TECAN.

Instructions for Use for

INFINITE F500

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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.

We would appreciate any comments on this publication.

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Copyright Information

Declaration for EU Certificate

See the last page of these Instructions for Use.

About the Instructions for Use

Original Instructions. This document describes the INFINITE F500 multifunctional microplate reader. It is intended as reference and instructions for use.

This document instructs 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 application has changed.

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- HTRF® is a registered trademark of CisBio International

Warnings, Cautions and Notes

The following types of notices are used throughout 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

INDICATES THE POSSIBLE PRESENCE OF BIOLOGICALLY HAZARDOUS MATERIAL. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.

ATTENTION

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.**

Symbols

	Manufacturer's address

Table of Contents

1. Safety

1.1 Instrument Safety

1. Always follow basic safety precautions when using this product to reduce the risk of injury, fire, or electrical shock.
2. Read and understand all information in the Operating Manual. Failure to read, understand, and follow the instructions in the manual may result in damage to the product, injury to operating personnel or poor instrument performance.
3. Observe all WARNING and CAUTION statements in the manual.
4. Never open the INFINITE F500 while the instrument is plugged into a power source.
5. Never force a microplate into the instrument.
6. The INFINITE F500 is intended as a general purpose laboratory instrument for professional use. Observe proper laboratory safety precautions, such as wearing protective clothing and using approved laboratory safety procedures.

Caution

Tecan Austria GmbH has taken great care when creating the stored Plate Definition Files that are received with the instrument software.

We take every precaution to ensure that the plate heights and well depths are correct according to the defined plate type. This parameter is 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 occurring to the measurement chamber as a result of small changes in plate height. This does not affect the performance of the instrument.

Users MUST ensure that the plate definition file selected corresponds to the actual plate being used.

Users should also take care that no potential fluorescent or luminescent contamination lies on top of the plate, for example droplets and also be aware that some plate sealers leave behind a sticky residue that should be removed before reading.

Caution

Before starting measurements, make sure that the microplate position A1 is inserted correctly. The position of well A1 has to be on the upper left side.

Caution

To insure the optimal working of Tecan instruments we recommend a service interval of 12 months.

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:

1. National industrial protection law
2. Accident prevention regulations
3. Safety data sheets of the reagent manufacturers

WARNING

DEPENDING ON THE APPLICATIONS, PARTS OF THE INFINITE F500 MAY COME IN CONTACT WITH BIOHAZARDOUS / INFECTIOUS MATERIAL. MAKE SURE THAT ONLY QUALIFIED PERSONNEL IS OPERATING THE INSTRUMENT. IN CASE OF SERVICE OR WHEN DISLOCATING OR DISPOSING THE INSTRUMENT, ALWAYS DISINFECT THE INSTRUMENT ACCORDING TO THE INSTRUCTIONS GIVEN IN THIS MANUAL.

2. General Description

2.1 Instrument

The Tecan INFINITE F500 is a multifunctional microplate reader with injector option. The INFINITE F500 provides high performance for the vast majority of today's microplate applications and research and is robotic compatible.

2.1.1 Intended Use

The INFINITE F500 is intended as a general purpose laboratory instrument for professional use, supporting common microplates conforming to the ANSI/SBS standards (see 2.1.2 Multifunctionality for further details).

Note
<u>System Validation by Operating Authority is Required</u>

The INFINITE F500 has been validated on a selected set of assays.

It is the responsibility of any operating authority to ensure that the INFINITE F500 has been validated for every specific assay used on the instrument.

2.1.2 Multifunctionality

The fully equipped INFINITE F500 provides the following measurement techniques:

- Fluorescence Intensity (FI) Top
- Fluorescence Intensity (FI) Bottom
- Fluorescence Time Resolved (TRF)
- Flash Fluorescence
- Fluorescence Polarization (FP)
- Absorbance
- Absorbance with injectors
- Glow Type Chemi- or Bioluminescence
- Bioluminescence Resonance Energy Transfer (BRET)
- Flash Luminescence

Any common microplate ranging from 6 to 384-well formats conforming to the ANSI/SBS standards (ANSI/SBS 1-2004; ANSI/SBS 2-2004, ANSI/SBS 3-2004 and ANSI/SBS 4-2004) may be measured with any of the above measurement techniques. The 1536-well formats conforming to the ANSI/SBS standards can also be used for all measurements techniques except Fluorescence Intensity Bottom and applications with injectors.

Switching between measurement techniques or plate formats is fully automated via software. It is not necessary to manually reconfigure the optics in order to switch between the reading modes supported by the INFINITE F500.

The instrument may be equipped with up to two injectors.

2.1.3 Performance

The INFINITE F500 has been designed to be sensitive while fast. Specifications of sensitivity or precision are related to the corresponding measurement time per microplate.

The INFINITE F500 provides a range of parameters for optimizing the measurement results according to the assay type (cell-based or homogeneous), the microplate type, and the dispensed volumes per well and dispensing speeds.

2.1.4 User Friendliness

The INFINITE F500 offers unparalleled flexibility for the customization of fluorescence and absorbance measurements; filter slides containing fluorescence and absorbance interference filters are easily accessible to the user.

While almost any dye could be measured using the integrated beam splitter (50% reflective mirror), a few common dyes profit from the built in dichroic mirrors. If your favorite dyes are not covered by the INFINITE F500 standard dichroics and filters, please contact your local Tecan representative. If necessary, a custom dichroic mirror can be easily mounted in the front of the mirror carrier.

Note

If the instructions given in this manual are not correctly performed, the instrument will either be damaged or the procedures will not be performed correctly and the safety of the instrument is not guaranteed.

2.1.5 System Requirements

Minimum

- Pentium PIII 1 GHz
- 20 GB HDD
- 512 MB RAM
- 1 x USB 1.1 or 2.0
- CD ROM Drive
- Screen Resolution: 1024 x 768

Recommended

- Pentium P4 2 GHz
- 40 GB HDD
- 1024 MB RAM
- 2 x USB 1.1 or 2.0, 1 x RS232
- CD ROM Drive
- Screen Resolution: 1280 x 1024

Operating System Requirements

- Windows XP Professional (English), Service Pack 2 (Minimum: Service Pack 1)
- Microsoft Excel 2003 (English) for i-control (Minimum: Excel 2000 or 2002)

2.2 Measurement Techniques

The following sections provide an introduction to the INFINITE F500 measurement techniques when fully equipped. To keep this compact, a few simplifications have been made. For details see the references.

2.2.1 Fluorescence

The INFINITE F500 offers the basic fluorescence measurement technique and some even more sophisticated variants:

A. Fluorescence Intensity (or simply Fluorescence)

B. Fluorescence Resonance Energy Transfer (FRET)

C. Time Resolved Fluorescence (TRF)

D. Homogeneous Time Resolved Fluorescence (HTRF®)

E. Fluorescence Polarization (FP)

Many assays rely on the measurement of the fluorescence intensity, either from spontaneous emission (FI), or a time delayed emission (TRF). However, the signal may be influenced by many factors: Inner filter effect, scattering due to sample turbidity, bleaching of the dye, volume and meniscus effects may all overlay the true signal and may lead to misinterpretation of the data. More robust are the ratiometric methods FP and HTRF where some of these effects do not occur.

TRF should not be confused with Fluorescence Lifetime Measurements.

Fluorescence Theory

Fluorescent molecules emit light of specific wavelength when struck by light of shorter wavelength (Stokes Shift). In particular, a single fluorescent molecule can contribute one fluorescence photon (quantum of light). This is a part of the energy, which has been absorbed before (electronic excitation), but could not be released quickly enough into thermal energy.

The average time it takes between excitation and emission is called the fluorescence lifetime. For many fluorescent molecular species, fluorescence lifetime is on the order of nanoseconds (prompt fluorescence). After excitation, fluorescence emission occurs with a certain probability (quantum yield), which depends on the fluorescent species and its environmental conditions.

For a detailed treatise on fluorescence techniques and applications see:

Principles of Fluorescence Spectroscopy by Joseph R. Lakowicz, Plenum Press.

A) Fluorescence Intensity (FI)

In many microplate applications, the intensity of fluorescence emission is measured to determine the abundance of fluorescent labeled compounds. In these assays, other factors having an influence on fluorescence emission need to be controlled experimentally. Temperature, pH-value, dissolved oxygen, kind of solvent etc. may significantly affect the fluorescence quantum yield and therefore the measurement results.

B) Fluorescence Resonance Energy Transfer (FRET)

Some microplate applications utilize a sophisticated dual labeling strategy. The FRET effect enables you to measure how many of two differently labeled compounds are in close proximity. This makes it suitable for binding studies.

Basically, FRET is a fluorescence intensity measurement of one of the two fluorescent labels (acceptor). However, the acceptor is not susceptible to the excitation wavelength of the light source being used. Instead, the acceptor may receive excitation energy from the other fluorescent label (donor), if both are spatially close together. As a prerequisite, the excitation wavelength has to apply to the donor. Secondly, the emission spectrum of the donor has to overlap the excitation spectrum of the acceptor (resonance condition). Nevertheless, the transfer of excitation energy from donor to the acceptor is radiation free.

Some FRET-based applications utilize suitable pairs from the fluorescent protein family, like GFP/YFP (Green/Yellow Fluorescent Protein, (ref. **Using GFP in FRET-based applications** by Brian A. Pollok and Roger Heim – trends in Cell Biology [Vol.9] February 1999). Overview is given in the Review Article – **Application of Fluorescence Resonance Energy Transfer in the Clinical Laboratory: Routine and Research** by J. Szöllösi et al. in Cytometry 34, page 159-179 (1998).

Other FRET-based applications take advantage from using TRF labels as the donor. For example see, **High Throughput Screening** – Marcel Dekker Inc. 1997, New York, Basel, Hong Kong, section 19 Homogeneous, Time-Resolved Fluorescence Method for Drug Discovery by Alfred J. Kolb et al.

C) Fluorescence Time Resolved (TRF)

TRF applies to a class of fluorescent labels (chelates of lanthanides like Europium, [ref. **Europium and Samarium in Time-Resolved Fluoroimmunoassays** by T. Stâhlberg et. al. - American Laboratory, December 1993 page 15]), some of them having fluorescence lifetimes in excess of 100 microseconds. The INFINITE F500 uses a flash lamp light source with flash duration much shorter than fluorescence lifetime of these species. This offers the opportunity to measure fluorescence emission at some time, when stray light and prompt fluorescence have already vanished (Lag Time). Thus, background can be significantly lowered while sensitivity is improved.

The benefits of TRF consequently apply to assays using multiple labels with different fluorescence lifetimes.

D) Homogeneous Time Resolved Fluorescence (HTRF®)

HTRF® technology (CIS bio international, France) combines both, time-gated fluorescence (commonly referred to as time-resolved fluorescence = TRF) and fluorescence resonance energy transfer (FRET). HTRF® is based on the energy transfer between two fluorescent labels, a long-lifetime Eu^{3+}-cryptate donor and the XL665 acceptor (chemically modified allophycocyanin). The main benefit of time gated measurement is the efficient reduction of background fluorescence by temporal discrimination. The addition of energy transfer further minimizes several undesired assay interferences and side effects (e.g. volume/meniscus, quenching, light scattering, autofluorescence, molecular size, etc.). Furthermore, the homogeneous format of these assays, so called ‘mix and measure’ protocols, satisfies the demand from the industry for one-step, non-separating applications for high throughput screening (HTS).

The measurement is based on sequential detection of donor intensity (620 nm) and acceptor intensity (665 nm) using the multilabeling setup as listed in section 7.3.5 Homogeneous Time Resolved (HTRF®). A ratio of the two intensities (acceptor: donor) is calculated and the relative energy transfer rate for each sample is determined as Delta F (%). The fluorescence ratio is a correction method developed by CIS bio international, which application is limited to the use of HTRF® reagents and technology, and for which CIS bio international has granted a license to Tecan. The method is covered by the US patent 5,527,684 and its foreign equivalents.

E) Fluorescence Polarization (FP)

Fluorescence Polarization (FP) measures rotational mobility of a fluorescent labeled compound. FP is therefore particular suitable for binding studies, because the tumbling motion of small molecules may be dramatically slowed down after binding to a larger molecule.

Fluorescence polarization measurements are based on the detection of the depolarization of fluorescence emission after excitation of a fluorescent molecule by polarized light. A fluorescent molecule can be visualized as an antenna. Such a molecule can absorb energy if and only if the polarization of the excitation light matches the orientation of the antenna. During the fluorescence lifetime, i.e. the time a molecule remains in the excited state, small molecules diffuse rotationally relatively rapidly. Hence they re-orient before they emit their photon. As a result and due to the random character of diffusion, a linearly polarized excitation light will be translated into a less polarized emission light. Thus, a high resultant mP value denotes the slow rotation of the labeled molecule, indicating that binding probably did occur. A resultant low mP value denotes a fast rotation of a molecule, indicating that binding probably did not occur.

The FP measurement result is calculated from two successive fluorescence intensity measurements. They differ in the mutual orientation of polarizing filters, one being placed behind the excitation filter, another ahead of the emission filter. By processing both data sets, it is possible to measure the extent of how much the fluorescent label has changed orientation in the time span between excitation and emission.

2.2.2 Absorbance

Absorbance is a measure for the attenuation of monochromatic light when transmitted through a sample. Absorbance is defined as:

$A = LOG_{10} (I_0/I_{SAMPLE})$,

Where I_{SAMPLE} is the intensity of the light being transmitted, I_0 the light intensity not attenuated by sample. The unit is assigned with Optical Density (O.D.)

Thus, 2.0 O.D. means $10^{2.0}$ or 100-fold attenuation (1% transmission),

1.0 O.D. means $10^{1.0}$ or 10-fold attenuation (10% transmission), and

0.1 O.D. means $10^{0.1}$ or 1.26-fold attenuation (79.4% transmission).

If the sample contains only one species absorbing in that narrow band of wavelengths, the background corrected absorbance (A) is proportional to the corresponding concentration of that species (Lambert-Beer's Law).

2.2.3 Luminescence

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Glow Type Chemi- or Bioluminescence

The INFINITE F500 provides measurement of glow type chemi- or bioluminescence. Glow type means that the luminescence assay glows much longer than a minute. Luminescence substrates are available, which provide stable enough light output over hours.

As an example, luminescence can be measured to determine the activity of an enzyme labeled compound (-peroxidase, -phosphatase). Light emission results from a luminescence substrate being decomposed by the enzyme. Under excess of substrate the luminescence signal can be assumed to be proportional to the abundance of the enzyme labeled compound. Additionally, when working with enzyme-based assays, the control of environmental conditions is very important (temperature, pH-value).

For practical aspects of luminescence assays see the following example:

Bioluminescence Methods and Protocols, ed. R.A. LaRossa, Methods in Molecular Biology 102, Humana Press, 1998

Bioluminescence Resonance Energy Transfer (BRET)

BRET is an advanced, non-destructive, cell-based assay technology that is perfectly suited for proteomics applications, including receptor research and the mapping of signal transduction pathways. BRET is based on energy transfer between fusion proteins containing *Renilla* luciferase (Rluc) and a mutant of the Green Fluorescent Protein (GFP). The BRETsignal is generated by the oxidation of p.a. DeepBlueC™, a coelenterazine derivative that maximizes spectral resolution for superior sensitivity. This homogeneous assay technology provides a simple, robust and versatile platform with applications in basic academic as well as applied research.

Flash Luminescence

In flash type luminescence assays the measurement is only done during the dispensing of the activating reagent or after a short delay time.

Over the past years luminescence substrates have been improved towards providing more stable signals. In so-called glow type luminescence assays the luminescence signal is spread over a wide time scale (e.g. a half-life of 30 min.)

Flash Type Luminescence with Injectors

Flash type luminescence is one of the measurement modes that can be performed with injectors.

Note

The plate detection sensor is only active if one of the injectors is in use (strips "injection" or "dispense").

Caution

During luminescence measurements it is necessary to close the lid covering the syringes and bottles of the reagent system.

2.3 Injectors

The INFINITE F500 can be optionally equipped with an injector module located system internally. The injector module consists of one or two syringe pumps (XCalibur, Tecan Systems), which feed one to three injector needles. The injector needles are designed to inject liquid into any well with a diameter equal to or larger than a standard 384-well plate.

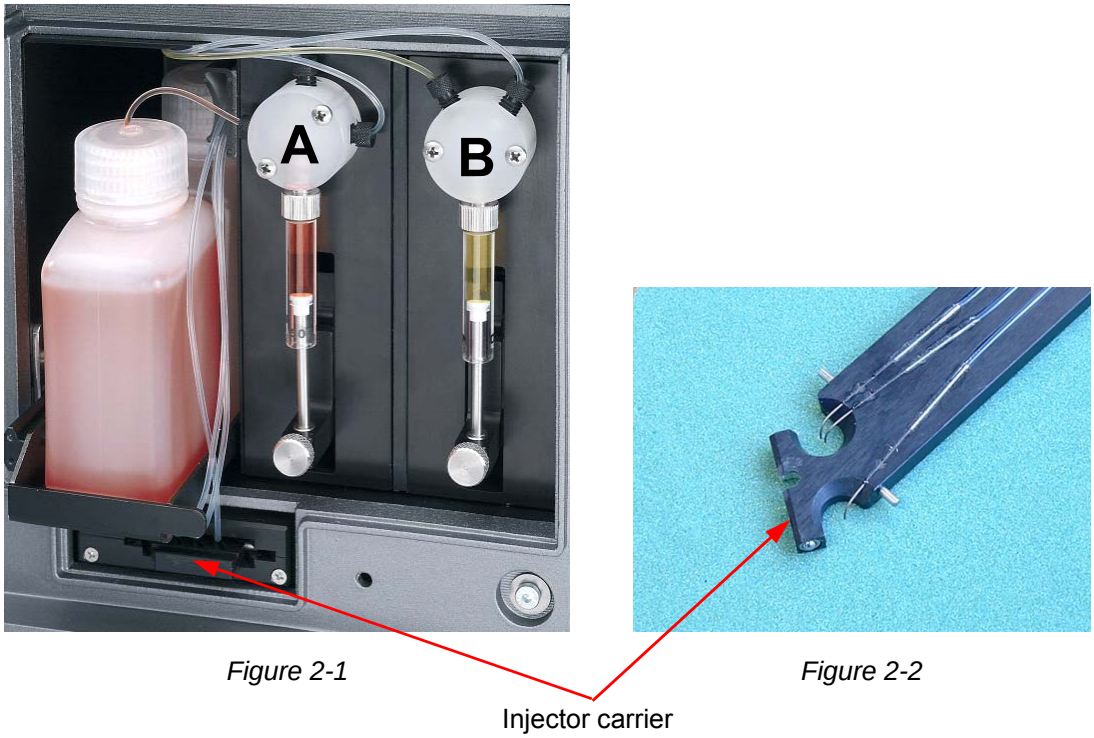


Figure 2-1 *Figure 2-2*

Figure 2-1,2-2: View of the injector module

The INFINITE F500 can be equipped with one pump (always pump A) or two pumps (pumps A and B) (see Figure 2-1 Figure 2-2 above).

One Injector Option (Injector A)

The INFINITE F500 is equipped only with Injector A permitting injections in SBS-conform microplate well types with a diameter equal to or larger than that of a 384-well plate. The injector A consists of one syringe with two injector needles: one for injection into plate formats up to 96-well and the second for the 384-well plates. This option is suitable for, for example, reactions requiring injection of only one liquid per well.

Two Injector Option (Injector A and Injector B):

Several reactions, such as flash luminescence reactions or dual reporter gene assays require the injection of two *independent* liquids into the same well. This is achieved by using the additional injector pump B.

By using pumps A and B, two independent liquids can be injected/dispensed into the same well with a diameter equal to or larger than that of a standard 384-well plate.

2.3.1 Measurement with Injectors

The injectors of the INFINITE F500 can be used with the following measurement modes: Fluorescence Intensity Top and Bottom, Time Resolved Fluorescence, Fluorescence Polarization, Absorbance, Flash and Glow Type Luminescence and Dual Color Luminescence.

For flash luminescence reactions, INFINITE F500 allows injection at the measurement position, i.e. no movement of the plate between injection and measurement, for both injectors, i.e. injector A and injector B, when using 96-well plates and only for injector A for luminescence measurements with a 384-well plate. The injection of liquids with the two injectors is performed sequentially.

Similar, Fluorescence Intensity Bottom measurements can be performed directly after the injection, i.e. without moving the plate between injection and measurement, for both injectors with 96-well plates, and in contrast to luminescence, only for injector B with 384-well plates.

In all other measurement modes used with injectors, i.e. Fluorescence Intensity Top, Time Resolved Fluorescence, Absorbance, Fluorescence Polarization, Luminescence (Injector B, 384-well plates) and Fluorescence Intensity Bottom (Injector A, 384-well plates), the measurement position is not the same as the injector position, and therefore a short time delay (approx. < 500 ms) between injection and reading occurs.

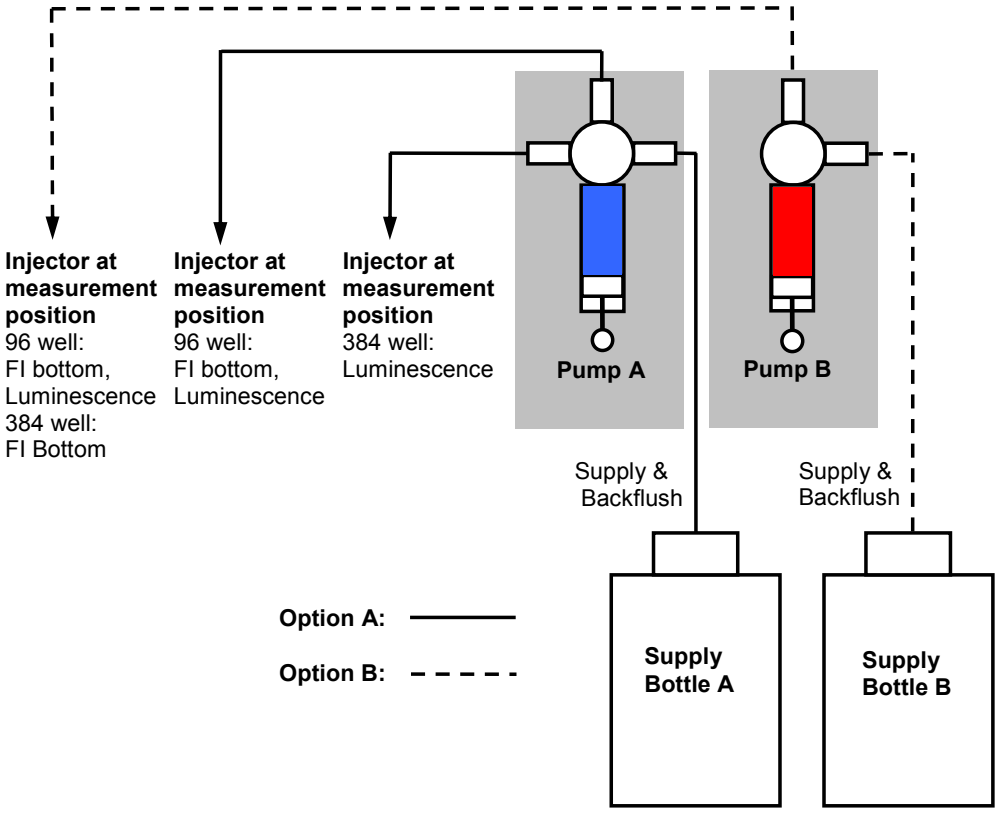


Figure 2-3: Liquid path in INFINITE F500 with two pumps

For details on how to set up a measurement with injectors please refer to chapter 2.4.1 i-control and Injectors.

2.3.2 Storage Bottles

The injector drawer may hold up to two 125 ml bottles. An adapter for smaller tubes allows using tubes of different size (1.5 ml, 15 ml, 50 ml tubes etc.)

The standard bottle set supplied with the Injector option consists of:

- Two 125 ml bottle and one 15 ml bottle for the “One Injector Option” (one pump) or
- Four 125 ml bottles and two 15 ml bottles for the “Two Injectors option” (two pumps).

Figure 2-4: Storage bottles and adapter for smaller tubes

2.3.3 Injector/Injector Carrier

The carrier which includes up to three injection needles and the tubing can be easily removed (by the customer) from the instrument for priming or washing the system and for optimizing the injection speed.

Figure 2-5: Injector carrier with injector in 'service position'. The injectors are removed from the carrier slot and rotated 180° so that the injector needles face away from the instrument.

When using the injector during a measurement or for just dispensing a plate the injector carrier must be inserted correctly into the instrument. The instrument contains an injector sensor that checks that the position of the injector carrier for the actions 'inject' and 'dispense' is correct.

If the injector carrier is not inserted correctly the injector sensor does not recognize the inserted carrier and neither dispensing nor injection is possible.

Note that the injector carrier is locked during measurements and automatically unlocked at the end of the measurement.

Caution
Never touch the injector pumps during operation.

Caution
The injector carrier must be in the service position for washing and priming. ‚Prime' and ‚Wash' must not be performed when the injector is in the instrument!

Caution
If the injector carrier is not inserted correctly in the injector port, the injector sensor will not detect the inserted injector and therefore washing and priming will be enabled, which can damage the instrument.

Injector A

The injector A consists of one syringe with two injector needles: one for injection into plate formats of up to 96-well and the second for injection into plate formats up to 384-well.

Injector B

The injector B consists of one syringe and one needle that can be used for all allowed plate formats.

Injection Speed

The injection speed can be adjusted via the software to allow for good mixing of reagents. The optimum injection speed depends on the assay parameters, such as viscosity of fluids, the plate format and the measuring behavior of the liquids.

Dead Volume

The dead volume of the injection system (injector needles, valves and tubing) without syringes is < 1.0 ml. The dead volume will correspondingly increase depending on which syringe used.

Before starting a measurement make sure that:

1. The tubes are clean. If not please refer to chapter Priming and Washing of the INFINITE F500 for details how to clean the injector system.
2. The injector tubes are correctly inserted into the storage bottles and fixed.
3. The injector system is primed. It is not possible to start a measurement without priming the system.

When priming the system:

1. Check the tubes for leaks.
2. Check the tubes for kinks.
3. Make sure that the injector needles are not twisted.

If the tubes require replacement for any reason, after the tubes have been changed do not forget to perform washing and priming before starting a measurement.

Priming and Washing of the INFINITE F500

Caution

The injector carrier must be in the service position for washing and priming. 'Prime' and ,Wash' must not be performed when the injector is in the instrument

Caution

The priming step has to be performed with the regents used for the measurement afterwards.

Caution

Make sure that the storage bottles are filled with sufficient amount of reagents needed for the measurement afterwards.

The initial filling step of the injector system (priming) as well as the cleaning step of the injector system (washing) must take place outside of the instrument. For these procedures the injector carrier is removed from the carrier slot and rotated 180° so that the injector needles face away from the instrument and then partially reinserted into the slot for support. For priming and washing 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 i-control software.

For the initial filling step of the injector system (priming) it is recommended to use at least 1500 µl to remove all air bubbles from the injection system when using a standard 500 µl injector syringe. The recommended minimum prime volume is therefore 1500 µl. To save precious reagents, this initial filling step can be performed with distilled water. To replace the water with the required reagent, a second priming step is needed. For this second priming step, the priming volume can be reduced to approx. 1000 µl.

Note that the prime volume depends on the syringe used and must be correspondingly increased for a 1 or 2.5 ml syringe.

Caution

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

If the injector carrier is not inserted correctly in the injector port, the injector sensor does not detect the inserted injector and therefore washing and priming is enabled which can damage the instrument. In addition to this, the actions 'dispense' and 'inject' will not be possible.

Priming

Before the injection system can be used, an initial filling step (priming) is needed to remove all air and to completely fill the system with liquid.

It is recommended to perform a washing step before priming.

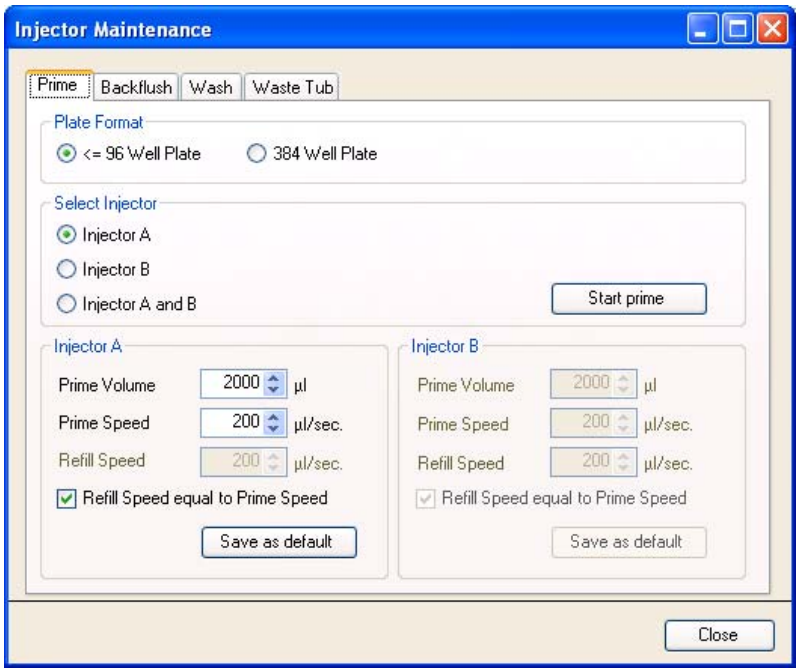
Priming is performed by using the i-control software:

Priming procedure (general):

1. Fill the storage bottles with the necessary reagents and insert the feeding tube(s). Make sure, that the tube(s) reaches the bottom of the bottle.
2. Remove the injector from the carrier slot and rotate it 180° so that the injector needles are facing away from the instrument and reinsert it partially into the slot for support (service position).
3. Put an empty container under the injector.

Priming procedure (i-control):

1. Adjust parameters at the prime tab of the injector maintenance dialog box in the settings menu
2. Activate the priming procedure by clicking the ‘Start prime’ button in the injector maintenance dialog box.
3. Visually inspect the syringes for air bubbles. Any bubbles should be removed after priming to ensure good injection performance.



Prime

Select one of the injectors **Injector A** or **Injector B** or **Injector A and B**:

Injector A: select the plate format (‘≤ 96-well Plate’ or ‘384-well Plate’)

Injector B: select ‘≤ 96 and 384-well Plate’

Injector A and B: select the plate format ‘≤ 96-well Plate’ or ‘384-well Plate’

Select the ‘**Prime Volume**’ (5 -10000 µl)

Select the ‘**Prime Speed**’ (100 - 300 µl/sec)

Select the ‘**Refill Speed**’ (100 – 300 µl/sec) or select ‘**Refill Speed equal to Prime Speed**’.

Start prime by clicking the ‘**Start prime**’ button.

Click the '**Save as default**' button to save the selected settings for the next priming procedure.

Select '**Close**' to exit the dialog box

Caution

Close the lid of the injector module completely before starting a measurement.

Priming Example – Operational Sequence of Pump System

The following example describes the operational sequence of the pump system when performing a prime step with 500 µl.

1) The system is already washed (syringe is empty; piston in upper position):
   - The first action is always that the syringe has to be filled completely with liquid. The piston therefore moves down to the lowest position to fill syringe completely (e.g. volume 500 µl for a 500 µl syringe).
   - Now the system prepares for the priming step. The piston makes space for the selected prime volume: The selected prime volume is ejected: 500 µl – the piston moves up.
   - The piston moves down again to prime the syringe with the selected volume. The syringe is now completely filled.
   - After finishing priming the last action is to eject ~ 5 µl. This step makes sure that the injection/dispense conditions are equal for each well (for details please refer to 'Waste tub')

Be aware that for selected prime volume of 500 µl, 1000 µl liquid are needed due to the initial filling step of the syringes.

2) The system is not washed (the syringe is partly filled with liquid, the piston is not in the upper position):
   - The syringe is always emptied first. The piston moves up to the highest position to empty the syringe.
   - The next actions are identical to 1), where the system is already washed.

Note

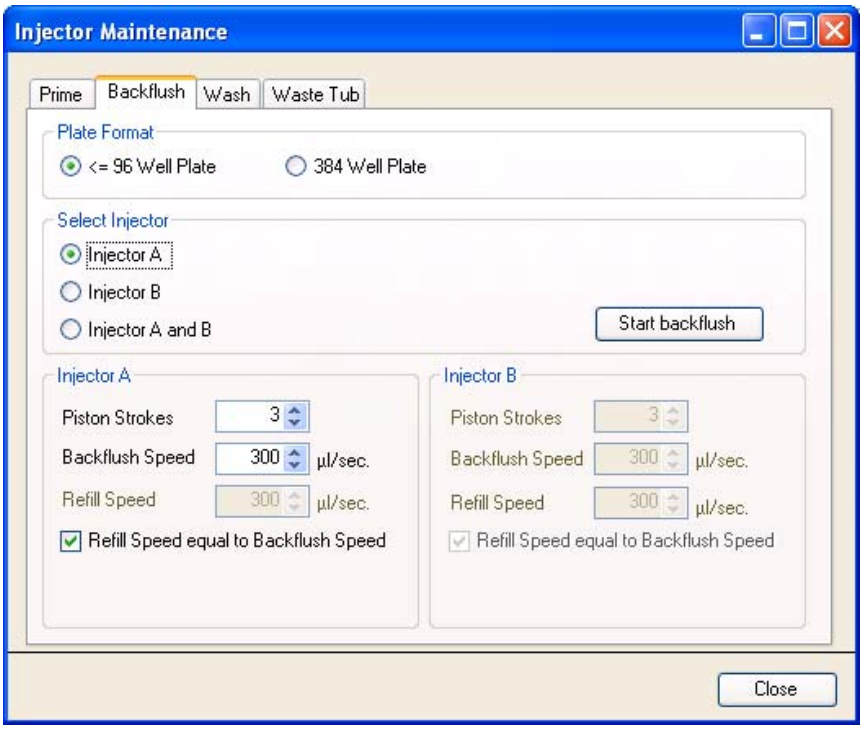
For a selected prime volume of 500 µl, a minimum volume of 1000 µl of liquid is required to perform a complete priming step when using a 500 µl syringe.

Reagent Backflush

The function of ***backflush*** is to return any unused reagent to the reservoir bottles. Reagent backflush allows reagents in the tubing system to be pumped back into storage bottles. This action can be performed optionally prior to washing the injector system to minimize the dead volume.

Reagent backflush procedure:

1. Remove the injector carrier from the instrument and insert the injector carrier into the service position.
2. Insert the feeding tubing into the appropriate storage bottle.
3. Adjust parameters on the **Backflush** tab of the **Injector Maintenance** dialog box in the **Settings** menu
4. Start the reagent backflush procedure by clicking **Start backflush**.



Backflush

Select one of the injectors **Injector A** or **Injector B or Injector A and B** (only 'primed' injectors are available for 'backflush'):

Injector A/Injector A and B: select the plate format '≤ 96-well Plate' or '384-well Plate'

Select the '**Piston Strokes'** (1 – 20)

Select the '**Backflush Speed'** (100 - 300 µl/sec)

Select the '**Refill Speed**' (100 – 300 µl/sec.) or select the '**Refill Speed equal to Backflush Speed'** check box.

Click '**Start backflush'** to start the reagent backflush procedure.

Click '**Close'** to exit the dialog box.

Caution

The injector carrier must be in the service position for the 'backflush' procedure.

Do not perform backflush when the injector is in the instrument!

Washing

Before the instrument is switched off, it is recommended to perform a wash procedure to clean the injector system.

Wash procedure:

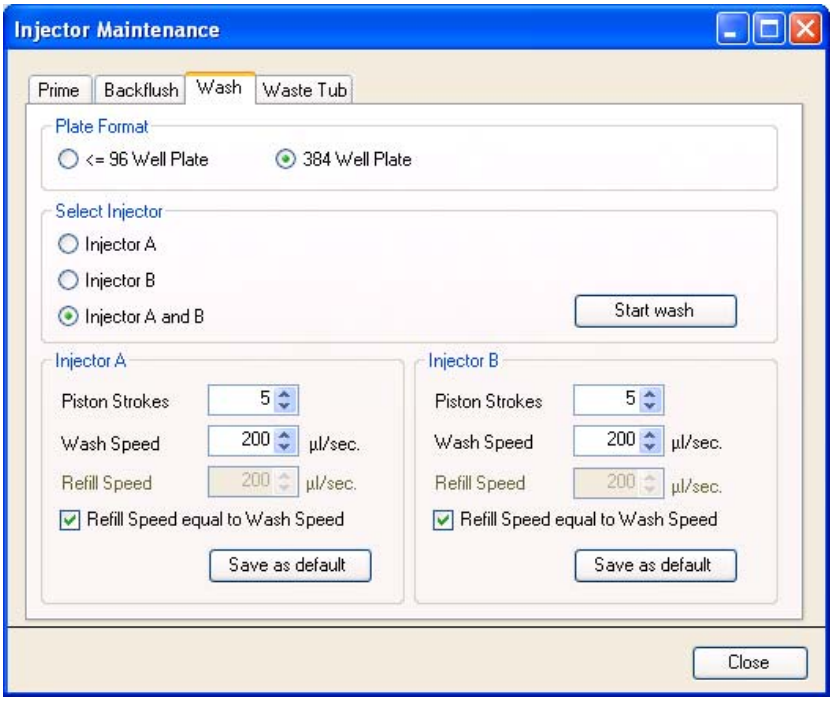
Washing can be performed by using the i-control software or by using the hardware buttons on the injector box.

Washing (general procedure):

1. Fill the storage bottles with the appropriate wash reagents (distilled water, 70 % ethanol, etc) and insert the feeding tube(s). Make sure, that the tube(s) reaches the bottom of the bottle.
2. Remove the injector from the carrier slot and insert it into the service position of the injector box.
3. Put an empty container under the injector.

Washing (i-control):

1. Adjust the parameters on the **Wash** tab of the **Injector Maintenance** dialog box in the **Settings** menu
2. Start the washing procedure by clicking the **Start wash**.



Wash

Select one of the injectors '**Injector A'** or '**Injector B'** or **'Injector A and B'**.

Select the '**Piston Strokes'** (1 – 20)

Select the '**Wash Speed'** (100 - 300 µl/sec)

Select the '**Refill Speed'** (100 – 300 µl/sec) or select '**Refill Speed equal to Wash Speed'**.

Click '**Start wash'** to start the wash procedure.

Click '**Close'** to exit the dialog box.

Caution

The injector carrier must be in the service position for the action 'wash'. Do not perform washing when the injector is in the instrument!

Important

Be sure to run a final wash procedure with distilled water and empty the injector system. For optimal care and long life fill the injector system with liquid (water) before turning off the instrument.

Important

Please see the corresponding reagent kit for advice on how to remove the substrate completely from the tubing system.

Important

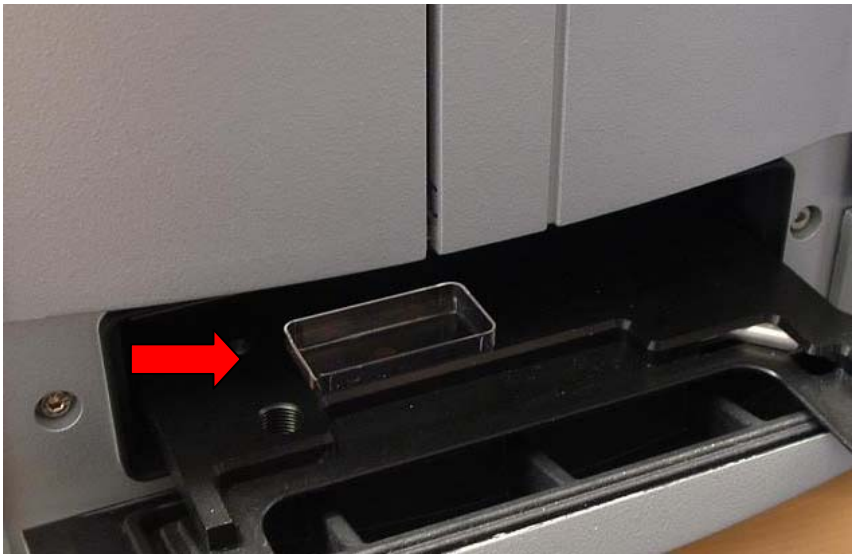
Take good care of the injectors, because if they are damaged the accuracy of dispensing may be affected. This can result in damage to the instrument.

Note

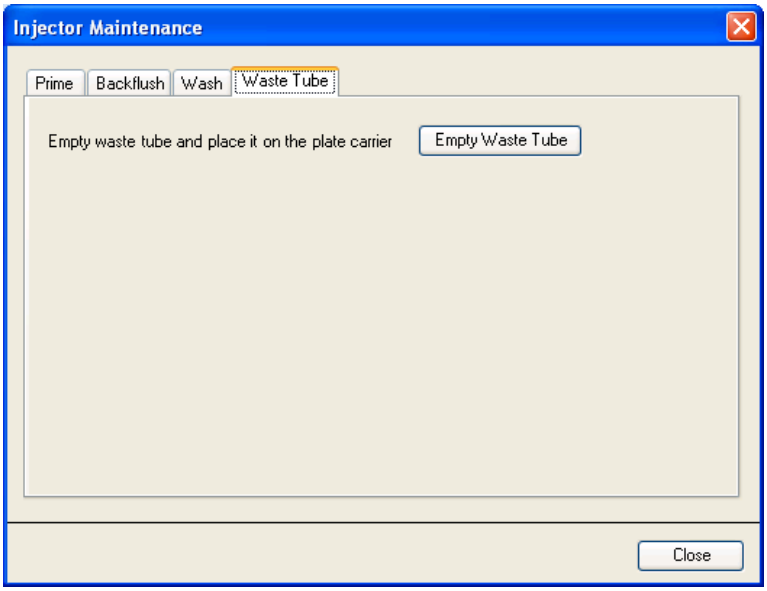
Injector needles can be replaced by exchanging the injector carrier together with the corresponding tubing.

Waste Tub

When starting a measurement with the actions ‘injection’ or ‘dispense’, 5 µl of liquid are dispensed into a disposable container on the plate carrier before starting ‘injection’ or ‘dispense’.
This initial dispense step makes sure that the injection/dispense conditions are equal for each well. This special dispense step depends on the selected refill mode selected on the injector or dispense strip (see chapter 2.4.1 i-control and Injectors for details).
When using ‘standard’ refill mode, the dispense step is performed after each refill.
When using ‘refill for every injection’ the dispense step is only performed once when starting the measurement.
The disposable waste container (waste tub) must therefore be emptied from time to time. The maximum filling volume is 3.0 ml. An internal counter checks the dispensed liquid volumes and the software alerts the user when it is time to empty the waste tub.



Picture 2-1: Waste tub on plate carrier



Waste tub:

Click the **'Empty Waste tub'** button and the plate carrier will move out automatically. Remove the waste tub and empty the contents. After the waste tub has been emptied place it back on the plate carrier. The i-control software will alert you when the waste tub needs to be emptied again.

Caution

Place the waste tub on the plate transport before starting a measurement with the actions 'injection' and/or 'dispense'.

Caution

It is recommended to empty the waste tub before starting a measurement and to empty it at least once a day.

WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF THE PROCESSES RUN ON THE INFINITE 500.

TREAT THE USED MICROPLATE, 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.

2.3.4 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.

Daily Maintenance:

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

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

Caution

Do not allow the pump(s) to run dry for more than a few cycles.

Weekly/Periodical Maintenance:

The injector system (tubing, syringes, injector needles) must be cleaned weekly to remove precipitates and eliminate bacterial growth:

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

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

2.4 Software

The INFINITE F500 is delivered with the *i-control* software, for operating the instrument and includes an online-help file and a printed manual. The software is formatted as a self-extracting archive on CD-ROM.

For advanced data reduction and full regulatory compliance with 21 CFR part 11, the ***Magellan Tracker*** software can be used to control the INFINITE F500. (For more information, contact your local Tecan representative).

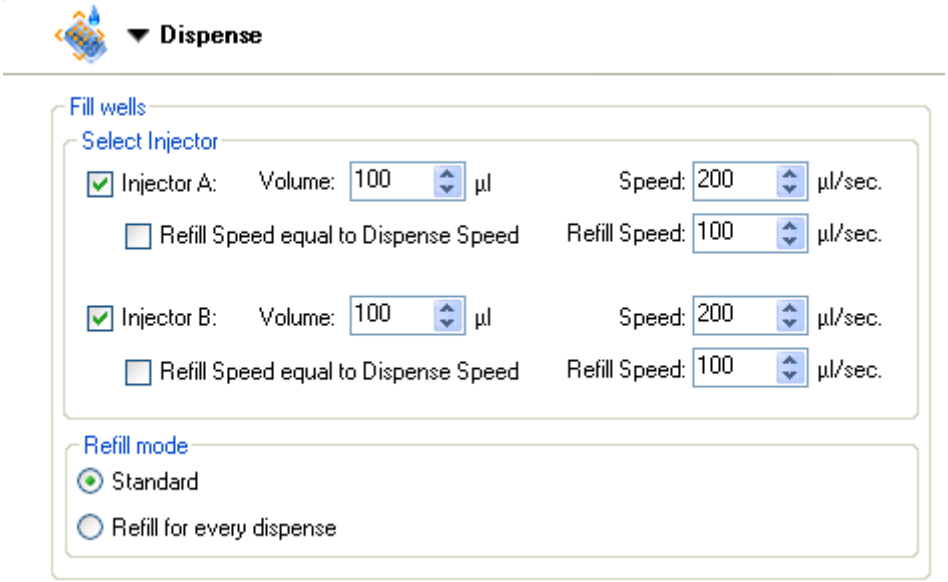
2.4.1 i-control and Injectors

When using the injector, two modes are available:

- **Dispense**: The dispense mode allows liquid to be dispensed plate-wise into the selected wells
- **Injection**: This mode must be used in combination with a measurement strip. The injection is performed in a well-wise mode.

Dispense Mode

The dispense settings can be adjusted via the software:



Dispense

Select Injector: Injector A and/or Injector B can be selected.

Speed: The injection speed is selectable from 100 – 300 µl/sec for each injector.

Select '**Refill speed**' from 100 – 300 µl/sec. For each injector or select '**Refill Speed equal to Dispense Speed**'.

Select refill mode '**Standard**', if refill should be performed when syringe is empty (multiple dispense steps before refilling).

Select '**Refill for every dispense**' if refill should be performed for every dispense step.

Using the Dispense Strip:

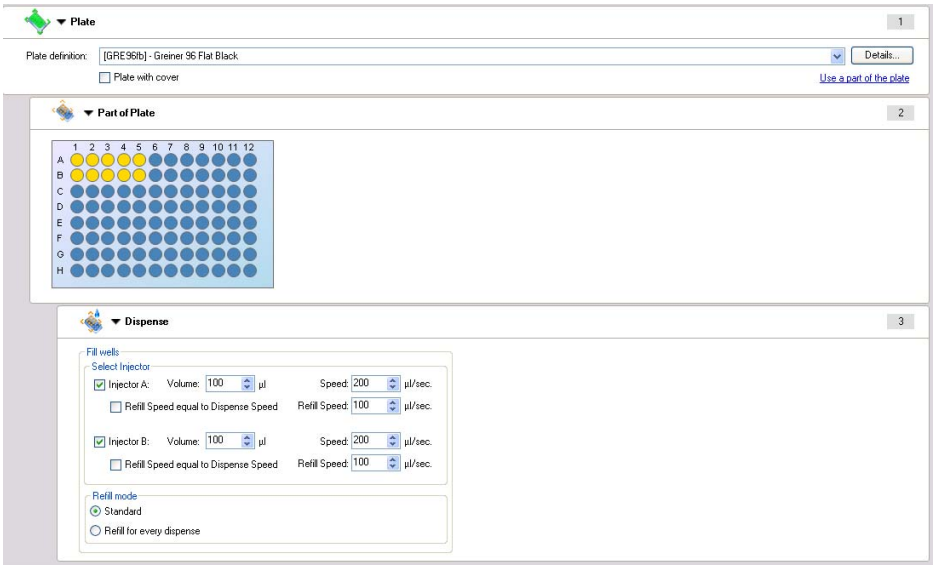
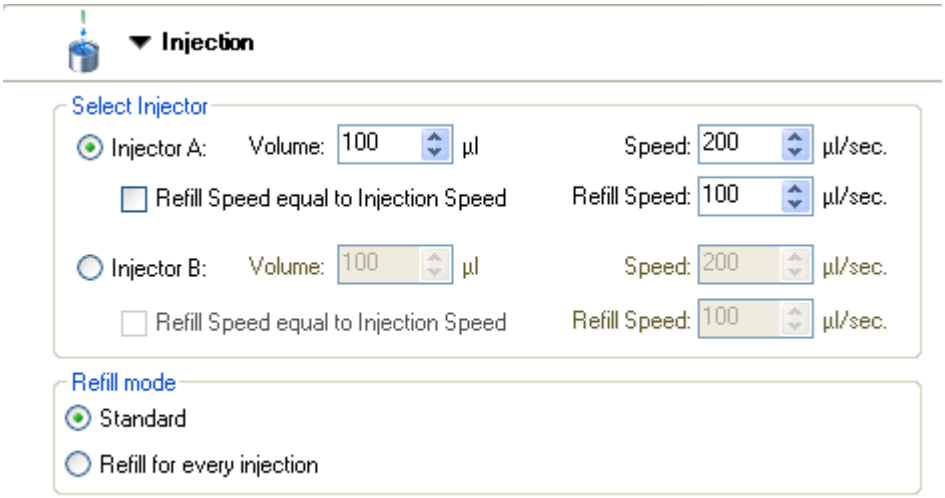


Plate	Select an appropriate plate type
Part of the plate	Optional; Select the wells to be dispensed
Dispense	Set up the dispense parameters. If both injectors are selected, all wells are first dispensed with Injector A and then with Injector B. The dispense strip does not require an additional measurement strip.
Dispense volume	The injection volume depends on the microplate type. The plate definition files include a so-called working volume. This working volume defines the maximum volume to be dispensed into the selected microplate. Therefore, always make sure that the selected plate definition file contains the correct setting for the working volume. The maximum dispense volume for a standard 500 µl syringe is 495 µl/dispense strip. If volumes greater than 495 µl are to be dispensed (e.g. into 6-well plates), more than one dispense strip has to be used.

Injection Mode

The injection settings can be adjusted via the software:



Injection

Select Injector:

Injector A or Injector B can be selected. It is not possible to select both injectors on one strip. If a measurement with two injectors is to be performed, two injector strips are necessary.

Speed: The injection speed is selectable from 100 – 300 µl/sec for each injector.

Select a '**Refill speed**' from 100 – 300 µl/sec. for each injector or check the 'Refill Speed equal to Injection Speed' box.

Select refill mode '**Standard**' if refill should be performed when syringe is empty (multiple injection steps before refilling). Select '**Refill for every injection**' if refill should be performed for every injection step.

Injection volume

The injection volume depends on the microplate type. The plate definition files include a so-called working volume. This working volume defines the maximum volume to be injected into the selected microplate. Therefore, always make sure that the selected plate definition file contains a correct setting for the working volume. The maximum injection volume for a standard 500 µl syringe is 495 µl/injection strip. If volumes greater than 495 µl are to be injected (e.g. into 6-well plates), more than one injection strip has to be used.

Using the Injection Strip:

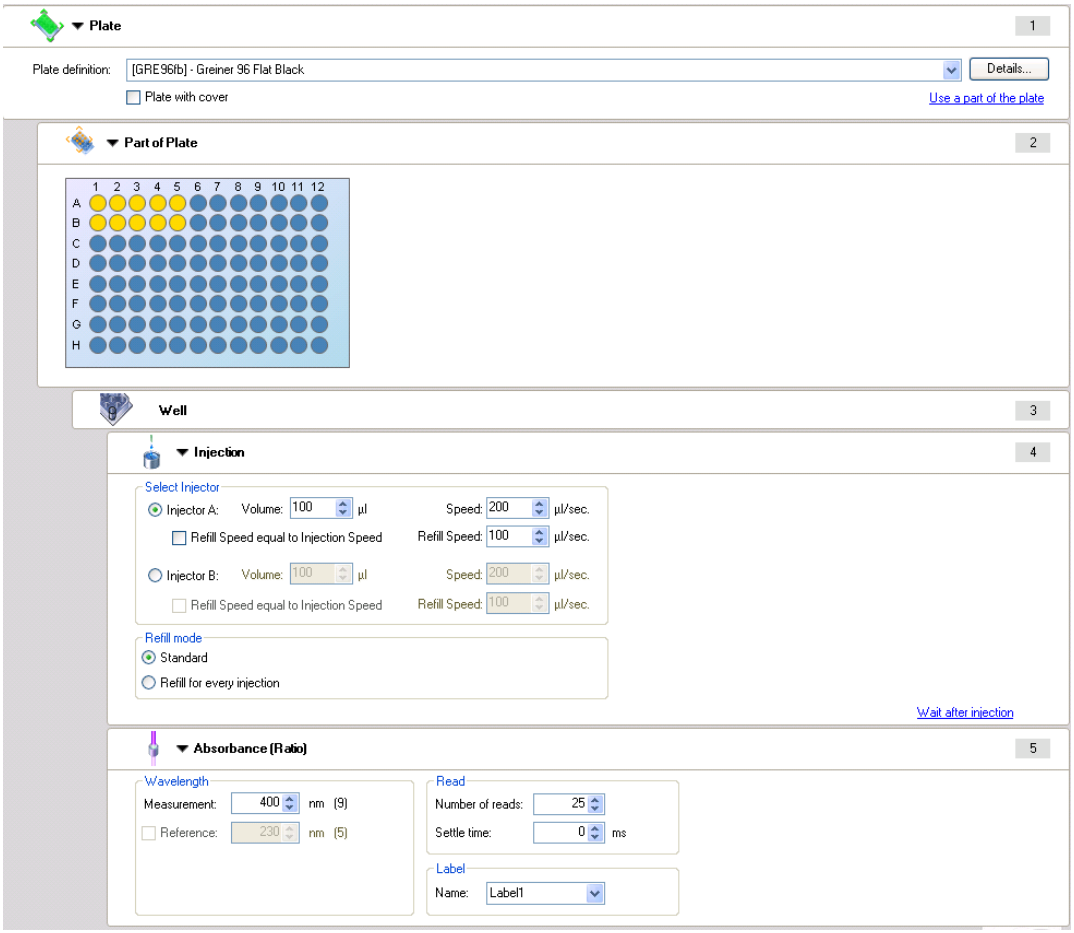


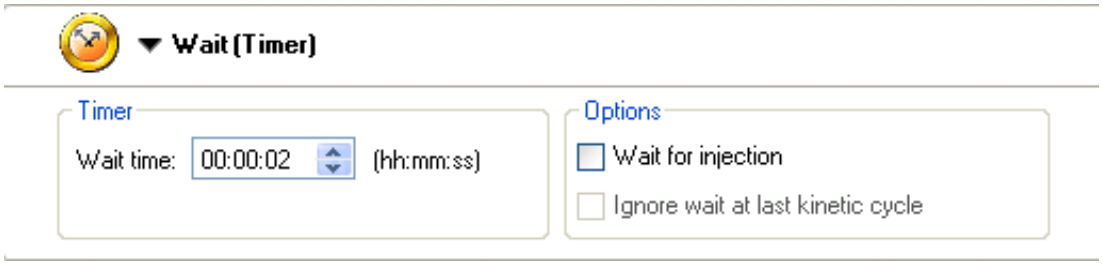
Plate	Select an appropriate plate type.
Part of the plate	Optional; Select the wells to be dispensed
Well	The well strip is mandatory. Injection is only possible with a ‘well’ strip. This strip ensures that the following indented strips are performed well-wise.
Injection	Set up the injection parameters. Only one injector can be selected per strip. If both injectors are required or one injector will perform two injections, an additional injection strip has to be inserted.
Measurement strip (Example Absorbance)	It is mandatory to use at least one measurement strip in combination with the injection strip. The position of the measurement strip(s) (before and/or after the injection strip) depends on the application and is therefore user-selectable.

Note

Make sure that the corresponding Working Volume value in your plate definition file is higher than the volume used for injection.

Wait Strip

A wait time (delay or settle time) can be inserted into the procedure.



Wait time	Select a time in hh:mm:ss from 00:00:01 up to 23:59:59
Options	If '**Wait for injection**' is selected, the wait time includes the injection time. If '**Wait for injection**' is NOT selected, the wait time is added to the injection time.

2.4.2 i-control Examples

Example 1: Dual Luciferase Reporter (DLR™) Assay (Promega Corp.)

For assay details please refer to www.promega.com.

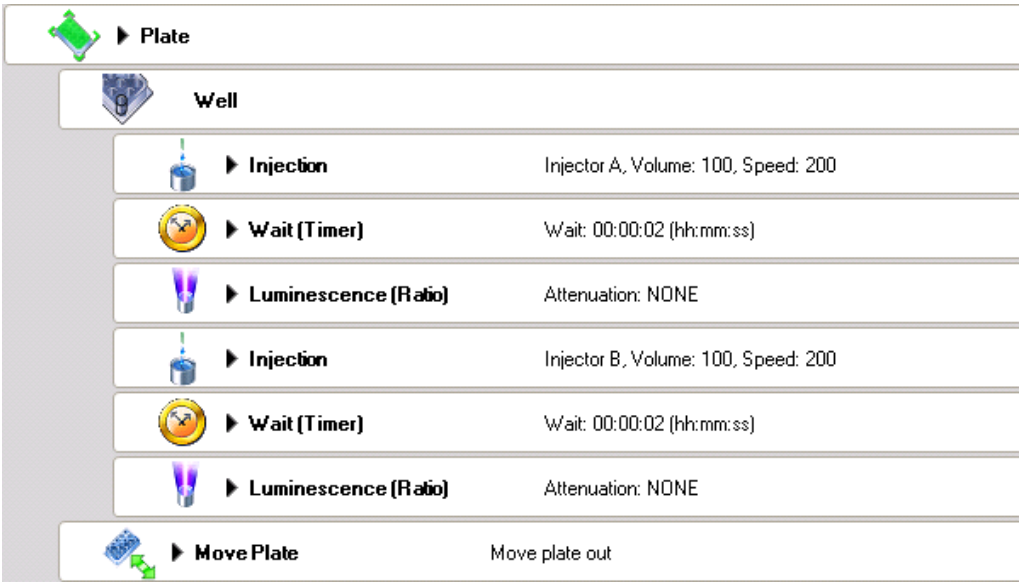


Plate	Select an appropriate plate type. For luminescence measurements, white microplates are recommended. For this example, a white 96-well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate is to be processed.
Well	Mandatory for measurements with 'injection'
Injection (1)	Injector A injects 100 µl with speed 200 µl/sec, refill mode: standard
Wait (Timer)	2 s wait time
Luminescence (1)	Luminescence measurement with 10 s integration time, attenuation 'none'
Injection (2)	Injector B injects 100 µl with speed 200 µl/sec, refill mode standard
Wait (Timer)	2 s wait time
Luminescence (2)	Luminescence measurement with 10 s integration time, attenuation 'none'
Move Plate	Plate is moved out after finishing all wells

Example 2: Enliten® ATP Assay System Bioluminescence Detection Kit for ATP (Promega Corp.)

For assay details please refer to www.promega.com.

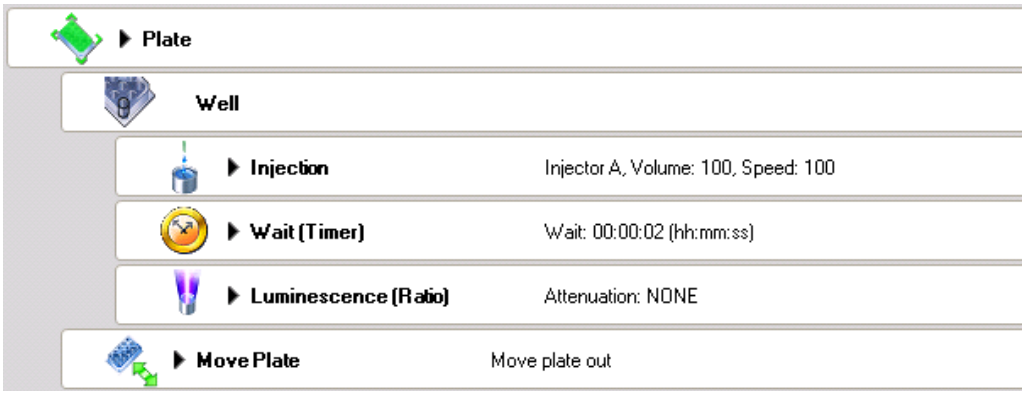


Plate	Select an appropriate plate type. For luminescence measurements, white microplates are recommended. For this example, a white 96-well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with ‘injection’
Injection	Injector A injects 100 µl with speed 100 µl/sec, refill mode: standard
Wait (Timer)	2 s wait time
Luminescence	Luminescence measurement with 10 s integration time, attenuation ‘none’
Move Plate	Plate is moved out after finishing all wells

Example 3: Measurement of Ca^{2+} sensitive probes using Fura-2

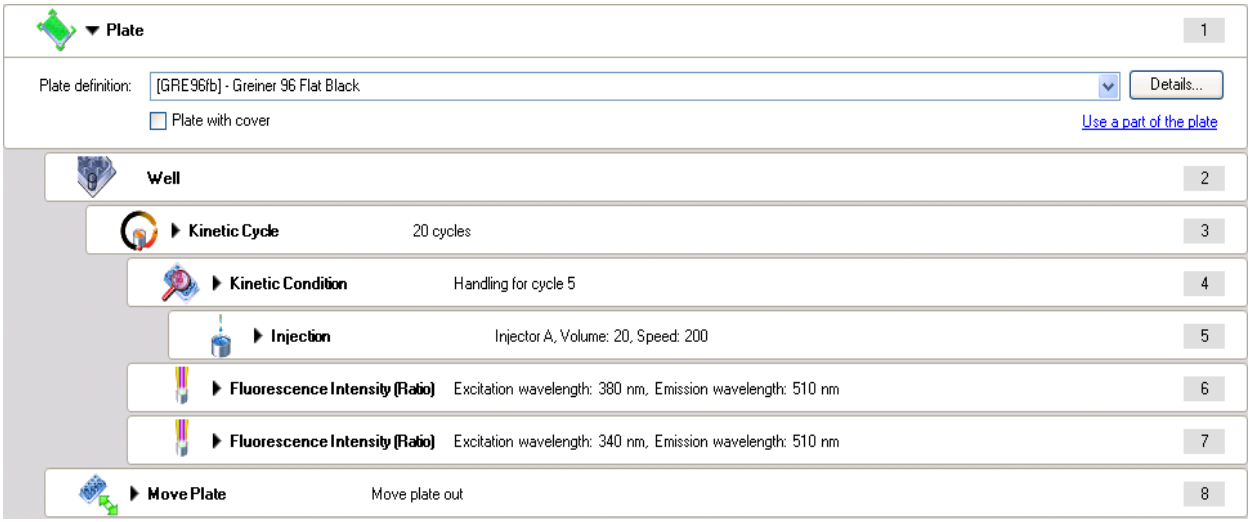


Plate	Select an appropriate plate type. For fluorescence measurements, black microplates are recommended. For this example, a black 96-well plate was selected.
Part of the plate	(Not shown), can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with 'injection'
Kinetic Cycle	Select the number of necessary cycles
Kinetic condition	This strip allows actions to be performed once in a kinetic run at a certain cycle. The intended injection strip below it is only processed once at the selected cycle.
Injection	Injector A injects 20 µl with speed 200 µl/sec, refill mode: not selectable; injection is performed at cycle 5 (defined by kinetic condition strip)
Fluorescence Intensity (1)	Select the appropriate parameters for the first label: Excitation wavelength: 380 nm, Emission wavelength: 510 nm; number of flashes: 10; integration time: 40; gain: manual
Fluorescence intensity (2)	Select the appropriate parameters for the second label: Excitation wavelength: 340 nm, Emission wavelength: 510 nm; number of flashes: 10; integration time: 40; gain: manual
Move Plate	Plate is moved out after finishing all wells

Example 4: Measurement of Ca^{2+} sensitive probes using Indo-1

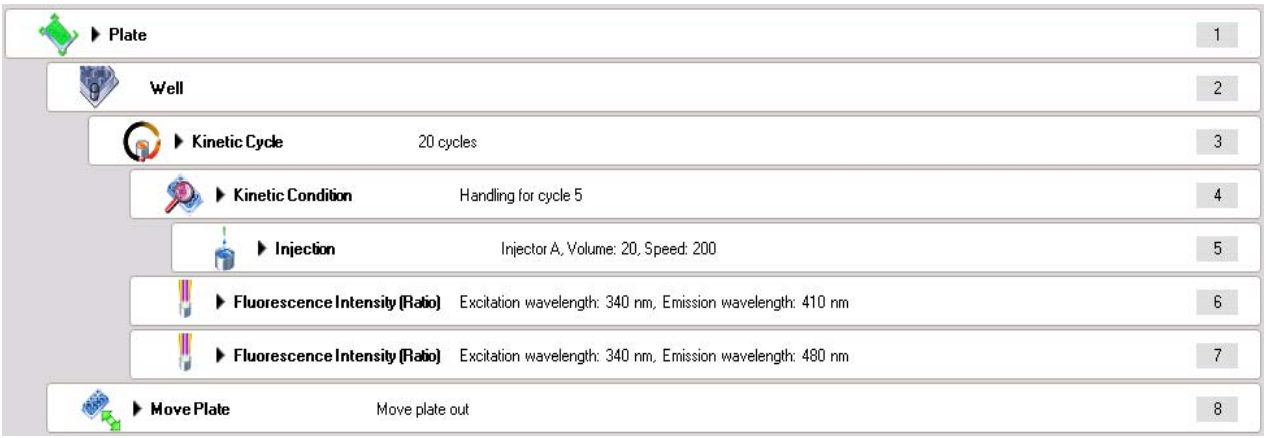


Plate	Select an appropriate plate type. For fluorescence measurements, black microplates are recommended. For this example, a black 96-well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with 'injection'
Kinetic Cycle	Select the number of necessary cycles
Kinetic Condition	This strip allows actions to be performed once in a kinetic run at a certain cycle. The intended injection strip below it is only processed once at the selected cycle.
Injection	Injector A injects 20 µl with speed 200 µl/sec, refill mode: not selectable; injection is performed at cycle 5 (defined by kinetic condition strip)
Fluorescence Intensity (1)	Select the appropriate parameters for the first label: Excitation wavelength: 340 nm, Emission wavelength: 410 nm; number of flashes: 10; integration time: 40; gain: manual
Fluorescence Intensity (2)	Select the appropriate parameters for the second label: Excitation wavelength: 340 nm, Emission wavelength: 480 nm; number of flashes: 10; integration time: 40; gain: manual
Move Plate	Plate is moved out after finishing all wells

3. Installation

3.1 Unpacking and Inspection

The delivered packaging includes the following items:

- CABLE USB 2.0 A/B 1.8 M Black with housing receptacle ferrite
- CDROM INFINITE F500
- OOB Quality Report
- Transport lock (mounted)
- 2 mm Allen key
- Operating Manual
- Final test protocol
- Accessory Box
- Filter stop rings (12)
- Filter assembly tool
- Plastic tweezers
- Filter slides
- FP Filter slides (only for option with Fluorescence Polarization module)

The injector module packaging for 1 injector includes the following items:

- Bottle holder
- Beaker for priming
- 125 ml bottle brown
- 125 ml bottle transparent
- 15 ml bottle
- Waste tube

The second injector comes with the following items:

- Bottle holder
- Beaker for priming
- 125 ml bottle brown
- 125 ml bottle transparent
- 15 ml bottle

3.1.1 Unpacking Procedure

1. Visually inspect the container for damage before it is opened.
2. *Report any damage immediately*.
3. 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. Ensure that the plate carrier cannot be accidentally hit when moved out. Ensure that the main switch and the main cable can be reached at all times and are in no way obstructed.
4. Place the carton in an upright position and open it.
5. 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.
6. Visually inspect the instrument for loose, bent or broken parts.
7. *Report any damage immediately.*
8. Compare the serial number on the rear panel of the instrument with the serial number on the packing slip.
9. *Report any discrepancy immediately.*
10. Check the instrument accessories against the packing list.
11. Save packing materials and transport locks (see next section) for further transportation purposes.

WARNING

THE INFINITE F500 IS A PRECISION INSTRUMENT AND WEIGHS APPROX. 37 KG WHEN FULLY EQUIPPED.

Caution

The maximum load for the INFINITE F500 cover is 37 kg; however the load must be distributed evenly across the entire surface of the cover.

Caution

The maximum load for the INFINITE F500 plate transport is 200 g.

Caution

Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment.

3.2 Removal of the Transport Locks

Caution
Remove the transport locks before operating the instrument.

3.2.1 Plate Transport Lock

The instrument is delivered with the plate carrier locked into place, so that it cannot be damaged. Before the instrument can be used, the transport locks must be removed using the following procedure:

1. Ensure that the instrument is disconnected from the main power supply.
2. Open the plate carrier door.
3. Using the 2 mm Allen key supplied, remove the screw that secures the red metal plate transport lock to the plate carrier. The transport lock must be saved and used to prevent damage to the instrument whenever it is moved or shipped.
4. Remove the Plate Transport Lock.

Caution
Save packing materials and transport locks for further transportation purposes. The INFINITE F500 must be shipped only with the original packaging and installed transport locks.

3.2.2 Bottle Drawer Lock

Note

The Bottle Drawer Lock is only in place if the INFINITE F500 instrument has the injector option installed.

If the instrument is equipped with the Injector option, the INFINITE F500 is delivered with the bottle drawer locked into place, so that it will not be damaged during transport. Before the instrument can be used, the bottle drawer lock must be removed.

1. Ensure that the instrument is disconnected from the main power supply.
2. Open the injection flap on the upper right of the instrument face. This door pivots up and back to reveal the injectors and the bottle drawer.
3. Remove the red thumb screw that secures the bottle drawer in place.
4. This screw must be saved and used to prevent damage to the instrument whenever it is moved or shipped.
5. Remove the Bottle Drawer Lock.

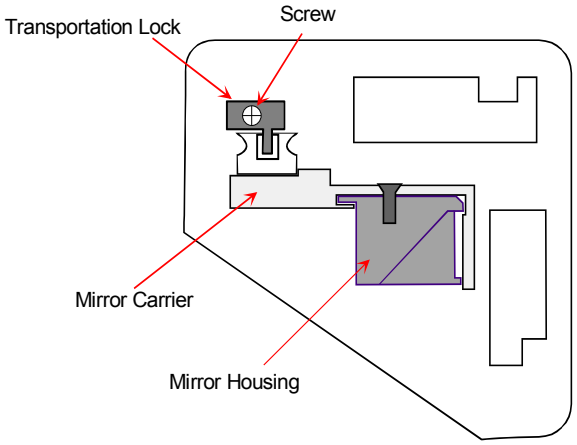
Caution

To prevent damage, the Bottle Drawer Lock must be saved and used whenever the instrument is moved or shipped.

3.2.3 Mirror Carrier Transport Lock

The INFINITE F500 is delivered with the mirror carriage locked into place, so that it will not be damaged during transport. Before the instrument can be used, the transport lock must be removed using the following procedure:

1. Ensure that the instrument is disconnected from the main power supply.
2. Open the filter slide compartment flap.
3. Using the 2 mm Allen key supplied, remove the screw holding the transport lock in place (see diagram below).
4. Remove the Mirror Carrier Transport Lock.



Caution

To prevent damage, the Mirror Carrier Transport Lock must be saved and used whenever the instrument is moved or shipped.

3.3 Transport and Storage

3.3.1 Transport

The INFINITE F500 must be shipped using the original packing and installed transport locks. Before shipping the instrument, it must be thoroughly disinfected (see 9.3 Instrument Disinfection).

3.3.2 Storage

Before storing the instrument the injectors must be rinsed using a wash procedure (see Priming and Washing of the INFINITE F500). Select a location to store the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents and acid vapors

Storage Specifications

Temperature	- 20 °C to + 60 °C -4 °F to + 140 °F
Relative Humidity	< 90 % non condensing

3.4 Power Requirements

The instrument is auto sensing and it is therefore not necessary 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 100-120/220-240V.

If the voltage is not correct, please contact your distributor.

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

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 be damaged.

WARNING

IF THE INSTRUCTIONS GIVEN IN THIS MANUAL ARE NOT CORRECTLY PERFORMED, THE INSTRUMENT WILL EITHER BE DAMAGED OR THE PROCEDURE WILL NOT BE PERFORMED CORRECTLY AND THE SAFETY OF THE INSTRUMENT IS NOT GUARANTEED.

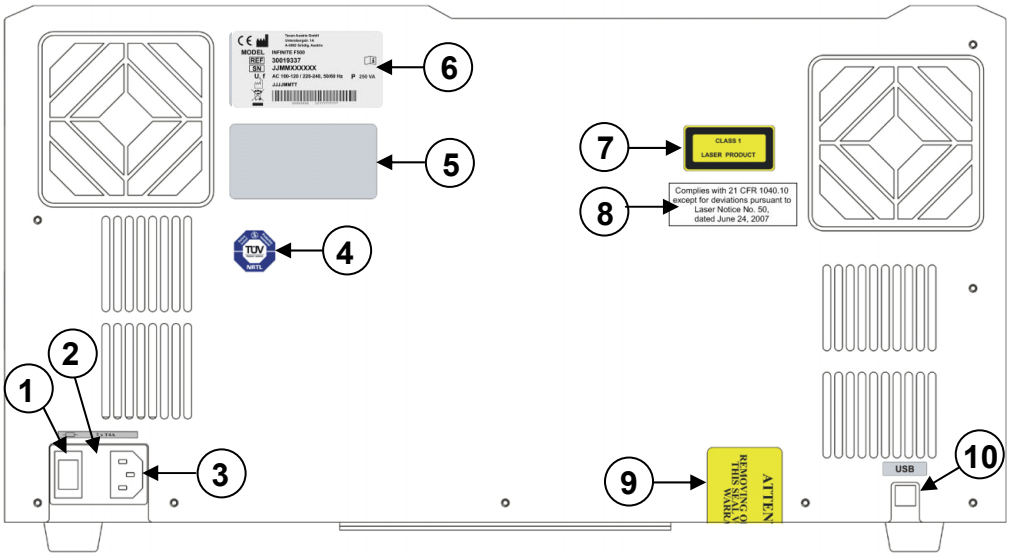
3.5 Switching the Instrument On

Caution

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

1. Ensure the computer is switched OFF and the instrument's main power switch on the back panel of the instrument is in the OFF position.
2. Connect the computer to the instrument with the delivered USB interface cable.
3. Insert the power cable into the main power socket (with protective ground connection) on the back panel of the instrument.
4. All connected devices must be approved and listed as per IEC 60950-1 Information Technology Equipment – Safety or equivalent local standards.
5. Switch the instrument ON using the main power switch on the back panel of the instrument.

Rear View



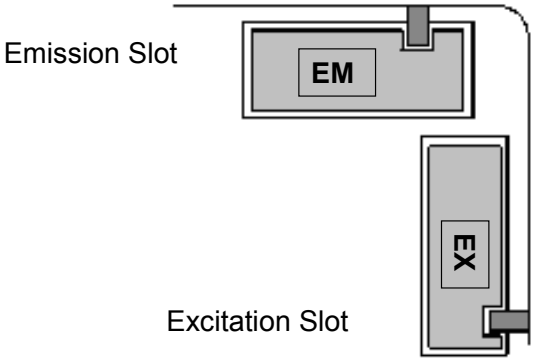
1 Main Power Switch
2 Fuse Compartment
3 Main Power Socket
4 Label – Technical Inspection Agency
5 Label – Options/Configuration
6 Name Plate
7 Label – Class 1 Laser Product
8 Complies with 21 CFR 1040.10
except for deviations pursuant to
Laser Notice No. 50, dated June 24, 2007
9 Warranty Label
10 USB Connection

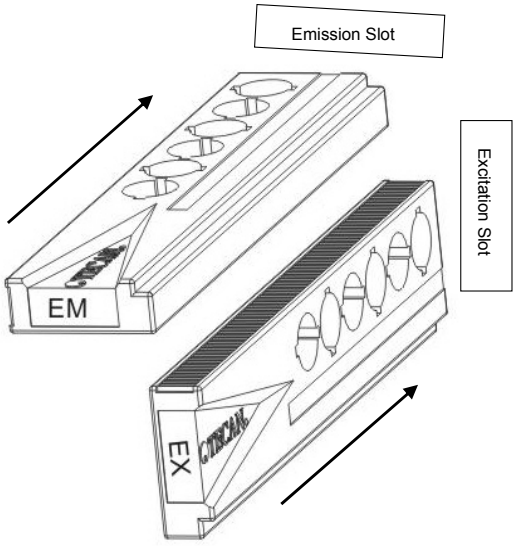
Caution
Only Tecan authorized service technicians are allowed to open the instrument. Removing or breaking the warranty seal voids the warranty.

3.6 Insert Filter Slides

To insert the filter slides open the upper door flap manually. For the ease of identification the excitation and emission filter slides are labeled different. Move the filter slides gently into the respective filter slot as indicated. The filter slides will be automatically retracted.

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





The instrument is now ready to be used with a suitable software program.

4. Defining Filter Slides and Mirrors

4.1 About Filters

4.1.1 Fluorescence Filters

The optical filters (bandpass style) in a filter slide are specially designed for fluorescence measurements. The spectral rejection 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.

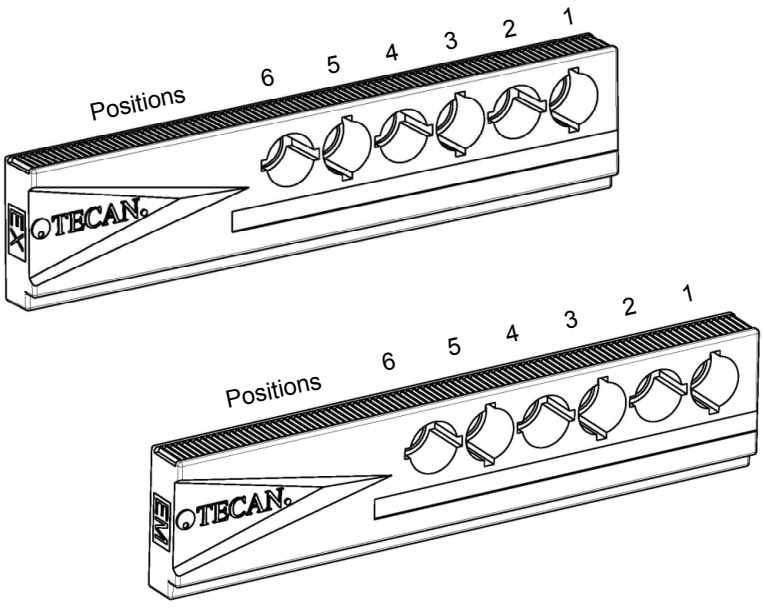
4.1.2 Absorbance Filters

Bandpass filters, which are commonly used in microplate readers for absorbance measurements, usually have a bandwidth of 10 nm. Therefore it is not recommended to use fluorescence filters for absorbance measurements because the bandwidth (FWHM) is usually larger than 10 nm. This could cause a bright value error or low OD values when measuring dyes with narrow peaks.

4.2 Filter Slide and Filter Orientation

4.2.1 Filter Slides

The INFINITE F500 works with two separate filter slides, i.e. an excitation and an emission filter slide. The filter slides enable the user to work with six independent excitation/emission filters, which can be defined on positions 1 to 6. Fluorescence excitation and emission filters, absorbance filters and polarizers for fluorescence polarization measurements can be mounted in the filter slides. The information about the inserted filters is saved on the microchip integrated into each filter slide.

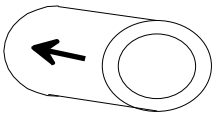


4.2.2 Filter Types

Caution

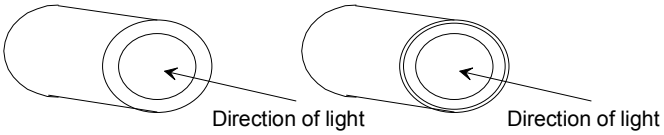
There are two types of filters. It is important that light travels through both types of filter in the correct direction. Before inserting a new filter carefully consider the filter and the direction of light through the filter slide.

1. Filters with an arrow on the side:



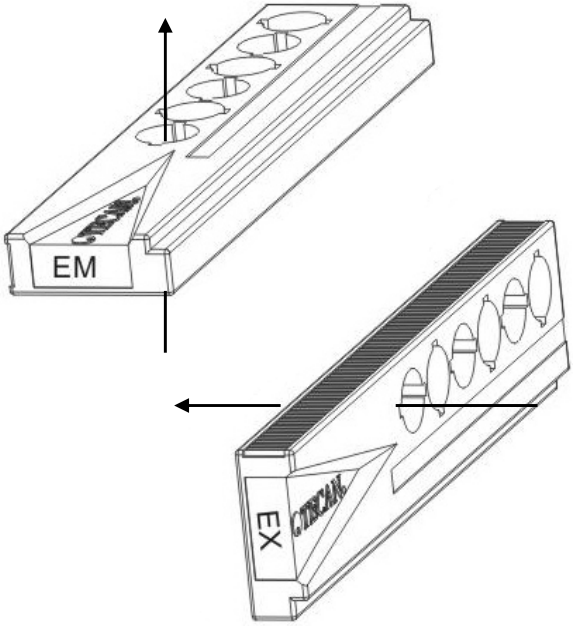
Light must travel in the direction of the arrow.

2. Filters without an arrow on the side:



The end of the filter with the metal lip must face away from the light source.

INFINITE F500: Filter slides - Direction of Light



Position of Polarization Filters

Note

Fluorescence polarization measurements on INFINITE F500 require one excitation and two identical emission filters placed together with the polarizers on the respective filter slide.

The INFINITE F500 excitation filter slide can be equipped with maximal three different fluorescence polarization filters placed on the positions 1, 3 and 6. Consequently, the INFINITE F500 emission filter slide can be equipped with maximal three different fluorescence polarization filters which are placed together with the polarizers either on the positions 1 and 2, 3 and 4 or 5 and 6.

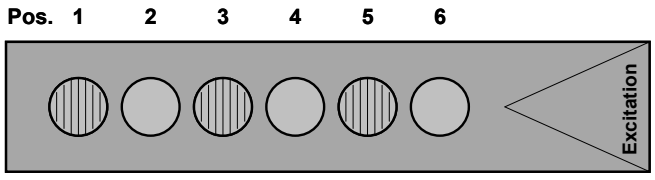
Caution

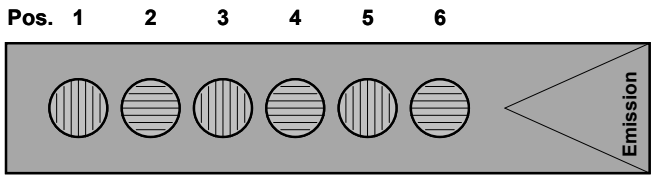
Fluorescence polarization mode on Position 1 (excitation filter slide) requires FP defined emission filters on Positions 1 and 2.

Fluorescence polarization mode on Position 3 (excitation filter slide) requires FP defined emission filters on Positions 3 and 4.

Fluorescence polarization mode on Position 5 (excitation filter slide) requires FP defined emission filters on Positions 5 and 6.

INFINITE F500: Filter slides with the indicated positions for fluorescence polarization filters and polarizers.





4.3 Installing a Custom Filter

When installing a new filter use the filter assembly tool included in the accessories case. For installing the polarizers use the soft tweezers (plastic).

4.3.1 Removing a Filter

1. Align the filter assembly tool with the notch of the stop-ring. Turn the tool and remove the stop-ring by pulling it out of the filter slot.

2. The filter will slide out of the filter slot when the filter carrier is turned over. Do not use the filter assembly tool to remove filters.

4.3.2 Mounting a Custom Filter

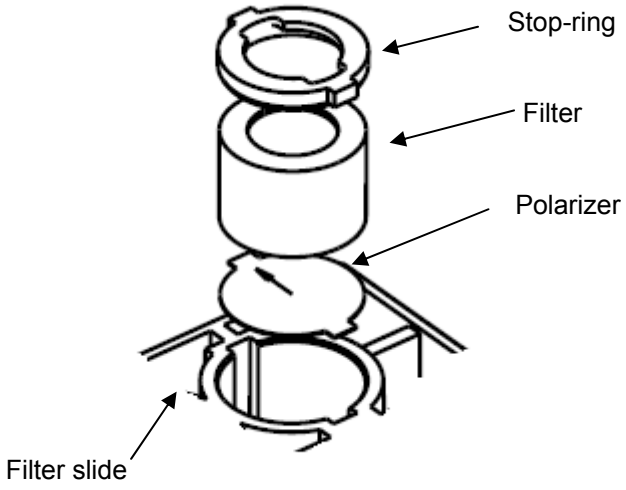
A new filter (and polarizer) must be inserted into the slide as shown below.

Note

Make sure that the filters are inserted correctly (see Filter Types). To ensure proper function, do not reuse the stop-rings more than 5 times.

Caution

Take care to insert the polarizers and the filters into the filter slide when working with fluorescence polarization.



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.

In order to install a custom filter do the following:

1. If required, carefully insert a polarizer at the excitation and emission half of the filter slide using tweezers, taking care not to scratch it or get fingerprints on it.
2. Carefully insert the filter into the opening, taking care not to scratch or get fingerprints on the filter.
3. Place the stop-ring on the end of the filter assembly tool and turn it so it cannot slip off.

Filter assembly tool with stop-ring

4. Using the filter assembly tool, push the stop-ring into the filter slot and press firmly into place.
5. Rotate the tool until the notch in the stop-ring is aligned with the end of the filter assembly tool and remove the tool.
6. If there are unused openings remaining after the required filters have been inserted (e.g. the emission part of an absorbance filter), filter dummies should be mounted in the holes that are still open.

4.4 Defining the Filters

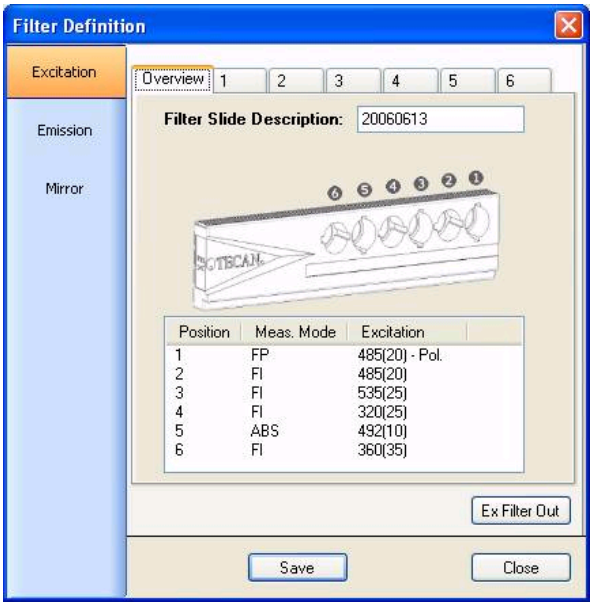
Caution

Any changes to the filters in the filter slide are to be carried out by trained personnel! The instrument is able to 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 (by a service engineer) or if a new undefined customized filter slide is to be used, the filter slides need to be defined.

Define a filter as follows:

1. Select **Filter Definitions** from the **Settings** menu.
2. The following dialog box is displayed showing an overview tab and six filter definition tabs for excitation and emission filter slide, respectively:



Overview: The overview provides the user with the current filter slide definition.

Filter Slide Description: Enter the filter slide description or the filter slide description will be generated automatically.

Note

No special characters (blank, ?, $, %, ., /, etc.) except '_' are allowed for the filter slide description.

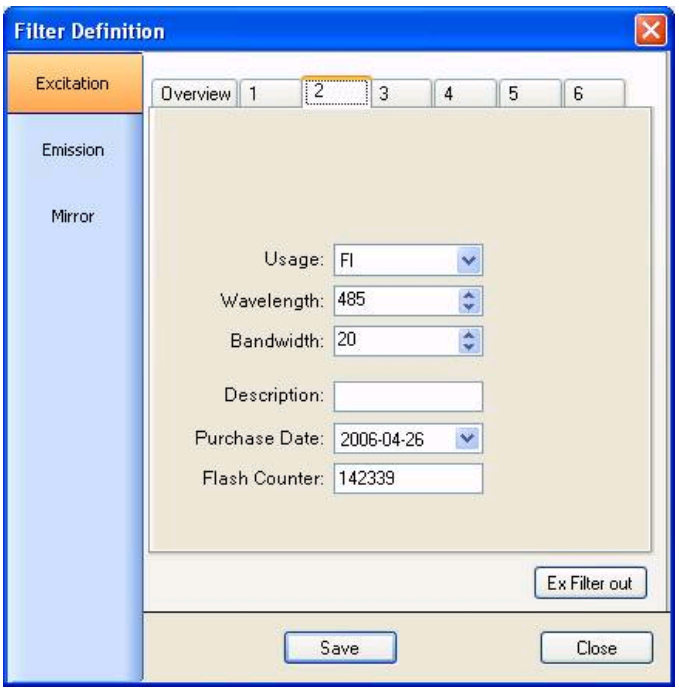
Caution

The filter slide description is part of the G-Factor key value. If manually entered, avoid using the same description for the different filter slides.

Position 1 - 6: Filter definition editor for the filters on positions 1, 2, 3, 4, 5 and 6.

Select the appropriate filter position and enter the new wavelength, bandwidth and measurement mode for each new filter:

Measurement Mode: choose from the dropdown list 'FI' for fluorescence intensity, 'ABS' for absorbance, 'FP' for fluorescence polarization (available only for positions 1, 3 and 5 on the excitation filter slide) and 'Empty' for filter-free positions

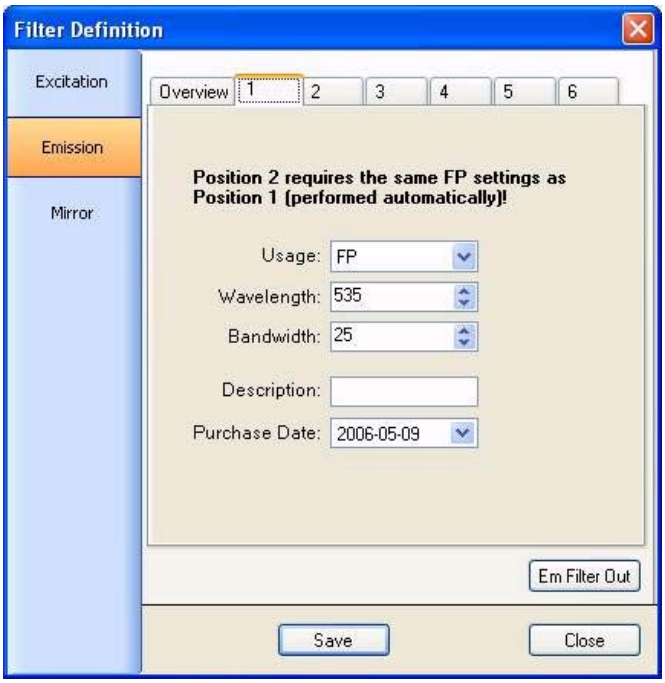


Caution

Fluorescence polarization mode on position 1 (excitation filter slide) requires FP defined emission filters on positions 1 and 2.

Fluorescence polarization mode on position 3 (excitation filter slide) requires FP defined emission filters on positions 3 and 4.

Fluorescence polarization mode on position 5 (excitation filter slide) requires FP defined emission filters on positions 5 and 6.



Note

EMISSION SLIDE: fluorescence polarization mode on Position 1 requires the same filter settings on Position 2 and vice versa. Fluorescence polarization mode on Position 3 requires the same filter settings on Position 4 and vice versa. Fluorescence polarization mode on Position 5 requires the same filter settings on Position 6 and vice versa. This is performed automatically.

Caution

Make sure that the filter slides contain polarizers together with the filters defined for fluorescence polarization.

Wavelength: Enter the filter wavelength within the following range:

(1) Fluorescence intensity mode: 230 to 900 nm (Excitation) and 280 to 900 nm (Emission)

(2) Fluorescence polarization: 300 to 750 nm (Excitation) and 330 to 750 nm (Emission)

(3) Absorbance mode: 230 to 1000 nm

Bandwidth: Enter the bandwidth (nm) of the filter

Description: This field can be used for individual user's remarks about the filter (e.g. filter name, application, etc.).

Note

No special characters (blank, ?, $, %, ., /, etc.) except '_' are allowed for the filter slide description.

Purchase Date: This option enables the user to enter the purchase or installation date of the filter

Flash Counter (available only for excitation filter slide): The flash counter monitors the number of flashes through an excitation/absorbance filter. The flash counter number provides the user only with additional information about the filter in use. The flash counter number is saved together with other information about the filter on the excitation filter slide microchip. If you replace a filter, this information will be lost unless the last filter flash number is manually documented by the user.

Caution

It is recommended to manually document the last flash counter number before replacing an excitation/absorbance filter; otherwise this information will be lost.

For a brand new filter set the counter to 0. For a previously used filter enter the last collected flash number if the number is available.

- Accept the new filter values by clicking **Save**. By closing the Filter Definition dialog the system is ready to collect data with the new filters.

4.5 Configuration of the Mirror Carrier

Along the mirror carrier, different types of (dichroic) mirrors are mounted. These mirrors are permanently mounted. The availability of the mirrors depends on the selected plate format.

Note

A dichroic mirror needs to match the selected fluorescence excitation and emission filters.

Plate formats up to 384-well

Fluorescence Mirror	Reflection (Excitation)	Transmission (Emission)
50% Mirror	230 – 900 nm	230 – 900 nm
Dichroic 510 (e.g. fluorescein)	320 – 500 nm	520 – 780 nm
Dichroic 560 (e.g. Cy3)	500 – 550 nm	570 – 900 nm
Dichroic 630 (e.g. Cy5)	560 – 625 nm	635 – 900 nm
User dichroic 1 (exchangeable)		
User dichroic 2 (exchangeable)		

If necessary, the mirror carrier can be extended with a custom type dichroic (User dichroic 1 and User dichroic 2).

Plate formats of 384-well and 1536-well

Fluorescence Mirror	Reflection (Excitation)	Transmission (Emission)
50% Mirror	230 – 900 nm	230 – 900 nm
Dichroic 510 (e.g. fluorescein)	320 – 500 nm	520 – 780 nm
Dichroic 560 (e.g. Cy3)	500 – 550 nm	570 – 900 nm
Dichroic 630 (e.g. Cy5)	560 – 625 nm	635 – 900 nm
50% Mirror for 1536 well	230 – 900 nm	230 – 900 nm
Dichroic 510 (e.g. fluorescein) for 1536 well	320 – 500 nm	520 – 780 nm
User dichroic 1: Dichroic 560 for 1536-well (exchangeable)	500 – 550 nm	570 – 900 nm
User dichroic 2: Dichroic 630 for 1536 (exchangeable)	560 – 625 nm	635 – 900 nm

If necessary, the dichroics 560 and 630 can be replaced with a custom type dichroic.

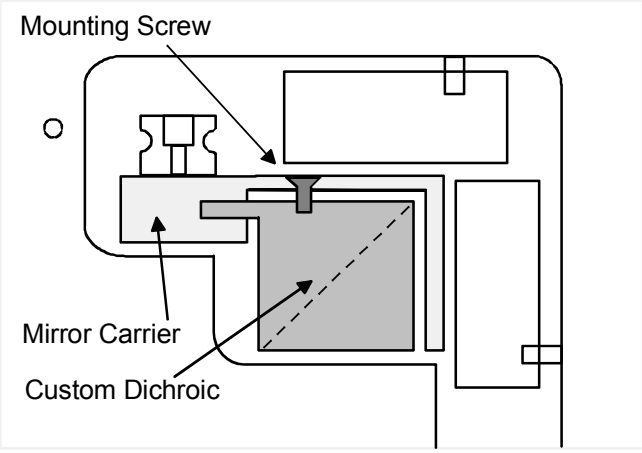
Please ask your local Tecan representative to find a suitable set of dichroic and filters for your application.

4.5.1 Mounting the Custom Dichroic

Caution
Mounting of the custom dichroic is to be carried out by a service engineer!

In order to install the custom dichroic do the following:

1. Select '**Movements'** from the **Instrument** menu. Click '**Mirrors Out'**. The Mirror Slide comes out of the instrument.
2. Keep the compartment flap open. Slide the custom dichroic into the Mirror Carriage as indicated in the figure. Apply and carefully tighten the mounting screw.



Caution
Do not apply too much torque to the Mirror Carrier.

3. Carefully release the compartment flap. Click '**Mirrors In'**. The Mirror Slide moves back into the instrument.
4. The custom dichroic is now ready to be defined.

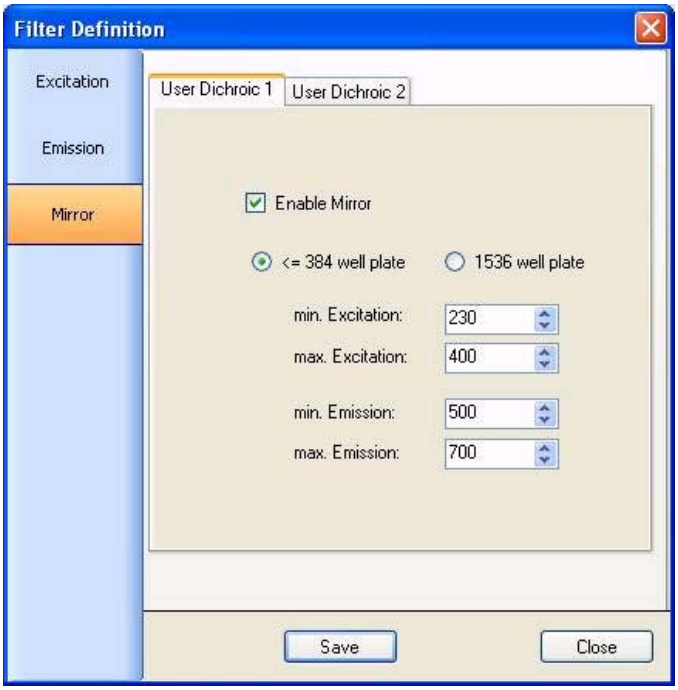
Caution
If the custom dichroic is mounted, it has to be defined.

4.5.2 Defining the Custom Dichroic

Caution
If a new undefined dichroic is to be used, it needs to be defined.

Define the custom dichroic as follows:

1. Select '**Filter Definitions'** from the **Settings** menu.
2. Select '**Mirror**' in the displayed Filter Editor Dialog and define a new dichroic as follows:



Select '**Enable Mirror'**

Select the plate format option '**≤ 384-well plate'** for dichroics that are recommended for plate formats ≤ 384-well or '**1536-well plate'** for dichroics that are recommended for usage with a 1536-well plate.

Caution
It is not recommended to use dichroics that are designed for use with plate formats ≤ 384 wells for 1536-well plate formats or vice versa. Always make sure that the inserted user dichroic matches the plate format selected in the mirror definition dialog.

Enter the minimal and maximal excitation wavelength (230 – 900 nm)

Enter the minimal and maximal emission wavelength (280 – 900 nm)

5. Optical System

5.1 Fluorescence System

Light Source System

Fluorescence applications usually require a specific range of excitation wavelengths. Additionally, linear polarization of excitation light might be required (Fluorescence Polarization) or pulsed light (TRF).

The INFINITE F500 light source system is built from the following components:

- Flash lamp
- Condensing Optics
- Excitation (or Absorbance) Filters
- Polarizing Filter (Optional)
- Flash lamp Monitor (Reference Diode)

Flash lamp

The INFINITE F500 utilizes a high energy Xenon arc discharge lamp (flash lamp). The flash sparks across a small gap between two electrodes. The lamp bulb contains a high pressure Xenon atmosphere. The flash decays within some microseconds.

The flash frequency is 40 Hz. A flash frequency of 400 Hz is applied only for 'on-the-fly' measurements in 1536-well plates.

The INFINITE F500 uses the flash lamp for fluorescence and for absorbance measurements, although pulsed illumination is a must only for TRF. The main benefits of this singular kind of lamp are:

1. High intensity from the deep UV to the near IR
2. Very long lifetime
3. Many applications - only one kind of lamp
4. No warm up time required

Condenser

Condenser type optics focuses the light through the entrance aperture to the fluorescence optical system.

Bandpass Filter

In both fluorescence and absorbance applications, optical filters of bandpass type are necessary to select the useful wavelengths from the flash lamp spectrum. Filters are mounted in removable slides (see 4.2 Filter Slide and Filter Orientation). In detail, filter requirements for fluorescence and absorbance measurements are slightly different.

Excitation Filter

Fluorescence emission spectra in many cases do not depend on the exact excitation wavelength. For a maximum total fluorescence signal, therefore, rather broad excitation band pass filters (10 – 40 nm) may be used.

Absorbance Filter

The absorbance of dyes sensitively depends on wavelength. This requires rather narrow bandpass filters (2 – 10 nm) with steep slopes.

Polarizing Filter

For fluorescence polarization applications some positions of the filter slides can be additionally equipped with polarizing filters (see 4.2.2 Filter Types). The polarizing filter on the excitation filter slide passes light of a specific plane of polarization, whatever the wavelength between 300 and 750 nm.

Flash Monitor

The light energy of single flashes may slightly fluctuate. To take these variations into account, a silicon photodiode monitors the energy of every single flash. Fluorescence measurement results are compensated correspondingly.

Optical System

The INFINITE F500 fluorescence optical system is sketched below. Arrows indicate paths of light. The system is composed of the Light Source System (1), the Fluorescence Optics (2), and the Fluorescence Detection (3).

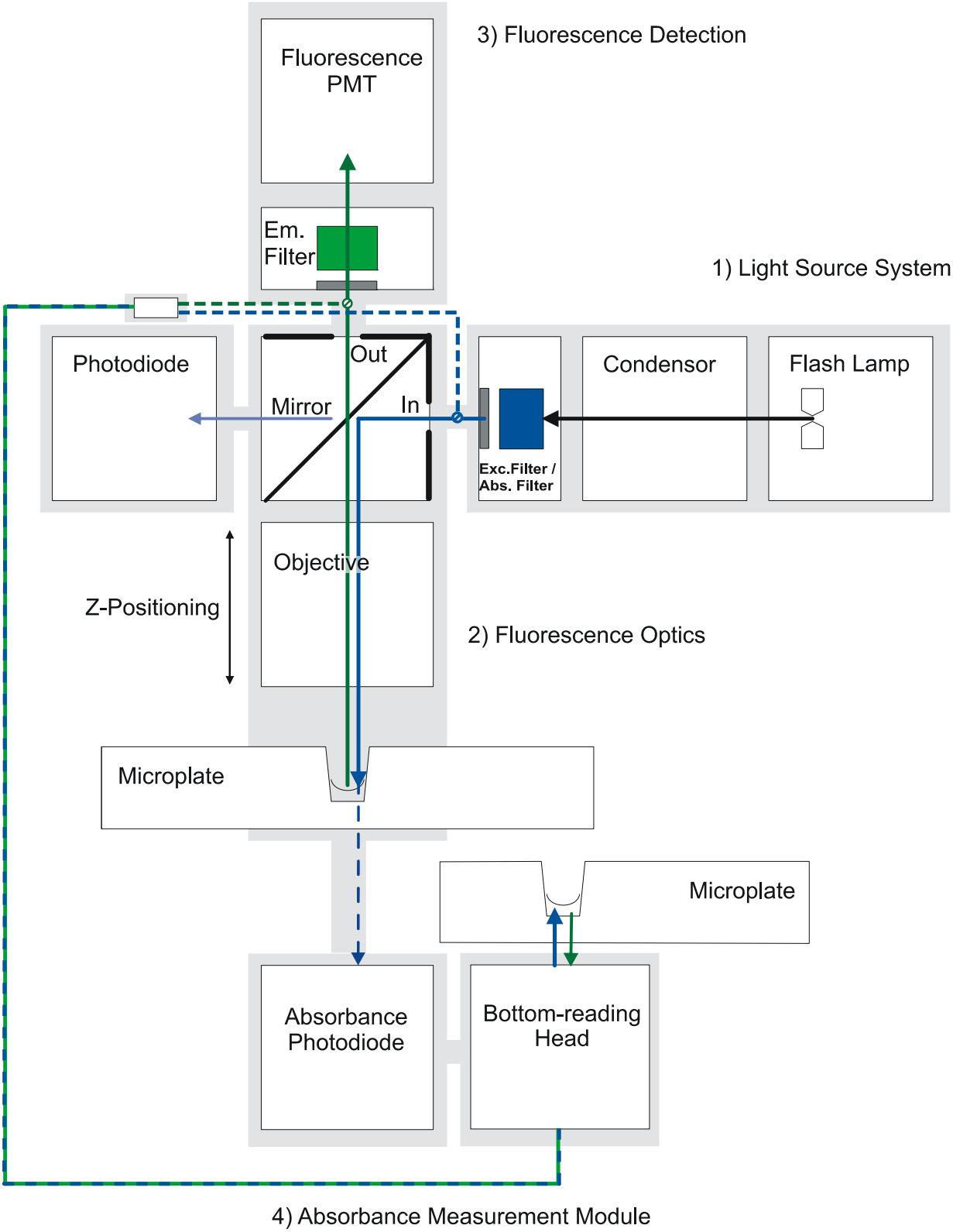


Figure 5-1: Fluorescence Optical System of the INFINITE F500

Fluorescence Intensity Top Reading

Flash light enters the optical system being focused through an aperture. This opening acts as a color specific light source, optionally polarized. By default, a semi-transparent mirror (beam splitter) reflects 50% of the light towards the microplate. The objective lens system focuses the light into the sample.

Fluorescence Emission is measured from above the well. Fluorescence light is collected by the objective, directed through the 50% mirror, and focused through the exit aperture for detection.

The Z-positioning stage of the objective allows adjusting for optimized fluorescence intensity depending on dispensed volume, shape of well, shape of meniscus, etc.

The size of the entrance aperture (“IN”) determines the diameter of the beam waist (spot size) in the microplate well. Depending on the required distance between measurement points the INFINITE F500 can automatically select between two available aperture diameters resulting in two different spot sizes: 1.8 mm (≤ 384-well microplates) or 1.0 mm (1536-well microplates).

Fluorescence Intensity Bottom Reading

When Bottom Reading Mode is selected, the mirror carrier is automatically moved to a position where light transmitted through the excitation filter is coupled into the bottom read fiber bundle (indicated by a blue circle). Fluorescence light is fed back through the emission filter (indicated by a green circle). The bottom read fiber bundle conducts light to and from the bottom read head (situated underneath the microplate). FI Bottom Reading can be used for measuring the microplate formats ≤ 384-well. The diameter of the bottom read excitation beam is approx. 3.5 mm.

Mirror Selection

The mirror carrier houses the 50% type mirror as well as different dichroic mirrors (see 4.5 Configuration of the Mirror Carrier). The advantage of the 50% mirror is that it works with any pair of excitation and emission wavelengths. However, 50% of excitation light and 50% of fluorescence light are lost.

A dichroic mirror is designed to reflect a range of wavelengths almost perfectly. This range is used for excitation. On the other hand, that dichroic does transmit most of the fluorescence light. This usually gives a better signal to noise ratio when compared with the 50% mirror.

According to the selected filter wavelengths, the appropriate mirror can be automatically set. A custom dichroic can easily be mounted and defined by the user. For details see 4.5.2 Defining the Custom Dichroic.

Objective Lens System

The objective is designed to collect as much of the fluorescent light from a well as possible and focus it through the exit aperture to the detection system.

The objective lenses are made from fused silica. This material provides high UV transmission and is virtually void of auto-fluorescence.

Z-Positioning

The Z-Position of the objective and thus the sample can be adjusted. As excitation light is reflected at the sample liquid interface, z-adjustment helps to maximize signal to noise ratio. For details see 6.3.1 Instrument Parameters.

5.1.1 Fluorescence Detection

Emission Filter

The emission filter suppresses scattered excitation light and unspecific fluorescence. The emission filter is part of a filter set containing excitation filter, emission filter and a 50 % mirror or optionally a dichroic.

Some general criteria for the selection of a suitable combination can be found in 6.3.3 Measurement Accessories.

Polarizing Filter

For fluorescence polarization applications some positions of the emission filter slides can be additionally equipped with polarizing filters. They are organized in pairs oriented “parallel” and “perpendicular” with respect to a single polarizing filter on the excitation filter slide (see 4.2.2 Filter Types). They pass light of a specific plane of polarization, whatever the wavelength between 330 and 750 nm.

PMT Detector

A photomultiplier tube (PMT) is used for the detection of such low light levels as involved with fluorescence. Adjusting the PMT gain allows a wide range of concentrations in lower or higher concentration domains to be measured.
For details see 6.3.1 Instrument Parameters.

5.2 Fluorescence Intensity Bottom Reading Option

The bottom reading option allows assays to be read through the clear bottom of a microplate that can be any format from 6 to 384 wells. No 1536-well plates can be measured with the bottom reading option. Fluorescence bottom reading can easily be selected via software.

See the optical system diagram in chapter 5.1 Fluorescence System.

With regard to the bottom fibers, the INFINITE F500 offers two Fluorescence Intensity Bottom Reading Options:

(1) FI Bottom Reading VIS - the INFINITE F500 is equipped with the glass fibers, allowing the FI Bottom measurements in the wavelength range between 380 - 900 nm.

(2) FI Bottom Reading UV - the INFINITE F500 is equipped with the quartz fibers, allowing the FI Bottom measurements in the wavelength range between 230 - 900 nm.

FI Bottom Reading VIS and FI Bottom Reading UV can be used for measuring the microplate formats ≤ 384-well. The diameter of the bottom read excitation beam is approx. 3.5 mm. Note, that 1536-well plates cannot be measured with the fluorescence bottom reading mode.

5.3 Absorbance System

For absorbance measurements a similar optical path is used as for fluorescence excitation. The absorbance measurement module is located underneath the plate carrier. It measures the light being transmitted through the sample. Before measurement of the microplate, a reference measurement is performed with the plate carrier moved out of the light beam (I_0).

5.3.1 Absorbance Optics

The mirror carriage has an absorbance position. A pair of small apertures forms a narrow and more collimated light beam when compared with fluorescence excitation. The diameter of absorbance light beam is approx. 0.4 mm for all plate formats.

Light being focused through the dispensed liquid is slightly refracted at the interfaces between air, liquid, and plate bottom. To accomplish a reliable measurement in the presence of the meniscus, a focusing lens recollects the rays of light, which might have been refracted too far away from the optical axis.

5.3.2 Absorbance Detection

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

For better absorbance performance when using 384- and 1536-well plates, we recommend using black microplates with transparent bottoms, such as Greiner µClear plates.

5.4 Luminescence System

For uncompromising performance, the INFINITE F500 luminescence detection system is separate from the fluorescence system. The luminescence optics are designed to meet different requirements than the dedicated fluorescence optics. Additionally, the much lower light levels produced by luminescence in comparison to flash lamp-induced fluorescence require the benefits of the photon counting detection technique.

5.4.1 Luminescence Optics

In luminescence measurement mode, the INFINITE F500 uses a fixed microplate position and a moveable luminescence measurement head (see Figure 5-2: Luminescence optics). The Z-position of the luminescence fiber bundle fixed onto the optics carrier is automatically adjusted for each measurement according to the selected microplate format. As light is refracted at the sample liquid surface, z-adjustment helps to maximize signal to background luminescence and minimize cross-talk. The INFINITE F500 is aware of the actual plate thickness once the user has selected the corresponding plate type in the software dialog.

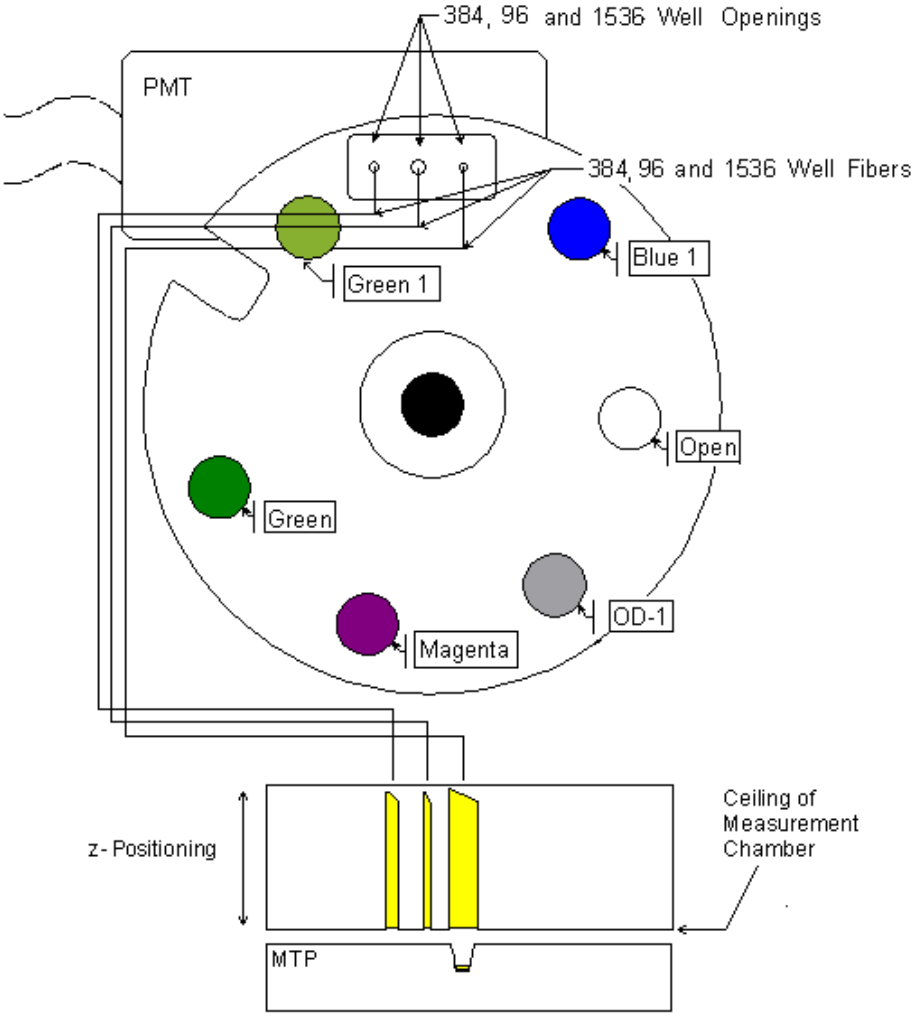


Figure 5-2: Luminescence optics

Fibers

Three fibers guide the light from the sample to the detection unit. The fibers are designed to measure 96-well plates, 384-well plates as well as 1536-well plates and are selected appropriately depending on the chosen plate format.

The apertures in the ceiling of the measurement chamber are designed to receive as much light as possible from samples in 96-, 384- or 1536-well plates, respectively, thus maximizing the luminescence signal. At the same time, cross talk is minimized because one particular aperture does not receive substantial amounts of light from neighboring wells.

The luminescence light is guided through the same fibers to the luminescence photomultiplier tube (PMT). The PMT is designed for applications in chemo- and bioluminescence providing a high dynamic range.
The exceptionally low noise and high sensitivity makes the detection of very low light levels possible.

Filter Wheel

A filter wheel with six filter positions in front of the PMT window is switched for the required luminescence channel. The sensitivity of the detection system makes it necessary to attenuate high luminescence light levels. Therefore, the filter wheel can also switch a neutral density filter across the selected fiber exit.

Filter Wheel Position	**Filter**
Position 1	Green *
Position 2	Magenta *
Position 3	OD 1 (neutral density filter)
Position 4	Empty (no attenuation)
Position 5	Blue 1 **
Position 6	Green 1 **

* recommended for BRET^{2} ™ and ChromaGlo™ assays

** recommended for BRET^{1} assays

Appropriate filters for other luminescence applications, e.g. dual color luminescence, may be chosen according to the spectra below. See figures 5-3 to 5-6 for the transmission spectra of each luminescence filter.

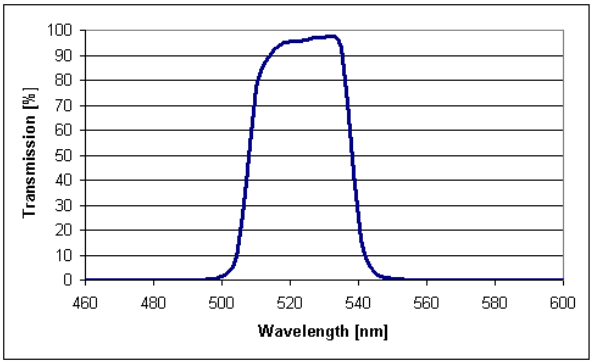


Figure 5-3: Transmission spectrum of ‘Green’ filter

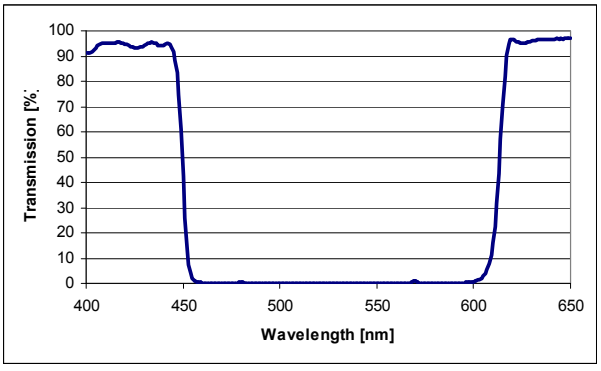


Figure 5-4: Transmission spectrum of ‘Magenta’ filter

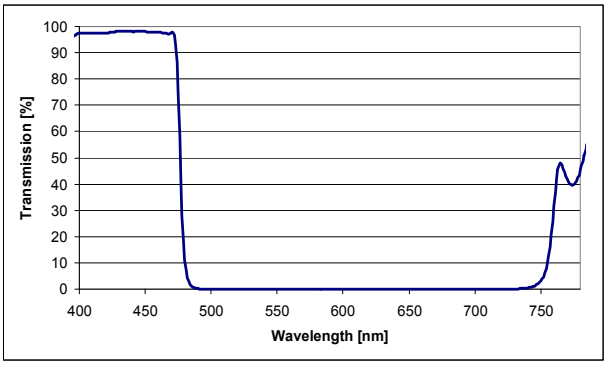


Figure 5-5: Transmission spectrum of 'Blue 1' filter

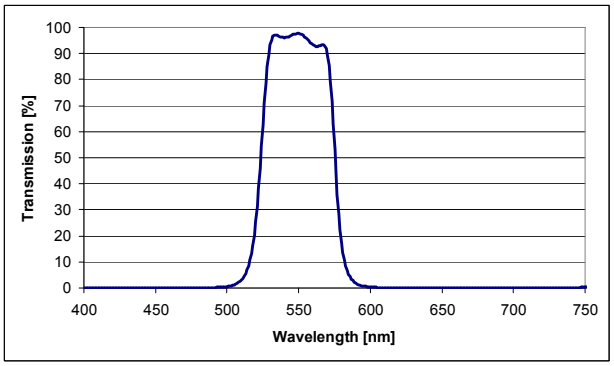


Figure 5-6: Transmission spectrum of 'Green 1' filter

5.4.2 Luminescence Detection

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Caution

Make sure that all instrument lids (injector module lid, plate transport lid and filter slides lid) are closed completely before starting a luminescence measurement.

The INFINITE F500 luminescence detection system utilizes the single photon counting measurement technique. This is based on a dedicated luminescence PMT with appropriate measurement circuitry. This technique is very robust against noise. It is preferred for measurement of very low light levels.

For best performance it is recommended to use white plates for luminescence measurements. For details see *6.3.3 Recommended Types of Microplates*.

Note

Results of luminescence measurements are always displayed in RLU (Relative Luminescence Units). 1 RLU corresponds to 1 count/s.

6. Operating the INFINITE F500

6.1 Introduction

The INFINITE F500 is operated using a personal computer based software control. *i-control* or *Magellan* software may be used as the user interface. For details see the corresponding software manual. This short introduction is for a general understanding of instrument parameters and operation. Suggestions are made on how to optimize instrument parameters for your applications.

Every effort has been made to ensure that the instrument will work correctly even if the default parameters are not appropriate for a particular application - with an important exception:

Caution

When placing a microplate into the plate carrier, always make sure that the correct plate definition file (plate height) has been selected in the software before you do anything else.

Maximum plate height is 23 mm (including lid).

Caution

Before starting measurements, make sure that the microplate position A1 is inserted correctly. The position of well A1 has to be on the upper left side.

Important

When operating the INFINITE F500 always work according to GLP guidelines.

Caution

The INFINITE F500 has a fan on the backside of the instrument that draws in air. The air filter has to be checked every 4 weeks and be replaced when dirty. The air filter must be replaced after 6 months.

6.2 General Operating Features

The INFINITE F500 has some general behavior and options, which are independent from a particularly selected measurement technique.

6.2.1 Instrument Start Up

Before the instrument is switched ON, check if the USB interface cable is connected.

Instrument Power On

When switching ON the instrument no initialization steps are performed.

Connect to Instrument

When the software connects to the instrument, communication is established between the instrument and the user interface.

The following steps are performed:

- Initialization of filter slides if inserted
- Initialization of mirror transport
- Initialization of luminescence filter wheel
- Initialization of z-transport of luminescence optics
- Initialization of plate transport
- Unlocking of injector carrier (if option is installed)
- The plate transport is not moved out automatically.

The current versions of firmware and software are displayed.

The instrument is ready to be operated.

Insert Filter Slides

Filter slides are automatically retracted to a reference position, when manually moved into the respective filter slot. In case a valid filter code cannot be identified, the slide will be rejected. To define a new filter slide, please refer to the chapter 4.4.

6.2.2 Finish a Measurement Session

Disconnect from Instrument

When disconnecting, communication between the instrument and the PC is terminated.

Note

Remove the microplate before disconnecting the instrument from the computer.

Instrument Shut Down

Upon shut down, the instrument activity is stopped immediately. Normally, you should disconnect before shut down. In the rare case of an unexpected hardware error, immediate instrument shut down will reduce the risk of possible damage.

6.2.3 General Options

The following options may be taken independently from the particular measurement technique.

Temperature Control

Some assays ask for an exact operating temperature. The INFINITE F500 can set up a specific temperature within a specific range, provide uniformity across the plate, and keep temperature constant above ambient. The main cooling fans stop ventilation.

Heating up 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.

Temperature range: 4°C above ambient to 42 °C.

Kinetic Measurements

i-control allows a plate to be measured repeatedly in equidistant time intervals. Fluorescence signal may significantly decrease over a longer period of time, especially when using low volumes. Depending on the amount of evaporation, the meniscus will shift to a lower position giving rise to slightly out of focus conditions. Usually, wells at the edges of the microplate are likely to evaporate faster. When measuring fluorescence, decreases in signal may also be a result of photo bleaching.

Microplate Shaking

The INFINITE F500 provides two shaking modes: linear and orbital. The shaking amplitude can be selected from 2 – 6 mm (linear shaking) and 1 – 6 mm (orbital shaking), respectively. The frequency is a function of the amplitude. The shaking duration is selectable from 1 – 999 s.

Multi Labeling

i-control provides a basic Multi Labeling capability. Up to four sets of instrument parameters can be edited. The corresponding plate measurements will be executed in the selected order. For example, when using more than one fluorescent label, different filter combinations could be selected. A multi labeling measurement can be set up by using a plate strip with/without a 'part of the plate'-strip and up to 4 measurement strips (absorbance fixed wavelength, fluorescence intensity, fluorescence polarization, luminescence).

6.3 Optimize Fluorescence Measurements

Fluorescence measurement results may be optimized by tuning instrument parameters on the one hand, and by selecting appropriate materials on the other hand.

6.3.1 Instrument Parameters

Z-Optimization

A basic feature of the INFINITE F500 is the z-optimization procedure. For a particular assay, this procedure should be performed once to determine the optimum working distance between the plate and the fluorescence optics. A user-selected well will be measured repeatedly while the objective is moved away from the closest to the farthest possible position.

Z-position can be determined as follows:

(1) **'Calculated from well'**:

When using the option '**calculated from well**', the INFINITE F500 will automatically identify the z-position of maximum raw signal for further measurements.

(2) **'Same as'** for multilabeling measurements:

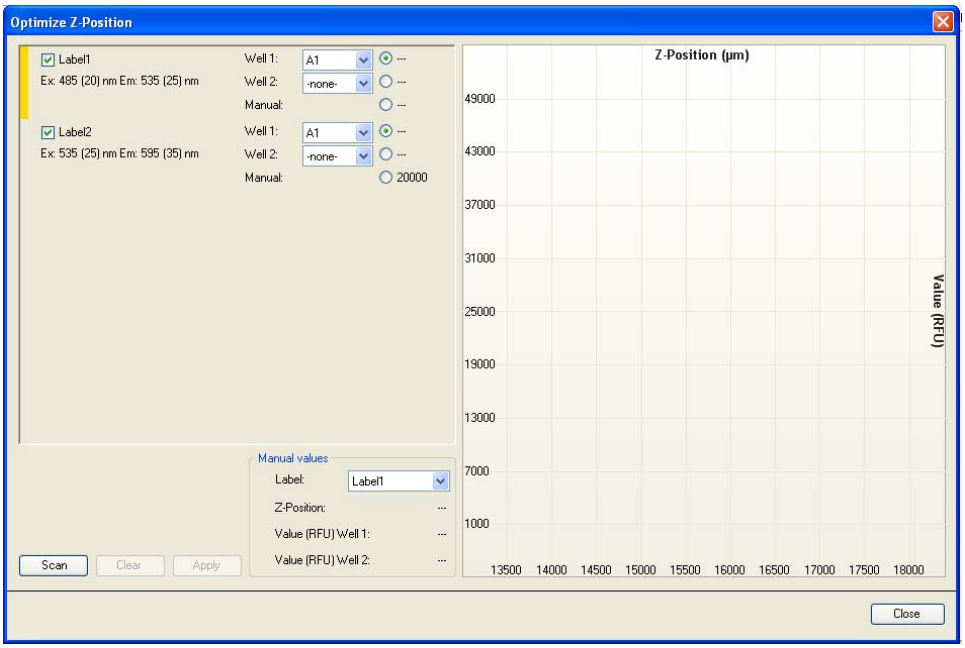
When using the option '**same as**', the INFINITE F500 will automatically use the same z-position as for a previously defined label.

E.g. in a script with 2 FI Top labels named as Label 1 and Label 2 the z-position of the Label 1 can also be used for the Label 2 by selecting the option 'Same as = Label 1'.

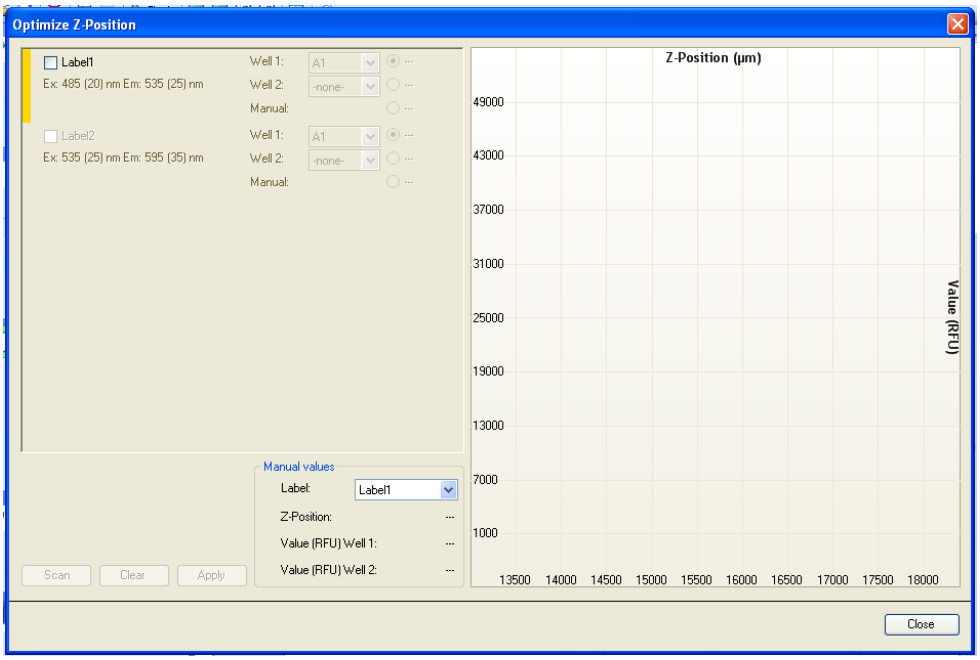
(3) '**Instrument' → 'Z-Position**':

When using the **'Z-Position**' from the instrument menu, the user can determine the appropriate z-position from a graphical plot with a mouse click. This value is used by the INFINITE F500 for further measurements:

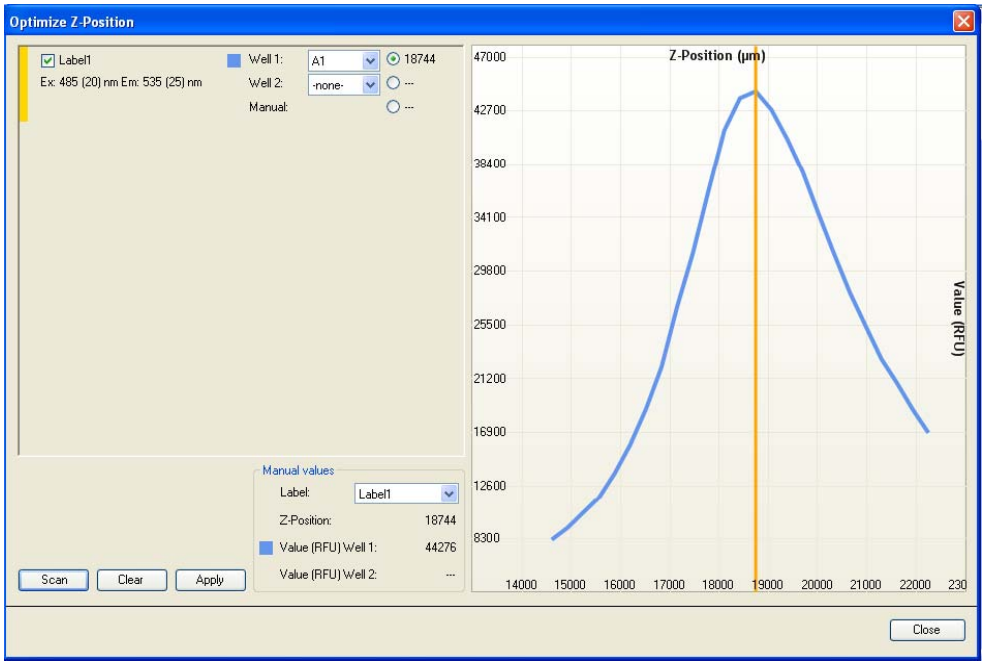
- Select '**Z-Position**' from the Instrument Menu:



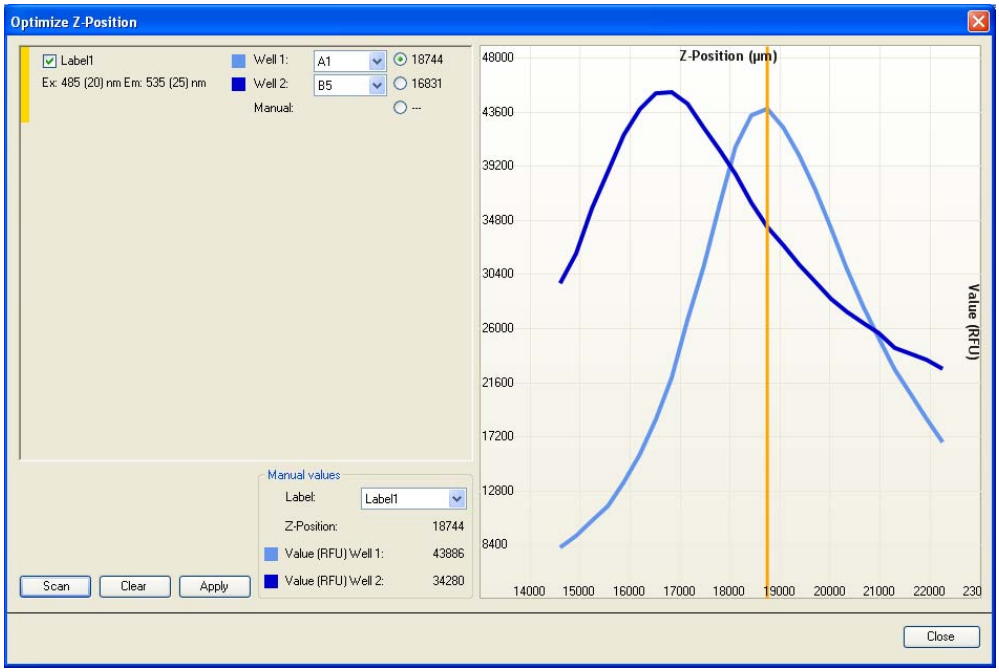
- Select the label(s) for which the z-position optimization should be performed. The optimal z-position can be simultaneously determined for up to 4 labels.
- The label selection/number of labels depends on the measurement script previously defined in the i-control. Additionally, if the z-position of one of the labels is defined as 'Same as', the label will be displayed but it cannot be selected for the z-optimization:



- For each selected label, one or two wells of the defined plate range can be used for the z-position optimization. Select the well(s) and click **'Scan'** for starting the z-optimization:



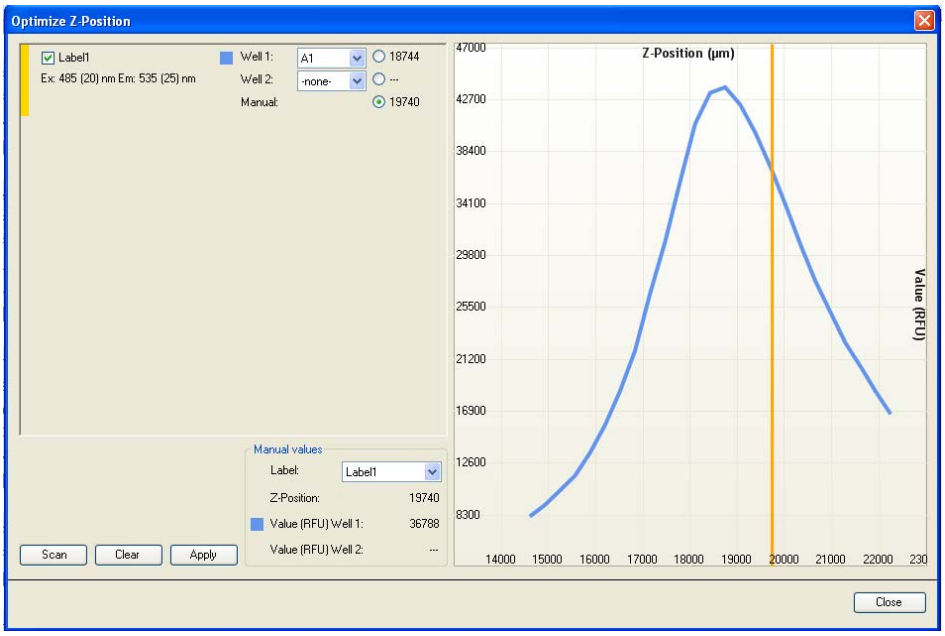
- If the z-optimization is performed with two wells, only one value can be applied to a defined script afterwards:



Note

When using two wells, the z-position optimization is performed with the optimal gain for each selected well, resulting in the optimal fluorescence signal for each z-position curve. Therefore, the signal intensity of the selected wells might not be directly comparable as the optimization measurement could be performed at two different gains.

- **Manual values:** the user can also define the z-position for each selected label manually. After the z-position scan a manual value can be determined by (i) clicking the option **'Manual'** and defining the new position by moving the yellow bar:



- Select the z-position value (optimal or manually defined) for each selected label. Clicking the **'Apply'** button, the selected values will be automatically applied to the script and used for the following measurement.

The z-position for maximum fluorescence raw signal depends on the dispensed volume, the plate height, the shape of the well, the shape of the meniscus, the wavelengths used, etc.:

(i) For homogeneous assays, the optimum z-position appears to be identical with the z-position where the fluorescence intensity raw data are maximal.

(ii) For non-homogeneous assays (for example: based on adherent cell layers) the optimum z-position may not be identical with the z-position, where the fluorescence intensity raw data are maximum. In such a case we recommend to employ z-optimization using option 2 (user interaction). The user may determine the z-position, where the difference between the raw data of a fluorescent SAMPLE and a BLANK well is greatest. Note: With option 2), two wells can be selected and data will be plotted into separate graphs.

Alternatively, the user may wish to switch to bottom reading fluorescence mode (if fitted) in the case of adherent cell-based assays.

Note

Z-position seems to be optimal where the net fluorescence signal is maximal.

PMT and Gain Settings

The INFINITE F500 fluorescence detection system comprises a Photo Multiplier Tube (PMT) and an A/D converter for conversion of the analogue PMT signal to a digital output.

The gain setting of the PMT controls the amplification of the incoming fluorescence light into electrical current. The signal amplification of the PMT depends on the supply voltage and is selectable as gain setting from 1 – 255. The relationship between gain setting and the voltage supply is described in Equation 6.3-1.

Equation 6.3-1:

$$U = \frac{Gain}{255} * 1250V$$

Where U is the voltage, Gain is the selected gain setting, 255 is the maximum possible gain and 1250 V is the maximum voltage supply of the PMT.

Example:

A gain of 100 corresponds to a voltage supply of 490 V:

$$U = \frac{100}{255} * 1250 = 490V$$

Gain settings influence the performance of the PMT and finally the assay signal to noise ratio (S/N) and the linearity range. In practice, e.g. for assay optimization the gain should be tuned carefully to get an optimal assay window, with the highest fluorophore concentration in a microplate well should give highest possible readout and the lower fluorophore concentrations separate well from background.

At low voltages the linearity range is limited, whereas at higher voltages a considerable noise can occur.

Tecan recommends working with gains of about 60 and higher because the PMT's behaviour is well characterized in this range. The user should therefore carefully evaluate measured data at lower gain settings to achieve an acceptable assay performance.

Note

If any well of interest is assigned "OVER" (overflow), you may manually reduce the gain, or select an automatic gain option (see the software manual).

Flash Settings

On the fly measurements with 1 flash (read) per well are possible for all plate types; however, measurement precision at low light levels depends on the reading time while fluorescence signal can be received.

Note

Increase the number of flashes (no. of reads) per well until noise of BLANK wells does not further improve, or until measurement time per well becomes unacceptable.

For prompt fluorescence it does not help to increase the default integration time, because the detector will not receive more signal once the flash has vanished.

Timing Parameters for Time Resolved Fluorescence

For TRF, signal integration parameters need to be adjusted according to the label. The start of the signal Integration Time is delayed against the preceding flash by a Lag Time. TRF timing parameters may be established with the following procedure:

1. As a starting point you may take the **Fluorescence Lifetime** of the label for both **Integration Time** and **Lag Time**.
2. Coarse tuning: With Integration Time being fixed reduces the Lag Time to maximize **Signal to Background (S/B)**.
3. Fine tuning: With Lag Time being fixed extends the Integration Time and check, if S/B further improves.
4. Optional Fine-tuning: With either timing parameter being fixed you may vary the other one and check, if S/B further improves.

Settle Time

Before measuring a well, a settle time may be set. Due to the stop and go motion of the plate carrier the meniscus of the dispensed liquid may still vibrate while signal is integrated. This can give rise to fluctuations of the measured values. The effect has been observed in wells of 96-well plates and larger wells. In particular, it is critical with absorbance measurements.

6.3.2 FI Ratio Mode

Ratio Mode

Up to 4 labels may be measured well-wise. This measurement mode is called ‘ratio mode’. Be aware that no ‘ratio’ calculation is performed after this measurement. The Excel™ result sheet shows the raw data. Further calculations have to be performed by the user.

FI Ratio Mode versus FI

Prior to each measurement a so called reference measurement has to be performed to compensate for fluctuations of the flash lamp. The sensitivity of the reference diode needs to be adjusted (automatically done by the software) before each measurement to make sure that the diode is working in an optimal sensitivity range and does not show overflow values. This reference measurement is performed differently for FI ratio measurements and normal fluorescence intensity measurements. For normal fluorescence intensity measurements the calibration of the reference diode is performed at the selected measurement wavelengths. In FI ratio mode, the reference measurement is performed with the label which gives the highest light intensity. This procedure has proven successful in avoiding overflow errors and in providing sufficient sensitivity. Therefore, the fluorescence intensity measurement and FI ratio measurement performed with the same measurement parameters (gain, number of flashes, z-position) may result in different RFU values.

Filter Switch Time

The INFINITE F500 can switch between two neighboring filters within 150 ms in case that the selected labels are measured with the same gain. Otherwise, the switching time is longer as the high voltage level at the PMT needs to be changed. The high voltage applied to the PMT needs some time to stabilize.

6.3.3 Measurement Accessories

Recommended Filters

Please ask your local Tecan representative for a recommended filter set. Filters designed for a different type of instrument will not necessarily perform well with the INFINITE F500.

Note

If the excitation and the emission maximum of a fluorescent species are close together, they should not be directly translated into center wavelengths for fluorescence filters.

To provide acceptable background, usually, the upper cutoff for excitation wavelengths on the one hand, and the lower cutoff for emission wavelengths on the other hand need to be separated. This compromise depends on the blocking properties of the filters. For many fluorescent molecules signal may be improved by expanding filter bandwidth away from the other band pass, respectively.

Recommended Type of Mirror

From the mirrors mounted to the mirror carrier the most appropriate can be automatically selected according to the selected filter wavelengths. Two custom dichroics can be optionally mounted on the mirror carrier.

Recommended Types of Microplates

Generally, for high fluorescence sensitivity, black microplates are recommended. For low concentrations of TRF labels, white microplates seem superior. You may check if white plates are superior with UV excitation wavelengths.

We do not recommend using volumes less than a third of the maximum volume. When using lower volumes, check the availability of a suitable plate type.

In order to ensure good performance for Fluorescence Bottom Reading, we recommend using black plates with transparent bottom.

All standard microplates from 6 to 1536 wells (maximum plate height 23 mm including lid) that conform to the following standards can be measured: ANSI/SBS 1-2004, ANSI/SBS 2-2004; ANSI/SBS 3-2004 and ANSI/SBS 4-2004.

When installing the operating software (i-control or Magellan), pre-defined plate definition files are installed. Please refer to the following list for the corresponding ordering numbers of the microplates. Please order microplates at your local microplate supplier.

Plate Definition File (*.pdfx)	Catalog Number	Manufacturer
GRE6ft	657 160 657 185	Greiner Bio-One, www.gbo.com/bioscience
GRE12ft	665 180 665 102	Greiner Bio-One, www.gbo.com/bioscience
GRE24ft	662 160 662 102	Greiner Bio-One, www.gbo.com/bioscience
GRE48ft	677 180 677 102	Greiner Bio-One, www.gbo.com/bioscience
GRE96ft	655 101 655 161	Greiner Bio-One, www.gbo.com/bioscience
GRE96fb_chimney	655 079 655 086 655 077 (Fluotrac 600) 655 076 (Fluotrac 200)	Greiner Bio-One, www.gbo.com/bioscience
GRE96fw_chimney	655 073 655 083 655 074 (Lumitrac 600) 655 075 (Lumitrac 200)	Greiner Bio-One, www.gbo.com/bioscience
GRE96ut	650 101 650 161 650 160 650 180 650 185	Greiner Bio-One, www.gbo.com/bioscience
GRE96vt	651 101 651 161 651 160 651 180	Greiner Bio-One, www.gbo.com/bioscience
GRE384fb	781 079 781 086 781 077 (Fluotrac 600) 781 076 (Fluotrac 200) 781 094 (µClear) 781 095 (µClear)	Greiner Bio-One, www.gbo.com/bioscience
GRE384ft	781 061 781 101 781 162 781 185 781 186 781 165 781 182	Greiner Bio-One, www.gbo.com/bioscience
GRE384fw	781 073 781 080 781 074 (Lumitrac 600) 781 075 (Lumitrac 200) 781 097 (µClear) 781 096 (µClear)	Greiner Bio-One, www.gbo.com/bioscience
GRE384sb	784 209	Greiner Bio-One, www.gbo.com/bioscience
GRE384st	784 201	Greiner Bio-One, www.gbo.com/bioscience
GRE384sw	784 207	Greiner Bio-One, www.gbo.com/bioscience
COS6ft	3506 3516	Corning, www.corning.com/lifesciences/
COS12ft	3512 3513	Corning, www.corning.com/lifesciences/

Plate Definition File (*.pdfx)	Catalog Number	Manufacturer
COS24ft	3524 3526 3527	Corning, www.corning.com/lifesciences/
COS48ft	3548	Corning, www.corning.com/lifesciences/
COS96fb	3916 (TC-Treated) 3915 (Non-Treated) 3925 (Treatment: High)	Corning, www.corning.com/lifesciences/
COS96ft	3370 3628	Corning, www.corning.com/lifesciences/
COS96fw	3362 (TC-Treated) 3912 (Non-Treated) 3922 (Treatment: High)	Corning, www.corning.com/lifesciences/
COS96rt	3360 3367 3788 3795 3358	Corning, www.corning.com/lifesciences/
COS96ft_half area	3690 (High Binding) 3695 (Non-Treated) 3697 (TC-Treated)	Corning, www.corning.com/lifesciences/
COS384fb	3708 (Treatment: High) 3709 (TC-Treated) 3710 (Non-Treated)	Corning, www.corning.com/lifesciences/
COS384ft	3680 (Non-Treated) 3700 (Treatment: High) 3701 (TC-Treated) 3702 (Non-Treated)	Corning, www.corning.com/lifesciences/
COS384fw	3703 (Treatment: High) 3704 (TC-Treated) 3705 (non-Treated)	Corning, www.corning.com/lifesciences/
COS384fb_assay plate clear bottom	3711 (Non-Treated) 3712 (TC-Treated)	Corning, www.corning.com/lifesciences/
COS384fw_assay plate clear bottom	3706 (Non-Treated) 3707 (TC-Treated)	Corning, www.corning.com/lifesciences/
COS384fb_low volume	3676 (NBS) 3677 (Med. Binding)	Corning, www.corning.com/lifesciences/
COS384fw_low volume	3673 (NBS) 3674 (Med. Binding)	Corning, www.corning.com/lifesciences/
NUN96ft	137 101 237 105 437 111 437 112 436 008 436 016 436 027 436 034	Nunc, www.nuncbrand.com
NUN96fb	137 101 237 105 437 111 437 112 436 008 436 016 436 027 436 034	Nunc, www.nuncbrand.com
NUN96fw	136 101 236 107 436 110 436 111 436 007 436 015 436 026 436 033	Nunc, www.nuncbrand.com
NUN384ft	436 009 436 017 436 028 436 036 164 555 152 030 242 757 460 440 464 718	Nunc, www.nuncbrand.com
NUN384fb	436 011 436 019 436 031 436 038 164 564 262 260 460 435 460 518	Nunc, www.nuncbrand.com
NUN384fw	436 010 436 018 436 029 436 037 164 610 262 360 460 420 460 372	Nunc, www.nuncbrand.com

Plate Definition File (*.pdfx)	Catalog Number	Manufacturer
GRE 1536fb	782 076 (FLUOTRAC 200, HiBase) 782 077 (FLUOTRAC 600, HiBase) 783 076 (FLUOTRAC 200, LoBase) 782 097 (µClear, HiBase) 782 096 (µClear, HiBase) 783 096 (µClear, LoBase)	Greiner Bio-One, www.gbo.com/bioscience
GRE1536fw	782 075 (LUMITRAC 200, HiBase) 782 074 (LUMITRAC 600, HiBase) 783 075 (FLUOTRAC 200, LoBase) 782 095 (µClear, HiBase) 782 094 (µClear, HiBase) 783 095 (µClear, LoBase)	Greiner Bio-One, www.gbo.com/bioscience
GRE1536ft	782 101 (HiBase) 782 061 (Microlon 600, HiBase) 783 101 (LoBase)	Greiner Bio-One, www.gbo.com/bioscience

Table 6.3-1: Plate definition files and the corresponding catalog numbers

6.4 FP Measurements

6.4.1 Fluorescence Polarization

Fluorescence Polarization (FP, P) is defined by the following equation:

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

where $I_{||}$ and $I_{\perp}$ equal the emission intensity of the polarized light parallel and perpendicular to the plane of excitation, respectively. Polarization is a dimensionless unit, generally expressed in mP units.

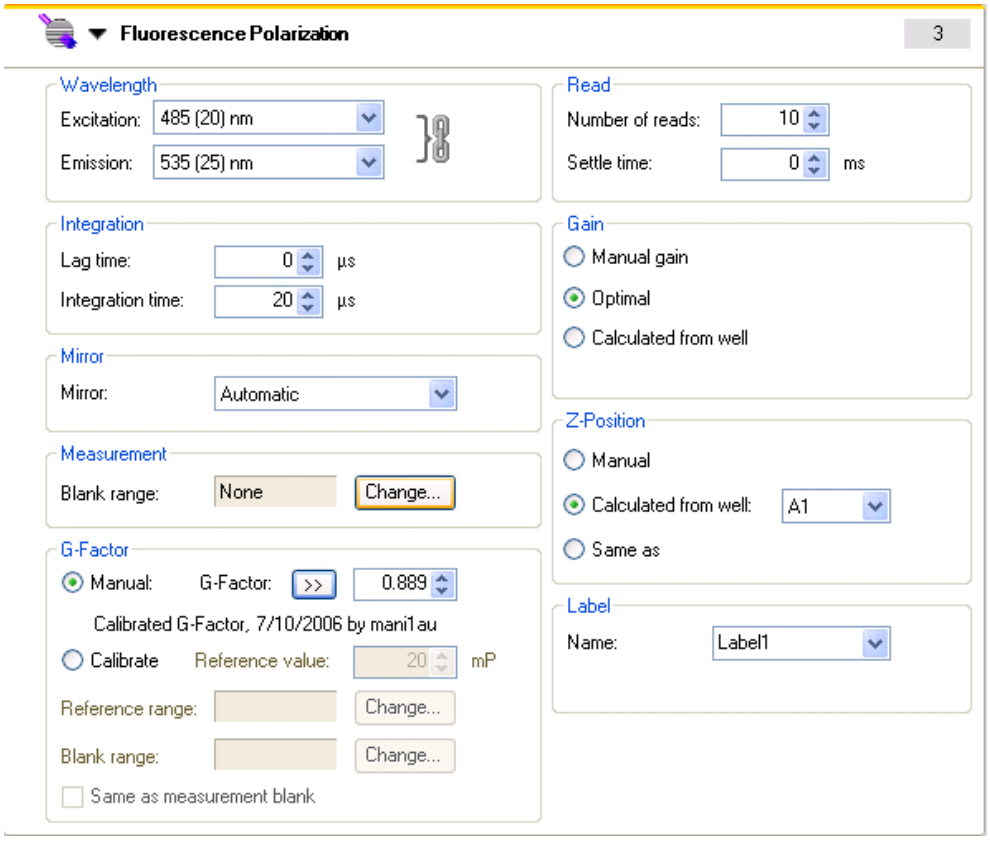
To start an FP measurement, the program strip must contain valid **G-Factor** settings.

6.4.2 Measurement Blank Range

If the measurement blank range is defined, the measurement blank reduction is performed automatically at each fluorescence polarization measurement; the mean value of the respective blank wells will be subtracted from each sample value (see 6.4.8).

Defining the measurement blank:

- In the **Measurement** group box, select the **Blank range** by clicking '**Change**' and then selecting the wells filled with the measurement (sample) blank.



6.4.3 G-Factor Settings

The given equation for calculation of fluorescence polarization assumes that the sensitivity of the detection system is equivalent for parallel and perpendicular polarized light. This is generally not the case and either the parallel or perpendicular intensity must be corrected by a so called 'G-Factor'. The G-factor compensates for differences in optical components between parallel and perpendicular measurement.

The G-Factor is the correction factor that can be determined for the wavelength of the fluorophore by measuring a sample with a known polarization value. A valid calibration of the instrument resulting in a G-factor is an important requirement for each fluorescence polarization measurement.

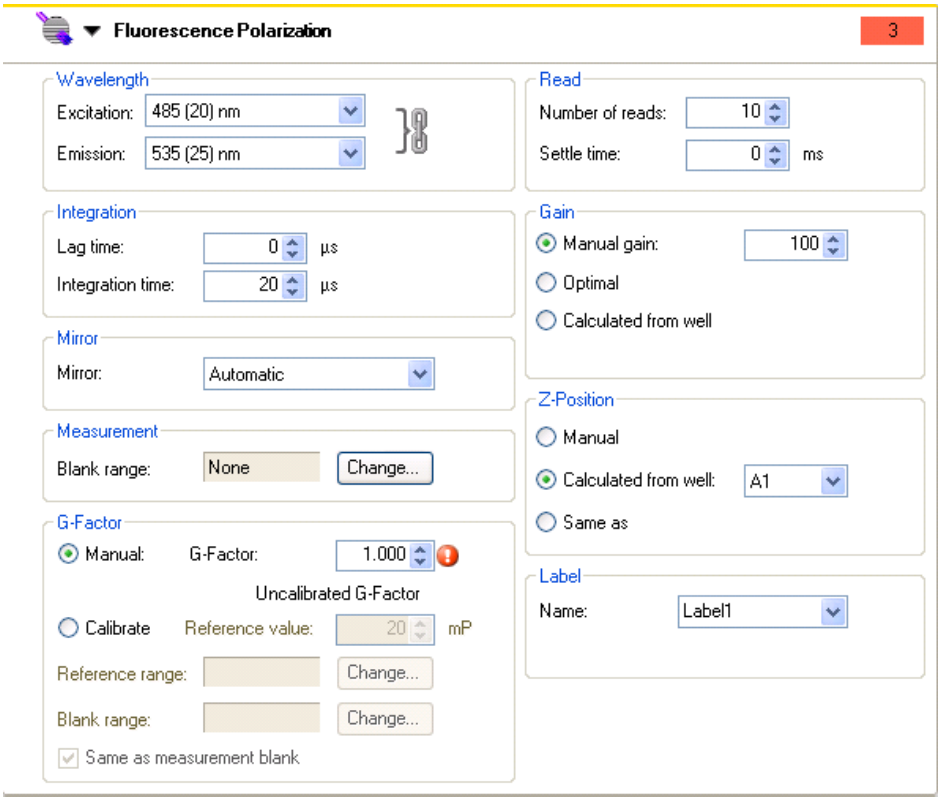
Caution

Make sure that the filter slides contain polarizers together with the filters defined for fluorescence polarization. Measurements without the polarizers will result in a false G-Factor and false measurement data.

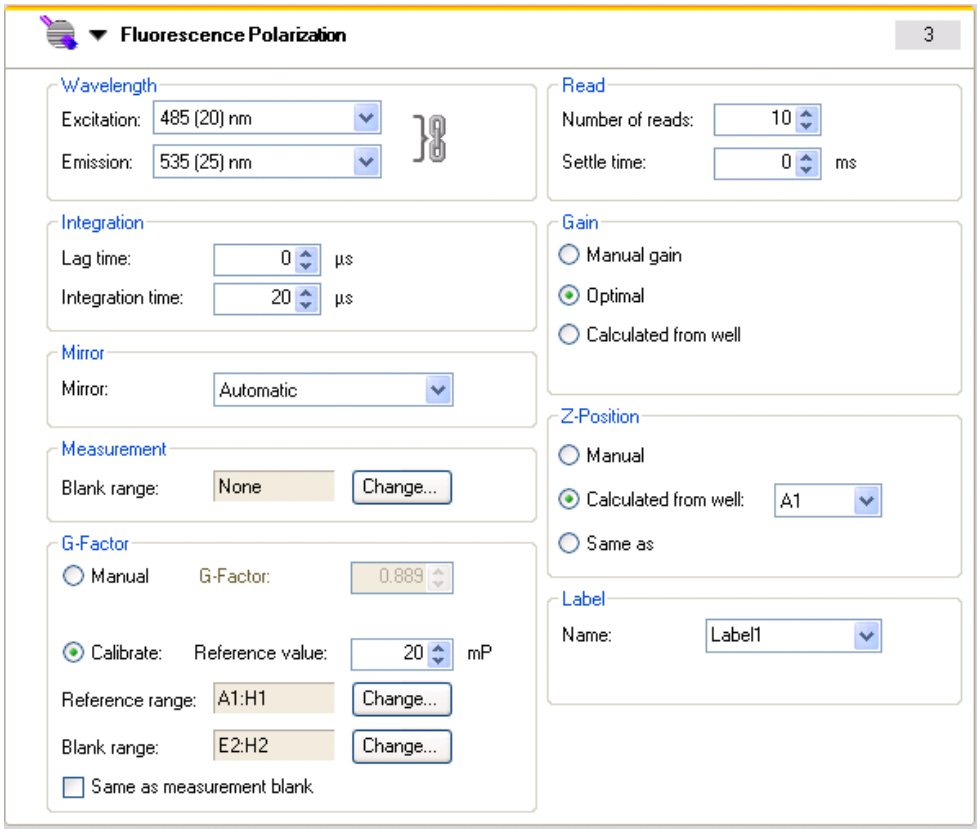
6.4.4 Measurement with an Uncalibrated G-Factor

If no calibrated G-factor is available, the default value of 1 will be displayed and marked as 'Uncalibrated G-Factor'. In order to enable the measurement, confirm this value or select a new one by either clicking the up and down arrows or entering a value in the **G-Factor** field.

For the G-Factor calibration see 6.4.5 Measurement with a Simultaneous G-Factor Calibration.



6.4.5 Measurement with a Simultaneous G-Factor Calibration



When **Calibrate** is selected, the G-factor is determined for the current measurement parameters and used for the following FP measurement. In order to perform the G-Factor calibration, please define:

- **Reference value**: select a polarization value for the reference used, e.g. 20 mP for a 1 nM fluorescein solution in 0.01 M NaOH.
- **Reference range**: click '**Change'** and select the wells filled with the reference.
- **Blank range**: click '**Change'** and select the wells filled with the reference blank. Select '**Same as measurement blank'** if the reference blank is the same as the measurement blank. In that case the measurement blank has to be defined as well.

Note

By filling in more than one well with polarization references and reference blanks, the mean values will be calculated and therefore the calibration result will be more accurate.

G-Factor Storage

The calculated G-Factor is automatically stored on the computer's hard drive. Each G-Factor entry corresponds to the filter pair selection as well as the filter slide description. There is always only one G-Factor available for the respective filter pair combination and filter slide description, unless the same filter pair has been used with the different filter slides and thus stored with the different filter slide descriptions.

Caution

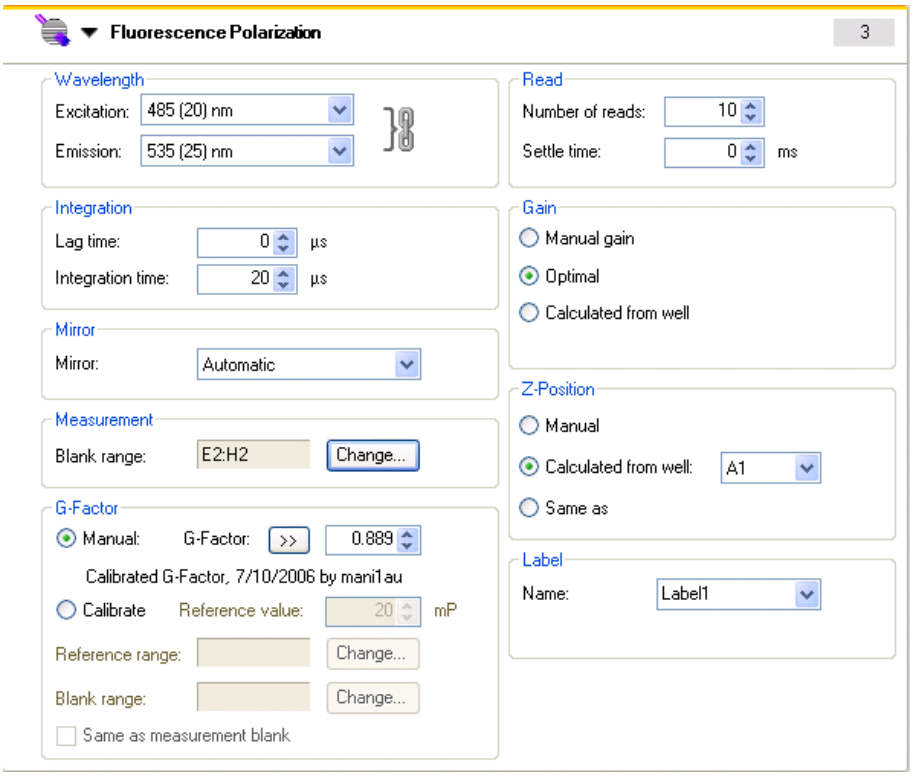
The filter slide description is part of the G-Factor key value. Avoid using the same filter slide description for different filter slides as this will affect the correct G-Factor recognition.

6.4.6 Measurement with a Calibrated G-Factor

Note

Once calibrated, the G-factor is shown and can be used immediately if it matches the Ex/Em wavelength pair and the filter slide description.

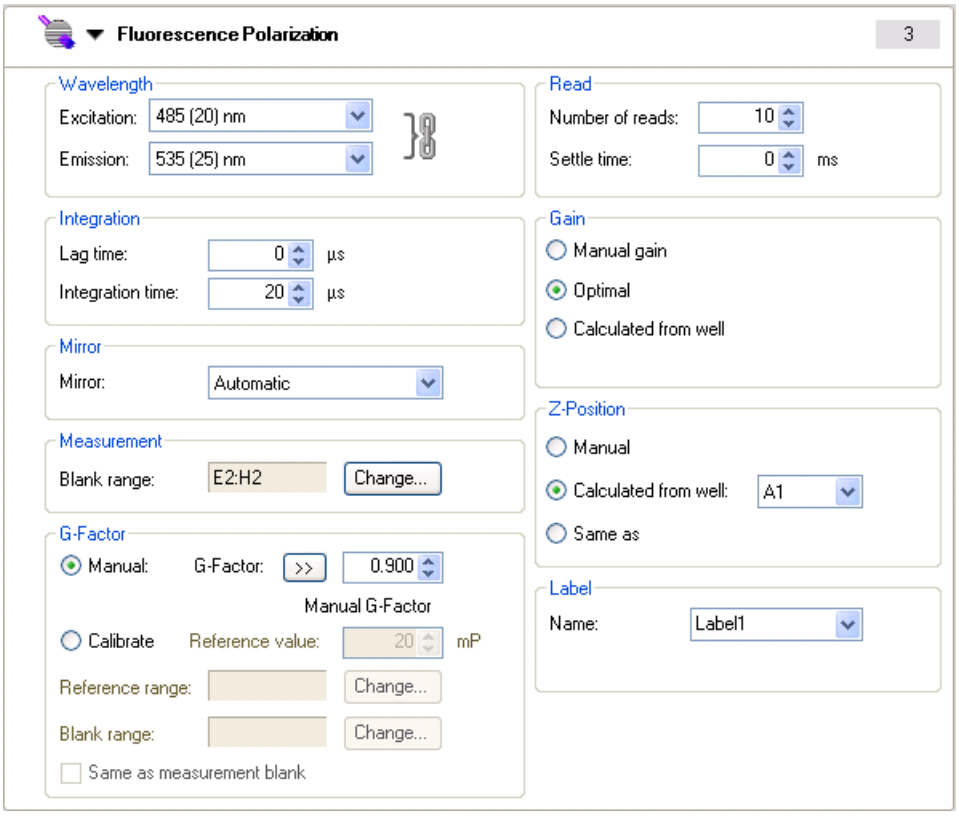
A calibrated G-factor will be displayed automatically or can be loaded by clicking the '>>' button only if it matches the selected fluorescence polarization filter pair and the filter slide description.



The calibrated G-Factor is marked as 'Calibrated G-Factor' with date and signature.

6.4.7 Measurement with a Manual G-Factor

If the displayed G-Factor does not match the calibrated value (e.g. the G-Factor has been manually changed or loaded with a method), the corresponding value will be marked as 'Manual G-Factor'.



The calibrated G-Factor can be restored by clicking the '**>>**' button on the left side of the displayed G-Factor.

Note

G-Factor adjustment via '>>' button is only possible, if a calibrated G-Factor is available for the corresponding wavelength.

6.4.8 Calculation of Fluorescence Polarization Parameters

G-Factor:

$$G = \frac{(1 + P_{ref})(\overline{RFU}^{cross}_{ref} - \overline{RFU}^{cross}_{buf})}{(1 - P_{ref})(\overline{RFU}^{par}_{ref} - \overline{RFU}^{par}_{buf})}$$

P_{ref} ... *Polarization value of reference* [P]

$\overline{RFU}_{ref}$ ... *Averaged relative fluorescence units of reference*

$\overline{RFU}_{buf}$ ... *Averaged relative fluorescence units of buffer*

Blank Reduction:

The mean value of the respective blank wells is subtracted from each value.

$$\Delta RFU^{par} = \begin{cases} RFU^{par}_{ref} - \overline{RFU}^{par}_{buf} \\ RFU^{par}_{buf} - \overline{RFU}^{par}_{buf} \\ RFU^{par}_{smp} - \overline{RFU}^{par}_{blk} \\ RFU^{par}_{blk} - \overline{RFU}^{par}_{blk} \end{cases} \quad for\ each\ well$$

$$\Delta RFU^{cross} = \begin{cases} RFU^{cross}_{ref} - \overline{RFU}^{cross}_{buf} \\ RFU^{cross}_{buf} - \overline{RFU}^{cross}_{buf} \\ RFU^{cross}_{smp} - \overline{RFU}^{cross}_{blk} \\ RFU^{cross}_{blk} - \overline{RFU}^{cross}_{blk} \end{cases} \quad for\ each\ well$$

Intensities:

Parallel and perpendicular intensities are calculated using the following formulas:

$$I^{par} = G * \Delta RFU^{par}$$

$$I^{cross} = \Delta RFU^{cross}$$

Polarization:

$$P = \frac{I^{par} - I^{cross}}{I^{par} + I^{cross}}$$

Anisotropy:

$$A = \frac{I^{par} - I^{cross}}{I^{par} + 2 * I^{cross}}$$

Total Intensity:

$$I_{tot} = I^{par} + 2 * I^{cross}$$

6.5 Optimize Absorbance Measurements

6.5.1 Measurement Parameters

Flash Settings

On the fly measurements with 1 flash (read) per well are possible for all plate types; however, measurement precision at low light levels depends on the reading time during which a fluorescence signal can be received.

Note

Increase the number of flashes (no. of reads) per well until noise of BLANK wells does not further improve, or until measurement time per well becomes unacceptable.

Settle Time

A settle time before measuring a well may be set (critical for absorbance measurements). Due to the stop and go motion of the plate carrier the meniscus of the dispensed liquid may still vibrate while signal is integrated. This can give rise to fluctuations of the measured values. The effect has been observed in wells of 96-well plates and larger wells.

6.5.2 Absorbance Ratio Mode

Ratio Mode

Up to 4 labels may be measured well-wise. This measurement mode is called ‘ratio mode’. Be aware that no ‘ratio’ calculation is performed after this measurement. The Excel™ result sheet shows the raw data. Further calculations have to be performed by the user.

Filter Switch Time

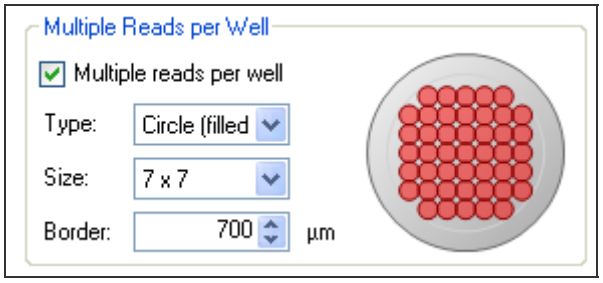
The INFINITE F500 can switch between two neighboring filters within 150 ms.

For conditions see 6.3.2 FI Ratio Mode.

6.6 Multiple Reads Per Well

The i-control software allows multiple reads per well (MRW) to be performed in absorbance, fluorescence top and fluorescence bottom mode.

The multiple reads per well functions can be activated on an absorbance or fluorescence intensity program strip by selecting the 'Multiple reads per well' check box (see Figure 6-1 below).



Note that the **Multiple reads per Well** function is not available for the 1536-well plate format.

Figure 6-1: **Multiple reads per Well**

Note

The function 'multiple reads per well' is only available for the reading modes 'absorbance', 'fluorescence intensity top' and 'fluorescence intensity bottom'.

6.6.1 MRW Type

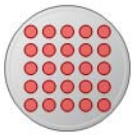
The MRW types define the pattern how the measurement will be performed. The software allows seven different MRW types to be selected:

1) Square
2) Square (filled)
3) Circle
4) Circle (filled)
5) X-line
6) Y-line
7) XY-line

Pattern examples:

Square:

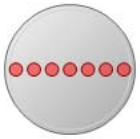
Square (filled):



Circle:

Circle (filled):

X-line:



Y-line:

XY-line:

6.6.2 MRW Size

The MRW size determines the number of points to be measured in a well. Depending on the microplate type the 'size' is selectable from 1 x 1 to a maximum of 15 x 15 points. The diameter of the single measurement points corresponds to the theoretically calculated diameter of the light beam at the focal point (see Table 6.6-1).

Measurement Mode	**≤ 384**
Fluorescence Intensity Top	1.8 mm
Fluorescence Intensity Bottom	3.5 mm
Absorbance (microplate optics)	0.4 mm

Table 6.6-1: Theoretically calculated beam diameter at the focal point.

The MRW type displayed in the software is therefore only a schematic overview of the measurement pattern. When measuring real samples the pattern can vary and the overlap of the single measurement points can be slightly different from the displayed pattern. It is therefore recommended to optimize the multiple reads per well parameters for every new application.

6.6.3 MRW Border

In addition to ‘Size’ and ‘Type’, a ‘Border’ function allows the user to select a certain distance between light beam and the wall of the microplate well (distance in µm). As already stated in chapter 6.6.2 MRW Size, the software displays only a schematic overview of the measurement pattern. The border is calculated from the theoretical beam diameter of the instrument. However, when measuring liquid samples, the light beam diameter is influenced by the type and amount of liquid in a well. In addition, the plate type (e.g. material of bottom of the microplate) also influences the characteristics of the light beam. Therefore the theoretical border displayed in the software might not correspond to the actual border when measuring a real sample. It is therefore strongly recommended to optimize the multiple reads per well parameters for every new application. Make sure that the selected border ensures sufficient distance between light beam and wall of the microplate well.

Caution

All absorbance and fluorescence intensity specifications given in this manual are only valid for single point measurements (one measurement point per well). When using the multiple reads per well option the specifications are not valid.

Caution

The software displays only a schematic view of the measurement pattern. Therefore optimize the multiple reads per well parameters for every new application. Make sure that the selected border is sufficient to avoid an overlap between the light beam and the well wall of the microplate.

Caution

A ‘border’ value that is too small may cause wrong measurement results due to overlap between the light beam and the well wall of the microplate.

6.6.4 Result Display in MS Excel™

The MS Excel™ result sheet generated by the i-control software displays a schematic graphical overview ('Multiple Reads Per Well – Alignment'; see Figure 6-2) of the measurement points. A number is assigned to each measurement point. The results are presented in list form: number of measurement point versus result value (OD or RFU; see Figure 6-3 for result of a fluorescence measurement). In addition, the standard deviation ('Stdev') and the average value ('Mean') of the measurements points/well are also displayed:

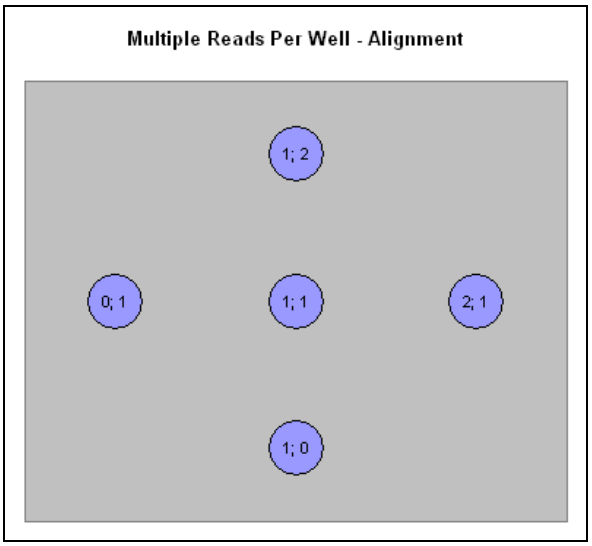


Figure 6-2: Alignment graphic (XY-Line, 3 x 3)

Well	Mean	StDev	1;2	2;1	1;1	0;1	1;0
A1	30	4	26	35	29	27	31
A2	28	3	28	31	23	28	30
A3	28	6	31	31	27	18	32
B1	33	5	29	35	30	41	30
B2	36	4	40	36	30	37	35
B3	32	8	30	41	22	29	39
C1	30	6	28	35	21	31	36
C2	35	5	30	36	31	37	41
C3	38	7	40	41	25	40	41

Figure 6-3: Example of MS Excel™ result list generated by i-control.

6.6.5 Miscellaneous Software Features of MRW

MRW is only available for the measurement modes ‘Absorbance’, ‘Fluorescence Intensity Top’ and ‘Fluorescence Intensity Bottom’.

MRW is not available for the 1536-well plate format.

The MRW feature is not active when performing well-wise measurements.

‘Reference Wavelength’ (located on the absorbance strip) is not available in combination with ‘Multiple Reads Per Well’.

6.7 Optimize Luminescence Measurements

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

6.7.1 Integration Time

At very low light levels, a PMT does not yield a continuous output current, which is necessary for a reliable analog to digital conversion. Instead, it produces a sequence of pulses the average rate of which can be measured using a counter. The advantage of the photon counting technique at such low light levels is that pulse height selection criteria allow electronic noise to be discriminated.

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

Note

The relevant signal to (shot) noise ratio can be improved by a factor when measurement time is multiplied with the square of the desired factor.

6.7.2 Light Level Attenuation

When using photon counting detection, optical attenuation of higher luminescence light levels (> 10,000,000 RLU) is necessary. In such a case, too many photons entering the PMT at a time cannot be distinguished as distinct exit pulses. Count rates would even fall behind values at lower light levels.

Therefore, values >10,000,000 RLU (without attenuation) are marked as “INVALID” on the result sheet.

The INFINITE F500 hardware can attenuate light levels by a fixed factor of either 1 (none) or 10 (1 OD). Correspondingly, the usable measurement range will be shifted to higher light levels (< 100,000,000 RLU). The attenuation of the emitted light and thereby the selection of the right attenuation factor can be also performed automatically (Attenuator → ‘automatic’).

7. Instrument Features

7.1 Introduction

Note

All specifications are subject to change without prior notification.

The following types of measurement are provided with the fully equipped INFINITE F500 microplate reader:

Measurement Type	Description
Fluorescence Intensity Top/Bottom	See 7.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)
Fluorescence Time Resolved	See 7.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)
Fluorescence Polarization	See 7.4 Fluorescence Polarization
Absorbance	See 7.5 Absorbance
Glow Type Luminescence	See 7.6 Glow Type Luminescence
Dual Color Luminescence	See 7.8 Dual Color Luminescence (e.g. BRET)
Flash Type Luminescence	See 7.7 Flash Type Luminescence

- All standard microplates from 6 to 384-wells that conform to the following standards can be measured in any of the above measurement types: ANSI/SBS 1-2004; ANSI/SBS 2-2004; ANSI/SBS 3-2004 and ANSI/SBS 4-2004.
- All standard microplates from 6 to 1536-wells that conform to the following standards can be measured in Fluorescence Intensity Top, Fluorescence Time Resolved, Fluorescence Polarization, Absorbance, Glow Type Luminescence and Dual Color Luminescence: ANSI/SBS 1-2004; ANSI/SBS 2-2004; ANSI/SBS 3-2004 and ANSI/SBS 4-2004.
- The instrument can perform kinetic measurements.
- Reading may be restricted to one part of the microplate.

7.2 Instrument Specifications

The table below lists the technical specifications of the instrument:

Parameters	**Characteristics**	
Measurement	Software controlled	
Interface	USB	
Filter Handling	External filter exchange	
Microplates to be measured	From 6-well to 1536-well plates (SBS standard formats)	
Plate Definition	Via scanning software	
Temperature Control	From 4 °C above ambient up to 42 °C	
Plate Shaking	Linear and orbital shaking, amplitude selectable from 0.5 to 6 mm	
Parameters	Characteristics	
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 photon-counting electronics	
Absorbance Detector	Silicon photodiode	
Power Supply	Auto-sensing: 100 – 120 V/220 – 240 V, 50/60 Hz	
Power Consumption	250 VA	
Main fuse	2 x T4A / 250 V	
Physical		
Outer Dimensions Basic instrument	Width: 515 mm Height: 280 mm Depth: 670 mm see	20.28 in. 11.02 in. 26.38 in.
Weight	38 kg	
Environmental		
Ambient Temperature		
Operation	+ 15 °C to + 30 °C	+ 59 °F to + 86°F
Non-operation	- 20 °C to + 60 °C	-4 °F to + 140 °F
Relative Humidity		
Operation	< 90 % non condensing	
Over-voltage Category	II	
Pollution Degree	2	
Usage	General Laboratory Instrument or Commercial Use	
Noise Level	< 60 dBA	
Method of Disposal	Electronic waste (infectious waste)	

7.2.1 Barcode Laser Scanner

The INFINITE F500 may be optionally equipped with a barcode scanner mounted on the right side of the plate transport.

When the instrument housing is closed, the INFINITE F500 reader with barcode scanner option is a **Class I / 1 Laser Product**.

Complies with 21 CFR 1040.10 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007

BCR: 14330052 Symbol Technologies SE_923_1000A

WARNING

LASER RADIATION – DO NOT STARE INTO THE BEAM!
CLASS I LASER PRODUCT.

The Class II / 2 Laser Scanner corresponds to the following norms:

- DIN EN 60825-1 : 2001
- CDRH 21 CFR 1040.10

PARAMETERS	CHARACTERISTICS
Classification	Class II / 2 Laser Product
Input power	5 V DC +/- 10%
Emission duration	> 0.25 s
Scan rate	42 +/- 3 Scans / second (bi-directional)
Laser power	< 1 mW
Definition of depth	Max. 40 cm
Resolution	0.15 mm
Min. print contrast	25% at 675 nm
Ambient light	Sunlight: 40000 Lux
	Halogen light: 1500 Lux

7.2.2 Barcode Labels

The INFINITE F500 barcode scanner supports following barcode types:

- Code 128
- Code 39
- UPC A, UPC E
- EAN 8

Used barcode labels must fulfill following norms:

- ISO/IEC 15416 Automatic identification and data capture techniques - Bar code print quality test specifications - Linear symbols (e.g. EN 1635)
- ANSI X3.182-1990 (R1995): Guideline for Bar Code Print Quality

Note

The barcode must have the quality Class A, B or C / ANSI/CEN/ISO standard. Dirty, folded, wet or damaged barcode labels must not be used. The adhesive labels must be flat and not peeling off at the edges.

Caution

Barcode labels on 6-well microplates cannot be read by the barcode scanner.

We recommend assuring the quality of the barcode labels by means of a local SOP.

The barcode has to be accurately aligned horizontally and centered on the right hand side of the plate. Position the barcode as near as possible to the bottom.

Parameters	Characteristics
Length	max 50 mm (3.5 in.)
Bars height	min 5 mm (0.2 in.)
Quiet zone	min 5 mm (0.2 in.)
Resolution	min 0.15 mm (6 mil) module width

7.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)

Parameters	Characteristics	
Wavelength Range	230 - 900 nm	
Standard Filter EX:	Pos1	320 (25) nm
	Pos2	485 (20) nm
	Pos4	340 (35) nm
Standard Filter EM:	Pos5	612 (10) nm
	Pos6	535 (25) nm
	Pos1	620 (10) nm
	Pos2	665 (8.5) nm
	Pos3	535 (25) nm
	Pos4	612 (10) nm
	Pos5	670 (25) nm
	Pos6	590 (20) nm

Gain Setting	Values	Measurement Range
Manual	1 - 255	0 - 60,000 RFU
Optimal	automatic	0 - 60,000 RFU
Calculated from Well	automatic	0 - 60,000 RFU

TRF Parameters	Characteristics
Integration Time	20 - 2000 µs
Lag Time	0 - 2000 µs

7.3.1 Definition of the Detection Limit

The detection limit is the fluorophore concentration where the background-subtracted signal equals 3 times the standard deviation of the background noise.

When selecting 1 flash per well, the plate carrier does not stop at the measurement position. Using more flashes per well may improve the detection limit, but the total measurement time will be longer.

7.3.2 Fluorescein (Fluorescence Intensity) Top

Plate Type (number of wells)	384	1536
Dispensed Volume [µl]	100	10
Flashes per Well	10	10
Fluorescein Detection Limit [pM]	< 0.8 pM	< 1.5 pM
Measurement Time per Plate [min:sec]	< 2:20	< 3:10 (3 flashes/well)

The measurement time per plate includes retracting and ejecting of the plate carrier.

7.3.3 Fluorescein (Fluorescence Intensity) Bottom

Plate Type (number of wells)	384 UV	384 VIS
Dispensed Volume [µl]	100	100
Flashes per Well	10	10
Fluorescein Detection Limit [pM]	< 15 pM	< 25 pM
Measurement Time per Plate [min:sec]	< 2:20	< 2:20
Wavelength Range:	230 - 900 nm	380 - 900 nm

7.3.4 Europium (Time Resolved Fluorescence)

Plate Type (number of wells)	384	1536
Dispensed Volume [µl]	100	10
Flashes per Well	10	10
Europium Detection Limit [fM]	< 60 fM	< 200 fM
Measurement Time per Plate [min:sec]	< 2:20	< 3:15 (3 flashes/well)

7.3.5 HTRF® (Homogeneous Time Resolved Fluorescence)

The measurement is set up using the 'multilabeling' function of i-control software. Excitation and emission filters are available as parts of the Tecan HTRF® upgrade kit (10122175) and must be defined in the software according to the kit description.

Parameters	Characteristics
Reading Mode	FI Top
Excitation wavelength	320 nm
Emission wavelength 1	620 (10) nm
Emission wavelength 2	665 (8.5) nm
Gain	Optimal
Z-Position	Label1 = Well A1 Label2 = Same as Label1
Flashes per Well	10
Integration Time	500 µs
Lag Time	150 µs
Mirror Selection	Dichroic 510 (e.g. FI)

Reagents: HTRF® Reader Control Kit 62RCLPEA (CisBio)

Delta F %:

	Low Calibrator		High Calibrator	
	INFINITE F500	Norm	INFINITE F500	Norm
DF% (18 h)	26 %	≥ 15 %	809 %	≥ 600 %

Signal to Background (S/B) and Standard 0 - CV % ratio:

	INFINITE F500	Norm
S/B (18 h)	94	≥ 40
CV % (18 h)	5.1 %	≤ 10 %

For detailed information, please refer to Tecan Technical Note: *Implementation of HTRF® on Tecan Ultra Evolution*.

7.4 Fluorescence Polarization

Parameters	Characteristics
Wavelength Range	Excitation: 300 – 750 nm
	Emission: 330 – 750 nm
Standard Filter	Ex filter slide: Pos1 485 (20) - parallel Em filter slide: Pos1: 535 (25) - parallel Pos2: 535 (25) - perpendicular

Gain Setting	Values	Measurement Range
Manual	1 - 255	0 - 60,000 RFU
Optimal	automatic	0 - 60,000 RFU
Calculated from Well	automatic	0 - 60,000 RFU

FP Parameters	Characteristics
Integration Time	20 - 2000 µs
Lag Time	0 - 2000 µs

7.4.1 Fluorescein (Fluorescence Polarization)

Plate Type (number of wells)	384	1536
Dispensed Volume [µl]	100	10
Flashes per Well	10	10
Precision at 1 nM Fluorescein [mP]	< 4 mP	< 5 mP
Measurement Time per Plate [min:sec]	< 6:10	< 13:20 (3 flashes/well)

7.5 Absorbance

Parameters	Characteristics
Wavelength Range:	230 - 1000 nm
Measurement Range	0 – 4 OD
Resolution	0.001 OD
Standard Filter: Excitation filter slide	 Pos3 492 (10) nm

The following specifications are valid for the wavelength of 492 nm:

Plate Type (number of wells)	MultiCheck Plate	384 / 1536
Dispensed Volume [µl]	Not applicable	100 / 10
Flashes per Well	10	10 / 10
Accuracy at 0 – 2 OD	< ± 1.0% + 10 mOD	
Accuracy at 2 – 3 OD	< ± 1.5% + 10 mOD	
Reproducibility at 0 – 2 OD	< ± 0.5% + 5 mOD	
Reproducibility at 2 – 3 OD	< ± 2.0% + 10 mOD	
Uniformity at 1 OD Orange G		< 3 % / < 5 %
Measurement Time per Plate [min:sec]		< 2:30 / < 3:40 (3 flashes/well)

7.6 Glow Type Luminescence

Caution
Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength Range	380 – 600 nm
Lin. Dynamic Range	6 orders of magnitude 7 orders of magnitude (extended dynamic range)
Integration Time/well	1 – 20000 ms
Cross Talk % (white plate)	
96-well plate	< 0.035 %
384-well plate	< 0.35 %
1536-well plate	< 5 %
Measurement range	> 6 orders of magnitude
Attenuation of Light	10, 1 (no attenuation)

7.6.1 ATP Glow Luminescence

Plate Type (number of wells)	384	1536
Dispensed Volume [µl]	100	10
Integration Time/well [ms]	1000	1000
ATP Detection Limit [fM]	< 0.5 fmol/well	< 0.8 fmol/well

7.7 Flash Type Luminescence

Caution
Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength Range	380 – 600 nm
Measurement Range	> 6 orders of magnitude
Integration Time/well	1 – 20000 ms
Cross talk % (white plate)	
96-well plate	< 0.035 %
384-well plate	< 0.35 %
1536-well plate	< 5 %
Attenuation of Light	10, 1 (no attenuation)

7.8 Dual Color Luminescence (e.g. BRET)

Caution
Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Parameters	Characteristics
Built-in Wavelength:	See 5.4.1.
Integration Time:	1 - 20000 ms. Different integration times are possible for each wavelength.
Plate Type:	96, 384 and 1536-well microplates
Dynamic Range	6 decades

7.9 “On the Fly” Measurements

“On the Fly” measurements are the fastest measurements possible using the INFINITE F500. These measurements are performed using only one flash (number of reads).

- 384-well plates (FI, Absorbance) Measurement time: < 35 s
- 1536-well plates (FI Top, Absorbance) Measurement time: < 40 s

7.10 Injector

Parameters	Characteristics
Injector Syringe Volumes	500 µl, 1000 µl, 2500 µl

7.10.1 500 µl Injector

Injector Syringe Volume	500 µl
Accuracy at 10 µl	± 3 %
Accuracy at 100 µl	± 0.8 %
Accuracy at 450 µl	± 0.8 %
Precision at 10 µl	± 3 %
Precision at 100 µl	± 0.6 %
Precision at 450 µl	± 0.3 %

8. Quality Control

8.1 Periodic Quality Control Tests

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

The tests described in the following sections 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 instrument parameters; 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 of the appropriate settings (filters, flashes, delays, etc.).

Caution

Before starting measurements, make sure that the microplate position A1 is inserted correctly. The position of well A1 has to be on the upper left side.

Caution

This section provides instructions on how to check the specifications of the instrument. If the results of these control tests do not lie within the official specifications of the instrument, please contact your local service center.

8.2 Specifications - Passed/Failed Criteria

Note

All specifications are subject to change without prior notification.

The following table gives an overview of the passed/failed criteria for the specification test of the INFINITE F500.

Specification	**Passed / Failed Criteria**	**Passed / Failed Criteria**
Plate type (number of wells)	384	1536
Fluorescence Top Sensitivity	< 0.8 pM Fluorescein	< 1.5 pM Fluorescein
Fluorescence Bottom Sensitivity UV	< 15 pM Fluorescein	not available
Fluorescence Bottom Sensitivity VIS	< 25 pM Fluorescein	not available
Time Resolved Fluorescence Sensitivity	< 80 fM Eu	< 200 fM Eu
Fluorescence Polarization Precision	< 4 mP	< 5 mP
Luminescence Sensitivity Glow Type	< 0.5 fmol/well	<0.8 fmol/well
Absorbance Accuracy (0 – 2 OD)	< ± (1 % + 10 mOD)	
Absorbance Accuracy (2 – 3 OD)	< ± (1.5 % + 10 mOD)	
Absorbance Reproducibility (0 – 2 OD)	< ± (0.5 % + 5 mOD) MultiCheck plate	
Absorbance Reproducibility (2 – 3 OD)	< ± (2 % + 10 mOD) MultiCheck plate	
Absorbance Uniformity at 1 OD	< 3%	< 5%

8.3 Specifications - Test Instructions

8.3.1 Fluorescence Top

For the INFINITE F500 with the option 'Fluorescence Top', the following tests may be performed to prove the specifications:

- Sensitivity
- Uniformity
- Reproducibility
- Linearity

Sensitivity

Perform the following measurement to determine the detection limit for Fluorescein:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Ex WL	485 (20) nm
Em WL	535 (25) nm
Number of Flashes	10
Integration Time	40 µs
Settle Time	0
Gain	Optimal
Z-position	Well A1
Mirror	Automatic
Plate Type	GRE384fb, GRE1536fb

Plate Layout 384-well plate:

Pipette 100 µl of 1 nM Fluorescein or the blank solution (0.01 M NaOH) into the appropriate wells according to the plate layout:

<>	1	3	5	7	9	11	13	15	17	19	21	23
A	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank
C												
E												
G												
I												
K												
M												
O												

Material/Reagents 384-well plate:

1 nM Fluorescein (e.g. Sigma-Adrich, F-6377) in 0.01 M NaOH

0.01 M NaOH (=Blank)

1 Greiner 384-well plate black

200 µl Pipette + tips

Plate Layout 1536-well plate:

Pipette 10 µl of 1 nM Fluorescein or the blank solution (0.01 M NaOH) into the appropriate wells according to the plate layout:

<>	1	5	9	13	17	21	25	29	33	37	41	45
A	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank	1 nM Fluorescein	Blank
E												
I												
M												
Q												
U												
Y												
AC												

Material/Reagents 1536-well plate:

1 nM Fluorescein (e.g. Sigma-Adrich, F-6377) in 0.01 M NaOH

0.01 M NaOH (=Blank)

1 Greiner 1536-well plate black

10 µl Pipette + tips

Calculation of Detection Limit (Sensitivity):

$$\text{DetectionLimit} = \frac{\text{Concentration}_F}{(\text{mean}_F - \text{mean}_B)} * 3 * \text{Stdev}_B$$

Concentration	Concentration of the fluorophore in pM units
mean_F	Average RFU value of wells filled with fluorophore
mean_B	Average RFU value of wells filled with blank
stdev_B	Standard deviation of RFU values of wells filled with blank

The result of the formula 'Detection Limit' determines the sensitivity in pM units.

Uniformity

Perform the following measurement to determine the Uniformity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Ex WL	Filter: 485 (20) nm
Em WL	Filter: 535 (25) nm
Number of Flashes	10
Integration Time	40 µs
Settle Time	0
Gain	Optimal
Z-position	Well A1
Mirror	Automatic
Plate Type	GRE384fb, GRE1536fb

Plate Layout:

See 8.3.1 Fluorescence Top: Sensitivity.

Material/Reagents:

See 8.3.1 Fluorescence Top: Sensitivity.

Calculation of Uniformity:

$$\text{Uniformity}(\%) = \frac{\text{stdev}_F * 100}{\text{mean}_F}$$

mean_F Average RFU value of wells filled with fluorophore

stdev_F Standard deviation of RFU values of wells filled with flurorophore

The result of the formula determines the uniformity in % CV.

Reproducibility

Perform the following measurement to determine the reproducibility:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Ex WL	485 (20) nm
Em WL	535 (25) nm
Number of Flashes	10
Integration Time	40 µs
Settle Time	0
Gain	Optimal
Z-position	Well A1
Mirror	Automatic
Plate Type	GRE384fb, GRE1536fb
Part of the Plate	A1
Kinetic Cycles	20
Interval Time	Minimal

Plate Layout:

See 8.3.1 Fluorescence Top: Sensitivity.

Material/Reagents:

See 8.3.1 Fluorescence Top: Sensitivity.

Calculation of Reproducibility:

$$CV\% = \frac{stdev_{wellA1} * 100}{mean_{wellA1}}$$

$mean_{wellA1}$ Average RFU value of well A1 over the 20 kinetic

$stdev_{wellA1}$ Standard deviation of RFU values of Well A1 over the 20 cycles

The result of the formula determines the Reproducibility in % CV.

Linearity

Perform the following measurement to determine the Linearity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Ex WL	485 (20) nm
Em WL	535 (25) nm
Number of Flashes	10
Integration Time	40 µs
Settle Time	0
Gain	Optimal
Z-position	Well A1
Mirror	Automatic
Plate Type	GRE384fb, GRE1536fb

Plate Layout 384-well plate:

Pipette 100 µl of the appropriate Fluorescein solution or the blank solution (0.01 M NaOH) into the appropriate wells according to the plate layout:

<>	1	3	5	7	9	11	13	15	17	19	21	23
A	**5 nM Fluorescein**	**1 nM Fluorescein**	**0.5 nM Fluorescein**	**0.25 nM Fluorescein**	**0.1 nM Fluorescein**	**0.05 nM Fluorescein**	**Blank**					
C												
E												
G												
I												
K												
M												
O												

Material/Reagents 384-well plate:

5 nM – 0.05 nM Fluorescein (e.g. Sigma-Aldrich, F-6377) in 0.01 M NaOH

0.01 M NaOH (=Blank)

1 Greiner 384-well plate black

200 µl Pipette + tips

Plate Layout 1536-well plate:

Pipette 10 µl of the appropriate Fluorescein solution or the blank solution (0.01 M NaOH) into the appropriate wells according to the plate layout:

<>	1	5	9	13	17	21	25	29	33	37	41	45
A	5 nM Fluorescein	1 nM Fluorescein	0.5 nM Fluorescein	0.25 nM Fluorescein	0.1 nM Fluorescein	0.05 nM Fluorescein	Blank					
E												
I												
M												
Q												
U												
Y												
AC												

Material/Reagents 1536-well plate:

5 nM – 0.05 Fluorescein (e.g. Sigma-Adrich, F-6377) in 0.01 M NaOH

0.01 M NaOH (=Blank)

1 Greiner 1536-well plate black

10 µl Pipette + tips

Calculation of Linearity:

Linearity is defined as the relationship between the concentration of the fluorophore/dye and the corresponding signal probability. For evaluation of linearity a dilution series of the appropriate dye is measured and the r-square value is assessed according to the following formula:

$$r^2 = \frac{SSR}{SST}$$

where SSR is the sum of squares of the regression:

$$\mathrm{SSR} = \sum_{i=1}^{n} \omega_i \left(\hat{y}_i - \bar{y}\right)^2$$

and SST is the total sum of squares:

$$\mathrm{SST} = \sum_{i=1}^{n} \omega_i \left(y_i - \bar{y}\right)^2$$

8.3.2 Fluorescence Bottom

For the INFINITE F500 with the option 'Fluorescence Bottom' the following tests may be performed to prove the specifications:

- Sensitivity
- Uniformity
- Reproducibility
- Linearity

Sensitivity

Perform the following measurement to determine the detection limit for Fluorescein:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Bottom
Ex WL	485 (20) nm
Em WL	535 (25) nm
Number of Flashes	10
Integration Time	40 µl
Settle Time	0
Gain	Optimal
Plate Type	GRE384fb

Plate Layout:

See 8.3.1 Fluorescence Top: 384-well plate.

Material/Reagents:

1 nM Fluorescein (e.g. Sigma-Adrich, F-6377) in 0.01 M NaOH
0.01 M NaOH (=Blank)
1 Greiner 384-well plate µClear, black with transparent bottom
200 µl Pipette + tips

Calculation of Detection Limit (Sensitivity):

See 8.3.1 Fluorescence Top.

Uniformity

Perform the following measurement to determine the Uniformity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Bottom
Ex WL	485 (20) nm
Em WL	535 (25) nm
Number of Flashes	10
Integration Time	40 µs
Settle Time	0
Gain	Optimal
Plate Type	GRE384fb

Plate Layout:

See 8.3.1 Fluorescence Top: 384-well plate.

Material/Reagents:

See 8.3.2 Fluorescence Bottom

Calculation of Uniformity:

See 8.3.1 Fluorescence Top

Reproducibility

Perform the following measurement to determine the reproducibility:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Bottom
Ex WL	485 (20) nm
Em WL	535 (25) nm
Number of Flashes	10
Integration Time	40 µs
Settle Time	0
Gain	Optimal
Plate Type	GRE384fb
Part of the Plate	A1
Kinetic Cycles	20
Interval Time	Minimal

Plate Layout:

See 8.3.1 Fluorescence Top: 384-well plate.

Material/Reagents:

See 8.3.2 Fluorescence Bottom

Calculation of Reproducibility:

See 8.3.1 Fluorescence Top.

Linearity

Perform the following measurement to determine the Linearity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Bottom
Ex WL	485 (20) nm
Em WL	535 (25) nm
Number of Flashes	10
Integration Time	40 µs
Settle Time	0
Gain	Optimal
Plate Type	GRE384fb

Plate Layout:

See 8.3.1 Fluorescence Top: 384-well plate.

Material/Reagents:

5 – 0.05 nM Fluorescein (e.g. Sigma-Adrich, F-6377) in 0.01 M NaOH
0.01 M NaOH (=Blank)
1 Greiner 96-well plate µClear, black with transparent bottom
200 µl Pipette + tips

Calculation of Linearity:

See 8.3.1 Fluorescence Top

8.3.3 Time Resolved Fluorescence

For the INFINITE F500 with the option 'Fluorescence Top' the following tests may be performed to prove the specifications:

- Sensitivity
- Reproducibility

Sensitivity

Perform the following measurement to determine the sensitivity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Ex WL	340 (35) nm
Em WL	612 (10 nm)
Number of Flashes	10
Integration Time	400 µs
Lag Time	200 µs
Settle Time	0
Gain	Optimal
Z-Position	Calculated from well A1
Mirror	Dichroic 510 (e.g. fluorescein)
Plate Type	GRE384fw, GRE1536fw

Plate Layout 384-well plate:

Pipette 100 µl of 0.1 nM Europium solution or the blank solution (enhancement solution) into the appropriate wells according to the plate layout:

<>	1	2	3	4	5	...
A	0.1 nM Europium	Blank	Blank	Blank	Blank	
B						
C						
D						
E						
F						
G						
H						
...						

Plate Layout 1536-well plate:

Pipette 10 µl of 0.1 nM Europium solution or the blank solution (enhancement solution) into the appropriate wells according to the plate layout:

<>	1	2	3	4	5	...
A	0.1 nM Europium	Blank	Blank	Blank	Blank	
B						
C						
D						
E						
F						
G						
H						
...						

Material/Reagents 384-well plate:

0.1 nM Europium

Enhancement Solution (=Blank)

1 Greiner 384-well plate white

200 µl Pipette + tips

Material/Reagents 1536-well plate:

0.1 nM Europium

Enhancement Solution (=Blank)

1 Greiner 1536-well plate white

10 µl Pipette + tips

Calculation of Detection Limit (Sensitivity):

See 8.3.1 Fluorescence Top

Reproducibility

Perform the following measurement to determine the reproducibility:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Ex WL	340 (35) nm
Em WL	612 (10 nm)
Number of Flashes	10
Integration Time	400 µs
Lag Time	200 µs
Time between Move and Flash	0
Gain	Optimal
Z-Position	Calculated from well A1
Mirror	Dichroic 510 (e.g. fluorescein)
Plate Type	GRE384fw, GRE1536fw
Part of the plate	A1
Kinetic Cycles	20
Interval Time	Minimal

Plate Layout:

See 8.3.3 Time Resolved Fluorescence

Calculation of Reproducibility:

See 8.3.1 Fluorescence Top

8.3.4 Fluorescence Polarization

For the INFINITE F500 with the option 'Fluorescence Polarization' the following test may be performed to prove the specifications:

- Precision

Precision

Perform the following measurement to determine the precision:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Polarization
Ex WL	485 (20) nm
Em WL	535 (25) nm
Number of Flashes	10
Integration Time	40 µs
Settle Time	0
Gain	Optimal
Z-Position	Well A1
Mirror	Automatic
Plate Type	GRE384fb, GRE1536fb
Part of the Plate	A1-C4
Blank Range	A4-C4

Plate Layout 384-well plate:

Pipette 100 µl of 1 nM Fluorescein solution into the wells A1 to C3 and 100 µl of 0.01 M NaOH (=blank) into the wells A4-C4.

Material/Reagents 384-well plate:

1 nM Fluorescein (e.g. Sigma-Aldrich, F-6377) in 0.01 M NaOH

10 mM NaOH

1 Greiner 384-well plate black

200 µl Pipette + tips

Plate Layout 1536-well plate:

Pipette 10 µl of 1 nM Fluorescein solution into the wells A1 to C3 and 10 µl of 0.01 M NaOH (=blank) into the wells A4-C4.

Material/Reagents 1536-well plate:

1 nM Fluorescein (e.g. Sigma-Aldrich, F-6377) in 0.01 M NaOH

0.01 M NaOH

1 Greiner 1536-well plate black

10 µl Pipette + tips

Calculation of Precision:

$$\boxed{\mathrm{Pr}ecision(mP) = stdev_{wellA1-C3}}$$

$stdev_{wellA1-C3}$ Standard deviation of Polarization values of Well A1 to C3

8.3.5 Glow Luminescence

For the INFINITE F500 with the option ‘Luminescence the following test may be performed to prove the specifications:

- Sensitivity

Sensitivity

Perform the following measurement to determine the Sensitivity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Luminescence
Integration Time	1000 ms
Settle Time	0
Plate Type	GRE384fw, GRE1536fw
Part of the Plate	A1 – D10

Plate Layout 384-well plate:

Pipette 100 µl of the Blank and 20 µl ATP reagents into the appropriate wells according to the plate layout:

	1	2	3	4	5	6	7	8	9	10	11	...
A	Bx	ATP	Bx	B	B	B	B	B	B	B		
B	Bx	ATP	Bx	B	B	B	B	B	B	B		
C	Bx	ATP	Bx	B	B	B	B	B	B	B		
D	Bx	ATP	Bx	B	B	B	B	B	B	B		
E												
F												
G												
...												

ATP 1 x 10^{-7} M ATP (2 x 10^{-8} M final concentration in well)
dilute ATP Standard with Tris-EDTA 1:100

B Blank
dilute ATP reagent with Tris-EDTA 1:5

Bx Blank wells used for cross-talk calculation
dilute ATP reagent with Tris-EDTA 1:5

To start the reaction, add 80 µl ATP Reagent to ATP wells and mix thoroughly by pipetting. Use a new tip for every well.

Material/Reagents 384-well plate:

Biothema ATP Kit (ATP Reagent, Diluent C, Tris-EDTA buffer,
ATP Standard [10^{-5} M])
1 Greiner 384-well plate white
200, 20 µl Pipette + tips

Plate Layout 1536-well plate:

Pipette 10 µl of the Blank and 2 µl ATP reagents into the appropriate wells according to the plate layout:

	1	2	3	4	5	6	7	8	9	10	11	...
A	**Bx**	**ATP**	**Bx**	**B**	**B**	**B**	**B**	**B**	**B**	**B**		
B	**Bx**	**ATP**	**Bx**	**B**	**B**	**B**	**B**	**B**	**B**	**B**		
C	**Bx**	**ATP**	**Bx**	**B**	**B**	**B**	**B**	**B**	**B**	**B**		
D	**Bx**	**ATP**	**Bx**	**B**	**B**	**B**	**B**	**B**	**B**	**B**		
E												
F												
G												
...												

ATP 1 x 10^{-7} M ATP (2 x 10^{-8} M final concentration in well)
dilute ATP Standard with Tris-EDTA 1:100

B Blank
dilute ATP reagent with Tris-EDTA 1:5

Bx Blank wells used for cross-talk calculation
dilute ATP reagent with Tris-EDTA 1:5

To start the reaction, add 8 µl ATP Reagent to ATP wells and mix thoroughly by pipetting. Use a new tip for every well.

Material/Reagents 1536-well plate:

Biothema ATP Kit (ATP Reagent, Diluent C, Tris-EDTA buffer,
ATP Standard [10^{-5} M])
1 Greiner 1536-well plate white
10 µl Pipette + tips

Calculation of the Sensitivity (Detection Limit):

$$DetectionLimit(\,fmol/well) = \frac{conc_{ATP} * 3 * Stdev_B}{mean_{ATP} - mean_B} * V * 10^{15}$$

$conc_{ATP}$	Concentration of ATP standard in well [M] (e.g. 2 x 10^{-8})
$Stdev_B$	Standard deviation of Blank
$mean_{ATP}$	Average of wells filled with ATP standard
$mean_B$	Average of Blank wells
V	Filling volume of wells [L] (e.g. 0.000001 L)
10^{15}	Conversion into fmol/well

The result of the formula determines the detection limit in fmol/well.

8.3.6 Absorbance

For the INFINITE F500 with the option 'Absorbance' the following tests may be performed to prove the specifications:

- Accuracy
- Uniformity at 1 OD
- Linearity
- Reproducibility

Accuracy

Use MultiCheck Plate – For details please refer to the MultiCheck plate operating manual.

Uniformity at 1 OD

Perform the following measurements (at 492 nm) to determine the uniformity:

Measurement Parameters (Orange G, 492 nm):

Parameter	Setting
Reading Mode	Absorbance
Measurement wavelength	492 nm
Number of Reads	10
Time between Move and Flash	0
Plate Type	GRE384ft

Plate Layout 384-well plate:

Pipette 100 µl of the Orange G solution (OG, 30 mg/l) into the appropriate wells according to the 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												

Material/Reagents:

Greiner, 384-well plate, flat bottom, transparent

Orange G solution (30 mg/l)

200 µl Pipette (8- or 12-channel) + tips

Calculation of Uniformity:

Calculate the uniformity:

$$\text{Uniformity}(\%) = \frac{\text{stdev}_F * 100}{\text{mean}_F}$$

mean_F Average OD value of wells filled with OG

stdev_F Standard deviation OD values of wells filled with OG

The result of the formula determines the uniformity in % CV.

Plate Layout 1536-well plate:

Pipette 10 µl of the Orange G solution (OG, 60 mg/l) into the appropriate wells according to the plate layout:

<>	1	5	9	13	17	21	25	29	33	37	41	45
A	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG
E												
I												
M												
Q												
U												
Y												
AC												

Material/Reagents:

Greiner, 1536-well plate, flat bottom, transparent

Orange G solution (60 mg/l)

10 µl Pipette + tips

Calculation of Uniformity:

Calculate the uniformity:

$$\text{Uniformity}(\%) = \frac{\text{stdev}_F * 100}{\text{mean}_F}$$

mean_F Average OD value of wells filled with OG

stdev_F Standard deviation OD values of wells filled with OG

The result of the formula determines the uniformity in % CV.

Linearity

Perform the following measurements to determine the linearity at 492 nm:

Measurement Parameters for Orange G (OG, 492 nm):

Parameter	Setting
Reading Mode	Absorbance
Measurement Wavelength	492 nm
Number of Reads	10
Time between Move and Flash	0
Plate Type	GRE384ft
Part of the Plate	A1 – H6

Plate Layout 384-well plate:

Pipette 100 µl of the Orange G solutions into the appropriate wells according to the plate layout:

<>	1	2	3	4	5	6	...
A	80 mg/l Orange G	60 mg/l Orange G	40 mg/l Orange G	20 mg/l Orange G	10 mg/l Orange G	Blank (Water)	
B							
C							
D							
E							
F							
G							
H							

Material/Reagents:

Greiner, 384-well plate, flat bottom, transparent

Orange G (80 mg/l; 60 mg/l, 40 mg/l, 20 mg/l, 10 mg/l)

Distilled/deionized water

100 µl 8-channel-pipette + tips

Calculation of Linearity:

Calculate the linearity for Orange G

See 8.3.1 Fluorescence Top

Plate Layout 1536-well plate:

Pipette 10 µl of the Orange G solutions into the appropriate wells according to the plate layout:

<>	1	5	9	13	17	21	25
A E I M Q U Y AC	80 mg/l Orange G	60 mg/l Orange G	40 mg/l Orange G	20 mg/l Orange G	10 mg/l Orange G	Blank (Water)	

Material/Reagents:

Greiner, 1536-well plate, flat bottom, transparent
Orange G (80 mg/l; 60 mg/l, 40 mg/l, 20 mg/l, 10 mg/l)
Distilled/deionized water
10 µl pipette + tips

Calculation of Linearity:

Calculate the linearity for Orange G
See 8.3.1 Fluorescence Top

Reproducibility

Perform the following measurements to determine the reproducibility at 492 nm:

Measurement Parameters for Orange G (OG, 492 nm):

Parameter	Setting
Reading Mode	Absorbance
Measurement Wavelength	492 nm
Number of Reads	10
Time between Move and Flash	0
Kinetic Cycles	20
Plate Type	GRE384ft
Part of the Plate	A1 – A6

Plate Layout 384-well plate:

Pipette 100 µl of the Orange G solutions into the appropriate well according to the plate layout:

<>	1	2	3	4	5	6	…
A	**20 mg/l OG**	**40 mg/l OG**	**60 mg/l OG**	**80 mg/l OG**	**120 mg/l OG**	**140 mg/l OG**	
B							
C							
...							

Material/Reagents:

Greiner, 384-well plate, flat bottom, transparent

20 mg/l, 40 mg/l, 60 mg/l, 80 mg/l, 120 mg/l and 140 mg/l Orange G

100 µl 8-channel-pipette + tips

Calculation of Reproducibility:

Calculate the reproducibility separately for each well.

$$CV\% = \frac{stdev_{wellx} * 100}{mean_{wellx}}$$

$mean_{wellx}$ Mean value of a well over 20 kinetic cycles

$stdev_{wellx}$ Standard deviation of a well over 20 cycles

The result of the formula determines the reproducibility in % CV.

Plate Layout 1536-well plate:

Pipette 10 µl of the Orange G solutions into the appropriate well according to the plate layout:

<>	1	2	3	4	5	6	…
A	**20 mg/l OG**	**40 mg/l OG**	**60 mg/l OG**	**80 mg/l OG**	**120 mg/l OG**	**140 mg/l OG**	
B							
C							
...							

Material/Reagents:

Greiner, 1536-well plate, flat bottom, transparent

20 mg/l, 40 mg/l, 60 mg/l, 80 mg/l, 120 mg/l and 140 mg/l Orange G

10 µl pipette+ tips

Calculation of Reproducibility:

Calculate the reproducibility separately for each well.

$$CV\% = \frac{stdev_{wellx} * 100}{mean_{wellx}}$$

$mean_{wellx}$ Mean value of a well over 20 kinetic cycles

$stdev_{wellx}$ Standard deviation of a well over 20 cycles

The result of the formula determines the reproducibility in % CV.

9. Cleaning and Maintenance

9.1 Introduction

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.

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

This section contains the following procedures:

- Liquid Spills
- Instrument Disinfection
- Safety certificate
- Instrument and Material Disposal

9.2 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 biohazardous spills, we recommend that the affected areas be disinfected using AREASDES B or DODACARNA (Schülke & Mayr GmbH, A-1070 Wien).
5. Wipe cleaned and disinfected areas dry.

WARNING

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.

9.3 Instrument Disinfection

WARNING

ALL PARTS OF THE INSTRUMENT THAT COME INTO CONTACT WITH POTENTIALLY INFECTIOUS MATERIAL, BIOLOGICAL SAMPLES, PATIENT SAMPLES, POSITIVE CONTROL SAMPLES 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.

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.

9.3.1 Disinfection Solutions

The outer surfaces and the plate transport of the instrument should be disinfected using a surface disinfection solution such as:

- B30 (Orochemie, Max-Planck-Str. 27, D-70806 Kornwestheim)

WARNING

PRIOR TO DISINFECTION DISCONNECT THE INSTRUMENT FROM THE MAIN POWER SUPPLY TO AVOID ANY RISK OF FIRE AND EXPLOSION.

9.3.2 Disinfection Procedure

WARNING

THE DISINFECTION PROCEDURE SHOULD BE PERFORMED IN A WELL-VENTILATED ROOM BY AUTHORIZED TRAINED PERSONNEL WEARING DISPOSABLE GLOVES AND PROTECTIVE GLASSES AND CLOTHING.

WARNING

THE DISINFECTION PROCEDURE SHOULD BE PERFORMED ACCORDING TO NATIONAL, REGIONAL, AND LOCAL REGULATIONS.

Caution

The surface disinfectant can negatively influence the performance of your 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.

Perform the disinfection procedure 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. Move the plate carrier into the load position.
4. If a microplate is on the plate carrier, remove it.
5. Disconnect the instrument from the main power supply and let it cool down to ambient temperature to avoid any risk of fire and explosion.
6. Disconnect the instrument from the computer and from any accessories.
7. Carefully apply the disinfectant solution according to the manufacturer's instructions for use on the plate transport of the instrument.
8. After the required contact time (according to the manufacturer's instructions for use) wipe the plate carrier using a soft paper towel moistened with a mild detergent or distilled water to remove all traces of the disinfectant.
9. Move the plate carrier into the instrument by gently pressing its front end (of the plate transport) until the front plate transport door is completely closed.
10. Carefully apply the disinfectant solution according to the manufacturer's instructions for use on all outer surfaces of the instrument.
11. After the required contact time (according to the manufacturer's instructions for use) wipe the instrument using a soft paper towel moistened with a mild detergent or distilled water to remove all traces of the disinfectant.
12. Wipe dry the outer surface of the instrument with a soft paper towel.

13. Repeat the disinfection procedure on any accessories which are being moved or returned.
14. Wash your hands with a mild detergent and then disinfect them.
15. Pack the instrument and any accessories.
16. Dispose of the container with the disposables according to the relevant national, regional and local laws and regulations.
17. Complete the safety certificate and attach it to the outside of the box so that it is clearly visible.

Caution

The plate transport should only be moved manually if the instrument is disconnected from the main power supply.

See below for the safety certificate, which must be completed before the instrument is returned to the distributor/ service center.

9.3.3 Safety Certificate

To ensure the safety and health of personnel, our customers are kindly asked to complete two copies of the **Safety Certificate** (which was delivered with the instrument) 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 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 9.3.2 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 should 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.

Your local Tecan customer support can send you a new copy of the Safety Certificate, if required.

9.4 Disposal

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

This chapter gives instructions on how to lawfully dispose of waste material accumulating in connection with the instrument.

Caution

Observe all federal, state and local environmental regulations.

ATTENTION

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.**

9.4.1 Disposal of Packing Material

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

Returning Packing Material

If you do not intend to keep the packing material for future use, e.g. for transport and storage purposes:

Return the packaging of the product, spare parts and options via the field service engineer to the manufacturer.

9.4.2 Disposal of Operating Material

WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF THE PROCESS RUN ON THE INFINITE F500.

TREAT THE USED MICROPLATE, 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.

9.4.3 Disposal of the Instrument

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

Caution
Always disinfect the instrument before disposal.

Pollution degree	2 (IEC/EN 61010-1)
Method of disposal	Contaminated waste

WARNING

DEPENDING ON THE APPLICATIONS, PARTS OF THE INFINITE F500 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.**

10. Troubleshooting

Error #	Error Text	Description
1	Command is not valid	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
2	Parameter out of range	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
3	Wrong number of parameters	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
4	Invalid parameter	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
5	Invalid Parameter at pos	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
6	[prefix] is missing	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
7	RS485 Timeout at module [module descr]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
8	Invalid module number [Nr]	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
9	Binary Transfer command: [cmd] at module [n]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
10	Error at command [cmd] at module [n],	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
11	LID open	Plate transport or filter slide lid were open during a measurement or the instrument was used in very bright environment (<< 500 LUX). Please check if the lid closes completely or if the environment was too bright.
12	LUMI FIBER broken	Hardware Failure Luminescence Module Please report this error to your local Tecan customer support office.

Error #	Error Text	Description
13	Z Motor out of Safety-Range	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
14	Filter is not defined	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
15	X drive init error	Hardware Failure Plate Transport Module Please report this error to your local Tecan customer support office.
16	Y drive init error	Hardware Failure Plate Transport Module Please report this error to your local Tecan customer support office.
17	z drive init error	Hardware Failure z-drive Module Please report this error to your local Tecan customer support office.
18	Injector A not available	Hardware Failure Injector A Please report this error to your local Tecan customer support office.
19	Injector B not available	Hardware Failure Injector A Please report this error to your local Tecan customer support office.
20	Injector Init Error:	Hardware Failure Injector Module Please report this error to your local Tecan customer support office.
21	Invalid Command: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
22	Invalid Operand: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
23	Invalid Command Sequence: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
24	N/A	N/A
25	Injector not init.: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
26	Plunger Overload:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.

Error #	Error Text	Description
27	Valve Overload:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
28	Plunger Move not allowed:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
29	Command Overflow	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
30	Prepare: [s]: Gain:[g], Counts: [cts]	Unspecific Hardware failure Please report this error to your local Tecan customer support office.
31	[ERR] at module [mod] (cmd:[cmd])	Unspecific Hardware failure Please report this error to your local Tecan customer support office.
32	"MTP is in Out-Position",	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
33	[val] ... not set at (Ratiolabel [n])	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
34	Injectors are not enabled	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
35	Invalid Parameter Length (max: [n] char allowed)	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
36	Checksum Error	Communication Error on USB interface. Please report this error to your local customer support office.
37	Init Error at module [mod#]	Unspecific Hardware Failure Please report this error to your local Tecan customer support office.
38	Instrument Initialization Error	Unspecific Hardware Failure Please report this error to your local Tecan customer support office.
39	Injector A Communication Timeout	Communication Error on Injector Interface Please report this error to your local customer support office.
40	Injector B Communication Timeout	Communication Error on Injector Interface Please report this error to your local customer support office.
41	Prime Wash Error	Injectors still priming or washing Please wait until prime or wash process is finished.

Error #	Error Text	Description
42	Instrument is locked	Instrument is locked after a serious hardware problem. For unlocking a reboot is necessary. Please report this error to your local customer support office.
43	Prepare: [channel]: Wavelength:[lambda] Gain:[g], Counts: [cts]	Unspecific Hardware failure Please report this error to your local Tecan customer support office.
44	Step loss Error	Actuator failure Please report this error to your local Tecan customer support office.
45	Sync Scan: Number of EX-Steps does not match EM-Steps	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
46	Handshake timeout at module	Unspecific Hardware Failure Please report this error to your local Tecan customer support office.
47	Motor Timeout	Unspecific Hardware Failure Please report this error to your local Tecan customer support office.
48	[Value] is not in defined a Range	Unspecific Hardware Failure Please report this error to your local Tecan customer support office.
49	Sensor is broken	Sensor Failure Please report this error to your local Tecan customer support office.
51	Optic Busy Timeout	Any optic head motor activity is not finished in Contemplated time Please report this error to your local Tecan customer support office.
52	RS485 Data Timeout	Data from ADC not received Please report this error to your local Tecan customer support office.

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Declaration of Conformity

We, TECAN Austria GmbH herewith declare under our sole responsibility that the product identified as:

Product Type:	**Microplate Reader**
Model Designation:	**INFINITE 500**
Variants:	**Infinite F500**
Options:	**Injector, Barcode**
Address:	Tecan Austria G.m.b.H. Untersbergstr. 1A A-5082 Grödig, Austria

is in conformity with the provisions of the following EC Directive(s) when installed in accordance with the installation instructions contained in the product documentation:

2006/95/EC – Low Voltage Directive
2004/108/EC – EMC Directive
2006/42/EC – Machinery Directive

and that the standards referenced below were taken in consideration:

EN 61010-1:2001	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements
EN 61010-2-081/A1:2003	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-081: Particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes
EN 60825-1/A1:2001	Safety of laser products – Part 1: Equipment classification, requirements and user's guide
EN 61326-1:2006	Electrical Equipment for Measurement, Control, and Laboratory Use - EMC Requirements - Part 1: General requirements
EN ISO 14121-1:2007	Safety of Machinery - Risk Assessment - Part 1:Principles

Year of CE-marking: 2010