TECAN.

## Instructions for Use – Basic Guide

# SPARK




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

### Notice

Every effort has been made to avoid errors in text and diagrams; however, Tecan Austria GmbH assumes no responsibility for any errors, which may appear in this publication.

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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### CE Declaration of Conformity

See the last page of these Instructions for Use.

### Area of Application – Intended Use

See chapter 2.2 Intended Use (Hardware and Software).

### About the Instructions for Use

Original Instructions. This document describes the SPARK multifunctional microplate reader. It is intended as reference and instructions for use. This document describes how to:

- Install the instrument
- Operate the instrument
- Clean and maintain the instrument

### 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.

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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.



**WARNING:** This symbol indicates the possible presence of biologically hazardous material. Proper laboratory safety precautions must be observed.



**WARNING:** This symbol indicates the possible presence of flammable materials and a risk of fire. Proper laboratory safety precautions must be observed.



**WARNING:** Negative environmental impacts associated with the treatment of waste (WEEE).

- Do not treat electrical and electronic equipment as unsorted municipal waste.
- Collect waste electrical and electronic equipment separately.



**WARNING:** Indicates laser. Do not stare into the beam!



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**WARNING:** This product can expose you to chemicals such as lead which is known to the State of California to cause cancer and birth defects or other reproductive harm. For more information go to: www.P65warnings.ca.gov/product.

# Symbols

| | |
|---|---|
| CE | CE conformity marking |
| UKCA | United Kingdom Conformity Assessed<br>marking shows that the labeled product is following the applicable regulation in Great Britain. |
|  | Date of manufacture |
|  | Manufacturer |
| REF | Catalogue number |
|  | Consult Instructions for use |
|  | China RoHS symbol |
| SN | Serial number |
|  | Single use only |
|  | TÜV SÜD MARK |
|  | USB symbol |
|  | Use by date |
|  | WEEE symbol |

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# 1 Safety

## 1.1 Introduction

- Always follow basic safety precautions when using this product to reduce the risk of injury, fire, or electrical shock.
- Read and understand all information in the Instructions for Use. Failure to read, understand, and follow the instructions in this document may result in damage to the product, injury to operating personnel or poor instrument performance.
- Observe all WARNING and CAUTION statements in this document.
- Never open the instrument while it is plugged into a power source.
- Never force a microplate into the instrument.
- Observe proper laboratory safety precautions, such as wearing protective clothing (gloves, lab coat, safety glasses, etc.) and using approved laboratory safety procedures.




**CAUTION:** To ensure the optimal operation of the SPARK, an annual maintenance procedure must be performed by a Tecan service engineer.



**WARNING:** Follow the instructions in this manual carefully to ensure the safety of the device. The device could be damaged by incorrectly performed procedures.

It is assumed that the instrument operators, because of their vocational experience, are familiar with the necessary safety precautions for handling chemicals and biohazardous substances.

Adhere to the following laws and guidelines:

- National industrial protection law
- Accident prevention regulations
- Safety data sheets of the reagent manufacturers



**WARNING:** Depending on the applications, parts of the instrument may come in contact with biohazardous/infectious material. Make sure that only qualified personnel operate the instrument. In case of service or when relocating or disposing of the instrument, always disinfect the instrument according to the instructions given in this manual.



**WARNING:** Do not open the instrument! Only Tecan authorized service technicians are allowed to open the instrument. Removing or breaking the warranty seal voids the warranty.

# 2 General Description

## 2.1 Instrument

The SPARK is a multifunctional microplate reader which is compatible with robots.

## 2.2 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-label measurements. The SPARK is equipped with the SparkControl software for reader control and data reduction.

The user 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 instrument 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.

## 2.3 User Profile

### 2.3.1 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 can recognize and avoid dangers.

The administrator has extensive skills and can 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.

### 2.3.2 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 can recognize and avoid dangers.

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

### 2.3.3 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 can 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

## 2.4 Multifunctionality

The fully equipped SPARK can perform the following measurement techniques (for detailed information, see chapter 5 SPARK Platform).

- Absorbance
- Absorbance Scan
- Absorbance Cuvette
- Absorbance Scan Cuvette
- Fluorescence Intensity Top (FRET)
- Fluorescence Intensity Bottom
- Time Resolved Fluorescence (TRF, TR- FRET)
- Fluorescence Scan
- Fluorescence Polarization
- Inject and Read (Injection incl. Fluorescence Intensity Bottom)
- Luminescence (Glow Type, Flash Type and Multicolor)
- Luminescence Scan
- Alpha Technology
- Bright field Imaging (Cell Counting, Cell Confluence) or
- Fluorescence Imaging (CYTO configurations)

The instrument can be equipped with up to two injectors, a heater/stirrer, and a microplate stacker. Special functionalities (such as cell counting, gas supply and lid lifting, temperature control - heating and cooling - and humidity control) support cell-based studies in particular.

### 2.4.1 SPARK CYTO Configurations

All instruments equipped with Fluorescence Imaging are denoted as SPARK CYTO and are available in four different configurations designed for the needs of various customers from academia to biopharma:

| SPARK CYTO 100 | SPARK CYTO 300 | SPARK CYTO 400 | SPARK CYTO 500 | SPARK CYTO 600 |
|---|---|---|---|---|
| | Absorbance (Standard) | Absorbance (Standard) | Absorbance (Enhanced) | Absorbance (Enhanced) |
| | Absorbance Scan | Absorbance Scan | Absorbance Scan | Absorbance Scan |
| Fluorescence Imaging | Fluorescence Intensity Top (Standard, Filter) | Fluorescence Intensity Top (Enhanced, Monochromator) | Fluorescence Intensity Top (Enhanced, Filter) | Fluorescence Intensity Top (Enhanced, Fusion Optics) |
| | Fluorescence Intensity Bottom (Standard, Filter) | Fluorescence Intensity Bottom (Enhanced, Monochromator) | Fluorescence Intensity Bottom (Enhanced, Filter) | Fluorescence Intensity Bottom (Enhanced, Fusion Optics) |
| | | Fluorescence Intensity Scan | | Fluorescence Intensity Scan |
| | TRF and TR-FRET (Filter) | TRF and TR-FRET (Monochromator) | TRF and TR-FRET (Filter) | TRF and TR-FRET (Enhanced, Fusion Optics) |
| | | Fluorescence Polarization | Fluorescence Polarization | Fluorescence Polarization |
| | Luminescence (Standard, Multicolor) | Luminescence (Standard, Multicolor) | Luminescence (Enhanced, Multicolor) | Luminescence (Enhanced, Multicolor) |
| | Luminescence Scan | Luminescence Scan | Luminescence Scan | Luminescence Scan |
| | | | | Alpha Technology |

The characteristics of the module options given in the table above are described in chapter 5 SPARK Platform.

All CYTO configurations come with environmental control:

- Temperature Control (up to 42 ° C)
- $CO_2$ and $O_2$ Control
- Integrated Lid Lifter

Additionally, the following optional functionalities are available for all CYTO configurations:

- Injectors
- Stacker
- Humidity Cassettes

**CAUTION:** SPARK CYTO is equipped with an internal USB drive which contains instrument specific calibration data for the optimal quality of fluorescence images. This drive is visible in the File Explorer under the name "USB DISK" or "SPARK CYTO". Do not eject or modify it to avoid a potential loss of functionality for SparkControl versions 4.0 or higher.

## 2.5 Microplate Requirements

Any common microplate ranging from 1- to 384-/1536 well formats compliant to the following ANSI/SBS standards can be measured with any of the above measurement techniques.

- ANSI/SBS 1-2004 (footprint dimensions)
- ANSI/SBS 2-2004 (height dimensions)
- ANSI/SBS 3-2004 (bottom outside flange dimensions)
- ANSI/SBS 4-2004 (well positions)

The SPARK supports microplates up to 384 wells; advanced modules support microplates up to 1536 wells.

The supported range of the plate heights is 10 mm (without lid) up to 24.5 mm (including lid). For bottom measurements, the elevation of the bottom of the well relative to the supporting plate rim must not be larger than 5.5 mm.

In addition to the above-mentioned microplate formats, cuvettes in an adapter, the Tecan NanoQuant Plate, the Tecan MultiCheck Plate and the Tecan Adapter for Cell Chips can be used with limitations for selected measurement techniques.

**CAUTION:** Tecan Austria GmbH has taken great care when creating the Plate Definition Files (.pdfx) that are delivered with the instrument.

Tecan Austria has taken every precaution to ensure that plate heights and well depths are correct according to the defined plate type. These parameters are used to determine the minimum distance between the top of the plate and the ceiling of the measurement chamber. Additionally, Tecan Austria adds a very small safety gap to prevent any damage that may occur to the measurement chamber as a result of small changes in plate height. This has no effect on the performance of the instrument.

Make sure that the selected plate definition file corresponds to the currently used microplate, so that the safety gap is correctly calculated, otherwise the instrument could become damaged.

**NOTE:** For instruments with the Spark-Stack module, additional microplate requirements apply, see chapter 15.2 Microplate Requirements for the Spark-Stack.

### 2.5.1 Filling Volumes/Smooth Mode

**CAUTION:** The following microplates can be processed **only** with the subsequent filling volumes:

- 1-well plates <= 15000 µl
- 4-well plates <= 4500 µl
- 6-well plates <= 2000 µl
- 12-well plates <= 1200 µl
- 24-well plates <= 1000 µl
- 48-well plates <= 400 µl
- 96-well plates <= 200 µl
- 384-well plates <= 100 µl
- 1536-well plates <= 10 µl

Larger filling volumes can lead to an overflow of liquids, which can result in cross-contamination. Additionally, the spillover can cause damage to the device (e.g., contamination of the optics and the centering clamp).

If the working volume in the plate definition file (pdfx) is smaller than the above defined volumes the smaller filling volumes must be used to avoid spilling (e.g., Corning 384-well plates have a working volume of only 80 µl).

For fluids that have a lower viscosity than aqueous solutions, the filling volume should additionally be optimized during method validation.

**Smooth mode** slows down the plate transport movements. **Smooth mode** is activated by selecting the appropriate checkbox in the **Plate** strip. Larger filling volumes than the ones defined above may be possible when **Smooth mode** is selected; however, the maximum filling volumes for each plate type and application must be optimized during method validation.

**CAUTION:** The maximum filling volumes for each plate type and application must be optimized even if **Smooth mode** is used.

**Smooth mode** is selected by default if a plate format with less than 96-wells is selected in the measurement method. **Smooth mode** is not available when using the onboard **Retract/Eject** button to move the plate in/out.

**CAUTION: Smooth mode** is not available when using the onboard **Retract/Eject** button to move the plate in/out.

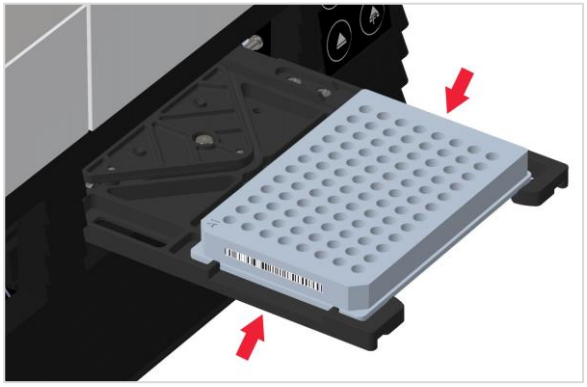
**NOTE:** The Filling volumes/Smooth mode parameters listed above also apply to microplates suitable for use with the Spark-Stack module, e.g. 6- to 1536-well formats (see chapter 15.2 Microplate Requirements for the Spark-Stack).

## 2.5.2 Microplates with Barcode

The SPARK multimode reader may be optionally equipped with a barcode reader mounted on the left or right side of the plate transport. For example, for a 96-well microplate, apply the barcode on the left (A) or right (H) side of the microplate (see picture below), depending on which side the barcode reader is mounted.

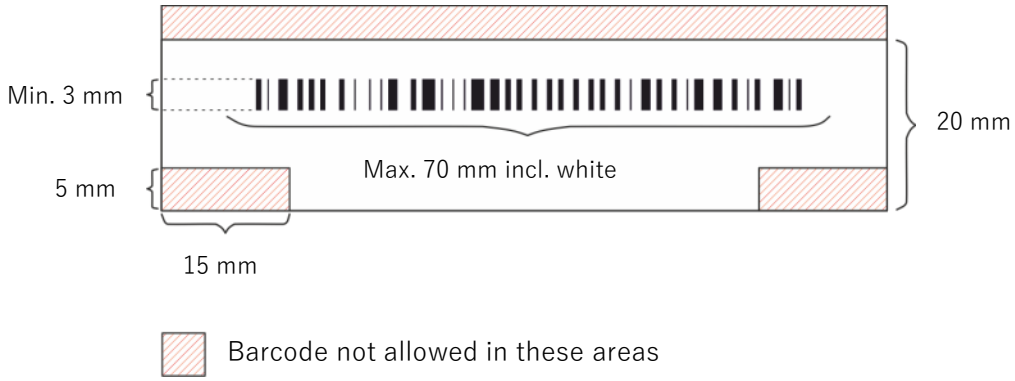
The minimum height of the barcode is 3 mm. At the start and end of the barcode, 2 mm of white space is required. The maximum length of the barcode is 70 mm including the white space at each end. The barcode must be mounted on the short side of the microplate with a minimum distance of 15 mm from the front as well as from the back edge and 5 mm above the lower edge of the microplate.

**Microplate on the plate carrier:**



Apply the barcode on the left or right side of the microplate.

**Side view of the microplate:**







**CAUTION**: Yellowed, dirty, folded, wet, or damaged barcode labels must not be used. The adhesive labels must be flat and without peeled edges. We recommend assuring the quality of the barcodes, by means of a local Standard Operating Procedure (SOP).




**CAUTION**: The barcode is not readable when hidden by the plate lid.

The specified barcode types are:

| | | |
|---|---|---|
| • CODE 39 | • UPC A | • UPC E |
| • EAN 8 | • EAN 13 | • CODE 128 |
| • CODE 2/5 Interleaved | • CODABAR | • CODE 93 |

## 2.6 Onboard Control Buttons

The SPARK has onboard control buttons to simplify some common tasks.



| | |
|---|---|
|  | The **On/Off** button on the front of the instrument is used to switch the instrument to STANDBY mode. |
|  | The **Onboard Start** button is used to start favorite SparkControl Methods directly from the instrument. It can also be used to stop a measurement, confirm user-defined user interventions, and to continue kinetic measurements already paused via the software. |
|  | The **Retract/Eject** button allows microplates to be inserted or removed from the instrument without software activation. |
|  | The **Eject Filter** button is used to move out the filter slides. The filter slides are moved in automatically at insertion. |



**NOTE:** To completely switch off the instrument, use the main power switch on the rear of the instrument.



**NOTE:** For functionality of onboard Control Buttons in combination with the installed microplate stacker module, see chapter 15 Spark-Stack Microplate Stacker .

## 2.7 Instrument LEDs

The SPARK is equipped with multi-color LEDs to optically signal the operation/activity state of the instrument. The table below gives an overview of possible signals that define which functionalities (onboard control buttons) are available at which instrument state.

| Led Status | Instrument State | Onboard Control Buttons | | |
|---|---|---|---|---|
| | | Retract/ Eject | Eject Filter | Onboard Start |
| - | OFF | O | O | O |
| - | STANDBY | O | O | O |
| BLUE | IDLE (not connected to SparkControl) | X | X | X |
| MAGENTA | IDLE (connected to SparkControl) | X | X | X |
| GREEN | RUN | O | O | X |
| RED BLINKING | ERROR | O | O | O |
| YELLOW BLINKING | USER INTERACTION | X | O | X |
| GREEN BLINKING | PAUSE | X | O | X |
| 5x CYAN BLINKING | ACTION NOT POSSIBLE | O | O | O |

Table of LED states and functionalities.
O = function not available. X = function available.

## 2.8 Rear View

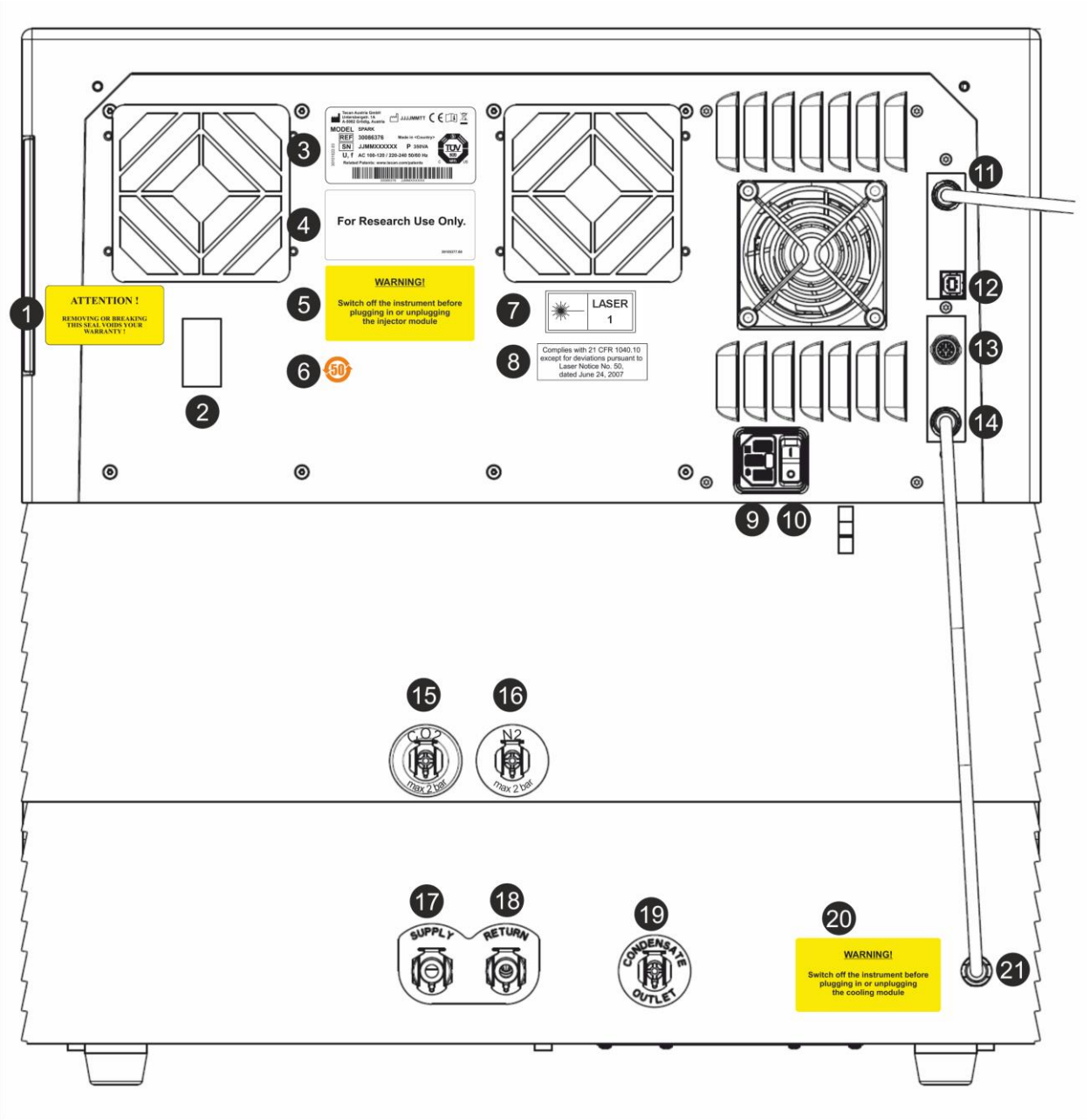
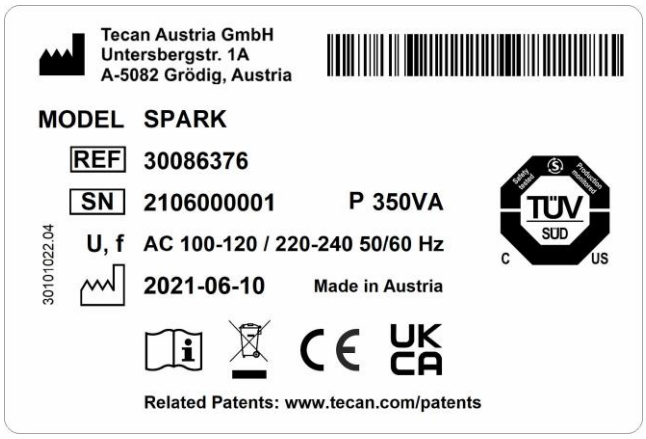



Figure 1: Rear view of the instrument

**NOTE:** This figure is only an example. The labels on the instrument depend on installed options and the destination country.

| | |
|---|---|
| 1 | Warranty Label: ATTENTION !<br>REMOVING OR BREAKING THIS SEAL VOIDS YOUR WARRANTY!<br>(also on bottom of instrument) |
| 2 | Temperature sensor cover |
| 3 | Name Plate (example) |
| 4 | Label: For Research Use Only. |
| 5 | Label: WARNING! Switch off the instrument before plugging in or unplugging the injector module |
| 6 | Label: China RoHS symbol |
| 7 | Label: Class 1 Laser Product |
| 8 | Label: Complies with 21 CFR 1040.10 except for conformance with IEC 60825-1 Ed.3, as described in Laser Notice No. 56, dated May 8, 2019. |
| 9 | Main Power Socket |
| 10 | Main Power Switch |
| 11 | USB 3.0 connection for camera |
| 12 | USB connection |
| 13 | Injector connection |
| 14 | CAN Cable to integrated cooling module (Te-Cool) |
| 15 | $CO_2$ connection (max 2 bar) |
| 16 | $N_2$ connection (max 2 bar) |
| 17 | Supply: liquid cooling |
| 18 | Return: liquid cooling |
| 19 | Condensate outlet |
| 20 | Label: WARNING! Switch off the instrument before plugging in or unplugging the cooling module |
| 21 | CAN Cable to Instrument |

## Example Name Plate




Contents of the name plate (e.g., model name and article number) may vary depending on the specific model.

# 3 Instrument Installation

## 3.1 Installing the SPARK

When installing, moving, or connecting the instrument, follow the instructions in this document. Tecan does not accept the responsibility for injury suffered by anyone attempting these operations nor for damage incurred to the instrument.

Make sure the laboratory meets all the requirements and conditions described in this chapter.

## 3.2 Installation Requirements for SPARK

### 3.2.1 Required Working Area

Select a location to place the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents, and acid vapors. Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment and 5 cm distance to any other equipment left and right to the instrument. See chapter 6 Instrument Specifications for further details regarding the environmental specifications.

The cell imaging performance of Spark's Cell Imager module is especially sensitive to external vibrations in the research laboratory, which can lead to blurred images and/or autofocus errors. Therefore, an appropriate location must be chosen to install the instrument, where external vibrations are kept to a minimum, or for best results use a vibration insulated laboratory table.

Ensure that the plate carrier and injector carrier cannot be accidentally hit when moved out. For the installation procedure for the Injector and the Heater/Stirrer, see 16 Injectors.

For the installation procedure for the Cooling Module (Te-Cool), see chapter 17.2 Cooling System .



**NOTE:** A service engineer is required to install the Spark-Stack microplate stacker module.

Ensure that the main switch and the main cable can always be reached and are in no way obstructed.




**CAUTION:** Install the instrument in a location that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents, and acid vapors. Ensure that the plate carrier and injector carrier cannot be accidentally hit when moved out.




**CAUTION:** Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment and 5 cm distance to any other equipment left and right to the instrument. Do not cover the instrument while it is in operation.




**CAUTION:** Do not place heavy objects on the instrument cover. The maximum load for the SPARK cover is 20 kg. However, the load must be distributed evenly across the entire surface of the cover.




**CAUTION:** Only use the supplied USB cable. The instrument has been tested with the USB cable delivered with the instrument. If another USB cable is used, Tecan Austria cannot guarantee the correct performance of the instrument.

## 3.3 Unpacking & Inspection

1. Visually inspect the container for damage before it is opened.
   Report any damage immediately.
2. Select a location to place the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents, and acid vapors. Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment and 5 cm distance to any other equipment left and right to the instrument. Ensure that the plate carrier and injector carrier cannot be accidentally hit when moved out. Ensure that the main switch and the main cable can always reached and are in no way obstructed.
3. Place the carton in an upright position and open it.
4. Lift the instrument out of the carton and place it in the selected location. Take care when lifting the instrument and ensure that it is held on both sides.
5. Visually inspect the instrument for loose, bent, or broken parts.
   Report any damage immediately.
6. Compare the serial number on the rear panel of the instrument with the serial number on the packing list.
   Report any discrepancy immediately.
7. Compare the contents of the subpackages to the packing list.
   Report any discrepancy immediately.
8. Save packaging materials and transport locks for further transportation purposes.



**WARNING:** The fully equipped SPARK is a precision instrument and weighs approximately 50 kg. At least two people must carefully lift the instrument from the box.




**CAUTION:** Do not overload the plate carrier. The maximum load for the plate transport is 275 g. Overloading the plate carrier can cause instrument damage which may require service.

## 3.4 Subpackages

**NOTE:** Always compare the contents of the subpackages with the delivered packing list.
Report any discrepancy immediately.

The instrument packaging includes the following items:

- Cables (USB 2.0 and main)
- Software (USB stick)
- Instruction for Use (optional)
- OOB Quality Report
- CE Declaration of Conformity
- Final Test Protocol (COC)
- RoHS Notice
- Cuvette adapter
- Transport Lock Install/Uninstall Procedure

Additional subpackages depend on the modules installed:

- Filter slide metal box (Fluorescence Filter/ Fusion Optics Module)
- Magnetic pad (Lid Lifter)
- Hose kit (Gas Control)
- Tecan Adapter for Cell Chips (cardboard box including 15 cell chips (Cell Counter))
- Injector dummy (Injector/Injector Ready)
- RoboFlask metal box (Centering clamp with set screw and spare screw)
- Metal box with user dichroic mirror (including Allen key for installation)

## 3.5 Options Packages

**NOTE:** Always compare the contents of the packaging with the delivered packing list.
Report any discrepancy immediately.

The injector module packaging for one injector (basic module) includes the following items:

- Injector cardboard box
- Injector carrier
- Bottle holder
- PVC clasps
- Carbon needle
- Beakers for priming (2 x 1 ml; 1 x 50 ml)
- 125 ml bottle (light protective)
- 15 ml bottle (light protective)

The injector module packaging for the second injector (extension module) includes the following items:

- Injector cardboard box
- Bottle holder
- PVC clasps
- Carbon needle
- Beakers for priming (2 x 1 ml)

- 125 ml bottle (light protective)
- 15 ml bottle (light protective)

The Heater/Stirrer option includes the following items:

- Heater/Stirrer module
- Main cable (basic module)
- Power supply (basic module)
- Beaker glass 100 ml (basic and extension module)
- Magnetic stirring bar (basic and extension module)
- Allen Key

The NanoQuant option includes the following items:

- NanoQuant storage box (aluminum case)
- NanoQuant Plate
- Pipetting Aid
- Safety Certificate

The Humidity Cassette standard option includes the following items:

- Humidity Cassette (cassette plus lid)
- Magnetic pad

The Humidity Cassette Cell Imager option includes the following items:

- Humidity Cassette Cell Imager (cassette plus lid)
- Magnetic pad

The Te-Cool option includes the following items:

- External liquid cooling device
- Tubing set
- Condensate tubing
- CAN-cable
- Stoppers
- Coolant concentrate

The Spark-Stack microplate stacker consists of the following items (according to the order):

- Stacker module option
- Short stack option
  - Set of 2 plate magazines for 30 plates per run
  - Dark covers and lids
- Long stack option
  - Set of 2 plate magazines for 50 plates per run
  - Dark covers and lids

The Cell Imager option includes a dedicated computer.

**NOTE:** A service engineer is required to install the Spark-Stack microplate stacker module.

**CAUTION:** All items delivered with the instrument and also all spare parts or supplemental parts for the instrument are intended for use with the instrument only and are not for general use.

## 3.6 Upgrades

The instrument consists of various modules and can be ungraded if required. Contact your local Tecan representative for more information.

## 3.7 Removal of the Transport Locks

### 3.7.1 Plate Carrier Transport Lock

**CAUTION:** Remove the transport lock before operating the instrument.

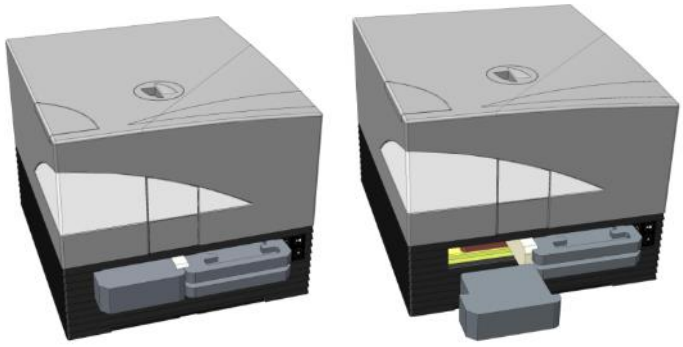
The instrument is delivered with the plate carrier locked into place, so that it cannot become damaged.

Before the instrument can be used, the transport locks (foam pieces) must be removed using the following procedure:

1. Ensure that the instrument is disconnected from the main power supply.
2. Remove the tape from the filter compartment doors.



3. Remove the piece of foam from the left plate carrier compartment (see picture below).



4. Move the plate carrier out manually by pulling on the pieces of foam in the right plate carrier compartment (see picture below).



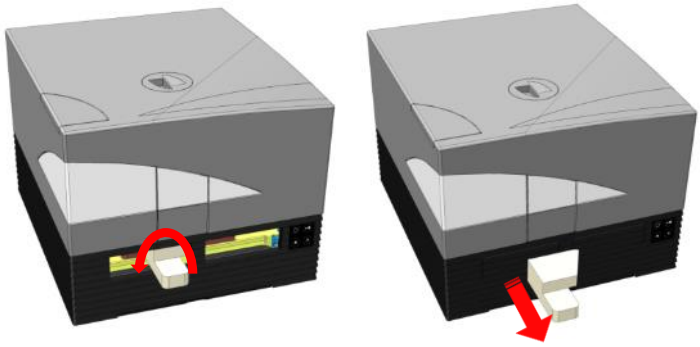
5. Remove the top piece of foam first and then the bottom piece (see picture below).



6. Move the plate carrier in carefully by hand. It must be pushed in far enough so that the plate carrier compartment door can close (see picture below).



7. Rotate the remaining piece of foam 90° counter-clockwise and pull it out of the instrument (see picture below).






**CAUTION:** Save packaging materials and transport locks (foam pieces) for further transportation purposes. The instrument must be shipped only with the original packaging and installed transport locks.

## 3.8 Power Requirements

The instrument is auto-sensing and it is therefore unnecessary to make any changes to the voltage range. Check the voltage specifications on the rear panel of the instrument and ensure that the voltage supplied to the instrument is correct to this specification.

The voltage range is from **100-120 V and 220-240 V**. If the voltage is not correct, please contact your distributor.

Connect the instrument only to an electricity supply system with a protective ground.

**CAUTION:** Do not use the instrument if the voltage setting is not correct. If the instrument is switched ON with the incorrect voltage setting it will become damaged.

**CAUTION:** Do not replace detachable main power supply cords with inadequately rated cords.

**NOTE:** This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules and CISPR 11/EN 55011. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates uses and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

## 3.9 Switching the Instrument On

**CAUTION:** Before the instrument is switched on for the first time, it should be left to stand for at least 3 hours, so there is no possibility of condensation causing a short circuit.

1. Make sure that the main power switch on the rear panel of the instrument is in the OFF position.
2. Connect the computer to the instrument only with the delivered USB interface cable.
3. Insert the power cable into the main power socket (with protective ground connection) on the rear panel of the instrument.
4. Connect the USB cable of the camera of the cell module (guided through the rear panel of the instrument) to the USB 3.0 port of the computer.

**CAUTION:** The cell module camera or the cell imager module camera, respectively, must be connected to the USB 3.0 port of the computer to avoid performance loss.

5. All connected devices must be approved and listed as per IEC 60950-1 Information Technology Equipment – Safety or equivalent local standards.
6. Connect the injector, if required.
7. Plug in the heater/stirrer, if required.

**CAUTION:** Switch off the instrument before plugging in or unplugging the injector module.

**CAUTION:** Switch off the instrument before plugging in or unplugging the cooling module.

8. Switch ON the instrument using the main power switch on the rear panel of the instrument.
9. Start the software to work with the instrument. For instrument control via software see chapter 8 Operation of SPARK with SparkControl Software.



**WARNING:** Do not reach into the instrument while it is in operation!

## 3.10 Switching the Instrument Off

1. Ensure that the plate transport is empty.
2. In the SparkControl software, disconnect from the instrument by selecting Exit in the File menu in the Method Editor (see the Reference Guide for more details) or Shut Down via the expandable Navigation bar on the left side of the Dashboard.
3. Switch OFF the instrument by using the main power switch on the rear panel of the instrument. Alternatively, switch the instrument to STANDBY mode by using the onboard control button (On/Off button) on the front of the instrument.



**NOTE:** To completely switch off the instrument at the end of the day or after completing the measurements, use the main power switch on the rear of the instrument.




**CAUTION:** When switched off wait at least 5 seconds until switching the instrument on again. Instrument errors can occur.

## 3.11 Preparing the Instrument for Shipping

Before shipping an instrument with integrated cooling module (Te-Cool) the cooling liquid must be removed from the cooling system. This procedure must be done by a service technician.




**CAUTION:** Do not ship an instrument with integrated cooling module! Only Tecan authorized service technicians are allowed to prepare instrument for transportation. Residual cooling fluid might damage the instrument.

Before shipping an instrument with the microplate stacker module (Spark-Stack), the stacker must be removed from the instrument. This procedure must be done by a service technician.




**CAUTION:** Do not ship an instrument with integrated stacker module! Only Tecan authorized service technicians are allowed to remove the stacker module for transportation of instrument or stacker module.

Before shipping the instrument, perform the parking procedure to avoid any damage to the optics and plate transport (see 3.11.1 Parking Procedure). After the parking procedure has been performed, the plate carrier transport locks must be installed (see 3.11.2 Installing the Plate Carrier Transport Locks).

Before shipping, the instrument (including the injector(s), heater/stirrer, humidity cassette, NanoQuant Plate and any other external optional components) must be thoroughly disinfected (see chapter 7.4 Instrument Decontamination/Disinfection). For injector maintenance, see chapter 16.3 Injector Cleaning and Maintenance).




**CAUTION:** Switch off the instrument before plugging in or unplugging the injector module.

**CAUTION:** Switch off the instrument before plugging in or unplugging the cooling module.

The instrument (including the injector(s), heater/stirrer, humidity cassette, NanoQuant Plate and any other external optional components) must be shipped in the original packaging.

**WARNING:** Always move the injector and the heater/stirrer separately, as the two units are not attached to each other. When carried together, one of the units can easily fall and become damaged.

### 3.11.1 Parking Procedure

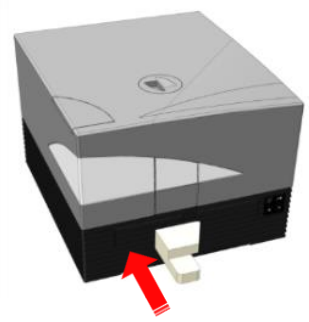
1. Ensure that the plate transport is empty.
2. Ensure that the injector (dummy) is removed from the injector port.
3. In the SparkControl software, disconnect from the instrument by selecting **Exit** in the File Menu in the Method Editor (see the Reference Guide for more details) or Shut Down via the expandable Navigation bar on the left side of the Dashboard.
4. Remove filter slides by using the onboard control button in the front of the instrument.
5. Move out the plate transport by using the onboard control button in the front of the instrument.
6. To start the parking procedure, press the On/Off button on the front of the instrument. Starting the parking procedure may take a few seconds.
7. Switch OFF the instrument by using the main power switch on the rear panel of the instrument.
8. Install the plate carrier transport lock (see chapter 3.11.2 Installing the Plate Carrier Transport Locks).

**CAUTION:** The parking procedure must be performed, and the transport lock must be mounted before shipping. If the instrument is shipped without these safety measures, the instrument guarantee is rendered null and void. Use original packaging for shipping.

### 3.11.2 Installing the Plate Carrier Transport Locks

The instrument must be shipped with the plate carrier locked into place, so that it cannot become damaged. Before the instrument can be shipped, the transport locks (foam pieces) must be inserted using the following procedure:

1. Ensure that the instrument is disconnected from the main power supply.
2. Hold the plate carrier compartment door down and insert the white piece of foam (shown below) into the left compartment.



3. With the foam piece inserted, turn it 90° clockwise, so that the pointed end sticks down into the space between the two compartment openings. This piece of foam holds the compartment doors open.



4. Move the plate carrier out carefully by hand until it lightly presses against the inserted white piece of foam from behind and cannot be moved out further.



5. Insert the bottom piece of foam first and then interlock the top piece into place (see picture below).



6. Move the plate carrier into the right compartment manually as far as it can go by pushing on the pieces of foam on the plate carrier.



7. Insert the piece of foam into the left plate carrier compartment (see picture below).



8. Tape the filter compartment doors shut (see picture below).



# 4 Plate Control

The plate transport can move horizontally (in the x- and y-directions) as well as vertically (in the z-direction), so that for each measurement mode, top or bottom, the optimal measurement position can be reached regardless of which plate type or filling volume is used. The movement speed is optimized according to the plate type and detection mode.

**NOTE:** For additional requirements when operating the instrument with the microplate stacker module, see chapter 15 Spark-Stack Microplate Stacker .

**CAUTION:** Before starting measurements, make sure that the microplate is inserted correctly. The position of well A1 must be on the upper left side.

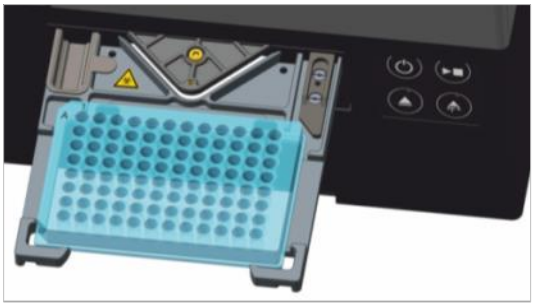


Figure 2: Microplate on the plate carrier with the A1 well in the upper left-hand corner

**CAUTION:** Tecan Austria GmbH has taken great care when creating the Plate Definition Files (.pdfx) that are delivered with the instrument.

We take every precaution to ensure that the plate heights and well depths are correct according to the defined plate type. These parameters are used to determine the minimum distance between the top of the plate and the ceiling of the measurement chamber. Additionally, Tecan Austria adds a very small safety gap to prevent any damage that may occur to the measurement chamber as a result of small changes in plate height. This does not affect the performance of the instrument.

Make sure that the selected plate definition file corresponds to the currently used microplate, so that the safety gap is correctly calculated, otherwise the instrument could become damaged.

**CAUTION:** Do not leave microplates inside the instrument overnight when working with aggressive solutions. Acids, bases, or cleaning solutions (bleach) will evaporate inside the reader and cause corrosion. This may lead to severe damage of the instrument and can impair its proper functioning. Tecan cannot take any responsibility nor be held liable if the reader is damaged due to improper plate handling.

**CAUTION:** Users should also take care that no potential fluorescent or luminescent contamination lies on top of the plate as droplets, and also be aware that some plate sealers leave a sticky residue that should be removed before measurement.

## 4.1 Z-Position

The height of the objective above the sample can be adjusted using the Z-position function. As excitation light is reflected by the sample fluid, the Z-adjustment helps to maximize the signal-to-noise ratio. For further details about Z-positioning, see the corresponding chapter in the Reference Guide.

## 4.2 Shaking

The SPARK is capable of plate shaking before start of a measurement or in between kinetic cycles. Three shaking modes are available: linear, orbital, and double orbital. The shaking amplitude can be selected from 1 to 6 mm in steps of 0.5 mm. The frequency is a function of the amplitude. The shaking duration is selectable from 3-3600 seconds.

## 4.3 Incubation/Cooling Position

The SPARK has a predefined incubation/cooling position with an optimum temperature distribution. These positions can be used for shaking or waiting steps within a measurement run.

## 4.4 Lid Lifter

The lid lifter option consists of a permanent magnet and a magnetic pad. The magnetic pad can be mounted on the lids of all commonly used microplate types with a maximum lid height of 11.5 mm. The magnetic mechanism is regulated by the software.

To attach the pad, peel the paper backing off from the metal disk and stick the pad onto the center of the lid.




**CAUTION:** The lid height must not exceed 11.5 mm.




**CAUTION:** Clean the lid with 70 % ethanol before attaching the magnetic pad.




**CAUTION:** Make sure that the magnetic pad is mounted on the plate lid if **Removable Lid** or **Humidity Cassette** is activated in the method.




**CAUTION:** Mount the magnetic pad in the middle of the appropriate plate lid to guarantee optimal performance.

The lid lifter option is used to temporarily remove the lid of the microplate to execute, e.g., injection steps or measurement steps within the workflow of a long-term experiment thus avoiding sample evaporation.

The lid lifter in combination with the gas module option can also be used to improve the gas exchange between the medium and the surrounding environment in case of cell-based studies. Ventilation steps can be inserted simply into the workflow and timed accordingly.

The lid lifter option can also be used in combination with Tecan's humidity cassette (refer to chapter 17 Environmental Control).

## 4.5 Securing the RoboFlask Cell Culture Vessels

A centering clamp is necessary to secure the RoboFlask Cell Culture Vessels (Corning, Inc.) onto the plate carrier. This centering clamp must be installed by the user before starting measurements using RoboFlask Cell Culture Vessels. Follow the given instructions.

- Move the plate transport out.
- Put the centering clamp on the plate fixing mechanism as indicated in the figure below.
- Fasten the screw, taking care to avoid putting pressure on the plate carrier.

**CAUTION:** Do not put pressure on the plate carrier when attaching the centering clamp. A bent plate carrier can negatively influence the performance of the instrument and may require service.

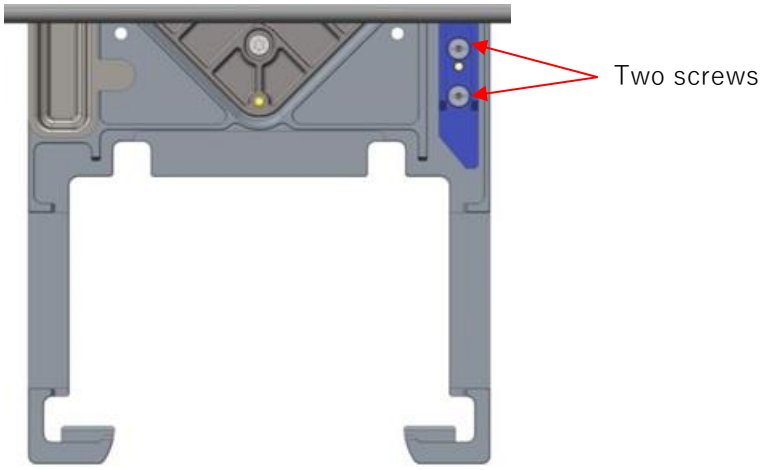



Figure 3: Centering clamp for the RoboFlask Cell Culture Vessels

**CAUTION:** Do not use the RoboFlask Cell Culture Vessels without the centering clamp. This can result in damage to the instrument.

**NOTE:** By using a higher number of flashes and/or a settle time for measurements with the RoboFlask, the result will be more accurate.

# 5 SPARK Platform

The SPARK is a multimode reader platform. Each instrument variant can be equipped with a large number of modules and functions. The following chapter provides an overview.

## 5.1 Overview of Available Modules and Functions

The SPARK  is compatible with plate formats from 1 well to 384 wells; advanced modules support plate formats up to 1536 wells.

| Module/Function | Characteristics |
|---|---|
| Absorbance | Absorbance (Fast absorbance scan included) or Absorbance Enhanced (Up to 1536-well) |
| NanoQuant Plate | For low volume nucleic acid samples. Ready to use Apps available for nucleic acid quantitation and labeling efficiency. |
| Cuvette module | For Absorbance measurements. Ready to use App available. |
| Luminescence Standard | Attenuation function (OD1 and OD2).<br>Up to 384-well. |
| Luminescence Enhanced | Attenuation function (OD1, OD2 and OD3).<br>Wavelength discrimination.<br>Luminescence scan included.<br>Up to 1536-well |
| Alpha Technology | AlphaScreen, AlphaLISA and AlphaPlex<br>Alpha Enhanced (Up to 1536-well) |
| Fluorescence Standard Top | Filter-only-, Monochromator-only- or Fusion Optics-System available.<br>Up to 384-well. |
| Fluorescence Standard Bottom | Filter-only-, Monochromator-only- or Fusion Optics-System available.<br>VIS or UV-VIS fiber.<br>Up to 384-well. |
| Fluorescence Standard Bottom Area Scan | Up to 100x100 data points/well |
| Fluorescence Standard Polarization | Filter-only-, Monochromator-only- or Fusion Optics-System available.<br>>300 nm or >390 nm fiber.<br>Up to 384-well. |
| Fluorescence Enhanced Top | Filter-only-, Monochromator-only- or Fusion Optics-System available.<br>More sensitive than Standard option.<br>Up to 1536-well |

| Module/Function | Characteristics |
|---|---|
| Fluorescence Enhanced Bottom | Filter-only-, Monochromator-only- or Fusion Optics-System available.<br>Equipped with UV-VIS fiber.<br>More sensitive than Standard option.<br>1536-well optional. |
| Fluorescence Enhanced Bottom Area Scan | up to 100x100 data points/well |
| Fluorescence Enhanced Polarization | Filter-only-, Monochromator-only- or Fusion Optics-System available.<br>Equipped with >300 nm fiber.<br>More sensitive than Standard option.<br>Up to 1536-well |
| Cell Module: Cell Counting and Confluence | Cell counting and viability in Tecan Cell Chips (Ready to use Apps).<br>Cell confluence in microplates. |
| Cell Imager | Bright field Imaging and Fluorescence Imaging in microplates. |
| Spark-Stack | Built-in microplate stacker designed for automated loading, unloading, and restacking of plates. |
| Injector (one or two injectors) | One or two injector options with different syringe sizes. |
| Heater&Stirrer | Both injector options can be equipped with Heater/Stirrer module. |
| Heating | 3 ° C above ambient up to 42 ° C. |
| Cooling (Te-Cool) | 18 ° C up to 42 ° C |
| Gas control | $CO_2$ only or $CO_2$ and $O_2$ |
| Humidity Control | Evaporation protection for different plate formats for long-term studies (with cells). |
| Lid lifter | Interactions during long-term studies (gas exchange, injection) |
| Barcode reader | Reads barcodes automatically. |

Fluorescence Standard and Fluorescence Enhanced options cannot be installed together in one instrument.

# 6 Instrument Specifications



**NOTE:** All specifications are subject to change without prior notification.

The table below lists the technical specifications of the basic instrument:

## General

| Parameters | Characteristics |
|---|---|
| Measurement | Software controlled |
| Interface | USB 2.0 or 3.0 (SPARK);<br>3.0 (SPARK CYTO) |
| Fusion optics system | Monochromator and Filter (external filter exchange) based |
| Microplates | From 1-well to 1536-well SBS plates |
| Temperature control | From 18 ° C up to 42 ° C (depending on installed modules) |
| Plate shaking | Linear, orbital, and double orbital shaking |
| Light source | High energy Xenon flash lamp |
| Optics | Fused Silica Lenses |
| Fluorescence detector | Low dark current photomultiplier tube |
| Luminescence detector | Low dark count photomultiplier tube |
| Absorbance detector | Silicon photodiode |
| Power supply | 100-120 V and 220-240 V, auto-sensing |
| Power consumption | Operation: 350 VA, Standby: 25 VA |

## Physical

| Parameters | Characteristics | | |
|---|---|---|---|
| Outer dimensions | Width: | 494 mm | (19.5 in.) |
| | Height: | 395 mm | (15.5 in.) |
| | Height (with Te-Cool): | 512 mm | (20.2 in.) |
| | Height (with cell imager): | 512 mm | (20.2 in.) |
| | Height (with injector carrier): | 455 mm | (17.9 in.) |
| | Depth: | 557 mm | (21.9 in.) |
| | Depth (carrier moved out): | 699 mm | (27.5 in.) |
| | Depth (with Spark-Stack): | 786 mm | (30.9 in.) |

## Weight

| Parameters | Characteristics | |
|---|---|---|
| Instrument | 40 kg | (88 lb.) |
| Instrument with Te-Cool | 50 kg | (110 lb.) |
| Instrument with Cell Imager<br>(for CYTO600, the heaviest configuration) | max. 50 kg | (max. 110 lb.) |
| Injector (2 channel) | 4.0 kg | (8.8 lb.) |
| Heater/Stirrer | 2.7 kg | (6 lb.) |
| **Spark-Stack module** | | |
| Stacker | 8.5 kg | (18.7 lb.) |
| Short Stack<br>(2 plate magazines, including dark covers and dark lids) | 4.5 kg | (9.9 lb.) |
| Long Stack<br>(2 plate magazines, including dark covers and dark lids) | 5 kg | (11 lb.) |

## Environmental

| Parameters | Characteristics | |
|---|---|---|
| Operating temperature | +15 ° C to +35 ° C | 59 ° F to 95 ° F |
| Operating temperature with active cooling | +15 ° C to +30 ° C | 59 ° F to 86 ° F |
| Transportation temperature | -30 ° C to +60 ° C | -22 ° F to +140 ° F |
| Operating humidity | 20 % to 90 % (non-condensing) | |
| Operating humidity with active cooling | 20 % to 80 % (non-condensing) | |
| Transportation humidity | 20 % to 95 % (non-condensing) | |
| Operating pressure | 700-1050 hPa | |
| Transportation pressure | 500-1100 hPa | |
| Overvoltage category | II | |
| Pollution degree | 2 | |
| Usage | Commercial | |
| Noise level | < 60 dBA | |
| Method of disposal | Electronic waste (infectious waste) | |

# 7 Cleaning and Maintenance

## 7.1 Introduction

- For maintenance of the NanoQuant, see 18.4 NanoQuant Maintenance and the corresponding chapter in the Reference Guide for more details.
- For injector maintenance, see 16.3 Injector Cleaning and Maintenance.
- For maintenance of the cell chip adapter, see 13.3.3 Maintenance and Cleaning of the Cell Chip Adapter and the corresponding chapter in the Reference Guide.
- For maintenance of the Cooling Module, see 17.2.7 Maintenance.
- For maintenance of the Spark-Stack, see 15.2.7 Cleaning and Maintenance of the Spark-Stack.

The cleaning and maintenance procedures are important to prolong the instrument's life and to reduce the need for service.

This section contains the following information:

- Preventive Maintenance (PM)
- Liquid Spills
- Instrument Disinfection
- Disinfection Procedure
- Safety Certificate
- Disposal




**CAUTION:** Keep the plate transport clean! Take special care of the clip mechanism that secures the microplates. Insufficient plate fixation can lead to instrument damage. Excessive soiling requires service.

## 7.2 Preventive Maintenance (PM)

To ensure and sustain optimal instrument performance and minimize wear and tear, annual preventive maintenance (PM) is strongly recommended.

The PM procedure includes (but is not limited to):

- Function and accuracy checks
- Cleaning of filters and fans
- Replacement of service parts
- Alignment of mechanical components



**NOTE:** This maintenance must be performed by a certified Tecan service representative.

## 7.3 Liquid Spills

1. Wipe up the spill immediately with absorbent material.
2. Dispose of contaminated material appropriately.
3. Clean the instrument surfaces with a mild detergent.
4. For biohazard spills, clean with B33 (Orochemie, Germany).
5. Wipe cleaned areas dry.



**WARNING:** Always switch off the instrument before removing any kind of spills on the instrument. All spills must be treated as potentially infectious. Therefore, always adhere to applicable safety precautions (including the wearing of powder-free gloves, safety glasses and protective clothing) to avoid potential infectious disease contamination.

Additionally, all resulting waste from the clean-up procedure must be treated as potentially infectious and the disposal must be performed according to the information given in chapter 7.5 Disposal.

## 7.4 Instrument Decontamination/Disinfection



**WARNING:** The disinfection procedure should be performed according to national, regional, and local regulations.



**WARNING:** All parts of the instrument that come into contact with potentially infectious or any hazardous material must be treated as potentially infectious areas.

It is advisable to adhere to applicable safety precautions (including the wearing of powder-free gloves, safety glasses and protective clothing) to avoid potential infectious disease contamination when performing the disinfection procedure.



**WARNING:** It is very important that the instrument is thoroughly disinfected before it is removed from the laboratory or before any service is performed on it.



**WARNING:** The disinfection procedure for the injector described in this chapter is valid only for the cover of the injector box. For cleaning and maintenance of the syringes, tubes and pumps, please refer to 16.3 Injector Cleaning and Maintenance.




**CAUTION:** Ensure that the microplate is removed from the instrument before it is prepared for shipment. If a microplate is left in the instrument, fluorescent solutions may spill onto the optical parts and damage the instrument.

Before the instrument is returned to the distributor or service center, all outer surfaces and the plate transport must be disinfected and a safety certificate must be completed by the operating authority. If a safety certificate is not supplied, the instrument may not be accepted by the distributor or service center, or custom authorities may hold it.

### 7.4.1 Disinfection Solutions

The instrument (Front, Cover, Plate transport) should be disinfected using the following solution:

- B33 (Orochemie, Germany)




**CAUTION:** The disinfection procedure should be performed by authorized trained personnel in a well-ventilated room wearing disposable gloves and protective glasses and clothing.



**WARNING:** The disinfection procedure for the injector is valid only for the cover of the injector box. For cleaning and maintenance of the syringes, please refer to 16.3 Injector Cleaning and Maintenance.

### 7.4.2 Disinfection Procedure




**CAUTION:** The surface disinfectant can negatively influence the performance of the instrument, if it is applied or accidentally gets inside the instrument.




**CAUTION:** Make sure that the microplate has been removed from the instrument before starting disinfection procedure.

If the laboratory has no specific disinfection procedure the following procedure should be used to disinfect the outside surfaces of the instrument:

1. Wear protective gloves, protective glasses, and protective clothing.
2. Prepare a suitable container for all disposables used during the disinfection procedure.
3. Disconnect the instrument from the main power supply.
4. Disconnect the instrument from any external components that are used.
5. Carefully wipe all outside surfaces of the instrument with a lint-free paper towel soaked in the disinfection solution.
6. Perform the same disinfection procedure on the plate carrier.
7. Perform the disinfection procedure on any external components that are used with the instrument.
8. Complete the Safety Certificate and attach it to the outside of the box so that it is clearly visible.

See below for information about the Safety Certificate, which must be completed before the instrument is returned to the distributor/ service center.




**CAUTION:** The plate transport should only be moved manually when the instrument is disconnected from the main power supply

### 7.4.3 Safety Certificate

The Safety Certificate must be requested from your local Tecan Customer Support (refer to http://www.tecan.com/ for contact information).

To ensure the safety and health of personnel, our customers are kindly asked to complete two copies of the **Safety Certificate** and attach one copy to the top of the container in which the instrument is returned (visible from the outside of the shipping container!) and attach the other copy to the shipping documents before shipping it to the service center for service or repair.

The instrument must be decontaminated and disinfected at the operating authority's site before shipping (see 7.4.2 Disinfection Procedure).

The decontamination and disinfection procedure must be performed in a well-ventilated room by authorized and trained personnel wearing disposable powder-free gloves, safety glasses and protective clothing.

The decontamination and disinfection procedure must be performed according to national, regional, and local regulations.

If a Safety Certificate is not supplied, the instrument may not be accepted by the service center.

## 7.5 Disposal

Follow laboratory procedures for bio-hazardous waste disposal, according to national, regional, and local regulations.

This section provides instructions on how to lawfully dispose of waste material accumulated in connection with the instrument.




**CAUTION:** Observe all federal, state, and local environmental regulations.



**CAUTION:** Directive 2012/19/EU on waste electrical and electronic equipment (WEEE)

Negative environmental impacts associated with the treatment of waste:

- Do not treat electrical and electronic equipment as unsorted municipal waste
- Collect waste electrical and electronic equipment separately

### 7.5.1 Disposal of Packaging Material

According to Directive 94/62/EC on packaging and packaging waste, the manufacturer is responsible for the disposal of packaging material.

#### Returning Packaging Material

If you do not intend to keep the packaging material for future use, e.g., for transport and storage purposes, return the packaging of the product, spare parts, and modules via the field service engineer to the manufacturer.

### 7.5.2 Disposal of Operating Material



**CAUTION:** Biological hazards can be associated with the waste material (i.e., microplate) of the processes run on the SPARK.

Treat the used microplate, cell chips, other disposables, and all substances used, in accordance with good laboratory practice guidelines.

Inquire about appropriate collecting points and approved methods of disposal in your country, state, or region.

### 7.5.3 Disposal of the Instrument

Please contact your local Tecan service representative before disposing of the instrument.




**CAUTION:** Always disinfect the instrument before disposing.

| | |
|---|---|
| Pollution Degree | 2 (IEC/EN 61010-1) |
| Method of Disposal | Contaminated waste |



**WARNING:** Depending on the applications, parts of the instrument may have been in contact with biohazardous material. Make sure to treat this material according to the applicable safety standards and regulations.

Always decontaminate all parts before disposal.

# 8 Operation of SPARK with SparkControl Software

## 8.1 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.

## 8.2 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 must 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:**<br>> 3 GHz (8 Core)<br>2 GB graphics card | |
| **Operating System** | Windows 10 (64-bit)<br>Windows 11 (64-bit)<br>Editions: Pro, Enterprise<br>**Windows RT NOT supported!** | |
| **Memory** | 8 GB RAM | 16 GB RAM |
| | **Cell Imager module:**<br>64 GB RAM | |

| | Supported | Recommended |
|---|---|---|
| **Free Hard Disk Space** | 6 GB<br>For Cell counting measurements: 40 GB<br>For Cell Confluence measurements, 500 GB is required. | 10 GB<br>For Cell counting measurements: 160 GB<br>For Cell Confluence measurements, 1000 GB is recommended. |
| | **Cell Imager module:**<br>512 GB SSD (system) +<br>8 TB HDD (archive) | |
| **Monitor** | Super VGA Graphics | **Cell Imager module:** 4 K Graphics |
| **Resolution** | 1280 x 1024 | 1680 x 1050<br>1920 x 1080 |
| **Color Depth** | 256 | |
| **Mouse** | Microsoft mouse or compatible pointing device | |
| **Communication** | USB 2.0<br>USB 3.0 | 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:**<br>USB 2.0 (instrument)<br>USB 3.0 (camera) | **Cell Imager module:**<br>USB 3.0 (instrument)<br>USB 3.0 (camera) |
| **Devices** | DirectX 9 graphics device with WDDM 1.0 or higher driver | |
| **.NET** | Microsoft.NET Framework 4.8<br>The required .NET version is installed automatically alongside any existing versions. | |
| **Microsoft Excel** | 2007, 2010, 2013, 2016, 2019, Excel 365<br>The export mechanism writes files according to the Office Open XML file format (.xlsx) | 2019, Excel 365 |

## 8.3 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.

### 8.3.1 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.

### 8.3.2 IoT Client

SparkControl supports remote monitoring of a registered, connected instrument (e.g. instrument/ measurement status) in the Tecan Connect Mobile App via the application interface provided by the Tecan IoT Client.

The IoT client is automatically installed if the option 'Install IoT Client' within the SparkControl setup is selected. If installed, the SparkControl will send the following messages:

| Event | Message |
|---|---|
| **Instrument state** | Idle (ready), not connected, etc. |
| **Measurement state** | Measurement started/paused/resumed/stopped |
| **Measurement progress** | Current data label or progress message<br>Current temperature and/or gas concentration value<br>Current cycle index (kinetic runs only)<br>Current plate index (stacker runs only)<br><br>Notification about required user interaction |
| **Warnings/Errors** | Error or warning message |




**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.

## 8.4 Starting the SparkControl

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

### 8.4.1 Connecting Instruments




**CAUTION:** Do not open the instrument lid during operation!

Each connected instrument is represented by a corresponding tile in the Dashboard (refer to chapter 8.6 Dashboard and to the corresponding chapter in the SparkControl manual).

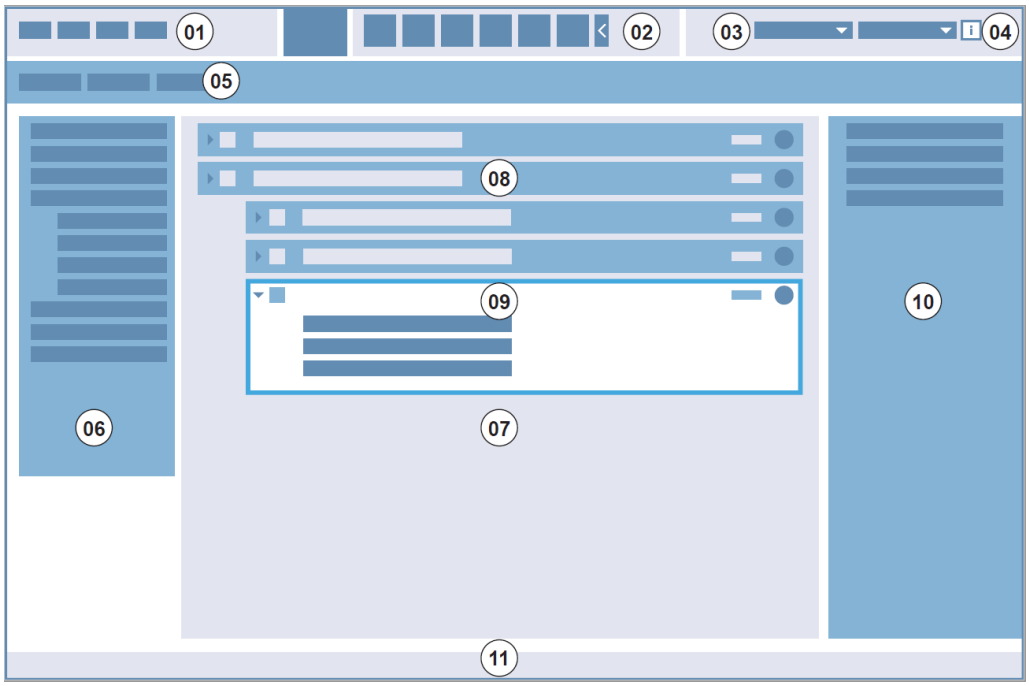


**NOTE:** The SparkControl supports the connection of a maximum of 4 instruments.

# 8.5 Method Editor

## 8.5.1 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.

We refer to the SparkControl manual for a detailed description of

- Control Bar
- Workflow Pane
- Menu Bar

- Toolbar
- Instrument
- Components and Apps



**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.



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




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



**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.



**NOTE:** Fluorescence intensity 3D scans 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 to obtain comparable results.



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



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



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



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

## 8.6 Dashboard

### 8.6.1 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:

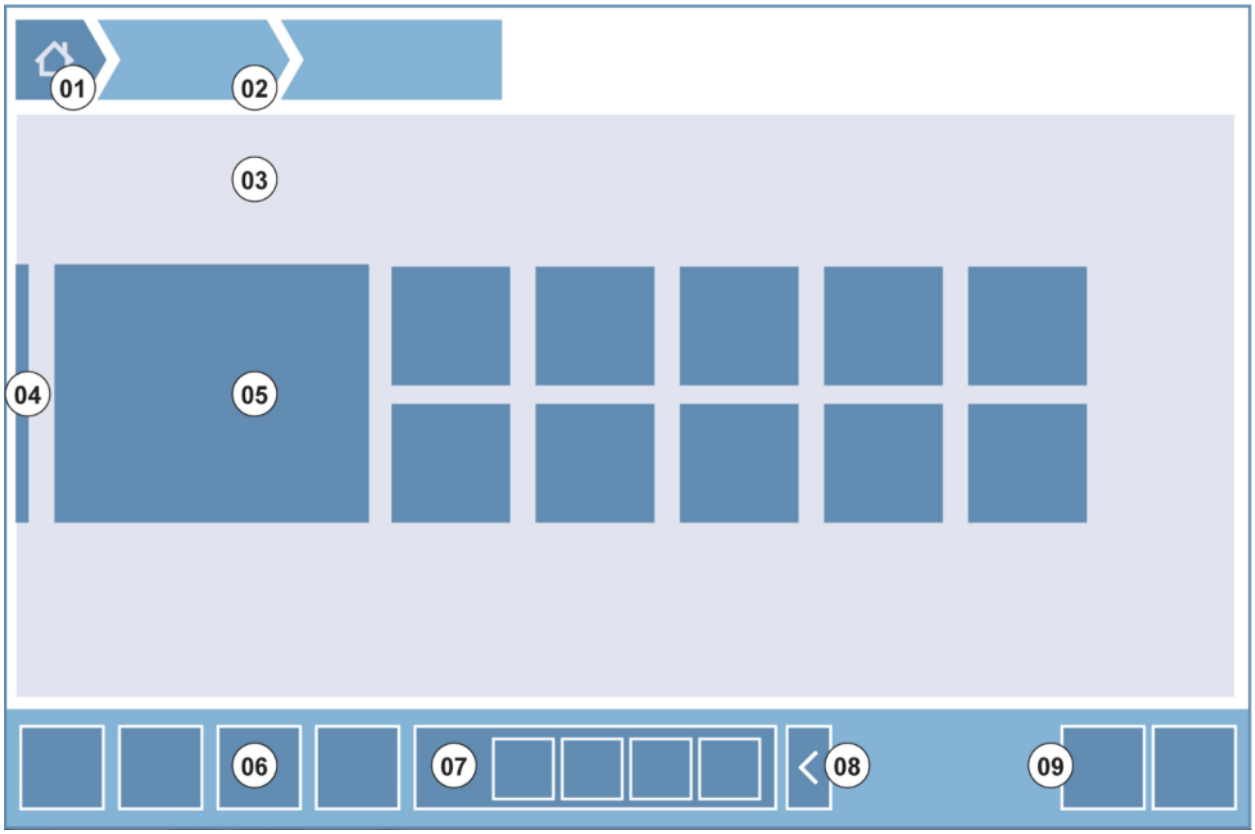



Figure 4: Structural elements of the Dashboard

| | |
|---|---|
| 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 except for tiles with multi-functionality.

For the multi-functional tiles, the clickable surface is always darker than the background color.
Example: Start tile (refer to chapter 8.7 Starting a Method and to the corresponding chapter in the SparkControl manual).

### Action Buttons

A group of buttons designed for

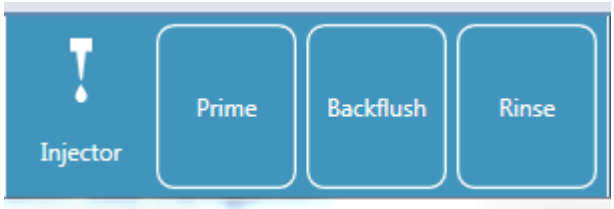
- Editing of method and instrument settings
- Confirmation/cancellation/stopping of workflow steps (OK/Cancel/Stop button)
- Searching/Alignment of listed elements

### 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 to change/prove the temperature before measurement start.

## 8.6.2 The Dashboard

The Dashboard window contains the following tiles:

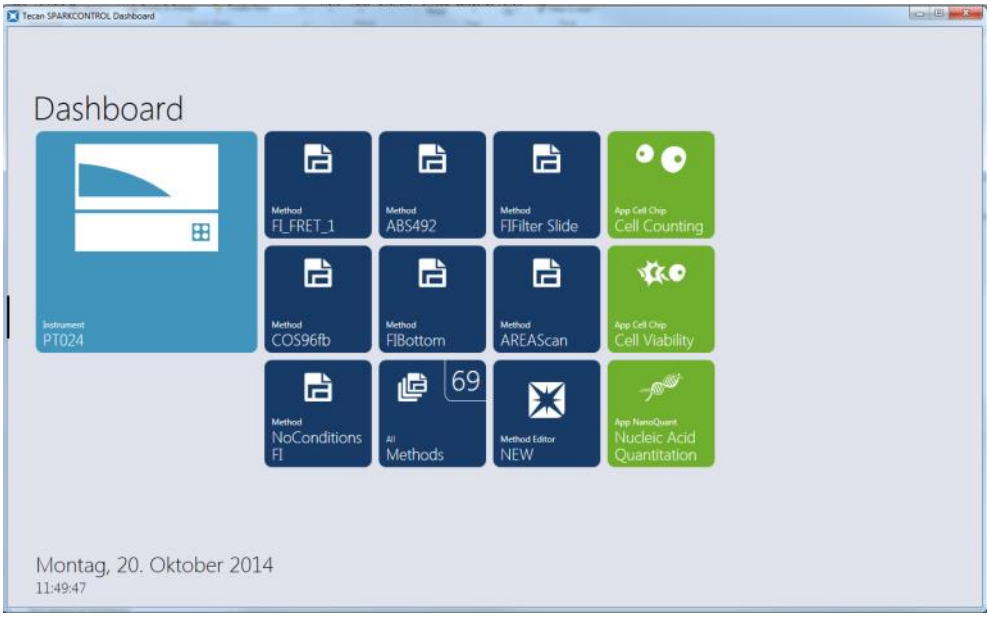



Figure 5: Instrument tiles, Method tiles and App tiles in Dashboard

| | |
|---|---|
| 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.<br>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.<br>The displayed group of method tiles is built up dynamically according to the following rules:<br>• Every new defined or every modified method is automatically displayed in dashboard and placed on the top of the group.<br>• Every executed method is displayed automatically in the Dashboard and placed on the top of the group.<br>• 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.<br>Select **NEW** to switch directly to Method Editor 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.<br>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.

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

**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.

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

**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

**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.

**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 must be deleted manually by 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.

## 8.7 Starting a Method



**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.

### 8.7.1 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.

### 8.7.2 Dashboard

A method can be started directly from the Dashboard by selecting the corresponding **Method** tile. See the corresponding chapter in the SparkControl manual.

### 8.7.3 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.

## 8.8 SparkControl Settings

### 8.8.1 Structure

The **Settings** component is designed to allow the user the customization of the system default settings. These settings can be modified by selecting the corresponding program tile for:

- Software: define the default plate type and the default pathlength correction values.
- Instrument: enter the height above sea level for instruments with the gas module.




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

- Data Handling: define the output settings of the measured results in Excel



**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.



**NOTE:** For kinetic measurements it is recommended to select **List** as export mode to facilitate data analysis in Excel.

- Plate Geometry: create plate definition files for not listed plates or to edit an existing 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.



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

- Images



**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 to open the selected image file format.

- Directory



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



**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.

The **Settings** component is optimized for working with touch using program tiles, tabs and buttons (refer to chapter 8.6 Dashboard and to the corresponding chapter in the SparkControl manual).

## 8.9 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 (refer to chapter Data Handling in the SparkControl manual), the results can be opened automatically after the measurement run in Excel.



**NOTE:** When 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:** 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.



**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.

# 9 Luminescence

**NOTE: Luminescence** is often used as umbrella term for all non-thermal emission types, such as fluorescence, phosphorescence, bio- and chemi-luminescence, etc.

At Tecan, however, the term **luminescence** is only used for emission types occurring without excitation.

## 9.1 Measurement Techniques

The following measurement techniques are available with the SPARK instrument:

- Glow Luminescence
- Flash Luminescence
- Multicolor Luminescence
- Luminescence Scan

The standard luminescence module enables the integral measurement of a luminescence signal, without distinguishing between emission wavelengths. The standard luminescence module can be used with all microplate formats up to 384 wells.

The enhanced luminescence module has the ability of performing all available multicolor applications, as well as fast and high-sensitive luminescence scans. Additionally, it can measure luminescence signals without wavelength discrimination and to attenuate strong signals like the standard luminescence module. The luminescence enhanced module can be used with all microplate formats supported by the instrument.

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.

For further details we refer to the Reference Guide.

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

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

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

**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 points 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 the user.

**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.

## 9.2 Luminescence Specifications

**NOTE:** All specifications are subject to change without prior notification.

### 9.2.1 General Specifications

| Parameters | Standard Luminescence Module | Enhanced Luminescence Module |
|---|---|---|
| Wavelength range | 370-700 nm | 370-700 nm |
| Wavelength range Luminescence scan | n.a. | 390-660 nm |
| Wavelength discrimination and multicolor luminescence | n.a. | via filter sets |
| Integration time/well | 10 - 60000 ms | 10 - 60000 ms |
| Attenuation | 1 OD, 2 OD | 1 OD, 2 OD, 3 OD |
| Dynamic range | $10^7$-$10^9$ | $10^7$-$10^{10}$ |

### 9.2.2 Performance Specifications

| **Glow Luminescence detection limit (Standard and Enhanced Module)** | | |
|---|---|---|
| **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| 96-well plate, white, 200 µl | Integration time/well: 1000 ms | ATP: < 50 pM (< 10 fmol/well) |
| 384-well plate, white, 100 µl | Integration time/well: 1000 ms | ATP: < 10 pM (< 1 fmol/well) |
| 1536-well plate, white, 10 µl | Integration time/well: 1000 ms | ATP: < 1 nM (< 10 fmol/well) |
| **Flash Luminescence detection limit (Standard and Enhanced Module)** | | |
| **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| 96-well plate, white, 200 µl | Integration time/well: 10000 ms | ATP: < 0.4 pM (< 80 amol/well) |
| 384-well plate, white, 100 µl | Integration time/well: 10000 ms | ATP: < 0.8 pM (< 80 amol/well) |

## 9.3 Quality Control of the Luminescence Module

### 9.3.1 Periodic Quality Control Tests

Depending on usage and application we recommend a periodic evaluation of the instrument on site at Tecan.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore, please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.




**CAUTION:** Before starting measurements, make sure that the microplate is inserted correctly. The position of well A1 must be on the upper left side.



**WARNING:** The following instructions explain how to perform the Quality Control to check the specifications of the instrument. If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

### 9.3.2 Detection Limit ATP 384-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.




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

#### Material:

- ATP Kit SL (BioThema AB, article no. 144-041)
- Greiner 384-well plate, flat bottom, white
- Pipettes and tips

#### Procedure:

Prepare reagents according to the manufacturer's instructions. Adjust ATP Standard to $10^{-7}$ M.

Pipette 100 µl of the Blank into the wells A4 – D10.

Pipette 20 µl of ATP standard $10^{-7}$ M into the wells A2 – D2, add 80 µl of ATP reagent and mix in well **(use fresh tip for each well); ATP reagent must NOT be contaminated with ATP standard!**

### Plate Layout:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | ... | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | ATP | | B | B | B | B | B | B | B | | | |
| B | | ATP | | B | B | B | B | B | B | B | | | |
| C | | ATP | | B | B | B | B | B | B | B | | | |
| D | | ATP | | B | B | B | B | B | B | B | | | |
| E | | | | | | | | | | | | | |
| ... | | | | | | | | | | | | | |
| P | | | | | | | | | | | | | |

ATP: 100µl, 2*10-8 M ATP (final concentration in well)
B: 100 µl Blank

### Measurement Parameters:

| | |
|---|---|
| Measurement mode: | Luminescence |
| Integration time: | 1000 ms |
| Plate definition file: | GRE384fw |

### Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(\text{fmol / well}) = \frac{2 \cdot 10^{-8} * 3 * SD_B}{\text{mean}_{ATP} - \text{mean}_B} * 0.0001 * \frac{1}{1e^{-15}}$$

| | |
|---|---|
| $2*10^{-8}$ | Concentration of ATP standard [M] |
| $SD_B$ | Standard deviation of Blank (B: A4 – D10) |
| $\text{mean}_{ATP}$ | Mean of wells filled with ATP standard |
| $\text{mean}_B$ | Mean of Blank wells (B: A4 – D10) |
| 0.0001 | Conversion into mol/well |
| $1/1e^{-15}$ | Conversion into fmol/well |

## 9.3.3 Detection Limit ATP 1536-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.




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

## Material:

- ATP Kit SL (BioThema AB, article no. 144-041)
- Greiner 1536-well plate, flat bottom, white
- Pipettes + tips

## Procedure:

Prepare reagents according to the manufacturer's instructions. Adjust ATP Standard to $10^{-7}$ M.

Pipette 10 µl of the Blank into the wells A4 – D10.

Pipette 2 µl of ATP standard $10^{-7}$ M into the wells A2 – D2, add 8 µl of ATP reagent and mix in well (use fresh tip for each well); ATP reagent must NOT be contaminated with ATP standard!

## Plate Layout:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | ... | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | ATP | | B | B | B | B | B | B | B | | | |
| B | | ATP | | B | B | B | B | B | B | B | | | |
| C | | ATP | | B | B | B | B | B | B | B | | | |
| D | | ATP | | B | B | B | B | B | B | B | | | |
| E | | | | | | | | | | | | | |
| ... | | | | | | | | | | | | | |
| P | | | | | | | | | | | | | |

ATP: 10µl, 2*10-8 M ATP (final concentration in well)
B: 10 µl Blank

## Measurement Parameters:

| | |
|---|---|
| Measurement mode: | Luminescence |
| Integration time: | 1000 ms |
| Plate definition file: | GRE1536fw |

## Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(\text{fmol / well}) = \frac{2 \cdot 10^{-8} * 3 * SD_B}{\text{mean}_{ATP} - \text{mean}_B} * 0.00001 * \frac{1}{1e^{-15}}$$

| | |
|---|---|
| $2*10^{-8}$ | Concentration of ATP standard [M] |
| $SD_B$ | Standard deviation of Blank (B: A4 – D10) |
| $\text{mean}_{ATP}$ | Mean of wells filled with ATP standard |
| $\text{mean}_B$ | Mean of Blank wells (B: A4 – D10) |
| 0.0001 | Conversion into mol/well |
| $1/1e^{-15}$ | Conversion into fmol/well |

# 10 Alpha Technology

## 10.1 Basic Principles

Amplified Luminescent Proximity Homogeneous Assays (AlphaScreen and AlphaLISA) are bead-based nonradioactive, homogeneous, and sensitive assays perfectly suited for the study of biochemical interactions. The interaction between an acceptor and donor bead leads to the light output: Upon illumination with a high-energy light source, the photosensitive molecules contained in the donor beads produce high level of oxyradicals. These oxyradicals travel to the acceptor beads and trigger a cascade of reactions that ultimately lead to the generation of a strong chemiluminescent signal.

By using multiple acceptor beads which emit at different wavelengths, multiple analytes can be detected in one well (AlphaPlex).

## 10.2 Alpha Module

The Alpha module is used for detection of assays based on the Alpha technology (AlphaScreen, AlphaLISA and AlphaPlex). The Alpha module consists mainly of luminescence enhanced and laser module coupled with a contactless IR temperature sensor.

### 10.2.1 Filter

Predefined filters for Alpha based applications are available. Each band pass filter is generated by combination of a long pass and short pass filter built into the filter wheels of the luminescence enhanced module. The following table shows the wavelength characteristic of the predefined band pass filter:

| Alpha Technology | Filter Choice | Central Wavelength / Bandwidth |
|---|---|---|
| AlphaScreen | Long pass filter: 520 nm,<br>Short pass filter: 620 nm | 570 nm/100 nm |
| AlphaLISA | Long pass filter: 610 nm,<br>Short pass filter: 635 nm | 622.5 nm/25 nm |
| AlphaPlex | Long pass filter: 610 nm,<br>Short pass filter: 635 nm<br>Long pass filter: 535 nm,<br>Short pass filter: 560 nm | 622.5 nm/25 nm<br>547.5 nm/25 nm |

### 10.2.2 Optics

As excitation light source for Alpha based assays a high-power laser [1] is used. The luminescence fiber [2] guides the light from the sample to the detector passing the filter wheels [4]. Long and short pass filters are installed on the filter wheels. Appropriate filter combinations result in dedicated band pass filters. The aperture wheel [3] adapts the light beam diameter to the used well size.

The Alpha module can be used with all microplate formats supported by the instrument.

Low light levels benefit from the single photon counting detector [5].

The Alpha module is combined with an IR-temperature sensor [6] to compensate for temperature-caused signal differences in every microplate well.

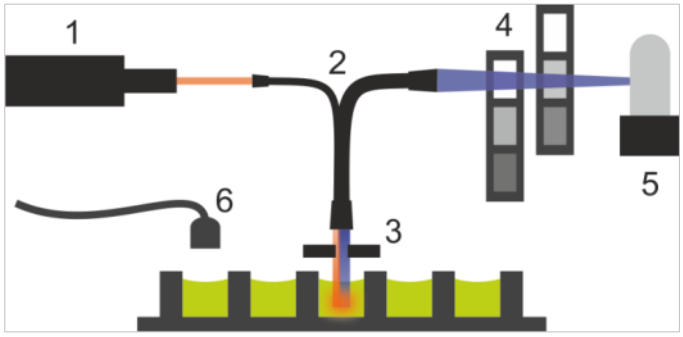
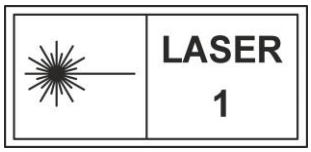


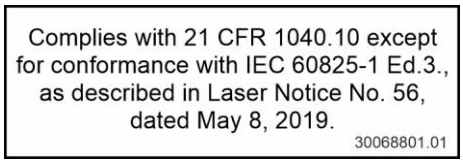

Figure 6: Optical system in Alpha module:
[1] laser module; [2] luminescence fiber; [3] aperture wheel; [4] filter wheels; [5] detection unit; [6] IR-temperature sensor

### 10.2.3 Laser

The laser module uses a high-power laser (680 nm/750 mW) as the excitation light source. A SPARK instrument equipped with an Alpha module is a LASER CLASS 1 product. The instrument complies with FDA radiation performance standards 21 CFR 1040.10 except for conformance with IEC 60825-1 Ed.3, as described in Laser Notice No. 56, dated May 8, 2019.

The following labels are attached to the rear of the instrument:






**WARNING:** Laser radiation Class IV inside the instrument - Keep the instrument lid closed during measurement.

### 10.2.4 Detection




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

The luminescence and Alpha module detection system utilizes the single photon counting measurement technique. This is based on a dedicated luminescence detector with appropriate measurement circuitry. This technique is very robust against noise, and is, therefore, the preferred method for performing measurements at very low light levels.




**CAUTION:** Use white or light grey plates for Alpha Technology based measurements. Never use black plates and don't measure empty wells to avoid damages caused by laser radiation.

### 10.2.5 Temperature Correction

To compensate for the temperature sensitive nature of Alpha based assays, the Alpha module offers a temperature correction system.

A contactless temperature sensor measures the temperature inside each well and the measured count rates are automatically normalized to a temperature of 22.5 ° C. Temperature and signal detection is performed in parallel. Due to the position of the temperature sensor the reading direction is from right to left (A12 to A1, B12 to B1 in case of a 96-well plate) if using the temperature correction function.



**NOTE:** To ensure the best performance for Alpha Technology based assays, the SPARK should be operated in a temperature-regulated environment (±1 ° C in the range of 20–25 ° C).

## 10.3 Defining Alpha Measurements

The SparkControl software provides a strip for measuring:

- AlphaScreen
- AlphaLISA
- AlphaPlex
- User-defined Measurements

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.

For further details we refer to the Reference Guide and the SparkControl manual.

## 10.4 Optimizing Alpha Technology based Measurements

### 10.4.1 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.

### 10.4.2 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.

### 10.4.3 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 magazines 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 15.1.2 Light Protection for Sensitive Assays/Dark Covers).

## 10.5 Alpha Specifications

**NOTE:** All specifications are subject to change without prior notification.

### 10.5.1 General and Performance Specifications

| Parameters | Specification |
|---|---|
| Excitation time/well | 10 - 1000 ms |
| Integration Time/well | 10 - 60000 ms |
| Predefined Filter | AlphaScreen, AlphaLISA, AlphaPlex |
| Temperature correction | available |
| Detection Limit 384-well plate, low volume (Omnibeads) | < 12.5 ng/ml |
| Uniformity 384-well plate, low volume (Omnibeads) | < 8 CV % |

## 10.6 Quality Control of the Alpha Module

### 10.6.1 Periodic Quality Control Tests

Depending on usage and application we recommend a periodic evaluation of the instrument on Tecan site.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore, please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.

We recommend adapting these tests and the acceptance criteria to the laboratory's primary application. Ideally these tests must be performed with the laboratory's own plates, fluorophore, buffers, volumes and all the appropriate settings (filters, flashes, delays, etc.).



**WARNING:** Before starting measurements, make sure that the microplate position A1 is inserted correctly. The position of well A1 must be on the upper left side.



**WARNING:** If the results of these control tests do not lie within the official specifications of the instrument, please contact your local service center for further advice.

### 10.6.2 Detection Limit AlphaScreen Omnibeads 384-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare instrument for measurement and start measurement immediately after pipetting.




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

**Material:**

- AlphaScreen Omnibeads
- Greiner 384-well plate, flat bottom, white
- Phosphate-buffered saline (PBS)
- Pipettes and tips

**Procedure:**

Dilute the Omnibeads stock solution 1:500 in PBS by adding 3 µl of the stock solution (5 mg/ml) to 1497 µl PBS yielding a solution of 10 µg/ml. Prepare 12 further dilutions in 1:2 steps by pipetting 750 µl
of the previous dilution step to 750 µl PBS. Use a new tip for each dilution step.

Pipette 100 µl of each dilution into 5 replicate wells of the microplate according to the plate layout. Use 100 µl PBS for the blank wells.

**CAUTION:** Use a fresh tip for each concentration and take care NOT to contaminate the blank with any Omnibeads dilution!

## Plate Layout:

| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | ... | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | 10.00 µg/ml | | | | | | | | | | | |
| B | | 5.00 µg/ml | | | | | | | | | | | |
| C | | 2.50 µg/ml | | | | | | | | | | | |
| D | | 1.25 µg/ml | | | | | | | | | | | |
| E | | 0.62 µg/ml | | | | | | | | | | | |
| F | | 0.31 µg/ml | | | | | | | | | | | |
| G | | 0.15 µg/ml | | | | | | | | | | | |
| H | | 0.08 µg/ml | | | | | | | | | | | |
| I | | 0.04 µg/ml | | | | | | | | | | | |
| J | | 0.02 µg/ml | | | | | | | | | | | |
| K | | 0.01 µg/ml | | | | | | | | | | | |
| L | | 0.005 µg/ml | | | | | | | | | | | |
| M | | 0.0025 µg/ml | | | | | | | | | | | |
| N | | PBS | | | | | | | | | | | |
| O | | | | | | | | | | | | | |
| P | | | | | | | | | | | | | |

100 µl of each Omnibeads concentration (5 replicate wells each)
100 µl PBS = Blank

## Measurement Parameters:

| | |
|---|---|
| Measurement mode: | AlphaScreen |
| Excitation time: | 100 ms |
| Integration time: | 300 ms |
| Temperature correction: | activated |
| Plate definition file: | GRE384fw |

## Evaluation:

Calculate the average and standard deviation for each Omnibeads concentration. Perform a blank reduction by subtracting the average signal of the blank wells from the average signal of each Omnibeads concentration.

Plot the average blank-corrected values against the final Omnibeads concentration in a XY scatter diagram. Add a linear trend line with intercept set to 0 and solve the trend line equation (y=kx) using the 3-fold standard deviation of the blank as y.

$$x = \frac{y}{k}$$

**y** = 3*standard deviation of the blank

Extrapolate the detection limit [ng/ml] by using the 3-fold standard deviation of the blank as y.

## 10.6.3 Uniformity AlphaScreen Omnibeads 384-Well Plate

The uniformity defines the well-to-well variations when measuring a multi-well plate. The uniformity is calculated as percentage deviation from the mean value.

Before pipetting the plate, prepare instrument for measurement and start measurement immediately after pipetting.




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

### Material:

- AlphaScreen Omnibeads
- Greiner 384-well plate, flat bottom, white
- Phosphate-buffered saline (PBS)
- Pipettes + tips

### Procedure:

Dilute the Omnibeads stock solution 1:2000 in PBS by adding 3 µl of the stock solution (5 mg/ml) to 5997 µl PBS yielding a solution of 2.5 µg/ml.

Pipette 100 µl of the Omnibeads dilution into the wells of the microplate according to the plate layout.

### Plate Layout:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | |
| B | | | | | | | | | | | | | | | | | | | | | | | | |
| C | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | |
| D | | | | | | | | | | | | | | | | | | | | | | | | |
| E | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | |
| F | | | | | | | | | | | | | | | | | | | | | | | | |
| G | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | |
| H | | | | | | | | | | | | | | | | | | | | | | | | |
| I | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | |
| J | | | | | | | | | | | | | | | | | | | | | | | | |
| K | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | |
| L | | | | | | | | | | | | | | | | | | | | | | | | |
| M | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | |
| N | | | | | | | | | | | | | | | | | | | | | | | | |
| O | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | | O | |
| P | | | | | | | | | | | | | | | | | | | | | | | | |

O: 100 µl/well Omnibeads dilution (2.5 µg/ml)

### Measurement Parameters:

| | |
|---|---|
| Measurement mode: | AlphaScreen |
| Excitation time: | 100 ms |
| Integration time: | 300 ms |
| Temperature correction: | activated |
| Plate definition file: | GRE384fw |

### Evaluation:

Calculate the Uniformity as follows:

$$\text{Uniformity} \quad (\text{CV}\%) = \frac{\text{SD}_O * 100}{\text{mean}_O}$$

| | |
|---|---|
| **$SD_o$** | Standard deviation of wells filled with 2.5 µg/ml Omnibeads solution |
| **$mean_o$** | Mean of wells filled with 2.5 µg/ml Omnibeads |

# 11 Absorbance

## 11.1 Absorbance Measurement Techniques

### 11.1.1 Absorbance

Absorbance signal is a measure for the attenuation of monochromatic light when transmitted through a sample.

### 11.1.2 Absorbance Scan

Absorbance scans measure the absorbance behavior of compounds under investigation within a selected wavelength range.

## 11.2 Cuvette Module

Cuvette based applications can be performed at any wavelength from 200 to 1000 nm. The optical path of the cuvette module is similar to the optical path of the absorbance standard module. A fiber bundle guides the light from the monochromator to the absorbance optics, which focuses the light into the cuvette. The transmitted light is detected by a photodiode.

### 11.2.1 Cuvette Optics

The absorbance cuvette module consists of the flash lamp, the monochromator, the absorbance fiber, and the photodiode (figure).

The light of the Xenon-flash lamp [1] (light source) passes an order sorting filter [2] and is focused onto the entrance slit of a single grating monochromator [3] by a condenser mirror. By moving the optical grating, the measurement wavelength is selected and focused onto the exit slit of the monochromator. There the light enters the absorbance fiber [4] which guides the light onto the sample in the cuvette [5]. A part of the light is reflected onto to the reference photodiode. The transmitted light is detected by the measurement photodiode [6]. At the focal point the spot diameter of the absorbance cuvette light beam is about 1 mm.

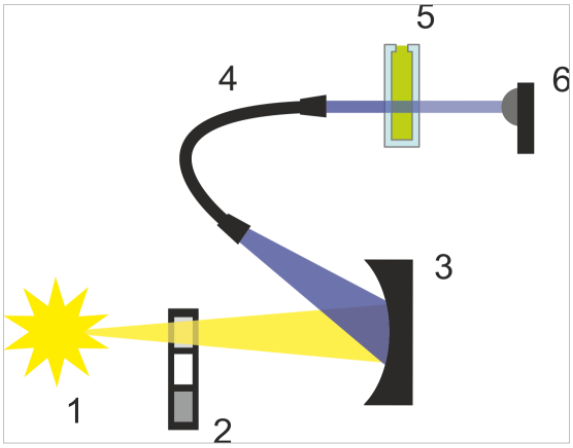



Figure 7: Optical system of the absorbance cuvette module
Xenon-flash lamp [1] (light source), order sorting filter [2], optical grating [3], absorbance fiber [4], cuvette [5], measurement photodiode [6]

### Detection

A silicon photodiode is used for the measurement of the transmitted light. It is sensitive to a wide range of wavelengths. The photodiode is well suited for the light levels of absorbance measurements below 4 OD.

## 11.3 Measurement Equipment

### 11.3.1 Microplates

Generally, for absorbance measurements, transparent or UV-transparent microplates are used. For high OD values, black microplates with transparent bottoms are superior. In general, to obtain accurate values measurements above OD3 are not recommended, especially when using 1536-well plates. Dilutions of the measurement samples will result in more accurate data.




**CAUTION:** Use UV compatible microplates for absorbance measurements in UV wavelength range.



**NOTE:** For absorbance measurements of nucleic acids in small volumes (2 µl) use Tecan's NanoQuant Plate. With this device it is possible to measure 16 different samples in one measurement.



**NOTE:** To obtain more accurate measurement data avoid OD values above 3.

### 11.3.2 Cuvette Adapter

The Tecan cuvette adapter can be used to measure four cuvettes in one measurement. For suitable cuvette dimensions, please refer to the table below. When using the cuvette adapter, the cuvette must be inserted horizontally and closed tightly to avoid any liquid leakage. Additionally, the cuvette must be filled with the maximum filling volume to prevent the formation of air bubbles at the measurement window.

The cuvette adapter was created to perform measurements with cuvettes that comply with the following dimensions (table):

| Dimension | Parameters |
|---|---|
| Absolute height (including lid) | 35 - 55 mm |
| Footprint (outer dimension) | 12.5 x 12.5 mm |
| Optical path | 10 mm* |

* If using a cuvette with different optical path measurement results must be corrected accordingly.




**CAUTION:** When performing measurements with the cuvette adapter, always use the maximum filling volume of the cuvette to prevent the formation of air bubbles at the measurement window. Close the cuvette tightly to avoid any liquid leakage.

### 11.3.3 Cuvette Port

Instead of in a microplate, absorbance measurement can be performed in a cuvette that is inserted into the cuvette port of the instrument. The cuvette port was created to perform measurements with cuvettes that comply with the following dimensions (table):

| Dimension | Parameter |
|---|---|
| Absolute height (including lid) | 35 - 55 mm |
| Footprint (outer dimension) | 12.5 x 12.5 mm |
| Optical path | 10 mm* |
| Central height | 15 mm |
| Measurement window | > 2 x 2 mm |

* If using a cuvette with different optical path measurement results must be corrected accordingly.




**CAUTION:** Always use a valid filling volume. Make sure that the liquid level in the cuvette exceeds 20 mm (height). Too low liquid levels lead to wrong measurement results.




**CAUTION:** The cuvette port has a measurement window of 2 x 2 mm and a central height of 15 mm.




**CAUTION:** The cuvette must be inserted into the carrier so that the measurement window of the cuvette lines up with the measurement window of the cuvette carrier. For correct insertion please follow the arrow on the cuvette port.




**CAUTION:** Close the cuvette port properly when it is not used. Contaminations lead to wrong measurement results.




**CAUTION:** Ensure that the cuvette is inserted properly into the cuvette port before starting a cuvette measurement. Misalignments lead to wrong measurement results.



**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. For further details we refer to the SparkControl manual.



**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.



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

## 11.4 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.

### Pathlength Correction:

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

For further details we refer to the SparkControl manual.



**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. The pathlength correction with cuvette will not compensate for this effect.



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

## 11.5 NanoQuant Application

Tecan provides a ready-to-use NanoQuant app for:

- Quantifying nucleic acids
- Labeling efficiency of nucleic acids
- Quantifying proteins

When using the app, the calculations of the nucleic acid, protein, and dye contents as well as the purity checks are performed automatically.

For details, see the chapter NanoQuant App in the SparkControl manual.

## 11.6 Absorbance Specifications

**NOTE:** All specifications are subject to change without prior notification.

### 11.6.1 General Specifications

| Parameters | Characteristics |
|---|---|
| Wavelength range | 200 - 1000 nm, selectable in 1 nm steps |
| Wavelength accuracy | ≤ 0.8 nm |
| Wavelength reproducibility | ≤ 0.5 nm |
| Bandwidth fixed wavelength | 3.5 nm |
| Measurement range | 0 - 4 OD |

### 11.6.2 Performance Specifications in Microplates

| Plate Type/ Filling Volume | Parameter | Specification | Criteria |
|---|---|---|---|
| 96-well plate, transparent, 200 µl | Flashes/well: 25 | Accuracy 0–0.8 OD | +/- 0.008 OD |
| 96-well plate, transparent, 200 µl | Flashes/well: 25 | Accuracy 0.8–2.5 OD | < +/- 1.0 % |
| 96-well plate, transparent, 200 µl | Flashes/well: 25 | Accuracy 2.5–3.0 OD | < +/- 1.5 % |
| 96-well plate, transparent, 200 µl | Flashes/well: 25 | Precision 0–1.2 OD | < +/- 0.006 OD |
| 96-well plate, transparent, 200 µl | Flashes/well: 25 | Precision 1.2–3.0 OD | < +/- 0.5 % |
| 96-well plate, UV transparent, 200 µl | Flashes/well: 25 | Linearity 0–3 OD at 260 nm | R2 > 0.999 |
| 96-well plate, transparent, 200 µl | Flashes/well: 25 | Uniformity at 1 OD | < 3 % |

### 11.6.3 Measurement Times

| Parameters | Measurement time |
|---|---|
| Measurement time 96-well, 1 flash | < 14 seconds |
| Measurement time 384-well, 1 flash | < 30 seconds |
| Fast Scan (200-1000 nm, 1 nm steps) | < 5 seconds |

Fast reading times are determined by using one flash only, plate-in and plate-out movements are not included in the measurement time.

### 11.6.4 Performance Specifications in Cuvettes (Cuvette port)

| Cuvette Type | Parameter | Specification | Criteria |
|---|---|---|---|
| Quartz cuvette, 1 cm light path | Flashes: 25<br>Wavelength: 260 nm | Detection limit (DNA) | < 0.2 ng/µl dsDNA |
| Quartz cuvette, 1 cm light path | Flashes: 25<br>Wavelength: 280 nm | Detection limit (Protein: BSA, IgG, Lysozyme) | < 0.1 mg/ml |
| Quartz cuvette, 1 cm light path | Flashes: 1 | Fast Scan (200-1000 nm, 1 nm steps) | < 5 seconds |

# 11.7 Quality Control of the Absorbance Module

## 11.7.1 Periodic Quality Control Tests

Depending on usage and application, we recommend a periodic evaluation of the instrument on Tecan site.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore, please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.




**CAUTION:** Before starting measurements, make sure that the microplate is inserted correctly. The position of well A1 must be on the upper left side.



**WARNING:** If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

## 11.7.2 Uniformity 96-Well Plate

Uniformity is a measure for the well-to-well variations when measuring a multi-well-plate. The uniformity is calculated as percentage deviation from the mean value.

### Material:

- Orange G [60 mg/l] diluted in distilled water (Sigma-Aldrich, O3756)
- Greiner 96-well plate, flat bottom, transparent
- Pipette and tips

### Procedure:

Pipette 200µl of the reagent into the wells of a Greiner 96-well plate (flat bottom, transparent) according to the plate layout.

### Plate Layout:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG |
| B | | | | | | | | | | | | |
| C | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG |
| D | | | | | | | | | | | | |
| E | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG |
| F | | | | | | | | | | | | |
| G | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG | OG |
| H | | | | | | | | | | | | |

OG: Orange G [60 mg/l]

### Measurement Parameters:

| | |
|---|---|
| Measurement mode: | Absorbance |
| Measurement wavelength: | 492 nm |
| Number of flashes: | 25 |
| Settle time: | 300 ms |
| Plate definition file: | GRE96ft |

### Evaluation:

Calculate the Uniformity (CV %) as follows:

$$\text{Uniformity} \quad (\text{CV}\%) = \frac{SD_{OG} * 100}{\text{mean}_{OG}}$$

| | |
|---|---|
| $SD_{OG}$ | Standard deviation of wells filled with OG |
| $\text{mean}_{OG}$ | Mean of wells filled with OG |

## 11.7.3 Quality Control of the NanoQuant Plate

### Material:

- Tris-EDTA buffer (BioThema, no. 21-103)
- Tecan NanoQuant Plate
- Pipette + tips

### Procedure:

Pipette 2 µl of the reagent onto all positions of the NanoQuant Plate.

### Measurement Parameters:

Start the NanoQuant application and perform average blanking procedure over all wells (16 positions).

### Evaluation:

The test is passed if the average blanking results at OD 260 are in the range of 10 % (CV). If average blanking is out of range, the failed wells are highlighted indicating that these wells are dirty due to lint, fingerprints, etc.

# 12 Fluorescence

## 12.1 Fluorescence Intensity Module

The fluorescence 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 and therefore provide a detection system with the maximum flexibility and maximum signal output. Furthermore, the fluorescence signals can be read from top and bottom.

### 12.1.1 Fluorescence Bottom Module Options

The SPARK can either be equipped with the Fluorescence Standard Module or the Fluorescence Enhanced Module. In general, the Enhanced Module is more sensitive than the Standard Module.

The Fluorescence Bottom Standard Module can either be equipped with a VIS- or a UV-VIS fiber. The Fluorescence Bottom Enhanced Module is equipped with a UV-VIS fiber by default.

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

## 12.2 Measurement Equipment

### 12.2.1 Filters

The optical filters (band pass filters) are mounted in the filter slides. The spectral transmission and the bandwidth of the fluorescence filters are optimized for achieving excellent sensitivity.

Contact Tecan for filters other than those supplied on the delivered filter slides.

### 12.2.2 Filter Slides

Two separate filter slides, an excitation and an emission filter slide, enable the user to work with six independent filter pairs for fluorescence measurements. The information about the inserted filters is saved on the microchip integrated into each filter slide.




**CAUTION:** There are two types of filters available. It is important that light travels through the filter in the correct direction. Before inserting a new filter, carefully consider the orientation of the filter and the direction of light through the filter slide.

For filters with an arrow on the side, the light must travel in the same direction as the arrow.



For filters without an arrow, the end with the lip must face away from the light source:

Filters have two different ends – one has a lip, and one doesn't have a lip.

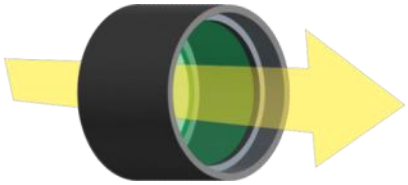
| End with lip | End without lip |
|---|---|
|  | |
| Direction of light through the filter: | |
|  | |

Figure 8: The light travels from the end without the lip towards the end with the lip.

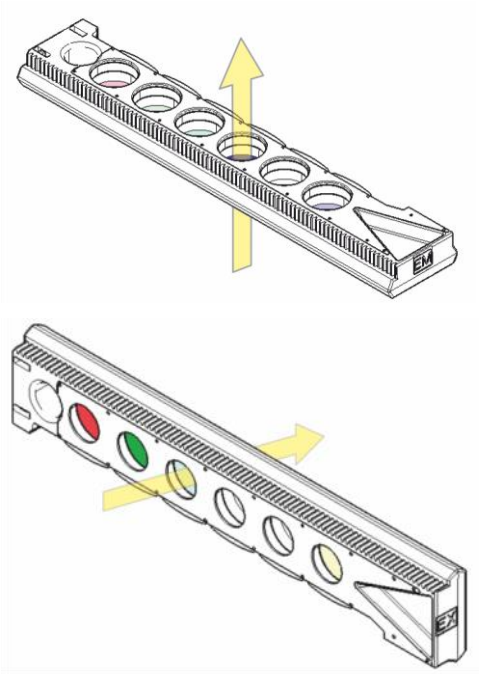


Figure 9: Direction of light through the filter slides

### 12.2.3 Installing and Removing Filters

No special tool is necessary to install or remove the filters of the excitation or emission filter slide.

To install a filter simply push the button next to the appropriate filter slot, insert the filter and release the button to secure the filter in the slot. Check that the filter is positioned firmly on the bottom of the filter slot.

**NOTE:** Make sure that the filters are inserted in the correct direction.

**CAUTION:** The filters are precision optical components, which should be handled by the edges and not scratched or stored face down in a drawer. Once the filters are installed in the slide, they are relatively well protected, but care should be exercised when handling or storing them.

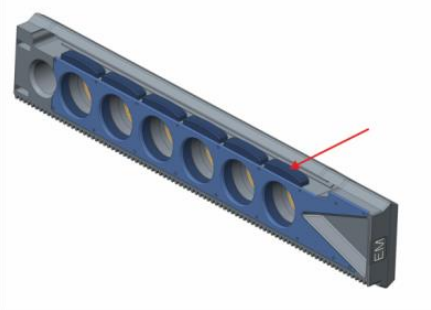



Figure 10: Remove the filter by pushing the button next to the appropriate filter slot (see picture above), turn the filter slide over and the filter will slide out of the slot.

### 12.2.4 Inserting Filter Slides

To insert the filter slides, open the door flap manually. For the ease of identification, the excitation and emission filter slides are labeled differently. Move the filter slides gently into the respective slots as indicated (chip side first) and push until the drive retract them automatically.

**CAUTION:** Do not push a filter slide further into the instrument when the drive has started to retract it.

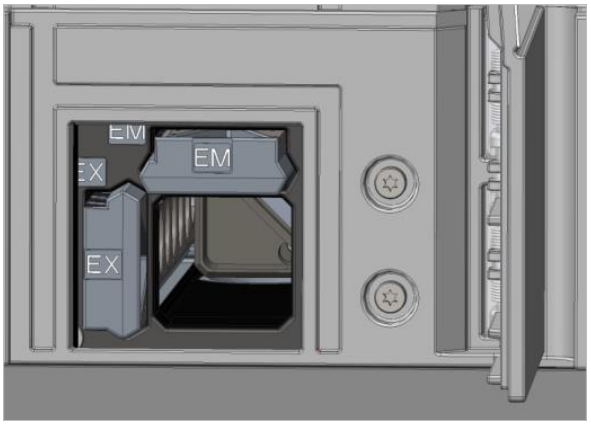



Figure 11: Inserting filter slides

Eject the filter slides via the software or by using the Onboard control button on the front of the instrument (refer to 2.6 Onboard Control Buttons).

### 12.2.5 Defining the Filters




**CAUTION:** Any changes to the filters in the filter slide are to be carried out by trained personnel! The instrument can recognize predefined filter slides and you should not attempt to change the filter values.

However, if the filters in the filter slide have been changed or if a new undefined customized filter slide is to be used, the filter slides need to be defined.

A custom filter can be defined via the **Filter Definition window** in the Dashboard or Method Editor.

For further details we refer to the Reference Guide.



**NOTE:** Alphanumeric Latin characters are allowed as well as defined special characters, including blank, ?, $, %, ., /.




**CAUTION:** It is recommended to manually document the last flash counter number before replacing a filter. Otherwise, this information will be lost.

### 12.2.6 Mirror Slides

Mirrors are used for all Fluorescence Top measurements to reflect the excitation light onto the samples. In case of the Fluorescence Top Standard module the mirror slide is equipped with two different mirror types, in case of the Fluorescence Top Enhanced module five mirror positions are available (one custom dichroic option).

For the performance characteristics of the different mirrors and their availability for the Standard or Enhanced module refer to the following table: The 50 % mirror can be used for all fluorescence measurements independent of the selected wavelength.

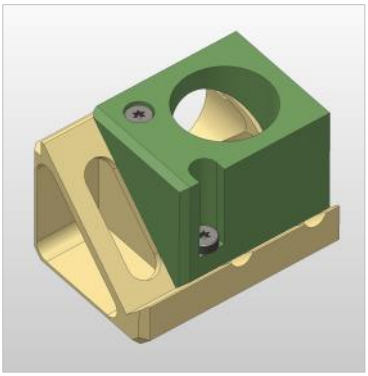
| Mirror | Reflection (Excitation) | Transmission (Emission) | Availability |
|---|---|---|---|
| 50 % Mirror | 230-900 nm | 230-900 nm | FI Top Standard and Enhanced |
| 510 Dichroic (e.g., Fluorescein, HTRF) | 320-490 nm | 515-750 nm | FI Top Standard and Enhanced |
| 560 Dichroic (e.g., Cy3) | 510-545 nm | 575-620 nm | FI Top Enhanced |
| 625 Dichroic (e.g., Cy5) | 565-610 nm | 640-700 nm | FI Top Enhanced |
| Custom Dichroic 410 | 360-395 nm | 425-470 nm | FI Top Enhanced |
| Custom Dichroic 430 | 380-415 nm | 445-490 nm | FI Top Enhanced |
| Custom Dichroic 458 | 350-450 nm | 470-900 nm | FI Top Enhanced |
| Custom Dichroic 593 | 350-585 nm | 605-900 nm | FI Top Enhanced |

| Mirror | Reflection (Excitation) | Transmission (Emission) | Availability |
|---|---|---|---|
| Custom Dichroic 660 | 350-650 nm | 670-900 nm | FI Top Enhanced |
| Custom Dichroic Mirror (Polka Dot Beam Splitter)<br><br>Beam Splitting Ratio: 50 % ± 5 % | 250-900 nm | 250-900 nm | FI Top Enhanced |

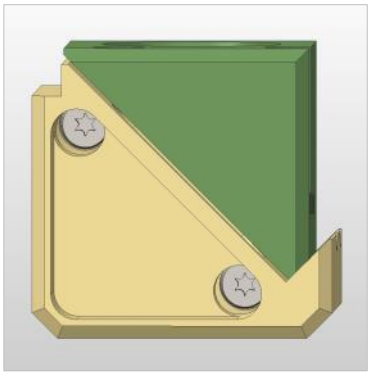
**NOTE:** A dichroic mirror needs to match the selected fluorescence excitation and emission wavelength.

### 12.2.7 Installing the Custom Dichroic Mirror

If desired, the mirror slide can be extended with a custom type dichroic mirror. The custom dichroic mirror is delivered separately with the subpackaging and needs to be installed and defined before usage.



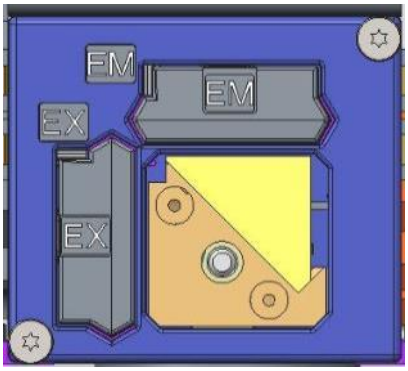
Inclined view



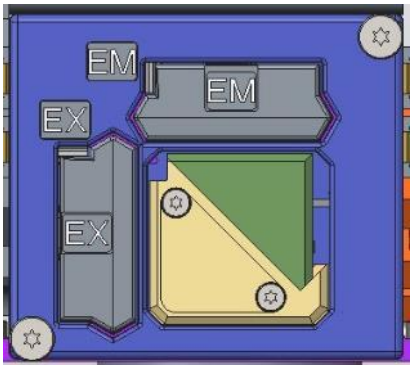
Front view

To install the custom dichroic mirror, follow these instructions:

1. Open the Mirror Definition window in the Dashboard or Method Editor and select **Mirror Out**. The mirror slide moves to the load position.
2. To install the Custom Dichroic Mirror, open the door flap manually. Slide the custom dichroic into the Mirror Carriage as indicated in the figure below. Apply and carefully tighten the mounting screws.




Load position




Installed custom dichroic




**CAUTION:** Do not apply too much torque to the mirror slide to avoid damages.

3. Carefully release the door flap and click **Mirror In**. The Mirror Slide moves back into the instrument.
4. The custom dichroic mirror is now ready to be defined (see chapter 12.2.8 Defining the Custom Dichroic Mirror).

### 12.2.8 Defining the Custom Dichroic Mirror

**CAUTION:** If a new dichroic is to be used, it needs to be defined in the software.

A custom dichroic can be defined via the Mirror Definition window in the Dashboard or Method Editor:

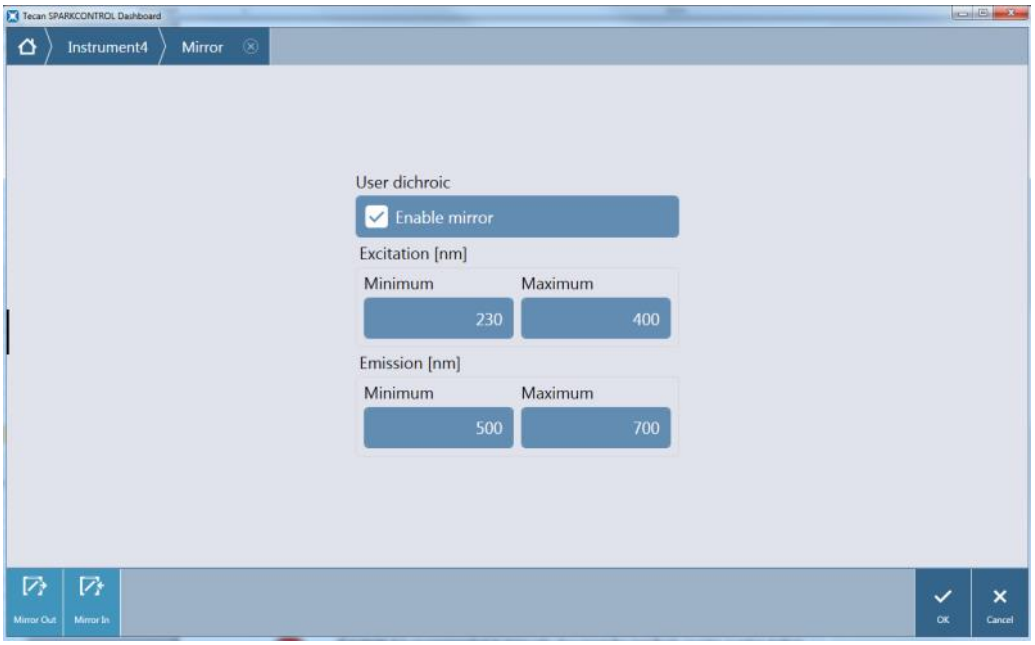



Figure 12: Mirror Definition window

Select **Enable mirror** and define the **Excitation** and the **Emission** range by entering the corresponding **Minimum** and **Maximum** wavelength.

## 12.3 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.

For further details we refer to the SparkControl manual.

**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.

**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.

## 12.4 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.

For further details we refer to the Reference Guide.



**NOTE:** By using more than one well filled with reference and reference blank, the mean values will be calculated and therefore the G-factor calibration result will be more accurate.



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



**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.
For further details we refer to the Reference Guide.

## 12.5 Optimizing Fluorescence and Fluorescence Polarization Measurements

Refer to the SparkControl manual for a detailed description.



**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).



**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.

### Scan Z-Position



**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.

## 12.6 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).

Refer to the SparkControl manual for a detailed description.



**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.



**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.



**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.



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



**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.



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

## 12.7 Fluorescence Specifications

**NOTE:** All specifications are subject to change without prior notification.

### 12.7.1 General Specifications of Fluorescence Intensity (Standard and Enhanced module)

If not otherwise stated, the specifications are valid for Standard as well as Enhanced module.

**Fluorescence Intensity Top:**

| **Parameters** | Monochromator | Filter |
|---|---|---|
| Wavelength range | Excitation: 230 – 900 nm<br>Emission: 280 – 900 nm<br>selectable in 1 nm steps | Excitation: 230 – 900 nm<br>Emission: 230 – 900 nm |
| Bandwidth Standard module | 20 nm | depends on the filter used |
| Bandwidth Enhanced module | 5, 7.5, 10, 15, 20, 25, 30, 50 nm | depends on the filter used |

**Fluorescence Intensity Bottom (Monochromator and Filter Option):**

| **Parameters** | **Standard VIS Bottom Fiber** | **UV-VIS enhanced Bottom Fiber** |
|---|---|---|
| Wavelength range | Monochromator and Filter:<br>390 – 900 nm, selectable in 1 nm steps (Monochromator only) | Monochromator:<br>Excitation: 230 – 900 nm<br>Emission: 280 – 900 nm, selectable in 1 nm steps<br>Filter:<br>Excitation: 230 – 900 nm<br>Emission: 230 – 900 nm |
| Bandwidth Standard Module - Monochromator | 20 nm | |
| Bandwidth Enhanced Module - Monochromator | 5, 7.5, 10, 15, 20, 25, 30, 50 nm | |
| Bandwidth Standard and Enhanced Module - Filter | depends on the filter used | |

**NOTE:** The UV-VIS enhanced bottom fiber is more sensitive than the standard VIS bottom fiber. Running assays below 400 nm with the standard VIS fiber leads to results with lower sensitivity.

### Gain Options

| Gain setting | Values |
|---|---|
| Manual | 1 – 255 |
| Optimal | Automatic |
| Calculated from well | Automatic |
| Extended dynamic range | Automatic |
| Use gain regulation | Automatic |

### TRF Parameters

| Parameters | Characteristics |
|---|---|
| Integration time | 20 µs – 2000 µs |
| Lag time | 0 µs – 2 ms |

### Performance Specifications of Fluorescence Intensity:

| Performance Specifications of Fluorescence Intensity Top Standard module | | | |
|---|---|---|---|
| **Module** | **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| Monochromator | 96-well plate, black, 200 µl | Flashes/well: 30 | Detection Limit: < 20 pM (1 nM Fluorescein) |
| Monochromator | 384-well plate, black, 100 µl | Flashes/well: 30 | Detection Limit: < 20 pM (1 nM Fluorescein) |
| Filter | 96-well plate, black, 200 µl | Flashes/well: 30 | Detection Limit: < 10 pM (1 nM Fluorescein) |
| Filter | 384-well plate, black, 100 µl | Flashes/well: 30 | Detection Limit: < 10 pM (1 nM Fluorescein) |
| Monochromator and filter | 96-well plate, black, 200 µl | Flashes/well: 30 | Uniformity: < 3 CV % (25 nM Fluorescein) |
| Monochromator and filter | 384-well plate, black, 100 µl | Flashes/well: 30 | Uniformity: < 5 CV % (25 nM Fluorescein) |

| Performance Specifications Fluorescence Intensity Top Enhanced module | | | |
|---|---|---|---|
| **Module** | **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| Monochromator | 384-well plate, black, 100 µl | Flashes/well: 30 | Detection Limit: < 3 pM (1 nM Fluorescein) |
| Monochromator | 1536-well plate, black, 10 µl | Flashes/well: 30 | Detection Limit: < 10 pM (1 nM Fluorescein) |
| Filter | 384-well plate, black, 100 µl | Flashes/well: 30 | Detection Limit: < 2 pM (1 nM Fluorescein) |
| Filter | 1536-well plate, black, 10 µl | Flashes/well: 30 | Detection Limit: < 7 pM (1 nM Fluorescein) |
| Monochromator and filter | 384-well plate, black, 100 µl | Flashes/well: 30 | Uniformity: < 3 CV % (25 nM Fluorescein) |
| Monochromator and filter | 1536-well plate, black, 10 µl | Flashes/well: 30 | Uniformity: < 5 CV % (100 nM Fluorescein) |

| Performance Specifications of Fluorescence Intensity Bottom Standard module | | | |
|---|---|---|---|
| **Module** | **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| Monochromator | 96-well plate, black, transparent bottom, 350 µl | Flashes/well: 30 | Detection Limit: < 45 pM (1 nM Fluorescein) |
| Monochromator | 384-well plate, black, transparent bottom, 100 µl | Flashes/well: 30 | Detection Limit: < 45 pM (1 nM Fluorescein) |
| Filter | 96-well plate, black, transparent bottom, 350 µl | Flashes/well: 30 | Detection Limit: < 35 pM (1 nM Fluorescein) |
| Filter | 384-well plate, black, transparent bottom, 100 µl | Flashes/well: 30 | Detection Limit: < 35 pM (1 nM Fluorescein) |
| Monochromator and filter | 96-well plate, black, transparent bottom, 200 µl | Flashes/well: 30 | Uniformity: < 3 CV % (25 nM Fluorescein) |
| Monochromator and filter | 384-well plate, black, transparent bottom, 100 µl | Flashes/well: 30 | Uniformity: < 5 CV % (25 nM Fluorescein) |

| Performance Specifications Fluorescence Intensity Bottom Enhanced module | | | |
|---|---|---|---|
| **Module** | **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| Monochromator | 96-well plate, black, transparent bottom, 350 µl | Flashes/well: 30 | Detection Limit: < 30 pM (1 nM Fluorescein) |
| Monochromator | 384-well plate, black, transparent bottom, 100 µl | Flashes/well: 30 | Detection Limit: < 30 pM (1 nM Fluorescein) |
| Monochromator | 1536-well plate, black, transparent bottom, 10 µl | Flashes/well: 30 | Detection Limit: < 40 pM (1 nM Fluorescein) |
| Filter | 96-well plate, black, transparent bottom, 350 µl | Flashes/well: 30 | Detection Limit: < 15 pM (1 nM Fluorescein) |
| Filter | 384-well plate, black, transparent bottom, 100 µl | Flashes/well: 30 | Detection Limit: < 17 pM (1 nM Fluorescein) |
| Filter | 1536-well plate, black, transparent bottom, 10 µl | Flashes/well: 30 | Detection Limit: < 40 pM (1 nM Fluorescein) |
| Monochromator and filter | 384-well plate, black, transparent bottom, 100 µl | Flashes/well: 30 | Uniformity: < 3 CV % (25 nM Fluorescein) |
| Monochromator and filter | 1536-well plate, black, transparent bottom, 10 µl | Flashes/well: 30 | Uniformity: < 5 CV % (100 nM Fluorescein) |

| Performance Specifications of Time Resolved Fluorescence (TRF) Standard Module | | | |
|---|---|---|---|
| **Module** | **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| Monochromator | 96-well plate, white, 200 µl | Flashes/well: 30 | Detection Limit: < 5 pM (1 nM Europium) |
| Monochromator | 384-well plate, white, 100 µl | Flashes/well: 30 | Detection Limit: < 5 pM (1 nM Europium) |
| Filter | 96-well plate, white, 200 µl | Flashes/well: 30 | Detection Limit: < 150 fM (1 nM Europium) |
| Filter | 384-well plate, white, 100 µl | Flashes/well: 30 | Detection Limit: < 150 fM (1 nM Europium) |

| Performance Specifications Time Resolved Fluorescence (TRF) Enhanced module | | | |
|---|---|---|---|
| **Module** | **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| Monochromator | 96-well plate, white, 200 µl | Flashes/well: 30 | Detection Limit:<br>< 750 fM<br>(1 nM Europium) |
| Monochromator | 384-well plate, white, 100 µl | Flashes/well: 30 | Detection Limit:<br>< 750 fM<br>(1 nM Europium) |
| Monochromator | 1536-well plate, white, 10 µl | Flashes/well: 30 | Detection Limit:<br>< 900 fM<br>(1 nM Europium) |
| Filter | 96-well plate, white, 200 µl | Flashes/well: 30 | Detection Limit:<br>< 75 fM<br>(0.1 nM Europium) |
| Filter | 384-well plate, white, 100 µl | Flashes/well: 30 | Detection Limit:<br>< 75 fM<br>(0.1 nM Europium) |
| Filter | 1536-well plate, white, 10 µl | Flashes/well: 30 | Detection Limit:<br>< 100 fM<br>(0.1 nM Europium) |

### 12.7.2 General Specifications of Fluorescence Polarization (Standard and Enhanced Polarization module)

If not otherwise stated, the specifications are valid for **Standard** as well as **Enhanced** module.

| **Parameters** | **>390 nm Fiber** | **>300 nm Polarization Fiber** |
|---|---|---|
| Wavelength range | **Monochromator and Filter:**<br>400 – 850 nm, selectable in 1 nm steps (Monochromator only) | **Monochromator and Filter:**<br>300 – 850 nm, selectable in 1 nm steps (Monochromator only) |
| Bandwidth Standard Polarization module - Monochromator | 20 nm | |
| Bandwidth Enhanced Polarization module - Monochromator | 5, 7.5, 10, 15, 20, 25, 30, 50 nm | |
| Bandwidth Standard and Enhanced Polarization module - Filter | depends on the filter used | |

## 12.7.3 Performance Specifications of Fluorescence Polarization

| **Performance Specifications Fluorescence Polarization Standard module (>300 nm and >390 nm)** | | | |
|---|---|---|---|
| **Module** | **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| Filter | 96-well plate, black, 200 µl | Flashes/well: 30 | Precision: < 5 mP (1 nM Fluorescein) |
| Filter | 384-well plate, black, 100 µl | Flashes/well: 30 | Precision: < 5 mP (1 nM Fluorescein) |

| **Performance Specifications Fluorescence Polarization Enhanced module (>300 nm and >390 nm)** | | | |
|---|---|---|---|
| **Module** | **Plate type/Filling Volume** | **Parameter** | **Criteria** |
| Filter | 96-well plate, black, 200 µl | Flashes/well: 30 | Precision: < 3 mP (1 nM Fluorescein) |
| Filter | 384-well plate, black, 100 µl | Flashes/well: 30 | Precision: < 3 mP (1 nM Fluorescein) |
| Filter | 1536-well plate, black, 10 µl | Flashes/well: 30 | Precision: < 5 mP (1 nM Fluorescein) |

### Fastest Measurement Time

Fastest measurement times are determined by using one flash only, manual gain, and manual Z-position. Plate-in and plate-out movements are not included in the measurement time.

| **Standard Module** | | |
|---|---|---|
| **Measurement Technique** | **Measurement Time** | |
| **Plate type** | **96-well** | **384-well** |
| Fluorescence intensity top filter | ≤ 13 seconds | ≤ 30 seconds |
| Fluorescence intensity top monochromator | ≤ 14 seconds | ≤ 32 seconds |
| Fluorescence intensity bottom monochromator | ≤ 21 seconds | ≤ 35 seconds |

| **Enhanced Module** | | | |
|---|---|---|---|
| **Measurement Technique** | **Measurement Time** | | |
| **Plate type** | **96-well** | **384-well** | **1536-well** |
| Fluorescence intensity top filter | ≤ 13 seconds | ≤ 22 seconds | ≤ 34 seconds |
| Fluorescence intensity top monochromator | ≤ 14 seconds | ≤ 23 seconds | ≤ 36 seconds |
| Fluorescence intensity bottom monochromator | ≤ 19 seconds | ≤ 24 seconds | ≤ 42 seconds |

## 12.8 Quality Control of the Fluorescence Module

### 12.8.1 Periodic Quality Control Tests

Depending on usage and application we recommend a periodic evaluation of the instrument on site at Tecan.

The tests described in the Reference Guide do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore, please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.

The following two chapters describe detection limits and uniformity for top/bottom measurements of 96-well plates. We refer to the Reference Guide for detection limits and uniformity of more plate types.




**CAUTION:** Before starting measurements, make sure that the microplate is inserted correctly. The position of well A1 must be on the upper left side.



**WARNING:** See the Reference Guide for a detailed description of detection limits and uniformity of different types of plates. These instructions explain how to perform the Quality Control to check the specifications of the instrument. If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

### 12.8.2 Detection Limit Top/Bottom 96-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

#### Material:

- Fluorescein, 1 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- 10 mM NaOH = Blank (NaOH pellets)
- Greiner 96-well plate, flat bottom, black (for top measurement)
- Greiner 96-well plate, flat transparent bottom, black (for bottom measurement)
- Pipettes and tips

#### Procedure:

Pipette 200 µl for top and 350 µl for bottom measurements of a 1 nM Fluorescein solution or the blank solution (10 mM NaOH) into the appropriate wells according to the plate layout.

## Plate Layout:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | F | B | F | B | F | B | F | B | F | B | F | B |
| B | F | B | F | B | F | B | F | B | F | B | F | B |
| C | F | B | F | B | F | B | F | B | F | B | F | B |
| D | F | B | F | B | F | B | F | B | F | B | F | B |
| E | F | B | F | B | F | B | F | B | F | B | F | B |
| F | F | B | F | B | F | B | F | B | F | B | F | B |
| G | F | B | F | B | F | B | F | B | F | B | F | B |
| H | F | B | F | B | F | B | F | B | F | B | F | B |

F: 200/350 µl 1 nM Fluorescein
B: 200/350 µl Blank (10 mM NaOH)

## Measurement Parameters:

| | Monochromator | Filter |
|---|---|---|
| Measurement mode | Fluorescence Top/Bottom | Fluorescence Top/Bottom |
| Excitation | 485 nm | 485 nm |
| Bandwidth excitation | 20 nm | 20 nm |
| Emission | 535 nm | 535 nm |
| Bandwidth emission | 20 nm | 25 nm |
| Flashes | 30 | 30 |
| Gain | Optimal | Optimal |
| Mirror | 510 Dichroic | 510 Dichroic |
| Z-position | Calculate from A1 | Calculate from A1 |
| Plate definition file | GRE96fb | GRE96fb |

## Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(pM) = \frac{(3 * SD_B * 1000)}{(mean_F - mean_B)}$$

| | |
|---|---|
| **$SD_B$** | Standard deviation of wells filled with Blank (10 mM NaOH) |
| **1000** | Concentration of Fluorescein in pM |
| **$mean_F$** | Mean of wells filled with 1 nM Fluorescein |
| **$mean_B$** | Mean of wells filled with Blank (10 mM NaOH) |

## 12.8.3 Uniformity Top/Bottom 96-Well Plate

The uniformity defines the well-to-well variations when measuring a multi-well plate. The uniformity is calculated as percentage deviation from the mean value.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

### Material:

- Fluorescein, 25 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- Greiner 96-well plate, flat bottom, black (for top measurement)
- Greiner 96-well plate, flat transparent bottom, black (for bottom measurement)
- Pipettes + tips

### Procedure:

Pipette 200 µl of Fluorescein solution into the appropriate wells according to the plate layout.

### Plate Layout:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | F | | F | | F | | F | | F | | F | |
| B | F | | F | | F | | F | | F | | F | |
| C | F | | F | | F | | F | | F | | F | |
| D | F | | F | | F | | F | | F | | F | |
| E | F | | F | | F | | F | | F | | F | |
| F | F | | F | | F | | F | | F | | F | |
| G | F | | F | | F | | F | | F | | F | |
| H | F | | F | | F | | F | | F | | F | |

F: 200 µl Fluorescein

## Measurement Parameters:

| | Monochromator | Filter |
|---|---|---|
| Measurement mode | Fluorescence Top/Bottom | Fluorescence Top/Bottom |
| Excitation | 485 nm | 485 nm |
| Bandwidth excitation | 20 nm | 20 nm |
| Emission | 535 nm | 535 nm |
| Bandwidth emission | 20 nm | 25 nm |
| Flashes | 30 | 30 |
| Gain | Optimal | Optimal |
| Mirror | 510 Dichroic | 510 Dichroic |
| Z-position | Calculate from A1 | Calculate from A1 |
| Plate definition file | GRE96fb | GRE96fb |

## Evaluation:

Calculate the Uniformity as follows:

$$\text{Uniformity } (CV\%) = \frac{SD_F * 100}{mean_F}$$

| | |
|---|---|
| **$SD_F$** | Standard deviation of wells filled with 25 nM Fluorescein |
| **$mean_F$** | Mean of wells filled with 25 nM Fluorescein |

# 13 Cell Module

## 13.1 Measurement Techniques

### 13.1.1 Cell Counting/Cell Viability

Tecan provides two fully automated apps for counting cells and determining cell viability in single-use cell chips. Both apps are optimized to perform a routine quality check of cell cultures on a daily basis.

### 13.1.2 Cell Confluence

Confluence indicates the size of surface covered by adherent cells. Cell confluence is displayed in percentage of the measured area. Confluence measurements can be performed in cell culture plates from 6- to 96-well format.

## 13.2 Bright Field Imaging

The cell module consists of an illumination module and a camera module. Samples are illuminated from the top and image acquisition is done from the bottom.

For further details we refer to the Reference Guide.

## 13.3 Measurements Equipment

### 13.3.1 Cell Chips

Tecan provides appropriate single-use cell chips consisting of two sample chambers each. The filling volume for one sample chamber is 10 µl and can be filled using an appropriate standard pipette. For optimal performance, avoid the formation of air bubbles in the sample chamber during the filling procedure.




**CAUTION:** Proper performance can only be guaranteed if Tecan's cell chips are used for cell counting and cell viability analysis. Avoid the formation of air bubbles when filling the sample chambers of the cell chip.




**CAUTION:** Before using the cell chips control their date of expiry. Optimal performance is not guaranteed if expiry date is exceeded.

### 13.3.2 Adapter for Cell Chips

Tecan's cell chip adapter is designed to hold up to four cell chips. The cell chips have cropped corners to prevent incorrect insertion and avoid wrong data acquisition. The slides must be inserted correctly for the adapter to close properly. The lid is automatically secured by a magnetic mechanism. The denotation (e.g., A1, A2) of the sample positions on the adapter corresponds to those presented in the software. Before starting measurements, make sure that the cell chip adapter is inserted correctly. The opening must be in front and chamber A1 must be on the upper left side.

The adapter can be cleaned by using 70 % ethanol.



**NOTE:** A cell chip adapter and 15 cell chips are included when the SPARK Cell Module (Option Cell Counting + Imaging) is ordered.

**CAUTION:** Before starting measurements, make sure that the cell chip adapter is inserted correctly. The opening must be in front and chamber A1 must be on the upper left side.

### 13.3.3 Maintenance and Cleaning of the Cell Chip Adapter

The cell chip adapter can be cleaned by applying the following procedure:

1. Wear protective gloves, protective glasses, and protective clothing.
2. Empty the cell chip adapter and carefully remove the springs installed on the inside of the adapter lid (see the Reference Guide for more details).
3. Carefully wipe all outside surfaces of the adapter and the springs with a lint-free paper towel soaked with 70 % ethanol.
4. Allow to dry.
5. Reinstall springs before using the adapter.

**CAUTION**: Do not use the cell chip adapter without springs! Measurement errors may result.

## 13.4 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.

For further details we refer to the SparkControl manual.

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., in 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 the result 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 yellow. 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.

## 13.5 Cell Counting Application

Tecan provides two ready-to-use cell counting apps for

- Cell counting
- Cell viability

When using these apps, the calculation of the cell concentration, cell size, and viability values are performed automatically.

## 13.6 Optimizing Cell Counting Measurements

### 13.6.1 Increase Number of Images

In general, cell counting, and cell viability is performed in very small volumes. Cell concentrations below $1x10^5$ cells/ml result in a low number of counted objects per image and often an irregular distribution of the cells. To improve counting rates and therefore absolute numbers of cells/ml, more than one image per sample can be taken and analyzed using the cell counting and cell viability apps. Choose between 4 and 8 images/sample.

## 13.7 Optimizing Cell Confluence Measurements

### 13.7.1 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 which include Well Border Detection take more time.

### 13.7.2 Live Viewer

The **Live Viewer** can be started from the cell confluence and cell counting strip, from the Instrument menu of the Method Editor or via the Check-and-Go window in the Dashboard 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.

For further details we refer to the SparkControl manual.




**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.



**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.

## 13.8 Cell Module Specifications

**NOTE:** All specifications are subject to change without prior notification.

### 13.8.1 General Specifications

| | |
|---|---|
| Illumination | LED |
| Image | Bright field |
| Objective | 4 x |
| Optical resolution | > 3 µm |
| Area/image | 2.2 $mm^2$ |

### 13.8.2 Specifications Cell Counting/Viability

| | |
|---|---|
| Disposable | Cell chips (Tecan brand) |
| Cell chips | 2 Sample chambers per cell chip |
| Adapter for cell chips | 4 Cell chips per adapter |
| Multiple images per sample | 1, 4, 8 |
| Cell size | 4-90 µm |
| Cell concentration | $1x10^4$-$1x10^7$ cells/ml |
| Reproducibility | < 10 % (1 Sigma), HeLa and CHO cell lines |
| Accuracy | ± 10 %, at $1x10^6$ cells/ml,<br>HeLa and CHO cell lines |

### 13.8.3 Measurement Time

Plate-in and plate-out movements as well as initialization steps are not included in the measurement time.

| Measurement Technique | Measurement Time |
|---|---|
| Cell counting/viability check | < 30 seconds/sample |
| Confluence, 96-well, whole well imaging | < 45 minutes |

# 13.9 Quality Control of the Cell Counting Module

## 13.9.1 Periodic Quality Control Tests

Depending on usage and application we recommend a periodic evaluation of the instrument on site at Tecan.

The tests described in the following chapter do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore, please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.




**CAUTION:** Before starting measurements, make sure that the Tecan Adapter for Cell Chips is inserted correctly. The position of chamber A1 must be on the upper left side.



**WARNING:** The following instructions explain how to perform the Quality Control to check the specifications of the instrument. If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

## 13.9.2 Cell Counting Accuracy

Accuracy is the ability of a system to give responses close to a true value. The accuracy is calculated as percentage deviation from the true value.

### Material:

- Cell suspension, approx. $1x10^6$ cells/ml
- Tecan Cell Chip
- Tecan Adapter for Cell Chips
- Cell counting chamber for manual counting (e.g., Neubauer chamber)
- Pipette and tips (10 µl)

### Procedure:

Adjust the cell suspension to a concentration of approximately $1x10^6$ cells/ml. Perform manual counting of cell suspension with, for example, a Neubauer chamber. Pipette 10 µl of cell suspension into the counting chambers (chamber A and B) of a Tecan Cell Chip and load the cell chip into the adapter (position 1). Start the Cell counting application.

### Measurement Parameters:

| | |
|---|---|
| Measurement | Cell counting app |
| Position | A1, B1 (define as duplicates) |
| Cell size | Depends on cell line |
| Images | 4 |

### Evaluation:

Calculate the difference between cell concentration (cells/ml) obtained from manual counting and automated counting and calculate the accuracy as follows:

$$\text{Accuracy}\,(\%) = \frac{concentration_{manual} - concentration_{automated}}{(\text{concentration}_{\text{manual}}/100)}$$

Accuracy data were collected by using HeLa and CHO cell lines. Cell lines with varying characteristics may not result in the same accuracy data.

# 14 Fluorescence Imaging (Cell Imager)

## 14.1 Bright Field Imaging

The Cell Imager offers an improved bright field illumination system, which captures a whole well of a 96-well plate in a single acquired image.

The detection of non-labeled cells, which exhibit very low optical density and are therefore barely visible, can be a troublesome issue in bright field imaging. The Cell Imager provides digital phase imaging, which produces a very high level of contrast and detail as well as an optimized sharpness. If bright field images are requested during a method, phase images are automatically generated, and the digital phase contrast is calculated by the software. Furthermore, the new astigmatism-based autofocus detection produces optimized results in less time. Samples are illuminated from the top and image acquisition is performed from the bottom.

### 14.1.1 Optics

The bright field illumination system consists of a light emitting diode (LED) (1) and two lenses (2). Homogenous illumination is achieved by acquiring the image at infinity, while high dynamic range imaging is performed simultaneously to compensate for any meniscus effects. The sample plane is imaged by a 2x, 4x or 10x microscope objective attached to a revolving objective turret (3) and is further guided via a tube lens (4) to the camera (5).

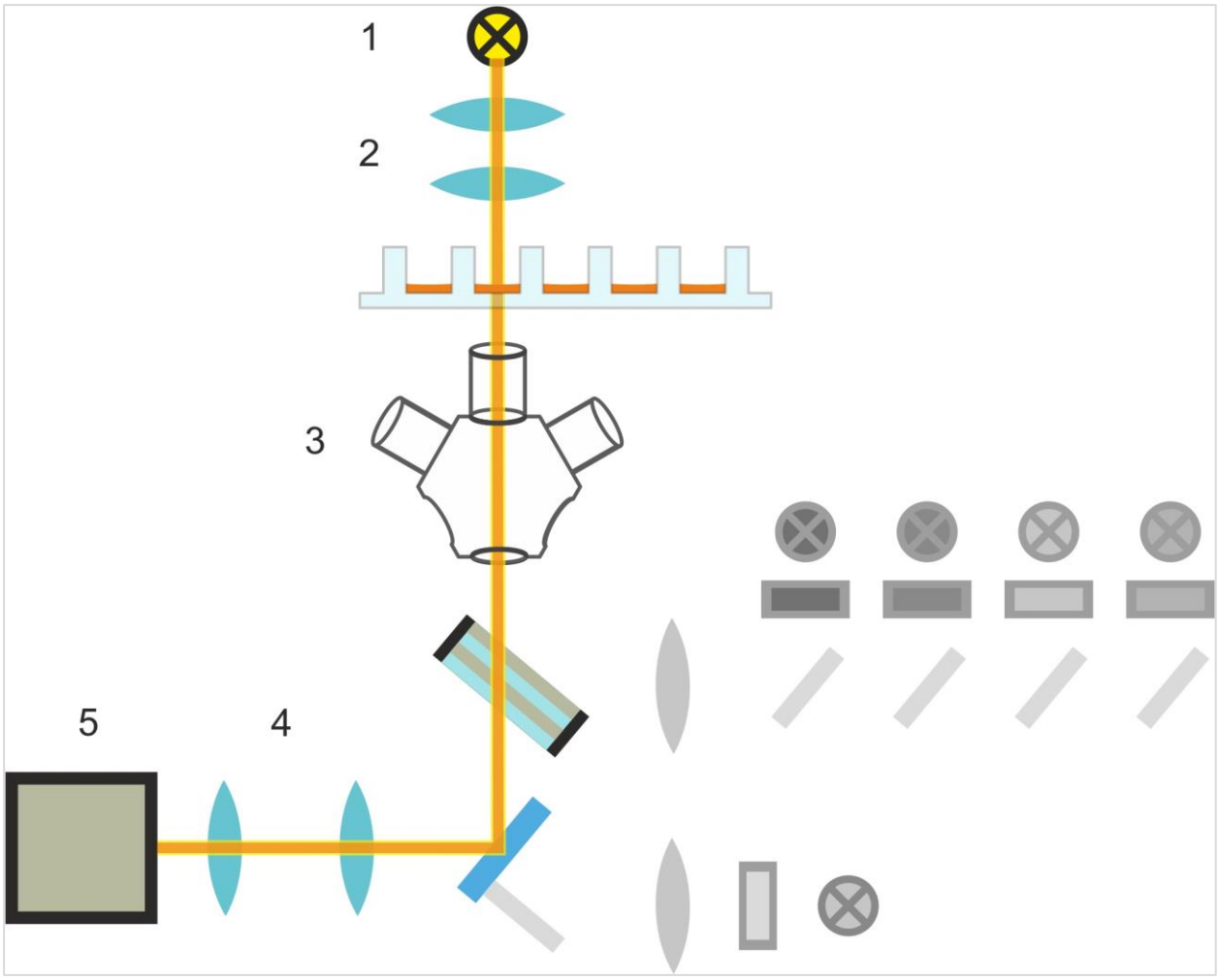



Figure 13: Schematic diagram of the bright field illumination system

### 14.1.2 Detection

An improved astigmatism-based autofocusing procedure (for a schematic diagram of the autofocusing system see figure below) allows for a stable, reliable and time efficient detection of objects in a microplate.

An LED (1) produces light, which is guided to the objective (2) and imaged onto the sample (3). The partial reflection of the autofocus light at the sample interfaces is imaged by the same objective, passes through the multiband dichroic filter (4) and is further transmitted via a tube lens (5) to the camera (6). For each measurement, a scan is performed along the optical axis to find the optimal position.

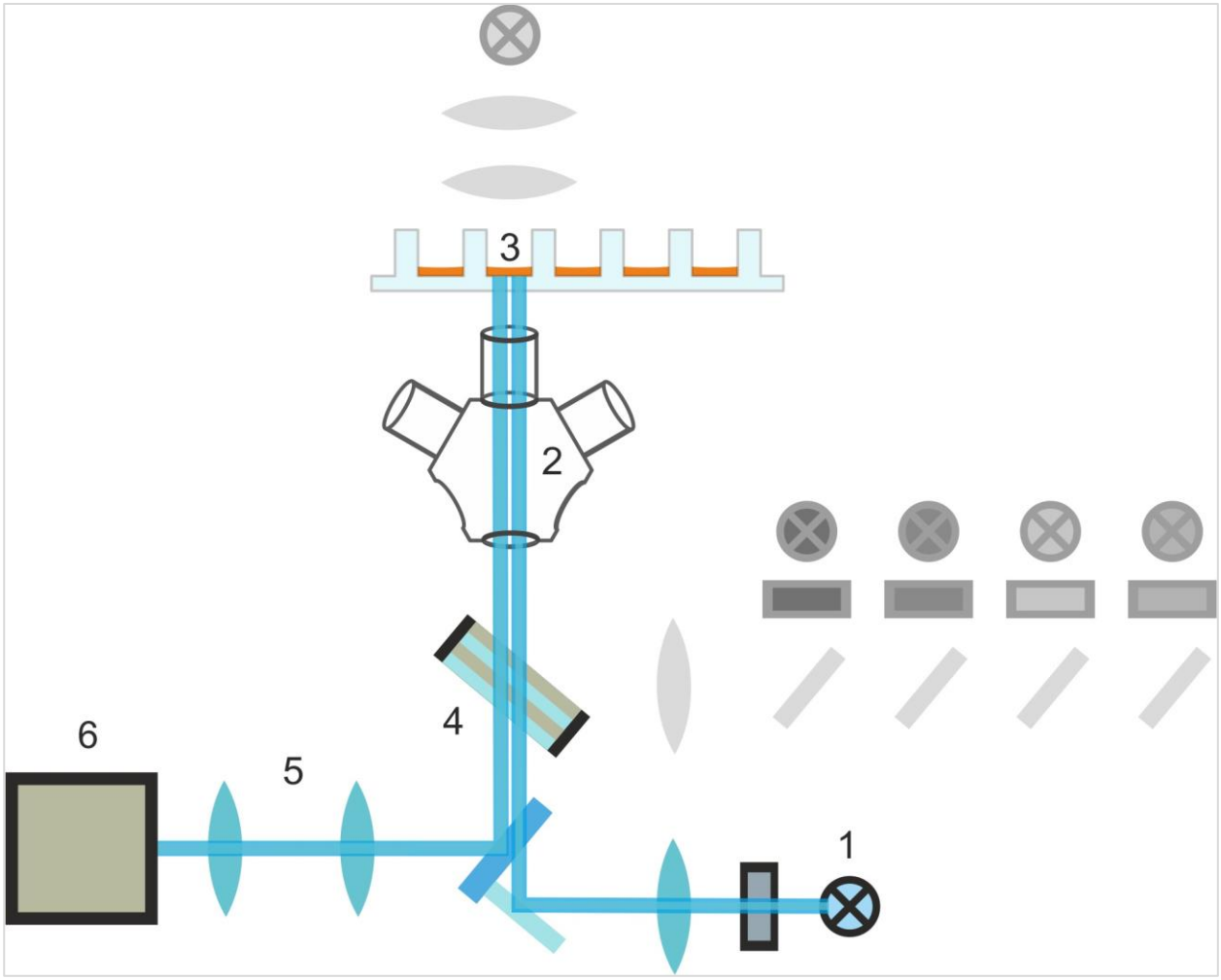



Figure 14: Schematic diagram of the auto-focusing system

### 14.1.3 Applications for Bright Field Imaging

See the Reference Guide for details.

**NOTE:** The roughness factor provides additional information about the cellular texture in a well. Changes in roughness factor are up to the user's interpretation.

## 14.2 Fluorescence Imaging

The fluorescence module utilizes four color channels, which correspond to the most commonly used dye classes DAPI/Hoechst, FITC, TIRTC and Cy5.

Owing to the Cell Imager module's innovative hardware architecture, samples are analyzed and both fluorescence and bright field images are acquired using the same astigmatism-based autofocus system, the same objectives, and the same camera. However, in contrast to the bright field module, fluorescent samples are illuminated and detected from the bottom.

### 14.2.1 Fluorescence Channels and their Excitation- and Emission-Profiles

Four different LEDs and their corresponding excitation filters may be selected in SparkControl.

The following table provides information about excitation and emission wavelengths, provided by the fluorescence module:

| **Channel** | $\lambda_{ex}$ | $\lambda_{em}$ |
|---|---|---|
| Blue | 381 - 400 nm | 414 - 450 nm |
| Green | 461 - 487 nm | 500 - 530 nm |
| Red | 543 - 566 nm | 580 - 611 nm |
| Far-red | 626 - 644 nm | 661 - 800 nm |

Exposure times and autofocus offset can be optimized by employing SparkControl's microscope mode, the Live Viewer.

### 14.2.2 Image Acquisition

If excited at the appropriate wavelength, the sample (1) emits a fluorescence signal, which is again passed through the multiband dichroic filter (2) and transmitted via the tube lens (3) to the camera (4).

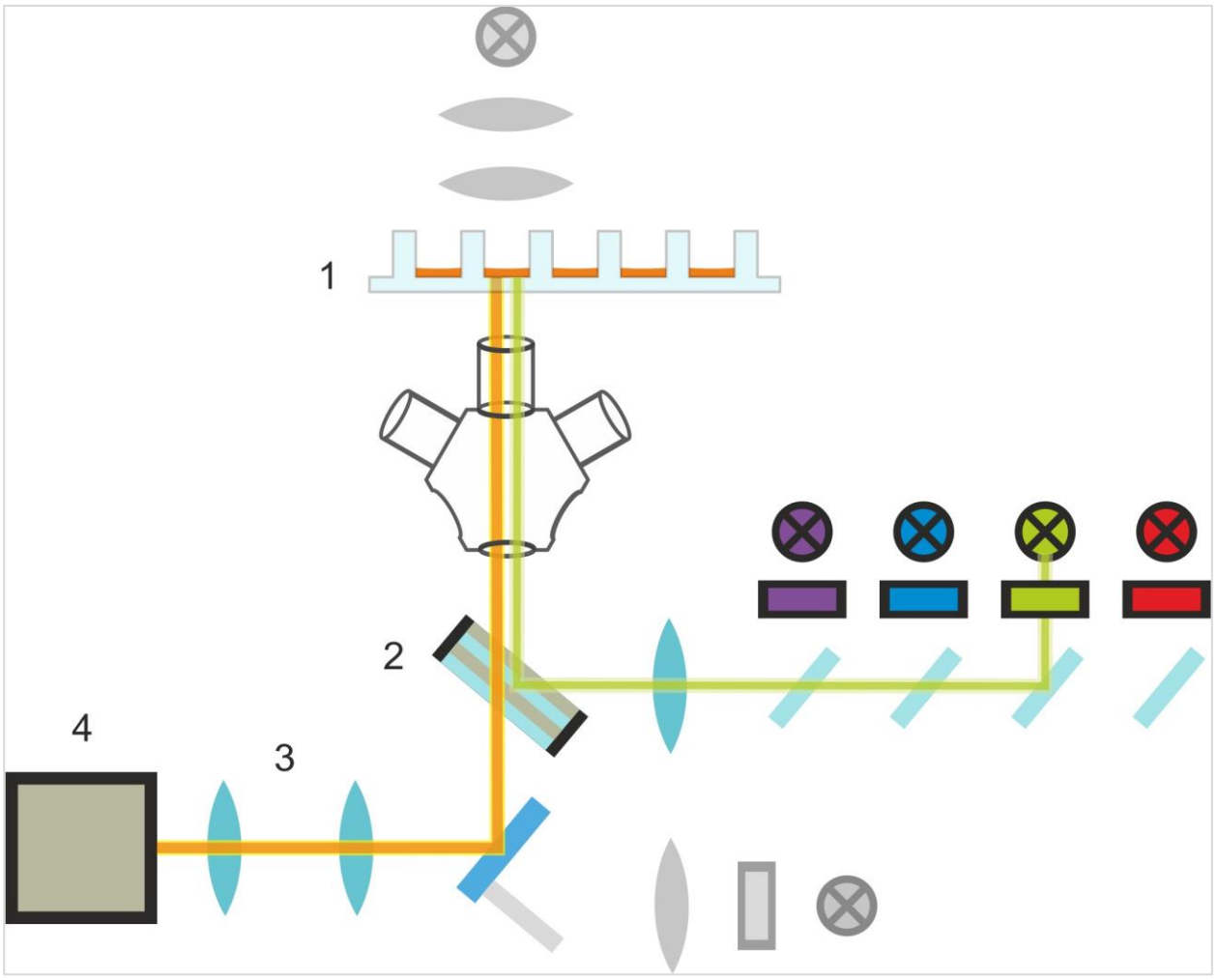



Figure 15: Schematic diagram of the fluorescence illumination system

## 14.3 Specifications for Cell Imager

### 14.3.1 General

| | |
|---|---|
| **Camera** | Sony IMX264 CMOS chip, 2456 x 2054 pixels (=5 megapixel), pixel size 3.45 µm |
| **Illumination** | Bright field LED,<br>four sets (LED + excitation filter) of different excitation- and emission wavelengths for fluorescence imaging |
| **Image** | Wide-field bright field, digital phase contrast and wide-field fluorescence |
| **Supported plate-formats** | 6-, 12-, 24-, 48-, 96- and 384-well plates |

### 14.3.2 Objectives

The following table summarizes the optical properties of the various selectable Olympus objectives:

| **Objective** | **2x** | **4x** | **10x** |
|---|---|---|---|
| Numerical aperture | 0.08 | 0.13 | 0.30 |
| Pixel resolution | 3.45 µm | 1.72 µm | 0.69 µm |
| Optical resolution | 4.50 µm | 2.77 µm | 1.20 µm |
| Field of view | 8.47 mm x 7.09 mm | 4.24 mm x 3.54 mm | 1.69 mm x 1.42 mm |

### 14.3.3 Full-multiband Filter Set

A Semrock full-multiband filter set, consisting of a full-multiband dichroic (FF409/493/573/652-Di01) and a specific emission filter set (FF01-432/515/595/730-25) is ideal for use with Hoechst, FITC, GFP, TRITC and Cy5.

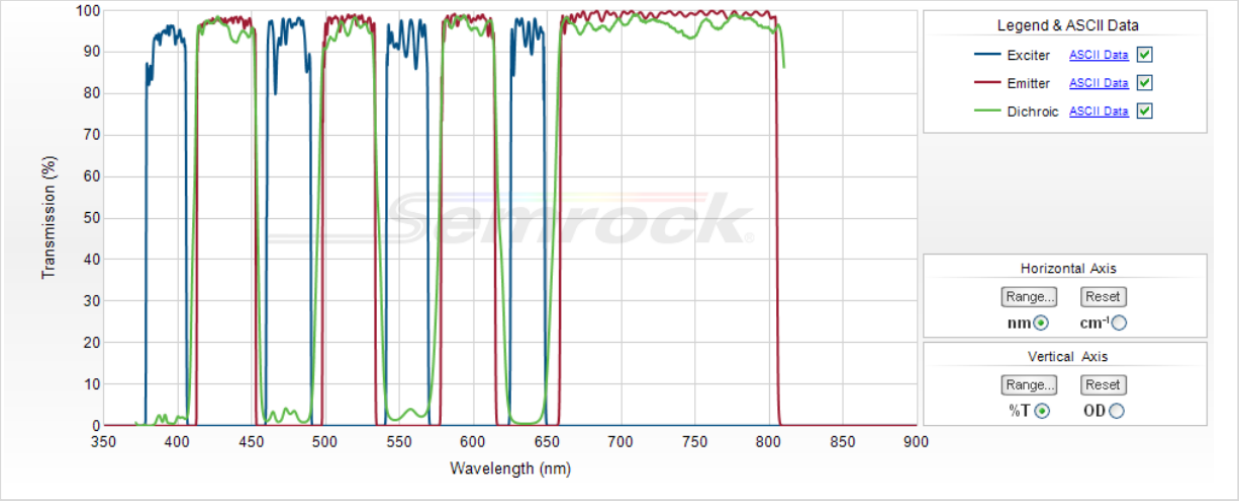



Figure 16: Transmission profile of the full-multiband filter set, built-in T-image (obtained from the official Semrock website: www.semrock.com.

### 14.3.4 Measurement Times

| Image Acquisition | Specified Measurement Time |
|---|---|
| 96-well, whole-well bright field & digital phase imaging, 2x objective | ≤ 12 min |
| 96-well, center, bright field, digital phase and one fluorescence channel, 10 x objective, default exposure time | ≤ 15 min |
| **Image Acquisition and Analysis for Standard Applications** | **Specified Measurement Time** |
| Confluence, 96-well, whole-well, 2x objective, sample within 60-80 % confluence range | ≤ 20 min (incl. analysis) |
| Nuclei Counting, 384-well, whole-well, 4x objective, sample within 60-80 % confluence range, optimized acquisition and analyzing settings | ≤ 45 min (incl. analysis) |
| Viability, 24-well, center, 10 x objective, sample within 60-80 % confluence range, optimized acquisition and analyzing settings | ≤ 10 min (incl. analysis) |

## 14.4 Standard Applications

The Cell Imager supports a wide range of applications in the area of imaging-based cytometry. See the Reference Guide for details. For details about defining the image analysis, see the Analysis Plugin instructions.

**NOTE:** To avoid fogged lids and indeterminate outcomes, use SPARK's temperature control to adjust the ambient temperature of the microplate to pre-measurement conditions.

**NOTE:** The given working-concentrations for the fluorescent dyes are only meant as guidelines and must be optimized for different cell lines by the user.

**NOTE:** For each application, an incubation time of 30 minutes is recommended to gain optimal fluorescence signals from treated cells. Additionally, incubation times must be optimized for different cells lines.

**NOTE:** Make sure to process samples in the dark, as fluorescence dyes may photobleach.

**NOTE:** If the 2x objective is used in combination with the Confluence application in a 96-well format, a filling volume of ≥ 200 µl is recommended, as otherwise the meniscus may cause undesirable circular artifacts.

**NOTE:** For whole-well confluence analysis, a border offset of 150 µm is recommended.

**NOTE:** If the 2x objective is used in combination with the cell counting in a bright field image, reduced cell detection might be observed in the well border area due to decreased cell visibility in that area. For more details see the Reference Guide.

**NOTE:** Low contrast of the unstained cells is one of the major obstacles for the bright field cell counting. Better results might be achieved by using dedicated imaging plates.

**NOTE:** Experimental conditions such as medium (composition, volume/well), autofocus offset values, plate bottom artefacts may have an impact on the bright field image and thus cell segmentation. For the best results use optimal experimental and acquisition settings.

## 14.5 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.
See the SparkControl manual for details.

**CAUTION:** Avoid working with SparkControl and ImageAnalyzer 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.

**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.

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

**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 ImageAnalyzer 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:** Increasing sensitivity settings (see Analysis Plugins instructions) leads to increased measurement times.

**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
- 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:** Histograms and heat maps, where applicable, are depicted in the generated PDF report. They are not included in the corresponding Excel file.

**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 one 3D Imaging strip. 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.

## 14.6 Optimizing Fluorescence Imaging Measurements

### 14.6.1 Live Viewer

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. See the SparkControl manual for details.

Note that the Live Viewer includes an option to enhance the contrast for single channel color images. By using this option (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.




**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.




**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:** The enhanced contrast option is available only in the Live Viewer view for single channel images.



**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.



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



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



**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.

**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.

**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.

**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).

### 14.6.2 ImageAnalyzer

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

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

#### Workspaces

Open the ImageAnalyzer and select a **workspace** to work with. If workspaces are not available at the predefined default path (C:¥Users¥Public¥Documents¥Tecan¥SparkControl¥Workspaces) go to **File/Directory** and define the new default path.

After opening a workspace, the software displays the corresponding image and the image analysis data, if available. These data always relate to the selected well, selected channel and in case of a kinetic measurement, to the selected kinetic cycle.

## Structure

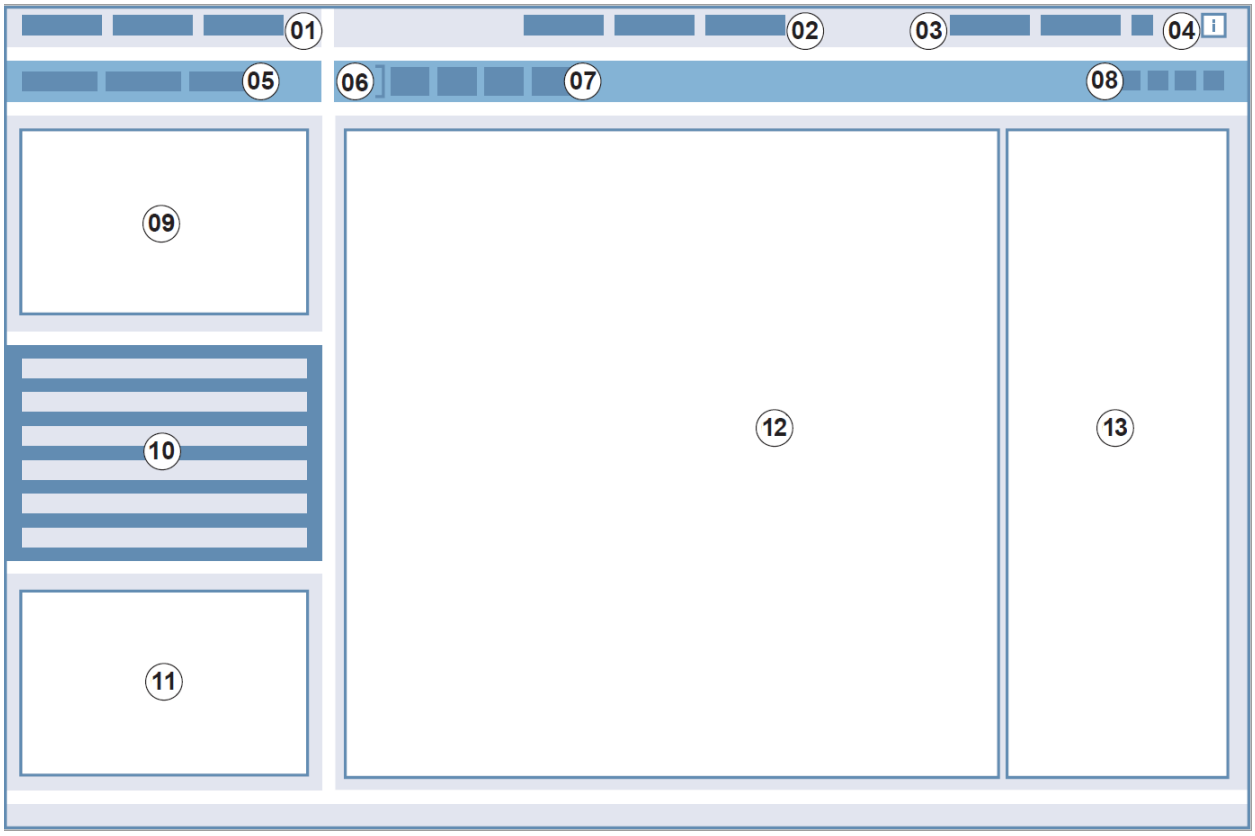



Figure 17: Structural elements of the ImageAnalyzer GUI
01 Menu bar; 02 Method definition tabs; 03 Toolbar; 04 Button Info pane; 05 Drop-down lists; 06 Selected well; 07 Dynamic image composition; 08 Context-sensitive toolbar; 09-12 Result area; 13 Split screen

| | | |
|---|---|---|
| Menu bar | **01** | Contains a drop-down menu of editor functions (File, View and Help) |
| Method definition tabs | **02** | Switch to Measurement (View mode), Processing and Analysis (Edit mode) of images and analysis results |
| Toolbar | **03** | Provides access to 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** | Includes channel-related icons for generation of user-defined composed images |
| Context-sensitive toolbar | **08** | 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**<br>**10**<br>**11**<br>**12** | 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. |
| Split screen | **13** | Opens a screen for defining/modifying Processing/Analysis settings |

See the SparkControl manual for details.



**NOTE:** 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 effects image content and should be performed before changing the Analysis and/or Gating settings.



**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:** Gates in ImageAnalyzer 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).



**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.

### 14.6.3 Analysis Plugins

For more information, see the corresponding Analysis Plugin instructions.



**NOTE:** For counting analysis with the Bright field channel, Sensitivity, Object length and width are not available.



**NOTE:** Sensitivity values beyond default settings are recommended for weak fluorescent signals only. The algorithm depends on signal intensity; if sensitivity values are set too high, background noise will be enhanced, and this may lead to the detection of artifacts.



**NOTE:** The algorithm does not exhibit linear behavior over the predefined range. Depending on object number, signal intensity and contrast, it may be necessary to use different sensitivity settings within one workspace.



**NOTE:** Increasing the sensitivity settings leads to an increase in measurement- and re-analysis time, respectively. Sensitivity values higher than default settings may have a significant impact on the recalculation time in ImageAnalyzer if workspaces contain multiple images per well.



**NOTE:** The 3D analysis is based on a deep learning algorithm, whose teaching for spheroids was performed on four selected cell lines (HeLa, A549, MCF-7 and MDA-MB-231). Single spheroids and multiple spheroids grown with and without matrix (Matrigel) were trained to allow for obtaining best possible results for these and similar cell lines. Teaching with organoids was conducted to identify in particular colon, lung and liver organoids.




**CAUTION:** SPARK CYTO supports 3D imaging with plates specialized for generation of large number of uniform spheroids/organoids (e.g., AggreWell™ Microwell Plates STEMCELL technologies, Corning® Elplasia® Plates). However, when using one of these plate formats for 3D imaging, the quality of analysis results is not guaranteed.

# 15 Spark-Stack Microplate Stacker

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 18: 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

## 15.1 Access to the Front Panel

By removing the plate magazines from the stacker module, the operator has full access to the front panel of the SPARK multimode reader, for:

- Exchanging dichroic mirrors
- Exchanging filter slides
- Manual loading of a single plate onto the plate carrier of the Spark reader
- Manual loading of the SPARK MultiCheck-QC plate for performing IQ/OQ

### 15.1.1 Onboard Control Buttons




If no plate magazines are installed on the Spark-Stack, all onboard control buttons are active, for more information, refer to chapter 2.6 Onboard Control Buttons.

When a plate magazine is installed on the Spark-Stack, only the Stop function of the Onboard-Start button  remains active. All other onboard control buttons are inactive. Pressing the Onboard-Start button during a stacker run will stop the stacker run after the current action is completed.




**CAUTION:** If a stacker run is interrupted using the Onboard-Start button, a microplate may remain in the reader. Make sure to remove the microplate from the reader before starting another stacker run.




**CAUTION:** In the event of a power failure, make sure to remove the microplate from the reader and to remove all processed plates from the OUTPUT plate magazine, before starting a new stacker run.

### 15.1.2 Light Protection for Sensitive Assays/Dark Covers

The Spark-Stack microplate stacker includes a set of light-protective front and top covers, which can be quickly inserted into place on the plate magazines.

These elements help to shield microplates containing light-sensitive contents, such as GFP-transfected cells, AlphaScreen, AlphaLISA, AlphaPlex assay plates, etc. from ambient light in the laboratory.



1. Place the front cover onto the magnetic strips of the plate magazine.



2. Slide the front cover down into position.



3. Place the top cover on the plate magazine.

## 15.2 Microplate Requirements for the Spark-Stack

Any common microplates (without lids) ranging from 6 to 1536-well formats conforming to ANSI / SLAS standards may be used for performing stacker runs with the Spark-Stack module:

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

**WARNING:** Do not use humidity cassettes in the Spark-Stack module.

**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.

## Specifications Spark-Stack

| Parameters | Characteristics |
|---|---|
| Microplates (without lids) | from 6 to 1536-well formats conforming to ANSI / SLAS standards |
| Restacking time | 15 seconds per plate (96-well microplate without smooth mode) |

## Required Microplate Dimensions

| Parameters | Characteristics |
|---|---|
| Overall plate height | From 10 mm to 23 mm |
| Footprint | Length = 127.76 mm ± 0.5 mm<br>Width = 85.48 mm ± 0.5 mm |
| Minimum difference between plate height and skirt height | ≥ 6.7 mm |



**WARNING:** Do not touch the inside of the input magazine or the output magazine during a stacker run.



**WARNING:** Do not insert or remove plates manually during a stacker run.

## Microplates with Barcodes

Microplates with barcodes for identifying plate IDs are especially helpful for stacker kinetic runs. A SPARK microplate reader equipped with the optional, integrated barcode reader module is required for barcode reading.

For more information, see chapter 2.5.2 Microplates with Barcode.

## Automated Processing of Cell Chips with the Spark-Stack Module:

The plate magazines of the Spark-Stack are compatible with the cell chip adapter of the SPARK reader.

Therefore, automated loading of Cell chips inserted in the cell chip adapter is possible using the Spark-Stack microplate stacker module.

For more information, refer to chapter 19 Cell Counting in Cell Chips.

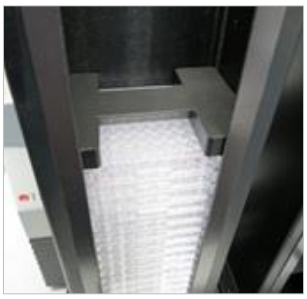
## Stabilizer Weights

The stacker comes with two H-shaped stabilizing weight elements (one for each plate magazine). These elements are designed to weigh down the microplates in the plate magazines for reliable stacking.

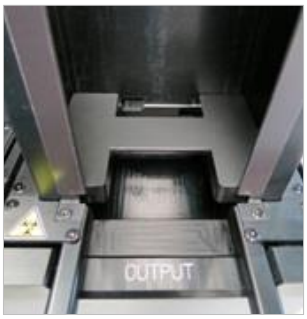


**NOTE:** The plate sensors in the plate magazines recognize the stabilizer weight; therefore, the stabilizer weights will not be loaded into the SPARK reader and do not need to be removed when restacking. Make sure that the stabilizer weight is always on top of the plates in the plate magazine.

1. Place a stabilizer weight on the plates in the input magazine. (The middle section is wedge-shaped. The wider part of the wedge should be facing up for easier gripping).



2. Place a stabilizer weight at the bottom of the output magazine.



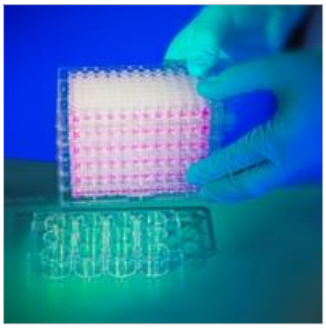

3. The Spark-Stack is now ready for operation.



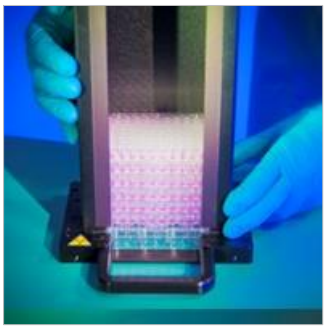
### 15.2.1 Loading Multiple Microplates into a Plate Magazine

It is possible to load several microplates at once into a plate magazine of the Spark-Stack module by using a standard 6-, 12-, or 24-well microplate, two 96-well microplates, or a standard half-deepwell or deepwell plate as a platform for the stack of microplates to be loaded into the plate magazine.

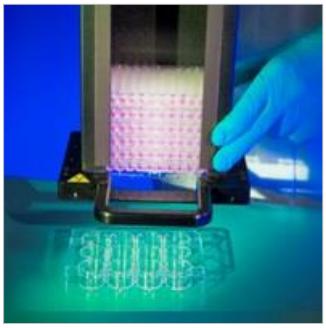
1. Prepare the full amount of microplates to be loaded into the plate magazine for the stacker run.
2. Place a portion of these microplates onto the platform plate. In this example, a standard 12-well plate is used as a platform plate. Make sure that the microplates are the same type and color and that well **A1** is in the upper left-hand corner (closest to **A1** label on the back left hand corner of the plate magazine).



3. Slide the plate magazine over the stack of microplates all the way down until the plate magazine makes contact with the surface of the lab bench.



4. Lift up the plate magazine. The stack of microplates has been added to the plate magazine. The platform plate remains on the lab bench.



Load the remaining microplates using the same procedure.




**CAUTION:** Ensure that no microplates are inserted upside-down.




**CAUTION:** Ensure that all microplates are inserted with the A1 label in the upper left-hand corner.



**CAUTION:** Always wear gloves when manually inserting microplates into the plate magazine. Fingerprints or smudges on the optical (bottom) surface of the microplate can adversely affect reader measurements.



**CAUTION:** Use only compatible plates. Flexible and non-level plates, such as PCR-plates are not suitable.



**CAUTION:** Do not use microplates that show any form of damage.



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



**CAUTION:** If the sealing films or foils are removed from microplates before measurement: Make sure that the top of the microplates are not sticky from residual adhesive, otherwise the plates can become stuck together and cause problems with retrieval from the plate magazine.

Additionally, make sure the microplates are level and have not become bent during the sealing process.



**NOTE:** To minimize evaporation during long time stacker kinetic runs, use empty plates at the first and the last position (top and bottom of the stack of plates to be measured) when loading microplates into the plate magazine.



**NOTE:** Condensation might have an impact on measurement quality. To avoid/minimize condensation effects on the sealing foil and/or bottom of the plate

- work in a temperature-controlled room,
- equilibrate plates to room temperature,
- centrifuge plates with sealing foils,
- use the SPARK's heating option and define measurement workflows with plate incubation before measuring each plate.

### 15.2.2 Loading a Single Microplate into a Plate Magazine

Before loading a single microplate into a plate magazine, please ensure that:

- **well A1** on the microplate is closest to the **A1** sticker on the back left hand corner of the plate magazine,
- the microplate is not upside down, its type and color correspond to the plate definition used in the method, and
- the microplate is not obviously damaged.



**CAUTION:** Always wear gloves when manually inserting microplates into the plate magazine. Fingerprints or smudges on the optical (bottom) surface of the microplate can adversely affect reader measurements.



1. Manually insert the microplate into the top of the plate magazine and carefully lower it towards the bottom of the stack.



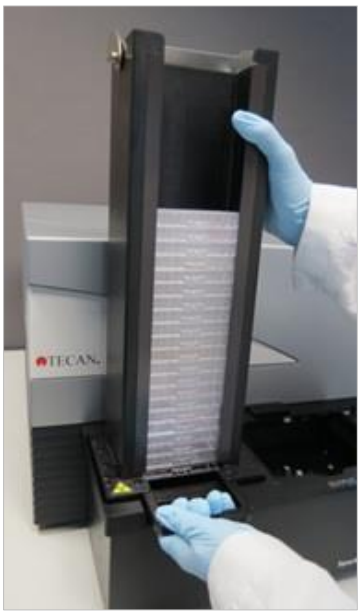
2. Gently release the microplate at the bottom of the plate magazine.

### 15.2.3 Loading the Plate Magazines onto the Spark-Stack Module

Lift the plate magazines using the handle at the bottom as shown below.

Position the plate magazine above the corresponding position on the Spark-Stack module and push it straight down.

1. Load the plate magazine with the microplates to be processed at the position labelled INPUT.
2. Press the plate magazine down firmly to click it into position.



3. Load the empty plate magazine at the position labelled OUTPUT.



**CAUTION:** If a plate magazine has not been inserted properly onto the Spark-Stack module, the Start Stacker button will be disabled. If this happens, press the plate magazine down to click it into place. Then start the stacker run.

**CAUTION:** If the output plate magazine is not empty, an error message will appear at the start of a stacker run. If this happens, clear the plates from the output magazine and restart the stacker run in the software.

**CAUTION:** If a plate has been forgotten on the plate carrier inside the SPARK reader, an error message will appear at the start of a stacker run. If this happens, remove the plate magazines from the Spark-Stack module. Move out the plate carrier from the SPARK reader and remove the microplate. Then move the empty plate carrier back into the SPARK reader. Re-load the plate magazine onto the Spark-Stack module and restart the stacker run.

**CAUTION:** Do not load additional microplates into the input magazine while a stacker run is in progress.

### 15.2.4 Inserting Microplates Directly into the SPARK Reader

By removing both plate magazines from the Spark-Stack module, it is possible to perform single plate measurements using standard microplates, the Spark MultiCheck plate or the NanoQuant plate.

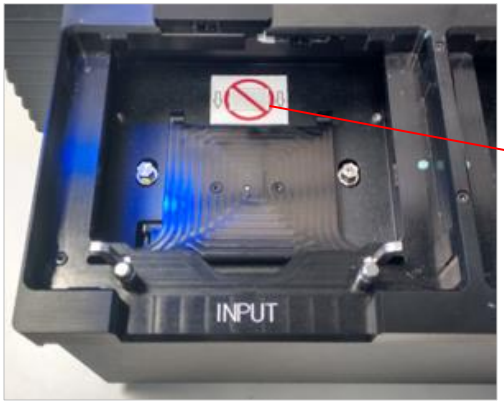
**WARNING:** Move the plate transport out first, before inserting a microplate. Place the microplate directly on the plate transport of the SPARK reader. Do not place the plate on the lifting table of the stacker when the **No microplates** label is visible, indicating that the plate transport is still located inside the reader. Otherwise, this will cause a collision with the plate when the plate transport is moved out of the reader.

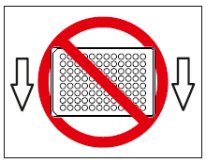
If this happens press the onboard Start/Stop button , or press the stop button in the software.

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

When loading a single plate by hand:

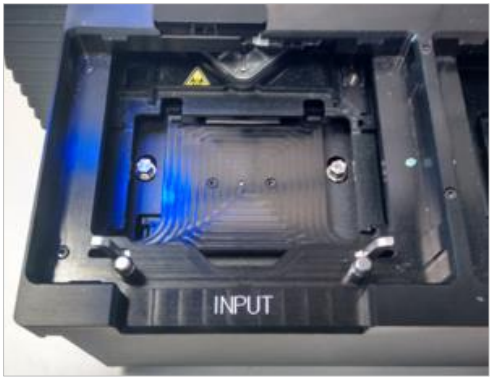
1. If the **No Microplates** label is visible when you try to load a single plate by hand, this indicates that the plate carrier is still inside the reader. Do not insert a microplate if this label is visible!



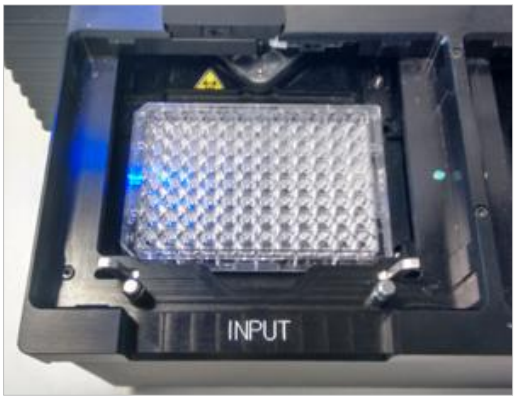



**No Microplates** label

2. Move the plate transport out first, before inserting a microplate. The **No Microplates** label is not visible when the plate carrier is moved out.




3. Place the microplate on the center of plate transport. Ensure that the **A1** label on the microplate is in the upper left-hand corner. Always place the microplate on the plate transport – never directly onto the lifting table of the stacker.



## 15.2.5 Unloading Processed Microplates Individually




**CAUTION:** Always wear gloves when unloading microplates from the plate magazine.

1. Gently remove the plate magazine from the Spark-Stack module. Avoid any tilting of the plate magazine. Place the plate magazine on the work bench.



2. Carefully grip the microplate at the top of the stack of plates in the plate magazine.



3. Gently slide the microplate towards the top of the plate magazine, and then remove the microplate. Avoid any spilling.

4. Discard the plate according to your laboratory procedures.

### 15.2.6 Unloading a Group of Processed Microplates

**CAUTION:** Always wear gloves when unloading microplates from the plate magazine.

1. Gently remove the plate magazine from the Spark-Stack module. Avoid any tilting of the plate magazine.
2. Place the plate magazine on the work bench.



3. Carefully slide one hand underneath the microplate at the bottom of the plate magazine and stabilize the group of microplates you want to unload, with the other hand.



4. Gently slide the group of microplates towards the top of the plate magazine, and then remove the group of microplates. Avoid any spilling.

5. Discard the microplates according to your laboratory procedures.

## 15.2.7 Cleaning and Maintenance of the Spark-Stack

### Liquid Spills



**WARNING:** Always switch off the instrument before removing any kind of spills on the microplate stacker. All spills must be treated as potentially infectious. Therefore, always adhere to applicable safety precautions, (including the wearing of powder-free gloves, safety glasses and protective clothing) to avoid potential infectious disease contamination.

Additionally, all resulting waste from the clean-up must be treated as potentially infectious and the disposal must be performed according to the information given in chapter 7.5 Disposal.

### Cleaning and Disinfection Procedure (including Liquid Spills)

The procedure for cleaning and disinfecting the Spark-Stack, including for liquid spills inside a plate magazine or on the Spark-Stack module is as follows:

1. Wear protective gloves, protective glasses, and protective clothing.
2. Prepare a suitable container for all disposables used during the disinfection procedure
3. Switch off the SPARK reader to shut down the instrument and the built-in Spark-Stack module.
4. Remove the plate magazines.
5. Remove the microplates from the plate magazine or remove the microplate from the lifting table of the Spark-Stack module.
6. Wipe up any spills immediately with absorbent material.
7. Clean the surfaces of plate magazines and the Spark-Stack module.
8. For biohazardous spills, carefully wipe all outside surfaces of the instrument with a lint-free paper towel soaked in the disinfection solution (B33 [Orochemie, Germany] or 70 % ethanol).
9. Wipe cleaned areas dry.
10. Dispose of contaminated material appropriately.

### Preventative Maintenance

No special preventative maintenance is required for the Spark-Stack module. For further information, refer to chapter 7 Cleaning and Maintenance.

## 15.3 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:** 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.

### 15.3.1 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 tool bar 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:

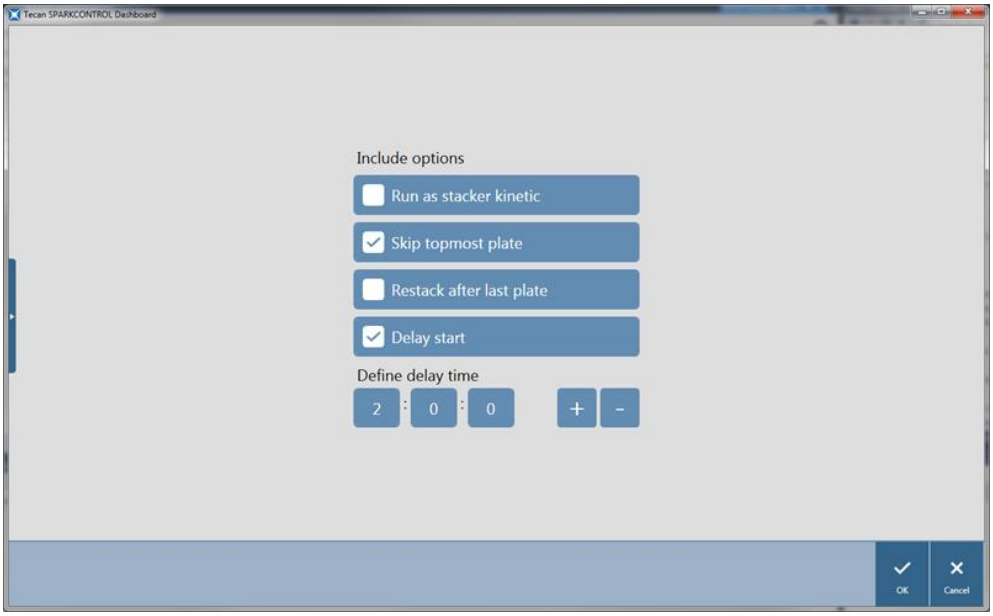



Figure 19: Stacker Operation window

| | |
|---|---|
| Runs as stacker kinetic | When selected, a method defined as a kinetic measurement will be executed as a stacker run. For more information refer to chapter 15.3.2 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.

### 15.3.2 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 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.

### 15.3.3 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 2.5.1 Filling Volumes/Smooth Mode). **Smooth mode** is recommended when using low weight microplates as e. g. 1536 well microplates.

# 16 Injectors

The injector module consists of one or two syringes contained in external units with lightproof covers. There are different syringe volumes, 500 µl, 1000 µl and 2500 µl. The injector needles are designed to inject liquid into any well of a 1 to 384-well microplate that complies with SBS standards (except for small volume 384-well plates).

**CAUTION:** Switch off the instrument before plugging in or unplugging the injector module.

## 16.1 Injector Carrier

The injector carrier can be easily removed (by the customer) from the instrument for actions such as injector priming, rinsing, or optimizing injection speed.

When using the injector during a measurement procedure, the injector carrier must be inserted correctly into the instrument. Remove the injector dummy and insert the injector carrier into the injector port. Press the injector carrier gently into the port to lock it in place.

The instrument is equipped with an injector sensor that checks the position of the injector carrier. If the injector is inserted into the instrument incorrectly, the sensor will not recognize the inserted injector carrier and injection will be disabled; however, actions such as rinsing, and priming will remain enabled. Performing rinse or prime procedures with an incorrectly inserted injector carrier can damage the instrument. Therefore, always make sure that the injector carrier is in the service position for rinsing and priming (see figure below).

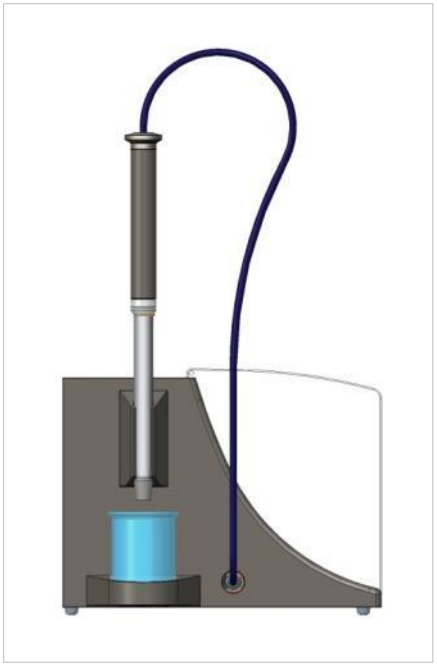


Figure 20: Injector carrier in service position

**CAUTION:** Never touch the syringes during operation.




**CAUTION:** The injector carrier must be in the service position for rinsing and priming. Do not perform rinse or prime procedures if the injector is inserted in the instrument. Performing rinse or prime procedures with an incorrectly inserted injector carrier can damage the instrument.




**CAUTION:** The injector carrier must be inserted correctly into the injector port, otherwise the injector will not be detected, and prime and rinse functions will remain enabled. Performing rinse or prime procedures with an incorrectly inserted injector carrier can damage the instrument.

The injection speed can be adjusted via the software. The optimal injection speed is dependent on the assay characteristics, such as the plate format and the viscosity and measuring behavior of the liquids. The removable injector carrier makes it possible to optimize this process outside of the instrument where a visual inspection can be easily performed.

### 16.1.1 Injector Dummy

All instruments possessing injector ports (instruments with injectors or instruments prepared to be upgraded for injectors) are delivered with injector dummies. The injector dummy replaces the injector if the injector itself is not in use. In such cases, the injector dummy ensures that the desired atmosphere in the instrument remains stable (temperature, gas concentration).

Always reinsert the injector dummy into the injector port after the injector carrier has been removed. Press the injector dummy gently into the port to lock it in place and close the lid. The injector dummy activates the injector sensor only if it is positioned correctly in the injector port.




**CAUTION:** Make sure that the injector dummy is inserted in the injector port every time the injector is not in use.




**CAUTION:** Be aware that the injector dummy also activates the injector sensor if correctly inserted into the injector port. Injection steps can be performed with the injector dummy inserted; however, the results will be unusable.

## 16.2 Priming and Rinsing



**CAUTION:** The injector carrier must be in the service position for rinsing and priming. Prime and rinse must not be performed when the injector carrier is in the injector port.

The initial filling step of the injector system (priming) as well as the cleaning step of the injector system (rinsing) must take place outside of the injector port. For these procedures the injector carrier is removed from the instrument and put into the service position of the injector module. For priming and rinsing steps of the injector system, a default setting for injection speed and volume dispensed is provided. If required, the priming parameters can be adjusted in the Injector Control window of the software.

The prime volume depends on the tubing length. Two types of injector tubing are available: **short** = 100 cm (39.37 in.) and **long** = 200 cm (78.74 in.).

The minimal priming volume is 1000 µl for an injector with short tubing and 1500 µl for an injector with long tubing.



**CAUTION:** Prime volumes that are too small may result in incomplete filling of the system, and therefore may negatively affect assay performance.



**CAUTION:** Do not touch the injector needles! They can become easily bent or misaligned, which can cause injection problems or damage the instrument.



**NOTE:** The selected settings for priming can be saved to the hardware buttons on the injector box by choosing the option **Save as default**. This function is only available via the Method Editor. Press the **Prime** button on the injector box to start the priming procedure.

For further details we refer to the Reference Guide.

### 16.2.1 Reagent Backflush

Prior to the cleaning of the injector system, reagent backflushing allows the remaining reagent in the liquid system (injector needles, syringes, valves, and tubing) to be pumped back into the storage bottles. This procedure is a cost-effective solution for minimizing reagent consumption. The dead volume of the injection system is approximately 100 µl.

For further details we refer to the Reference Guide.



**WARNING:** Hold the injector carrier only by the handle provided for this purpose.



**CAUTION:** The injector must be in the service position for the **Backflush** action. Do not perform **Backflush** when the injector is in the instrument.



**NOTE:** The selected settings for rinsing can be saved to the hardware buttons on the injector box by choosing the option **Save as default**. This function is only available via the Method Editor. Press the **Rinse** button on the injector box to start the rinsing procedure.




**CAUTION:** The injector carrier must be in the service position for the **Rinse** action. Do not perform **Rinse** when the injector is in the instrument.




**CAUTION:** Be sure to run a final rinse procedure with distilled water.




**CAUTION:** Take good care of the injectors! If they are damaged, the accuracy of dispensing may be affected. This can result in damage to the instrument.

## 16.3 Injector Cleaning and Maintenance

The required maintenance may vary with your application. The following procedures are recommended for optimal performance and maximum life of the injector system.




**CAUTION:** To avoid reagent mixing and cross-contamination, rinse the whole injector system thoroughly between different applications requiring the injector(s).

### Daily Maintenance:

If not otherwise stated by the manufacturer of the kit used, the following tasks must be performed daily:

- Inspect the syringes(s) and tubing for leaks.
- Flush the whole system thoroughly with distilled or deionized water after each use and when the syringe is not in use. Failure to do so can result in crystallization of reagents. These crystals can damage the syringe seal and valve plug, which can result in leakage.




**CAUTION:** Do not allow the syringes(s) to run dry for more than a few cycles.

### Weekly/Periodical Maintenance:

The injector system (tubing, syringes, inject needles) must be cleaned weekly to remove precipitates such as salts and eliminate bacterial growth.

Follow these steps to clean the syringe/injector system with 70 % EtOH (ethanol):

1. Depending on the user's application thoroughly flush the system with buffer or distilled water before rinsing with 70 % EtOH.
2. Rinse the fully lowered syringes with 70 % EtOH for 30 minutes.
3. After the 30-minute period, pump all the fluid from the syringe and tubing into a waste container.
4. Rinse the syringe/injector system with 70 % EtOH.
5. Rinse the syringe/injector system with distilled or deionized water. Leave the fluid pathway filled for storage.
6. Clean the end of the injector needles carefully with a cotton swab soaked in 70 % ethanol or isopropanol.



**WARNING:** Risk of fire and explosion!

Ethanol is flammable and when improperly handled can lead to explosions.
Proper laboratory safety precautions must be observed.




**CAUTION:** Syringes must only be replaced by a service technician, otherwise the performance of the instrument cannot be guaranteed.

## 16.4 Injector: Reagent Compatibility

The injector system consists of the following materials:

- PTFE, TFE, FEP: Tubing, valve plug, seal
- PEEK: Needle head, coupler tubing/injector
- KelF: Valve body
- Parylene coating: Injector needles

Please refer to the following list for reagent compatibility. Rating **A** indicates a good compatibility with the injector system. Chemicals with the rating **D** must not be used with the injector system. They will severely damage it.

| A-Rated Chemicals | D-Rated Chemicals |
|---|---|
| Acetic Acid < 60 % | Acetonitrile |
| Dimethyl Formamide | Butyl Amine |
| Ethanol | Chloroform |
| Methanol (Methyl Alcohol) | Carbon Tetrachloride (dry) |
| Water, Deionized | Diethyl Ether |
| Water, Distilled | Ethanolamine |
| Water, Fresh | Ethylene Diamine |
| Potassium Hydroxide (Caustic Potash) | Furfural |
| Potassium Hypochlorite (aqueous) | Hexane |
| Sodium Hydroxide (< 60 %, aqueous) | Hydrofluoric Acid |
| Sodium Hypochlorite | Monoethanolamine |
| | Sulfuric Acid (diluted or concentrated) |
| | Tetrahydrofuran |




**CAUTION:** Use only **A**-rated reagents with the injector system. **D**-rated reagents must not be used with the injector system.

The information listed in this table has been created by Tecan Austria according to available material compatibility information and provides only a general guideline for the selection of compatible reagents.



**WARNING:** Approved chemicals must be stored and handled properly. Environmental factors such as temperature, pressure and concentration can result in undesired chemical behavior, which can damage the instrument.



**WARNING**: Be aware that the improper handling of chemicals may result in serious injury. Follow safe laboratory practices and wear protective clothing when handling chemicals.

## 16.5 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 12.6 Inject and Read.

For further details we refer to the SparkControl manual.




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

## 16.6 Heater and Magnetic Stirrer

The injector module can additionally be equipped with a heater and magnetic stirrer option.

For further details we refer to the Reference Guide.



**NOTE**: The selected temperature equates the temperature of the surface of the heating plate. The temperature of the injection solution in the container must be controlled explicitly by the user.




**CAUTION**: If heating is activated be aware that the basic as well as the expansion module are equally tempered!

### 16.6.1 Laboratory Flask and Magnetic Stir Bar

The heating plate is designed to accommodate a laboratory flask of up to 100 ml volume. The standard set for each heater and magnetic stirrer module consists of one 100 ml laboratory flask and an appropriate magnetic stir bar.

## 16.7 Injector Specifications

**NOTE**: All specifications are subject to change without prior notification.

### 16.7.1 Technical Specifications for the Injector

| Parameters | Characteristics |
|---|---|
| Plate type | 1- to 384-well plates |
| Injector syringe volumes | 500 µl, 1000 µl, 2500 µl |

### 16.7.2 Performance Specifications for the Injector

| **500 µl syringe** | | |
|---|---|---|
| **Inject Volume** | **Accuracy** | **Precision** |
| 10 µl | ≤ 10 % | ≤ 10 % |
| 100 µl | ≤ 1 % | ≤ 1 % |
| 450 µl | ≤ 0.5 % | ≤ 0.5 % |
| **1000 µl syringe** | | |
| **Inject Volume** | **Accuracy** | **Precision** |
| 20 µl | ≤ 10 % | ≤ 10 % |
| 200 µl | ≤ 1 % | ≤ 1 % |
| 900 µl | ≤ 0.5 % | ≤ 0.5 % |
| **2500 µl syringe** | | |
| **Inject Volume** | **Accuracy** | **Precision** |
| 50 µl | ≤ 10 % | ≤ 10 % |
| 500 µl | ≤ 1 % | ≤ 1 % |
| 2250 µl | ≤ 0.5 % | ≤ 0.5 % |

### 16.7.3 Specifications for the Heater / Stirrer

| Parameters | Characteristics |
|---|---|
| Power supply | 24 V, max. 60 Watt, external plug-in |
| Temperature regulation | 20-42 ° C |
| Stirring speed regulation | 50-1000 rpm |

# 16.8 Quality Control of the Injector Module

## 16.8.1 Periodic Quality Control Tests

Depending on usage and application, we recommend a periodic evaluation of the instrument on Tecan site.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore, please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.




**CAUTION:** Before starting measurements, make sure that the microplate is inserted correctly; well A1 must be on the upper left side.



**WARNING:** The following instructions explain how to perform the Quality Control to check the specifications of the instrument. If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

## 16.8.2 Injector Accuracy

Accuracy is the ability of a system to give responses close to a true value. The accuracy is calculated as percentage deviation from the true value.

### Material:

- Distilled water
- Greiner 96-well plate, flat bottom, transparent
- Scales with accuracy specification of 1 mg

### Procedure:

Prime the injector with distilled water. Weigh the empty plate and note. Inject 20 µl into 20 wells of a Greiner 96-well plate (flat bottom, transparent) plate and immediately weigh the plate again (take care of evaporation effects). Perform procedure at room temperature (25 ° C).

### Injection Parameters:

| | |
|---|---|
| Injector | Select Injector A or B |
| Speed | 200 µl/s |
| Refill speed | Same as injection speed |
| Refill mode | Standard |
| Refill volume | Default |
| Plate definition file | GRE96ft |
| Part of plate | D2-E10 |

### Evaluation:

The weight of 400 µl distilled water (20 x 20 µl) at 25 ° C is 398.8 mg (mass density of water is 0.997 mg/µl). Calculate the Accuracy (%) as follows:

$$\text{Accuracy}\,(\%) = \frac{398.8 - measured}{(398.8/100\ )}$$

# 17 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.

## 17.1 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.

### 17.1.1 Temperature Control Software Settings

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

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

**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**: 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 refer to chapter Manual Temperature Control in the SparkControl manual.

## 17.2 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.

The cooling system consists of two main components: the external liquid cooling device and the integrated cooling module (Te-Cool). The two components form a closed circulation system.

The liquid cooling device is an external unit, which pumps cooled liquid into the integrated cooling module to cool the air and the heated liquid returns to the external liquid cooling device to be cooled again.

The integrated cooling module is mounted on the bottom of the SPARK multimode reader. It cools the air and blows the air into the measurement chamber of the reader. The warm air flows back to the integrated cooling module to be cooled again.

Tecan recommends and supports the following liquid cooling device exclusively: **Thermoelectric Re-circulating Liquid Chiller MRC 150/300 (Laird Technologies GmbH, Germany)**. Tecan assumes no responsibility for any other product or liquid cooling solution. Prior to operating the SPARK reader in combination with the integrated cooling module and the liquid cooling device, read and follow the instructions provided by the manufacturer of the liquid cooling device (Laird Technologies, Operational Manual).



**WARNING:** Tecan assumes no responsibility for any liquid cooling system other than the one recommended in this document.



**WARNING:** Carefully read and follow the instructions given in the operating manual of the external liquid cooling device as well.




**CAUTION:** To ensure optimal operation of the cooling system, an annual maintenance procedure must be performed by a Tecan service technician.

### 17.2.1 Setting up the Liquid Cooling System




**CAUTION:** If the external liquid cooling device is used after it has been placed in storage or transported, it should be left to stand for at least 3 hours, to allow for temperature adjustment.

Before activating the cooling control option, ensure that the designated site meets the following requirements. Select a location for the external liquid cooling device that is flat, level, vibration free, away from direct sunlight or heat sources, and free from dust, solvents, and acid vapors. Leave sufficient distance behind the instrument for access to the rear panel.



**NOTE**: The ambient temperature sensor is located on the inside of the rear panel of the instrument and may be influenced by nearby heat sources.

The external liquid cooling device has an air-cooled refrigeration system. The liquid cooling device must be positioned so that the air flow is not restricted. Supply and return flow connections must be easily accessible and all tubes must be installed without sharp bends. A minimum clearance of 0.3 meters on all vented sides is necessary for adequate ventilation.




**CAUTION:** Leave enough space between the liquid cooling device and adjacent objects: 0.3 meters on all vented sides. Inadequate ventilation will cause a reduction in cooling capacity and compressor failure.

### Coolant

Only distilled water-propylene-glycol mixture may be used as a coolant. A propylene-glycol concentrate is obtainable from Tecan. This concentrate (0.25L concentrate) must be diluted with 0.75L distilled water prior to use to obtain 1L of coolant. Never use any other coolant or tap water to avoid instrument damages caused by pollution and corrosion!




**CAUTION:** Only use the recommended coolant in the cooling system, otherwise the integrated cooling module or the external liquid cooling device could become damaged (lime scale, impermeability of tubing).




**CAUTION:** Never operate the liquid cooling device without coolant in the reservoir!

## 17.2.2 Connection Procedure




**CAUTION:** Use only cooling tubing with no signs of damage.

The following information details the connection procedure:

- SPARK reader and external liquid cooling device: Make sure that the main power cables are unplugged, and the main power switch is in the OFF position.
- Connect the coolant OUTLET of the liquid supply of the external liquid cooling device to the instrument's SUPPLY port on the back of the integrated cooling module. Use the provided tube (see figures below).
- Connect the coolant INLET of liquid return of the external liquid cooling device to the instrument's RETURN port on the back of the cooling module. Use the provided tube.
- Connect the integrated cooling module to the cooling port of the SPARK reader using the provided CAN cable. (See figures below).
- Connect the condensate tube from the CONDENSATE OUTLET on the rear panel of the instrument (integrated cooling module). Place a condensate collector at the end of the tube. A condensate collector is not delivered with the instrument. (See figures below).
- Open the coolant reservoir of the external liquid cooling device by removing the cap. (See figure below).
- Fill the coolant reservoir about 2/3 full of coolant.
- Close the coolant reservoir of the external liquid cooling device by replacing the cap. (See figure below).

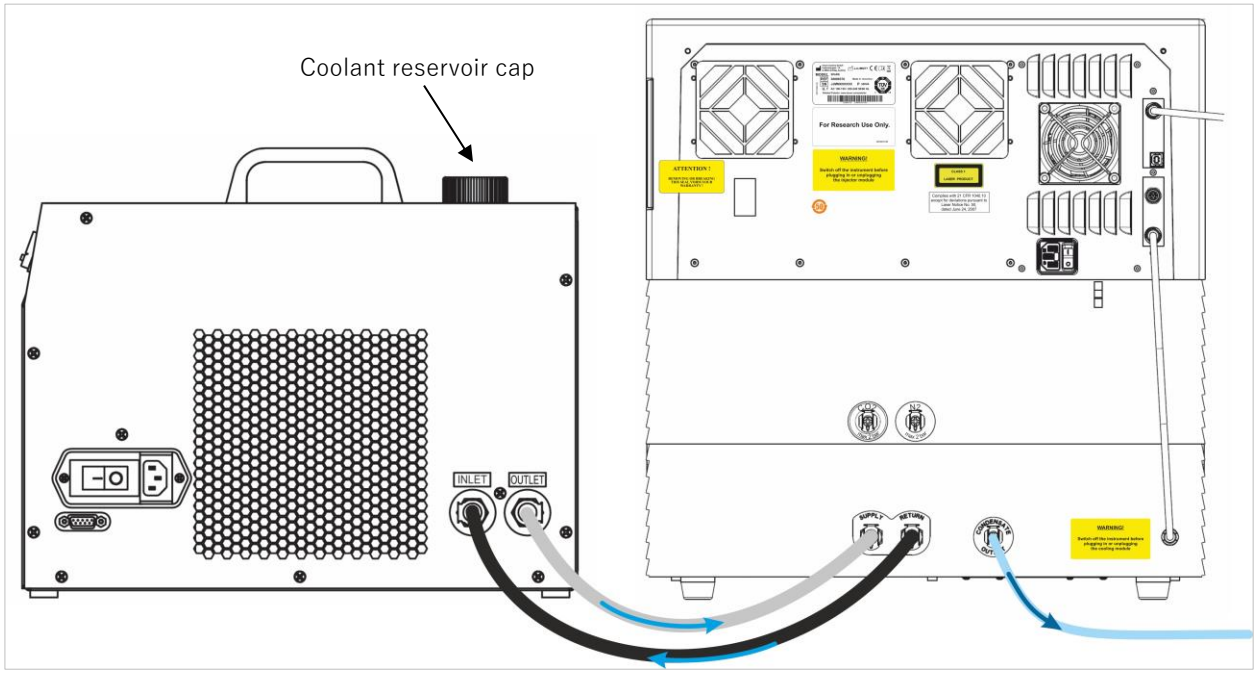



Figure 21: SPARK with integrated cooling module connected to the external liquid cooling device

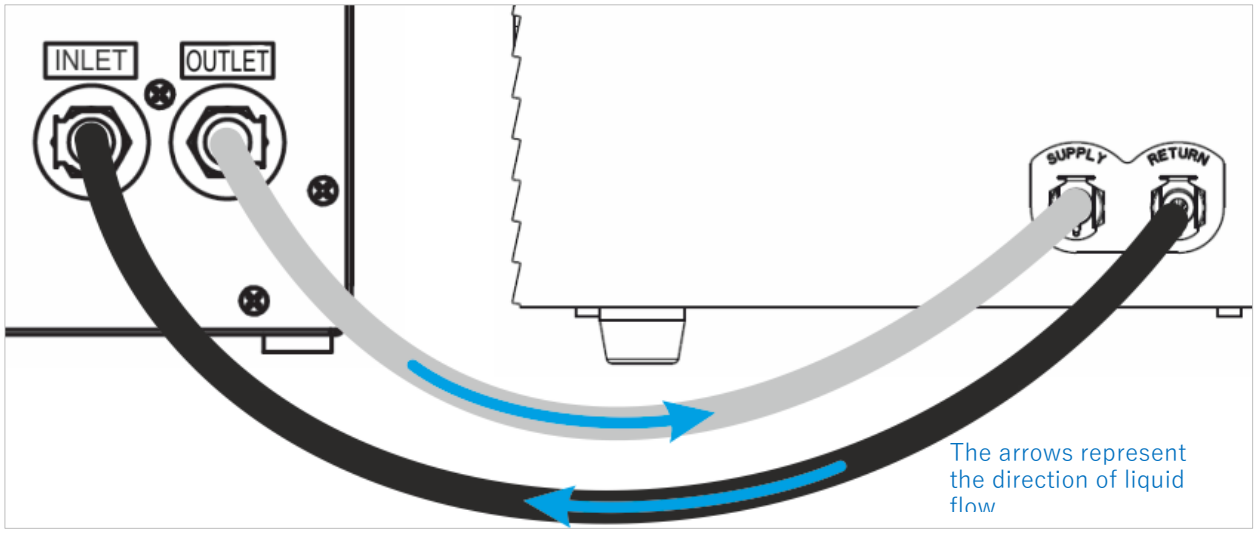



Figure 22: Connections between the integrated cooling module and the external liquid cooling device

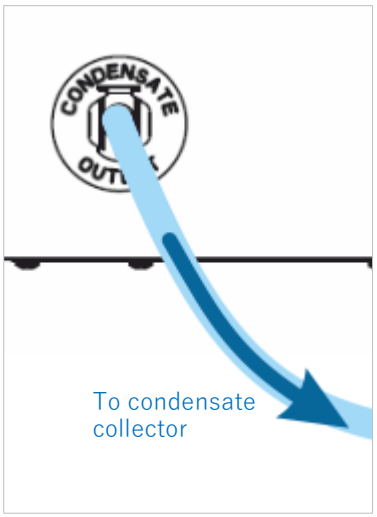



Figure 23: Condensate outlet

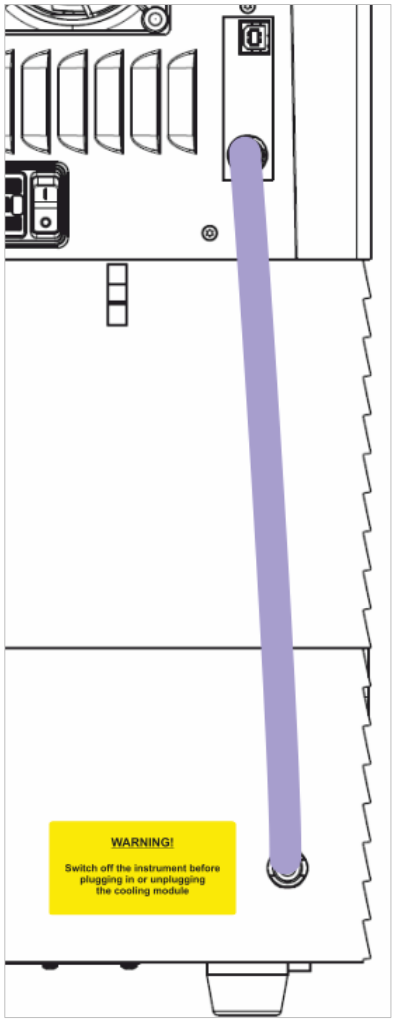



Figure 24: CAN cable

### 17.2.3 Switching on the External Liquid Cooling Device

1. Make sure that the coolant reservoir is about 2/3 full of coolant.
2. Connect the main power cable of the liquid cooling device to an appropriate AC power source.
3. Switch on the device and let it run for about 10 minutes to fill and vent the cooling system. Continuously check the filling level during this procedure. If required add coolant.
4. Check the compliance with the operational parameters. (See the operating manual of the liquid cooling device).
5. Set digital controller to 12 ° C (see the operating manual of the water-cooling device).
6. Replace the cap on the coolant reservoir.
7. The device is now ready for operation.

**NOTE**: For daily start-up, switch on the liquid cooling device for an appropriate amount of time prior to usage, depending on the ambient temperature of the laboratory.

**CAUTION:** Place the liquid cooling device near the instrument being cooled so that the tubing is straight and without bends or kinks.

### 17.2.4 Operating the Integrated Cooling Module (Te-Cool)

Switch on the main power switch of the external liquid cooling device and set target temperature to 12 ° C. To set temperature refer to the Operating Manual, Laird Technologies, Thermoelectric Re-circulating Liquid Chiller MRC 150/300.

Wait for coolant equilibration before starting a measurement by using the cooling function of the SparkControl Software. Depending on target temperature settings, the ambient conditions, and the current temperature of the measurement chamber; this will take 30 to 90 minutes.

Two condensation prevention stoppers are delivered with the instrument (see figure below). They fit into the slots on the left and the right side of the integrated cooling module. They should not be installed by default. If installed, the cooling module will heat up and target cooling temperature may not be achieved. They must be installed if the cooling function operates at full capacity (large difference between ambient temperature and target temperature) to prevent condensation. Otherwise, an accumulation of water might be observed.

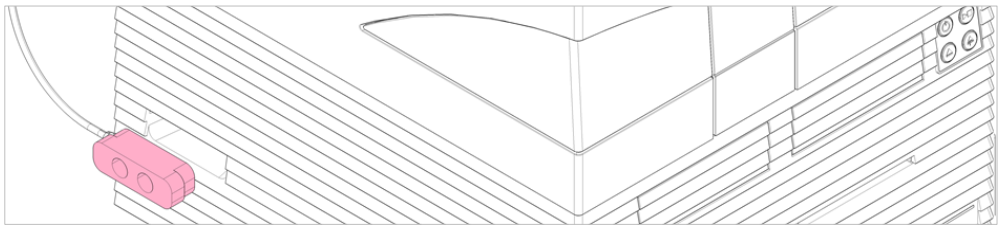


Figure 25: Condensation prevention stoppers (both sides of the instrument)

**NOTE**: The condensation prevention stoppers must only be installed by the user if a large difference between ambient temperature and target temperature is expected.

## 17.2.5 Cooling Control Software Settings

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

For the software settings please refer to 17.1 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:

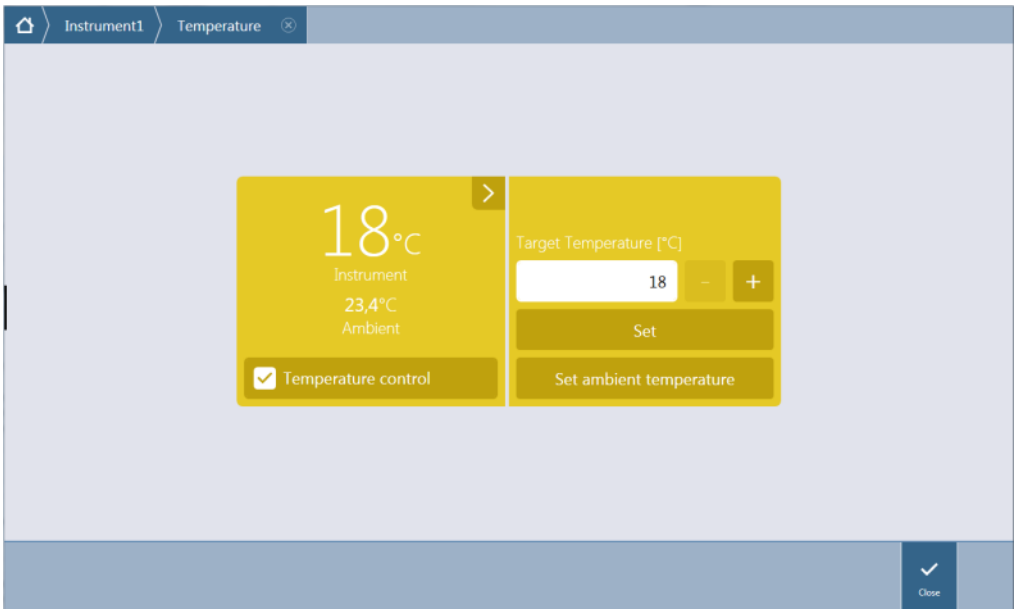



Figure 26: Temperature Control window for instruments with the cooling module

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.

## 17.2.6 Alarm Function/Troubleshooting

For the external liquid cooling device alarm functions and for troubleshooting, refer to the Operating Manual, Thermoelectric Re-circulating Liquid Chiller MRC 150/300 (Laird Technologies GmbH).

For further technical support and services, contact your local Tecan Customer Support organization.

## 17.2.7 Maintenance

For the external liquid cooling device maintenance refer to the Operating Manual, Thermoelectric Re-circulating Liquid Chiller MRC 150/300 (Laird Technologies GmbH).

For daily maintenance inspect the tubes for kinks and leaks and check that all tubes are connected properly.
Check that the external liquid cooling device is filled up with coolant. Check the level of the condensate collector and empty if required.

## 17.3 Gas Control

The gas control module offers a comprehensive solution for a variety of cell-based applications for the SPARK multimode reader. Two integrated gas inlets allow the control of $CO_2$ and $O_2$ to help maintain stable culture conditions and improve cell growth. Carbon dioxide concentration is regulated by an inflow of $CO_2$ gas, whereas oxygen reduction is achieved by supplying $N_2$ gas.

When equipped with the gas control module, the instrument can be used for in vitro studies of eukaryotic cell lines as well as for the study of anaerobic or facultative anaerobic bacteria.

The gas control module is available in two configurations:

| | |
|---|---|
| **$CO_2$ configuration** | $CO_2$ concentration can be regulated inside the measurement chamber |
| **$CO_2$ and $O_2$ configuration** | $CO_2$ and/or $O_2$ concentrations can be regulated inside the measurement chamber. |

### 17.3.1 Gas Safety

Adhere to the following guidelines:

- Always follow basic safety precautions when using the gas control module to reduce the risk of injury, fire, or electrical shock.
- Read and understand all information in this chapter. Failure to read, understand, and follow the instructions in this chapter may result in damage to the instrument or gas control module, injury to operating personnel or poor instrument performance.
- Observe all WARNING and CAUTION statements in this chapter. Ensure that this safety information is accessible for every employee working with the gas control module.
- Furthermore, it is assumed that instrument operators, due to their vocational experience, are familiar with the necessary safety precautions for handling gas and biohazardous substances.
- Precautions must be taken when working with potentially infectious material. Make sure to treat biohazardous material according to applicable safety standards and regulations as well as good laboratory practice guidelines.
- Wear protective glasses when using compressed gases outside of the instrument when the instrument is open.

**WARNING:** The gas control option is designed for $CO_2$ (carbon dioxide) and $N_2$ (nitrogen) supply only. The gas control option must only be used by trained personnel.

**NEVER USE A FLAMMABLE OR CRYOGENIC GAS SUPPLY!**

**WARNING:** Adequate ventilation must be provided for the room in which $CO_2$ and $N_2$ are used.

**WARNING:** Follow the security measures for working with compressed gas (transportation, storage, handling, and use)!

The $CO_2$ and $N_2$ gas cylinders must ALWAYS be securely fastened upright to a large, stationary object.

Always protect the gas cylinder from falling! A compressed gas cylinder which falls and is damaged can easily become a lethal projectile!

## 17.3.2 Gas Connection

Operate the gas control module in a well-ventilated, temperature and humidity controlled (air-conditioned) environment. Before activating the gas control option, ensure that the designated site meets the following requirements:

**Temperature:** 15 ° C (59 ° F) – 35 ° C (86 ° F)

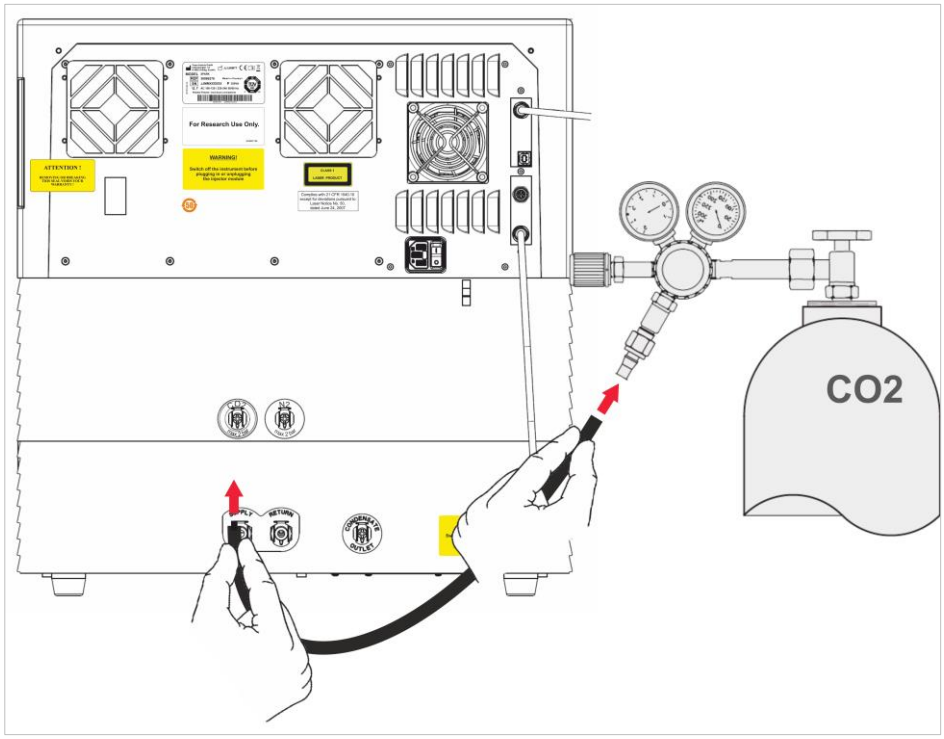
Do not expose or locate the instrument near direct sunlight or heat sources.
Maintain a low-dust environment. Keep liquids and vapors away from the instrument.
Leave sufficient distance behind the instrument for access to the rear panel. Make sure that all gas tubes are accessible and in no way obstructed.

**WARNING:** Follow appropriate gas handling precautions and safety regulations when setting up the $CO_2$ and/or $N_2$ supply. Read all label information and material safety data sheets (MSDS) from the manufacturer or supplier.

**WARNING:** Always use a regulator approved for the specific gas with high- and low-pressure gauges.

The following information describes details of the gas connection procedure:

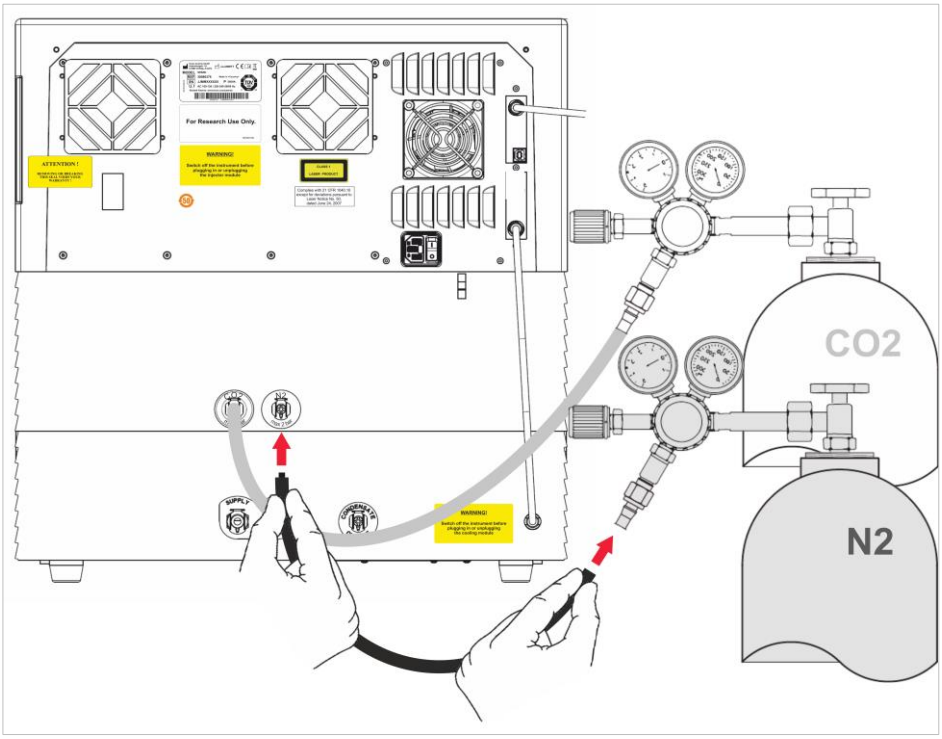
Connect the pressure regulator's outlet of the $CO_2$ gas cylinder or laboratory gas handling system to the instrument's inlet port (**$CO_2$**) on the back. Use the provided tube with quick connector and attach the tube to the regulator of the cylinder with a plastic clamp, as depicted in the figure below.




Start the SparkControl software and enter the sea level height of your location (see the Reference Guide and the SparkControl manual for more details).

**NOTE**: Before starting work with the gas module, the sea level height of your location must be entered via the SparkControl software.

If the gas control module is configured for **$CO_2$ and $O_2$**, nitrogen gas can be used to regulate the amount of oxygen, in addition to $CO_2$ regulation. Connect the pressure regulator's outlet of the $N_2$ gas cylinder or central gas supply to the instrument's inlet port ($N_2$) on the back. Use the provided tube with quick connector and attach the tube to the regulator of the cylinder with a plastic clamp, as depicted in the figure below.




### 17.3.3 $CO_2$ and $N_2$ Gas Cylinders (Not Supplied)

To control the gas concentration, gas cylinder(s) or a laboratory gas handling system with pressure reduction valves are required.

Gases: Carbon Dioxide ($CO_2$) to regulate $CO_2$ concentration; Nitrogen ($N_2$) for the reduction of $O_2$ concentration (e.g., 50 Liter cylinder). It is recommended that the gases meet the following gas purity levels:

| Gas | Gas Purity |
|---|---|
| $CO_2$ | ≥ 99.0 % |
| $N_2$ | ≥ 99.9 % |

The pressure reduction valve must have two gauges, one for the pressure in the bottle (high pressure gauge) and one for the reduced pressure of max 2 bar (max 29 psi; low pressure gauge). Take care that the display for regulating the pressure has a range of 5 bar (72.5 psi) or maximum 15 bar (217.5 psi) to allow regulation from 1 – 2 bar. Make sure that the pressure reduction valve is designed for use with biological applications (ask manufacturer).

The connection from gas cylinder to pressure reduction valve is different for each country. **Check with a gas cylinder company in your country for the proper connection!** Check that the connection piece of pressure reduction valve matches the inner diameter of the gas tube to the instrument. The inner diameter of this tube is approx. 6 mm. The tube on the connector to the pressure reduction valve must be secured with a plastic clip. A pair of pliers will be necessary to perform this task.

Make sure that there are no bends or kinks in the tubing.

If necessary, convert bar into psi: bar x 14.5 = psi (pounds per square inch), e.g., 2 bar = 29.0 psi.

To protect the gas cylinder from falling, a cylinder stand, or table mount (with a securing chain or strap), or gas cylinder cradle can be bought from a gas cylinder company or ordered from a laboratory catalog.

**WARNING:** Before opening the main valve, ensure that the regulator and the shut-off valves are closed.

**WARNING:** Make sure that the gas ($CO_2$ and $N_2$) to the instrument does not exceed a maximum pressure of 2 bar.

**WARNING:** Keep the injector port closed during gas supply. Insert the injector dummy if the injector is not in use.

**WARNING:** Before running a method with gas supply check gas tubes and connectors for leaks and ensure that tubes and connectors are fixed properly.

### 17.3.4 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.

### 17.3.5 Manual Gas Control

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

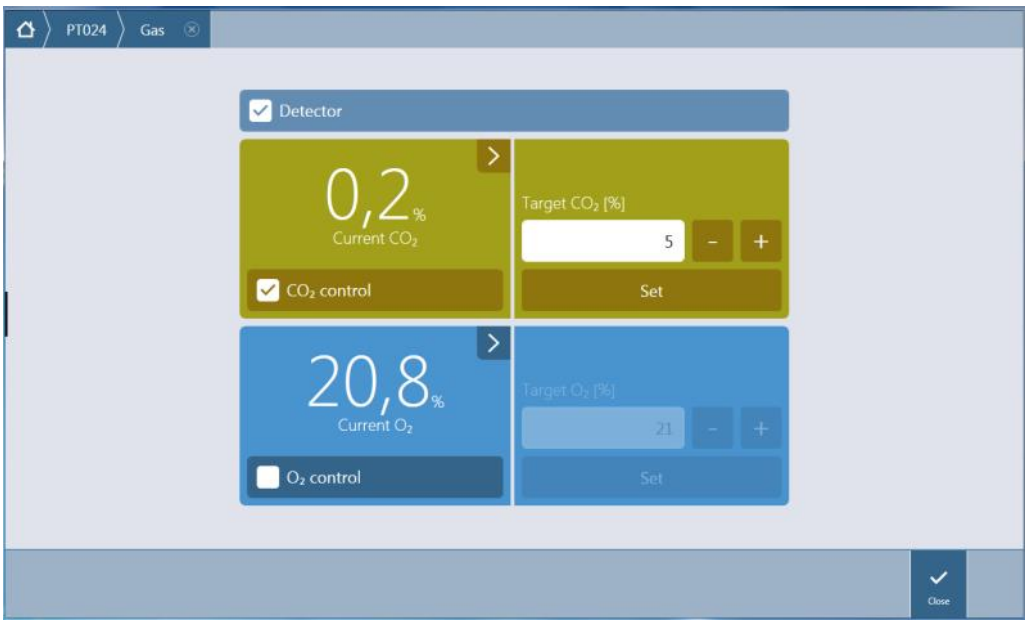



Figure 27: Gas Control window

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.

## 17.3.6 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, refer to 17.3.5 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.

For further details we refer to the SparkControl manual.




**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.

### 17.3.7 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.

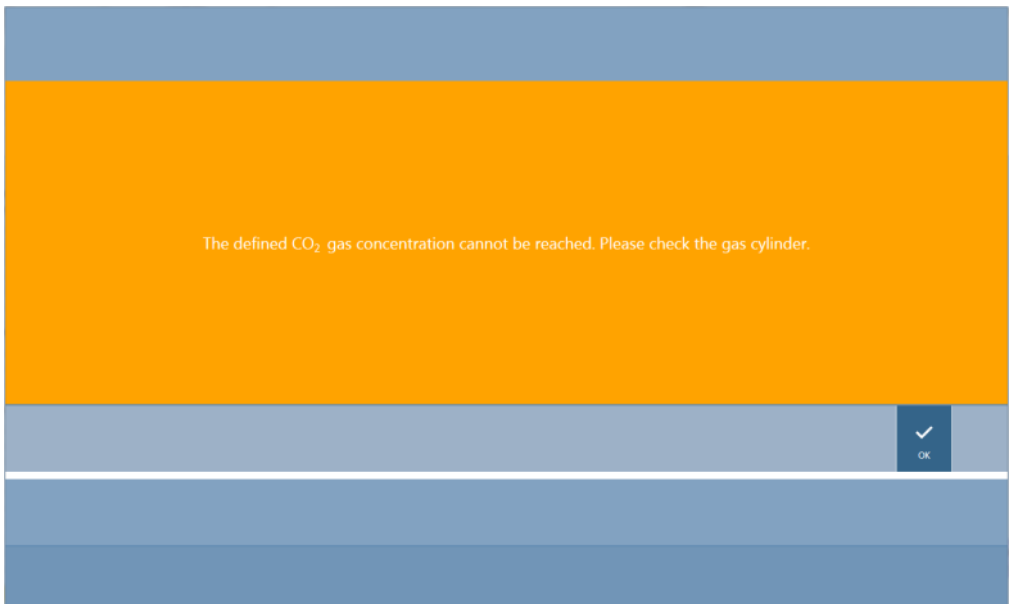



Figure 28: Stopping the gas alarm

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

## 17.4 Humidity Control

Evaporation is most pronounced when carrying out long-term studies (3 days or more). Especially when performing live-cell experiments over long periods of time significant evaporation effects may occur, affecting the outer wells of the microplate and the corner wells in particular. When the water evaporates the concentrations of substances in the medium will increase, which can influence cell growth and performance, causing heterogeneous or biased results.

The humidity cassette passively stabilizes the humidity and reduces evaporation for lengthy incubations. The humidity cassette is combinable with all plate formats from 1 to 384 wells complying with the SBS standard. It also allows for simultaneous incubation, signal detection in all measurement modes. Gas exchange (ventilation), signal detection as well as injection steps are supported in combination with the lid lifting option. Shaking in combination with the humidity cassette is restricted to orbital and double orbital mode.

**NOTE:** The humidity cassette is always combined with the lid lifting option.

SPARK CYTO configurations require specific humidity cassettes with altered dimensions, which are marked as Cyto on the packaging label. In comparison to standard humidity cassettes, the maximum filling levels for the reservoirs are different. All plate formats (6-well to 384-well) are compatible and user handling remains the same.

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

### 17.4.1 Humidity Cassette Standard / Cyto

The humidity cassette consists of water reservoirs and a lid with magnetic foil to facilitate lid lifting. The lid is closed to prevent evaporation. To allow gas exchange, the lid lifting option (ventilation) must first be selected in the software.

**WARNING:** Humidity cassettes are not compatible in the Spark-Stack module.




Figure 29: Humidity cassette

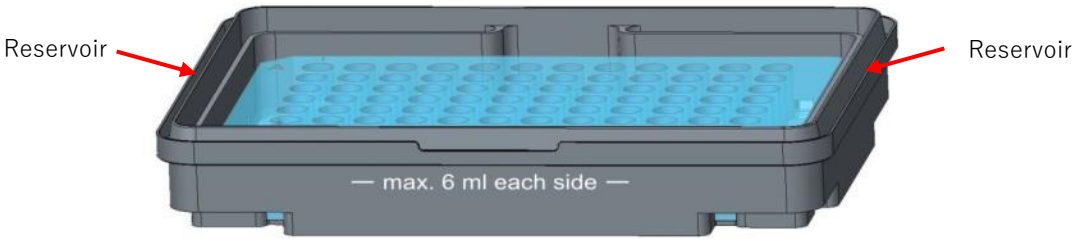



Figure 30: Basic part of the humidity cassette which holds the microplate and contains the water reservoirs

#### Standard Humidity Cassettes

Two different cassette types, a large one and a small one, are offered to shelter different types of microplates.

**Humidity Cassette - Small:** Usable for 96- and 384-well plates without plate lid. The maximum height is 16 mm. By using the lid lifting option in the software, all detection modes can be combined with the low humidity cassette. Maximum filling level of 4 ml in each reservoir.

**Humidity Cassette - Large:** Usable for 6- to 384-well plates with or without plate lid with a maximum height of 23 mm (including lid). By using the lid lifting option in the software, all detection modes except luminescence can be combined with the high humidity cassette. Maximum filling level of 6 ml in each reservoir.

### Cyto Humidity Cassettes

The humidity cassettes delivered with the Cell Imager module have different maximum filling levels compared to the standard humidity cassettes.

**Humidity Cassette – Cyto Small:** Usable with 96- and 384-well plates without a plate lid. The maximum height is 16 mm. By using the lid lifting option in the software, all detection modes can be combined with the low humidity cassette. Maximum filling level of 3 ml in each reservoir.

**Humidity Cassette – Cyto Large:** Usable with 6- to 384-well plates with or without a plate lid with a maximum height of 23 mm (including lid). By using the lid lifting option in the software, all detection modes except luminescence can be combined with the high humidity cassette. Maximum filling level of 5.2 ml in each reservoir.

**WARNING:** Select the correct humidity cassette type (small or large) in the software to avoid instrument damages.

## 17.4.2 Handling Procedure

1. Fill each reservoir with 3-4 ml distilled water in case of the small cassette and with 6 ml water in case of the large cassette type by using a pipette.
2. Insert the microplate (with or without lid) containing samples to be investigated into the basic part of the humidity cassette. Check that the orientation is correct, the cassette is labeled accordingly.
3. Place the lid on the cassette to properly close the humidity cassette, match A1 position of the microplate with the A1 position of the cassette lid.
4. Put the humidity cassette on the plate carrier. Take care of correct orientation; the position of well A1 must be on the upper left side.

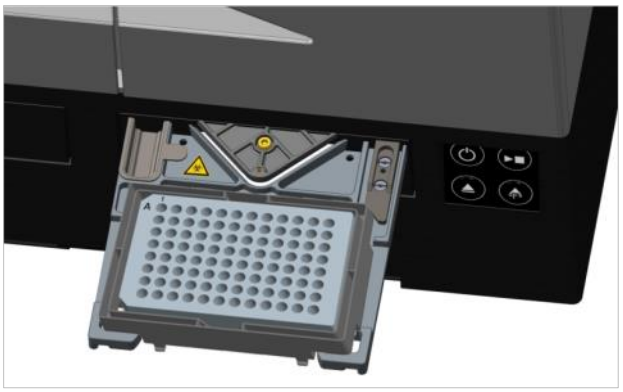


Figure 31: Microplate on the plate carrier with the A1 well in the upper left-hand corner

5. Start method.

**CAUTION:** Before starting measurements by using the humidity cassette, make sure that the microplate position and the cassette position A1 is inserted correctly. The position of well A1 must be on the upper left side.

**WARNING:** Do not fill more water into the reservoirs than recommended to avoid spill over.

**WARNING:** Before the humidity cassette is placed on the plate transport ensure that the cassette lid closes properly.

6. After the end of the run and the plate carrier has been moved out, the humidity cassette containing the sample microplate can be easily removed from the plate carrier. Remove the lid of the cassette and put the lower part of the cassette containing the microplate on the unloading tool to easily remove the plate from the cassette.

The humidity cassette can be cleaned by using 70 % ethanol or sterilized at maximum 125 ° C.

The unloading tool is located in the original packaging of the humidity cassette underneath the lower part of the humidity cassette. It has been cut from the material of the packaging, but not removed. Remove the foam piece by pushing it out.

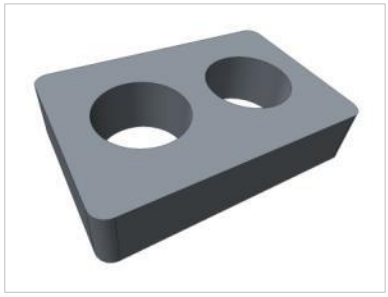


Figure 32: Unloading tool (Part of packaging)

### 17.4.3 Software Settings

The humidity cassette can be selected within the Plate strip.

**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.

#### 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.

## 17.5 Environmental Control Specifications

**NOTE**: All specifications are subject to change without prior notification.

### 17.5.1 Heating

| Parameters | Characteristics |
|---|---|
| Heating range | +3° C above ambient up to +42 ° C |
| Heating range with active gas control | +3° C above ambient up to +42 ° C |
| Heating uniformity | < 0.5° C between 30° C and 37° C at incubation position |
| Environmental operating conditions | +15 ° C to +35 ° C |

### 17.5.2 Cooling

| Parameters | Characteristics |
|---|---|
| Cooling range | +18° C up to +42° C |
| Cooling uniformity over a 96-well plate | < 1° C at a plate temperature between 18° C and 37° C |
| Environmental operating conditions | +18° C above ambient up to +30° C |

### 17.5.3 Gas Control

| Parameters | Characteristics |
|---|---|
| $CO_2$ concentration range | 0.04 % to 10 % volume |
| $CO_2$ concentration accuracy | < 1 % |
| $O_2$ concentration range | 0.1 % to 21 % volume (imprecise regulation below 0.5 % and below 0.8 % with active cooling) |
| $O_2$ concentration accuracy | < 0.5 % |

**NOTE**: Measurement accuracy of the $CO_2$ sensor below 0.1 % of gas concentration gets imprecise.
Sensitivity of the $CO_2$ sensor below 0.1 % of gas concentration is imprecise.

### 17.5.4 Humidity Control

| Parameters | Characteristics |
|---|---|
| 96-well plate with lid, 4 days incubation at +37 ° C with 5 % $CO_2$ | Evaporation < 10 % (excluding the outside wells; first and last column, first and last row) |
| Operating condition | +18 ° C to +42 ° C |

# 18 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.

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.

For further details we refer to the SparkControl manual.

## 18.1 Nucleic Acid Quantitation App

**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.

**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.

**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 must be repeated.

### 18.1.1 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 must be repeated and the measurement of the samples is prevented. The wells displaying values exceeding the allowed CV-threshold are highlighted.

### 18.1.2 Repeat Blanking

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

**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.

### 18.1.3 Start Measurement

For further details we refer to the SparkControl manual.

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

## 18.2 Labeling Efficiency App

For further details we refer to the SparkControl manual.

## 18.3 Protein Quantitation App

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

**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:** 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 must be repeated.

### 18.3.1 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.

### 18.3.2 Repeat Blanking

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




**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.

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

## 18.4 NanoQuant Maintenance

In 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:

### 18.4.1 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.

### 18.4.2 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 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!

# 19 Cell Counting in Cell Chips

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 must 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 homogenous. Avoid any dye particles because they can influence data analysis.

We refer to the SparkControl manual for a detailed description.




**CAUTION:** Cell chips are disposables 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.




**CAUTION:** Do not use the cell chip adapter without springs! Measurement errors may result.




**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.



**NOTE:** A smaller cell size speeds up the image analysis time.



**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.




**CAUTION:** Recalculated data is not saved automatically. Select **Export** in the action bar after the recalculation procedure to avoid data loss.

# 20 Cuvette App

The Cuvette App is designed for routine absorbance and absorbance scan endpoint measurements performed in a cuvette within a cuvette port.

For further details we refer to the SparkControl manual.



**NOTE:** The Prepare Instrument measurement must be performed every time a measurement with new measurement parameters is started. Please make sure that the cuvette port is empty.



**NOTE:** The selection Edit parameters will close the current measurement session. The Prepare Instrument measurement must be repeated.

# 21 Troubleshooting

## 21.1 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.<br>Switch off/on device and retry. |
| Steploss error for motor 'motor' | Actuator failure; checked after measurement | Report to Tecan (results not trustworthy).<br>Switch off/on device and retry. |
| Motor 'motor' not initialized | Actuator failure; checked before measurement | Report to Tecan.<br>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 |

| Error | Description | Possible solution / workaround |
|---|---|---|
| SCI timeout, Submodule 'sub-module' | Device error (SCI) | Report to Tecan |
| Injector timeout | Timeout while communication to injector module | Report to Tecan.<br>Switch off device. Check injector cabling. Switch on device and retry. |
| Injector communication error | Communication error device - injector module | Report to Tecan.<br>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 |
| 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.<br>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.<br>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).<br>Switch off/on device.<br>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 |

| Error | Description | Possible solution / workaround |
|---|---|---|
| Instrument not free | Device blocked by other process | Be sure that no other program uses the device.<br>Eventually restart computer. |
| Instrument could not be acquired | Device blocked by other process | Be sure that no other program uses the device.<br>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 |
| 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 |

| Error | Description | Possible solution / workaround |
|---|---|---|
| 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.<br>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 |

| Error | Description | Possible solution / workaround |
|---|---|---|
| **General errors** | | |
| Database doesn't exist! | Cannot open database | Re-install program |
| 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).<br>Switch off/on device.<br>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).<br>Switch off/on device.<br>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).<br>Switch off/on device.<br>Restart services by clicking the tray icon "SPARKCONTROL Agent" with right mouse button (context menu) and selecting "Restart Services".<br>If problems persist, contact Tecan. |
| Instrument 'device' is defective. | Defective device detected | Report to Tecan |

| Error | Description | Possible solution / workaround |
|---|---|---|
| **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) |
| 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) |

| Error | Description | Possible solution / workaround |
|---|---|---|
| **Spark-Stack related errors** | | |
| Input column 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 column 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 magazines are 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. |
| No plate detected during stacker run in input column or for restacking in output column.<br>(Error:...Stacker get/stack column_Input/Output ⋯) | There is no plate on the lifting table of the stacker or the plate transport. | Report to Tecan.<br>Switch off the instrument.<br>Remove input and output magazine s.<br>If necessary, remove the plate from the lifting table of the stacker.<br>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.<br>Make sure that microplates are not damaged.<br>Restart the stacker run. |

| Error | Description | Possible solution / workaround |
|---|---|---|
| Initialization error<br>Steploss error | Actuator failure while initializing the stacker. | Report to Tecan.<br>Switch off the instrument.<br>Remove input and output magazine s.<br>If necessary, remove the plate from the lifting table of the stacker.<br>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.<br>Restart the stacker run. |
| Power Failure | Power supply interrupted | Report to Tecan.<br>Switch off the instrument.<br>Remove input and output magazines.<br>If necessary, remove the plate from the lifting table of the stacker.<br>After power is available again:<br>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.<br>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.<br>Restart services. See chapter 21.2 Spark Services Manager. |

## 21.2 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.

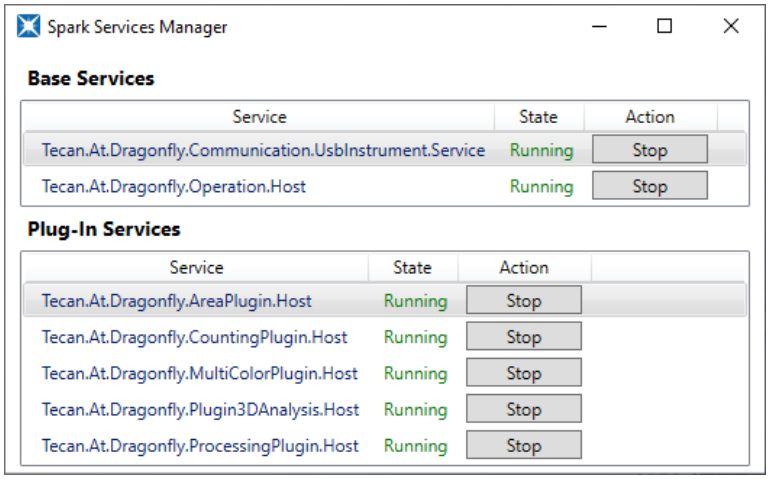
In Windows 10 and Windows 11, the System-Tray (also known as the Notification Area) is located typically on the right side of the taskbar:




Figure 33: 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.

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# 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.)

TECAN AUSTRIA GMBH, Untersbergstrasse 1a, A-5082 Grödig / Salzburg, Austria
T +43 62 46 89 33, F +43 62 46 72 770, office.austria@tecan.com, www.tecan.com

# Declaration of Conformity

We, TECAN Austria GmbH herewith declare under our sole responsibility that the product identified as:

**Product Type:** **Microplate Reader**
**Model Designation:** **SPARK**

**Article Numbers:** **30086376**

Address: Tecan Austria GmbH
Untersbergstr. 1A
A-5082 Grödig, Austria

is in conformity with the provisions of the following European Directive(s) when installed in accordance with the installation instructions contained in the product documentation:

- **EMC Directive**
- **Machinery Directive**
- **RoHS Directive**

is in conformity with the relevant U.K. legislation for UKCA-marking when installed in accordance with the installation instructions contained in the product documentation:

- **Electromagnetic Compatibility (EMC) Regulations**
- **Supply of Machinery (Safety) Regulations**
- **The Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment Regulations**

The current applicable versions of the directives and regulations as well as the list of applied standards which were taken in consideration can be found in separate CE & UK declarations of conformity.