TECAN.

Instructions for Use

SparkControl



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WARNING: Carefully read and follow the instructions provided in this document before operating instrument and software.

Notice

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It is the policy of Tecan Austria GmbH to improve products as new techniques and components become available. Tecan Austria GmbH therefore reserves the right to change specifications at any time with appropriate validation, verification, and approvals.

We would appreciate any comments on this publication.

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Area of Application – Intended Use

See chapter 1.2 Intended Use (Hardware and Software).

About the Instructions for Use

Original Instructions. This document describes the SparkControl software for the SPARK multifunctional microplate reader. It is intended as reference and instructions for use. This document describes how to install and use the SparkControl software, including different Apps.

Remarks on Screenshots

The version number displayed in screenshots may not always be the one of the currently released version. Screenshots are replaced only if content related to the application has changed.

Remarks on Analysis and Processing Plugins

The use and functionality of Analysis and Processing Plugins as part of the SparkControl software are described in additional instructions to this document.

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- Spark-Stack™
- NanoQuant Plate™
- ResultAnalyzer™
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Warnings, Cautions, and Notes

The following types of notices are used in this publication to highlight important information or to warn the user of a potentially dangerous situation:



NOTE: Gives helpful information.



CAUTION: Indicates a possibility of instrument damage or data loss if instructions are not followed.



WARNING: Indicates the possibility of severe personal injury, loss of life or equipment damage if the instructions are not followed.

Symbols

	CE conformity marking
	United Kingdom Conformity Assessed marking shows that the labeled product is following the applicable regulation in Great Britain.
	Date of manufacture
	Manufacturer
	Catalogue number
	Consult Instructions for Use
	China RoHS symbol
	Serial number
	USB symbol
	Use by date

Table of Contents

1 General Description 9

1.1 Instrument 9
1.2 Intended Use (Hardware and Software) 9
1.3 User Profile 10
1.3.1 Professional User – Administrator Level 10
1.3.2 End User or Routine User 10
1.3.3 Service Technician 10

2 Operation of SPARK with SparkControl Software 11

2.1 Area of Application 11
2.2 System Requirements 11
2.3 Software Installation 13
2.3.1 Uninstall/Repair Installation 13
2.4 Licensing SparkControl's Plugin 14
2.4.1 Licensing Client Module 14
2.4.2 Online Request 15
2.4.3 Offline Request 17
2.5 Starting the SparkControl 20
2.5.1 Connecting Instruments 20
2.6 Method Editor 21
2.6.1 Structure 21
2.6.2 Method Definition Tabs 22
2.6.3 Control Bar 22
2.6.4 Workflow Pane 22
2.6.5 Menu Bar 22
2.6.6 Toolbar 24
2.6.7 Instrument 24
2.6.8 Select Component 24
2.6.9 Select App 24
2.7 Control Bar 25
2.7.1 Plate 26
2.7.2 Detection 30
2.7.3 Action 31
2.7.4 Kinetic 34
2.8 Workflow Pane 35
2.8.1 Hierarchy of Strips 35
2.8.2 Info Pane 37
2.8.3 Measurement Types 37
2.8.4 Multiple Reads per Well 40
2.9 Dashboard 42
2.9.1 Structure 42
2.9.2 Software Navigation 44
2.9.3 The Dashboard 45
2.9.4 Instrument Control 46
2.9.5 Starting a Method 47
2.9.6 Measurement Progress 50
2.9.7 Data 51
2.9.8 Scans 53
2.9.9 Images 54
2.9.10 Batch Processing 55
2.9.11 Analysis 56
2.9.12 Curves 56
2.10 Starting a Method 57
2.10.1 Method Editor 57

2.10.2 Dashboard 57
2.10.3 Onboard-Start 57
2.11 Measurement Results 57
2.12 Method File Explorer 57
2.13 SparkControl Settings 58
2.13.1 Structure 58
2.13.2 Instrument 59
2.13.3 Data Handling 60
2.13.4 Software 62
2.13.5 Plate Geometry Editor 63
2.13.6 Images 64
2.13.7 Directory 64
2.14 Screencasts 65
3 ResultAnalyzer 67
3.1 Workspaces 67
3.2 Evaluate Results 69
3.2.1 Changing the Analysis Method 69
3.2.2 Mask Value 70
3.3 Export Results 71
3.3.1 Export Analysis 71
4 Luminescence 73
4.1 Defining Luminescence Measurements 73
4.1.1 Luminescence Strip 73
4.1.2 Multicolor Luminescence Strip 75
4.1.3 Luminescence Scan Strip 76
4.1.4 Corrected Spectra 76
4.1.5 Measurement Mode 77
4.2 Optimizing Luminescence Measurements 77
4.2.1 Integration Time 77
4.2.2 Light Attenuation 77
5 Alpha Technology 79
5.1 Defining Alpha Measurements 79
5.1.1 Alpha Technology 79
5.1.2 Measurement Mode 80
5.2 Optimizing Alpha Technology based Measurements 80
5.2.1 Integration Time 80
5.2.2 Excitation Time 80
5.2.3 Dark Covers for Light Protection 80
6 Absorbance 81
6.1 Defining Absorbance Measurements 81
6.1.1 Absorbance Strip 81
6.1.2 Absorbance Scan Strip 83
6.2 Absorbance Measurement Modes 84
6.2.1 Optimizing Absorbance Measurements 84
7 Fluorescence 85
7.1 Defining Fluorescence Measurements 85
7.1.1 Fluorescence Intensity Strip 85
7.1.2 Time-Resolved Fluorescence Intensity Strip 88
7.1.3 Fluorescence Intensity Scan Strip 90
7.1.4 Measurement Mode 91
7.2 Fluorescence Polarization Module 91
7.2.1 Fluorescence Polarization Optics 92
7.2.2 Fluorescence Polarization Detection 92

7.2.3 Defining Fluorescence Polarization Measurements 93
7.2.4 Calculation of Results 95
7.3 Optimizing Fluorescence and Fluorescence Polarization Measurements 96
7.4 Inject and Read 99
7.4.1 Defining **Inject and Read** Measurements 100
7.4.2 Measurement Mode 102
7.4.3 Optimizing **Inject and Read** Measurements 102
8 Cell Module 103
8.1 Defining Cell Counting & Confluence Measurements 103
8.1.1 Cell Counting Strip 103
8.1.2 Cell Confluence Strip 104
8.1.3 Measurement Mode 105
8.2 Optimizing Cell Confluence Measurements 106
8.2.1 Use Well Border Detection 106
8.2.2 Live Viewer 106
9 Fluorescence Imaging (Cell Imager) 109
9.1 Defining Bright field and Fluorescence Imaging Measurements 109
9.1.1 Imaging Strips 109
9.1.2 Analysis Results 112
9.1.3 Measurement Progress View 113
9.1.4 Image Types 113
9.1.5 Measurement Mode 113
9.2 Optimizing Fluorescence Imaging Measurements 115
9.2.1 Live Viewer 115
9.2.2 ResultAnalyzer 121
10 Spark-Stack Microplate Stacker Module 125
10.1 Software 126
10.1.1 Start Stacker Run 127
10.1.2 Stacker Kinetics 128
10.1.3 Restacking 129
11 Injectors 131
11.1 Performing Measurements with Injectors 131
11.1.1 Injector Strip 131
11.1.2 Injector Strip in Measurement Flow 131
11.1.3 Z-Drive Injector Port 132
11.1.4 Optimizing Measurements with Injection 132
12 Environmental Control 133
12.1 Heating Module 133
12.1.1 Manual Temperature Control 133
12.1.2 Temperature Control via Method 134
12.2 Cooling System 135
12.2.1 Cooling Control Software Settings 135
12.3 Gas Control 136
12.3.1 Gas Control Software Settings 136
12.3.2 Manual Gas Control 136
12.3.3 Gas Control via Method 137
12.3.4 Acoustic Alarm 138
12.4 Humidity Control 139
12.4.1 Software Settings 139
13 NanoQuant App 141
13.1 Nucleic Acid Quantitation App 141
13.1.1 Applying Samples 142
13.1.2 Starting the Nucleic Acid Quantitation App 142

13.1.3 Validation Criteria for Blanking Results 145
13.1.4 Repeat Blanking 145
13.1.5 Start Measurement 145
13.2 Labeling Efficiency App 148
13.3 Protein Quantitation App 149
13.3.1 Starting the Protein Quantitation App 149
13.3.2 Validation Criteria for Blanking Results 150
13.3.3 Repeat Blanking 151
13.3.4 Start Measurement 151
13.4 Calculations – Detection of Nucleic Acids 153
13.4.1 Calculation of Nucleic Acid Concentration 153
13.4.2 Blanks 153
13.4.3 Samples 154
13.4.4 Labeled Samples 154
13.5 Calculations – Detection of Proteins 155
13.5.1 Calculation of Protein Concentration 155
13.5.2 Blanks 155
13.5.3 Samples 155
13.6 NanoQuant Maintenance 156
13.6.1 Cleaning Procedure with Ultrasonic Bath 156
13.6.2 Cleaning Procedure with Kimwipe 156
14 Cell Counting in Cell Chips 157
14.1 Sample Preparation 157
14.2 Cell Viability 159
14.2.1 Starting the Cell Viability App 159
14.2.2 Sample Selection 159
14.2.3 Measurement Results 161
14.2.4 Cell Histogram 164
14.2.5 Duplicates 164
14.3 Cell Counting App 164
15 Cuvette App 165
15.1 Prepare Instrument and Blanking 165
15.2 Measurement 166
16 Troubleshooting 169
16.1 SparkControl Errors and Warnings 169
16.2 Spark Services Manager 175
Index 177
Tecan Customer Support 179

1 General Description

1.1 Instrument

The SPARK is a multifunctional microplate reader platform and is robotic compatible.

1.2 Intended Use (Hardware and Software)

The SPARK microplate multimode reader with a modular design is intended for use in research laboratories. Depending on the configuration, the instrument is intended for the measurement and data analysis of absorbance, fluorescence, time resolved fluorescence, fluorescence polarization and luminescence of biological and non-biological samples, as well as for the acquisition and analysis of bright field and fluorescence images.

Additionally, the reader is suited for both endpoint and kinetic measurements with either single or multi-labels. The SPARK is equipped with SparkControl software for reader control and data reduction.

Users must evaluate this instrument and any associated data reduction packages with their specific assays to ensure specified performance characteristics of the assay are met. The performance characteristics of the reader have not been validated for specific assays.

The SPARK multimode reader is for research use only.



CAUTION: A system validation by the operating authority is required. It is the responsibility of any operating authority to ensure that the SPARK has been validated for every specific assay used on the instrument.

1.3 User Profile

1.3.1 Professional User – Administrator Level

The administrator is a person who has suitable technical training and corresponding skills and experiences. If the product is used as intended, the person is able to recognize and avoid dangers.

The administrator has extensive skills and is able to instruct the end user or the routine user in assay protocols in connection with a Tecan product within the bounds of the intended use.

Computer application skills and good English skills are required.

1.3.2 End User or Routine User

The end user or routine user is a person who has suitable technical training and corresponding skills and experiences. If the product is used as intended, the person is able to recognize and avoid dangers.

Computer application skills and good language skills in the respective national language at the installation site and English are required.

1.3.3 Service Technician

The service technician is a person who has suitable technical training and corresponding skills and experiences. If the product needs to be serviced or maintained, the person is able to recognize and avoid dangers.

Computer application skills and good English skills are required.



NOTE: Training dates, their duration and frequency are available at your customer support.

Address and phone number can be found on the web:
http://www.tecan.com/customersupport

2 Operation of SPARK with SparkControl Software

2.1 Area of Application

The SparkControl software is an easy-to-use and flexible tool, which gives the user control over Tecan SPARK multimode reader.

NOTE: Depending on the instrument connected and the modules installed, certain SparkControl features may be disabled or not visible.

2.2 System Requirements

NOTE: A SPARK instrument with Cell Imager module is always delivered with a dedicated stand-alone computer, which meets the required memory- and video-card demands. The operating system language of this PC is set to English.

NOTE: The SparkControl software does not support 32-bit versions of compatible Windows operating systems.

CAUTION: If the operating PC has an internet access, it is the user's responsibility to take the necessary precautions to protect the system from cybersecurity threats.

To prevent the system from being used/modified by unauthorized users, Tecan recommends using the Windows user management system. When installing virus protection software or security-related updates of the operating system please follow the recommendations of the local IT department.

The following hardware requirements and operating system requirements have to be met to use the SparkControl software:

	Supported	**Recommended**
PC	Windows compatible PC with a Pentium compatible processor running at 2 GHz (Dual Core)	2.4 GHz (Quad Core)
	Cell Imager module: > 3 GHz (8 Core) 2 GB graphics card	
Operating System	Windows 11 (64-bit) Editions: Pro, Enterprise **Windows RT NOT supported!**	
Memory	8 GB RAM	16 GB RAM
	Cell Imager module: 64 GB RAM	
Free Hard Disk Space	6 GB For Cell counting measurements: 40 GB For Cell Confluence measurements: 500 GB is required.	10 GB For Cell counting measurements: 160 GB For Cell Confluence measurements: 1000 GB is recommended.
	Cell Imager module: 512 GB SSD (system) + 8 TB HDD (archive)	

	Supported	Recommended
Monitor	Super VGA Graphics	**Cell Imager module:** 4 K Graphics
Display scale settings	100% Developed and tested at 100% display scaling. Users should verify proper GUI display when using other settings.	100%
Resolution	1280 x 1024	1680 x 1050 1920 x 1080
Color Depth	256	
Mouse	Microsoft mouse or compatible pointing device	
Communication	USB 2.0 USB 3.0 **Cell Imager module:** USB 2.0 (Instrument) USB 3.0 (Camera)	The dedicated cable for the Cell Module must be plugged into a USB 3.0 port to ensure optimal performance, ideally on a separate host controller. **Cell Imager module:** USB 3.0 (Instrument) USB 3.0 (Camera)
Devices	DirectX 9 graphics device with WDDM 1.0 or higher driver	
.NET	Microsoft.NET Framework 4.8 The required .NET version is installed automatically alongside any existing versions.	
MATLAB	MathWorks MATLAB Runtime Version 2025a (25.1) The required MATLAB version is installed automatically with the Evaluation plugin.	
Microsoft Excel	2013, 2016, 2019, Excel 365 The export mechanism writes files according to the Office Open XML file format (.xlsx)	2019, Excel 365

2.3 Software Installation

NOTE: You must have administrative rights to install the software.

NOTE: Install the software before plugging the instrument into the computer.

NOTE: Before upgrading the SparkControl software, make sure that the instrument, the camera, and all accessories are unplugged from the computer.

CAUTION: Always finish all open kinetic runs before uninstalling or upgrading the software, otherwise open kinetic data will be lost.

The SparkControl software is installed using the following procedure:

1. Insert the installation USB stick.
2. Open the Windows Explorer and browse to folder **Software/<article number>SparkControl Vx.y** on the installation stick. Double-click **SparkControl <version>_Setup.exe** to start the installation procedure.
3. The software will be installed to C:\Program Files\Tecan. The installation destination can be changed optionally.
4. Select **Install** to start the software installation.

For the Installation of Plugins see the corresponding Analysis Plugin instructions.

2.3.1 Uninstall/Repair Installation

If for any reason the current version of the SparkControl software needs to be reinstalled, proceed as follows:

1. Insert the installation USB stick.
2. Open the Windows Explorer and browse to folder **Software** on the installation stick.
3. Double-click **SparkControl <version>_setup.exe** to start the installation procedure.
    - Select **Uninstall** to uninstall the current software version, or
    - Select **Repair** to repair the installation and restore the original program files.

2.4 Licensing SparkControl's Plugin

The licensing of plugins requiring registration is performed with the **Licensing Client Module**. The Licensing Client Module is accessible via Help/Activate License in the Menu bar of SparkControl.

CAUTION: If a plugin requiring a license has not been registered, the corresponding strip(s) will not be visible in SparkControl and ResultAnalyzer.

The licensing request can be set using one of two options:

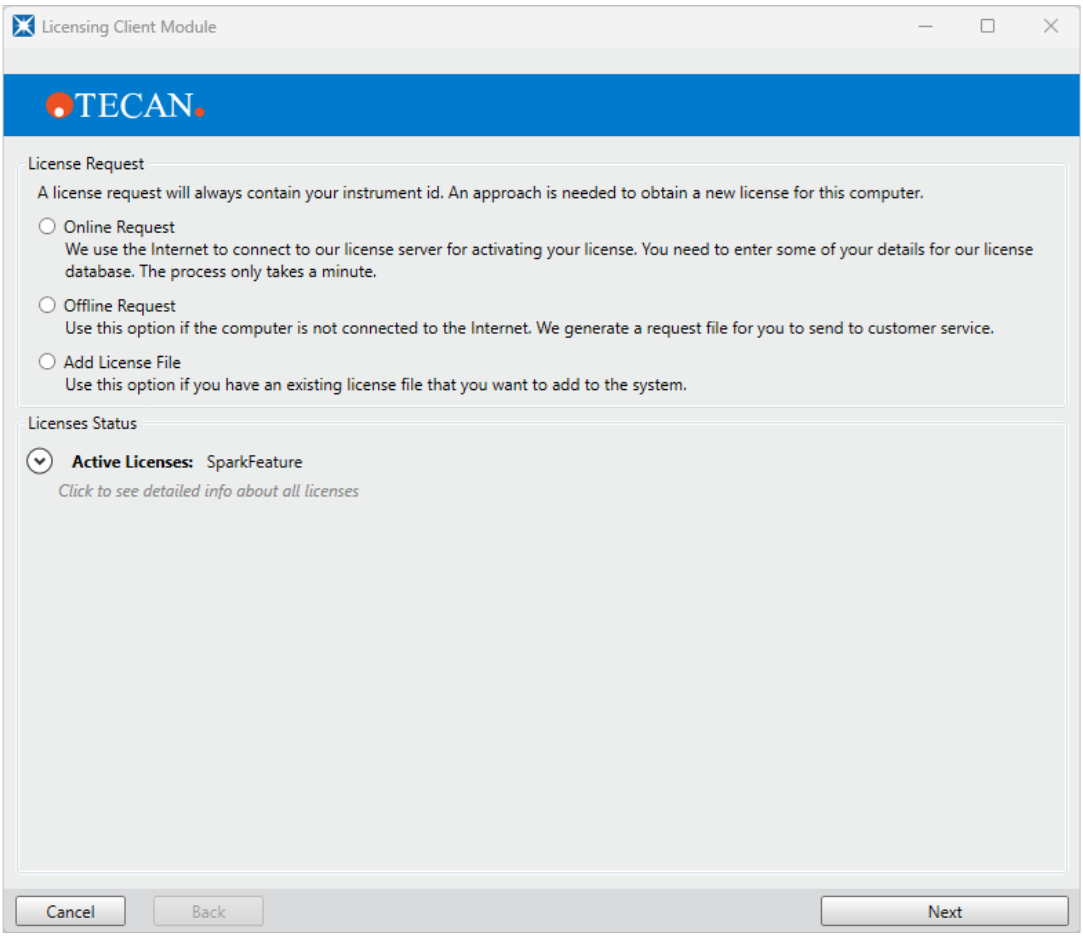
- **Online Request:** Instrument computer is online and has an internet connection
- **Offline Request:** Instrument computer is offline and has no internet connection

NOTE: It is very important that the person who registers the software also has administrator rights for the operating system on the computer.

NOTE: The license file is linked to the serial number of a specific instrument. It is possible to download the license file multiple times. When replacing the instrument computer, create a new license request and download the license file for the new computer.

2.4.1 Licensing Client Module

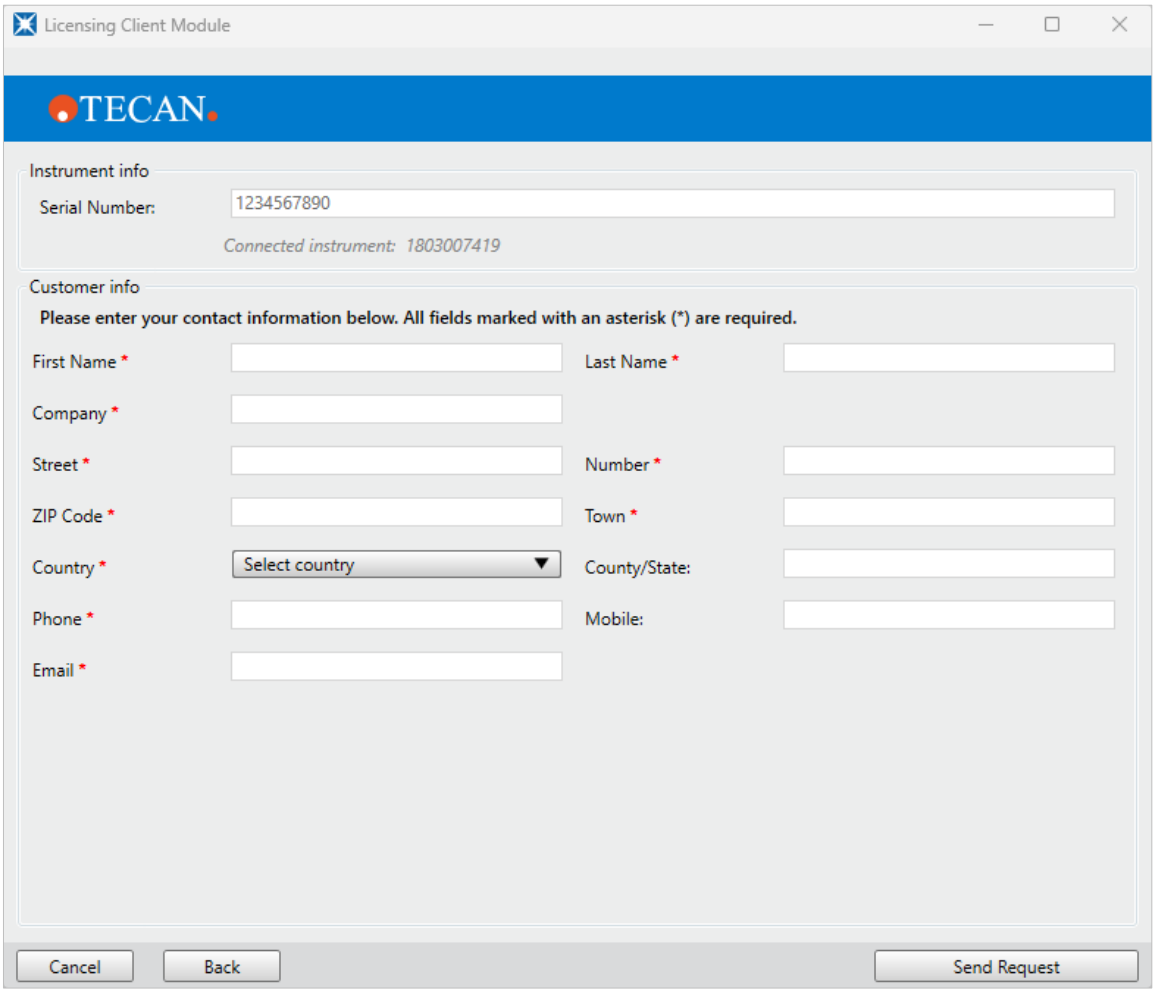
To start the Licensing Client Module, select Help/Activate License in the menu bar of SparkControl. The License Request window appears allowing for selection of the licensing type, i.e. **Online Request** or **Offline Request**. This window also includes the license status of all active licenses:



2.4.2 Online Request

Use this option, if the instrument computer is online and has an internet connection.

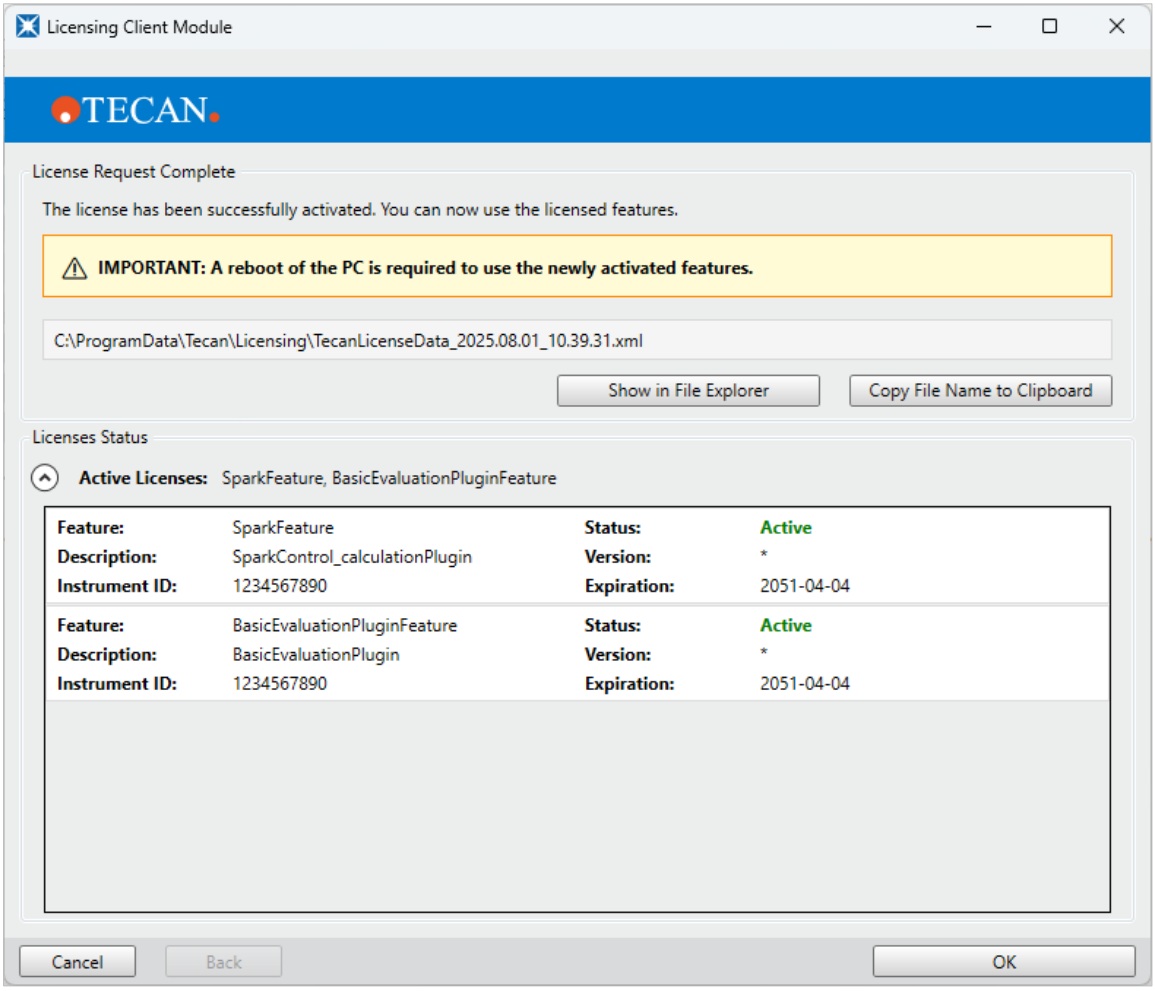
1. Select **Online Request**. Click **Next** and complete the registration form. Fields marked with an asterisk are mandatory:





CAUTION: For registration in SparkControl, the instrument must be connected to the PC. The corresponding serial number is automatically filled in.

2. After having clicked **Send Request**, the license will be activated and automatically shown in the License Request Complete window/**Licenses Status**:

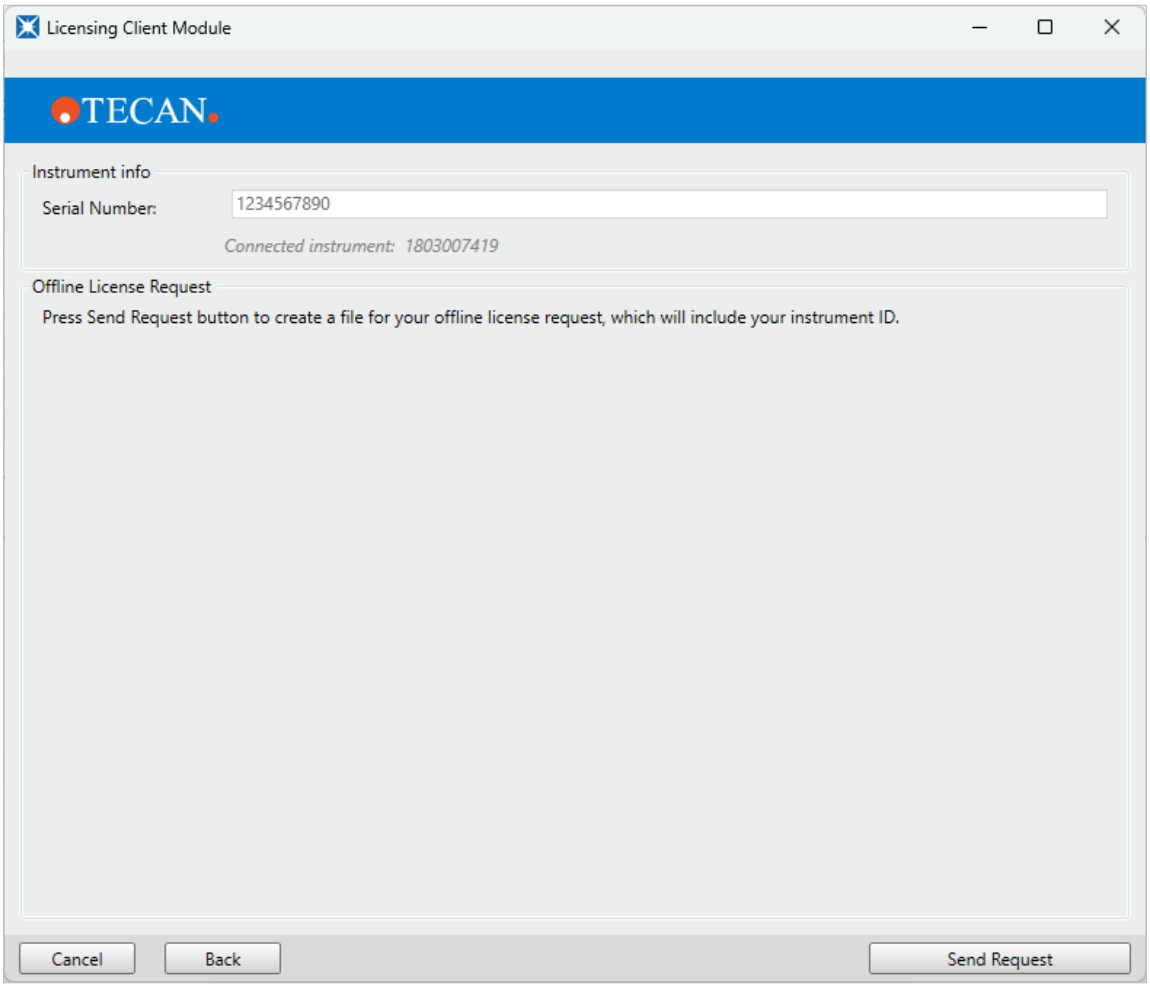


CAUTION: A reboot of the PC is required to use the new plugin features.

2.4.3 Offline Request

Use this option if the instrument computer is offline and has no internet connection.

1. In the License Request window, select **Offline Request**. Click **Next** to enter the registration form:

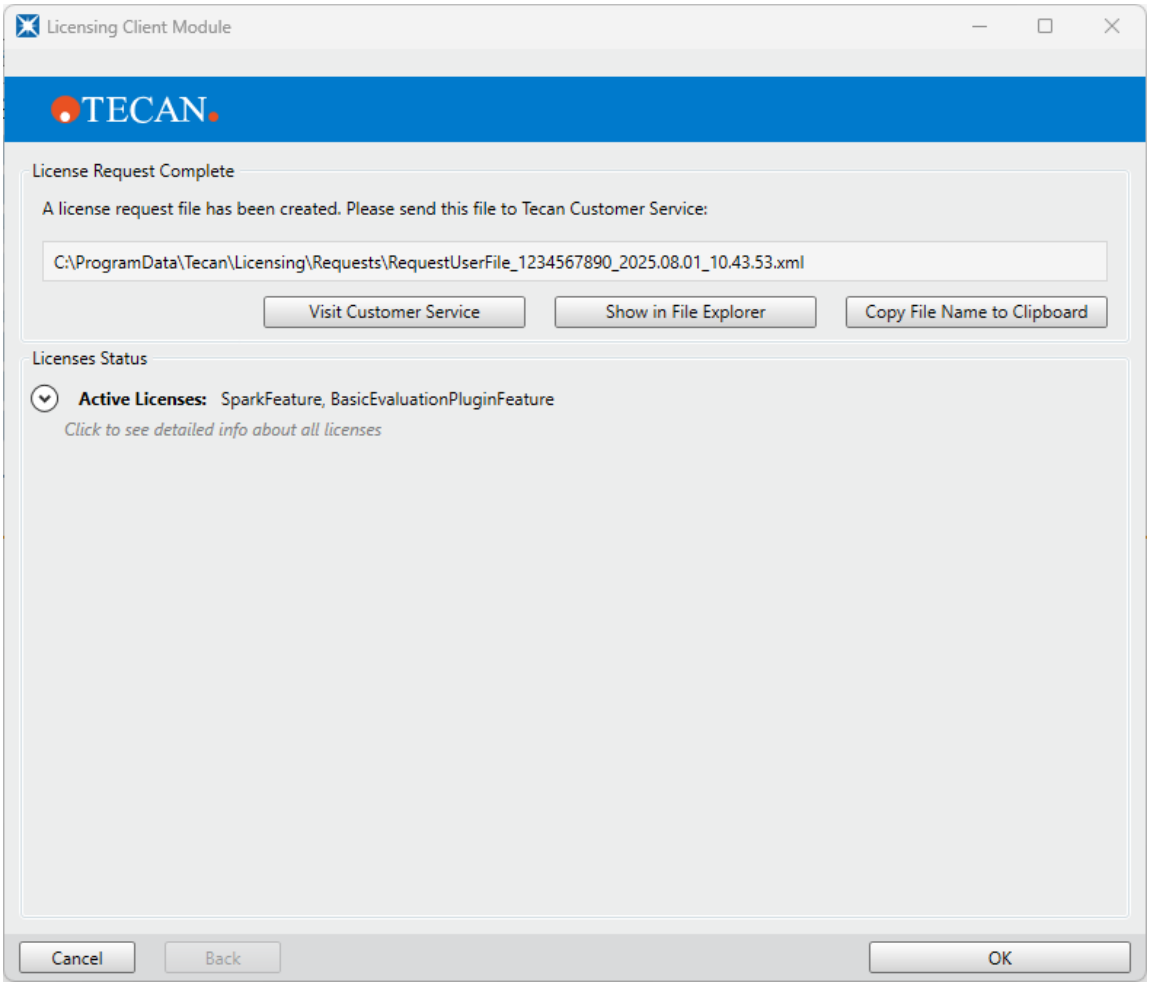


2. Complete the registration form by filling in the instrument serial number.

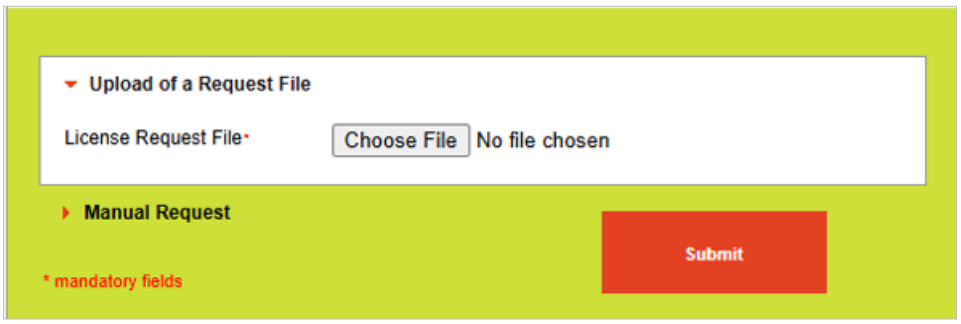


CAUTION: For registration in SparkControl, the instrument must be connected to the PC. The corresponding serial number is automatically filled in.

3. After having clicked **Send Request**, a license request file is generated. The License Request Complete window containing the path to your license request file is displayed:

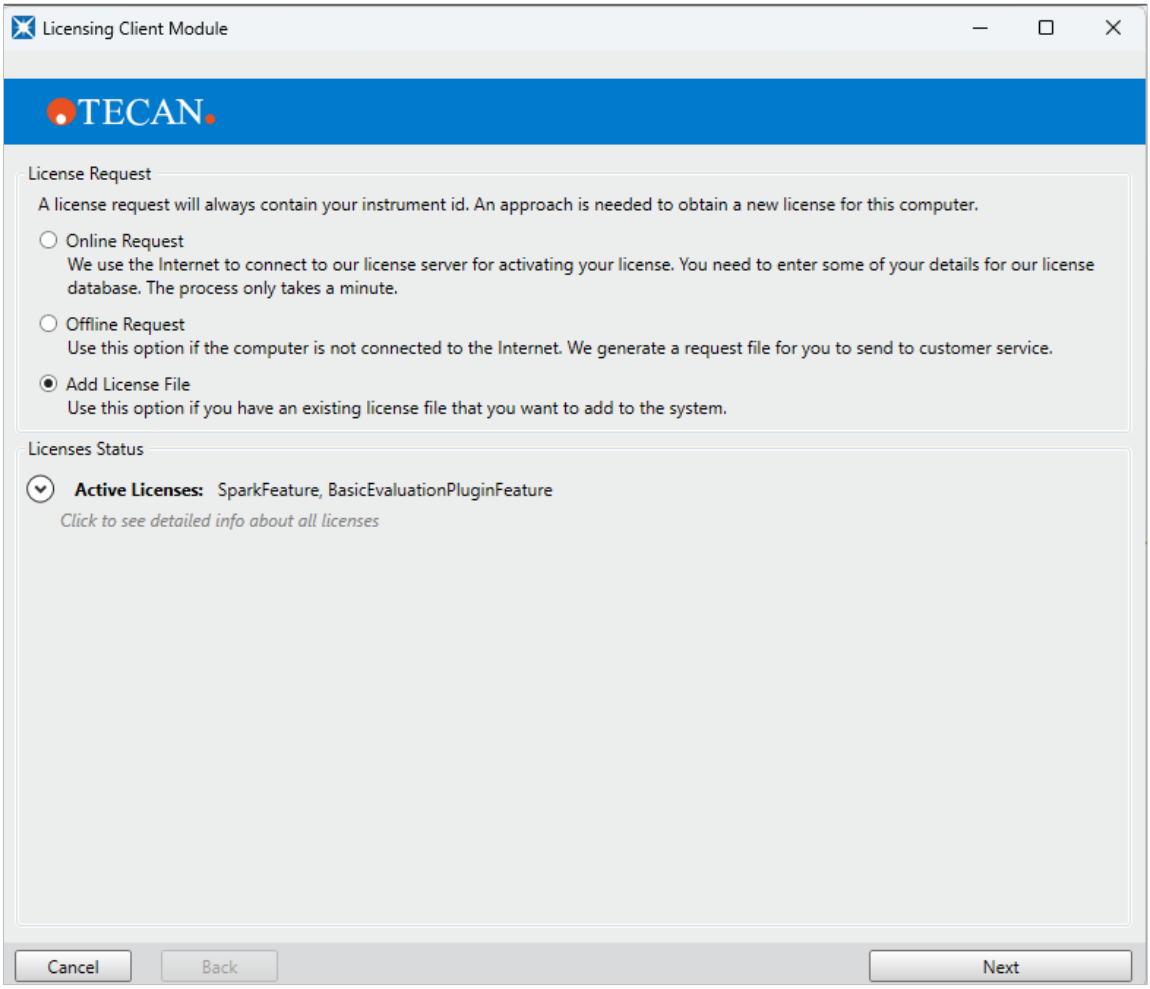


4. Copy the license request file to a USB storage device.
5. Use another computer which has internet access to complete the next steps.
6. Open the licensing webpage, which can be accessed at the URL below. The URL is also available by clicking the **Visit Customer Service** button in the above window.
   URL: https://license.tecan.com/lsprovider/licensingrequest.do
7. Complete the registration form. Fields marked with an asterisk are mandatory.
8. Upload and submit your license request file or click **Manual Request** to manually enter your request key that can be found in the license request file:

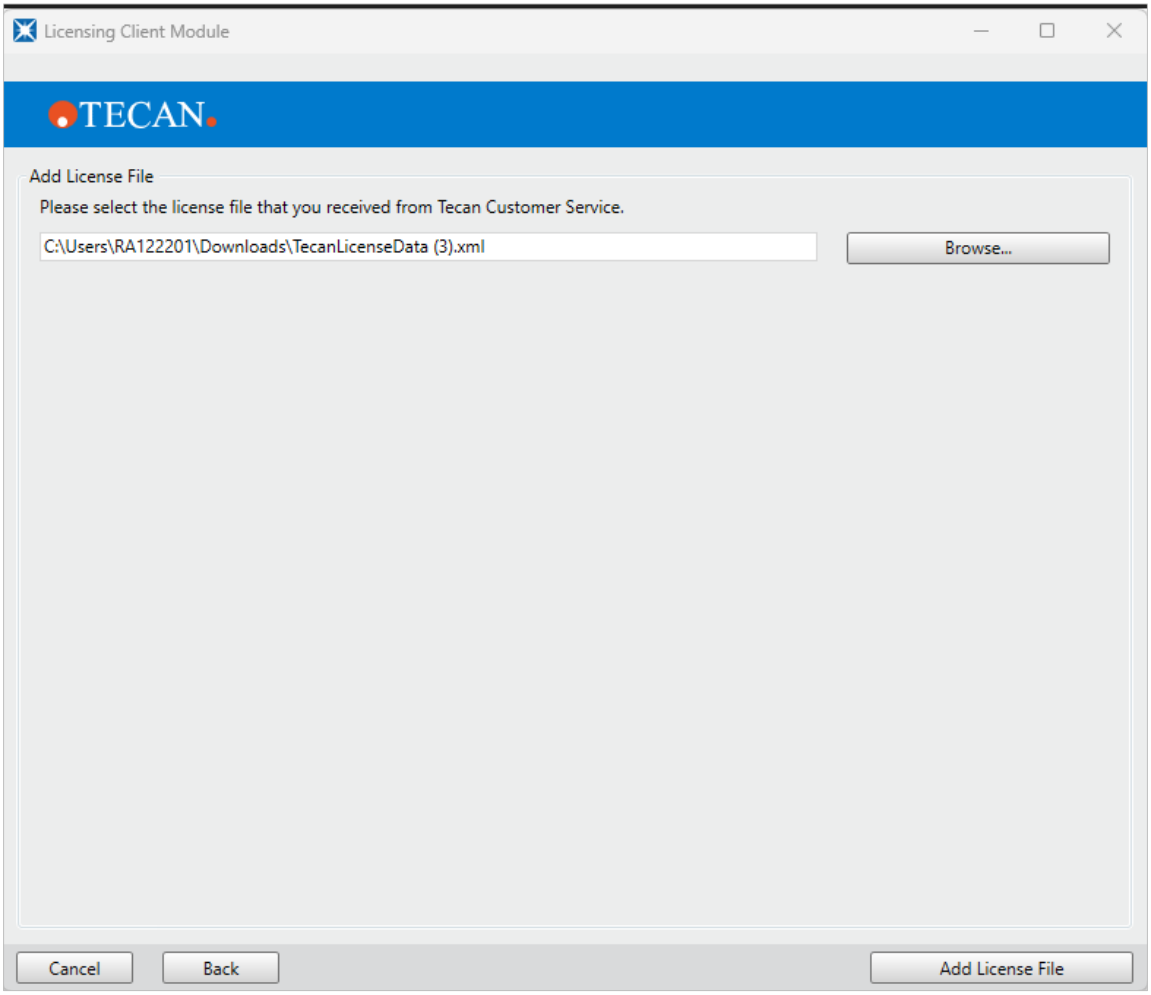


9. A license data file specific to your instrument will be available for download. Copy this file to a USB storage device.
10. Return to the instrument computer with the copy of your license data file.

11. Open the Licensing Client Module. Select **Add License File** followed by **Next**:



12. Select the downloaded license data file and click the **Add License File** button:



13. Restart the PC. You now have access to the licensed features.

2.5 Starting the SparkControl

From the Windows Start menu, select Tecan>SparkControl Dashboard or Method Editor to start the program.

2.5.1 Connecting Instruments

CAUTION: Do not open the instrument lid during operation!

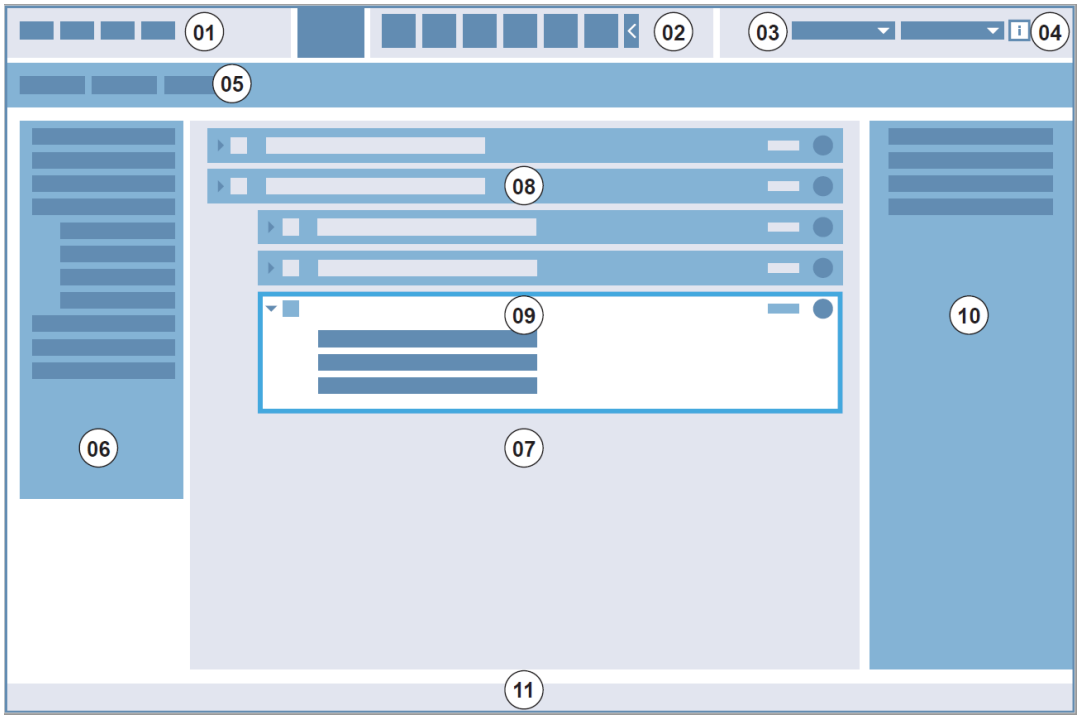
Each connected instrument is represented by a corresponding tile in the Dashboard (see chapter 2.9 Dashboard).

NOTE: The SparkControl supports the connection of a maximum of 4 instruments.

2.6 Method Editor

2.6.1 Structure

The Method Editor is used to set up workflows.



01 Menu bar; 02 Toolbar; 03 Drop-down list; 04 Button for opening the Info pane;
05 Method definition tabs; 06 Control bar; 07 Workflow pane; 08 Collapsed strip;
09 Expanded strip; 10 Info pane; 11 Status bar

Menu bar	**01**	Contains a drop-down menu of editor and reader functions (e.g. File, Edit, Settings)
Toolbar	**02**	Contains icons for commonly used editor functions (e.g. New, Save)
Drop-down lists	**03**	Select and start functions related to the respective software application or instrument connected (e.g. Select app)
Method definition tabs	**05**	Tabs for defining methods with available analysis tools (e.g. fluorescence imaging method)
Control bar	**06**	Contains strips for defining workflows
Workflow pane	**07**	Insert strips into this pane to define the workflow. Default settings can also be adjusted here
Info pane	**10**	Displays additional information about the workflow
Status bar	**11**	Displays information about the connected instrument (e.g. name, temperature)

Each workflow can be created easily by dragging and dropping the process steps into a sequence according to the application. The application workflow is then visible to the user in the Workflow pane and can be saved for future use.

2.6.2 Method Definition Tabs

Measurement

Addresses the creating of a method without any user-defined analysis steps. See chapter 2.7 Control Bar for available definition elements.

Processing

Provides the definition of processing steps for images before being subject to analysis. For more information, see the corresponding Processing Plugin instructions.

Analysis

Contains strips of analysis plugins (e.g. Counting, Area, Multi-color for image analysis). For more information, see the corresponding Analysis Plugin instructions.

2.6.3 Control Bar

See chapter 2.7 Control Bar for details.

2.6.4 Workflow Pane

See chapter 2.8 Workflow Pane for details.

2.6.5 Menu Bar

File

New	Open a new measurement workflow. If an empty document is to be opened, you will be asked to save the current workflow. Click **Yes** to save the current workflow or click **No** to create a new workflow without saving the previous one. Click **Cancel** to leave the dialog box.
Open	Open an existing SparkControl method from the selected folder. If you want to open an existing method while another one is still open, you will be asked if you want to save the workflow. Click **Yes** to save the current workflow to a certain destination or click **No** to create a new workflow without saving the previous one. Click **Cancel** to leave the dialog box.
Save	Save the current workflow as a SparkControl method.
Save as	Save the current workflow as a SparkControl method under a different name.
Onboard-Start	Save the current workflow as a SparkControl method that can be started via the instrument Onboard-Start button.
Export	Export a SparkControl method from the Method File Explorer.
Import	Import a SparkControl method to the Method File Explorer.
Exit	Exit and close the program.

Edit

Cut	Cut the selected strip.
Copy	Copy the selected strip.
Paste	Paste the copied strip.
Delete	Delete the selected strip.
Outdent	Outdent the selected strip.
Indent	Indent the selected strip.

View

Info pane	Show or hide the Info pane.
Status bar	Show or hide the Status bar.
Collapse all	Collapse all strips in the Workflow pane to view only one line of text per strip.
Expand all	Expand all strips in the Workflow pane to show all visible parameters.

Instrument

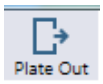
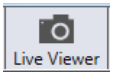
Movements	Define plate and filter movements: Click **Plate Out Left** to move the plate carrier out left. Click **Plate Out Right** to move the plate carrier out right. Click **Plate In** to move the plate carrier in. Click **Ex Filter** to move the excitation filter slide out. Click **Em Filter** to move the emission filter slide out. When a measurement is started, the plate is moved into the instrument automatically.
Temperature	Set the target temperature of the instrument manually.
Gas	Set the target gas concentration of the instrument manually.
Z-Position	Define the Z-position for measurement prior to measurement start.
Filter	Define filters located on a filter slide.
Mirror	Define a custom dichroic mirror.
Injector	Prime/rinse/backflush injectors.
Live Viewer	Check the autofocus/acquisition settings prior to measurement start.
Stacker	Restack plates without performing a measurement (instruments with Spark-Stack module only).

Help

View Help	Open the online help file and allows you to browse through the different topics.
Tecan Homepage	Open your favorite browser and navigate automatically to the Tecan homepage.
Contact Tecan	Open a direct contact link to Tecan.
Exception History	Open the Exception History dialog with a list of exceptions (i.e. instrument & software failures) resulting from a measurement. Every time an exception occurs and an error is displayed, all relevant information is collected and saved in a zip-file. Each of these zip-files leads to an entry in this list. Select a zip-file and send it to Tecan as this information is helpful to the customer support to track problems.
About Box	Display information about SparkControl including the software version number.

2.6.6 Toolbar

The following commands are accessible via the toolbar:

	Open a new measurement workflow
	Open an existing file
	Save the current workflow
	Start the measurement
	Start a stacker run (instruments with Spark-Stack module only)
	Move plate out
	Move plate in
	Move Ex Filter out
	Move Em Filter out
	Open the Gas Control window
	Open the Temperature Control window
	Open the Z-position window
	Open the Live Viewer

2.6.7 Instrument

Change the working instrument if more than one instrument is connected.

2.6.8 Select Component

Switch to the SparkControl components, **Dashboard, Settings** and **Screencasts**.

2.6.9 Select App

Start one of the available Tecan apps. Select an app to start it automatically.

2.7 Control Bar

This chapter describes the Control bar of the Method Editor/Measurement tab.

The Control bar is divided into four sections. Each section contains strips used to create an individual workflow. Depending on the instrument connected and the modules installed, the available strips may vary; e.g. if the instrument is not equipped with a fluorescence polarization module, the fluorescence polarization strip is not visible in the detection section.

Create a workflow by double-clicking the selected strip or by dragging and dropping it into the Workflow pane.

The following strips are available:

Plate	Cuvette Plate Part of Plate Well
Detection	Absorbance Absorbance Scan Alpha Technology Cell Counting Cell Confluence 2D Imaging 3D Imaging Fluorescence Intensity TR Fluorescence Intensity Fluorescence Intensity Scan Fluorescence Polarization Inject and Read Luminescence Luminescence Multicolor Luminescence Scan
Action	Shaking Wait Injector Condition Temperature Gas Move Plate User Intervention Comment
Kinetic	Kinetic Loop

2.7.1 Plate

Plate Strip

This strip is used to define the plate format used for the measurement.

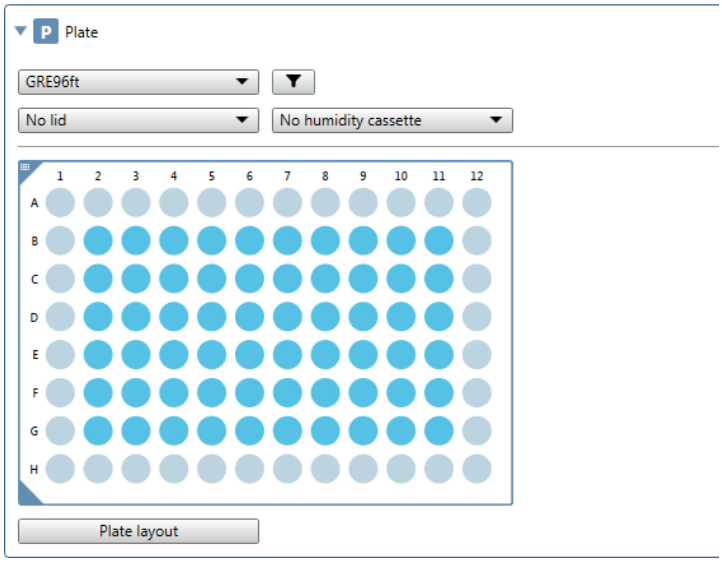


Figure 1: Plate strip

The **Plate** strip contains the following elements:

Plate definition	Select the plate to be used for the measurement. The list of available plate files can be filtered according to the **Number of wells**, **Catalog number**, **Manufacturer**, **Material** and **Cell** (plates for cell based assays).
Plate lid	• Select **No lid** if using a plate without a cover • Select **Lid** if using a plate with a cover • Select **Removable lid** (this option is only available for instruments with the lid lifting module)
Humidity cassette	This option is only available for instruments with the lid lifting module • Select **No humidity cassette** if using a plate without a humidity cassette • Select **Humidity cassette–Large** if using a large humidity cassette or **Humidity cassette–Small** if using a small humidity cassette (SPARK without Cell Imager module) • Select **Humidity cassette – Cyto Large** if using a large humidity cassette or **Humidity cassette – Cyto Small** if using a small humidity cassette (SPARK with Cell Imager module)
Read barcode	This option is only available for instruments with a barcode module. If selected, the plate barcode is read.
Smooth mode	Select **Smooth mode** to avoid sample spilling.
Plate	Select the plate area used for the measurement. The measurement will automatically measure all selected wells of the plate.

NOTE: Use the **Fit to window** control located at the left edge of the plate when defining the plate area for a 1536-well plate.

CAUTION: A removable lid is used in combination with the lid lifter. Please make sure to attach a magnetic pad to the plate lid before use.

Defining the Plate Layout

Click **Plate Layout** to view and change the plate layout.

Identifiers	**None** to delete defined identifiers **BL** (Blank) **SM** (Sample) **ST** (Standard) **PC** (Positive control) **NC** (Negative control) **LPC** (Low positive control) **HPC** (High positive control) **CL** (Calibrator) **RF** (Reference) **BF** (Blank for polarization reference) **CMR** (Reference compound for dose-response curve) **CM** (Compounds CMA-CMK for dose-response curves)
Identifier No.	The identifier number is used to assign the same identifier to replicates that belong together. The identifier number is available only for the samples and standards.
No. of replicates	Define the number of replicates for the selected identifier type. There are two options available: • Define a fixed number of replicates. This number defines how many replicates are intended for this identifier. The selected wells are then filled with this number of replicates. Therefore the number of selected wells must be a multiple of the entered number of replicates. The fixed number definition is only available for the **Samples** and **Standards**. The number of replicates for the **References**, **Controls** and **Blanks** is always identical to the number of selected wells. • Select **All** to define all selected wells as replicates of one identifier. This selection is automatically active for the **References**, **Controls** and **Blanks**.
Direction	Define the direction for filling the selected well area with the respective identifier: • **Horizontal** (i.e. the fill order is horizontal) • **Vertical** (i.e. the fill order is vertical) The fill direction is available for the **Identifier No.** and **No. of replicates**.

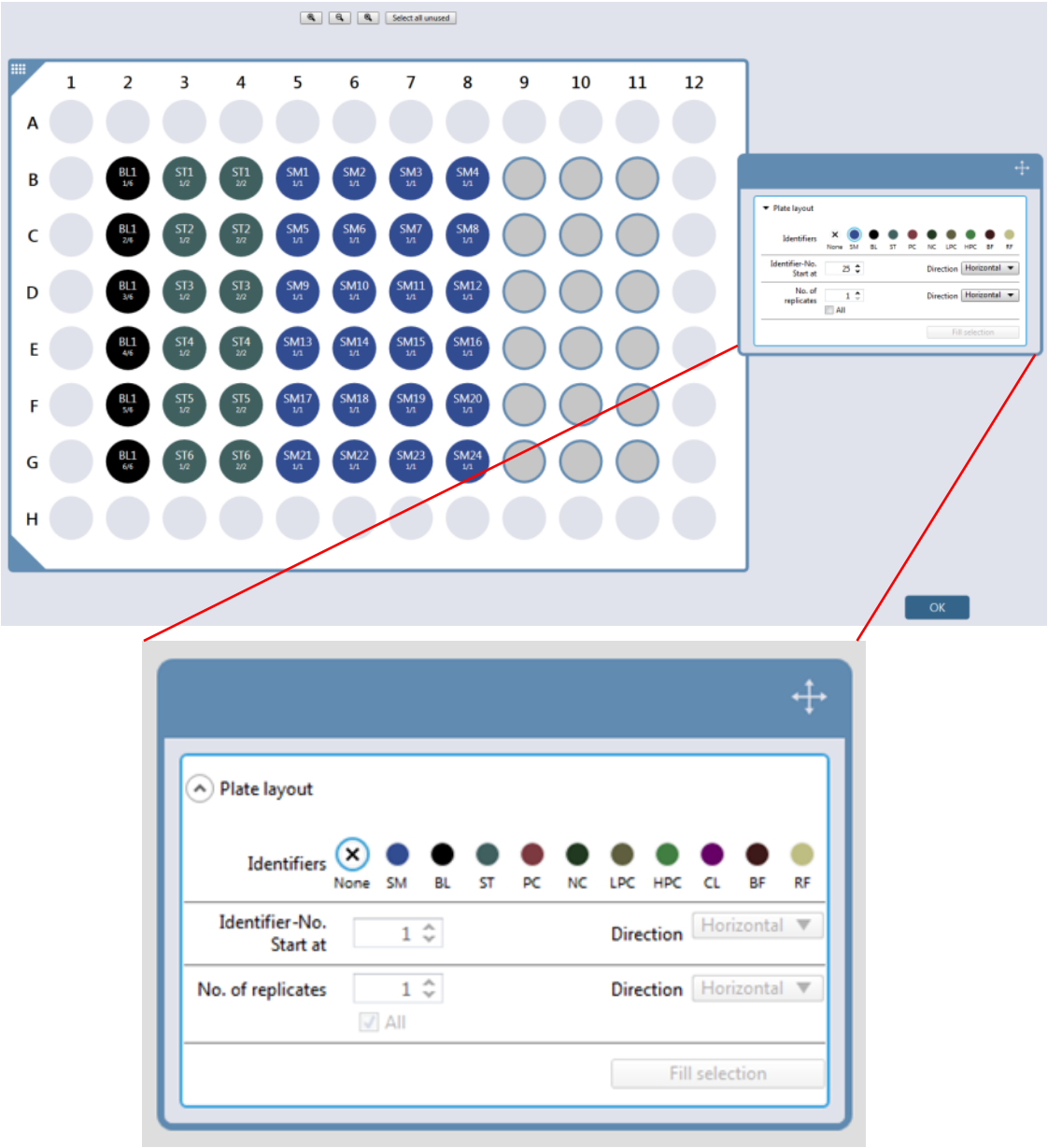


Figure 2: Defining the plate layout

An identifier can be assigned to a well or a well area as follows:

1) Select the plate area.
2) Select **Plate Layout**.
3) Select the wells on the plate to be filled with the selected identifier.
4) Select the identifier.
5) Select **Fill selection**: the identifiers and their colors are displayed in the plate layout. (It is also possible to fill the selection by double-clicking the selected identifier).

Optional:

- Define the **Identifier No.** or use the default settings.
- Define the **No. of replicates** or use the default settings.

Concentration/Dilution Values

This table displays all wells in the Identifier column, which match the selected identifier (as from the Select identifier list). The corresponding dilutions, concentrations values are inserted and listed in the Concentration/Dilution column.

If dilution factors are defined, they are considered by the calculation of the unknown sample concentration. E.g. entering a dilution factor of 2 means that the sample has been diluted by half. The calculated concentration will therefore be multiplied by 2. For further information, see the Evaluation Plugin instructions.

NOTE: Concentration and dilution values have to be inserted manually. To save time, copy and paste values from an existing Excel table. In case of multiple dose-response curves, the inserted values for the first compound can be copied and pasted in the tables of the subsequent identifiers if applicable.

Part of Plate

Use **Part of Plate** to define sub-areas within the previously selected plate area.

Measurement strips below each sub-area will be applied only to the respective sub-area.

Example:

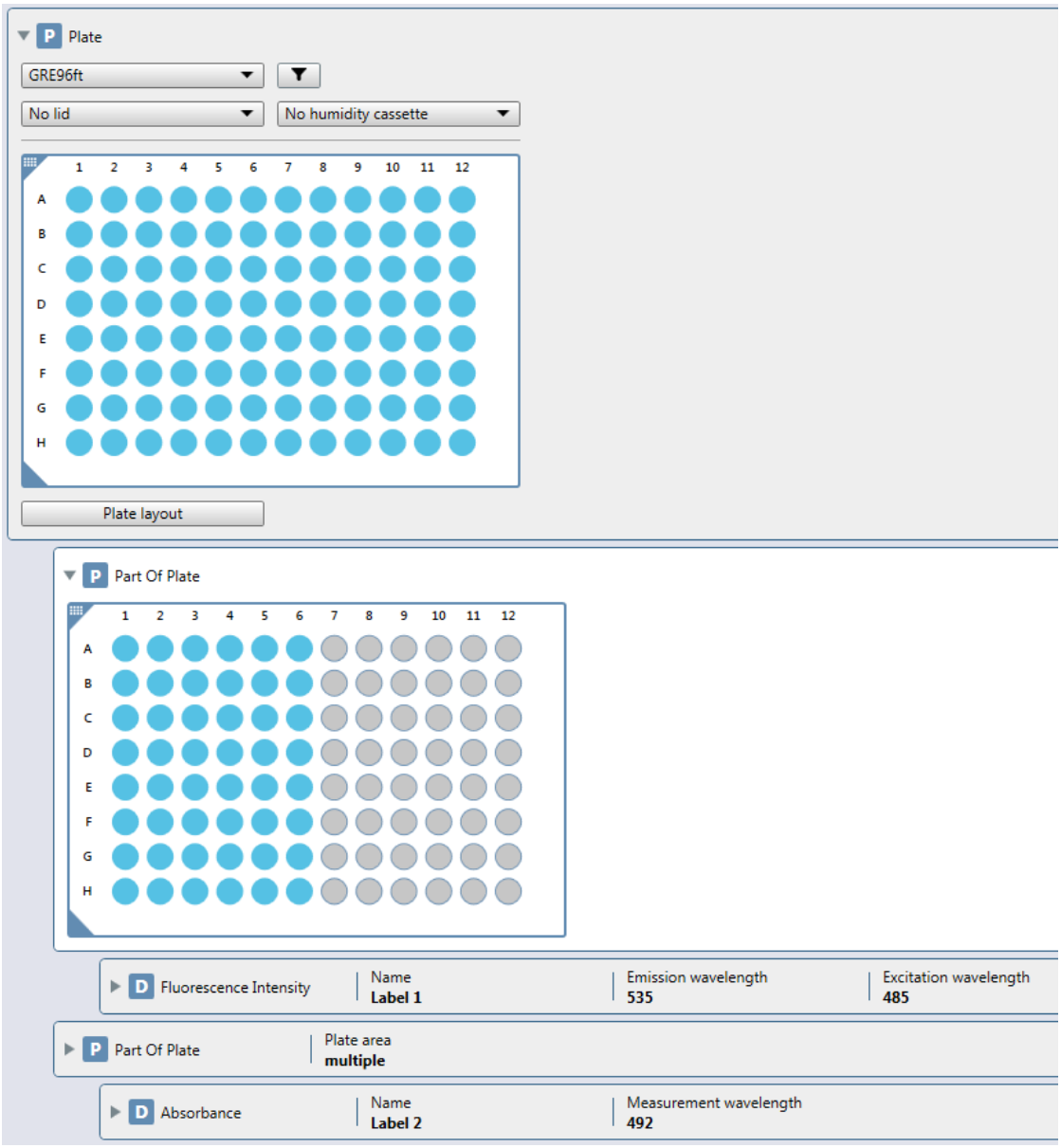


Figure 3: (Plate: A1-H12; Part of Plate: A1-H6; FI; Part of Plate: A7-H12; ABS)

In this workflow, fluorescence intensity is measured only in the wells A1-H6, followed by an absorbance measurement for the wells A7-H12.

Cuvette Strip

This strip is used to define a measurement in a cuvette that is inserted into the cuvette port of the instrument.

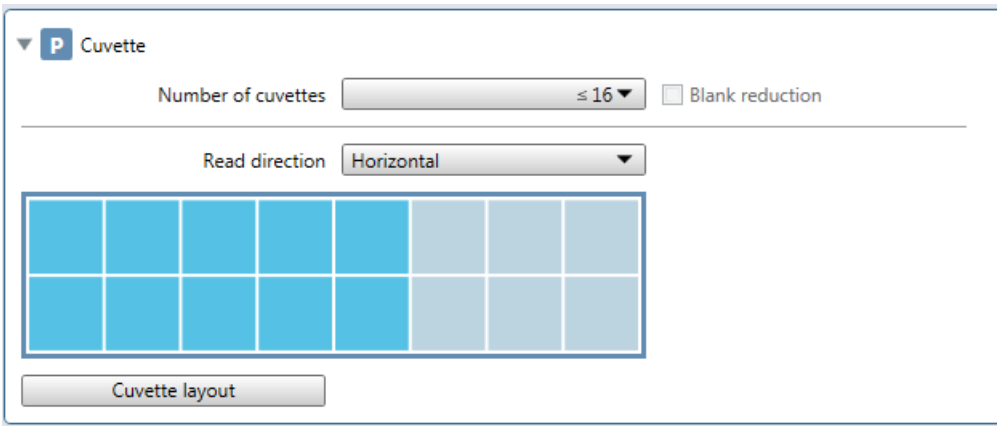


Figure 4: Cuvette strip

The **Cuvette strip** contains the following elements:

Number of cuvettes	Select the maximum possible number of cuvettes. Define the exact number of cuvettes used for the measurement by selecting the corresponding cuvettes within the cuvette holder displayed.
Blank reduction	Select **Blank reduction** to perform the automatic blank correction for all measured values. The Blank reduction is selectable only if the cuvette layout contains at least one cuvette defined as Blank (BL).
Read direction	Define the direction for the consecutive cuvette reading and the corresponding prompting with respect to the definition on the cuvette holder: • **Horizontal** (i.e. horizontal order) • **Vertical** (i.e. vertical order)
Cuvette layout	See above, chapters **Plate Strip** and **Defining the Plate Layout**.

NOTE: When working with the Tecan cuvette adapter, select the corresponding plate definition file within the Plate strip and define a measurement.

Well Strip

Use the **Well** strip to perform measurements well by well. Without this strip, all measurement steps are done plate-wise.

2.7.2 Detection

For detailed information on detection strips, see the corresponding chapter for the detection mode, e.g. 4.1.1 Luminescence Strip, 5.1.1 Alpha Technology, 6.1.1 Absorbance Strip, 7.1.1 Fluorescence Intensity Strip, etc.

2.7.3 Action

Shaking Strip

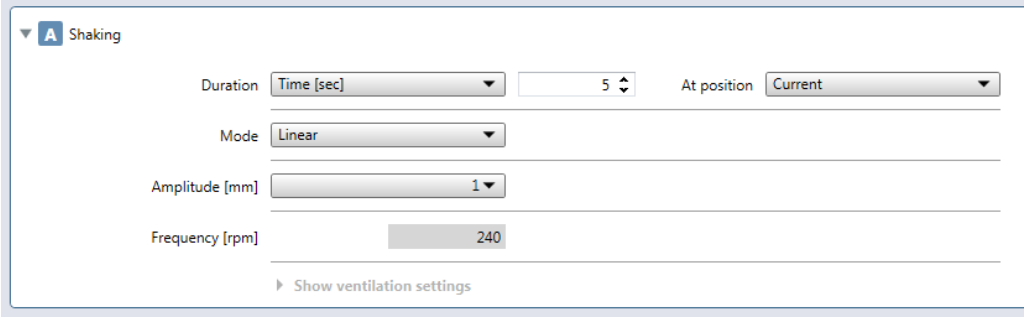


Figure 5: Shaking strip

Use the **Shaking** strip to define the following parameters:

Duration	Define **Time [sec]** for shaking. **Continuous shaking** can be selected and used within a kinetic measurement with a fixed interval time. Only in that case is the option **Continuous shaking** enabled and available. Select it to perform shaking for the remaining time within consecutive cycles at any of the available positions. At position: • **Current** • **Incubation** • **Current with ventilation** (if removable lid or humidity cassette is selected) • **Incubation with ventilation** (if removable lid or humidity cassette is selected)
Mode	Linear, Orbital, Double orbital (humidity cassette not selected) Orbital, Double orbital (humidity cassette selected)
Amplitude [mm]	1-6 mm (humidity cassette not selected) 2.5-6 mm (humidity cassette selected)
Frequency [rpm]	The number of revolutions per minute (rpm) is displayed.
Show ventilation settings	Select the **Interval time** for ventilation in minutes. Select the **Duration** of ventilation in seconds.

Wait Strip

This strip can be used to define a specific time period before the next step within a workflow is executed.

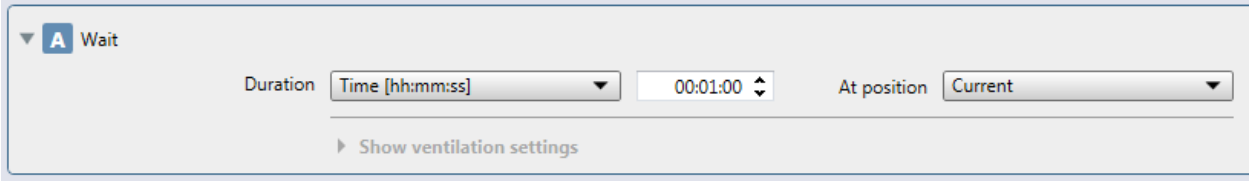


Figure 6: Wait strip

Use the **Wait** strip to define the following parameters:

Duration	Define **Time [hh:mm:ss]** for waiting. **Continuous waiting** can be selected and used within a kinetic measurement with a fixed interval time. Only in that case is the option **Continuous waiting** enabled and available. Select it to perform waiting for the remaining time within consecutive cycles at any of the available positions. At position: • **Current** • **Incubation** • **Current with ventilation** (if removable lid or humidity cassette is selected) • **Incubation with ventilation** (if removable lid or humidity cassette is selected)
Show ventilation settings	Select the **Interval time** for ventilation in minutes. Select the **Duration** of ventilation in seconds.

Injection Strip

For detailed information about the **Injection** strip, see chapter 11.1.1 Injector Strip.

Condition Strip

Use this strip to define measurements with conditions.

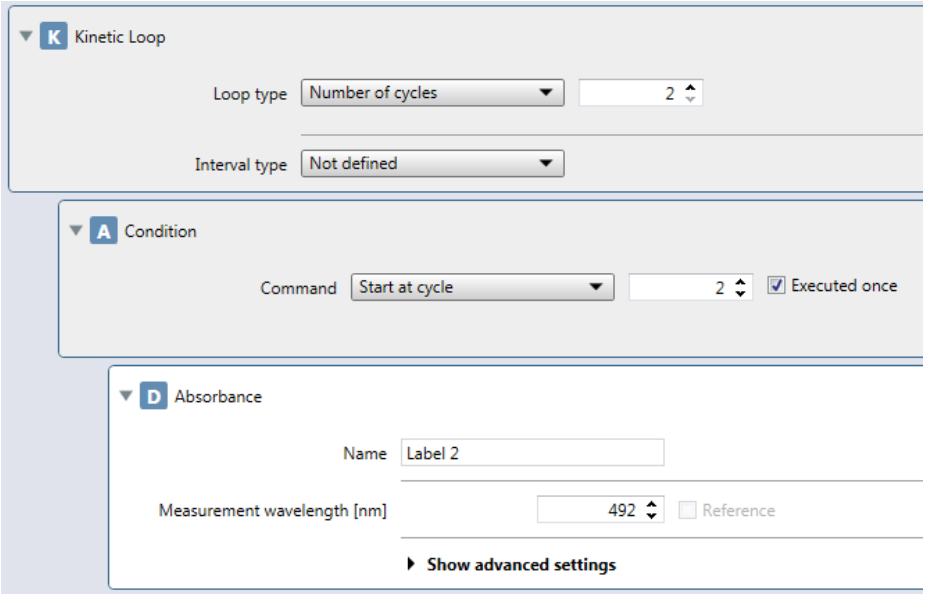


Figure 7: Condition strip

The **Condition** strip contains the following elements:

Command	• **Start at cycle** to perform the conditional step at a defined cycle. • **Start at value** to execute the conditional step at a defined raw data value. Define the **Input data** (i.e. Label name the reference value refers to), **Reference well** and **Value** at which the conditional step should be started. • **Stop at value** to stop the conditional step at a defined raw data value. Define the **Input data** (i.e. Label name the reference value refers to), **Reference well** and **Value** at which the conditional step should be stopped.
Executed once	Selected by default. If selected, the conditional step is executed only once.



CAUTION: When defining values with decimal places always use the decimal symbol as defined in the Region and Language settings of the PC's operating system.

Temperature Strip

For detailed information about the **Temperature** strip, see chapter 12.1.2 Temperature Control via Method.

Gas Strip

For detailed information about the **Gas** strip, see chapter 12.3.3 Gas Control via Method.

Move Plate Strip

Use the **Move Plate** strip to move the plate out or into the instrument within a workflow.

User Intervention Strip

The **User Intervention** strip informs the operator of the instrument to execute a particular action during the workflow at a specific time. A message appears and the measurement process stops until **OK** is clicked.

Comment Strip

Use the **Comment** strip to enter a remark or statement for the current measurement in the text field. This text is shown together with the measurement in the result output sheet. Comments have no impact on the measurement workflow.

2.7.4 Kinetic

Kinetic Loop Strip

Use this strip to define kinetic measurements.

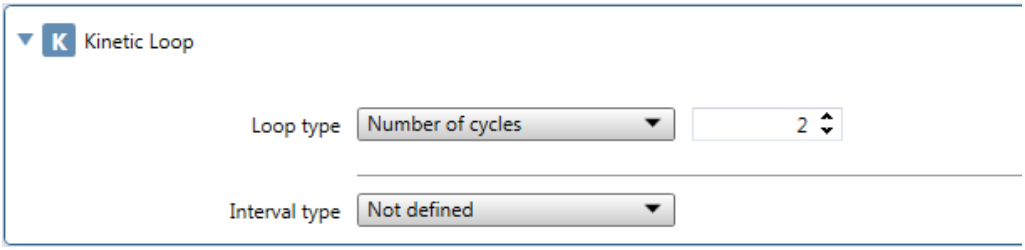


Figure 8: Kinetic Loop strip

The Kinetic Loop strip contains the following elements:

Loop type	Select **Number of cycles** and define a cycle value. Select **Duration [hh:mm:ss]** and define duration of the kinetic measurement.
Interval type	Select **Not defined** for measurements without a kinetic interval time, measurements are performed as fast as possible. Select **Fixed** for measurements with a kinetic interval time and define the interval time.

NOTE: To enable the options **Continuous shaking** and **Continuous waiting** define a kinetic measurement with a **Fixed** interval time.

2.8 Workflow Pane

The Workflow pane is the central window of the Method Editor, in which the workflow is visible and available for defining and editing measurement parameters.

There are two ways to insert a strip from the Control bar into the Workflow pane:

- Select a strip from the Control bar; double-click it to insert it into the Workflow pane directly after the previous strip.
- Click the strip in the Control bar and drag it into the Workflow pane to the respective position.

The strips are numbered according to their sequence. Once a strip has been inserted into the Workflow pane, the settings and parameters for this strip can be entered or edited.

Single strips inside the Workflow pane can be collapsed to display the most important information or expanded to access all editable functions. Click the expand button next to the title of the strip to switch between the two view modes.

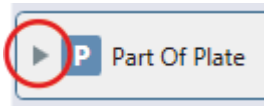


Figure 9: Expand button

By default, SparkControl starts with the Plate strip in the Workflow pane.

The currently selected strip within the Workflow pane is highlighted. If a strip contains an erroneous item, the item is highlighted in red and the strip number is colored red. If a strip is invalid within the current workflow, the whole strip is marked red. All errors are automatically displayed in the Info pane. If the workflow contains errors, the workflow can be neither saved nor started.

It is recommended to always save the workflow before starting a measurement.

2.8.1 Hierarchy of Strips

The hierarchy of strips in the Workflow pane is as follows:

1. Plate
2. Part of Plate (range)
3. Well

Any desired measurement step can be inserted directly after a **Plate**, **Part of Plate** or **Well** strip. Use the **Outdent** and **Indent** options in the Edit menu to modify the sequence of execution of the single strip component or select a strip in the Workflow pane, click the right mouse button and select **Outdent** or **Indent**.

Indenting and Outdenting Strips

The decision to indent/outdent a measurement strip will modify the workflow of the instrument during measurements.

The actions of all strips with the same indentation are performed sequentially. The only dependence between these strips is that the next action starts directly after the previous action is finished.

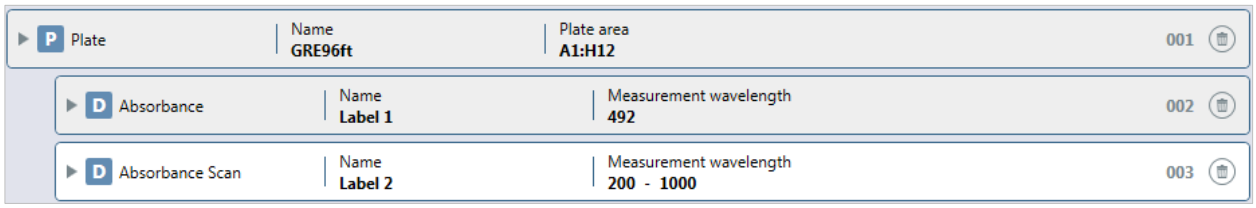
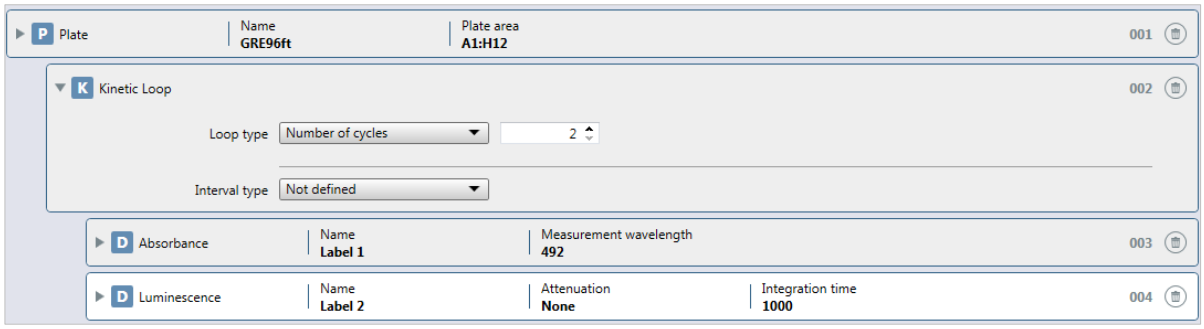


Figure 10: A series of independent measurement steps

A strip that is indented more than the previous strip shows dependence between the two strips. This means that the parameters defined in the first strip are also active for the second (indented) strip.

The following is an example of how to define a multi-label kinetic measurement with an **Absorbance** and a **Luminescence** strip. In this example, both strips depend on the **Kinetic Loop** strip, which depends on the **Plate** strip.

The **Workflow** pane appears as follows:

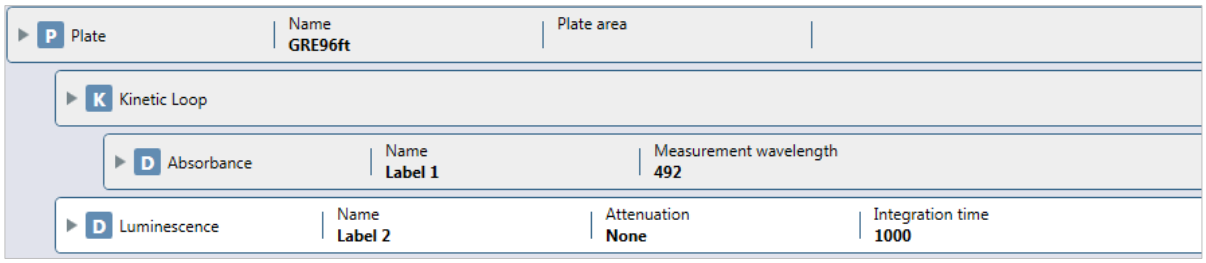


The above definition results in the following workflow:

The **Absorbance** of all wells of a 96-well plate is measured first following by **Luminescence** measurements of all wells. Both measurements are performed in 2 kinetic cycles.

Outdenting the **Luminescence** strip to a level with a **Kinetic Loop** item changes the workflow. Select **Luminescence** and click the right mouse button. Select **Outdent** from the context sensitive menu.

The **Workflow** pane appears as follows:



In this workflow, an **Absorbance** kinetic measurement with 2 cycles is performed first. After this loop has finished, the **Luminescence** endpoint measurement starts.

2.8.2 Info Pane

The **Info** pane on the right side of the screen displays information that is relevant for the currently selected strip. Any warnings and errors are shown.

2.8.3 Measurement Types

Plate-wise Measurements

Each detection strip is performed on all selected wells. Plate-wise measurements may contain a maximum of 25 independent detection strips that do not need to be of the same detection type.

Well-wise Measurements

Each detection strip is performed in one well before continuing to the next well. Well-wise measurements may be composed of a maximum of five detection strips of the same type, e.g. five absorbance strips.



NOTE: Only measurement steps of the same detection mode are allowed within a Well strip (e.g. two absorbance steps with different wavelengths). Exception of that rule: multi-label kinetic measurements performed well-wise (e.g. Kinetic loop/Well/Absorbance/ Fluorescence Intensity).



NOTE: The action strips Move plate and User intervention are not allowed within a Well strip.

Endpoint Measurements

An endpoint measurement is a measurement executed only once without repetition. Endpoint measurements can be performed plate-wise and well-wise.

Kinetic Measurements

A kinetic measurement consists of several consecutive measurements, which may be executed in certain intervals. Kinetic measurements can be performed plate-wise and well-wise.



NOTE: Fluorescence intensity 3D scan are not allowed within a kinetic measurement.



NOTE: The action strips Temperature and Gas are not allowed within a kinetic measurement loop except within a kinetic condition.



NOTE: Users are advised to set up suitable methods prior to measurements and to use the same method for all similar kinetic measurements in order to obtain comparable results.

Single-label Measurements

Single-label measurements are measurements with only one detection strip, e.g. with absorbance or fluorescence. Single-label measurements can be performed plate-wise and well-wise as endpoint or kinetic measurements.

Multi-label Measurements

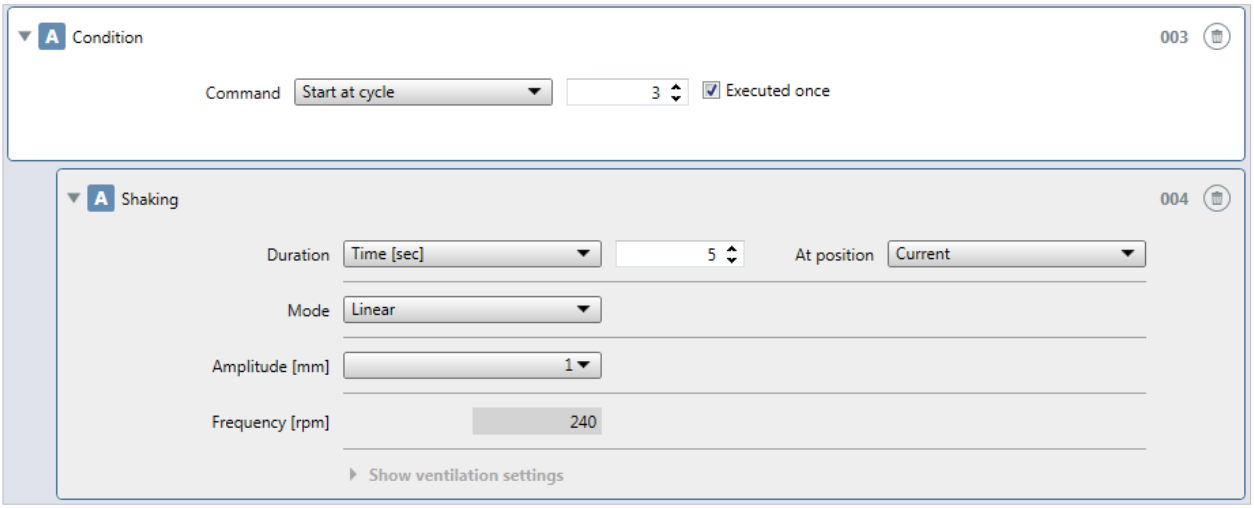
Multi-label measurements are measurements with multiple detection strips of the same or different consecutive detection modes, e.g. with multiple absorbance, fluorescence, luminescence labels or with mixed measurements. Multi-label measurements can be performed plate-wise and well-wise as endpoint or kinetic measurements.

Conditional Measurements

Time-triggered measurements:

Time-triggered conditional measurements are a group of the measurements in which a measurement or an action is executed or stopped at a certain cycle.

Example:



If **3** is entered for **Command: Start at cycle** within a kinetic measurement containing, e.g. a **Shake** step, shaking is performed only at cycle 3, if **Executed once** is selected.

NOTE: Kinetic conditions such as **Shake** and **Inject** should be inserted right after a **Kinetic Loop** strip in order to ensure optimal result reproducibility.

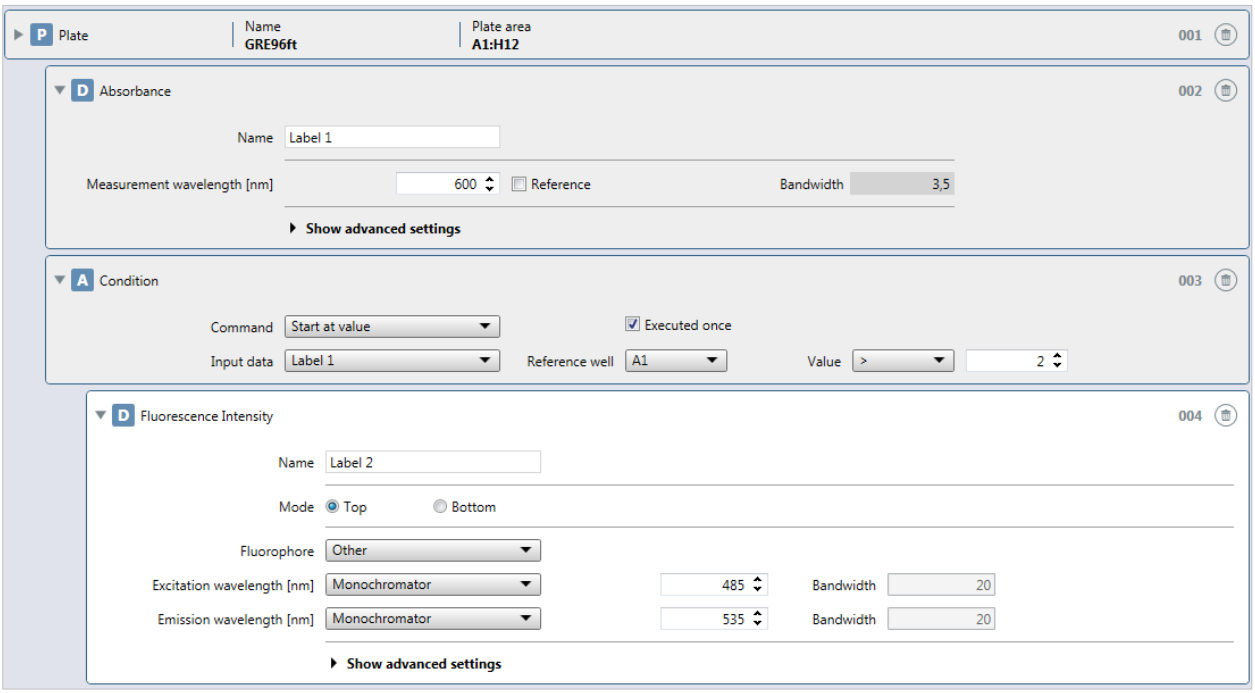
Signal-triggered Measurements:

Signal-triggered conditional measurements are measurements in which a measurement or an action is triggered by a measured value while running a method. The method steps can be started or stopped after the pre-defined reference value for a specific detection label has been reached.

CAUTION: When defining values with decimal places always use the decimal symbol as defined in the Region and Language settings of the PC's operating system.

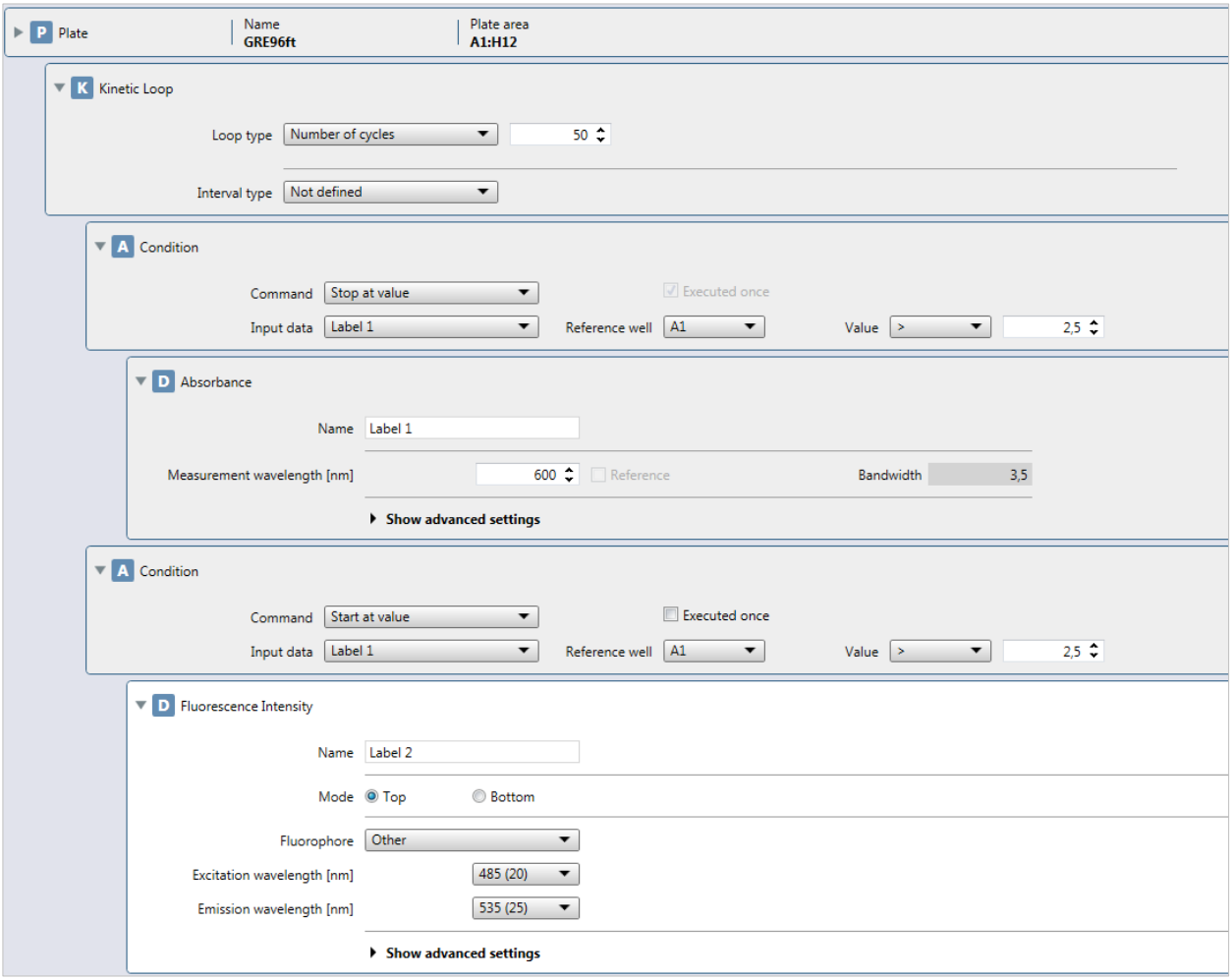
Examples:

1. Kinetic fluorescence measurement:



Absorbance is measured at 600 nm. A fluorescence measurement is executed only if an OD value > 2 has been detected in a pre-defined reference well.

2. Kinetic multi-label measurement:



A kinetic multi-label measurement with absorbance and fluorescence as labels; the absorbance is measured as long as the reference well reaches an OD value of 2.5 OD. After that, the absorbance measurement is stopped and the method proceeds with the execution of the fluorescence label.

2.8.4 Multiple Reads per Well

The multiple reads per well option (MRW) is available for absorbance, fluorescence top and bottom fixed wavelength measurements in order to achieve maximum well illumination. The function is not available for scan measurements. This option is especially applicable for cell based assays, since the distribution of the cells in the well is often not homogeneous.

NOTE: The feature **Multiple Reads per Well** is not available for well-wise measurements.

NOTE: The reference wavelength in the **Absorbance** strip is not selectable in combination with **Multiple Reads per Well**.

When working with multiple reads per well, select one of the following options:

User Defined

Select a MRW pattern type to be used for the measurement. The following MRW types can be selected:

- Square
- Square (filled)
- Circle
- Circle (filled)
- X-Line
- Y-Line
- XY-Line

Select the MRW size that defines the number of measurement points per well. The size values available depend on the plate format used.

Area Scan

The **Area scan** is a high density option of MRW and enables enhanced signal resolution within a measured well. Select one of the following sizes: 20x20, 30x30, 40x40, 50x50, 60x60, 70x70, 80x80, 90x90 and 100x100.



NOTE: It is recommended to perform area scan measurements with one flash.

Optimal Read

The Optimal Read is available only for fluorescence intensity bottom measurements. Similar to the MRW read mode, multiple, spatially separated spots inside the well are measured. The spots are arrayed to cover the whole well area in order to achieve maximum well illumination. The total number of individual measurement spots per well is reflected by the size of the Fluorescence Intensity Bottom fiber and is optimized for plate formats from 12 to 96 wells (see table below). 384-well plates are optimally illuminated by a single-spot read.

Plate format	Size	Number of spots
96 wells	3x3	5
48 wells	5x5	21
24 wells	7x7	37
12 wells	9x9	61

Table: Optimal Read spot patterns in different plate formats. Pattern used: Circle (filled)

Changing the total number of flashes per well (1-100) will result in the automatic adjustment of the number of flashes per spot, giving the user the possibility to obtain representative results in each well.

The total number of flashes is automatically distributed over all measured spots. A minor imprecision occurs if an entered flash number is not divisible by the default number of spots for the used plate format. In this case, the next possible flash distribution that is divisible by the number of spots per well is calculated, e.g. a measurement with a total of 26-30 flashes in a 96-well plate is performed with 6 flashes per spot, whereas a total flash number of 31 results in 7 flashes per spot.

2.9 Dashboard

2.9.1 Structure

The Dashboard of the SparkControl software is used for

- Communicating with connected instruments
- Starting measurements
- Monitoring measurement progress

The Dashboard is designed to work with a touchscreen. Fingers can be used for interaction.

The Dashboard contains the following structural elements:

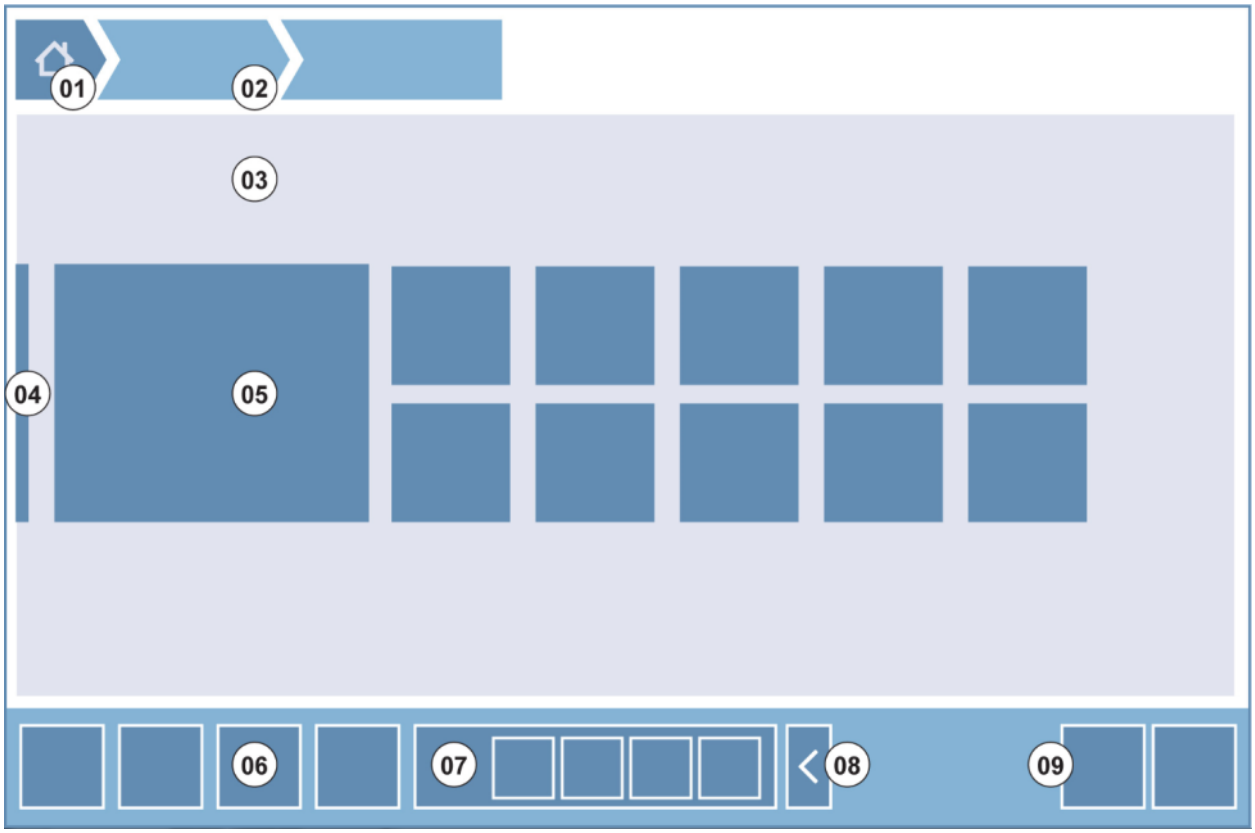


Figure 11: Structural elements of the Dashboard

01 Home button
02 Breadcrumbs
03 Workflow pane
04 Navigation bar
05 Tiles
06 Action bar with action buttons
07 Expandable action button
08 Expand button (to show more action buttons)
09 Action buttons (OK, Cancel, Stop)

Tiles

Tiles start user selected process steps, e.g. a ‘Method’ tile starts the selected method.
The clickable surface is always the whole tile area with the exception of tiles with multi-functionality.

For the multi-functional tiles, the clickable surface is always darker than the background color.
Example: Start tile (see chapter 2.9.5 Starting a Method).

Action Buttons

A group of buttons designed for

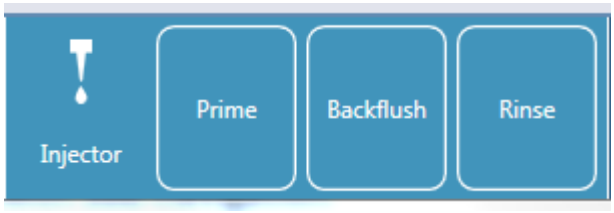
- Editing of method and instrument settings
- Confirmation/cancellation/stopping of workflow steps (OK/Cancel/Stop button)
- Searching/Alignment of listed elements

Expandable Action Buttons

Expandable action buttons are used for a group of action buttons that refers to the same action group (e.g. Filter, Injector).

Tap an expandable action button to reveal all action buttons for the corresponding group.

Example: The action group Injector contains the sub-action buttons Prime, Backflush and Rinse.



Expand Buttons

Expand buttons are used for expanding/collapsing of grouped elements.

Action Bar

The action bar is the Dashboard area with action buttons.

Navigation Bar

The expandable Navigation bar on the left side of the Dashboard is used to switch to other SparkControl components (e.g. Method Editor).

Breadcrumbs / Navigation history

Breadcrumbs are used as guides within the different application levels and are placed on the top of the screen. They track the navigation history of the previous windows and include a home button. Select the home button to return to the Dashboard window.

Example:



I.e. a method called ELISA has been selected first, following by opening the Temperature Control window in order to change/prove the temperature before measurement start.

2.9.2 Software Navigation

Application Levels

The levels are based on a hierarchical navigation system with a maximum of three different application levels.

- The **first application level** is the entry point of each application in the dashboard, e.g. instrument settings, start a measurement run or monitor the measurement process.
- The **second application level** is displayed whenever the selection on the first level requires further user interaction, e.g. final user confirmation in order to start a method.
- The **third application level** reveals the details for a selected element in the second level, e.g. defining filters of the excitation filter slide.

Switching Levels

Breadcrumbs	The breadcrumbs include an integrated close and cancel button (x) for each application level displayed. Select this button to close the current application level and to return to the next higher level. Closing the application level via the breadcrumb navigation automatically discards all changes made within the respective application level.
OK and Cancel	Select **OK** to confirm all changes, select **Cancel** to discard the changes. Both selections automatically close the respective application level.

2.9.3 The Dashboard

The **Dashboard** window contains the following tiles:

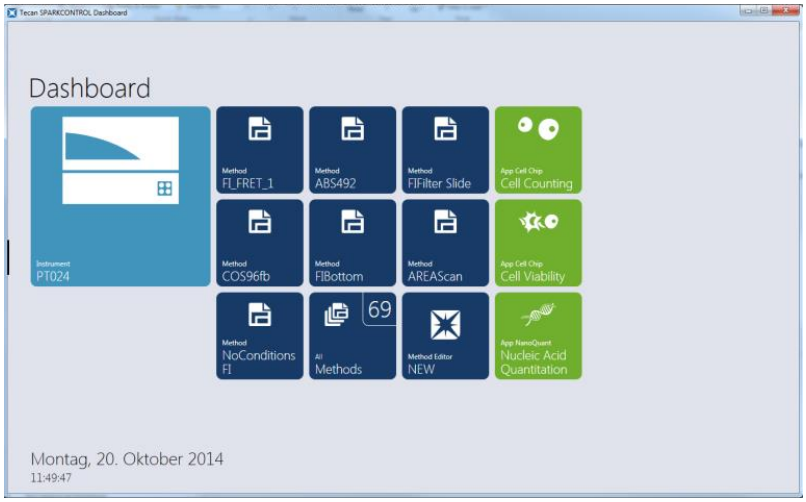


Figure 12: Instrument tiles, Method tiles and App tiles in Dashboard

Instrument	Light blue **Instrument tiles** stand for connected instruments. Select an instrument tile to access the Instrument Control window.
Method	Dark blue **Method tiles** stand for methods valid for the connected instrument. Select a method tile to start the method. The maximum number of method tiles is limited to eight. If more than eight methods are available, use the **All methods** tile to open the list of all methods. The displayed group of method tiles is built up dynamically according to the following rules: • Every new defined or every modified method is automatically displayed in dashboard and placed on the top of the group. • Every executed method is displayed automatically in the Dashboard and placed on the top of the group. • All other method tiles are moved accordingly. In case of more than eight available methods, the previously last method of the group is removed from the dashboard. Select **NEW** to switch directly to Method Editor in order to define a new method.
App	Light green **App tiles** stand for apps provided by Tecan. Select an App tile to start the corresponding app.
Open Workspaces	Olive green **Open Workspace** tiles stand for uncompleted kinetic measurements as results of an open kinetic run. Select an open workspace tile to continue the kinetic measurement. The maximum number of open workspace tiles is limited to eight. If more than eight methods are available, use the **All open workspaces** tile to open the list of all methods.

NOTE: To delete an open workspace tile and hence interrupt an open kinetic run before its complete execution, select the **All open workspaces** tile and mark the corresponding workspace(s) for deletion.

To switch to **Method Editor**, **Settings** or **Screencasts** use the expandable Navigation bar on the left side of the Dashboard start window. Select **Shut down** to close the SparkControl application.

2.9.4 Instrument Control

The **Instrument Control** window can be opened by tapping an instrument tile. It provides the user interface for the direct communication to the instrument (e.g. injector maintenance, filter definition).

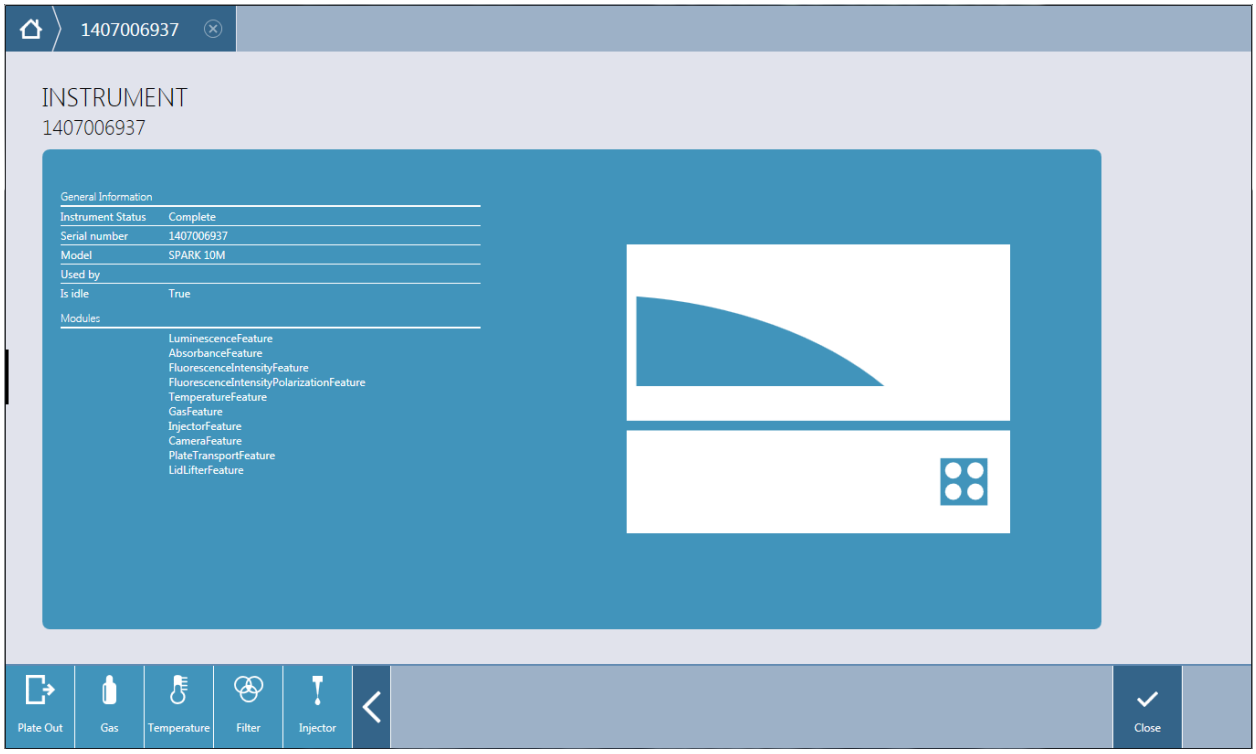


Figure 13: Instrument Control window

NOTE: The availability of action buttons depends on the instrument configuration.

The window contains the following action buttons:

Plate In/Out	Select **Plate In** to move the plate carrier into the instrument. Select **Plate Out** to move the plate carrier out of the instrument.
Gas	Select **Gas** to open the Gas Control window.
Temperature	Select **Temperature** to open the Temperature Control window.
Filter	Select **Excitation** to open the Filter Definition window for the excitation filter slide. Select **Emission** to open the Filter Definition window for the emission filter slide.
Mirror	Select **Mirror** to open the Mirror Definition window.
Injector	Select **Prime** to open the Injector window for priming. Select **Rinse** to open the Injector window for rinsing. Select **Backflush** to open the Injector window for backflushing.
Stacker	Select **Stacker** to restack plates without performing a measurement (instruments with Spark-Stack module only).

Multiple Instruments

NOTE: The SparkControl supports the connection of a maximum of 4 instruments. However, working in parallel is not possible. Only one instrument can be used at a time.

Each connected instrument is represented by a corresponding tile. The largest instrument tile represents the working instrument, i.e. the instrument that can be currently used for measurements.

To switch working instruments, select the instrument tile and close the Instrument Control window by clicking **OK**.

2.9.5 Starting a Method

After selecting a method, the **Check-and-Go** window is opened.

NOTE: A method can be selected from the Dashboard start window or from the list of all methods via the **All Methods** tile**.**

The **Check-and-Go** window contains the following tiles and buttons:

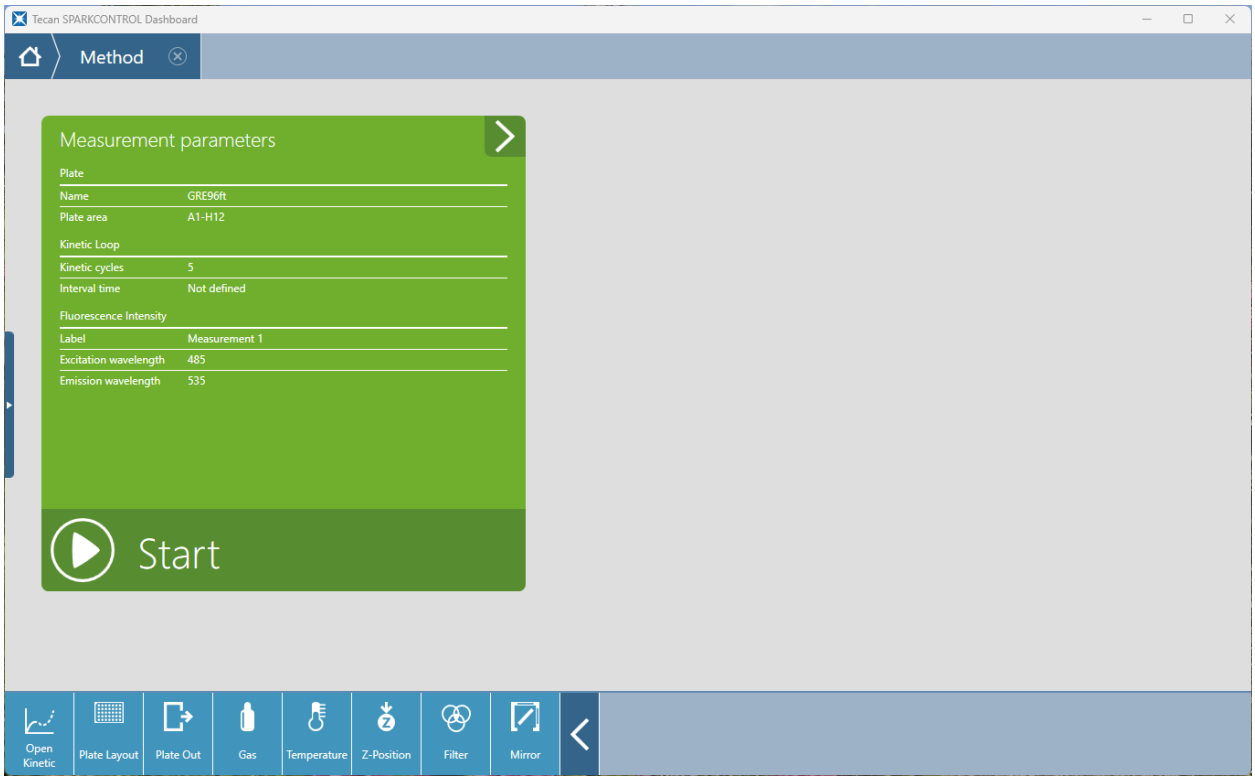


Figure 14: Check & Go window

NOTE: The availability of action buttons depends on the instrument configuration and method definition.

Start	The **Start tile** is a multi-functional tile. Select the expand button top right to view detailed information about the selected method (e.g. plate area, plate layout). Select **Start** to start the method.
Start Stacker	This tile is displayed only for instruments with the Spark-Stack module and the input and the output magazines inserted. The **Start Stacker tile** is a multi-functional tile. Select the expand button on the top right to view detailed information about the selected method (e.g. plate area, plate layout). Select **Start Stacker** to start a stacker run.
Plate Layout	Select **Plate Layout** to perform changes in the plate layout. Changing the well's position: Select well(s) on the plate layout first. To change a well's position, select **Cut**. Select the new well position on the plate and click **Exchange**. Wells cannot be exchanged if the direction of their position (horizontal, vertical) does not match. Delete samples (SM): Select sample(s) on the plate layout first. For deletion, select **Delete**.
Open Kinetic	Select **Open Kinetic** to start a method as a discontinuous kinetic run.
Plate In/Out	Select **Plate In** / **Plate Out** to move the plate carrier into or out of the instrument.
Gas	Opens the Gas Control window.
Temperature	Opens the Temperature Control window.
Filter	Select **Excitation** / **Emission** to define the corresponding filters.
Mirror	Opens the Mirror Definition window.
Injector	Select **Prime** / **Rinse** / **Backflush** to open the Injector window to adjust the corresponding settings.
Z-Position	Select to perform Z-position optimization prior to measurement start.
Live Viewer	Select to check the autofocus settings prior to measurement start.
Stacker	Select **Stacker** to restack plates without performing a measurement (instruments with Spark-Stack module only).

NOTE: Changes in the plate layout are valid only for the subsequent run and do not overwrite the original method. They are stored only in the corresponding workspace.

NOTE: Changing the temperature or gas settings prior to measurement will not overwrite the temperature or gas settings defined in a method.

Start Open Kinetic

By selecting the **Open Kinetic** action button a kinetic method can be executed discontinuously, i.e. split into several kinetic runs which results are finally merged within one result worksheet.

NOTE: Run kinetic measurements with long interval times as open kinetics. Optimize the instrument usage and perform short-term measurements in between.

NOTE: Only kinetic measurements with the loop type **Number of cycles** can be run as open kinetic.

NOTE: Only **plate-wise** kinetic measurements can be run as open kinetic. Exception of that rule: multi-label kinetic measurements performed well-wise (e.g. Kinetic loop/Well/Absorbance/ Fluorescence Intensity).

NOTE: Kinetic measurements with time or value triggered gas and/or temperature settings cannot be run as open kinetic.

NOTE: An open kinetic run can be started via Dashboard only.

In addition to the **Check-and-Go** window the **Open Kinetic** windows contains the following tiles and buttons:

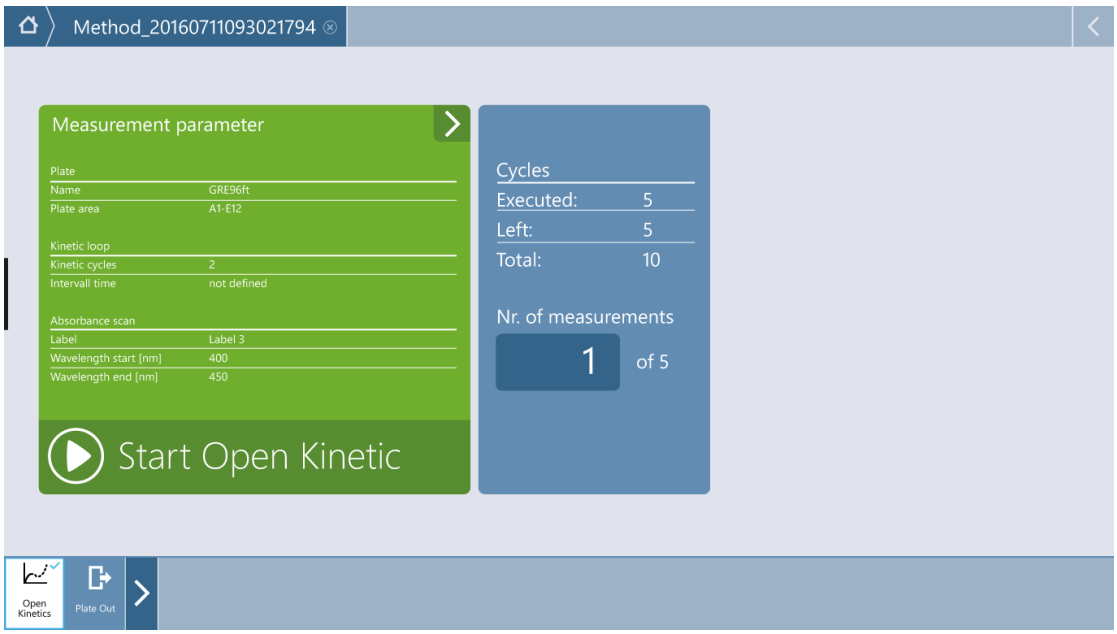


Figure 15: Open Kinetic window

Start Open Kinetic	Select **Start Open Kinetic** to start the method.
Nr. of measurements	Define the number of cycles to be executed within the current run.
Executed cycles	Displays the number of cycles already executed.
Left cycles	Displays the number of cycles still left for execution.
Total cycles	Displays the total number of cycles according to the method definition.

To start a measurement as open kinetic proceed as follows:

1. Define a kinetic measurement via the Method Editor and save it.
2. Select the corresponding method tile in dashboard.
3. Select **Open Kinetic** action button.
4. Define the number of measurements for execution within the current run.
5. Select **Start Open Kinetic**.
6. To continue an open kinetic run, select the corresponding **Open Workspace** in Dashboard and repeat the steps 4, 5 (and 6).



NOTE: Select the corresponding Open Workspace in the Dashboard to continue an open kinetic run. An open workspace must be processed with the same instrument that was used for the first open kinetic measurement, otherwise it will not be visible in the Dashboard.



NOTE: Changing the method used for an open kinetic run will not have any effect on the started open kinetic run. The original method is saved together with the open workspace and, thus, used for all subsequent open kinetic runs.



CAUTION: Breaking an open kinetic run via the **Stop** button will interrupt not only the current measurement run but also the open kinetic run as whole. After stopping the method run, no future continuation of the open kinetic run is possible.



CAUTION: Open kinetic workspaces lose their validity after a service inspection and have to be deleted manually by the user.



CAUTION: Changing the workspace path will disable further execution of open workspaces until the original workspace path is restored.



CAUTION: Do not delete an open workspace folder as long as the corresponding open workspace is still pending. The workspace folder includes information needed for further method execution.



CAUTION: Always finish all open kinetic runs before uninstalling or upgrading the software, otherwise open kinetic data will be lost.

2.9.6 Measurement Progress

Starting a measurement run opens the **Measurement Progress** window with the following elements:

Analysis	Display analysis data. Plate view: Select **Values** to display numeric values. Select **Graphics** to display kinetic curves. For detailed information about the data display, see chapter 2.9.7 Data.
Curves	Display calculated curves (standard curve, dose-response curve).
Data	Data is selected by default. The incoming raw data from the instrument is monitored. Plate view: Select **Values** to display numeric values. Select **Graphics** to display images or kinetic curves. For detailed information about the data display, see chapter 2.9.7 Data.
Data as Colors	Raw data values are correlated to colors and displayed as such in the plate.

Monitor	Monitor temperature and gas distribution during a measurement run.
Stop	Interrupt the current measurement run. The data acquired prior to measurement stop are automatically saved.
Plate Out/In	Disabled during a measurement run. The button becomes enabled after the measurement has been finished or while pausing a kinetic run.
Pause/Continue	Available for plate-wise kinetic measurements only. Select **Pause** to interrupt the measurement and subsequently **Continue** to proceed with the measurement.

NOTE: Selecting the **Pause** button will not immediately pause the current measurement run. The measurement will not be paused before the current kinetic cycle has been completed. Please also note that the interval time is part of a cycle; therefore a kinetic measurement with an interval time will not be paused until after the interval time has expired.

2.9.7 Data

Measured data are displayed in two different views: the **Plate** view and the **Graph** view. The following chapters describe the general rules for data display depending on the measurement mode. Scan measurements, Images and Batch Processing are excluded from the general rules and described separately (see chapters 2.9.8 Scans, 2.9.9 Images and 2.9.10 Batch Processing). For display of analysis data (e.g. fluorescence imaging) refer to chapter 2.9.11 Analysis.

Single-label Endpoint Measurements

Plate View: Includes all measured data. Only single well selection is possible. After the measurement the value of the selected well is displayed in the information header on top of the screen.

Graph View: Includes all measured data. The well selected in the Plate view is highlighted.

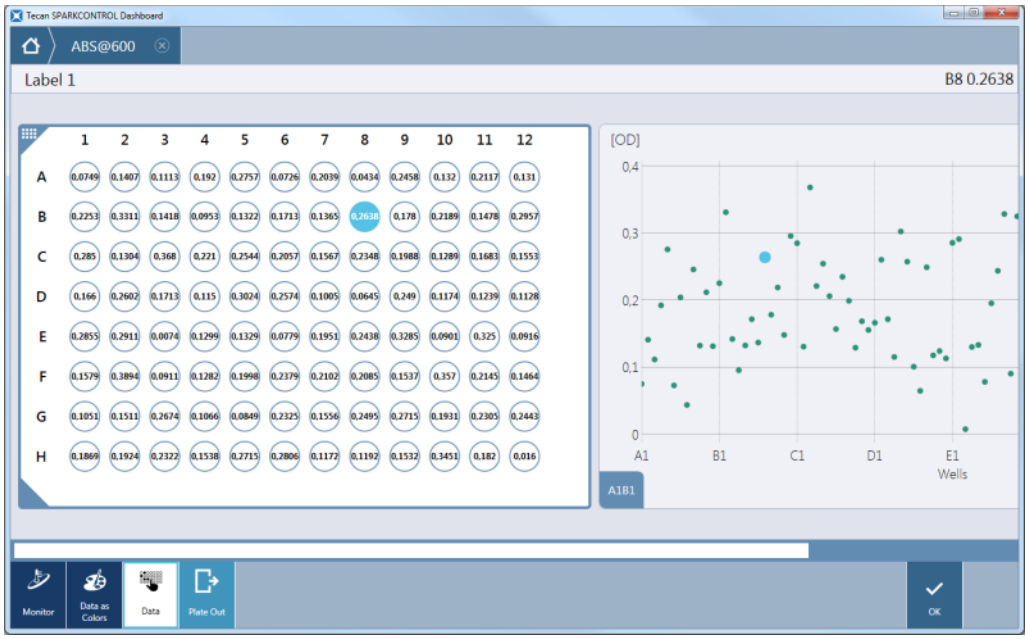


Figure 16: Monitoring a single-label endpoint measurement

Single-label Kinetic Measurements

Plate View / Values:: Includes measured data of the last measured kinetic cycle. Multi well selection is possible. After the measurement the last cycle value of the selected wells is displayed in the information header on top of the screen.

Plate View / Graphics: Includes kinetic curve per well.

Graph View: Includes kinetic curves for the last ten wells selected in the Plate view. Fade in and out the single curves by selecting/deselecting the corresponding check boxes.

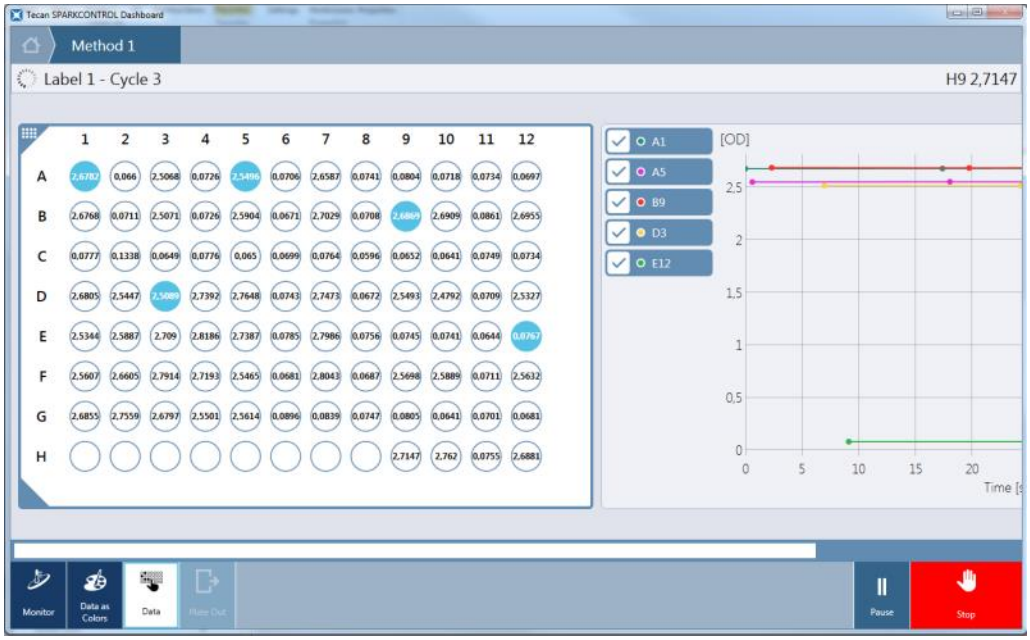


Figure 17: Monitoring a single-label kinetic measurement

Multi-label Endpoint Measurements

Plate View: Includes measured data for all labels available. Move between the labels by applying the **>** and **<** controls placed below the plate. Only single well selection is possible. After the measurement, the value of the corresponding label for the selected well is displayed in the information header on top of the screen.

Graph View: Includes all measured data for the selected well. It is possible to move to any of adjacent wells by tapping the corresponding **well control** placed left, right, top and bottom of the graphic display. Fade in and out the single-labels by selecting/deselecting the corresponding check boxes.

Multi-label Kinetic Measurements

Plate View / Values: Includes measured data of the last measured kinetic cycle for all labels available. Move between the labels by applying the **>** and **<** controls placed below the plate. Only single well selection is possible. After the measurement, the last cycle value of the corresponding label for the selected well is displayed in the information header on top of the screen.

Plate View / Graphics: Includes kinetic curve per well.

Graph View: Includes kinetic curves for all labels of the selected well in the Plate view. It is possible to move to any of adjacent wells by tapping the corresponding **well control** placed left, right, top and bottom of the graphic display. Fade in and out the single curves by selecting/deselecting the corresponding check boxes.

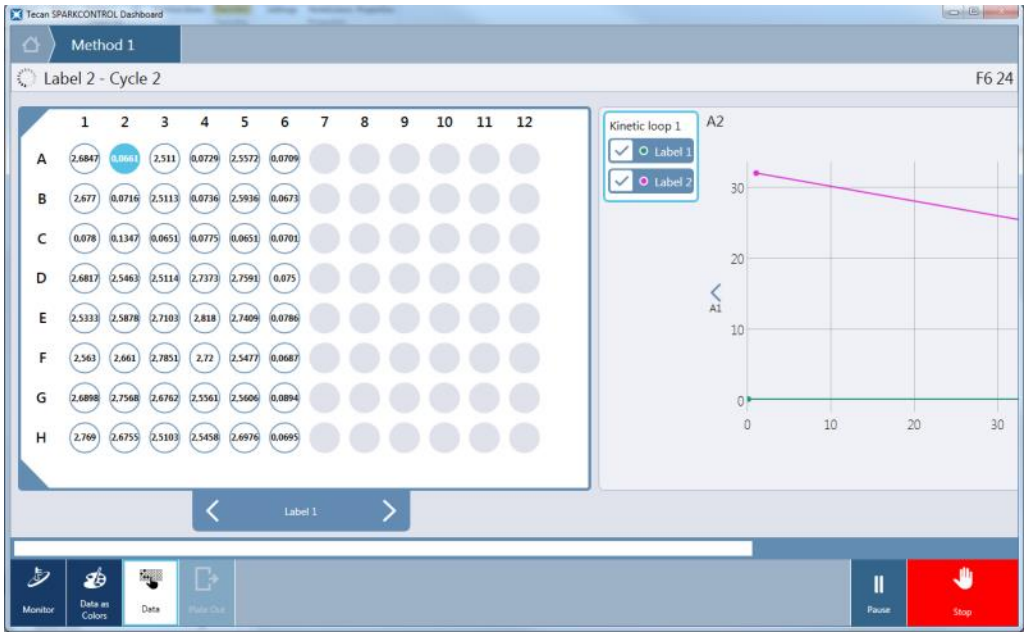


Figure 18: Multi-label kinetic measurement

2.9.8 Scans

Single-label Endpoint Measurements

Plate View: Measured data are indicated by an **S**. Multi-well selection possible. Exception of that rule: 3D scans support only single well selection.

Graph View: Includes scans for the last ten wells selected in the Plate view. Fade in and out the single scans by selecting/deselecting the corresponding check boxes.

Multi-label Endpoint Measurements

Plate View: Measured data are indicated by **S**. Only single well selection is possible.

Graph View: Scans are displayed separately, not in parallel with other measured data. Select a scan tile for its display. It is possible to move to any of adjacent wells by tapping the corresponding **well control** placed left, right, top and bottom of the graphic display.

Single-label Kinetic Measurements

Plate View: Measured data are indicated by **S**. Multi well selection possible.

Graph View: Includes scans for the last ten wells selected in the Plate view according to the kinetic cycle selected. Use the **+** and **–** controls included in the graphic to change the kinetic cycle number. Fade in and out the single curves by selecting/deselecting the corresponding check boxes.

Multi-label Kinetic Measurements

Plate View: Measured data are indicated by **S**. Only single well selection is possible.

Graph View: Scans are displayed separately, not in parallel with other measured data. Select a scan tile for its display. It is possible to move to any of adjacent wells by tapping the corresponding **well control** placed left, right, top and bottom of the graphic display. Use the **+** and **–** controls included in the graphic to change the kinetic cycle number.

3D Scan

Plate View: Measured data are indicated by **S**. Only single well selection is possible.

Graph View: Scans are always displayed separately, not in parallel with other measured data if available. Select a scan tile for its display. It is possible to move to any of adjacent wells by tapping the corresponding **well control** placed left, right, top and bottom of the graphic display.

2.9.9 Images

Single-label Endpoint Measurements

Plate View (Cell Module): Values for the Cell Module depend on the measurement type and may include cell concentration, cell viability or cell confluence values.

Plate View (Cell Imager): Includes images per well. Analysis values for the Cell Imager are displayed in **Analysis** view. Only single well selection is possible.

Graph View: Includes the image of the selected well.

Multi-label Endpoint Measurements

Plate View (Cell Module): Values for the Cell Module depend on the measurement type and may include cell concentration, cell viability or cell confluence values.

Plate View (Cell Imager): Includes images per well. Analysis values for the Cell Imager are displayed in **Analysis** view. Only single well selection is possible.

Graph View: Images are displayed separately, not in parallel with other measured data. Select an image tile for its display. It is possible to move to any of adjacent wells by tapping the corresponding **well control** placed left, right, top and bottom of the graphic display.

Single-label Kinetic Measurements

Plate View (Cell Imager): Includes cell confluence values for the last kinetic cycle. Only single well selection is possible.

Plate View (Cell Imager): Includes image per well for the last kinetic cycle. Analysis values for the Cell Imager are displayed in **Analysis** view. Only single well selection is possible.

Graph View: Includes the image of the selected well. It is possible to move to any of adjacent wells by tapping the corresponding **well control** placed left, right, top and bottom of the graphic display. Use the **+** and **–** controls included in the graphic to change the kinetic cycle number.

Multi-label Kinetic Measurements

Plate View (Cell Module): Includes cell confluence values for the last kinetic cycle. Only single well selection is possible.

Plate View (Cell Imager): Includes image per well for the last kinetic cycle. Analysis values for the Cell Imager are displayed in **Analysis** view. Only single well selection is possible.

Graph View: Images are displayed separately, not in parallel with other measured data. Select an image tile for its display. It is possible to move to any of adjacent wells by tapping the corresponding **well control** placed left, right, top and bottom of the graphic display. Use the **+** and **–** controls included in the graphic to change the kinetic cycle number.



NOTE: Make use of an optional export of images displayed in the plate view after the measurement. The captured picture is saved as a file in the corresponding measurement workspace.

2.9.10 Batch Processing

The measured data for each microplate is displayed according to the rules described above.

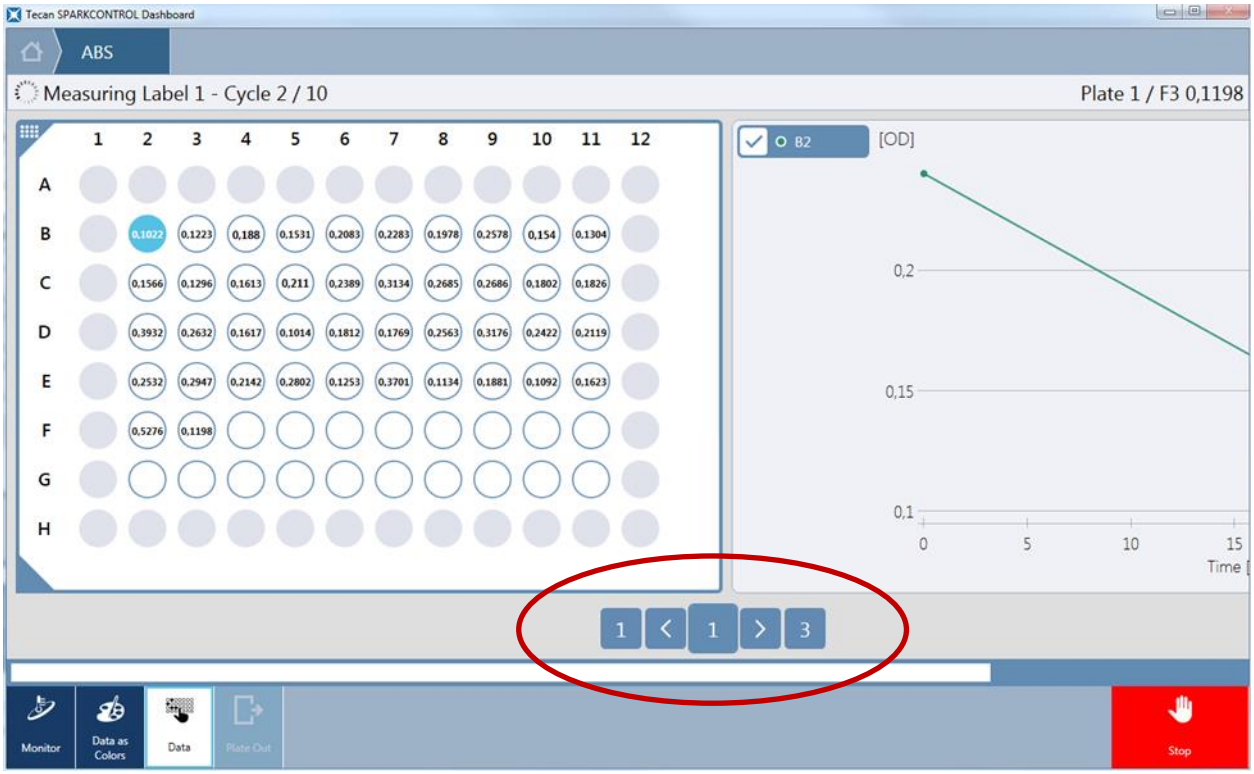


Figure 19: Plate Control

The plate control consists of the following sub-controls:

Plate Control Left: Displays the number of the first measured plate (i.e. Plate 1), click this button to move automatically to Plate 1.

Left Arrow: Click this button to move to the previous plate.

Plate Control Middle: Displays the number of the currently selected plate. To switch to a specific plate, select this control and enter the corresponding plate number.

Right Arrow: Click this button to move to the next plate.

Plate Control Right: Displays the number of the last measured plate, click this button to move automatically to the last plate.

2.9.11 Analysis

Analysis and calculated data are displayed in two different views: the **Plate** view and the **Graph** view. The display of data follows the same general rules as described in chapter 2.9.7 Data.

The analysis labels may also contain different sublabels, e.g. object size within the Counting analysis. For selecting/deselecting the display of the **sublabels** of a label, select the more icon ••• in the upper right corner of the **Graph** view.



Figure 20: Analysis screen

2.9.12 Curves

Graphs such as standard curve or dose-response curve are displayed as graphic under the **Curves**.

2.10 Starting a Method

2.10.1 Method Editor

A method can be started directly from the Method Editor by clicking the **Start** button. After starting a method, the software will switch to Dashboard view.

2.10.2 Dashboard

A method can be started directly from the Dashboard by selecting the corresponding **Method** tile. See chapter 2.9.5 Starting a Method.

2.10.3 Onboard-Start

A method can be started directly by pressing the **Onboard-Start** button on the instrument.

Define a method for the Onboard-Start as follows:

- Define a method and save it
- Select **Onboard-Start** via File menu of the Method Editor

Or

- Open a method
- Select **Onboard-Start** via File menu of the Method Editor

For watching the measurement progress of a measurement started via the Onboard-Start button, open the Dashboard and select the instrument tile of the working instrument.

2.11 Measurement Results

The export mechanism writes files according to the Office Open XML file format (.xlsx). The results are saved automatically and can be found under default path or under the path defined by the user.

Default path:
SparkControl version < 4.0: C:\Users\Public\Documents\Tecan\SparkControl\Workspaces

SparkControl version ≥ 4.0: C:\Users\Public\Documents\Tecan\SparkControlStore\Workspaces

Dependent on Result presentation settings (see chapter 2.13.3 Data Handling), the results can be opened automatically after the measurement run in Excel.

2.12 Method File Explorer

The Method File Explorer includes a list of all available methods files. The Method File Explorer can be accessed via the Method Editor.

2.13 SparkControl Settings

2.13.1 Structure

The **Settings** component is designed to allow the user the customization of the system default settings. These settings can be modified for

- Software
- Instrument
- Data Handling
- Plate Geometry
- Images
- Directory

by selecting the corresponding program tile.

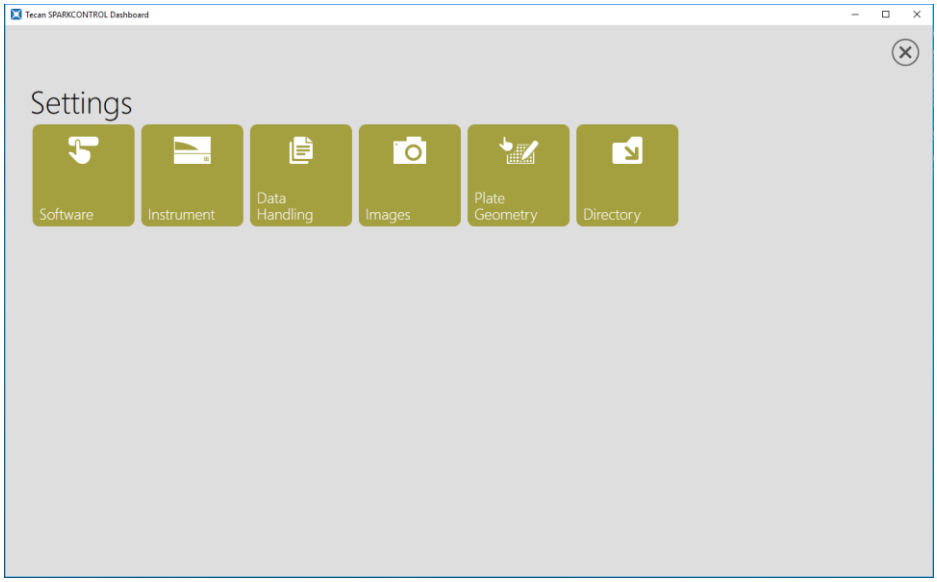


Figure 21: Settings window

The **Settings** component is optimized for working with touch using program tiles, tabs and buttons (see chapter 2.9 Dashboard).

2.13.2 Instrument

The following settings per connected instrument can be modified:

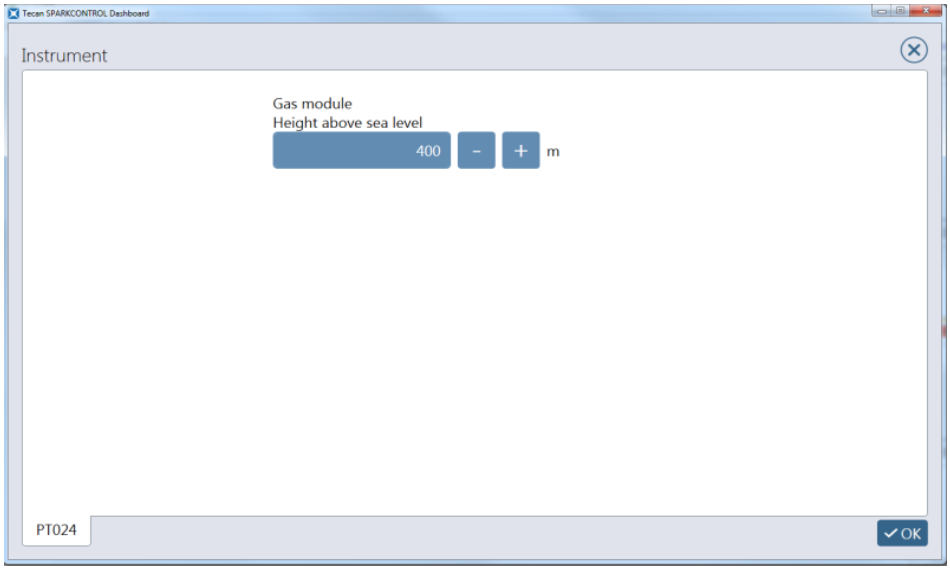


Figure 22: Instrument settings

Gas module	Available only for instruments with the Gas module. Enter the height above sea level.



CAUTION: Before the first use of the Gas module, enter the height above sea level.

2.13.3 Data Handling

Default Export to Excel

This window determines the output settings of the measured results in Excel.

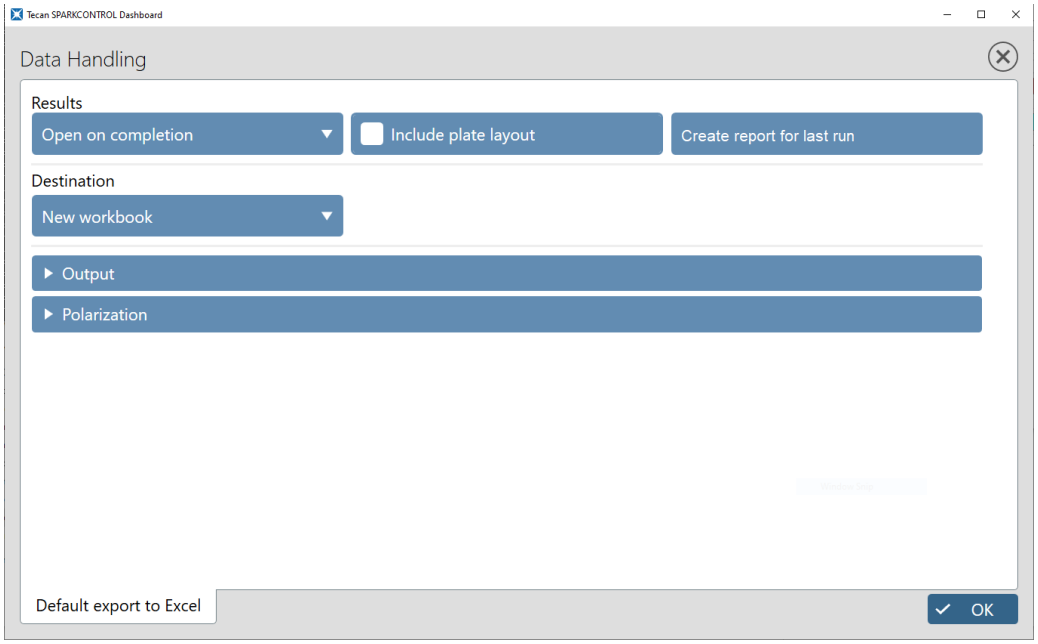


Figure 23: Data Handling window

Results

For export handling after the method execution, select one of the following destinations:

Open on completion	If selected, the result sheet is opened after a SparkControl method is performed. This option is combinable only with the result destination **New workbook.**
Open on completion with Excel	If selected, the result sheet is opened in Excel after a SparkControl method is performed. This option is combinable with all result destinations.
Do not open on completion	If selected, the result sheet is not opened after a SparkControl method is performed.

Include plate layout

If selected, the plate layout is included in the result sheet.

Create report for last run

If selected, the Excel export file of the last executed measurement is created.

Destination

For result presentation, select one of the following destinations:

New workbook	If **New workbook** is selected, a new workbook is generated every time a measurement script is performed.
New worksheet	If **New worksheet** is selected, results are written into a new worksheet that is added to the workbook automatically generated by SparkControl after the first measurement. A new workbook is generated daily. However, it is possible to generate another workbook by selecting **Create new** button, i.e. after its selection the software starts adding worksheets into a new workbook.

Existing workbook	If **Existing workbook** is selected, a new worksheet will be added into a pre-defined workbook (an Office Open XML file format (.xlsx)). Select the workbook, the results shall be placed into.



NOTE: Destinations **New worksheet** and **Existing workbook** are combinable only with the Results settings **Open on completion with Excel**.



NOTE: Destination settings will be ignored when performing stacker runs with the integrated Spark-Stack. Each stacker run will create a new workbook with single worksheets, each containing the measured data of the corresponding plates.

Output Design

Measurement mode: Select between Endpoint, Endpoint scan, Kinetic and Kinetic scan.

Endpoint:

Show	Select between **All** and **Measured**. If **All** is selected, the whole plate geometry, including all possible rows and columns, is displayed. If **Measured** is selected, only the results of the measured wells are displayed.
Export mode	Select between **Matrix** and **List**. If **Matrix** is selected, the data alignment corresponds to the microplate.
List orientation (for export mode List only)	Select between **Horizontal** or **Vertical**. If **Vertical** is selected, the wells are aligned in a column (in the Excel sheet). If **Horizontal** is selected, the wells are displayed in a row (in the Excel sheet).
Well order (for export mode List only)	Select between **A1A2** or **A1B1**. If **A1A2** is selected, the results are arranged in rows (of the microplate). If **A1B1** is selected, the results are arranged in columns (of the microplate).
Time (for export mode List only)	Select between **No time** or **Time per well**. If **No Time** is selected, only the values are displayed. If **Time per well** is selected, a timespan for each value is displayed.

Endpoint scan:

Well order (for export mode List only)	Select between **A1A2** or **A1B1**. If **A1A2** is selected, the results are arranged in rows (of the microplate). If **A1B1** is selected, the results are arranged in columns (of the microplate).
Time (for export mode List only)	Select between **No time** or **Time per well**. If **No Time** is selected, only the values are displayed. If **Time per well** is selected, a timespan for each value is displayed.
Scan charts	If selected, the scan charts are included in the result sheet.

Kinetic:

Show	Select between **All** and **Measured**. If **All** is selected, the whole plate geometry, including all possible rows and columns, is displayed. If **Measured** is selected, only the results of the measured wells are displayed.
Export mode	Select between **Matrix** and **List**. If **Matrix** is selected, the data alignment corresponds to the microplate.
List orientation (for export mode List only)	Select between **Horizontal** or **Vertical**. If **Vertical** is selected, the wells are aligned in a column (in the Excel sheet). If **Horizontal** is selected, the wells are displayed in a row (in the Excel sheet).
Well order (for export mode List only)	Select between **A1A2** or **A1B1**. If **A1A2** is selected, the results are arranged in rows (of the microplate). If **A1B1** is selected, the results are arranged in columns (of the microplate).

Time (for export mode List only)	Select between **Time per cycle** or **Time per well**. If **Time per cycle** is selected, a timespan per cycle is displayed. If **Time per well** is selected, a timespan for every well is displayed.



NOTE: For kinetic measurements it is recommended to select 'List' as Export mode in order to facilitate data analysis in Excel.

Kinetic scan:

Well order (for export mode List only)	Select between **A1A2** or **A1B1**. If **A1A2** is selected, the results are arranged in rows (of the microplate). If **A1B1** is selected, the results are arranged in columns (of the microplate).
Cycle display	Select between **Horizontal** or **Vertical**. If **Vertical** is selected, the cycles are displayed in a column (in the Excel sheet). If **Horizontal** is selected, the cycles are displayed in a row (in the Excel sheet).
Time	Select between **Time per cycle** or **Time per well**. If **Time per cycle** is selected, a timespan per cycle is displayed. If **Time per well** is selected, a timespan for every well is displayed.

Polarization

Select the output for fluorescence polarization measurements, e.g. polarization, anisotropy, total intensity, etc.

Inject and Read

Select the output for Inject and Read measurements by defining:

Show	Select between **All** and **Measured**. If **All** is selected, the whole plate geometry, including all possible rows and columns, is displayed. If **Measured** is selected, only the results of the measured wells are displayed.
List orientation	Select between **Horizontal** or **Vertical**. If **Vertical** is selected, the wells are aligned in a column (in the Excel sheet). If **Horizontal** is selected, the wells are displayed in a row (in the Excel sheet).
Well order	Select between **A1A2** or **A1B1**. If **A1A2** is selected, the results are arranged in rows (of the microplate). If **A1B1** is selected, the results are arranged in columns (of the microplate).

2.13.4 Software

The following startup behavior for the SparkControl can be modified:

Method Editor

Default plate	Select the plate type to be shown as default in the plate strip when defining a new method. It is possible to define up to four default plates. These plates are listed as favorites on top of the list of the available plate files in the **Plate** strip.
Default strip values	**Absorbance** Define default **Pathlength correction** values: **Test wavelength** and **Correction factor**

2.13.5 Plate Geometry Editor

SparkControl offers a wide selection of predefined plate definition files. Use Plate Geometry Editor to create plate definition files for not listed plates or to edit an existing plate definition file. Select an existing plate file and modify it according to the plate demands of your choice. After editing save the plate definition file under a different name.

General

Enter the information about the plate manufacturer, color and material.

Plate

Enter the plate dimensions (e.g. number of rows, columns, length, size, height, etc.) including the bottom thickness and refractive index.



Figure 24: Editing a plate definition file

NOTE: Measure with a caliper ruler or better use values from the plate design drawings, given by the plate manufacturer.

CAUTION: When you manually measure the plate height, be aware that any plate tolerances caused by the production process of the plate will not be covered.

Well

Edit the well dimensions (form, size, depth, working volume, etc.).

NOTE: Be careful with settings of µm and µl values.

2.13.6 Images

Use this window to modify the file format of acquired images in combination with the Cell Module.

File format

Select jpg (compressed image file) or tiff (uncompressed image file).



NOTE: If images cannot be opened because the User Account Control (UAC) of the operating system is disabled, enable the UAC or choose another default program for opening the selected image file format.

2.13.7 Directory

For every executed measurement, SparkControl generates a workspace folder. Each workspace folder may contain four subfolders, the contents of which, however, depend on the measurement performed:

Database	A database file with raw data is created for every measurement.
Export	**xlsx:** Excel compatible result files are generated for every measurement and, depending on the Data handling settings, automatically saved in the **xlsx** folder. **pdf:** For fluorescence imaging measurements and Tecan apps (e.g. Cell counting, NanoQuant), a pdf report is created and saved in the **pdf** folder. **Images:** If images are exported from ResultAnalyzer, they are saved in the **Images** folder. **csv:** Includes object list reports resulting from 2D and 3D imaging measurements as csv files. **Video:** Includes time-lapse videos generated via ResultAnalyzer. **PlateView:** Includes files with images displayed in plate view if exported after measurement. **StandardCurve:** Includes generated standard curve(s) as html file. **DoseResponseCurve:**Includes generated dose-response curve(s) as html file.
Images	Contains acquired original images of measurements with the Cell Module and the Cell Imager module.
Methods	Contains methods(s) after they have been exported from the corresponding workspace via ResultAnalyzer.
Plugin folder	Contains plugin related data in corresponding subfolders. For more information see the corresponding Plugin Instructions.
ResultAnalyzer	Contains ResultAnalyzer database and is created after a workspace has been opened in ResultAnalyzer.



CAUTION: Never change the name of the workspace subfolders. Changing the subfolder name, especially the **Images** subfolder, will break compatibility with ResultAnalyzer due to failed allocation of images for the respective workspace file.

Define the export path for measurement results. Make sure that the NETWORK SERVICE account has Full control or at least Special permission for the selected folder.

Default settings:

SparkControl version < 4.0: C:\Users\Public\Documents\Tecan\SparkControl\Workspaces
SparkControl version ≥ 4.0: C:\Users\Public\Documents\Tecan\SparkControlStore\Workspaces

NOTE: When the Method Editor or Dashboard is not present at the time that the data export runs (i.e. software closed) and the method is started via the **Onboard-Start** button, the option **Existing workbook** will be ignored and treated as the option **New workbook**.

NOTE: When defining a user-defined path, always make sure that the NETWORK SERVICE account has Full control or at least Special permission for the selected folder.

2.14 Screencasts

The **Screencasts** component is designed to allow the user to view recorded example workflows for working with SparkControl.
For starting the display, select the corresponding record tile.

3 ResultAnalyzer

The ResultAnalyzer software is designed for analyzing measurement results after a method execution with SparkControl. Raw data, evaluation data and evaluation parameters can be viewed, and data can be re-analyzed. For further information, see the instructions of the analysis plugins.

ResultAnalyzer allows the user to add new analysis strips or modify the strips already included in the originally executed method.



NOTE: Every modification of the method will trigger a re-calculation of evaluated data. These changes are saved in the workspace but will not modify the original method itself. They have to be exported from the ResultAnalyzer separately.



NOTE: ResultAnalyzer supports post-analysis of measured data for all detection modes (absorbance, imaging, etc.). Few exceptions, though, are related to the applied measurement mode and selected method type: Tecan Apps (e.g. NanoQuant App, Cell Counting/Viability App) as well as workspace with scan data or multiple reads per well (incl. area scan) cannot be opened and subjected to further evaluation in ResultAnalyzer.



CAUTION: Avoid working with ResultAnalyzer and SparkControl in parallel to ensure maximum performance of the ResultAnalyzer software.

3.1 Workspaces

Open ResultAnalyzer and select a **workspace** to work with. If workspaces are not available at the predefined default path go to **File/Directory** and define the new default path (see chapter 2.11 Measurement Results).

When opening a workspace in ResultAnalyzer, all measured and analyzed data are shown under **Results/Measurement**. Depending on the executed method, these are raw data, image analysis data, calculated data and graphs.

To view acquired images and their analysis only, select **Images/Measurement**.



NOTE: In measurements with imaging, analysis strips for (re)analysis of images are located in the **Images** tab. They have to be considered in a separate workflow before making changes in the **Result** screen. The generated image analysis data (e.g. cell count, fluorescence intensity) are available as Input data for use in the evaluation strips.

Structure

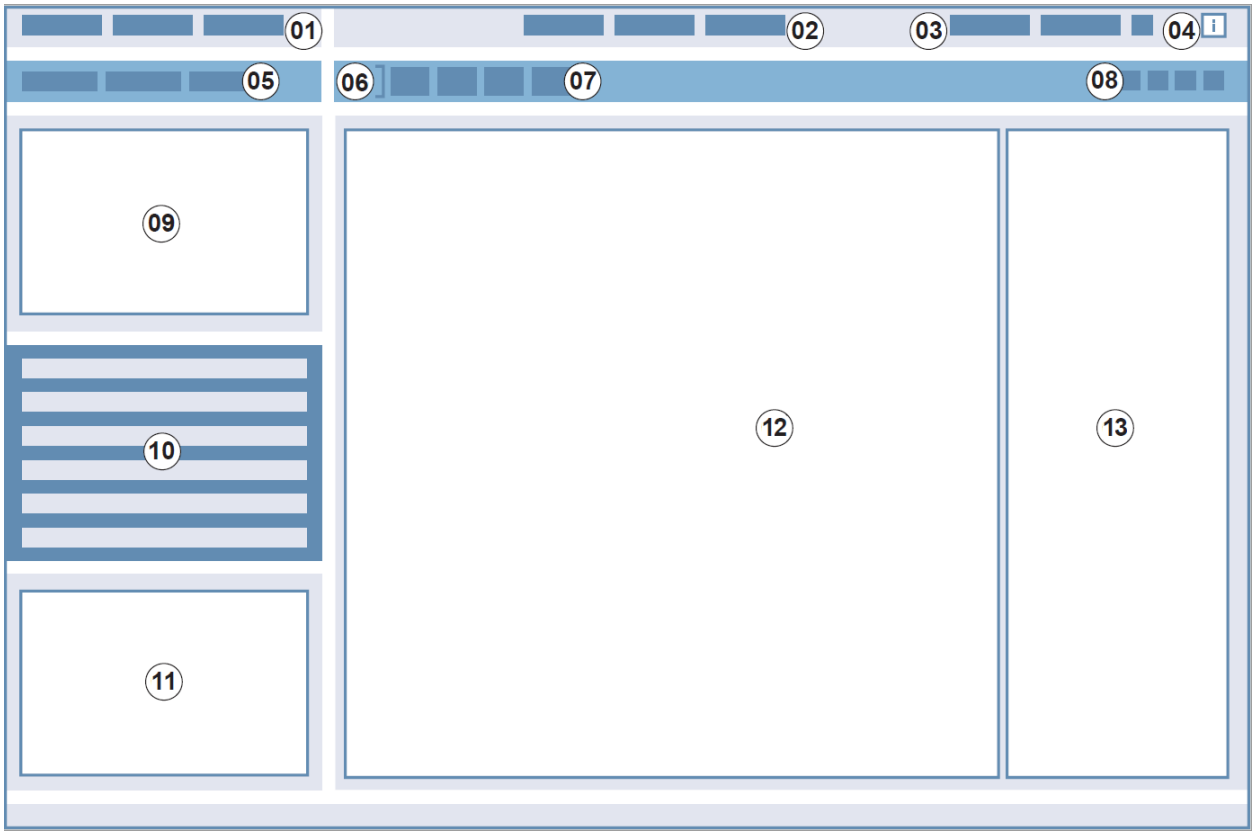


Figure 25: Structural elements of the ResultAnalyzer
01 Menu bar; 02 Method definition tabs; 03 Toolbar; 04 Button Info pane; 05 Drop-down lists; 06 Selected well; 07 Dynamic image composition; 08 Context-sensitive toolbar; 09-12 Result area; 13 Split screen

Menu bar	**01**	Contains a drop-down menu of editor functions (File, View and Help)
Method definition tabs	**02**	Contains **Images** tab (workspace incl. imaging): switch to Measurement (View mode), Processing and Analysis (Edit mode) for images and image analysis results and Edit method (Edit mode) to define/modify the analysis of data **Results** tab: switch to Measurement (View mode) for raw data, image analysis data, calculated data and graphs and Edit method (Edit mode) to define/modify the analysis of data
Toolbar	**03**	Provides access to user interface buttons (e.g. Apply, Preview) and image-related functions (e.g. export of composed images, capture image and selection of scale bar)
Button Info pane	**04**	Opens the info pane and displays the workflow-relevant information
Drop-down lists	**05**	Select e.g. label, sublabel, kinetic cycle, plate number for display in the Result area
Selected well	**06**	Displays the selected well and, if expanded, the information about the method settings
Dynamic image composition	**07**	Images tab only: Includes channel-related icons for generation of user-defined composed images
Context-sensitive toolbar	**08**	Images tab only: Contains icons for image adjustment and analysis area (e.g. region of interest (ROI), time-lapse videos, brightness and contrast, mask outline)

Result area	**09** **10** **11** **12**	Images tab: Includes **Image(s)** per selected well and analysis results displayed in a **Plate**, **List** and **Graphic** view. Contains the central, enlarged area and three minimized areas. Results tab: Includes analysis results displayed in a **Plate**, **List** and **Graphic** (e.g. standard curve, dose-response curve) view. Contains the central, enlarged area and minimized areas.
Split screen	**13**	Images tab only: Opens a screen for defining/modifying Processing/Analysis settings

3.2 Evaluate Results

All available data are displayed in the **Measurement** section. In the Result area, there are several different viewing options:

- Selected data that presents a value for each well is displayed in the plate view. If supported, it can be viewed in pseudo-colors together with a colors scale.
- Kinetic data can be viewed as a small graph in each well.
- Selected data that presents a data collection which cannot be assigned to a single well is displayed in a single well only. (e.g. calculated Z' value, average value).
- Graphic presentation of a standard curve and dose-response curve.
- Images (see also chapter 9.2.2 ResultAnalyzer).

The Plate, List and Graphic views contain round navigation controls at the bottom of the window, if multiple result sets per view are available. Use the controls to move through the different result sets.

3.2.1 Changing the Analysis Method

To change to the currently used analysis method and its settings select

- **Images/Analysis** for viewing and analyzing images per selected analysis strip
- **Images/Edit method** for adding/modifying the analysis method for image
- **Results/Edit method** for adding/modifying the analysis method of numeric values (measured data and analyzed image values). For further information, see the Evaluation Plugin instructions.

These changes can be saved in the workspace, however, will not be applied to the originally inserted method file itself.

Images Tab

For recalculation of data, the ResultAnalyzer offers the following options:

Preview	Modified parameters are applied to the image analysis (whole image or area of interest within an image) of the selected well but **not saved**. Available in the Analysis view.
Apply to well	Modified parameters are applied to the image analysis of the selected well and **saved**. Available in the Analysis view.
Apply to plate	Modified parameters are applied to the image analysis of the whole measurement area and **saved**. Available in the Analysis view.
Apply to all plates	Stacker runs: modified parameters are applied to the image analysis of the whole measurement area for all measured plates and **saved**. Available in the Analysis view.
Analyze and apply	Images are analyzed with modified analysis settings and the results automatically applied (saved). Available in the Edit method view.
Add strips and apply	Newly defined strip(s) are added and saved without executed analysis. Available in the Edit method view.

Reset	Modified analysis parameters are cleared and the last saved analysis parameters restored.

Results Tab

For recalculation of data, the ResultAnalyzer offers the following options:

Preview	Defined analysis parameters are applied to the subject of analysis but **not saved**.
Apply	Defined analysis parameters are applied to the subject of analysis and **saved**.
Reset	Modified analysis parameters are cleared and the last saved analysis parameters restored.



NOTE: Recalculated data are automatically saved by selecting the corresponding **Apply** button. The **Preview** function will only recalculate but not save the data.

3.2.2 Mask Value

To exclude the value of a well from the calculation, select **Results/Measurement**. In the Measured Results view, right-click on the well and select **Mask well**. Data will then be shown in brackets. The remaining values are automatically reanalyzed and displayed in Preview mode. To save changes, select Apply.

To undo masking, right-click on the well and select **Unmask well**. Select Apply to save changes.



NOTE: Masking can be applied only to raw measurement values and image analysis values. Each selected well must be masked individually. For measurements that include kinetic cycles, masking must be applied separately to each cycle. When an image analysis value is masked, all image analysis values from all imaging channels for the selected well will be excluded.



NOTE: Use the Mask function to exclude wells that contain an invalid value (such as values out of range, indicated by ‘OVER’, or wells with an autofocus error) from calculations that include these wells (e.g. calculating an average value or generating a standard curve). Otherwise the calculation cannot be executed.

3.3 Export Results

After the re-evaluation of data, the new results are not exported automatically to Excel and/or PDF report. Select **Export Results** in the File menu of the ResultAnalyzer to export the corresponding files. Exported file(s) are generated and saved according to the Workspace path defined in **File/Directory**.

The content of the analysis results is defined for each application and consequently cannot be changed by the user.

3.3.1 Export Analysis

If the analysis settings have been modified, the new parameters can be transferred and saved to the corresponding method. To use a modified method in SparkControl, the method has to be exported from the ResultAnalyzer and then imported to SparkControl.

To export a method select **File/Export Analysis from well to method** (the analysis parameters will be exported from the currently selected well; available only for workspaces with imaging) or **Export Analysis method**. The method is exported to the Methods folder as defined in **File/Directory**. Note that the export path for the Methods folder depends on the Workspace path and is not editable by the user. Finally, open the Method Editor of SparkControl and import the method via **File/Import**.

4 Luminescence

4.1 Defining Luminescence Measurements

The software provides three separate strips for defining measurement parameters:

- Luminescence
- Luminescence Multicolor
- Luminescence Scan

The availability of the strips depends on the configuration of the instrument connected.

4.1.1 Luminescence Strip

The **Luminescence** strip is used to perform luminescence measurements with or without luminescence filters.

Instrument with Standard Luminescence Module

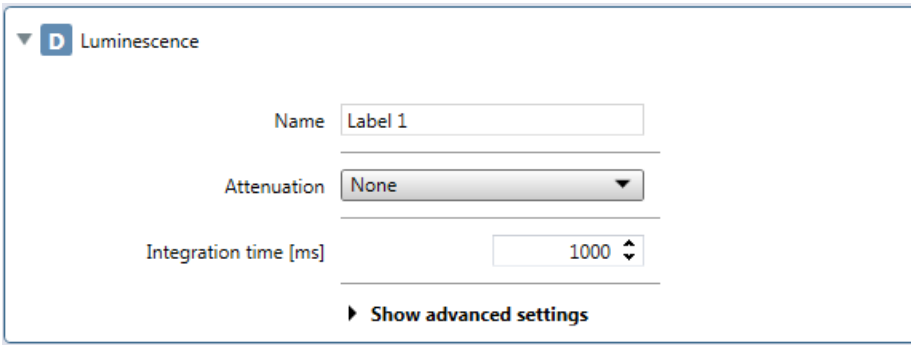


Figure 26: Luminescence strip for the standard luminescence module

The **Luminescence** strip includes the following elements:

Name	Enter a label name.
Attenuation	Select the attenuation settings for the measurement: • **None:** no attenuation applied • **OD1:** signal intensity is attenuated by the factor 10 • **OD2:** signal intensity is attenuated by the factor 100 • **Automatic:** attenuation settings are defined and set automatically for each well
Integration time [ms]	Define the duration time for the signal integration. Enter a value within the valid instrument range.

NOTE: Luminescence signals measured with the attenuation filter OD1 and OD2 are automatically corrected by a factor of 10 and 100, respectively.

Settle time [ms]	Define the time delay between plate movement and the start of signal integration. Enter a value within the valid instrument range.
Output	Define the signal readout. Select one of the following options: • **Counts:** the readout is displayed as total counts measured per well • **Counts/s:** the readout is displayed as counts per second measured per well
Show advanced settings	Click here to access **Settle time** and **Output** settings.

Instrument with Enhanced Luminescence Module

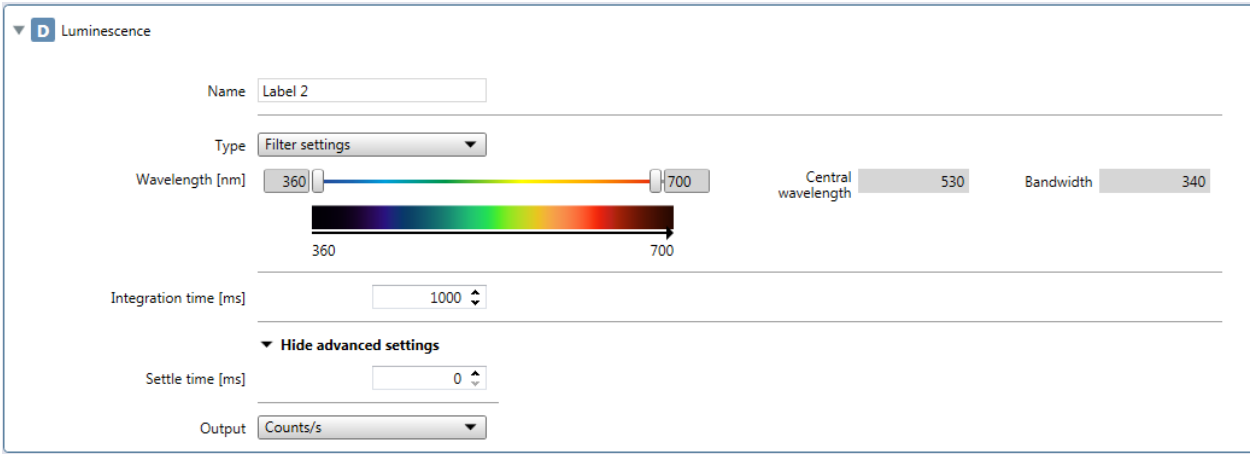


Figure 27: Luminescence strip with interactive graphic for the enhanced luminescence module

The **Luminescence** strip includes the following elements:

Name	Define a label name.
Type	Select the measurement type: • **Attenuation** for measurement signals with no wavelength discrimination • **Filter settings** for measurement signals with wavelength discrimination
Attenuation	Define the attenuation settings for the measurement. Select one of following options: • **None:** no attenuation applied • **OD1:** signal intensity is attenuated by the factor 10 • **OD2:** signal intensity is attenuated by the factor 100 • **OD3:** signal intensity is attenuated by the factor 1000 • **Automatic:** the appropriate attenuation settings are defined and set automatically for each well

NOTE: Luminescence signals measured with the attenuation filters OD1, OD2 and OD3 are automatically corrected by the factor 10, 100, and 1000, respectively.

Filter settings	Define the band pass filter for signal detection. Select the **From** and **To** wavelength by moving the respective sliders. Switch to the numeric interface by clicking the List button in the upper right-hand corner of the strip. Select the appropriate wavelength values from the corresponding drop-down lists.
Integration time [ms]	Define the duration time of the signal integration. Enter a value within the valid instrument range.

NOTE: When working with band pass filters, the central wavelength with the bandwidth resulting from the corresponding filter settings is automatically displayed.

Settle time [ms]	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.
Output	Define the signal readout. Select one of the following options: • **Counts:** the readout is displayed as total counts measured per well • **Counts/s:** the readout is displayed as counts per second measured per well
Show advanced settings	Click here to access the **Settle time** and **Output** settings.

4.1.2 Multicolor Luminescence Strip

Select the strip to set-up a user-defined multicolor luminescence measurement or use the strip to select one of the pre-defined settings optimized for common commercial applications.

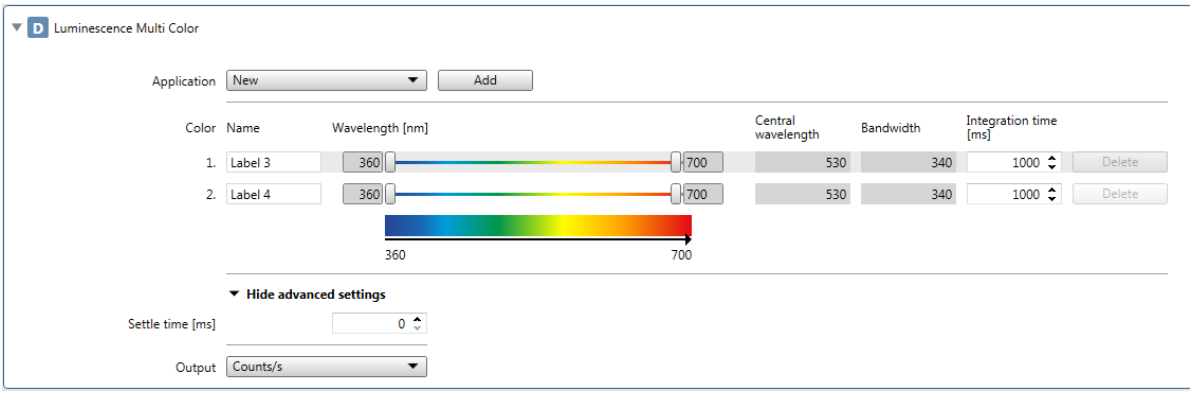


Figure 28: Multicolor Luminescence strip with interactive elements

The **Multicolor Luminescence** strip includes the following elements:

Application	Select **New** to define a new application. Select the application name (e.g. BRET1, BRET2, BRET3 or Chroma-Glo) to work with the predefined filter settings optimized for each respective application.
Add	Click the **Add** button to add a label to the list.
Color	Indicates a number of labels to be measured. A minimum of two and a maximum of five can be defined.
Name	Define a name for each label.
Filter settings	Define the band pass filter used for the signal detection of each label: • Select the **From** and **To** wavelengths by moving the respective sliders • Switch to the numeric interface by clicking the List button in the upper right-hand corner of the strip. Define the **From** and **To** wavelengths by selecting the appropriate wavelength values from the corresponding drop-down lists
Integration time [ms]	Define the duration time of the signal integration for each label. Enter a value within the valid instrument range.
Delete	Click the **Delete** button to remove a label from the list.

NOTE: When working with band pass filters, the central wavelength and bandwidth are automatically displayed according to the corresponding filter settings.

Settle time [ms]	Define a time delay between the plate movement and the start of signal integration. Enter a value within the valid instrument range.
Output	Define the signal readout. Select one of the following options: • **Counts:** the readout is displayed as total counts measured per well • **Counts/s:** the readout is displayed as counts per second measured per well
Show advanced settings	Click here to access the **Settle time** and **Output** settings.

4.1.3 Luminescence Scan Strip

Select the strip to perform luminescence scans.

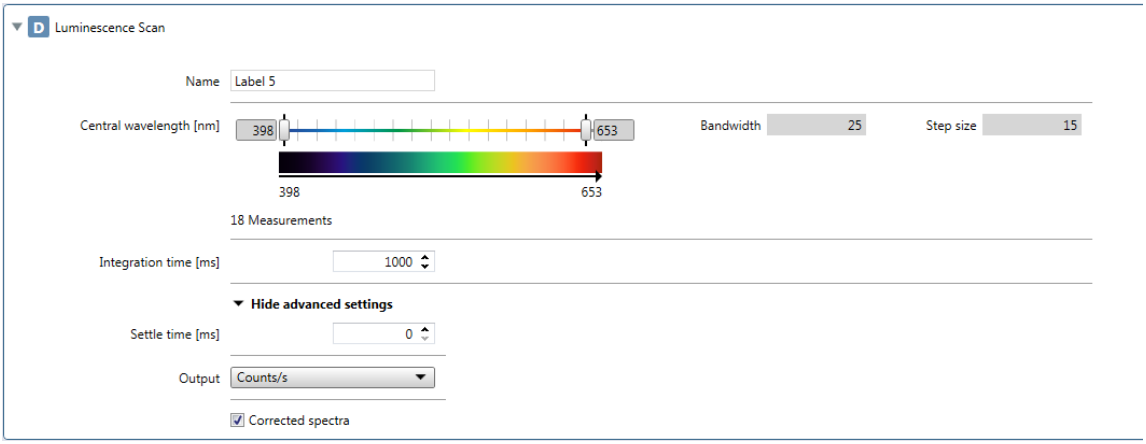


Figure 29: Luminescence Scan strip

The **Luminescence Scan** strip includes the following elements:

Name	Define a label name.
Central wavelength [nm]	Define the central wavelength range to be used for the luminescence scan. • Select the **From** and **To** central wavelengths by moving the respective sliders. • Switch to the numeric interface by clicking the List button in the upper right-hand corner of the strip. Select the appropriate wavelength values from the corresponding drop-down lists.
Integration time [ms]	Define duration time for the signal integration for each label. Enter a value within the valid instrument range.

NOTE: The luminescence scan is performed at discrete central wavelengths resulting from the combination of the luminescence filters. The wavelength range is defined by the first and the last central wavelength that also represent the starting and the end point of the scan. All remaining measurement points are automatically derived from the range settings.

NOTE: The bandwidth and step size of the luminescence scan measurements are fixed and cannot be changed by user.

4.1.4 Corrected Spectra

The luminescence values are influenced by instrument components as well as by the selected wavelengths and therefore may distort the measured spectra. Calibration data is saved on the instrument and intensity value corrections are performed automatically.

4.1.5 Measurement Mode

Luminescence measurements can be defined and performed as:

- Single-label endpoint or kinetic measurements
- Multi-label endpoint or kinetic measurements

All measurements, except luminescence scans, can be carried out plate-wise or well-wise. Luminescence scans can only be performed in a plate-wise manner only.

4.2 Optimizing Luminescence Measurements

For optimal performance, it is recommended to use white plates for luminescence measurements.

For enzymatic reactions, care has to be taken to ensure stable environmental conditions for the measurement.



CAUTION: Switch on the instrument at least 15 minutes before starting a luminescence measurement to ensure stable conditions for the measurement.

4.2.1 Integration Time

At very low light intensity, the measured counts per second are proportional to the light intensity. Increasing the measurement time per well yields more accurate values because of the irregular photon impact (photon statistics). The photonic noise (shot noise) cannot be reduced further by technical means.



NOTE: If a luminescence measurement results in an OVER in one or more wells because the measured signal was too high, the luminescence detector may need a certain amount of time to return to the equilibrium baseline count level.

4.2.2 Light Attenuation

If the photons entering the photon counting module cannot be distinguished as distinct single exit pulses, results are marked as OVER. The signals can be attenuated by moving neutral density filters into the light path. The OD1 and OD2 neutral density filters, as well as the OD3 option, serve to attenuate high light levels by a factor of 10, 100, and 1000, respectively.

5 Alpha Technology

5.1 Defining Alpha Measurements

The SparkControl software provides a strip for measuring:

- AlphaScreen
- AlphaLISA
- AlphaPlex
- User-defined Measurements

5.1.1 Alpha Technology

The **Alpha Technology** strip is available only for instruments with the Alpha module that includes luminescence enhanced and laser module. Select the strip to define methods based on Alpha Technology.

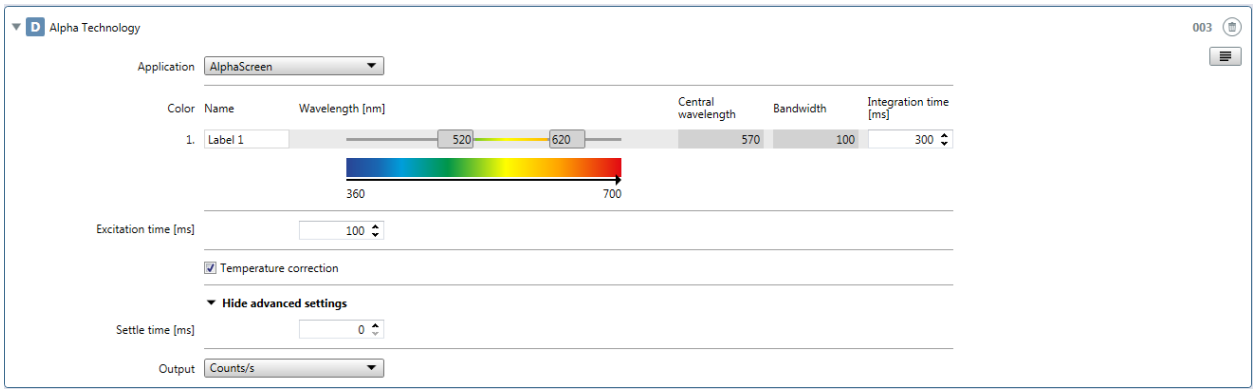


Figure 30: Alpha Technology strip

The **Alpha Technology** strip includes the following definitions:

Application	Select **New** to define a new application. Select the application name **AlphaScreen**, **AlphaLISA** or **AlphaPlex** to work with the predefined filter settings optimized for each respective application.
Add	Click the Add button to add a label to the list (available for the application **New** only).
Color	Indicates a number of labels to be measured. A minimum of one and a maximum of two can be defined.
Name	Define a name for each label.
Wavelength [nm]	Define the band pass filter used for the signal detection of each label: • Select the **From** and **To** wavelengths by moving the respective sliders (Central wavelength and Bandwidth are displayed accordingly) • Switch to the numeric interface by clicking the **List** button in the upper right-hand corner of the strip. Define the **From** and **To** wavelengths by selecting the appropriate wavelength values from the corresponding drop-down lists
Integration time	Define duration time for the signal integration. Enter a value within the valid instrument range.
Delete	Click the **Delete** button to remove a label from the list (available for the application **New** only).
Excitation time	Define duration time of excitation. Enter a value within the valid instrument range.
Temperature correction	Select the check box to activate the temperature correction function.

Settle time	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.
Output	Define the signal readout. Select one of the following options: • **Counts:** the readout is displayed as total counts measured per well • **Counts/s:** the readout is displayed as counts per second measured per well
Show advanced settings	Click here to access the **Settle time** and **Output** settings.

5.1.2 Measurement Mode

Alpha Technology based measurements can be performed as

- Single-label endpoint measurements
- Multi-label endpoint measurements

All measurements can be carried out plate-wise only.

5.2 Optimizing Alpha Technology based Measurements

5.2.1 Integration Time

Due to irregular photon statistics during signal integration longer integration times per well result in more accurate values. The photonic noise (shot noise) cannot be reduced technically but optimized in pretest experiments by applying different integration times.



NOTE: The relevant signal to (shot) noise ratio can be improved by longer integration times per well resulting in increased measurement times of the whole plate.

5.2.2 Excitation Time

The excitation time defines the duration of the sample illumination by the laser. Optimizing the excitation time for Alpha Technology based assays may help to minimize sample bleaching and improve the signal-to-noise ratio.

5.2.3 Dark Covers for Light Protection

For SPARK readers equipped with the optional Spark-Stack microplate stacker module, a set of dark covers for light protection (front cover and top cover) for the plate cassettes is available. These easily inserted elements help to protect plates with light-sensitive contents inside the plate magazines, from the ambient light in the lab. Therefore, we recommend using these dark covers for walk-away automation of Alpha Technology based measurements using the Spark-Stack plate stacker module (see chapter Light Protection for Sensitive Assays/Dark Covers in the SPARK – Reference Guide).

6 Absorbance

6.1 Defining Absorbance Measurements

The SparkControl software provides two separate strips for measuring:

- Absorbance
- Absorbance Scan

The availability of the strips depends on the configuration of the instrument connected.

6.1.1 Absorbance Strip

This strip is used for absorbance measurements.

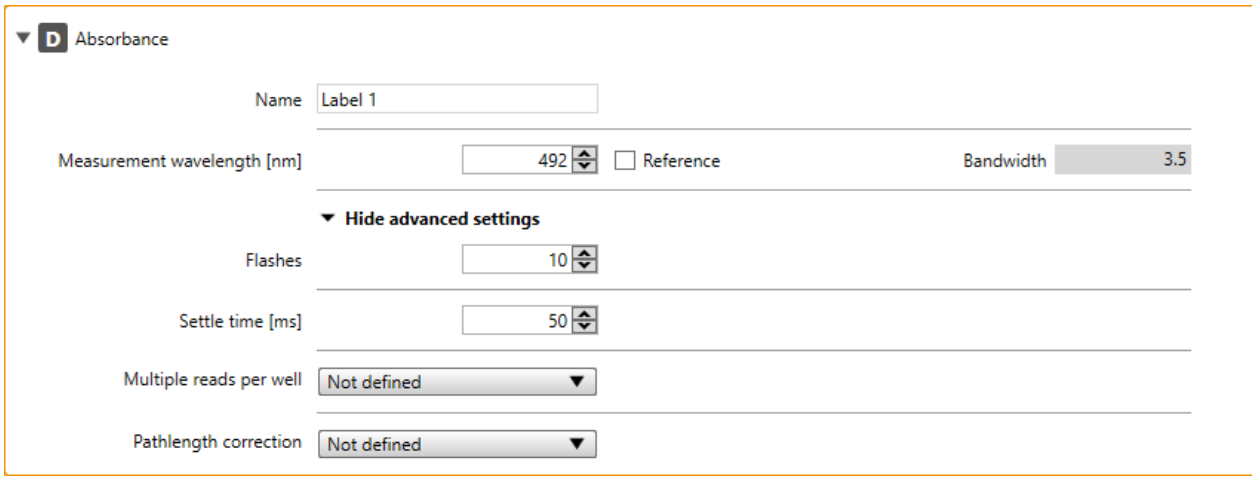


Figure 31: Absorbance strip for absorbance module

The **Absorbance** strip includes the following elements:

Name	Define a label name.
Measurement wavelength [nm]	Enter the measurement wavelength.
Reference	Enter the reference wavelength if required by the application.
Flashes	Enter the number of flashes. For optimal performance, use the default number of flashes.
Settle time	Define a time delay between the plate movement and the start of signal integration. Enter a value within the valid instrument range.
Multiple reads per well	Select **User defined** in order to define the **Type**, **Size** and **Border**, the multiple reads per well shall be performed with. Use **Area Scan** to produce a fine granulated picture of the absorbance distribution within a well.
Pathlength correction	If selected, all measured absorbance values are corrected to 1 cm pathlength.
Show advanced settings	Click this expand button to access the following parameters: **Flashes, Settle time, Multiple reads per well** and **Pathlength correction.**

Pathlength correction

Pathlength correction can be used to correct the measured absorbance values of samples in microplates to 1 cm pathlength, in order to compare the measurement results to those read with cuvettes or to perform quantitative analysis of samples based on their extinction coefficient.

According to the Lambert-Beer's law, the amount of absorbed light is proportional to the concentration of the sample and to the pathlength of the light passing the sample. Unlike a standard cuvette with a pathlength of 1 cm, the pathlength of the light in a microplate is unknown and depends on the filling volume of wells. For aqueous solutions, the pathlength can be calculated from the absorbance values for water recorded in the near infrared wavelength range (900 nm to 1000 nm) by using a cuvette and the respective microplate.



NOTE: The absorption of water is temperature dependent. Please make sure that all measurements are performed at exactly the same temperature.



NOTE: Any light absorption of assay components between 900 and 1000 nm will interfere with pathlength correction.



NOTE: Please be aware that buffers (salt concentration), organic solvents, meniscus and plate characteristics can affect the pathlength correction measurement.



CAUTION: Turbid samples can lead to a false estimation of pathlength due to scattering of light. Pathlength correction with cuvette will not compensate for this effect.

If **Pathlength correction** is selected, define the following parameters:

Test wavelength [nm]	Define a wavelength within the available wavelength range to determine the specific absorption of water in the aqueous sample. Recommended wavelengths: 977, 997, 998 and 1000 nm.
Reference wavelength [nm]	The background of water in the aqueous sample is measured at a **Reference wavelength** of 900 nm.
Correction factor	The **Correction factor** is defined as the absorbance value of water at the **Test wavelength** corrected by the absorbance value of water at the **Reference wavelength** for a pathlength of 1 cm. **Manual:** Define a manual value that has been previously calculated via water or sample buffer measurements at the **Test** and **Reference wavelengths** with a cuvette. **Cuvette:** If selected, the measurement with a cuvette filled with water or sample buffer in a cuvette port will be executed at the defined **Test** and **Reference wavelengths** for the aqueous sample.



NOTE: Please make sure that the manual correction factor matches the selected Test and Reference wavelengths of the aqueous sample and was determined with the corresponding sample buffer.

The **Pathlength** calculation of the sample is performed as follows:

$Pathlength_{Sample} = (A_{TW} – A_{RW})/(A_{Water}) * 1\ cm$

A_{TW} = Absorption of aqueous sample at Test wavelength

A_{RW} = Absorption of aqueous sample at Reference wavelength

A_{Water}= A_{TW} minus A_{RW} of water in a 1 cm cuvette (= Correction factor)

The calculated pathlength is finally used to correct the absorbance of sample (A_{Sample}) at specific dye wavelength to 1 cm ($A_{SampleCorrected}$):

$A_{SampleCorrected} = A_{Sample}/Pathlength_{Sample}$

6.1.2 Absorbance Scan Strip

This strip is used to perform absorbance scans.

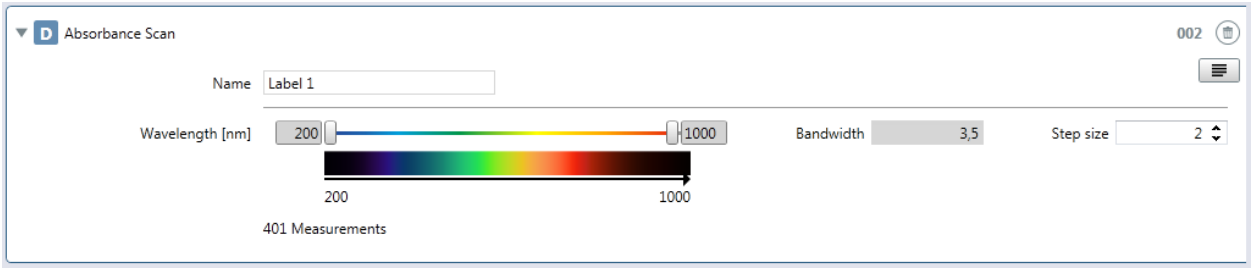


Figure 32: Absorbance scan strip

The **Absorbance Scan** strip includes the following elements:

Name	Define a label name.
Wavelength range	Define the wavelength range to be used for the absorbance scan. Select **From** and **To**, i.e. the lower and the upper wavelength limit, respectively. Optionally, use the graphic interface for the wavelength range. Switch to the graphic interface by clicking the ‘Graph’ button placed top right in the strip. Select the **From** and **To** central wavelength by moving the respective slider.
Bandwidth	3.5 nm
Step	Define a step size.

NOTE: To achieve maximum speed, absorbance scans are performed with one flash. Up to a step size of 4 nm a proportional increase of speed can be expected. If larger step sizes are defined the increase of measurement speed is no longer proportional to the selected step size.

6.2 Absorbance Measurement Modes

Absorbance measurements can be defined and performed as:

- Single-label endpoint or kinetic measurements
- Multi-label endpoint or kinetic measurements

All measurements, except absorbance scans, can be carried out plate-wise or well-wise. Multiple reads per well and absorbance scans can be performed plate-wise only.

6.2.1 Optimizing Absorbance Measurements

Flash Settings

Measurements with one flash per well are possible for all plate types. However, measurement precision at low light levels depends on the reading time over which the absorbance signal is being collected. By increasing the number of flashes, more accurate results can be achieved.



NOTE: Increase the number of flashes per well until the noise of the blank wells does not improve further, or until the measurement time per well becomes unacceptable.

Settle Time

Due to the stop and go motion of the plate carrier the liquids meniscus may vibrate during signal integration. Vibrations can cause fluctuations in the measured values, therefore to minimize this effect and to obtain optimal performance, it is recommended to select a settle time between move and flash between 100 and 300 ms.



NOTE: To obtain accurate measurement data apply a settle time for plate formats between one- and 96-well.

7 Fluorescence

7.1 Defining Fluorescence Measurements

The software provides three separate strips for defining measurement parameters:

- Fluorescence Intensity strip
- Time-Resolved Fluorescence Intensity strip
- Fluorescence Intensity Scan strip

The availability of the strips depends on the configuration of the instrument connected.

7.1.1 Fluorescence Intensity Strip

This strip is used for fluorescence intensity measurements.

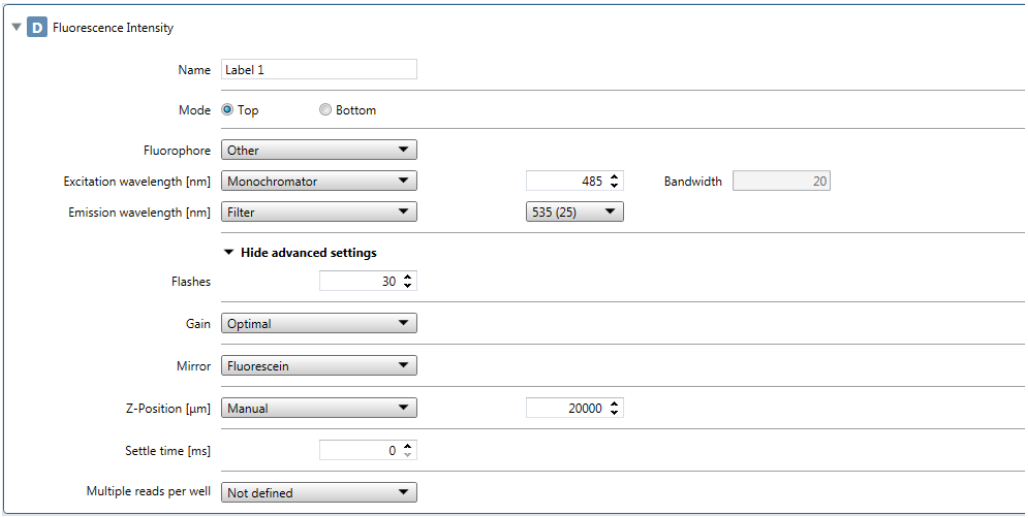
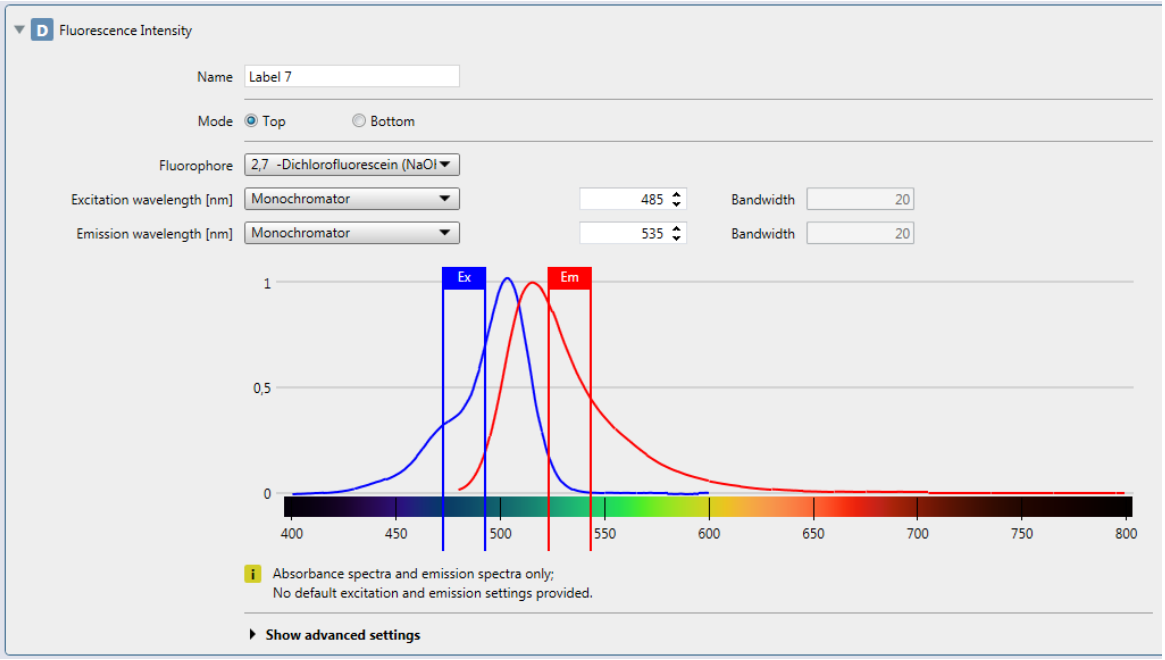


Figure 33: Fluorescence Intensity strip for the fluorescence module

The **Fluorescence Intensity** strip includes the following elements:

Name	Define a label name.
Mode	Select **Top** for fluorescence intensity top reading and **Bottom** for fluorescence intensity bottom reading.
Fluorophore	Select a fluorophore and define the excitation and the emission settings by • Moving the sliders within the graphic display of the corresponding absorption and emission spectra (monochromator systems only) or • Selecting/entering the settings manually for the excitation and the emission wavelength (monochromator and filter systems). Select **Other** if working with a fluorophore that is not included in the list of fluorophores.



NOTE: Tecan provides a list of commercially available fluorophores with their absorption and emission spectra. Fluorophores are not displayed with a recommended wavelength combination for the excitation and emission. The excitation and the emission wavelengths for each respective fluorophore need to be defined by the user.

Excitation wavelength	Define the excitation wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Emission wavelength	Define the emission wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Bandwidth	For the monochromator, select the bandwidth for excitation and emission if supported by the connected instrument.
Flashes	Enter the number of flashes. For optimal performance use the default number of flashes for each respective instrument.
Gain	Select one of the following modes:
	• **Manual gain:** define a gain value to be used for the measurement (range: 1-255).
	• **Optimal gain:** the optimal gain value is calculated automatically by the instrument according to the highest signal within the selected well range. Optimal gain determination is performed in a pre-measurement. It is recommended to use the optimal gain function for all applications that produce results with unknown RFU values.
	• **Calculated from well:** the optimal gain value is calculated for the selected well. The resulting gain value is applied to all other wells within the selected well range.
	• **Extended dynamic range:** The optimal gain measurement is done in two consecutive parts, one with a high and one with a low gain. The results of both measurements are automatically correlated and displayed within one single data set. Select this option to optimally adjust the gain settings for very high and very low signals on a microplate within one single measurement.

	• Use the option **% RFU** in combination with gain calculation from well (endpoint and kinetic measurements) or optimal gain calculation (kinetic measurements only) to apply only a percentage of the initial gain for the measurement. The percentage may be set individually from 10-100 %, with 100 % as the default value.
	• **Use gain regulation:** This option is available for plate-wise kinetic measurements only. By activating this option, fluorescence kinetic measurements with increasing signals are prevented from running into "OVER" once the samples produce RFU values that are too high. Instead the initial gain settings (manual/ optimal/ calculated from well) is automatically reduced in order to permit the measurement of even very high signals. Results obtained with different gain settings are highlighted accordingly. All RFU values with different gain settings are automatically correlated, allowing the evaluation of the entire kinetic data within one and the same graph.
Mirror	• The availability of mirrors depends on the types of (dichroic) mirrors that are installed. Depending on the selected wavelength settings, the appropriate mirror may be set by the instrument (**Automatic**) or select manually. The selection is possible only for fluorescence intensity top measurement.
Z-position	Select one of the following options: • **Manual:** define a value that will be used for the measurement • **Calculated from well:** select a well for the calculation of the Z-position. The optimal Z-position for the selected well is calculated and the corresponding value applied to all other wells within the selected well range. • **Same as**: Select a label in order to set the Z-position of the current label equal to that of the selected label. This option is available for measurements with more than one fluorescence intensity label.
Settle time	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.
Multiple reads per well	Select **User defined** in order to define the **Type**, **Size** and **Border**, the multiple reads per well shall be performed with. Use **Optimal** in combination with fluorescence intensity bottom measurement to perform a measurement on multiple, spatially separated spots inside the well. For the instrument with the option Area Scan: Use **Area Scan** in combination with fluorescence intensity bottom to produce a fine granulated picture of the fluorescence intensity distribution within a well.
Show advanced settings	Click this expand button to access the following parameters: **Flashes**, **Gain**, **Z-position**, **Mirror**, **Settle time** and **Multiple reads per well**.

7.1.2 Time-Resolved Fluorescence Intensity Strip

This strip is used for time resolved fluorescence intensity measurements.

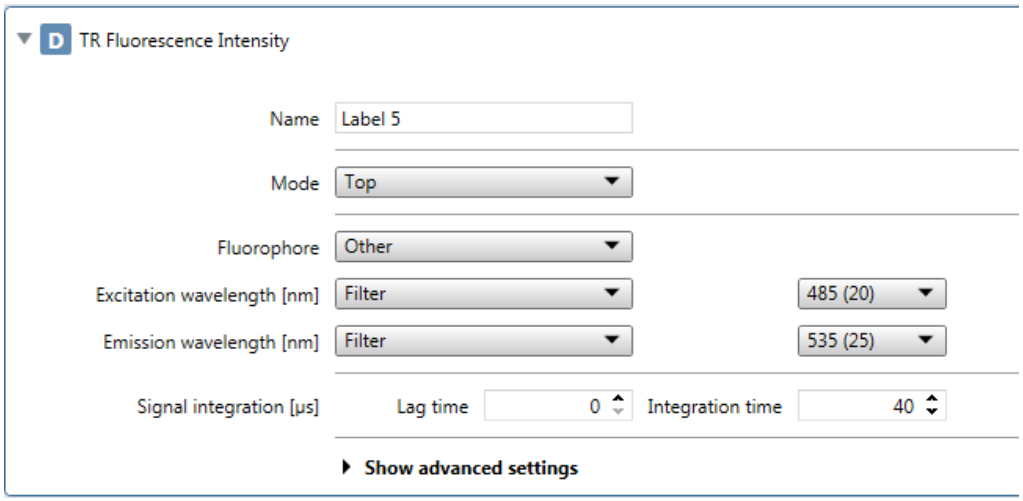


Figure 34: Time-Resolved Fluorescence Intensity strip for fluorescence module

The **Time-Resolved Fluorescence Intensity** strip includes the following elements:

Name	Define a label name.
Mode	Select **Top** for fluorescence intensity top reading and **Bottom** for fluorescence intensity bottom reading.
Fluorophore	Select a fluorophore and define the excitation and the emission settings by • Moving the sliders available within the graphic display of the corresponding absorption and emission spectra (monochromator systems only) or • Selecting/entering the settings manually using the numeric definition for the excitation and the emission wavelength (monochromator and filter systems). • Select **Other** if working with a fluorophore that is not included in the list of fluorophores.

NOTE: Tecan provides a list of commercially available fluorophores with their absorption and emission spectra only. Fluorophores are not displayed with a recommended wavelength combination for the excitation and emission. The excitation and the emission wavelengths for each respective fluorophore need to be defined by the user.

Excitation wavelength	Define the excitation wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Emission wavelength	Define the emission wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Bandwidth	For the monochromator, select the bandwidth for excitation and emission if supported by the connected instrument.
Integration time	Integration time stands for duration of signal recording per well. Select a value within the valid instrument range.
Lag time	Lag time stands for time between flash and the start of signal integration. Select a value within the valid instrument range.

NOTE: While lag time is an optional function, the integration time is a mandatory parameter that determines the duration of signal recording. The default values for standard fluorescence intensity measurements are 0 µs lag time and 40 µs integration time. Time resolved fluorescence measurements typically require a lag time and increased integration time according to the particular application.

Flashes	Enter the number of flashes. For optimal performance use the default number of flashes for each respective instrument.
Gain	Select one of the following modes:
	Manual gain: define a gain value to be used for the measurement (range: 1-255)
	Optimal gain: the optimal gain value is calculated automatically by the instrument according to the highest signal within the selected well range. Optimal gain determination is performed in a pre-measurement. It is recommended to use the optimal gain function for all applications that produce results with unknown RFU values.
	• **Calculated from well:** the optimal gain value is calculated for the selected well. The resulting gain value is applied to all other wells within the selected well range.
	• **Extended dynamic range:** The optimal gain measurement is done in two consecutive parts, one with a high and one with a low gain. The results of both measurements are automatically correlated and displayed within one single data set. Select this option to optimally adjust the gain settings for very high and very low signals on a microplate within one single measurement.
	• Use the option **% RFU** in combination with gain calculation from well (endpoint and kinetic measurements) or optimal gain calculation (kinetic measurements only) to apply only a percentage of the initial gain for the measurement. The percentage may be set individually from 10-100 %, with 100 % as the default value.
	• **Use gain regulation:** This option is available for plate-wise kinetic measurements only. By activating this option, fluorescence kinetic measurements with increasing signals are prevented from running into "OVER" once the samples produce RFU values that are too high. Instead the initial gain settings (manual/ optimal/ calculated from well) is automatically reduced in order to permit the measurement of even very high signals. Results obtained with different gain settings are highlighted accordingly. All RFU values with different gain settings are automatically correlated, allowing the evaluation of the entire kinetic data within one and the same graph.
Mirror	The availability of mirrors depends on the types of (dichroic) mirrors that are installed. Depending on the selected wavelength settings, the appropriate mirror may be set by the instrument (**Automatic**) or selected manually. The selection is possible only for fluorescence intensity top measurements.
Z-position	Select one of the following options: • **Manual:** define a value that will be used for the measurement • **Calculated from well:** select a well for the calculation of the Z-position. The optimal Z-position for the selected well is calculated and the corresponding value applied to all other wells within the selected well range. • **Same as:** select a label in order to set the Z-position of the current label equal to that of the selected label. This option is available for measurements with more than one fluorescence intensity label.

Settle time	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.
Multiple reads per well	Select **User defined** in order to define the **Type**, **Size** and **Border**, the multiple reads per well shall be performed with. Use **Optimal** in combination with fluorescence intensity bottom measurement to perform a measurement on multiple, spatially separated spots inside the well. For the instrument with the option Area Scan: Use **Area Scan** in combination with fluorescence intensity bottom to produce a fine granulated picture of the fluorescence intensity distribution within a well.
Show advanced settings	Click this expand button to access the following parameters: **Flashes**, **Gain**, **Z-position**, **Mirror**, **Settle time** and **Multiple reads per well**.

7.1.3 Fluorescence Intensity Scan Strip

This strip is used to perform fluorescence intensity scans: excitation, emission and 3D scans.

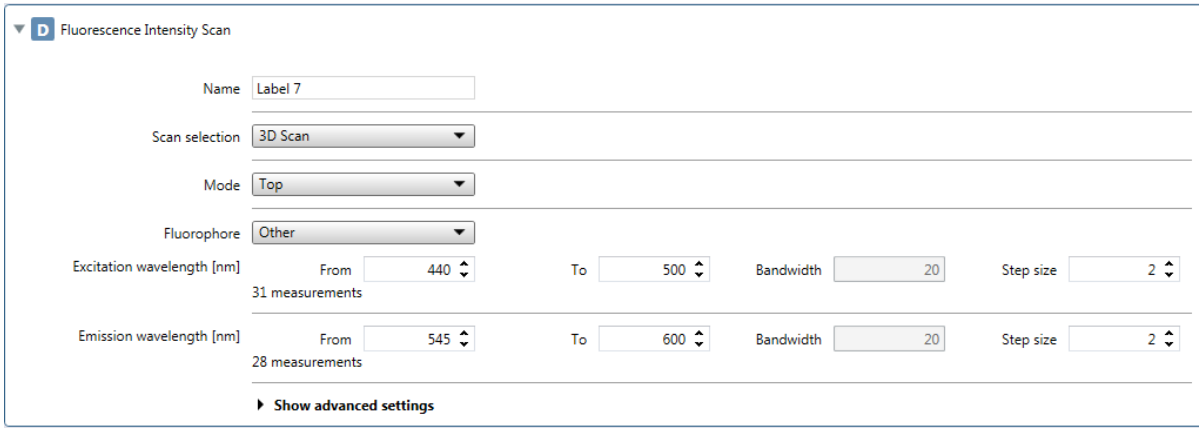


Figure 35: Fluorescence Intensity Scan strip for the fluorescence module

The **Fluorescence Intensity Scan** strip includes the following elements:

Name	Define a label name.
Scan selection	Select • **Excitation** to perform an excitation scan • **Emission** to perform an emission scan • **3D** to perform a 3D scan
Mode	Select **Top** for fluorescence intensity top reading and **Bottom** for fluorescence intensity bottom reading.
Excitation wavelength	**Excitation scan/3D scan:** Define the excitation range by entering From and To values. **Emission scan:** Define the respective excitation wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Emission wavelength	**Excitation scan:** Define the respective emission wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode. **Emission scan/3D scan:** Define the emission range by entering **From** and **To** values.
Bandwidth and Step	For the monochromator, select the bandwidth for excitation and emission if supported by the connected instrument. Define a step size within the valid instrument range to be used for the scan measurement.

Flashes	Enter the number of flashes. For optimal performance use the default number of flashes for each respective instrument.
Gain	**Excitation, emission and 3D scan:** Define a **Manual** gain value to be used for the measurement (range: 1-255) **3D scan:** By selecting **Calculated from well** the optimal gain value is calculated for the selected well. The resulting gain value is applied to all other wells within the selected well range.
Mirror	The availability of mirrors depends on the types of mirrors that are installed. Depending on the selected wavelength settings, the appropriate mirror may be set by the instrument (**Automatic**) or select manually. The selection is possible only for fluorescence intensity top measurement.
Z-position	Select one of the following options: **Manual:** define a manual value that will be used for the measurement **Calculated from well:** select a well for the calculation of the Z-position. The resulting Z-position value is applied to all other wells within the selected well range. **Same as:** select a label in order to set the Z-position of the current label equal to that of the selected label. This option is available for measurements with more than one fluorescence intensity scan label.
Settle time	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.
Signal integration	**Integration time** is the duration of signal recording per well. Select a value within the valid instrument range. **Lag time** is the time between flash and the start of signal integration. Select a value within the valid instrument range.
Show advanced settings	Click this expand button to access the following parameters: **Flashes**, **Gain**, **Z-position**, **Mirror**, **Settle time** and **Signal integration**.

7.1.4 Measurement Mode

Fluorescence measurements can be defined and performed as

- Single-label endpoint or kinetic measurements
- Multi-label endpoint or kinetic measurements

All measurements, except fluorescence scans, can be carried out plate-wise and well-wise. Multiple reads per well as well as fluorescence scan are supported only in the plate-wise manner.

7.2 Fluorescence Polarization Module

The fluorescence polarization module is designed as a Fusion Optics system. The wavelength selection for excitation and emission can be performed by either the monochromator or the filter option. The monochromator and the filter mode are independently combinable for the excitation and the emission side and therefore provide a detection system with the maximum flexibility and maximum signal output. The polarization option is available for top measurements only.

The fluorescence polarization Standard Module is available in two versions: a >300 nm or a >390 nm version. The fluorescence polarization Enhanced Module is equipped with the >300 nm version by default.

For further differences between Fluorescence Standard and Fluorescence Enhanced Module, see chapter Fluorescence Top Module in the SPARK – Reference Guide.

7.2.1 Fluorescence Polarization Optics

Optics

The polarization module consists of a flash lamp as light source, monochromators and/or filters for wavelength selection, a measurement head, and a photomultiplier tube as detector (see chapter Fluorescence Top Module Optics in the SPARK – Reference Guide). Polarizers are installed on the measurement head and switched in automatically when performing polarization measurements.

Z-Positioning

The Z-position defines the distance between the measurement head and the surface of the microplate. The Z-position can be adjusted by moving the plate transport up and down. As light is reflected onto the sample liquid surface, a Z-adjustment helps to maximize signal to noise ratio.

7.2.2 Fluorescence Polarization Detection

See chapters Fluorescence Polarization (FP) and Fluorescence Detection in the SPARK – Reference Guide.

G-Factor Calculation

The equation for calculation of fluorescence polarization assumes that the sensitivity of the detection system is equivalent for parallel and perpendicular polarized light. This is generally not the case. Therefore the so called G-factor calculation has to be performed. The G-factor compensates for differences in response of the optical components to the parallel and perpendicular polarized light. The G-factor calculation is an important requirement for each fluorescence polarization measurement.

The G-factor depends on the selected wavelength and bandwidth. Its calculation requires at least one well containing the fluorophore used in the assay as reference and at least one well for blanking containing the (assay) solution without the fluorophore.

In the plate layout, define the RF identifier as reference fluorophore or use an identifier other than BL (Blank) or BF (Blank fluorophore) for referencing the fluorophore. For blanking the assay system, the identifier BF (Blank Fluorophore) or BL (Blank) can be selected.



NOTE: By using more than one well filled for referencing the fluorophore and more than one well without fluorophore for blanking, mean values will be calculated and the G-factor calibration becomes more accurate.

Once the G-factor was calculated for a certain fluorophore (assay type) it can be set manually for all further measurements using the same fluorophore. Recalculation of the G-factor has to be performed under following conditions:

- The wavelengths/filters have changed
- New filters are installed on the filter slide
- New assay type with different fluorophore is used



NOTE: It is recommended to use a free fluorophore or a fluorophore with a low polarization value for the G-factor calibration.

7.2.3 Defining Fluorescence Polarization Measurements

The availability of the Fluorescence Polarization strip depends on the configuration of the instrument connected.

Fluorescence Polarization Strip

This strip is used for fluorescence polarization measurements.

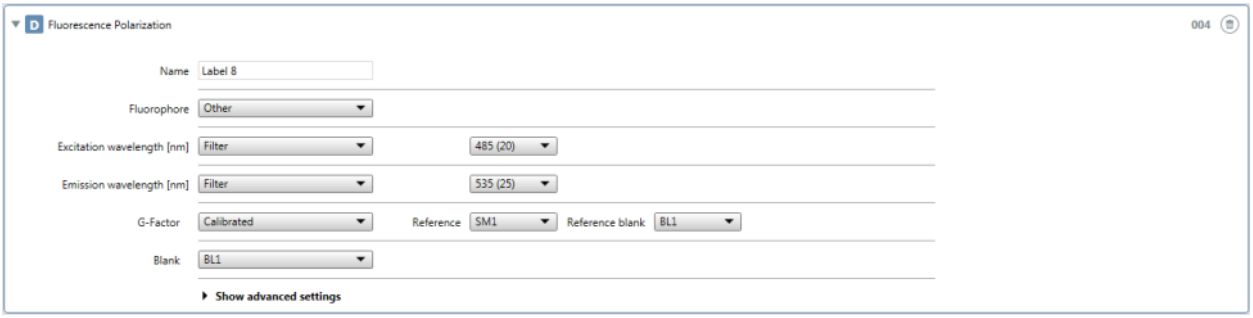


Figure 36: Fluorescence Polarization strip

The **Fluorescence Polarization** strip includes the following elements:

Name	Define a label name.
Fluorophore	Select a fluorophore and define the excitation and the emission settings by • Moving the sliders available within the graphic display of the corresponding absorption and emission spectra (monochromator systems only) or • Selecting/entering the settings manually using the numeric definition for the excitation and the emission wavelength (monochromator and filter systems). • Select **Other** if working with a fluorophore that is not included in the list of fluorophores.

NOTE: Tecan provides a list of commercially available fluorophores with their absorption and emission spectra only. Fluorophores are not displayed with a recommended wavelength combination for the excitation and emission. The excitation and the emission wavelengths for each respective fluorophore need to be defined by the user.

Excitation wavelength	Define the excitation wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Emission wavelength	Define the emission wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Bandwidth	For the monochromator, select the bandwidth for excitation and emission if supported by the connected instrument.

G-Factor	Select **Calibrated** for automatic calculation of the G-factor by the instrument. Use the **Plate layout** button and select the wells for referencing the fluorophore (e.g. RF, SM). Select a blank identifier to be used for blanking (BL or BF). For defining the Plate Layout, see chapter 2.7.1 Plate. Select **Manual**, if the measurement is carried out with a G-factor value manually defined by the user or with a G-factor already available for the selected fluorophore and assay type. If no calibrated G-factor is available, the default value of 1 will be displayed and marked as **Uncalibrated G-Factor**. Otherwise, a previously calibrated value will be displayed and marked as calibrated value with the corresponding date of calibration (e.g.: 3/15/2019). Both the uncalibrated and the calibrated value can be manually changed. Use the **Reset** button to recall the original value.
Blank	Select the blank identifier to be used for blanking according to the plate layout as defined in the **Plate** strip Select **Not defined** if the measurement will be performed without blanking.
Flashes	Enter the number of flashes. For optimal performance use the default number of flashes for each respective instrument.
Gain	Select one of the following modes: • **Manual gain:** define a gain value to be used for the measurement (range: 1-255) • **Optimal gain:** the optimal gain value is calculated automatically by the instrument according to the highest signal within the selected well range. Optimal gain determination is performed in a pre-measurement. It is recommended to use the optimal gain function for all applications that produce results with unknown RFU values. • **Calculated from well:** the optimal gain value is calculated for the selected well. The resulting gain value is applied to all other wells within the selected well range. • Use the option **% RFU** in combination with gain calculation from well (endpoint and kinetic measurements) or optimal gain calculation (kinetic measurements only) to apply only a percentage of the initial gain for the measurement. The percentage may be set individually from 10-100 %, with 100 % as the default value.
Mirror	The availability of mirrors depends on the types of mirrors that are installed. Depending on the selected wavelength settings, the appropriate mirror may be set by the instrument (**Automatic**) or select manually.
Z-position	Select one of the following options: • **Manual:** define a manual value that will be used for the measurement • **Calculated from well:** select a well for the calculation of the Z-position. The optimal Z-position for the selected well is calculated and the corresponding value applied to all other wells within the selected well range. • **Same as:** select a fluorescence polarization label in order to set the Z-position of the current label equal to that of the selected label. This option is available for measurements with more than one fluorescence polarization label.
Settle time	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.
Show advanced settings	Click this expand button to access the following parameters: **Flashes**, **Gain**, **Z-position**, **Mirror**, and **Settle time**.

7.2.4 Calculation of Results

Fluorescence polarization measurements use parallel and perpendicular fluorescence intensity values for calculation of the following data:

Polarization*:

$$P = \frac{(I_{II} - I_{\perp})}{(I_{II} + I_{\perp})}$$

Anisotropy*:

$$r = \frac{(I_{II} - I_{\perp})}{(I_{II} + 2 \cdot I_{\perp})}$$

*The polarization and anisotropy values are displayed as calculated values multiplied by 1000.

Total Intensity:

$$I_T = (I_{II} + 2 \cdot I_{\perp})$$

Intensity parallel:

$$I_{II} = g \cdot (RFU_{II}^{meas} - RFU_{II}^{blank})$$

Intensity perpendicular:

$$I_{\perp} = RFU_{\perp}^{meas} - RFU_{\perp}^{blank}$$

RFU_{II}^{meas} and $RFU_{\perp}^{meas}$ refer to measured parallel and perpendicular relative fluorescence unit (RFU) values of a well; g is the G-factor defined manually or calculated by the G-factor calibration.

7.3 Optimizing Fluorescence and Fluorescence Polarization Measurements

Gain

The gain defines the amplification factor of the detector when converting light into electrical current.

To provide a proper signal-to-noise ratio and linearity signals must have a suitable input range. Therefore, the gain should be tuned to obtain highest possible signal intensities from the highest concentrated samples allowing to clearly distinguishing lower concentrated samples from the background noise.

To avoid measurements performed outside of the linear range of the detector (PMT), which could lead to compromised results, gain settings below 40 should not be applied.

NOTE: If any well of interest is assigned OVER (overflow), you may manually reduce the gain, or select an automatic gain option (optimal gain, gain from well).

Scan Z-Position

The Z-position can be optimized in the Instrument menu of the Method Editor or via Dashboard. Results of the Z-position scan are shown in a graphical plot.

Select the label(s) for which the Z-position optimization is to be performed. The label selection/number depends on the measurement previously defined in the Method Editor.

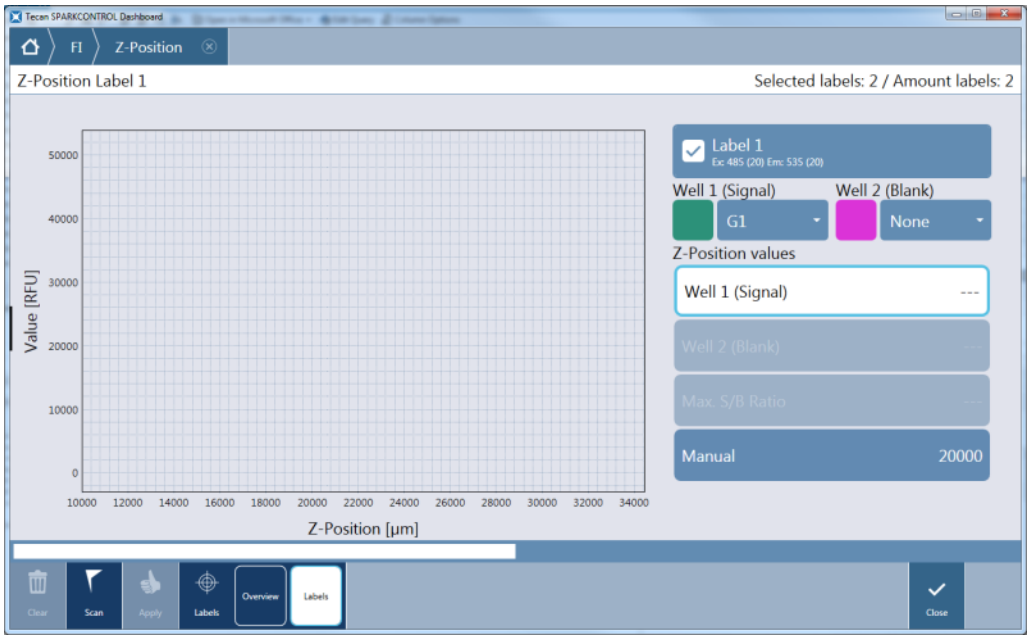


Figure 37: Z-Position window – define parameters

For each selected label, one or two wells of the defined plate range can be used for the Z-position optimization. Select the well(s) and click **Scan** to start the Z-optimization. After the measurement the resulting Z-position curve(s) and the corresponding Z-position value(s) are displayed. Select one of the Z-position values. Upon clicking **Apply**, the selected Z-position will be automatically applied to the method used for the subsequent measurement.

The option **Max. S/B Ratio** is available for fluorescence intensity and fluorescence intensity scan measurements only. It requires the measurement of two wells, one filled with the fluorophore of interest (signal) and one filled with buffer (blank). Both wells are scanned and the resulting signal and blank curves are shown in the graph. The Z-position may now be set to the maximum signal-to-blank (S/B) ratio.

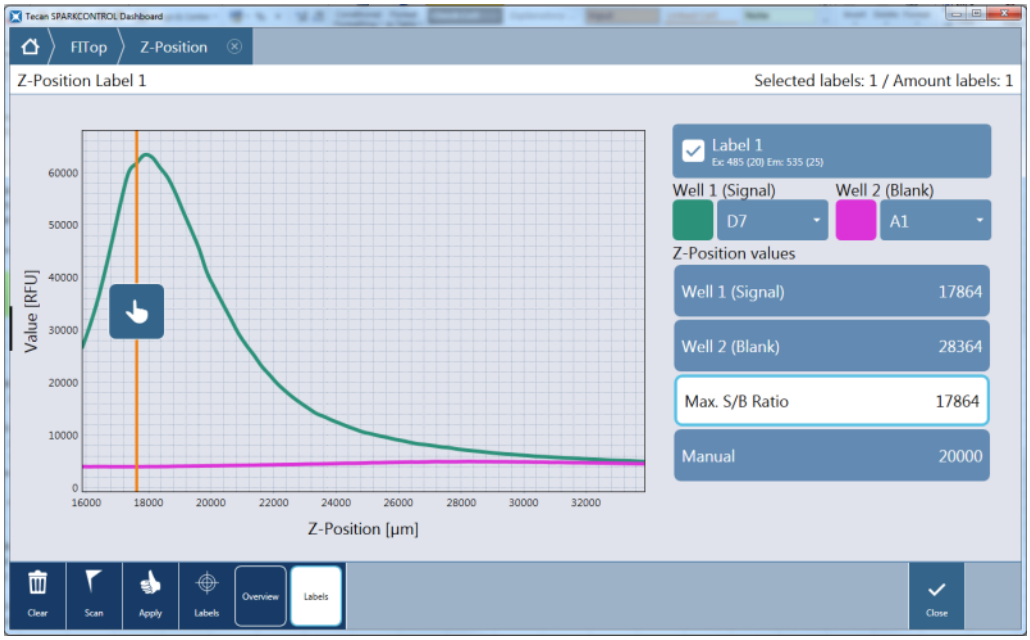


Figure 38: Z-Position window – display of Z-position curves

NOTE: When the option **Max. S/B Ratio** is used, the sample well is first measured with optimal gain. The same gain value is applied to the second measurement with the blank well. Therefore, both signal and blank curves are directly comparable.

The Z-position for each selected label can also be selected within the depicted scan curve manually. Move the vertical yellow bar of the graph to the desired Z-position.

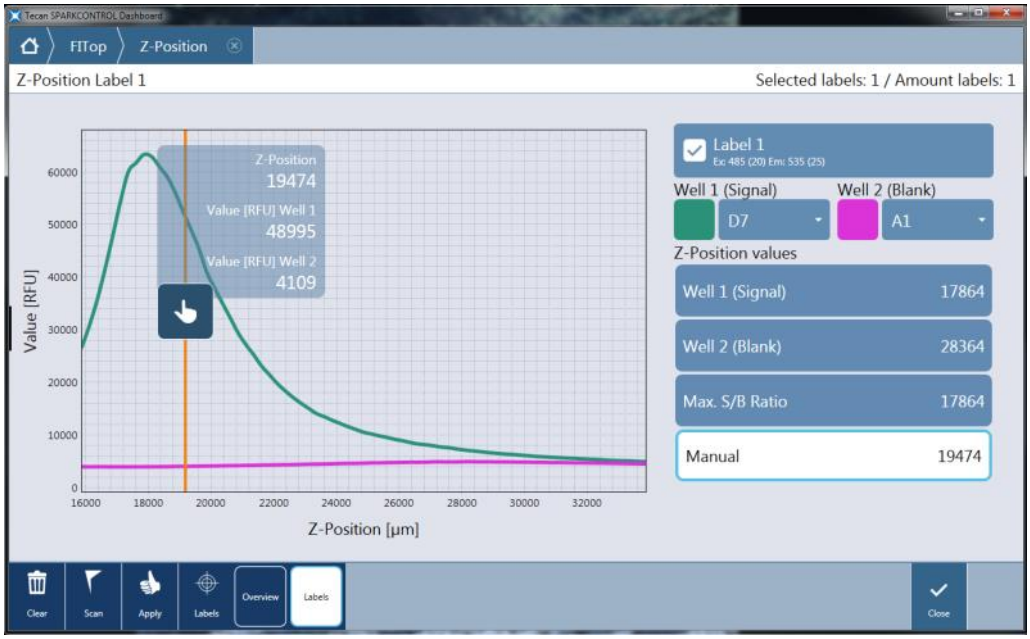


Figure 39: Z-Position window – changing Z-position value manually

Flash Settings

Measurement with one flash per well is possible for all plate types. Measurement precision at low light levels depends on the total signal integration time. However, increasing the signal integration time will not always result in better signal, as the signal integration might be limited by the length of the fluorescence signal (life time).

Improve the measurement data by increasing the number of flashes instead. The measured intensities of all flashes are averaged resulting in more accurate measurement data.



NOTE: Increase the number of flashes per well until noise of blank wells does not improve further, or until the measurement time per well becomes unacceptable.

Settle Time

Due to the stop and go motion of the plate carrier the dispensed liquids meniscus may vibrate during signal integration. Vibrations can cause fluctuations in the measured values therefore a settle time can be defined to minimize this effect and to optimize performance. The settle times describes the time between move and flash. A settle time between 100 and 300 ms may be applied to fluorescence polarization measurements and fluorescence measurements in plate formats with less than 96 wells to improve data.

G-Factor

See chapter 7.2.2 Fluorescence Polarization Detection / G-Factor Calculation.

Multiple Reads per Well

The multiple reads per well option (MRW) is available for fluorescence top and bottom fixed wavelength measurements in order to achieve maximum well illumination. This option is especially applicable for cell based investigations, since the distribution of the cells in the well is often not homogeneous.

7.4 Inject and Read

The **Inject and Read** measurement mode is designed to support application with the need of simultaneous injection and fluorescence bottom reading, such as measurement of intracellular Ca^{2+} concentration with calcium sensitive non-ratiometric dyes (e.g. Fluo-4).

Inject and Read combines the benefit of the injection module with the Fluorescence Intensity Bottom reading within one measurement strip. The measurement is automatically executed as a well-wise kinetic measurement at user-defined interval timestamps.

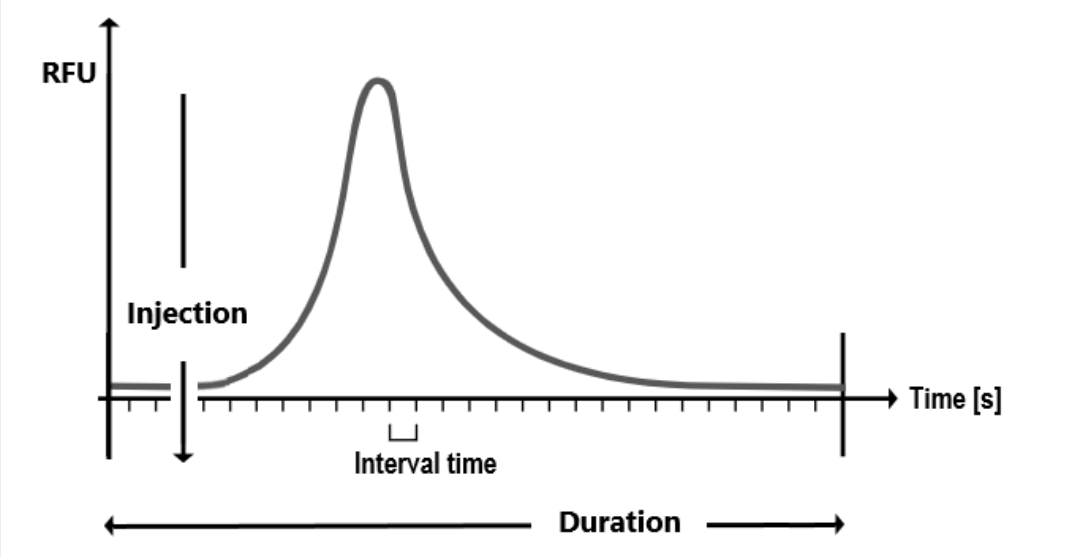


Figure 40: Measurement setup of an **Inject and Read** measurement

The availability of the **Inject and Read** strip depends on the configuration of the instrument connected.

NOTE: The **Inject and Read** measurement mode is not compatible with Spark Cyto and therefore, it cannot be used in combination with the Cell Imager module.

7.4.1 Defining **Inject and Read** Measurements

Inject and Read Strip

This strip is used for simultaneous injection and fluorescence intensity bottom measurements.

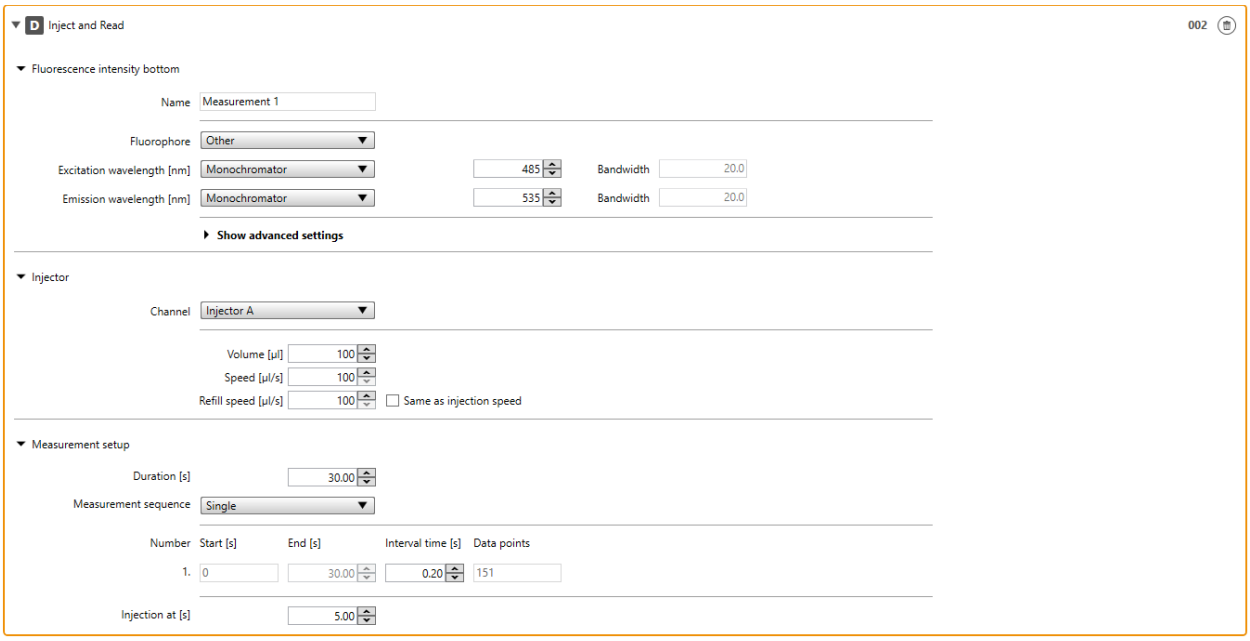


Figure 41: Inject and Read strip

The **Inject and Read** strip includes the following elements:

Fluorescence intensity bottom	
Name	Define a label name.
Fluorophore	Select a fluorophore and define the excitation and the emission settings by • Moving the sliders available within the graphic display of the corresponding absorption and emission spectra (monochromator systems only) or • Selecting/entering the settings manually using the numeric definition for the excitation and the emission wavelength (monochromator and filter systems). • Select **Other** if working with a fluorophore that is not included in the list of fluorophores.

NOTE: Tecan provides a list of commercially available fluorophores with their absorption and emission spectra only. Fluorophores are not displayed with a recommended wavelength combination for the excitation and emission. The excitation and the emission wavelengths for each respective fluorophore need to be defined by the user.

NOTE: The **Inject and Read** strip supports workflows with non-ratiometric fluorescent dyes only.

Excitation wavelength	Define the excitation wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Emission wavelength	Define the emission wavelength. For the Fusion Optics system, select either **Monochromator** or **Filter** mode.
Bandwidth	For the monochromator, select the bandwidth for excitation and emission if supported by the connected instrument.
Gain	Define a manual gain value to be used for the measurement (range: 1-255)

Z-position	Select one of the following options: • **Manual:** define a manual value that will be used for the measurement • **Calculated from well:** select a well for the calculation of the Z-position. The optimal Z-position for the selected well is calculated and the corresponding value applied to all other wells within the selected well range.
Settle time	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.
Show advanced settings	Click this expand button to access the following parameters: **Gain**, **Z-position** and **Settle time**.



NOTE: To aid fast fluorescence intensity bottom reading, the number of flashes is set to 1 and cannot be changed by the user. Multiple Reads per Well are further not supported.

Injector	
Channel	Select injector A or B. The selection depends on the instrument configuration.
Volume	Define the volume to inject into a single well.
Speed	Define the speed of liquid flow during the injection.
Refill speed	Define the speed for liquid refill during the method run. **Same as injection speed:** Select the check box if the refill speed will be the same as the injection speed.



NOTE: Refilling of the syringe is executed always before every injection.

Measurement setup	
Duration [s]	Define duration of the kinetic measurement.
Measurement sequence	Select **Single** to work with one fixed user-defined interval time for the entire duration. Select **Multiple** to work with up to three different user-defined interval times for the entire duration. For every measurement sequence define the **Interval time [s]** and in addition, **Start [s]** and **End [s]** time values for multiple measurement sequences.
Injection at [s]	Define a timestamp within the duration time at which the injection will be executed.



NOTE: An **Inject and Read** measurement can contain an overall value of 1000 measurement points, i.e. **Data points** per well. The number of data points results from the values defined for the duration and interval time. If the value of 1000 points is exceeded increase the interval time and/or decrease the duration.



NOTE: Depending on SPARK's fluorescence bottom module, the minimal **Interval time** value is 10 ms (Fluorescence Enhanced) and 20 ms (Fluorescence Standard), respectively.

7.4.2 Measurement Mode

Inject and Read measurements are always performed in the well-wise kinetic manner. This inherent combination of a well strip with a kinetic strip makes an additional use of these strips in relation to the **Inject and Read** measurements obsolescent. However, the **Inject and Read** strip can be used in

- Single-label measurements
- Multi-label measurements

according to the implemented workflow rules.

7.4.3 Optimizing **Inject and Read** Measurements

Inject and Read measurements are often accompanied with a steep increase of the fluorescence intensity signal after binding of ions to the indicator dye. This characteristic brings a risk of signal overflow that can be reduced by some preliminary optimization work. Using a well with the highest expected fluorescence intensity value (positive control), it is recommended to determine an optimal gain and a Z-position value first and apply these values to the final method definition afterwards. Alternatively, the user can perform a series of **Inject and Read** measurements, applying different gain values manually, in order to optimize the gain, avoiding overflow values, but keeping the maximum dynamic signal range.

Gain

The gain defines the amplification factor of the detector when converting light into electrical current.

To provide a proper signal-to-noise ratio and linearity signals must have a suitable input range. Therefore, the gain should be tuned to obtain highest possible signal intensities from the highest concentrated samples allowing to clearly distinguishing lower concentrated samples from the background noise.

To avoid measurements performed outside of the linear range of the detector (PMT), which could lead to compromised results, gain settings below 40 should not be applied.



NOTE: If any well of interest is assigned OVER (overflow), reduce the gain.

Scan Z-Position

See chapter 7.3 Optimizing Fluorescence and Fluorescence Polarization Measurements / Scan Z-Position.

8 Cell Module

SPARK can be equipped with either the Cell Module described in this chapter, or with the enhanced fluorescence imaging module, the Cell Imager. Only one of these imaging modules can be installed in the SPARK instrument.

Tecan's Cell Module is based upon the bright field illumination technique which is a commonly used tool for visualization of cells. Bright field cellular visualization requires a difference in contrast between cells and their surrounding medium. This contrast is caused by the different levels of light absorption of cells and their media. Counting of cells with low or no contrast can be improved by using various staining procedures.

8.1 Defining Cell Counting & Confluence Measurements

The SparkControl software provides two separate strips for measuring:

- Cell Counting
- Cell Confluence

The availability of the strips depends on the configuration of the instrument connected.

8.1.1 Cell Counting Strip

This strip is used for cell counting measurements in the Tecan cell chip adapter.

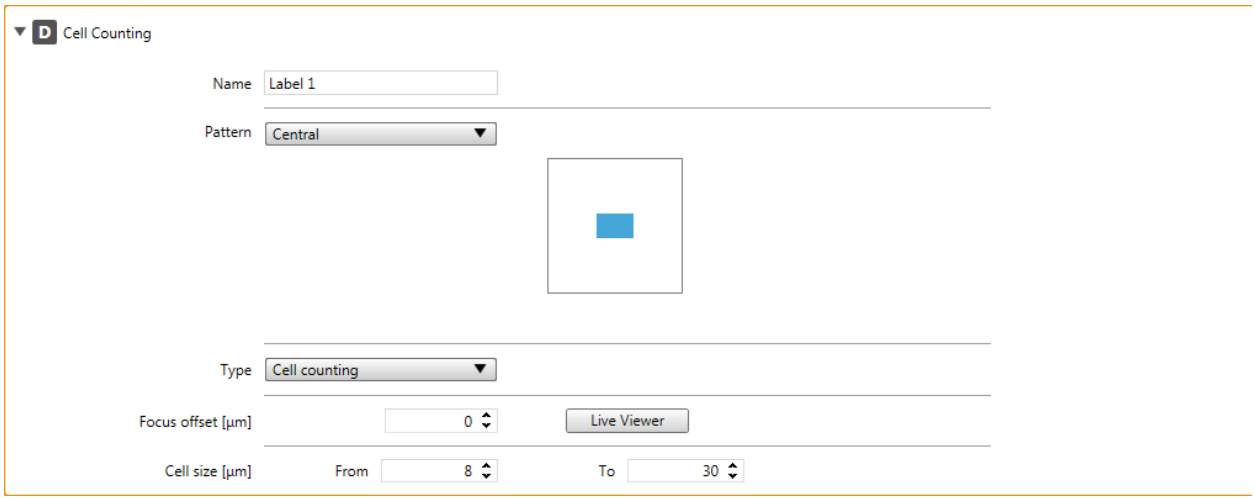


Figure 42: Cell Counting strip

The **Cell Counting** strip includes the following elements:

Name	Define a label name.
Type	Select **Cell counting** or **Cell viability**
Pattern	Select **Center** to acquire an image in the center of a chamber. Select **Whole well** to acquire images within the whole chamber area. Select **User defined** and define positions within a chamber.
Focus offset [µm]	Enter a value manually or use the Live Viewer (see chapter 8.2.2 Live Viewer) for its definition. If defined, the focus offset value is automatically added to the autofocus value.
Cell size [µm]	Define the cell size of the counted cells.

8.1.2 Cell Confluence Strip

This strip is used for cell confluence measurements.

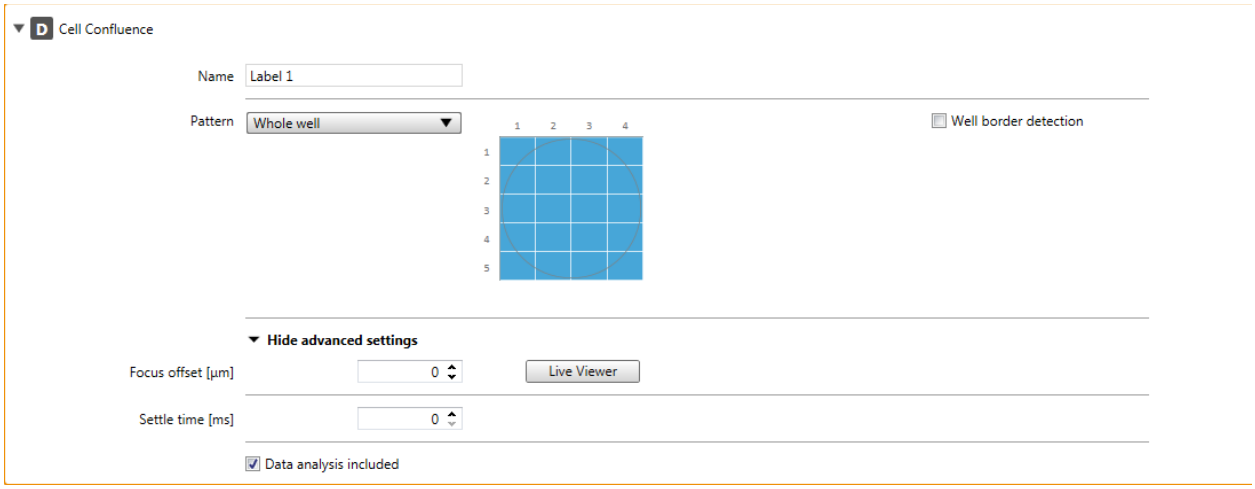


Figure 43: Cell Confluence strip

The **Cell Confluence** strip includes the following elements:

Name	Define a label name.
Pattern	Select **Center** to acquire an image in the center of a well. Select **Whole well** to acquire images within the whole well. To detect the well border, perform measurements with **Well border detection** selected. Select **User defined** and define positions within a well without intersections with the well border. It is possible to define only the area within the well without the well border intersection.
Focus offset [µm]	Enter a value manually or use the Live Viewer (see chapter 8.2.2 Live Viewer) for its definition. If defined, the focus offset value is automatically added to the autofocus value.
Settle time [ms]	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.
Data analysis included	If selected (default settings), the acquired images are analyzed and the results available for the user.
Show advanced settings	Click this expand button to access the following parameters: **Focus offset**, **Settle time** and **Data analysis included**.

The automated cell confluence determination is optimized for 96-well tissue culture microplates. Depending on the characteristics of certain microplates, cell confluence in blank wells, i.e. wells with no cells, may result in confluence signals greater than 10%. The confluence value for these wells depends on the composition of the well bottom. We recommend an individual evaluation of your preferred combination of tissue culture plate and cell type.

NOTE: Confluence values are displayed on the analyzed images in the left upper corner. Values ≤ 10% and ≥ 90% are in red whereas all other values are in blue. Red colored values might not be compatible with linear growth curves or with data collected using an alternative method.

CAUTION: Confluence measurements for wells with no cells may result in confluence values > 10%. It is the responsibility of any operating authority to take into consideration the confluence signal of empty wells when performing the system validation.

8.1.3 Measurement Mode

Cell counting measurements can be defined and performed plate-wise only as

- Single-label endpoint measurements
- Multi-label endpoint measurements

Cell confluence measurements can be defined and performed as

- Single-label endpoint and kinetic measurements
- Multi-label endpoint and kinetic measurements

All measurements can be carried out plate-wise and well-wise.

8.2 Optimizing Cell Confluence Measurements

8.2.1 Use Well Border Detection

Confluence detection requires exact plate transport movements and positioning. To compensate for plate dimension variances, activate the Well Border Detection function in the software. This option enables accurate confluence analysis of adherent cells up to the well border. Without Well Border Detection, contrast changes in the field of the well border will be included in the data analysis and may result in false confluence values.



CAUTION: Be aware that measurements that include Well Border Detection take more time.

8.2.2 Live Viewer

The Live Viewer can be started from cell confluence and cell counting strips, from the Instrument menu of the Method Editor or via the Check-and-Go window in the Dashboard in order to check the autofocus settings prior to measurement start.

In addition, the Live Viewer is available as an individual app for a quick quality check of the cell culture in a microplate.

In all cases, the Live Viewer provides an image of the cells in live mode. When using the Live Viewer for the method definition or before the method execution, the automatically found autofocus position can be corrected by a manually set offset value.

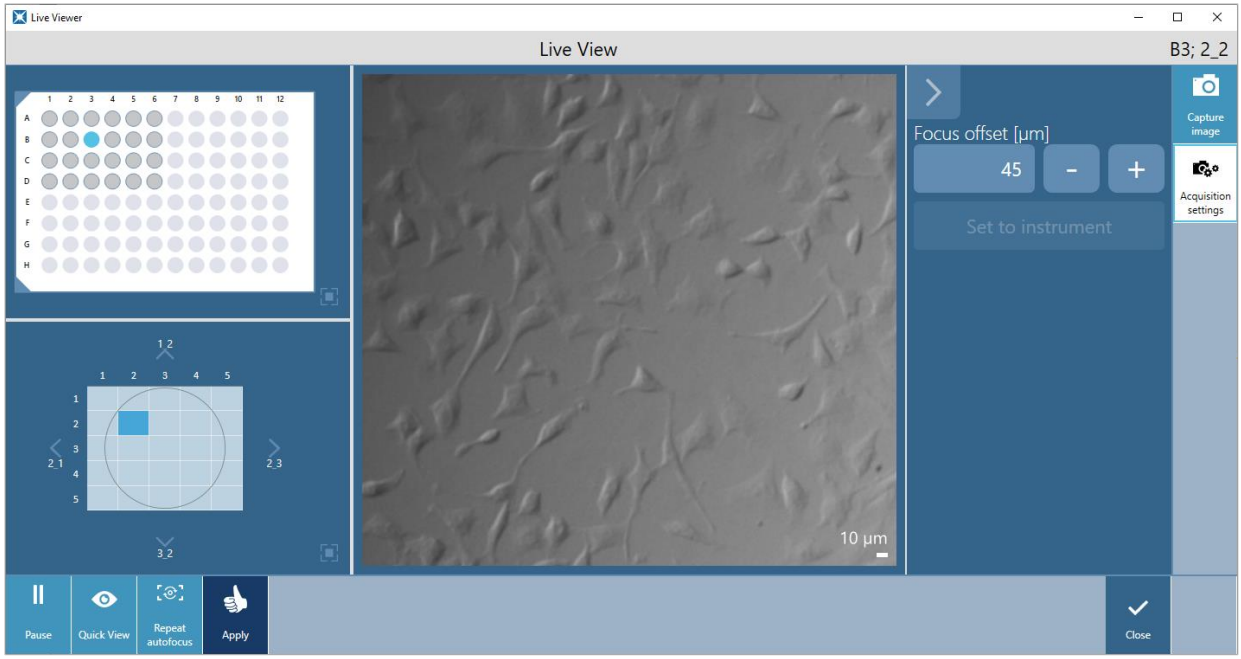


Figure 44: Live Viewer

Plate Selection

The Live Viewer, when started from a strip or via the Check-and-Go window, always expects the plate format as defined in the current method.

When working with the Live Viewer app, the plate format must be selected first. Please note that microplates with removable lids and the use of humidity cassettes are not supported by the app.

CAUTION: Always use the microplate according to the method definition or plate format selection in the Live Viewer app, otherwise the image acquisition may result in errors.

Graphic Areas

The Live Viewer window is divided into the **Image**, **Plate**, and the **Intrawell Position** graphic areas. The **Image** is displayed enlarged by default. Use the magnification button located at the lower right edge of the minimized areas to enlarge the corresponding graphic area.

When opening the Live Viewer, a well is automatically selected for image acquisition. Use the **Plate** area to select a new well and the **Intrawell Position** area to change the position for the image acquisition within a well.

Autofocus and Focus Offset

The Live Viewer automatically provides an image for the selected well by using the autofocus function of the SparkControl software. The autofocus is newly calculated every time the well selection changes. For a rapid image check in different wells without performing the autofocus repetition per well, use the **Quick View** function. In that case, all images will be acquired with the last valid autofocus settings.

If needed, the autofocus calculation can be restarted by selecting the **Repeat Autofocus** action button. The autofocus value can also be adjusted by defining a focus offset value. Enter a value and confirm it by selecting the **Set to instrument** button. The software will adjust the autofocus position accordingly and acquire a new image. Upon clicking **Apply**, the defined focus offset will be automatically applied to the method used for the subsequent measurement, i.e. the focus offset will be added to the each autofocus value calculated per well.

NOTE: The **Apply** button, for adopting the autofocus values, is available only in the Live Viewer connected to the method definition/execution but not in the Live Viewer app.

NOTE: If the focus offset within the Check-and-Go/Live Viewer screen is changed, this new value will be applied to the current measurement run only and will not overwrite the original method definition.

Image

Images are displayed in real time mode and will therefore be periodically updated. To pause this live stream, select the **Pause** button. To continue the live stream, select the **Live** button.

To save an image, select the **Capture image** button located at the upper right edge of the screen. The saved image can be found in the LiveViewer folder under the default path destination.

9 Fluorescence Imaging (Cell Imager)

9.1 Defining Bright field and Fluorescence Imaging Measurements

For instruments equipped with the Cell Imager module, the SparkControl software provides a single detection strip that can be used for measurements based on bright field and/or fluorescence imaging.

The availability of the strip depends on the configuration of the connected instrument.



CAUTION: Avoid working with SparkControl and ResultAnalyzer in parallel to ensure maximum performance of the SparkControl software.



CAUTION: Do not connect or disconnect any USB devices (e.g. USB stick, external SSDs, etc.) during fluorescence imaging measurements.



CAUTION: A correct plate definition file is essential for the quality of bright field and fluorescence imaging measurements. Always work with plates that match the selected plate definition file in the Plate strip. If an imaging plate is not part of the Plate Definition Files (.pdfx) delivered with the instrument, use the Plate Geometry Editor to define a user-defined pdfx file or contact Tecan.



NOTE: Cell counting and Cell viability in cell chips that is based on bright field illumination is not supported by the Cell Imager module.

9.1.1 Imaging Strips

Method definition with a 2D Imaging strip or 3D Imaging strip includes settings for image acquisition. For image processing and analysis, refer to the corresponding Plugin instruction. If the analysis is defined, images are analyzed and the results are displayed in real-time during method

execution. For the post-analysis of images after the measurement, see chapter 9.2.2 ResultAnalyzer.

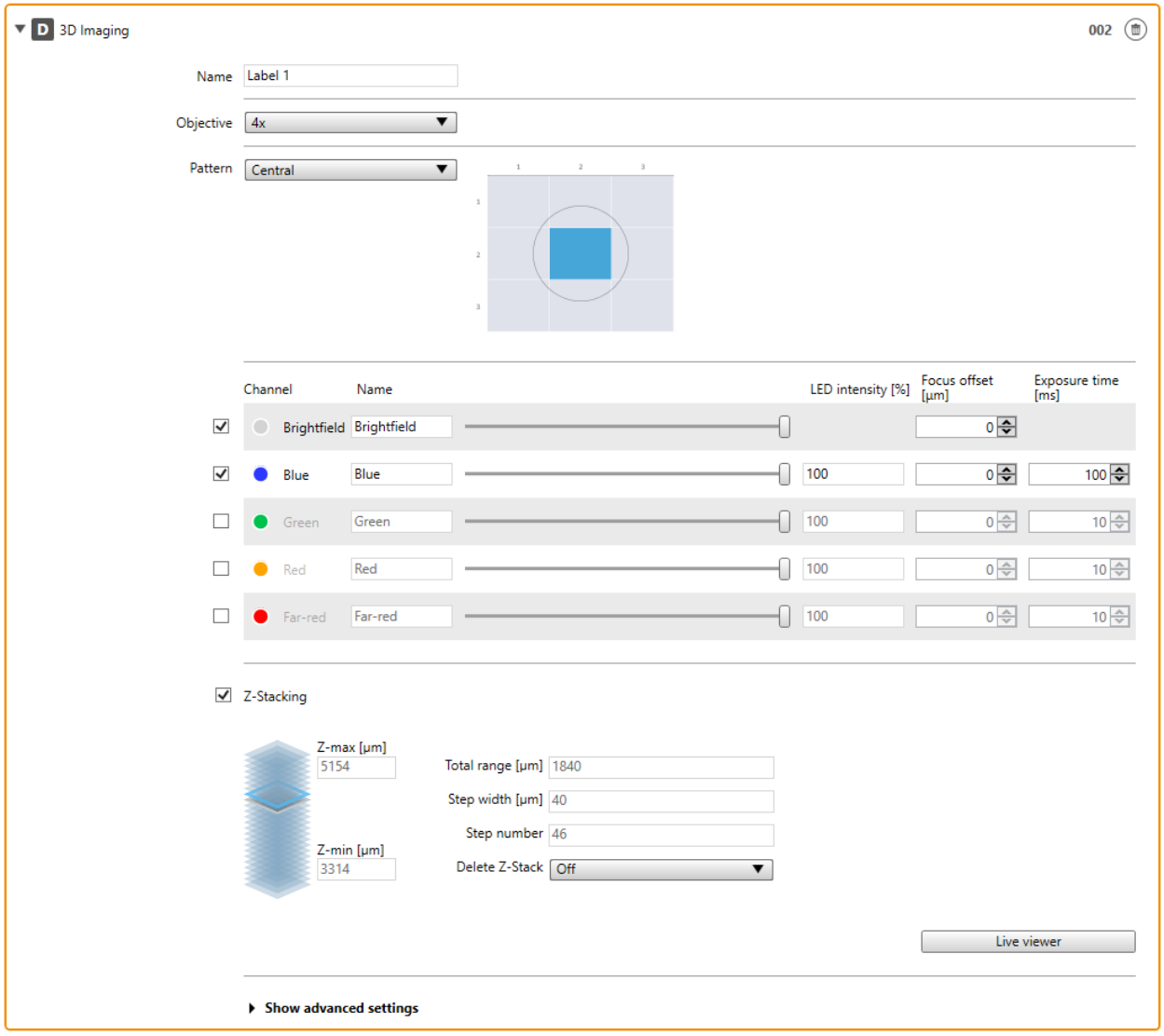


Figure 45: 3D Imaging strip

The **Fluorescence Imaging** strip includes the following elements:

Name	Define a label name.
Objective	Select one of the following objectives: • 2x • 4x • 10x
Pattern	Select **Center** to acquire an image in the center of a well. Select **Whole well** to acquire images within the whole well, including the well border intersection. Note that the selection **Whole well** is not available, if the number of intrawell imaging positions exceeds 25. Select **User defined** and define positions within a well without intersections with the well border. Select **Center grid** to position the grid exactly through the center of a well. For the wells with more than 25 available intrawell positions, a maximum of 25 images can be selected within a bounding box.

NOTE: The bounding box is a rectangular area within a well, in which a maximum of 25 imaging positions can be manually selected. The selection range changes dynamically depending on the selected positions. To select positions outside of the highlighted selection range, some of the already selected imaging positions must first be deselected.

NOTE: If the pattern **Whole well** is unavailable for the currently selected objective, please select an objective with a lower resolution that will result in an increased area per image.

Channels	Select **Bright field** illumination to acquire images for measuring cell confluence, counting cells, creating an overlay with a fluorescence image, or simply controlling the cell quality. Select **Blue channel** when cells are stained with a fluorescent dye characterized with an excitation wavelength between 381 – 400 nm and an emission between 414 – 450 nm. Select **Green channel** when cells are stained with a fluorescent dye characterized with an excitation wavelength between 461 – 487 nm and an emission between 500 – 530 nm. Select **Red channel** when cells are stained with a fluorescent dye characterized with an excitation wavelength between 543 – 566 nm and an emission between 580 – 611 nm. Note that it is not possible to combine the **Red** and the **Far-red** channel within one application. Select **Far-red channel** when cells are stained with a fluorescent dye characterized with an excitation wavelength between 626 – 644 nm and an emission between 661 – 680 nm.

NOTE: It is not possible to combine the **Far-red** and the **Red** channel within one application.

Channels: Image acquisition settings	The settings collection depends on the selected channel: **LED intensity [%]:** enter a value manually or use the Live Viewer (see chapter 9.2.1 Live Viewer) for its definition. **Focus offset [µm]:** enter a value manually or use the Live Viewer (see chapter 9.2.1 Live Viewer) for its definition. If defined, the focus offset value is automatically added to the autofocus value. **Exposure time [ms]:** enter a value manually or use the Live Viewer (see chapter 9.2.1 Live Viewer) for its definition.
Z-Stacking	Available for 3D imaging only. If defined, images are acquired at multiple focal positions within the Z-range and used for composition of a 2D projected images. To define the Z-stack settings open the Live Viewer (see chapter 9.2.1 Live Viewer). If option **Delete Z-stack** is set to **Off**, the acquired Z-stack images are saved in the corresponding workspace folder and can be used in the process of further data analysis in e.g., ResultAnalyzer.
Live viewer	See chapter 9.2.1 Live Viewer.
Settle time	Define a time delay between a plate movement and the start of signal integration. Enter a value within the valid instrument range.

Show advanced settings	Click this expand button to access the following parameters: **Settle time**.



NOTE: If the **Exposure time** or **LED intensity** is set too high, there is a risk of photobleaching of samples and images might be too light or too dark.



CAUTION: If the focus offset is defined, always check the value via the Live Viewer. If the focus offset is not in a valid calculated range during the measurement, the corresponding wells will be marked with an autofocus error. In this case, readjust the defined focus offset value.



NOTE: When performing 3D Imaging in U-shaped plates, the autofocus scan range (mainly of 2x objective) may exceed the supported instrument range. The image is acquired but the image quality may be affected. In such cases, it is recommended to use the 4x objective.



NOTE: Image acquisition including real-time data analysis will result in longer measurement times. Perform the image analysis by using the ResultAnalyzer afterwards, if measurement time is a limited factor.



NOTE: For the best system performance use the C: drive. Due to its larger capacity it is recommended to use the DATADRIVE disk for long-term fluorescence imaging kinetic measurements.



NOTE: When conducting kinetic measurements, time intervals between recorded time stamps may deviate slightly due to increasing data base size and varying memory consumption. This effect can be minimized by:

- defining sufficiently large interval times
- reducing the number of images per well
- working with default sensitivity settings for image analysis
- deferring data analysis
- making sure that there is enough memory available (i.e. do not run programs in parallel while performing measurements and make sure to restart the PC after very long and extensive measurements).



NOTE: 3D imaging measurements may result in longer measurement times if executed with a large Z-stack range and multiple images per well (e.g., whole well imaging in a 96-well plate with 4x or 10x objective).

This effect can be reduced by:

- decreasing the number of images per well
- reducing the Z-stack range or increasing the step width if suitable
- deferring data analysis

9.1.2 Analysis Results

The 2D image analysis in SparkControl takes advantage of three underlying analysis types called **Area, Counting and Multi-Color.** The algorithms have been developed to optimally cover the goal of the supported applications, i.e. either the detection of single objects or the localization of

fluorescence or confluence areas in an image. The 3D image analysis with **3D Analysis** tool is based on object segmentation and subsequent optional analysis of fluorescence signal(s) within the segmented area.

Depending on the selected analysis, SparkControl calculates different data as a result of image analysis. This data is grouped into different sets. The selection of a set defines the quantity of results that are calculated and included with the written result files (Excel sheet, PDF report).

For more information, see Analysis Plugins instructions.

9.1.3 Measurement Progress View

During method execution, the calculated data and images are displayed according to the rules described in chapter 2.9.9 Images and 2.9.11 Analysis.

9.1.4 Image Types

Depending on the selected fluorescence imaging application, SparkControl will produce different types of images. All images are stored in the defined Image folder (see chapter 2.13.7 Directory) and named according to the image type as follows:

Type	Description	Example
Original image	• 16-bit monochrome image in tiff format • Related to a single channel • High Dynamic Range (HDR) image for Bright field channel & Digital phase contrast • Non-digitally processed image for fluorescence channels • Use for internal (SparkControl & ResultAnalyzer) and external (third-party software) image processing	O_Bf (Bright field) O_Ph (Phase) R_B (Blue) R_G (Green) R_R (Red) R_F (Far-red)
Z-stack images	• 32-bit monochrome image (HDR for Bright field) • 16-bit monochrome image (fluorescence channels) • Projected image • Related to a single channel	zValue_O_Bf (Bright field) zValue_R_B (Blue) zValue_R_G (Green) zValue_R_R (Red) zValue_R_F (Far-red) Projected image: O_Bf (Bright field) R_B (Blue)
Processed images for fluorescence channels	• 16-bit monochrome image in tiff format • Related to a single channel • Cross-talk corrected image • Digitally processed image	R_B_Processed (Blue) R_G_Processed (Green) R_R_Processed (Red) R_F_Processed (Far-red) R_B_Processed3D (Blue) R_G_Processed3D (Green)
Cross-talk mask	• Related to a single channel • Use for internal cross-talk correction workflow	R_B_Mask (Blue) R_G_Mask (Green)

9.1.5 Measurement Mode

Measurements performed with the Cell Imager module can be defined as:

- Single-label endpoint and kinetic measurements

- Multi-label endpoint and kinetic measurements: fluorescence imaging and the real-time image analysis can be combined with other detection modes, such as fluorescence intensity or luminescence



NOTE: A multi-label measurement with 2D imaging can contain maximum four 2D Imaging strips. A multi-label measurement with 3D imaging can contain maximum four 3D Imaging strips. It is not possible to combine 2D and 3D imaging in a method.



NOTE: If the method includes more than one selected imaging channel within one imaging strip, the corresponding image acquisition is always executed in a well-wise mode.

9.2 Optimizing Fluorescence Imaging Measurements

9.2.1 Live Viewer

The Live Viewer for the Cell Imager module can be started from the corresponding strip, from the Instrument menu of the Method Editor or via the Check-and-Go window in the Dashboard in order to optimize the image acquisition settings prior to measurement start.

In addition, the Live Viewer is available as an individual app for a quick quality check of the cell culture in a microplate.

In all cases, the Live Viewer provides an image of the cells in live mode. When using the Live Viewer for method definition or before method execution, the optimized image acquisition settings can be automatically applied to the corresponding method.

Plate Selection

The Live Viewer, when started from a strip or via the Check-and-Go window, always expects the plate format as defined in the current method.

When working with the Live Viewer app, the plate format must be selected first. Please note that microplates with removable lids and the use of humidity cassettes are not supported by the app.



CAUTION: Always use the microplate according to the method definition or plate format selection in the Live Viewer app, otherwise image acquisition may result in errors.

Graphic Areas

The Live Viewer window is divided into the following graphic areas: **Image**, **Plate** and **Intrawell Position**. The **Image** is displayed enlarged by default. Use the magnification button located at the lower right edge of the minimized areas to enlarge the corresponding graphic area.

When opening the Live Viewer, a well is automatically selected for image acquisition. Use the **Plate** area to select a new well and the **Intrawell Position** area to change the position for the image acquisition within a well.

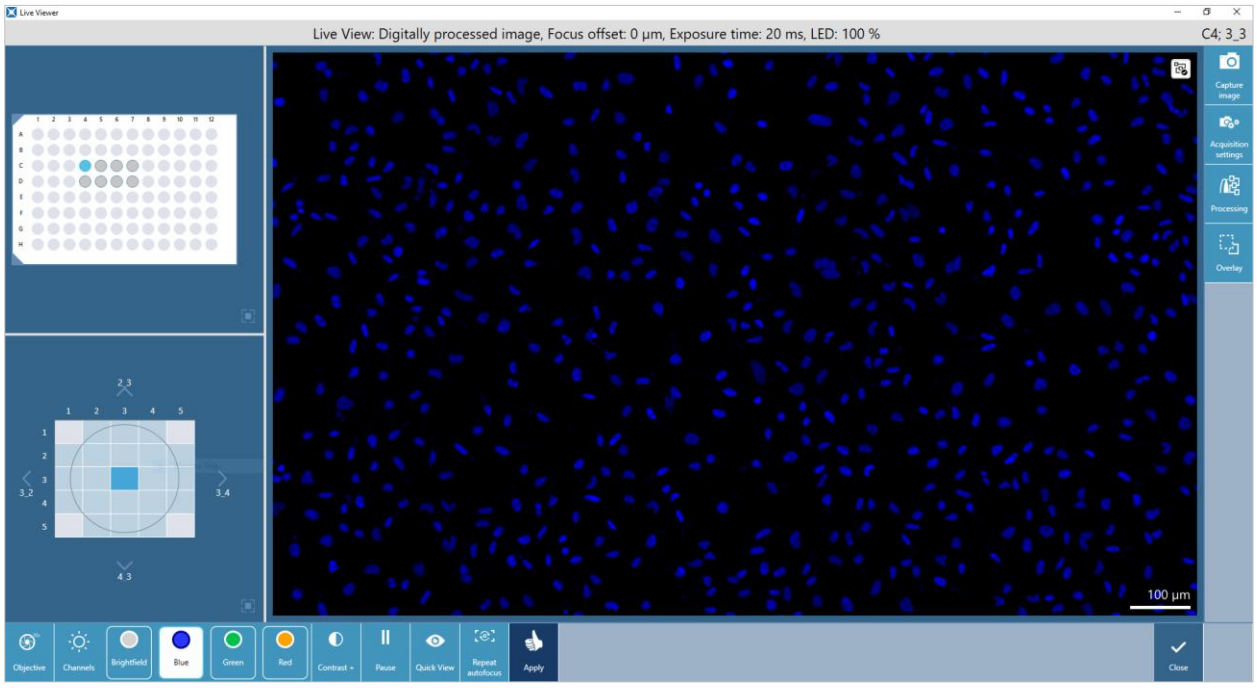


Figure 46: Live Viewer

Objective Selection

- Live Viewer started from the Method Editor or the Check-and-Go window: the objective is defined in the corresponding Fluorescence Imaging strip.
- Live Viewer app: select the objective 2x, 4x, or 10x.

Channel Selection

- Live Viewer started from the Method Editor or the Check-and-Go window: the availability of channel(s) depends on the definition in the corresponding fluorescence imaging strip. In case of multiple channels, select a channel to work with.
- Live Viewer app: all fluorescence channels including bright field are available. Select a channel to work with.

Contrast+

The Live Viewer includes an option to enhance the contrast for single channel color images. Cross-talk and overlay images do not have this feature.

By selecting **Contrast+,** the software displays an image with enhanced contrast. This image may reveal objects with a weak signal, which in the original image may have lower contrast, but are still recognized in the process of image analysis.

NOTE: The **Contrast+** option is available only in the Live Viewer view for single channel images.

Region Of Interest (ROI)

When performing 3D imaging, the definition of ROI, which can be a rectangle or circle area, can be used to limit the analysis area during the measurement or during the post-analysis of images in ResultAnalyzer.

NOTE: The **Region Of Interest (ROI)** is applied to all measurement channels for image analysis only. The ROI does not affect the imaging pattern defined in the corresponding 3D Imaging strip.

For 2D imaging, the ROI is accessible only in ResultAnalyzer.

Acquisition Settings

Select **Acquisition settings** in the action bar located on the right side of the Live Viewer window to edit the default settings:

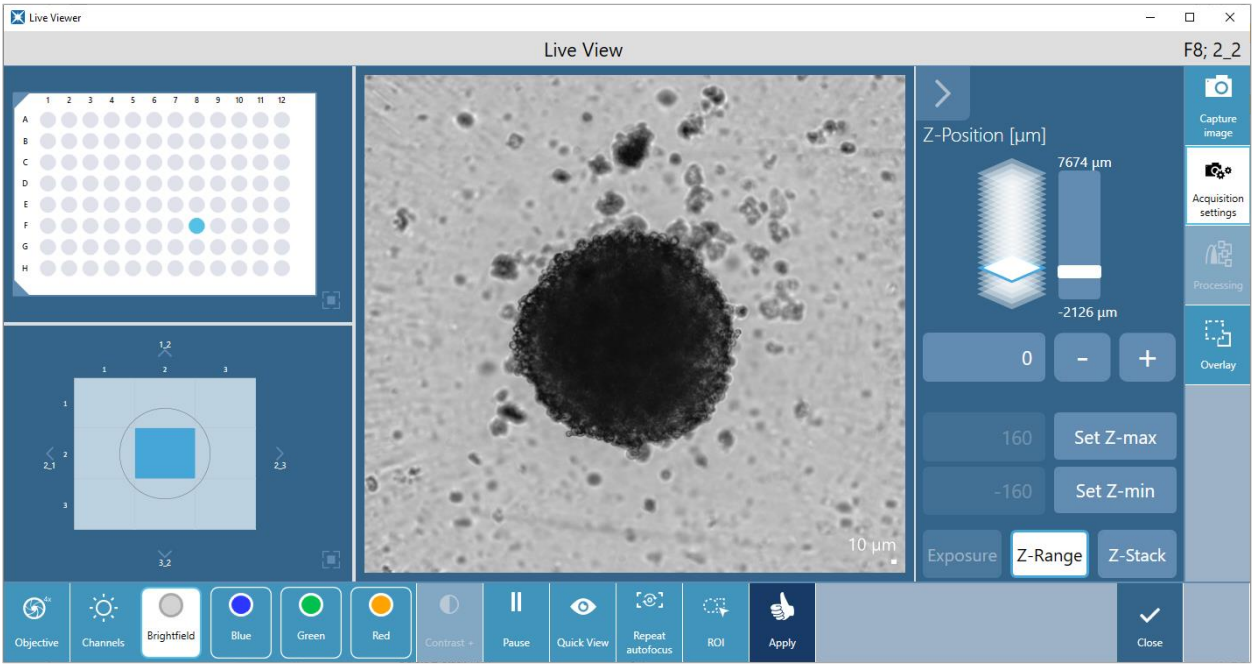


Figure 47: Image acquisition settings for 3D imaging

LED Intensity [%]	Define the optimal LED intensity (not available for bright field channel).
Focus offset [µm]	The Live Viewer automatically provides an image for the selected well by using the autofocus function of the SparkControl software. The autofocus will be automatically re-calculated every time the well selection changes. For a rapid image check in different wells without performing the autofocus repetition per well, use the **Quick View** function. In that case, all images will be acquired with the last valid autofocus settings. If needed, the autofocus calculation can be restarted by selecting the **Repeat Autofocus** action button. It is also possible to define a **Focus offset** value that is added to the calculated autofocus value. Enter an offset value and confirm it by selecting the **Set to instrument** button. The software will adjust the autofocus position accordingly and acquire a new image. Upon clicking **Apply**, the defined focus offset will be automatically applied to the method used for the subsequent measurement, i.e. the focus offset will be added to the each autofocus value calculated per well.
Exposure time [ms]	Define the optimal exposure time (not available for bright field channel) or select **Auto exposure.** Saturated pixel intensity values of 1 RFU are marked white additionally within the corresponding image.
Z-Range	Available for 3D imaging only. To set the **Z-Range** for imaging, defined by Z-max and Z-min position, use the **Z-Position** slider or type in a valid Z-position value. The software will display the corresponding image, which Z-position can be set as **Z-min** or **Z-max**.
Z-Stack	Available for 3D imaging only. Define the **Step width** or **Step number** used for the image acquisition at different focal positions within the selected Z-range. Recommended step width for supported objectives: 20 µm (10x) 40 µm (4x) 120 µm (2x)

After changing the acquisition settings, select the **Apply** button to transfer the modified settings to the method (the Apply option is available only in the Live Viewer connected to the method definition/execution but not in the Live Viewer app).

NOTE: When working in Acquisition Settings mode with the activated digital image processing in the Processing Settings, the display of the corresponding image can be switched between the digitally processed and non-digitally processed image, respectively.

NOTE: The **Apply** button, for the transfer of modified acquisition settings into the method acquisition settings, is available only in the Live Viewer connected to the method definition/execution but not in the Live Viewer app.

NOTE: If the acquisition settings within the Check-and-Go/Live Viewer screen are changed, these new values will be applied to the current measurement run only and will not overwrite the original method definition.

If more than one color imaging channel is in use, apply the **Cross-talk correction** to avoid cross-talk in the selected channels. Check the final camera setup by using the **Overlay** function that generates an overlap of images gained from different channels.

NOTE: When imaging with multi channels, adjust the acquisition settings per channel and afterwards perform the cross-talk correction.

Processing

Digital Image Processing

Digital image processing is an optional function in the SparkControl and ResultAnalyzer software, which allows to optimize the display and analysis of fluorescence images for common 2D cell applications. Specifically, the object segmentation and the resulting object counting are improved for applications such as nucleic counting, viability analysis or the determination of transfection efficiency. It is therefore recommended to select digital image processing for the analysis of 2D cell applications, including multi-color analysis.

For applications that require quantification of total pixel intensity and/or analysis of images with 3rd party software, digital image processing can be deselected. Also, for imaging of 3D cell applications, digital image processing is turned off by default to avoid overcorrection of extended objects (i.e. spheroids and organoids).

In Live Viewer, the digital image processing can be turned on/off and transferred to the corresponding method by selecting the **Apply** button.

If the digital image processing is turned on, the graphic area **Image** contains an additional icon placed top right. This icon enables the user to make a quick visual comparison of the corresponding images.

NOTE: When defining the digital image processing settings for an underlying fluorescence imaging method, use the **Processing** tab or the corresponding setting in the **Image Processing** strip.

Turning on and off the digital image processing icon in the Image graphical area does not have any impact on the digital image processing settings in the method itself. They solely provide the possibility of a quick visual inspection.

CAUTION: In 3D imaging, turning on the digital image processing might result in overcorrection of extended objects (i.e. spheroids and organoids).

Cross-talk correction

When imaging cells labeled with multiple fluorescent markers, the signal detection may be subjected to signal cross-talk. In that case, a fluorescence marker produces a signal in more than just one selected channel, e.g. a green signal is detected in the blue and the green channel. It is recommended to check and correct the signal cross-talk before the start of a measurement. However, the cross-talk settings can also be determined or modified after the measurement via the ResultAnalyzer.

NOTE: It is strongly recommended to perform cross-talk correction via Live Viewer if more than one color channel is used.

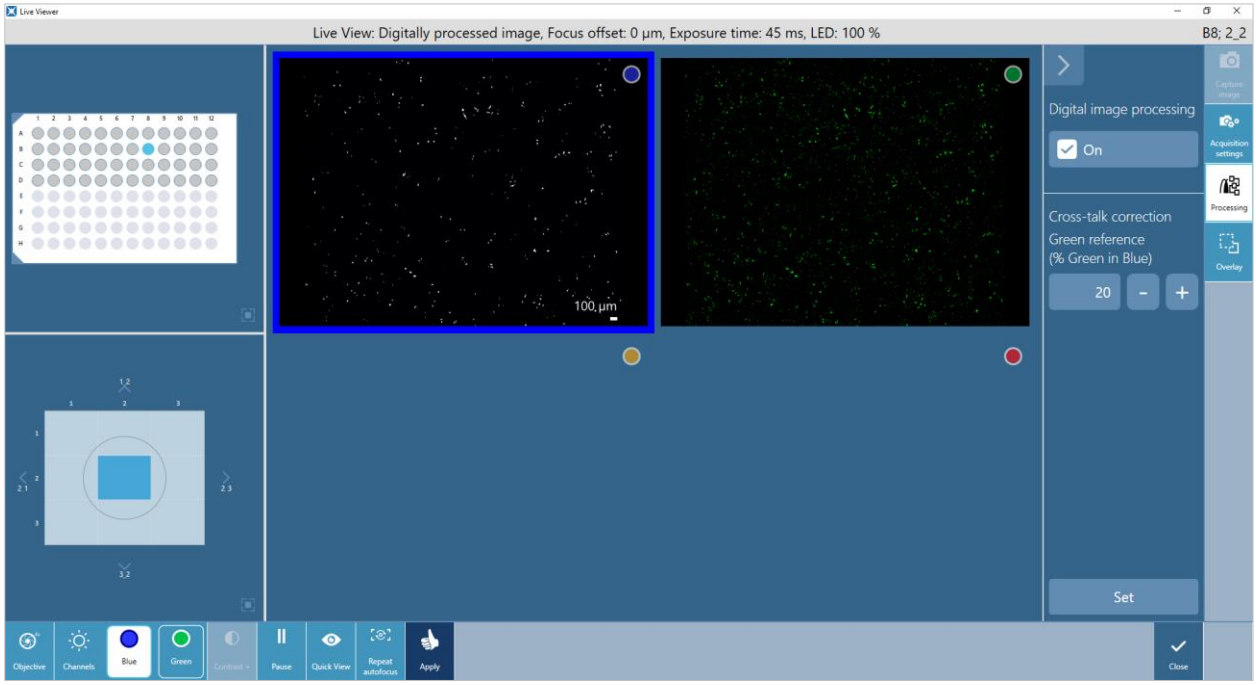


Figure 48: Cross-Talk settings with overcorrection of the Blue channel depicted in white

Cross-talk correction via the Live Viewer is based on the following approach:

1. Cross-talk correction requires a plate with control wells. A control well must contain cells stained with a single fluorescent dye that might cause cross-talk. For example, when running an application with blue and green signals, the plate should contain control wells with the green fluorescence signal only, as the green signal might be detected not only in the green but also in the blue channel.
2. The selected control well is imaged in all relevant channels. In our example application, an image of the control well is acquired with the blue and the green channel, respectively.
3. If there is no cross-talk between the channels, the signal will be recorded only in the dye-specific channel.
4. In case of cross-talk, the cell signal will also be detected in a channel or channels that should not receive the signal of the relevant dye. Continuing with the example above, cross-talk is removed by subtracting the green signal by the % of the signal detected in the blue channel.

NOTE: Cross-talk correction requires control wells with a single fluorophore only.

NOTE: Cross-talk correction strongly depends on LED intensity and exposure time. Always repeat the cross-talk correction, if the LED or exposure time settings have been changed.

NOTE: Cross-talk correction can only be done for excitation cross-talk. Dyes with a broad excitation spectrum should be corrected in all underlying channels. Propidium iodide, for example, might be visible in the blue and green channel, in addition to the dye-specific red one; therefore, a cross-talk correction should be performed in the blue and green channel selecting a reference well stained with propidium iodide only.

Select **Cross-talk correction** and proceed as follows:

1. Select a control well and the corresponding channel. The software displays the corresponding images.
2. Evaluate the images for cross-talk.
3. If cross-talk is detected, correct the respective channel by defining a percentage of the signal to be subtracted from the image. The image is corrected according to the defined value and displayed.
4. Re-evaluate the corrected images and, if needed, repeat the cross-talk adjustment.
5. Select **Apply** to apply the cross-talk settings to the method (available only in the Live Viewer connected to the method definition/execution but not in the Live Viewer app)

CAUTION: Too large correction value(s) in % can result in overcorrection of the respective channel. The parts of overcorrected images are depicted in white. To avoid overcorrection reduce the corresponding correction value.

Image

Images are displayed in real time mode and will therefore be periodically updated. To pause this live stream, select the **Pause** button. To continue the live stream, select the **Live** button.

To save an image, select the **Capture image** button located at the upper right edge of the screen. The saved image can be found in the LiveViewer folder under the default path destination (see chapter 2.11 Measurement Results).

Use the **Overlay** function from the action bar right to view an overlap of images captured in different acquisition channels.

9.2.2 ResultAnalyzer



CAUTION: Avoid working with ResultAnalyzer and SparkControl in parallel to ensure maximum performance of the ResultAnalyzer software.

The ResultAnalyzer software is used to open images, set their analysis parameters and evaluate their content after a method execution. The ResultAnalyzer works with **workspaces** that are created by SparkControl as a result of an imaging measurement.

To view acquired images and their analysis only, select **Images/Measurement**.

For general information and additional functionality of ResultAnalyzer, see chapter 3 ResultAnalyzer.

Result area

The **Result area** is reserved for the display of acquired images and the corresponding analysis results. It is divided into the **Image**, **Plate**, **List** and the **Graphic** views:

Image	Display the acquired and analyzed image(s) for selected channel, well, cycle (kinetic measurements) and, in the case of multiple fluorescence imaging strips, selected label.
Plate	Display the calculated data allocated to the measured area on a plate; User interface for **selecting a well.**
List	Display the calculated data in the list form.
Graphic	Display statistical graphic elements such as histograms and heat maps.

The Image view is enlarged by default and displayed in the central screen area. The remaining views are displayed on the right of the central area in minimized form. To enlarge a minimized view use the magnification button located at the lower right edge of a view.

The Plate, List and Graphic views contain **round navigation controls** at the bottom of the window, if multiple result sets per view are available. Use the controls to move through the different result sets.

Save image

Select **Save image** to export and save the images composed by dynamic image composition. The export can be done per **Well** or per **Plate** for all measured wells. The exported images are located in the Export/Images folder of the corresponding workspace.

Context-sensitive toolbar

The context-sensitive toolbar is an integrated part of the **enlarged view** area. Depending on the view it can reveal the following functions:

Region of interest (ROI)	Selectable only when the Analysis dialog is opened: For quick preview select a region of interest, modify analysis parameters and perform a preview for the selected ROI only. For final analysis select a region of interest, modify analysis parameters and apply it to well/plate. The analysis is performed within the selected ROI only.
Show grid lines	Activate a grid within the Image view.
Capture image	Make a screenshot of the current image view. The image will be saved to the corresponding workspace folder.
Time-lapse	Generate time-lapse video(s) at the selected framerate: Slow (3), Medium (7) or Fast (10). Timestamps can be added optionally.
Adjust image	Modify brightness and contrast settings of selected image.
Mask outline	Modify the line thickness of the object mask.

Measurement

The **Measurement** screen is used to display saved data of an open workspace (View mode). The selected workspace determines the content of the **Result area.**

The image(s) itself can be composed by selecting/deselecting the available image building blocks, i.e. the corresponding channel, and the object mask (Dynamic Image Composition). The selection of the imaging channel(s) **Bright field, Blue, Green, Red** and **Far-red** depends on the method setup of the currently opened workspace and is therefore restricted to the channel(s) that were used for image acquisition.

To modify the contrast and/or brightness settings of an image, select the **Adjust** icon. The contrast algorithm will alter the contrast of visible objects with the background in a linear way. For visualization of very week signals, adjustment of brightness might be needed in addition. **Contrast+**, on the other hand, automatically enhances the signals of all objects. Thus, it represents a straightforward way to reveal objects with a weak signal, which in the original image may have lower contrast, but are still recognized in the process of image analysis. Contrast+ is available for each individual fluorescence channel and can be selected in the process of dynamic image composition.

The selected channel determines the content of the **Result area.** Select an **imaging channel** to view and/or evaluate the channel-specific image(s) and calculated results. Select additional channels to view the overlay of images and the corresponding evaluation results.

Processing

Select the Processing tab to edit the settings for digital image processing and cross-talk correction.

For further information, see chapter 9.2.1 Live Viewer and the corresponding Processing Plugin instruction.



NOTE: Cross-talk correction requires control wells with a single fluorophore only.



CAUTION: Too large correction value(s) in % can result in overcorrection of respective channel. The parts of overcorrected images are depicted in white. To avoid overcorrection reduce the corresponding correction value.



NOTE: Cross-talk correction affects image content and should be performed before changing the Analysis and/or Gating settings.

Analysis

Image analysis is a crucial part of every cell imaging workflow. The analysis result strongly depends on selected analysis parameters. Therefore, their optimization is an indispensable step in every imaging application.

The ResultAnalyzer in SparkControl offers several tools for getting the most out of an image: re-calculation of images based on modified algorithm- and/or cross-talk settings, gating of objects by their size or signal and last but not least, selection of objects by their intrawell localization (Border offset to exclude cells at the well border).

Modification of the **analysis (algorithm) settings** will have an impact on the algorithm used. Therefore, changes will result in a re-analysis of an image with the newly defined values taken into account. In contrast to the analysis settings, object **gating** does not influence the present analysis algorithm. Object gating uses the current image and removes/includes objects from/into the image results in relation to their size or signal intensity.

Work in Analysis mode to edit defined image analysis (e.g. Counting, Area, Multi-color) or perform gating of calculated data. For recalculation, the ResultAnalyzer offers the following options:

Preview	Modified parameters are applied to the image analysis (whole image or area of interest within an image) of the selected well but **not saved.**
Apply to well	Modified parameters are applied to the image analysis of the selected well and **saved.**
Apply to plate	Modified parameters are applied to the image analysis of the whole measurement area and **saved.**
Apply to all plates	Stacker runs: modified parameters are applied to the image analysis of the whole measurement area for all measured plates and **saved.**



NOTE: The cancelation of a recalculation is available only for modifications that can be applied well-wise and not only plate-wise. However, the recalculation of data can be canceled only if the modification is applied to plate. After cancelation, the already recalculated wells will contain the new, recalculated data, whereas the data for the remaining wells will remain unchanged.



NOTE: When using a region of interest (ROI) in combination with the Preview function, the calculated preview results are shown only for the selected ROI within the image of the selected well.



NOTE: Recalculated data are automatically saved by selecting **Apply to well/Apply to plate/Apply to all plates**. The **Preview** function will only recalculate but not save the data.



NOTE: Cross-talk correction affects image content and should be performed before changing Analysis and/or Gating settings.



NOTE: Gates in ResultAnalyzer can have two states: **inactive** (solid line, no gates set) and **active** (dashed line, gates effective). Please consider these states when applying gates to well and/or plate(s).

Edit Method

Work in **Edit method** mode to edit/add/delete defined strips for image analysis (e.g. Counting, Area, Multi-color).For recalculation, the ResultAnalyzer offers the following options:

Analyze and apply	Edit method: images are analyzed with modified analysis settings and the results automatically applied (saved).
Add strips and apply	Edit method: newly defined strip(s) are added and saved without executed analysis.
Reset	Modified analysis parameters are cleared and the last saved analysis parameters restored.



NOTE: Strips for image analysis can be added and saved to the method without automatic analysis execution. This workflow might be of advantage for an application, where the analysis parameters still need to be optimized in **Analysis** mode.

For more information on available analysis strips, see the corresponding Analysis Plugins instructions.

Export Results

After the re-evaluation of images, the new results are not exported automatically to Excel and/or PDF report. Select **Export Results** in the File menu of the ResultAnalyzer to export the corresponding files. Exported file(s) are generated and saved according to the Workspace path defined in **File/Directory**.



NOTE: To export and save the images generated by dynamic image composition, use the Save image function. The Export Result function will export only analysis results.

To export a method select **File/Export Analysis from well to method** (the analysis parameters will be exported from the currently selected well; available only for workspaces with imaging) or **Export Analysis method**. The method is exported to the Methods folder as defined in **File/Directory**. Note that the export path for the Methods folder depends on the Workspace path and is not editable by the user. Finally, open the Method Editor of SparkControl and import the method via **File/Import**.

10 Spark-Stack Microplate Stacker Module

The Spark-Stack is an integrated microplate stacker module, which is available as an option for the SPARK multimode reader. It is designed for automated loading, unloading and restacking of plates for walk-away automation of up to 50 non-lidded microplates per run.



Figure 49: The built-in Spark-Stack microplate stacker for automated loading, unloading and restacking of up to 50 plates per run.

The built-in microplate stacker module uses plate magazines (stacks) as storage containers. The plate magazines are compatible with non-lidded 6- to 1536-well plates and are provided with light-protection covers for light-sensitive assays.

The microplates in the plate magazine located at the INPUT position of the Spark-Stack module are loaded into the SPARK reader one after the other. After the measurement has been performed, the processed plates are collected in the plate magazine at the OUTPUT position.

The grippers of the plate magazines are spring loaded to remain closed in the event of a power failure, holding the plates in position inside the plate magazines despite the lack of power.

Two different heights of plate magazines are available:

- Two short stacks with a capacity of up to 30 plates (standard 96-well plates) per run
- Two long stacks with a capacity of up to 50 plates (standard 96-well plates) per run

10.1 Software

If the Spark-Stack is connected to SparkControl, the defined SparkControl method will be performed on each of the available plates in the input magazine.



CAUTION: Do not use microplates with lids in the Spark-Stack module.



NOTE: Humidity cassettes are not supported when using a SPARK with the Spark-Stack module installed. Please select 'No humidity cassette' if it is applicable to the assay.



NOTE: Open kinetic is not supported when using the Spark-Stack.



NOTE: Optimization of Z-Position via the Z-Position window, Live Viewer and actions Gas, User Request and Move Plate In/Out are not supported in stacker measurements.



NOTE: Tecan Apps are not supported in stacker measurements.



NOTE: The Evaluation plugin is not supported in stacker measurements.



NOTE: A kinetic measurement cannot be paused when executed within a stacker run.



NOTE: A stacker run cannot be started via the Onboard-Start.



NOTE: Temperature settings can only be maintained when the plate is located inside the instrument, not in the input/output magazine.

10.1.1 Start Stacker Run

Once a method has been defined, batch processing can be started from the Method Editor by selecting the **Start Stacker button** in the toolbar or from the Dashboard by selecting the corresponding **Method** tile **and** clicking the **Start Stacker tile** in the Check-and-Go window of the Dashboard. The output magazine of the Spark-Stack must be empty before staring a stacker run.

NOTE: When the input and output magazines are inserted, the Method Editor contains an enabled **Start Stacker** button and a disabled **Start** button. Remove the input and the output magazines to perform a run without the stacker.

CAUTION: Make sure that the microplate matches the plate definition in the method to prevent problems during a stacker run. Always use microplates of the same type and color.

Stacker Operations Window

After starting a stacker run, the Stacker Operations window appears:

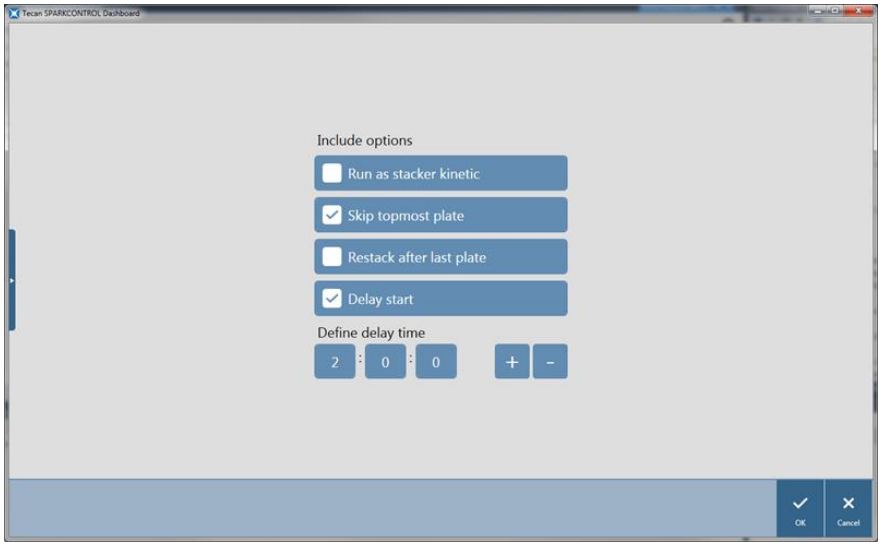


Figure 50: Stacker Operation window

Run as stacker kinetic	When selected, a method defined as a kinetic measurement will be executed as a stacker run. For more information, see chapter 10.1.2 Stacker Kinetics.
Skip topmost plate	Select **Skip topmost plate** to not perform measurements on the topmost plate. The topmost plate will be directly transported through the SPARK reader, without measurement, to the output magazine.
Restack after last plate	Select **Restack after last plate** to return all plates to the input magazine in their original order after processing.
Delay start	Define a time to delay the start of the stacker run. The start of the stacker run will be paused for the defined delay time.
Number of plates	To check the free disc space, enter the number of plates used for measurement (bright field and fluorescence imaging only).

NOTE: Delay start of the Stacker run: this function can be used to perform a room-temperature incubation step for microplates in the plate magazine, before the stacker run is started. A set of dark covers and lids for the plate magazines are available to protect light sensitive assays.

10.1.2 Stacker Kinetics

In contrast to kinetic measurements on one plate, stacker kinetics allows for the analysis of multiple plates in a time-dependent manner. After all of the plates in the input magazine have been measured in cycle 1, the plates are automatically restacked in their original order and measured again until the user-defined number of cycles has been completed on all of the plates.

To facilitate data evaluation, a separate results sheet is generated for each plate and named according to the plate number or barcode (if installed and selected in the method). Results of subsequent cycles are automatically added to the corresponding results sheet.

Stacker kinetics can be used with any plate-wise kinetic measurement script, and can be combined with all available kinetic conditions. A maximum of 300 cycles is possible.

To perform a stacker kinetic measurement, the workflow / method can be set up in the same way as a usual kinetic measurement and started using the **Start Stacker** button. The **Stacker Operations** window opens to provide access to additional functions specific for stacker measurements. By selecting **Run stacker kinetic**, the script is automatically executed as a stacker kinetic measurement.



NOTE: Plate-wise kinetic measurements with one kinetic strip and a maximum of 300 cycles can be executed as stacker kinetics.



NOTE: Only kinetic measurements with the loop type **Number of cycles** can be run as stacker kinetic.



NOTE: The actions **Wait** and **Shake** can be used in a stacker kinetic, however **Continuous Waiting** and **Continuous Shaking** are NOT supported as a single plate does not remain in the instrument between two subsequent kinetic cycles.



NOTE: Fluorescence imaging is not supported in Stacker kinetic runs.

10.1.3 Restacking

Use the restack function of SparkControl to restack plates without performing a measurement. Restacking can be started in the Instrument menu of the Method Editor or via Instrument control or Check-and-Go window of the Dashboard by selecting the **Stacker** button.

Before restacking, define the plate format of the plates in the output magazine. Use **Smooth mode** according to plate format or filling volume (see chapter Filling Volumes/Smooth Mode in the SPARK – Reference Guide). **Smooth mode** is recommended when using low weight microplates as e. g. 1536 well microplates.

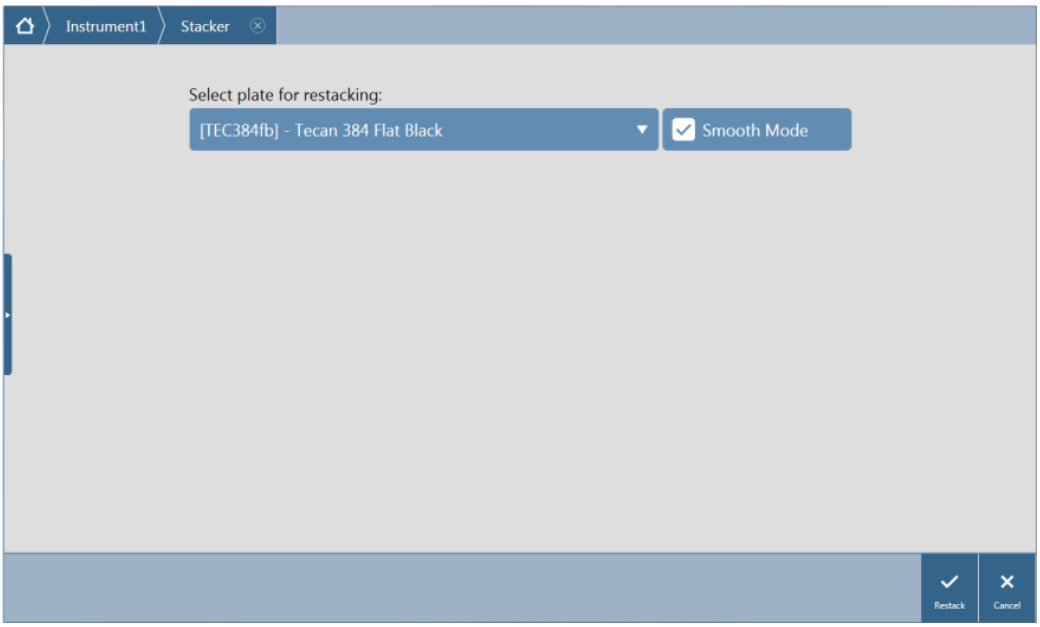


Figure 51: Restacking window

11 Injectors

11.1 Performing Measurements with Injectors

The injectors can be used alone or in combination with the following detection modes: Fluorescence Intensity top and bottom, Time Resolved Fluorescence, Fluorescence Polarization, Absorbance, Luminescence as well as Multicolor Luminescence. However, as the measurement position is not the same as the injection position, a short time delay (approx. < 0.5 s) between injection and reading occurs. For exception, see chapter 7.4 Inject and Read.

11.1.1 Injector Strip

This strip is used to perform injections.

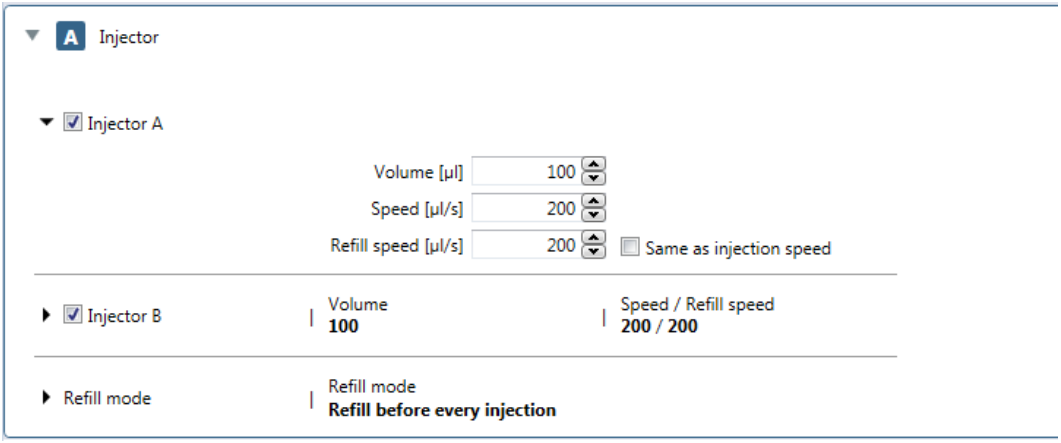


Figure 52: Injector strip

The **Injector** strip includes the following elements:

Injector A **Injector B**	Select injector A, B or both. The selection depends on the instrument configuration.
Volume	Define the volume to inject into a single well.
Speed	Define the speed of liquid flow during the injection.
Refill speed	Define the speed for liquid refill during the method run. **Same as injection speed:** Select the check box if the refill speed will be the same as the injection speed.
Refill mode	Select one of the following options: **Standard:** Injection occurs as long as the syringe contains enough liquid. As soon as the liquid in the syringe is used up, the syringe is refilled with the defined refill volume. **Refill before every injection:** Refilling of the syringe occurs before each injection step.

11.1.2 Injector Strip in Measurement Flow

Injection can be performed plate-wise or well-wise. The plate-wise injection means that the liquid is first injected into all selected wells of the plate, and the whole plate is measured thereafter.

For the well-wise injection, the injection strip must be inserted below a Well strip. In that case, the liquid is injected into the first well, and then this well is measured as defined, before the liquid is injected into the second well and so on.

11.1.3 Z-Drive Injector Port

The injector port is equipped with a Z-drive to optimize injection performance for every plate format and height. The Z-optimization is available for measurements in absorbance, luminescence and fluorescence mode and adjusted automatically by the software.



CAUTION: Make sure that the selected plate definition file corresponds to the currently used microplate, otherwise the instrument could become damaged.

11.1.4 Optimizing Measurements with Injection

The measurements with an injection step might be optimized by waiting and/or shaking after the injection step in order to homogenize the liquid mixture in the well before starting the next workflow.

For the corresponding strips (Wait and Shake), see chapter 2.7.3 Action.

12 Environmental Control

The heating, gas and humidity control of Tecan's multimode reader, SPARK, provides an optimal system for the regulation of environmental conditions during a measurement run. Stable environmental conditions are demanded by many assays to ensure optimal performance. Especially when performing live-cell experiments – a constant temperature, pH-value and humidity are required to maintain cell health and to minimize cellular response to environmental changes.

12.1 Heating Module

The heating module enables temperature control within a range from 3 °C above ambient temperature to 42 °C. Heating of the measurement chamber will take some time. Please check the temperature control display. If not incubated externally, the microplate should be left for equilibration before the measurement is started.

NOTE: To keep the temperature constant and provide uniformity across the plate, the plate must be placed in incubation position while shaking or waiting. When the heating function is used during shaking, the temperature may vary slightly.

The temperature control in the software can be activated manually or during the execution of a method.

12.1.1 Manual Temperature Control

The temperature control can be switched on manually via the **Temperature Control** window in the Dashboard or the Method Editor:

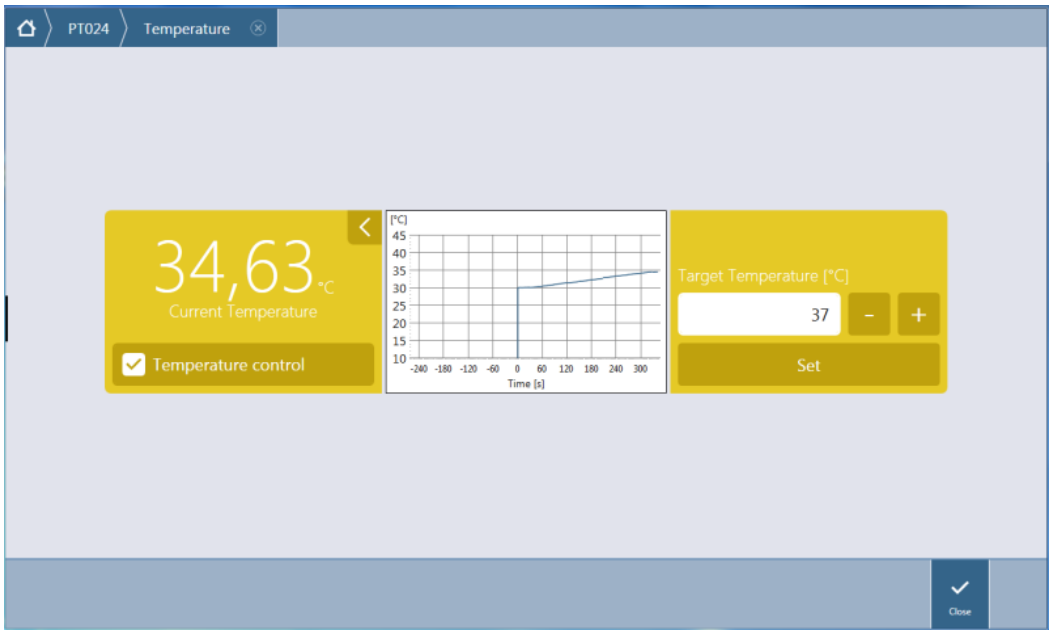


Figure 53: Temperature Control window

Select **Temperature control**. Enter the **Target Temperature** and click **Set** to start heating. View the current temperature inside the instrument by selecting the expand button at the top right of the Temperature Control tile. Clear the **Temperature** control check box to stop heating.

CAUTION: When defining values with decimal places always use the decimal symbol as defined in the Region and Language settings of the PC's operating system.

NOTE: When starting a method with temperature control, the method settings will always overrule the manual settings if their definitions do not match.

12.1.2 Temperature Control via Method

NOTE: The heating of the instrument starts when starting the method. If **Wait for temperature** is selected, the measurement will not start until the current instrument temperature is within the specified range. For pre-heating of the instrument, see chapter 12.1.1 Manual Temperature Control.

Temperature Strip

This strip is used for temperature control.

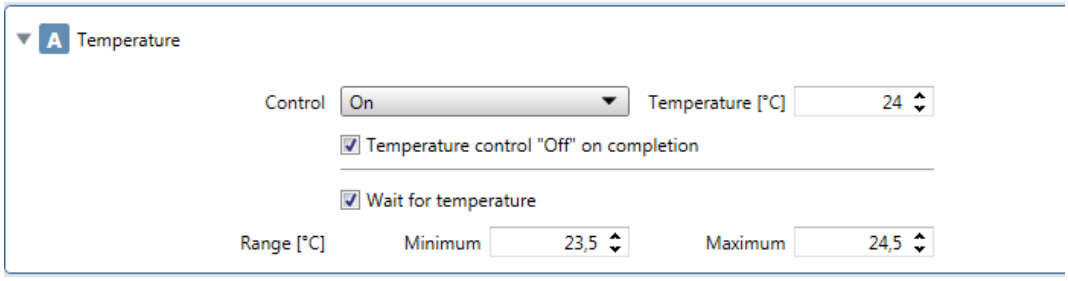


Figure 54: Temperature strip

The **Temperature** strip includes the following elements:

Control	Select On to enter a target temperature value.
Wait for temperature	Select Wait for temperature to define the Minimum and/or Maximum temperature values.
Temperature control 'Off' on completion	Select this option to turn off the temperature control after the measurement execution.

CAUTION: When defining values with decimal places always use the decimal symbol as defined in the Region and Language settings of the PC's operating system.

12.2 Cooling System

The cooling system of the SPARK multimode reader enables temperature control in a range from 18 °C up to ambient temperature.

Preparing the instrument for cooling and the cooling of the measurement chamber itself will take some time. Please follow these instructions and check the temperature control display. If not incubated externally, the microplate should be left for equilibration before the measurement is started.

12.2.1 Cooling Control Software Settings

NOTE: Always switch on the external liquid cooling device when working with temperature control.

For the software settings, see chapter 12.1 Heating Module.

Ambient Cooling Mode

The ambient cooling mode is designed to easily set the room temperature as target temperature for the instrument. It can be activated via the **Temperature Control** window in the Dashboard or the Method Editor:

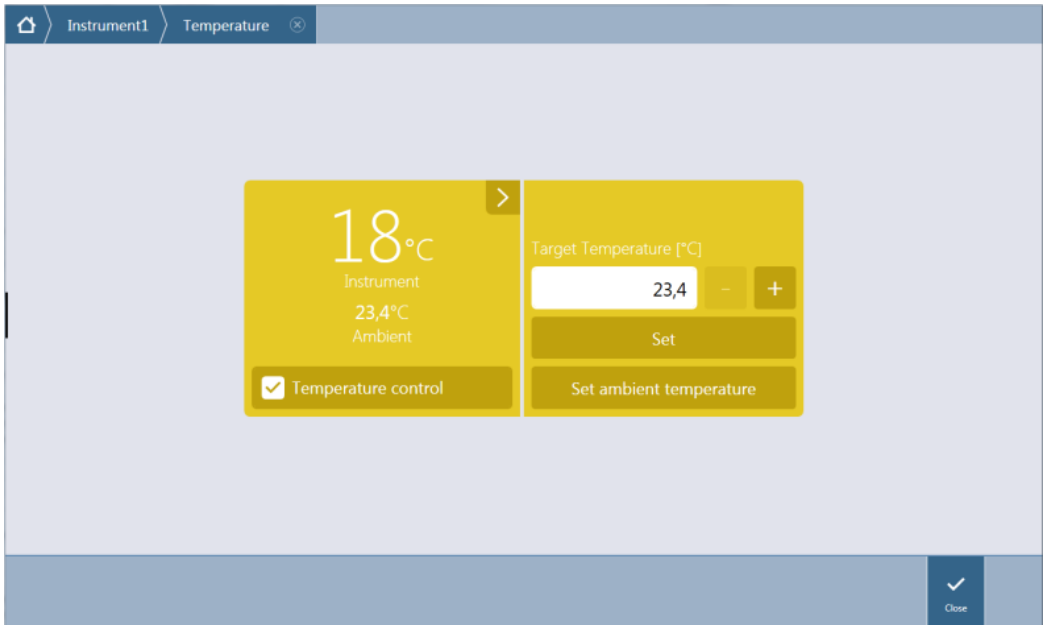


Figure 55: Temperature Control window for instruments with the cooling module (Te-Cool)

Select **Temperature control** and click **Set ambient temperature.** The current ambient temperature will be set automatically as the target temperature. View the current temperature inside the instrument by selecting the expand button at the top right of the Temperature Control tile. Clear the **Temperature control** check box to stop cooling.

NOTE: Always switch on the external liquid cooling device when working with temperature control.

12.3 Gas Control

12.3.1 Gas Control Software Settings

The gas control can be activated manually or within a method execution.

NOTE: When starting a method with gas control, the method settings will always overrule the manual settings, if their definitions do not match.

NOTE: Before starting work with the Gas module, the sea level height of your location must be entered via the Instrument settings (see chapter 2.13.2 Instrument).

12.3.2 Manual Gas Control

The gas control can be switched on manually via the **Gas Control** window in the **Dashboard** or the **Method Editor**.

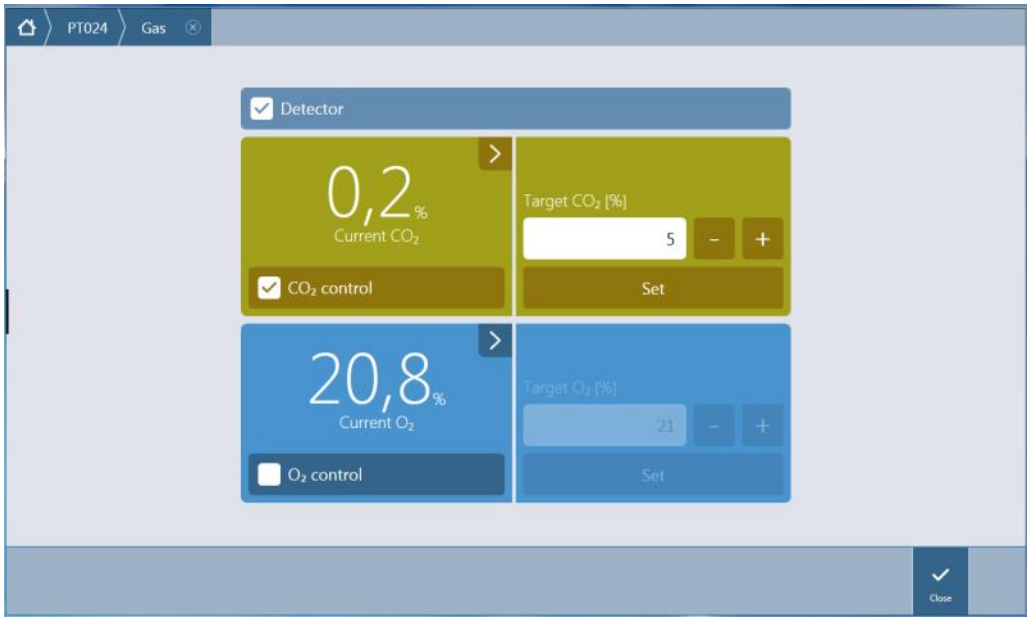


Figure 56: Gas Control window

Select **Detector** to switch on the gas detector(s). Select **CO_2 control** or/and **O_2 control**. Enter the target gas concentration and click **Set** to start gas regulation. View the current gas concentration inside the instrument by selecting the expand button top right in the control tile(s). Clear the gas control check box(es) to stop gas regulation. Clear the **Detector** check box to switch off the gas detectors.

CAUTION: When defining values with decimal places always use the decimal symbol as defined in the Region and Language settings of the PC's operating system.

NOTE: Switching on the gas detector(s) might take a few minutes.

12.3.3 Gas Control via Method

NOTE: Gas regulation starts when the method starts. If **Wait for gas** is selected, the measurement will not start until the current gas concentration is within the specified range. For information about adjusting the gas settings prior to performing measurements, see chapter 12.3.2 Manual Gas Control.

NOTE: Turning on the gas detector(s) might take a few minutes. We recommend turning on the detector(s) before starting a measurement with gas control.

Gas Strip

This strip is used for gas control.

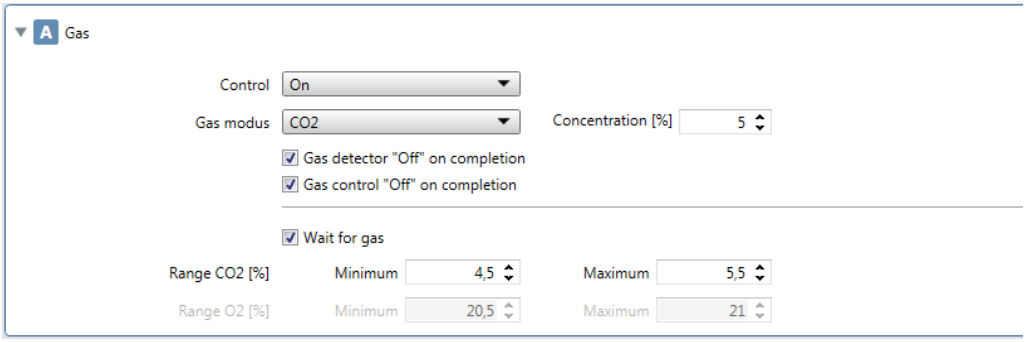


Figure 57: Gas strip

The **Gas** strip includes the following elements:

Control	Select **On** to start the gas control
Gas module	Select one of the following options: **CO_2**, **O_2**, **CO_2** and **O_2**, **Monitoring.** Use the last one to monitor the **CO_2** and/or **O_2** concentration without turning on the internal gas regulation.
Wait for gas	Select it this option to define the **Minimum** and/or **Maximum** gas concentration values.
Gas control 'Off' on completion	Select this option to turn off the gas control after the measurement execution.
Gas detector 'Off' on completion	Select this option to turn off the gas detector after the measurement execution.

CAUTION: When defining values with decimal places always use the decimal symbol as defined in the Region and Language settings of the PC's operating system.

WARNING: Ensure that a sufficient supply of CO_2 or N_2 is provided during incubation. Running out of gas or failure of gas supply may negatively affect or harm your cell application.

WARNING: Make sure to apply a suitable gas-permeable adhesive foil, tape, or cover to the microplate. Sealing the plate facilitates the gas exchange (ventilation) of cultures while simultaneously acting as a barrier to reduce evaporation during gas supply.

NOTE: Always include appropriate positive and/or negative controls in your assay to reflect effects on cell viability during incubation.

WARNING: Treat bio-hazardous material according to applicable safety standards and regulations.

12.3.4 Acoustic Alarm

If the target concentration is not reached within 20 minutes after the initial activation of a gas mode or when it deviates for more than 10 minutes during operation, i.e. with a deviation (> +/- 20 %), an acoustic alarm will sound. This will help you to recognize, for example, when the gas supply has run out (tank is empty). A message appears specifying which gas is affected and to check the corresponding gas cylinder. Click **OK** to stop the acoustic alarm and continue the method.

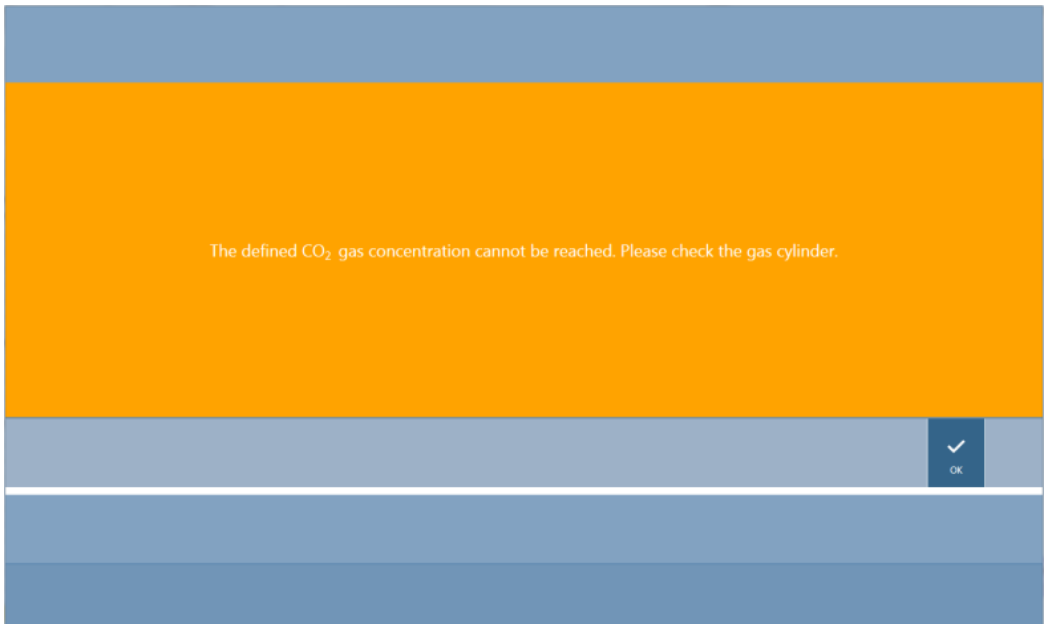


Figure 58: Stopping the gas alarm

If power is lost, the gas valves will close automatically.

12.4 Humidity Control

12.4.1 Software Settings

The humidity cassette can be selected within the Plate strip (see chapter 2.7.1 Plate).



NOTE: A humidity cassette is used in combination with the lid lifter. Please make sure to attach a magnetic pad to the cassette lid before use.



NOTE: The option Removable Lid cannot be used with the Humidity cassette. If a plate cover is used, select the option Lid in the software.



WARNING: Always use the Cyto humidity cassettes in combination with the Cell Imager module, otherwise the instrument could become damaged.

Ventilation

The ventilation settings, i.e. the duration and interval time, can be defined within the **Shake** and **Wait** strips.

Shaking

Shaking in combination with the humidity cassette is restricted to orbital and double orbital mode to avoid liquid spilling.

13 NanoQuant App

The NanoQuant Plate is intended to quantify nucleic acids and proteins in a small volume of 2 µl by using absorbance as detection mode. Up to 16 samples can be measured at a time.

Tecan provides two ready-to-use apps for the routine analysis of nucleic acids: the NanoQuant Quantitation App, which is used for the quantitation of nucleic acids at 260 nm and to enable quick access to the information about the concentration and purity of applied samples. The Labeling Efficiency App additionally provides information on the concentration of the marker(s) used in the labeling procedure.

For the quantitative determination of proteins, Tecan offers the NanoQuant Protein Quantitation App. Quantitation of proteins is performed by measuring their specific absorbance at 280 nm.

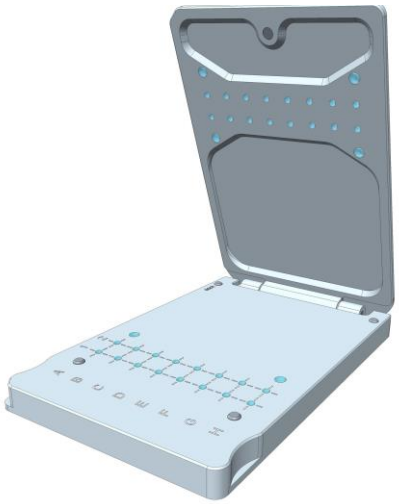


Figure 59: The NanoQuant Plate

13.1 Nucleic Acid Quantitation App

Measuring the inherent absorbance of nucleic acid samples at 260 nm forms the basis for this app. The concentration levels of the samples are calculated from the values obtained. To determine the purity of the nucleic acid, additional measurements at 280 nm and 230 nm are performed followed by an absorbance scan in the range of 200 to 400 nm as default. The measurement at 280 nm is performed to check for the presence of proteins in the sample. The corresponding absorbance value is used for the calculation of the index of purity, which is a ratio between the measured values at 260 and 280 nm. The measurement at 230 nm is performed to check the sample for contamination by carbohydrates, salts or organic solvents. In this case, a ratio of the measured signals at 260 and 230 nm is calculated and used as an indicator for the purity of the sample.

NOTE: Pure DNA samples show a 260/280 ratio between 1.8 and 1.9, while pure RNA samples have a ratio of about 2.0. Lower ratio values may indicate the presence of proteins or other contaminants. If this is the case, an additional purification step is recommended.

NOTE: Pure nucleic acids show a 260/230 ratio in the range of 2.0 – 2.2. If this ratio is appreciably lower than expected, it may indicate the presence of, for example, salts or organic solvents. If this is the case, an additional purification step is recommended.

13.1.1 Applying Samples

The fastest way to apply samples onto the NanoQuant Plate is by using an 8 channel pipette. The precise and consistent application of the samples is ensured by the use of optimal tips for the pipette.

If help with the application of the samples is needed, the pipetting aid is recommended. The pipetting aid has to be placed onto the quartz lenses with the indentation downward. After applying the samples onto the quartz lenses, the pipetting aid has to be removed carefully without touching the sample drops. Close the lid carefully and put the plate onto the plate carrier.

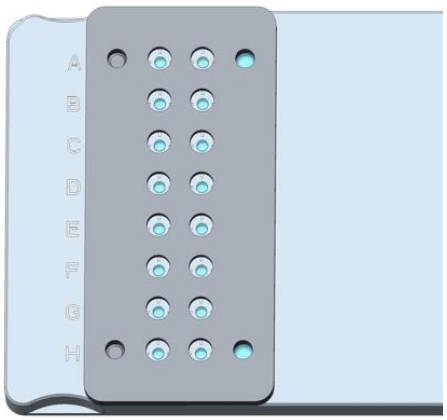


Figure 60: The NanoQuant pipetting aid

Optionally, a single pipette can be used for the application of samples to the NanoQuant Plate. To increase precision and to avoid cross contamination with other samples, it is highly recommended to use a new tip for every sample. Work in a timely manner – otherwise samples may quickly evaporate leading to false results.

13.1.2 Starting the Nucleic Acid Quantitation App

The Nucleic Acid Quantitation application can be started via:

- The Dashboard by selecting the corresponding application tile or
- The Method Editor by selecting the application from a drop-down list placed top right (-> Select app).

Blanking

Before quantifying the nucleic acids, a blanking measurement must be performed.

Perform blanking as follows:

1. Start the Nucleic Acid Quantitation application tile.
2. Select the wells to be used for blanking (by default all wells are selected).
3. Select Sample in the action bar to select the sample types if another sample other than ds DNA is measured (by default **ds DNA** is selected, see figure below)
   The following selections are possible:
   - ds DNA
   - ss DNA
   - RNA
   - or select Edit Sample in the Action bar
4. Optionally, select **Absorbance Scan** to change the wavelength range for absorbance scan. Default settings: 200 – 400 nm.

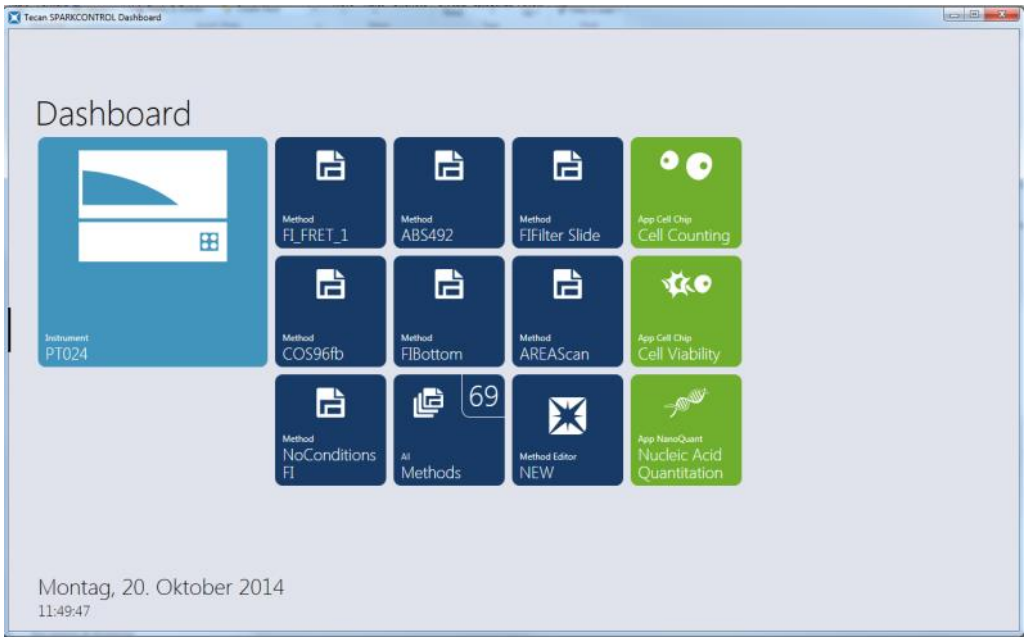


Figure 61: Start the app via Dashboard

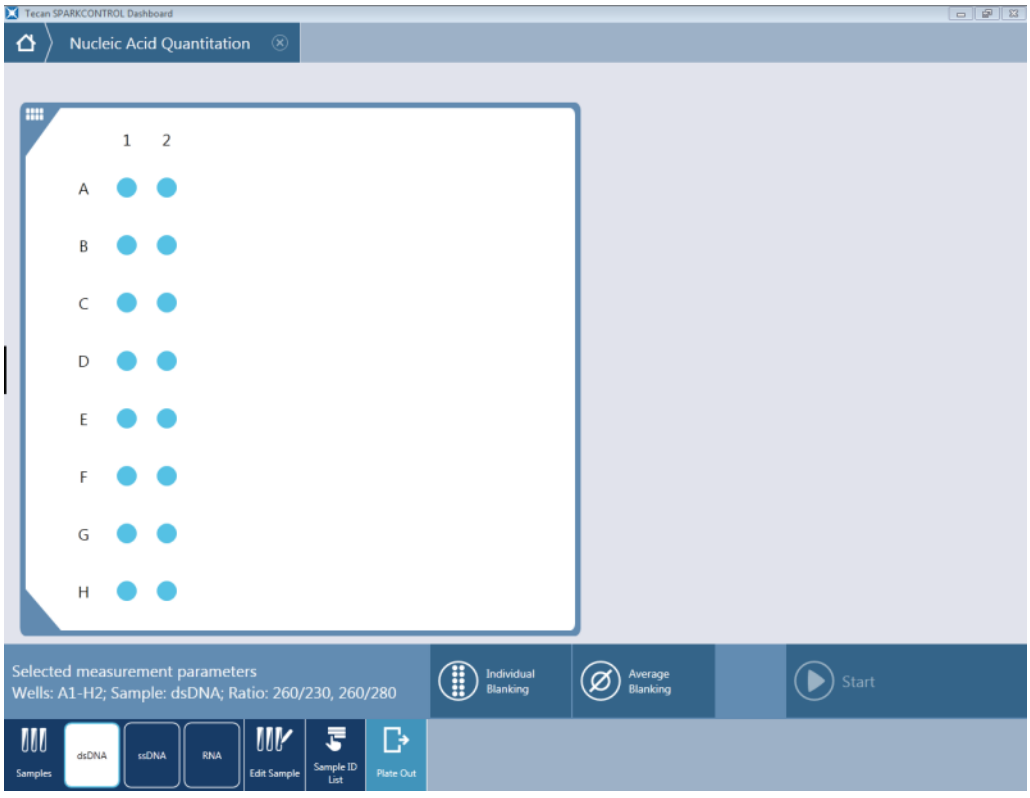


Figure 62: Start Blanking window

5. Load the blanks onto the selected positions for blanking and close the lid of the NanoQuant Plate carefully.
6. Select **Individual Blanking** to start the individual blanking procedure or **Average Blanking** to start the average blanking procedure.

NOTE: Individual blanking requires blanking for all wells that are to be used for subsequent measurements. The blank correction of the samples is performed by using the single blanking value of the corresponding well on the NanoQuant Plate. For individual blanking, the selection of at least one well is required.

NOTE: Average blanking: The selection of at least two wells is required, independent of the number of wells used for the subsequent sample measurement. The measured blank values are averaged and the calculated average value is then used for correcting the sample measurement values.

7. After selecting Individual Blanking or Average Blanking, the plate transport moves out and the user is requested to insert the NanoQuant Plate with the respective blanking samples.
8. Start the blank measurement by selecting **OK**. The incoming values/wells are shown in the information header of the Measurement Status window.
9. After blanking, the blanking results are displayed in a list (see figure below). Enlarge the list by double-tapping.

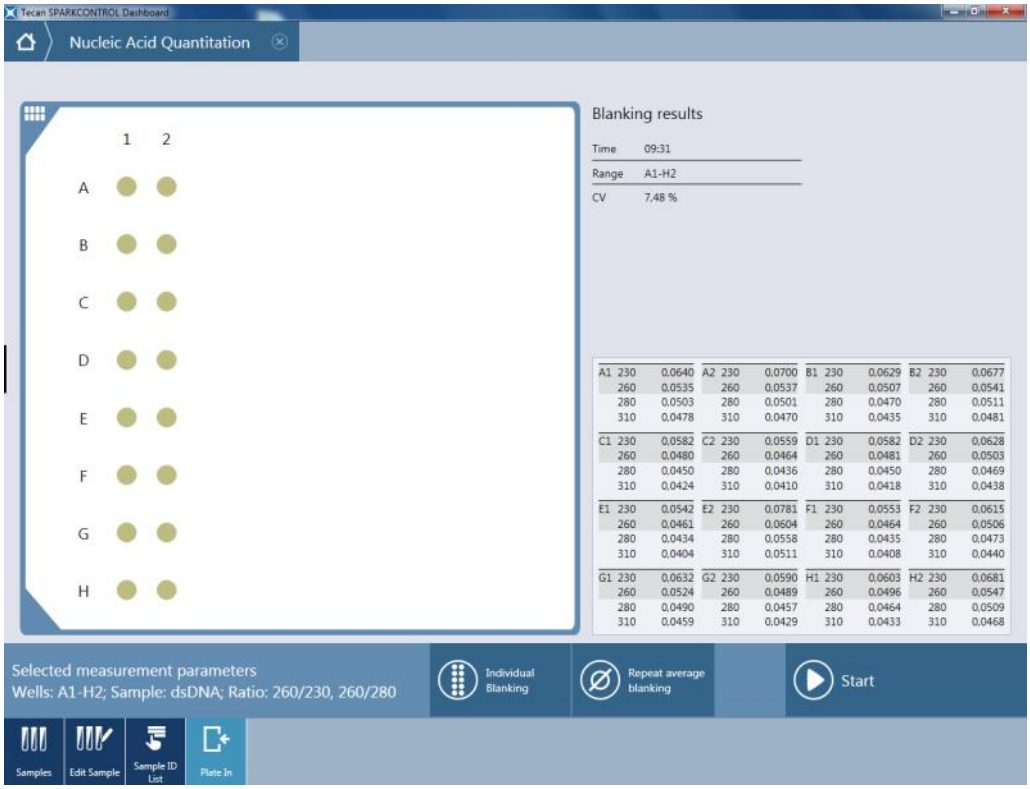


Figure 63: Start Measurement window with blanking results

NOTE: The blanking results will be stored with respect to the blanking parameters, wavelength settings and sample type. If one of these parameters is changed, the blanking procedure has to be repeated.

13.1.3 Validation Criteria for Blanking Results

NOTE: Individual blanking requires no validation criteria.

NOTE: Average blanking: A blanking result is valid if the CV (coefficient of variation) of the raw OD values at 260 nm is below a threshold of 10 %. If this criterion is not fulfilled the blanking procedure has to be repeated and the measurement of the samples is prevented. The wells displaying values exceeding the allowed CV-threshold are highlighted.

13.1.4 Repeat Blanking

NOTE: Repeat the blanking in case of an incorrect blanking measurement or when using new blanking samples.

To repeat individual or average blanking, select the corresponding action button. If necessary, reselect wells that will be used for blanking.

CAUTION: If blanking is repeated the current blanking results will be discarded.

CAUTION: Opening and closing the NanoQuant application does not lead to loss of the blanking results. By disconnecting the instrument or restarting the software the existing blanking results are discarded.

13.1.5 Start Measurement

1. Remove any remaining blanking buffer from the sample positions by wiping the quartz spots with a piece of lint free paper and apply 2 µl of your samples.
2. After individual blanking has been performed, as default the same area used for blanking is selected for measurement. It is possible to reduce the number of wells for the sample measurement, but the number of wells cannot be increased.
3. After average blanking has been performed, the blanking selection is automatically inverted, unless the whole plate was selected. In this case, the whole plate remains selected as default. It is possible to reduce the number of wells or increase the number of wells for the sample measurement beyond the blanking range (max. 16 wells).
4. Insert the plate into the plate carrier.
5. Select **Start**. The measurement is started and the incoming values can be retrieved from the information header of the Measurement Progress window.

6. After the measurement the results are displayed and the plate is moved out automatically (see figure below).
7. To perform a new measurement without closing the app, select **Next Plate**. Click **OK** to close the window. The software returns to the Start Measurement window and a new measurement can be started.

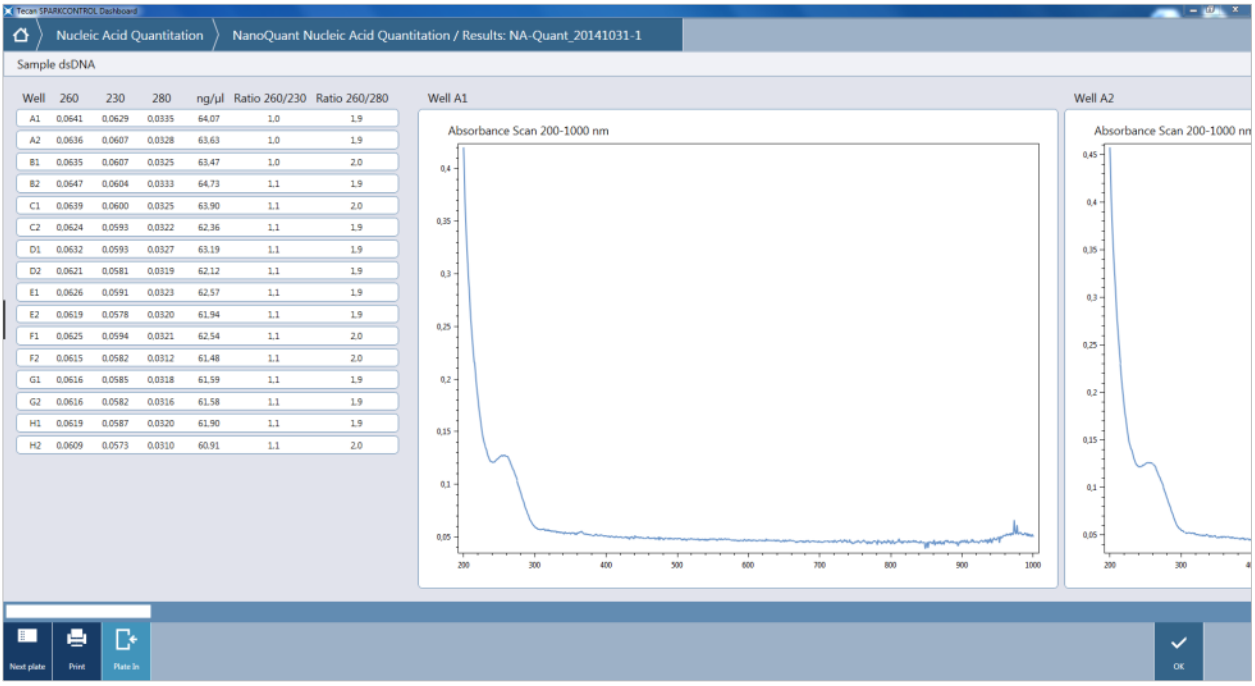


Figure 64: Result window

Measurement Results

The result window includes the following results:

- Sample type (information header)
- Well position
- Well-specific OD correction values at 260, 280 and 230 nm
- Nucleic acid concentration in ng/µl
- Ratio 260/230 nm
- Ratio 260/280 nm
- Sample ID (if sample IDs are defined)
- Absorbance Scan values according to the defined wavelength range. Use the slider to obtain all of the absorbance scans being measured (see figure below).

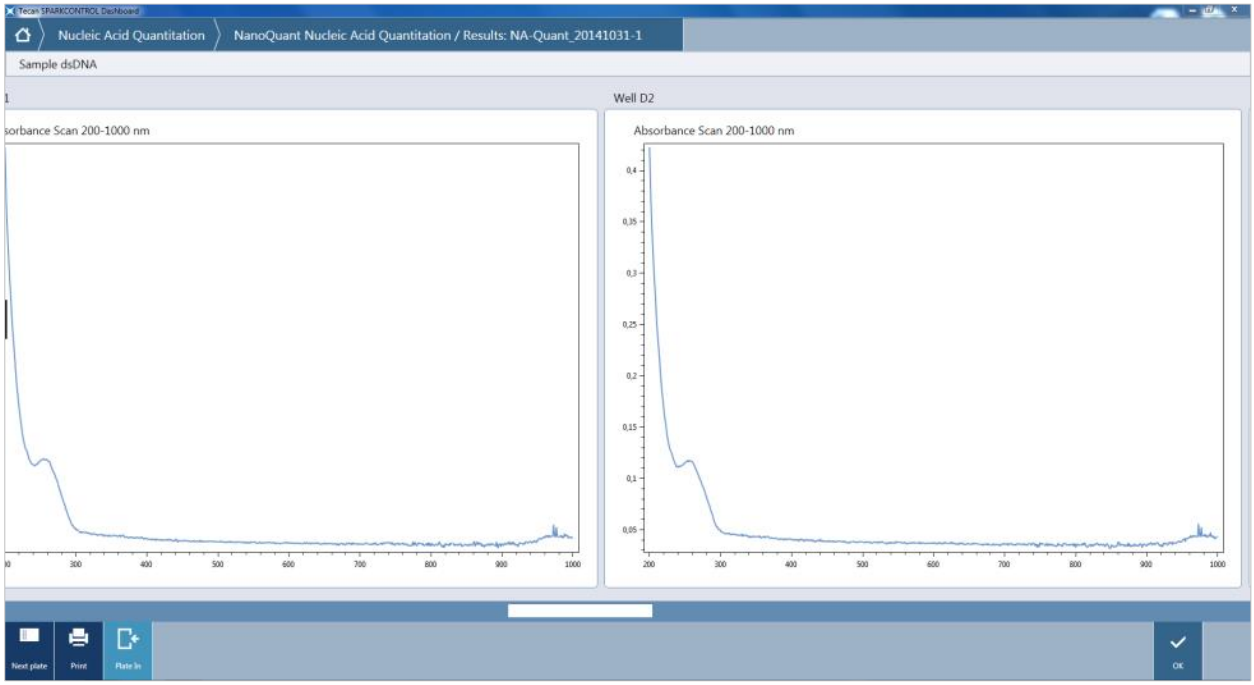


Figure 65: Absorbance scans in the Result window

The final report can be printed in form of a pdf file by selecting **Print** in the action bar.

NOTE: All result data is automatically exported to Microsoft Excel.

Edit Sample

To define or edit a user defined sample select **Edit Sample** in the action bar.

It is possible to edit user defined samples only. To define a new sample the name, the extinction coefficient and the ratio wavelength (the wavelength at 280 and/or 230 nm is available for ratio measurements) must be assigned (see figure below).

To add a new sample, select the **+** button. To delete a sample, select the trashcan button.

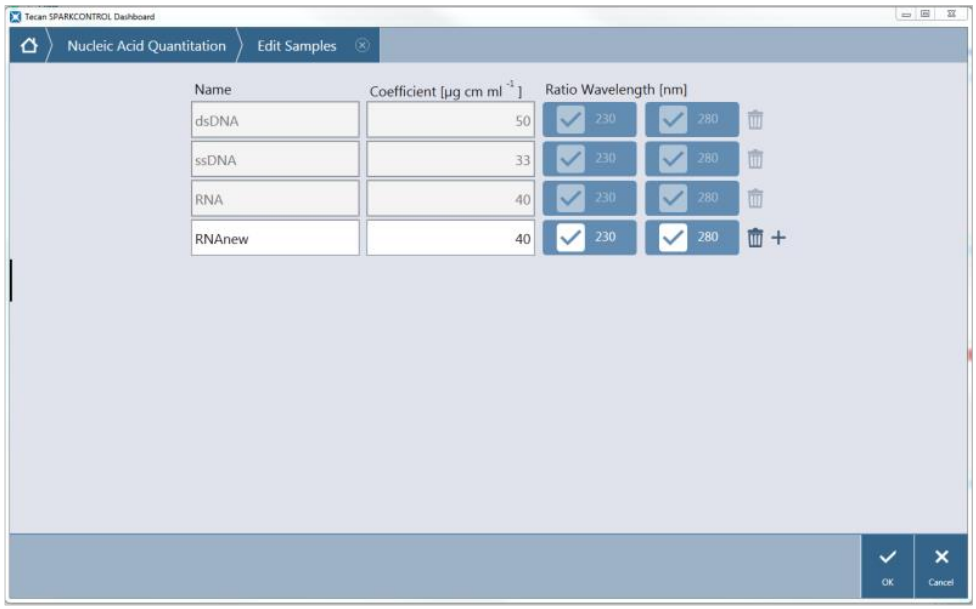


Figure 66: Edit/Add sample

Sample ID List

With the sample ID list it is possible to assign an ID to each sample on the NQ Plate. To add a sample ID list, select **Sample ID List** in the action bar. Define the sample ID (15 characters at maximum). A sort function enables the ordering of the samples (e.g. A1, A2,... or A1, B1,... ; see figure below). To accept the entries select **OK**, to discard the changes, click **Cancel**.

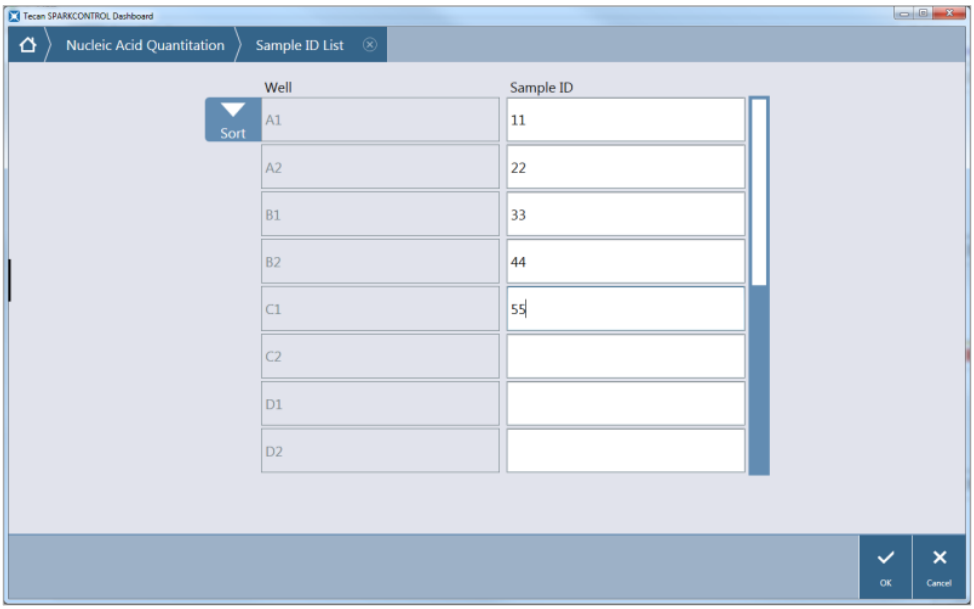


Figure 67: Define a sample ID list

Absorbance Scan

To edit the wavelength range for automatically performed absorbance scan select **Absorbance Scan**.

Default settings: 200 – 400 nm; Valid range: 200 – 1000 nm.

13.2 Labeling Efficiency App

The Labeling Efficiency app is designed for working with nucleic acids labeled with fluorescent markers. It provides not only the information about the quantity and the purity of measured samples but also their labeling efficiency by measuring absorbance at the wavelength of the corresponding marker(s). The labeling efficiency measurement can be performed with one or two markers that can be easily selected out of the list of different markers (e.g. Cy3, Cy5, Alexa 555, Alexa 647, etc.) included in the app.

The basic set-up and functionality of the Labeling Efficiency App is similar to the Nucleic Acid Quantitation App, see chapter 13.1 Nucleic Acid Quantitation App. In addition, please consider the following content specific for the Labeling Efficiency app only.

Select marker(s)

Select **Markers** in the action bar of the starting window and define a maximum of two markers used for the measurement. The selected marker(s) are provided with a check mark.

Edit marker

To define or edit a user defined marker select **Edit Marker** in the action bar.

It is possible to edit user defined markers only. To define a new marker the name, the wavelength (nm), the extinction coefficient and the correction factor for 230, 260 and 280 nm must be assigned.

To add a new marker, select the **+** button. To delete a sample, select the trashcan button.

Measurement Results

The result window includes the following results:

- Sample type (information header)
- Well position
- Well-specific OD correction values at 260, 280 and 230 nm
- Nucleic acid concentration in ng/µl
- Marker (dye) concentration in pmol/µl
- Ratio 260/230 nm
- Ratio 260/280 nm
- Sample ID (if sample IDs are defined)
- Absorbance Scan values according to the defined wavelength range. Use the slider to obtain all of the absorbance scans being measured (Figure 65: Absorbance scans in the Result window).

13.3 Protein Quantitation App

Measuring the inherent absorbance of proteins at 280 nm forms the basis for this app. The concentration levels of the samples are calculated from the values obtained. To determine the purity of the protein samples, absorbance scans are executed in addition.

13.3.1 Starting the Protein Quantitation App

The Protein Quantitation application can be started via:

- The Dashboard by selecting the corresponding application tile or
- The Method Editor by selecting the application from a drop-down list placed top right (-> Select app).

Blanking

Before quantifying protein samples, a blanking measurement must be performed.

Perform blanking as follows:

1. Start the Protein Quantitation App.
2. Select the wells to be used for blanking (by default all wells are selected).
3. Select Sample type in the action bar
   The application provides the following default samples:
   - BSA
   - IgG
   - Lysozyme



NOTE: Working with default samples will result in calculating the corresponding protein concentration in mol/L. To obtain the concentration values in mg/ml, see chapter Edit Sample.

4. Optionally, select **Blanking Threshold** and change the default value of 0.3.
5. Optionally, select **Absorbance Scan** to change the wavelength range for absorbance scan. Default settings: 200 – 400 nm
6. Load the blanks onto the selected positions for blanking and close the lid of the NanoQuant Plate carefully.
7. Select **Individual Blanking** to start the individual blanking procedure.

NOTE: Individual blanking requires blanking for all wells that are to be used for subsequent measurements. The blank correction of the samples is performed by using the single blanking value of the corresponding well on the NanoQuant Plate. For individual blanking, the selection of at least one well is required.

8. After selecting **Individual Blanking**, the plate transport moves out and the user is requested to insert the NanoQuant Plate with the respective blanking samples.
9. Start the blank measurement by selecting **OK**. The incoming values/wells are shown in the information header of the Measurement Status window.
10. After blanking, the blanking results are displayed in a list. Enlarge the list by double-tapping.

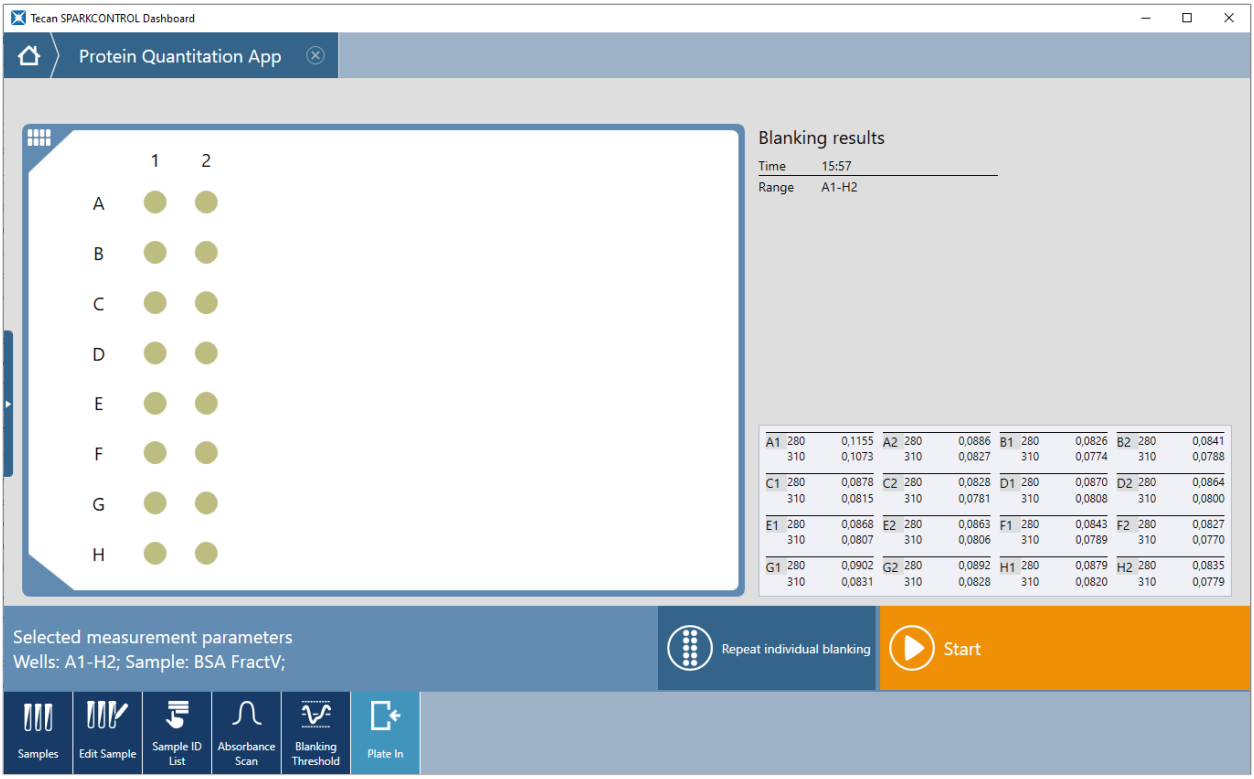


Figure 68: Start Measurement window with blanking results

NOTE: The blanking results will be stored with respect to the blanking parameters, wavelength settings and sample type. If one of these parameters is changed, the blanking procedure has to be repeated.

13.3.2 Validation Criteria for Blanking Results

NOTE: Individual blanking requires no validation criteria.

NOTE: If the defined threshold value has been exceeded, the system provides a warning. The measurement of samples can be executed yet.

13.3.3 Repeat Blanking

NOTE: Repeat the blanking in case of an incorrect blanking measurement or when using new blanking samples.

To repeat individual blanking, select the corresponding action button. If necessary, reselect wells that will be used for blanking.

CAUTION: If blanking is repeated the current blanking results will be discarded.

CAUTION: Opening and closing the NanoQuant application does not lead to loss of the blanking results. By disconnecting the instrument, restarting the software or starting another NanoQuant app, the existing blanking results are discarded.

13.3.4 Start Measurement

1. Remove any remaining blanking buffer from the sample positions and the lid by wiping the quartz spots with a piece of lint free paper. Apply 2 µl of your samples onto the selected positions and close the lid of the NanoQuant Plate carefully.
2. After individual blanking has been performed, as default the same area used for blanking is selected for measurement. It is possible to reduce the number of wells for the sample measurement, but the number of wells cannot be increased.
3. Insert the plate into the plate carrier.
4. Select **Start**. The measurement is started and the incoming values can be retrieved from the information header of the Measurement Progress window.
5. After the measurement the results are displayed and the plate is moved out automatically.
6. To perform a new measurement without closing the app, select **Next Plate**. Click **OK** to close the window. The software returns to the Start Measurement window and a new measurement can be started.

Measurement Results

The result window includes the following results:

- Well position
- Well-specific OD correction values at 280 nm
- Protein concentration in mg/ml and/or mol/L depending on Sample definition
- Sample ID (if sample IDs are defined)
- Absorbance Scan values according to the defined wavelength range. Use the slider to obtain all the absorbance scans being measured.

The final report can be printed in form of a pdf file by selecting **Print** in the action bar.

NOTE: All result data is automatically exported to Microsoft Excel.

Edit Sample

To define or edit a user defined sample select Edit Sample in the action bar.

It is possible to edit user defined samples only. To define a new sample the name, the extinction coefficient (molar extinction coefficient Ɛ [L mol-1cm-1] or percent extinction coefficient Ɛ 1% [g/100ml]) must be assigned (see figure below). If the molecular weight of a protein is part of sample definition, the concentration values will be reported as [mol/L] and [mg/ml].

To add a new sample, select the **+** button. To delete a sample, select the trashcan button.

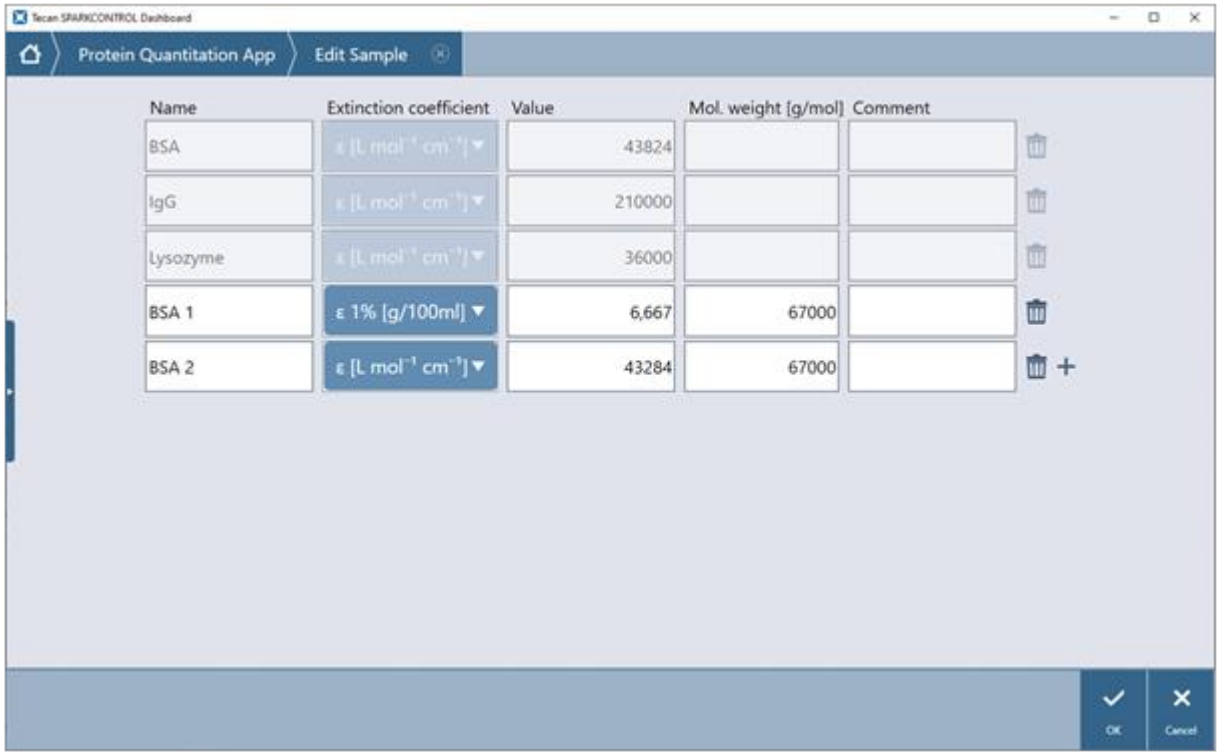


Figure 69: Edit/Add sample

Sample ID List

With the sample ID list it is possible to assign an ID to each sample on the NanoQuant Plate. To add a sample ID list, select **Sample ID List** in the action bar. Define the sample ID (15 characters at maximum). A sort function enables the ordering of the samples (e.g. A1, A2,... or A1, B1,...) To accept the entries select **OK**, to discard the changes, click **Cancel**.

Absorbance Scan

To edit the wavelength range for automatically performed absorbance scan select **Absorbance Scan**.

Default settings: 200 – 400 nm; Valid range: 200 – 1000 nm.

13.4 Calculations – Detection of Nucleic Acids

Valid for chapters 13.1 Nucleic Acid Quantitation App and 13.2 Labeling Efficiency App.

13.4.1 Calculation of Nucleic Acid Concentration

The calculation of nucleic acid concentration is based on the Lambert-Beer law:

$A = \varepsilon * d * c$

A Absorbance
ε Molarity Extinction Coefficient [µg/1 cm*1 ml]
d Distance (path length in cm)
c Concentration [µg/ml]

To correct the OD values due to dirt on the outer surfaces of the quartz lenses, an additional measurement at a reference wavelength is performed automatically with each measurement. A reference wavelength of 310 nm is used.

13.4.2 Blanks

Correction of Average Blanking value

The average absorbance value at 310 nm measured over all blank wells is subtracted from the average absorbance value at 230 nm, 260 nm and 280 nm. The relative variation of the wells used for average blanking at 260 nm must be below 10 % in order to be able to start a measurement.

$$\text{E.g. Abs}_{\text{blank average 260 nm}} = \text{Abs}_{\text{260 average}} - \text{Abs}_{\text{310 average}} \text{ [OD]}$$

Correction of Individual Blanking value

The well-specific absorbance value at 310 nm is subtracted from the corresponding absorbance value at 230 nm, 260 nm and 280 nm. Individual blanking information is stored for each well used and blank-correction of the samples is done with the corresponding single blanking values instead of one average blank. Every well that is to be used for sample measurement needs to be blanked beforehand.

$$\text{E.g. Abs}_{\text{blank A1}} = \text{Abs}_{\text{260 A1}} - \text{Abs}_{\text{310 A1}} \text{ [OD]}$$

13.4.3 Samples

Calculations based on Average Blanking: Each well used for sample measurement is blanked with the average blanking value.

$$Abs_{A1} = (Abs_{260\,A1} - Abs_{310\,A1}) - Abs_{blank\ average}\ [OD]$$
$$Abs_{A2} = (Abs_{260\,A2} - Abs_{310\,A2}) - Abs_{blank\ average}\ [OD]$$

Calculations based on Individual Blanking: Each well used for sample measurement is blanked individually with the corresponding blanking value.

$$Abs_{A1} = (Abs_{260\,A1} - Abs_{310\,A1}) - Abs_{blank\,A1}\ [OD]$$
$$Abs_{A2} = (Abs_{260\,A2} - Abs_{310\,A2}) - Abs_{blank\,A1}\ [OD]$$

The absorbance values at 230 nm and/or 280 nm are also corrected by the corresponding absorbance values at 310 nm. The corrected absorbance values are used for the 260/230 and 260/280 ratio calculations.

13.4.4 Labeled Samples

The calculation of the concentration of the marker is based on the Lambert-Beer law:

$$A = \varepsilon * d * c$$

A Absorbance
ε Extinction Coefficient for the wavelength of the marker [L mol-1 cm-1]
d Distance (path length in cm)
c Concentration [pmol/µl]

13.5 Calculations – Detection of Proteins

Valid for chapter 13.3 Protein Quantitation App.

13.5.1 Calculation of Protein Concentration

The calculation of protein concentration is based on the Lambert-Beer law:

$A = \varepsilon * d * c$

A Absorbance
Ɛ Molar Extinction Coefficient [L $mol^{-1}cm^{-1}$]
D Distance (path length in cm)
C Concentration [mol/L]

To display concentrations in mg/ml, the molar extinction coefficient Ɛ [L $mol^{-1}cm^{-1}$] can be converted to percent extinction coefficient Ɛ 1% [g/100ml] as follows:

$\varepsilon * 10 = \varepsilon\ 1\% * \text{molecular weight of protein}$

To correct the OD values due to dirt on the outer surfaces of the quartz lenses, an additional measurement at a reference wavelength is performed automatically with each measurement. A reference wavelength of 310 nm is used.

13.5.2 Blanks

Correction of Individual Blanking value

The well-specific absorbance value at 310 nm is subtracted from the corresponding absorbance value at 280 nm. Individual blanking information is stored for each well used and blank-correction of the samples is done with the corresponding single blanking values. Every well that is to be used for sample measurement needs to be blanked beforehand.

$\text{E.g. } Abs_{\text{blank A1}} = Abs_{\text{280 A1}} - Abs_{\text{310 A1}}\ [OD]$

13.5.3 Samples

Each well used for sample measurement is blanked individually with the corresponding blanking value.

$Abs_{A1} = (Abs_{\text{280 A1}} - Abs_{\text{310 A1}}) - Abs_{\text{blank A1}}\ [OD]$
$Abs_{A2} = (Abs_{\text{280 A2}} - Abs_{\text{310 A2}}) - Abs_{\text{blank A1}}\ [OD]$

13.6 NanoQuant Maintenance

For achieving optimal measurement results, the cleaning of the NanoQuant Plate is one of the most essential parts of the entire measurement procedure. There are two procedures for cleaning the NanoQuant Plate:

13.6.1 Cleaning Procedure with Ultrasonic Bath

1. Fill an ultrasonic bath with water and place a suitable beaker filled with distilled water into the ultrasonic bath.
2. Switch on the ultrasonic and immerse the lid of the NanoQuant Plate into the beaker, with bobbing movements for about 20 seconds. Take care not to immerse the hinge of the plate.
3. Repeat the procedure with the bottom part of the NanoQuant Plate.
4. Remove any surplus water from the NanoQuant Plate with dry and oil-free compressed air.

13.6.2 Cleaning Procedure with Kimwipe

1. Moisten a laboratory Kimwipe with 70 % ethanol and clean the inner and outer surfaces of the NanoQuant Plate.
2. Moisten a piece of cotton or Kimwipe with distilled water and clean both sides of each quartz lens on the NanoQuant Plate.
3. Wipe off any excess liquid with a dry Kimwipe.

After cleaning, store the plate in a dirt-free and lint-free place. No lint, nor any kind of dirt or streaks, should be on the quartz lenses. Any contamination can lead to false measurements. When measuring many different samples one after the other, the quartz wells can be cleaned with a (wet) Kimwipe. The cleaning and maintenance procedures are important in order to prolong the NanoQuant Plate's lifespan and to reduce the need for servicing. It is recommended to store the cleaned NanoQuant Plate in the original storage box.



CAUTION: Lint, dirt or fingerprints on the quartz lenses may alter the OD values significantly! Avoid getting dirt on the spacers as this can lead to a change in the length of the light path of the NanoQuant Plate and thus alter the OD values. Apply samples only onto clean quartz lenses!

14 Cell Counting in Cell Chips

Cell based assays are an important tool in basic research of academic and life science laboratories as well as in the pharmaceutical industry. The quality check and the counting of cells before cell seeding are common steps for all cell-based applications. This includes identification of live and dead cells that can be easily performed by staining the cells with Trypan blue. Only the dead cells or the cells with a damaged cell membrane will be stained blue and a viable cell will have a clear cytoplasm. Therefore, it is possible to count the cells and determine the viability of a cell culture in one step.

Two ready-to-use apps are available:

- **Cell viability:** Cell counting and viability checks are performed simultaneously in one measurement. To check the viability Trypan blue has to be added to the cell suspension sample in a ratio of 1:1. This dilution step is automatically considered when calculating results.
- **Cell counting:** Cell counting is performed only, no additive to the cell solution is necessary.



CAUTION: Take care that the Trypan blue solution is homogeneous. Avoid any dye particles because they can influence data analysis.

14.1 Sample Preparation

1. Unpack the cell chip immediately before use. The cell chips are sterile and individually packaged.
2. The cell chip consists of two sample chambers with a filling volume of 10 µl each. Fill each chamber with a cell sample by using standard pipettes and tips. Avoid liquid drops on the cell chip, air bubbles and scratches in order to guarantee optimal performance.
3. For cell viability checks, Trypan blue has to be added to the cell suspension sample in the ratio of 1:1. This dilution step is automatically taken into account when calculating results.

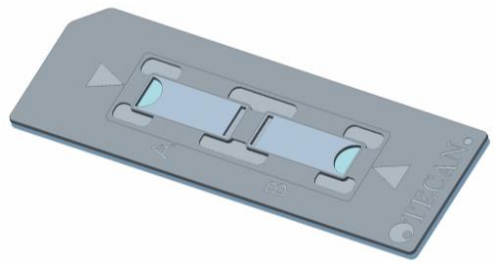


Figure 70: Cell chip



CAUTION: Cell chips are disposable and are single-use only. Do not use after the **Use by** date found on the bottom of the packaging.



CAUTION: Always wear gloves when handling the cell chip. Avoid any contamination or scratches to guarantee optimal performance.

4. Load up to four cell chips onto the adapter. The cropped corner of the cell chip prevents incorrect orientation.
5. Close the adapter lid. The lid is fixed by a magnetic mechanism. Before closing the adapter lid, visually check that the springs are installed on the inside of the lid to secure the cell chips (see figure below).



Figure 71: Cell chip adapter with springs (dark gray)

CAUTION: Don't use the cell chip adapter without springs! Measurement errors may result.

6. Place the cell chip adapter onto the plate carrier of the instrument with the opening in front and the sample position A1 on the upper left side.

CAUTION: Before starting measurements, make sure that the adapter for cell chips is inserted correctly with the opening in front and well A1 on the upper left side.

For the cleaning procedure of the adapter and the handling of springs; see chapter Maintenance and Cleaning of the Cell Chip Adapter in the SPARK – Reference Guide.

14.2 Cell Viability

14.2.1 Starting the Cell Viability App

The Cell Viability App can be started via:

- The **Dashboard** by selecting the corresponding application tile or
- The **Method Editor** by selecting the application from a drop-down list placed top right (→ Select app).

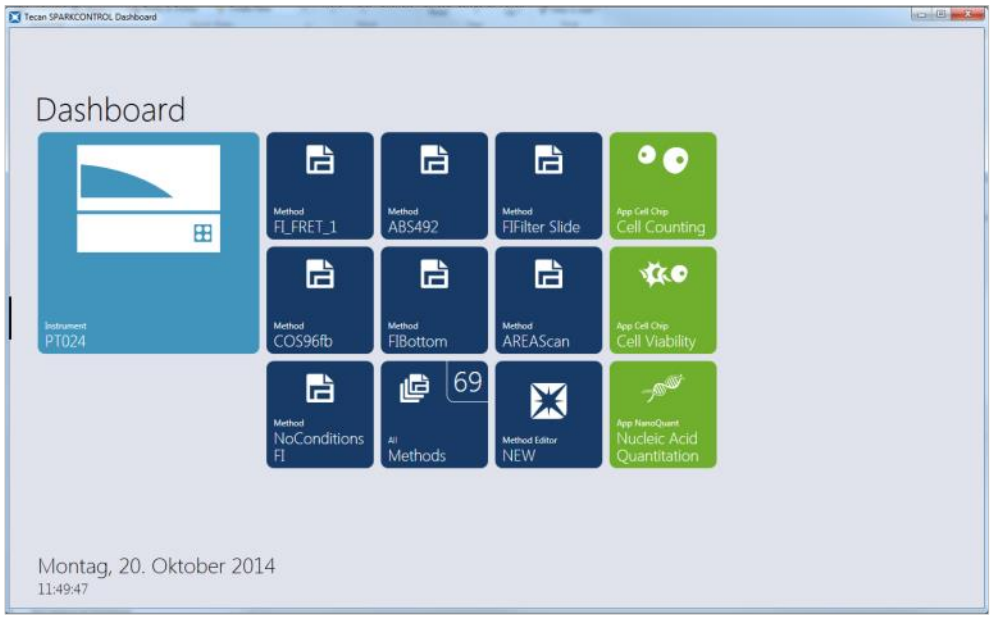


Figure 72: Start the app via Dashboard

14.2.2 Sample Selection

1. Select the **Cell Viability** application tile and the start window appears (Figure 73: Cell Viability start window).
2. Select the wells/sample chambers to be used for measurement.
3. Select **Cell size** in the action bar to change the cell size (by default 8-30 µm is selected). Sizes within 4-90 µm can be defined.

NOTE: Smaller cell size ranges speed up the image analysis time.

4. Select **Images** in the action bar if more than one image per sample should be taken (by default one image per sample is selected). Alternatively 4 or 8 images per sample can be taken and analyzed.

NOTE: At low cell concentrations (smaller $5x10^5$ cells/ml) and therefore small number of counted cells per taken image, it is recommended to take more than one image to compensate for irregular cell distribution, resulting in more accurate counting data.

5. Mark samples as duplicates by selecting the corresponding cell chip number action button – a symbol appears below the chambers to show that the cell chip contains duplicates (see figure below). Note that duplicates can be defined within one cell chip only, e.g. the two chambers 1A and 1B can be duplicates, but chambers 1A and 2A cannot be duplicates. Marking samples as duplicates will result in the calculation of the corresponding average values for all analyzed parameters.

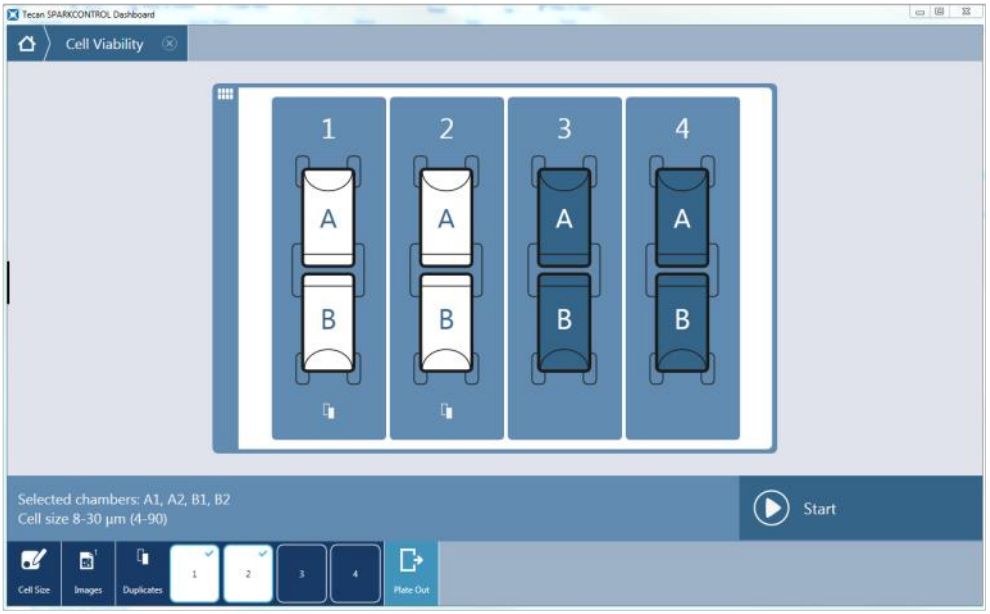


Figure 73: Cell Viability start window

6. Start the cell viability measurement by selecting **Start**. The incoming images and data are displayed as soon as they are available (see figure below).

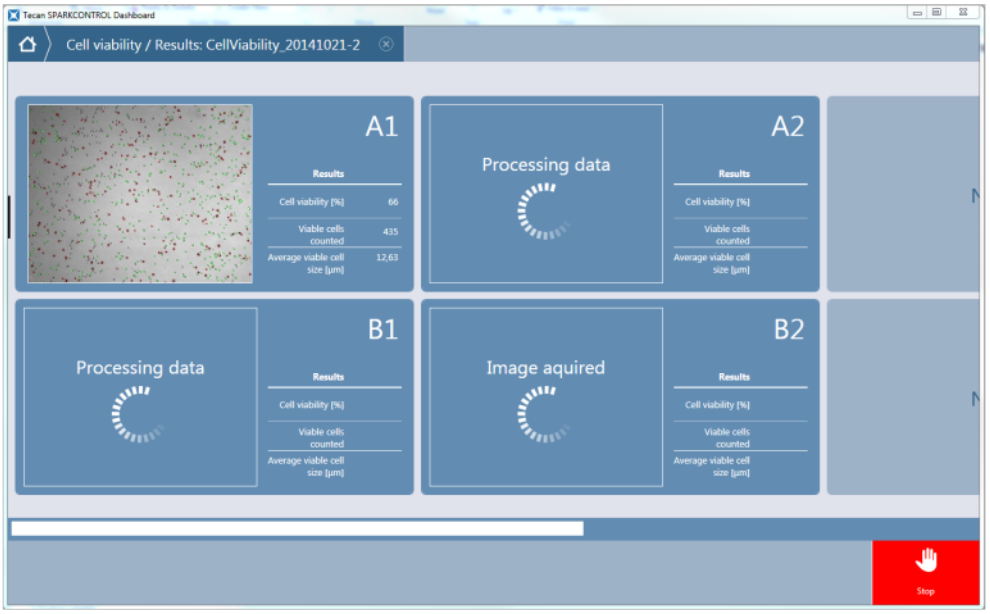


Figure 74: Measurement progress window

14.2.3 Measurement Results

1. Images and data are displayed in the result window (see figure below). Live cells are displayed in green whereas dead cells are shown in red. A summary of the corresponding data (cell concentration, counted cells and the average cell size) is displayed on the right side of the image.

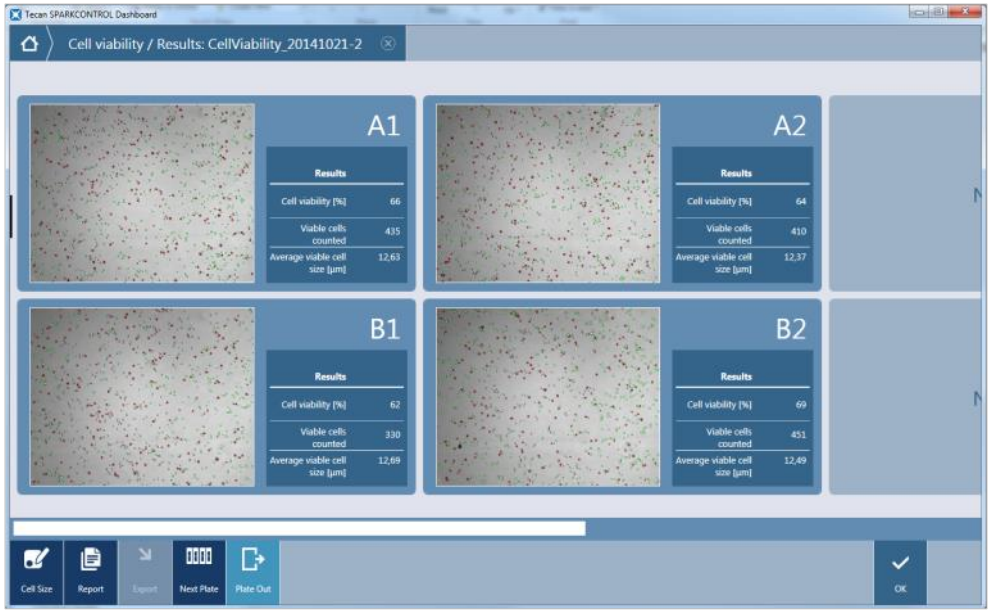
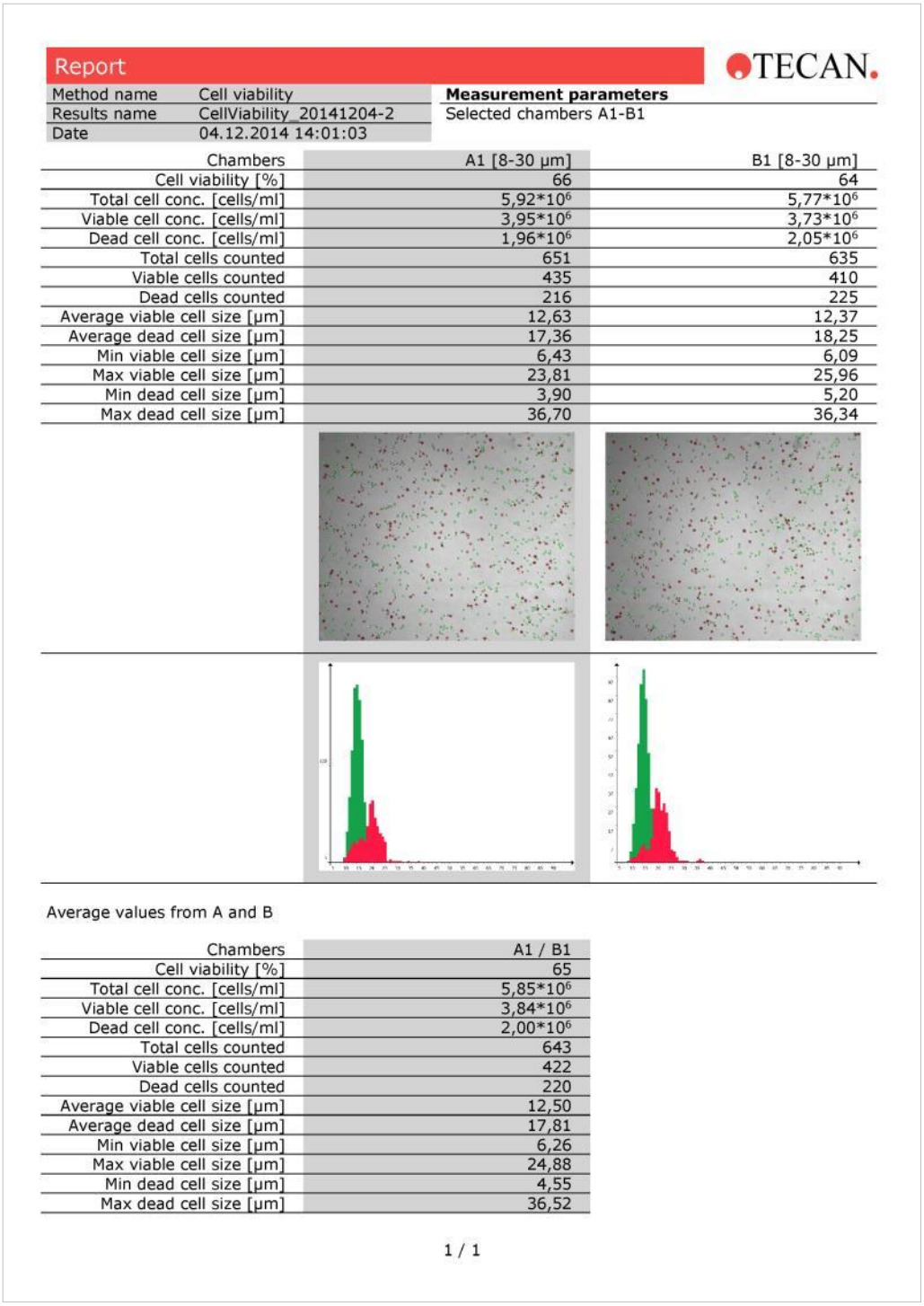


Figure 75: Cell Viability result window

2. Select **Cell Size** in the action bar to choose a new cell size range and recalculation of data based on the already taken images is performed. After recalculation the new data is presented in the result window.

3. Select **Report** in the action bar to view the report. The report includes all calculated data for each measured sample, the images as well as the histograms of all samples. If duplicates are defined the average data is shown too. The report is automatically saved after the measurement to the corresponding workspace folder. Click **Print** to send the report to the default printer (pdf-format, see figure below).



Report

TECAN.

Method name	Cell viability
Results name	CellViability_20141204-2
Date	04.12.2014 14:01:03

Measurement parameters
Selected chambers A1-B1

Chambers	A1 [8-30 µm]	B1 [8-30 µm]
Cell viability [%]	66	64
Total cell conc. [cells/ml]	$5,92*10^6$	$5,77*10^6$
Viable cell conc. [cells/ml]	$3,95*10^6$	$3,73*10^6$
Dead cell conc. [cells/ml]	$1,96*10^6$	$2,05*10^6$
Total cells counted	651	635
Viable cells counted	435	410
Dead cells counted	216	225
Average viable cell size [µm]	12,63	12,37
Average dead cell size [µm]	17,36	18,25
Min viable cell size [µm]	6,43	6,09
Max viable cell size [µm]	23,81	25,96
Min dead cell size [µm]	3,90	5,20
Max dead cell size [µm]	36,70	36,34

Average values from A and B

Chambers	A1 / B1
Cell viability [%]	65
Total cell conc. [cells/ml]	$5,85*10^6$
Viable cell conc. [cells/ml]	$3,84*10^6$
Dead cell conc. [cells/ml]	$2,00*10^6$
Total cells counted	643
Viable cells counted	422
Dead cells counted	220
Average viable cell size [µm]	12,50
Average dead cell size [µm]	17,81
Min viable cell size [µm]	6,26
Max viable cell size [µm]	24,88
Min dead cell size [µm]	4,55
Max dead cell size [µm]	36,52

1 / 1

Figure 76: PDF report

4. After a measurement, an Excel file with all data is generated and saved according to the export path to the corresponding measurement workspace folder (for default path, see chapter 2.11 Measurement Results). This workspace folder also contains all images as well as the generated PDF report.
5. If a recalculation of data is performed after the measurement, new data is not exported automatically. Select the **Export** button to trigger the export of the data including the images and the report.



CAUTION: Recalculated data is not saved automatically. Select Export in the action bar after the recalculation procedure to avoid data loss.

6. To perform a new measurement without closing the app select the action button **Next Plate** before leaving the window by selecting **OK**. In that case, the software returns to the start window and a new measurement can be started.
7. For a full-screen view of the image only, double-click the desired image. For a detailed view of image and data, click the **Results**. The view is enlarged and all available result data is displayed.

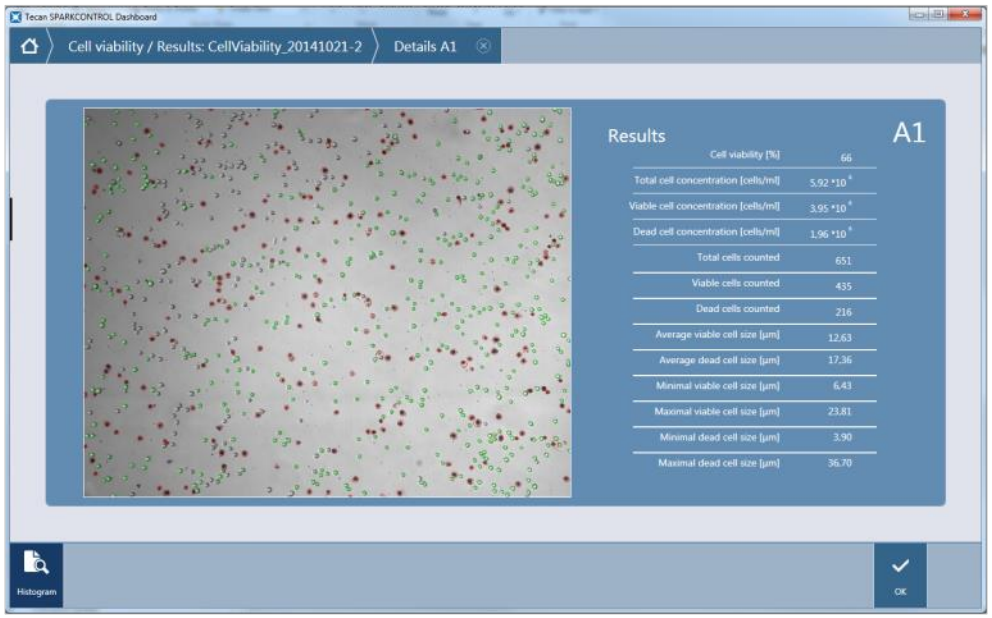


Figure 77: Detailed result window

14.2.4 Cell Histogram

By selecting **Histogram** in the action bar the correlated histogram is displayed. The cell size is plotted against the number of cells. Live cells are presented by the green bars and dead cells by the red bars.



Figure 78: Histogram

Modify the histogram view by selecting one of the following action buttons: **All**, **Viable** or **Dead**.

Move the sliders to change the cell size and select **Refresh Calculation** in the action bar. Displayed data as well as histogram will be refreshed accordingly.

14.2.5 Duplicates

Data calculation is performed for each sample separately. In the detailed result view of a sample with an existing duplicate (e.g. for slide one: A1 or B1) beside the single data the average data of the duplicates is shown too.

14.3 Cell Counting App

The Cell Counting App is designed for counting cells without further classification into viable and dead cells. In an image, all the counted cells are highlighted in blue. The Cell Counting App contains the same functional graphical elements as the Cell Viability App (see chapter 14.2 Cell Viability).

15 Cuvette App

The Cuvette App is designed for routine absorbance and absorbance scan endpoint measurements performed in a cuvette within a cuvette port. Each measurement starts with the measuring of a blank value that can be used for the automatic blank correction of the measured samples.

15.1 Prepare Instrument and Blanking

1. Select the **Cuvette** application. Within the **Absorbance** window (default selection) define the measurement wavelength and the number of flashes used for the measurement (see figure below) For absorbance scan, select the **Absorbance Scan** action button and define the measurement wavelength range.



Figure 79: Selecting detection mode

2. Select **Blanking**. A user intervention window for emptying the cuvette port is displayed.
3. Empty the cuvette port and select **OK**.

NOTE: The Prepare Instrument measurement has to be performed every time a measurement with new measurement parameters is started. Please make sure that the cuvette port is empty.

4. Insert a cuvette for blanking and confirm the corresponding user intervention by selecting **OK**. The blanking measurement is performed and the blanking result is part of the Result window (Figure 80: Result window during a measurement).

15.2 Measurement

1. After blanking, remove the cuvette for blanking from the cuvette port and insert a cuvette with sample. De-select **Apply blanking** if the automated blank correction should not be performed automatically.
2. Start the measurement by selecting **Start**. The measured result is displayed within the Result window (Figure 80: Result window during a measurement). To measure a new sample remove the previous cuvette from the cuvette port, insert the new one and continue with **Start**. The new measured result is added to the Result window.

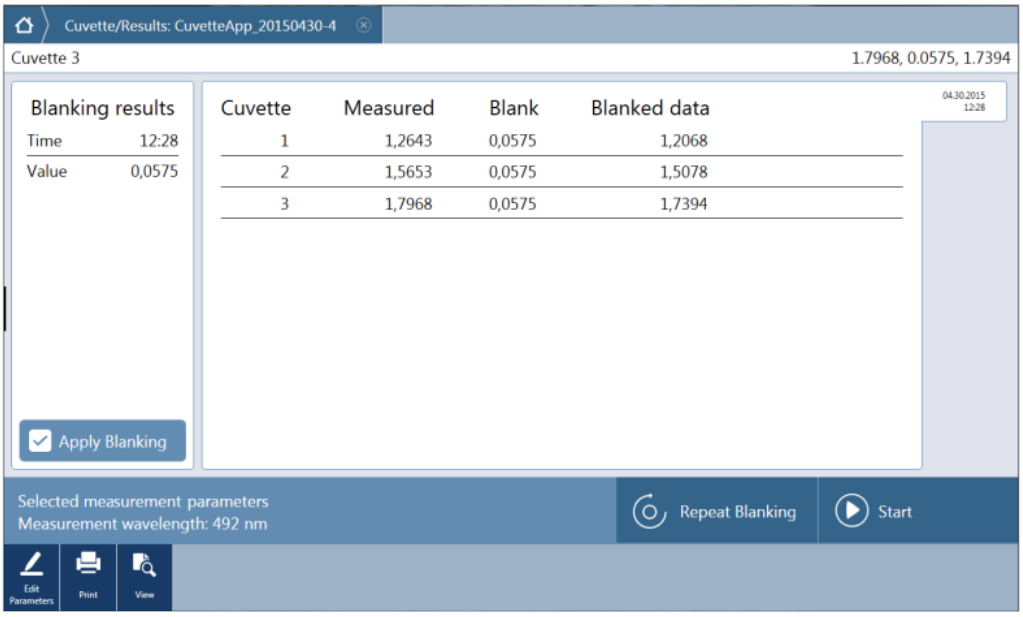


Figure 80: Result window during a measurement

3. Select **View** to change between the list and the graphic view of the measured results.

4. If needed, repeat blanking during the current measurement session via **Repeat blanking**. The old blanking value is discarded and the new blanking value is valid for all the following measurement. For each repetition, a new result sheet is generated indicated by the tabs placed on the right side of the Result window (Figure 81: Result window with multiple result sheets). Each tab is provided with the date and time of the respective blanking readout.

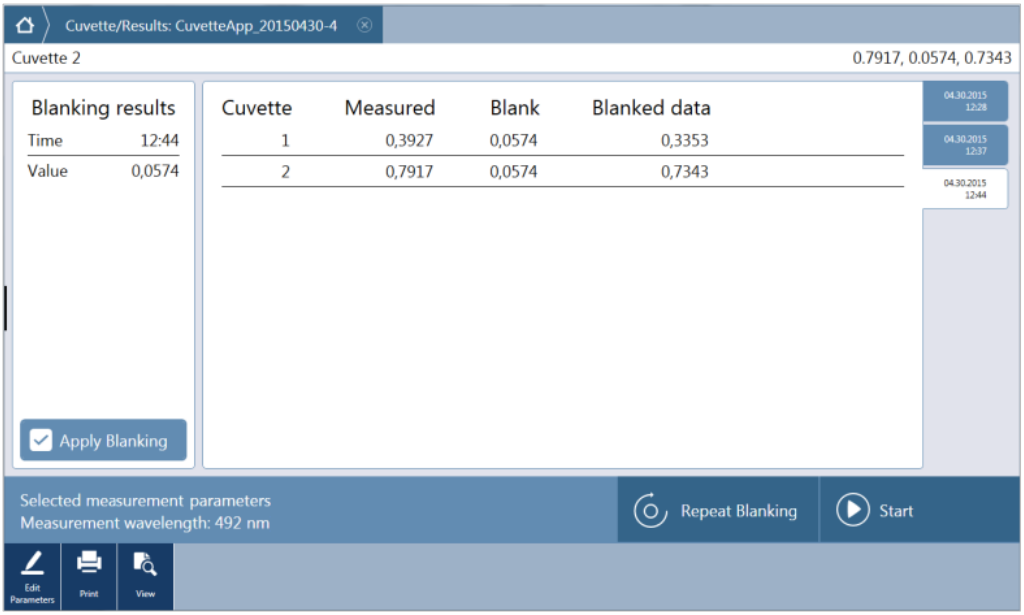


Figure 81: Result window with multiple result sheets

5. After a measurement, an Excel file with all data is generated and saved under default path or the path defined by the user (see chapter 2.11 Measurement Results).
6. The final report can be printed as a pdf file. Select Print to send the report to the default printer.
7. To edit the measurement parameters select **Edit Parameters**. The software returns to the Cuvette App starting screen, a new measurement session can be started.

NOTE: The selection Edit Parameters will close the current measurement session. The Prepare Instrument measurement has to be repeated.

16 Troubleshooting

16.1 SparkControl Errors and Warnings

If an error cannot be resolved or reoccurs regularly, contact your local Tecan service representative.

Also check this page for further support:
https://www.tecan.com/knowledge-portal/microplate-reader#spark-troubleshooting.

Error	Description	Possible solution / workaround
Device related errors		
Initialization error for motor 'motor'	Actuator failure while initializing	Report to Tecan. Switch off/on device and retry.
Steploss error for motor 'motor'	Actuator failure; checked after measurement	Report to Tecan (results not trustworthy). Switch off/on device and retry.
Motor 'motor' not initialized	Actuator failure; checked before measurement	Report to Tecan. Switch off/on device and retry.
Movement position 'position' not found	Logical position not found; configuration error	Report to Tecan
Movement for motor 'motor' timed out!	Actuator failure	Report to Tecan
Error reading temperature sensor	Temperature sensor failure	Report to Tecan
Command 'command' is not valid	Error in computer - device communication protocol	Report to Tecan
Parameter 'parameter' is missing	Error in computer - device communication protocol	Report to Tecan
Module 'module' with number 'number' had an error|'add. text'	Device error (module)	Report to Tecan
Submodule 'module' had an error|'add. text'	Device error (sub-module)	Report to Tecan
CAN Receive timeout from Module 'module'	Device error (Timeout on CAN bus)	Report to Tecan
CAN communication error	Device error (CAN bus)	Report to Tecan
SPI timeout	Device error (SPI)	Report to Tecan
I2C timeout	Device error (I2C)	Report to Tecan
SCI timeout, Submodule 'sub-module'	Device error (SCI)	Report to Tecan
Injector timeout	Timeout while communication to injector module	Report to Tecan. Switch off device. Check injector cabling. Switch on device and retry.
Injector communication error	Communication error device - injector module	Report to Tecan. Switch off device. Check injector cabling. Switch on device and retry.
Answer 'answer' from internal Command 'command' wrong|'add. text'	Device error	Report to Tecan
Buffer 'buffer' is out of memory|'add. text'	Device error	Report to Tecan

Error	Description	Possible solution / workaround
Buffer 'buffer' is out of memory|'add. text'	Device error	Report to Tecan
Sending the data over USB failed ('number' retries)	Device error while sending data over USB channel to computer	Report to Tecan. Switch off device. Check USB cabling. Switch on device and retry. If error is related to heavy USB traffic or heavy load on computer it may be helpful to close other applications.
Communication related errors (computer to device)		
Not able to connect to the communication service	Unable to connect to service	Switch off/on device. Restart services by clicking the tray icon "SPARKCONTROL Agent" with right mouse button (context menu) and selecting "Restart Services"
Lost connection to Instrument Server. Terminate application	Device connection lost	Close application (Dashboard or Method Editor). Switch off/on device. Restart services by clicking the tray icon "SPARKCONTROL Agent" with right mouse button (context menu) and selecting "Restart Services"
No instrument found	Device not present	Switch on device
Instrument not free	Device blocked by other process	Be sure that no other program uses the device. Eventually restart computer.
Instrument could not be acquired	Device blocked by other process	Be sure that no other program uses the device. Eventually restart computer.
Instrument is busy	Device busy	Wait until device becomes free.
Error occurred: 'command'	Device reports an error on 'command'	Report to Tecan
Unexpected message received: 'response'	Unexpected response from device received	Report to Tecan
Unexpected response format: 'response'	Unexpected response format detected	Report to Tecan
Checksum mismatch in received command	Checksum of response message from device not correct	Report to Tecan
No configuration found	Device not configured correctly	Report to Tecan
Measurement related errors		
Instrument has no lid lifter defined	Device not configured correctly	Report to Tecan
Optimal Gain could not be found	Unable to find optimal gain	Use manual gain
Strongest well signal could not be found	Unable to find optimal gain	Use manual gain
Signal too low. Gain could not be calculated	Unable to find optimal gain	Use manual gain

Error	Description	Possible solution / workaround
Unable to find optimal Z-position after n retries	Unable to find optimal Z-position	Use manual Z-Position
No reference blank selected	No reference blank well for FP measurement given	Select reference blank well
Blank well 'Id' is not selected in the Plate strip	No reference blank well for FP measurement given	Select reference blank well
No reference well selected	No reference well for FP measurement given	Select reference well
Signal well 'Id' is not selected in the Plate strip	Signal well for FP measurement not given	Select signal well
Signal of reference well too low, choose another one	Signal of reference well too low	Use another well
Invalid G-Factor, signal of reference well is too low.	Unable to determine G-factor	Choose another well
Dark counts too high	Dark counts too high	Report to Tecan
Dark value too high: Darkvalue='value', Limit='limit'	Dark counts too high	Report to Tecan
Lid Check error	Lid check error	Device gets too much light (from direct sun light or from sample)
The lid check had an error! Value='value', Limit='limit'	Lid check error	Device gets too much light (from direct sun light or from sample)
Low 'add. Text' signal error	Lamp low error (or too low signal)	Report to Tecan. Switch off/on device and retry.
'Add. Text' signal overflow error	Overflow error	Too much signal; could be a device error. Or: too much signal from sample (reduce gain)
Cancel of method failed	Unable to stop measurement	Try again
Pause of method failed.	Unable to pause a measurement (kinetic)	Retry; Report to Tecan.
Method can't be started because method 'method' is still pending on instrument 'device'.	Unable to start a method because another one is still pending	Wait until device is free
Method can't be started because instrument 'device' is in use.	Unable to start a method because device is in use	Wait until device is free
Error occurred executing method 'method'	Unspecific error occurred while executing a method	Retry; Report to Tecan.
Lid already taken	Lid already taken by lid lifter	Move plate-out and in again
Autofocus Error: No peak found!	Application/Device error	Check the plate/Report to Tecan
General errors		
Database doesn't exist!	Cannot open database	Re-install program

Error	Description	Possible solution / workaround
WCF call failed after 'n' retries	Unspecific error occurred while sending a message from Dashboard or Method Editor to server	Close application (Dashboard or Method Editor). Switch off/on device. Restart services by clicking the tray icon "SPARKCONTROL Agent" with right mouse button (context menu) and selecting "Restart Services"
Not able to find given printer	Unable to find printer	Check printer settings
There is not enough memory available for image processing	Memory allocation error while image processing	Close other applications. Equip computer with more memory
Memory allocation failed	Memory allocation error while image acquisition or image processing	Close other applications. Equip computer with more memory
Imaging Server not found	Unable to connect to imaging server	Close application (Dashboard or Method Editor). Switch off/on device. Restart services by clicking the tray icon "SPARKCONTROL Agent" with right mouse button (context menu) and selecting "Restart Services"
The PDFX directory: 'directory' doesn't exist	Directory for the plate definition files not existent (or not accessible)	Re-install program
Camera initialization failed	Unable to initialize camera module	Close application (Dashboard or Method Editor). Switch off/on device. Restart services by clicking the tray icon "SPARKCONTROL Agent" with right mouse button (context menu) and selecting "Restart Services". If problems persists, contact Tecan.
Instrument 'device' is defective.	Defective device detected	Report to Tecan
Injector related errors		
Injector carrier is inserted	Injector carrier inserted (but should not be)	Remove injector carrier
Injector carrier is not inserted	Injector carrier is not inserted (but should be)	Insert injector carrier
Plate is not inserted	No plate detected	Insert plate
The injection volume would be greater than the maximum capacity of the wells of the selected microplate. Injection aborted.	Fill-volume too high	Decrease volume
Injection is not possible with a plate cover.	Injection not possible	Remove plate cover (and adjust setting in Plate strip)

Error	Description	Possible solution / workaround
Injector 'injector' is not primed. Please prime the injector.	Injector not primed	Prime injector before usage
Filter related errors		
Filter 'filter' - Maximum characters of filter description is 'n'	Filter description too long	Reduce text
Maximum characters of filter slide description is 'n'	Filter description too long	Reduce text
Filter 'filter' - Bandwidth must be in the range of 5 - 100 nm	Bandwidth out of range	Define correct bandwidth
Filter 'filter' - Wavelength must be in the range of 230 - 900 nm	Wavelength out of range	Define correct wavelength
Defined filter was not found.	Requested filter could not be found	Equip filter slide with requested filter
Filter not found 'filter'	Requested filter could not be found	Equip filter slide with requested filter
Filter 'filter' not inserted!	Required filter not inserted	Insert correct filter
Defined mirror was not found.	Mirror could not be found	Report to Tecan (if user-defined filter: Equip and define with correct mirror)
Spark-Stack related errors		
Input magazine is empty	There is no plate in the input magazine when starting a stacker run.	Insert plate/plates into the input magazine before starting a stacker run. Restart the stacker run.
Output magazine is not empty	There is a plate in the output magazine before starting a stacker run.	Remove the plate from the output magazine. Restart the stacker run.
Plate carrier is not empty	The plate carrier must be empty before starting a stacker run.	Remove the plate from the plate carrier. Restart the stacker run.
Start of method as stacker run not possible	No magazine is loaded or a magazine is tilted.	Mount the input magazine (with plates) and output magazine (without plates) properly. Press the plate magazine down to click it into place.

Error	Description	Possible solution / workaround
No plate detected during stacker run in input magazine or for restacking in output magazine. (Error:...Stacker get/stack magazine _Input/Output ...)	There is no plate on the lifting table of the stacker or the plate transport.	Report to Tecan. Switch off the instrument. Remove input and output magazines. If necessary remove the plate from the lifting table of the stacker. Move out the plate carrier from the SPARK reader, if necessary remove microplate, move the empty plate carrier back into the SPARK reader. Re-load the plate magazines onto the Spark-Stack. Make sure that microplates are not damaged. Restart the stacker run.
Initialization error Steploss error	Actuator failure while initializing the stacker.	Report to Tecan. Switch off the instrument. Remove input and output magazines. If necessary remove the plate from the lifting table of the stacker. Move out the plate carrier from the SPARK reader, if necessary remove microplate, move the empty plate carrier back into the SPARK reader. Re-load the plate magazines onto the Spark-Stack. Restart the stacker run.
Power Failure	Power supply interrupted	Report to Tecan. Switch off the instrument. Remove input and output magazines. If necessary remove the plate from the lifting table of the stacker. After power is available again: Move out the plate carrier from the SPARK reader, if necessary remove microplate, move the empty plate carrier back into the SPARK reader. Re-load the plate magazines onto the Spark-Stack. Restart the stacker run.
Stacker communication error	Unable to connect the stacker; no communication with the stacker.	Close application (Dashboard or Method Editor). Switch off/on the device. Restart services. See chapter 16.2 Spark Services Manager.

16.2 Spark Services Manager

The Spark Services Manager can be found in the System-Tray of Microsoft Windows. The System-Tray gives users quick access to system functions like network, volume, battery status and to your Tecan Spark Services Manager.

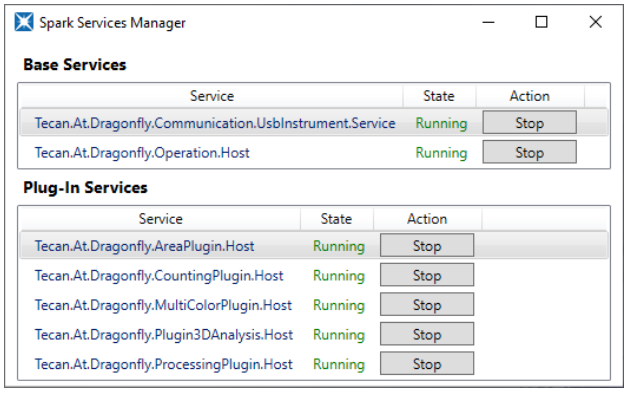
In Windows 10 and Windows 11, the System-Tray (also known as the Notification Area) is located typically on the right side of the taskbar:



Figure 82: Example for the Spark Services Manager

Right-click on the SparkControl icon to open the menu, then select Spark Services Manager.





Services can be manually started or stopped as needed.

Index

3

3D Scan 53

A

Action Bar 43
Action Buttons 43
  Expandable 43
Actions 31
Analysis Settings 122
Area Scan 41

B

Breadcrumbs 43

C

Camera Settings 116
Cell counting 103
  in cell chips 157
Channels 122
Check-and-Go 47, 49
Comment Strip 34
Condition Strip 33
Control bar 25
Cooling System 135

D

Dashboard 42
Data Display 56
Data Handling 60
Detection Strips 30
Display 51

E

Environmental Control 133
Expand Button 43
Export 71, 124

F

File menu 22
Fluorescence Bottom Module 91
Fluorescence Imaging
  Analysis Results 113
  Image Types 113
  Live Viewer 115
  Measurement Mode 113
  Measurement Progress View 113
  ResultAnalyzer 121
Fluorescence Intensity Scan Strip 90
Fluorescence Polarization Module 91
Fluorescence Polarization Optics 92

G

Gas Control
  Manual 136
G-Factor Calculation 92
Graph View 51, 56

H

Heating module 133
Help menu 23

I

Image Analysis 122
  Analysis Settings 122
  Object Gating 122
Images 64
Imaging Strips 109
Incubation position 31, 32, 133
Info Pane 37
Inject and Read 62, 99
Injection Strip 32
Instrument Control 46
Instrument menu 23
Integration Time 77

K

Kinetic Loop Strip 34

L

Licensing SparkControl's Plugin 14
Light Attenuation 77
Live Viewer 106, 115

M

Measurement Progress 50
Measurement Results 57
Measurement Types
  Conditional
    Signal-triggered 39
    Time-triggered 38
  Endpoint 37
  Kinetic 37
  Multi-label 38
  Plate-wise 37
  Single-label 38
  Well-wise 37
Menus 22
Method
  Starting 47, 57
Method File Explorer 57
Move Plate Strip 33
Multiple instruments 47
Multiple Reads per Well 40

N

NanoQuant App 141
Navigation Bar 43

O
Object Gating 122
Open Kinetic 49
Optimal Read 41

P
Pathlength correction 82
Plate 26, 30
Plate Geometry Editor 63
Plate layout 27
Plate Strip 26, 30
Plate View 51, 56

R
Result Area 121
ResultAnalyzer 67, 121
  Channels 122
  Result Area 121

S
Scans 53
Screencasts 65
Shaking Strip 31
Software
  Installation 13
  Starting 20
  System Requirements 11
  Uninstall/Repair 13
Spark Services Manager 175
SparkControl Settings 58, 65
Spark-Stack 125
Start tile 48
Strip / Luminescence 73
Strip / Luminescence Scan 76
Strip / Multicolor Luminescence 75
Strips
  Hierarchy of 35
  Indenting and Outdenting of 36
System Requirements 11

T
Tiles 43
Time-Resolved Fluorescence Intensity Strip 88
Toolbar 24

U
User Intervention Strip 33
User Profile 10

V
View menu 22

W
Wait Strip 32
Well Strip 30
Workflow Pane 35

Tecan Customer Support

If you have any questions or need technical support for your Tecan product, contact your local Tecan Customer Support organization. Go to: http://www.tecan.com/customersupport for contact information.

Prior to contacting Tecan for product support, prepare the following information for the best possible technical support (see name plate):

- Model name of your product
- Serial number (SN) of your product
- Software and software version (if applicable)
- Description of the problem and contact person
- Date and time when the problem occurred
- Steps that you have already taken to correct the problem
- Your contact information (phone number, fax number, e-mail address, etc.)