



# voyager

SOFTWARE MANUAL

## **Software Manual Voyager**

Version 2405



*The Microplate Reader Company*

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## About this document

**Note:** Make sure to carefully read and follow the instructions in this document before running the software

This document was designed to guide customers, who purchased a microplate reader device from BMG LABTECH, through the Voyager software and its features. It is solely intended for the use by qualified and instructed personnel having knowledge of relevant standards, provisions, regulations and operating conditions.

## Contact Addresses

<b>Manufacturer:</b>	<b>Germany</b>
	BMG LABTECH GmbH Allmendgrün 8 77799 Ortenberg
	<a href="http://www.bmglabtech.com">www.bmglabtech.com</a> <a href="mailto:techsupport@bmglabtech.com">techsupport@bmglabtech.com</a> +49-781-969680
<b>Subsidiaries:</b>	<b>France</b>
<b>Australia</b> BMG LABTECH Pty. Ltd. 2/24 Carbine Way Mornington, Victoria 3931 Phone: +61 3 5973 4744 Fax: +61 3 5973 4711 Email: <a href="mailto:australia@bmglabtech.com">australia@bmglabtech.com</a>	BMG LABTECH SARL 7, Rue Roland Martin 94500 Champigny s/Marne Phone: +33 1 48 86 20 20 Fax: +33 1 48 86 47 07 Email: <a href="mailto:france@bmglabtech.com">france@bmglabtech.com</a>
	<b>UK</b>
<b>Japan</b> BMG LABTECH Japan Ltd. 2F TS-1 Building, 1-6-2, Shimo-cho Omiya-ku, 330-0844 Saitama City Phone: +81 48 647 7217 Fax: +81 48 647 7218 Email: <a href="mailto:japan@bmglabtech.com">japan@bmglabtech.com</a>	BMG LABTECH Ltd. 8 Bell Business Park Smeaton Close Aylesbury, HP19 8JR Phone: +44 1296 33 66 50 Fax: +44 1296 33 66 51 Email: <a href="mailto:uksales@bmglabtech.com">uksales@bmglabtech.com</a>
	<b>USA</b>
	BMG LABTECH, Inc. 13000 Weston Parkway Suite 109 Cary, North Carolina 27513-2250 Phone: +1 877 264 5227 Fax: +1 919 678 1640 Email: <a href="mailto:usa@bmglabtech.com">usa@bmglabtech.com</a>

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## Notes

**Note:** A Note contains additional information.

**Attention:** A notice that when ignored this might lead to unintended behavior or unforeseen results

## Information on IT Security

The customer's computer or laptop (data processing system) is connected to the microplate reader by a USB interface.

The reader is controlled via this interface and information and data are transmitted.

The operator bears sole responsibility for providing and guaranteeing a secure and functional connection between the reader and the data processing system.

The operator must bring about and maintain suitable measures (e.g. by installing firewalls, using authentication measures, data encryption, installing anti-virus programs; etc.) to protect the operator's data processing system, network and the interface from any security gaps, unauthorized access, disruption, intrusion, loss and/or misappropriation of data or information.

BMG LABTECH is not liable for any damage and/or loss caused by such security breaches, any unauthorized access, interference, intrusion or loss and/or misappropriation of data or information.

The devices and evaluation software enable access to Internet pages for help, support, error tracking or for training material, such as videos. These accesses only take place after selecting the corresponding buttons or links. No access to the Internet is necessary for the normal function of the microplate reader. The software packages from BMG LABTECH only send data to the Internet if actively requested by the customer.

BMG LABTECH may provide a support tool to support the customer and for troubleshooting. With the help of this support tool, data and information can be collected according to the customer's decision and compressed into a data archive. Details on the data and information are given in the description and in the notes in the support tool.

The data archive can then be made available to BMG LABTECH for analysis, for example as an email attachment. By submitting the data, the customer agrees that BMG LABTECH may analyze the data for the purpose of customer support and contact the customer in this context.

## Disclaimer

Although these instructions were carefully written and checked, BMG LABTECH cannot accept responsibility for problems encountered when using them or for additional material related to these instructions. Suggestions for improvement are welcome.

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

# 1 Installation and setup

## 1.1 Installing the software

Make sure your computer system meets the minimum computer requirements (see section 1.2 on page 8).

Make sure to have elevated rights (e.g. administrator rights) when installing the software. If your Windows user does not have elevated rights, you will be asked for corresponding credentials. Start the Voyager setup file "VoyagerSetup\_vXX.XX.XX.exe" (XX.XX.XX is a placeholder and corresponds to the release version number) from the installation media. Perform the following steps to install the Voyager software:

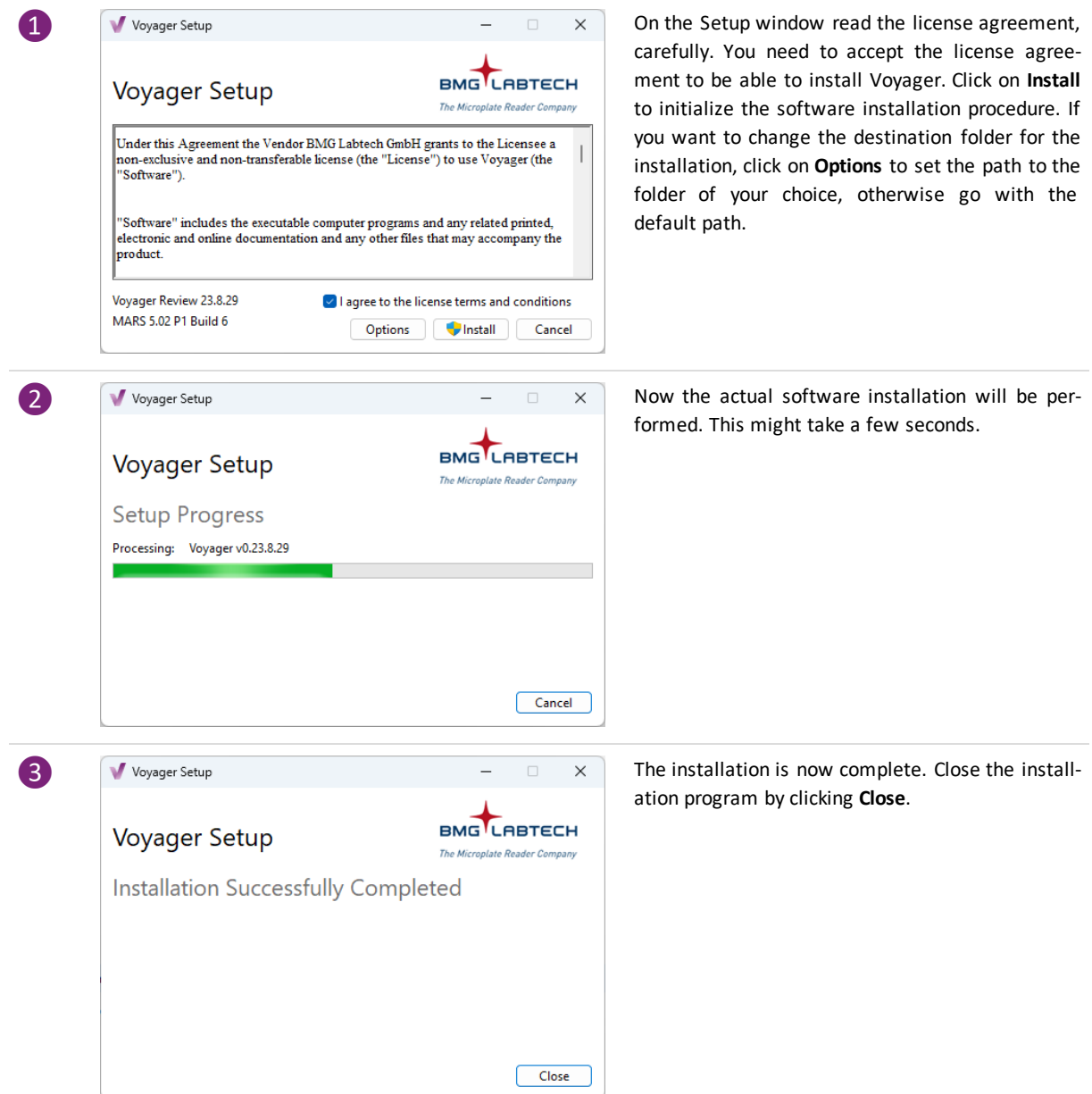


Figure 1.1: Steps to install the Voyager software.

## 1.2 Minimum computer system requirements

Item	Minimum Requirements
Processor	x64-based processor
Operating System	Microsoft Windows 10 / 11 64-Bit
Memory	4 GB RAM (8 GB RAM recommended)
Hard Drive	2 GB (4 GB of available space or more recommended)
Minimum Screen Resolution	1680 x 1050 Pixel
Connection	1 free USB 2.0 / 3.0 port

The following screen resolutions were tested and validated with the Software:

- 1680 x 1050 Pixel with Scaling 100%
- 1680 x 1200 Pixel with Scaling 100%
- 1920 x 1080 Pixel with Scaling 100%
- 1920 x 1280 Pixel with Scaling 100% - 125%
- 1920 x 1440 Pixel with Scaling 100% - 125%
- 2048 x 1536 Pixel with Scaling 100% - 125%
- 2560 x 1440 Pixel with Scaling 100% - 150%
- 2560 x 1600 Pixel with Scaling 100% - 150%
- 2560 x 1920 Pixel with Scaling 100% - 150%
- 2880 x 1800 Pixel with Scaling 100% - 175%
- 3840 x 2160 Pixel with Scaling 100% - 225%

We recommend to turn off all sleep and hibernation settings in Windows to prevent data loss. Also automatic Windows updates can interrupt active measurements. We thus recommend to turn automatic Windows updates off and to perform them manually at an appropriate time point.

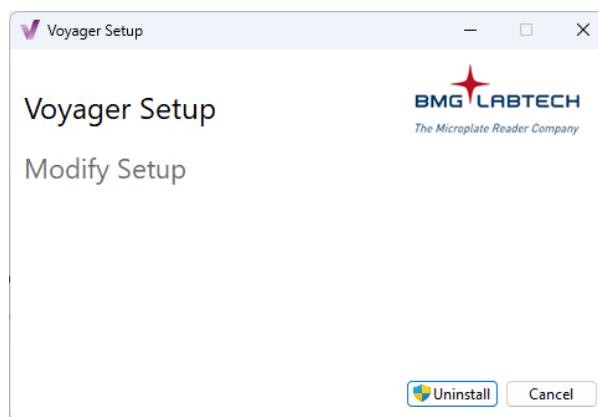
## 1.3 Uninstalling the software

You can either use the Voyager setup file or the control panel Windows to remove Voyager from your PC.

**Note:** A backup of your data (user database, protocols, measurement data) will be placed in C:\ProgramData\BMG Labtech\Voyager\Backup.

To remove the Voyager program files from your PC, start the Voyager setup file "VoyagerSetup\_vXX.XX.XX.exe" (XX.XX.XX is a placeholder and corresponds to the release version number) from the installation media. The **Voyager Setup Wizard** appears (see section 1.1 on page 7). Click on **Uninstall** to start the deinstallation (see Figure 1.2).

**Figure 1.2:** Uninstalling the software.



Alternatively, you can also use the **Installed apps** control panel in Windows to remove Voyager.

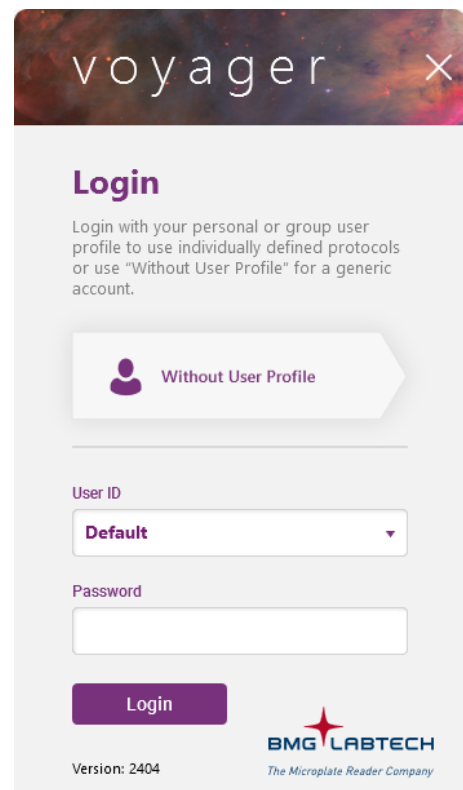
## 2 Running the Voyager software

### 2.1 Starting the software

When Voyager is installed correctly, you can open it by double-clicking the Voyager icon on the desktop in Windows or by opening it from the Windows Start menu. Either use the app search in the Start menu to search for Voyager or go to **All Apps**, search for the BMG LABTECH folder and search for the **Voyager** app in this folder.

### 2.2 Log on to Voyager

**Figure 2.1:** The **Login** window to open Voyager **Without User Profile** or to **Login** with a dedicated user profile.



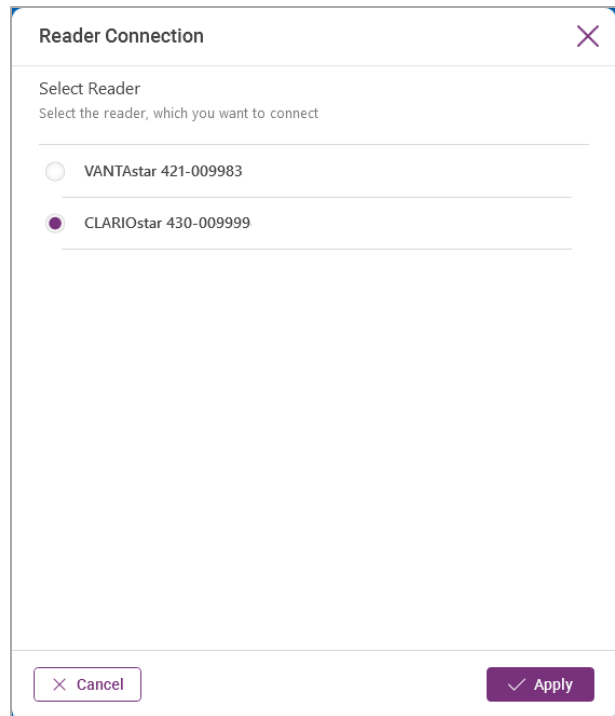
At the start of Voyager, the login screen appears where you can either open the software without a dedicated user profile or you can log on as a dedicated user if you have already created a user profile. Refer to section see section 4.5 on page 33 for more details on how to create a user profile and the different login options.

To log on **Without User Profile**, click on the corresponding  button on the login screen. Otherwise, select your **User ID** and enter your **Password** and then click on **Login**.

## 2.3 Select an instrument

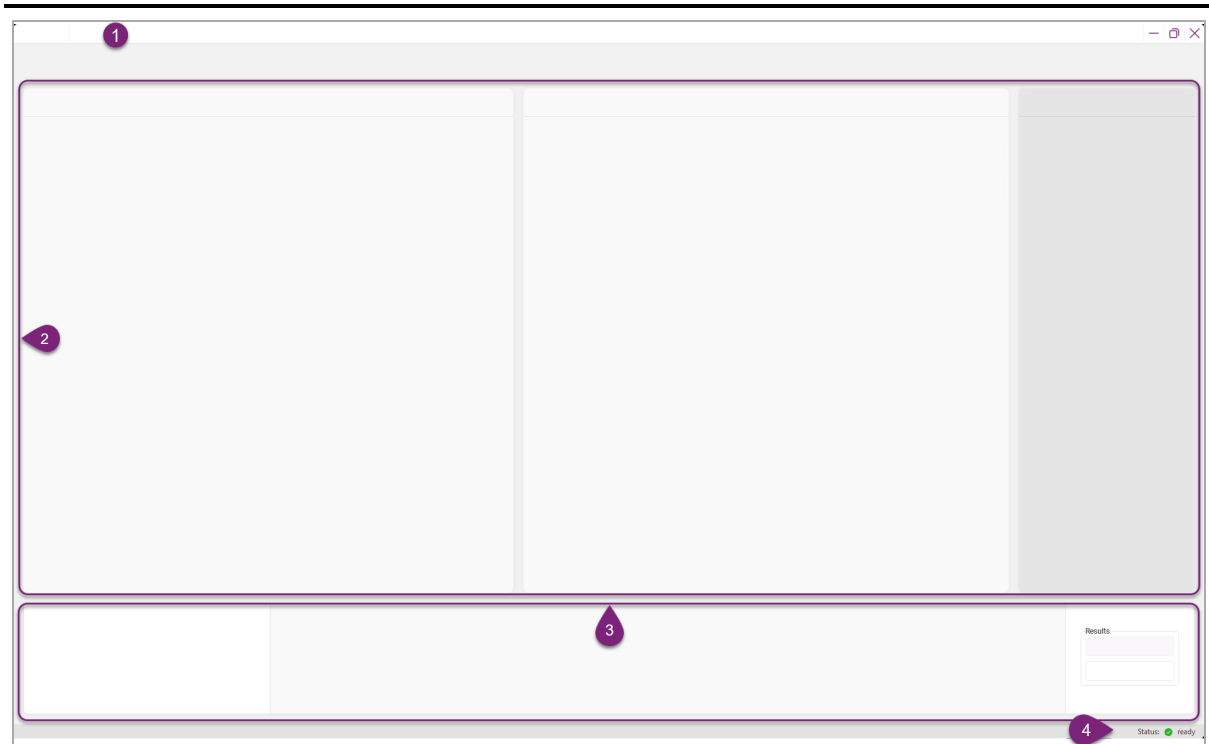
If only one compatible reader is connected to the PC, Voyager will automatically recognize it and establish the connection. If multiple compatible readers are connected, a **Reader Connection** dialogue will appear after login from which you can select and connect to the reader of interest. Select the corresponding reader of interest and click **Apply** (see Figure 2.2).

**Figure 2.2:** If multiple instruments are connected, select the one to work with.



### 3 Voyager in a nutshell

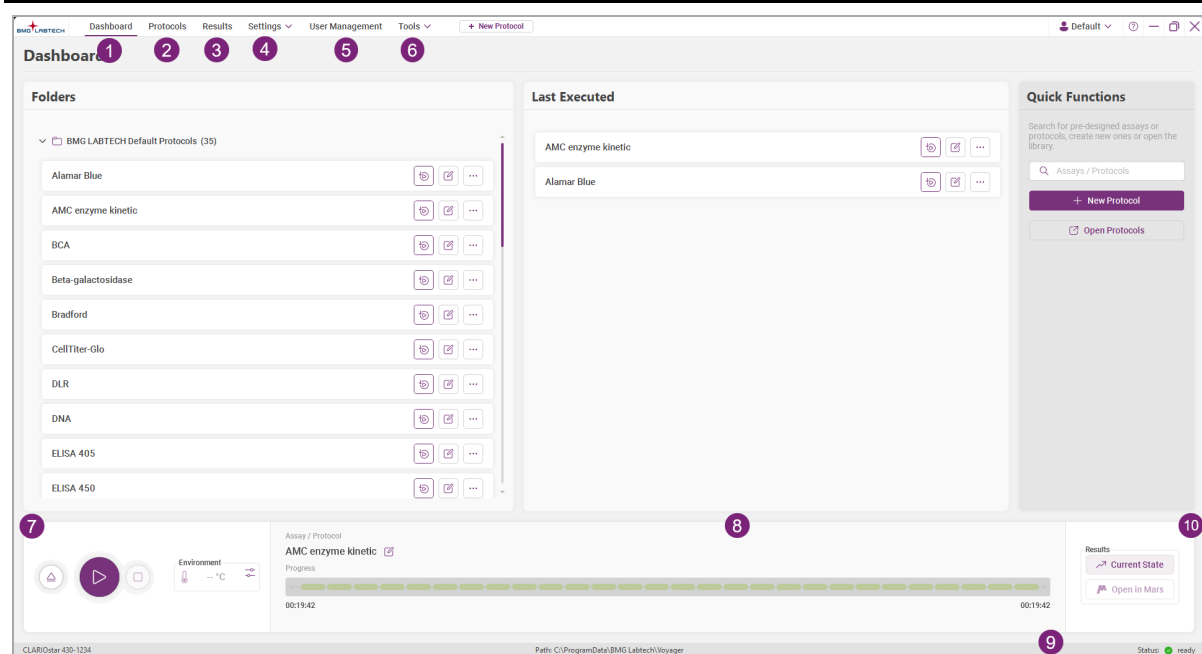
Voyager is divided into four different panes: (1) the menu bar at the top, (2) the main pane, (3) the measurement pane and (4) the status bar at the bottom.



**Figure 3.1:** Panes in the Voyager Software.

- 1 The menu bar has six menus: **Dashboard**, **Protocols**, **Results**, **Settings** and **User Management** (see also Figure 3.2).
- 2 Voyager's main pane. The content displayed here depends on which page/menu is opened. E.g. clicking the **Protocols** menu opens the **Protocol Library** page.
- 3 From the measurement pane you can start and follow the progress of a measurement and also set **Environment** conditions, preview data by opening the **Current State** window or **Open Results**.
- 4 In the status bar, the instrument status, the current user path and the currently connected reader are listed.

Next, the different menus and panes in Voyager are described in more detail.

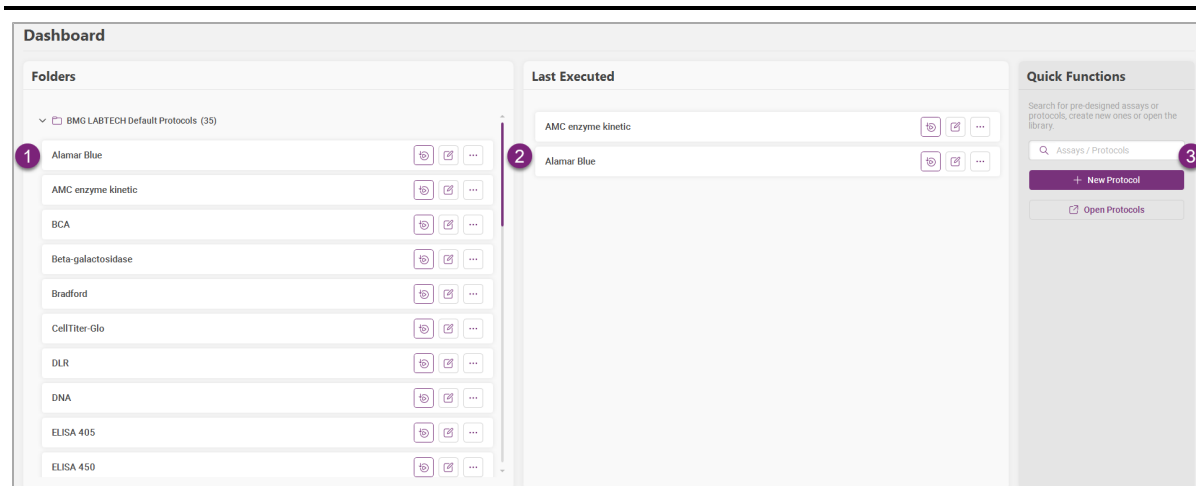


**Figure 3.2:** Menus and panes in the software.


- 1 Clicking the **Dashboard** button opens the Dashboard page. This allows you to not only **load** and run or **edit** recently executed protocols, but also to open the **Protocol Library** or to create a new protocol.
- 2 Clicking the **Protocols** button opens the Protocol Library, i.e. a list of existing protocols that can be edited, deleted or exported for sharing with other users. The entries are listed in alphabetical order.
- 3 The **Results** button opens the Results Library, i.e. a list of measurement results from protocols that have already been run. The entries are listed in chronological order.
- 4 The **Settings** button contains various sub-menus including **Connection** (for use when multiple readers are connected), **Filters** and **Manage Microplates**.
- 5 Clicking the **User Management** button opens a **User Account List** with all users and their access rights listed therein.
- 6 Tools menu to access the LVIS cleanliness check and LVIS calibration wizard, but also the Support Tool and information on the Software and connected reader.
- 7 Dashboard player containing buttons to eject/insert a microplate, to run or stop a measurement and a sub-pane to set **Environment** conditions such as temperature control for incubation.
- 8 If a protocol is loaded or being executed, a measurement progress bar is shown here. However, if no protocol is loaded, there are two buttons: one to create a **New Protocol** and one to **Open Protocols**.
- 9 Status bar with the currently connected reader's serial number (on the left), with the working directory and the reader status.
- 10 Open the **Current State** window (if measurement is running) or **Open Results** of the last executed measurement.

## 4 Voyager overview




### 4.1 Dashboard – Your entry point to Voyager



**Figure 4.1:** The Dashboard as an entry point to Voyager.

- 1 The left pane of the Dashboard lists available protocols in the **Folders** section. By default, the **BMG LABTECH Default Protocols** folder is selected, but user-defined folders can be selected, too. You can even load the protocol from here or edit it.
- 2 The **Last Executed** protocols list contains all protocols that were executed recently. The top entry corresponds to the most recently executed protocol. You can even load the protocol from here or edit it. Click on the 3-dots button to see options to **Export** the protocol, to get a **Protocol Overview** or to **Open Results** related to this protocol.
- 3 The **Quick Functions** section has a search bar to search for existing protocols. Existing protocols can be edited from the search list (simply click on the protocol name) or can be loaded from there by clicking . Click on **Open Protocols** to open the **Protocols Library** listing all existing and user-specific protocols. Or create a **+ New Protocol** from here.

Once logged on to Voyager, the **Dashboard** appears and gives an overview of recently executed protocols (if they exist). If not in the list, you can search for a protocol from the **Search** bar on the right, or you can simply **Open Protocols** or create a **+ New Protocol** from here. From the **Dashboard** you can also monitor and set **Environment** conditions such as **Temperature**, atmospheric conditions (if applicable) or control the **Heater** and **Stirrer** (if applicable). The **Dashboard** is designed to guide you to your point of interest as quickly as possible.

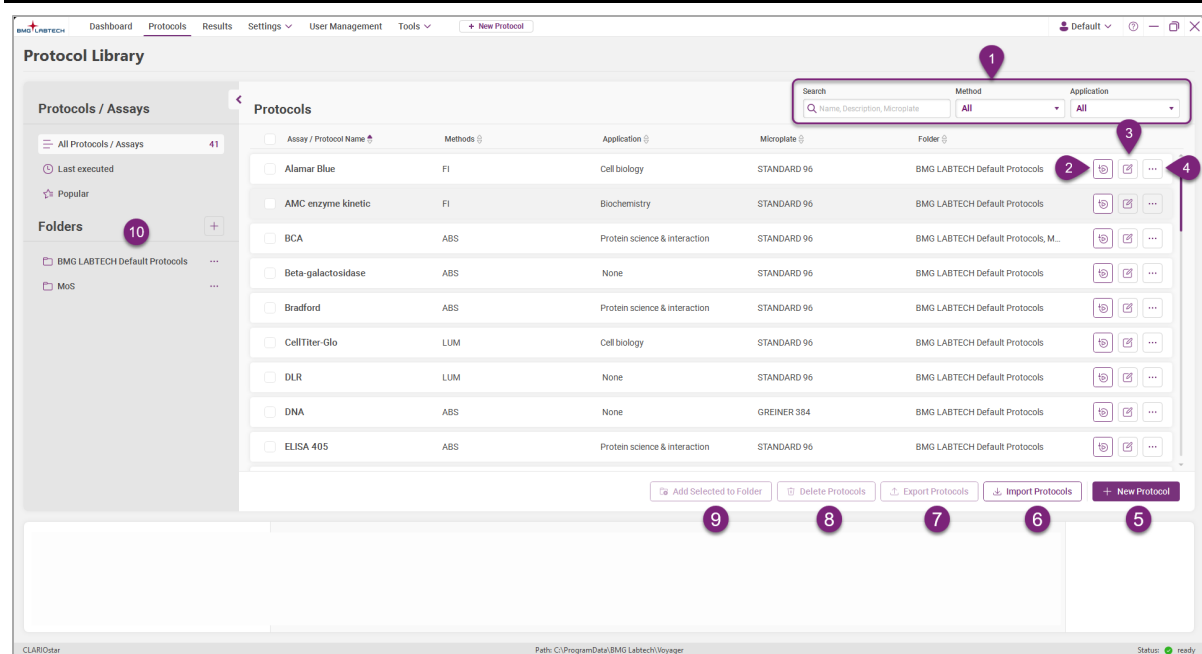
The **Dashboard** player allows you to quickly execute an existing protocol from the **Last Executed** protocol list. From the **Last Executed** list, click on **load** to load the corresponding protocol and to enable the play button. If needed, move the plate-carrier out by clicking the eject button , then insert your microplate and move it back in by clicking the eject button  once again. Click the **Play** button  to open the start measurement window (see section 4.6 on page 35) and start your measurement.

### 4.2 Protocols

Protocols store the entire information on events, settings, microplate layouts and action loops required by the reader to execute a measurement appropriately. Voyager comes with commonly used, pre-defined protocols accessible via the **Protocols** page. It also allows you to create a **+ New Protocol**.

#### 4.2.1 Load and run existing protocols

By default Voyager comes with many pre-defined measurement protocols that are assigned to a folder called *BMG LABTECH Default Protocols* that can be either accessed from the Dashboard or from the **Protocol Library**. The whole list (i.e. **Protocol Library**) can be viewed and edited from the **Protocols** page.



**Figure 4.2:** The Protocol Library

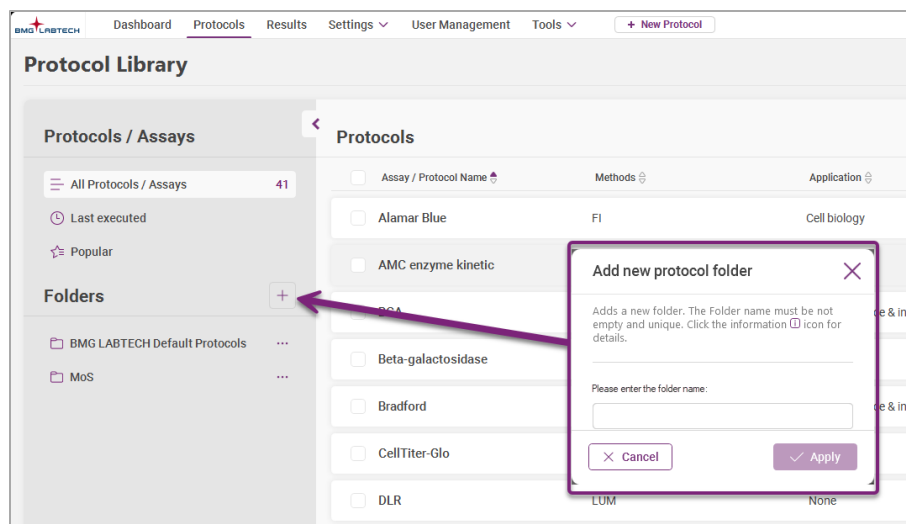
- 1 The **Protocol Library** can be searched for various entries or filtered by detection **Methods** or by **Application**.
- 2 Here you can **load** a protocol.
- 3 You can also **edit** the protocol and adjust settings in the protocol editor.
- 4 Clicking **more** allows you to **Duplicate** or **Delete** a protocol, or to get an **Overview** of the settings defined in the protocol.
- 5 From **+ New Protocol** you can create a new protocol.
- 6 Or **Import Protocols** by clicking this button.
- 7 Select one or more protocols to export using **Export Selected Protocols**.
- 8 Or **Delete Selected Protocols** from the library.
- 9 Klick on **Add Selected to Folder** to assign selected protocols to a folder that needs to be set after clicking the button.
- 10 You can create your own folder by clicking on **+ New folder** and assign one or more protocols to this folder (see step 9). Or you can filter for **Popular** or **Last executed** protocols.

If you want to see only those protocols that were executed just recently, click on **Last executed** to filter for only these protocols. The protocol on the top of the list is the last executed protocol. The second on the list is the second last executed and so on. By selecting **Popular** the most used protocols are shown. The list is ordered by the number of executions of the protocol. The top entry of the list is the protocol the most often executed. The second entry is the second most executed protocol and so on.

## Folders

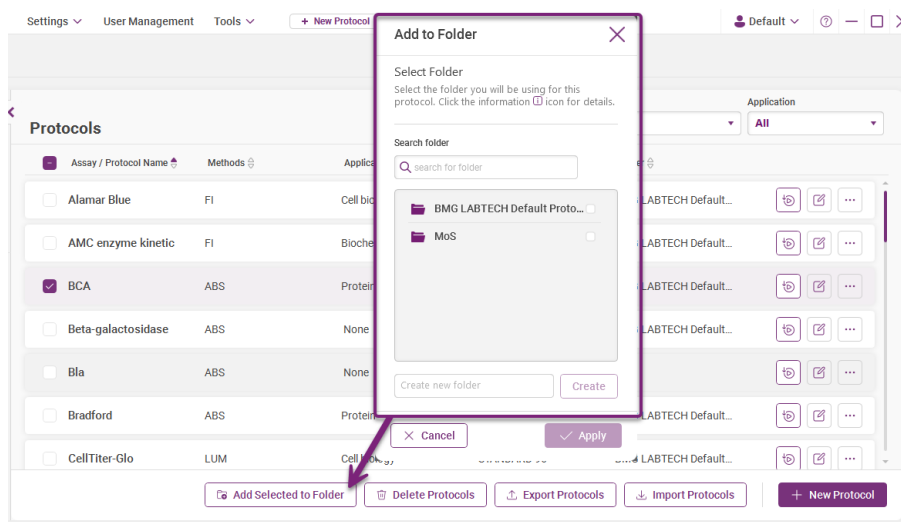
Folders can be used to group protocols (e.g. according to assay type). You can create a new folder by clicking **+ New Folder**. The new protocol folder dialog opens where you can enter a folder name. After clicking **Apply**, the folder is listed in the **Folders** section of the **Protocol Library**.

**Figure 4.3:** How to create a new protocol folder.



To add one or more protocols to a folder, check the protocols in the Protocol Library and click the **Add Selected to Folder** button. The **Add to Folder** dialog opens where you can select one or more target folders.

**Figure 4.4:** Add Protocol(s) to a folder.



You can also create a new folder directly from this dialog. Enter the name for the new folder in the text field and click **Create**. Clicking **Apply** will assign the selected protocols to the folders chosen. To see this you can click on the dedicated folder in the **Protocol Library** to filter for the protocols that are only in this folder. At any time you can rename or delete a folder that you created. Therefore, click the ... button next to the folder name to either edit its name or delete it.

#### 4.2.2 Create new protocols

The protocol editor is one of the key panes of the Voyager software and the main window of interaction for the user to create or edit a new measurement protocol. The protocol editor is an easy-to-use interface to select the microplate, its corresponding layout, workflow, and to add different action elements to be executed by the reader. It allows to set one or multiple detection modes, wait actions, injections, shaking actions or even temperature rampings. Setting-up an endpoint protocol, or a plate-wise / well-wise kinetic measurement is straightforward from the workflow. The steps required to set-up a protocol with the protocol editor are shown in Figure 4.5.

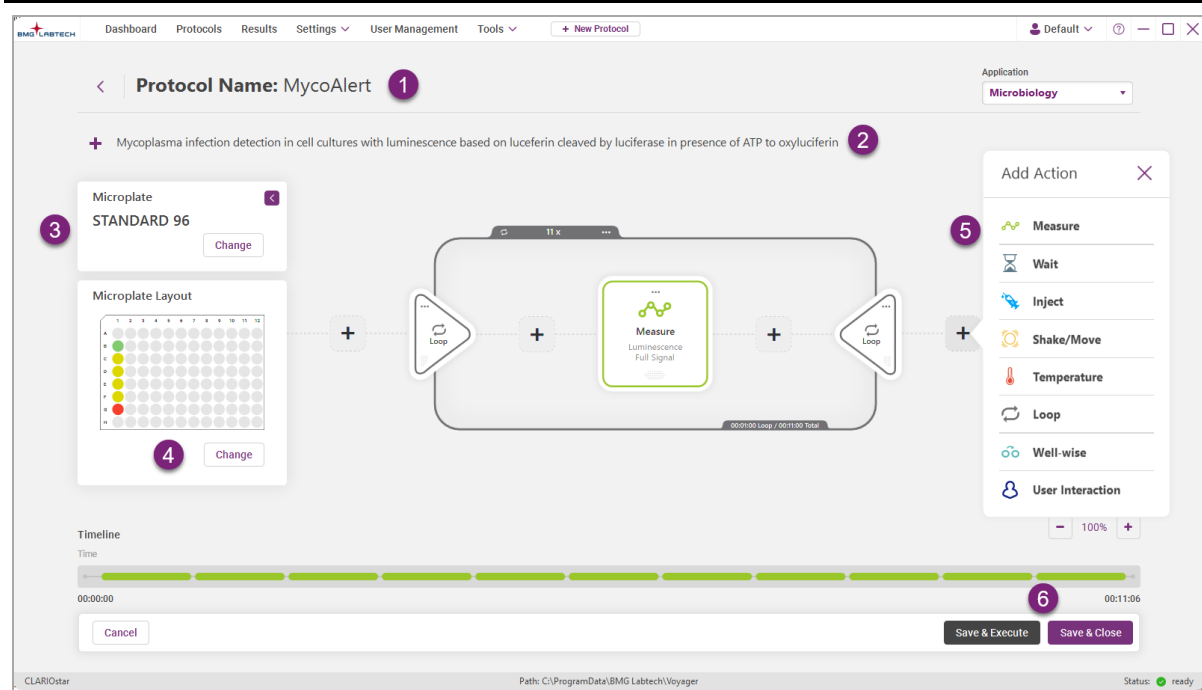


Figure 4.5: Overview of the protocol editor.

- 1 Enter a meaningful **Protocol Name**.
- 2 Enter an optional concise description of your protocol.
- 3 Choose your **Microplate** or leave it as the default (SLAS Standard 96 Wells).
- 4 **Change** your **Microplate Layout** (if needed) or measure the whole plate by default.
- 5 Add a new action (e.g. Measure action) to the workflow.
- 6 **Save & Execute** the protocol directly or simply click **Save & Close** to store it for future use.

### 4.2.3 Choose microplate and microplate format

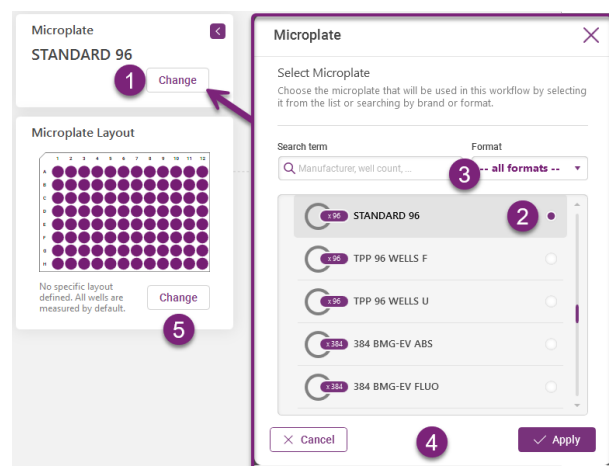



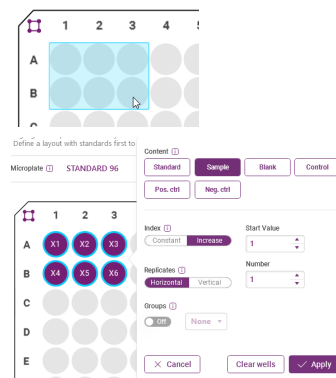
Figure 4.6: Changing the microplate.


- 1 If you do not want to use the predefined microplate format click on **Change**.
- 2 Select your **Microplate** from the list.
- 3 You can search for microplates (e.g. search by the manufacturer ) or filter for dedicated microplate formats.
- 4 Click **Apply** to store your selection or **Cancel** when your microplate or at least a similar microplate is not in the list. You can create your own microplate (see section 4.4.3 on page 30).
- 5 You can (optionally) **Change** the **Microplate Layout** and adapt it according to the wells occupied on your microplate. The whole plate will be measured by default if nothing is changed and wells will be labeled as samples starting from 1 to the number of wells.

### 4.2.4 Assign or modify microplate layout

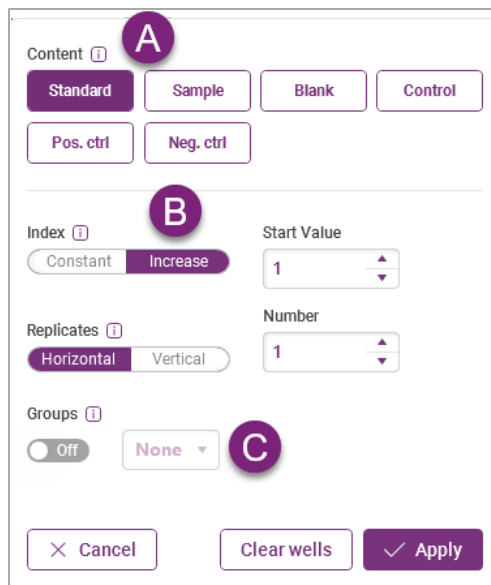
Voyager offers multiple ways to modify your **Microplate Layout**. You can access the modification menu in the protocol editor by clicking the **Change** button on the **Microplate Layout** tile. Now you have multiple options to select and define the microwells on the used microplate. If you change the layout, you will start with an empty plate. Select wells of interest and assign contents to them.

Figure 4.7: Selecting wells to create a microplate layout.



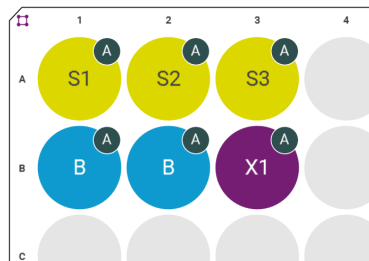
Select individual or multiple wells by clicking and dragging, respectively, over selected wells (Figure 4.7, top). The corresponding wells become samples by default indicated by a purple fill and X as identifiers (Figure 4.7, bottom). Use the **Clear wells** button to remove wells from your microplate layout. You can also select all microwells on a plate simultaneously by clicking on the  icon in the upper left corner of the microplate layout. This allows you to define all microwells at once according to the selected **Content** or to use the **Clear Wells** command on the whole plate. You may click dedicated column or row identifiers (e.g. row A) to select whole columns or rows.

**Figure 4.8:** The pop-up window for defining the microplate layout including contents (A), content index and replicates (B) and groups (C, if required).



The pop-up window that appears when selecting new wells or when selecting existing wells in the layout allows you to set the content type of these wells, i.e. **Standard**, **Sample**, **Blank**, **Control**, **Positive Control** or **Negative Control** (see Figure 4.8, A). Setting an appropriate **Content** not only helps with documentation but also with the data analysis in MARS afterwards (see section 4.3 on page 27). You can also set the start value of the content **Index** (see Figure 4.8, B). **Replicates** can be defined by increasing the Number setting to the desired number of **Replicates**, e.g. 2 for duplicates, 3 for triplicates, etc. Voyager will automatically adjust the layout depending on whether **Vertical** or **Horizontal Replicates** are chosen. **Replicates** are used later on in the MARS analysis software to calculate various statistics like averages over replicate wells. By setting the **Groups** slider from **Off** to **On**, microwells can be organised in groups. Other groups can be chosen for subsequent microwells by selecting a different letter in the selection slider. This allows you to selectively perform calculations for Samples of a specific group in the MARS data analysis software. Groups are indicated as superscript-like objects at the top right of the content of each well.

**Figure 4.9:** Microplate Layout with **Groups**.



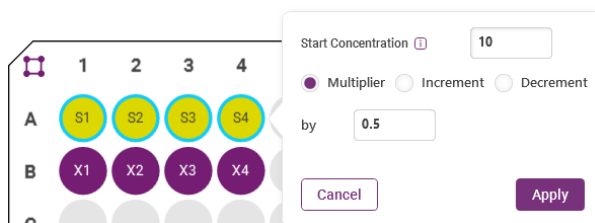
Content	Identifier	Remarks
Sample	X	Wells whose content have unknown concentrations.
Blank	B	Wells containing solvent like water or buffer. These wells are typically used as background reference.
Standard	S	Wells containing liquids with known concentrations of analytes. These are typically used to create calibration curves in the data analysis software.
Negative Control	N	Wells whose contents typically have no or a negligible amount of analyte but are otherwise identical to the actual sample.
Positive Control	P	Wells whose contents have a known and typically high amount of analyte and thus result in a good signal for the assay. Otherwise identical to the actual sample.
Control	C	Other type of control.

**Well IDs, Standard Concentrations and Dilutions**

After defining the **Layout** of your microplate, you can switch to the **Well IDs** tab window to assign descriptions for each content type present on the microplate in the **Values Table**.

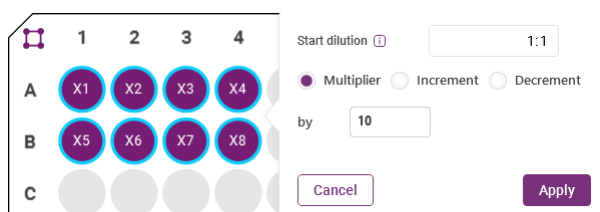
**Figure 4.10:** Entering Well IDs into the Values Table.

Concentrations of Standard wells can be defined under the **Concentrations** tab. This can be done by selecting the standard wells on the microplate and setting the corresponding values in the concentration pop-up window (see Figure 4.11) or by clicking the **Define Concentrations** button (on the right) or by typing the concentrations of the standards into the **Values Table** (available if concentrations have already been edited before).

**Figure 4.11:** Setting Concentrations.

To set concentrations, enter a **Start Concentration** and either choose whether to **Increment** or **Decrement** the concentration series with a constant increment or whether to create a geometric growing/decaying concentration series, based on a user-defined **Multiplier**. 10, 15, 20, 25 is an example of a concentration series with a constant increment of 5. 10, 5, 2.5, 1.25 is an example of a concentration series with geometrically decaying concentration values according to  $(\text{Start Concentration}) * \text{Multiplier}^{(n-1)}$  where n is nth standard.

Similarly, **Dilutions** can be assigned to samples, or controls on a microplate by clicking the **Dilutions** tab on the **Microplate Layout** window. Dilutions can be assigned manually, by typing in the dilutions into the **Values Table**, or by selecting the corresponding wells and using the resulting pop-up window to automatically assign dilution values. Therefore, set a **Start Dilution** by entering a corresponding dilution **Factor** and either choose a **Multiplier** or **Increment/Decrement** that the dilution series shall follow. The dilution value you enter manually or the values that are automatically entered from the dilution pop-up window are in the format 1:Z, where Z denotes the dilution factor of the corresponding sample. E.g. to automatically create a dilution series with dilutions 1:1, 1:10, 1:100 etc. enter 1:1 as **Start dilution** and use a multiplier of 10 (Figure 4.12).

**Figure 4.12:** Setting Dilutions.

**Note:** Defining dilutions in a test protocol allows you to recalculate the concentrations of the sample stock solution in the MARS data analysis software, e.g. in protein quantification assays.

#### 4.2.5 Description of actions

Click on the big button in the protocol editor to get access to various **Actions**. These are briefly described below.



Use **Measure** action element to include measurements in the workflow for selected detection modes (refer to detection mode-specific sections for detailed description of relevant settings). You can combine several measure elements containing the same detection modes and use different settings for each in a workflow. For example, you can measure several fluorescent labels in one protocol.



Use the **Wait** action element in protocols where a pause between workflow steps is required. This function can be used for example in assays requiring incubation steps.

### Add Wait ✕

**Settings**

Values entered here will set the duration of the waiting period. Click the information ⓘ icon for details.

---

Duration ⓘ

1

↑  
↓

seconds
▼

✕ Cancel
✓ Apply



Use the **Injection** action element for adding reagents into wells with the installed injectors (optional). Select the injector which will be used for adding the reagents (A). Choose the speed for injection (B). Select wells in the layout where the injection should be applied (C). Enter the injection volume in the popup dialog (D) if the same volume should be used for all wells. If you want to inject different volumes, use the **Individual Volumes** table (E) and enter the volumes manually. Smart Dispensing (F) will enable a faster injection for protocols where timing is critical. In this case, the injector will be filled completely and emptied in subsequent steps at the defined injection volumes.

Click **Apply** when all settings are made.

### Add Injection ✕

**Injection Settings**

Mark wells for injection in the plate layout below. Choose injector, speed and enter volumes. Click the information ⓘ icon for details.

Injection Volume (µl) ⓘ

20

↑  
↓

Cancel
Apply

Injector ⓘ

A
Injector 1
▼

Injection Speed ⓘ

B
350 µl/s
▼

Use Smart Dispensing ⓘ

F

On  
Off

Individual Volumes ⓘ

Sample	X1	Volume (µl)									
Standard	S1	20									
Standard	S2	Volume (µl)									
Standard	S3	Volume (µl)									
Standard	S4	Volume (µl)									
Standard	S5	Volume (µl)									
Control	C1	Volume (µl)									
Neg. ctrl	N	Volume (µl)									

Clear

✕ Cancel
✓ Apply

Use the **Shake/Move** action element to introduce shaking steps into the workflow. Set the **Shake Duration** (A) either in seconds or in the time format mm:ss ([zero-padded minute]:[zero-padded second]), e.g. 02:30 means 2 minutes and 30 seconds). Change the default **Shake Mode** (B) from **Double Orbital** to **Linear** or **Orbital** (if necessary). Set the shake **Frequency** (C) in rotations per

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minute (rpm).



Use the **Temperature** action element to include incubation steps in the workflow.

**Constant Incubation** will keep the interior of the reader at a selected target temperature. Set the required temperature in the **Set Temp.** field (A).

Activate **Temperature Change** if you wish to define a temperature ramp in the workflow. Enter values with “+” for temperature increase or values with “-” for temperature decrease into the **Temp. Increment** field (B).

Activate **Wait until temperature is reached** (C) if the workflow needs to be paused until the selected temperature is reached. When the box is deactivated, the reader will proceed with the workflow elements and incubate in parallel.



Add **Loop** to the workflow if you wish elements to be repeated (e.g. kinetic measurements).

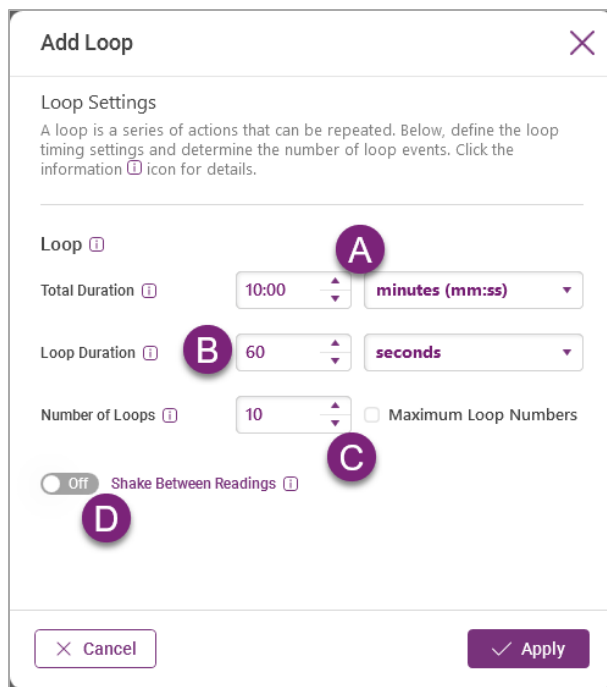
Define the **Total Duration** for the whole period where the elements need to be repeated (A).

Either set the time in milliseconds or seconds, or select and set the time format in mm:ss or hh:m-m:ss ([zero-padded hour]:[zero-padded minute]:[zero-padded second], e.g. 01:30:25 corresponds to 1 hour, 30 minutes and 25 seconds).

The **Loop Duration** corresponds to the time periods when loop repetitions are performed (B). The minimum loop duration is calculated automatically according to the number and duration of single elements in the loop and according to the plate layout selected.

The **Number of Loops** corresponds to the number of repetition steps for all elements in the loop (C). Number of loops depends on loop duration, minimum loop time and total duration of the loop. Activate **Maximum Loop Numbers** if you wish to repeat all elements in the loop as often as possible in the selected total duration. Here an automatically calculated minimum loop time is used as the loop duration.

Activate **Shake Between Readings** if you wish to include shaking between execution of single loops (D).



If **Shake Between Readings** (A) is activated but **Shake Interval** (B) is deactivated, the reader will shake all the time when no measurements or other workflow steps are executed.

Activate **Shake Interval** (B) if you wish to select shaking and waiting periods. Enter the time for shaking step duration in seconds in the **On-Time** field. (C). Enter the time for a pause between shaking steps in seconds in the **Off-Time** field (C).

### Add Loop ✕

**Loop Settings**  
A loop is a series of actions that can be repeated. Below, define the loop timing settings and determine the number of loop events. Click the information ⓘ icon for details.

---

**Loop ⓘ**

Total Duration ⓘ

Loop Duration ⓘ

Number of Loops ⓘ   Maximum Loop Numbers

Shake Between Readings ⓘ **A**

Shake Mode ⓘ

Frequency ⓘ

Shake Interval ⓘ **B**  On

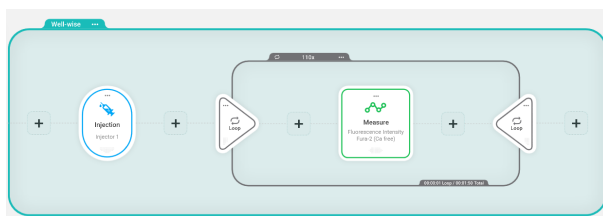
On-Time  Off-Time

**C**

✕ Cancel ✓ Apply



The **Loop** action element described above is a repetition of elements within the loop for the entire selected plate layout. In special cases where reactions and signals change quickly (in the range of seconds), you can use the **Well-wise** action element. If well-wise is activated, all action elements within the well-wise bracket (including loop and injection) will be executed on one well. This feature enables monitoring of fast reactions.



Use the **Atmosphere** action element to include incubation steps under altered atmospheric conditions in the workflow.

**Constant O<sub>2</sub>** or **Constant CO<sub>2</sub>** will keep the interior of the reader at selected target oxygen and carbon dioxide concentrations, respectively. Adjust the required O<sub>2</sub> or CO<sub>2</sub> concentration in the corresponding O<sub>2</sub> or CO<sub>2</sub> field using the up-down arrows. (A)

Activate **O<sub>2</sub> Change** or **CO<sub>2</sub> Change** if you wish to define an oxygen or carbon dioxide ramp in the workflow. Enter values with “+” for gas increase or values with “-” for gas decrease (B).

Activate **Wait until concentrations are reached** if running the protocol shall be paused until the selected gas concentrations (O<sub>2</sub>, CO<sub>2</sub>) are reached. When the box is deactivated, the reader will proceed with the workflow elements and incubate in parallel (C).

### Add Atmosphere ✕

**Atmosphere Settings**  
Please define your settings for the Atmosphere in the selection below. Click the information i icon for details.

---

**Oxygen** i

Constant O<sub>2</sub>       O<sub>2</sub> Change

O<sub>2</sub>      O<sub>2</sub>

19.6% **A**      +0.0% **B**

---

**Carbon dioxide** i

Constant CO<sub>2</sub>       CO<sub>2</sub> Change

CO<sub>2</sub>      CO<sub>2</sub>

0.1% **A**      +0.0% **B**

---

Wait until concentrations are reached **C**

✕ Cancel      ✓ Apply



The user interaction can be used to make the protocol stop at a certain point in the workflow, e.g. to manually pipette a reagent into certain wells.

Add a meaningful **Interaction name** (A) and choose whether the plate should be moved out of the reader (B) when the protocol has reached the interaction in the workflow. You can (optionally) add a description in the **Notification** field (C).

The **Interaction name** and the **Notification** will be prompted to the user while the protocol is executed and reaches the point of interaction.

**Add User Interaction** ✕

**User Interaction Settings**  
User interaction element will pause the workflow and wait for an action and confirmation from the user. Click the information i icon for details.

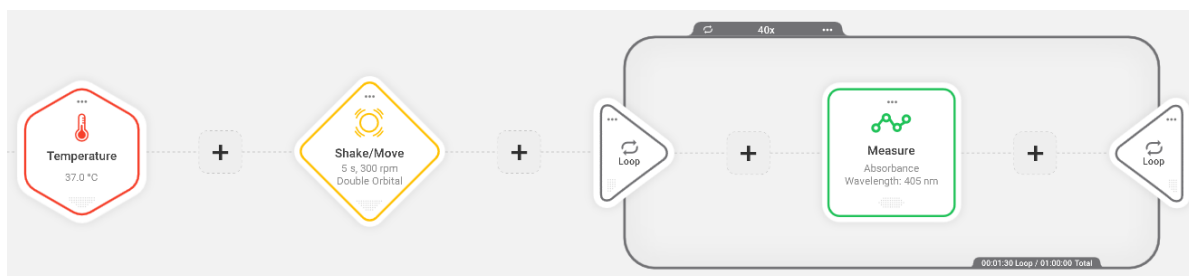
Interaction name  
Plate out A

Automatic Plate Out i B On

Notification  
Pipette 10 µl of reagent into wells A1-A8 C

✕ Cancel ✓ Apply

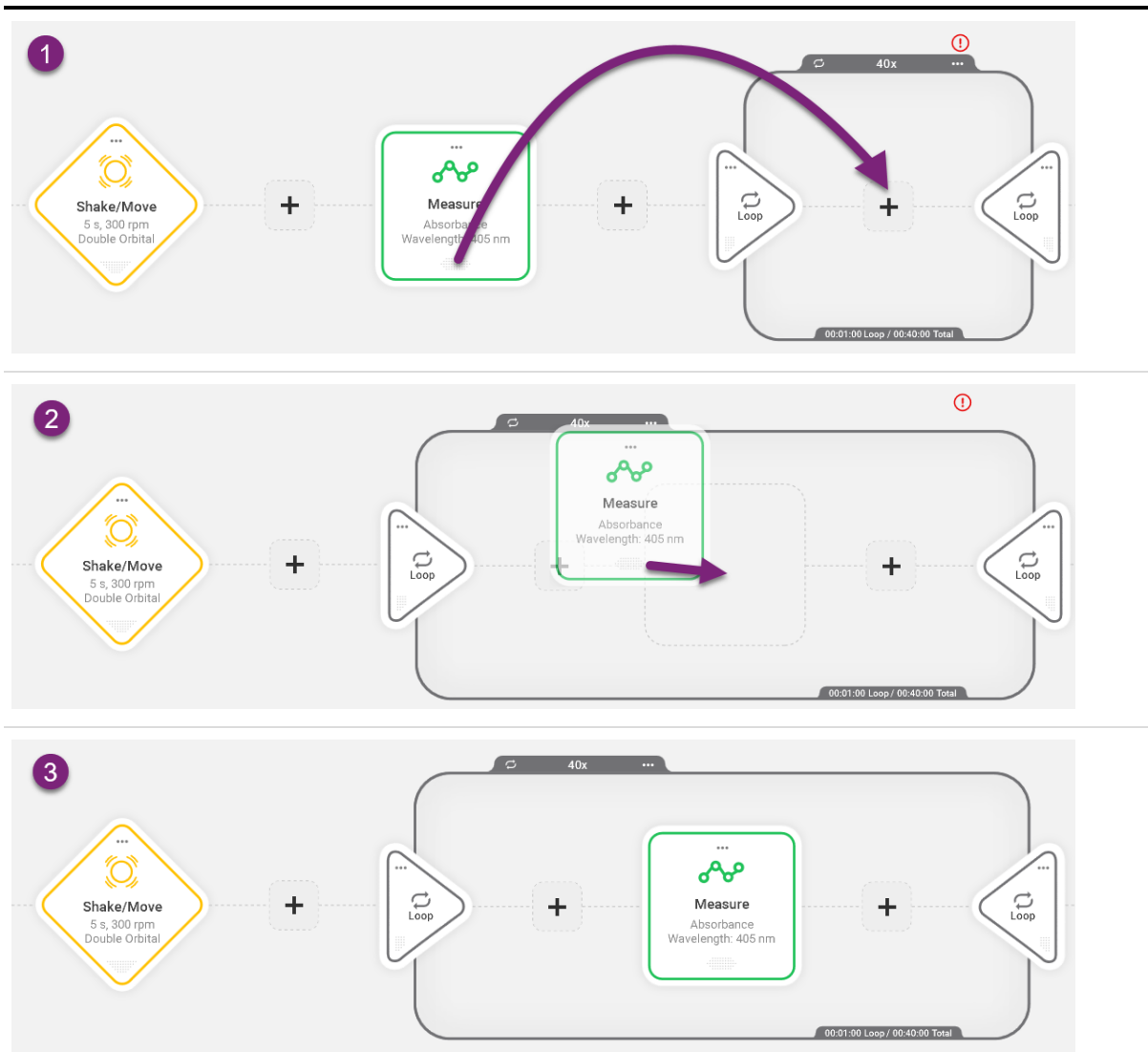
The sequence of action elements forms a workflow which can be read from left to right. The workflow shown below (see Figure 4.13), starts with an incubation at 37°C followed by a 5-second double-orbital shaking which is then followed by an absorbance measurement with 40 repetitions (loops) and a total duration of 1 hour.



**Figure 4.13:** Example workflow with Temperature, Shake/Move and a kinetic measurement defined within a Loop.

### Create a loop in the workflow

Select the microplate and define your microplate layout (see section 4.2.4 on page 17). Add all action elements required for your experiment to the workflow including a **Measure** and a **Loop** action and follow the next steps (see Figure 4.14) to create a kinetic measurement with a Loop in the workflow:



**Figure 4.14:** How to drag the Measure action inside the Loop.

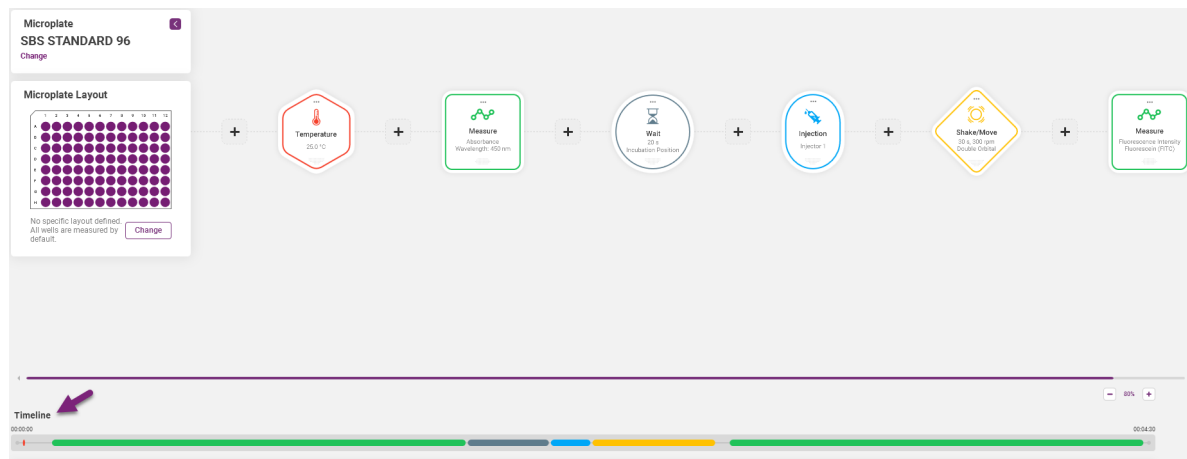
- 1 Drag the **Measure** element into the **Loop** towards the plus button by holding down the left mouse button.
- 2 When the mouse cursor moves inside the loop, an additional squared space will appear where you can drop the **Measure** element.
- 3 Move further action elements to be repeated into the loop by drag-and-drop.

**Note:** Injection elements cannot be dragged into the loop. This would create a protocol with injection repetitions equal to the number of loops and consequently overshoot the maximum volume per well. If you wish to perform several injections in one workflow, duplicate or create several injection elements outside of a loop.

It is worth mentioning that you do not necessarily have to create the **Measure** and **Loop** actions upfront to subsequently drag the **Measure** action into the loop element. You can also create the **Loop** element first, click on the plus sign inside the **Loop** and add a **Measure** action from the action window popping up. The action elements will have varying durations which is represented by the **Timeline** at the bottom of the protocol editor.

#### 4.2.6 Timeline

The timeline indicates how long the entire experiment will take and includes all selected actions. The timeline is displayed from the beginning at 00:00:00 to the completion of the last action (00:04:30 in the following example).



**Figure 4.15:** The color-coded timeline shows the duration of actions.

The various actions are color-coded. These colors are also represented in the timeline. The color code in the timeline shows which actions are carried out, when they are carried out and how long they will take. The following color-code is used:

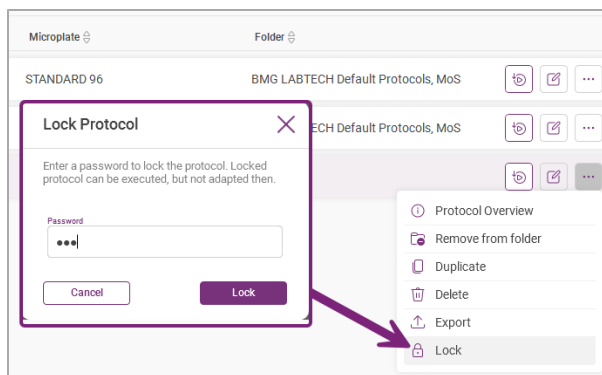
- Red: Temperature
- Green: Measurement
- Gray: Wait
- Blue: Injection
- Yellow: Shaking
- Gray: Loop
- Green: Well-wise

The **Timeline** is not only visible in the protocol editor but will also be seen on the **Dashboard** when a measurement has started (see section 4.1 on page 13). In this case the timeline will additionally show the progress of the measurement.

### 4.2.7 Lock protocols

A locked protocol is read only. It cannot be edited or deleted but it can be duplicated. If you duplicate a locked protocol the duplicate is not locked. To lock a protocol, click on **...** **more** and then on the **Lock** button. The lock protocol dialog opens and asks for a password.

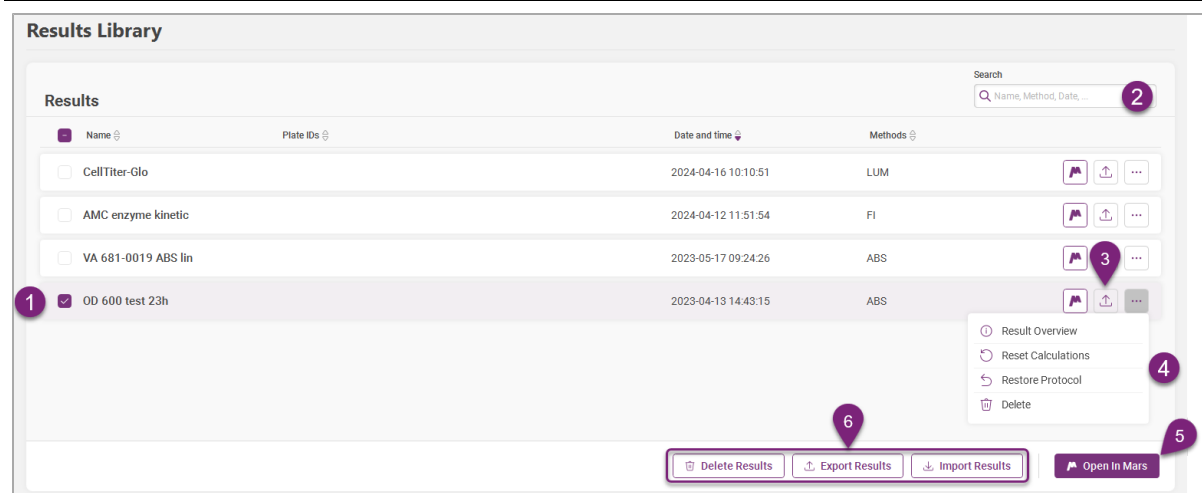
**Figure 4.16:** How to lock a protocol.



A padlock icon in the Protocol Library indicates that a protocol is locked. To unlock a protocol, click on **...** **more** and then **Unlock**. You need to enter the corresponding password to unlock the protocol.

## 4.3 Results

The **Results Library** contains the results of all measurements performed with the Voyager software. You can access the **Results Library** by clicking the **Results** menu. In the **Results Library** each line represents an individual test run:



**Figure 4.17:** Example of a **Results Library**.

- 1 **Open** a dedicated Result of a measurement.
- 2 **Search** and filter for a specific result (e.g. search for a specific date).
- 3 **Export** dedicated Results to a file. The file has extension \*.vmr.
- 4 Clicking ... opens a menu to **Delete** the corresponding result, to **Reset Calculations** (if available), to get a **Results Overview** and to **Restore Protocols**.
- 5 Use **+ Open Results** to open one or more results.
- 6 Additionally, you can **Delete Selected Results**, **Export Selected Results** and **Import Results**. Each of these options can work on multiple (selected) results.

Restoring protocols from results can be useful if the underlying protocol has been deleted and can now be recovered from corresponding results. Or if a result file from another user can be imported, it is now possible to restore this user's protocol from the results.

By default, the **Results Library** is sorted by **Date and time** of measurement. It can also be sorted by **Name** or **Methods** via the up and down arrows  $\updownarrow$  in the headings. You can also search for specific result files using the **Search** function  $\text{Q}$  at the top right of the window by entering a keyword and/or a number. To open result files in the MARS analysis software, click the  $\text{M}$  button for the result of interest, or select one or several results files by checking the box(es) at the beginning of each line and click **Open Results** at the lower right corner of the window.

To export result files from Voyager, click the button on the right end of each result file line or use the button at the lower end of the window after selecting individual result files via the check box(es). Both will guide you to a window allowing you to save the results in a dedicated Voyager measurement result format.

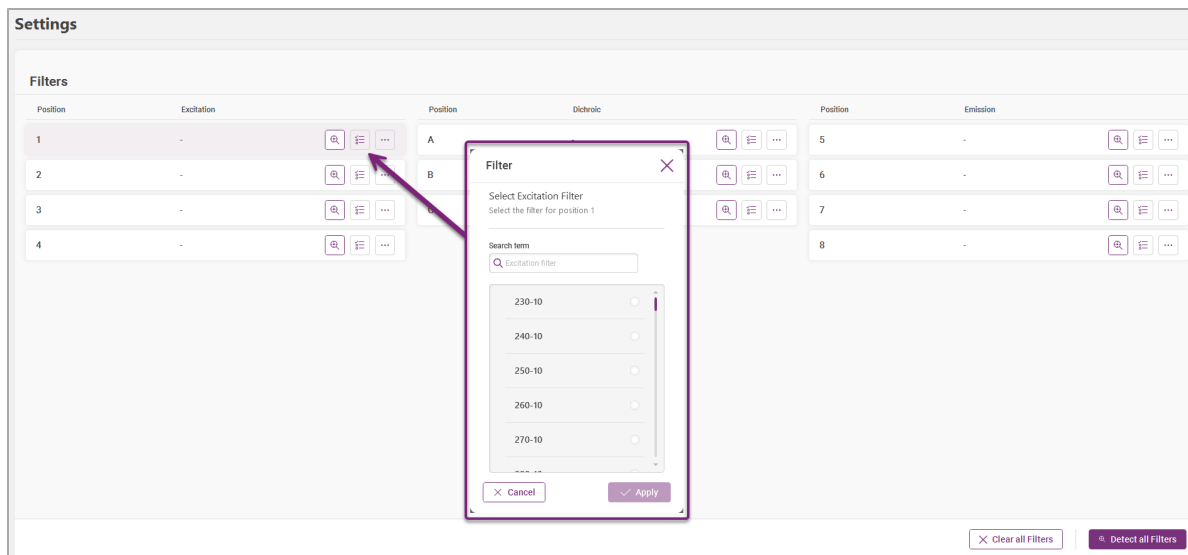
To delete result files, click on the ... button on the right end of each result file and select delete or use the button at the bottom of the window after selecting individual result files via the check box(es).

To reset the settings/calculations of a result file, click on the ... button on the right end of each result file and select **Reset Calculations**. From here, you can also **Delete** the selected test run or get a **Result Overview**.

## 4.4 Settings


### 4.4.1 Filter selection and detection

If you want to use filters in your measurement protocol, go to **Settings** and open the **Filters** window. Enter or select your filters in their respective positions. Instruments with a monochromator can automatically **Detect All Filters** or **detect**  $\text{+}$  filters.



**Figure 4.18:** The Manage Filters window to set excitation, dichroic and emission filters.

The settings in the **Manage Filters** table are only related to user-editable filters. Note the monochromator settings are independent and set explicitly in the measurement protocols.

Each position in the table corresponds to a filter position on the filter slides. The filter properties can be determined automatically or selected by clicking  **select**. In the latter case, a window appears listing commonly used filter. Select your filter and **Apply** the settings or **Cancel** editing.

After clicking the **X Clear** button, the table will be emptied.

Please note that when clicking **Detect All Filters** or **Detect** disables the corresponding button and the reader becomes busy. It might take some time to **Detect All Filters**. When detecting a filter, it will be scanned by the reader and the results will be compared with a database of known filters. The filter, fitting to the obtained key parameters, will be entered into the filter table automatically. It is anyway possible to select another filter from the pull down list or to edit the filter name or parameters.

**Note:** The filter table applies to all users.

**Note:** If the reader is not equipped with a spectrometer and LVF monochromator, an automatic detection of the dichroics is not possible. The automatic detection of filters is also not available for filter-only readers.

#### 4.4.2 Priming the injection system

Before using injections in a test protocol, it is necessary to prime the injectors. To do so, open the **Settings** menu and click on **Priming**.

**Note:** The Priming menu is only available if the reader has injectors installed.

The Prime Injection System window appears. It is partitioned based on the number of injectors installed. Having 2 injectors, the window is divided into two sections: **Injector 1** and **Injector 2**. Having only one injector installed, the window will be similar to the one below (see Figure 4.19). Follow these steps to prime the injector:

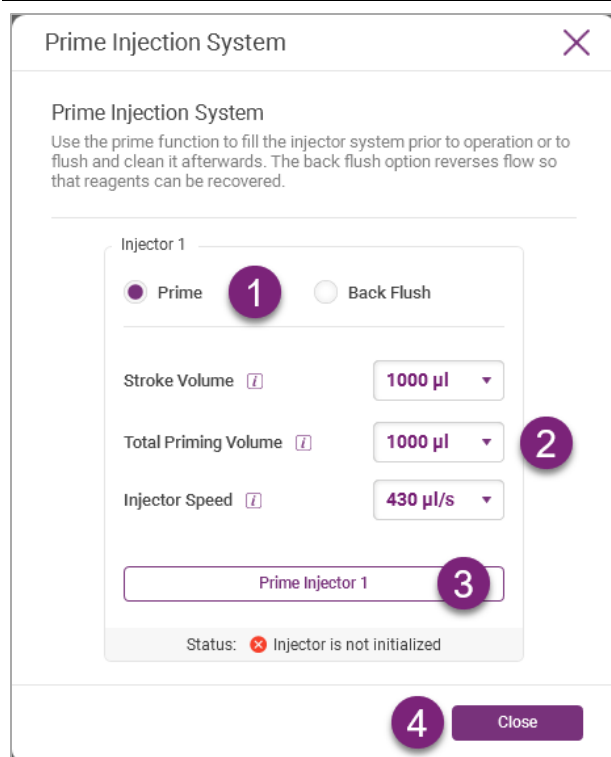


Figure 4.19: The Priming window.

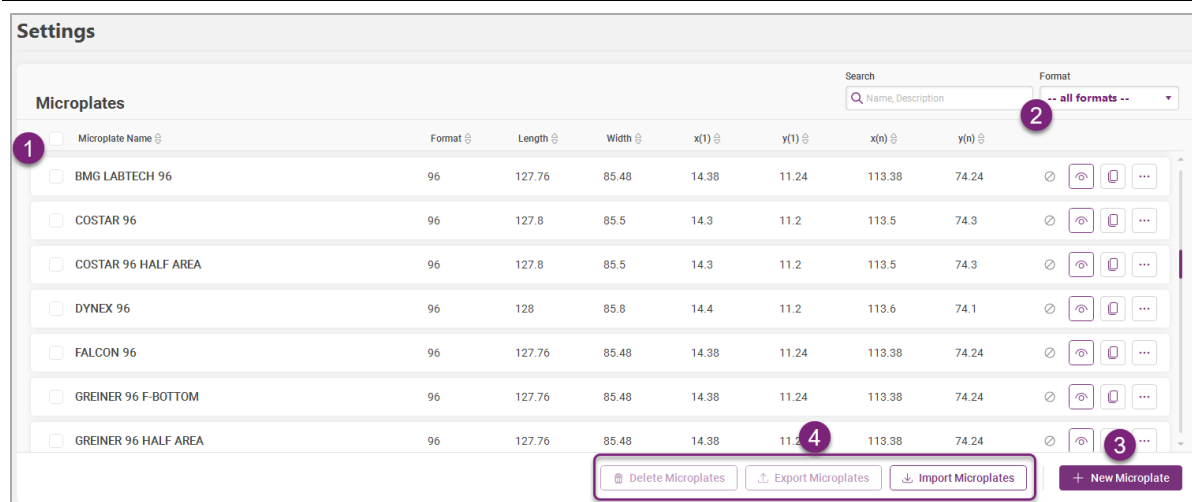
- 1 Make sure the **Prime** option is selected (default).
- 2 Select the **Stroke Volume**, the **Total Priming Volume** and the **Injector Speed** according to your needs.
- 3 Click on the **Prime Injector 1** button to prime the first injector. When finished, the **Status** will change from 'Injector is not initialized' to 'Injector is initialized'. The injector is then ready to be used.
- 4 Click on **Close** to close the Prime window.

**Note:** Use the **Back Flush** feature to recover reagent from the injector by pumping it back into the original reservoir.

Once the injector is initialized, it can be used within a measurement protocol (see section 4.2.2 on page 15).

#### 4.4.3 Manage microplates


The Voyager software has a comprehensive library of pre-installed microplates. You can access a corresponding microplate library through the **Settings** menu. In the Settings menu, click on **Manage Microplates** to open the **Manage Microplates** page (see Figure 4.20).



**Figure 4.20:** Manage Microplates page.

- 1 Select your microplate from the list.
- 2 Search for your microplate (e.g for a dedicated manufacturer) or filter for a dedicated microplate format. You can also sort for **Length**, **Width** or other parameters using the up-down arrows.
- 3 If your microplate is not yet part of the microplate library and there is no similar one with suitable dimensions, define your own microplate by clicking **Add Plate**.
- 4 You can also **Import Microplates** exported previously, or you may **Delete Selected Microplates** from the library.

Use the **view** button next to the microplate's name to see the dimensions of the selected microplate in the list.

**Note:** Pre-defined microplates can neither be changed nor deleted. This is indicated by the following symbol .

When adding a new microplate to the list, it is helpful not to start from scratch but duplicate an existing microplate and edit the copy. Pre-installed microplates are not editable but copies of them are. Clicking **+ New Microplate** opens the Add Microplate window. To create a new microplate type follow these steps:

**Figure 4.21:** Adding a new microplate to the library.

- 1 Enter a **Microplate name**.
- 2 Select the **Plate Format** (e.g. 96).
- 3 Select the **Well Shape**.
- 4 Enter the **Well Diameter** (typically published by the microplate manufacturer).
- 5 You can optionally enter a **Comment** such as catalogue number of the manufacturer.
- 6 Choose between **Symmetric Plate** and **Asymmetric Plate**. The former one is more common.
- 7 Enter the **Length** and the **Width** of the microplate as well as **Top Left x** and **Top Left y** distances (typically published by the microplate manufacturer).
- 8 Click **Save & Close** to store the microplate and its dimensions.

Click the **SLAS Standard Values** button to import the dimensions for the corresponding SLAS standard format (formerly known as SBS standard). Note that for an Asymmetric Plate there are two additional dimensional parameters to be set: the **Bottom Right x(n)** value and the **Bottom Right y(n)** value. The 'n' in brackets corresponds to the nth well. Please note that the microplate drawing is just a sketch to illustrate the meaning of the x and y-value(s). As 96 well-plates are most commonly used, a 96-well microplate is drawn. The drawing will not adapt with other plate formats.

**Attention:** If the dimensions of a microplate are edited, the changes will apply to all users.

#### 4.4.4 Connection

The **Connection** menu is only of interest if multiple readers are connected to the same PC. From the **Reader Connection** window you can select the corresponding reader to work with. The Reader Connection window is described in more detail elsewhere (see section 2.3 on page 10).

## 4.5 User account settings and user management

### 4.5.1 Permission models


Voyager offers fine-grained permission models that are linked to dedicated user-profiles. The following table gives an overview of the available permissions.

Item	Permission	Remark
<b>Accounts</b>	None	User can only view own permissions
	Edit	Create new users and edit permissions of existing users
	Edit & Delete	Additionally, delete users
<b>Test Runs</b>	Read Only	Can only view test runs
	Edit	Change evaluation settings in MARS
	Edit & Delete	Additionally, delete test runs
<b>Protocols</b>	Run only	Execute protocols
	Run & Adjust	Execute, adjust gain and focus
	Layout only	Execute, adjust layout
	Layout & Adjust	Execute, adjust gain, focus and layout
	Edit	Additionally, create new and edit existing protocols
	Edit & Delete	Additionally, delete existing protocols
<b>Microplates</b>	Read only	Use pre-defined microplates
	Edit	Change user-defined microplates
	Edit & Delete	Additionally, delete user-defined microplates

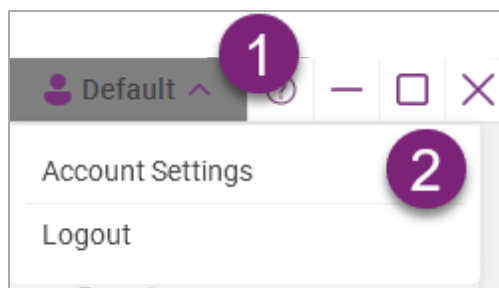
**Note:** Users cannot modify their own permissions.

Voyager comes with a **Default** user having full permissions.

### 4.5.2 User account settings

To examine your own permissions or change your password, click on your username or the user icon  in the upper right corner of the main window and then click on **Account Settings**.

**Figure 4.22:** To access the user **Account Settings** click on the user icon (1) and then click on **Account Settings** (2).



The Edit User window appears listing various options that can or cannot be changed by the current user depending on the permission model of that user.

**Edit User** [X]

Define User Settings  
Define your user settings down below. Click the information ⓘ icon for details.

---

**User Account Settings**

---

**User Settings**

User Name ⓘ  
Default

**Password Settings** 1

New Password ⓘ      Confirm Password ⓘ      Change Password

---

**Access Rights** 2

Manage Accounts ⓘ	Edit & Delete ▾
Manage Testruns ⓘ	Edit & Delete ▾
Manage Protocols ⓘ	Edit & Delete ▾
Manage Microplates ⓘ	Edit & Delete ▾

[X] Cancel      [✓] Apply

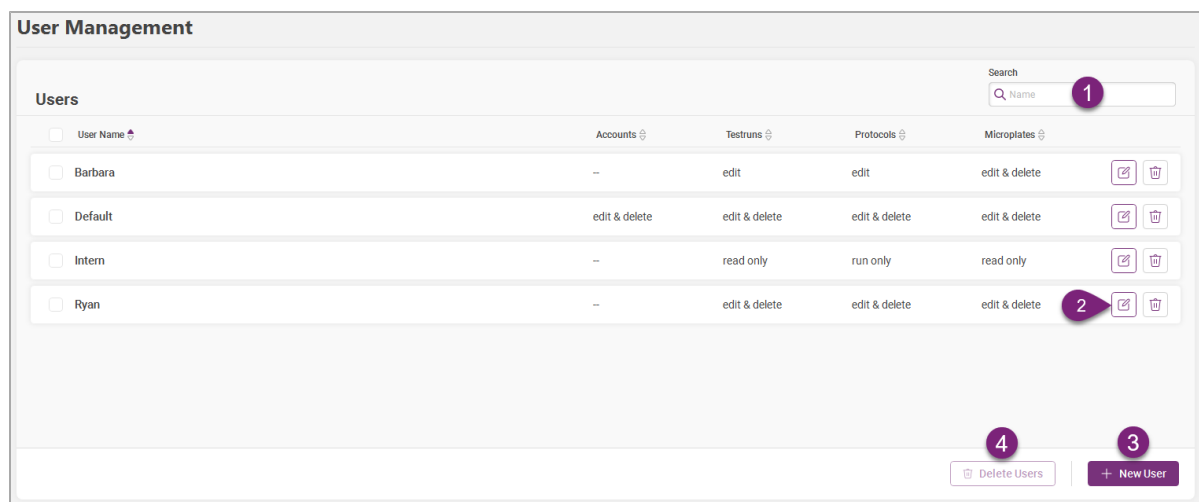
**Figure 4.23:** Editing User Settings

- 1 You can change your password under **Password Settings**. To do so, enter your **Old Password** and then enter your **New Password**. Re-enter your new password into the **Confirm Password** text field and click on **Change Password** to confirm it.
- 2 The current user's permissions are shown in the **Access Rights** section.

Make sure to save changes by clicking on **Apply**.

### 4.5.3 User management

Users with corresponding permissions will see a **User Management** menu in Voyager. Clicking on it will open a page where all users are listed including their permissions.



**Figure 4.24:** User Management

- 1 If your installation has many users, you might find it helpful to filter the user list by using the **Search** text box in the upper right corner. Only users having names that match the entered text will then be shown in the user list. Click the **X** in the text box to remove the filter afterwards.
- 2 To edit the settings of an existing user, click the edit button on the right. The options here are the same as described in the previous section (see section 4.5.2 on page 33).
- 3 To add a new user, click the **+ New user** button in the lower right corner. The Edit User window will appear (see section 4.5.2 on page 33), where you have to enter the username of the new user. Optionally, you can set a password for the new user, otherwise the password field will be empty, i.e., no password will be required to log in. Set the **Access Rights**, or leave them at default. New users will have full rights by default with the exception of the right for account administration.
- 4 To delete a single user, click on the username or the corresponding check box in the user list and click the delete button. A confirmation dialog will appear where you have to confirm the action.

**Note:** The currently logged in user cannot be deleted to make sure that there is always a user available for account administration.

## 4.6 Start measurement window

### 4.6.1 Open the start measurement window

To start the measurement of a protocol, go to the **Protocols**, load the protocol you want to execute and click the **Start measurement** button ► at the lower left side of the page. The **Start Measurement** window will appear. You can also enter this window directly from the protocol editor after clicking **Save and Execute**. The three **Plate ID** fields in the **Start Measurement** window allow you to enter specific information for each measurement run, like incubation time, plate number, etc.

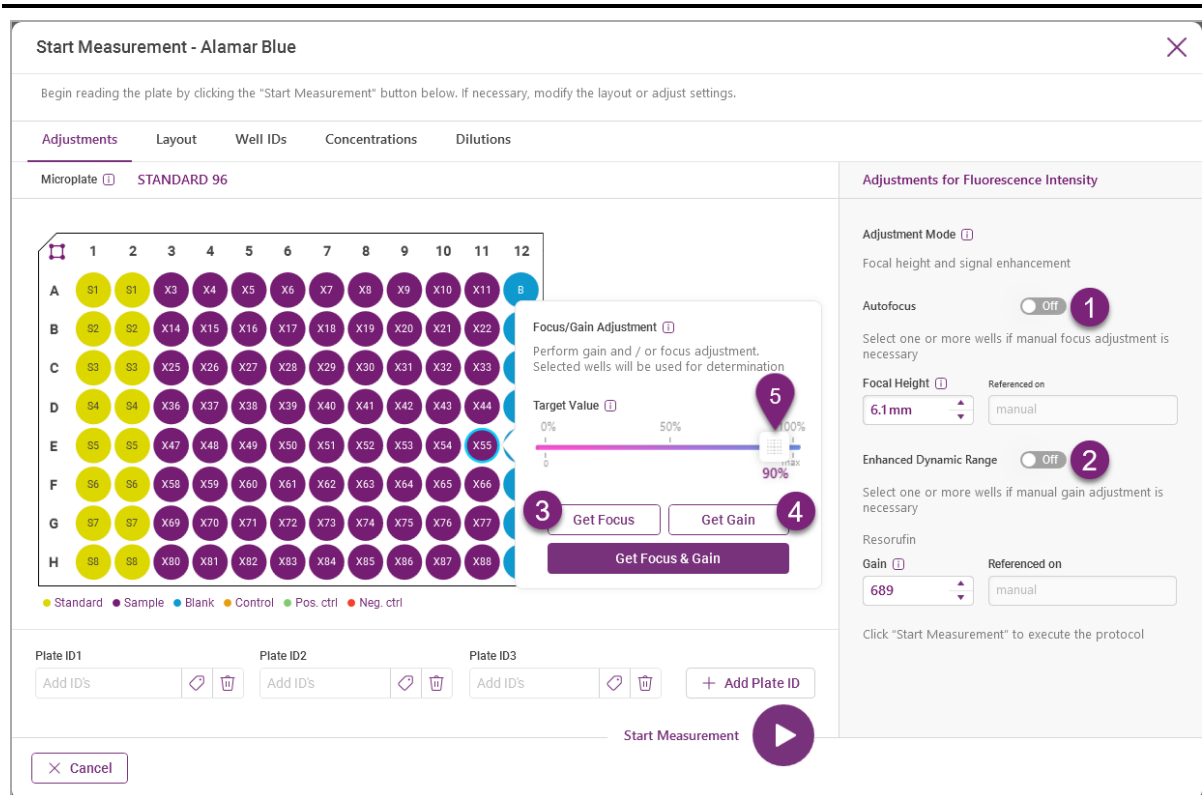


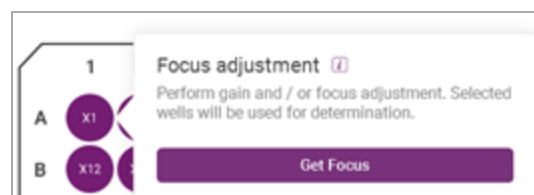
Figure 4.25: Gain and Focus adjustment.

- 1 For manual focus adjustment, deactivate the **Autofocus** switch and set it to **off**.
- 2 For manual gain adjustment, deactivate the Enhanced Dynamic Range (EDR) switch and set it to **off**.
- 3 Select one or more wells and click **Get Focus** to find the best-suited well for focus adjustment.
- 4 Select one or more wells with the highest signal to be expected, adjust the **Target Value** according to your experiment and click **Get Gain**.
- 5 Set the **Target Value** according to the current and expected maximum signal. E.g when the signal is expected to increase 10-fold in the course of a kinetic measurement, set the **Target Value** below 10%. For end point measurements, the **Target Value** is typically set to 90 %.

### 4.6.2 Focus adjustment

Measurements using Fluorescence intensity/FRET, Luminescence/BRET, TRF/TR-FRET can be performed using the **Autofocus** and the **Enhanced Dynamic Range** feature (Figure 4.25). For manual focus adjustment, deactivate the **Autofocus**, select one or more wells and click **Get Focus** (Figure 4.26).

Figure 4.26: Manual focus adjustment from the **Start Measurement** window.



**Note:** For luminescence measurements the VANTastar performs an automatic focal height adjustment and moves an aperture into the light path for crosstalk reduction. Manual adjustment of the focal height is not required for luminescence measurements.

### 4.6.3 Gain adjustment

Fluorescence intensity/FRET, Luminescence/BRET or TRF/TR-FRET measurements can be performed using the **Enhanced Dynamic Range** feature.

When measuring in the FRET, BRET or TR-FRET mode, manual gain adjustment can be performed individually for each emission wavelength. For FP measurements, gains have to be adjusted manually.

**Figure 4.27:** Example of the focus and gain adjustment window for TR-FRET.

**Adjustments for TR-FRET**

**Adjustment Mode** ⓘ  
Focal height and signal enhancement

**Autofocus**  Off  
Select one or more wells if manual focus adjustment is necessary

Focal Height ⓘ Referenced on  
-- mm --

**Enhanced Dynamic Range**  Off  
Select one or more wells if manual gain adjustment is necessary

**Ex: Ex TR Em: 665-10**  
Gain ⓘ Referenced on  
-- --

**Ex: Ex TR Em: 620-10**  
Gain ⓘ Referenced on  
-- --

To determine the gains for FP emission wavelengths, use wells with unbound fluorophore for the gain adjustment. The default target mP of 35 mP is typical for Fluorescein and can be changed for other fluorophores. By activating the **Advanced** switch, you can adjust the target value according to your experiment. Click **Get Gain** to perform the gain adjustment.

**Figure 4.28:** Example of the focus and gain adjustment window for FP.

**Gain Adjustment** ⓘ  
Perform gain and / or focus adjustment.  
Selected wells will be used for determination

**Target mP for Gain Adjustment** ⓘ  
35 mP

**Advanced**  Target Value ⓘ  
0% 50% 100%  
0 max  
10%

**Get Gain**

### 4.6.4 Plate IDs

Plate identifiers (Plate IDs) can be added on the **Start Measurement** window to add specific textual information to the Protocol and Results. Use up to three Plate IDs by entering any text or by selecting pre-defined tags like **Date**, **Time**, **SerialNumber** or barcode-related tags (for readers with integrated barcode scanners). By default no Plate ID field is shown, but they can be added by clicking the **+ Add Plate ID** button.

Figure 4.29: Up to 3 Plate IDs can be defined.



### 4.6.5 Start a measurement


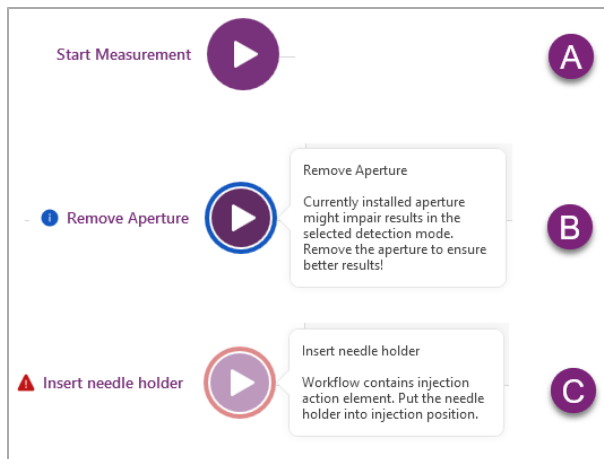
Click the **Start Measurement** button  to start microplate reading. If the text Start Measurement next to the play button is something else there is user action required. E.g. if a fluorescence intensity protocol is measured and an aperture is still inserted from a former luminescence measurement, the user will be prompted towards removing this aperture to assure optimal results in fluorescence. Some prompts are only informative and the user can still start the measurement, but some prompts do not allow the start the measurement. If, for instance, there is no or the wrong injection needle holder installed, a protocol with injection cannot be executed. In these cases the play button has a coloured contour either in blue, when you can still execute the protocol although not recommended without having changed what was recommended, or in red, when you need to change what is said in the hint text to be able to execute the protocol. In the latter case the play button will be disabled and grayed out. Figure 4.30 shows 3 examples of how the Start Measurement button may look like.

Figure 4.30: Examples of the states of the Start Measurement button. (A) Start Measurement without prompt, (B) It is recommended to remove the aperture (e.g. when executing FI protocols) and then run the measurement, (C) Protocol cannot be executed until an injection needle holder is inserted.

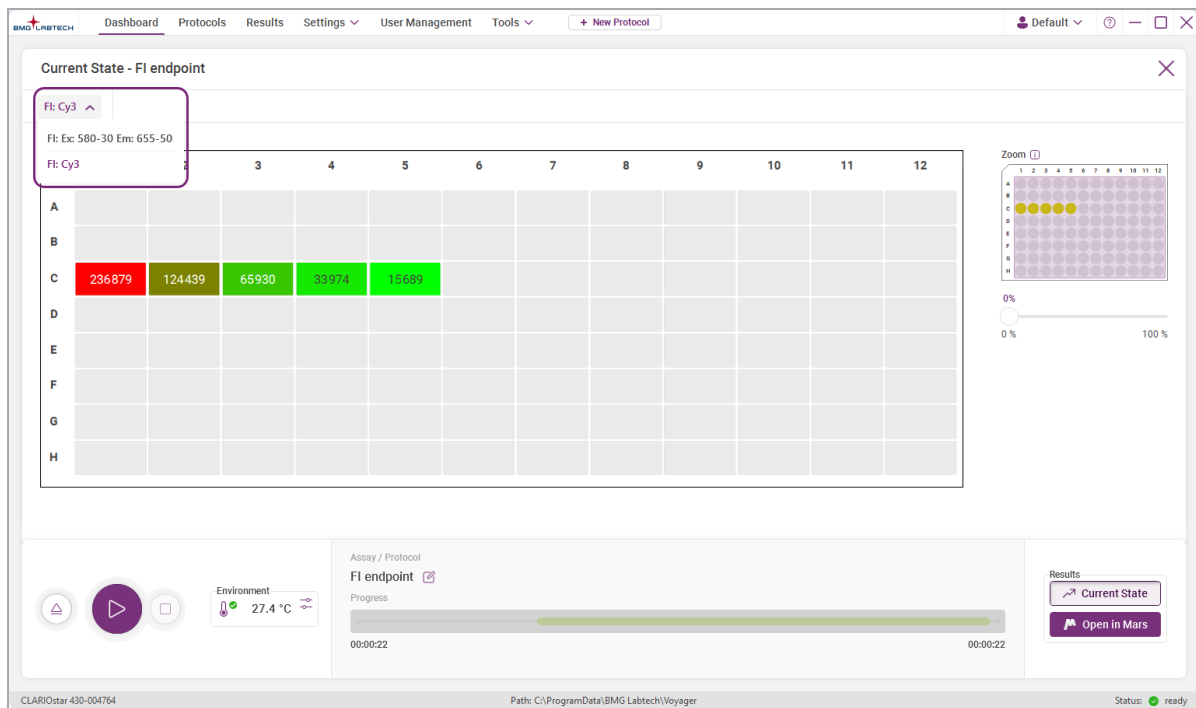


When the measurement starts, the **Current state** window that shows the measured values in real-time will automatically appear.

## 4.7 Current state window – Viewing your results in real-time

### 4.7.1 View endpoint measurements

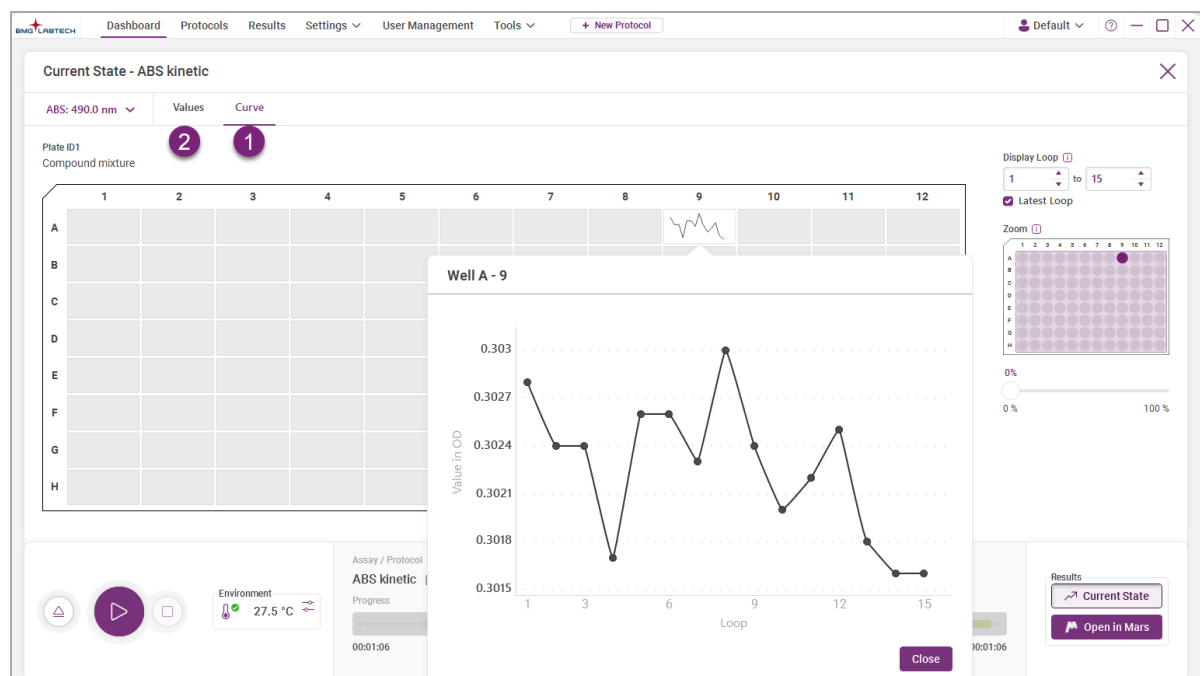
The **Current State** window, which automatically appears after a measurement has been started, allows you to view the measurement values generated by the reader in real time (A). A heatmap indicates high (red) and low (green) measurement values on the plate. The plate overview on the right side shows the selected plate layout. The slider below the layout plate allows you to zoom in and out the plate, and to view the displayed values and signal curves (for kinetics) in more detail. Please note that when measuring multiple discrete wavelengths, the **Current State** view has a drop-down menu just above the microplate to display the measurement values of the corresponding wavelength of interest (see figure below).



**Figure 4.31:** Current State view with heatmap and values. In this case, the measurement was performed at two dedicated wavelengths (shown at the top left in the drop-down menu)

#### 4.7.2 View kinetic measurements

When a kinetic measurement is performed, the Current State window opens. The Current State window shows curve insets for the microplate wells that display the measurement values of the current loop and the previous loops as a kinetic curve. The inset graphs can be zoomed by simply clicking on the corresponding well as shown below.



**Figure 4.32:** Current State View of a kinetic measurement.

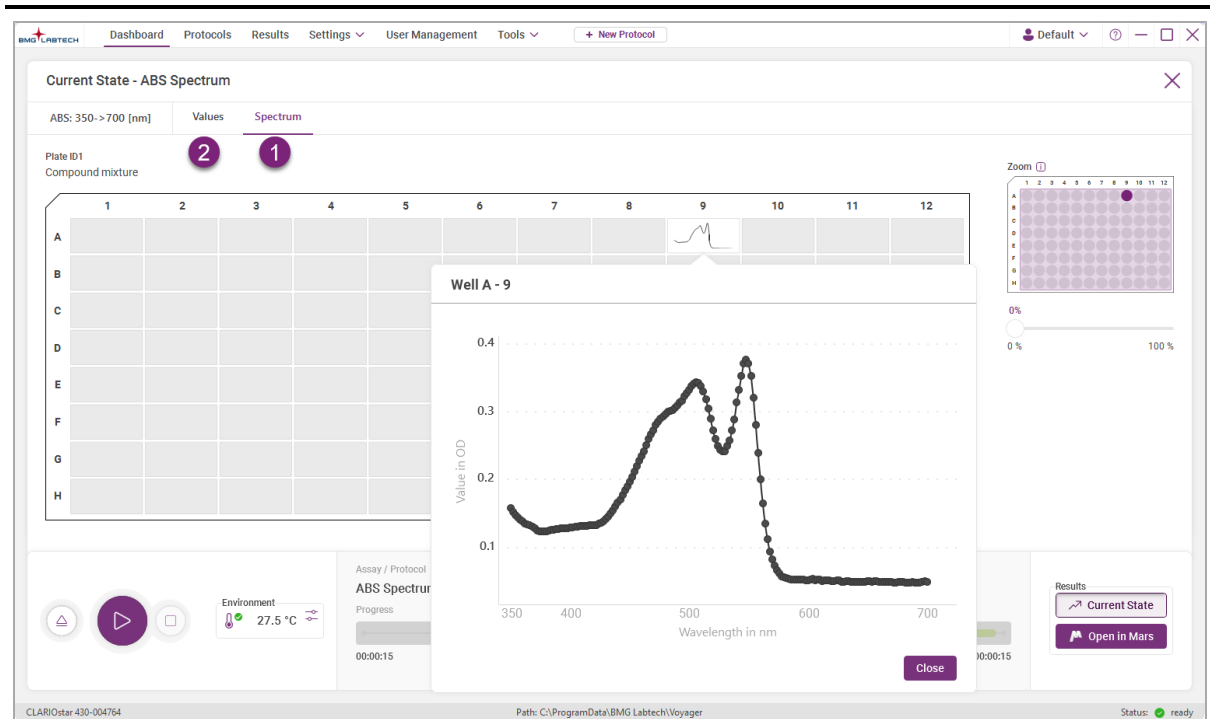
- 1 Clicking on **Curve** allows you to view the kinetic course of a measurement including the current and previous loops.
- 2 From the **Values** tab, you can display a heatmap and measurement values of either the **Latest Loop** or of a previous loop.
- 3 You can adjust the loop ranges to be displayed in the chart insets and in the detailed view by setting the lower loop limit and upper loop limit using the corresponding spinners.

Please note that data points are added to the curves with each new loop. For well-wise kinetic measurements, the graph is first fully generated for the first well, then the second etc. Here all loops are first measured for the first well, then for the second and so on. For "conventional" kinetic measurements, the curves are generated with every new measurement loop of the plate.

In additions to the overall progress, the timeline (see section 4.2.6 on page 26) at the bottom of the **Current State** view will show not only show the progress per well for well-wise kinetic measurements but also the progress per plate for "conventional" kinetic or endpoint measurements.

### 4.7.3 View spectral scan measurements

When running a spectral scan measurement (e.g. an absorbance scan), you can see the corresponding spectrum of the processed well. This will occur very quickly for absorbance measurements and moderately fast for monochromator-based measurements. The options in the current state view for spectral scan measurements are comparable to those of kinetic measurements, but instead of a kinetic curve, you will see a spectrum on a dedicated **Spectrum** tab and instead of a dedicated loop, you will be able to set a wavelength on the **Values** tab.

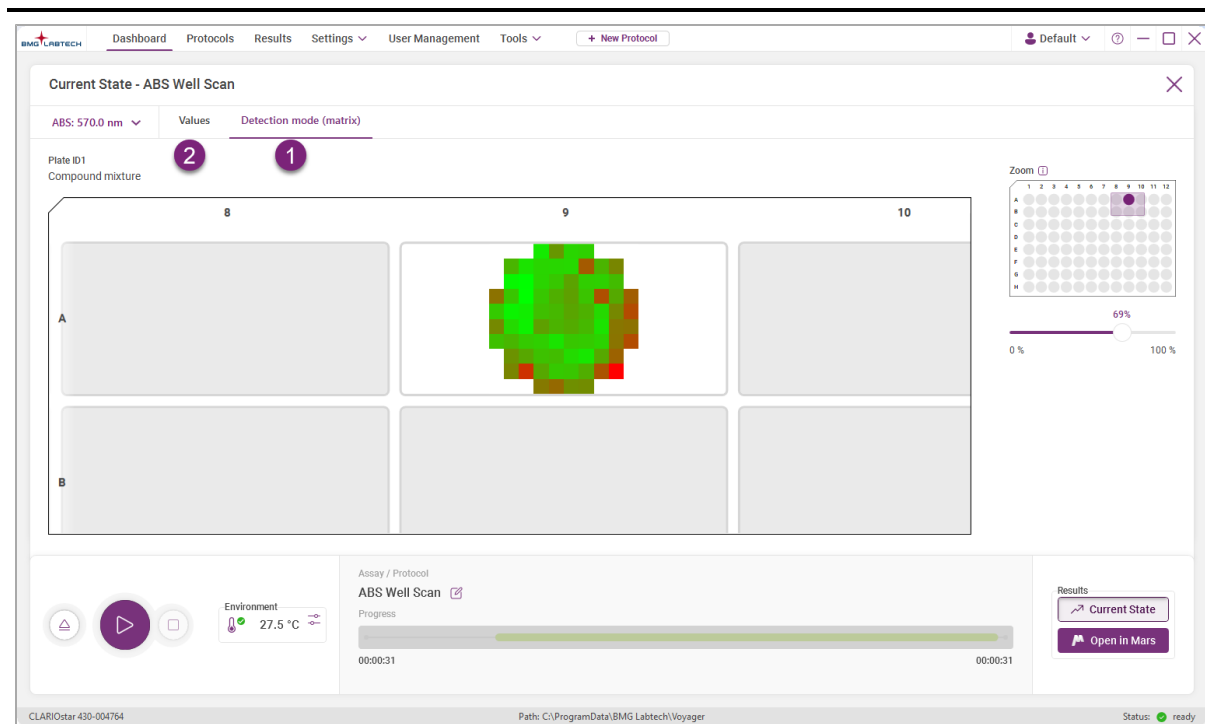


**Figure 4.33:** Current State View for a spectral scan measurement.

- 1 Clicking on **Spectrum** allows you to view the spectrum of a measurement.
- 2 From the **Values** tab you can display a heatmap and measurement values at a dedicated wavelength.

#### 4.7.4 Display of matrix well scan data

When performing a protocol using matrix well scan, the individual scan values can be seen in a color gradient display mode.



**Figure 4.34:** Current State View for a matrix well scan measurement.

- 1 Clicking on **Detection mode (matrix)** allows you to view individual matrix scan points.
- 2 From the **Values** tab you can display a heatmap and measurement values at a dedicated wavelength corresponding to the average of all matrix scan points.

If running a multichromatic protocol, the values of the chromatic selected in the drop-down menu on top of the current state microplate view will be displayed. Use zooming (either with the mouse wheel or the zoom-view to the right) to zoom into the matrix scan view of a dedicated well.

## 5 Getting started

### 5.1 Create an absorbance protocol

#### 5.1.1 Absorbance read mode

Absorbance is the attenuation of light passing through matter whose molecules become excited and can convert the absorbed energy into other forms like heat. The capability of molecules to absorb light of a specific wavelength depends on their chemical structure. Absorbance in a microplate reader is measured by vertically illuminating the sample through the transparent bottom of a microplate well, with light of known intensity  $I_0$ . The light  $I$  that passes through the sample and is not attenuated is detected from top by a spectrometer. The fraction  $I/I_0$  is the so-called transparency. It is more convenient to transform the transmittance into the so-called optical density (OD):

$$OD = -\log_{10} \left( \frac{I}{I_0} \right)$$

In general the OD is wavelength dependent. In the UV/Vis-range a typical chromophore has a maximum absorbance at a dedicated wavelength but can also have multiple local maxima at other wavelengths. Scanning the OD over many wavelengths reveals the absorbance spectrum of that chromophore which can be useful for assay optimization purposes.

Furthermore the OD is concentration and path length dependent. In a microplate well the path length of a sample can be estimated based on the geometry of the well given the sample volume is known or by measuring the NIR-absorbance of water near 977 nm (given the sample is aqueous):

$$d = \frac{OD_{near\ 977\ nm} - OD_{900}}{WPC}$$

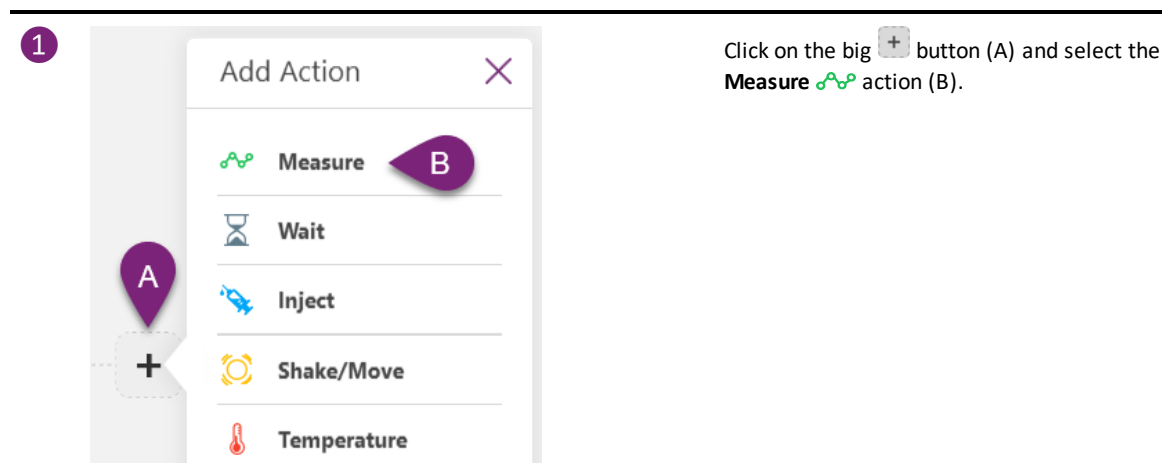
WPC denotes a correction factor and is essentially the absorbance of pure water near 977 nm minus the absorbance at 900 nm measured in a 1 cm cuvette. The value is already stored in Voyager and doesn't need to be entered by the user. Path length-corrected values can finally be obtained by dividing the OD measured at the actual wavelength divided by the measured path length.

For more details on the basics of absorbance and absorbance measurements, scan the following QR-code:



#### 5.1.2 Set up an absorbance protocol

Click on **+ New Protocol** on the Dashboard to open the protocol editor. From there, setting up a simple absorbance protocol is straightforward.



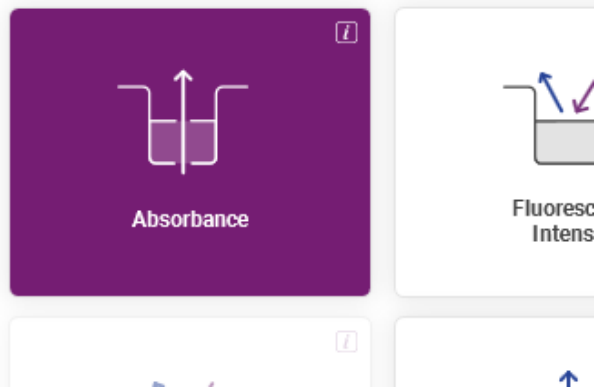
Click on the big **+** button (A) and select the **Measure** action (B).

2

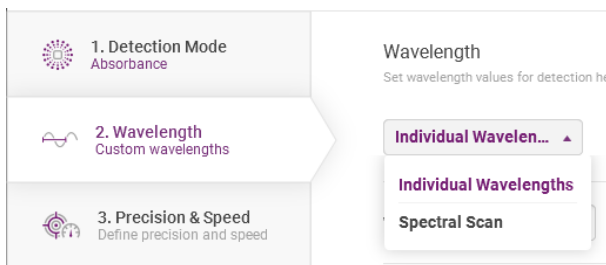
### Detection Mode


Select a detection mode for this measurement. Click the informati

In the following window choose **Absorbance** as detection mode.



3



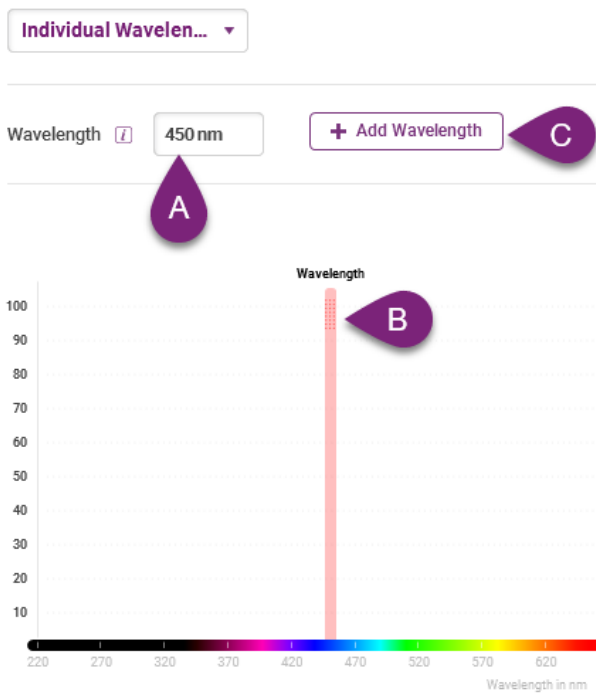
Click on  **Wavelength** and choose between measuring individual wavelengths or measuring an absorbance spectrum.

4

### Wavelength

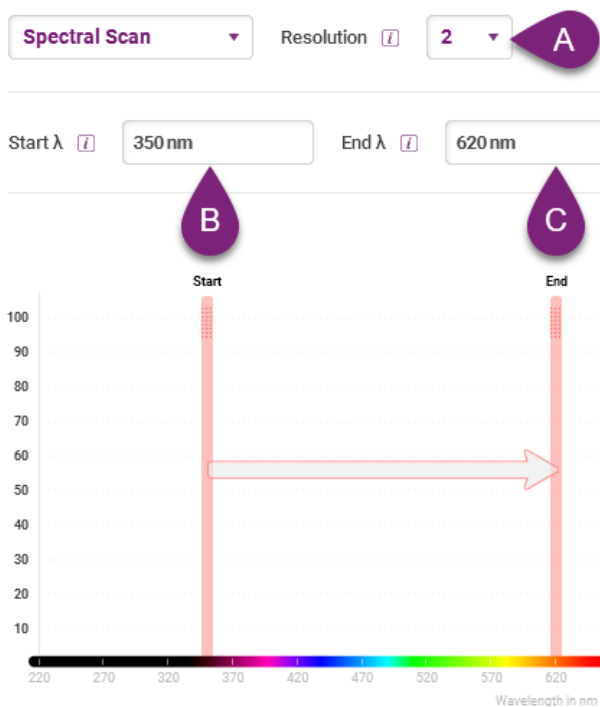
Set wavelength values for detection here. To adjust values, drag the elements in the

When **Individual wavelengths** is selected, type in the wavelength directly (A) or drag the red Wavelength bar in the chart (B). Add further wavelengths by clicking the **Add Wavelength** button (C).



## 5 Wavelength

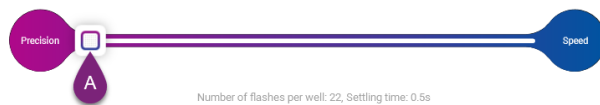
Set wavelength values for detection here. To adjust values, drag the elements in the



When **Spectral Scan** is selected, choose the wavelength **Resolution** between spectrum data points in nanometer (A). Enter **Start** (B) and **End** wavelength (C) directly or use the corresponding red bars in the chart and drag them accordingly.

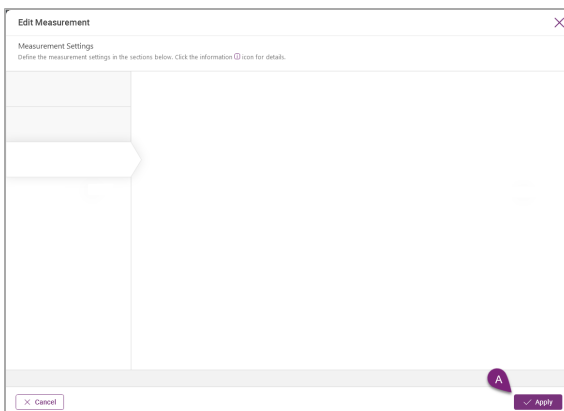
## 6 Precision & Speed

Set the slider for a faster or more precise measurement. Click the information icon for details.



Click on **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.

## 7



Click **Apply** (A) to save the absorbance measurement settings and to go back to the workflow from which you can either **Save & Execute** the protocol directly or **Save & Close** for later use.

Please note that you might want to choose a different measurement pattern like matrix scanning or you want to perform path length correction to normalize the OD values to 1 cm path length. To do so, enable the **Advanced Settings** by toggling the corresponding switch to **On**. For more details on the advanced settings, see section 5.9 on page 60

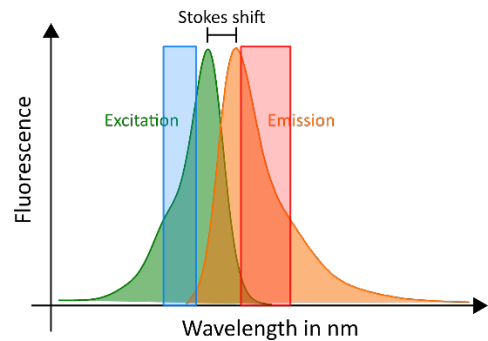
**Attention:** For absorbance measurements, microplates with a transparent bottom are mandatory. If lids or sealers are used, make sure they are also transparent.

## 5.2 Create a fluorescence protocol

### 5.2.1 Fluorescence intensity read mode

Fluorescence is created by the absorption of light by fluorescent molecules (fluorophores), that become excited and emit a photon at a red-shifted wavelength (compared to the incoming light). Fluorophores have well-defined spectra with an excitation and emission maximum. Excitation and emission spectra look like mirror-images. The shift of their respective maxima is often called Stokes-shift (see Figure 4). This shift enables the effective separation of excitation light from the emission light (i.e. the light of interest). Spectra of common fluorophores are well-known. They are included in the fluorophore library of Voyager and are linked to recommended filter and Linear Variable Filter (LVF) settings. Additional fluorophores can be scanned for their excitation and emission spectra when using monochromator-based readers. The generated spectra can then be used to identify optimum wavelength settings for the detection of the respective fluorophore.

**Figure 5.1:** Fluorescence excitation and emission spectra with suitable fluorescence filters.



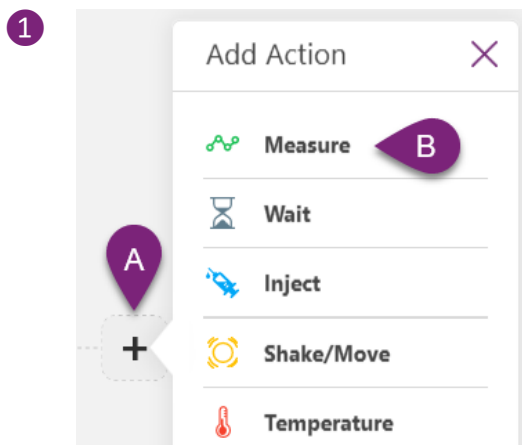
The emission light intensity is proportional to photophysical parameters of the fluorophore (like quantum yield, extinction coefficient), to the detection efficiency of the measurement system, and to the concentration of the fluorophore in solution.


For more details on the basics of fluorescence measurements, scan the following QR-code:



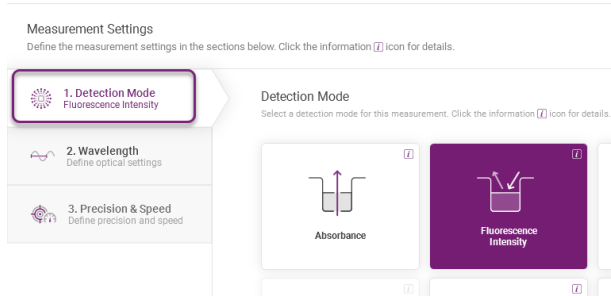
### 5.2.2 Set up a fluorescence measurement protocol

For the definition of a fluorescence measurement protocol, go to the **Protocols** tab in the Voyager software and click the **New Protocol** button. After selecting your microplate and defining your microplate layout, you can add actions to your workflow.



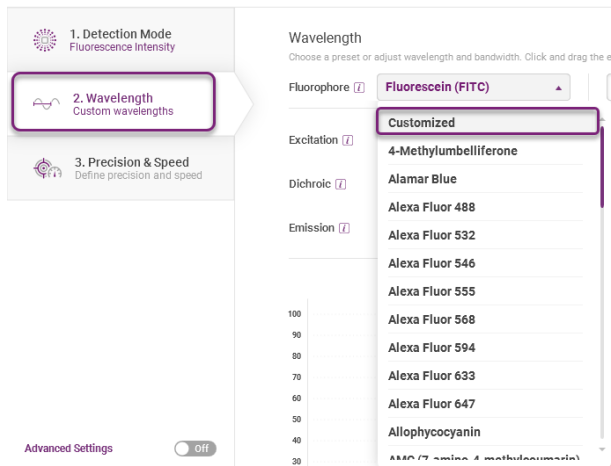
Click on the big **+** button (A) and select the **Measure**  action (B).

2 Add Measurement



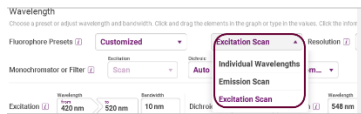
In the following window choose **Fluorescence intensity** as detection mode.

3



In the **Wavelength** window you can select your **Fluorophore** from the **Fluorophore Presets** list or add a customized fluorophore and create individual wavelength settings.

4



You can choose between the measurement at **Individual Wavelengths** or measure an **Excitation Scan** or **Emission Scan**.

5



When measuring at an **Individual wavelength**, you can either use the **Monochromator** or **EasyClick Filters** (if available).

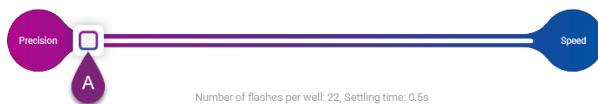
EasyClick filters, installed in the reader, can be selected from the drop-down menu. Monochromator settings can be chosen freely in the range of 320-740 nm with bandwidths of 8-100 nm. You can change the LVF monochromator settings by dragging the elements in the graph or typing a value directly in the respective fields.


6

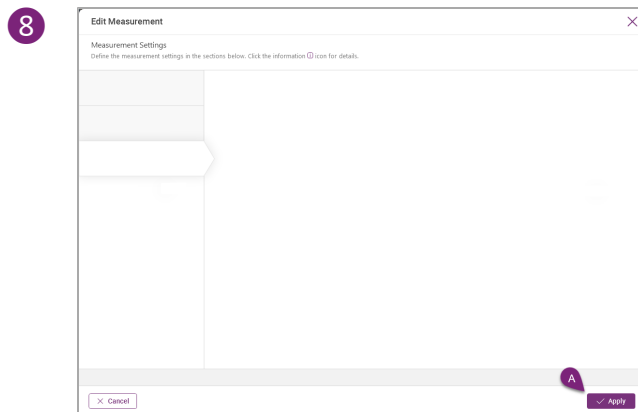


When scanning emission with the monochromator, the excitation wavelength is kept constant. You can change the emission scan range by dragging the elements in the graph or typing a value directly in the respective fields. For Excitation scans, emission is kept constant.

- 7 Precision & Speed  
Set the slider for a faster or more precise measurement. Click the information  icon for details.



Click on  **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.



Click **Apply** (A) to save the fluorescence measurement settings and to go back to the workflow from which you can either **Save & Execute** the protocol directly or **Save & Close** for later use.

## 5.3 Create a time-resolved fluorescence (TRF) protocol

### 5.3.1 Time-resolved fluorescence read mode

Fluorescence detection can be mostly divided into two types of measurements: steady-state (“common” fluorescence intensity) and time-resolved fluorescence. The main difference between these two methods is based on the nature and properties of the fluorescent molecules (fluorophores) used and the ensuing time of detection.

Time-resolved fluorescence (TRF) is monitored as a function of time upon excitation. In contrast to steady-state fluorescence intensity, time-resolved fluorescence is based on the detection of intensity decays and/or on the delayed detection of the emission signal upon excitation. In time-resolved fluorescence measurements, the excitation light pulse is shorter than the decay time of the fluorescent signal. Time-resolved fluorescence detection can only be achieved when the emission signal of the fluorophore is prolonged to the micro- or even millisecond range and not short-lived within nanoseconds as for common labels.

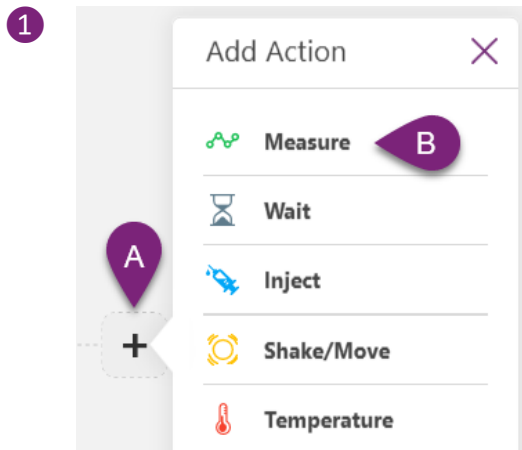
For the definition of a time-resolved fluorescence measurement protocol, go to the “Protocols” tab in the Voyager software and click the **+ New Protocol** button. After selecting your used microplate and defining your used plate layout you can add actions to your workflow.

For more details on the basics of TRF measurements, scan the following QR-code:

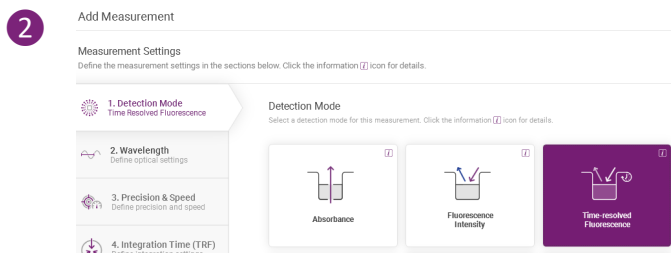


### 5.3.2 Set up a TRF measurement protocol

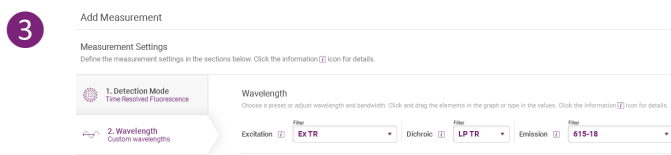
For the definition of a TRF measurement protocol go to the **Protocols** tab in the Voyager software and click the **+ New Protocol** icon. After selecting your used microplate and defining your used plate layout you can add actions to your workflow.



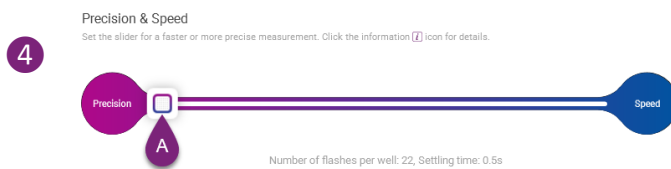
Click on the big **+** button (A) and select the **Measure** action (B).



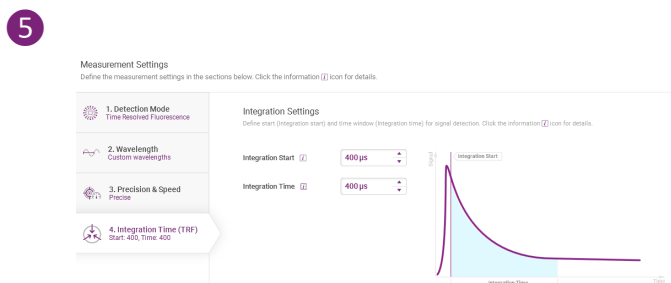
In the following window choose **Time-resolved Fluorescence** as detection mode.



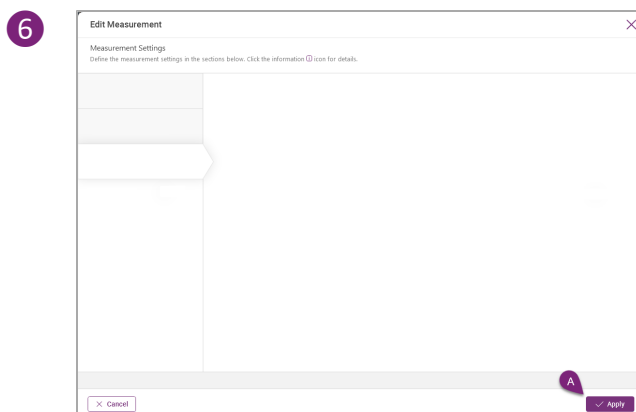
In the **Wavelength** tab you can select **EasyClick filters** specific for your assay. Working with time-resolved fluorescence requires filters that are specific for the TR- fluorophores employed for the assay (Ex TR, LP TR and dedicated emission filters).



Click on **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.



Define **Integration Start** and **Integration Time** in the **Integration Time** tab. Integration Start defines the delay between stopping excitation and starting signal monitoring. Integration Time determines the period for which the signal will be recorded. These settings are specific for TRF fluorophores used.



Click **Apply** (A) to save the TRF measurement settings and to go back to the workflow from which you can either **Save & Execute** the protocol directly or **Save & Close** for later use.

## 5.4 Create a Förster's resonance energy transfer (FRET) protocol

### 5.4.1 FRET read mode

The FRET theory (FRET stands for Förster resonance energy transfer) describes the energy transfer of a donor fluorophore that has been excited by light of an appropriate wavelength and de-excited by energy transfer onto an acceptor fluorophore that is close to the donor. The efficiency of energy transfer between donor and acceptor decreases disproportionately with the distance between these fluorophores. A typical measure for the FRET efficiency is the ratio of acceptor emission intensity to donor emission intensity. Thus, FRET measurements record the donor signal and one or more acceptor signal(s). If the donor and the acceptor are each placed on different molecules, FRET is often used to examine binding events. Placing them on the same molecule for example allows tracking different folding states of proteins.

For more details on the basics of FRET and FRET measurements, scan the following QR-code:



### 5.4.2 Set up a FRET measurement protocol

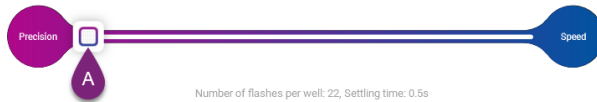
**1**


Click on the big **+** plus button (A) and select the **Measure** action (B).

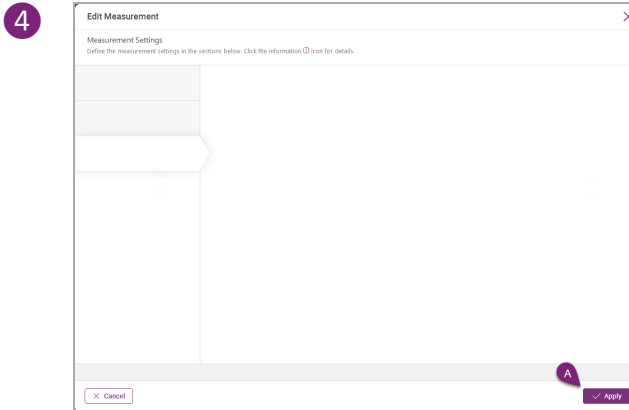
**2**

In the following window choose **FRET** as detection mode and go to **Wavelength** . Either choose a Fluorophore Preset or use **Customized** settings. Presets are wavelength settings for commonly used donor-acceptor pairs. You can either start with those settings and adapt them accordingly or you can start from scratch with **Customized** settings. In any case select whether to measure **Over Well** or **Over Plate**. Either select **Monochromator** or **EasyClick filters** for **Excitation** wavelength. Do the same for the **Dichroic** and for the donor and acceptor emission (i.e. Emission 1 and Emission 2, respectively). Set the exact wavelengths for excitation including the corresponding bandwidth and for Emission 1 and Emission 2. You can drag the bars in the spectrum chart.

- 3 Precision & Speed  
Set the slider for a faster or more precise measurement. Click the information  icon for details.



Click on  **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.



Click **Apply** (A) to save the FRET settings and to go back to the workflow from which you can either **Save & Execute** the protocol directly or **Save & Close** for later use.

## 5.5 Create a time-resolved FRET (TR-FRET) protocol

### 5.5.1 TR-FRET read mode

Time-Resolved FRET (TR-FRET) is a detection technology that combines Time-Resolved Fluorescence (TRF) with Förster Resonance Energy Transfer (FRET). TR-FRET relies on resonance energy transfer taking place between a lanthanide and short-lived fluorophore being in close proximity. Donor and acceptor are either covalently bound to the interacting partners or a specific antibody against each of the two targets (or tags) is labelled with either the donor or the acceptor. By exciting the donor, energy will be transferred to the acceptor, which will emit because of fluorescence resonance energy transfer, if the targets are in close proximity. Thus, TR-FRET measurements record the donor signal and one or more acceptor signal (s). The output is proportional to the amount of binding taking place. As for FRET, the efficiency of energy transfer between donor and acceptor decreases disproportionately with the distance between these fluorophores.

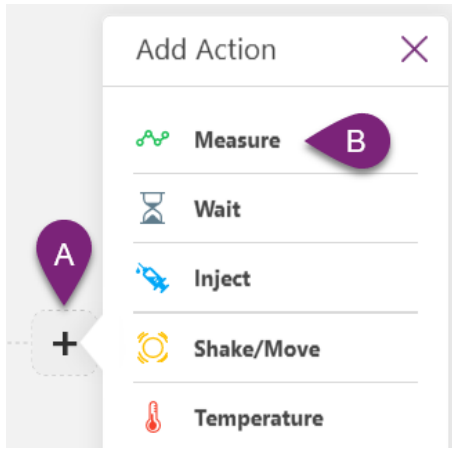
For more details on the basics of TR-FRET measurements, scan the following QR-code:




### 5.5.2 Set up a TR-FRET measurement protocol

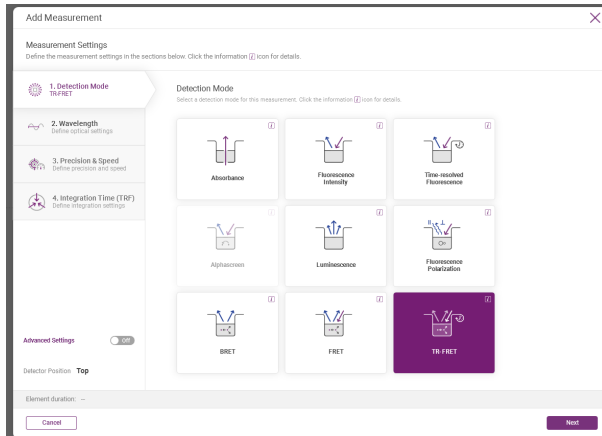
For the definition of a TR-FRET measurement protocol, go to the **Protocols** tab in the Voyager software and click the **+ New Protocol** button. After selecting your used microplate and defining your used plate layout, you can add actions to your workflow.

1



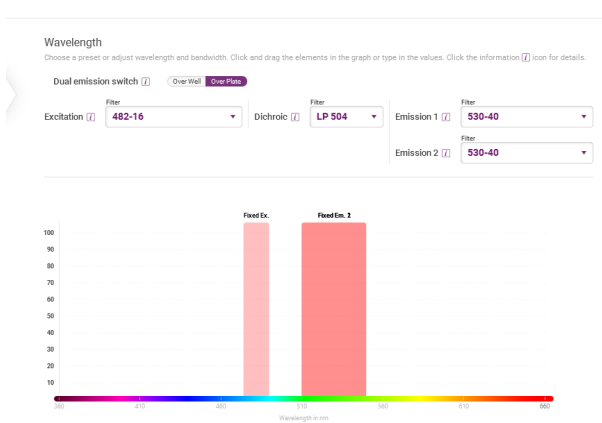
Click on the big **+** button (A) and select the Measure action  (B).


2



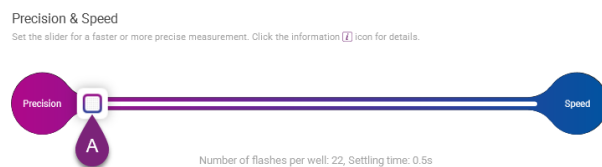
In the window choose **TR-FRET** as detection mode.


3

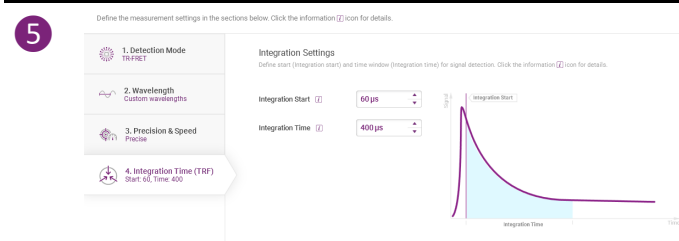


Go to Wavelength . Select whether to measure **Over Well** or **Over Plate**. Select from the EasyClick filters installed on your device for excitation wavelength selection. Do the same for the **Dichroic** mirror and for the donor and acceptor emission (i.e. Emission 1 and Emission 2, respectively).

4

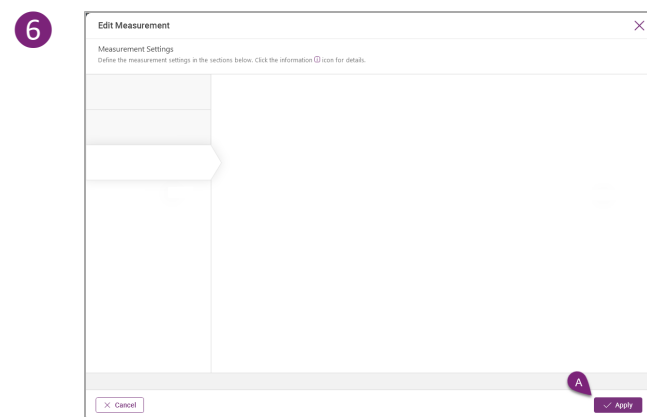


Click on  **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.



Select the **Integration Start** and the **Integration**

**Time** of your TR-FRET measurement according to assay-specific recommendations.



Click **Apply** (A) to save the TR-FRET measurement settings and to go back to the workflow from which you can either **Save & Execute** the protocol directly or **Save & Close** for later use.

## 5.6 Create a fluorescence polarization protocol

### 5.6.1 Fluorescence polarization read mode

Fluorescence polarization (FP) is a fluorescence-based detection method that is widely used to monitor molecular interactions in solution. Unlike fluorescence intensity which focuses on the quantification of emission intensity at a specific wavelength and neglects its polarization, fluorescence polarization specifically analyses as output the emission intensity of different polarization planes.

FP is typically used to assess biomolecular interactions such as protein-protein and protein-DNA binding, as well as enzyme activity. It has been adopted in basic research as well as high-throughput screening.

Initially, fluorescence polarization was mainly used in the diagnostic field. An FP immunoassay was first described in the 1960s. In life science research, applications were at first limited due to the lack of sensitive instruments. Currently, the demand for FP is increasing due to its popularity in biological research. This is mainly due to the increased performance and sensitivity of modern microplate readers that can deliver robust results with minimal variability and larger assay windows.

The polarization is calculated according to the following equation:

$$\text{mP (Polarization)} = 1000 \cdot \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Another commonly used measure is the anisotropy:

$$\text{mA (Anisotropy)} = 1000 \cdot \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

Where do the intensities  $I_{\parallel}$  and  $I_{\perp}$  come from?

The VANTASTAR employs polarization foils for this purpose. The excitation light will be parallel polarized. The emission will be monitored in parallel and perpendicular channels. The selectivity of emission channels for the polarized light will be achieved by positioning of polarization foils in front of emission filters.

The readout is the fluorescence intensity in parallel  $I_{\parallel}$  and perpendicular  $I_{\perp}$  channels. The respective intensities are put into the first formula. In the case of highly polarized fluorescence, the parallel polarized excitation light “transfers” its polarization to the emission light. Consequently, if the relaxation time is long (big, slow rotating conglomerate) the  $I_{\parallel}$  is significantly higher than  $I_{\perp}$  and the whole formula provides higher result = higher polarization value in mP.

In the context of polarization detection, also the G-factor is also referred to. The G-factor compensates the reader’s contribution of the reader’s hardware components to the difference in the  $I_{\perp}$  and  $I_{\parallel}$ .

This difference comes from slight differences in the transmission of emission filters, differences between detectors in simultaneous dual detection systems etc. This factor needs to be evaluated and considered in calculations. Other vendors have

these parameter in their software. Therein, the user can either evaluate the G-factor from measuring a real sample, put a G factor known from earlier measurements in or simply set it to “1” if no information is available.

The polarization formula is adapted in the following way:

$$mP = 1000 \frac{I_{\parallel} - G \cdot I_{\perp}}{I_{\parallel} + G \cdot I_{\perp}}$$

BMG systems evaluate this factor for each measurement automatically. This is the target mP-based gain adjustment routine that is needed for a free, unbound fluorophore or a free, labeled tracer. This procedure provides two parameters: the G-factor and the lowest mP value achievable in a real experimental setup, i.e. the “lowest edge” of the assay window.

The reader excites the free, unbound fluorophore with parallel polarized light. The gain target value is set at 10%. This means the reader adjusts the gain so that the parallel emission channel provides a signal of 26.000 RFUs.

Then the software calculates how high the intensity of the perpendicular channel must be for achieving the mP target value (e.g. 35 mP for fluorescein). This intensity will be used as a target intensity value for the second gain adjustment in the perpendicular channel. In this way both gain values are corrected for the fine differences driven by the reader's contribution to the result.

For more details on the basics of fluorescence polarization and fluorescence polarization measurements, scan the following QR-code:



### 5.6.2 Set up a fluorescence polarization protocol

**1**

Click on the big **+** button (A) and select the **Measure** action (B).

**2**

In the following window choose **Fluorescence Polarization** as detection mode.

**3**

Under **Wavelength**, select **Excitation** and **Emission** filters as well as a dichroic mirror as appropriate for the fluorescent dye you are planning to use for your assay.

**4**

**Precision & Speed**  
Set the slider for a faster or more precise measurement. Click the information icon for details.

Click on **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.

**5**

Click **Apply** (A) to save the absorbance measurement settings and to go back to the workflow from which you can either **Save & Execute** the protocol directly or **Save & Close** for later use.

**Note:** Only EasyClick filters can be used for fluorescence polarization. An LVF monochromator is not suitable for this detection mode. Make sure, that appropriate EasyClick filters are installed and defined under **Settings** → **Filters** (see section 4.4 on page 28).

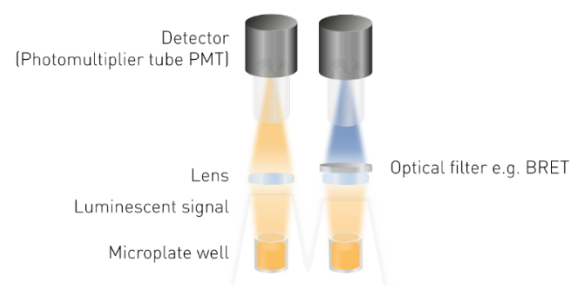
**Note:** Plate bottom material can have negative impact on fluorescence polarization results in bottom reading mode. Plates made of polystyrene are not suitable for assays operating with wavelengths < 350nm.

## 5.7 Create a luminescence protocol

### 5.7.1 Luminescence read mode

Luminescence is the production of a luminous signal through energy conversion. Luminous signals can be generated during energy conversion from a broad range of energy sources. This process turns invisible energy into visible radiation. Luminescence is easier to detect than fluorescence or absorbance as no excitation is required. This means neither a light source, nor an excitation wavelength selection are required. The minimum components needed are a lens to collect the luminous signal and a detector (see Figure 5.2). However, specific luminescence applications, such as BRET (bioluminescence resonance energy transfer), require a wavelength selection tool which allows you to differentiate between specific emission wavelengths. This can be achieved either by using optical filters or the LVF monochromator. The Voyager software contains a selection of pre-sets for commonly used luminophores you can choose from.

**Figure 5.2:** Schematic illustration of a luminescence detection system in a microplate reader.



A luminescent signal lasts a second or longer and is therefore clearly different from fluorescence that decays in nano-seconds. Accordingly, the signal is typically detected over a time period between 0.1 - 1 seconds. On BMG LABTECH microplate readers this is defined as the "integration time" which you can adjust in the measurement protocol.

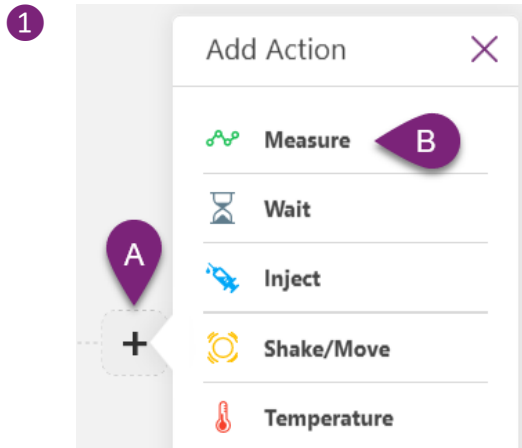
For luminescence assays it is often convenient to take cross-talk into account. One can do so by choosing an appropriate plate layout, by using an appropriate crosstalk reduction aperture or by reading the wells in a smart order by using the interlaced reading mode ( See "Advanced settings" on page 60).


For more details on the basics of luminescence and luminescence measurements, scan the following QR-code:

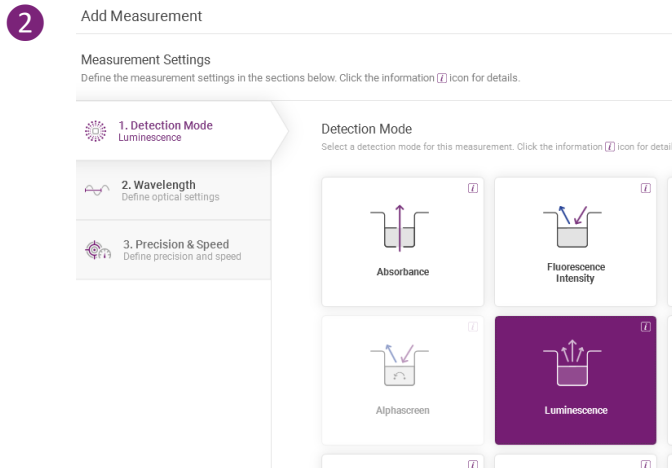


### 5.7.2 Set up a luminescence measurement protocol

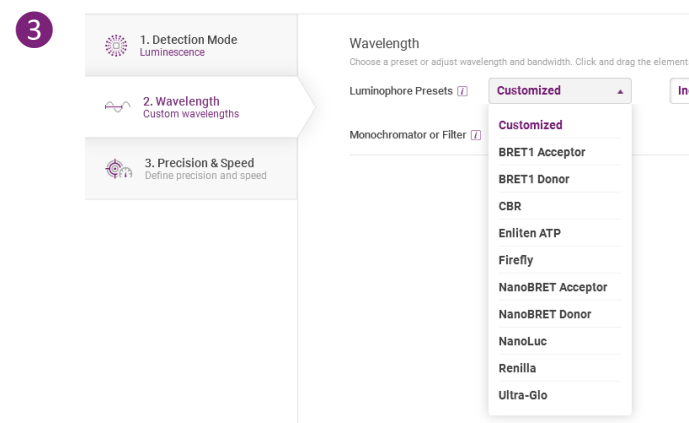
For the definition of a luminescence measurement protocol, go to the **Protocols** menu and click the **+ New Protocol** button. You can add actions to your workflow after selecting your microplate and after defining your microplate layout.



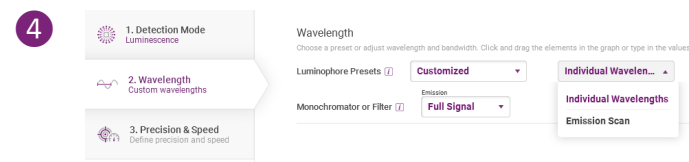
Click on the big **+** plus button (A) and select the **Measure**  action (B).



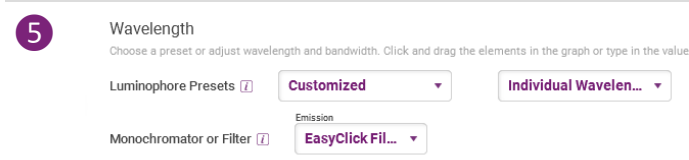
In the window choose **Luminescence** as detection mode.



In the **Wavelength** window, you can select your **Luminophore** from the Luminophore Presets list or add a **Customized** luminophore and create **Individual Wavelength** settings

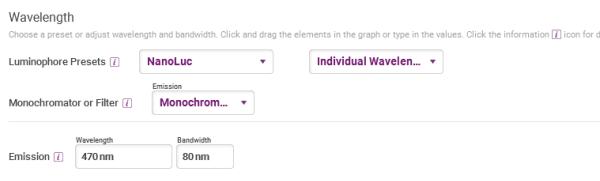


You can choose between the measurement at **Individual Wavelengths** or **Emission Scan**.

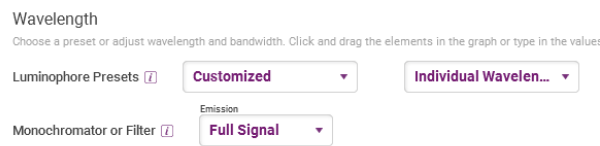
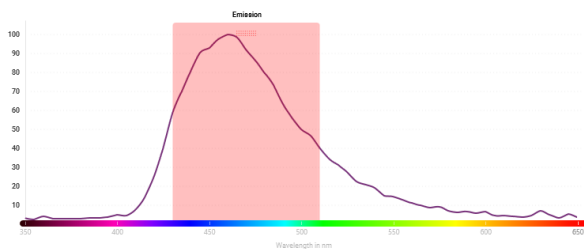


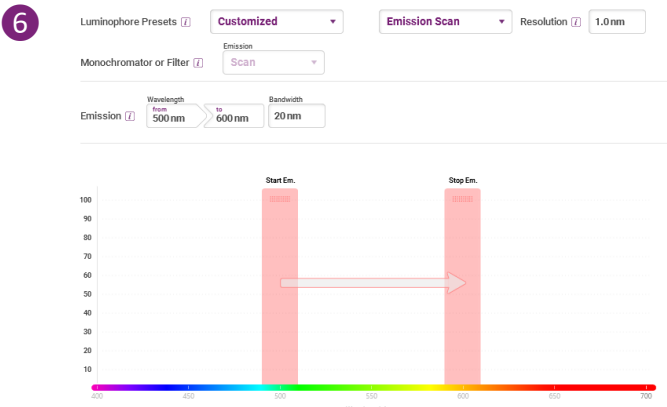
When measuring at an Individual Wavelength, you can either use the EasyClick Filters, Monochromator, or Full Signal, depending on your reader options.

Easy Click filters, installed in the reader, can be selected from the drop down list.

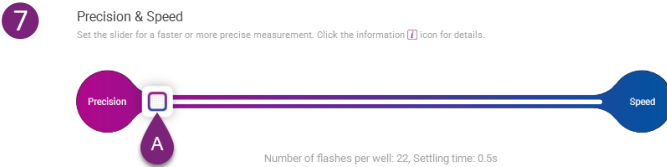


Monochromator settings can be chosen freely in the range of 320-740 nm with bandwidths of 8-100 nm. You can change the LVF monochromator settings by dragging the elements in the graph or typing a value directly in the respective fields. With Full Signal the microplate reader will measure all emitted light of a sample independent of wavelength.

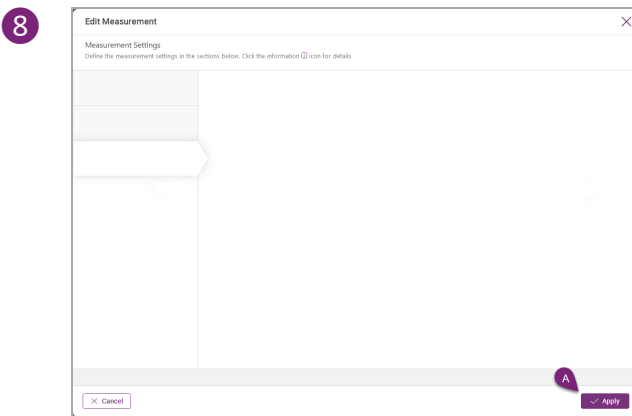




When scanning the emission with the monochromator, you can change the emission scan range by dragging the elements in the graph or typing a value directly in the respective fields.



Click on **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.



Click **Apply** (A) to save the absorbance measurement settings and to go back to the workflow from which you can either Save & Execute the protocol directly or Save & Close for later use.

## 5.8 Create a bioluminescence resonance energy transfer (BRET) protocol

### 5.8.1 BRET read mode

Resonance energy transfer describes the transfer of energy from an electronically excited donor molecule to an acceptor fluorophore. The process excites the acceptor fluorophore which in turn emits photons. When the donor energy is generated by bioluminescence, the process is called bioluminescence resonance energy transfer (BRET). For the detection of BRET (emission of the donor and acceptor), no external excitation event is needed. Various conditions must be met to allow the transfer: the emission spectrum of the donor molecule needs to overlap with the excitation spectrum of the acceptor fluorophore. Additionally, donor and acceptor need to be in proximity (typically 1-10 nm), as the transfer decreases with distance. Consequently, BRET is commonly used to measure the interaction of two biomolecules. The output of BRET is the intensity of the acceptor fluorophore related to the intensity of the donor, which is known as the BRET ratio.

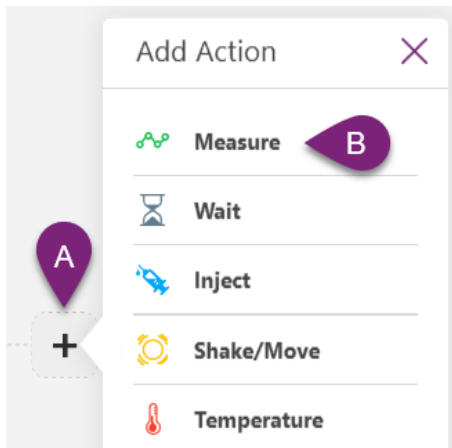
For more details on the basics of BRET and BRET measurements, scan the following QR-code:




### 5.8.2 Set up a BRET measurement protocol

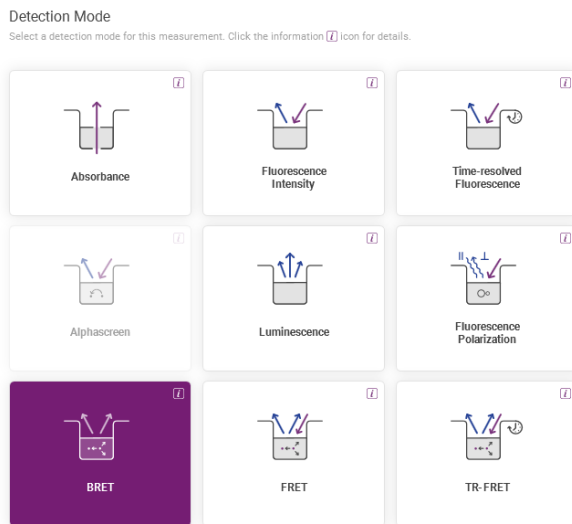
For the definition of a BRET measurement protocol, go to the **Protocols** tab in the Voyager software and click the **New Protocol** icon. After selecting your microplate and defining your microplate layout you can add actions to the workflow.

1



Click on the big **+** button (A) and select the **Measure**  action (B).


2



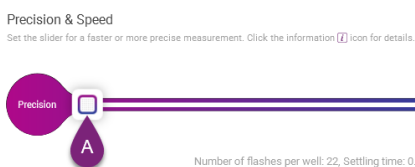
In the following window choose **BRET** as detection mode.


3



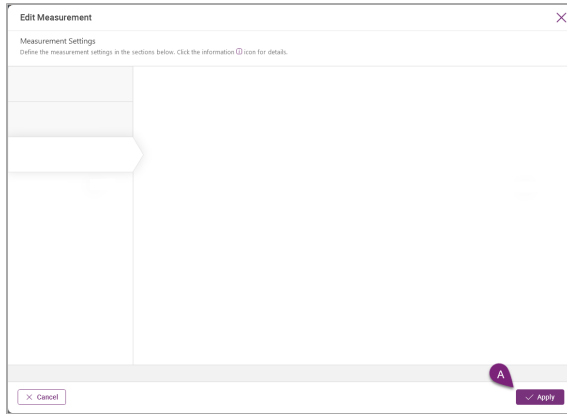
Go to **Wavelength** . Either choose a Luminophore Preset or use **Customized** settings. Presets are wavelengths settings for commonly used luminophores. You can either start with those settings and adapt them accordingly or you can start from scratch with Customized settings. In any case select whether to measure **Over Well** or **Over Plate**. Either select **Monochromator** or **EasyClick** filters for the **Dichroic** mirror and for the donor and acceptor emission (i.e. Emission 1 and Emission 2, respectively). Set the exact wavelengths for Emission 1 and Emission 2 including the corresponding bandwidth. You can drag the bars in the spectrum chart.

4



Click on  **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.

5



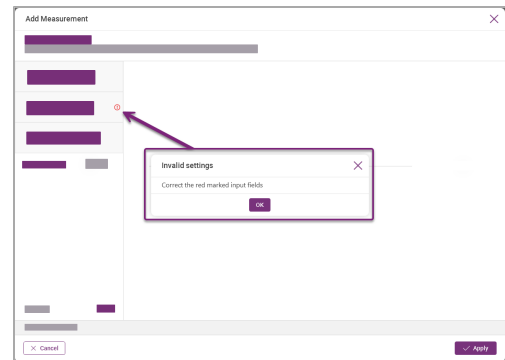
Click **Apply** (A) to save the BRET measurement settings and to go back to the workflow from which you can either **Save & Execute** the protocol directly or **Save & Close** for later use.

## 5.9 Advanced settings

### 5.9.1 Advanced speed & precision settings

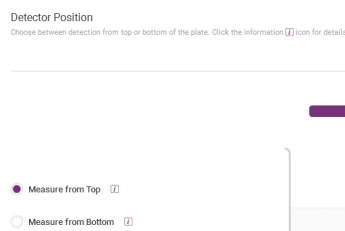
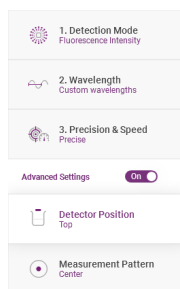
Adding a measurement action to a workflow requires to set all mandatory parameters (Detection Mode, Wavelength etc.). Most parameters have defaults but others need to be set by the user explicitly. E.g. if filters are not set in an AlphaScreen protocol, a window as shown in (Figure 5.3) will appear. When clicking **Apply** while there are still mandatory parameters not set, Voyager will point towards these missing settings indicated by an exclamation mark.

**Figure 5.3:** Missing Measurement Settings are indicated by an exclamation mark.



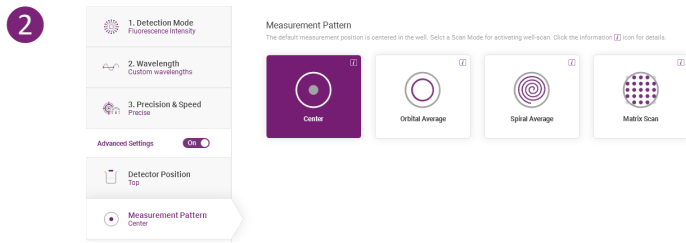
Beside basic setting, there are advanced settings whose default values are set to work for most use-cases. Nevertheless, these can be adjusted if needed. These include the settings listed below.

1



### Detector Position

determines if measurement is performed from the top or bottom of the well. Measurement from the bottom should be considered for lidded or sealed plates or in prolonged kinetic assays where condensation cannot be avoided.



**Measurement Pattern** is used for wells with non-homogenous signal. Instead of measuring in the well center, the Xenon lamp flashes will be distributed over well surface. Several patterns can be selected:



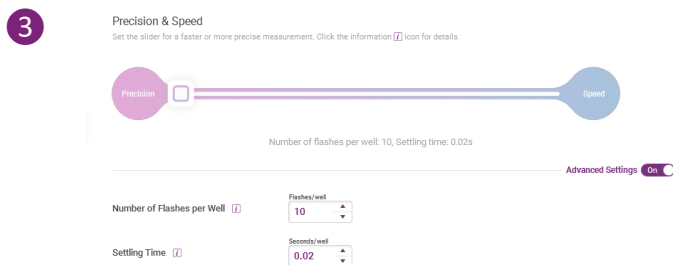
**Orbital Averaging** – flashes will be distributed in a circular pattern with selectable diameter. The result is an arithmetic average of all single flashes.



**Spiral Averaging** – flashes will be distributed in a spiral-shaped pattern with selectable diameter. The result is an arithmetic average of all single flashes.



**Matrix Scan** – measurement will be performed in a matrix pattern with selectable diameter and number of dots. Each dot is an independent measurement where defined number of flashes and EDR parameters will be applied. Output is a scan matrix over the well.



**Number of Flashes per Well** – defines the number of flashes which the Xenon lamp will perform for each well during the measurement. The higher the number the better is the stability of the data output. An increased number of flashes has an impact on the speed of the measurement!

**Settling Time** – defines the period for which the reader will wait between plate movement and start of the lamp action and detection. A prolonged settling time has a positive impact on the data stability but impairs the speed of the measurement.

4

1. Detection Mode  
Absorbance

2. Wavelength  
Custom wavelengths

3. Precision & Speed  
Precise

Advanced Settings On

Measurement Pattern  
Center

Pathlength Correction  
On

Pathlength correction  
Activate pathlength correction if your experiment

---

Activate Pathlength Correction On

Waterpeak-based i  
 Volume-based i

**Path length correction** can be used with absorbance protocols to normalize the OD values to 1 cm path length in order to make microplate measurements comparable to cuvette measurements. You can choose between 2 methods for the path length correction:

**Waterpeak- based:** Normalizes the path length by measuring the specific absorption of water near 970 nm. This method is recommended for most applications except those samples that are not mainly aqueous or samples that are highly scattering like bacterial or yeast cells.

**Volume- based:** The volume-based path length correction estimates the path length based on the geometry of the well and the volume of the sample inside the well. This method can be applied in cases where the waterpeak-based shall not be applied. E.g. the volume-based method can be appropriate when the sample solution contains a non-negligible amount of organics (e.g. above 20 %) or the sample is highly scattering or is absorbing near 970 nm. The volume of the sample needs to be entered and the corresponding path length will be calculated automatically by the software.

5

1. Detection Mode  
Luminescence

2. Wavelength  
Custom wavelengths

3. Precision & Speed  
Precise

Advanced Settings On

Detector Position  
Top

Measurement Pattern  
Center

Time Referencing  
1 seconds

Time Referencing  
Use this function for referencing of results to information i icon for details.

---

Activate Time Referencing On

Seconds/well

**Time Referencing (Luminescence protocols):** Normalizes each RLU value towards 1 second integration time. This is useful to make luminescence measurements comparable that were recorded at different integration times. E.g. a luminescence measurement performed with 0.02 integration time can be normalized to 1 second per well by simply multiplying the RLUs by a factor of 50. Thus it is comparable to measurements normalized or actually performed at 1 second integration time.

6

1. Detection Mode  
AlphaScreen

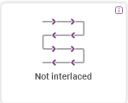
2. Wavelength  
Custom wavelengths

3. Precision & Speed  
Precise

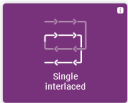
Advanced Settings On

Interlacing  
Single

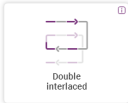
Interlacing  
Choose between no interlacing or single or double interlacing. Click the information icon for details.



Not interlaced



Single interlaced



Double interlaced

**Interlacing** is an advanced setting for AlphaScreen measurements to determine the order in which the microplate wells are read. If set to **Not interlaced**, wells will be read in a meandering pattern starting at well A1 (upper left corner). The **Single interlaced** mode is the default one where wells are read in some sort of nested meandering pattern in which every second row (or

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cross talk effects is the **Double interlaced** mode. Herein, the rows and columns are interlaced.

---

## 5.10 Create an AlphaScreen protocol

### 5.10.1 AlphaScreen read mode

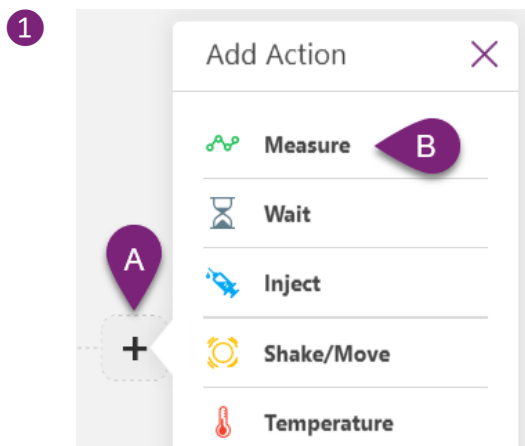
AlphaScreen® is a luminescent-based assay used to study biomolecular interactions. It applies a donor and an acceptor bead carrying specific chemical groups. The chemical groups of the donor release singlet oxygen upon excitation at 680 nm. Singlet oxygen is an excited form of the oxygen molecule (half-life in the lower microsecond range) that can transfer its energy to the acceptor bead if it is close enough to reach the acceptor without being depleted. The chemical energy of the singlet oxygen is thus converted into radiative energy and the acceptor emits light of a specific wavelength. Overall, this is a chemiluminescent process with an oxygen-mediated energy transfer from the donor to the acceptor. The light emitted by the acceptor only occurs if it is in close proximity to the donor. Given a receptor is bound to the donor and its corresponding ligand is bound to the acceptor, the amount of emitted light from the acceptor is a measure of binding. The excitation can be done with a Xenon flash lamp or a laser with a dedicated wavelength at 680 nm. In a simple AlphaScreen assay the emission of a single acceptor is measured. In a multiplexed AlphaScreen assay two or more acceptors can be measured.

As AlphaScreen is a luminescence assay it is often convenient to take cross-talk into account. One can do so by choosing an appropriate plate layout, by using an appropriate crosstalk reduction aperture or by reading the wells in a smart order by using the interlaced reading or even double-interlaced reading mode ( See "Advanced settings" on page 60).

For more details on the basics of AlphaScreen and AlphaScreen measurements, scan the following QR-code:

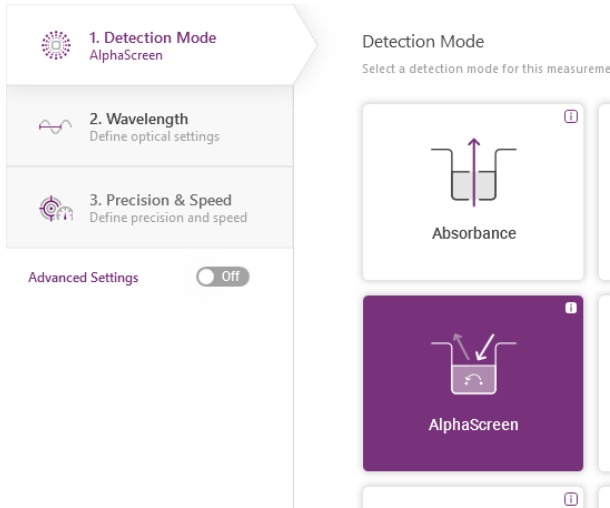


### 5.10.2 Set up an AlphaScreen protocol



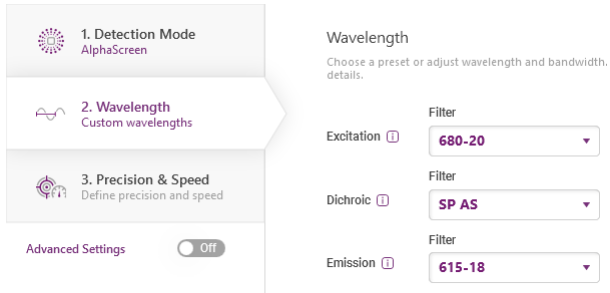
Click on the big **+** button (A) and select the **Measure** action (B).

2



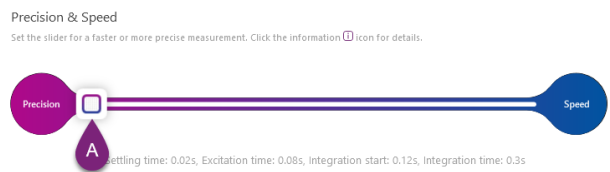
In the following window choose **AlphaScreen** as detection mode.

3



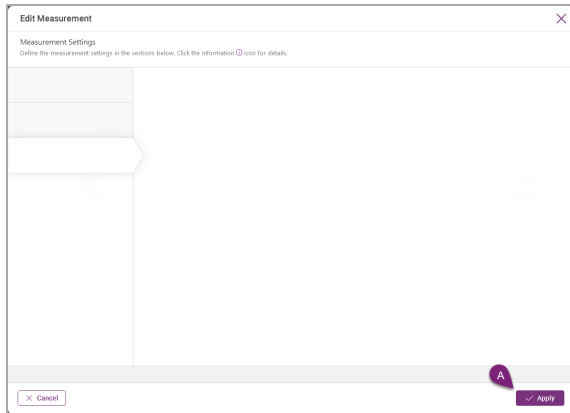
Under **Wavelength**, select **Excitation** and **Emission** filters as well as a dichroic mirror as appropriate for the dye you are planning to use for your assay.

4



Click on **Precision & Speed** to balance between precision and speed by dragging the slider (A) accordingly.

5

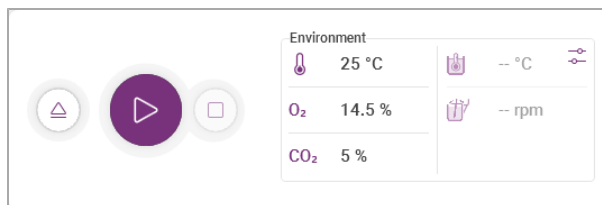



Click **Apply** (A) to save the absorbance measurement settings and to go back to the workflow from which you can either **Save & Execute** the protocol directly or **Save & Close** for later use.

## 6 Environment settings – Incubation, ACU and Heater/Stirrer

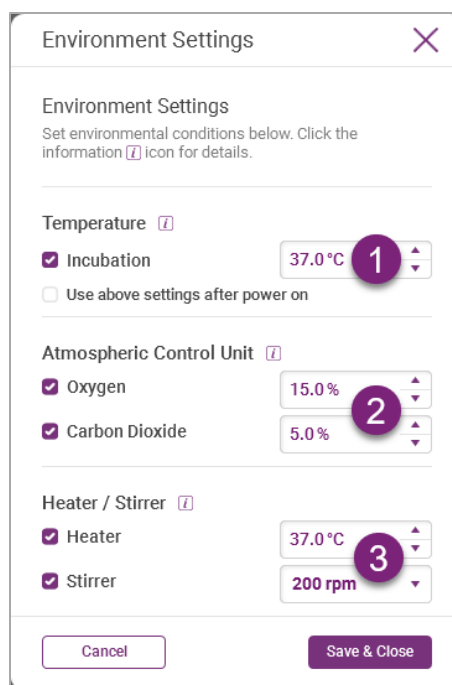
Environment settings can be edited from the player-like section at the bottom of the Voyager window.

**Figure 6.1:** The Dashboard player (left) together with the **Environmnet** display (right).






By clicking the settings symbol  you get access to the Temperature settings, to the Atmospheric Control Unit settings (optional) and to the Heater/Stirrer settings (both optional).

**Figure 6.2:** The Environment settings window.



The settings for the atmospheric control unit (ACU) are only visible if an ACU is connected. The same is true for the Heater/Stirrer settings. They are only visible if a VANTAstAr dispenser module with heater and stirrer is connected to the reader.

### 6.1 Incubation

Under **Environment Settings**, activate the **Incubation** and set the temperature in degree Celsius (1 in Figure 6.2). You can also activate to use the incubation setting for future use, after power on of the reader. The status of the temperature is indicated by a small arrow next to the thermometer icon. When the arrow is red and points upwards , the reader is currently heating to reach the target temperature. When the arrow is blue and points downwards , the inner temperature of the reader is higher than the target temperature. When the reader has reached the target temperature it will balance between heating and not heating to maintain this temperature. This is indicated by a green check mark next to the thermometer icon .

Please note that the temperature setting made here is a static one. If temperature ramps are needed, set the corresponding temperature action in your protocol (see section 4.2.5 on page 19).

## 6.2 Atmospheric Control Unit (ACU)

Activate **Oxygen** and set the oxygen concentration in the incubation chamber in percent ( **2** ). You can additionally activate **Carbon Dioxide** and set its concentration in the incubation chamber.

For more details on the ACU, refer to its operating manual.

## 6.3 Heater and Stirrer

The VANTAstar can (optionally) be equipped with a reagent dispenser module which itself can (optionally) have a Heater and Stirrer included. Activate the Heater to set its temperature in degrees Celsius ( **3** ). Activate the Stirrer and select the stir frequency from the drop-down in rotations per minute (rpm).

For more details on the VANTAstar's reagent dispenser module, refer to the dedicated operating manual.

## 7 LVIs Plate Measurements

BMG LABTECH's Low-volume (LVIs) microplate is used to perform low volume absorbance measurements on samples like DNA, RNA, Protein etc.

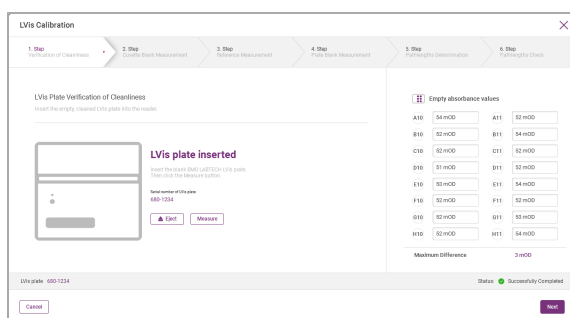
The LVIs Plate is selectable in the microplate section of the protocol (See "Choose microplate and microplate format" on page 17). Upon purchase of an LVIs Plate, The LVIs Plate installer software installs the corresponding LVIs plate path lengths values of the 16 measurement micro drop sites which were determined by the manufacturer. Calibrating the LVIs Plate can also, however, be done using the **LVIs Calibration** wizard. It is recommended to perform the calibration on a regular basis. The **LVIs Calibration** wizard can be found under the **Tools** menu in Voyager. The wizard guides the user through various measurement steps to determine the path lengths values of the micro drop sites. Under **Tools** the user can perform a cleanliness check on an empty LVIs Plate to check for purity of the 16 micro drop sites. E.g. samples not being thoroughly removed from the spots can impede further measurements and lead to flawed results. Therefore it is required to thoroughly clean the measurement spots afterwards (for details, refer to the LVIs Plate operating manual).

### 7.1 LVIs Plate Calibration

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

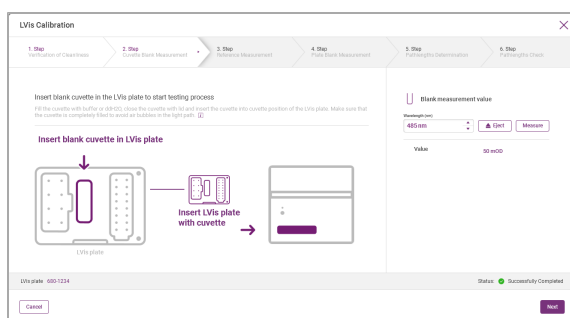
Calibrating the exact path length for every micro drop site is achieved by comparing the optical densities of two reference solutions with a known concentration ratio. Two reference solutions of Orange G are needed. Orange G can be obtained e.g. from Sigma-Aldrich ([www.sigmaaldrich.com](http://www.sigmaaldrich.com); for microscopy or electrophoresis). Follow the steps below to perform the path length calibration.

1



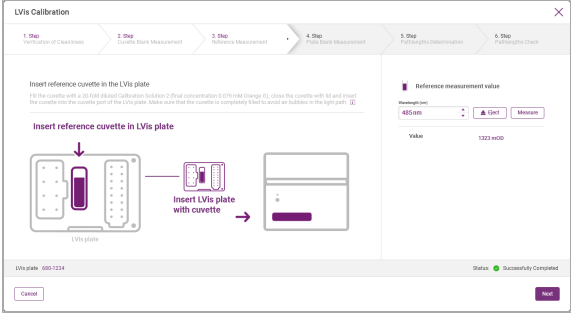
The LVIs Plate Calibration wizards starts by checking the cleanliness of the LVIs Plate. Therefore, insert an empty LVIs Plate and start the first measurement by clicking the **Measure** button. If the cleanliness check is passed, the status will display 'Successfully Completed', otherwise it will display 'Completed with Warnings'. To get more details on the failure, you can click on the exclamation mark beside the problematic micro drop sites. Warnings will be displayed if at least one measurement value is above 65 mOD or if the difference between the maximum value and the minimum value exceeds 10 mOD. If successful, click **Next** to proceed.

2



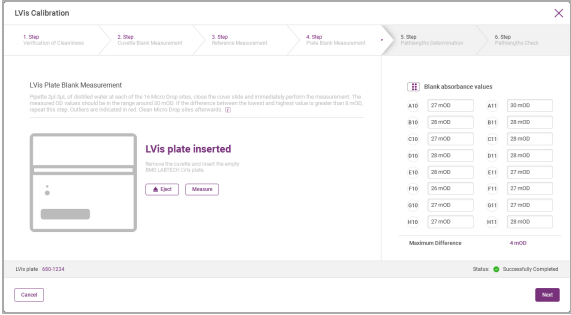
Fill a cuvette with the appropriate amount of distilled water, insert the cuvette into the BMG LABTECH LVIs Plate and perform a blank measurement by clicking **Measure**. If the blank measurement is passed, the status will display 'Successfully Completed', otherwise it will display 'Completed with Warnings'. To get more details on the failure, you can click on the exclamation mark beside the problematic micro drop sites. Warnings will be displayed if the difference between the maximum value and the minimum value exceeds 8 mOD. If successful, click **Next** to proceed.

**3**



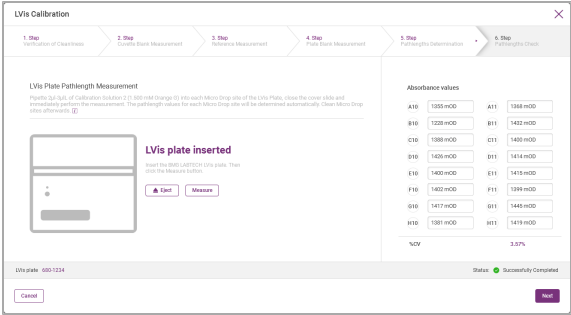
Fill a cuvette with the appropriate amount of 0.075 mM Orange G solution and perform a measurement at a wavelength of 485 nm. The status message reports if an action has been completed or not. If completed successfully, click the **Next** button. Note that the result should be approximately in the range of 1.1 to 1.9 OD.

**4**



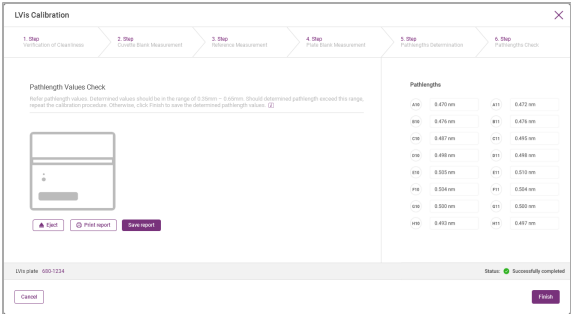
Pipette 3  $\mu$ L of distilled water at each of the 16 micro drop sites using a single or multi-channel pipette, close the lid of the LVIS Plate and immediately perform the measurement. The measured OD values should be in the range around 0.025 OD. If the difference between the lowest and highest value is greater than 8 mOD, a warning message appears and you should repeat this step. Outliers are indicated in red. Click **Next** to proceed.

**5**



Remove the LVIS Plate, and repeat the cleaning procedure as described in the LVIS Plate Operating Manual. Then pipette 2 - 3  $\mu$ L of 1.5 mM 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 below 5%. If the %CV is higher, repeat this step. If successful, click **Next** to proceed.

**6**



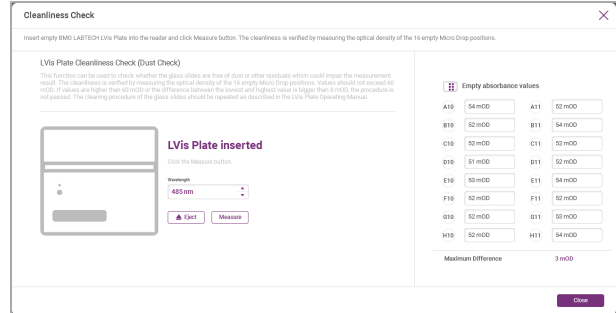
The individual pathlengths of each micro drop site of the LVIS Plate are calculated automatically. Examine the path length values (denoted in mm). If one of the pathlength values is outside the typical range around 0.5 mm, a warning appears. A report of the calibration procedure can be printed or exported as \*.txt or Excel file for your records.

**Figure 7.1:** Steps to perform the LVIS Plate path length calibration.

## 7.2 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 0.065 OD. If a value is greater than 0.065 OD or the difference between the lowest and highest value is greater than 10 mOD a warning message occurs, and the cleaning procedure of the glass slides should be repeated as described in the LVis Plate Operating Manual.

**Figure 7.2:** The LVis cleanliness check can be used to check for impurities on the LVis plate glass slides.



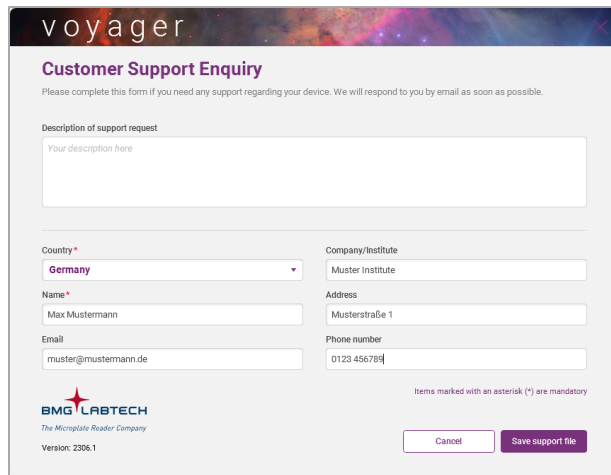
## 8 Tools

The **Tools** menu contains multiple sub-menus including the **LVis Calibration** wizard, the LVis **Cleanliness Check**, the **Support Tool** and the **About** window.

### 8.1 Support Tool

The Support Tool can be used to gather information (error-logs, reader information, software and firmware versions etc.) that can be sent to BMG LABTECH's support in case of a problem. Under the **Tools** menu click on **Support Tool** to open a window where you can fill the customer support enquiry. Add a comprehensive description to help our support team to reproduce the issue you have. Add your Name, company/institute name and address and your Email. You can optionally add a phone number. Click on **Save support file** and select a destination folder on your local drive to save a zip-file that contains files and information for the support team. Once saved, send this zip-file by e-mail to BMG LABTECH's support team ([support@bmglabtech.com](mailto:support@bmglabtech.com)).

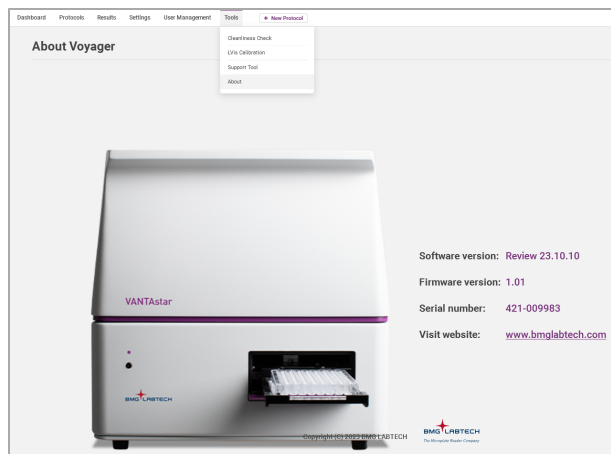
**Figure 8.1:** The **Support Tool** window in Voyager to gather information for support enquiries.



### 8.2 About window

The **About** window can be accessed through the **Tools** menu and displays useful information about software version, firmware version and serial number of the reader in case you need to contact BMG LABTECH's support. You can also find a link to BMG LABTECH's website that contains a lot of useful information including application notes, how-to notes and much more.

**Figure 8.2:** The **About** window in Voyager contains useful information.



## Glossary

### Aperture

An aperture is an accessory with a hole in it that is placed above the microplate. Through the hole, the signal of the well of interest reaches the detector, while all light coming from its surrounding is physically blocked which reduces cross-talk from adjacent wells in luminescence detection.

### Autofocus

The Autofocus is a function in the “start measurement” window which first scans the whole microplate for the well with the highest signal intensity. This well is then used for focal adjustment, selecting the Z-plane with the highest signal available in the well. This focal height is then used to measure all wells selected in the layout.

### Cross-talk

Cross-talk arises when a signal produced by a certain well interferes with signals of its adjacent wells, resulting in artificially increased signals and reduced signal-to-blank values.

### EasyClick filters

An optical filter selectively transmits light of a wavelength with a defined bandwidth while blocking light of other wavelengths. Dichroic filters transmit light below a specific wavelength while blocking light above this wavelength. EasyClick filters are available for the VANTastar and the CLARIOstar. Thanks to their magnetic component, they can be easily installed or changed by simply clicking them in.

### Enhanced dynamic range

The enhanced dynamic range (EDR) function available on the VANTastar and CLARIOstar readers can be used for a continuous and automatic adjustment of the gain setting throughout the measurement. Gain adjustment is performed for each well individually. This ensures that each well is read with optimal gain to allow the maximum dynamic range and highest sensitivity.

### Excitation/Emission Scan

An Excitation Scan measures the fluorescence emission at a single, fixed wavelength across a spectrum of excitation wavelengths. An Emission Scan measures the fluorescence emission across a spectrum of wavelengths using a single, fixed excitation wavelength.

### Focal adjustment

With manual focal adjustment, a well is scanned for maximum signal intensity along the Z-axis. The Z-

plane with the highest signal intensity is selected as focal height for the upcoming measurement.

### Focal height

The focal height is the Z-position in the well on which the light beam is focused. The focal height setting regulates the distance between the detection system and the microplate. With the optimal distance, the detection is focused on the optimal plane of a sample which means the plane at which the signal intensity is highest.

### Gain

Gain is a factor that defines the voltage of the photomultiplier tube (PMT) detectors which are responsible for recording the incoming light signal of a sample. Depending on the gain the incoming signal is more or less amplified. Low intensity samples need a higher amplification and vice versa.

### LVF monochromator

A monochromator is an optical device that selects a wavelength of light or a range thereof. In an LVF monochromator the light passes through a linear variable short pass and long-pass filter, which by sliding against each other, create the rising and falling edge of the filter. In this way, the LVF monochromator can separate the light into distinct wavelengths as well as into continuously adjustable bandwidths. In contrast to conventional grating-based monochromators, the LVF monochromator shows filter-like sensitivity.

### Optical Density (OD)

Optical density is the fraction of incoming light attenuated by a sample either by absorption of molecules or by scattering (e.g. of turbid samples).

### Over Well/Over Plate

'Over Well' and 'Over Plate' options are available for dual-emission measurements like FRET. Select 'Over Well' if emission 1 and 2 should switch in each well. Select 'Over Plate' if whole layout should first be measured with emission 1 and then with emission 2. The switch 'Over Well' is advantageous for assays with fast reactions. The switch 'Over Plate' contributes to faster reading time for the whole plate.

### Photomultiplier Tube (PMT)

A Photomultiplier tube consists of a vacuum tube and functions as an extremely sensitive detector of light. Incoming photons produce a current that is amplified in a multitude of dynode stages.

### Plate ID

A Plate ID is measurement-related information that can be assigned by the user to label and distinguish different measurements. The “Start Measurements”

window offers to assign up to three plate IDs per measurement.

**Protocol**

A protocol is the entire combination of events, settings, layouts and loops.

**Target mP**

The target mP describes the initial polarization value expected in a FP assay. Usually, this value corresponds with the polarization of free fluorophore or unbound, labeled ligand.

**Target value**

Target value describes the signal intensity that should be used for gain adjustment. For assays with stable signals to be expected, a target value of 90 % is recommended. If the signal is expected to increase during the measurement (e. g. by 10-fold), define a lower percentage to allow the signal to increase within the dynamic range (e. g. <10%).

**Workflow**

A workflow consists of a sequence of action elements selectable from the protocol editor.

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