



SMART Control

SOFTWARE MANUAL

Part II: Control Software

Version 6.20

This manual was designed to guide SMART Control 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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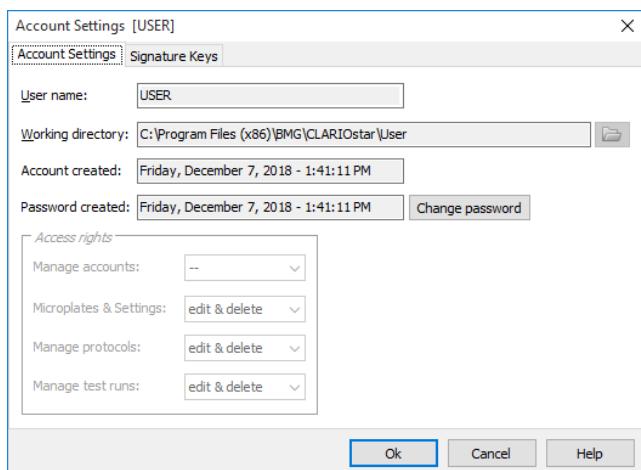
1 Login Dialogue

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.



1.1 User Functions

After clicking the 'Account Settings' button in the login dialogue the 'Account Settings' dialogue showing the properties of the selected user account will appear:



Here the user can see **information about his account**, e.g. the working directory, the account creation and the password creation date and the access rights (see also chapter 1.1).

In addition, the user can **change the password** and **manage signature keys** (see chapter 1.1.1).

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).

1.1.1 Signature Keys

To digitally sign data records a pair of RSA keys is necessary: 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.

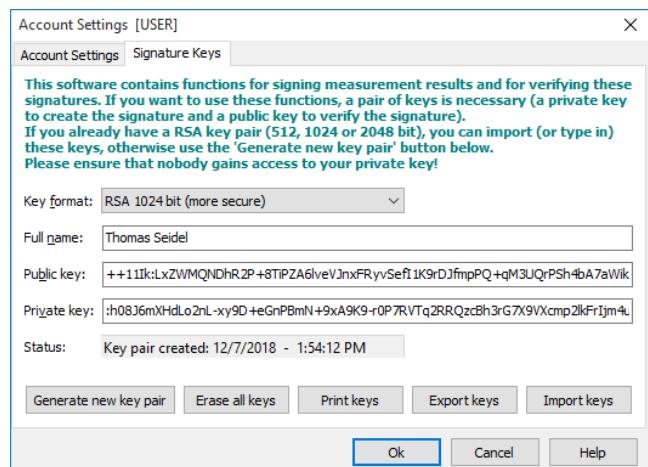
When logging in as the standard user 'USER', by default no password is necessary.

By clicking the Account settings button, the user can view the rights assigned to the account, change the password and manage signature keys.

There is one initial administrator account ADMIN. The default password for this account is 'bmg'. When an administrator is logged in, new users can be created or changes for existing user entries can be made using the Account settings dialogue (see chapter 1.1).

Hints: The Login Screen function can be switched off, see chapter 3.3.1.

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



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

If the user already has an RSA key pair, it can be **imported** or typed in. An example of the expected file format is shown below.

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

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

Example of an exported key file:

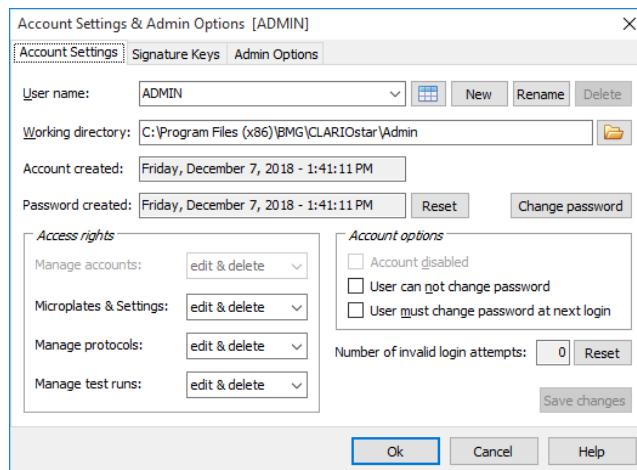
```
[CurrentKeys]
Name=Thomas Seidel
Created=2013-08-14 - 16:22:13
Key Format=RSA, 1024 bit
PrivateKey=:QKUrng1p3zpjFky+3O-
M0QssGnRYRRJ9hb8wEdY08RDlw15PtWpJnzc ...
PublicKey=++11Ik:h+BJtQm6NxBMDbIh4fpGZEs4ujF1GgOr
Cq+RWjaCygf3GcCee+6S ...
NumberOfOldPublicKeys=2
```

```
[OldKey1]
Name= Thomas Seidel
Created=2013-08-12 - 16:16:54
Key Format=RSA, 512 bit
PublicKey=++11Ik:h+BJtQm6NxBMDbIh4fpGZEs4ujF1GgOr
Cq+RWjaCygf3GcCee+6S ...
```

```
[OldKey2]
Name= Thomas Seidel
Created=2013-08-12 - 16:12:38
Key Format=RSA, 512 bit
PublicKey=++11Ik:p4SIFP6ngf1vxM9v+CB5OF00zb3dNeOx
cdOwG9OGmDybeUF01xX6 ...
```

1.2 Administrator Functions

When clicking the 'Account Settings' button in the login dialogue as an administrator the 'Account Settings & Admin Options' dialogue will appear:



Here the administrator can **create**, **rename** and **delete user accounts**.

For each account the **access rights** for microplates, protocols and test runs can be defined.

The **protocols access rights** can be set to different levels between 'run only', 'edit layout only' and full access ('edit') with or without the right to delete existing protocols. The 'run only' and 'layout only' access rights can be combined with the right to perform focus and gain adjustments (e.g., 'layout & adjust'). An overview of the different protocols access rights can be found in chapter 1.1.

Hint: Instead of changing the protocol access right option every time a protocol change is necessary, two user entries can be created using the same directory, one of these entries with edit option and the other one without this option.

The 'microplate... access right' also includes other settings which are valid for all users (and not only for the user currently logged in), e.g. the filter table, the firmware download function and the access to the offset determination function.

Any user with the **right to edit accounts** is considered to be an **administrator**. More than one administrator account can be defined. It is possible to rename the default administrator account ADMIN. All administrator accounts besides the last one can be deleted.

Using the Account options the administrator can disable or enable user accounts, force password changes at the next login or select an option to not allow password changes. In addition, the counter for invalid login attempts can be reset here.

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).

After clicking the table button a table listing all user accounts appears:

Account List					
User Name	Working Directory	Accounts	Testruns	Protocols	Microplates
ADMIN	C:\Program Files (x86)\BMG\CLARIOstar\Admin	edit & delete	edit & delete	edit & delete	edit & delete
Tom	C:\Program Files (x86)\BMG\CLARIOstar\Tom	--	edit & delete	edit & delete	edit & delete
USER	C:\Program Files (x86)\BMG\CLARIOstar\User	--	edit & delete	edit & delete	edit & delete

Note: Exporting the user table into an Excel format (.xls / .xlsx), text or HTML file is possible after right clicking on the table.

1.3 Administrator Options

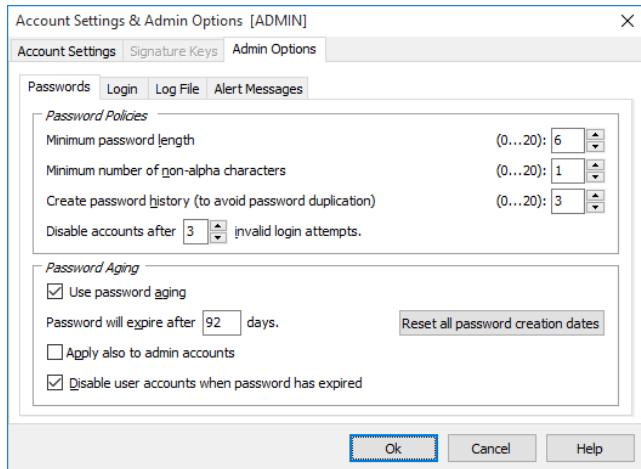
1.3.1 Password Policies and Password Aging

In the first sub tab ('Passwords') of the 'Admin Options' tab, the administrator can define the policies for passwords. It is also possible to set password aging (i.e., passwords will be valid for a defined time forcing users to change passwords after a specified period of days).

Password Policies

The required **minimum length of passwords** and the **minimum number of non-alphabetical characters** (numbers, special characters) can be specified under Password Policies. Entering 0 in both fields permits user accounts without requiring passwords.

Note: These requirements are not valid for password changes done by the administrator.



The **create password history** setting defines how frequently old passwords can be reused. To disable this feature, set the value to 0.

To **disable an account after a defined number of invalid login attempts** (i.e., login attempts using an incorrect password), select a number in the drop-down menu 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 requiring at least one non-alphabetical character. In this case, disable the auto login function and choose to disable account after 3 invalid login attempts. In addition, change the initial administrator password to something other than 'BMG' and also define a password for the default user 'USER'. See also Software manual part IV: FDA 21 CFR part 11.

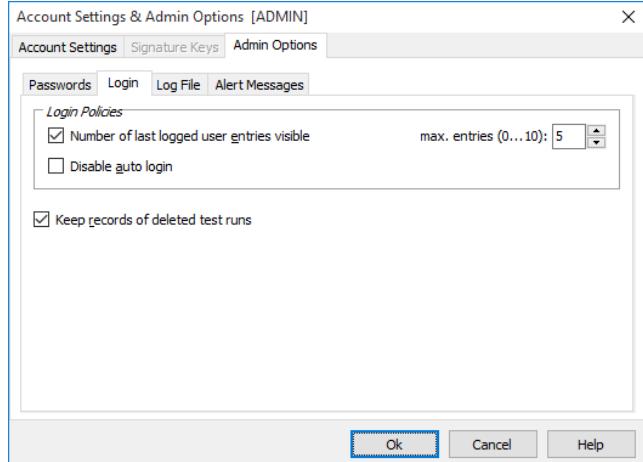
Password Aging

Use the password aging function to prompt password change after a specified period of time. User will be prompted to change password several days before password expiration, after the period of time defined by the administrator. Logging on is not possible after the defined period of time. If the option '**Disable user accounts when password has expired**' has been selected, only the administrator can reactivate the account. (Reactivate an account by deselecting the 'Account disabled' check box in the 'Account Properties' dialogue (see chapter 1.1 *Administrator Functions*); alternatively, the user can reactive his account by changing the password (see chapter 1.1 *User Functions*). When changing the password aging settings, use the '**Reset all password creation dates**' button to avoid the problem of existing passwords that have already expired.

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

1.3.2 Login Policies

In the second sub tab ('Login') of the 'Administrator Options' tab, the administrator can change the login policies.



Login Policies

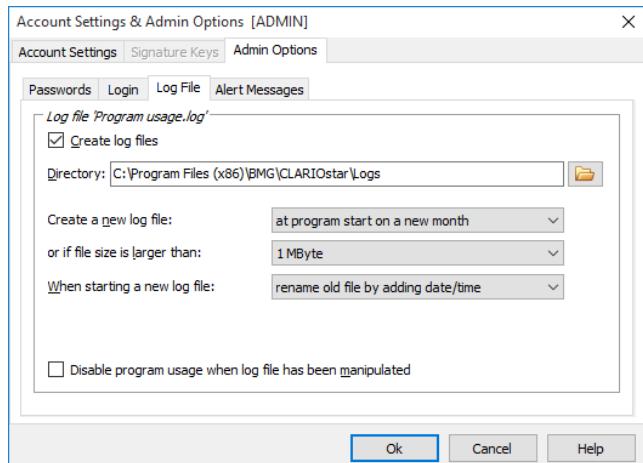
If the first **check box** is not checked, always all existing user accounts will be offered in the 'User name' pull down box in the Login dialogue (see chapter 1). If this check box is checked, only the defined **number of last logged users** will be shown there. To avoid that any user name is shown using the pull down box (= increased security), make sure that the check box is checked and set the number of max. entries to 0.

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

If the check box '**Keep records of delete test runs**' checked, certain information of deleted test runs (e.g., the Audit trail) will be kept even after deleting the test run itself. See also Software manual part IV: FDA 21 CFR part 11.

1.3.3 Program Usage Log File

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



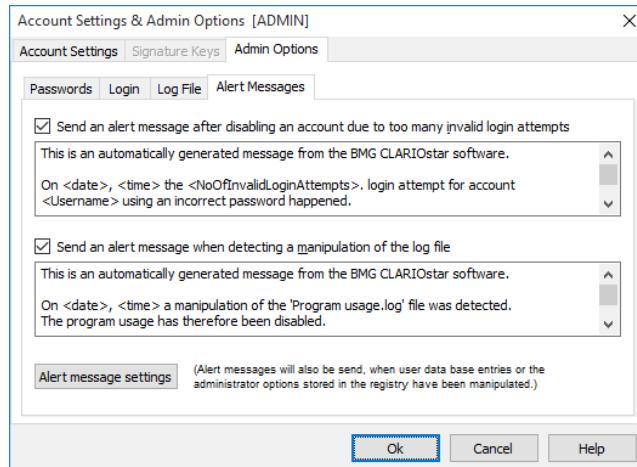
Here the directory where this file is to be stored and under which conditions a new file should be opened can be specified. When starting a new log file, the old file might be renamed or erased. The log file is protected against manipulation by calculating a cryptographically secure hash value. The integrity 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 must log in. It is also possible to use the 'Check Data Integrity' tool (see also Software manual part IV: FDA 21 CFR part) to check the integrity of the log file.

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

Note: There will be an additional run log file (see chapter 3.1.8) which contains the communication between reader and computer. This file is intended to deliver background information in case of problems.

1.3.4 Alert Messages

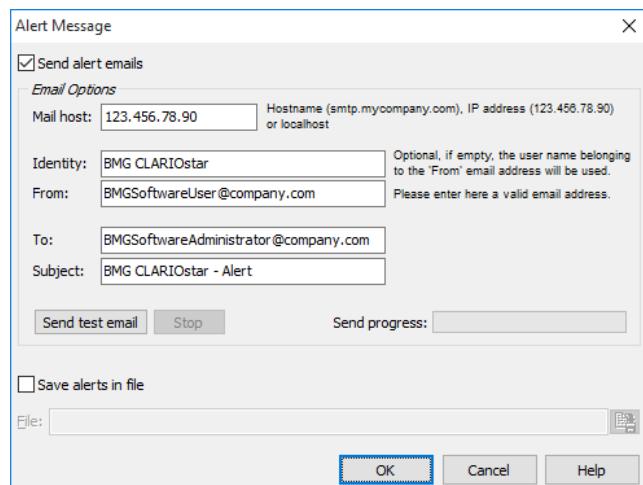
By using the fourth sub tab ('Alert Messages') of the 'Administrator Options' tab, it is possible to define alert messages to be sent under certain circumstances via email (e.g. to the administrator) or saved into an alert message file.



It is possible to define a message to be sent after disabling an account due to too many invalid login trials. Furthermore, it is also possible to define a message to 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 in the 'Administrator Options' dialogue, a dialogue will appear to set definitions for these messages:

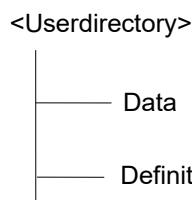


In order to send alert messages as emails, the computer needs to be connected to the internet. For email options, use the same settings as in the regular email program. If unsure about these settings, ask the system administrator.

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

1.4 User Directories

After the first login of a new user, two subdirectories will automatically be created under the defined user directory:

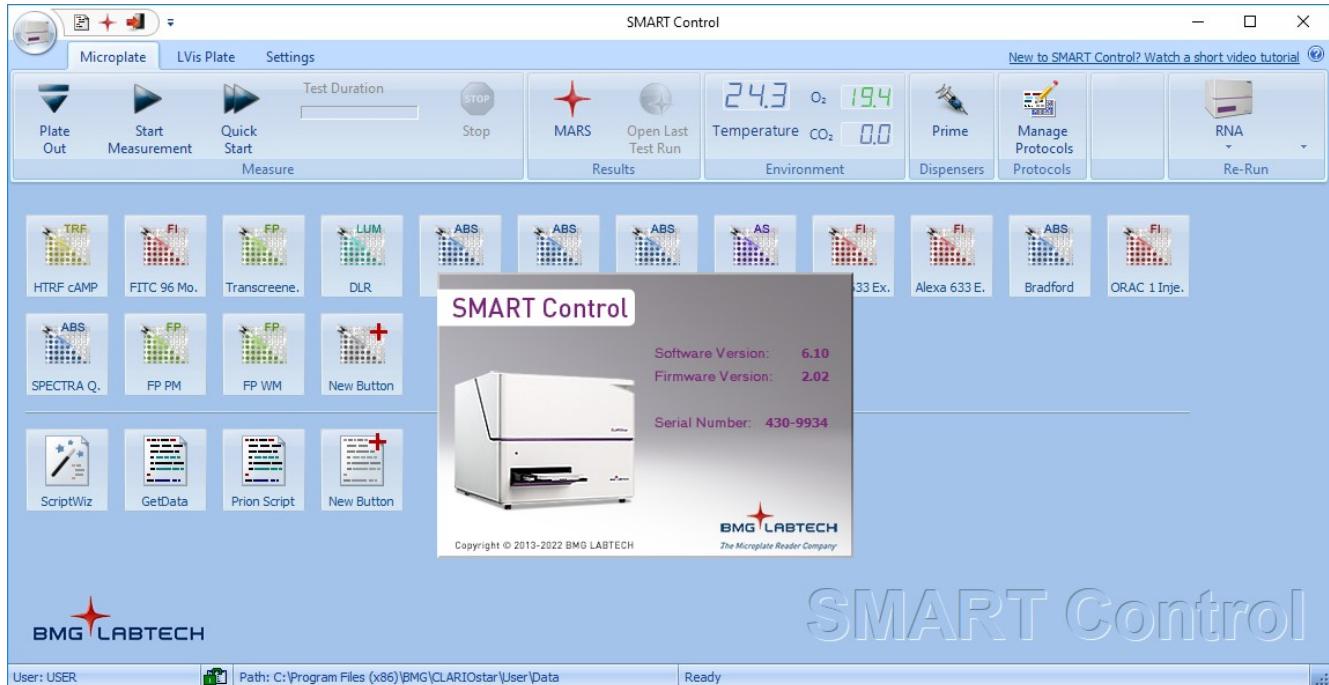


It is possible to redirect the path of data and store information in another directory. To change data storage location, select 'Login' under the application button in the top left corner the control software (see also chapter 2.2.2 *Application Button Menu*).

Definit: The test protocols are stored in this directory.

Data: The measurement data is stored in this directory.

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. To close this window, click on it.

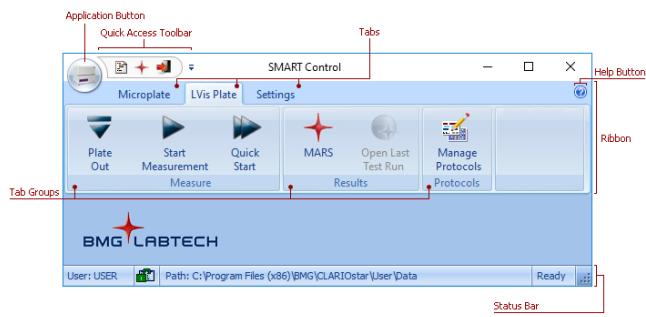
Note: Make a note of the software version and firmware version so it is easily accessible if technical support is needed. This information is also accessible by clicking on the application button in the top left corner and then selecting '**About SMART Control**'.

The status bar at the bottom of the main screen shows the user logged in and the path used for storing the measurement results. These settings can be changed by clicking the respective field of the status bar.

In the right part of the status bar, the current reader status will be shown. If a stacker is attached to the reader, a plate counter appears at the right end of the status bar. This counter will show the number of the plate which is currently being processed (during active batch mode measurements) or the number of plates which have been processed during the last batch mode measurement.

2.2 Menu Commands

The SMART Control software uses a ribbon style menu, commonly seen in newer Windows software:



The small icon right of the user field shows the user access rights:

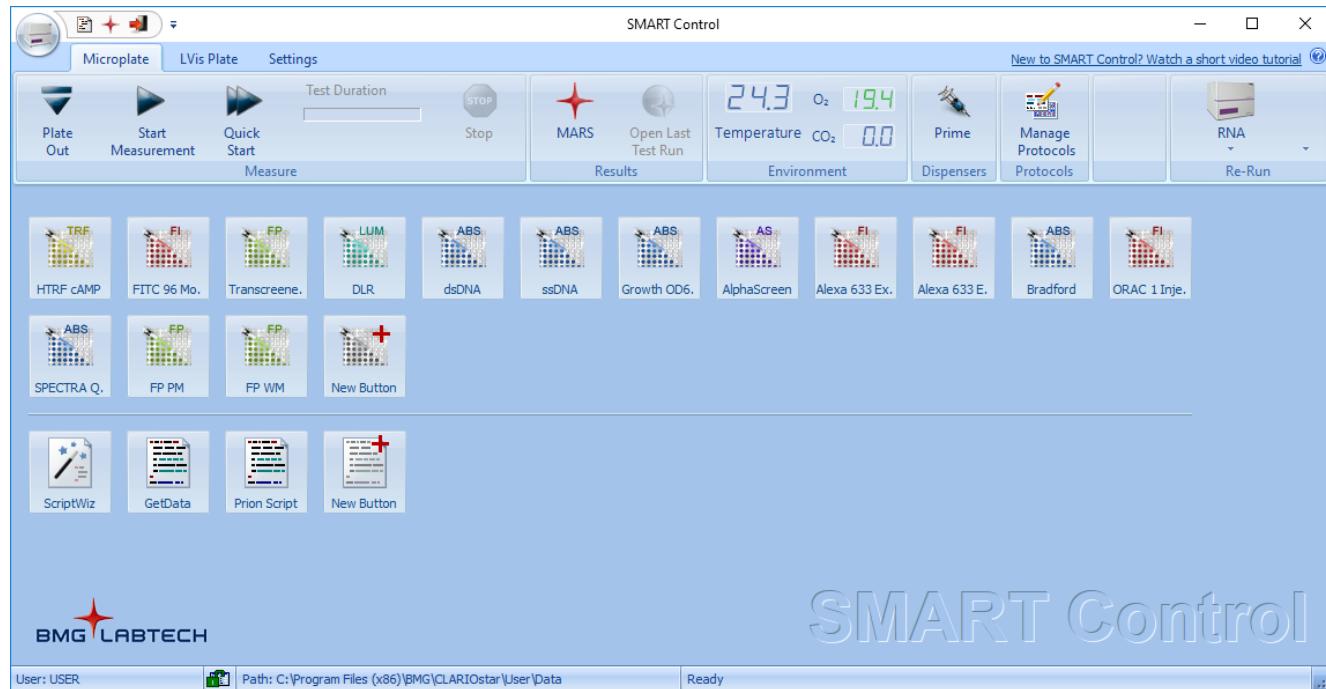
Icon	Meaning	Description
	edit & delete	The user is allowed to edit and delete test protocols.
	edit	The user is allowed to edit test protocols.
	layout & adjust	The user is only allowed to change the layout of test protocols and to adjust gain and focus settings.
	layout only	The user is only allowed to change the layout of test protocols, but cannot change gain and focus settings.
	run & adjust	The user is only allowed to execute existing test protocols, but can adjust gain and focus settings.
	run only	The user is only allowed to execute existing test protocols.

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



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].

2.2.1 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 Command	Icon	Function
Measure	Plate Out	Moves microplate out of the instrument. ¹
	Plate In	Moves microplate into the instrument. ¹
	Start Measurement	Performs 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 the need to define a test protocol.
	Current State Graphics	Opens the current state display.
	Pause	Interrupts the active test run after finishing the current cycle / well. ²
	Stop	Stops the active test run or the execution of a script.
	Barcode List	Opens the Barcode List. ³
Results	MARS	Opens BMG LABTECH's MARS Data Analysis software.
	Open Last Test Run	Opens the last test run performed using MARS.

Microplate Tab Command	Icon	Function	
Environment	Temperature		Shows the current temperature. A click opens the Temperature dialogue to change incubator settings.
	O ₂ / CO ₂ Concentration		Shows the current O ₂ and CO ₂ concentrations. A click opens the Environment Settings dialogue to change the concentration settings. ⁴
Dispensers	Prime / Reagent Dispensers		Prepares the dispenser pumps for injection. VANTAstar: Also change heater and stirrer settings. (Only available if pumps are installed.)
	Manage Protocols		Opens the manage test protocol dialogue. Here you can edit existing test protocols or define new ones.
Re-Run	Re-Run <Protocol Name>		A click on the top part of this button will repeat the execution of the last used test protocol.
	Re-Run List (Down arrow)		A click on the bottom part will open a popup menu which offers functions to change IDs, to edit this protocol, to assign this protocol to a user button, or to delete the protocol from the re-run list. ⁵
			Shows a list of up to the 10 last used test protocols. Clicking one of the entries will repeat the execution of this protocol. ⁵

- ¹ This option is not available when the reader is connected 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\CLARIOstar\CLARIOstar.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.

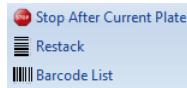
- ² The ‘Pause After Current Cycle / Well’ function is not available when using a stacker or in script mode. Due to technical reasons this pause function is also not available when performing absorbance spectra plate mode measurements.

To use the pause function for well mode protocols a firmware version 1.20 or newer is necessary.

- ³ The ‘Barcode List’ function is only available for CLARIOstar Plus readers (with built-in barcode readers) or when using the reader in combination with a stacker, which is equipped with a barcode reader.
- ⁴ These functions are only shown when an ACU (Atmospheric Control Unit) is attached to the reader.
- ⁵ When a Stack is attached, the Re-Run function will always measure the whole batch of plates available in stacker magazine 1.

Stacker Functions

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

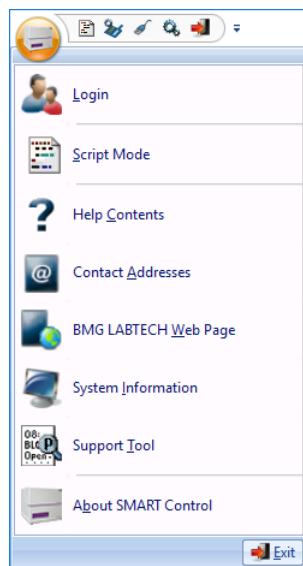


Microplate Tab Command	Icon	Function
Measure	Stop After Current Plate	This function is only available in batch mode (reader with a stacker attached) or in script mode when a script containing batch measurements is used. After selecting this function, the measurement of the current plate will be finished 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 been read.

Please see chapter 1 for a description of microplate measurements.

2.2.2 Application Button Menu



The application button located in the top left corner can be used to access certain software features, which are not available via the ribbon menu tabs.

Application Button Command	Icon	Function
Login		Opens the login window.
Script Mode		Opens the script mode window. ¹
Help Contents		Opens the online help. <i>Hint: Hit F1 on any tab or dialogue to call up a specific online help page.</i>
Contact Addresses		Shows BMG LABTECH's contact addresses.
BMG LABTECH Web Page		Starts an internet browser and connects to the BMG LABTECH web page.
System Information		Shows information about the operating system, the CPU speed, and the amount of memory available.
Support Tool		Starts the BMG LABTECH Support Tool.
About SMART Control		Shows the software and firmware version.

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

2.2.3 LVis Plate Tab



This tab provides the same functions as the Microplate Tab (see chapter 2.2.1) for the BMG LABTECH LVis Plate (micro drop section).

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.

The LVis Plate Tab does not contain the Re-Run function.

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

Hints: The LVis Plate Tab can be switched off using the 'Settings | Program Configuration' function.

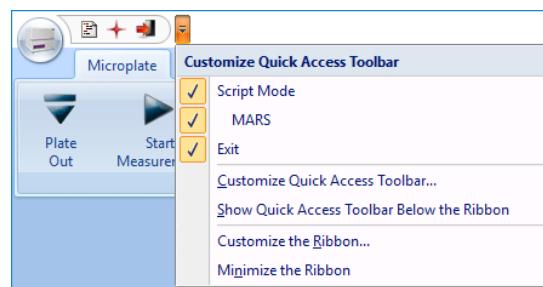
It is also possible to use the Microplate Tab to perform measurements using the LVis Plate. This also allows the re-run function to be used. (When the LVis Plate Tab has been switched, the LVis Plate menu group can be found in the Settings Tab.)

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.

2.2.4 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 Command	Icon	Function
Script Mode		Opens the script mode window. ¹
MARS		Opens BMG LABTECH's MARS Data Analysis software.
Exit		Exits SMART Control Control software.

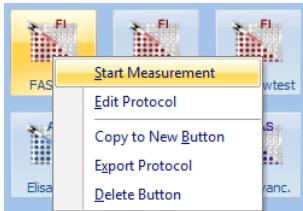
¹ The script mode is not available when in ActiveX or DDE mode, e.g., as part of a robotic system.

Hint: Right click on most icons will give the option to 'Add to Quick Access Toolbar'. After selecting the 'Customize Quick Access Toolbar...' entry, changes can be made to more menu settings and the order of the buttons in the quick access toolbar can also be changed.

The functions of the different tabs are described on the following pages.

2.2.5 User-Definable Buttons

It is possible to create up to 36 user 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’ or by copying an existing user button. It is also possible to 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 that allows you to start the measurement immediately (without opening the Start Measurement dialogue) and to edit the test protocol.

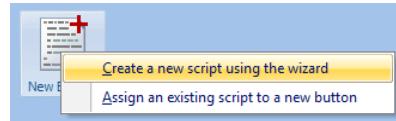
It is possible to change the position of a user button by dragging it to its new position (drag-and-drop).

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

The user-definable buttons feature can be switched off using the program configuration dialogue. Since the user-definable buttons use the user-specific protocol data bases, each user can create his own set of buttons and can define the protocols independently. Users without the appropriate manage protocol rights (see chapter 1 *Login Dialogue*) are not permitted to create or delete user-definable buttons or to edit the associated test protocols.

2.2.6 Script Buttons

Up to 12 script buttons can be created in the Microplate and LVis Plate tab of the main program window (see also chapter 8 *Script Mode*).



There are two options: (1) assign an existing script to a button for faster access or (2) 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 options 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. It is possible to 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.

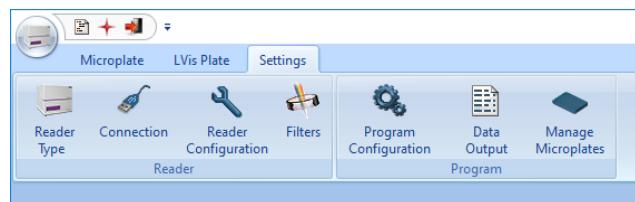
The script buttons feature can be switched off using the program configuration dialogue.

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

Users without the edit test protocol right (see chapter 1 *Login Dialogue*) are not permitted to create or delete script wizard buttons.

3 Settings

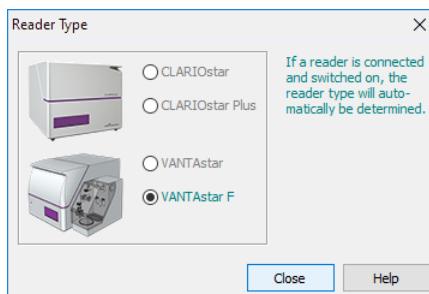
To change the settings of the instrument or the software use the 'Settings' Tab:



3.1 Reader Configuration

3.1.1 Reader Type

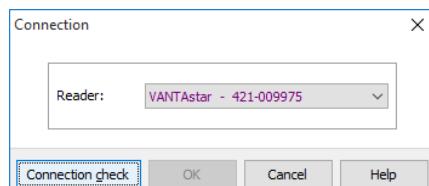
If a reader is connected to the computer and switched on (and has been assigned to this software installation via 'Settings | Connection'), this window (accessible via 'Settings | Reader Type') will show the type of reader which was automatically recognized. Depending on the reader type different measurement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



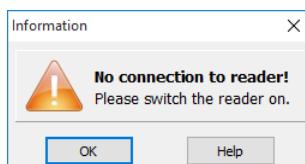
The software remembers the last reader type used and will use this type if no reader is connected, but in this case, it is possible to change the reader type using this dialogue box. This might be useful if you want to define a test protocol for a reader currently not available.

3.1.2 Connection

After installing the software, connect the reader and 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.



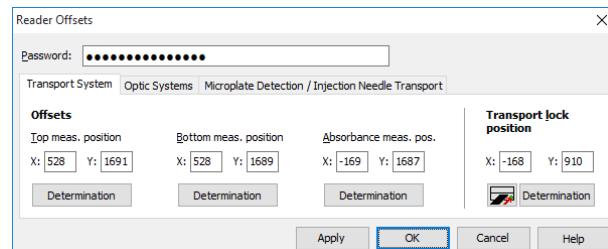
Check the connection by clicking the 'Connection check' button. If there is no communication between the computer and the instrument, a message box will appear:



In this case, check that the power to the instrument is switched on.

3.1.3 Reader Offsets

Every reader is accurately calibrated after production and has individually defined offset values. The offset values correspond to the home position of the microplate carrier or of the optic units. 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 Offsets' in the 'Reader Configuration' dialogue, which is under the 'Settings' tab.

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

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

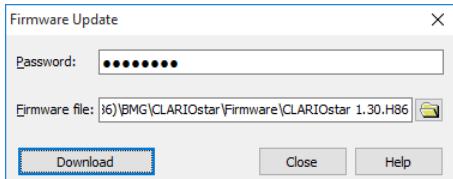
Click 'OK' to save all changed offset values into the reader EEPROM.

Do not perform an 'Offset determination' unless you are qualified to do so!

Note: Users, where the 'Microplates & Settings' access right (see chapter 1 *Login Dialogue*) has been set to 'read only', are not allowed to change offset values.

3.1.4 Firmware Update

This function allows update of the reader firmware (content of the built in Flash-EPROM). Use the 'Firmware update' sub function of the 'Settings | Reader Configuration' menu command to open the firmware download dialogue.



To install a new firmware file received from BMG LABTECH, copy this file into the folder `~:\Program Files\BMG\CLARIOstar\Firmware`.

Make sure the reader is connected to the computer and turned on. After typing in the correct password (which will be included with the new firmware), select the firmware file using the button, then press 'Download'. The SMART Control program will switch the reader into the download mode and then start the download program. Downloading the new firmware is an automatic process which lasts a few minutes. Do not interrupt this process by switching the reader or the computer off or by closing the software!

After the download is completed, the download window will close automatically. Then the reader will be switched off and on again to activate the new firmware.

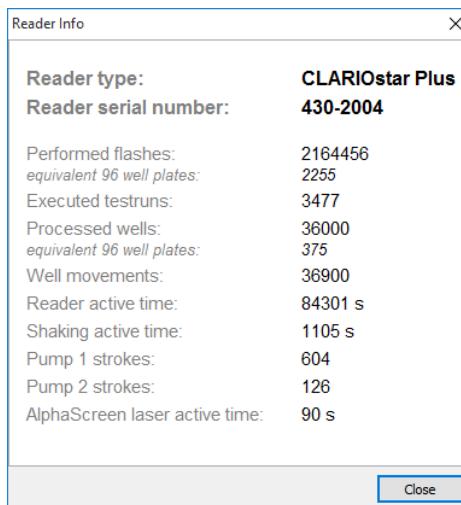
If the download process is interrupted, the reader will not work, but even in this state it is usually possible to repeat the download process.

It is also possible to download a new version of the measurement controller, monochromator or spectrometer firmware using this dialogue. (For newer readers select the appropriate peripheral firmware file, for older readers change the file type to 'Measurement controller firmware files', 'Monochromator controller firmware files' or to 'Spectrometer firmware files').

Note: Users, where the 'Microplates & Settings' access right (see chapter 1 *Login Dialogue*) has been set to 'read only', are not allowed to perform a firmware upgrade.

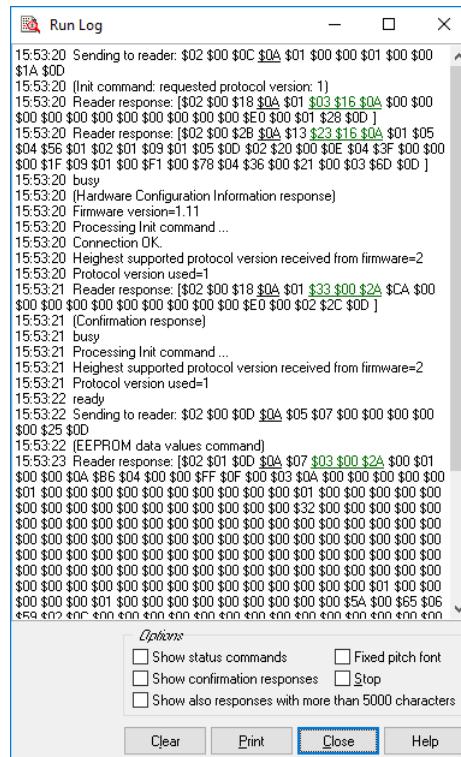
3.1.5 Reader Info

This function opens a window with statistic information, like number of test runs performed and reader active time.



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.



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.

'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:

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 something is selected 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. 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 'CLARIOstar.log' in the SMART Control main directory (usually '~\Program Files\BMG\CLARIOstar\'). The generation of the run log file can be stopped by changing the line 'RunLog=true' in the configuration file '~\Program Files\BMG\CLARIOstar\CLARIOstar.ini' (section [Debug]) into 'RunLog=false'.

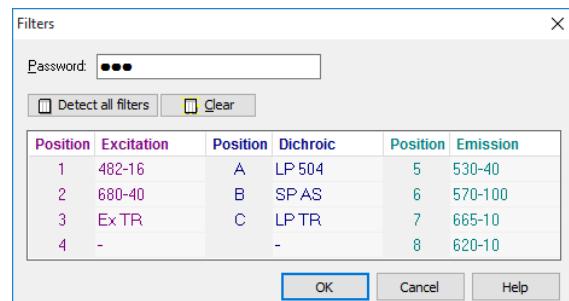
Hint: If support is needed due to a malfunction, please send BMG LABTECH this log file together with a description of the problem before restarting the program or this will be erased.

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

3.1.9 Filters

Besides using the monochromator (all reader types beside the VANTAstar F) also filters can be used. To achieve the best performance possible in fluorescence polarization (FP), HTRF, AlphaScreen®, AlphaLISA®, and other more specific applications, BMG LABTECH offers dedicated filter sets, specific for each application.

The reader can be equipped with up to 4 excitation filters, up to 3 dichroic filters and up to 4 emission filters (VANTAstar F: maximum 6 each). To install or change a filter open the instrument lid, remove the filter cover and click the filter access button (yellow button 1, see Operating Manual). Each filter slide can be accessed by repeatedly pushing the filter access button so that each respective filter slide emerges in succession. Click on 'Settings | Filters' to open the filter dialogue.



The settings in this table are only for the user changeable filters. The monochromator settings are independent and are set in the protocols.

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

Each position in the table corresponds to a filter position on the filter slides. The filter values can be determined automatically, selected from pull down lists or entered manually.

Detect all filters Clicking this button initiates all filters to be measured by the reader; in succession the table is filled out automatically. After right clicking this button, it is possible to only detect excitation, dichroic or emission filter.

Stop During an active filter detection process the 'Detect all filters' button will be changed into a 'Stop' button. Clicking this button will stop the detection process. Results obtained so far will not be discarded.

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

OK Saves the table entries and returns to the main program window.

Cancel Closes this window without saving changes. If this button is clicked while a filter detection procedure is active this will be aborted.

Besides detecting all filters, it is also possible to scan only one selected filter position. To do so use the pull down box of the respective table cell.

When detecting a filter, it will be measured by the reader and the results will be compared with a data base of known filters. The filter where the obtained values match best will be entered into the filter table automatically. It is possible to select another filter from the pull down list or to edit the filter name or parameters (see below).

The pull down boxes contain as last entry 'no filter' or 'no dichroic'. Select this entry to clear a table position.

Notes: The table is common to all users.

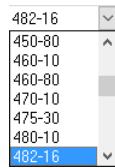
If the reader is not equipped with a spectrometer an automatic detection of the fixed dichroics is not possible.

The automatic detection is also not available when using a VANTAstar F reader.

Filters on position 6/F/12 of the VANTAstar F are not user changeable, therefore, it is also not possible to change the entries in the last line of this filter table.

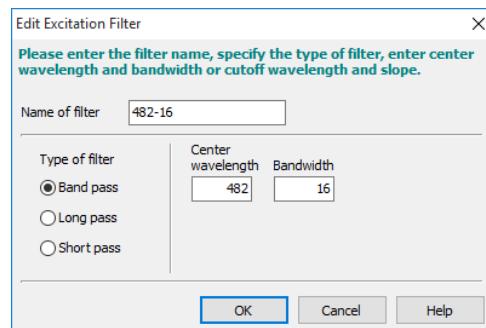
Exporting the filter table to an ASCII or Excel format file is possible after right clicking the table.

Users, where the 'Microplates & Settings' access right (see chapter 1 *Login Dialogue*) has been set to 'read only', are not allowed to change the filter settings.



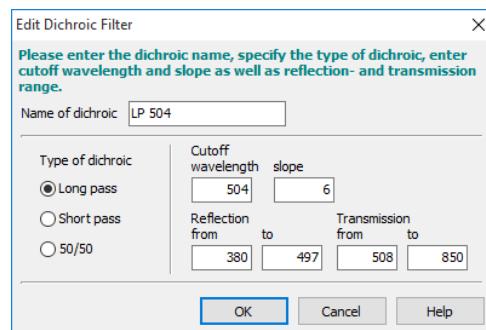
Filter Edit

Use the 'edit' function of the pull down box from any filter table position to open an 'Edit Filter' dialogue.



Select the filter type. Filters used as excitation or emission filters are usually band pass filters. Enter a filter name and the filter wavelength and bandwidth values in the corresponding fields.

For defining a dichroic filter, it is necessary to specify the dichroic type, e.g., short pass (for AlphaScreen measurements) or long pass (for other measurement methods). Besides the cutoff wavelength and slope the reflection wavelength range and the transmission wavelength range need to be defined.



Additional Filters

Please contact BMG LABTECH if a special filter is needed for a specific application.

3.2 Stacker Configuration

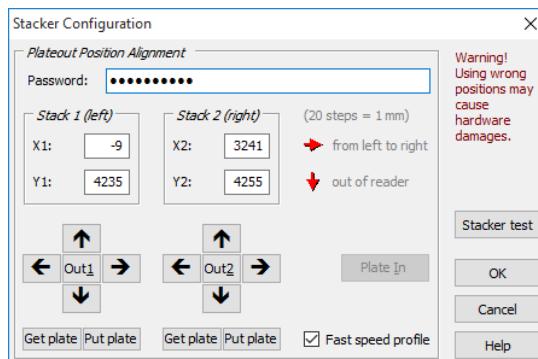


To open the Stacker Configuration dialogue, use the menu command 'Settings | Stacker Configuration'.

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).

Repeat this procedure for the stack 2 position.

Using the '**Get plate**' and '**Put plate**' buttons it is possible to test the positions immediately.

Beginning with Stacker firmware version 2.16 you can choose between a slow and a **fast speed profile**.

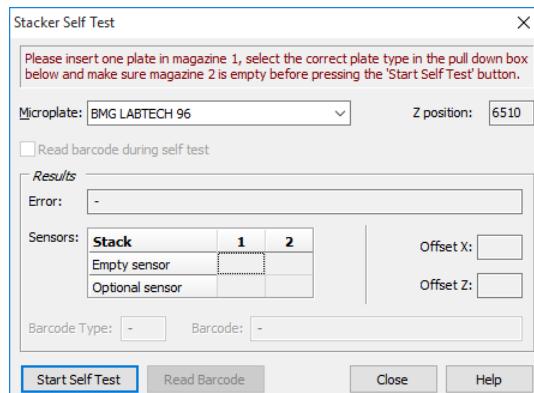
After clicking the 'Stacker test' button you can perform a stacker self test.

Note: Users, where the 'Microplates & Settings' access right (see chapter 1.1 *Login Dialogue*) has been set to 'read only', are not allowed to change the Stacker Configuration.

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.

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 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 read the barcode of the inserted plate.

When the '**Read Barcode**' button is clicked, the barcode reader will be triggered. Use this function to align the barcode reader or test its function (a red laser beam will appear). 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



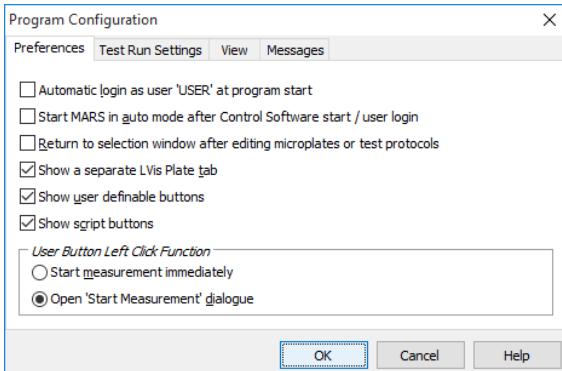
To open the Program Configuration dialogue, use the menu command 'Settings | Program Configuration'.

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 some general 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 Dialogue*).

Start MARS in auto mode after Control Software start / user login

If this option is chosen for the user currently logged in, MARS will automatically be started in auto mode (see MARS online help).

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 are not using an LVis Plate, it is possible to switch off the LVis Plate Tab using this function.

It is also possible to use the Microplate Tab to perform measurements using the LVis Plate.

Note: The special LVis Plate functions are only available if the reader is equipped with a spectrometer.

Show user definable buttons

It is possible to 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. Deselect this box if you do not wish to use this feature.

Show script buttons

It is possible to 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. Deselect this box if you do not wish to use this feature.

User Button Left Click Function

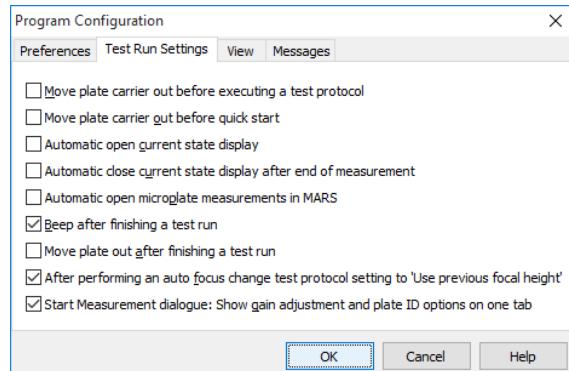
This selection allows the user to decide if, after left clicking, a measurement will start immediately or if the user will be directed to a dialogue to change Plate and Sample IDs.

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

If the second option is selected ('Open Start Measurement Dialogue'), a left click onto a user-definable button will prompt a dialogue to appear, where IDs can be changed. An immediate measurement start is still possible by using the first menu selection of the popup menu which appears after right clicking the button ('Start Measurement').

3.3.2 Test Run Settings Tab

Use this tab to change how the program handles measurements.



Move plate carrier out before executing a test protocol

Select this option for the plate carrier to be moved out automatically when starting the execution of a test protocol. The plate carrier will be moved out after selecting the test protocol and opening the 'Start Measurement' dialogue. Then insert the microplate.

Notes: This movement happens when opening the 'Start Measurement' dialogue (see chapter 6.3). As this dialogue will not be shown during test runs started via script (see chapter 8) or via ActiveX or DDE (remote controlled mode = robotic integration) this option is not relevant for these operating modes.

This option is not active if the reader is attached to a stacker with source magazine inserted.

Move plate carrier out before quick start

Select this option for the plate carrier to be moved out when opening the 'Quick Start' dialogue box.

Note: This option is not active if the reader is attached to a stacker with source magazine inserted.

Automatic open current state display

Select this option to automatically open the Current State display will automatically 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.

Notes: As long as the Current State display is open no new measurement can be started.

In script mode, in remote controlled mode (robotic integration) and when performing Stackert batch measurements the Current State display will always be closed automatically after finishing a measurement to avoid delays.

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 for producing an audible signal after a test run is finished.

Move plate out after finishing a test run

Select this option for the plate to 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.

Notes: This option is not active if the software is in ActiveX, DDE or script mode (see chapter 8).

When using a stacker the plate will always move out to stacker magazine 2 after the measurement.

After performing an auto focus change test protocol setting to 'Use previous focal height'

When the 'auto focus' feature is used, the well with the highest signal will be searched and a focal height adjustment will be performed on this well before the measurement itself starts. Therefore, the use of this feature requires some extra time. When this program configuration option is selected, the software will automatically switch from 'auto focus' to 'use previous focal height' (see chapter 6.3) after performing the auto focus once (to save this extra time when executing the same protocol using the same plate type with similar liquid levels consecutively).

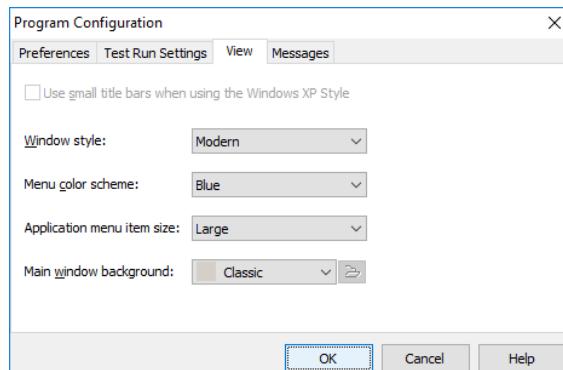
Notes: The auto focus feature is only available when using a CLARIOstar Plus, VANTAstar or VANTAstar F. Therefore, this option only exists when using such a reader.

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

By default, the gain and focus adjustment and plate ID options will be shown on a single tab. Deselect this check box if you prefer separate tabs for gain adjustment and plate IDs.

3.3.3 View Tab

Change the look of the program using this tab.

**Use small title bars when using the Windows XP Style**

Use this option to choose small or large title bars for all program sub windows.

Note: This option is only available when Windows XP is used and the Windows XP style has been selected.

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

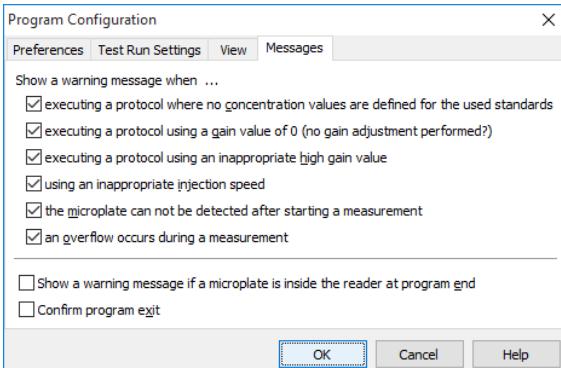
Select either normal or large for the application menu size.

Main window background

Select the background for the main window by choosing one of the predefined background styles or by selecting a bitmap file. When using a bitmap file, the dimensions will be scaled to fit.

3.3.4 Messages Tab

Use this tab to change the configuration of messages.



Example: select a warning message to be displayed when trying to execute a test protocol with an inappropriate gain value or injection speed.

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, an optional warning message can be set to indicate if a microplate is inside the reader at program end. User will be prompted to move the plate out or not.

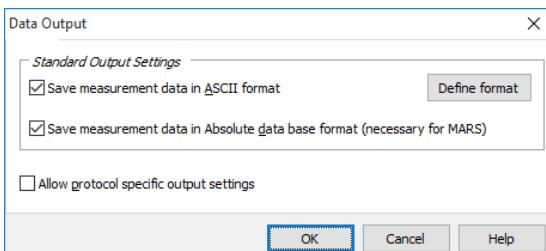
Confirm program exit

Deselect this option to eliminate 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 1.4). BMG LABTECH's Data Analysis software, MARS, uses this file format. To use a different data analysis software package, which will not work with Absolute data bases, it is also possible to store the data in ASCII format. If this option is selected, the 'Define Format for ASCII Export' window becomes available; here specify the file name and format for the ASCII files (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 MARS)

If this option is selected, all measurement data is stored in an Absolute data base file in the data directory of the user logged in. This format can be used with the BMG LABTECH Data Analysis software. To use a different data analysis software package, which will not work with Absolute data base formats, it is also possible to get an ASCII file (see above).

Note: It is possible to use both formats simultaneously.

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.

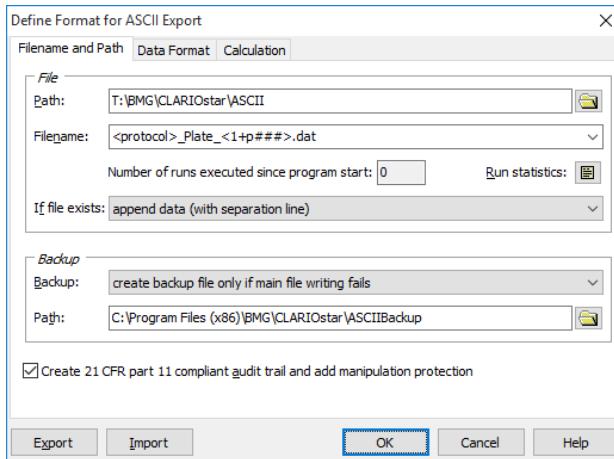
Allow protocol specific output settings

If this option is checked, there will be an extra tab 'Output' in all protocol editors (see chapter 4.3.22). Using this tab, you might define specific output settings (e.g., a special ASCII output directory or file format) for each test protocol. All protocols where no specific settings have been defined will use the standard settings defined here inside the Data Output dialogue.

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.

On the first tab of the appearing window define the filename and path.



File

Path

In this field, specify the data path for storing the measurement results in ASCII format files. This can be a network directory too. To create a new directory type in the path. Use the special functions <protocol>, <method>, <ID#>, <date>, <time>, <ser_number> or any of the consecutive number options (<1+##>) as part of the directory name to sort protocols (see the description of these options under **Filename**).

Note: When characters are used in the <date> or <time> format or in the <ID#> options, which are not allowed for path names, these characters will be replaced automatically ("/", "\" => "-"; ":" => ".")

Filename

The filename can be a constant name (typed in) or can be automatically generated using the following options:

- <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 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##>. See Run Statistics after pressing the button.

In batch mode (using a stacker) or in script mode add a ‘B’ before the '#' to get the plate numbers of the current batch run.

To count the number of batch runs use ‘S’ or ‘U’. <1+S##> inserts the number of Stacker batch runs after program start. (Opposite to <1+B##>, which inserts the counted plate number of the current batch run). <1+U##> inserts the number of Stacker batch runs after program installation. If more than one reader is used, this value will be counted separately for each reader (serial number specific).

To change a number only after e.g. every tenth plate add this value in parenthesis before the > character, e.g. <1+##(10)>.

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>

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

dddddd – date in the format defined as “Short Date Format” under windows (‘Settings | Control Panel | Regional Settings’)

ddddddd – date in the format defined as “Long Date Format” under windows

Example: <date:yyyy_mm_dd>

If format is not specified, “yyymmdd” 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...)
	<i>Example:</i> <time:hh.mm.ss> If format is not specified, "hhmmss" will be used.
<ser_number>	Serial number of the reader used
<barcode_front>, <barcode_right>	Barcode attached to the front / right side of the current plate (this option will use the internal barcode readers of the CLARIOstar Plus and is, therefore, only available when using a CLARIOstar Plus reader).

<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).
-----------	---

All these things can be mixed (Example: <protocol>_Plate <1+##>_<date>.dat => 'QC Test_Plate 01_20132811.dat').

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

If characters are used which are not allowed in file names (e.g. ":"), these characters will be removed automatically.

Number of executed runs

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

To see a **Run Statistics** press the  button. 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. These numbers will be used 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. This number will be used with the <1+T#> or <A+T#> option.

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.

The last two versions can be used to save the data from different test runs into one file. The data from different test runs can be separated by a line of '---'. See also the 'Separate data blocks with an empty line' option described in the next chapter.

Backup

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

Backup options:

- **no backup file.** Show an error message when creation / writing into the defined ASCII file failed
- **create backup file only if main file writing fails.** If creation / writing into the defined ASCII file failed, the ASCII file will be stored in the specified backup directory (same file name and behavior if file already exists as defined above). If writing into the backup file fails too, then there will be an error message. This setting is recommended if the main directory is on a network drive.
- **always create backup file.** The ASCII file will be stored in the specified main directory. A second copy will be stored in the backup directory (same file name and behavior if file already exists as defined above). There will be no error message when writing into the main file failed, but there will be an error message if writing into the backup file fails.
- **always create backup file (no error message when this fails).** 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

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

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

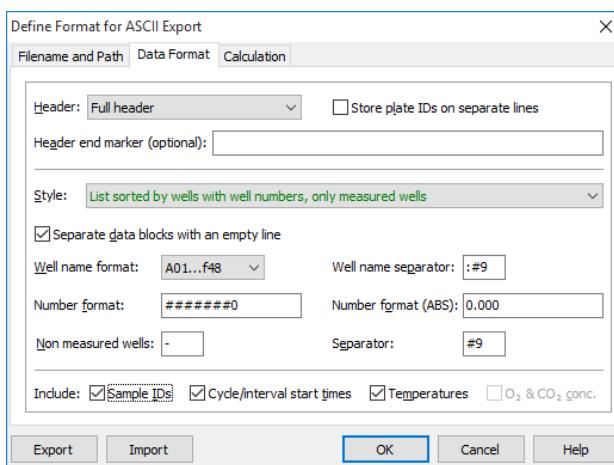
Hint for FDA 21 CFR part 11 compliance: BMG LABTECH recommends using this option if the ASCII files are to be used in accordance with the 21 CFR part 11 rule.

Export / Import

It is possible to export and re-import the whole set of ASCII settings. This might be especially useful when using protocol specific output settings (see chapter 4.3.22) 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 6.5).
- **Short header** uses 5 lines (plus an additional empty line as separator) 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, the used monochromator / filter settings and gain settings (8 additional lines).
- **Long header + basic parameter** contains everything from the long header and the most important protocol settings, e.g. reading mode, microplate, number of flashes...
- **Full header without layout...** contains all protocol settings (see below), but without the layout, concentrations and volume tables.
- **Full header** contains all protocol settings including layout, target temperature, the firmware and software versions, and the reader serial number.

Hint: 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).

- **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.

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

Store plate IDs on separate lines

By default the three plate IDs will be stored in one single line:

i.e. ID1: FAST TEST ID2:
07.08.2013, 21:17:19 ID3: 430-0042

Check this box if you prefer to get the IDs on separate lines.

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 used, followed by the data from the second chromatic, etc.

Hint: When using ‘Table with well numbers in plate layout style (optimized for human reading)’, 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, for example, a comma would appear 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), use the option ‘Table with well numbers in plate layout style (optimized for import)’.

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 chromatic, a separate data block for each chromatic (for each monochromator / filter setting or wavelength used) will be given. Depending on the calculation method selected (see Data Calculation during ASCII Export) you will also get separate data blocks for each channel and / or a data block for the polarization values when using fluorescence polarization method.

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 chromatic / wavelength, it will be added to the same line. Depending on the protocol executed and on the selection in the Calculation sheet (see below), the line will contain first the values of the first cycle/interval for the first chromatic (for a fluorescence polarization protocol this might be one value for channel A, one value for channel B and a polarization value) and then the values for the second chromatic and so on. After this, the values of the second cycle/interval will follow and so on.

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 or when using multichromatics 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 entering ‘#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 for absorbance measurements, because the OD values can range from 0.000 to 3.500 (without pathlength 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 (empty 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 6.6) in the ASCII file (after the measurement value).

Include cycle/interval start times

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 temperatures

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

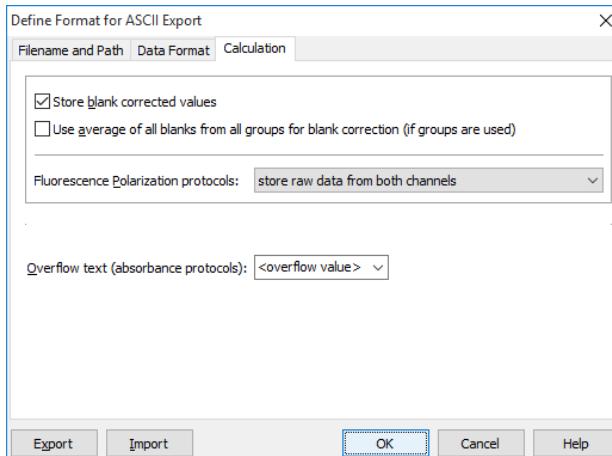
Note: The built in incubator needs to be switched on or the temperature monitoring function needs to be used (see chapter 5.1 *Incubation*).

Include O2 and CO2 concentrations

Store the gas concentration values which were measured during the measurement of each cycle (plate mode) or during the measurement of each well (well mode).

Note: This option is only available when an ACU is connected. The ACU needs to be switched on or the monitoring function needs to be used (see chapter 5.2 *ACU Control*).

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 4.3.13 *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 protocols you can decide, whether you want the raw data of the two channels, the polarization or the anisotropy values to be stored. It is also possible to get 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'.

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

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

Overflow text

Define how overflow measurement values are displayed:

- *overflow*
- *ov.*
- *<empty>*
- *<overflow value>* - show the maximum possible measurement value = measurement range end value.

It is also possible to type in any text you want to use, e.g. '-'.

Notes: This function is currently only available for absorbance measurements. When using any other measurement method the maximum possible measurement value will be shown, e.g. 260.000.

When using the *<overflow value>* option in combination with pathlength correction 99.999 OD or 99999 mOD will be displayed. These values might appear if an overflow occurred at the actual measurement wavelength(s) or at one of the water peak reference wavelengths or if the absorbance at these reference wavelengths is equal.

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

3.5 Manage Microplates

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

To open this dialog box select ‘Manage Microplates’ in the ‘Settings’ tab.

Microplate	Length	Width	X (1)	Y (1)	X (m)	Y (n)	Plate Format	User def.
LABSYSTEMS NANOPlate 384	127.60	85.60	12.10	9.00	115.60	76.50	384	
MATRIX 384	127.53	85.33	12.09	8.97	115.42	76.38	384	
MICROTITER 384	127.78	85.91	12.15	9.18	115.75	76.75	384	
NUNC 384	127.60	85.50	12.10	9.00	115.50	76.50	384	
PACKARD 384	127.70	85.80	12.00	9.00	115.60	76.60	384	
PACKARD PROXIPATEL 384 F	127.76	85.48	12.13	8.99	115.63	76.49	384	
PE AlphaPlate 384	127.76	85.47	12.10	9.00	115.60	76.50	384	
PE AlphaPlate 384 shallow well	127.76	85.48	12.13	8.99	115.63	76.49	384	
PE OptiPlate 384	127.76	85.47	12.10	9.00	115.60	76.50	384	
» SBS STANDARD 384	127.76	85.48	12.13	8.99	115.63	76.49	384	
AURORA LOBASE 1536	127.76	85.48	11.01	7.87	116.75	77.61	1536	
BMG EV Plate 1536	127.75	85.45	11.00	7.85	116.75	77.60	1536	
CORNING 1536	127.51	85.27	10.98	7.77	116.56	77.49	1536	
GREINER 1536 HI-BASE	127.75	85.50	11.00	7.90	116.75	77.60	1536	

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. After clicking the ‘User def.’ part 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).

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.

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

Users, where the ‘Microplates & Settings’ access right (see chapter 1 Login Dialogue) has been set to ‘read only’, are not allowed to edit, copy or import microplate definitions. To be able to delete a microplate definition, the access right needs to be set to ‘edit & delete’.

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.

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 microplate entry from the data base.

Close button exits this window.

3.5.1 Define a New Microplate or Edit an Existing One

Using this dialogue existing microplate definitions can be changed or new plates can be added to the database. Enter a name for the plate and choose the plate format (number of wells). 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, 864 and 1536 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

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.

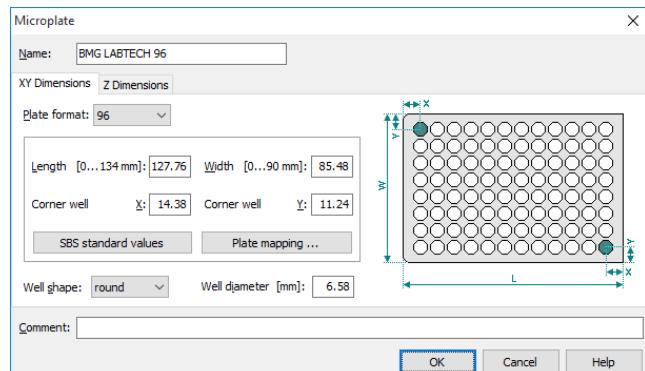


Plate format selects the total number of wells in the plate – 6, 12, 24, 48, 96, 384, 864 or 1536 wells.

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

Use the second sheet to define the Z dimensions. All dimensions need to be entered in mm. If these dimensions are not provided by the plate manufacturer, an accurately calibrated caliper can be used to obtain the dimensions. See the diagrams for the correct measurement dimensions.

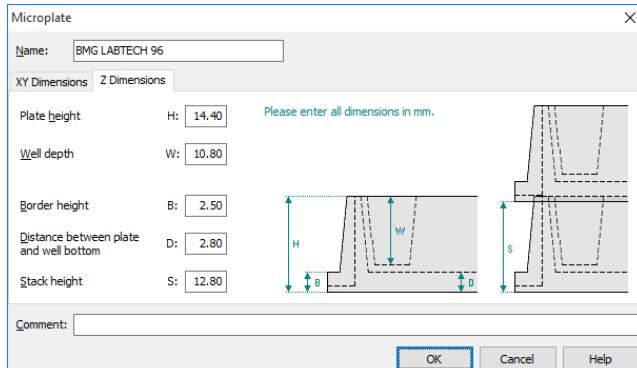


Plate height is the total height of the microplate.

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 is 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.

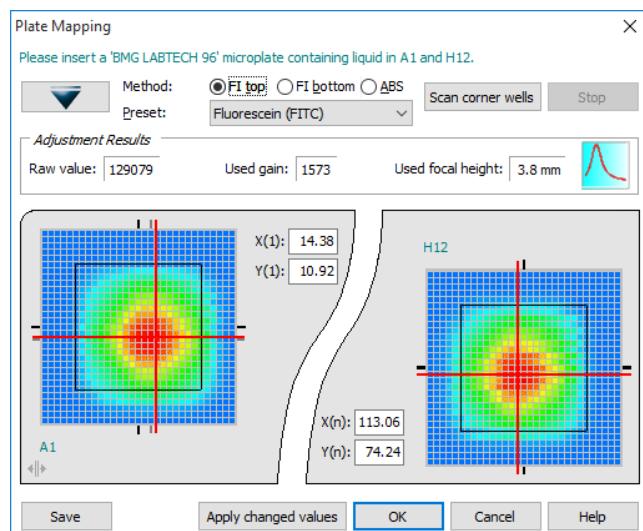
Note: If an original BMG LABTECH plate definition is changed, there will be a backup created ('Original Name_ORG'). To retrieve the original definitions, delete the changed definition; then either rename the _ORG definition the original name, or copy the _ORG definition and name the copy the original 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. Next, put the microplate into the reader (or insert the plate into magazine 1 if your reader is attached to a stacker).

Choose between using FI top or bottom or absorbance measurement method to perform the plate mapping. After selecting an appropriate preset or defining the wavelength for absorbance measurement, click the 'Scan corner wells' button. For FI measurements the instrument will first perform a gain and focus adjustment. The instrument will then scan the top left and the 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. Optimum X and Y positions will be calculated automatically, but it is also possible to change these values manually. **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, the automatically determined new positions will be displayed using gray markers.

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

When holding down 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 X and Y values currently specified (automatically determined or manually changed) will be stored without closing the dialogue box. A new scan can then 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: VANTAstar and VANTAstar F readers can only measure microplates with up to 384 wells.

It is possible to 'Save' the display in bitmap (*.BMP) or JPEG format.

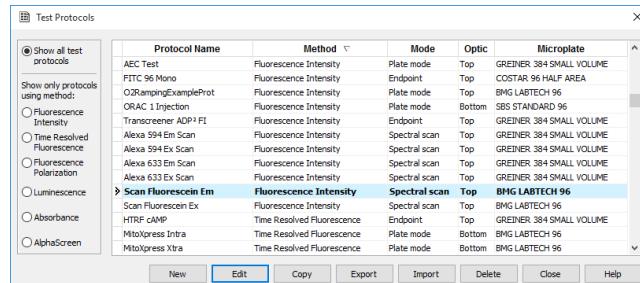
Use the key combination [Shift]+[Ctrl]+[P] to **print out** the dialogue box. A standard print dialog box will appear.

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.

The ‘Test Protocols’ window lists all previously defined protocols and gives options for creating new test protocols or modifying existing ones.



By default, the protocols are sorted by method first and then alphabetically by name. To sort by name, click the ‘Protocol Name’ heading box. To switch back to the original sorting, click ‘Method’ heading box. It is also possible to sort by mode or microplate used. Simply click on the ‘Mode’ or ‘Microplate’ heading box.

Additional filtering is possible (by detection mode/measurement method) by clicking one of the radio buttons to the left side of the dialogue box. CLARIOstar and VANTAstar readers can measure fluorescence intensity, time resolved fluorescence, fluorescence polarization, luminescence, and absorbance. CLARIOstar readers can also measure AlphaScreen.

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

New

Define a new test protocol. See section 4.2.

Edit

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

Copy

Select the protocol to copy. A new dialogue appears asking for a name of the copy. The duplicate will appear on the test protocol list under the new name. Making a duplicate protocol allows modification of a test protocol without changing the original.

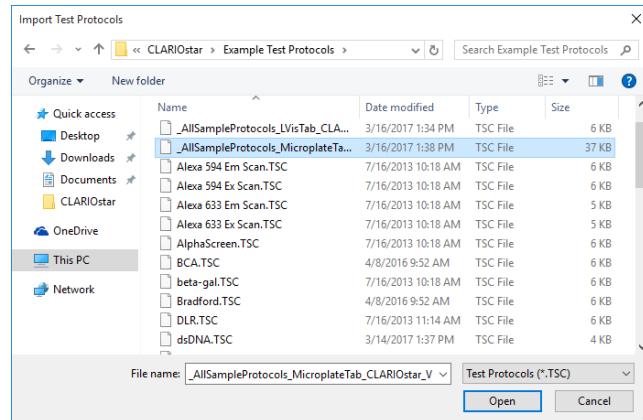
Export

Using this function test protocols can be exported onto a USB stick or a hard drive folder. Select the protocol(s) to export, then click on ‘Export’. A dialogue box will ask for the destination drive

and folder and a name for the file. The exported file will get the file name extension ‘.TSC’.

Import

Import a test protocol by clicking on ‘Import’. Choose the folder. A list of files with the extensions ‘.TSC’ and ‘.TST’ will appear:



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

Note: It is only possible to import test protocols created using the SMART Control or CLARIOstar software.

Delete

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

Close

Close the test protocol selection window.

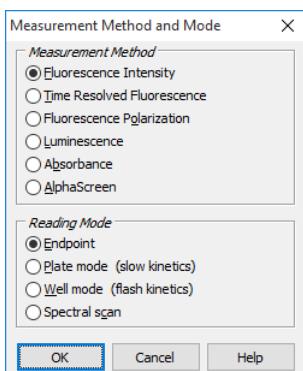
Notes: The test protocol selection table can be exported into an Excel format (.xls / .xlsx), text or HTML file by right clicking on the table.

The context menu (accessed by right clicking a protocol entry) offers functions to convert protocols from one reading mode into another reading mode, e.g. from endpoint into plate mode.

This menu also offers functions to copy the layout from one protocol and to paste it into other protocols.

Users, where the ‘Manage protocols’ access right (see chapter 1) has been set to ‘read only’, are not allowed edit, copy or import test protocols. To be able to delete a protocol, the access right needs to be set to ‘edit & delete’.

4.2 Creating a New Test Protocol



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

After the method and mode is selected, test protocol parameters can be defined in the next dialogue box.

Notes: Spectral scan is only available for fluorescence intensity and luminescence measurements. For AlphaScreen, only endpoint mode can be used.

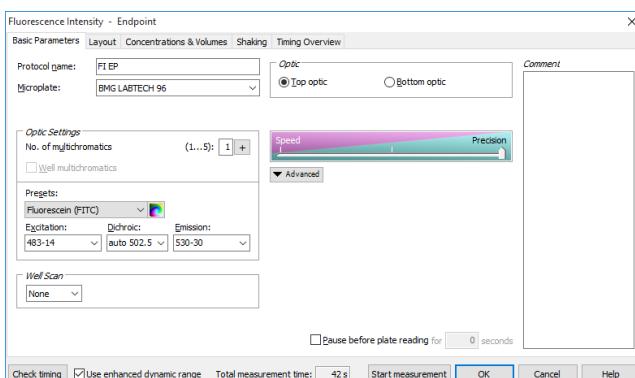
AlphaScreen is not available when using VANTAstar or VANTAstar F readers.

4.3 Fluorescence Intensity Protocols

Fluorescence intensity can be measured in endpoint, plate mode, well mode or spectral scan mode. Multichromatic measurements are possible.

4.3.1 Basic Parameters – Endpoint

To measure each well only once (if kinetic reaction is not expected) and well scan or spectral scan is not required, define the test protocol in endpoint mode.



Protocol name

Assign a test name to appear on the list of test protocols.

Microplate

Select the microplate used in the assay. All microplates defined under 'Setup | Microplates' will be listed. To select the first microplate, beginning with, for example, 'B', simply press the key [B] after opening the drop-down box (using [\downarrow]).

Optic Settings

Number of multichromatics

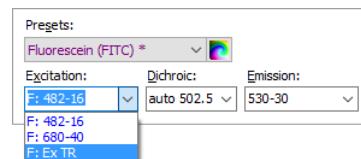
There is the possibility to analyze 5 fluorophores in one test protocol. Enter the number of fluorophores to be analyzed, then click on the arrow button (or on the newly appeared 'Multichromatic' tab) to select the settings for each fluorophore to be analyzed. When using only one fluorophore all optic settings are defined directly in this window.

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 settings in between) before moving to the next well.

Presets

The BMG LABTECH software contains a library of commonly used fluorophores. Select either one of the presets from the fluorophore preset drop down list or use the option '<user defined settings>'. When selecting a preset, the necessary monochromator settings for Excitation, Dichroic and Emission will be set automatically. It is possible to modify these settings here or in the Fluorophore Toolbox (click the button; see chapter 4.3.4). When using a modification of the pre-defined wavelength setting, the preset name will be shown in purple color and marked with a star.



Excitation / Dichroic / Emission

The excitation filter / monochromator is used to select the excitation light produced by the flash lamp. The emission filter / monochromator is used to select the light emitted by the sample. The dichroic mirror is used to separate the excitation and emission light.

To use the monochromator simply type in the desired wavelength in nm. The monochromator can be used in the wavelength range from 320 nm to 740 nm. When an extended IR PMT is installed wavelengths up to 840 nm can be selected (dichroic 760 nm). For the excitation and emission monochromators the bandwidths can also be defined (separated from the wavelength by a hyphen). If you do not enter a bandwidth value, a standard value will be entered automatically (for fluorescence intensity measurements 15 nm for excitation and 20 nm for emission). The bandwidth can be defined between 8 and 100 nm.

Alternatively use the pull down boxes to select any of the filters which have been installed and defined using the Filters dialogue box (see chapter 3.1.9). Filter entries are preceded by 'F:' and displayed in blue.

It is possible to combine filter (e.g. for excitation) and monochromator (e.g. for emission).

By default, the dichroic wavelength will be determined automatically. It is also possible to manually define a dichroic wavelength, or to select an appropriate fixed dichroic from the dichroic pulldown list. The dichroic wavelength needs to be between the excitation and emission wavelength.

Note: As VANTAstar F readers are not equipped with a monochromator, there is no Presets pull down box available when using such a reader. Only filters can be used.

Well Scan

When using microplates with 6 to 384 wells, instead of measuring one point in the middle of the well, use the orbital or spiral averaging function (see chapter 4.3.7) or use matrix well scan (see chapter 4.3.8). This is useful if you use large wells and if the probe is not equally distributed, e.g. when using cell based assays.

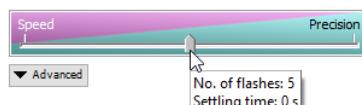
Note: Well scan cannot be combined with Flying mode (see below).

Optic

It is possible to select top or bottom optic.

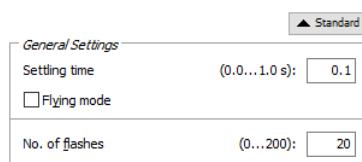
Speed and Precision

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



Hints: The automatically selected parameters will be shown in a hint box, if you move the mouse cursor to the thumb of the slider.

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



General Settings

Settling time

Define a waiting period after a well of the microplate moves to the measurement position before the measurement begins. The settling 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 settling time of 0.1 s. For fluorescence measurements of cell

based assays, a settling time of 0.5 s is adequate. Liquid movements and hence settling time are influenced by viscosity.

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

Flying mode

This is a time optimization option for endpoint and 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 by the measurement position. Injection will be performed in non flying mode.

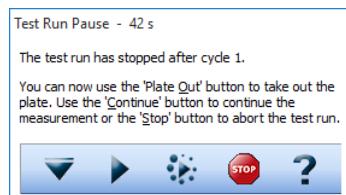
Number of flashes per well

Up to 200 flashes per well can be defined. 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 will be. For fluorescence measurements, 10 flashes are usually adequate.

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

Pause before plate reading

It is possible to define a pause before the measurement starts. If 0 is defined as pause time, the Test Run Pause window will appear, otherwise the measurement will automatically be continued after the defined pause time. A pause time of up to 3600 s (one hour) can be defined.



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

A pause can be used to incubate the plate before starting a measurement.

When using the button, the measurement will be continued automatically when the defined target temperature and the defined target concentrations (if an ACU is used) are reached.

Note: The pause function is not available when using a stacker or in script mode (see chapter 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, click the button '**Check timing**'. If the Use enhanced dynamic range option is selected, the time necessary for the usage of this feature will be taken into account (see chapter 6.4). 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 4.3.21).

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

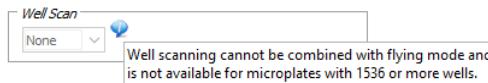
Click the Start Measurement button to immediately start the measurement after defining all necessary settings. This will open the 'Start Measurement' window (see chapter 6.3).

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.

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 additional *Kinetic Settings*, **No. of cycles** and **Cycle time**.

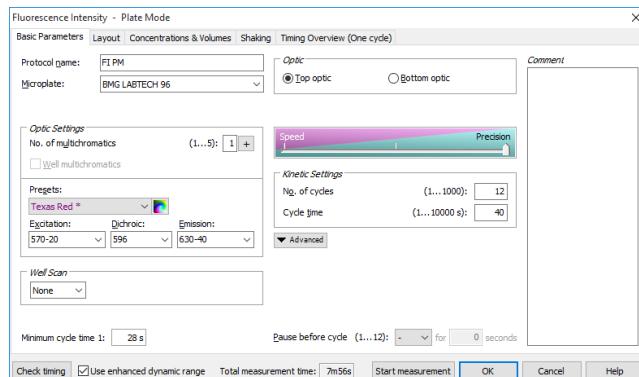


Plate mode can be used for slow kinetics, i.e. the reaction lasts for an extended period of time. All wells defined in the layout are read once during a plate cycle; it is possible to read up to 1000 cycles.

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 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 = 900 s.

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

General Settings

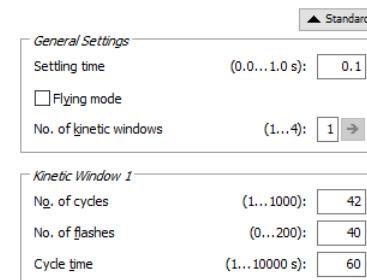
Settling time

See chapter 4.3.1.

Number of kinetic windows

It is possible to split the measurement into up to 4 kinetic windows and to 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.

To use more than one kinetic window and define the various parameters, click the arrow button or on the Kinetic Windows tab. If using only one kinetic window, all necessary parameters can be defined 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 - this 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 4.3.9).

Number of flashes per well and cycle

Up to 200 flashes per well and cycle can be defined. 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 will be. For fluorescence intensity 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

Define the duration of each cycle from 1 to 10000 seconds. When the instrument is switched on, click the ‘Check timing’ button and the cycle time is automatically validated by the instrument. (The minimum cycle time will be displayed below this group box. If the defined cycle time is smaller than this minimum cycle time, automatic correction to the minimum time will occur.).

To increase the cycle time (i.e., for a delay between cycles), manually enter a time greater than the minimum cycle time.

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

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

Pause before cycle

To define a pause, enter the cycle number before which the reader should pause the measurement. It is also possible to select pause before each cycle. If 0 s is defined as pause time, the Test Run Pause window will appear; otherwise the measurement will automatically be continued after the defined pause time. A pause time of up to 3600 s (one hour) can be defined.

A pause can be used to take a plate out and incubate or inject before continuing the measurement.

During an active pause it is also possible to change incubator settings and to change ACU settings (when a firmware version >= 1.11 is installed).

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

You can also pause after a measurement has started (for plate mode protocols only if more than one cycle has been defined). Go to the ‘Measure’ menu group and click the ‘Pause’ button.

To use the option ‘Pause before each cycle’ a firmware version 1.20 or newer is necessary.

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, click the button ‘**Check timing**’. If the **Use enhanced dynamic range** option is selected, the time necessary for the usage of this feature will be taken into account (see chapter 6.4). The shortest possible cycle time will be displayed. If a cycle time has been defined that is shorter than

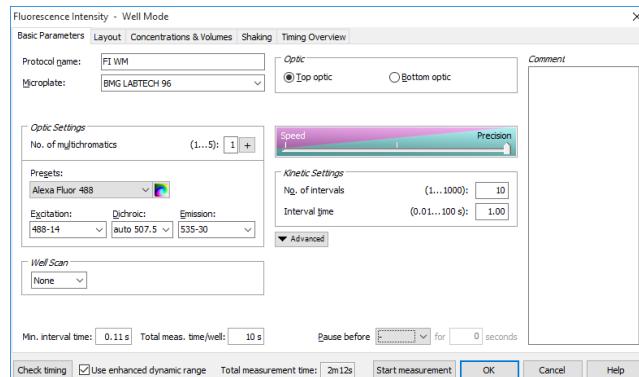
this minimum time, automatic correction will occur. At the bottom of the test protocol definition window, the calculated ‘**Total measurement time**’ for the entire plate will be displayed.

After pressing the ‘Check timing’ button, the ‘Timing Overview’ sheet will become available (see chapter 4.3.21).

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

4.3.3 Basic Parameters – Well Mode

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



For example, a test can be defined with 3 intervals and an injection. Therefore, well A1 will be moved to the measurement position, a measurement is performed, then an injection, followed by two more measurements. When all defined measurement intervals have been performed on a well, 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 are possible.
Interval time	The time in seconds that each measurement interval should last. This includes the flashes and the time for measuring the emission light.
Total measurement time per well	The total time necessary to perform all the measurement intervals on one well. This time will be calculated using the measurement start time, the defined interval time, the number of intervals, and the minimum interval time.

Parameters not described in this chapter are identical to endpoint protocols (see chapter 4.3.1).

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 (i.e., the number of readings on each well). It is possible to 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.

Interval time

Define the duration of each interval from 10 ms to 100.0 seconds. When the instrument is active, click the '**Check timing**' button and the interval time is automatically validated by the instrument. To increase the interval time (to define a delay between the intervals), manually enter a time. If the defined interval time in any of the kinetic windows is shorter than the respective **minimum interval time** it will automatically be corrected up to the minimum interval time. 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 it is possible to 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, the interval time 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 advanced button, the group boxes General Settings and Kinetic Window 1 become available.

General Settings

General Settings	
Settling time	(0.0...1.0 s): 0.2
No. of kinetic windows	(1...4): 1 →

Kinetic Window 1	
Measurement start time	(0...1200 s): 0.0
No. of intervals	(1...1000): 10
No. of flashes	(0...200): 40
Interval time	(0.01...100 s): 0.20
End time of kinetic window 1	(s): 2

Number of kinetic windows

It is possible to split the measurement into up to 4 kinetic windows and to 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.

To use more than one kinetic window and define the various parameters, click the arrow button or on the Kinetic Windows tab (see chapter 4.3.9). If using only one kinetic window, all necessary parameters can be defined on the 'Basic Parameters' sheet.

Kinetic Window 1

Measurement start time

Define 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, for example, injection or shaking is performed before the measurement.

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.

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). It is possible to define up to 1000 intervals – this 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

Define up to 200 flashes per well and interval. All the measurement values from the flashes for an interval are averaged for one intensity. Therefore, the greater the number of flashes the greater the accuracy will be. For fluorescence measurements, 10 flashes are usually adequate.

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

Interval time

Define the duration of each interval from 10 ms to 100 seconds. When the instrument is active, click the '**Check timing**' button and the interval time is automatically validated by the instrument. To increase the interval time (to set a delay between the intervals), manually enter a time. If an interval time is defined which is shorter than the minimum interval time, it will automatically be corrected to the shortest possible time.

The minimum interval time depends on the number of flashes and on the usage of multichromatics.

For example, the instrument gives a minimum interval time of 0.4 seconds, this can be changed 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.

Pause before ...

It is possible to define a pause before the measurement of the first well, each well or a specific well. If 0 s is defined as pause time, the Test Run Pause window will appear; otherwise the measurement will automatically be continued after the defined pause time. A pause time of up to 3600 s (one hour) can be defined.

A pause can be used to take a plate out and incubate or inject before continuing the measurement. During an active pause it is also possible to change incubator settings and to change ACU settings.

Notes: You can also pause after a measurement has started. Click the 'Pause' button in the 'Measure' menu group.

To use the pause function for well mode protocols a firmware version 1.20 or newer is necessary.

The pause function is not available when using a stacker or in script mode (see chapter 8).

Check timing

Minimum interval time 1

The minimum interval times can only be calculated by the reader. After defining all necessary settings, click the button 'Check timing'. If the Use enhanced dynamic range option is selected, the time necessary for the usage of this feature will be taken into account (see chapter 6.4). The shortest possible interval time will be displayed. If an interval time has been defined which is shorter than the respective 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.

Total measurement time per well

This time will be calculated using the settings in the protocol, i.e., the interval time and the number of intervals and the measurement start time. 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, since 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 an injection has been defined 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.)

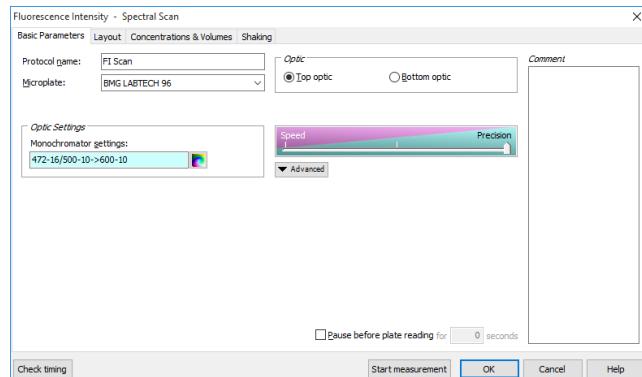
After pressing the 'Check timing' button, the 'Timing Overview' sheet will become (see chapter 4.3.21).

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

4.3.4 Basic Parameters – Spectral Scan

To measure spectra instead of discrete wavelengths, define a spectral scan protocol. As VANTAstar F readers are not equipped with a monochromator, these readers cannot measure spectra.

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.

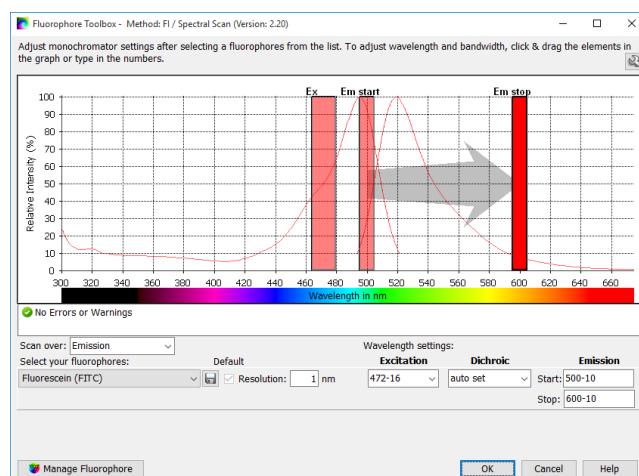


Optic Settings

Click the button to open the Fluorophore Toolbox (see chapter 4.3.5). Using this dialogue, it is possible to define a scan over an excitation or emission wavelength range. A combined excitation and emission scan is also possible.

4.3.5 Fluorophore Toolbox

The Fluorophore Toolbox window is used to find the optimal wavelength settings for the monochromator. Depending on the measurement method it is possible to define the excitation wavelength and bandwidth and the emission wavelength and bandwidth. If a spectral scan over a wavelength range is selected, the start wavelength, the stop wavelength and the resolution for the selected spectral scan can be defined.



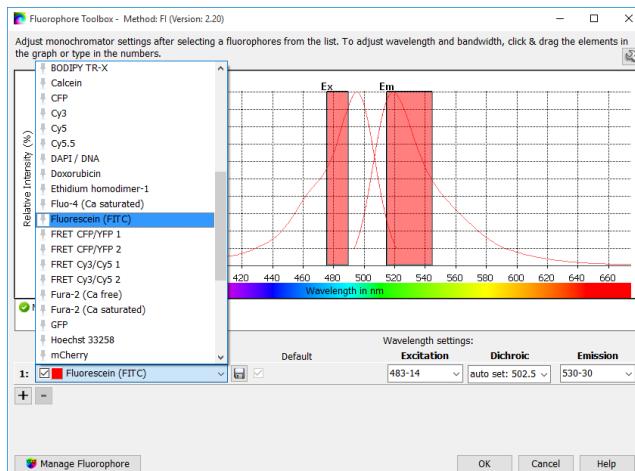
A given recommendation for the monochromator can be used for a set of fluorophores and luminophores. These settings can be adjusted or unique settings may be defined by the user.

The current settings are displayed in the chart together with the spectral curve of a selected fluorophore / luminophore. A band for the excitation filter and a band for the emission filter is shown. If a spectral scan is selected, two bands for either the excitation or the emission are shown to indicate the start and the stop value of the scanned wavelength range.

The following sections describe the usage of the Monochromator Settings dialog for fluorescence measurements (Fl). The definition for luminescence measurements is the same but without the definition for the excitation wavelength.

Define the Wavelengths Settings

Select the fluorophore / luminophore: If the used fluorophore or luminophore is in the list, select it to see its excitation and emission spectral curves in the chart.

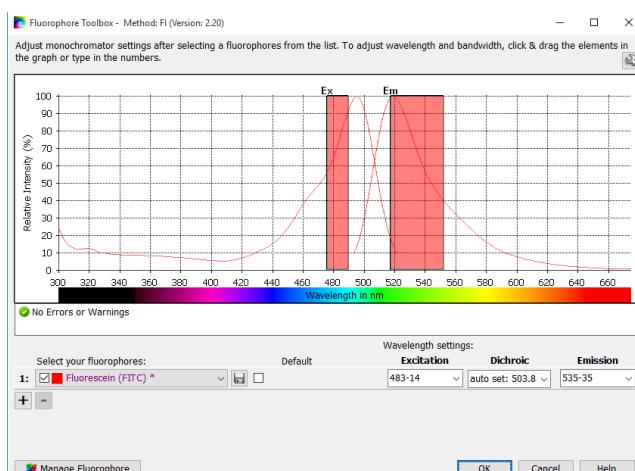


Using Recommended Settings

For each fluorophore / luminophore in the list, there are recommendations for the filter wavelengths and bandwidths that give the best measurement results in most cases. To set the values for the wavelengths and bandwidths to recommended settings select the desired fluorophore in the list and the recommended settings will automatically be entered into the Excitation, Dichroic and Emission controls.

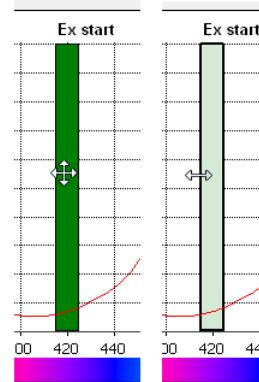
Using User Defined Settings

If the desired fluorophore / luminophore is not in the list, or if the recommended settings should be adjusted, they can be overwritten. The small 'Default' check box control shows if the recommended settings were used. After changing the settings, the check box is not checked and the fluorophore in the list is marked with an * to indicate that the default settings were changed. To go back to the recommended settings, click on the check box.



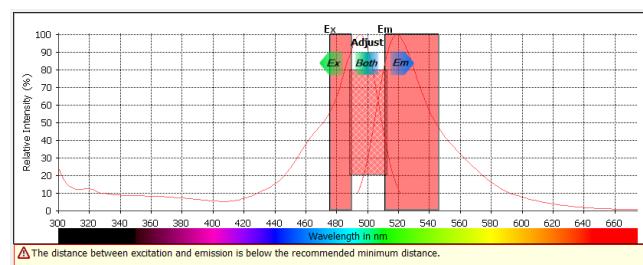
The filter settings can also be changed in the chart by using the mouse. Move the mouse over the filter settings and click and hold the left mouse button. The filter band can now be to the

desired position. To change the bandwidth, click on the left or right border of the filter band and increase or decrease the wavelength by moving the border. When pressing and holding the shift key on the keyboard, it is possible to move the border of the filter band to increase/decrease the bandwidth in both directions (left and right). In this case the center wavelength of the filter will not be changed.



Moving center wavelength (left) and changing the bandwidth of the filter (right) using the mouse

The distance between the excitation and the emission filter must be adhered to a minimum distance value. If the distance between the two filters goes below this value, a distance warning is shown in the graph:



The red band shows the minimum required distance between the right side of the excitation filter band and the left side of the emission filter band.

The filter settings are easily corrected to fulfill the distance rule. Simply click on one of the three adjust buttons: Ex - Both – Em

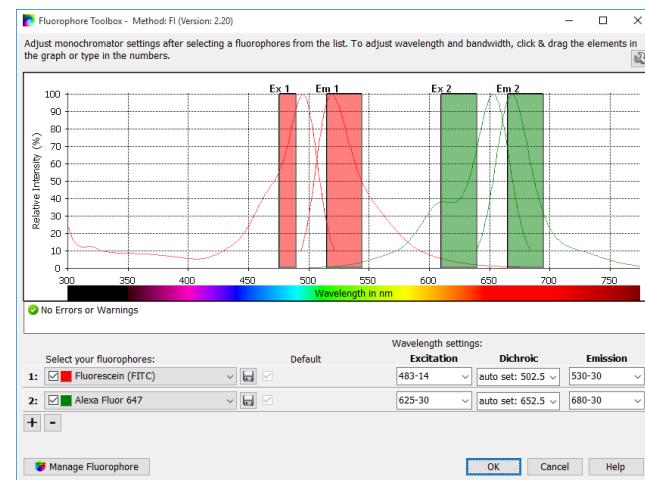
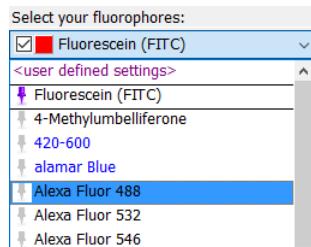
It is also possible to change the bandwidth of the filters until the distance is above the required minimum.

Note: It is possible to apply and use the settings for the filters even if the distance is below the required minimum, but this can lead to poor measurement results.

If you want to use the changed settings not only for this protocol, you can save these settings as new recommended settings for this fluorophore. Press the save button on the right side of the fluorophore pulldown list. If you want to overwrite the current settings for this fluorophore, select 'save'. If you want to keep the old settings and add new settings, select 'save as' and enter a unique name for the new settings. The save function is only available for fluorophores from the users fluorophore list (they can be recognized as blue entries in the list). Black entries in the list are read-only fluorophores. With the save as function, you will create a copy of this fluorophore in the users fluorophore list which will not be read-only. To see and manage the users fluorophore list, click on the Manager Fluorophore button to open the fluorophore manager.

Make a fluorophore your favorite

The list with the fluorophore is sorted in alphabetical order. If a fluorophore is used often, you can make it a favorite to place it always on the top of the list. Click on the gray pin left beside the fluorophore. The pin will change its color to purple and the fluorophore moves to the top of the list. Click the pin again to move the fluorophore back to its old position:



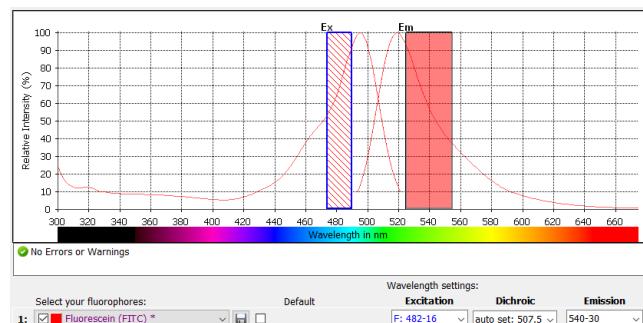
Dichroic and fix filter

For the defined excitation and emission wavelength the monochromator will be used. Alternatively, you can use the pull down boxes to select any of the filters which have been installed and defined using the Filters dialogue box (see chapter 3.1.9). Filter entries are preceded by 'F:' and displayed in blue.

The dichroic mirror is used to separate the excitation and emission light. The dichroic wavelength will be determined automatically if auto set is selected from the pulldown list. The automatically determined dichroic is in the middle between the excitation and emission wavelength. If a different value is needed, it can be defined manually. The wavelength should be between the excitation and emission wavelength. You also can select an appropriate fixed dichroic from the dichroic pulldown list.

If the list with the fix filter is empty, no fix filters are installed / defined. Use the Filters dialogue box to define filters.

Fix filters are displayed in a transparent filter band with small diagonal lines:



Define settings for up to 5 fluorophores

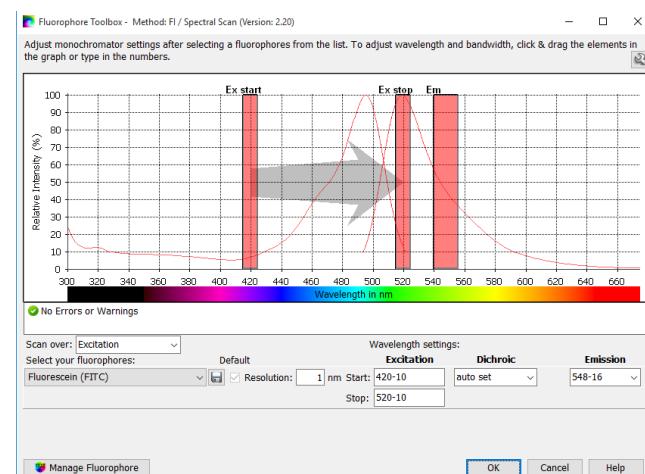
As there is the possibility to analyze 5 fluorophores in one test protocol you can add up to 5 fluorophore settings in the fluorophore toolbox.

Click on the plus button to add a further fluorophore setting definition:

Use the check box control in the fluorophore pulldown list to display or hide the fluorophore in the chart.

Define the Settings for a Spectral Scan

For the definition of a spectral scan, a start, a stop wavelength, and the resolution of the scan in nm needs to be defined.



Scan over: Select the type of scan.

Excitation:

the wavelength for the excitation filter changes from the start value to the stop value, in steps defined by resolution. The emission wavelength is fixed.

Emission:

the wavelength for the emission filter changes from the start value to the stop value, in steps defined by resolution. The excitation wavelength is fixed.

Excitation / Emission:

two scans are defined: one excitation scan and one emission scan. Both scans will be measured into one test run.

The resolution for the scan can only be changed, if 'user defined setting' is selected (see *Using user defined settings* above).

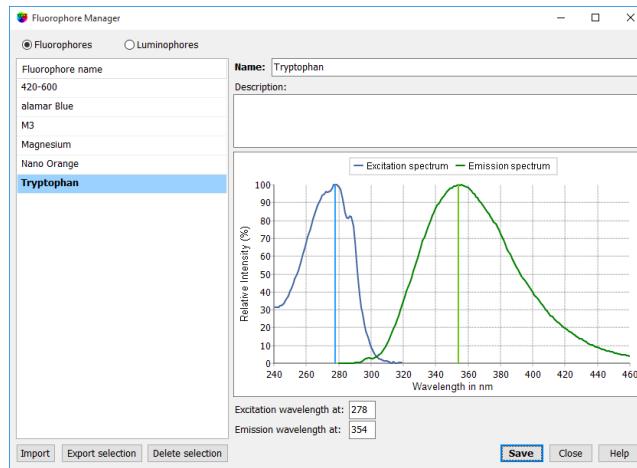
Note: A maximum of 1000 measurement points can be measured. Therefore, when using a resolution of 0.1 nm define a wavelength range of less than 100 nm.

4.3.6 Fluorophore Manager

The **Fluorophore Manager** holds a list with fluorophores. Every user has his own list. This list is separated from the fluorophores delivered by BMG together with the installation. The users fluorophores can be recognized on their blue color in the fluorophore pull down list of the fluorophore toolbox.

For each fluorophore in the list, you can change the name, the description and the wavelength of interest for the excitation and the emission. This wavelength will be used to select a recommendation for the monochromator settings. For luminophores you see only an emission spectrum and the emission wavelength to define.

You can also change the wavelength if you move the wavelength line in the chart (move the mouse-cursor over the line, press the left mouse button and move the mouse. If the line is at the desired position, let go of the mouse button).



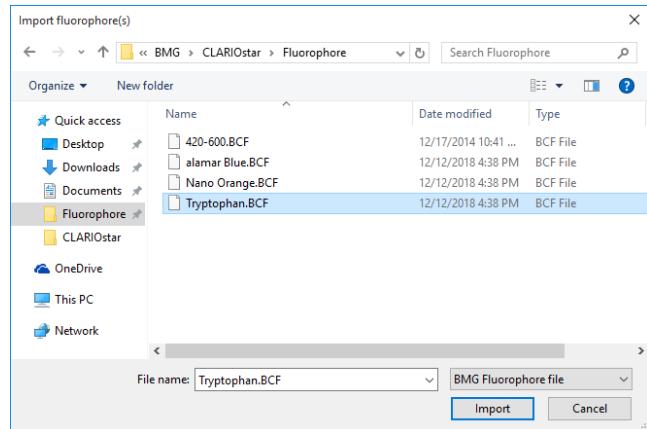
Add new fluorophores

There are two ways to add fluorophores or luminophores to the list:

1. If you change the recommended settings for a fluorophore with the fluorophore toolbox and use the 'save as' function. A new fluorophore with the name you define will be added to the list.
2. You can use the import function to import fluorophores (see section Import fluorophores below)

Import fluorophores

You can import fluorophores from three different sources: Fluorophores exported from the fluorophore manager (files with the extension BCF), fluorophores from a third party source saved in a text file (extension TXT or CSV) or fluorophores in an excel file format (extension XLS XLSX or CSV).

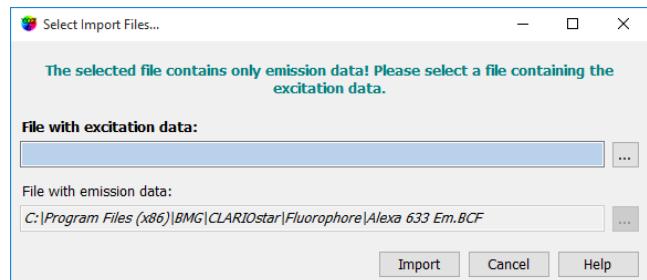


Click the import button and select the type of file you want to import.

Importing from BMG fluorophore files (BCF):

BMG fluorophore files can be created with the fluorophore manager using the export function. Alternatively, fluorophores can be created with MARS by exporting spectral scans (read the chapter **Export a Fluorophore** in the MARS software manual).

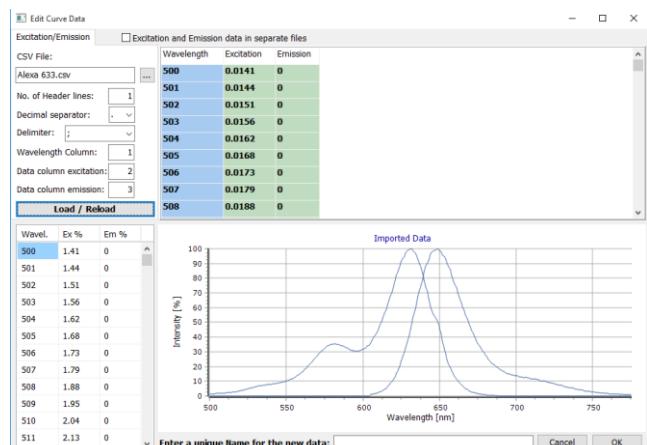
If the exported fluorophore was created with MARS, the manager checks if the file contains an excitation and emission scan (for luminophores, only an emission scan is needed). If one of the scans is not in the file, a dialog pops up:



You will be asked to select a second file with the missing data. You can import the data without selecting a second file. The imported fluorophore will then show only the imported spectra.

Importing from text files:

After selecting a text file, a new dialog to define the import format opens:



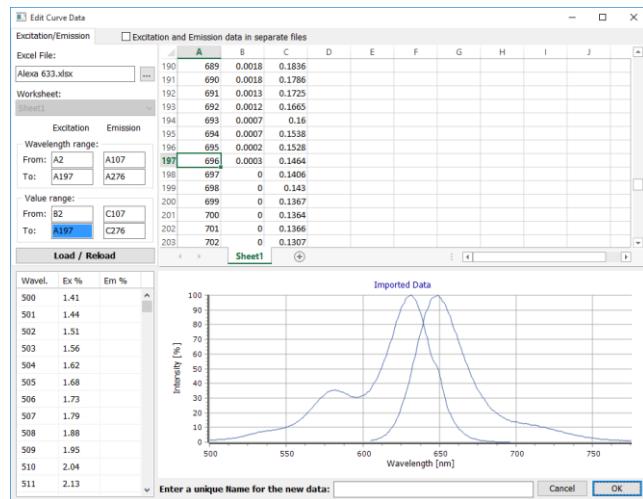
On the top right side of the window, you see the content of the text file. Use the correct settings for the number of header lines, the decimal separator and the delimiter between the single numbers. The wavelength and the values for excitation and/or emission must be arranged in columns to be able to import them. Define the column for the wavelength values, the excitation and the emission values.

Press on the **Load/Reload** button to see if the import will work. You can see a preview of the imported data in the chart and the table on the lower half of the window. If the data are imported as expected, enter a Name for the new fluorophore and click OK to save it in the users fluorophore list.

If the excitation and emission data is saved in two different files, check the '**Excitation and Emission data in separate files**' control. A second tab appears and you can define the import settings for the excitation and the emission data for each file.

Importing from Excel files:

The import dialog for excel files looks a little bit different:



On the upper half of the window, you see the excel file and you can define the cells with the wavelength and the values for excitation and emission.

You can enter cell names of the start and stop cell in the entry fields, or you can set the cursor into the From field and mark with the mouse in the excel sheet the cells for the desired area.

With the **Load / Reload** button you can again check the result of the import. If the data are imported as expected, enter a Name for the new fluorophore and click OK to save it in the users fluorophore list.

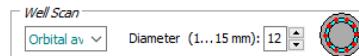
If the excitation and emission data is saved in two different files or worksheets, check the '**Excitation and Emission data in separate files**' control. A second tab appears and you can define the import settings for the excitation and the emission data for each file.

4.3.7 Orbital and Spiral Averaging

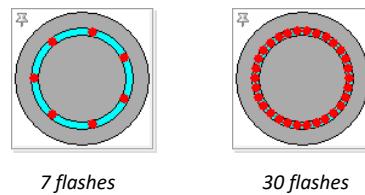
Orbital Averaging

With 6 to 384 well microplates, use the orbital averaging function instead of measuring one point in the middle of each well when using large wells and in instances when the probe is not equally distributed (for example, in cell based assays).

Using this mode, the measurement takes place on an orbit with definable diameter. Using the **fluorescence intensity** and **absorbance** measurement methods the defined number of flashes will be equally distributed throughout 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 4.3.8).



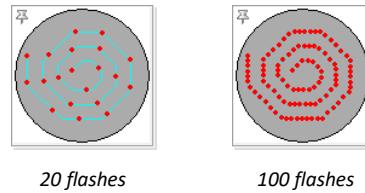
A small icon inside the orbital averaging box will illustrate how the measurement points are distributed over the well:



7 flashes 30 flashes

Spiral Averaging

Instead of orbital averaging it is also possible to use spiral averaging. Here instead of one orbital movement a spiral movement will be used. This covers a larger part of the well, but also last longer.



20 flashes 100 flashes

Notes: As it is possible to use different measurement interval times in different kinetic windows (see chapters 4.3.7 and 4.3.10), 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 4.3.8). There will be no positioning delay between the measurements of single scan points.

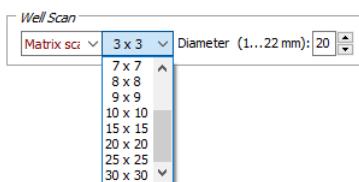
To use orbital averaging in combination with spectrometer based absorbance measurements a firmware version 1.10 or newer is necessary.

To use spiral averaging a firmware version 1.20 or newer is necessary.

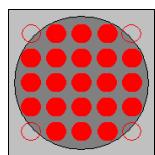
4.3.8 Matrix Well Scan

With 6 to 384 well microplates, use the matrix scan function instead of measuring one point in the middle of each well when using large wells and in instances when the probe is not equally distributed (for example, in cell based assays). Matrix 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 enables the user to see measurement values from the single scan points in the Current State Display (see chapter 6.9.5) and in the SMART Control Data Analysis software (see software manual part III).

Well Scanning is not available in time resolved fluorescence, fluorescence polarization and AlphaScreen mode. It is not possible to measure more than one cycle or interval in combination with matrix scanning.



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 on the right 21 out of 25 possible points):



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

Notes: Matrix scanning is slower than orbital or spiral averaging (see chapter 4.3.7), but opposite to these modes the measurement values of the single scan points can be obtained.

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.

4.3.9 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. This might be useful in combination with injections.

The screenshot shows the 'Fluorescence Intensity - Plate Mode' dialog box. Under 'Kinetic Windows', there is a table with four rows for cycles. The columns are: Number, No. of cycles (1...1000), Meas. start time (0...1200 s), No. of flashes (0...200), Cycle time (1...10000 s), and Minimum cycle time. Row 1 has 5 cycles at 0.0s start, 10 flashes, and 26s cycle. Row 2 has 20 cycles at 0.0s start, 40 flashes, and 41s cycle. Row 3 has 3 cycles at 0.0s start, 10 flashes, and 26s cycle. Row 4 has 0 cycles at 0.0s start, 10 flashes, and 1s cycle. At the bottom left is a checked checkbox for 'Equisistant kinetic cycles'. At the bottom right are 'OK', 'Cancel', and 'Help' buttons.

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. It is possible to 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.

Number of flashes (all measurement methods except luminescence)

You can define up to 200 flashes per well and cycle (for fluorescence polarization measurements up to 500). All the measurement values obtained for all flashes of a cycle sent to one well are averaged for one intensity value. Therefore, the greater the number of flashes the greater the accuracy will be. 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 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

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 (You will see the minimum cycle times on the right. If a cycle time you have defined is smaller than the 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 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.

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.

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.

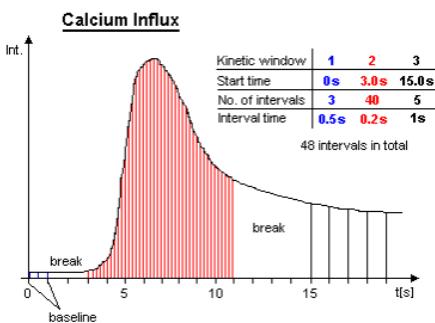
If you do not use this option, the time for cycles with injection can be different from cycles without injection and the minimum cycle time for each kinetic window can be different. This allows very short sampling rates in kinetic areas with a fast change in signal.

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.

4.3.10 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. This might be useful in combination with injections.

Example:



Fluorescence Intensity - Well Mode

Basic Parameters		Kinetic Windows		Layout	Concentrations & Volumes	Shaking	Timing Overview
Number	Start time (0...1200 s)	No. of intervals (1...1000)	No. of flashes (0...200)		Interval time (0.01...100 s)	End time (s)	Minimum interval time
1	0.0	10	20		0.50	5	0.11s
2	6.1	20	20		0.50	16.1	0.11s
3	16.1	10	20		0.50	21.1	0.11s
4	21.1	20	20		0.50	31.1	0.11s

Total meas. time/well: 31.1s

Check timing Use enhanced dynamic range Total measurement time: 5m18s Start measurement OK Cancel Help

Start time

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

Number of intervals

This is the amount of times a measurement will be successively taken on each well. You can define up to a total of 1000 intervals.

Number of flashes (all modes except luminescence)

You can define up to 200 flashes per measurement interval (for fluorescence polarization measurements up to 500). All measurement values obtained for all flashes defined for one interval are averaged to produce one intensity value per well. Therefore, the greater the number of flashes the greater the accuracy will be. For fluorescence measurements, 10 flashes are usually adequate.

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

Measurement interval time (only luminescence mode)

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

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 0.01 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.

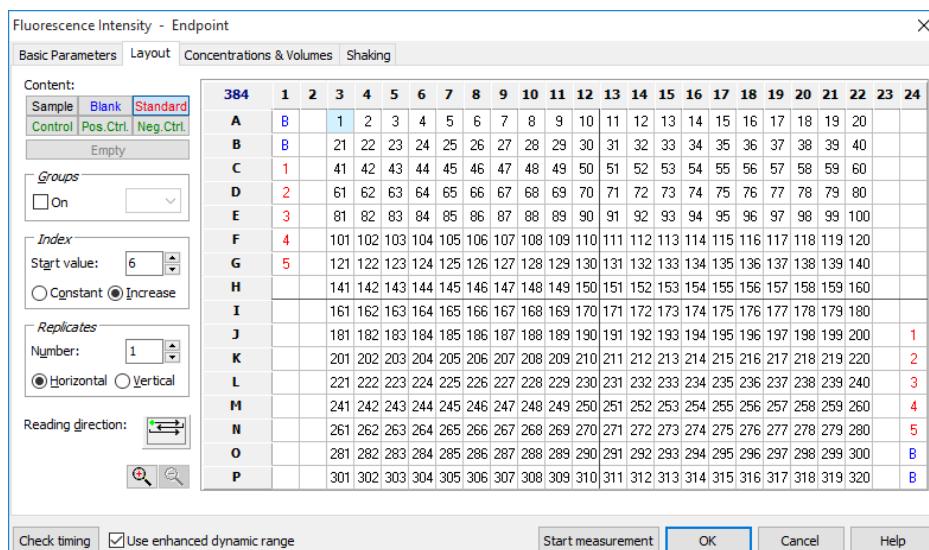
Note: The 'Check timing' function is only available if the reader is switched on.

Total measurement time per well

This time will be calculated using all the settings, i.e. the interval time, the number of intervals and the start time for all kinetic windows. If the defined interval time for the last interval (in the last kinetic window) is higher than the minimum interval time, the calculated total measurement time per well will show a value slightly too high, since the reader, when the last interval is finished, will not wait for the defined interval time before it goes on to the next well. (The total measurement time calculated by the reader will always be correct.)

4.3.11 Layout Definition

When editing a 384, 864 or 1536 well layout, 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 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.

This sheet contains a grid representing the wells of the microplate that has been selected. Define wells containing samples, blanks, standards and controls.

Replicates

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

Methods of Labeling (Auto Fill Function)

There are several ways to label the plate:

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. '384') in the top left corner.

Notes: It is possible to **export** and **re-import** a complete layout (containing also the standard concentration values, see chapter 4.3.14) using the context menu of the layout grid (right click on the grid). Exporting only the layout grid into an Excel (.xls / .xlsx), text or HTML file is also possible.

To **undo** the last 5 layout changes use the context popup menu or use the key combination [Ctrl]+[Z].

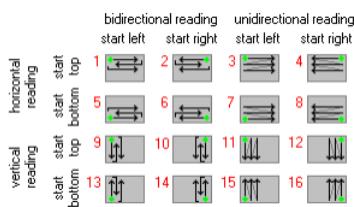
Reading direction

After pressing the reading direction button, 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**.

Possible reading modes:

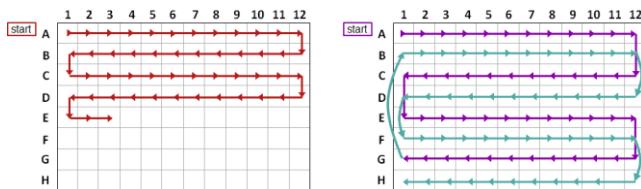


Note: The reading direction has no bearing on the measurements; it is intended to optimize the time it takes to read the plate.

4.3.12 Interlaced Reading Mode

When using a CLARIOstar Plus, VANTAstar or VANTAstar F reader it is possible to select an interlaced reading mode for luminescence well mode and for AlphaScreen protocols to reduce cross talk effects.

If this option is selected for **AlphaScreen** protocols first only every second row is measured (purple marked in the right picture below) and afterwards the other rows (teal marked).

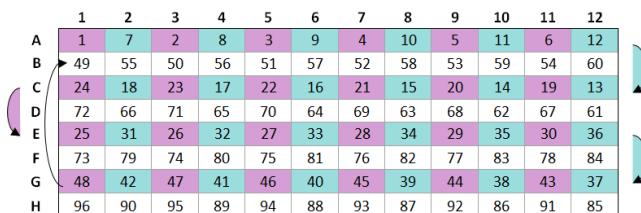


Reading mode 1 with (left) and without (right) interlaced option

When using a reading mode 9 ... 16 the measurement is performed with interlaced columns instead of interlaced rows.

Note: AlphaScreen measurements are not available when using a VANTAstar or VANTAstar F reader.

If the interlaced option is selected for **luminescence** protocols a double interlaced mode (row + column interlace) will be used.



Reading order for well mode measurements in luminescence (reading mode 1 with interlaced option)

4.3.13 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).



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

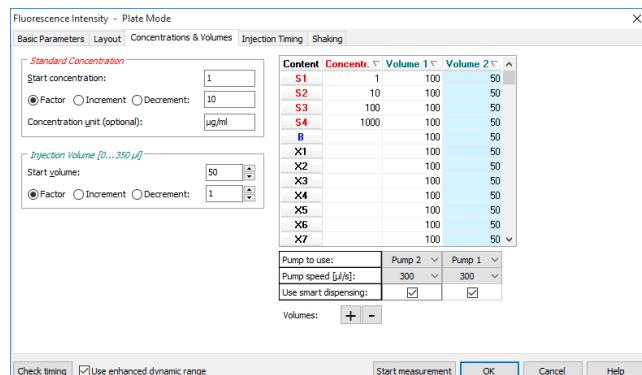
Notes: When switching on the group mode, all existing layout entries will be converted to group A.

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

4.3.14 Concentrations & Volumes

To define the standard concentrations and the injection volumes go to the '**Concentrations & Volumes**' tab sheet. In this tab a table that lists contents and their reference numbers is shown. 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 and volumes for injection can be entered manually into each space or by using the auto fill function.



The **Auto fill 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 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’. It is also possible to select a specific set of wells by finding the first (last) well and clicking and scrolling down (or up) with the left mouse button or using the cursor keys [\downarrow] and [\uparrow] together with [Shift]. The calculated values will automatically be entered into the table.

Standard Concentration

When using standards in the plate layout it is necessary to 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 MARS (see the separate MARS online help).

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

Injection Volume

It is possible to 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, it is also possible to use the auto fill function (see above).

It is possible to enter the volumes in steps of 0.5 μ l. If you prefer steps of 0.166 μ l (1/6 μ l = minimum step of the injectors) add the following line to the [Configuration] section of the ‘CLARIOstar.ini’ file: MinVolumeStep=6, for minimum steps of 1 μ l use MinVolumeStep=1. Use the key combination [Shift]+[Ctrl]+[I] from the main screen of the Control software to open the CLARIOstar.ini file. If the section [Configuration] does not yet exists, please add it to the end of the file.

The up / down buttons for the start volume and increment / decrement volume input box will also work using this step size, unlike you use these buttons together with [Ctrl] (= 1 μ l steps) or with [Shift] (= 10 μ l steps). When the factor option is used, these buttons will work in 0.1 steps.

Limitations for Injection

The maximum amount per injection is 500 μ l (6, 12, 24 and 48 well plates), when using a standard 500 μ l syringe or a larger syringe. When using a 250 μ l syringe or a 100 μ l 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 μ l for 96 well plates and 100 μ l for 384 well plates respectively. Injection is not possible when using 864 or 1536 well plates.

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

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 μ l / second. The default speed is 300 μ l / second which should be appropriate for most assays. There are pre-defined speeds available by using the pull-down menu.

Factors determining pump speed:

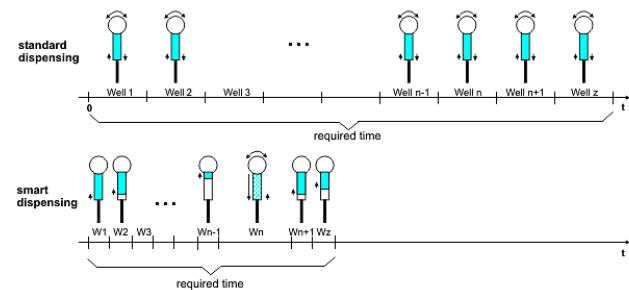
Viscosity: Liquids, such as water and buffer, can be dispensed at higher speeds. For highly viscous solutions you should use a slower speed to ensure higher precision and lower the risk of air bubbles.

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 μ l) to ensure the best performance.

Use smart dispensing

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



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

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

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

For well mode protocols smart dispensing can only be used in combination with an injection needle holder H3, but never for absorbance measurements (due to the different measurement position).

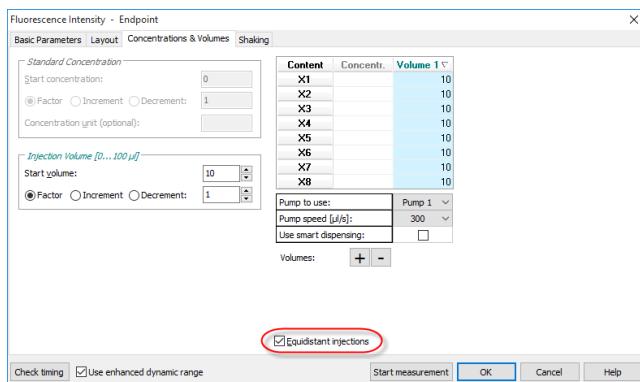
Shaking after Injection without Injection

If a shaking is needed at a certain time for a well mode protocol, but no injection at this time, it is possible to use any of the four volume groups. Please select inside the 'Concentrations & Volumes' tab the entry "Shake only" in the 'Pump to use' pull down box. The shaking duration can then be specified inside this tab (see chapter 4.3.19). The start time this shaking procedure can be defined in the 'Injection Timing' sheet (see chapter 4.3.15– 4.3.18).

Note: It is necessary to prime all used pumps before performing a test with injections (see chapter 5.3).

4.3.15 Injection Timing – Endpoint

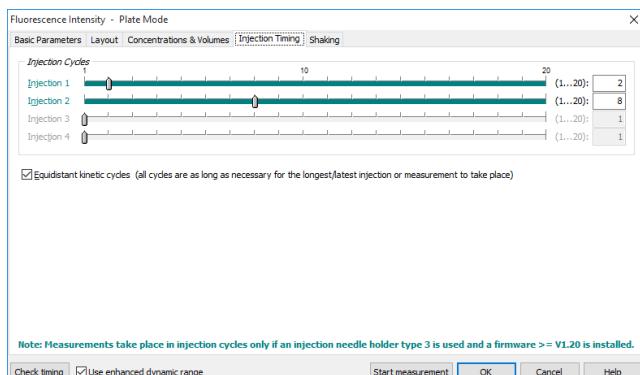
When using injections in endpoint test protocols, these injections happen before the measurement. One invisible injection cycle per injection group will be performed before the measurement.



As long as the option **Equidistant injections** is selected, the software will ensure that the injections and the measurements are equidistant (same time difference between injection and measurement for each well). When this option is not selected, the measurement can be performed faster than the injections. There are no other injection timing parameters for endpoint protocols, therefore, a separate Injection Timing tab does not exist for these protocols.

4.3.16 Injection Timing – Plate Mode

If reagent dispensers are installed in your reader, it is possible to define injection timing parameters in this tab sheet. In plate mode define in which cycle injections should be performed.



Injection Cycles

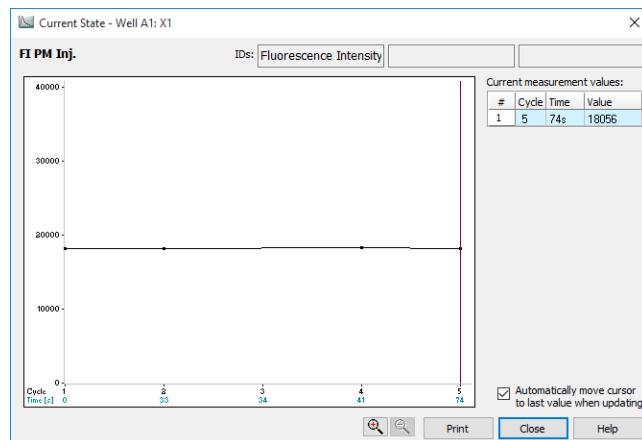
The cycles, in which the injection of volume group 1...4 will be performed. The defined cycle numbers cannot be greater than the number of cycles defined.

Equidistant kinetic cycles

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

Note: Only the input elements for volume groups, where a pump is defined in the corresponding layout definition, are available (see chapter 4.3.14).

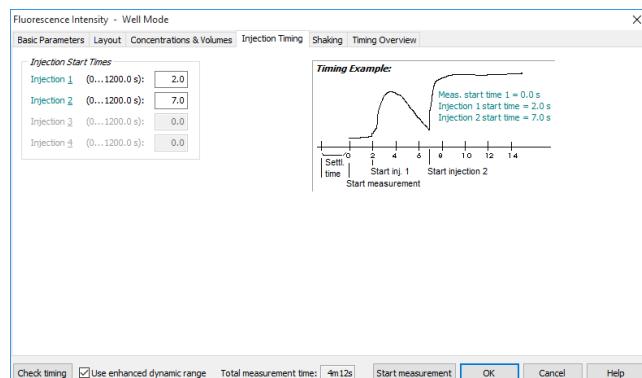
As the reader use different positions for measurement and injections (besides when using the needle holder type 3), simultaneous injections and measurements are not possible. Therefore, in injection cycles no measurement takes place. This means, for example, if a protocol is defined with 5 cycles and an injection in cycle 3, there will be no measurement values created in this cycle. In this example only 4 measurement values per well will be generated:



To use both pumps at the same time an injection needle holder type 2 needs to be installed.

4.3.17 Injection Timing – Well Mode

In well mode it is possible to define the injection start times using the Injection timing tab.

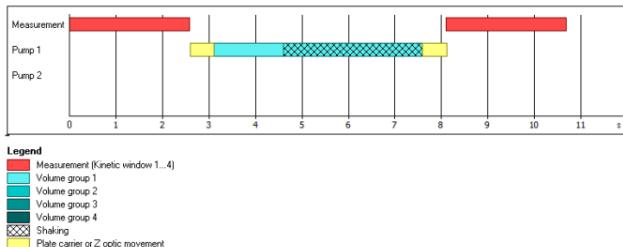


Injection Start Times

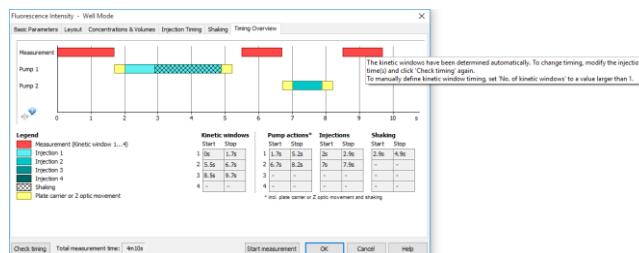
Defines the time the injection for volume group 1, 2, 3 or 4 is initiated, relative to the time when the measurement position is reached, plus settling time. The time can be before the beginning of the measurement (Measurement start time > Volume group X injection time), it can be during or after the measurement.

Note: Only the input elements for volume groups, where a pump is defined in the corresponding layout definition, are available (see chapter 4.3.14).

Due to the fact that the measurement position is not located at the same place as the injection positions, injection and measurement cannot take place at the same time. 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. When using **well mode** this takes place for each well. During this movement times (yellow bars in the picture below) no other actions can take place.



If only one kinetic window has been defined, the "Auto kinetic windows" feature will be active. This means the defined measurement range will be intermittent during the injection time:

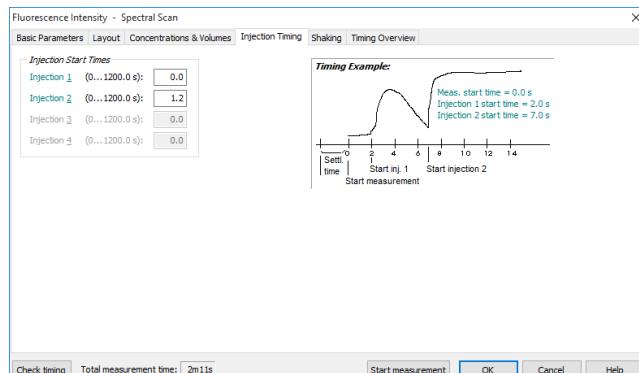


When using this Auto kinetic windows feature it is not possible to change the timing by dragging the measurement bars, but it is possible to change the timing of the injections - either by dragging an injection bar or by changing the injection start times inside the Injection timing tab. After changing an injection time click 'Check timing' to update the timing overview display.

Defining the kinetic windows (see chapter 4.3.10) manually is possible after activating the advanced mode inside the basic parameters tab.

4.3.18 Injection Timing – Well Scan / Spectral Scan

In well scan and spectral scan mode it is possible to define the injection start times using the Injection timing tab.

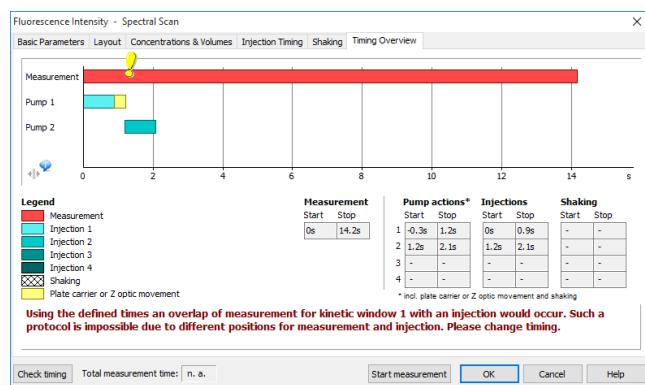


Injection Start Times

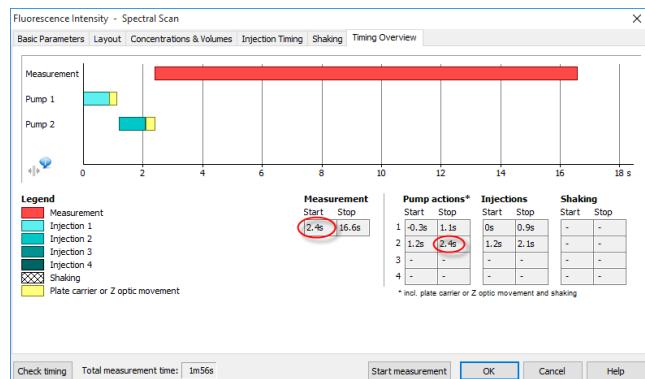
Defines the time the injection for volume group 1, 2, 3 or 4 is initiated, relative to the time when the measurement position is reached, plus settling time. The time can be before the beginning of the measurement (Measurement start time > Volume group X injection time) or after the measurement.

Note: Only the input elements for volume groups, where a pump is defined in the corresponding layout definition, are available (see chapter 4.3.14).

Since the measurement position is not located at the same place as the injection positions, injection and measurement cannot take place at the same time. 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. When using spectral scan (see chapter 4.3.4) mode this takes place for each well. During this movement times (yellow bars in the picture below) no other actions can take place. After clicking the **Check timing** button, the **Timing Overview** tab becomes available:



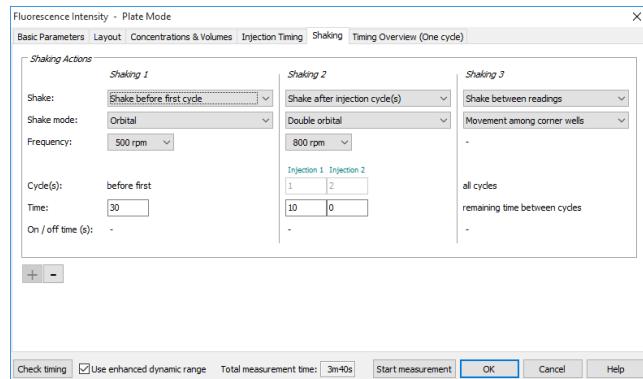
To avoid such an overlap, define, for example, a measurement start time equal or larger than the stop time of the latest pump action. This can be done either by moving the measurement bar (drag and drop) or by specifying the measurement start time inside the basic parameters sheet (switch on the advanced mode there; see chapter 4.3.4).



Note: To use both pumps at the same time with a CLARIOstar reader an injection needle holder type 2 needs to be installed. Simultaneous injections are not possible using VANTAstar readers.

4.3.19 Shaking

To define shaking actions, go to the 'Shaking' tab sheet.



Shake

When defining endpoint or well mode protocols shaking can be performed **before plate reading**. For well mode protocols also a shaking **after injections** is possible. In well mode this shaking happens immediately after each injection, which means for each well used.

When defining plate mode protocols shaking can be performed **before each cycle**, **before the first cycle**, **before defined cycle(s)** or **after injection cycle(s)**. In addition, it is also possible to **shake between readings**. This shaking action will happen during the idle time of each cycle. E.g., if the measurement lasts 10 seconds and the cycle time is defined as 60 seconds, there will be a shaking for 50 seconds.

Notes: When opening a test protocol, which was defined with an older software version, options like 'shake after first cycle' or 'after each cycle' might be defined. For compatibility reasons these options will not be changed. When defining a new test protocol use 'shake before defined cycle: 2' or 'before each cycle'.

When using the option to shake before defined cycle(s) it is possible to specified up to 4 cycles. Enter '-' if less than 4 cycles should be used.

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.



Movement among corner wells: The plate carrier will move between the 4 corner wells (linear X and Y movement). This option is only available when shake between readings has been selected.

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.

Cycle(s)

When using the **shake before defined cycle(s)** option it is possible to enter up to 4 cycles, before which the shaking should occur.

Time

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

On / off time

These options will become available when using a shaking between reading. If a value larger than 0 is entered as on time, the shake movement will be interrupted periodically. Define as on time the time of the active phase for the intermittent movement. Define as off time the time of the pause phase for the intermittent movement, e.g. use 20 s shaking and 40 s pause.

Notes: To use on / off times other than 0 a firmware version 1.40 or newer is necessary.

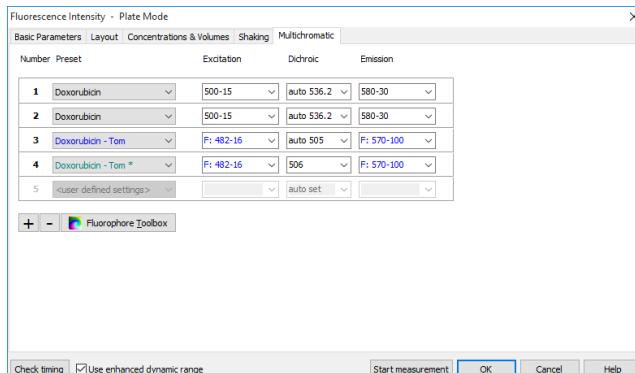
Shaking between reading will only become active if the idle time is larger than 15 s.

Shaking after Injection without Injection

If a shaking is needed at a certain time for a well mode protocol, but no injection at this time, it is possible to use any of the four volume groups. Please select inside the 'Concentrations & Volumes' tab the entry "Shake only" in the 'Pump to use' pull down box. The shaking duration can then be specified inside this tab (see chapter 4.3.19). The start time this shaking procedure can be defined in the 'Injection Timing' sheet (see chapter 4.3.15– 4.3.18).

4.3.20 Multichromatic Measurements

When you choose to analysis more than one fluorophore per well, you must define filter settings for each fluorophore. You can use the monochromator or filters or a mix of these. First enter the number of fluorophores to be analyzed under 'No. of Multichromatics' in the 'Basic Parameters' sheet. Then click on the 'Multichromatic' tab to switch to the following sheet:



Here you can use the pull-down boxes in each row to select a preset or to select a filter or just type in the monochromator wavelength and bandwidth. The filters shown in the pull-down boxes are listed as they are defined in the 'Settings | Filters' function (see chapter 3.1.9).

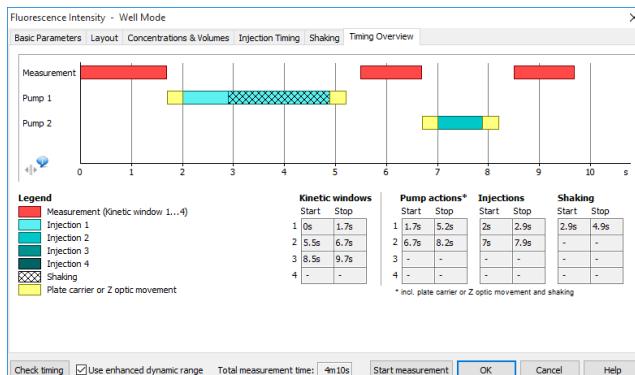
The number of chromatics can also be changed inside this tab using the **[+]** **[-]** buttons.

When defining fluorescence intensity or luminescence protocols the Fluorophore Toolbox can be accessed using the Fluorophore Toolbox button.

See chapter 4.4.1 for multichromatics / multitimings parameters for time resolved fluorescence protocols.

4.3.21 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 sheet will become available:



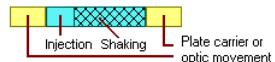
In this sheet, a graphic overview of the measurement and all injections for one well is displayed. (For plate mode tests, all actions are displayed in the timing overview of one cycle. The displayed injections may occur in different cycles, as defined under 'Injection Timing'. The cycle used will be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

It is possible to 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). When the auto kinetic window option is used (well mode protocols), you can only move the injection bars. The measurement (kinetic) windows will here be determined automatically (click 'Check timing' again after moving a bar).

An injection action can contain different steps:



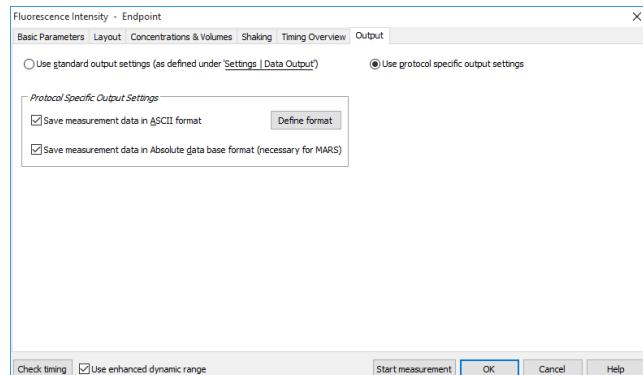
Note: The pump action times listed include the time for shaking and for plate carrier and optic movement *.

- * Depending on the setting of an EEPROM parameter and on the focal height used the measurement optic will be moved up before an injection and down again afterwards to avoid contamination of the optic.

Injections are not possible during shaking.

4.3.22 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:



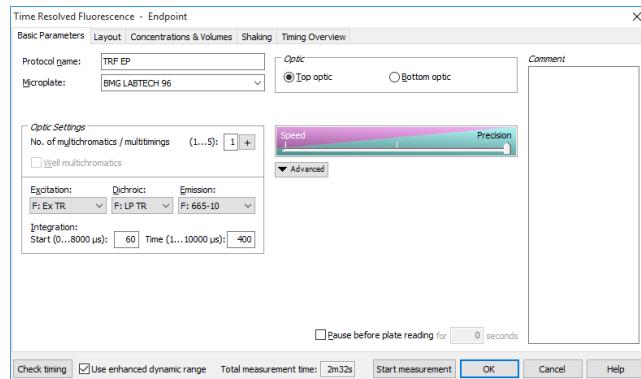
Here you can choose between using the standard output settings and using protocol specific output settings.

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 involves a delay time between flash and measurement.

Time resolved fluorescence can be measured in endpoint, plate or well mode. Well or spectral scan is not possible in time resolved fluorescence mode. It is possible to perform multichromatic / multitimings measurements. Most parameters are similar to fluorescence intensity protocols (see chapter 4.3).



Optic Settings

No. of multichromatics / multitimings

There is the possibility to analyze 5 fluorophores or to use 5 different integration windows in one test protocol. Enter the number of fluorophores to be analyzed, then click the arrow button or on the 'Multichromatic' tab to define the settings for each chromatic (see chapter 4.4.1).

Excitation / Dichroic / Emission

Select filters appropriate for the assay here. The pull down boxes show all filters which have been installed and defined using the Filters dialogue box (see chapter 3.1.9). The use of the monochromator is not possible using this measurement method.

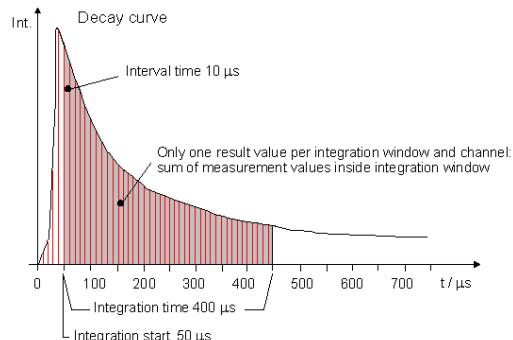
Integration start

Enter the time after the flash when the measurement should begin.

Integration time

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

The measurement values of the selected integration range of the decay curve will be integrated by the reader. There will be one (raw) measurement value per well transmitted from the reader.



Example of a measurement

4.4.1 Multichromatics / Multitimings for Time Resolved Fluorescence

Using the Multichromatics / Multitimings function, you can determine the optimal integration time for a time resolved fluorescence assay. Using one time resolved fluorophore, you can define to use the same filters for all 5 chromatic settings and define 5 different integration times. You can keep the integration time constant and vary the integration start time in order to find the optimal start time or you can keep the integration start time constant and vary the integration time to find the optimal measurement time.

Time Resolved Fluorescence - Plate Mode					
Basic Parameters Layout Concentrations & Volumes Shaking Multichromatic / Multitiming Timing Overview (One cycle)					
Number	Excitation	Dichroic	Emission	Integr. start (0...8000 μs)	Integr. time (1...10000 μs)
1	F: Ex TR	F: LP TR	F: 615-18	60	300
2	F: Ex TR	F: LP TR	F: 615-18	60	400
3	F: Ex TR	F: LP TR	F: 615-18	60	500
4				60	400
5				60	400

+ -

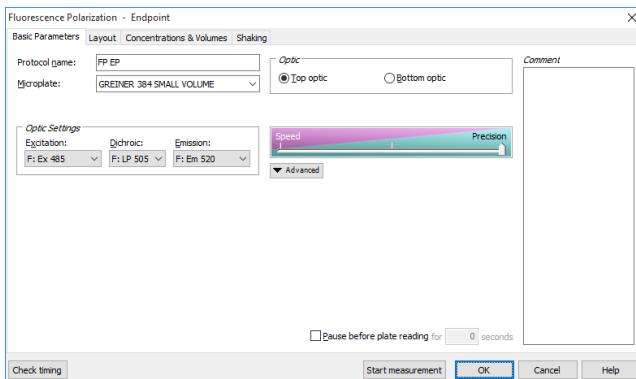
It is also possible to use totally different integration windows or to use different filters to measure different wavelengths.

4.5 Fluorescence Polarization Protocols

In fluorescence polarization mode, two light channels need to be measured. Channel A measures parallel polarized light and channel B measures perpendicular polarized light. The CLARIOstar and VANTAstar readers measure these channels sequentially.

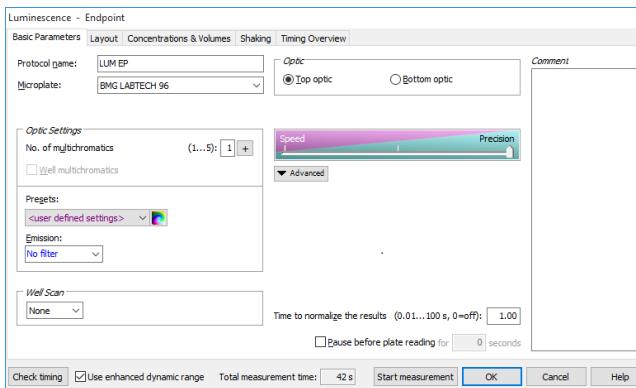
Fluorescence polarization can be measured in endpoint, plate or well mode. Multichromatic measurements are not possible.

Most parameters are similar to fluorescence protocols (see chapter 4.3).



4.6 Luminescence Protocols

Luminescence can be measured in all reading modes (endpoint, plate mode, well mode and spectral scan mode). Multichromatic measurements are possible. Most parameters are similar to fluorescence intensity protocols (see chapter 4.3).



Optic Settings

Presets

The BMG LABTECH software contains a library of commonly used luminophores. Select either one of the presets from the luminophore preset drop down list or use the option '**<user defined settings>**'. When selecting a preset the necessary monochromator setting will be set automatically. It is possible to modify this setting here or in the Fluorophore Toolbox (click the  button, see chapter 4.3.5). When using a modification of the pre-defined wavelength setting, the preset name will be shown in **purple** color and marked with a star.

Optic Settings

Excitation / Dichroic / Emission

Select filters appropriate for the assay here. The pull down boxes show all filters which have been installed and defined using the Filters dialogue box (see chapter 3.1.9). The use of the monochromator is not possible using this measurement method. The polarization filters necessary to measure the two channels are selected automatically.

Settling time

The settling time in fluorescence polarization tests is recommended to be at least 0.3 seconds, so that the surface of the liquid is stable before measurement.

No. of flashes

For fluorescence polarization protocols up to 500 flashes can be used. BMG LABTECH recommends 50 for accurate results.

Emission

The emission filter / monochromator is used to select the light emitted by the sample.

To use the monochromator simply type in the desired wavelength in nm. The monochromator can be used in the wavelength range from 320 nm to 730 nm. When an extended IR PMT is installed wavelengths up to 840 nm can be selected. For the monochromator the bandwidth can also be defined (separated from the wavelength by a hyphen). If you do not enter a bandwidth value, a standard value will be entered automatically (20 nm). The bandwidth can be defined between 8 and 100 nm.

Alternatively use the pull down boxes to select any of the filters which have been installed and defined using the Filters dialogue box (see chapter 3.1.9). Filter entries are preceded by '**F:**' and displayed in **blue**. Alternatively select '**No filter**' to measure all emitted light wavelength independent.

Note: As VANTAstar F readers are not equipped with a monochromator, there is no Presets pull down box available when using such a reader. Only filters can be used.

Settling time

At least 0.1 seconds are recommended for luminescence measurements.

Measurement interval time

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

Interval time (only in well mode)

You can define the duration of each interval from 10 ms 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 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 change the filters or the monochromator settings.

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 which is shorter than the minimum interval time, it will automatically be corrected to the shortest possible time.

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.

Time to normalize the results

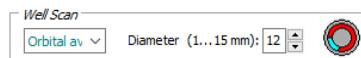
As the measurement values depend on the measurement interval time, it is possible to normalize the measurement values. This will allow the user to compare the measurement values of test runs using different measurement interval times. To switch off the normalization enter 0 here.

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

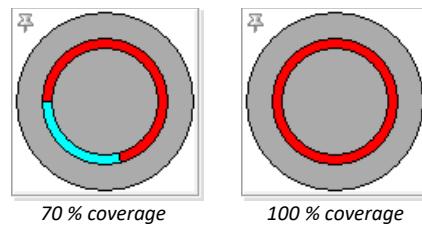
4.6.1 Orbital and Spiral Averaging for Luminescence Protocols

Orbital Averaging

Using this mode, the measurement takes place on an orbit with a definable diameter. Using the luminescence measurement method, 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 matrix well scanning, see chapter 4.3.8).



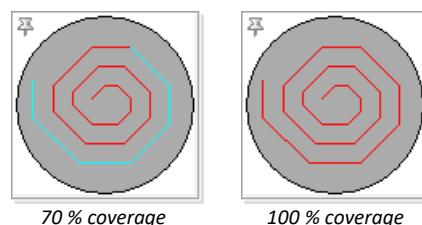
As the time necessary for one orbital movement only depends on the selected diameter, the measurement may occur only during a part of the orbital movement. The small icon in the orbital averaging box will show you which part of the orbit is used for the measurement.



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.

Spiral Averaging

Instead of orbital averaging it is also possible to use spiral averaging. Here instead of one orbital movement a spiral movement will be used. This covers a larger part of the well, but also last longer.

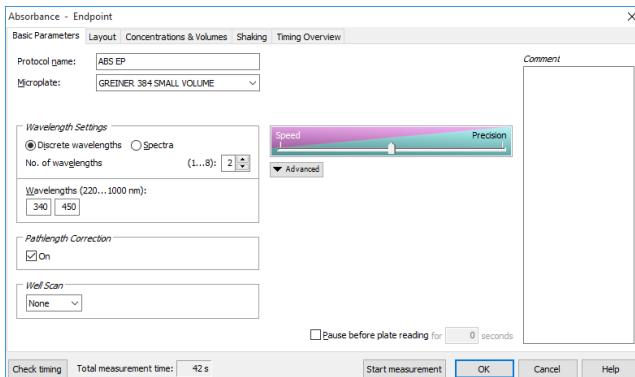


Notes: The defined settling time occurs only once for each well in orbital and spiral averaging mode (opposite to matrix well scanning, see chapter 4.3.8).

To use spiral averaging a firmware version 1.20 or newer is necessary.

4.7 Absorbance Protocols

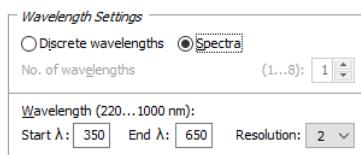
Absorbance test protocols can be performed in all reading modes. Multichromatic measurements are possible. Besides measuring discrete wavelengths, you can also measure whole spectra (however, this is only in endpoint and plate mode possible), see chapter 6.9.4). Most parameters are similar to fluorescence intensity protocols (see chapter 4.3).



Wavelength Settings

Specify here **up to 8 discrete wavelengths** to be measured.

Alternatively, it is also possible to measure whole **spectra** (for endpoint or plate mode protocols). For spectra measurements, 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, only measure a targeted wavelength range.

Pathlength Correction

By applying this function, the absorbance values will be normalized to unit pathlength (1 cm) using a water peak-based correction method.

This correction might not be appropriate in case of:

- Measuring samples with a significant amount of organic solvents (e.g. > 20 % DMSO).
- Measuring samples whose absorption is in the near IR wavelength range (e.g., Lowry assay).
- Measuring turbid samples (e.g., microbial growth assays measuring the OD at 600 nm).
- Measuring wells whose sample volume is far below the recommended working volume of the microplate or which are empty.

Note: The pathlength correction box is not available when using the BMG LABTECH LVis plate. Here the pathlength values determined using the LVis Pathlength 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.

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.

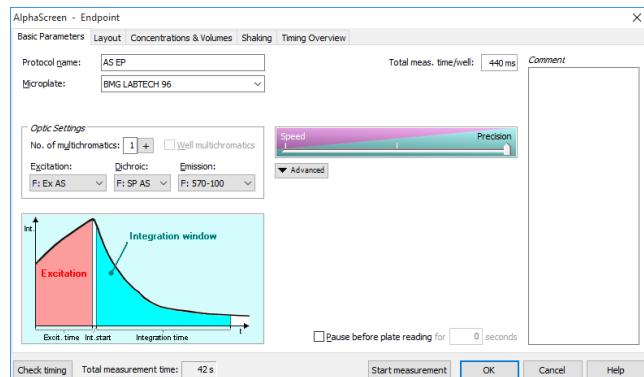
4.7.1 Sequential Blanking

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*).

4.8 AlphaScreen Protocols

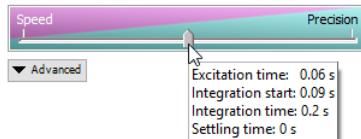
In AlphaScreen™ (Amplified Luminescence Proximity Homogeneous Assay) mode the samples are excited at a wavelength of 680 nm for a defined time (excitation time) and the emission in the range of 520 nm to 620 nm is measured. Well scanning and multichromatic or kinetic measurements are not possible in AlphaScreen mode. Most parameters are similar to fluorescence intensity protocols (see chapter 4.3).

AlphaScreen measurements are not available when using VANTAstar or VANTAstar F readers.



Speed and Precision

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



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



Excitation time

Defines how long there should be excitation light.

Integration start

Defines the time when the measurement (integration) of the emission light should begin. The best results are usually achieved if the integration of the emission light starts approximately 40 ms after the end of the excitation, but nevertheless, it is also possible to start the integration already during the excitation phase.

Integration time

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

Notes: Longer excitation and longer emission (integration) times result in higher measurement values.

It does not make sense to define an excitation time which is higher than the integration end time (Integration start + Integration time).

5 Incubation / ACU / Reagent Dispenser

5.1 Incubation

5.1.1 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 5.1.2 *Temperature Monitoring Feature* below).

Enter the temperature manually or select 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 color remains red until the selected temperature is reached, then it turns green.

It is possible to perform a measurement before the target temperature is reached. In the BMG LABTECH Data Analysis software MARS (see software manual part III) the current temperature for each kinetic point during the measurement can be displayed.

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

5.1.2 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 is cyan. The temperature will also be stored with the measurement data and displayed in the Data Analysis software.

5.1.3 Auto Power On Incubation

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

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

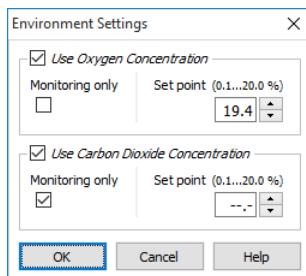
By using a target temperature of 00.1 °C (selected e.g., by clicking the 'Temperature monitoring without incubation' button), only the temperature monitoring function is switched on by default, the heating plates will not be used.

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.

5.2 ACU Control

 The Environment Settings dialogue can be opened by selecting the 'O₂' or 'CO₂' concentration menu command.

These menu commands are only available when an ACU (Atmospheric Control Unit) is attached to the reader. The ACU provides a complete solution to fully regulate and independently control both oxygen and carbon dioxide gas levels within the microplate reader chamber.



The control range for oxygen is 0.1 % to 20 % (CLARIOstar readers) respectively 1 % to 20 % (VANTAstar readers). The control range for carbon dioxide is 0.1 % to 20 %. The target concentrations can be set in 0.1 % increments. Enter the concentration values manually or select the desired values using the arrow buttons.

The concentration values can also be monitored without activating the regulation.

Click on 'OK'. The concentration display in the toolbar of the control software will be activated. The indicator color remains red until the selected concentration is reached, then it turns green. It is possible to perform a measurement before the target concentration values are reached. In the BMG LABTECH Data Analysis software MARS the current concentration values for each kinetic point (plate mode) or each well (well mode) during the measurement can be displayed.

When using the 'Monitoring only' option, the display color is cyan. The concentration values will also be stored with the measurement data and displayed in the Data Analysis software.

Click 'Cancel' to close this dialogue without applying any changes.

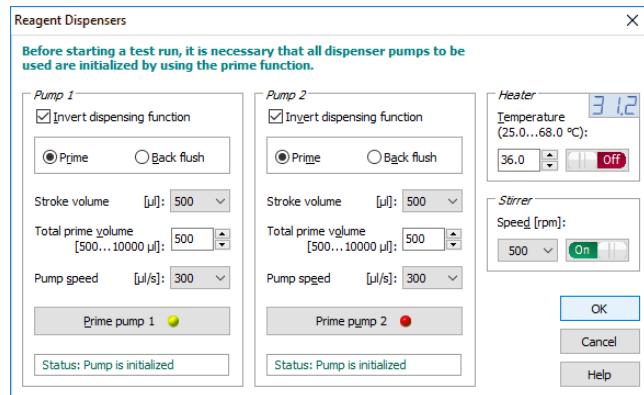
Note: Due to technical reasons, it will take a few seconds after switching on the ACU until the first concentration values are shown.

5.3 Reagent Dispensers

The reader may have up to 2 reagent dispenser pumps. Before starting a test run, it is necessary that all pumps to be used are initialized by using the prime function. Priming fills the tubing and needles of the reagent system with solution.



To open the prime window, use the ‘Prime’ (CLARIOstar) or the ‘Reagent Dispensers’ (VANTAstar) menu command.



Prime Pump 1 / Pump 2

Invert dispensing function

If this option is not selected (= standard 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 then 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.

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 conservation expensive reagents, since the entire syringe can be emptied after use.

Stroke and Prime volume

The volume of the syringe can be 100 µl, 250 µl, 500 µl, 1 ml or 2.5 ml. The **total prime volume** can be selected in steps of the defined stroke volume. A total 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.

To save on reagents the priming can also be performed with **stroke volumes** smaller than the syringe size. Besides using 100% of the syringe size (e.g., 500 µl) also a volume equal to 50% (e.g. 250 µl), 20% (e.g. 100 µl) or 10% (e.g. 50 µl) of the syringe size can be used. This feature is only available when using invert dispensing. A firmware version 1.20 or newer is necessary to use this feature.

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’ sheet of the test protocol definition window (see chapter 4.3.14).

Priming Procedure

To start priming, place the injection needle holder over a waste container, a compound solution into the compound vessel, and insert the tubing into the solution. Priming is then initiated either via the control software or by a double click of the respective color-coded priming button.

Priming is also used to clean the tubing and to check that the solution comes straight out of the needle tips. If the fluid stream comes out at an angle this indicates that some debris is partly clogging the needle tip. In this case, cleaning of the tubing and the needle is required. Refer to the CLARIOstar or VANTAstar Operating Manual for the cleaning procedure.

End priming by re-placing the needle holder in its operating positions. Close the lid before executing a measurement run.

Please refer to Operating Manual for a detailed description.

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

It is also possible to prime the pumps without using the software. After opening the reagent door, you will see two buttons. To prime a pump double click the respective button (see Operating Manual for details).

Heater

Using this box, a target temperature for the reagent dispenser module can be defined and the heater can be switched on and off.

Stirrer

Using this box, the speed for the stirrer of the reagent dispenser module can be defined and the stirrer can be switched on and off.

Note: The heater and stirrer features are only available when using a VANTAstar or VANTAstar F reader with attached reagent dispenser module.

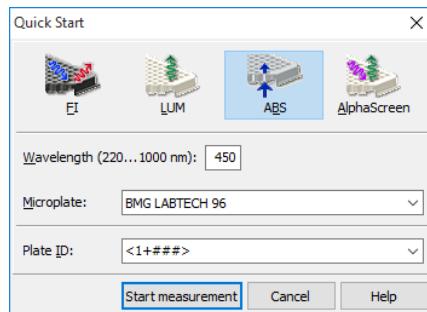
6 Microplate Measurements

6.1 Quick Start

The ‘Quick Start’ function can be used to measure a plate without defining a test protocol using the fluorescence intensity, luminescence, absorbance, or AlphaScreen method. Using this function the complete plate will always be measured as an endpoint test. Please use the standard measurement method based on pre-defined protocols (see chapter 4) to measure only a part of the microplate, to perform a kinetic measurement, to use multichromatics, for shaking, or to measure using the fluorescence polarization or time resolved fluorescence method.



After clicking the ‘Quick Start’ button the following dialogue will appear:



Select the **measurement method** first by clicking the appropriate button.

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

The **preset (or filter / wavelength)** and **microplate** selection is specific for the measurement method.

It is possible to add one **plate identifier**. Here use the same special functions as when executing a pre-defined protocol (see chapter 6.5).

When using the Quick Start function of the LVis Plate tab it is possible to 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 using the blank measurement function in the LVis Plate menu group (see chapter 7.2.3).

CLARIOstar: After clicking ‘Start measurement’ an automatic gain adjustment and a focal height adjustment will be performed for fluorescence intensity measurements. In luminescence mode a fixed gain of 3400 and a focal height of 10 mm will be used.

CLARIOstar Plus: A focal height adjustment (Auto Focus) will be performed for fluorescence intensity measurements. For luminescence measurements a focal height of 10 mm will be used. For both measurement methods the enhanced dynamic range feature will be used (see chapter 6.4).

VANTAstar and VANTAstar F: A focal height adjustment (Auto Focus) will be performed for fluorescence intensity measurements. For luminescence measurements the automatic focus mode will be used. For both measurement methods the enhanced dynamic range feature will be used.

All reader types: When using the absorbance method, the gain settings will be determined automatically during the measurement. For AlphaScreen measurements, a fixed gain of 3600 and a focal height of 12 mm will be used.

AlphaScreen measurements are not available when using VANTAstar or VANTAstar F readers.

As soon as the measurement is started, measurement values can be viewed in Current State display (see chapter 6.9). Also possible is to set up the program to automatically open the Current State display (see chapter 3.3.2).

Notes: For Quick Start measurement, a settling time of 0.1 s will be used. Fluorescence intensity measurements will use 10 flashes per well, absorbance measurements will use 20 flashes per well. For luminescence measurements, a measurement interval time of 1 s will be used. For AlphaScreen measurements, the excitation will last 0.3 s, the integration will start at 0.34 s and will last 0.3 s.

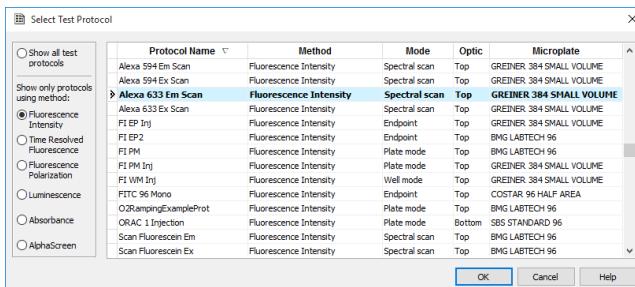
6.2 Executing Pre-Defined Test Protocols



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 the defined protocols.



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

Additional filtering is possible (by detection mode / measurement method) by clicking one of the radio buttons to the left side of the dialogue box.

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

After selecting the protocol, the next window, which appears after clicking OK or double clicking the protocol name, allows to define the focus and dynamic range mode / to perform a focus and gain adjustment, addition of identifiers to the test run, definition of sample IDs and dilution factors, and the option to start the measurement. (see next chapters).

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

6.3 Focus and Dynamic Range

Start Measurement - Lumi Plate Mode

Focus and Dynamic Range / Plate IDs Sample IDs / Dilution Factors Crosstalk Determination

Change layout

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

Focus: New focal height...

Dynamic range: Fixed range (gain)...

Monochromator / Filter Settings Gain
1 No filter 3600

Focus Adjustment
Focal height (0...25.0 mm): 15.0

Gain Adjustment
Target value: 90%
 Selected well(s) Full plate

Raw result: 230951

Status: Ready

Plate Identification

ID1: ID2: ID3:

Automatically enter the plate IDs previously used with this protocol

No. of executed runs since program start: 0 Total no. of executed runs: 304

Run statistics:

Needle holder H2 and aperture 96/384 recommended

Delay: 0 s

The height adjustment for the focal point of the optical system ensures the best signal-to-noise ratio for every plate, every application and every volume.

The purpose of a gain adjustment is to optimize the signal amplification for maximum sensitivity. The gain adjustment is usually performed on the well with the standard of highest concentration. This sets the gain of the PMT (photo multiplier tube) of the reader so there is no overflow in the most intense well. (An overflow means the signal of a well exceed the maximum range, i.e. 260,000 rfu.) When using a CLARIOstar Plus, VANTAstar or VANTAstar F reader it is possible to use the enhanced dynamic range feature for fluorescence and luminescence measurement methods (see chapter 6.4).

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

Zoom Feature

For 384, 864 and 1536 well formats, a zoom function is available. To zoom in and out of the screen use the zoom buttons.

6.3.1 Focus

Using this combo box, it is possible to choose between performing an **auto focus** before the measurement, to use the **focal height previously determined** (or a **default focal height**) or to define a **new focal height** by performing an automatic focal height adjustment (see below) or by entering a focal height value manually.

When auto focus is selected, the well with the highest signal will be searched and a focal height adjustment will be performed on this well before the measurement itself starts. Therefore, the use of this feature requires some extra time. There is an option to automatically switch from 'auto focus' to 'use previous focal height' after performing the auto focus once in the program configuration dialogue (to save this extra time when executing the same protocol using the same plate type with similar liquid levels consecutively) (see chapter 3.3.2).

Note: The auto focus feature is only available when using a CLARIOstar Plus, VANTAstar or VANTAstar F. Therefore, this combo box only exists when using such a reader.

6.3.2 Dynamic Range

Using this combo box it is possible to choose between **enhanced dynamic range (EDR)** (see chapter 6.4) and **fixed range (gain)**. When using the enhanced dynamic range feature the gain will be adapted automatically for each measurement point instead of using a fixed gain for the whole test run. When selecting fixed range the gain needs to be determined or entered manually (see below).

Notes: The enhanced dynamic range feature is only available when using a CLARIOstar Plus, VANTAstar or VANTAstar F. Therefore, this combo box only exists when using such a reader.

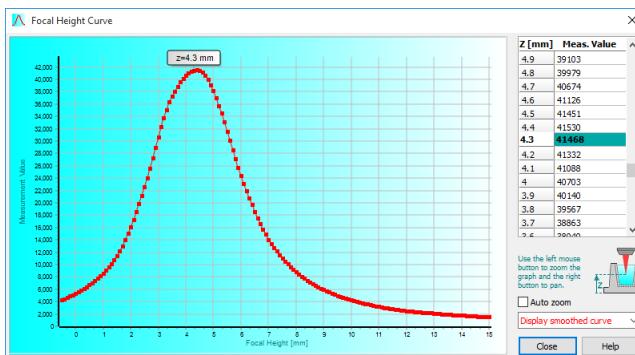
The enhanced dynamic range feature can only be used for fluorescence and luminescence measurements, but not for spectral scan protocols. It can also not be used if the test protocol uses the flying measurement mode or orbital/spiral averaging or if the timing does not allow it.

6.3.3 Focus Adjustment

To achieve optimal measurement results when using different microplate types and different liquid levels it is necessary to adjust the Z position of the measurement optics.

Note: The focus and gain adjustment section does not exist in absorbance mode. Absorbance measurements are performed using a spectrometer, and a focal height adjustment is not necessary.

If the ‘Focus Adjustment’ check box is selected, a focus adjustment procedure will be performed after clicking ‘Start Adjustment’. The instrument will measure the selected well at different heights. After the procedure has finished, the focal height, which shows the highest signal, will be automatically entered into the ‘Focal height’ input box. A curve showing the signal strength as a function of the focal height will be displayed inside the focus adjustment box. Click on the small graph to open a zoomed view of this curve:



The focal height (Z) values describe the distance between plate (carrier) bottom and focal point.

If the **Auto zoom** checkbox is not checked the scaling of the measurement value axis starts at 0, otherwise it starts at the lowest measured value. It is possible to change the size of this window and to zoom (using the left mouse button) and pan the graph (using the right mouse button). Double click the graph to return to a full curve display after zooming or panning.

By using the pull down box on the lower right part of the dialogue box, select a smoothed version of the focal height curve (calculated using a 5 point average), the original (unsmoothed) version or both curves.

The table on the left lists the measurement values at different focal heights.

Notes: It is possible to print out this curve display. Use the key combination [Shift]+[Ctrl]+[P] to open a print dialogue box. Choose portrait or landscape orientation. The image will be proportional scaled to fit on one page.

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

6.3.4 Gain Adjustment

In **absorbance** mode, the gain adjustment is automatic as part of the measurement, so this gain adjustment is not an option when using this method.

In **AlphaScreen** mode, automatic gain adjustment is not available, but the gain value can be manually changed.

In **fluorescence polarization** mode, the appearance of the gain adjustment box is different, please see chapter 6.3.6.

The monochromator / filter settings used in the test protocol selected are displayed in the table on the top left. If the protocol is multichromatic, then it is necessary to perform a gain adjustment for each chromatic (for each filter pair). Select the desired chromatic before clicking the ‘Start Adjustment’ button. When using a spectral scan protocol, it is possible to select the wavelength which should be used for the adjustment procedure. When using a fluorescence polarization protocol, there will be two columns in this table containing the gain values for channel A and B, otherwise there will be only one gain column.

Automatic Gain Adjustment for one Well

Select the well for gain adjustment. Start the automatic gain adjustment by clicking the button ‘Start Adjustment’. The instrument measures the well up to eleven times to find the optimal gain. The optimal gain value then appears in the table next to the monochromator / filter setting. It will also be stored in the test protocol.

Automatic Gain Adjustment for the entire Plate

When a gain adjustment for one well is not possible, for example, when the well with the highest intensity is not known, it is possible to perform a gain adjustment on the entire plate. Select the ‘**Full plate**’ radio button or click on the blue microplate format number in the top left corner of the layout. This selects all wells. Click the ‘**Start Adjustment**’ button. The instrument will measure all used wells and perform the gain adjustment using the well with the highest signal. The resulting gain value(s) will be displayed in the table on the top left of the sheet. The well which showed the highest intensity, will be selected after finishing the adjustment procedure.

Automatic Gain Adjustment using Selected Wells

Besides using one well or the entire plate it is also possible to perform the gain adjustment with a set of selected wells. To select more than one well hold down the [Ctrl] key and click onto the desired wells or select a range of wells by drawing a rectangle while holding down the left mouse button.

See also the chapter Target Value.

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. 500,000,000 for 100 s). To avoid an overflow, it is necessary to set the gain so that the values stay within the measurement range of the instrument.

In the Gain Adjustment group box you can specify 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.

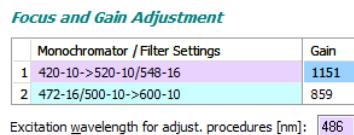
If the measurement is an endpoint test, then the target value can remain at the default value of 90%.

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 dynamic range for higher intensity values and to prevent an overflow.

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

6.3.5 Gain Adjustment for Spectral Scan Protocols

When a spectral scan protocol is used, a wavelength inside the scan range needs to be selected for the adjustment procedure. For an excitation scan select an excitation wavelength, for an emission scan select an emission wavelength. It is suggested to use a wavelength at around two thirds of the excitation scan range respective around one third of the emission scan range. These values will be calculated by the Control Software and entered automatically into the wavelength input box as a suggestion:



When executing a dual scan protocol (a protocol using an excitation and emission scan combination), it is necessary to perform the gain adjustment for both, the excitation and the emission scan. Select the scan by clicking the related table line, check the suggested wavelength and click 'Start Adjustment'. Repeat the procedure for the second scan.

It is recommended to use a target value of not more than 10% to prevent an overflow during the measurement of the full scan range.

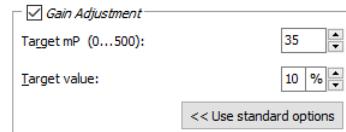
6.3.6 Gain Adjustment for Fluorescence Polarization Protocols

When a fluorescence polarization protocol is used, the gain adjustment box appearance is different compared to the other modes:



There are two measurements for each well necessary for measuring the fluorescence polarization, one for channel A (parallel polarized light) and one for channel B (perpendicular polarized light). The CLARIOstar and VANTAstar readers will perform the measurement of these two channels consecutively. As with standard fluorescence, perform the gain adjustment on the well with the highest concentration of free fluorophore. The relationship between channels A and B determines the fluorescence polarization value, so it is important that the two channels are optimized for the best results. Select the desired well and click on 'Start 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 target value (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 entered 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 polarization value for free fluorescein is 35 mP. Enter this value in the field 'Target mP'. If a different fluorophore or a labeled biomolecule is used, it is necessary to use the theoretical mP value of this molecule as 'Target mP'.

Subsequently, when a gain adjustment is performed 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 using a different fluorophore or a fluorescein-labeled peptide (bound fluorescein), the 'Target mP' value must be changed. If it is not known what this value should be, start with, for example, 35.

Perform a gain adjustment as described above.

Start the measurement. Use the current state display (see chapter 6.9) or the SMART Control data analysis software (see software manual part III) to check the measurement values. If the results are negative, then 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.

6.3.7 Performing the Adjustments

Start Adjustment

After clicking the 'Start Adjustment' button, all selected adjustment procedures are performed. It is possible to perform the focus and gain (selected well) determination simultaneously, but it is not possible to combine focus adjustment and a full plate (auto search) gain adjustment.

Stop Adjustment

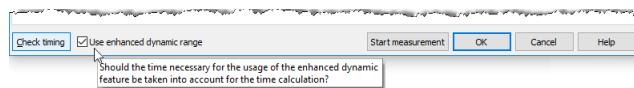
Use this button to terminate an ongoing adjustment procedure.

6.4 Enhanced Dynamic Range (EDR)

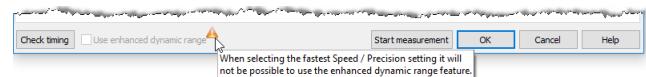
CLARIOstar Plus, VANTAstar and VANTAstar F readers offer an enhanced dynamic range feature in combination with fluorescence and luminescence measurement methods. When using this feature with fluorescence measurements the dynamic range (range of possible measurement values) is increased from 0 ... 260.000 up to 0 ... 700 million. For luminescence measurements the maximum count with EDR is 200 million per seconds measurement interval time instead of 10 million.

This measurement range enhancement is realized by adapting the gain of the PMT (photo multiplier tube) for each measurement point instead of using a fixed gain for the whole test run.

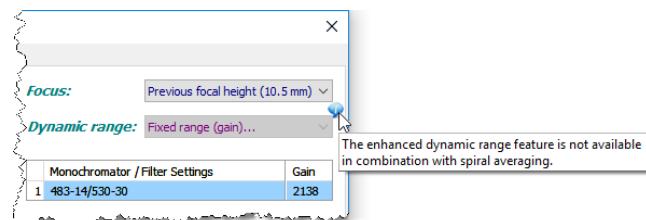
Executing a test run with the enhanced dynamic range feature might last a few seconds longer than executing the same test run with a fixed range (depending on the test protocol settings). Therefore, there is an option to take the time necessary for the usage of the enhanced dynamic range feature into account when using the Check timing function inside a protocol editor:



In combination with certain protocol settings (e.g. when using flying mode) the enhanced dynamic range feature cannot be used. In such cases a small icon providing the explanation will be shown inside the protocol editor:



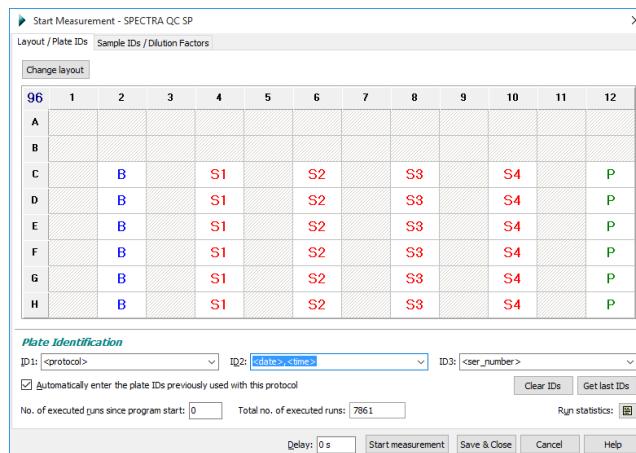
... and in the Start Measurement dialogue:



The enhanced dynamic range feature cannot be used when running spectral scan protocols.

6.5 Plate Identification

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.



Note: To see the plate ID options and the focus and dynamic range functions on separate tabs, define this using the Program Configuration dialogue (see chapter 3.3.2).

Several options for each identifier are available from the drop down menu for Plate IDs or descriptions may be typed in manually.

ID Options

<barcode_front>, <barcode_right>

Barcode attached to the front / right side of the current plate (this option will use the internal barcode readers of the CLARIOstar Plus and is, therefore, only available when using a CLARIOstar Plus reader).

<barcode> Barcode of the current plate (only available if the reader is connected 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). See also chapter 6.8.5.

<protocol>

Name of the used test protocol.

<method>

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

<1+#>

Automatically incremented numbers: The Plate ID for the first measurement run will contain '1', for the second run '2' and so on. Changing the start number is possible. Using <5+#> counting will start with 5. For each # one number will be inserted (<1+##> → '001'). The counting of the test run will start with 1 after each program start.

For a number to be changed only after, for example, every tenth plate, add this value in parenthesis before the > character, e.g. <1+##(10)>.

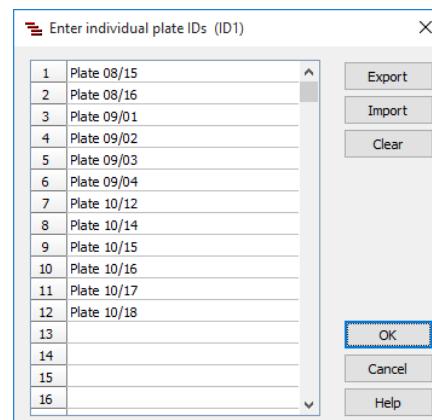
In batch mode (using a stacker connected to the reader) or in script mode (see chapter 8) add a 'B' before the '#' to get the plate numbers of the current batch run, e.g. <1+B###>.

To use the 'Total number 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##>. Run Statistics is shown after pressing the button.

In batch mode (using a stacker connected to the reader) or in script mode (see chapter 8) add a 'B' before the '#' to get the plate numbers of the current batch run, e.g. <1+B###>.

	Counting down is also possible, use a '-' instead of the '+'. <A+##> Same concept as consecutive numbers using the alphabet. <i>Example:</i> <A+##> → AAA, AAB, AAC ...																								
<date>	Current date Specifying the date format is possible by adding a format description after '<date:>'. Use yy or yyyy for the year, m or mm for the month and d or dd for the day: <table border="0"> <tr><td>yy</td><td>Year with two digits (1999 → 99, 2000 → 00)</td></tr> <tr><td>yyyy</td><td>Year with four digits</td></tr> <tr><td>m</td><td>One or two digits for the month (January → 1, December → 12)</td></tr> <tr><td>mm</td><td>Month with two digits (January → 01)</td></tr> <tr><td>mmm</td><td>Abbreviated name of the month (January → Jan.)</td></tr> <tr><td>mmmm</td><td>Full name of the month</td></tr> <tr><td>d</td><td>Day with one or two digits</td></tr> <tr><td>dd</td><td>Day with two digits</td></tr> <tr><td>ddd</td><td>Abbreviated name of the day (Monday → Mo.)</td></tr> <tr><td>dddd</td><td>Full name of the day</td></tr> <tr><td>ddddd</td><td>Date in the format defined as "Short Date Format" under windows ('Settings Control Panel Regional Settings')</td></tr> <tr><td>ddddddd</td><td>Date in the format defined as "Long Date Format" under windows</td></tr> </table> <i>Example:</i> <date:yyyy_mm_dd> If the format is not specified, the date format defined as "Short Date Format" under windows will be used.	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')	ddddddd	Date in the format defined as "Long Date Format" under windows
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')																								
ddddddd	Date in the format defined as "Long Date Format" under windows																								
<time>	Test run start time Specify the time format by adding a format description after '<time:>': <table border="0"> <tr><td>h or hh</td><td>For the hour</td></tr> <tr><td>m or mm</td><td>For the minute</td></tr> <tr><td>s or ss</td><td>For the second</td></tr> <tr><td>t</td><td>Time in the format defined as "Short Time Format" under windows ('Settings Control Panel Regional Settings')</td></tr> <tr><td>tt</td><td>Time in the format defined as "Long Time Format" under windows</td></tr> <tr><td>am/pm or a/p or AM/PM or Am/Pm</td><td>use 12 hours format and show am or pm (a or p...)</td></tr> </table> <i>Example:</i> <time:hh.mm.ss> If the format is not specified, the time format defined as "Long Time Format" under windows will be used.	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...)												
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...)																								
<ser_number>	Serial number of the reader used.																								
<table>	Only for Stacker batch measurements: use an individual identifier for each plate: After selecting <table>, 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.

Special ID Options

These options are not available in the drop down menu (as they are probably only seldom used), but you can manually type in these options.

<O1> ...	Optic settings for chromatic 1 ... 5 of the protocol used (settings string, as shown in the Monochromator / Filter Settings table). When using a dual spectral scan protocol use <O1> for the first scan and <O2> for the second scan.
<O5>	
<Gain1> ... <Gain5>	Gain value for chromatic 1 ... 5 of the protocol used (obtained via automatic gain adjustment or manually defined). When using a dual spectral scan protocol use <Gain1> for the first scan and <Gain4> for the second scan.
<Gain1B>	Gain value for the B channel of FP protocols (obtained via automatic gain adjustment or manually defined).
<GainA>	Gain value obtained during last automatic gain adjustment
<GainB>	Gain value for the B channel of FP protocols obtained during last automatic gain adjustment
<FH>	Focal height

The above identifiers can be combined. If the resulting ID is longer than 100 characters it will be truncated in the Absolute database, 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. Identifiers can still be edited or use the 'Clear IDs' button.

6.6 Sample IDs / Dilution Factors

Start Measurement - FI EP

		Sample ID	Dilution
Well	Content		
A1	X1	Sample 008/1	1
A2	X2	Sample 008/2	1
A3	X3	Sample 008/3	1
A4	X4	Sample 008/4	1
A5	X5	Sample 008/5	1
A6	X6	Sample 008/6	1
A7	X7	Sample 008/7	1
A8	X8	Sample 008/8	1
B1	X9	Sample 009/1	2
B2	X10	Sample 009/2	2
B3	X11	Sample 009/3	2
B4	X12	Sample 009/4	2
B5	X13	Sample 009/5	2
B6	X14	Sample 009/6	2
B7	X15	Sample 009/7	2
B8	X16	Sample 009/8	2
C1	X17	Sample 010/1	2.5
C2	X18	Sample 010/2	2.5
C3	X19	Sample 010/3	2.5

Options

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

Dilution Factor Auto Fill Function

Start dilution:
 Factor Increment Decrement

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

Print ID list
Import IDs
Export IDs

Delay:
Start measurement
Save & Close
Cancel
Help

Enter identification values for each well under this tab. These values will be shown in the MARS 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 (see software manual part III: Data Analysis software). A dilution factor cannot be smaller than 1.

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

Clear IDs

Clicking this button will clear all ID fields.

Get last IDs

Clicking this button recalls 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

Sort by rows, columns or well content. The selected sort order will also be used for printing or exporting the sample ID / dilution factors list.

Print ID list

Clicking this button will print out the ID list on any available printer.

Import IDs

It is possible to import sample IDs / dilution factors from a text file (created using the export function or using a text editor like Notepad) or from an 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: 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 Excel format (.xls / .xlsx) file.

Options

Automatically enter sample IDs previously used with this test protocol

If this option is checked, the last used sample IDs for the selected test protocol will be reentered automatically 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 will 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 large 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.

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

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

6.7 Crosstalk Correction

6.7.1 Principle of the Crosstalk Correction

Optical crosstalk between the wells of a microplate is explained by two reasons: 1) light transmission through the sides of a well into adjacent wells, and 2) light transmission from the top of the well that bleeds into the detector. The first type of crosstalk, light transmission through the plate wall, depends on the plate material or the structure of the wells. This effect can be corrected mathematically with the provided crosstalk correction function. The second type of crosstalk is caused by light transmission from the top of neighboring wells that bleed into the detector. This effect can be eliminated or greatly reduced utilizing adequate apertures (Aperture Spoons) positioned at the top of the microplate (see Operating Manual).

Two different crosstalk effects can be corrected:

- Luminescence: glow crosstalk
- Alpha Technologies: afterglow crosstalk and glow crosstalk

Afterglow effect: Only for Alpha Technology measurements. The well measured subsequently the previously excited Alpha well shows typically the biggest amount of crosstalk called afterglow crosstalk. This effect strongly depends on the measurement time / integration time as the Alpha signal afterglows (depending on the chemistry) some seconds. The correction of the afterglow effect happens in reading direction of the plate for the directly previously measured well.

Glow effect in Alpha Technology: For this effect the Alpha well didn't get excited before. A small amount of excitation light during the measurement transmits the microplate side wall, excites the neighbor well and the retransmitted Alpha emission leads to an increased measurement value.

Glow effect in Luminescence: During the measurement a certain amount of luminescence light of the neighbor well is transmitted

through the microplate side wall and increases the measurement value.

The glow crosstalk correction corrects the measurement values of all wells by taking into account the crosstalk from the 4 directly adjacent neighbor wells. This effect is independent of the reading direction.

The determination of the crosstalk correction factors has to be done once before the measurement for a new setup using a defined layout.

Important: The determination of the crosstalk correction factors needs to be made using the aperture spoon, the same type of microplate, same chemistry, same filling volume and for Alpha Technology measurements additionally identical measurement settings (measurement times).

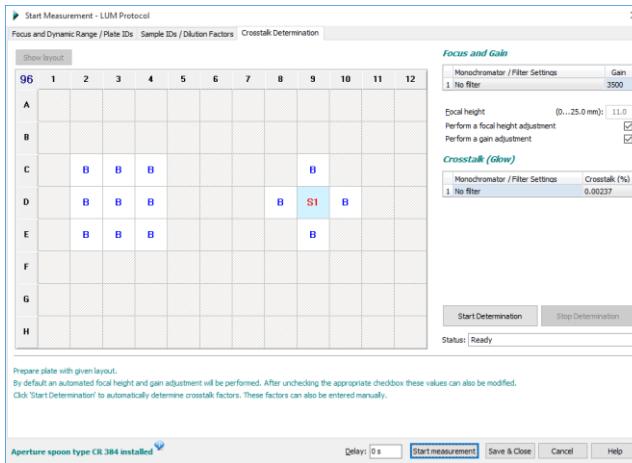
During following measurements the raw data will be automatically corrected.

6.7.2 Crosstalk Correction Factor Determination Procedure

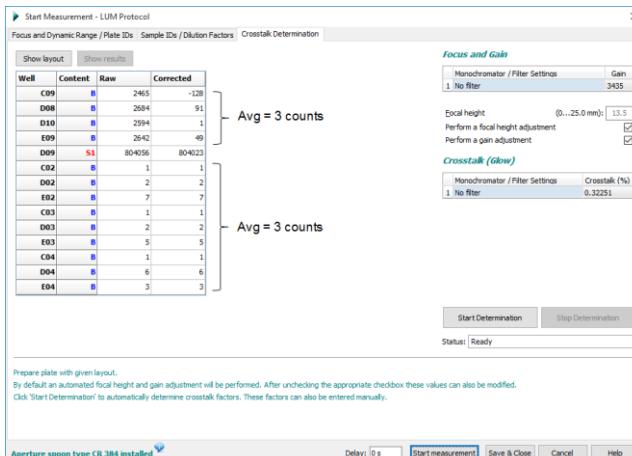
The crosstalk correction factors can be determined using the 'Crosstalk Determination' tab, which is a part of the 'Start Measurement' dialog.

The tab is only available for AlphaScreen and luminescence endpoint and plate mode protocols and only when using a CLARIOstar Plus, VANTAstar or VANTAstar F reader.

To determine the glow correction factor for **luminescence** the following layout with one standard well and some buffer wells needs to be prepared. This layout is for 96 up to 3456 well plates at the same position in the top left plate corner (for plate formats less than 96 wells no crosstalk correction is available and usually not necessary).



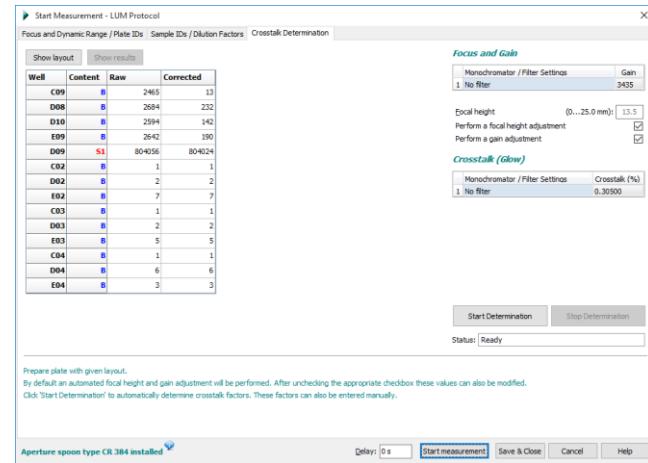
The four buffer wells adjacent to the standard well (C9, D8, D10 and E9) show a higher signal than the nine buffer wells (C2 to E4) without standard well in close proximity. After starting the determination procedure by clicking the button 'Start Determination' the window changes. Instead of the layout now a table with the measured raw and corrected values will be displayed:



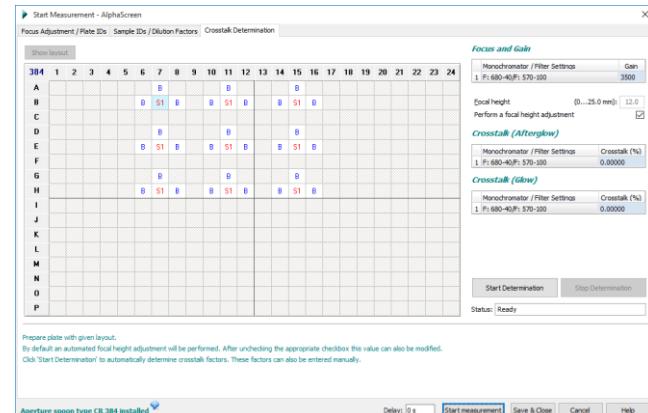
Typically the mathematical correction will cause also negative values. After the correction the average of the buffer wells with crosstalk is the same as the average of the buffer wells without crosstalk. To avoid negative values the crosstalk factors can manually be reduced slightly. The following weightings of the factors might be used:

- Luminescence: glow correction factor with a weighting of **90%**
- Alpha Technologies: glow and afterglow correction factors both with a weighting of **80%**

Using the manually optimized crosstalk factor the corrected values in the table will now change. There are no more negative values:



For the crosstalk determination for **Alpha Technology** measurements the layout for 384 well up to 3456 well plates consists of 9 identical blocks each containing one standard well and 3 buffer wells surrounding the standard well. For 96 well plates the last 3 blocks (with standard well B15, E15 and H15) are omitted.



The determination procedure for Alpha Technology is the same as described in the procedure above for luminescence.

For the crosstalk determination it is important to have high signal intensity for the luminescence or Alpha well. If the signal is too low the crosstalk effects cannot be seen and they will get lost in the noise of the buffer measurements. Therefore, for such low signals no crosstalk correction is needed.

To achieve an accurate crosstalk correction factor following relations should be considered:

Crosstalk	Signal intensity relative to buffer intensity
1 %	1.000 times buffer intensity
0,1 %	10.000 times buffer intensity
0,01 %	100.000 times buffer intensity

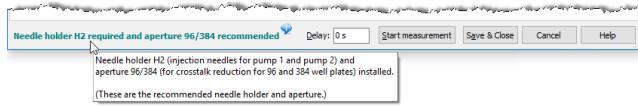
6.8 Executing Measurements

6.8.1 Start the Measurement

After defining the focus and dynamic range settings (see chapter 6.3) and after defining the plate and sample IDs (see chapter 6.5 and 6.6), the following options are possible:

Needle Holder / Aperture Information

If an injection needle holder is required, the suitable types will be shown in the lower left corner of the start measurement dialogue. If an aperture is recommended to reduce crosstalk, the recommended type will also be shown there. The text will be displayed in green if the required or recommended types are already installed and in red if not. When moving the mouse cursor to this text a hint with additional information will appear:



Notes: Which injection needle holders can be used depend on which pump is used in the protocol or whether both pumps are used.

The use of an aperture is only recommended for luminescence and AlphaScreen measurements with microplates with 96 or more wells.

If an injection needle holder is required but not installed, the Start measurement will be disabled.

If the recommended aperture is not installed, it is nevertheless possible to execute the measurement.

Delay

Optionally a delay (an incubation time) before the start of the measurement can be specified. This delay time can be entered in seconds (s), minutes (m) or hours (h). During this incubation period status information in the last section of the status bar will be shown.

Note: When performing a Stacker batch measurement this delay happens before measuring the first plate.

Start measurement

Begins the measurement using the defined focus and dynamic range settings.

Save & Close

Saves the focal height and gain values (entered manually in the 'Focus and Gain Adjustment' sheet or calculated using the automatic adjustment functions), the plate IDs, the sample IDs and the dilution factors, but does not start a measurement.

Cancel

Closes this window without saving IDs, focal height and gain values or dilution factors and without starting a measurement.

6.8.2 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 no stacker were attached, meaning that the microplate must be moved into the reader using the plate reader in/out button or the software menu function 'Measure | Plate In' / 'Measure | Plate Out'.

When a stacker is attached to the reader, there is an additional check box 'Don't measure the last plate' and a 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 to 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 1.1 *Stacker Configuration*.

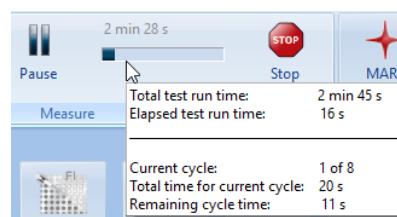
To re-order the microplates back as they were before starting the measurement, use the menu command 'Measure | Restack'. This command will transfer all plates from magazine 2 back to magazine 1.

6.8.3 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.



By moving the mouse cursor to the time gauge, a small window with additional timing information will appear:

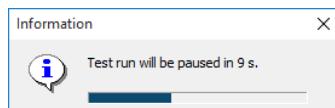


If performing a plate mode kinetic or well mode measurement, the quick start button  will change into the pause button . Click this button to pause the active test run after finishing the measurement of the current cycle (plate mode) or current well (well mode). A pause window will appear (see chapter 4.3.2). It is then possible to take out the plate for incubating the plate or injecting a compound.

Notes: The pause function is not available when using a stacker or in script mode.

Due to technical reasons the pause function via menu command is also not available when performing absorbance spectra plate mode measurements.

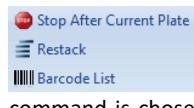
A count down window will appear after using the ‘Pause’ command, showing the remaining time until the current cycle / well is finished.



6.8.4 Stop 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), use this function to immediately stop any activity. Depending on the state of the system when the stop function is employed, it might be necessary to reinitialize the stacker and the reader (the software will indicate this). If, during this moment, a plate is inside the reader, a dialogue will appear asking whether this plate should be moved out to stacker magazine 1 or 2.



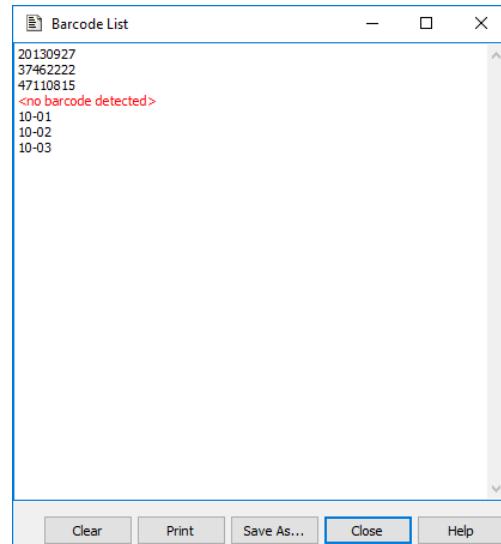
In Stacker batch mode, there is also an additional menu command ‘Measure | **Stop After Current Plate**’ available. When this command is chosen, the measurement of the current plate will be finished and the plate will be moved out to magazine 2 before the batch processing is terminated.

Note: To re-order the microplates back 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.

6.8.5 Barcode List

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

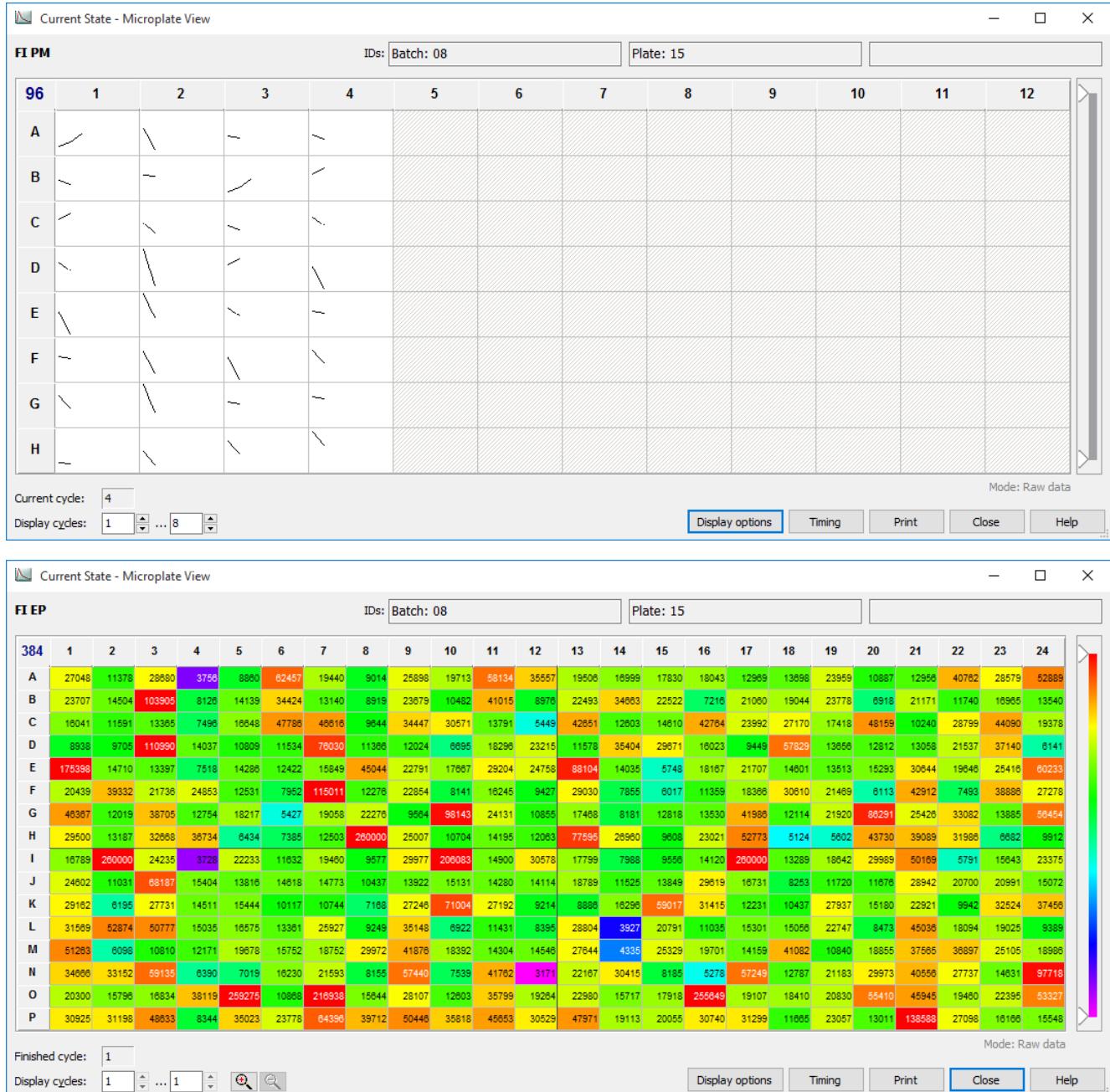
The barcode list is editable. It is possible to save the barcode list as a pure text file or to print out the list.



When using the internal barcode readers of the CLARIOstar Plus each line will contain two entries: the barcodes from the front and right side of a plate. If a barcode reader has not been used the corresponding entry will contain -. If a barcode could not be read it will contain <no barcode detected>.

6.9 Current State Graphics

6.9.1 Current State – Microplate View



When the measurement has started, the 'Start Measurement' button will change to the 'Current State Graphics' button . This function allows measurement data to be viewed 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. It is possible to 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 6.9.2 *Current State Display Options*).

When using a color display mode (two colors, three colors, color gradient), use the slider on the right of this window to change the displayed color range or the thresholds.

When using 384, 864 or 1536 well plates it is possible to zoom in and out using the zoom buttons.

Current / Finished cycle Shows the number of the cycle currently being measured (only for plate mode tests). If the test run is paused this field will change to show the number of the next cycle ('Pause before cycle:').

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.

Display options	Opens the 'Current State Display Options' dialogue box (see chapter 6.9.2).
Timing	Opens the 'Timing Overview' window (see chapter 6.9.6).
Print	Prints the screen on any available printer.
Close	Closes the current state window.

When you move the mouse pointer over a well, the well content (e.g. 'SA1') will be shown for a few seconds. If using layout groups (see chapter 4.3.13), the layout grid will be displayed using the background colors belonging to the layout groups used.

To save this display as bitmap (windows BMP or JPEG format) use the key combination [Shift]+[Ctrl]+[S]. It is possible to change the size of this window.

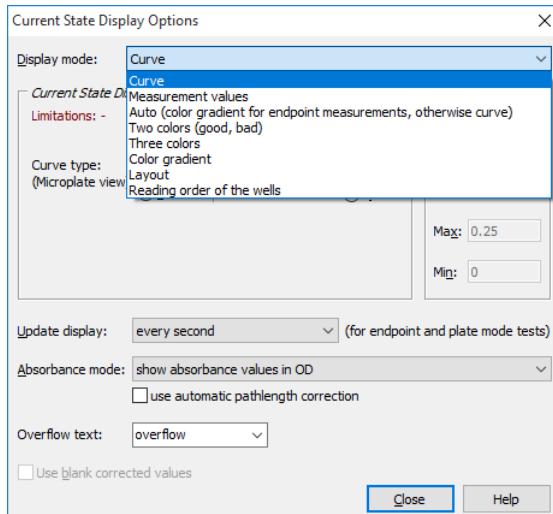
When performing a spectra measurement, the spectrum of each well can be viewed (see chapter 6.9.4 *Display of Spectra Data*).

For test protocols with well scan (see chapter 4.3.8 *Matrix Well Scan*), the single scan points will be displayed, if the two or three colors display mode or the color gradient display mode has been selected (see chapter 6.9.4).

Double click on a well to get a zoomed view of the measurement values (see chapter 6.9.3 *Current State Well View*).

6.9.2 Current State Display Options

After clicking the 'Display options' button in the Current State – Microplate View window, the following dialog box appears:



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), the results of all chromatics will be displayed together.

Measurement values of last measured cycle / interval

The last measurement values are shown in numeric style. Due to space limitations, only the results for up to two chromatics will be shown (the first two). To see values from an earlier cycle /

interval, change the number in the Cycles / Intervals input box of the Current State window.

Note: When using 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.

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

'CLARIOstar.ini' file:

ClearBeforeUpdateInMValuesMode = False.

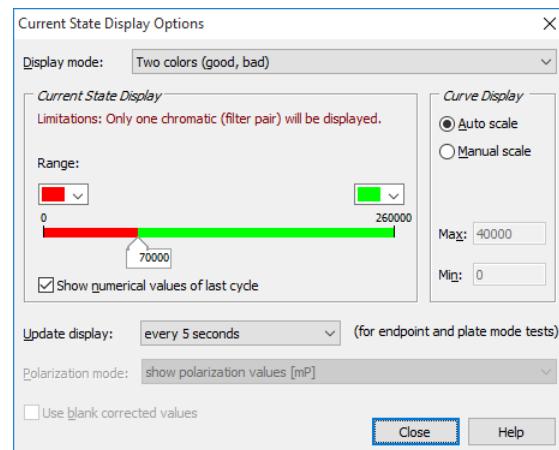
Use the key combination [Shift]+[Ctrl]+[I] from the main screen of the Control software to open the CLARIOstar.ini file. If the section [Configuration] does not yet exists, please add it to the end of the file.

Auto

If this option is chosen, for all tests with only one cycle / interval (endpoint tests) the color gradient option will be used (see below) and a curve display will be shown for all other tests.

Two colors

To show a good / bad (pass / fail) decision, choose this option to display a color for all values under a certain threshold and to display a different color for all values above the selected threshold. Change the threshold value using the thumb of the color slide. Besides moving the thumb using the mouse it is also possible to type in a value. If the measurement values use only a part of the total measurement range, it might be useful to change the start and/or end value of the slider. This can be done by typing in the requested value. Only the results from one chromatic / channel will be displayed, but if the test run uses multichromatics you can decide which chromatic should be used for the current state display.



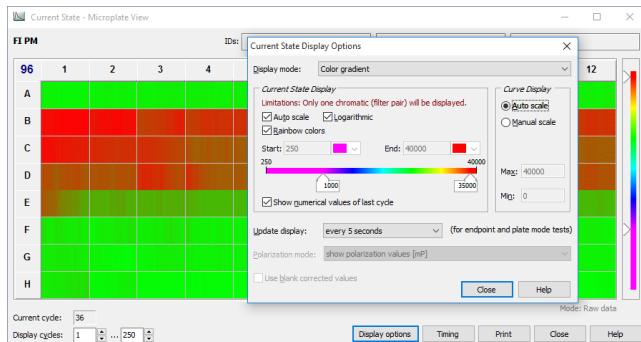
The slider in the Display Options dialogue is synchronized with the slider in the Current State - Microplate View window (see chapter 6.9.1).

Three colors

Same concept as 'Two colors', but here it is possible to 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. Select a **start** and an **end color** or select **rainbow colors**.



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

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 option allows manual specification of graph limits. Use this function to enlarge parts of the graph of special interest.

Max: Maximum value displayed

Min: Minimum value displayed

Update display

Specify here 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 any of the 'Update every X seconds' options is selected, 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 measurements with a large number of cycles / intervals or multichromatics. Therefore, we do not recommend using the highest update frequencies on slow computers. 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 endpoint or plate mode tests and well 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 fluorescence polarization protocols) will be transmitted.

When using the matrix 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.

When performing fluorescence or luminescence spectral scan measurements the display will always be updated 'After a well is completed'.

Absorbance mode

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

- *OD units*
- *mOD units*

Use automatic pathlength correction

By applying this function, the absorbance values will be normalized to unit pathlength (1 cm) using a water peak-based correction method.

This correction might not be appropriate in case of:

- Measuring samples with a significant amount of organic solvents (e.g. > 20 % DMSO).
- Measuring samples whose absorption is in the near IR wavelength range (e.g. Lowry assay).
- Measuring turbid samples (e.g. microbial growth assays measuring the OD at 600 nm).
- Measuring wells whose sample volume is far below the recommended working volume of the microplate or which are empty.

Notes: This function is only available for absorbance measurements.

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

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

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

Overflow text

Define how overflow measurement values are displayed:

- *overflow*
- *ov.*
- *<empty>*
- *<overflow value>* - show the maximum possible measurement value = measurement range end value.

It is also possible to type in any text you want to use, e.g. '-'.

Notes: This function is currently only available for absorbance measurements. When using any other measurement method, the maximum possible measurement value will be shown, e.g. 260.000.

When using the *<overflow value>* option in combination with pathlength correction 99.999 OD or 99999 mOD will be displayed. These values might appear if an overflow occurred at the actual measurement wavelength(s) or at one of the water peak reference wavelengths or if the absorbance at these reference wavelengths is equal.

The overflow text will not be used in combination with the Curve and Spectra display modes and not in the Current State - Well view measurement values table.

Use blank corrected values

If this option is chosen, all results will be blank corrected before display.

Notes: If using 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 4.3.11) when using 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 using 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').

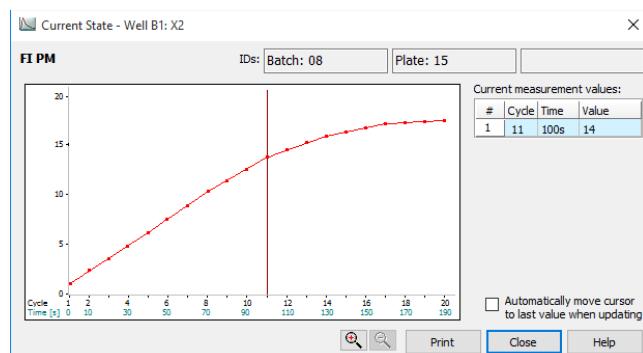
Use average of all blanks from all groups for blank correction

This option is only available when different layout groups (see chapter 4.3.13 *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 this option is selected, 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 the preferences independently.

6.9.3 Current State – Well View

After double clicking on a well in the Current State – Microplate View window, zoomed view of the measurement values is shown. Here the measurement values are always displayed as a curve (when using well scan the display might look different, depending on the selected display mode, see chapter 6.9.4). There is also a table on the right side of the window where measurement results for all used chromatics can be seen in numeric style. When the mouse pointer is moved to the module setting number, a small hint box containing the details of the monochromator / filter settings, will appear.



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

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

Using the zoom 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 using a multichromatic or FP protocol, the table on the right of the zooming display will show the measurement values of all chromatics / channels. If color display mode is used (two colors, three colors, color gradient), only one channel / chromatic will be displayed in the Current State – Microplate View display (see chapter 6.9.1). The selected chromatic / channel will be marked inside the table by a blue bar. Besides using the Current State Options dialogue (see chapter 6.9.2), the chromatic / channel selection can also be changed by moving the blue bar to a different row.

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

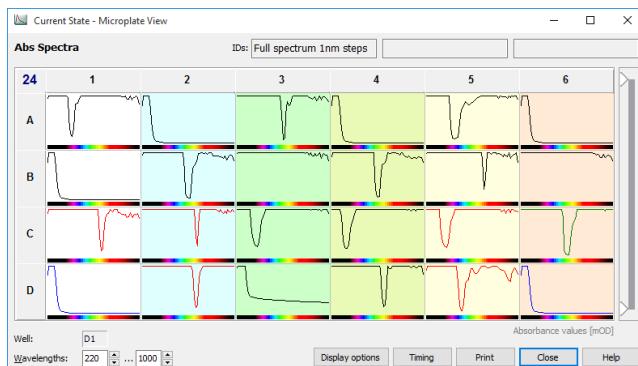
To save this display as bitmap (windows BMP or JPEG format) use the key combination [Shift]+[Ctrl]+[S].

Print Prints the screen on any available printer.

Close Closes the Current State – Well View window.

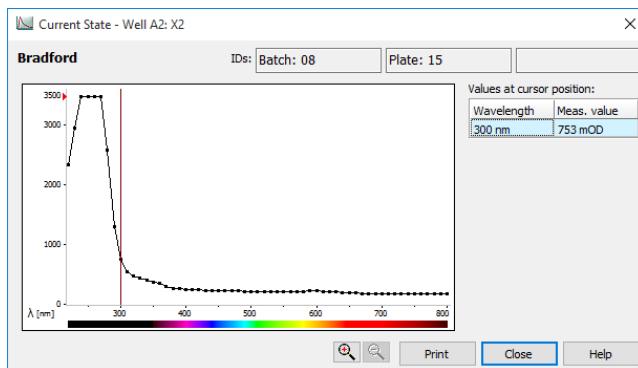
6.9.4 Display of Spectra Data

When performing a protocol in absorbance spectra mode or when performing a fluorescence intensity or luminescence spectral scan, the spectra of all wells used can be seen:



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

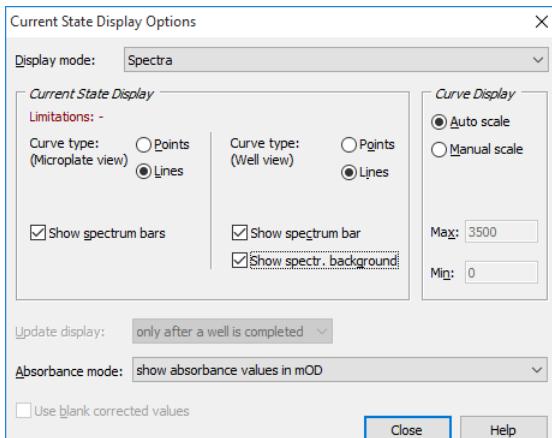
Clicking on one well will open the Current State – Well View window:



By moving 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: [, , , , , , , and].

Use the zoom buttons or the [+/-] keys to zoom into the spectrum curve and out again.

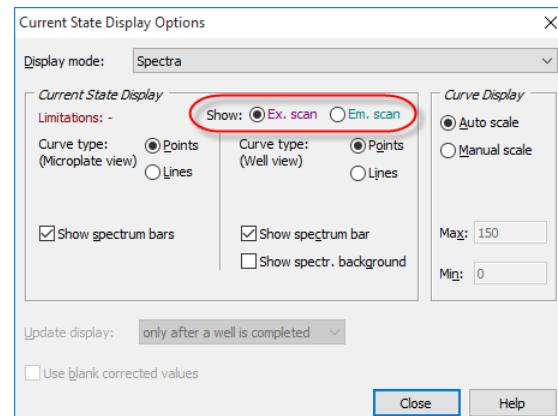
Inside the Options dialogue, choose whether to display a spectrum bar below each spectrum or not. This bar might help with the orientation of the spectrum.



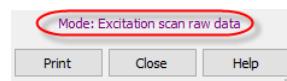
For the well view display, it is also possible to display the background in colors according to the measured wavelengths.

Dual Scan Protocols

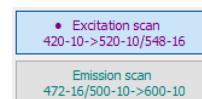
When performing a dual scan protocol (a protocol using an excitation and emission scan combination), the spectrum to be displayed can be changed inside the Current State - Options dialogue:



or by clicking the mode label inside the Current State - Microplate View window:

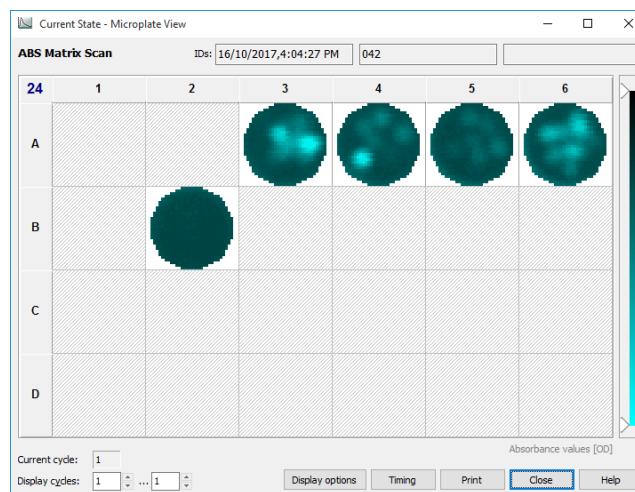


or by clicking the corresponding button inside the Current State - Well View window:

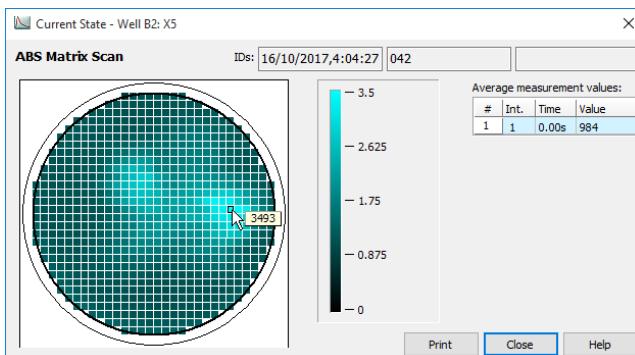


6.9.5 Display of Matrix Well Scan Data

When performing a protocol using well scan, the single scan values can be seen in two and three colors and color gradient display mode (see chapter 6.9.2 Current State Display Options). Example using the color gradient display mode:



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

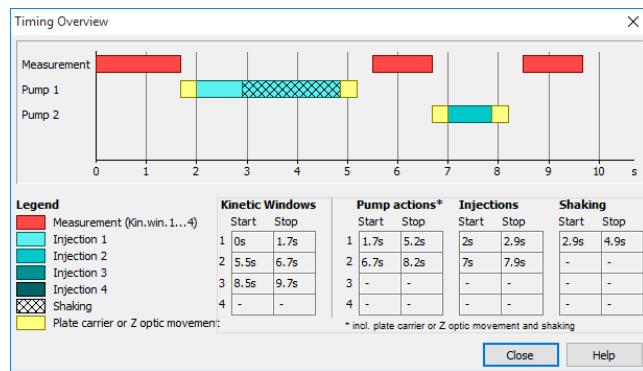


Move the mouse cursor over the zoomed well display to see the measurement value of the selected scan point. The table on the right shows the average measurement value of the well.

If using a multichromatic or FP 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. Changing the chromatic / channel selection is also possible by moving the blue bar to a different row.

6.9.6 Timing Overview

After clicking the ‘Timing’ button in the Current State – Microplate View window, a window showing an overview of the timing of the test currently running appears.

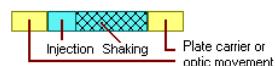


In this window, a graphic overview of the measurement and all injections for one well is shown. (For plate mode tests all actions are displayed in the timing overview of one cycle. The displayed injections may occur in different cycles. The used cycle will be listed behind each injection bar.)

In addition, tables containing the measurement and the injection times are shown.

Note: The listed pump action times include the time for shaking after injection and for plate carrier a Z optic movement.

An injection action can contain different steps:

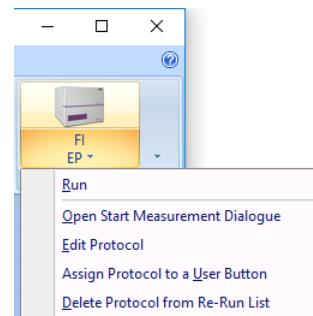


6.10 Repeating the Execution of a Test Protocol (“Re-run”)

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 focal height and 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 1) for the BMG LABTECH LVIS Plate (micro drop section measurements only).

The LVIS Plate Tab uses a separate protocol data base. This allows defining protocols with the same names as protocols from the Microplate Tab. Using the LVIS Plate Tab it is only possible to define protocols using the BMG LVIS Micro Drop plate and it is only possible to use the absorbance measurement method.

Hints: This LVIS plate tab only exists if the reader is equipped with a spectrometer for absorbance measurements.

The LVIS Plate Tab can be switched off using the 'Settings | Program Configuration' dialogue (see chapter 3.3.1).

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!

SMART Control

The LVIS Plate Tab does not contain the Re-Run function. (see chapter 6.10). It is also possible to use the Microplate Tab to perform measurements using the LVIS Plate. This also allows the re-run function to be used. (When the LVIS Plate Tab has been switched, the LVIS Plate menu group can be found in 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.

Well Scanning is not possible when using the BMG LVIS Micro Drop plate.

For more information see the Operating Manual for BMG LABTECH's LVIS Plate.

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.

Simply select the desired **Wavelength** to be read (between 220 and 1000 nm) and add an optional **Plate ID** (see chapter 6.5).

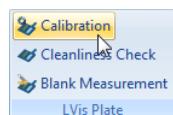
When using the Quick Start function of the LVIS Plate tab it is possible to use **sequential blanking**. In this mode the individual blank measurement value of each well will be subtracted from the measurement values. To use this function the blank values need to be measured before using the quick start function. This

can be done using the blank measurement function of the LVIS Plate menu group (see chapter 7.2.3).

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

Use the standard measurement method based on pre-defined protocols to measure a spectrum, to measure part of the BMG LVIS Plate or to perform a kinetic measurement (see chapters 4 and 6.2).

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.

7.2.1 LVis Plate Calibration

By using the LVis Plate installation CD the correct pathlengths 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 pathlength for every micro drop site is achieved by comparing the optical densities of two reference solutions with a known concentration ratio.

Two reference solutions of Orange G are needed. Orange G can be obtained from Sigma-Aldrich (www.sigmapelabtech.com; Cat. No. 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 pathlength 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 pathlength of 0.5 mm.

When "Solution 2" is measured at a nominal pathlength 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 pathlength 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 pathlength of 0.5 mm.

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

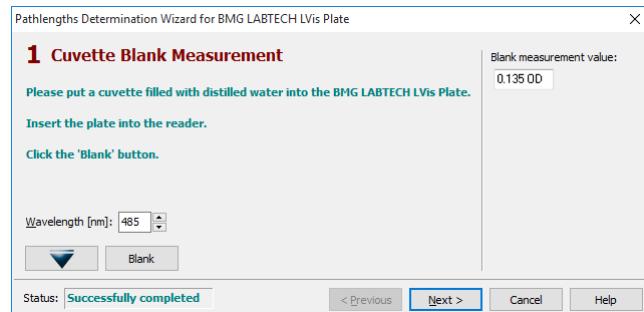
The exact pathlength for each Micro Drop site can be derived from the following equation:

$$\text{Exact PathLength}_{\text{(Micro Drop)}} = \frac{0.5 \times \text{OD}_{\text{(Micro Drop)}}}{\text{OD}_{\text{(Cuvette)}}}$$

Open the SMART Control 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.

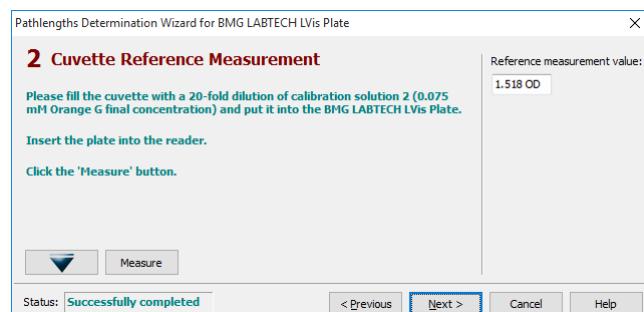
Step 1: Cuvette Blank Measurement

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



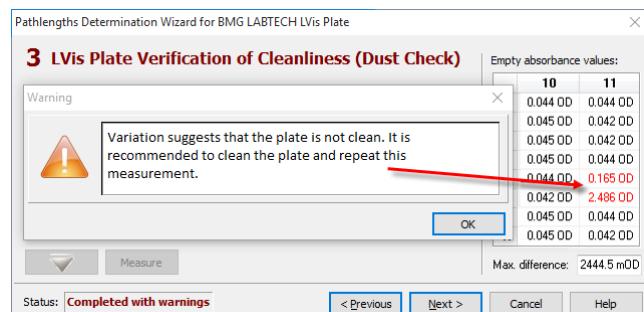
Step 2: Cuvette Reference Measurement

Fill a cuvette with the appropriate amount of "Solution 1" 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, enter the serial number which can be found on the back side of the LVis Plate and insert the empty LVis Plate into the reader. Click Measure. 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, it is necessary to repeat the cleaning procedure as described in the LVis Plate Operating Manual. Outliers are indicated in red.

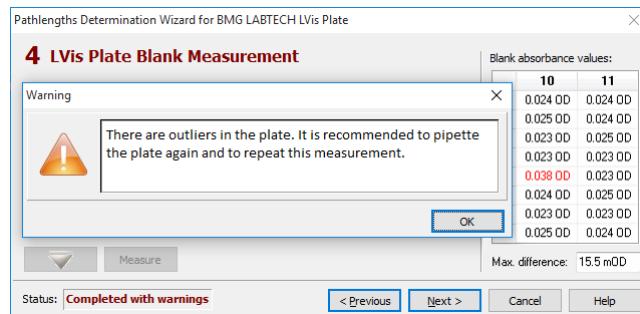


Repeat the cleaning procedure of the LVis Plate until it passes this dust check verification. Click 'Next'.



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'.

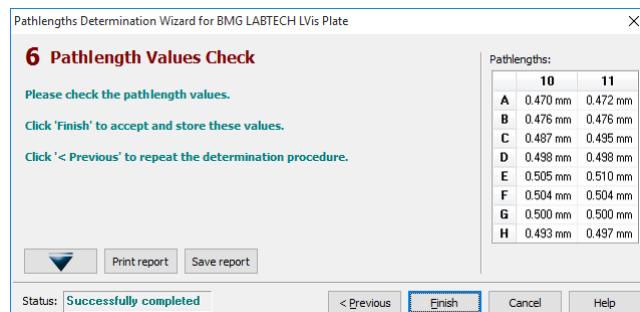
Step 5: LVis Plate Pathlength Determination

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 Pathlength Values

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

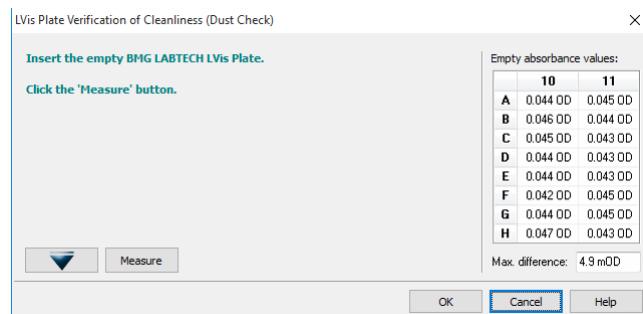


Observe the pathlengths (denoted in mm). A report of the calibration procedure can be printed or exported as *.txt or Excel file for your records. Thus, it is recommended to always include the serial number of the LVis Plate at step 3.

Click 'Finish' to end the calibration procedure. These pathlength values are stored in the 'BMGLVis.pl' file and will now be used for further measurements with the LVis Plate until a new pathlength 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.



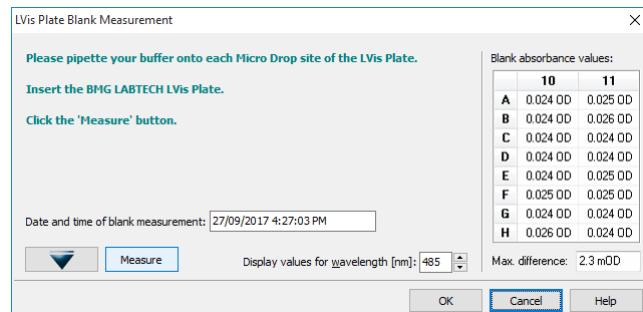
The OD values should be below 0.060 OD. If a value is greater than 0.060 OD or the difference between the lowest and highest value is greater than 8 mOD a warning message pops up, and the cleaning procedure of the glass slides as described in the LVis Plate Operating Manual should be repeated.

Note: For this measurement a wavelength of 485 nm will be used.

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

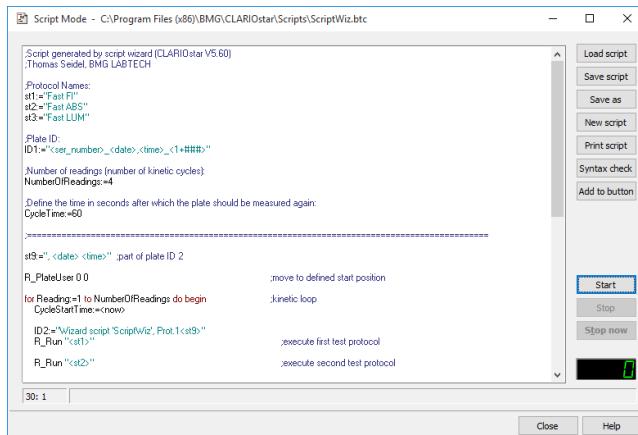
 Use the script button of the application button menu or of the quick access toolbar to open the script mode window to define scripts, which give **unlimited flexibility**. Use this function, for instance, to use different test protocol definitions in one batch run or to perform more than one measurement 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! Use this function with caution.

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. 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. There will be a range check performed for all numeric parameters. 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 only get a warning message, as the value of a variable is only known at run time. <i>Hotkey:</i> [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. <i>Hotkey:</i> [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.

Use the system menu function 'Default position and size' to 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. Double clicking the form's background also activates 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.

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

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.

8.1.2 Editor Functions

To **undo** the last change of the script use the key combination [Ctrl]+[Z] 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 something has been selected before using the [Ctrl]+[Q], [F] / [Ctrl]+[F] key combination, this selection will be used as default search string, otherwise if the cursor is positioned onto a word this word will be taken as default value.

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

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

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

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

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 NumberOfReadings do ;loop for multiple readings
begin
  ID1:="Reading " 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 using 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.

8.2 Script Auto Run

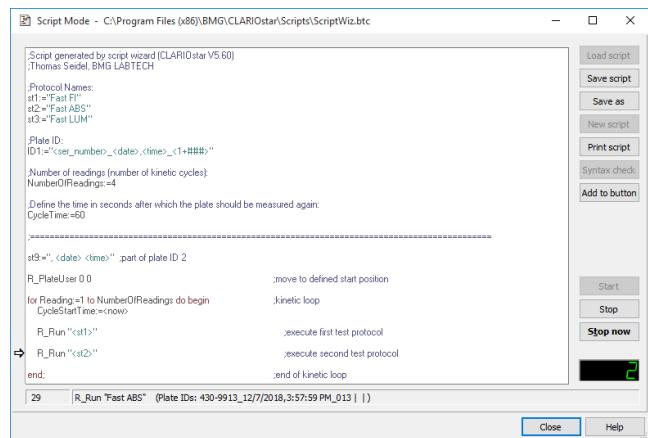
It is possible to optionally specify a script which will automatically be executed after starting the program (immediately after initialization and reading the EEPROM content of reader and stacker). To use this feature, specify the name of the script in the command line after the program name preceded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

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

If **/v** is added 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 is 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 will not be executed.

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.



The number of the script line currently processed is shown at the far left of the status section. The remaining part of the status section shows the currently processed command and, for many commands, additional information.

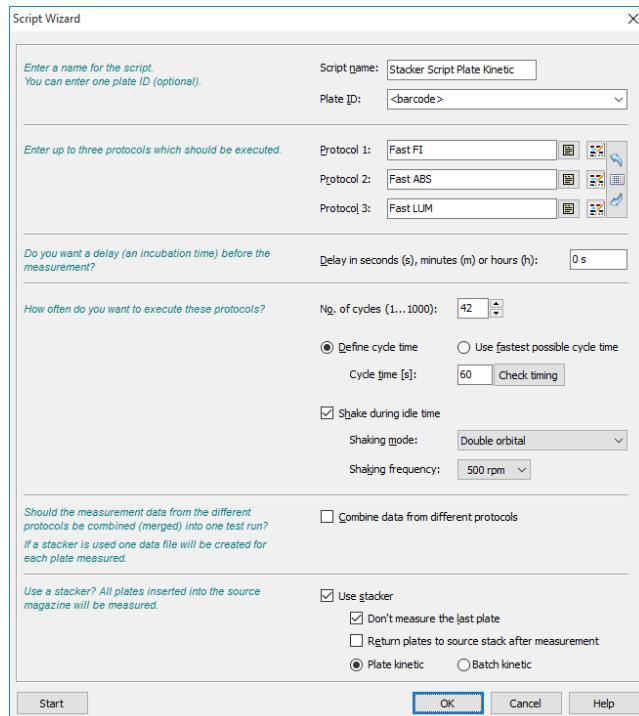
All script commands are listed in the run log file / run log window (see chapter 3.1.8).

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 using nested loops, this loop counter will show the value of the most inner loop.

Notes: If no path is specified, the script will be searched inside the '\ExeDII' subfolder of the SMART Control main directory. It is possible to omit the file extension '.btc', but it is not possible to use files with extensions different from '.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), users can choose between assigning an existing (file) script to a button or using the script wizard to get a script created automatically.



Using the wizard multi measurement method measurements can be performed. These measurements can include a kinetic.

Select up to three **protocols** to be executed. It is possible to edit a protocol using the button. As long as no protocol name has been entered this button will allow to create a new protocol.

Use this button to copy the layout from one test protocol to the other protocols.

Optionally a **delay** (an incubation time) before the start of the measurements can be specified.

The measurements can include a kinetic. When using 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.

Note: When defining a cycle time, which is shorter than the time necessary for executing the test protocols, the measurements will happen as fast as possible. There will be no warning message.

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.

Shaking 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, use a lower frequency (slower speed) while a higher frequency (faster speed) should be used 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 also use a lower frequency.

Volume: A higher frequency is suggested for smaller volumes.

The measurement data of the different cycles will always be merged together (Absolute database format, see chapter 3.4 *Data Output*). When using more than one test protocol, it is possible to also **combine the data from the different protocols** into one test run. The data from the second and third protocol will appear as additional chromatograms in the merged test run. Please note: in the data analysis software only the protocol settings from the first test protocol are shown. 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 the 'Merge data' selection box is unchecked, one test run per protocol will be created.

When a **stacker** is attached to the reader, it is possible to measure a whole batch of plates. In this case, 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. 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 'CLARIOstar.btc' in the SMART Control temp folder (usually c:\Program Files\BMG\CLARIOstar\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 focus or gain adjustment).

Example scripts can be found in the subfolder Scripts of the SMART Control installation directory (usually c:\Program Files\BMG\CLARIOstar\).

The script language is case insensitive. However, 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, enter a / at the end of the line and continue in the next line, e.g.:

```
R_GainWell "FI Protocol" /
"C:\Users\Tom\Definit" /
2 5 80 0 1
```

9.2 Batch Control Commands

9.2.1 Wait Commands

wait for {n} s

Wait for {n} seconds before proceeding with the next line. It is possible to specify the time in 0.1 s steps. Instead s for seconds also m for minutes and h for hours can be used.

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 (the temperature can be specified in 0.1 °C steps).

Use this command after switching on the incubator using the R_Temp command.

Example:

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

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_PrepForPlate 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_PrepForPlate 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 the reader, use an S_DisableMotors command at the end of the script, to switch off the stepper motors, to save energy and prevent heat built-up.

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.

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 5.1.2) by sending an 'R_Temp 0.1' command.

wait for O2Conc >= {n} wait for O2Conc <= {n} wait for CO2Conc >= {n} wait for CO2Conc <= {n}

Wait for the O2 / CO2 concentration of the reader to reach the specified level before proceeding with the next line.

Use this command after setting the ACU (Atmospheric Control Unit) target concentrations to a specific value using the R_ACU command (see chapter 9.4.6).

Note: For these commands a firmware version 1.10 or newer is necessary. The commands can only be used if an ACU is connected.

wait for ConcReached
wait for TempReached
wait for ConcAndTempReached

Wait for the O₂ / CO₂ concentration or the incubator temperature of the reader to reach the currently used target values before proceeding with the next line.

Use this command after setting the ACU (Atmospheric Control Unit) target concentrations to a specific value using the R_ACU command or after setting the incubator's target temperature to a specific value using the R_Temp command (or after using the Temperature Control or the Environment Settings dialogues, see chapter 1).

wait for file {file name}

See chapter 9.12.

wait for ready

Stops the script execution until the reader becomes ready. This might be useful in combination with reader activities caused by direct reader interactions, e.g. by using the plate or prime button.

wait for busy

Stops the script execution until the reader becomes busy.

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, it is possible to use <All_Plates> as end value. If using 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 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 start 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.)

continue

The continue command causes the flow of control to proceed to the next iteration of the enclosing for statement.

9.2.3 Comparisons

if <Barcode_String>="value" then ...

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

Examples:

```
if <Barcode_String>="<no barcode detected>" then
  ID1:="unknown plate"
if <Barcode_String> ="008" then
  R_Run "PROTOCOL 008"
```

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 to 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 to 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 it is also possible to use one of the ID values.

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

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>="430-0000" then
  ShowMessage "Reader type is CLARIOstar."
```

if {variable}=value then ...

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

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

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

To perform more than one command after the comparison, 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;
```

Note: Without begin ... end block only a single line command after then or in the next line is allowed!

Not allowed:

```
if i=1 then
  if j=2 then
    beep
```

Possible:

```
if i=1 then
  if j=2 then beep
```

Better:

```
if i=1 then
  begin
    if j=2 then
      beep
  end;
```

Use the **else branch** for commands, which should be performed if the condition is not met.

Examples:

```
a:=42
if a=42 then b:=1
  else b:=2
```

```
PerformMeasurement:=True
if PerformMeasurement then begin
  R_Run "FI"
end
else begin
  R_PlateOut
end;
```

The 'begin' after 'for ... to ... do' or after 'if ... then' or after 'else' can also be written in the next line (to ease code reading):

```
if PerformMeasurement then
  begin
    R_Run "FI"
  end
else
  begin
    R_PlateOut
  end;
```

Before or after 'else' or between 'then' / 'else' and 'begin' also empty lines or comment lines are allowed:

```
if PerformMeasurement then
;Execute the test protocol:
begin
  R_Run "FI"
end

else

;No measurement, just move the plate out:
begin
  R_PlateOut
end;
```

Also single commands after 'then' or after 'else' can be moved to the next line(s):

```
a:=42
if a=42 then
  b:=1
else
  b:=2
```

'end else begin' in one line is not allowed!

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

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 is 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}}

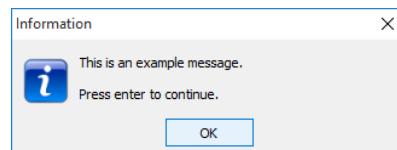
Shows the defined message in a small window. Depending on the {Type} (Info, Warning, Error), the window will contain an appropriate icon. Optional: specify the window title (caption). If a title is not specified, 'Information', 'Warning' or 'Error' will be used (depending on the specified {Type}).

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})

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

Optional: Enter a title; otherwise 'Question' will be used.

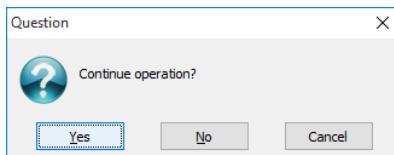
When the user clicks the 'Cancel' button, the script operation will be terminated.

After Yes : it is possible to enter a command to be performed when the user clicks the 'Yes' button.

Similarly, it is possible to enter a command to be performed after clicking the 'No' button after No : .

Example:

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

**9.3.2 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, optionally specify a window title, otherwise, otherwise 'Script Memo' will be used. The following options can be used to format the text:

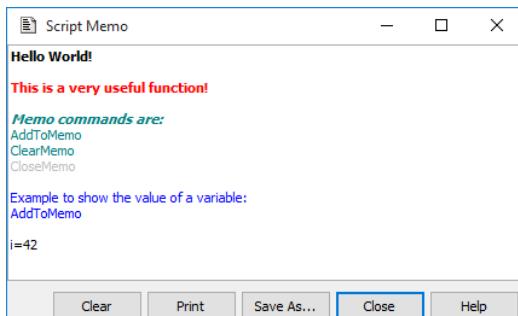
	use a bold font style
<i>	use an italic font style
<bi>	use bold italic
 or </i> or </bi>	use normal font style
<c:{ColorName}>	use the defined color, possible values are: Black, Red, Maroon, Lime, Green, Yellow, Olive, Blue, Navy, Fuchsia, Purple, Aqua, Teal, Silver, Gray

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

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

Example:

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

**SaveMemo {Filename}**

Use this command to 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.

9.3.3 Input Commands**{Variable}:={MenuSelection {Label|Item 1|Item 2 ...} {{Title}} {{Default selection}}**

This command shows 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 '&' is used inside the item text, the following letter will be used as a hot key.

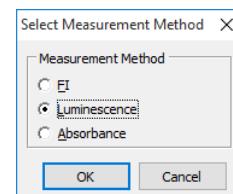
Specifying a title or defining a default selection is optional. If a title is not specified, 'Please select' will be used. If a default selection is not specified, the first item will be pre-selected.

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
```

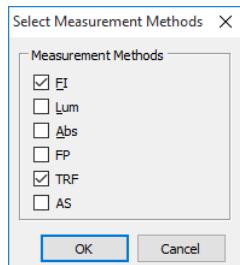


{Variable}:=**MultiSelection** {Label|Item 1|Item 2 ...} ({Title})

The purpose and syntax is very similar to the MenuSelection command. This command is using check boxes instead of radio buttons and allows multiple selections. The user input will be returned into a field of boolean variables (see chapter 9.8). The variable for the first option need to be specified as part of the command:

Example:

```
UseMeasMode1:=MultiSelection /
"Measurement Methods|&FI|&Lum|&Abs|FP|TRF|AS" /
"Select Measurement Methods"
```



If the user selects the first checkbox, this variable will be set to True. The selection of the second check box will be returned in variable UseMeasMode2, the third option as UseMeasMode3 and so on. Instead of a start index 1 any other start value (max. 190000) can be used.

If no start index is specified, 1 will automatically be used.

Default values can be specified by setting the appropriate variable before using the MultiSelection command, e.g.:

```
UseMeasMode1:=true
UseMeasMode2:=true
UseMeasMode3:=false
```

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

This command shows an input box for integer values. Using the first parameter, it is possible to specify the text in front of the input field. Similar to the Ask and ShowMsg command, use ‘&’ inside the text for a line break.

If a title is not specified, ‘Input’ will be used.

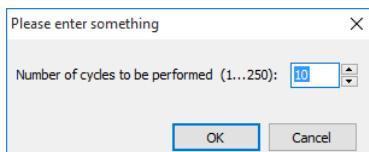
If minimum and maximum values are specified, only input values in this range will be accepted. If the range between minimum and maximum value is not larger than 250, up/down scroll buttons will appear beside the input field.

Optionally, 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
```

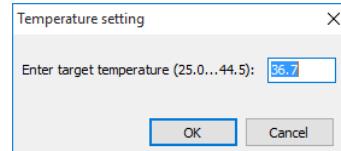


{Variable}:=**InputFloat** {Label} ({Title}) ({Min}) ({Max}) ({Default value})

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})

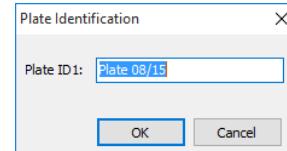
This command is similar to the IntegerInput command, but will be used for string values. As target variable, only a string variable (st0 ... st100) is allowed.

Examples:

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

or

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



{Stringvariable}:=**SelectProtocol** ({Title}) ({Method pre-selection}) ({“LVis”})

This command will open a test protocol selection window, showing all available test protocols of the user currently logged in from the Microplate tab or from the LVis Plate tab (when “LVis” is added as parameter).

Optionally, define the caption of the selection dialogue. If a caption is not specified, “Select Test Protocol” will be used.

For the microplate tab protocol selection window, it is also possible to define which method should be pre-selected. Use “Method: followed by the method name abbreviation and ”. The following values can be used here: All, FI, ABS, FP, LUM, TRF, AS. If you do not specify a method, the last method that was selected will be used.

Examples:

```
st1:=SelectProtocol
st1:=SelectProtocol "Please select any protocol" /
"Method:All"
st1:=SelectProtocol "Please select a lum. protocol" /
"Method:LUM"
st1:=SelectProtocol "Method:FI"
st2:=SelectProtocol "LVis"
st2:=SelectProtocol "Please select a LVis Tab protocol" /
"LVis"
```

See the chapter 9.12 File Commands for reading variables from files.

9.4 Reader Commands

9.4.1 Init Command

R_Init {{InitMode}}

If the optional {InitMode} parameter is not used, 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.

Using the optional {InitMode} parameter changes the behavior of the reader upon initialization. This parameter is binary coded (see table for the high byte). The TestMode from the configuration file will be added to the low byte.

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 be 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 $0C00 ;perform plate search, but no incubator
;reset
```

9.4.2 Plate Carrier Movement

R_PlateOut

This command moves the plate carrier out of the reader to stack position 1, if the reader is attached to a stacker. Otherwise, the plate carrier will be just moved out. See also S_PlateOut.

R_PlateOutR

This command moves the plate carrier out and to the right side (to stack position 2, if the reader is attached to a stacker). See also S_PlateOut.

R_PlateIn

This command moves the plate carrier into the reader. See also S_PlateIn.

R_PlateInB {BarcodeReaderSelection} {Barcode reading height}
or
R_PlateInB {BarcodeReaderSelection} {MP:Microplate Name}
or
R_PlateInB {BarcodeReaderSelection} {Protocol Name} ({Path to protocol data base})

Move plate carrier into the reader and read the selected plate barcodes (only for CLARIOstar Plus and only available if the instrument is equipped with plate barcode readers).

BarcodeReaderSelection: F=Front; R=Right; A=Auto: automatic selection of the necessary barcode values based on the defined plate IDs or the ASCII export settings, can be combined with F, R.

The barcode reading height can be entered directly (in mm, e.g. 8.5) or can be taken from the microplate data base. By specifying a protocol name the script interpreter will use the microplate name defined as part of the test protocol and will then use the barcode reading height belonging to this microplate.

This command can also be used to read the plate barcodes when the plate carrier is already inside the reader (e.g. after using an S_PlateIn command).

Examples:

```
R_PlateInB "LR" "Tom's Protocol"
R_PlateInB "LA" "MP:BMG LABTECH 96"
R_PlateInB "L" 8.5
R_BarcodeData
```

R_BarcodeData

Use this command to transfer the barcode strings obtained using the R_PlateInB command from the reader to the computer. This command has no parameters. The barcode data can be used using <Barcode_Front_String>, <Barcode_Right_String>, <Barcode_Front_Value>, <Barcode_Right_Value> or using the <barcode_front> and <barcode_right> options for plate IDs.

Notes: If you want to use the barcode reader of a Stackers use the commands S_PlateInB and S_BarcodeData (see chapter 9.5.2).

The plate barcodes will automatically be read by the R_Run command if the barcode values are necessary for the defined plate IDs or the ASCII export. Use the R_PlateInB / R_BarcodeData combination if a barcode value is needed inside a script before executing the R_Run command, e.g. to use different protocols based on the barcode string.

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 (extended incubator up to 65 °C).

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 Focus and Gain Adjustment

Notes: Focus and gain adjustment is not necessary / possible for absorbance protocols. Gain adjustment is not available for AlphaScreen™ protocols. Gain adjustment over the whole plate (`R_GainPlate`) is not available for fluorescence polarization protocols.

R_FocusPlate {Protocol name} {[Path to protocol definition]} {Chromatic number} {Channel}

Perform a focus adjustment by first searching the well with the highest signal and then searching the optimum focal height on that well. Specify the chromatic number and the channel (for the CLARIOstar and VANTAstar only channel "A" exists) to be used.

When adding **Flying** to the command line, the measurement will be done in flying mode to save time. This option is only available for fluorescence method protocols.

Example:

```
R_FocusPlate "FI Protocol" 1 "A" Flying
```

Note: This command is only available when using a CLARIOstar Plus with firmware V1.30 or newer or when using a VANTAstar or VANTAstar F.

R_FocusWell {Protocol name} {[Path to protocol definition]} {Well column} {Well row} {Chromatic number} {[Wavelength for gain adjustment for spectral scan protocols]} {Channel}

Perform a focus adjustment using the defined well, chromatic number and channel (for the CLARIOstar and VANTAstar only channel "A" exists).

When using a spectral scan protocol optionally the wavelength to be used for the adjustment procedure can be specified. This wavelength should be inside the scanning range. If 0 is entered for this parameter (or if the parameter is omitted) a default value is used. If a value smaller than the start wavelength of the scan range is defined, the start wavelength will be used instead of this value. If a value larger than the end wavelength of the scan range is defined, the end wavelength will be used.

Example:

```
R_FocusWell "FAST TEST" /
"C:\Program Files\BMG\CLARIOstar\User\Definit" /
3 4 1 "A"
```

Note: The old name of this command (`R_FocusAdjust`) can still be used for compatibility reasons.

R_GainPlate {Protocol name} {[Path to protocol definition]} {Desired raw result for channel A} {Desired raw result for channel B} {Chromatic number} {[Focus adjustment channel]}

This command performs a gain adjustment for all measurement points of the plate using the defined parameters.

The parameter {Path to protocol definition} is optional. This permits use of a test protocol belonging to a different user account.

Please specify the desired raw result (required value) in % of the measurement range.

Add an optional parameter to select the channel for the focus adjustment procedure ("A"). If this parameter exists, a focus adjustment procedure will be performed before the gain adjustment procedure.

Example:

```
R_GainPlate "QC Test" 80 0 1
```

Notes: To use the optional focus adjustment parameter a CLARIOstar Plus with firmware V1.30 or newer or a VANTAstar / VANTAstar F is necessary.

The parameter {Desired raw result for channel B} exists only for compatibility reasons and should always be 0.

R_GainWell {Protocol name} {[Path to protocol definition]} {Well column} {Well row} {Target value for channel 1} {Target value for channel 2} {Chromatic number} {[Target mP value for fluorescence polarization protocols / wavelength for gain adjustment for spectral scan protocols]} {[Focus adjustment channel]}

This command performs a gain adjustment on the specified well using the declared parameters.

The parameter {Path to protocol definition} is optional.

When using a spectral scan protocol specify the wavelength to be used for the adjustment procedure. This wavelength should be inside the scanning range. If 0 is entered for this parameter (or if the parameter is omitted) a default value is used (see chapter 6.3.5 *Gain Adjustment for Spectral Scan Protocols*). If a value smaller than the start wavelength of the scan range is defined, the start wavelength will be used instead of this value. If a value larger than the end wavelength of the scan range is defined, the end wavelength will be used.

Add an optional parameter to select the channel for the focus adjustment procedure ("A"). If this parameter exists, a focus adjustment procedure will be performed before the gain adjustment procedure.

Example:

```
R_GainWell "FAST TEST" 5 2 80 0 1 "A"
```

(Perform focus adjustment using channel A and gain adjustment for channel A (req. value 80%) with chromatic 1 on well B5.)

R_GainWell in Fluorescence Polarization Mode

You should perform one GainWell command to adjust the gain value for the first channel (chromatic 1), e.g. using a target value of 10. After this you should perform a GainWell command for the second channel (chromatic 2). Here the target value should be set according to the known target mP value of the fluorophore used (for fluorescein-based chemistry (35 mP) use 9.32).

After performing the second gain command in Fluorescence Polarization mode a K-Factor for fine-adjustment of the two measurement channels will automatically be calculated (based on the obtained raw results and the used target mP parameter). But to achieve maximum accuracy it is recommended to send a separate GetKFactor command after performing the gain adjustment.

Example:

```
R_GainWell "FP EP" 2 1 10 0 1 35 "-"
R_GainWell "FP EP" 2 1 9.32 0 2 35 "-"
```

and optional:

```
R_GetKFactor "FP EP" 2 1 1 35 "-"
```

R_GetKFactor {Protocol name} {[Path to protocol definition]} {Well column} {Well row} {Chromatic No.} {Target polarization value}

This command calibrates the ratio between the two measurement channels (fine adjustment for fluorescence polarization tests) using the declared parameters.

Note: It is recommended to execute this command after performing the gain adjustment (R_GainWell command) for fluorescence polarization protocols.

R_SetGain {Protocol Name} ({Path to protocol data base})
{Chromatic} {channel} {gain value}

This command changes the gain value defined in a test protocol to the specified value (range 0...4095).

Example:

```
R_SetGain "FP PLATE" 1 "A" 222  
(Sets the gain value for chromatic 1, channel A to 222.)
```

R_SetFocalHeight {Protocol Name} ({Path to protocol data base})
{focal height value}

This command changes the focal height value used by the defined test protocol. The focal height can be changed in increments of 0.1 mm, the maximum is 25 mm.

Example:

```
R_SetFocalHeight "FP PLATE" 10.5  
(Change the focal height value to 10.5 mm)
```

Note: If the path to the protocol definition is not included, the path to the microplate tab protocol data base of the user currently logged in will be used. If using an LVis Plate tab protocol, either enter (1) the complete path to this data base (e.g. "c:\Program Files\BMG\CLARIOstar\User\DefLVis") or (2) use "LVis" (the software will then use the path belonging to the user currently logged in).

9.4.5 Run Commands

n:=R_CalculateTestDuration {Protocol name} ({Path to protocol definition}) ({Source magazine}) ({Destination magazine}) ({EDR mode}) ({Calculation mode})

This command calculates the duration of executing a test protocol and store this value (in seconds) in variable n.

If a Stacker is connected (and when using a reader firmware version 1.10 or newer), it is optionally possible to specify a source and a destination magazine (see also the description of the R_Run command below). If stacker magazines are specified here, the time for fetching a plate from the stacker source magazine and / or for inserting the plate into the destination magazine will be taken into account for the calculation of the test duration.

If a CLARIOstar Plus, VANTAstar or VANTAstar F is used it is possible to specify an EDR (enhanced dynamic range see chapter 6.4) mode. Add **UseEDR** to take the time necessary for the usage of the enhanced dynamic range feature into account. When adding **DontUseEDR** the time calculation will be done without the extra time for the enhanced dynamic range feature. Without any of these options the setting will be used, which was used the last time a check timing or an execution of the protocol was done. There will be an error message if you try to use EDR but the protocol settings do not allow it.

If this command is used without the optional calculation mode parameter, the script execution will be cancelled in case of timing errors (e.g. a too small cycle time).

When using **cmCheckOnly** as calculation mode parameter, in case of a timing error a negative value (= the quit code obtained from the reader firmware multiplied by -1) will be returned.

When using the mode **cmCheckAndCorrect** too small cycle / interval times will be corrected. Should a correction be necessary, the return value will be negative. It is recommended to repeat the command with the option **cmCheckOnly** when the test run duration value is needed.

When using the mode **cmOptimize** all cycle / interval times will be set to the smallest possible value. For well mode protocols with more than one kinetic window also the start times of kinetic window 2 ... 4 will be set to the minimum. When using the command in this mode the return value will always be negative. In this mode the command can also be used without specifying a variable for the result. Repeat the command with the option **cmCheckOnly** to get the test duration.

The mode **cmCheckFilters** works very much like the mode **cmCheckOnly**. The only difference is: if a filter, which is used by the test protocol, is currently not installed, the script execution will not be canceled with an error message. Instead of this the return value will be set to -1000. This option allows to check a protocols for missing filters before executing it and to react inside the script accordingly.

Example:

```
R_CalculateTestDuration "FI Protocol" cmOptimize  
n:=R_CalculateTestDuration "FI Protocol" cmCheckOnly  
UseEDR
```

ID1:=, ID2:=, ID3:=

This command changes the ID values used by the R_Run command. Enter <Loop> to use the current value of the loop variable (For ...To command), e.g.: **ID1:="Batch: <Loop>"**.

It is also possible to combine numerical variables with strings, e.g.: **ID1:="Temperature=" Temp1**.

Here it is possible to use all special functions that are described in the chapter 6.4.

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 / .xlsx) format). The file content should use the same syntax as for the 'Import IDs' function of the Sample IDs sheet of the Start Measurement dialogue. It is possible to use this dialogue to create the sample IDs file.

Examples:

```
R_SetSampleIDs "FI TEST" "E:\SIDs.txt"  
R_SetSampleIDs "TOM'S TEST" /  
"c:\BMG\CLARIOstar\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** is used to clear the sample IDs associated with the specified protocol.

R_ClearDilutionFactors {Protocol name} ({Path to protocol definition})

This command resets 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}) ({Last plate mode}) ({EDR mode})

This command starts a measurement using the declared parameters.

Specifying the path to the protocol definition and specifying the path for measurement data is optional (useful, if performing a protocol belonging to a different user), but it is not possible to only specify the path for measurement data.

Note: If the path to the protocol definition is not included, the path to the microplate tab protocol data base of the user currently logged in will be used. If using an LVis Plate tab protocol, either enter (1) the complete path to this data base (e.g., "c:\Program Files\BMG\CLARIOstar\User\DefLVis") or (2) use "LVis" (the software will then use the path belonging to the user currently logged in).

If a CLARIOstar Plus, VANTAstar or VANTAstar F is used it is possible to specify an EDR (enhanced dynamic range, see chapter 6.4) mode. Add **UseEDR** to use the enhanced dynamic range feature. When adding **DontUseEDR** the enhanced dynamic range feature will not be used. Without any of these options the setting will be used, which was used the last time a check timing or an execution of the protocol was done. There will be an error message if you try to use EDR but the protocol settings do not allow it.

If a Stacker is connected (and when using a reader firmware version 1.10 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_PrepForPlate** 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
```

When adding **NoCalibration** to the **R_Run** command line, the calibration step of the transport system axes will be omitted. In addition the transport system motors will not be automatically

disabled afterwards to ensure that the current positions are not lost. To disable the motors (e.g., at the end of a script) use the command **R_DisableMotors** or use an **R_Run** command without this option. The usage of the **NoCalibration** option will save one or two seconds per test run. If the motors are disabled during the usage of this option (e.g., when executing the first **R_Run** command), the necessary calibration will be performed even in combination with this option.

9.4.6 Additional Reader Commands

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

This command performs 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.

The Shake frequency can be defined in the range 100 ... 700 rpm, when high speed shaking is activated up to 1100 rpm (VANTAstar: 1300 rpm). For linear shaking a maximum of 800 rpm (when high speed shaking is activated) and for meander corner well shaking a maximum of 300 rpm can be used.

The Shake time can be anything between 1 and 3600 seconds.

It is possible to define the x position in the range 250 ... 3100 and the Y position in the range 125 ... 800. When no shake position is specified (or when 9999 for X or Y is used) 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
```

Notes: Modes 3 and 4 are no longer supported by firmware versions beginning V2.00 and not for VANTAstar readers.

Mode 4 (orbital corner well shaking) is only available for readers equipped with a special plate carrier.

R_IdleMove {IdleMove mode} {IdleMove frequency} {IdleMove duration} ({IdleMove On-time} {IdleMove Off-time})

This command can be used to perform an individually defined stand-alone idle movement. The mode parameter is used to set the position / kind of movement:

- 1 = linear x and y movement between the 4 corner wells
- 2 = wait at the incubation position (a dedicated waiting position where the microplate is fully covered by the incubator heating plates)
- 3 = meander corner well shaking
- 4 = orbital corner well shaking
- 5 = orbital shaking
- 6 = double orbital shaking

Using mode 3, the idle move shaking frequency can be defined in the range 100 ... 300 rpm, using modes 4 ... 6. the range can be defined in the range = 100 ... 700 rpm (having high speed shaking activated up to 1100 rpm, VANTAstar: 1300 rpm). In mode 1 and 2 this parameter is not evaluated.

The idle move duration defines the entire movement in the range of 1 ... 65535 seconds. Within this duration, the on- an off-time can be assigned optionally (not assigned or off-time set to 0 will perform a permanent idle movement during the full duration; having on-time set to zero whilst off-time is >0, no movement will be active (just wait at the defined position); having both on- and off-time set to a value in the range of 1 ...

3600 seconds, the movement will be active for on-time seconds and pause for off-time seconds repetitive during the full duration).

Examples:

```
R_IdleMove 1 100 60      ;perform a linear movement for
R_IdleMove 3 200 30      ;60 s
R_IdleMove 3 300 30 7    ;perform a meander corner well
R_IdleMove 3 300 30 7 4  ;shaking with 200 rpm for 30 s
                          ;shaking with 300 rpm for 30 s,
                          ;whilst moving for 7 s and
                          ;pausing for 7 s repetitive.
                          ;perform a meander corner well
                          ;shaking with 300 rpm for 30 s,
                          ;whilst moving for 7 s and
                          ;pausing for 4 s repetitive.
```

Notes: For this command a firmware version 1.20 or newer is necessary.

Modes 3 and 4 are no longer supported by firmware versions beginning V2.00 and not for VANTAstar readers.

Mode 4 (orbital corner well shaking) is only available for readers equipped with a special plate carrier.

R_ACU {Sub command} {Parameter 1} {Parameter 2}

Using this command, the atmospheric control unit (ACU) can be controlled.

Use sub command code 0 to start or stop the ACU regulation dependent on the values defined by parameter 1 and 2. Parameter 1 is used for the O₂ channel and parameter 2 for the CO₂ channel. Here a target concentration in 1/10% can be defined, the allowed range is 1 ... 200 (when using a VANTAstar the O₂ range is 10 ... 200). When using 0 here the regulation for the respective channel will be switched off. Use 255 to only monitor the concentration.

The other possible sub command codes are intended for internal use.

Examples:

```
R_ACU 0 0 0      ;switch ACU off
R_ACU 0 255 255 ;monitor the O2 and CO2 concentration
                  ;(no regulation)
R_ACU 0 100 200 ;switch on the ACU regulation with a
                  ;target concentration of 10% for O2 and
                  ;20% for CO2
```

Notes: For this command a firmware version 1.10 or newer is necessary.

The command can only be used if an ACU is connected.

R_Fan {Fan number} {Speed in %} ({On time in seconds})

This command supports a direct access to the speed controllable fan (fan number 2, which is the additional cover fan that usually is only available when an ACU is connected to the system). The command allows to temporarily or permanently set the speed of this fan. In combination with the R_ACU command this allows also a ramping functionality for the ACU concentrations.

The speed can be defined between 0 and 100 (percent), but currently the hardware allows only a speed up to 93%.

The on time can be define between 1 and 3600 (seconds). The defined fan speed will be applied temporarily for this time. While the fan is running on the temporarily set speed it will anyway react on other internal fan events (ACU, start of testrun, door open,...). In this case the originally set fan speed will be overwritten by the internal fan event. So when the speed has been overwritten by a door open event and the door will be

closed again, the speed will be set back to the originally set fan speed until the defined on time has elapsed.

When no on time is defined or when using 0 for this parameter, the defined fan speed will be applied permanently (until another internal fan event will be triggered or until the fan speed will be set to another value via the R_Fan command).

Examples:

```
R_Fan 2 93 5      ;switch on the fan for 5 seconds with 93%
                  ;speed
R_Fan 2 50        ;switch on the fan with 50% speed until a
                  ;new R_Fan command or an internal fan event
R_Fan 2 0         ;switch off the fan
```

Note: For this command a firmware version 1.10 or newer is necessary.

R_Stirrer {Target temperature} {Stirrer speed}

This command controls the heater and the stirrer of the optional reagent dispenser module of the VANTAstar / VANTAstar F readers.

The temperature range in °C is: 25.0 ... 45.0; use 0 for off. For readers with an extended incubator the temperature can be defined between 10.0 and 60.0 °C.

The stirrer speed can be defined between 200 and 1500 (rpm), use 0 for off.

Examples:

```
R_Stirrer 36 500 ;switch on the heater with a target
                  ;temperature of 36 °C and the stirrer
                  ;with a speed of 500 rpm
R_Stirrer 0 500   ;only use the stirrer but not the
                  ;heater
R_Stirrer 0 0     ;switch off the heater and the stirrer
```

Note: This command is only available when using a VANTAstar or VANTAstar F reader with attached reagent dispenser module.

9.4.7 Protocol Names / Edit Protocol Layout

{String variable}:=R_GetProtocolNames ({separator}) ({Path to protocol definition})

Use this command to get a list of all existing protocols. The protocol names are separated by the specified separator or by a tab character (#9), if no separator is defined. If no protocol path is specified the microplate tab protocols of the user currently logged in will be listed. If "LVis" is used as protocol path parameter the LVis tab protocols of the user currently logged in will be listed.

Example:

```
st1:=R_GetProtocolNames "LVis"
```

R>EditLayout {Protocol name} ({Path to protocol definition}) {Layout change action}

Use this command to edit the layout of the specified test protocol.

{Layout change action} is a string of one or more {well name}={well content} pair(s), e.g. "A1=X1 H5=S5". To empty a well use {well name}=Empty or {well name}=-.

Example:

```
R>EditLayout "FI Fast" "A1=S1 A2=Empty A3=B"
```

When using layout groups include the group specifier as part of the well content.

Example:

```
R>EditLayout "FI Fast" "A1=SA1 A2=Empty A3=BA B1=SB1"
```

To delete the complete layout before defining new well contents use the command **EmptyLayout** as part of the layout change action string.

Example:

```
R>EditLayout "FI Fast" "EmptyLayout A1=X1 A2=X2 A3=X3"
```

R>EditConcAndVol {Protocol name} ({Path to protocol definition}) {Concentrations/volumes change action}

Use this command to edit the concentrations and/or injection volumes of the specified test protocol.

{Concentrations/volumes change action} is a string of one or more {content}: C={concentrations} V1={injection volume 1} V2={injection volume 2} V3={injection volume 3} V4={injection volume 4} entries. It is not necessary to specify all properties. Unspecified properties will not be changed.

Example:

```
R>EditConcAndVol "FI Fast" "SA1: C=10000 V1=10 V2=0 SA2: C=1000"
```

R_ExportLayout {Protocol name} ({Path to protocol definition}) {Layout file name}

This command exports the layout of the specified test protocol into a layout file using the *.lac file format.

Example:

```
R_ExportLayout "FI TEST" "E:\Layout384.lac"
```

R_ImportLayout {Protocol name} ({Path to protocol definition}) {Layout file name}

This command imports a layout into the specified test protocol.

9.5 Stacker Commands

9.5.1 Init Command

S_Init

This command initializes the stacker device.

9.5.2 Plate Handling Commands

S_PrepForPlate {Protocol Name} ({Path to protocol data base})

or

S_PrepForPlate {MP:Microplate Name}

This command takes the microplate name from the specified test protocol (or takes the microplate name specified directly using the second syntax version) and reads the Z dimensions from the microplate data base. Based on these dimensions, the Z value will be calculated, at which the Stacker opens its stoppers when inserting a plate into a magazine or closes the stopper when retrieving 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: Always use this command in scripts before the first stacker plate in / out action!

S_PlateIn {n} (NotLastPlate)

This command retrieves a plate from magazine {n} and moves it into the reader.

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

Examples:

```
R_ImportLayout "FI TEST" "E:\Layout384.lac"
```

```
R_ImportLayout "TOM'S TEST" / "c:\BMG\CLARIOstar\Tom\Definit" / "C:\BMG\CLARIOstar\Extern\Layout384.lac"
```

Layout files can be created using the **R_ExportLayout** command or using the layout export function inside a protocol editor (right click onto the Layout grid or the Concentrations & Volume table to access the context menu, see chapters 4.3.11 and 4.3.14).

The script command **R_ImportLayout** also accepts *.lb (= Layout BMG) files. The content of the layout file should follow the syntax of the layout change action parameter of the **R>EditLayout** command (see above). Line breaks (e.g. between the different contents) are allowed. Any file with a file name extension other than .LAc will be treated as a .lb file.

R_ImportConcAndVol {Protocol name} ({Path to protocol definition}) {Conc and vol file name}

This command imports only concentration and/or volume changes into the layout of the specified test protocol.

The content of the concentration and volumes file should follow the syntax of the Concentrations/volumes change action parameter of the **R>EditConcAndVol** command. Line breaks (e.g. between the different contents) are allowed.

As file name extension .cvb (= Concentrations and Volumes BMG) is suggested.

Example:

```
R_ImportConcAndVol "FI TEST" "E:\CV384.cvb"
```

See also the stacker options of the **R_Run** command.

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

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

S_PlateInB {n} (NotLastPlate)

This command retrieves a plate from magazine {n}, reads the barcode from this plate (using the Stacker barcode reader) and moves the 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.

Use the **R_PlateInB** and **R_BarcodeData** commands (or the **R_Run** command in combination with appropriate plate ID settings) to use the plate barcode readers built in into the CLARIOstar Plus.

S_PlateOut {n}

This command moves the plate out of the reader and inserts it into magazine {n}.

S_GetPlate {n}

This command retrieves 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}

This command retrieves a plate from magazine {n} and reads 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}

This command places a plate from the stacker table (or from the reader plate carrier, if this has been moved under the target stack) into the 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}

This command transfers {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

This command shows the number of counted plates (counted during an S_MovePlate command) and moves the plates back to the count stack.

9.5.3 Other Stacker Commands

S_MoveTable {n}

This command moves the 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

9.6 System Constants and Variables

There are some predefined constants and variables that can be used in scripts, e.g. for the S_MoveX command.

9.6.1 System Constants

<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> <48 then begin
    ShowMessage "For using this script you need a newer
    software version!" Error
    halt
end;
```

S_MoveX {n}

This command moves the stacker table to X position {n}.

S_MoveZ {n}

This command moves the stacker table to Z position {n}.

S_DisableMotors

Disable the stepper motors.

Enter 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

This command reads (scans) the barcode using the Stackter barcode reader.

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

This command transfers 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. See also R

S_SysTest {mode}

This command starts the stacker system self-test command.

S_GetSysTestData

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

Note: If error 32 occurs 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.

<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	<PMT_Config>	0: Single PMT System, PMT1 is standard PMT.
<Zpos_Put_CloseStp>	Z position to close stopper when inserting plate into stack		1: Single PMT System, PMT1 is Extended IR PMT.
<Zpos_Get_OpenStp>	Z position to open stopper when fetching plate from stack		2: Dual PMT system (PMT1 is Extended IR PMT in a standard dual PMT system)
<Zpos_Get_CloseStp>	Z position to close stopper when fetching plate from stack		3: Cross-over dual PMT system (PMT1 is Extended IR PMT in a cross-over dual PMT system)

9.6.2 Numerical System Variables

<Barcode_Value>	If the plate barcode (read by the BMG LABTECH Stacker) 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.)	<PMT_Config>	<i>Example:</i> if <PMT_Config>>=2 then IsDualPMT:=True else IsDualPMT:=False
<Barcode_Front_Value>, <Barcode_Right_Value>	If the plate barcode (read by the internal barcode readers of the CLARIOstar Plus) contain a valid integer or floating point number, this numeric variables will return this number, otherwise they will return 0. (All non-numerical characters will be removed before converting the barcode string into an integer or float value.)		<i>Note:</i> This variable is only available when using a firmware version 1.20 or newer.
<Temp>	Current temperature of the incubator built into the reader (floating point value with 0.1 °C resolution). <i>Note:</i> 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).	<GainA>	gain value for channel A obtained during gain adjustment
<ReagentDispenserModuleTemp>	Temperature inside the reagent dispenser module of the VANTAstar or VANTAstar F.	<GainB>	gain value for channel B obtained during gain adjustment (for fluorescence polarization protocols)
<O2Conc>	Current O ₂ concentration in percent. <i>Note:</i> This value is only available when an ACU is connected and the regulation or the monitoring has been switched on. (R_ACU command).	<RawResultA>	raw result obtained during gain adjustment for channel A
<CO2Conc>	Current CO ₂ concentration in percent. <i>Note:</i> This value is only available when an ACU is connected and the regulation or the monitoring has been switched on. (R_ACU command).	<RawResultB>	raw result obtained during gain adjustment for channel B (for fluorescence polarization protocols)
		<GainAdjWellColA>	column of well with highest signal found during R_GainPlate (gain adjustment over the whole plate) for channel A
		<GainAdjWellRowA>	row of well with highest signal found during R_GainPlate for channel A
		<KFactor>	K factor (fine adjustment between the two channels) obtained during R_GainWell or R_GetKFactor in fluorescence polarization mode (floating point value)
		<FocalHeight>	optimum focal height obtained with R.FocusAdjust (focus adjustment) or with R_GainWell when using the focus adjustment option
		<RawResult>	row result obtained during focus adjustment for the used channel at the optimum focal height
			<i>Note:</i> 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 System Variables

<Barcode_String> Contains the barcode value, which was read by the BMG LABTECH Stacker last, in string format

Note: The command **ID1:="<Barcode_String>"** takes the barcode string 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 string 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 automatically be updated after using an **R_Run "<Protocol name>" 1 2 ReadBarcode** command.

<Barcode_Front_String>, <Barcode_Right_String>

Contain the barcode values, which were read by the internal barcode readers of the CLARIOstar Plus last, in string format.

Notes: The command **ID1:="<Barcode_####_String>"** takes 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 **R_BarcodeData** command (The **R_BarcodeData** command should be used after an **R_PlateInB**, **S_GetPlateB** or **S_ReadBarcode** command.). It will automatically be updated after using an **R_Run** command.

<User> Contains the name of the user currently logged in (BMG software user).

<Windows_User> Contains the name of the user currently logged in into Windows.

<User_Root_Path> Contains the root path of the user currently logged in.

<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 Database 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 1.4). If an **R_Run** command is used inside the script in combination with the optional path parameters (e.g. **R_Run "FAST" "D:\My Files\CLARIOstar\Definit" "D:\My Files\CLARIOstar\Data"**) these system variables will show afterwards the paths used for the last run command. These variables are especially useful in combination with the Call and Execute commands (see chapter 9.13).

Example:

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

<ASCIIPath>

Contains the current path for storing the measurement data in ASCII format (as defined using 'Settings | Data Output | Save measurement data in ASCII format | Define format', see chapter 3.4.1).

When using protocol specific output settings, it contains the last used value (e.g. after an **R_Run** command).

<InstallPath>

Contains the installation path of the SMART Control Control Software.

<ScriptPath>

Contains the path of the current script file (empty for new scripts, which have not yet been saved).

<Ser_Number>

Contains the serial number of the reader currently connected.

9.6.4 Boolean System Variables

<ABSBuiltIn>

true, if the reader is equipped with a measurement system for absorbance measurements

<SpectrometerBuiltIn>

true, if the reader is equipped with a spectrometer for absorbance measurements

<FlbuiltIn>

true, if the reader is equipped with a measurement system for fluorescence intensity measurements

<LUMbuiltIn>

true, if the reader is equipped with a measurement system for luminescence measurements

<TRFbuiltIn>

true, if the reader is equipped with a measurement system for time-resolved fluorescence measurements

<FPbuiltIn>

true, if the reader is equipped with a measurement system for fluorescence polarization measurements

<ASbuiltIn>

true, if the reader is equipped with a measurement system for AlphaScreen measurements

<MonochromatorBuiltIn>

true, if the reader is equipped with a monochromator

<PlateCarrierOut>

true, if the reader plate carrier is outside the reader

<ReagDoorOpen>

true, if the reagent door of the reader is open

<PlateInserted>

true, if a microplate is inserted (see also **<MPSensorValid>**)

<MPSensorValid>

true, if the microplate sensor has been read (e.g. after a plate in)

<StackerAttached>

true, if a stacker is attached to 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

These boolean variables can be used in combination with the 'if ... then' command.

9.7 Numerical Variables

It is possible to 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 9.9). The variables do not need to be declared. 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. Letters and numbers can be used 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, else, flying, for, halt, ID1, ID2, ID3, if, next, no:, NoCalibration, NotLastPlate, plate, ReadBarcode, restart, StopperZPosIsDifferent, TestPlateOnReader-Carrier, 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, Include, InputFloat, InputInteger, InputString, MenuSelection, MultiSelection, SelectProtocol, 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 ... st100, as these are the predefined names for the string variables. Do not use the constants True and False as variable name.

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:

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 also possible to use these boolean system variables in assignments or in calculations, e.g.:

```
PlateCarrierIn:=Not(<PlateCarrierOut>)
```

```
b := 3*a
for i:=1 to b do begin
```

Also allowed:

```
for i:=4*c to d do begin
  ...
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)
RemainingTime:=335
NoOfShakingIntervals:=RemainingTime / 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)>"
```

```
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.

It is possible to use the **<IntToStr(n)>** and **<FloatToStr(n)>** functions in scripts on all places where a string is used. These functions need to be inserted inside the quotation marks.

9.8 Boolean Variables

Any numerical variable (see chapter 9.7) can also be used as boolean variable. The variables do not need to be declared. A variable will be automatically initialized with a value of 0.0 = False when it is used first time.

Constants True and False

True (=1)

False (=0)

These constants can be used for assignments:

```
bool1:=True
```

and comparisons:

```
if bool1=True then beep
if bool2=False then beep
```

Not(..) operator

For boolean comparisons also a shorter syntax can be used. Here also the operator 'Not(..)' can be used:

```
if bool1 then beep
if Not(bool2) then beep
```

For all boolean comparisons any value <>0 will be considered to be True.

```
A:=42
if A then beep
```

The Not(###) operator can also be used for assignments:

```
bool2:=Not(bool1)
```

Example:

```
bool42:=true
if Not(bool42) then beep
bool42:=Not(bool42)
if Not(bool42) then beep
```

Operators or, and

For comparisons in combination with two boolean variables the operators 'or' and 'and' can be used.

Example:

```
if UseMeasMode1 or UseMeasMode2 then ...
```

The 'or' and 'and' operators can also be used for calculations using boolean variables.

Examples:

```
PerformMeasurement:=UseMeasMode1 or UseMeasMode2
a:=b and c
```

In addition the operators 'nor', 'nand' and 'xor' can be used.

It is not allow to use more than one Boolean operation in one comparison (e.g. 'if Option1 and Option2 and Option3 then begin'). This will lead to an error message.

9.9 String Variables

It is possible to 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). The variables do not need to be declared. 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 "."
st4:="The value of n is <FloatToStr(n)>."
st5:="The barcode label is <Barcode_String>."
```

A string variable can be used for all commands where a string parameter is expected. 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 performed with string variables can also be performed with the three plate identifiers ID1 ... ID3. The only difference is that some special functions can be used for the plate IDs, as for example ID1:="Date:<date>" (see chapter 6.5).

9.10 Measurement Data

9.10.1 R_GetData

To get access to the measurement data, use the **R_GetData** command. This is useful when perform different actions in the script depending on the measurement values.

Syntax:

```
Variable := R_GetData {well name} {[cycle/interval]} {[chromatic]}
{[channel]} {[Return value for unused wells]}
```

or

```
Variable := R_GetData {well column} {well row} {[cycle/interval]}
{[chromatic]} {[channel]} {[Return value for unused wells]}
```

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.

Optionally a return value can be specified, which will be send back if the specified well was not used during the measurement.

If such a value is not specified the script processing will be cancelled with an error message.

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 Database format measurement files and will therefore only work if the 'Save measurement data in Absolute Database format' option has not been switched off (menu command 'Settings | Data Output')!

9.11 Date and Time Functions

9.11.1 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 "Tom's Protocol"
Tend:=<now>
DeltaT:=Tend-Tstart
DeltaT:=DeltaT * 86400 ;convert fractions of a day into s
WaitTime:=600 - DeltaT
wait for WaitTime s

```

This example script will perform one measurement and will then wait the remaining time to 600 seconds before continuing with the following commands. A wait time of 10 minutes can be set 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.

9.10.2 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 9.14), which performs some operations with the measurement data, e.g. a merge of different measurement runs into a single run. 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}}

Wrong: DeltaT:=<now>-Tstart

Correct: Tend:=<now>
DeltaT:=Tend-Tstart

or

```

DeltaT:=<now>
DeltaT:=DeltaT-Tstart

```

9.11.2 Using Date and Time in String Variables

It is 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 that time this script line is processed. The date and time format can be specified in the same way <date> and <time> are used for plate IDs. If the date and time format is not specified, the "Short 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>, Plate: " N

```

Notes: If <date> or <time> is assigned to any of the plate IDs, the date and time value will be assigned in the moment, when this ID is used, e.g. when performing an R_Run command (and not when the ID1:= script line is processed!).

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>").

9.12 File Commands

9.12.1 Input From Files

{String variable}:=**ReadStringFromFile** {File name}

This command reads any text file and assigns its content to a string variable. The text file can also contain CR/LF characters.

Example:

```
st1:=ReadStringFromFile "E:\Test\HelloWorld.txt"
showmsg "<st1>"
```

ReadVariablesFromFile {File name}

This command reads any text file and parses this for assignments to numerical, boolean and string variables.

Example:

```
ReadVariablesFromFile "E:\Test\MyVariables.txt"
```

As file content variable assignments are expected:

```
VarA=21 VarB=42 st1="Hello world!"
```

These assignments can be in one line or in different lines. Instead of = also := can be used (Pascal syntax). Also == (C syntax) is possible.

One space before and after =/:= can be used.

All empty lines and lines without an assignment (without =) will be ignored.

It is possible to uncomment entries using ; (this is valid per assignment, not per line!).

If you want to include a line break in a string (e.g. for usage with ShowMsg), use &.

All values read will be listed in the RunLog.

Example file:

```
St0 := "Hello World"
A=42
B :=21
C := 42 D:=43 E := -44.44
PerformRun = True PerformShaking=False
C2= 42E9 D2=43E-5 St1 = "Hello World!" St2 = "The answer
is 42."
```

Wrong:

```
A22=
99=98
MyStringVariable := "Hello World"
A := Hello World
A:=44-123
A:=4
St9 =: "Hello World"
```

No error:

```
St5 :=
```

(An empty string will be assigned to St5.)

Do not use more than one space before or after =/:=.

9.12.2 File Handling

FileExists {File name}

Checks whether the specified file exists.

Examples:

```
FileFound:=FileExists "E:\Test\Test2.csv"
FileMissing:=Not(FileExists "E:\Test\Test2.csv")
if FileExists "E:\Test\Test.csv" then beep
if Not(FileExists "E:\Test\Test.csv") then beep
```

In assignments the combination with a boolean variable using a boolean operator like and, or, xor, nor and nand is possible, but it is not allowed to use two FileExists commands in one code line.

DeleteFile {File name}

Deletes the specified file. There will be no error message, if the file not exists (in this case there will be only an entry in the Run Log file: 'DeleteFile command: specified file "<name>" does not exists.').

{String variable}:=**SelectFile** ({Title}) ({File type filter})

Opens a file selection dialogue box. Optionally a title (dialogue caption) and a file type filter can be specified:

Examples:

```
st1:=SelectFile
st2:=SelectFile "Please select a file"
st3:=SelectFile "Please select a script file" /
"Script files|*.btc"
```

If no title is specified, "Select File" will be used. If no file type filter is specified, all files will be listed.

wait for file {File name}

The script processing will pause until the specified file appears. This command can be used to synchronize the script operation with other programs.

9.13 Include (Call) Other Scripts

Include {Sub script file name}

This command will include (call) the specified script. This function can be used as a kind of sub routine call.

Examples:

```
include "C:\BMG\CLARIOstar\Scripts\IncludeTest1.btc"
include "IncludeTest1"
```

After processing the included script the script execution continues with the next command inside the calling script.

Notes: All variables are global. This allows transferring of data between the different script files. But you need to be careful when using the same variable name for different purposes in different included script files.

Do no split begin ... end blocks (for ... to, if ... then ...) over different script files.

When using break inside a sub script (and there outside a loop) the processing of the sub script will be finished and execution of the calling script will be continued.

If no file extension is specified as part of the sub script file name, '.btc' will be added automatically. If no path is specified as part of the sub script file name, the script file to be included will be searched:

1. in the directory of the current main script file
2. in the directory of the last included sub script
3. inside <Installation directory>\Scripts\

It is also possible to use relative paths, e.g.:

```
include "SubRoutines\MySubRoutine1"
```

A maximum of 100 nested sub scripts is allowed.

9.14 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 SMART Control installation directory is used (usually c:\Program Files\BMG\CLARIOstar\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 SMART Control program).

It is optional to 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 "MergeReadings.exe <DataPath> <User> H ID1" Hidden
```

Note: It does not make sense to combine the options Activated, Minimized and Hidden, therefore, only one of these options per Call command is permitted.

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. Execute employs the same parameters as described above for the Call command.

Example:

```
Execute "MergeReadings.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 "CLARIOstar.chm" ;opens the online help
```

Note: If a command with parameters is used were there are spaces in one of the parameters use stars (*) to enclose the parameters. These stars will be replaced by quotation marks ("") when sending the command to windows.

If a star is necessary as part of a parameter replace this by two stars.

10 Known Problems and Solutions

10.1 Disabled Plate In / Out Buttons

If the plate button (▼) is disabled while the reader is not attached to a stacker or a robotic system 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\CLARIOstar\CLARIOstar.ini’. The value behind

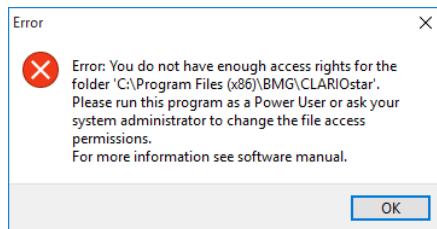
‘DisablePlateCmds=’ should be ‘False’. This parameter will be set to ‘True’ if the reader is used in a robotic system or in combination with Stacker Control, as here the plate in/out movement will be only controlled using the robotic software.

10.2 Access Rights

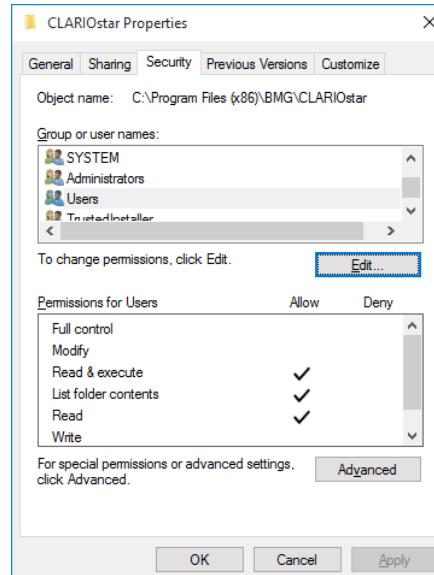
10.2.1 File Access Permissions

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\CLARIOstar’).

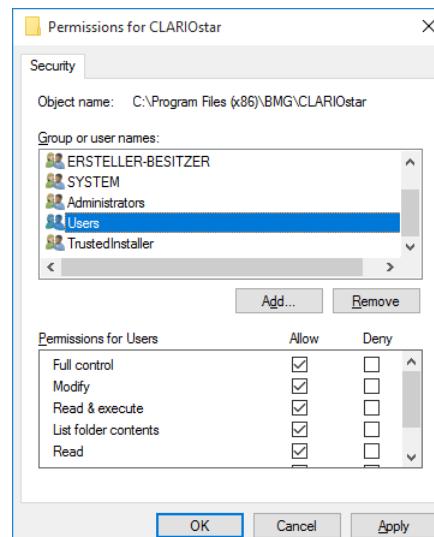
If the option ‘**Program should be usable also for non power users**’ was chosen during installation (see Software manual part I), the write access rights to the necessary files will be set during the installation. If this option was not chosen or if new users / user groups were added after installation, the following error might appear:



Default settings:



Customized settings:

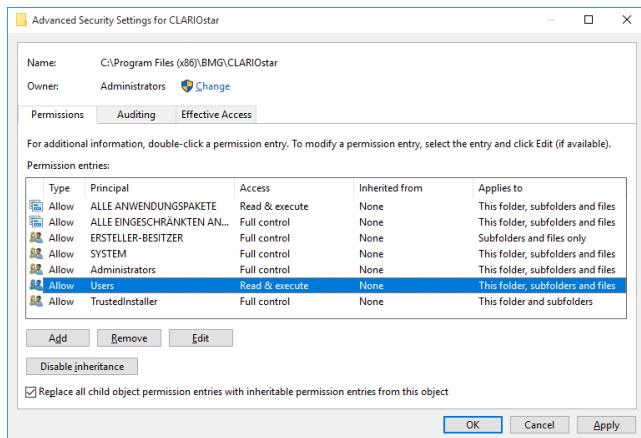


Solution

Logon as administrator (or user with administrative rights).

Run the program ‘**SetPermissions**’ from the SMART Control USB stick (folder ‘~:\SMART Control Vx.xx’) or from the local hard disk (folder ‘~:\Program Files\BMG\SharedComponents’).

Alternatively, manually change the permissions by opening Windows Explorer and right-clicking the folder where the BMG LABTECH software is installed (usually ‘~:\Program Files\BMG\CLARIOstar’). 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 box). Now all users should be able to write to the BMG LABTECH directory and work with the software.



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	pdoxusr.net	Paradox network control file

You can use the BDE Administrator to change the location of the pdoxusr.net file (Configuration | Drivers | Native | Paradox | Net Dir).

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\CLARIOstar\	CLARIOstar.ini CLARIOstar.rns CLARIOstar.log CLARIOstar.bak	Configuration file Run Statistics File Log file Backup of log file (= log file from last session)
~:\Program Files\BMG\CLARIOstar\Temp		Directory for temporary files
~:\Program Files\BMG\CLARIOstar\Stamm		Directory for files shared by all users of the CLARIOstar software, e.g. microplate data base, filter table, LVis pathlength and LVis blank values file, ...
~:\Program Files\BMG\CLARIOstar\User		Subdirectories for test protocol definitions and measurement data of standard user
~:\Program Files\BMG\CLARIOstar\Admin		Subdirectories for test protocol definitions and measurement data of administrator
~:\Program Files\BMG\CLARIOstar\<UserName>		Subdirectories for test protocol definitions and measurement data of defined users
~:\Program Files\BMG\CLARIOstar\Scripts		Subdirectories for example scripts
~:\Program Files\BMG_Flashtools		Used by Flash-EPROM Update Program

10.2.2 Registry Access

There are two places in the registry where the BMG LABTECH software stores information:

All user specific parameters 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, are 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 SMART Control USB stick (folder '`~:\SMART Control Vx.xx\`') or from the local hard disk ('`~:\Program Files\BMG_SharedComponents\`').
- or
- Start the program '**Regedit**' (use the 'Run' command from windows start menu).
 - Navigate to '`HKEY_LOCAL_MACHINE\Software\BMG Labtechnologies`'.
 - Select 'Permissions'.
 - Add read and write rights for everyone to this key and all sub keys.

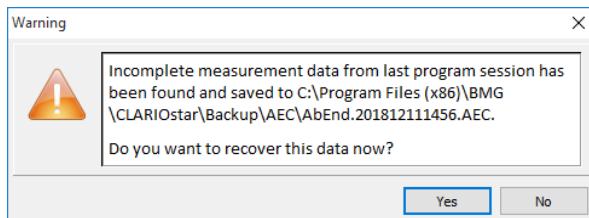
10.3 Disabled Script Mode

The script mode will be disabled when the Control Software is remote controlled, e.g. when the reader is used in a robotic environment. In such an environment complex action sequences are controlled by the robotic software. If the script mode button () is disabled while the software is not operated in remote

control mode, then it is most likely that there is an incorrect setting in the configuration file ‘~\Program Files\BMG\CLARIOstar\CLARIOstar.ini’. The value behind ‘AsDDEserver=’ should be ‘False’. This parameter will be set to ‘True’ if the reader is used in a robotic system.

10.4 Recovery of Temporary Measurement Data

If the Control Software has been terminated unexpectedly during an active measurement, e.g. due to a power loss or due to a computer failure, the measurement data obtained so far will automatically be saved at the next Control software start.



After clicking 'Yes' the data will be recovered and stored into the measurement data base belonging to the user which has performed the interrupted test run.

If 'No' has been selected, the data can be obtained later using the import function of MARS. Select 'Test Runs Recovery Set (*.AEC)' as file type.

Please see the chapter 11 *Support* for all other problems.

11 Support

For any problems / questions regarding the software / the instruments, visit our web page (<http://www.bmglabtech.com>) and read the information on the Support page. If an answer cannot be found on the website or in the ‘Known Problems and Solutions’ chapter in this manual, please use our on-line bug report form:

<http://www.bmglabtech.com/en/support/technical-support/>

Alternatively contact BMG LABTECH using the following email addresses:

- Problems / questions regarding software:
support@bmglabtech.com
- Problems / questions regarding the instruments:
techsupport@bmglabtech.com

Note: For support due to a software / firmware malfunction, please send the run log file (see chapter 3.1.6) together with the error description. Please always tell us the serial number of the reader and the software and Windows versions (see ‘System Information’). It is recommended to use the BMG LABTECH support tool, accessible via the application button menu (see chapter 2.2.2).