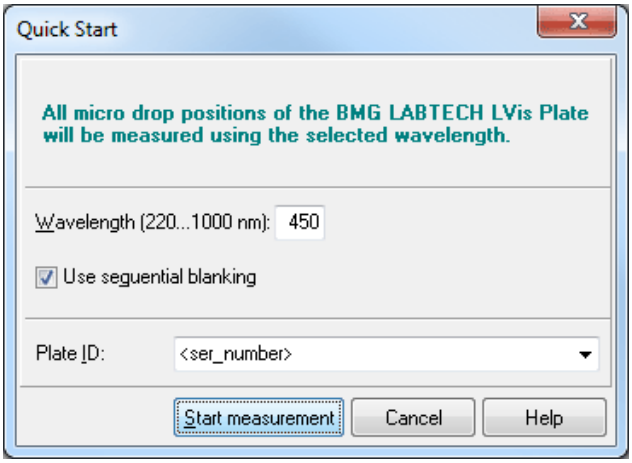
See also the Operating Manual for BMG's LVis Plate for more information on the optional Low Volume and internal standard microplate to perform low volume measurements like DNA.

7.1 Using LVis Tab Quick Start Function

The '**Quick Start**' button function can be used to measure a plate without defining a test protocol. Using this function all micro drop positions of the BMG LVis Plate will always be measured as an endpoint test at a specified wavelength.

Note for Advanced Assays: Use the standard measurement method based on pre-defined protocols to measure a spectrum, to measure part of the microplate, to perform a kinetic measurement or to add shaking (see chapters 4 and 6.2).

After clicking the '**Quick Start**' button the following dialogue box will appear:



Simply select the desired **Wavelength** to be read (between 220 and 1000 nm) and add an optional **Plate ID** (see chapter 6.3).

Sequential Blanking

When using the Quick Start function of the LVis Plate tab you can use sequential blanking. In this mode the individual blank measurement value of each well will be subtracted from the measurement values. To use this function the blank values need to be measured before using the quick start function. This can be done using the blank measurement function of the LVis Plate menu group (see chapter 7.2.3 *LVis Plate Blank Measurement*).

Start measurement button initiates the measurement after closing the Quick Start window.

Use the Current State display to view the measurement values in real time (see chapter 6.7).

Notes: The software can automatically open the Current State display (see chapter 3.2).

The default settings use a settling time of 0.5 s and 20 flashes per well (see chapter 4.3.1)

7.2 Specific Functions for the LVis Plate

There is a special menu group ‘LVis Plate’ containing functions to calibrate the LVis Plate, to perform a cleanliness check and to determine the blank values for sequential blanking.

Hints: This LVis plate tab only exists if the reader is equipped with a spectrometer for absorbance measurements, e.g. it does not exist when using a NEPHELOstar Plus.

If the reader is attached to a Stacker, please remove stacker magazine 1 to insert the LVis plate manually. The LVis plate cannot be handled by the Stacker!

7.2.1 Path Length Calibration of the Micro Drop Sites

By using the Installation CD the correct path lengths of the LVis Plate micro drop sites are copied onto the PC and calibration is not necessary.

If a cleaning procedure was done, in particular when the glass slides were taken out of the LVis Plate for cleaning, we recommend recalibrating the micro drop sites as described below.

Calibrating the exact path length for every micro drop site is achieved by comparing the optical densities of two reference solutions with a known concentration ratio.

You need two reference solutions of Orange G. Orange G can be obtained from Sigma-Aldrich (www.sigmaaldrich.com; Cat. Nr. 75380) and is dissolved in distilled water. The Material Safety Data Sheet (MSDS) for Orange G is located on the Installation CD.

Use the “Micro Drop Calibration Solution 2” (if provided in the LVis Plate box) or prepare an Orange G solution at a concentration of 1.5 mM and denote it as reference “Solution 2”.

Dilute the reference “Solution 2” by a factor of 20 to yield a concentration of 0.075 mM. Denote this as reference “Solution 1”. A simple way to establish the minimum amount of “Solution 1” is done by pipetting 50 µL of “Solution 2” into the cuvette and add 950 µL of distilled water.

“Solution 1” is the reference for the cuvette and should have an OD of ca. 1.5 at a path length of 1 cm.

“Solution 2” is the reference for the micro drop sites. It has a 20x higher concentration compared to “Solution 1” and thus will have an OD of 1.5 at a path length of 0.5 mm.

When “Solution 2” is measured at a nominal path length of 0.5 mm with the Micro Drop sites the result will be equal to the result of “Solution 1” measured with a standard cuvette at a nominal path length of 1 cm.

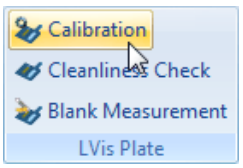
The ratio of the value of every Micro Drop and the value in the cuvette provides the coefficient to correct for the nominal path length of 0.5 mm.

The calibration needs to be performed to yield an individual path length for each of the 16 Micro Drop sites.

The exact path length for each Micro Drop site can be derived from the following equation:

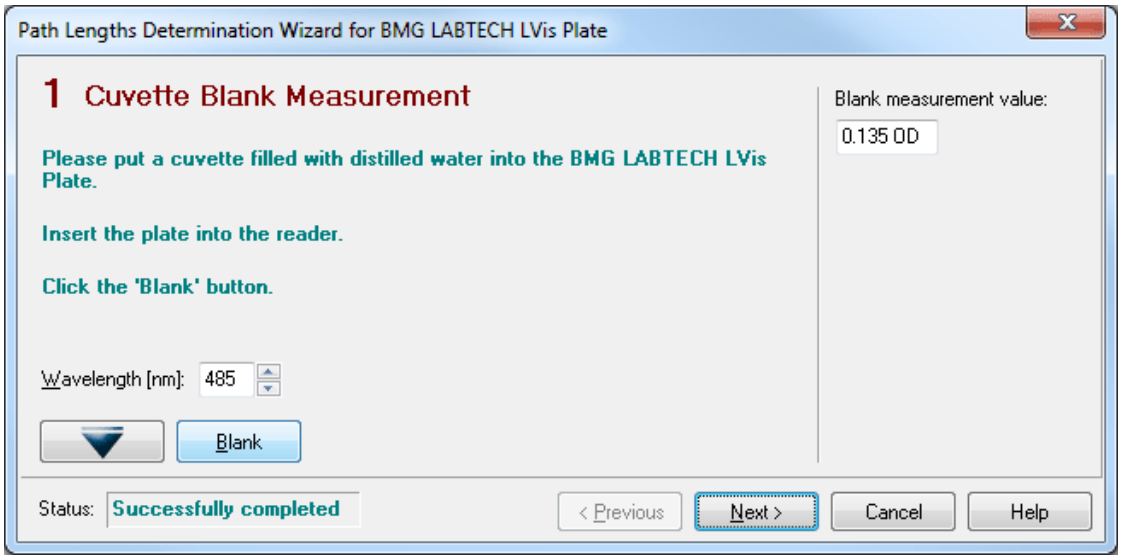
$$Exact\ Path\ Length\ (Micro\ Drop) = \frac{0.5\ x\ OD\ (Micro\ Drop)}{OD\ (Cuvette)}$$

Open the Omega Control Software, and click on the ‘Calibration’ button in the LVis Plate menu group. A “Calibration Wizard” will lead you through the calibration procedure step-by-step. Simply follow the instructions on the screen.



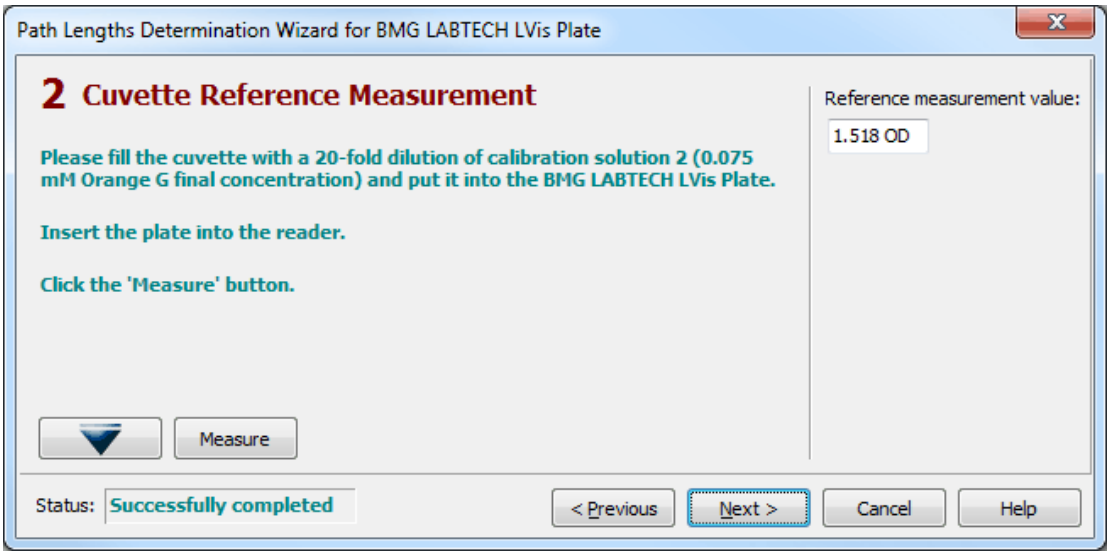
Step 1: Cuvette Blank Measurement

Fill a cuvette with the appropriate amount of distilled water, insert the cuvette into the BMG LABTECH LVis Plate and perform a blank measurement.



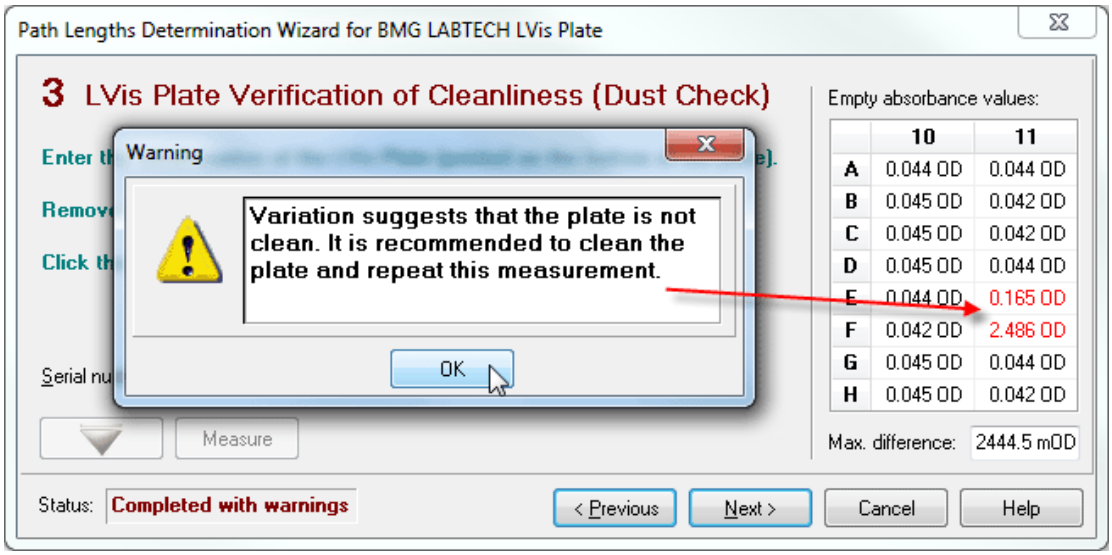
Step 2: Cuvette Reference Measurement

Fill a cuvette with the appropriate amount of “Solution 1” (a 20-fold dilution of calibration “Solution 2”, 0.075 mM Orange G final concentration) and perform a measurement at a wavelength of 485 nm. The status message reports if an action has been completed or not. If completed successfully, click the ‘Next’ button. Note that the result should be approximately 1.5 OD.

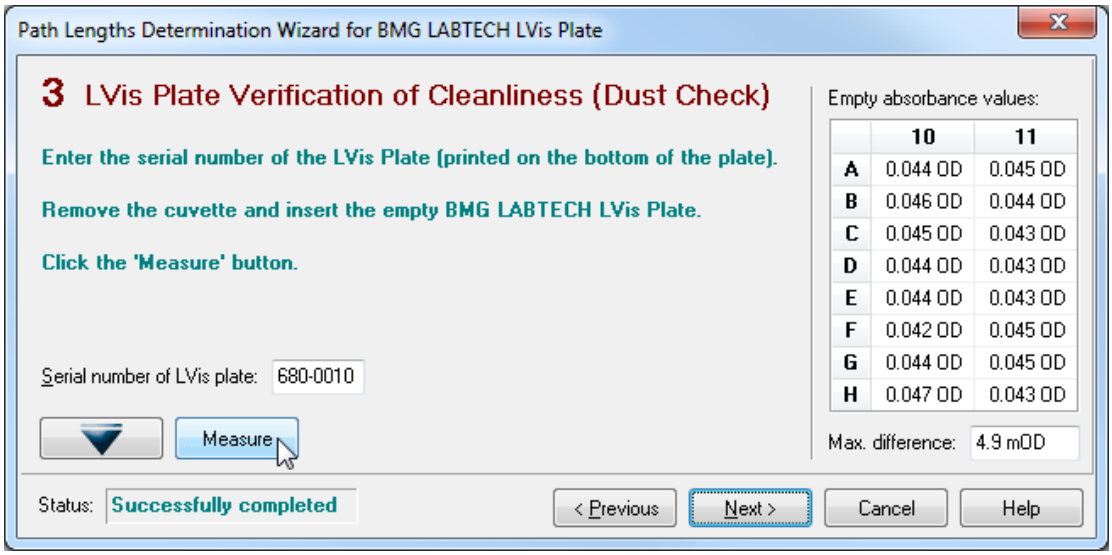


Step 3: LVis Plate Verification of Cleanliness

Remove the cuvette, insert the empty LVis Plate into the reader and enter the serial number which can be found on the back side of the LVis Plate. The measured OD values for the empty and clean Micro Drop Sites should be below 0.060 OD. If the LVis Plate glass slides are not clean a warning message appears, and if at least one value is greater than 0.060 OD or the difference between the lowest and highest value is greater than 8 mOD you are asked to repeat the cleaning procedure as described in the LVis Plate Operating Manual. Outliers are indicated in red.

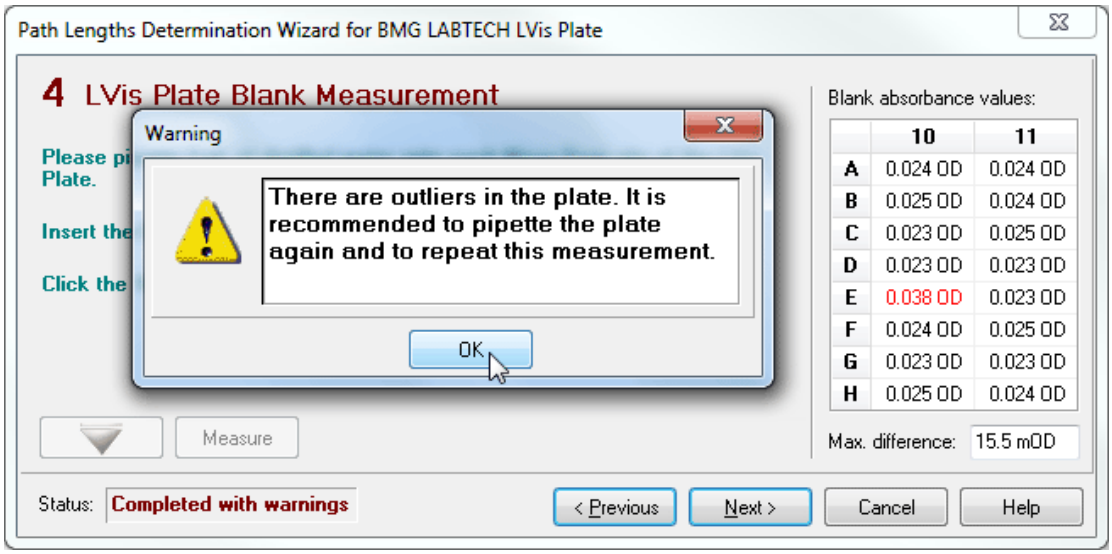


Repeat the cleaning procedure of the LVis Plate until it passes the dust check verification. Click ‘Next’.



Step 4: LVis Plate Blank Measurement

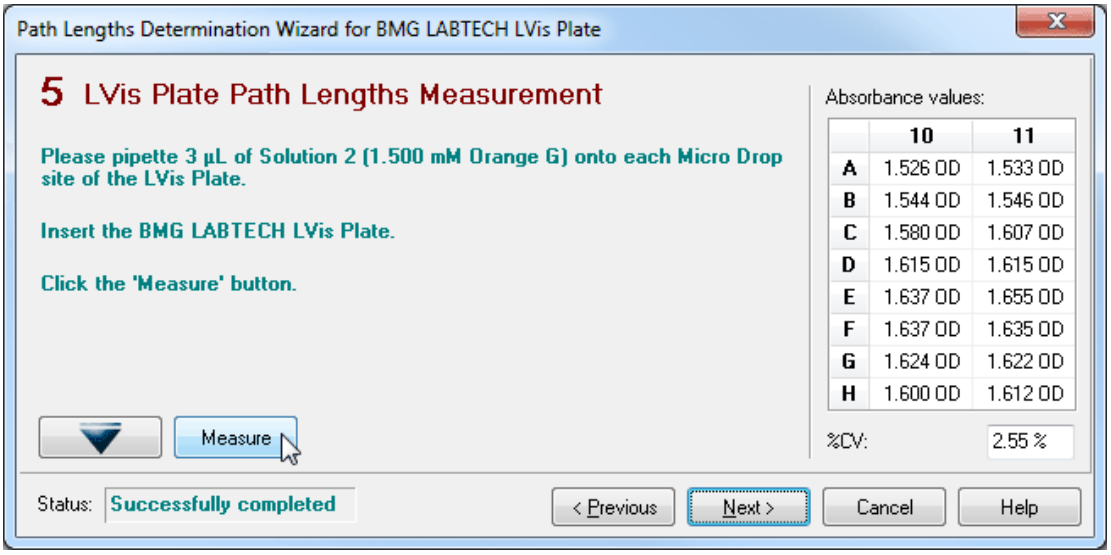
Pipette 3 µL of distilled water at each of the 16 Micro Drop sites using a single or multi-channel pipette, close the lid of the LVis Plate and immediately perform the measurement. The measured OD values should be in the range of 0.025 OD. If the difference between the lowest and highest value is greater than 8 mOD a warning message pops up and you are asked to repeat this step. Outliers are indicated in red.



Repeat the cleaning procedure of the LVis Plate and again pipette 3 µL of distilled water at each of the 16 Micro Drop sites and repeat the measurement until there are no more warnings. Click ‘Next’.

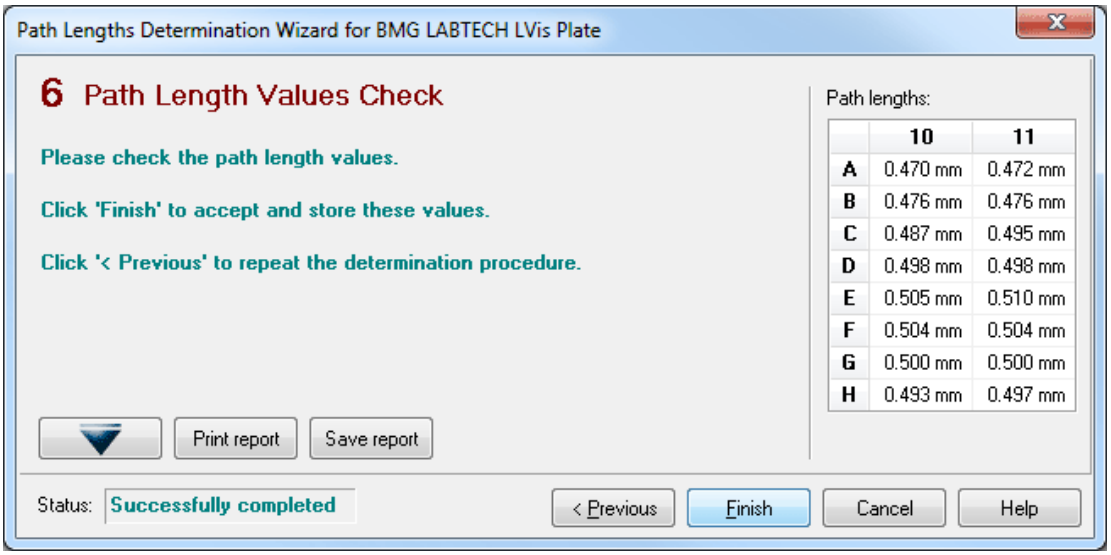
Step 5: LVis Plate Path Length Determination

Remove the LVis Plate, and repeat the cleaning procedure as described in the LVis Plate Operating Manual. Pipette 3 µL of “Solution 2” onto each micro drop site, insert the LVis Plate into the reader and immediately perform the measurement. The OD values should be in the range of 1.500 OD and the associated Coefficient of Variation (%CV) should be < 5%. If the %CV is higher, repeat this step. Click ‘Next’.



Step 6: Determination of the Path Length Values

The individual path lengths of each micro drop site of the LVis Plate are calculated automatically.

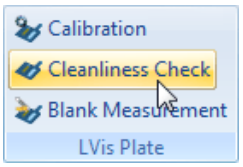


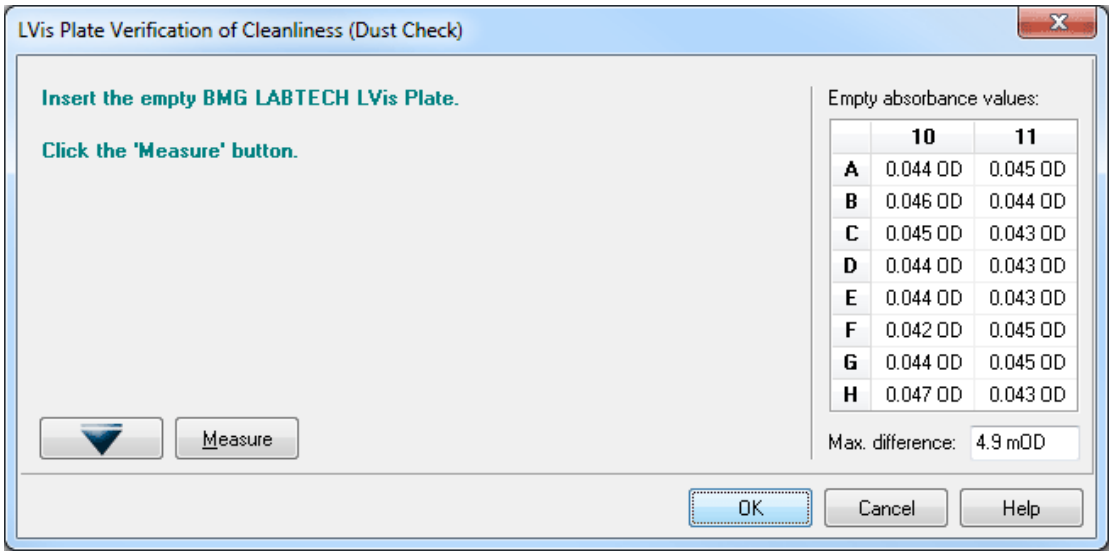
Observe the path lengths (denoted in mm). A report of the calibration procedure can be printed or exported as *.txt or *.xls file for your records. Thus, it is recommended to always include the serial number of the LVis Plate at step 3.

Click ‘Finish’ to end the calibration procedure. These path length values are stored in the ‘BMGLVis.pl’ file and will now be used for further measurements with the LVis Plate until a new path length calibration is performed.

7.2.2 LVis Plate Cleanliness Check

This function can be used to check whether the glass slides are free of dust or any other related particles which could lead to a false measurement result. The cleanliness is verified by measuring the optical density of the 16 empty micro drop positions.

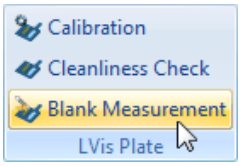




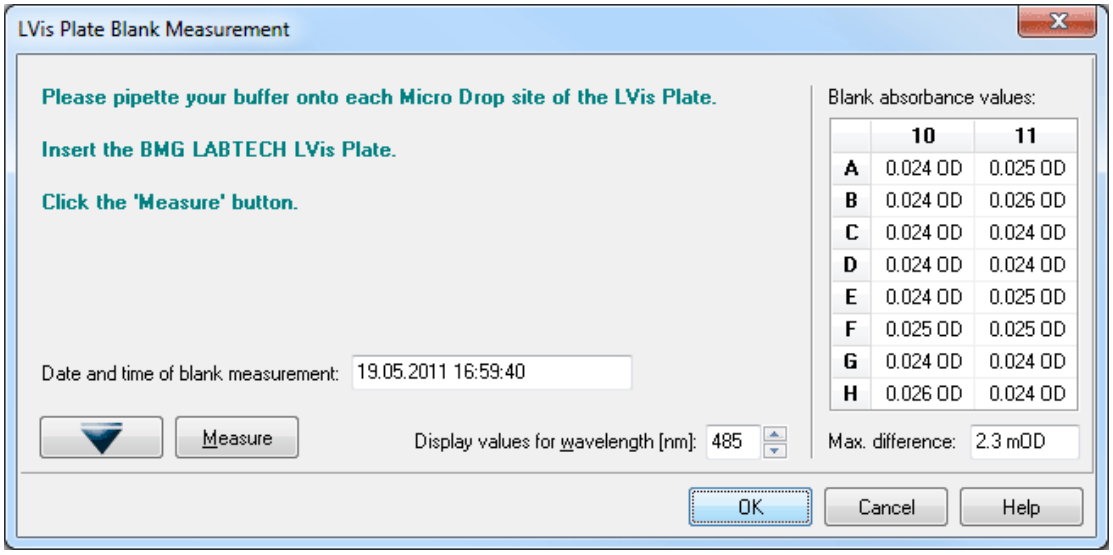
The OD values should be below 0.060 OD. If a value is greater than 0.060 OD or the difference between the lowest and highest value is greater than 8 mOD a warning message pops up and you should repeat the cleaning procedure of the glass slides as described in the LVis Plate Operating Manual.

7.2.3 LVis Plate Blank Measurement

For LVis Plate measurements which use sequential blanking (see chapter 4.7.1) the blank values need to be measured before executing the test protocol. This function can be accessed via the 'Blank Measurement' entry in the LVis Plate menu group.



When performing the blank measurement the whole spectrum is measured. You can select any wavelength to check the results.



When you reopen this dialogue the last measured blank values will be displayed.

8 Script Mode

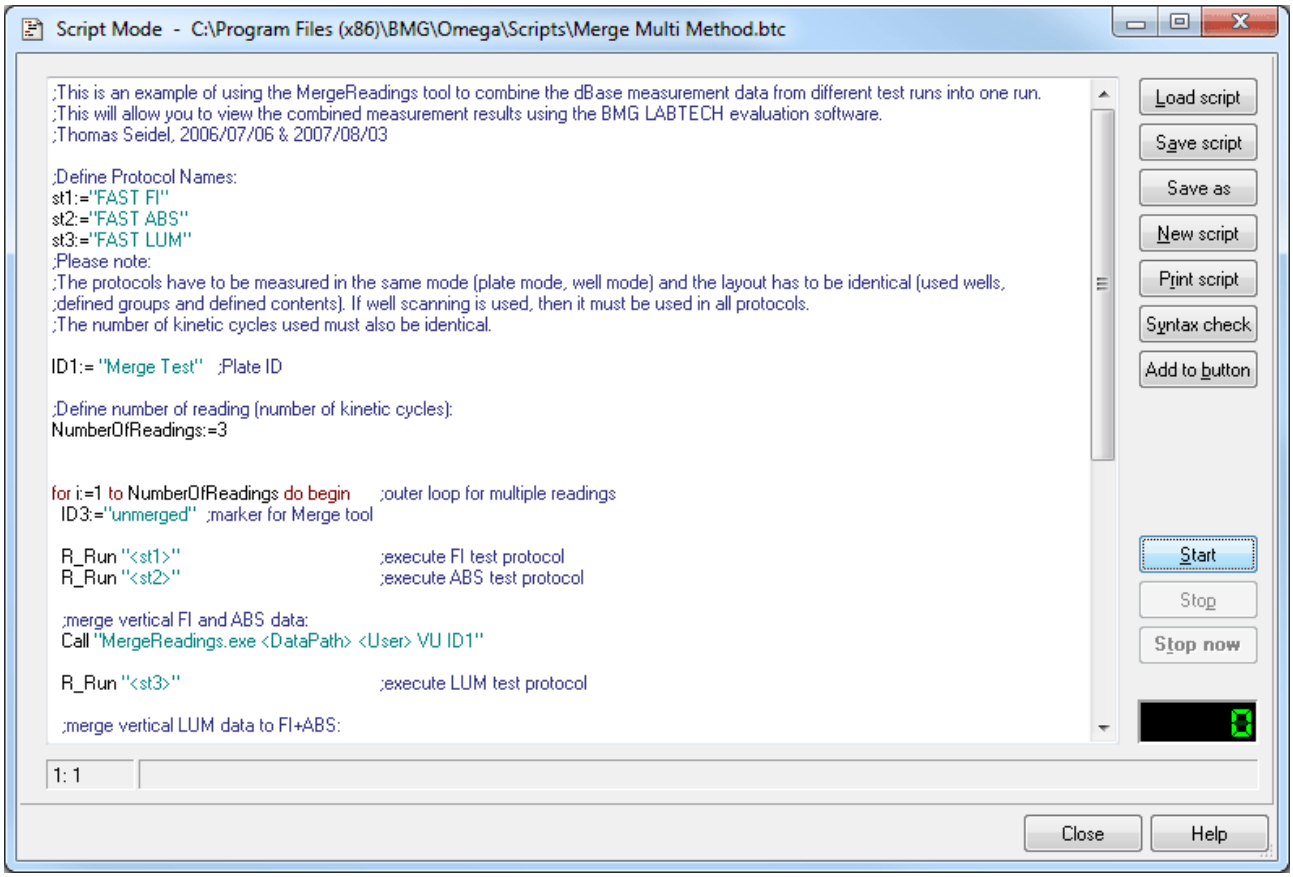
8.1 Script Mode Window

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. The script mode function can be used for both a reader combined with a stacker or for a standalone reader. All available script language commands are described in chapter 9 *The Script Language*.

Warning: It is possible to move the stacker table and the reader plate carrier into a collision position using the script language provided! Therefore, you should use this function only if you are absolutely sure about what you are doing.

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.

8.1.1 Control Elements



Load script	Loads a script file. By 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. If you use variables for such parameters you will get a warning message, as the value of a variable is only known at run time. *Hotkey:* [Ctrl]+[F9]
Add to button	Adds the current script to a script button (see chapter 2.2.6).
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.

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.

8.1.2 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.

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).

8.1.3 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.

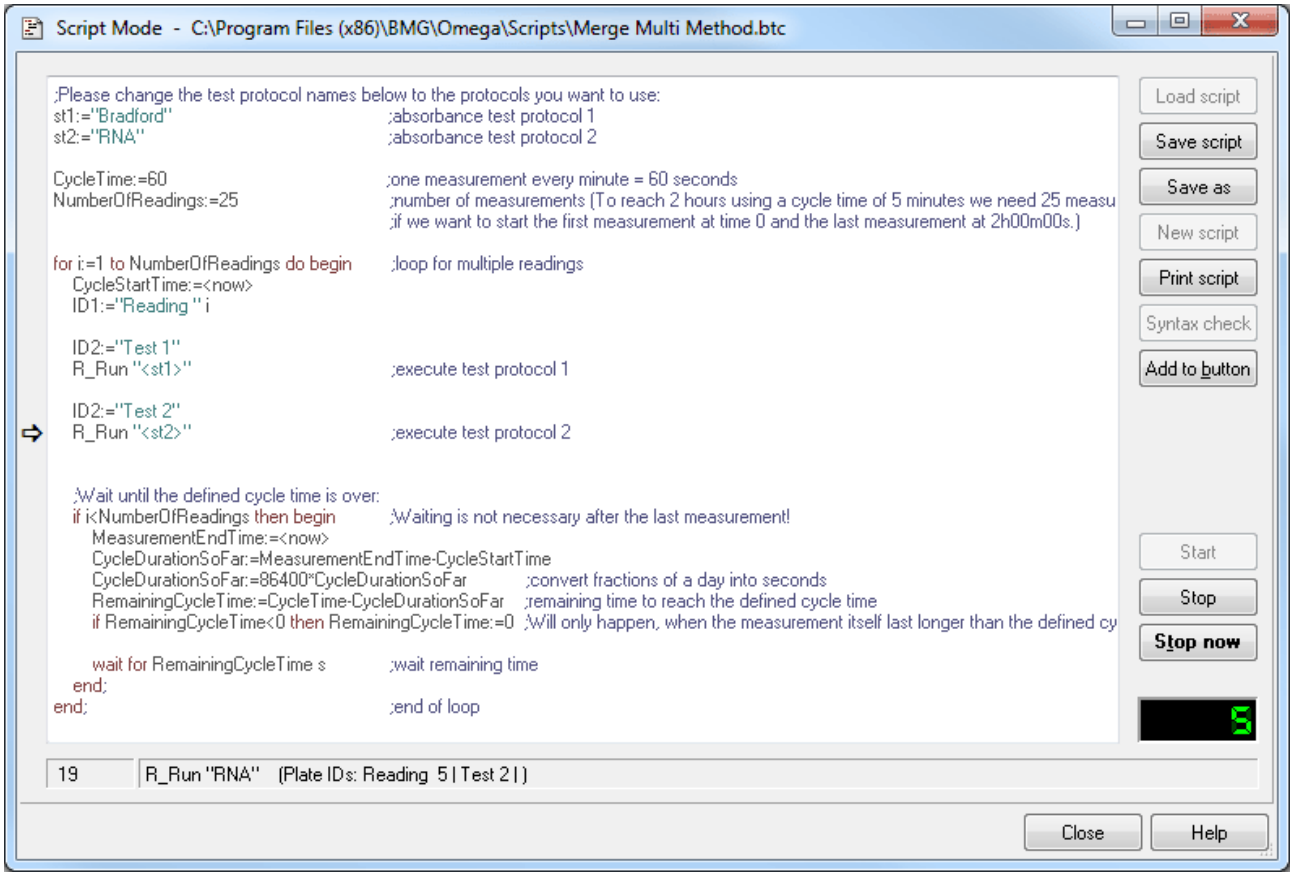
Example:

```
for i:=1 to NumberOfPlates do begin      ;loop for reading of multiple plates
  ID1:="Plate " i                        ;define plate ID
  S_PlateIn 1                            ;get plate from stacker magazine #1
  R_Run "TOM´S PROTOCOL"                 ;execute test protocol
  S_PlateOut 2                           ;put plate into stacker magazine #2
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.

8.1.4 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 10.3).

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 9.2.2). If you use nested loops, this loop counter will show the value of the inner most loop.

8.2 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.

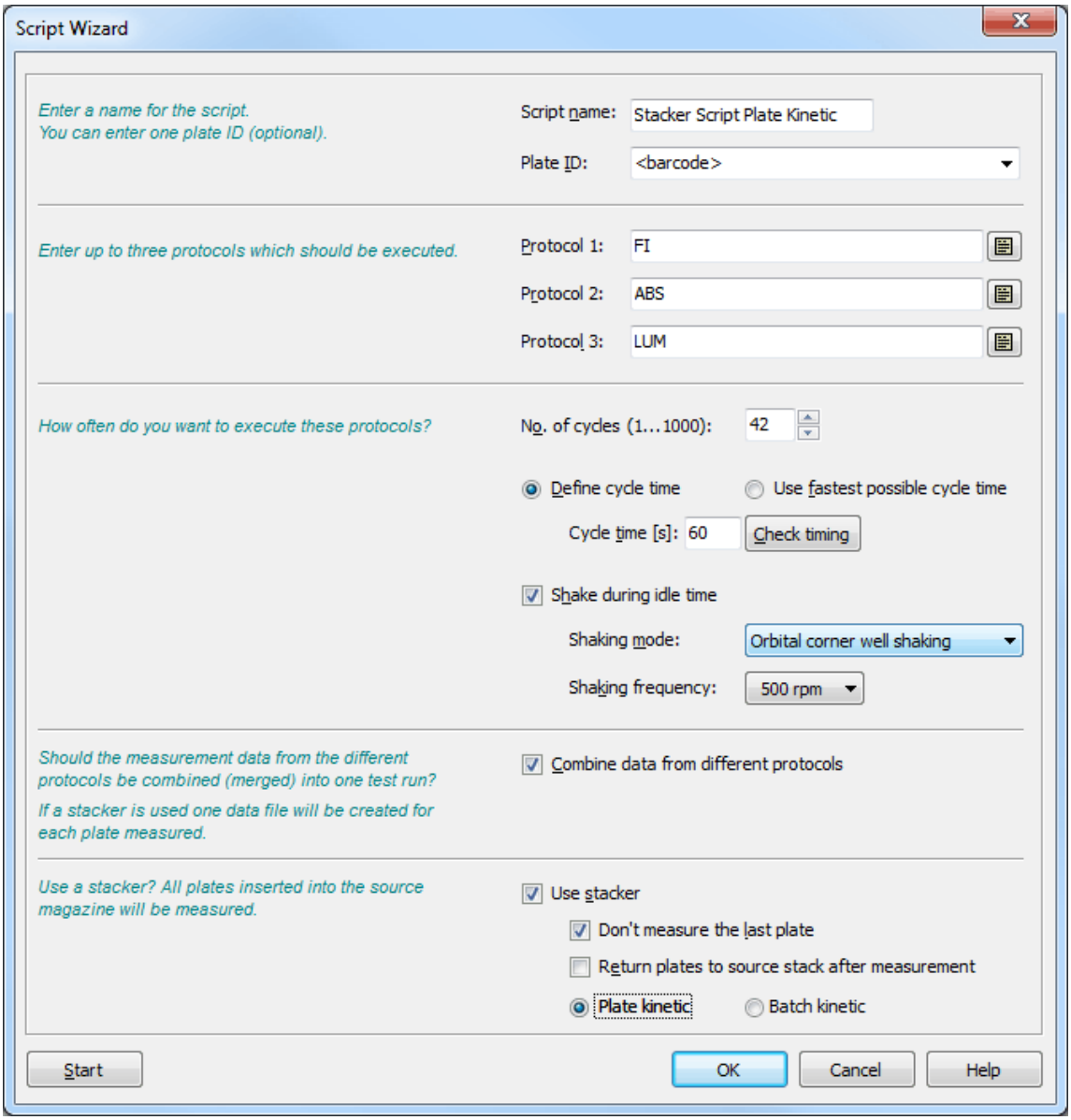
```
Omega.exe /s"C:\Program Files\BMG\Omega\Scripts\Autorun.btc" /v.
```

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 Omega 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 (").

8.3 Script Wizard

When using script buttons (see chapter 2.2.6) you can choose between assigning an existing (file) script to a button or you can use the script wizard to get a script created automatically.

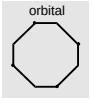


Using the wizard you can perform multi measurement method measurements. These measurements can include a kinetic.

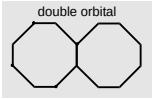
If you define a **cycle time** which is longer than the time necessary to execute all test protocols define, during the remaining cycle time (= idle time) a **shaking** can be performed. The shaking will happen on a randomly selected position. When using longer shaking times this position will be changed every 60 s to prevent the transport system of the reader from damage.

Shaking mode

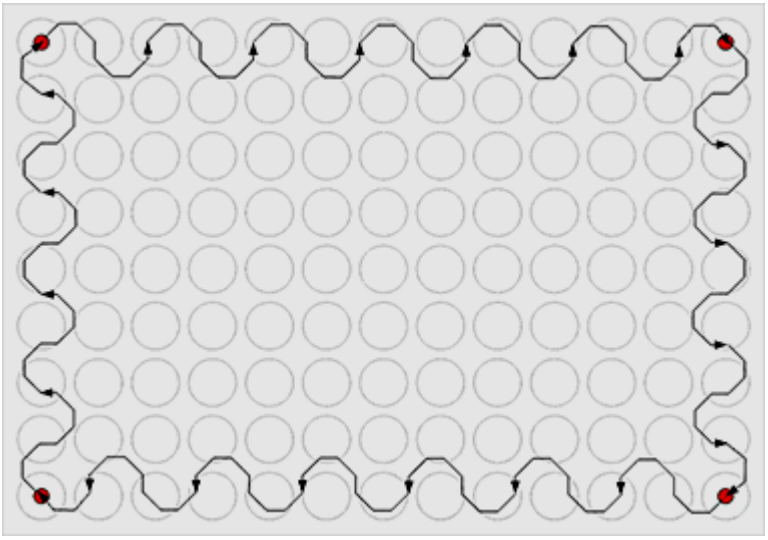
Linear:	Shaking mode is from right to left.
Orbital:	Shaking mode is circular; mixing is more complete, especially around the edges of the microplate.



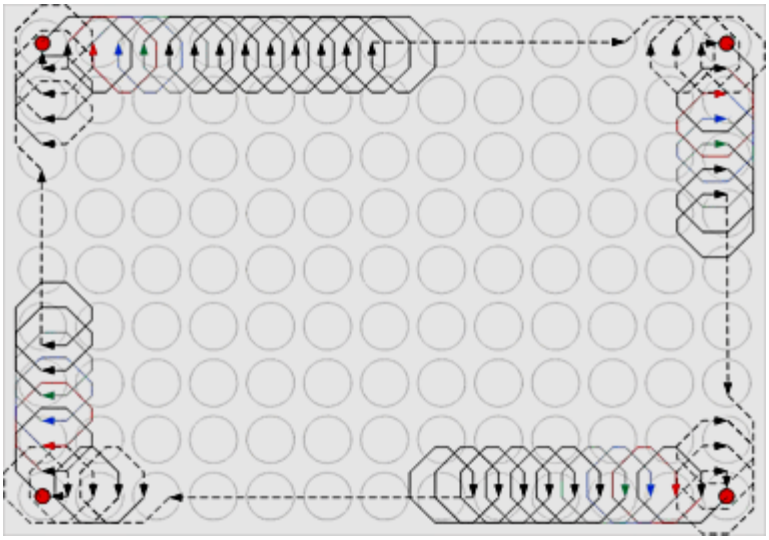
Double orbital: The shaking function is performed as orbital movement. The plate carrier makes a figure eight movement.



Meander corner well shaking: Meander shaking movement overlaid with a movement between the 4 corner wells of the plate.



Orbital corner well shaking: Orbital shaking movement overlaid with a movement between the 4 corner wells of the plate.



Note: To use shaking a firmware version 1.30 or newer is necessary. When using 'Meander corner well shaking' or 'Orbital corner well shaking' the firmware version needs to be at least 1.40.

The measurement data of the different cycles will always be merged together (Absolute Data Base format, see chapter 3.4 *Data Output*). When you use more than one test protocol you can also **combine the data from the different protocols** into one test run. The data from the second and third protocol will appear as additional chromatics in the combined (merged) test run. Please note: in the data analysis software you will only see the protocol settings from the first test protocol.

Merging will only work when the selected test protocols use an identical layout, the same reading mode (e.g. endpoint or plate mode) and the same number of kinetic points (cycles or intervals).

If you uncheck the 'Combine data from different protocols' box you will get one test run per protocol used.

When a **stacker** is attached to the reader you can measure a whole batch of plates. In this case you can select between '**Plate kinetic**' and '**Batch kinetic**'.

In plate kinetic mode all cycles will be read on one plate before continuing to the next plate. Opposite to this in batch kinetic mode all plates will be read at each kinetic cycle.

9 The Script Language

9.1 Introduction

The script language can be used for controlling the stacker and the reader. Scripts can either be automatically generated or written by the user using the 'Script Mode Window' (see chapter 8.1).

The automatically generated scripts will be saved in the file '`Omega.btc`' in the Omega temp folder (usually `c:\Program Files\BMG\Omega\Temp`). It might be a good idea to have a look at the automatically generated scripts for batch measurements. You can copy these scripts and modify them according to your needs, but be aware of the fact that there will be different batch run scripts generated, depending on the usage of <barcode> as one of the plate IDs or depending on whether or not the last plate is measured. For the automatically generated scripts, it will also be taken into account whether or not the first plate has already been moved in into the reader (for gain adjustment).

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

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\Omega\Tom\Definit″ /
           2 5 80 40 1
```

9.1.1 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)>.

9.1.2 Advices

Before using an S_PlateIn(B), S_GetPlate(B) or S_MovePlate command (or before using an R_Run command with stacker parameters) send an S_PrepareForPlate command, to ensure that the stacker knows the Z dimensions of the microplate used (different plate types require different handling). If you use different microplates in one script run, you need to send the S_PrepareForPlate command each time the plate type will be changed; otherwise it is enough to send this command once at the beginning of your script.

- If a stacker is connected to your reader, you should use an **S_DisableMotors** command at the end of your script, to switch off the stepper motors, to save energy and prevent heat built-up.

9.2 Batch Control Commands

9.2.1 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.

9.2.2 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;
```

For stacker batch measurements you can use **<All_Plates>** as end value. If you use this special term as part of the loop command (for i:={n} to <All_Plates> do begin), all commands between the for...to- and the end-line will be repeated as long as an S_PlateIn, S_PlateInB, S_GetPlate, S_GetPlateB or S_MovePlate command (or an R_Run command with stacker parameters) inside the loop finds a plate in the used source stack.

If used in this context, there will be no error message if the source stack is empty, instead the script execution will be continued with the first line after the loop. This allows you to program scripts which will measure every plate available, without needing to know the plate number upfront.

Example:

```
for Plate:=1 to <All_Plates> do begin
  S_PlateIn 1
  R_Run ”MY TEST”
  S_PlateOut 2
end;
```

Note: There will be an error message if the S_PlateIn, S_PlateInB, S_GetPlate, S_GetPlateB command (or the R_Run command with stacker parameters) already fails for the first plate (loop counter = 1, occurs only if the loop does not starts with 2), which means there has not been a single plate inserted.

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.)

9.2.3 Comparisons

if <Barcode_String>=“value” **then** ...

Perform the command after ‘then’ only if the last read barcode is equal the declared (string) value. *

Example:

```
if <Barcode_String>=”<no barcode detected>” then ID1:=”unknown plate”
```

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 ... st100) 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 Ser_Number=“value” **then** ...

Perform the command after ‘then’ only if the serial number of the reader currently connected is equal to the declared value. *

Example:

```
if Ser_Number>=”415-1000” then ...
```

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”.

9.2.4 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 8.2).

9.3 Dialogue Commands

9.3.1 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
```

9.3.2 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.

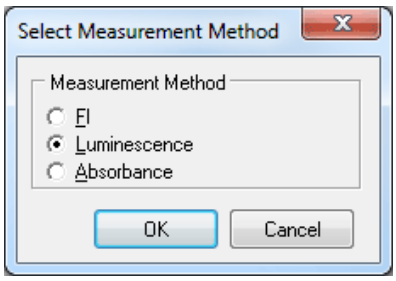
Specifying a title or defining a default selection is optional. If you do not specify a title, 'Please select' will be used. If you do not define a default selection, the first item will be preselected.

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.

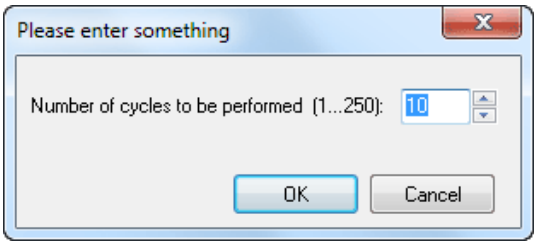
If you specify a minimum and maximum value, only input values in this range will be accepted. If the range between minimum and maximum value is not larger than 250, up/down scroll buttons will appear beside the input field.

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
```

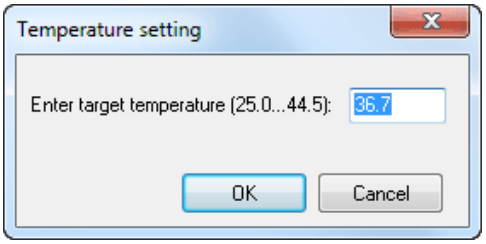


{Variable}:=**InputFloat** {Label} ({Title}) ({Min}) ({Max}) ({Default value})

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})

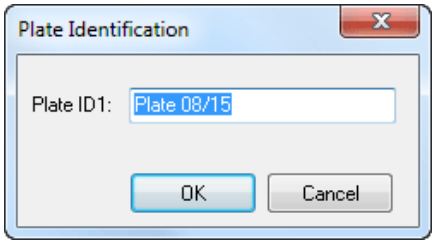
This command is similar to the IntegerInput command, but will be used for string values. As target variable, only a string variable (st0 … st100) is allowed.

Examples:

```
st1:=InputString ”Plate ID1:”
```

or

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



9.3.3 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:

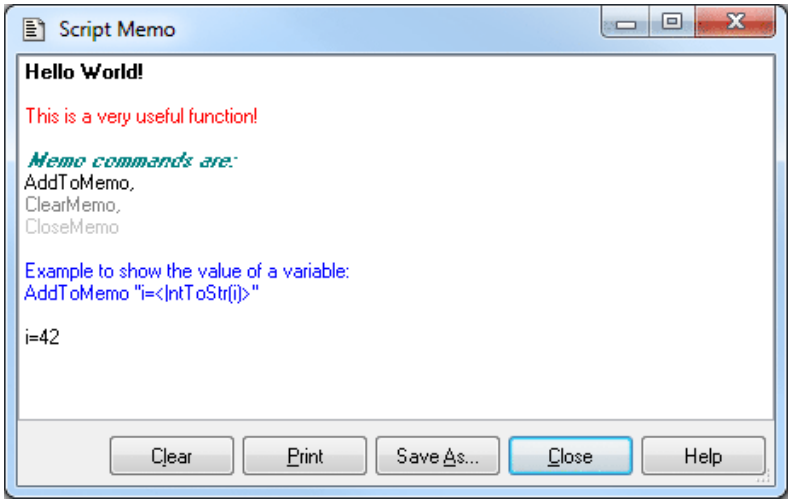
	use a bold font style
	use an italic font style
<bi>	use bold italic
or or </bi>	use normal font style
<c:{ColorName}>	use the defined color, possible values are: Black, Red, Maroon, Lime, Green, Yellow, Olive, Blue, Navy, Fuchsia, Purple, Aqua, Teal, Silver, Gray

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 !!!"
```



SaveMemo {Filename}

Using this command you can save the memo content into a file.

Examples:

```
SaveMemo "C:\Memo.txt"
SaveMemo "C:\Data.csv"
```

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.

9.4 Reader Commands

9.4.1 Init Command

R_Init ({InitMode})

If you do not use the optional {InitMode} parameter, this command will initialize the reader without resetting the incubator. If a stacker is connected to the reader, the stacker will also be initialized and a search for "lost" microplates will be performed.

By 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	disable reader plate in/out button
Bit 1	do not perform plate search (only if a stacker is attached to the reader)
Bit 2	*not used*
Bit 3	do not reset the incubator
Bit 4	put plate into magazine 1 (instead of 2), if a plate will been found during plate search

Examples:

`R_Init 0` *full initialization including plate search (if a stacker is attached) and incubator reset*

`R_Init $0200` *no plate search, but including incubator reset*

`R_Init $0800` *perform plate search, but no incubator reset*

9.4.2 Plate Carrier Movement

R_PlateOut

Move plate carrier out of the reader to stack position 1, if the reader is attached to a stacker. Otherwise this will just move out the plate carrier.

R_PlateOutR

Move plate carrier out and to the right side (to stack position 2, if the reader is attached to a stacker).

R_PlateIn

Move plate carrier into the reader.

R_PlateUser {x} {y}

Move plate carrier to position x, y.

9.4.3 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.

9.4.4 Gain Adjustment

Notes: Gain adjustment is not necessary / possible for absorbance protocols. Gain adjustment over the whole plate (R_GainPlate) is not available for fluorescence polarization 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 calculated 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.)

9.4.5 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_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\Omega\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}) ({Source magazine}) ({Destination magazine}) (NotLastPlate) (ReadBarcode)

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.

Note: If you do not include the path to the protocol definition the path to the microplate tab protocol data base of the user currently logged in will be used. If you want to use an LVis Plate tab protocol you can either enter the complete path to this data base (e.g. "c:\Program Files\BMG\Omega\User\DefLVis") or you can just use "LVis" (the software will then use the path belonging to the user currently logged in).

If a Stacker is connected (and when using a reader firmware version 1.40 or newer), it is optionally possible to specify a source and a destination magazine. If the source magazine value is set to 1 or 2 a plate will be fetched from this stacker magazine before the measurement. If the destination magazine value is 1 or 2 the plate will be inserted into this stacker magazine after the measurement. When specifying 0 for one of the stacker magazine parameters, there will be no movement from or to the respective stacker magazine.

When adding **NotLastPlate** to the command line, the last plate will not be taken (this might be useful, if the top plate is used as cover).

Note: Use the following command line to leave a loop after detecting the last plate:

```
if LastPlateDetected then break
```

If you add **ReadBarcode** to the command line, the barcode of the plate will be read during the plate in procedure (only possible when using magazine 1 as source magazine). Opposite to using the S_PlateInB command the barcode value will be transferred automatically. This value can be used as plate ID (use "<barcode>" or as part of the ASCII file name. Use the S_PlateInB and S_BarcodeData commands if you need access to the barcode before the R_Run command (e.g. for conditional branches).

Please use the S_PrepareForPlate command before using the R_Run command in combination with stacker parameters!

Examples:

```
R_Run "Tom's Protocol"  ;measurement without using a Stacker
R_Run "Tom's Protocol" 1 0  ;get a plate from stacker magazine 1 and measure it
R_Run "Tom's Protocol" 1 2 NotLastPlate ReadBarcode  ;get a plate from stacker magazine 1 (but don't take the last plate), read its barcode, perform the measurement and insert the plate afterwards into magazine 2
```

9.4.6 Shake Command

R_Shake {Shake mode} {Shake frequency} {Shake time} ({Shake position X}) ({Shake position Y})

Performes a shaking.

Use 0 = Orbital, 1 = Linear, 2 = Double Orbital, 3 = Meander corner well shaking or 4 = Orbital corner well shaking as Shake mode parameter. You can define the Shake frequency in the range 100 ... 700 rpm, when high speed shaking is activated up to 1100 rpm. For linear shaking a maximum of 800 rpm (when high speed shaking is activated) and for orbital corner well shaking a maximum of 300 rpm can be used. The Shake time can be anything between 1 and 3600 seconds.

You can define the x position in the range 120 ... 3600 and the Y position in the range 1 … 800. When no shake position is specified (or when you use 9999 for X or Y) a random position will be used.

Examples:

```
R_Shake 0 100 2 333 444
R_Shake 1 500 3
R_Shake 2 1000 3 9999 9999
```

Note: This command requires a firmware version 1.30 or newer. When using shake mode 3 or 4 at least firmware version 1.40 is necessary.

9.5 Stacker Commands

9.5.1 Init Command

S_Init

Use this command to only initialize the stacker device.

9.5.2 Plate Handling Commands

S_PrepareForPlate {Protocol Name} ({Path to protocol data base}) or

S_PrepareForPlate {MP:Microplate Name}

This command will take the microplate name from the specified test protocol (or will take the microplate name specified directly using the second syntax version) and read the Z dimensions from the microplate data base. Based on these dimensions the Z value will be calculated, at which the Stacker should open its stoppers when inserting a plate into a magazine or should close the stopper when fetching a plate. The calculated Z value will be compared with the Z values stored inside the Stacker EEPROM. If different, the new Z value will be written into two positions of the EEPROM.

Note: You should always use this command in your scripts before the first S_PlateIn command!

S_PlateIn {n} (**NotLastPlate**)

Get a plate from magazine {n} and move this plate into the reader.

If you add NotLastPlate to the command line, the last plate will not be taken (this might be useful, if the top plate is used as cover).

See also the stacker options of the R_Run command.

Note: You can use the following command line to leave a loop after detecting the last plate:

```
if <LastPlateDetected> then break
```

S_PlateInB {n} (**NotLastPlate**)

Get a plate from magazine {n}, read the barcode from this plate and move this plate into the reader.

Note: Barcode reading is only possible when magazine 1 is used as source (and when a barcode reader is installed). Use S_BarcodeData after this command to transfer the barcode value to the computer.

S_PlateOut {n}

Move the plate out of the reader and insert it into magazine {n}.

S_GetPlate {n}

Get a plate from magazine {n}. If the reader plate carrier has been moved to the selected stack position, the plate will be inserted into the reader plate carrier, otherwise it will stay on top of the stacker table.

Recommendation: Use the command S_PlateIn instead of this command to move a plate into the reader.

S_GetPlateB {n}

Get a plate from magazine {n} and read the barcode from this plate (barcode reading is only possible when using stacker magazine 1 as source).

Recommendation: Use the command S_PlateInB instead of this command to move a plate into the reader.

S_PutPlate {n}

Put a plate from the stacker table (or from the reader plate carrier, if this has been moved under the target stack) into stacker magazine {n}.

Recommendation: Use the command S_PlateOut instead of this command to move a plate from the reader into a stacker magazine.

S_MovePlate {src} {dest} {no}

Move {no} plates from stacker magazine {src} to magazine {dest}.

Note: When using **<All_Plates>** as third parameter all plates available in the source magazine will be transferred (max. 127). In this case there will be no error message, if the source magazine is empty.

S_RestackAfterCount

Show number of counted plates (counted during an S_MovePlate command) and move the plates back to the count stack.

9.5.3 Other Stacker Commands

S_MoveTable {n}

Move stacker table to a predefined position:

n	Position
0	stack 1
1	stack 2
2	position of plate carrier sensor
3	position for barcode reading

S_MoveX {n}

Move stacker table to X position {n}.

S_MoveZ {n}

Move stacker table to Z position {n}.

S_DisableMotors

Disable the stepper motors.

You should send this command at the end of your scripts or whenever the stacker will not be used for a long time to save energy.

Note: The stepper motors will automatically be enabled by the stacker firmware when a stacker action is started.

S_ReadBarcode

Read (scan) the barcode.

Note: This command will only trigger the barcode reader (useful for testing purposes). To read the barcode of a plate use S_PlateInB or S_GetPlateB.

S_BarcodeData

Transfer the scanned barcode data (obtained using the S_PlateInB, S_GetPlateB or S_ReadBarcode command) to the computer. The barcode data can be used using **<Barcode_String>**, **<Barcode_Value>** or using the **<barcode>** option for plate IDs.

S_SysTest {mode}

Start the system self test command.

S_GetSysTestData

Transfer the data from the last **S_SysTest** command to the computer.

Note: If error 32 happens during the self test ('The plate is still on the stacker table (no magazine inserted or solenoid problem)!'), the script execution will be terminated and the system will be reinitialized.

9.6 System Constants and Variables

There are a few predefined constants and variables you can use in scripts, e.g. for the S_MoveX command.

9.6.1 System Constants

<MaxX>	maximum X position for S_MoveX command
<Xpos_Stack[0]>, **<Xpos_Stack[1]>**	X position for stack 1 and 2
<Xpos_MPsensor>	X position to detect a microplate using the microplate carrier sensor
<Zpos_MPsensor>	Z position to detect a microplate using the microplate carrier sensor
<Zpos_PlateUp>	Z position to move plate up (when inserting in stack)
<Zpos_PlateDown>	Z position to move plate down (when inserting in reader plate carrier)
<Zpos_Scan>	Z position to scan barcode of microplate
<Zpos_Put_OpenStp>	Z position to open stopper when inserting plate into stack
<Zpos_Put_CloseStp>	Z position to close stopper when inserting plate into stack
<Zpos_Get_OpenStp>	Z position to open stopper when fetching plate from stack
<Zpos_Get_CloseStp>	Z position to close stopper when fetching plate from stack

9.6.2 Numerical System Variables

<Barcode_Value>	If the plate barcode contains a valid integer or floating point number, <Barcode_Value> will return this number, otherwise <Barcode_Value> will return 0. (All non-numerical characters will be removed before converting the barcode string into an integer or float value.)
<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).
ScriptLanguageVersion	Contains the version of the script language. This value can be used when writing scripts which use new commands to check whether these are supported.

Example:

```
if ScriptLanguageVersion<43 then begin
  ShowMessage ”For using this script you need a newer software version! ” Error
  halt
end;
```

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
<GainAdjWellColB>	column of well with highest signal found during R_GainPlate for channel B
<GainAdjWellRowB>	row of well with highest signal found during R_GainPlate for channel B

<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**)>**.

9.6.3 String Type System Variables

<Barcode_String> contains the barcode value, which was read last, in string format

Note: The command **ID1:="<Barcode_String>"** will take the barcode value available at the moment of execution of this script line as a value for plate ID 1. This value will not be changed before a new ID1:=... command appears.

Contrary to this command, the **ID1:="<barcode>"** command means, that the last available barcode value should always be used. The barcode value will be updated with every S_BarcodeData command (The S_BarcodeData command should be used after an S_PlateInB, S_GetPlateB or S_ReadBarcode command.). It will also be automatically updated after using an R_Run "<Protocol name>" 1 2 ReadBarcode command.

<User> This system variable contains the name of the user currently logged in (BMG software user).

<Windows_User> This system variable contains the name of the user currently logged in into Windows.

<ProtocolPath> and **<DataPath>** These variables contain the path to the test protocol definition data base and the path for storing the measurement data in Absolute Data Base format (measurement data base). Before using the R_Run command (see chapter 9.4.5) these paths are the default paths belonging to the user currently logged in (as defined in the login screen, see chapter 1). If you use an R_Run command inside the script in combination with the optional path parameters (e.g. R_Run "FAST" "D:\My Files\Omega\Definit" "D:\My Files\Omega\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 9.11).

Example:

```
Execute "Merge.exe <DataPath> <User> H ID1"
```

<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 3.4.1 or as defined as protocol specific value, see chapter 4.3.15).

<Ser_Number> This variable contains the serial number of the reader currently connected.

9.6.4 Boolean System Variables

<PlateCarrierOut>	true, if the reader plate carrier is outside the reader
<Magazine1In>	true, if the stacker magazine 1 is inserted and locked
<Magazine2In>	true, if the stacker magazine 2 is inserted and locked
<PlatesIn1>	true, if there is at least one microplate in stacker magazine 1
<PlatesIn2>	true, if there is at least one microplate in stacker magazine 2
<LastPlateDetected>	true, if the last microplate has been found during an S_PlateIn or an R_Run command with NotLastPlate parameter

You can use these boolean variables 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(<PlatesIn1>)` **`then …`**

(perform the command after 'then' only if there are no plates in magazine 1)

Note: It is not possible to use these boolean variables anywhere else than in comparisons; it is not possible to use these variables 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).

9.7 Numerical Variables

You can use up to 1000 numerical variables (type double float, 8 bytes precision) in your scripts. In addition, up to 101 string variables can be used (see chapter 9.8). 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, ReadBarCode, 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, InputFloat, InputInteger, InputString, MenuSelection, 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;
```

9.7.1 Rounding

To round a numerical value, use the **Round(**n**)** or **Trunc(**n**)** function.

The Round function rounds a real-type value to an integer-type value. Round returns a value that is the value of n rounded to the nearest whole number. If n is exactly halfway between two whole numbers, the result is always the even number.

The Trunc function truncates a real-type value to an integer-type value. Trunc returns a value that is the value of n rounded toward zero.

Examples:

```
i:=41.6
i:=Round(i)

RemainingCycleTime:=335
NoOfShakingIntervals:=RemainingCycleTime / 60       ;Number of 60 s intervals
NoOfShakingIntervals:=Trunc(NoOfShakingIntervals)
```

9.7.2 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.

9.8 String Variables

You can use up to 101 string variables in your scripts. The name of these variables need to start with ‘st’ followed by a number ‘0’... ‘100’ (st0, st1, ... st100). 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)>.″
st5:=″The barcode label is <Barcode_String>.″
```

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).

9.9 Measurement Data

9.9.1 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 Absolute Data Base format measurement files and will therefore only work if the ‘Save measurement data in Absolute Data Base format’ option has not been switched off (menu command ‘Setup | Program Configuration’)!

9.9.2 R_GetRawDataFileNumber

To get the number (= name) of the latest raw data table, use the **R_GetRawDataFileNumber** command. This might be useful in combination with the call of an external program (see chapter 9.11), 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. It is also an option to specify a data path for this command as parameter. If the path is not specified, this command will return the last raw data table number from the MeasurementData.abs data base, 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 MeasurementData.abs data base inside the current measurement data path (see <DataPath> in chapter 9.6.3).

Syntax:

Variable := **R_GetRawDataFileNumber** {(Path to measurement data)}

9.10 Date and Time Functions

9.10.1 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 ”My Protocol”
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
```

9.10.2 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 an 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>").

9.11 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 Omega installation directory is used (usually c:\Program Files\BMG\Omega\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 Omega 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.

It is also possible to store the exit code of the program called into a variable:

Example:

```
i:=Call ″SetCBus 999-0001 3 3 1 0″
```

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 ″Omega.hlp″          ;opens the Omega online help
```

10 Known Problems and Solutions

1. Disabled Plate In / Out Buttons
2. Access Rights Problems

For solutions see the following chapters.

10.1 Disabled Plate In / Out Buttons

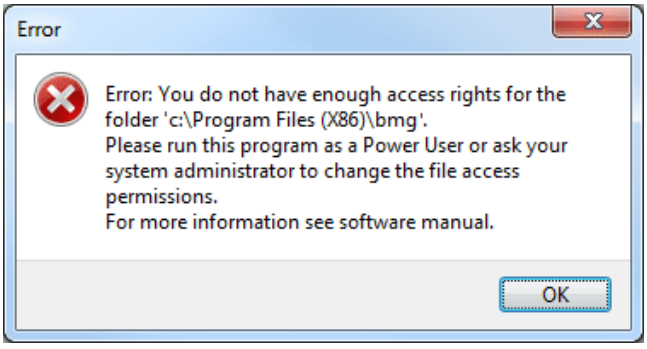
If the plate button ( ) is disabled and there is no test run active and the communication to the reader is OK, then it is most likely that there is a wrong setting in the configuration file '`~\Program Files\BMG\Omega\Omega.ini`'. The value behind ´`DisablePlateCmds=`´ should be ´`False`´. This parameter 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.

10.2 Access Rights

10.2.1 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\Omega`).

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:



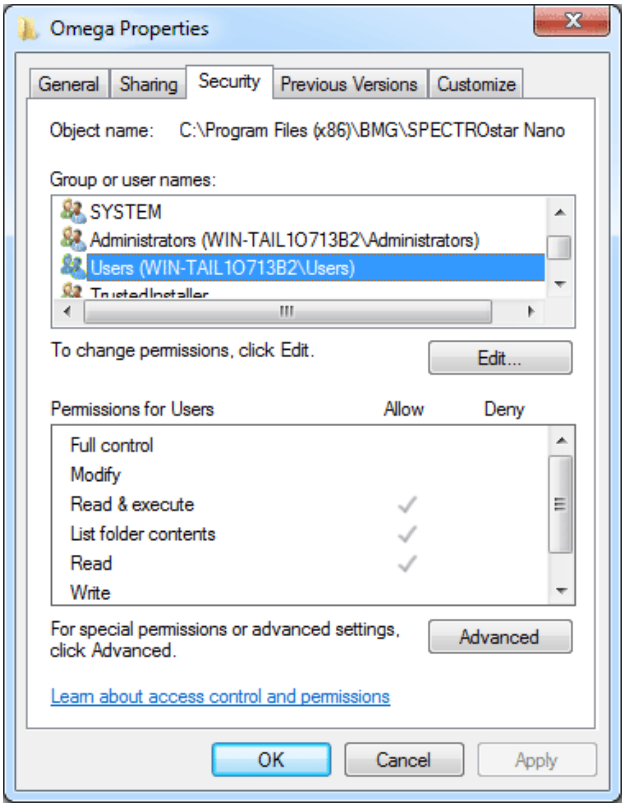
Solution

Logon as administrator (or user with administrative rights).

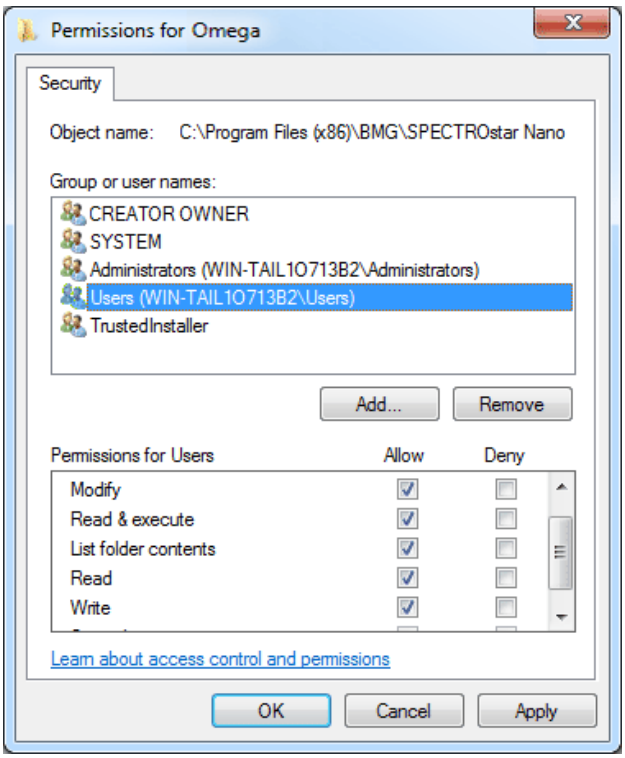
Run the program '**SetPermission**' from the Omega CD-ROM (folder `~:\Omega Vx.xx\`).

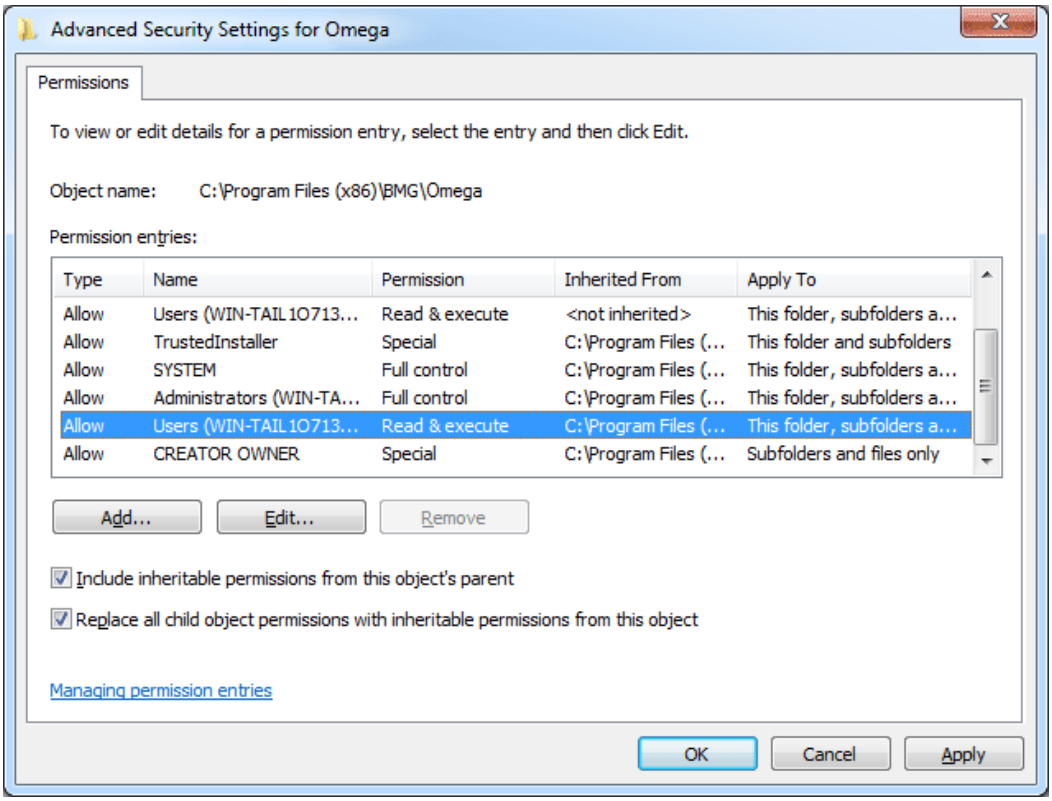
Alternatively, you can also manually change the permissions: Open the Windows Explorer and right-click the folder where the BMG LABTECH software is installed (usually `~:\Program Files\BMG\Omega`). Choose 'Properties', then 'Security'. Check the 'Modify' permission, 'Write' permission will be checked automatically. Uncheck the box 'Allow inheritable permissions from parent to propagate to this object' or 'Inherit from parent the permission entries that apply to child objects.' (Microsoft is using a slightly different naming in different Windows versions, in older Windows versions this option is in the first dialogue, in newer Windows versions you will find it inside the 'Advanced' dialogue). 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\Omega\	Omega.ini Omega.rns Omega.log Omega.bak	Configuration file Run Statistics File Log file Backup of log file (= log file from last session)
~:\Program Files\BMG\Omega\Temp		Directory for temporary files
~:\Program Files\BMG\Omega\Stamm		Directory for files shared by all users of the Omega software, e.g. microplate data base, filter table, LVis path length and LVis blank values file, …
~:\Program Files\BMG\Omega\User		Subdirectories for test protocol definitions and measurement data of standard user
~:\Program Files\BMG\Omega\Admin		Subdirectories for test protocol definitions and measurement data of administrator
~:\Program Files\BMG\Omega\<UserName>		Subdirectories for test protocol definitions and measurement data of defined users
~:\Program Files\BMG\Omega\Scripts		Subdirectories for example scripts
~:\Program Files\BMG_Flashtools		Used by Flash-EPROM Update Program

Borland Database Engine		
Directory	**File**	**Usage**
~:\Program Files\Borland\Common Files\BDE\	idapi32.cfg idapi32.bak	BDE properties Backup of idapi32.cfg
~:\Program Files\BMG_SharedComponents 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

10.2.2 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 Windows 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

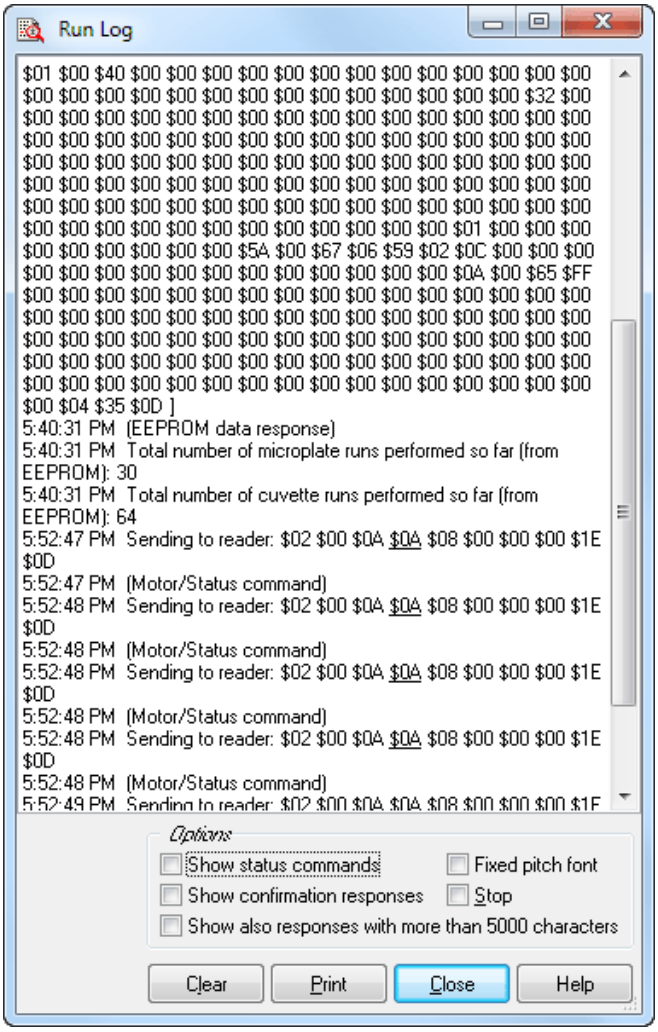
- Logon as Administrator (or a user with administrative rights).
- Run the program '**SetPermission**' from the Omega CD-ROM (folder `~:\Omega Vx.xx\`).

or

- Start the program '`Regedit`' (Windows 2000: '`Regedt32`', 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.

10.3 Run Log Window

The Run Log window shows all commands sent to the reader and all responses. You can open the Run Log window using the 'Show run log window' sub function of the 'Settings | Reader Configuration' menu command or using the key combination [Shift]+[Ctrl]+[L] from the main window of the control program.



It is possible to stop the process of adding new entries to the run log by checking the '**Stop**' checkbox.

As listing very long responses (e.g. the measurement data responses of absorbance spectra plate mode test runs) will slow down the data transfer process, by default only the first bytes of such responses will be shown. Check the last check box of this window to see the complete responses.

You can **print** the entire run log or a marked part of the run log.

10.3.1 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.

10.3.2 Run Log File

The run log will automatically be saved into the file '`Omega.log`' in the Omega main directory (usually '`~\Program Files\BMG\Omega\`'). You can switch off the generation of the run log file by changing the line '`RunLog=true`' in the configuration file '`~\Program Files\BMG\Omega\Omega.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.

11 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

Note: When you need support from BMG LABTECH due to a software / firmware malfunction, you should send us the run log file (see chapter 10.3.2) together with the error description.