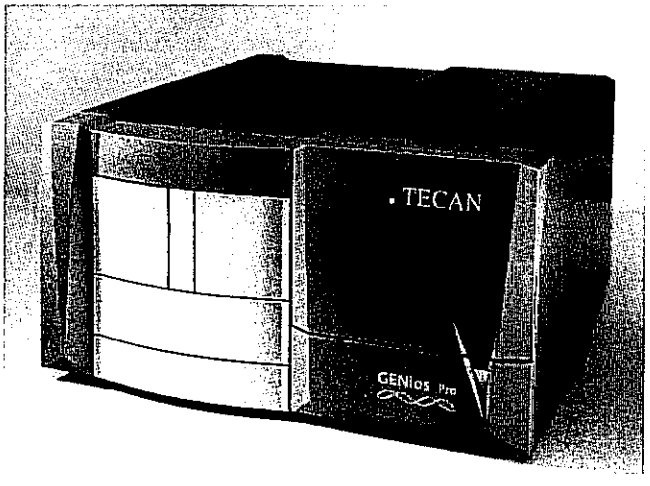
TECAN.

Instructions for Use for

GENios Pro



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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 back of this document.

About the Instructions for Use

This document describes the GENios Pro 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

Warnings, Cautions and Notes

There are various types of notices used in this publication. These notices highlight important information or warn the user of a potentially dangerous situation. The notices used in this publication appear below:

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.

WARNING
INDICATES THE POSSIBLE PRESENCE OF TOXIC MATERIAL. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.

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 GENios Pro while the instrument is plugged into a power source.
5. Never force a micro plate or filter slide into the instrument.
6. GENios Pro is intended for laboratory research use only. Observe proper laboratory safety precautions, such as wearing protective clothing and using approved laboratory safety procedures.

Caution

Tecan Austria GmbH have taken great care when creating the stored Plate Definition Files (.pdf) that are received with the instrument.
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. The safety gap cannot be calculated by GENios Pro if the plate used does not match the pdf selected.

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.

For details of how to modify a pdf, please refer to Plate Definition Editor Operating Manual, chapter 2.2 Defining a Plate

Caution

Before starting measurements, make sure that the microplate position A1 is inserted correctly.

Caution

To insure the optimal working of Tecan instruments we recommend a service interval of 6 month.

2. Installation

2.1 Unpacking and Inspection

The delivered packaging includes the following:

- Instrument with excitation and emission filter slides
- Operating and software manual
- Software (disk or CD-ROM)
- Cables (interface and mains)
- Transport lock
- Spare fuses
- 2 mm allen key
- disposable container for plate carrier when injector option is installed

1. Visually inspect the container for damage before it is opened.
   Report any damage immediately.
2. Select a location to place the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents and acid vapors. Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment. Ensure that the plate carrier and injector carrier cannot be accidentally hit when moved out. Ensure that the main switch and the main cable can be reached at all times and are in no way obstructed.
3. Place the carton in an upright position and open it.
4. Lift the instrument out of the carton and place it in the selected location. Take care when lifting the instrument and ensure that it is held on both sides.
5. Visually inspect the instrument for loose, bent or broken parts.
   Report any damage immediately.
6. Compare the serial number on the rear panel of the instrument with the serial number on the packing slip. *Report any discrepancy immediately.*
7. Check the instrument accessories against the packing list.
8. Save packing materials and transport locks (see next section) for further transportation purposes.
9. Insert and define the filters (see 2.5 Insert Filter Slides).

WARNING

The GENios Pro is a precision instrument and weighs approx. 38.5 kg. At least two people must carefully lift the instrument from the box.

Caution

The maximum load for the GENios Pro cover is 38.5 kg, however the load must be distributed evenly across the entire surface of the cover.

2.2 Removal of the Transport Locks

Caution

Save packing materials and transport locks for further transportation purposes. The GENios Pro must be shipped only with the original packing and installed transport locks.

2.2.1 Plate Transport Lock

The GENios Pro is delivered with the plate transport in place, so that it will not be damaged during transport. Before the instrument can be used, the plate transport lock must be removed.

1. Ensure that the instrument is disconnected from the main power supply.
2. Open the lower flap which leads to the plate carrier.
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

To prevent damage to the instrument, the transport lock must be saved and used whenever the instrument moved or is shipped.

2.2.2 Bottle Drawer Lock

Note:

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

If the instrument is equipped with the Injector option, the GENios Pro 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. This screw must be saved and used to prevent damage to the instrument whenever it is moved or shipped.
4. Remove the Bottle Drawer Lock.

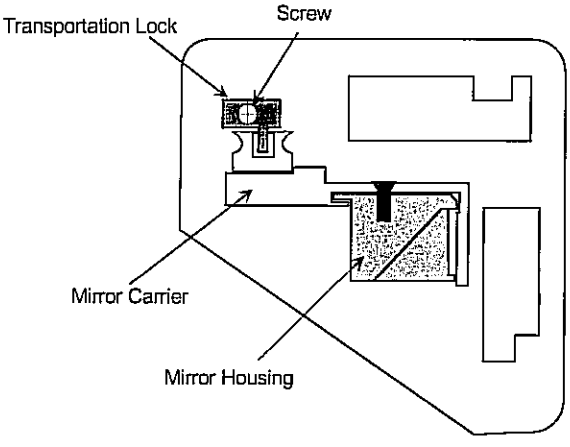
Caution

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

2.2.3 Mirror Carrier Transport Lock

The GENios Pro 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.

2.3 Power Requirements

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

The voltage range is 100-120 / 220-240V.

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

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.

Ensure that the correct type and rating of fuse is fitted.

Fuse = T 6,3 A / 250 V (slow blow)

WARNING

IF THE INSTRUCTIONS GIVEN IN THIS MANUAL ARE NOT FOLLOWED, DAMAGE TO THE INSTRUMENT MAY RESULT, AND THE SAFETY OF THE INSTRUMENT CANNOT BE GUARANTEED.

2.4 Switching the Instrument ON

Caution

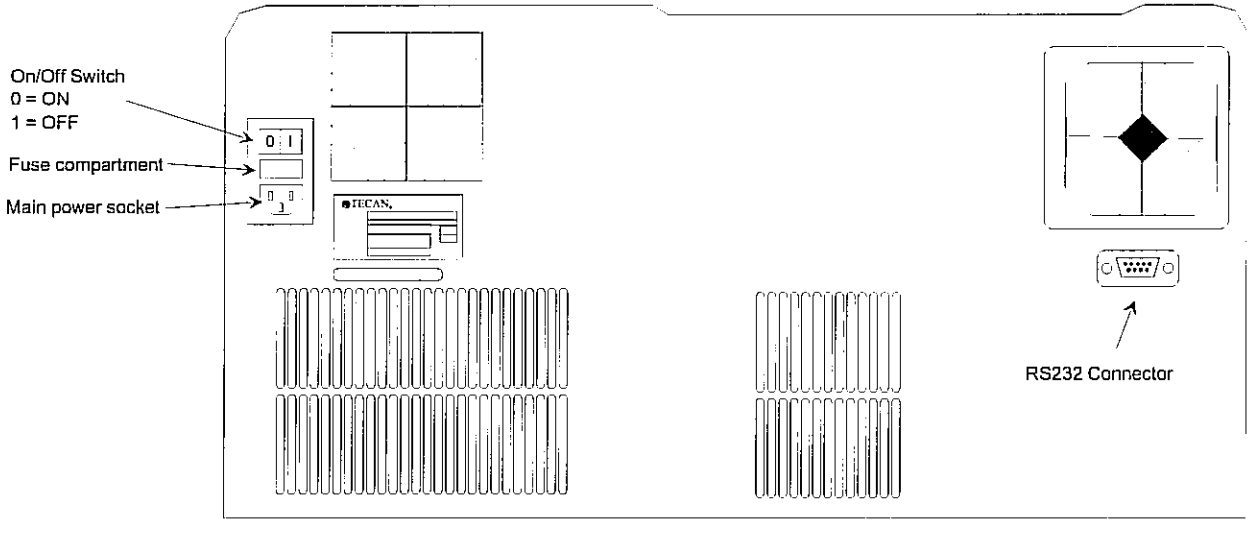
Before the instrument is switched on for the first time after installation, it should be left to stand for at least 3 hours, so there is no possibility of condensation causing a short circuit.

- Ensure the computer is switched OFF and the instrument's mains power switch in the back panel of the instrument is in the OFF position.
- Connect the computer to the instrument with the delivered RS 232 interface cable.
- Insert the power cable into the mains power socket (with protective earth connection) in the back panel of the instrument.
- All connected devices must be proved and listed with regulations EN 60950, UL 1950 or CSA C22.2 No. 950 for Data Processing Devices
- Switch the instrument ON using the switch in the back panel of the instrument.

Caution

When installing or uninstalling the instrument ensure that the instrument and the computer are both switched off and disconnected from the mains before the RS 232 interface cable is connected or removed.

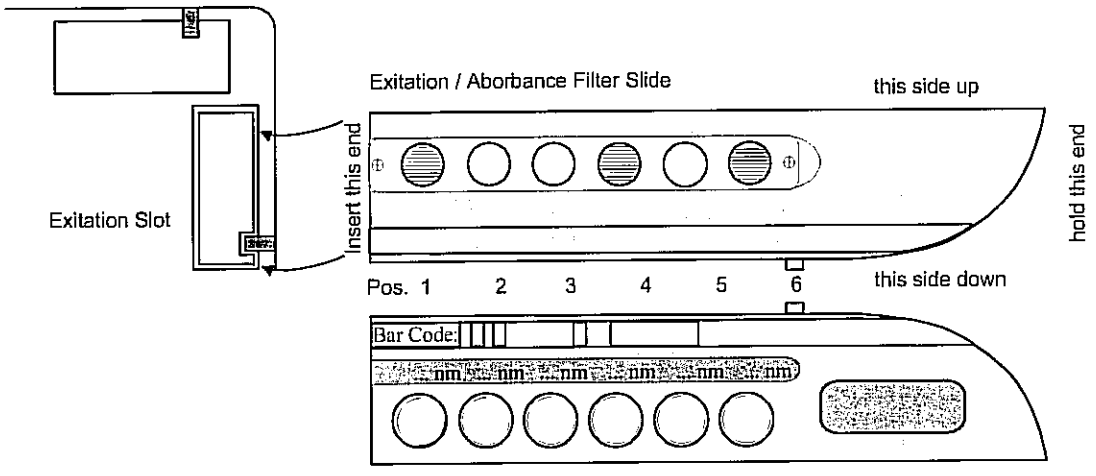
Rear View



WARNING

IF THE INSTRUCTIONS GIVEN IN THIS MANUAL ARE NOT FOLLOWED, DAMAGE TO THE INSTRUMENT MAY RESULT, AND THE SAFETY OF THE INSTRUMENT CANNOT BE GUARANTEED.

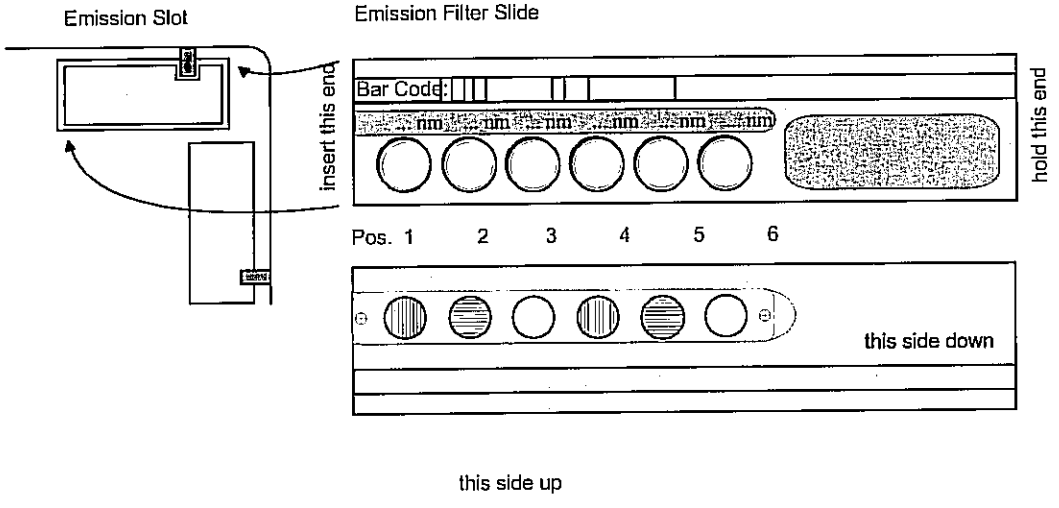
2.5 Insert Filter Slides



To insert the filter slides open the upper door flap manually. For the ease of identification the excitation and emission filter slides are different in shape (see below). They also have a different cross profile such that they only fit into the appropriate filter slot. 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.

3. General

3.1 Instrument Features

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

3.1.1 Multifunctionality

The following measurement techniques are supported by the GENios Pro:

1. Fluorescence Intensity (FI) Top
2. Fluorescence Intensity (FI) Bottom
3. Time Resolved Fluorescence (TRF)
4. Fluorescence Polarization (FP)
5. Flash Fluorescence
6. Absorbance
7. Absorbance with injectors
8. Glow Type Chemi- or Bioluminescence
9. Bioluminescence Resonance Energy Transfer (**BRET^{2TM}**)
10. Flash Luminescence

Any common microplate ranging from 6 to 384 well format may be measured with any of the above measurement techniques. 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 GENios Pro.

3.1.2 Performance

The GENios Pro has been designed for optimum sensitivity and speed of measurement. Specifications of sensitivity or precision correlate with the corresponding measurement time per microplate. (See also chapter 6. Instrument Features).

The GENios Pro 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.

3.1.3 User Friendliness

The GENios Pro offers unparalleled flexibility for the customization of fluorescence and absorbance measurements: 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 GENios Pro's 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.

3.2 Measurement Techniques

The following sections provide an introduction to the GENios Pro's measurement techniques. To keep this chapter compact, a few simplifications have been made. For details see the references.

3.2.1 Fluorescence

The GENios Pro offers the basic fluorescence measurement technique and some even more sophisticated variants

A) Fluorescence Intensity (FI)

B) Time Resolved Fluorescence (TRF)

C) Fluorescence Polarization (FP)

FI may also be used to measure Fluorescence Resonance Energy Transfer (FRET). For some microplate applications, FP and FRET offer advantage over FI and TRF, because they simplify assay preparation. These preferably apply for **mix and measure** binding studies. While FP needs only one binding partner to be labeled, FRET needs both being labeled in a suitable way. Other FRET-based applications take advantage of TRF labels for increased sensitivity; these are referenced as HTRF (Homogeneous TRF).

Time Resolved Fluorescence (TRF) should not be confused with Fluorescence Lifetime Measurements.

Fluorescence (An Abstract)

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 quick enough into thermal energy.

For a detailed treatise on fluorescence techniques and applications see, for example:

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

Fluorescence Intensity (FI)

In many microplate applications, the intensity of fluorescence emission is measured to determine the abundance of fluorescently 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 effect the fluorescence quantum yield and therefore the measurement results.

Fluorescence Resonance Energy Transfer (FRET)

Some microplate applications utilize a sophisticated dual labeling strategy. Fluorescence Resonance Energy Transfer effect (FRET) allows one 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. And second, 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 less.

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 of using TRF labels as the donor, (for example: see. **High Throughput Screening** – Marcel Dekker Inc 1997 New York, Basel, Hong Kong – see section 19 Homogeneous, Time-Resolved Fluorescence Method for Drug Discovery by Alfred J. Kolb et al.).

Time Resolved Fluorescence (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 GENios Pro uses a flash lamp light source with flash duration much shorter than the 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.

Homogenous 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 minimises

several undesired assay interferences and side effects (eg. volume/meniscus, quenching, light scattering, autofluorescence, molecular size, etc.). Furthermore, the homogenous 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 6.2.1 (*Fluorescence Intensity, Time Resolved (TRF) and Homogenous Time Resolved (HTRF®) – Top Reading*). 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 licence to Tecan. The method is covered by the US patent 5,527,684 and its foreign equivalents.

Fluorescence Intensity Bottom Reading (Optional)

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. Fluorescence light is fed back through the emission filter. The bottom read fiber bundle conducts light to and from the bottom read head (situated underneath the microplate). The diameter of the bottom read excitation beam can be selected for both 96 and 384 well plate formats. An automated aperture selects either the full or the core diameter of the bottom fiber bundle.

Flash Fluorescence and FI Kinetic (Optional)

For high sensitivity Flash Fluorescence assays, the measurement is done just after dispensing the activating reagent or after a short delay time.

96 Well position: Measurement position is identical to the injector position.

384 Well position: Measurement position is not the same as the injector position The instrument injects and then moves to the read position; this takes 0.5 sec.

Fluorescence Polarization (FP) (Optional)

Fluorescence Polarization measures rotational immobility of a fluorescently labeled compound due to its environment.

Fluorescence Polarization is defined by the following equation:

$$P = \frac{(I_v - I_h)}{(I_v + I_h)}$$

where P equals polarization, I_v equals the emission intensity of the vertically polarized light parallel to the plane of excitation and I_h equals the emission intensity of the horizontally polarized light perpendicular to the plane of excitation.

FP is suitable for binding studies, because tumbling of molecules may be dramatically reduced after binding to a much larger site, and vice versa. For more information see:

- **High Throughput Screening** – Marcel Dekker Inc 1997 New York, Basel, Hong Kong – see section Fluorescence Polarization by J.R. Sportsman et al.
- **Polarization De La Lumière De Fluorescence Vie Moyenne Des Molécules Dans L'etat Excité** by M. Francis Perrin (Journal de Physique No:12, 1926).

For a simplified picture of FP, fluorescent molecules may be visualized as antennae, which need suitable orientation to pick up light waves of excitation successfully. Using planar polarized light, only a specifically oriented subset of the randomly oriented molecules is susceptible to excitation.

The FP measurement result will be 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. 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.

3.2.2 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 **O.D.** (Optical Density).

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).

Note:
In absorbance mode, the two syringes can only be used simultaneously.

3.2.3 Luminescence (Optional)

Glow Type Chemi- or Bioluminescence

The GENios Pro 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. As with enzyme based assays, control of environmental conditions is rather critical (temperature, pH-value).

For practical aspects of luminescence assays see for example:

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

Bioluminescence Resonance Energy Transfer [2] (BRET^{2TM})

(Bioluminescence Resonance Energy Transfer) 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^{2TM} is based on energy transfer between fusion proteins containing *Renilla* luciferase (Rluc) and a mutant of the Green Fluorescent Protein (GFP). The BRET^{2TM} signal 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.

Dual Colour Luminescence

Some assays send out light of two different wavelengths at the same time. For these assays wavelength discrimination during luminescence detection may be required. Tecan luminescence filters are optimised for the [1] Chroma-Glo™ Luciferase assay system and for [2] BRET^{2TM}. Filters are built into the luminescence filter wheel and cover a wavelength range of 370 - 450 nm, 610 – 700 nm and 510 - 540 nm, selected according to the demands of the applied assay.

The Chroma-Glo™ luciferase assay generates red and green (dual-color) luminescence from two luciferases within a single well and upon a single reagent addition. This homogenous dual-reporter gene assay permits each reporter to be measured independently by detecting one well at two different wavelengths (red and green).

Flash Type Luminescence with Injectors (Optional)

In high sensitivity 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. These so-called glow type reagents will always have one advantage, sensitivity. In the glow type the luminescence signal is spread over a wide time scale (for example half life 30min) and only a portion of it is read 0.1 sec / well), so a lot of the light is not detected. In Flash Luminescence assays most of the light is counted (half-life 5 s).

Flash type luminescence is one of the measurement modes that can be performed with injectors. Both injection and measurement without time delay can be performed for reaction kits up to the range of milliseconds.

Note:
The plate detection sensor is only activated if one of the injector is activated or in "dispensing only" mode.

Note:
During luminescence measurements it is important to close the lid covering the syringes and bottles of the reagent system to minimize background signal.

[1] Chroma-Glo™ is a trademark of Promega Corporation.

[2] BRET$^{2\,TM}$ is a proprietary technology from BioSignal Packard. Patents pending

3.3 Injectors (Optional)

The GENios Pro can be optionally equipped with two syringe pumps, (XP3000 plus, Tecan System), which feed three injector needles. Two of these needles are completely independent for the injection of two liquids into the same well of a 96-well plate. The third needle is for the injection into 384-well plates.

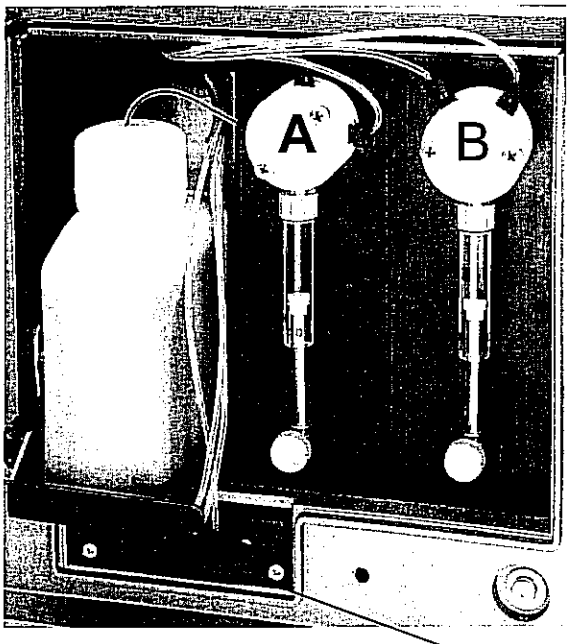


figure 3-1

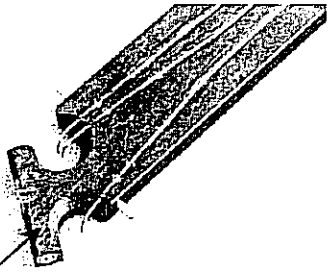


figure 3-2

Injector carrier

- Pump A (figure 3-1) feeds two injector needles (for 96-and 384-well plates),
- Pump B (figure 3-1) feeds a second needle at the 96-well position.

One Injector Option (one pump): A GENios Pro equipped with one pump allows injections in both 96-and 384-well plates using the same liquid for both plate formats. All reactions requiring injection of only one liquid per well (such as reporter gene assays) can be performed with this option.

Two Injector Option (two pumps): 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 dispensed into the same well of a 96-well plate and one liquid can be dispensed into a well of a 384-well plate.

3.3.1 Measurement with Injectors

For flash luminescence reactions, time is especially critical, therefore the GENios Pro allows injection at the measurement position for both 96-and 384-well plates. This means no movement of the plate must be carried out between injection and measurement. Also, a continuous measurement can be started at some time interval before the injection to obtain a background reference signal . These readings can be performed to obtain a baseline reading of a reaction and may be handled prior to the dispense step.(for details see Luminescence Example for Fast Kinetic Measurements page 3-18).

Similar, Fluorescence-bottom measurements in 96-well plates (fast flash fluorescence reactions such as ion studies) can be performed during the injection, because the plate must not be moved from the injection position to the measurement position.

The injectors of the GENios Pro can also be used with all other measurement modes: Fluorescence Intensity top (96-and 384-well plates), Fluorescence Intensity bottom (384-well plates), Time Resolved Fluorescence, Absorbance (96- and 384-well plates), Flash and Glow Type Luminescence and Dual Color Luminescence. However, as the measurement position is not the same as the injector position, a short time delay of approx. 0.5 s between injection and reading occurs.

Schematic Diagram of the Two Injector Option:

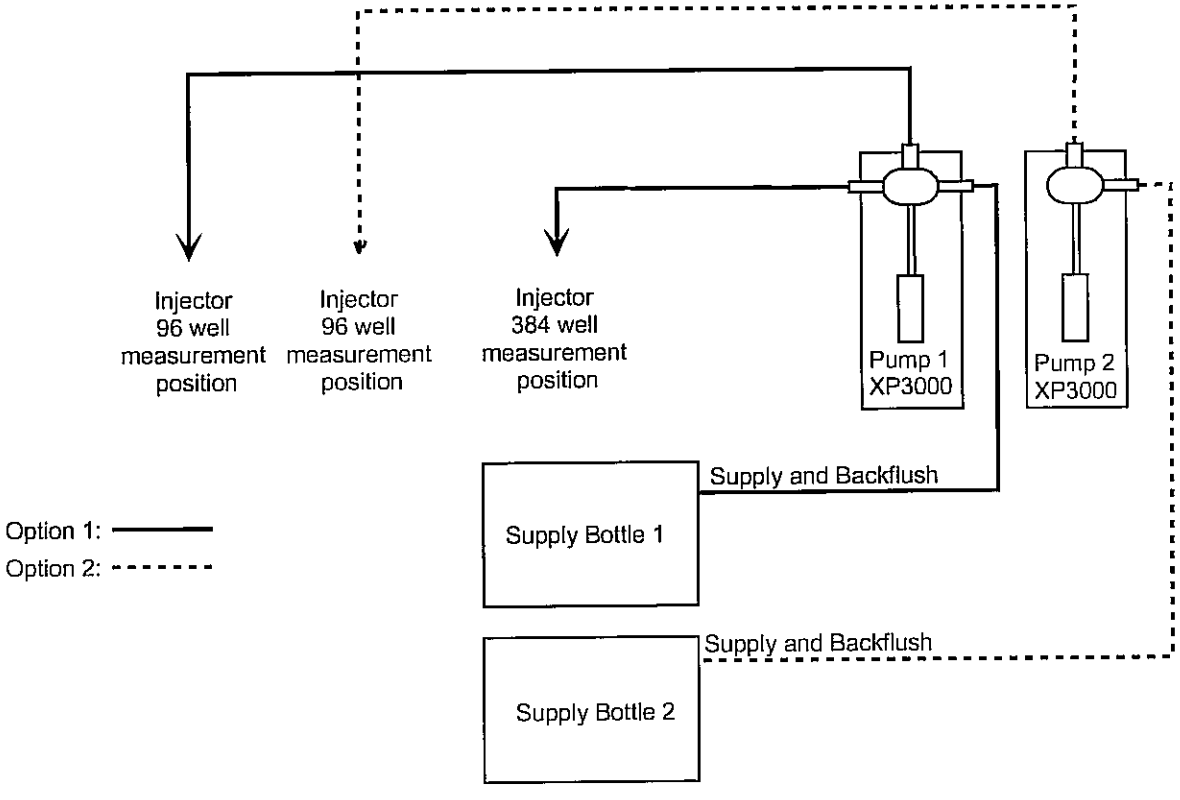


figure 3-3: Liquid path in GENios Pro with two pumps

3.3.2 Storage Bottles

In a drawer in the instrument up to two bottles with a volume of 125 ml or two Falcon tubes can be inserted. Additionally, a smaller container may be used via an adapter that can be mounted into the drawer of the GENios Pro.

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

- Two 125 ml bottles (1 translucent and 1 opaque), for the "One Injector option" (one pump) or
- Four 125 ml bottles (2 translucent and 2 opaque), for the "Two Injectors option" (two pumps).

The bottles are available in translucent and opaque versions (for light-sensitive reagents). In cases in which very small volumes of reagent are required, 15 ml *Falcon* tubes can be used. The 125 ml bottles can be used as a support for the *Falcon* tubes.

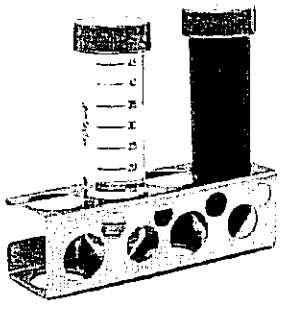


figure 3-4: Adapter for smaller tubes

3.3.3 Injector Carrier

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

Note that the injector carrier is locked during measurements. The injector carrier is automatically unlocked at the end of a measurement when the plate carrier returns to the loading position outside the reader.

Additionally, the injector carrier can be unlocked by actively driving the plate carrier out of the reader using the command buttons in the " movements"-window of XFluor.

Caution
The injector carrier must be in the service position (extended position – see figure 2.3) for washing und priming.

The dead volume of the injection system (injector needles, syringes, valves and tubing) is approximately 1,5 ml. By using ***reagent backflush*** to return any unused reagent to the reservoir bottles, the dead volume can be reduced by approximately 80µl per syringe. The injection speed can be adjusted via the software to allow for good mixing of reagents. The optimum injection speed is dependent on the assay parameters, such as viscosity of fluids, the plate format and the measuring behavior of the liquids. The removable injector carrier allows this process to be done outside of the instrument where a visual inspection can be easily performed.

3.3.4 Priming and Washing of the System

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. The priming parameters can be adjusted, if required, using the injector control window in the XFluor software.

For the initial filling step of the injector system (priming) a prime volume of 1,5 ml is recommended to completely remove air from the injection system. 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 800 µl.

Caution

A prime volume below 1,5 ml in an empty system may result in incomplete filling of the system, and therefore may negatively affect assay performance.

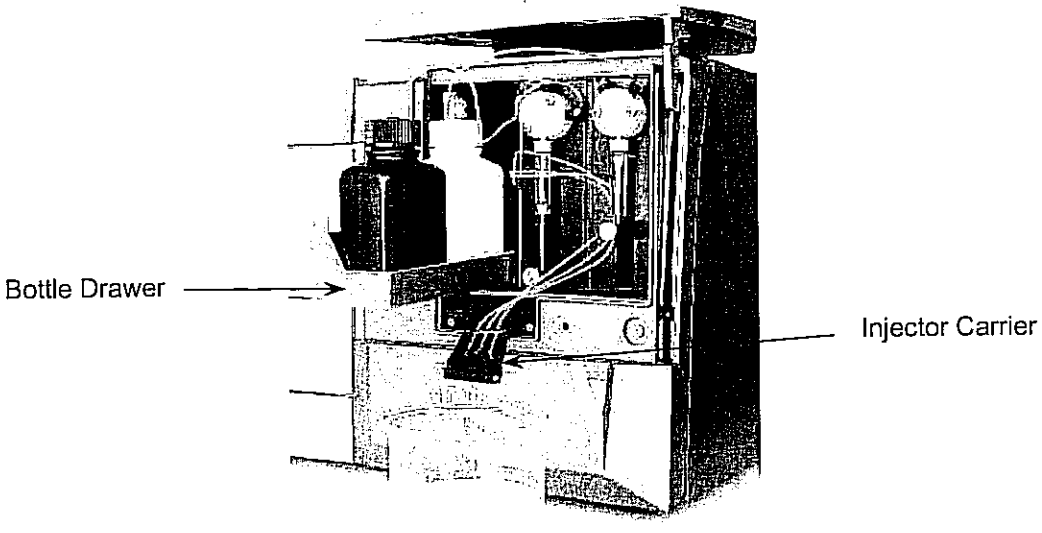


figure 3-5: "Service Position" of the injectors. The injectors are removed from the carrier slot and rotated 180° so that the injector needles face away from the instrument.

The injector carrier includes up to three injection needles and the tubing.

Caution

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

Be careful when inserting the injector carrier into the instrument, to fully load it into its home position (it must click into place).

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.

To perform the priming procedure:

1. Fill the storage bottles with the necessary reagents and insert the feeding tubes. Make sure, that the tube reaches the bottom of the bottle.
2. Remove the injector carrier from the carrier slot, rotate it 180° so that the injector needles are facing away from the instrument and reinsert it partially into the slot for support (see *figure 2.3*).
3. Position an empty container under the injector needles.
4. Adjust parameters if required
5. Activate the priming procedure via the injection control window of the XFluor software
6. Make absolutely sure that no air bubbles are in the system before starting the measurement!!!

After the procedure has been successfully performed, rotate the injector carrier 180° and reinsert it into the instrument (injector needles facing towards the instrument).

Prior to the first dispense step into a well of a microplate, a small dispense step into a disposable container on the plate carrier is performed automatically to condition the injector needle. After this step, the instrument is ready to use.

Reagent Backflush

Prior to the cleaning of the injector system, reagent backflushing allows the remaining reagent in the liquid system (injector needles, syringes, valves and tubing) to be pumped back into the storage bottles. This procedure is a cost effective solution for minimizing reagent consumption, because the dead volume of the injector system is reduced to approx. 80µl per syringe.

To perform the reagent backflushing procedure:

1. Put injectors in the service position (see figure 3-5: "Service Position" of the injectors. The injectors are removed from the carrier slot and rotated 180° so that the injector needles face away from the instrument.)
2. Place tubing in the storage bottle.
3. Adjust parameters if required
4. Select **start backflush** from the injector control menu.

Washing

Before the instrument is switched off, a wash procedure should be run to clean the injector system. Note that no prime step is needed as the wash step includes a priming step. To perform a typical wash procedure:

1. Remove injector carrier and bring it into the "service position" (see figure 2.3).
2. Perform a backflush procedure to feed unused reagent back into the storage bottle.
3. Fill bottle with distilled or deionized water and insert feeding tubes of the injector system.
4. Start wash procedure with distilled or deionized water (this will automatically flush the injector system three times).
5. Prepare a container with 70% ethanol as wash solution and prime the injectors with this solution.
6. Start wash procedure with a 70% ethanol wash solution (this will automatically flush the injector system three times).It is preferable to allow the injector system to soak in a 70% ethanol solution for a few minutes, prior to removing the wash solution with water
7. **To clean the ends of the injector needles** from outside, a cotton swab soaked in 70% ethanol may be used.
8. Start final wash procedure with distilled or deionized water, to completely remove the ethanol wash solution.
9. For extended cleaning procedures it is recommended to use the **TECAN CLEAN-SYSTEM** with **DAILY SYSTEM CLEAR** (Order number: H002001) for all day cleaning or for protein proteolysis the **PROTOLYSE** (Order number: H002002) cleaning system. Make sure that these cleaning solutions are thoroughly removed from the injector system using distilled or deionized water!

Important

Be sure to run a final wash procedure with distilled water and empty the injector system 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 injector needles, 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.

3.4 Software

GENios Pro is delivered with the *XFluor* software including online-help 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 CFR 21 part 11 guidelines, ***Magellan Tracker*** software can be used to control the GENios Pro. (For more information, contact your local Tecan representative).

3.4.1 XFluor

The *XFluor* software contains the following key components (list not exhaustive):

1. *XFluor* is a user interface to operate the GENios Pro from within Microsoft Excel™. *XFluor* is intended to control stand alone operation by the user.
2. *RdrOle* is the Reader Server. The GENios Pro and many other Tecan microplate readers are operated under software control through the Reader Server. *RdrOle* is a powerful tool which enables the GENios Pro to be integrated into a robotic platform via a defined interface.

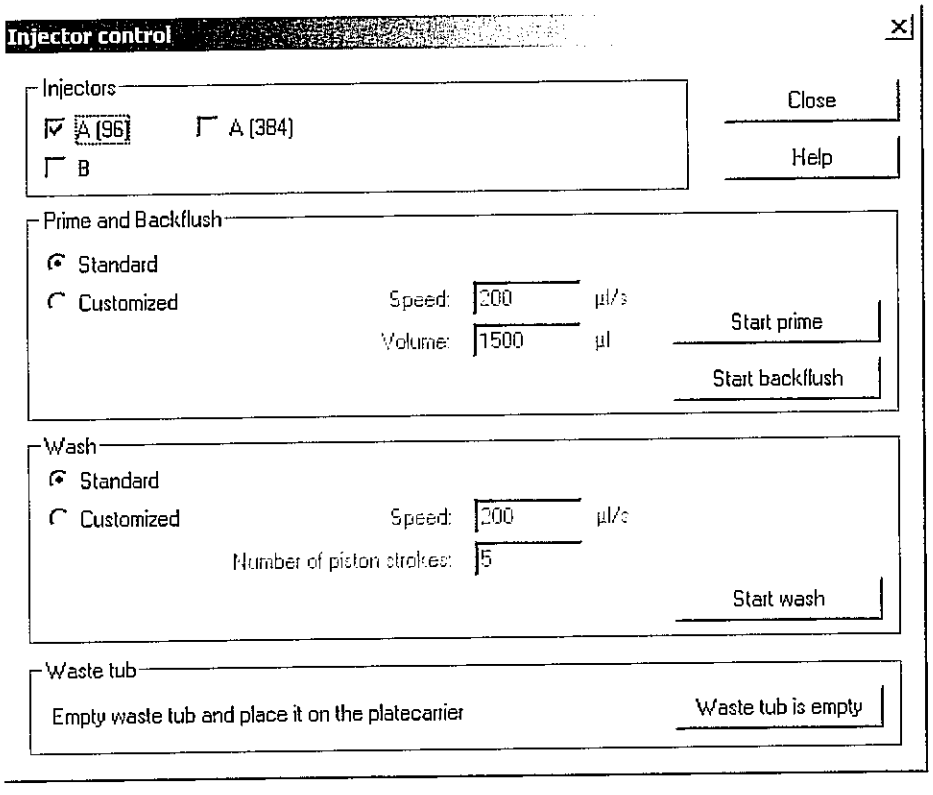
XFluor uses *RdrOle* to control operation of the GENios Pro. *RdrOle* may be called explicitly for service purposes (see 7. Quality Control). We do not recommend to use *RdrOle* in this way for daily operation.

Benefits of *XFluor*

1. Measurement raw data are formatted into a Worksheet, where they can be processed directly using Microsoft Excel™ spreadsheet functions. Fluorescence Intensity and Luminescence readings are displayed as relative units. Absorbance and Fluorescence Polarization values are evaluated into physical units.
2. Instrument operation is provided by a single and well organized pull down menu within Microsoft Excel™. *XFluor* can be used to acquire measurement data.
3. *XFluor* may be customized or extended through Microsoft Visual Basic for Applications programming.

3.4.2 How to Use XFluor for Measurements with Injectors

Injector Control



Injectors:	Select one of the injectors **A(96)** or **A(384)** and injector **B**, if desired. Injector **A(384)** for use with 384 Microtiterplates.
Prime and Backflush:	Select **Standard** for most applications. The settings should be: **200 µl /s** in the *Speed* box and **1500 µl** in the *Volume* box. When **Customized** is selected, custom *Speed* (1 - 475 µl /s) and *Volume* (1 to 9999 µl) parameters can be entered.
Wash:	Select **Standard** for most applications. The settings should be: **200 µl /s** in the *Speed* box and **5** in the *Number of piston strokes* box. When **Customized** is selected, custom *Speed* (1 - 475 µl /s) and *Number of piston strokes* (1 to 99) parameters can be entered.
Waste tub:	Click **Waste tub is empty**, only if the waste tub on the plate carrier is empty. The program will then alert you if the waste tub needs to be emptied again.

Dispensing Only Mode

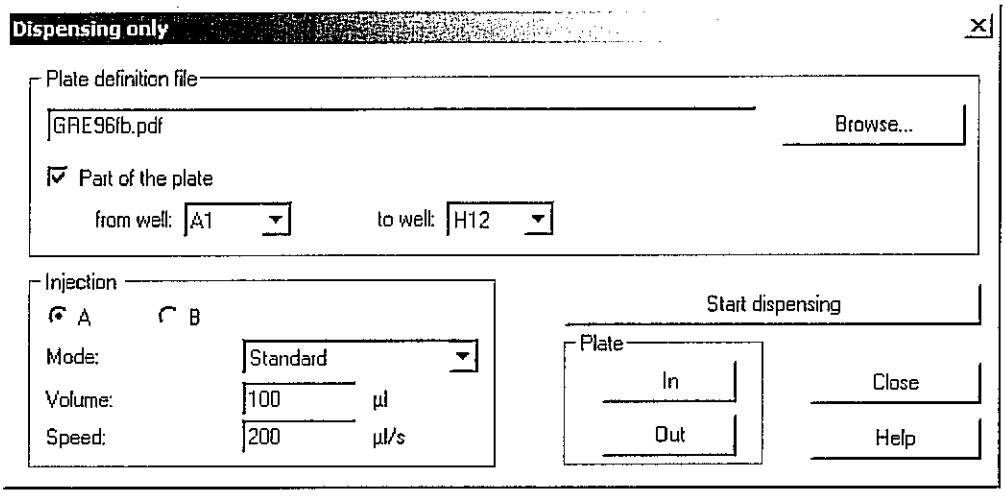
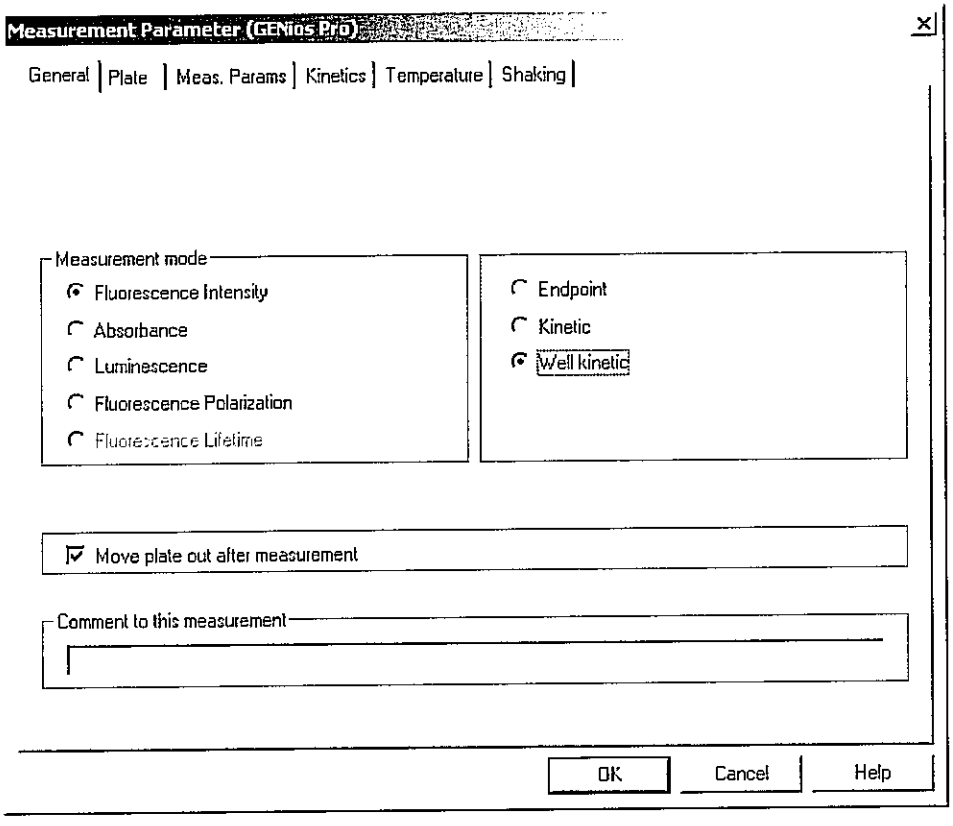


Plate definition file:	Choose plate definition and the part of the plate you wish to fill.
Injection:	Choose Injector A or B; Filling **Mode**: *Standard* (the syringe is refilled when all liquid is dispensed) or *Every injection refill* (the syringe is refilled after every injection step); **Volume** range from 1 to 495 µl ; **Speed** range from 1 to 495 µl /s;

Click **Start dispensing** to proceed the filling of the MTP wells. After a sensor has checked if a plate has been loaded, the filling will be proceed.

Note:
In Standard Filling Mode, multiple wells are dispensed before the syringe is refilled. The number of dispense steps that can be performed before refilling of the syringe take place is dependent on the selected dispense volume per well

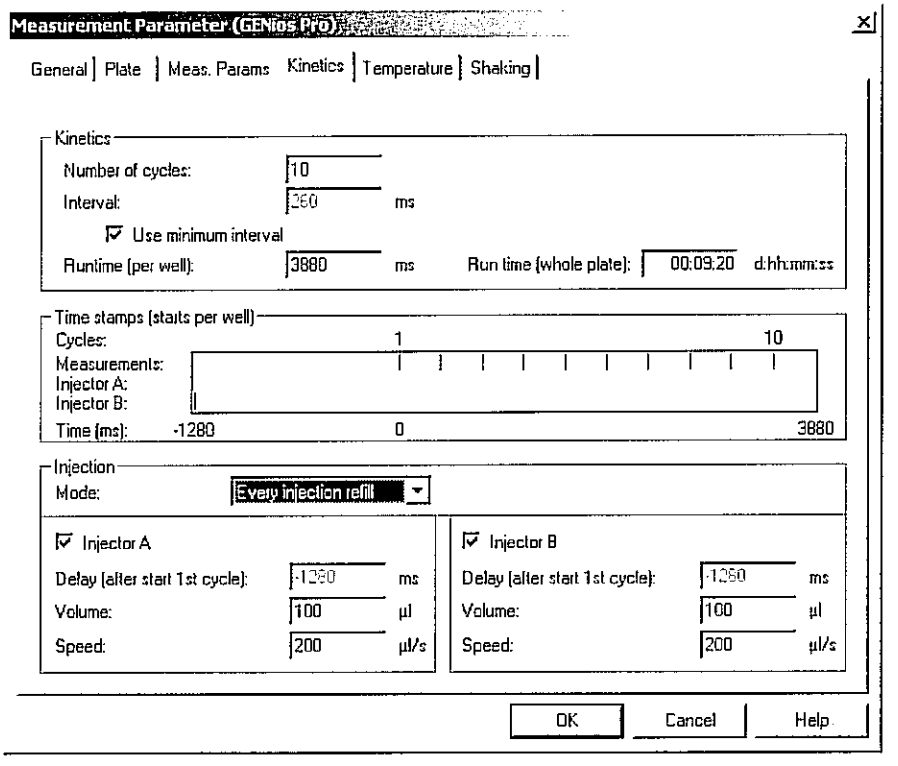
Measurement Parameter



In the *Measurement Parameter* dialog box, select **Well kinetic** to activate the use of injectors to dispense reagents.

This mode is available in all measurement modes (FI top and bottom, TRF, Absorbance, Flash and Glow Luminescence, FP)

Fluorescence / Fluorescence Polarization / Absorbance / Luminescence



Under *Kinetics*, set the **Number of Cycles** (1 to 9999) and the **Interval** (minimum interval is calculated by the program maximum interval is 60000 ms (1 minute). The **Runtime (per well)** and **Runtime (whole plate)** are calculated automatically.

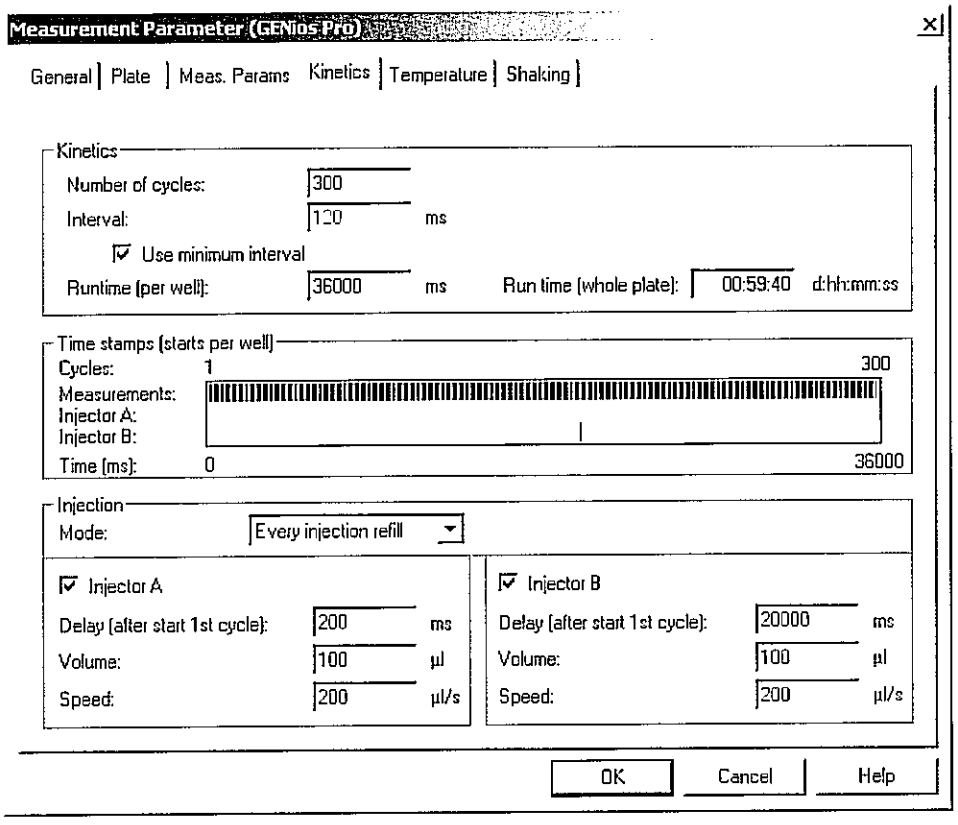
Time stamps (start per well) graphically shows the used *time stamps* and sequence of measurement for both injections. The time scale is in milliseconds.

Under *Injection*, select the **Mode**, select *Standard* (multiple dispense steps before the syringe is refilled) or *Every injection refill* (the syringe is filled after every injection).

Under *Injector A* and *Injector B*, the **Delay (after 1st cycle)** is shown. The delay time can be set for luminescence measurements and is always counted from the beginning of the 1st cycle. If injection before the measurement is needed use "negative time" value (ex : "-1000" means that the injection is performed 1000 ms before the measurement). Use 0 ms to start injection and measurement step at the same time. Set the **Volume** (1 - 495 µl) and the **Speed** (1 - 495 µl/s). For standard liquids (aqueous liquids) 200 µl/s is recommended.

Note:
In absorbance mode both injectors are only usable simultaneously. Only valid for XFluor!

Luminescence Example for Fast Kinetic Measurements



Under *Kinetics*, set the **Number of Cycles** to 300 to 1200 number of cycles to get a high density of measurement points during the reaction use.

For the **Interval**, select the **Use minimum interval** check box.

Runtime (per well) and **Runtime (whole plate)** are calculated automatically.

Time stamps (start per well) graphically shows the used *time stamps* and sequence of measurement for both injections. The time scale is in milliseconds.

The injection of the first liquid is performed after collecting some reads that can be used as a pre-injection baseline. The second injection is performed 20 sec (20000 ms) after the reaction has started.

Under *Injection*, select the **Mode**, select *Standard* (multiple dispense steps before the syringe is refilled) or *Every injection refill* (the syringe is filled after every injection).

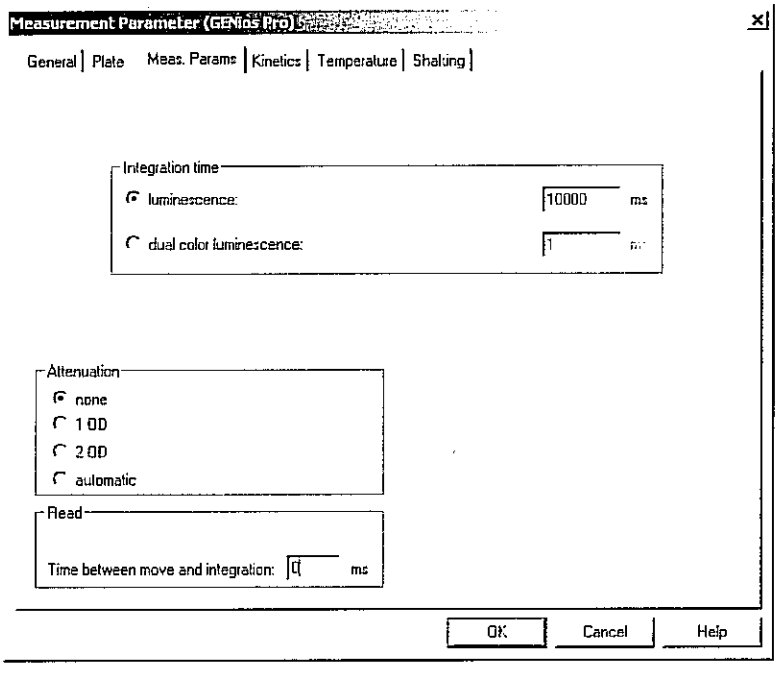
Under *Injector A* and *Injector B*, set the **Delay (after 1st cycle)** to 200 - 1000 ms to inject during the measurement that has already started.

Set the **Volume** (1 - 495 µl) and the **Speed** (1 - 495 µl/s).

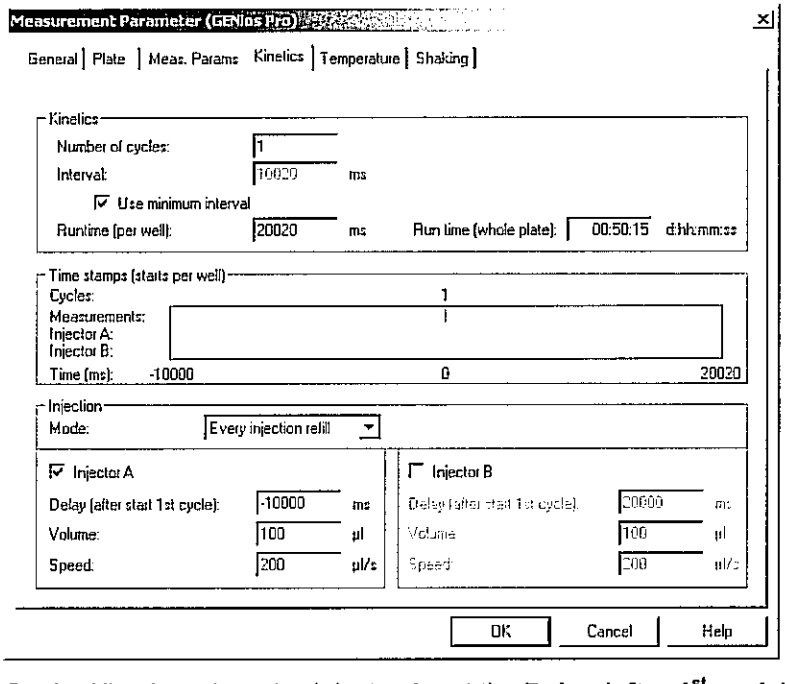
Using the well wise kinetic measurement shown above (such as flash luminescence) integration time starts prior to the injection step, so that the collection of data for the baseline signal is possible before triggering the signal increase due to the dispensing of the first reagent.

Luminescence Example of One Point Measurements (Glow Type)

This example of a one point measurement with a 10 second delay after injection and 10 second integration time for the luminescence signal is a procedure commonly used by Glow luminescence kits that measure ATP concentrations.



On the *Measurement Parameters* tab, under *Integration time*, set luminescence to 10000 ms.



On the Kinetics tab under *Injector A*, set the **Delay (after 1st cycle)** -10000 ms.

Set the **Number of Cycles** to 1. For the **Interval**, select the **Use minimum interval** check box. **Runtime (per well)** and **Runtime (whole plate)** are calculated automatically.

Time stamps (start per well) graphically shows the used *time stamps* and sequence of measurement for both injections. The time scale is in milliseconds.

Under *Injection*, select the **Mode**, select *Standard* (multiple dispense steps before the syringe is refilled) or *Every injection refill* (the syringe is filled after every injection).

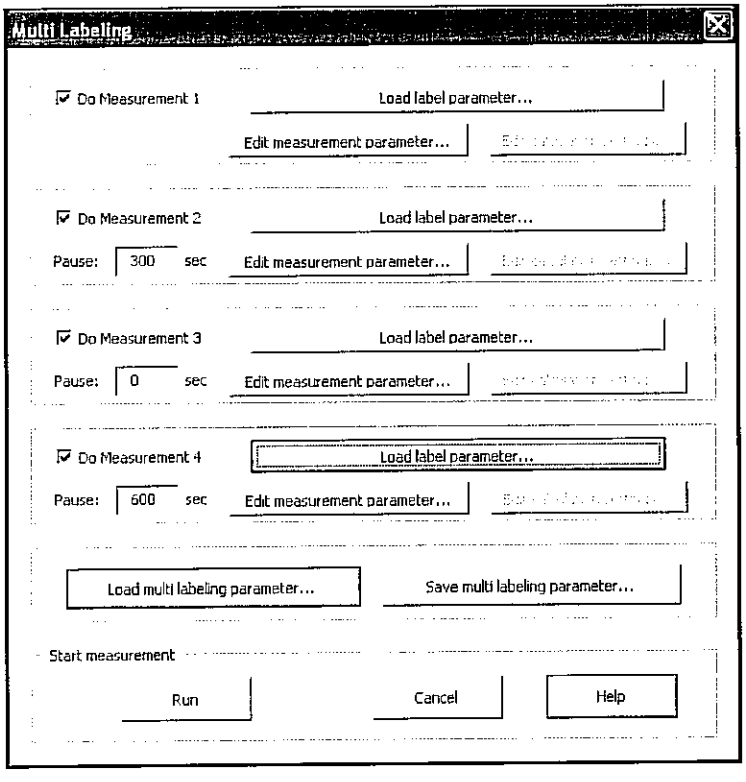
Under *Injector A* and *Injector B*, set the **Delay (after 1st cycle)** to 200 - 1000 ms to inject during the measurement that has already started.

Set the **Volume** (1 - 495 µl) and the **Speed** (1 - 495 µl/s).

For details about "Attenuation" and "Read between move and integration" see the XFluor Instructions for Use.

Multilabel Measurement

In this measurement mode there is the possibility of a plate-wise use of injector via multilabel reading.



Label measurement mode can be performed with or without injectors.

For example, it is possible to use a combination of first injection and plate wise reading (Do Measurement 1), then 300 sec of delay and then the entire plate is read again (Do Measurement 2), second injection and plate reading (Do Measurement 3) and after a 600 second delay the whole plate is read again (Do Measurement 4).

3.4.3 Multilabeling Example using XFluor for GENios Pro Software

Introduction

Plate-wise use of injectors for typical glow-type lumi-assays (end-point reading) in which on-board injectors are a convenient tool to dispense reagents:

Examples of glow-type lumi-assays :

Luciferase – based assays that work via detection of ATP

- Cell viability
- Cytotoxicity
- Apoptosis
- Mycoplasma detection: quality check for cell-culture media
- etc...

Customer benefit:

Automated dispensing of reagents – no manual pipetting necessary.

Multilabel pipetting: Example of Glow Lumi-Assay using injectors for dispensing using * Mycoplasma – assay / Cambrex:

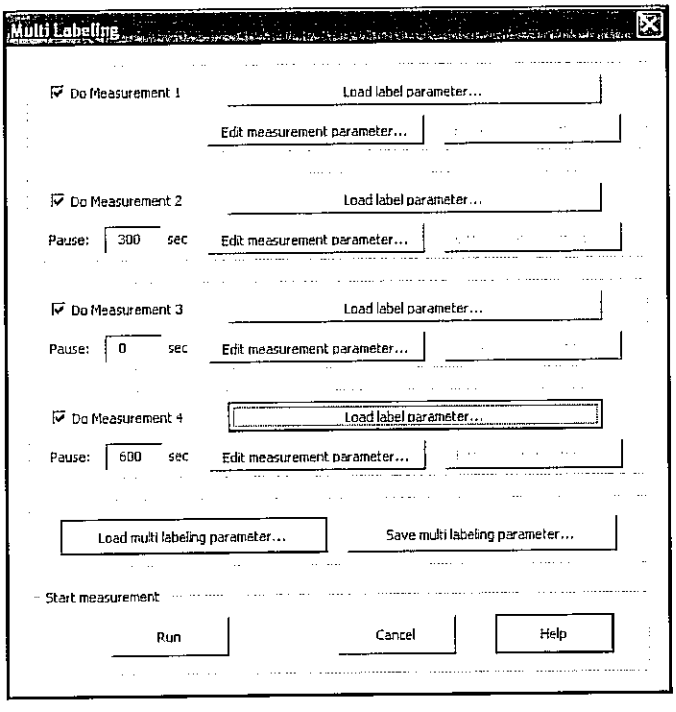
Overview of assay procedure:

1. Dispense 100 µl of reagent 1 into each well of a white 96 well plate
2. Incubate 5 min. and perform endpoint reading: result A
3. Dispense 100 µl of reagent 2 into each well of the plate
4. Incubate 10 min. and make endpoint reading: result B
5. Calculate ratio for each well (result B divided by result A)

Start XFluor for GENios Pro and connect instrument.

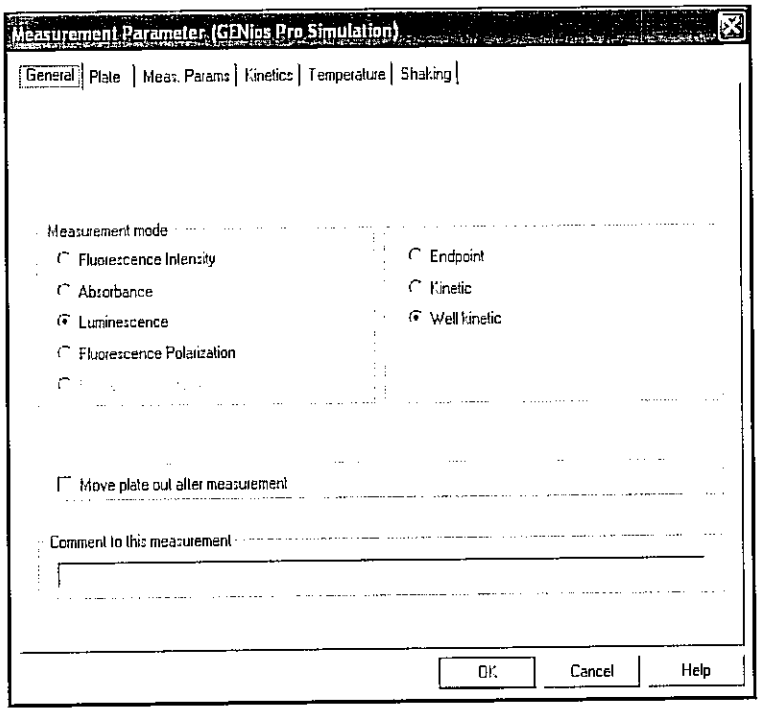
Select **Multi Labeling** from the XFluor GENios Pro menu.

The following dialog box appears:



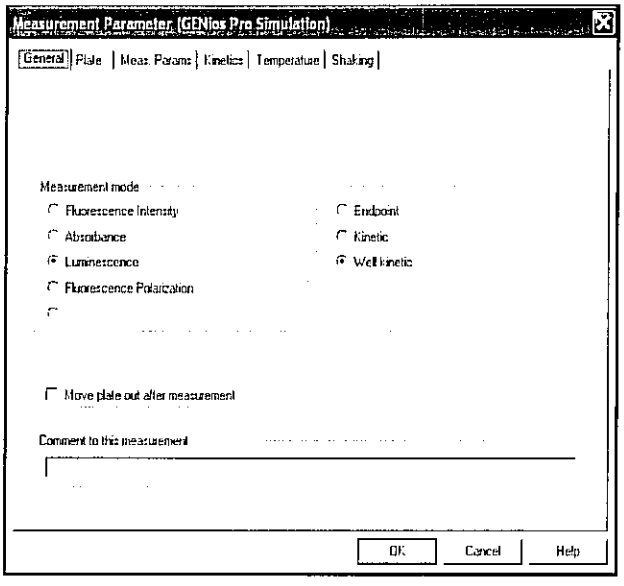
Select **Do Measurement 1**.

Click **Edit measurement parameter** for measurement 1 and the following dialog box appears:



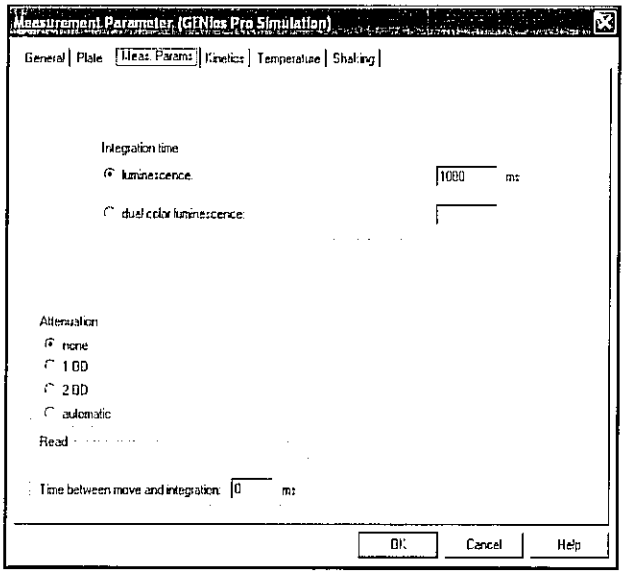
Measurement for Label 1:

Step 1 of assay procedure:
Disp. 100µl of reagent 1 into all wells of a 96 well plate.



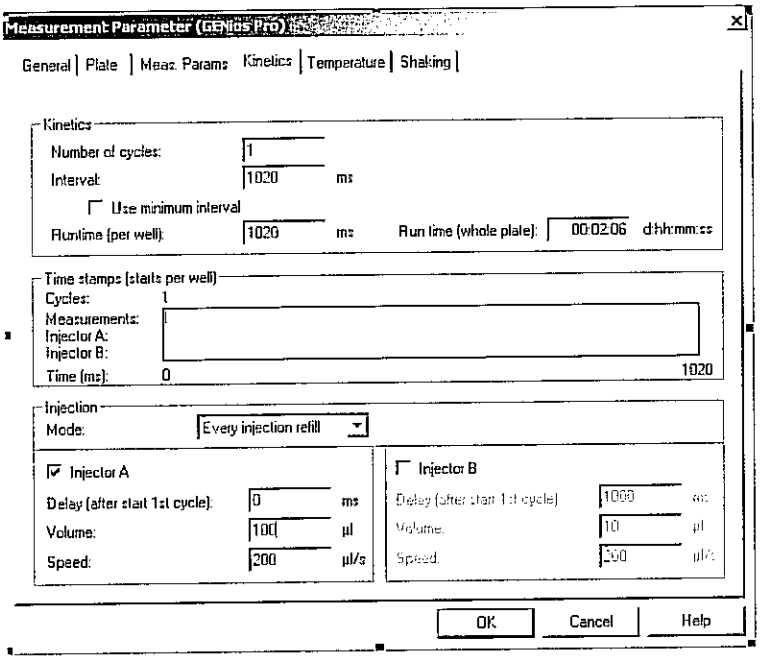
General tab: Select **Luminescence** and **Well kinetic** to enable injectors for luminescence reading

Plate tab: Select white, flat-bottom 96-well plate



Meas. Params tab: Select the following settings:

- Integration time: **luminescence, 1000ms**
- Attenuation: **none**
- Read: **Time between move and integration, 0 ms**



Kinetics tab: select the following settings:

- number of cycles: **1**
- interval: **1020 ms**
- Injection Mode: **Every injection refill**
- **Injector A** for Reagent 1:

  Delay: **0 ms**

  Volume: **100 µl**

  Speed: **200µl/sec**

Click **OK** to close Measurement Parameter dialog box for label 1.

Measurement for Label 2:

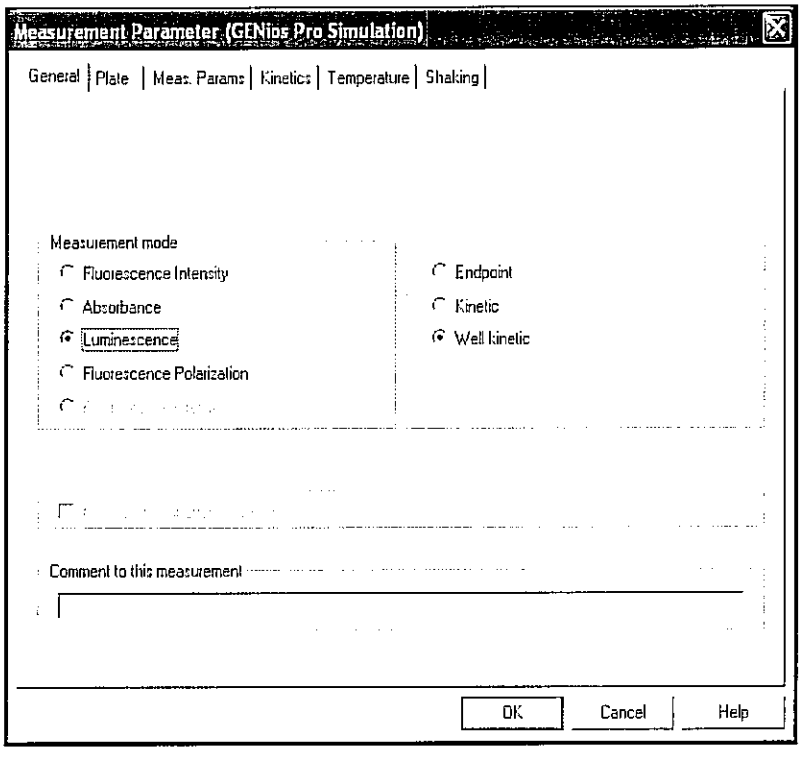
Step 2 of assay procedure:

5 min incubation time followed by endpoint read (result A).

Select **Do Measurement 2.**

Select Pause (incubation step) of 5 min (= 300 sec.)

Click **Edit measurement parameter** for measurement 2 and the Measurement Parameter dialog box appears:



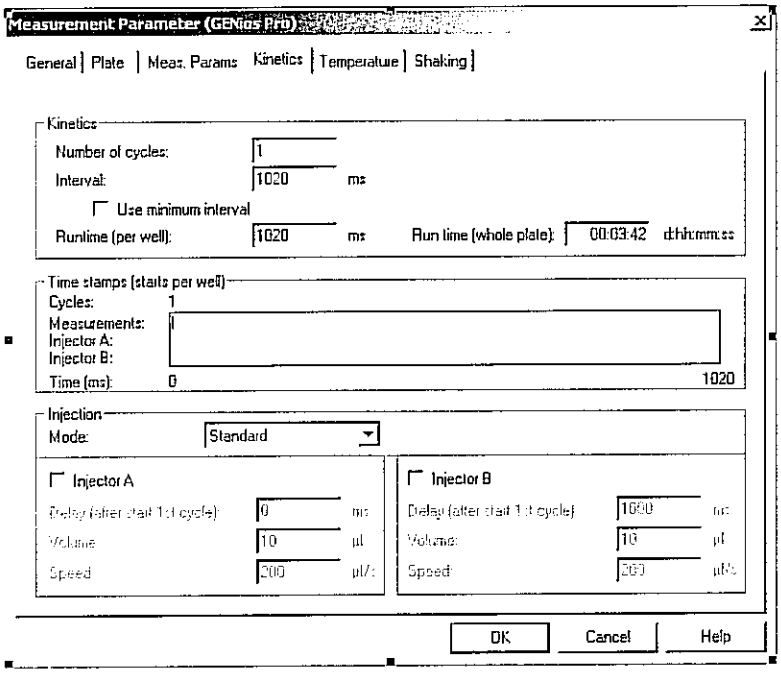
General tab: Select **Luminescence** and **Well kinetic**

Measurement Parameters tab: select the following:

- Luminescence integration time (offset): 1000ms
- Attenuation: none
- Time between move and integration: 1000 ms to compensate offset between label 1 (dispense and read) and label 2 (read only).

Comment:

In this example, offset is caused by the time needed to refill the syringe.



Kinetics tab: select the following settings:

- number of cycles: 1
- interval: 1020 ms
- inject. mode: default setting
- Injectors: not activated as read step only

Click **OK** to close Measurement Parameter dialog box for label 2.

Measurement for Label 3:

Step 3 of assay procedure:

Dispense 100µl of reagent 2 into all wells of 96 well plate

Select **Do Measurement 3**.

Click **Edit measurement parameter** for measurement 3 and the Measurement Parameter dialog box appears.

General tab: select again Luminescence and well kinetic

Measurement Parameters tab: use same settings as for label 1:

- Integration time: 1000 ms
- Attenuation: none
- Time between move and integration: 0 ms

Kinetics tab:

- number of cycles: 1
- interval: 1020 ms
- inject. mode: every inject. refill
- **Injector B for Reagent 2:**

  delay: 0 ms

  volume: 100 µl

  speed: 200µl/sec

Click **OK** to close Measurement Parameter dialog box for label 3.

Measurement for Label 4:

Step 4 of assay procedure:

Incubate 10min and perform endpoint reading (result B)

Select **Do Measurement 4**.

Select Pause (incubation step) of 10 min (= 600 sec.)

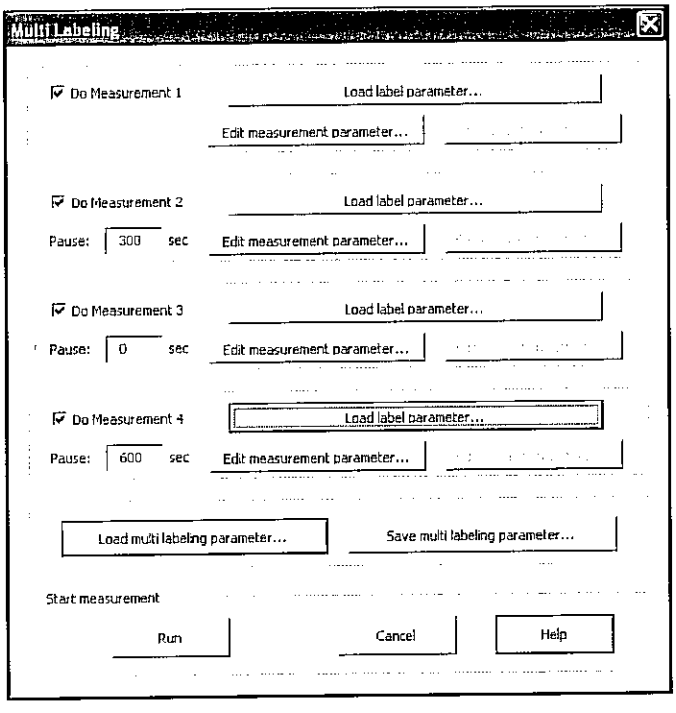
Click **Edit measurement parameter** for measurement 4 and the Measurement Parameter dialog box appears.

General tab: select again Luminescence and well kinetic

Measurement Parameters tab: use same settings as for label 2:

- Integration time: 1000 ms
- Attenuation: none
- Time between move and integration: 1000 ms

Click **OK** to close Measurement Parameter dialog box for label 4.



Click **Run** to start the multilabeling measurement.

Step 5 of assay procedure:

Calculate result (ratio B / A) in XFluor:

- Measurement data will be shown in „matrix-format" for each of the four labels
- Use data for label 2 (result A) and data for label 4 (result B) to calculate ratio

4. Optical System

4.1 Fluorescence System

The GENios Pro fluorescence optical system is sketched below. Paths of light are indicated by arrows. The system consists of the Light Source System (1), the Fluorescence Optics (2), and the Fluorescence Detection (3).

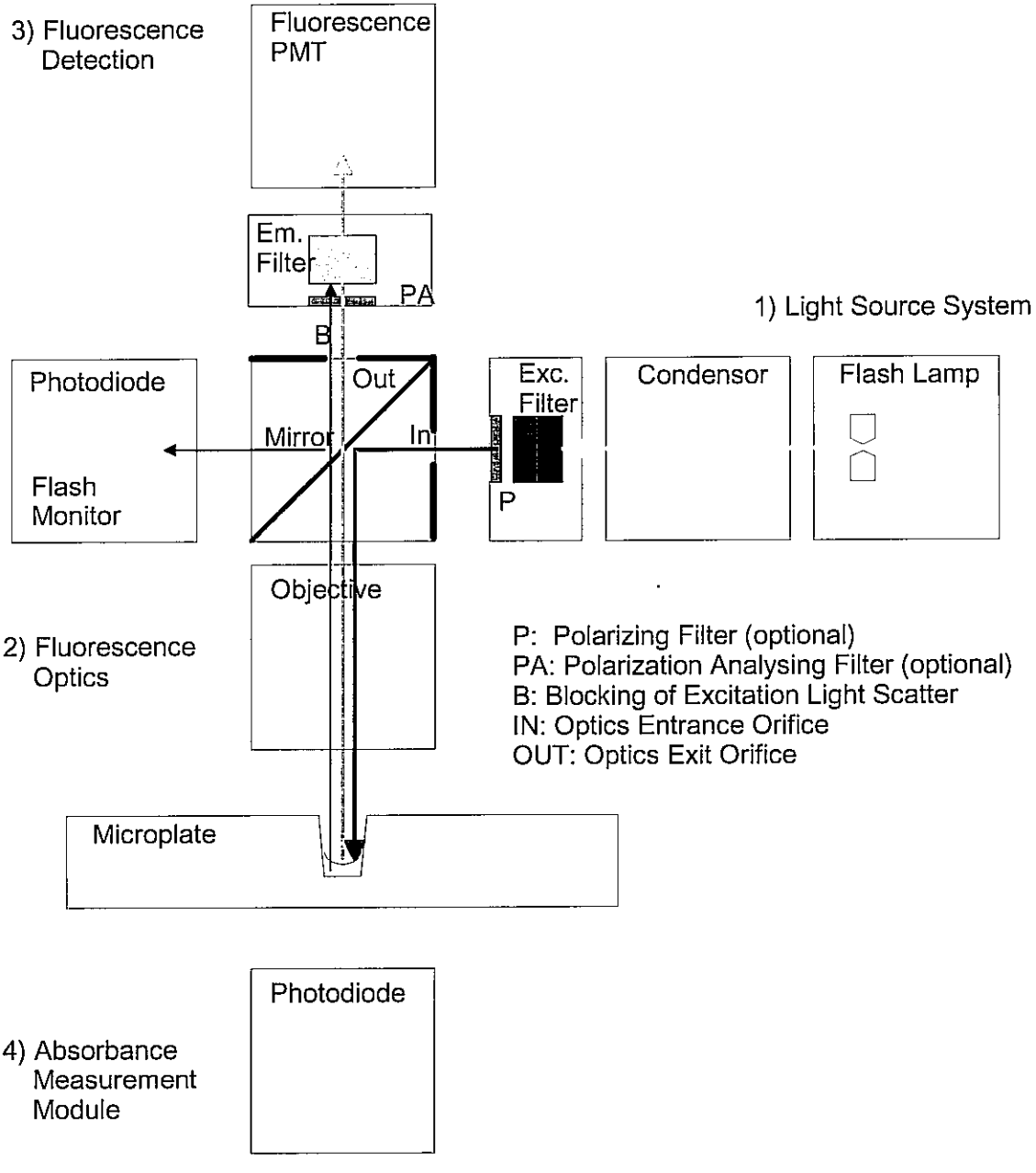


Figure 4-1 Optical System

4.2 Light Source System

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

The GENios Pro light source system consists of the following components.

1. Flash Lamp
2. Condensing Optics
3. Excitation (or Absorbance) Filter Slide
4. Polarizing Filter (Optional)
5. Flash Lamp Monitor

Flash Lamp

The GENios Pro 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 GENios Pro 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

a) High intensity from the deep UV to the near IR

b) Very long lifetime

c) Many applications - only one kind of lamp

d) No warm up time required

Condensor

Condensor type optics focus the light through the entrance orifice ("IN", see *Figure 4-1*, page 4-1) to the fluorescence optical system.

Band pass Filter

In both fluorescence and absorbance applications, optical filters of band pass type are necessary to select the useful wavelengths from the flash lamp spectrum. Filters are mounted in removable slides (see 6.1.1 Configuration of Filter Slides).

Absorbance Filter

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

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.

Polarizing Filter (P)

For fluorescence polarization applications some positions of the filter slides can be additionally equipped with polarizing filters (see 6.1.1 Configuration of Filter Slides). The polarizing filter on the excitation filter slide (P) passes light of a specific plane of polarization, whatever the wavelength between 275 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 and Absorbance measurement results are compensated correspondingly.

4.2.1 Fluorescence Optics

Flash Light enters the optical system being focused through an orifice **IN**. This opening acts as a color specific light source, optionally polarized ("P"). By default, a semi-transparent mirror 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 orifice ("OUT") for detection.

Mirror Selection

The mirror carrier houses the 50% type mirror as well as different dichroic mirrors (see 6.1.2 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 8. Defining Filter Slides and Custom Dichroic Mirror.

Objective Lens System

The objective is designed to collect as much of the fluorescent light from a well and focus it through the exit orifice ("OUT") to the detection system. Due to the confocal like arrangement of the orifices, the background due to detection of scattered excitation light is efficiently reduced.

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

4.2.2 Fluorescence Detection

Emission Filter

The emission filter discriminates scatter of excitation light ("B") and unspecific fluorescence. The emission filter is part of a filter set containing excitation filter, emission filter, and optionally a dichroic. Some general criteria for the selection of a suitable combination can be found in 5.2.5 Measurement Accessories.

Polarization Analyzing Filter (PA)

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 6.1.1 Configuration of Filter Slides). They pass light of a specific plane of polarization, whatever the wavelength between 275 and 750 nm.

PMT Detector

A photomultiplier tube (PMT) is used for the detection of such low light levels as involved with fluorescence. The GENios Pro's dedicated fluorescence PMT is sensitive up to the near infrared (NIR) while still having low dark current. Electronic circuitry uses analog to digital conversion of PMT output current. Adjusting the PMT gain allows a wide range of concentrations in lower or higher concentration domains to be measured. For details see 5.4 Optimize Luminescence Measurements.

4.3 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, compare 3.2.2 Absorbance).

4.3.1 Absorbance Optics

The mirror carriage has an absorbance position. A pair of small orifices forms a narrow and more collimated light beam when compared with fluorescence excitation.

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.

4.3.2 Absorbance Detection

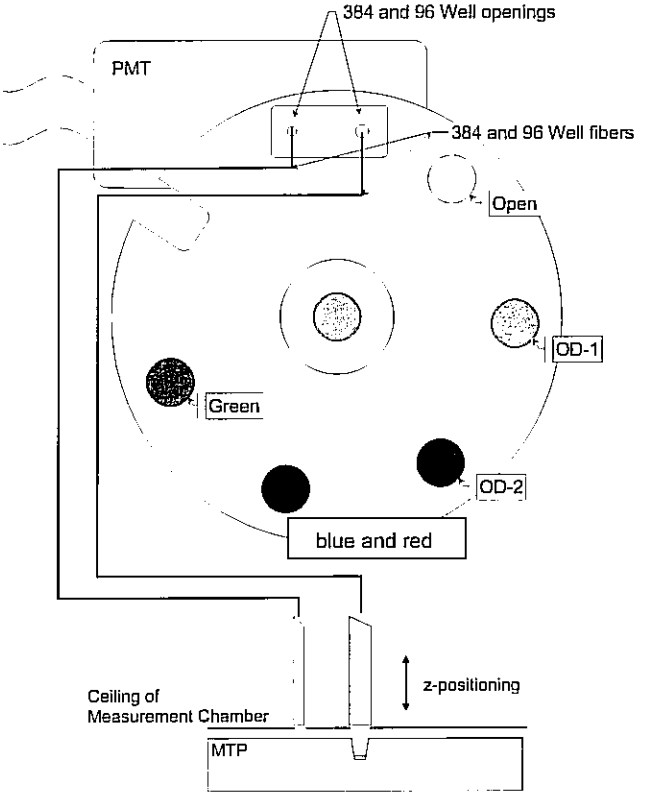
A silicon photodiode is used for the measurement of the light beam. It is sensitive to a wide range of wavelengths. The photodiode is well suited for the light levels being encountered with absorbance measurements below 4 O.D.

4.4 Luminescence System (Optional)

For uncompromising performance, the GENios Pro luminescence detection system is a unit separate from the fluorescence system. The luminescence optics are designed to meet requirements that are different from those of the fluorescence optics. The much lower light levels involved, when compared to flash lamp induced fluorescence, require the advantages of using a photon counting detection technique.

4.4.1 Luminescence Optics

In luminescence measurement mode, GENios Pro uses fixed microplate position and a moveable luminescence measurement head (see figure below). The GENios Pro is aware of the actual plate thickness once the user has selected the corresponding plate type in the software dialog (see the software manual).



In the ceiling of the measurement chamber there are two orifices of different diameter. Depending on the well diameter of the selected microplate, luminescence light will be automatically measured through the most appropriate channel:

1) The orifices are designed to receive as much light as possible from wells of either 96, or 384, well plates, respectively. Thus, luminescence signal is maximized.

2) A particular orifice does not receive substantial amounts of light from neighboring wells. Thereby, cross talk is minimized.

Fiber light guides pass the luminescence light to the luminescence photo multiplier (PMT). A selector wheel 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 selector wheel can also switch neutral density filters across the selected fiber exit.

4.4.2 Luminescence Detection

The GENios Pro 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 (such as Greiner) for luminescence measurements . For details see 5.4 Optimize Luminescence Measurements

5. Operating the GENios Pro

5.1 Introduction

The GENios Pro is operated under personal computer based software control. *XFluor* or Magellan software may be used as the user interface. For details see the corresponding software manual. This chapter is for a general understanding of instrument parameters and operation. Suggestions are made how to optimize instrument parameters for your applications.

Every effort has been made 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 including lid is 23,3 mm.

Caution

Before starting measurements, make sure that the microplate position A1 is inserted correctly.

5.2 General Operating Features

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

5.2.1 Instrument Start Up

Before the instrument is powered ON, check if the serial interface cable is connected.

Caution

When the serial interface cable is being plugged or unplugged, the instrument and the PC should be powered off.

Instrument Power On

When switching the instrument ON, the following actions are induced:

- The inserted filter slides move into a reference position and are identified The mirror carrier moves into a reference position
- The microplate transport moves into a reference position
- The injector carrier is unlocked (if option is installed)
- The cooling fans start to ventilate
- The light source of the GENios Pro does not need a warm up time. But if exact operating temperature is an issue, we recommend to let the instrument warm up for 2 minutes before performing a measurement (see also Temperature Control, page 5-2).

Connect to Instrument

When the software connects to the instrument, communication is established between the instrument and the user interface. 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. Compare the figures in chapter 2. Installation. To define a new filter slide, please refer to chapter 8. Defining Filter Slides and Custom Dichroic Mirror.

5.2.2 Finish a Measurement Session

Disconnect from Instrument

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

Note:
Remove the microplate before disconnect.

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.

5.2.3 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 GENios Pro can set up a specific temperature within some 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

XFluor 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 in the corner evaporate faster, the next at the edges of the microplate. When measuring fluorescence, decrease in signal may also result from photo bleaching.

Microplate Shaking

The GENios Pro is capable of plate shaking before start of a measurement or in between kinetic cycles.

Multi Labeling

XFluor provides a basic Multi Labeling capability. Up to four sets of instrument parameters can be edited. The corresponding plate measurements will be executed in order. For example, when using more than one fluorescent label, different filter combinations could be selected.

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.

5.2.4 Instrument Parameters

Gain Settings

The GENios Pro fluorescence detection system uses analog to digital (A/D convert) conversion of PMT signal. The gain setting controls the amplification of the PMT when converting fluorescence light into electrical current. The A/D converter needs a suitable input range of PMT current to provide a proper signal to noise ratio (S/N) on the one hand, and linearity on the other hand. Therefore, the gain should be tuned to make highest concentration microplate wells give highest possible readings. Then, readings of lower concentration microplate wells separate from background - as far as the background noise level allows for that.

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).

Extended Dynamic Range

The Extended Dynamic Range procedure is an automatic gain option. When the range of concentrations exceeds three orders of magnitude, it may be useful to measure the assay at both a high and a low gain. Wells assigned OVER from the high gain measurement in many cases can be extrapolated from the low gain measurement. For details see the software manual.

Flash Settings

On the fly measurements with 1 flash per well are possible for all plate types.

However, measurement precision at low light levels depends on the reading time during which fluorescence signal can be received.

Note:
Increase the number of flashes 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 reduce the Lag Time to maximize **Signal to Background (S/B)**.

3) Fine tuning: With Lag Time being fixed extend 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.

Comparing S/B at different timing parameters is valid if the gain is fixed. For dual TRF labels, establish the procedure for the label with the shorter fluorescence lifetime (label 1). Compromise the Integration Time of label 1 with the Lag Time of label 2.

Time between Move and Flash

When selecting more than one flash per well, a time delay between move and flash 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.

5.2.5 Measurement Accessories

Recommended Filters

Please consult your local Tecan dealer for a recommended filter set for the dye you are using. Filters designed for a different type of instrument may not necessarily perform well with the GENios Pro.

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 needs 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 and luminescence measurements labels white microplates seem superior. You may check if white plates are superior with UV excitation wavelengths.

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

Make sure when using plates with well strips that all strips are inserted during the use of the plate to avoid the malfunction of the plate detection sensor.

5.3 Optimize Absorbance Measurements

Flash Settings

On the fly measurements with 1 flash 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 per well until noise of BLANK wells does not further improve, or until measurement time per well becomes unacceptable.

Time between Move and Flash

When selecting more than one flash per well, a time delay between move and flash 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.

5.4 Optimize Luminescence Measurements

5.4.1 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. It rather 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 to discriminate electronic noise.

At very low light levels the measured counts per second are proportional to the light intensity (see Figure 5-1). 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.

5.4.2 Linearization (Automatic Calibration)

At intermediate luminescence light levels the measured count rate deviates from a perfect linear response curve (see figure below). In this domain, PMT output pulses have fused when they originated from two photons striking the photocathode within too short time. The electronic counter registers less photons than what is actually impacted.

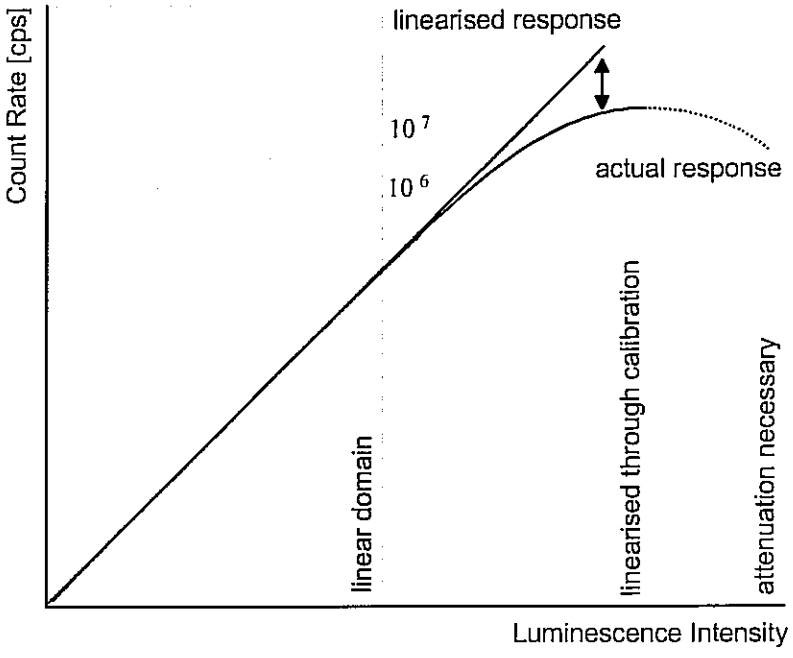


Figure 5-1

The nonlinearity of the photon counting technique results from the time resolution of the PMT. By PMT construction it cannot be reduced without compromising the sensitivity. The GENios Pro detection system will be automatically calibrated to compensate for this effect.

Calibration of the photon counting system is performed by determination of the particular PMT time resolution. For this purpose, a built-in LED is used to illuminate a silicon photodiode in its linear response domain, while a small fraction of the light is measured simultaneously by the PMT. Comparing both signals at different LED light levels yields the desired factor that is used to correct the actually measured luminescence count rates.

5.4.3 Light Level Attenuation

Optical attenuation of higher luminescence light levels is necessary when using photon counting detection. 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.

The GENios Pro hardware offers to attenuate light levels by a fixed factor of either 1 (none), 10 (1 OD), or 100 (2 OD). Correspondingly, the usable measurement range will be shifted to higher light levels.

If much different light levels are expected for the same assay, a dynamical choice of attenuation depending on a test measurement is useful ("automatic"). This dynamical measurement mode will extend rather than shift the measurement range.

6. Instrument Features

6.1 Introduction

The following types of measurement are provided with the GENios Pro microplate reader.

Measurement Type	
Fluorescence Intensity	(see 6.2 Fluorescence Intensity and Time Resolved (TRF))
Time Resolved Fluorescence	(see 6.2 Fluorescence Intensity and Time Resolved (TRF))
Fluorescence Polarization	(see 6.3 Fluorescence Polarization)
Absorbance	(see 6.4 Absorbance)
Glow Type Luminescence	(see 6.5 Glow Type Luminescence)
Dual Color Luminescence	(see 6.6.1 Dual Color Luminescence (BRET2TM)
Injector options for Fluorescence, Luminescence and Absorbance	(see 3.3 Injectors (Optional))

- All standard microplates from 6 to 384 wells may be measured in any of the above measurement types.
- Injection is possible for all standard microplates from 6 to 384 wells in any of the above measurement types.
- The instrument allows for kinetic measurements.
- Reading may be restricted to one part of the microplate.
- Up to 4 measurement parameters sets can be queued using the *XFLUOR*.

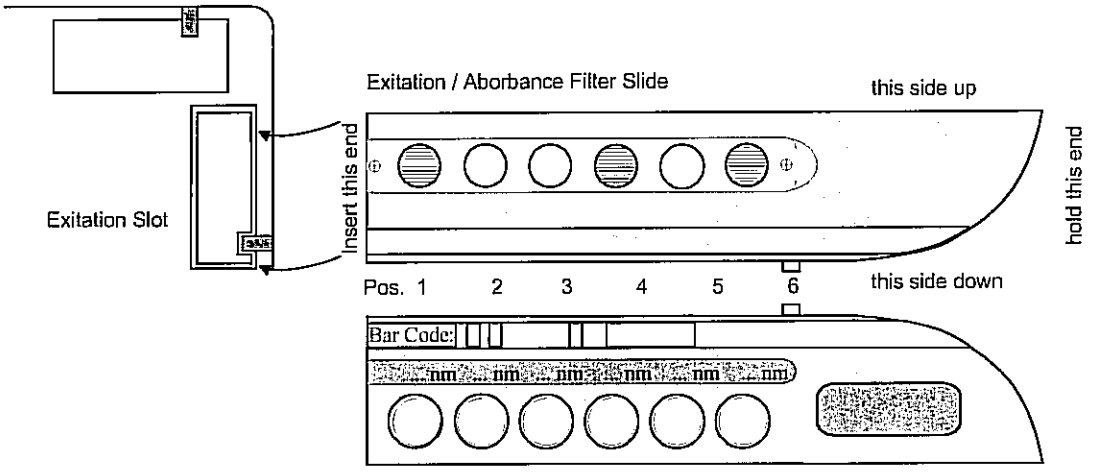
The table below lists the technical specifications for the instrument.

Parameters	Characteristics
Measurement	Software controlled
Interface	Serial interface: RS 232
Filter handling	*External filter exchange* see 6.1.1 Configuration of Filter Slides
Microplates to be measured	From 6 well to 384 well plates
Plate definition	Via scanning software
Temperature control	From 4° C above ambient up to 42°C
Plate shaking	Linear shaking

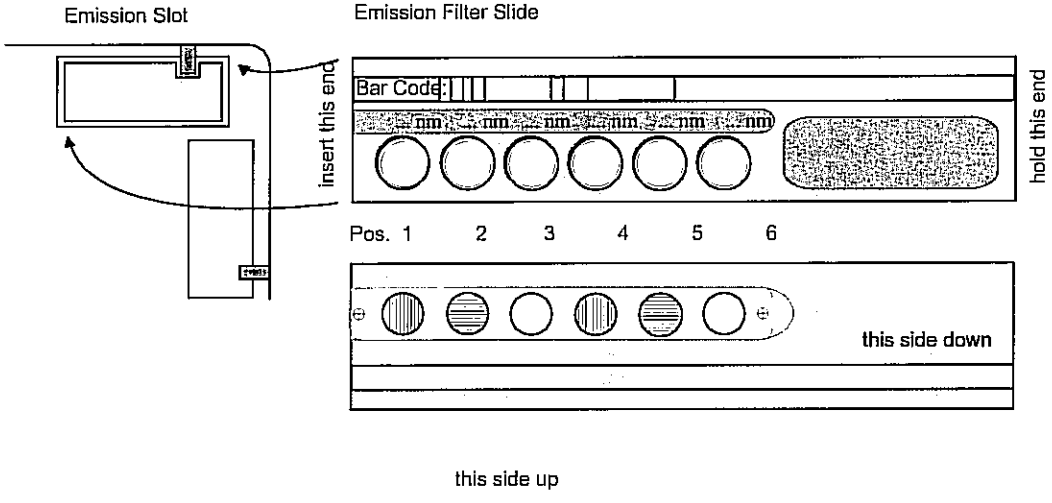
Parameters	Characteristics	
Light source	High energy Xenon flash lamp	
Optics	Fused Silica Lenses	
Fluorescence Detector	Low dark current photomultiplier tube	
Luminescence Detector	Low dark count photomultiplier tube	
Absorbance Detector	Silicon photodiode	
Power supply	Auto-sensing:100-120V / 220-240V 50/60 Hz	
Power consumption	250 VA	
Main fuse	2x T 6,3 A/ 250 V	
Physical		
Outer dimensions	Width: 514mm	20,24 inches
	Height: 280 mm	11,02 inches
	Depth: 545mm	21,46 inches
Weight	38,5 kg	
Environmental		
Ambient temperature		
Operation	15°C - 30°C	59°F - 86°F
Non-operation	-20°C - +60°C	-4°F - +140°F
Relative humidity		
Operation	90% non-condensing	
Overvoltage category	II	
Pollution degree	2	
Usage	Commercial	
Noise level	< 60 dBA	
Method of disposal	Electronic waste (infectious waste)	

6.1.1 Configuration of Filter Slides

General



Fluorescence excitation and emission filters, absorbance filters, and polarizing filters are mounted in removable filter slides.



Up to 6 fluorescence excitation and absorbance filters can be mounted in an excitation filter slide. Up to 6 fluorescence emission filters can be mounted in an emission filter slide.

Up to 8 excitation and up to 8 emission filter slides can be defined. When defined, they are automatically identified by the GENios Pro reading the bar code.

Caution

Fluorescence and absorbance filters should only be replaced by a service engineer.

Specific positions on the filter slides can be additionally equipped with polarizing filters. Polarizing filters are put onto the slide. Polarizing filters can be reversibly removed. When mounted that position is reserved for a fluorescence polarization application.

Note:
For a fluorescence polarization application, one excitation filter, but two identical emission filters are needed. The polarization filters in the emission slide are perpendicular and parallel respectively.

For the customization of filter slides please consult your local Tecan dealer.

Filter Slides (Type A)

Excitation Slide A

Pos.	Center Wavelength[1] [nm]	Bandwidth[2] [nm]	Purpose
1	485*	20	fluorescence polarized
2	485	20	fluorescence
3	492	10	absorbance
4	340	35	fluorescence
5	612	10	fluorescence
6	535	25	fluorescence

Emission Slide A

Pos.	Center Wavelength [nm]	Bandwidth [nm]	Purpose
1	535*	25	fluor. polarized perpendicular
2	535*	25	fluor. polarized parallel
3	535	25	fluorescence
4	612	10	fluorescence
5	670	25	fluorescence
6	590	20	fluorescence

**Part of the FP option for GENios Pro.*

[1] Center wavelength for filters is accurate within a 5nm interval

[2] Bandwidth for filters may have some tolerance 10%

6.1.2 Configuration of the Mirror carrier

Along the mirror carrier, different types of (dichroic) mirrors are mounted. These mirrors are permanently mounted.

If necessary, the mirror carrier can be extended with a custom type dichroic.

Fluorescence Mirror	Reflection (Excitation)	Transmission (Emission)
50% Mirror	230 – 800 nm	230 – 800 nm
Dichroic 1 (e.g. fluor.)	320 – 500 nm	520 – 800 nm
Dichroic 2 (e.g. Cy5)	560 – 625 nm	635 – 850 nm
Dichroic 3 (exchangeable)	500 – 550 nm	570 – 850 nm

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

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

6.2 Fluorescence Intensity and Time Resolved (TRF)

Parameters	Characteristics
Wavelength Range	230 - 900 nm
Standard filters	see 6.1.1 Configuration of Filter Slides

Gain setting	Values	Measurement range
Manual	1 - 255	0 - 60,000 RFU
Optimal	automatic	0 - 60,000 RFU
Extended Dynamic Range	automatic	0 - 6,000,000 RFU
Calculated from Well	automatic	0 - 60,000 RFU

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

6.2.1 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 (fast mode). Using more flashes per well may improve the detection limit, but the total measurement time will be longer.

6.2.2 Fluorescein (fluorescence intensity) Top

Plate type (number of wells)	96	384
Dispensed Volume [µl]	200	100
Flashes per Well	10	10
Measurement Time per Plate [min:sec]	1:05	3:00
Fluorescein Detection Limit [pM]	2 pM	5 pM

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

6.2.3 Fluorescein (fluorescence intensity) Bottom

Plate type (number of wells)	96	384
Dispensed Volume [µl]	200	100
Flashes per Well	10	10
Measurement Time per Plate [min:sec]	1:05	3:00
Fluorescein Detection Limit [pM]	25 pM	25 pM

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

6.2.4 Europium (time resolved fluorescence)

Plate type (number of wells)	96	384
Dispensed Volume[µl]	200	100
Flashes per Well	10	10
Measurement Time per Plate [min:sec]	1:05	3:00
Europium Detection Limit [fM]	100 fM	100 fM

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

HTRF®

The measurement is set up using the 'multilabeling' function of Tecan XFluor 4 software. Excitation and emission filters are available as parts of the Tecan HTRF® upgrade kit (B122175) and must be defined in RdrOLE4 according to the kit description.

Measurement 1	
Ex Filter	320 nm
Em Filter	620 nm
Mirror	Dichroic2 (eg FI 96)
Lag time	150 µs
Integration time	500 µs
Number of flashes	10
Optimal gain	
Optimal z-pos	

Measurement 2	
Ex Filter	320 nm
Em Filter	665 nm
Mirror	Dichroic2 (eg FI 96)
Lag time	150 µs
Integration time	500 µs
Number of flashes	10
Optimal gain	

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

6.3 Fluorescence Polarization

Parameters	Characteristics
Wavelength Range	275 - 750 nm
Standard Filters	485 excitation, 535 emission (compare 6.1.1 Configuration of Filter Slides)
Gain setting	see 6.2 Fluorescence Intensity and Time Resolved (TRF)

6.3.1 Fluorescein 1nM (fluorescence polarization)

Plate type (number of wells)	96	384
Dispensed Volume[µl]	200	100
Flashes per Well	10	10
Measurement Time per Plate [min:sec]	2:10	5:45
Fluorescein 1nM Precision [mP]	4mP	5mP

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

6.4 Absorbance

The specifications below only apply to 96 well plates, 10 flashes per well.

Parameters	Characteristics		
Wavelength range	230 - 999 nm		
Meas. range	0 - 4 OD		
Resolution	0.0001 OD		
Accuracy	+/-1% and +/- 0,010 OD	0 - 2.0 OD	492 nm
Precision	+/- 0,5 %and +/-0,005OD	0 - 2.0 OD	492 nm
Standard filters	492 nm (compare 6.1.1)		

6.5 Glow Type Luminescence

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength range	400 - 700 nm
Lin. Dynamic Range	8 decades
Cross Talk (96 well)	< 0.01%
Integration Time / well	1 – 30000 ms
Attenuation of Light	100 (2OD), 10 (1OD), 1 (No attenuation)

6.5.1 ATP Glow Luminescence

Plate type (number of wells)	96	384
Total Dispensed Volume[µl]	200	100
Integration Time / well [ms]	1000	1000
Measurement Time per Plate [min:sec]	2:30	8:00
ATP Detection Limit [fM]	1 fM	1 fM

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

6.6 Flash Type Luminescence

Plate type (number of wells)	96	384
Total Dispensed Volume [µl]	200	100
Integration Time / well [ms]	1000	1000

6.6.1 Dual Color Luminescence (BRET^{2TM})

Note:
All specifications are subject to change without prior notification.

Parameters	Characteristics
Built-in Wavelength:	475 / 550 nm
Integration time:	1- 30000 ms. Different integration times are possible for each wavelength.
Plate type:	96 and 384 well microplates
Dynamic range	6 decades

6.7 Injector Specifications

Dispense Volumes	100µl	450µl
Accuracy	0.8%	0.3%
Precision	0.6%	0.15%

6.8 "On the Fly" Measurements

"On the Fly" measurements are the fastest measurements possible using the GENios Pro. These measurements are performed using only one flash.

- **96-well plates (FI, TRF, Absorbance) Measurement time: 35 s**
- **96-well plates (FP) Measurement time: 70 s**
- **384-well plates (FI, TRF, Absorbance) Measurement time: 55 s**
- **384-well plates (FP) Measurement time: 85 s**

7. Quality Control

7.1 Periodic Quality Control Tests

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

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

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

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

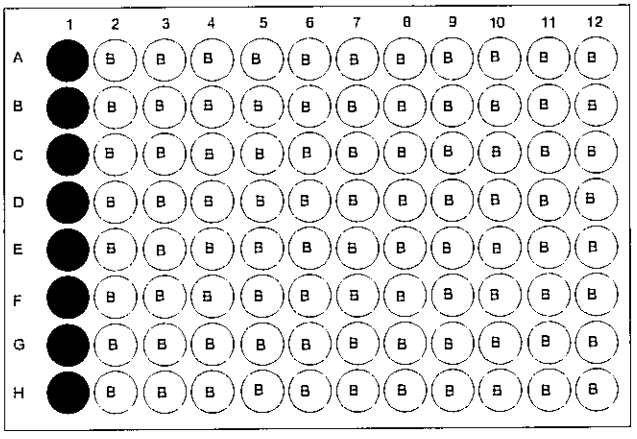
Caution

Before starting measurements, make sure that the microplate position A1 is inserted correctly.

7.2 Fluorescence

7.2.1 Sensitivity Test

- Use a solid black 96-well plate, for example: Greiner flat bottom, black microplate, (no strip plates).
- Apply 200 µl of 1 nM Fluorescein (Sigma-Aldrich, F-6377) in 0.01 M NaOH solution to wells A1 to H1.
- All other wells are blank (**B**), for example: 200 µl 0.01 M NaOH solution without fluorescein.
- Use 30 flashes with gain adjusted to a well of column 1.



The procedure to calculate the sensitivity (MDL = minimum detection limit) is as follows:

Divide the standard deviation of the blanks by the blanked average RFU (Relative Fluorescence Units) of column 1.

Multiply with a factor 3 to achieve statistical reliability.

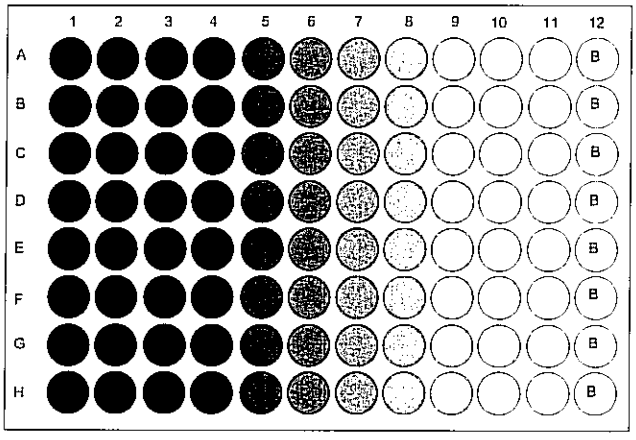
Arrive at physical units (pM) by multiplication with the concentration (1000 pM). To obtain the unit (pg/well) multiply with the volume per well (0,002 l) and the molecular weight (376.3 g/mol).

The sensitivity is expected to be 2 pM or 0.15 pg/well fluorescein per well.

$$MDL\,(pM) = \frac{3 * sdev\,(\text{blank})}{average\ RFU\ (\text{sample}) - average\ RFU\ (\text{blank})} * [\text{Fluorescein (pM)}]$$

$$MDL\,(pg/well) = \frac{3 * sdev\,(\text{blank})}{averageRFU\ (\text{sample}) - averageRFU\ (\text{blank})} * [\text{Fluorescein (pM)}] * Mw\,(\frac{g}{mol}) * Vol\,(l)$$

7.2.2 Linearity Test



- Use a black 96-well plate, for example: a Greiner flat bottom, black microplate.
- Prepare a 1:1 dilution series of fluorescein in 0.01 M NaOH solution column-wise in replicates of 8.
- Highest concentration = 1 µM fluorescein, max. volume per well = 200 µl.
- Provide column 12 as blank (**B**), that is fill with pure NaOH solution.
- Use 10 flashes, adjust gain to a well of column 1.

Calculate the blanked average RFU for each of the columns 1 to 11. Compute the coefficient of correlation (r^2) using the blanked average RFUs and the corresponding concentrations.

$$r^2 = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x2 - (\sum x)2][n\sum y2 - (\sum y)2]}}$$

The coefficient of correlation is expected to be ≥ 0.995.

7.2.3 Precision Test

- Use the same plate as mentioned in **7.2.2**.
- Perform 10 measurements with number of flashes set to 10.

Compute the CV (Coefficient of Variation) value of each well in column 4.

The CV value should be <=2.5%.

$$CV\,(\%) = \frac{sdev\,(\text{sample})}{average\,(\text{sample})} * 100$$

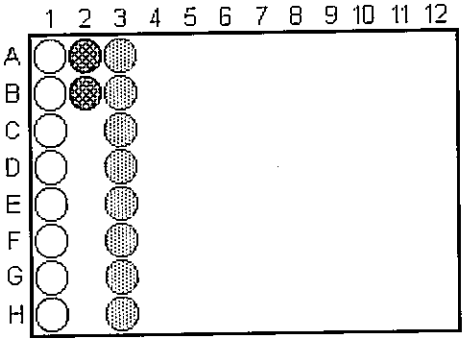
7.3 Fluorescence Polarization

The reagents used in this test are available from PanVera Corporation:

FP Standardization Kit	PanVera Part # P2581
BGG/Phosphate Buffer	PanVera Part # P2580
High Polarization Standard	PanVera Part # P2579
Low Polarization Standard	PanVera Part # P2578

The test is performed in a 96-well black microplate with wells filled to 200µl. Tecan's specifications are established using a Greiner flat-bottom black 96-well plate. The pipetting procedure is as follows:

- The first column wells (A1-H1) are filled with PanVera's BGG/Phosphate Buffer.
- Wells A2 and B2 in the 2nd column are filled with PanVera's High Polarization Standard.
- The third column wells (A3 to H3) are filled with PanVera's Low Polarization Standard.



KEY to Plate Layout:

- BGG/Phosphate Buffer (PanVera)
- High Polarization Standard (PanVera)
- Low Polarization Standard (PanVera)
- Empty wells (unused)

- Use 10 flashes with optimal gain adjusted to a well of column 3.
- The first column (wells A1 to H1) has to be defined as buffer, the third column (wells A3 to H3) as reference with 20mP (at room temperature).

Calculate the average mP value for the High Polarization Standard and the standard deviation of the Low Polarization Standard's mP values. The reader is performing correctly, if the observed results are within the limits below.

	Standard Deviation of Low Polarization Standard	Milli-Polarization Value of High Polarization Standard
Limits:	<5 mP	400+/-30 mP

Note:
The FP Standardization Kit from PanVera is now available as Invitrogene Green Polarized Standard Kit .

7.4 Glow Type Luminescence

7.4.1 Sensitivity Test

Material:

ATP Kit SL 144-041, BioThema AB

Greiner 384 well plate, flat bottom, white

200 µl Pipette + tips

Plate Layout:

- 100 µl Blanks in wells A1-D1, A3 – D10
- 20 µl ATP standard 10^{-7} M in wells A2 – D2, 80 µl ATP

	1	2	3	4	5	6	7	8	9	10	11	12
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												
H												

ATP....$2*10^{-8}$ M ATP

B....Blank

Bx...Blank for cross talk measurement

A2 – D $2*10^{-8}$ M ATP

D1, A3 – D10 Blank

100 µl/well

Measurement Parameters

Use the following measurement parameters:

Measurement mode:	Luminescence
Integration time:	1000 ms
Attenuation:	none
Plate definition file:	GRE384fw.pdf
Part of the plate:	A1 – D10

Start the measurement directly after mix of the solutions

Handling

Use only white MTP from Greiner. During the measurement the MTP shall be removed from light .

The ATP Kit consist of ATP Reagent, Diluent C, Tris EDTA puffer and the ATP-Standard with a concentration of 10^{-5} M.

Please make sure that all Reagent are at RT before starting the measurement.

Evaluation:

Calculation of the detection limit in fmol/well:

$$DetectionLimit(fmol/well) = \frac{ConcATP * 3 * Stdev_B}{mean_{ATP} - mean_B} * 0.0001 * \frac{1}{1e^{-15}}$$

Conc ATP concentration of ATP Standard = $2*10^{-8}$ [M]

$Stdev_B$ Standard deviation Blanks (B: A4 – D10)

$mean_{ATP}$ Mean of wells that are filled with ATP

$mean_B$ Mean of the Blanks (B: A4 – D10))

0.0001Conversion into mol/well

$1/1e^{-15}$ Conversion into fmol/well

detection limit 1 fmol ATP/well

8. Defining Filter Slides and Custom Dichroic Mirror

8.1 About Filters

8.1.1 Fluorescence Filters

The optical filters (band pass style) in the EX and EM filter slides 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 standard filter slides.

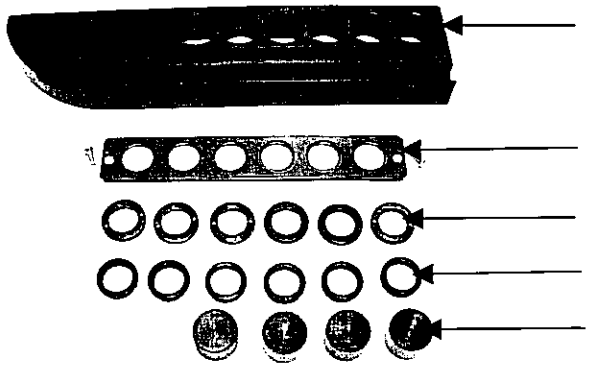
8.1.2 Absorbance Filters

Band pass 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.

8.2 Installing Custom Filters

8.2.1 Mounting the Custom Filter and Polarizers

Excitation Filter Slide :

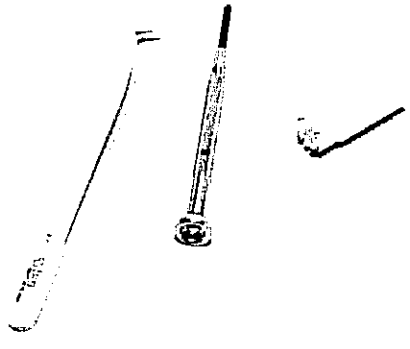


Note:
In order to use the FP option it is necessary to buy the entire option. It is not sufficient to buy only the polarization filters.

Emission Filter Slide:

similar to Excitation filter slide

Toolset (included in accessories case):



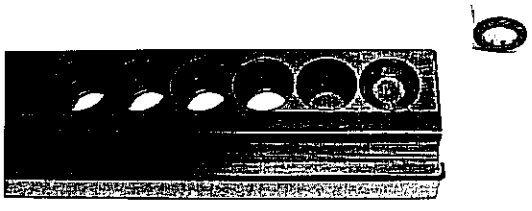
Filter dummy

Caution

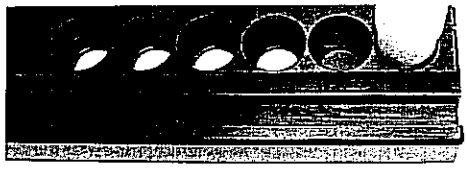
The filters are precision optical components, which should be handled by the edges, and not scratched or stored face down in a drawer and so on. Once the filters are installed in the slide, they are relatively well protected, but care should be exercised when handling or storing them.

In order to install a custom filter do the following:

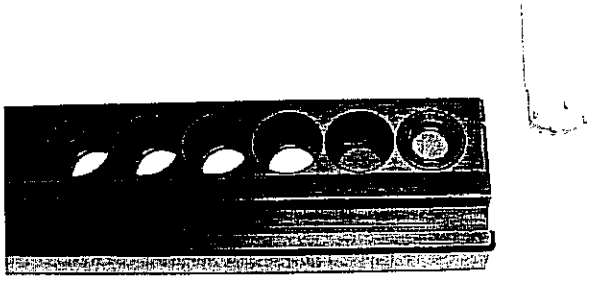
1. Carefully insert the filter into the opening, taking care not to scratch or get fingerprints on the filter.



2. Using the mounting tool, place the O-ring above the filter and fix it. Make sure that it is not screwed too tightly.



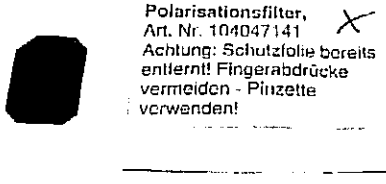
3. Remove the mounting tool.



4. If there are unused openings remaining after filter insertion, filter dummies should be mounted in the holes that are still open.
5. Affix the barcode that came with the filter to the back of the filter slide. This is done by first removing the current barcode if one is present, and then affix the new barcode ensuring that the barcode is in position in the deep groove. The hole of the label must be placed on the centering pin of the filter slide.

8.2.2 Description and Installation of the Polarizers

Each polarizer comes in bag and is ready to be used.

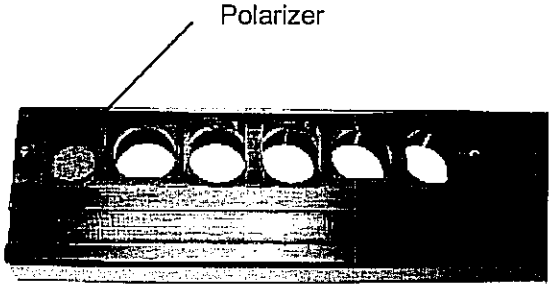


Caution

Fingerprints will influence performance. In order to avoid fingerprints, use the soft plastic tweezers supplied with the toolset.

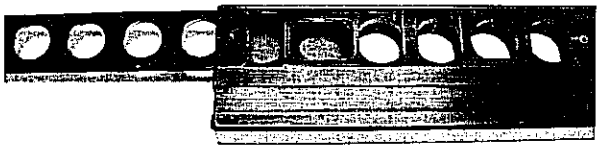
Installation of the Polarizers

1. Put polarizer(s) into the slot using the tweezers.

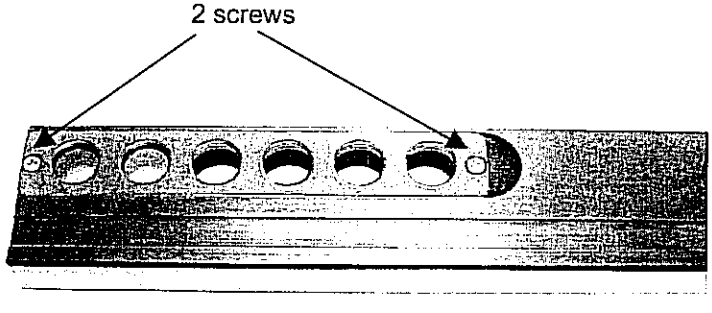


Caution
Take care not to scratch or get fingerprints on the filter.

Note:
For the excitation slide,
the polarizers are defined at positions 1, 4 and 6 in the software.



2. Slide the beveled securing plate into the groove above the polarizers.



3. The securing plate is held in place by two screws.

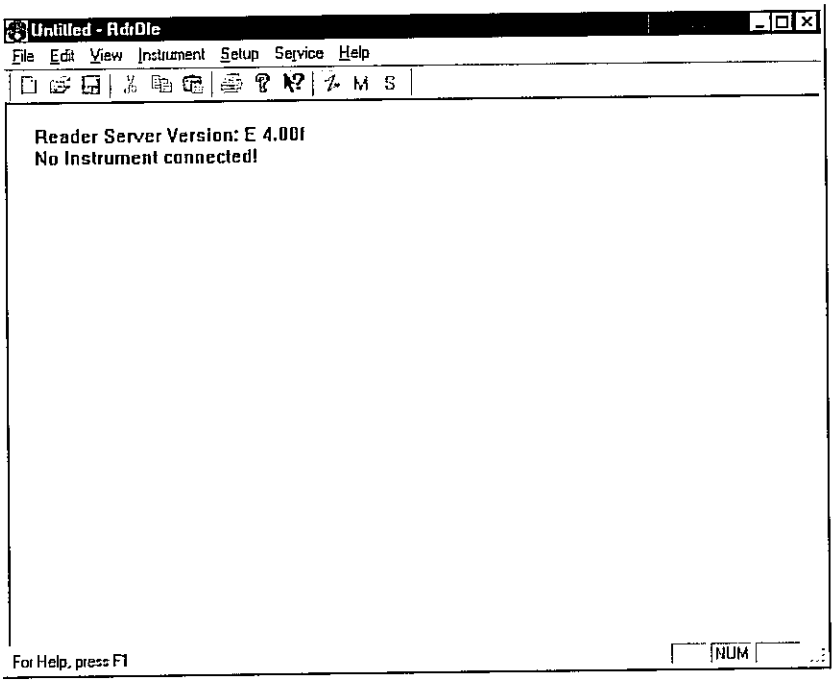
8.2.3 Defining the Filter

Caution

Any changes to the filters in the filter slide are to be carried out by the service engineer! 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.

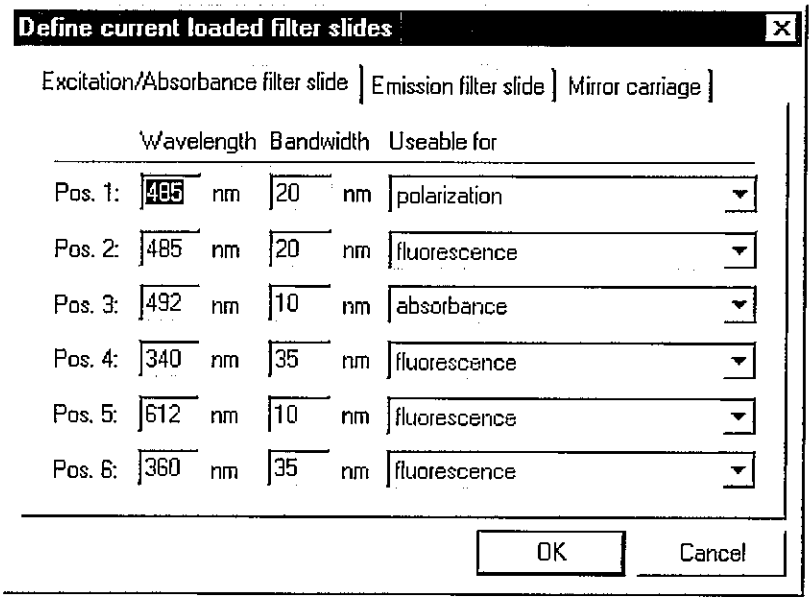
Define a filter as follows:

1. Close XFluor and/or Magellan.
2. Click the *RdrOle* icon in the Tecan group, and the *RdrOle* screen is displayed.



3. Select Connect from the Instrument menu. You are now connected to the GENios Pro.
4. Select Define Filter from the Setup menu.

5. The following dialog box is displayed showing 3 tabs: The first two tabs list the filter values for the excitation / absorbance and the emission filter slide:



6. Enter the new wavelength, bandwidth and measurement type for each new filter. Ensure that the wavelength corresponds with the filter position.
7. Accept the new filter values by clicking **OK**. You are now ready to collect data with the new filters.
8. Exit *RdrOle* by clicking **Exit** in the *File* menu and restart *Magellan*.
9. This completes the installation procedure.

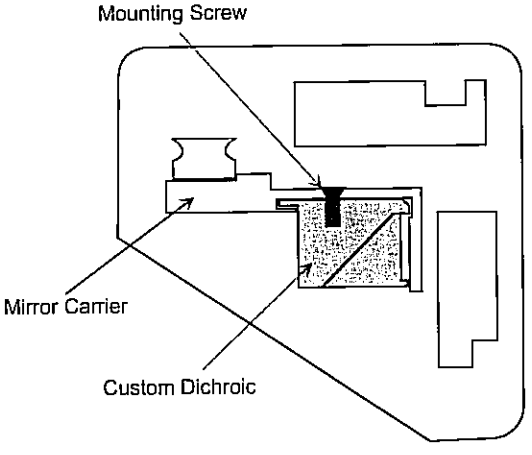
8.3 How Do I Install the Custom Dichroic?

8.3.1 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. Close *XFluor and/or Magellan*.
2. Click the **RdrOLE** icon in the **Tecan** group, and the RdrOLE screen is displayed.
3. Select **Connect** from the **Instrument** menu. You are now connected to the *GENios Pro*.
4. Select **Define Filter** from the **Setup** menu. Manually open the filter compartment flap. Click **Move Out** and the Mirror Carriage will appear
5. 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 Carriage.

6. Carefully release the compartment flap. Click **Move In**, then **OK**. The Mirror Slide goes back into the instrument.

7. You are now ready to define the custom dichroic.

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

8. We recommend that you immediately continue with defining the custom dichroic (next section; step 5). If custom dichroic has already been defined following the instructions in the next section, exit ***RdrOle*** by clicking **Exit** in the **File** menu and restart *XFluor*.

8.3.2 *Defining the Custom Dichroic*

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

Define the custom dichroic as follows:

1. After mounting the custom dichroic, GENios Pro may still be connected within the *RdrOle*. Continue with step 5.
2. Close *XFLUOR*.
3. Click the **RdrOle** icon in the **Tecan** group, and the RdrOle screen is displayed.
4. Select **Connect** from the **Instrument** menu. You are now connected to the *GENios Pro*.
5. Select **Define Filter** from the **Setup** menu.
6. The following dialog box is displayed showing 3 Tabs. Click the third Tab named Mirror Carriage. This Tab shows a table with the spectral characteristics of the defined dichroics. 4 wavelengths define the dichroic reflection and transmission range.
7. Enter the corresponding data provided with the custom dichroic into the row assigned with **User defined**.
8. Decide if you enable **Prefer Custom Dichroic**. This option is to settle a decision in the special case that
   a) both a built in and the custom dichroic match the selected filter wavelengths, and
   b) automatic selection of dichroic (the default) is selected in the software (XFluor).
9. Accept the new dichroic values by clicking OK. You are now ready to collect new data with the new dichroic.
10. Exit ***RdrOle*** by clicking **Exit** in the **File** menu and restart *XFluor*.
11. This completes the installation procedure.

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 (See 9.2).
- Replacing the Fuse (See 9.3).
- Instrument Disinfection (See 9.4).

9.2 Liquid Spills

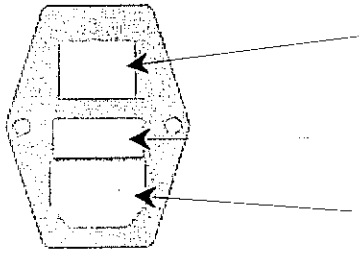
1. Wipe up the spill immediately with absorbent material.
2. Dispose of contaminated material appropriately.
3. Clean the instrument surfaces with a mild detergent.
4. For biohazard spills, clean with a 5-10 % solution of bleach in de-ionized water.
5. Wipe cleaned areas dry.

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 Replacing the Fuse

- Turn the instrument OFF and unplug the mains cable from the instrument. The fuse is located on the right hand side of the mains cable connection in the rear of the instrument.
- Open the fuse compartment by inserting a screwdriver into the slot in the top of the cover and push the fuse holder out.



- Pull the fuse holder out and replace the defective fuse(s) with the spare fuse(s).
- Ensure that the fuse(s) has/have the correct rating.
- **T 6,3A / 250V**

Caution
Risk of Fire
For continued protection replace only with the same type and rating of fuse.

- Replace the fuse holder.
- Reconnect the mains cable.
- Turn the instrument on.

Note:
If the fuse continues to blow, call for service.

9.4 Instrument Disinfection

All parts of the instrument that come into contact with the patient samples, positive control samples or hazardous material must be treated as potentially infectious areas.

Caution

It is very important that the instrument is thoroughly disinfected before it is removed from the laboratory or any servicing is performed on it.

Before the instrument is returned to the distributor for servicing, it must be disinfected and a disinfection certificate completed. If a disinfection certificate is not supplied, the instrument may not be accepted by the servicing center or it may be held by the customs authorities.

9.4.1 Disinfection Solutions

If the laboratory has no specific disinfection procedure, the following procedure should be used to disinfect the instrument.

The instrument should be disinfected using a solution such as:

Lysetol Manufacturer: Schülke and Mayr Ges.m.b.H.

Aseptisol Manufacturer: Bode Chemie Hamburg

If neither of these solutions are available 70% ethanol should be used as an alternative.

Caution

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

Please note that the disinfectant can influence the performance of your instrument if it applied inside the instrument.

9.4.2 Disinfection Procedure

The following procedure should be used to disinfect the outside surfaces of the instrument.

1. Disconnect the instrument from the mains power supply.
2. Disconnect the instrument from any accessories that are used.

   Ensure that you are wearing disposable gloves.
3. Carefully wipe all the outside surfaces of the instrument with a wad of cotton wool, which has been soaked in the disinfecting solution.
4. Ensure that the same disinfection procedure is performed with the plate carrier.
5. Repeat the disinfection procedure on any accessories which are also being moved or returned.
6. After the disinfection procedure has been performed, ensure that the disinfection certificate is completed.

See 9.5 Disinfection Certificate for an example of the disinfection certificate, which must be completed before the instrument is returned to the distributor for servicing.

9.5 Disinfection Certificate

This disinfection certificate must be completed before the instrument is returned to the distributor for servicing.

The certificate must be attached to the top of the outer package in which the instrument is returned.
It must be visible from the outside of the shipping container!

I declare that the instrument in this package has been decontaminated or disinfected to remove or inactivate any biological material, which could be dangerous to the service personnel, or that it has never been exposed to any hazardous biological material.

Name:.....

Firm:.....

Address:.....

.....

.....

Country:.....

Signature:.....

✂----------

I declare that the instrument in this package has been decontaminated or disinfected to remove or inactivate any biological material, which could be dangerous to the service personnel, or that it has never been exposed to any hazardous biological material.

Name:.....

Firm:.....

Address:.....

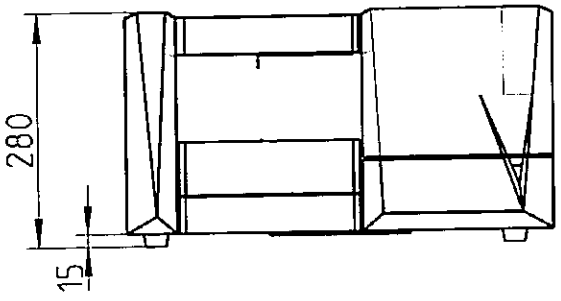
.....

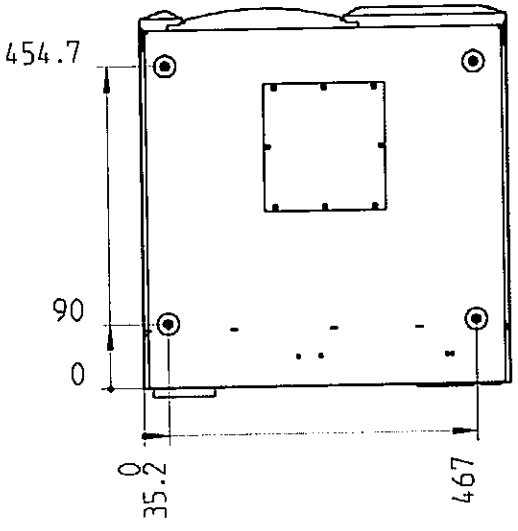
.....

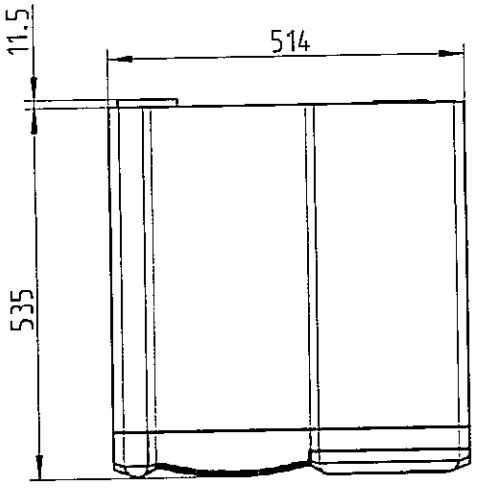
Country:.....

Signature:.....

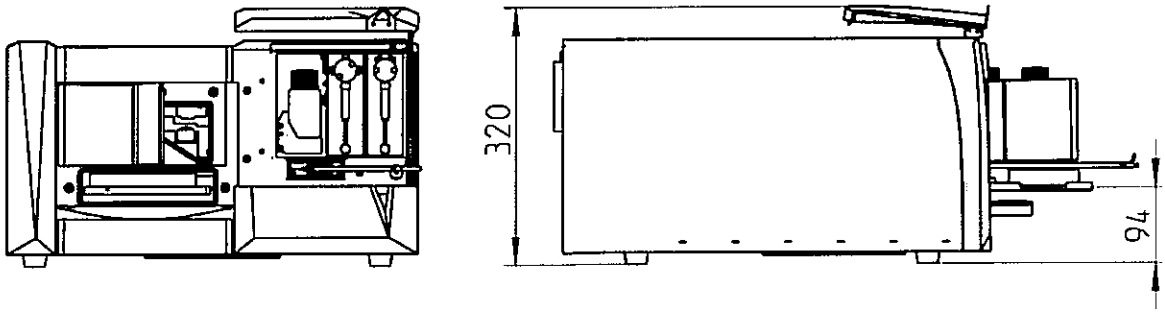
9.6 Outer Dimensions of the GENios Pro

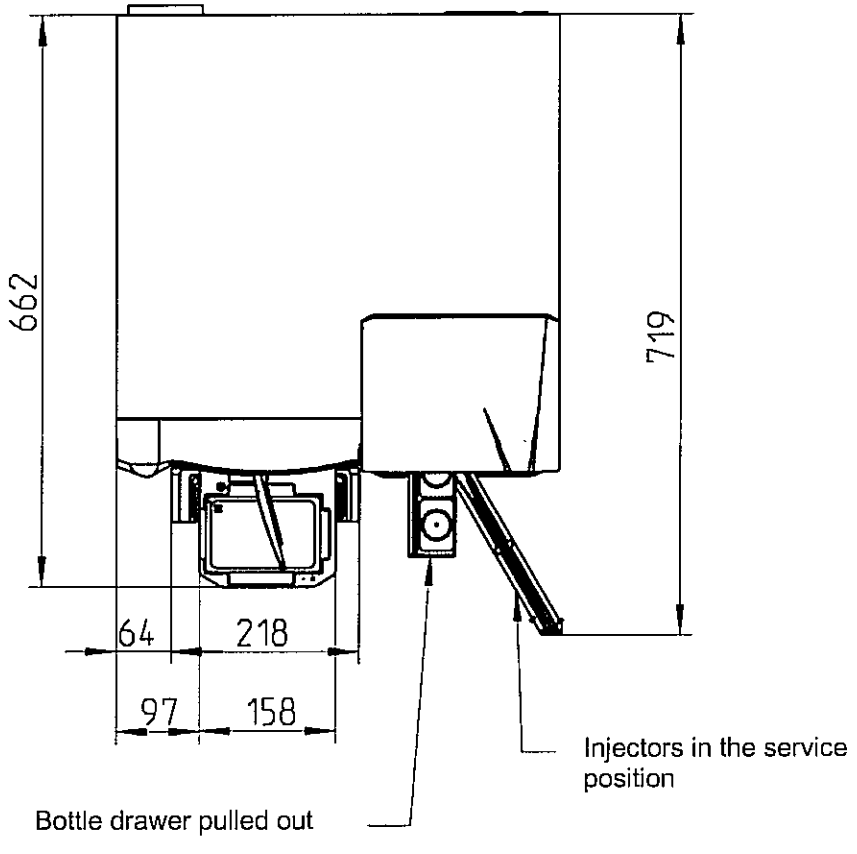






Note: all dimensions are in millimeters.





Note: all dimensions are in millimeters.

!

Index

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TECAN.

Declaration of Conformity

Product Type:	**Multimode micro plate reader**
Model Designation:	**GENios Pro**
Manufacturer:	TECAN AUSTRIA G.M.B.H. Untersbergstraße 1A 5082 Grödig Austria/ Europe

The product complies with the requirements of the following European directives:

- **Low Voltage Directive 73/23/EEC incl. amendments 93/68/EEC**

  Applied Standards:

  EN 61010-1:2001

- **EMC Directive 89/336/EEC incl. amendments 92/31/EEC + 93/68/EEC**

  Applied Standards:

  EN 61326-1:1997 + A1:1998 + A2:2001
  EN 61000-3-2:2000
  EN 61000-3-3:1995

Furthermore, the product complies with the following Standards, which are not within the scope of the above EC-Directive:

National Recognized Testing Laboratory (by OSHA – USA):
UL 61010A-1
CAN/CSA-C22.2 No. 1010.1-92
CAN/CSA-C22.2 No. 1010.1 B-97

Place, Date: Grödig, 7th of March 2003

Legally binding signature:		
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Function:	**Head of Research & Development**	**Head of Finance & Administration**