TriStar^2 LB 942

Modular Multimode Microplate Reader

Operating Manual

56550BA2

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Not for use in in-vitro diagnostic (IVD) procedures.

The information in this guide is subject to change without notice.

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This instrument is not designed or intended for use with installations or equipment in hazardous environments. Servicing of the instrument must only be performed by Berthold Technologies Field Service Engineers or service staff authorized by Berthold Technologies.

Please contact our Service Center at service@berthold.com if you have any operational issues.

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1. Prefatory Comments

1.1 Explanation of LED's and Beeps

LED	*Instrument status*
lights up **green**	Instrument OK and connection to PC OK
lights up **yellow**	Instrument OK, no connection to PC
flashes **yellow** + 1 short beep	New CAN is installed after power on of instrument
lights up **yellow** + 1 long beep	CAN correctly installed
lights up **red**	Shortly after power on of the instrument (during initialization)
flashes **red** + 2 short beeps	Error after power on of instrument / CAN module not correctly installed

1.2 Operating manual

This Operating Manual is structured as follows:

The manual covers all manipulations in a work flow order starting from installation via regular operation to maintenance.

In each section you are guided through the respective procedures step by step. The steps are consecutively numbered in each section. Explanations on the individual steps are added in small type font.

Explanations on the various types of operations are highlighted specifically.

For your convenience, illustrations are placed directly next to the respective text.

1.3 Typographical conventions

<**Add formula**>, <**OK**>, <**Close**>	**Buttons** are printed inside angular brackets in bold typeface
Menu **File**, **Open** dialog box	**Menu titles** and **dialog boxes** are printed in bold type
File | **Open**, **Options** | **Read**	**Menu items** are also printed in bold type; menu and submenu item are separated by a vertical line.

2. Safety Instructions

2.1 Safety Instructions

Hot surface: Care while touching the cover or the lamp, they can be hot.

Caution! This sign alerts you to important operating procedures with a potential danger of damaging the equipment and endangering your safety on disobeying. Refer to the user and instrument manuals for precautionary instructions.

This operating manual includes information and warnings that have to be observed by the user in order to ensure safe operation of the instruments.

Please do always act according to the following safety instructions, before as well as during operation of the system! Before set up and operation of the instrument it is necessary to read the instructions below as neither safe operation of the instrument nor safety of the user are guaranteed otherwise. Failure to follow the instructions may invalidate the warranty.

The instrument has been manufactured in accordance with the safety requirements for electrical measuring systems. If the law lays down regulations on the installation and/or operation of sample measuring system, then it is the operator's responsibility to adhere to them.

The manufacturer has done everything possible to guarantee that the equipment functions safely, both electrically and mechanically. The user has to make sure that the instrument will be set up and installed properly to guarantee safe operation.

The instruments are tested by the manufacturer and supplied in a condition that allows safe and reliable operation.

- This equipment must be installed and used in accordance with the manufacturer´s recommendations. Installation must be performed by properly trained and authorized personnel.
- The instrument may only be operated by personnel who have been trained on the use of the system. It is strongly recommended that all users read this manual prior to use.

- **Never put parts of your body or other devices into the instrument while the unit is in operation.**
- **Remove the transportation lock before switching on the instrument.**
- Use the instrument only for the designated application.
- The instrument is designed for indoor use only.
- BERTHOLD TECHNOLOGIES assumes no liability for any damages, including those to third parties, caused by improper use or handling of the instrument.

- ❑ The user is responsible for connecting the instrument in accordance with the valid regulations for electrical instruments.
- ❑ Set the instrument up to ensure easy access to the mains switch.
- ❑ The mains supply voltage fluctuations must not exceed +/-10 % of the nominal voltage. Maximum voltage to be applied is 253 VAC.
- ❑ The instruments are designed according to these standards:
  IEC / EN 610 10-1: 2001 (2ed)
  CAN / CSA C22.2 No 61010-1-04
  UL 61010-1, 2nd Edition

- ❑ To disconnect the instrument from power the appliance coupler has to be removed from the mains.
- ❑ Do not open any instrument doors as long as the instrument is in operation.
- ❑ Service and repair work may be carried out by qualified personnel only.
- ❑ The operator may only perform the maintenance work described in this user guide.
- ❑ There are no exchangeable electrical components in the instrument. In case of malfunction call authorized service personnel.
- ❑ Use only parts described in this manual for servicing.
- ❑ Disconnect power supply before opening the instrument.
- ❑ Pull the power cord to disconnect instrument from power supply.
- ❑ Upon removal of the front and top parts of the housing no safety measures are in effect. Be aware of any moving parts. The interior of the instrument may reach temperatures that can cause burns. Some parts of the instrument may remain hot without visual indication for some time after the power has been turned off.
- ❑ The electronic unit of the detector generates high voltage. Do not touch it during operation!
- ❑ If you can see that the instrument has become unsafe to use, switch it off and disconnect it from power supply.

- ❑ If liquid gets inside the instruments, pull the power cord. Clean the unit or have it cleaned by an authorized service center.
- ❑ Protect yourself from electrostatic charge, as discharge could damage sensitive instrument parts, especially sensitive parts of the computer and electronics boards.
- ❑ When the lid is opened (e.g. filter change) ESD can no longer be guaranteed. To avoid any damages to the electronic parts it is recommended to take precautions (touching the metal case of a safety grounded object, wearing a grounding strap, etc.).
- ❑ The system always has to be primed with solutions recommended by the kit manufacturer.
- ❑ Use only reagents recommended by the kit manufacturer.

- Use reagents only in accordance with the kit manufacturer's instructions.
- Do not use any flammable or explosive solutions or liquids whose mixture is flammable or explosive.
- Waste (when priming/washing the tubing) always has to be disposed properly: if a waste pump is installed, a bottle has to be connected. If no waste pump is present, a suitable prime plate has to be placed below the injectors during priming/washing.
- Injector solutions may be pumped back only if the appropriate reagent bottle is connected.
- Observe all statutory requirements for handling biological waste, reagents and samples.
- **The operator is responsible for the use of reagents.**
- **The units are not for use in in-vitro diagnostic (IVD) procedures.**
- The instrument should be shipped in its own case. For transport all transportation locks (e.g. for the plate carrier) have to be installed.
- For instrument cleaning, please refer to the respective sections in this manual.
- Reliable instrument function can be guaranteed only when original spare parts are used.

The tests and service work recommended by the manufacturer has to be performed to make sure that the operator remains safe and that the instrument continues to work correctly. Any service and maintenance work not described in this user guide has to be performed by authorized service personnel. Use the instruments only for the designated application.

2.2 Consignes de Sécurité

Surface chaude: Attention en touchant la couverture ou la lampe – danger de se brûler!

Attention! Ce symbole d'alarme, vous avertit de prêter attention aux consignes opératoires. En effet si vous ne suivez pas ces instructions, il peu y avoir un risque d'endommagement du matériel et également vous faire ecourir des risques pour votre propre sécurité. Il est impératif de respecter les instructions du mode d'emploi et de les respecter.

Ce mode d'emploi contient des informations et avertissements qui doivent être suivis par l'utilisateur afin de garantir un fonctionnement sûr des instruments.

Il est impératif de respecter les consignes de sécurité suivantes non seulement avant la mise en service mais aussi pendant le fonctionnement de l'appareil! Avant l'installation et mise en service de l'instrument tous les utilisateurs des appareils sont tenus de lire d'abord ces instructions de service, autrement ni le fonctionnement correct de l'appareil ni la sécurité de l'utilisateur peuvent être garantis. Ne pas suivre ces instructions de service peut invalider la garantie.

Le appareil a été fabriqué conformément aux prescriptions de sécurité en vigueur pour les appareils de mesure électrique. Si l'installation et/ou l'utilisation des appareils de mesure de prélèvements-échantillons sont/est soumise(s) à des réglementations prescrites par la loi, il appartient à l'utilisateur de les respecter.

Le constructeur a fait tout le nécessaire pour assurer le fonctionnement sûr des appareils (du point de vue électrique, électronique et mécanique). L'utilisateur est tenu de veiller à ce que les appareils soient installés correctement afin d'éviter toute altération de leur utilisation sûre.

Les appareils sont contrôlés à l'usine et livrés dans un état assurant la sécurité de fonctionnement.

- Les appareils doivent être mis en service et utilisés strictement conformément aux recommandations du constructeur. La mise en service est réservée au personnel formé et autorisé.
- Les appareils ne doivent être utilisés que par des personnes autorisées et leur utilisation est réservée au personnel compétent. Tous les utilisateurs des appareils sont tenus de lire d'abord ces instructions de service.

- Ne mettez jamais des parties de votre corps ou des objets dans l'appareil lorsque celui-ci est en fonctionnement.
- Enlevez le verrouillage de transport avant la mise sous tension de l'appareil.

- Utilisez l'instrument uniquement pour les applications désignées compatibles.
- L'appareil est destiné uniquement pour une utilisation en intérieur de bâtiments.
- BERTHOLD TECHNOLOGIES décline toute responsabilité de dommages résultant d'une utilisation non conforme à l'emploi prévu, y compris les dommages causés à des tiers.
- Les variations sur la tension du secteur ne doivent pas dépasser +/- 10% de la valeur nominale (max. 253 VAC).
- L'utilisateur porte la responsabilité de la mise en service de l'appareil selon les prescriptions électriques en vigueur.
- Les appareils correspondent aux prescriptions de la norme:
  IEC / EN 610 10-1: 2001 (2ed)
  CAN / CSA C22.2 No 61010-1-04
  UL 61010-1, 2nd Edition

- Pour arrêter et débrancher l'instrument la fiche doit être retirée hors de la prise.
- Ne pas ouvrir le couvercle lors du fonctionnement de l'appareil. Arrêtez l'instrument avant.
- Les travaux d'entretien et de réparation devront être confiés exclusivement à des spécialistes dûment formés.
- Les travaux d'entretien uniquement décrits dans le manuel peuvent être effectués par l'utilisateur.
- Pour les travaux d'entretien, utiliser exclusivement les pièces mentionnées.
- Avant d'ouvrir l'appareil, couper l'alimentation en courant.
- Arrêter l'appareil avant de retirer la fiche.
- Si vous ouvrez l'appareil, les sécurités ne sont plus activées (capôt et parties de la façade de l'appareil). Faites attention aux parties mobiles. L'intérieur de l'appareil et certaines pièces peuvent atteindre des températures pouvant provoquer des brûlures si il y a contact. Appareil éteint, des parties peuvent rester chaudes alors qu'il n'y a pas d'indication visible de température élevée.
- Positionner l'appareil de manières à ce que les interrupteurs soient accessibles.
- Si vous vous apercevez que le fonctionnement de l'appareil n'est plus sûr, il faut alors l'arrêter et le débrancher de la prise secteur.

- Si du liquide a pénétré dans l'appareil il faut immédiatement le débrancher. Ouvrir l'appareil et le nettoyer ou bien le faire nettoyer par une agence de service après vente autorisée.
- Protégez vous des charges électrostatiques afin d'éviter de provoquer des décharges qui pourraient endommager des parties sensibles de l'appareil telles que les cartes électroniques ou PC.
- Ne pas utiliser des liquides inflammables ou explosifs ni de liquides dont le mélange est inflammable ou explosif.

- Respecter toutes les prescriptions légales concernant la manipulation des déchets biologiques, des réactifs et des échantillons.
- **L'utilisateur assume la responsabilité exclusive de l'utilisation des réactifs.**
- **Le dispositif n'est pas destiné à être utilisé dans In Vitro Diagnostic.**
- Transporter l'appareil uniquement dans son emballage d'origine. Lors du transport, bloquer le support de plaques à l'aide de la vis d'arrêt.
- Pour le nettoyage de l'instrument veuillez vous référer au paragraphe correspondant dans ce mode d'emploi.
- Le fonctionnement correcte ne peut être garantit qu'à la condition que des pièces de rechange appropriées sont utilisées.

Afin d'assurer la sécurité de l'utilisateur et le bon fonctionnement des appareils, effectuer les travaux d'inspection et d'entretien recommandés par le fabricant. Toutes les mesures d'entretien et de réparation allant au-delà de celles spécifiées dans ce manuel sont réservées aux techniciens autorisés.

2.3 Sicherheitshinweise

Heiße Oberfläche: Vorsicht beim Berühren der Abdeckung bzw. der Lampe, sie können heiß sein.

Die vorliegende Bedienungsanweisung enthält Informationen und Warnungen, die vom Benutzer befolgt werden müssen, um einen sicheren Betrieb der Geräte zu ermöglichen.

Dieses Zeichen weist den Benutzer auf wichtige Punkte hin, deren Beachtung unerlässlich ist.

Die folgenden Sicherheitshinweise sind sowohl vor der Inbetriebnahme als auch während des Betriebs des Gerätes unbedingt zu beachten. Vor Inbetriebnahme des Gerätes ist es zwingend erforderlich, die Bedienungsanleitung zu lesen, da ansonsten die Sicherheit des Gerätes und des Benutzers nicht gewährleistet wird.

Das Gerät wurde in Übereinstimmung mit den Sicherheitsanforderungen für elektrische Messgeräte hergestellt. Bestehen für die Errichtung und/oder den Betrieb von Probenmessgeräten gesetzlich vorgeschriebene Regelungen, so ist es die Aufgabe des Errichters und Betreibers, diese einzuhalten.

Der Hersteller hat alles unternommen, um ein sicheres Arbeiten der Geräte (bezüglich Elektrik, Elektronik und Mechanik) zu gewährleisten. Der Benutzer muss dafür sorgen, dass die Geräte so aufgestellt und installiert werden, dass ihr sicherer Gebrauch nicht beeinträchtigt wird.

Die Geräte sind werkgeprüft und wurden in betriebssicherem Zustand ausgeliefert.

- ❑ Die Geräte dürfen nur von autorisierten Personen in Betrieb genommen und nur von eingewiesenem Personal bedient werden. Alle Benutzer, die mit den Geräten arbeiten, müssen zuerst diese Bedienungsanleitung lesen.
- ❑ Die Geräte dürfen nur von dafür geschultem Personal betrieben werden. Es wird allen Anwendern empfohlen, diese Bedienungsanleitung vor Benutzung zu lesen.

- ❑ Während des Betriebes nicht in die Geräte faseen oder andere Teile in die Geräte einführen.
- ❑ Transportsicherungen vor dem Einschalten entfernen.
- ❑ Die Geräte dürfen nur für den vorgesehenen Zweck eingesetzt werden.
- ❑ Berthold Technologies übernimmt keinerlei Gewährleistung, auch für Schäden gegenüber Dritten, die durch unsachgemäße Handhabung der Geräte hervorgerufen werden.
- ❑ Die Geräte dürfen nur innerhalb geschossenen Räumen betrieben werden.
- ❑ Die Stromversorgung darf nicht mehr als ±10 % des Nominalwertes aufweisen. Maximal sind 253 V Wechselstrom erlaubt.
- ❑ Es liegt im Verantwortungsbereich des Anwenders, dass die Geräte nach den lokalen elektrischen Vorschriften installiert werden.

- ❑ Die Geräte entsprechen den Vorschriften der:
  IEC / EN 610 10-1: 2001 (2ed)
  CAN / CSA C22.2 No 61010-1-04
  UL 61010-1, 2nd Edition
- ❑ Nicht öffnen, wenn das Gerät in Betrieb ist.
- ❑ Service- und Reparaturarbeiten dürfen nur von Fachleuten ausgeführt werden.
- ❑ Es dürfen nur die im Handbuch beschriebenen Wartungsarbeiten vom Anwender ausgeführt werden.
- ❑ Bei Wartungsarbeiten dürfen nur die angegebenen Teile verwendet werden.
- ❑ Vor dem Öffnen des Gerätes ist die Stromzufuhr zu unterbrechen.
- ❑ Um das Gerät vollkommen vom Netz zu trennen, kann das Netzkabel gezogen werden.
- ❑ Wenn das Gerät geöffnet ist sind Sicherheitsmaßnahmen nicht mehr in Betrieb. Auf bewegliche Komponenten achten! Das Innere der Geräte kann Temperaturen erreichen, die Verbrennungen verursachen können. Einige Teile können heiß bleiben ohne sichtbare Zeichen, auch nachdem das Gerät abgeschaltet worden ist.
- ❑ An der Multiplier-Einheit liegt Hochspannung an. Nicht berühren, wenn das Gerät läuft.
- ❑ Stellen Sie das Gerät so auf, dass Sie es leicht ein- und ausschalten können.
- ❑ Bei Beeinträchtigung der Betriebssicherheit sind die Geräte abzuschalten und vom Netz zu trennen.
- ❑ Ist Flüssigkeit in das Innere des Gerätes gelangt, Netzstecker ziehen. Das Gerät öffnen und reinigen bzw. durch eine autorisierte Servicestelle reinigen lassen.
- ❑ Elektrostatische Aufladungen (z.B. durch Teppichböden) müssen beim Öffnen des Gerätes verhindert werden, da Entladungen am Gerät zur Beschädigung elektronischer Teile führen können.
- ❑ Das System muss immer ausreichend mit den vom Reagenzienhersteller empfohlenen Lösungen gespült werden.
- ❑ Es dürfen nur Reagenzien verwendet werden, die vom Reagenzhersteller empfohlen werden.
- ❑ Reagenzien dürfen nur in der vom Reagenzienhersteller vorgeschriebenen Art und Weise verwendet werden.

- ❑ **Es dürfen keine entzündlichen oder explosiven Flüssigkeiten oder keine Flüssigkeiten, deren Mischung entzündlich oder explosiv ist, verwendet werden.**
- ❑ Es ist immer auf eine korrekte Entsorgung des Abfalls (beim Füllen/Spülen der Leitungen zu achten: Bei integrierter Abfallpumpe ist ein Behälter anzuschließen. Wenn keine Abfallpumpe vorhanden ist, muss beim Spülen/Füllen der Leitungen eine entsprechende Auffangwanne unter den Injektoren plaziert sein.

- Das Zurückpumpen der Injektorflüssigkeit darf nur dann vorgenommen werden, wenn die entsprechenden Reagenzbehälter angeschlossen sind.
- Beachten Sie alle gesetzlichen Vorschriften für den Umgang mit biologischem Abfall, Reagenzien und Proben.
- **Die Anwendung der Reagenzien liegt im alleinigen Verantwortungsbereich des Benutzers.**
- **Die Geräte sind nicht für den Einsatz in der In Vitro Diagnostik bestimmt.**
- Das Gerät sollte nur in der eigenen Verpackung transportiert werden. Beim Transport ist darauf zu achten, dass alle Transportsicherungen eingesetzt werden (z.B. die Sicherung für den Plattenträger).
- Zum Reinigen des Gerätes bitte den entsprechenden Teil dieser Bedienungsanleitungen beachten.
- Ordungsgemäße Funktionalität kann nur bei Verwendung der Originalersatzteile garantiert werden.

Für die Sicherheit des Benutzers und die Funktionsfähigkeit der Geräte sind die vom Hersteller empfohlenen Überprüfungen und Wartungsmaßnahmen durchzuführen. Alle über die Betriebsanleitung hinausgehenden Wartungs- und Instandhaltungsmaßnahmen dürfen nur von autorisierten Technikern ausgeführt werden.

3. Warranty and Technical Issues

3.1 Special spare parts

The following spare parts are safety parts: Use the original part from the manufacturer or direct agent only.

Power supply	input 100 - 240 VAC, 4 A output 24 VDC, 9.2 A, max 221 W	GST220A24-R7B part no. 59048

3.2 Warranty statement

The instrument is sold in accordance with the general conditions of sale of Berthold Technologies GmbH & Co KG and its affiliates and representatives.
Berthold Technologies warrants this product to be free of defects in material and workmanship for a period of 12 months from the date of delivery, ex works Bad Wildbad.
Berthold Technologies or its authorized representative will repair or replace, at its option and free of charge, any product that under proper and normal use proves to be defective during the warranty period.

Berthold Technologies shall in no event be liable or responsible for any incidental or consequential damage, either direct or indirect.

The above warranty shall not apply if:

a) the product has not been operated in accordance with the operating manual
b) the product has not been regularly and correctly maintained
c) the product has not been repaired or modified by a Berthold Technologies authorized representative or user
d) parts other than original Berthold Technologies parts are used
e) the product and parts thereof have been altered without written authorization from Berthold Technologies GmbH & Co KG
e) the product has not been returned properly packed in the original Berthold Technologies packaging

This warranty does not apply to any third party product involved in the application.

Berthold Technologies reserves the right to refuse to accept the return of any product that has been used with radioactive or (micro)biological substances, or any other material that may be deemed hazardous to employees of Berthold Technologies. Such products have to be properly decontaminated and marked. Before returning products to Berthold Technologies ensure the devices are properly decontaminated and the form "**Confirmation on decontamination**" is properly filled in and will be accompanying the product. (See appendix for a blank form)

Before returning products to Berthold Technologies, a returns/repair number must be obtained and clearly identified on the packing and documents. Call Berthold Technolo-

gies to get this number. Retain the original packaging for use if the instrument needs to be returned to Berthold Technologies.

3.3 Customer service

Customer service will be provided in the first instance by the network of Berthold Technologies representatives. In the event of any problem experienced with your instrument, the first recourse should be **your local Berthold Technologies representative**. For further problems requiring hardware or software expertise, the Technical Support group at Berthold Technologies GmbH & Co KG will be available by phone, fax or email to deal with your queries. Here is their address, phone, fax and e-mail:

Berthold Technologies GmbH & Co KG
Technical Support
Calmbacher Str. 22
75323 Bad Wildbad
Germany
Phone: +49 7081 177 114
Fax: +49 7081 177 301
Email: service@berthold.com

At the end of this manual you will find a Customer Reply Form (Appendix section). If a problem arises with the instrument which you are not able to resolve, please fill in this form. This form should then be transmitted to your Berthold Technologies representative or to Technical Support at Berthold Technologies, where it will receive early attention.

Please also make sure that you have the relevant information available before contacting Berthold Technologies. Helpful information would include:

- serial numbers, part number, revision:
  see production label on instrument
- software and firmware versions
- monitor and log files (refer to the respective service manuals)

4. Introduction

4.1 Intended use

The **TriStar²** is a modular multi-technology microplate reader for different types of fluorescent, luminescent and absorbance applications.

The units are not for use in in-vitro diagnostic (IVD) procedures.
These units are not designed for use in hazardous areas.

4.2 Description

The **TriStar²** microplate reader is distinguished by its exceptionally high sensitivity allowing detection limits in scientifically relevant magnitudes with low reagent consumption.

Detector sensitivity and stability are the result of Berthold Technologies´ experience with thousands of photon counters. **True photon counting** has the benefit that no user parameters need to be set, ensuring the same conditions are used for every measurement during the instrument´s entire life time. The fast photon counting circuitry provides a dynamic range in excess of six orders of magnitude, which complements the range of the latest assays.

A proprietary design of the optical system achieves absolute minimisation of cross-talk down to 10^{-6} (depending on the type of microplate).

The PC based **ICE operating software** has been designed with the basic research scientist in mind and offers straight forward access to the respective parameter settings. Besides raw data measurements the user can select dual measurements with ratio calculation or kinetic and scanning.

The instrument can read solid plates as well as strip plates from 6 to 384 well formats with a height **not** exceeding 21 mm (respective adapter frames need to be applied).

4.3 Recommendations for proper handling

To obtain good and consistent results please **follow these recommendations** :

- Do not expose instrument to direct sunlight
- Set up instrument in dry rooms
- Open lid for loading filter/microplates or cleaning only to keep light and dust out
- Keep plate carrier free from dirt
- Remove spilled reagents immediately with damp cloth or optical grade tissue
- Very bright samples may cause saturation of the PMT (indicated by an “Overload” message); let the PMT recover for a few seconds

To avoid damages to mechanical, electrical and optical components **obey to these rules**:

- Load microplates correctly
- Do not use microplates or strip plates with heights exceeding 21 mm
- Do not fill the microplates above their specified maximum volume
- Do not shake completely filled microplates in the instrument

- Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system; take special care when ice in the trough starts to melt

5. Installation

Read this part completely prior to starting with the first steps and make sure that all pre-requisites are met as described below.

5.1 Unpacking and Set up

1. Unpack TriStar² and accessories
2. Put TriStar² onto an appropriate laboratory desk

3. Remove external power supply from its box and connect to power cord

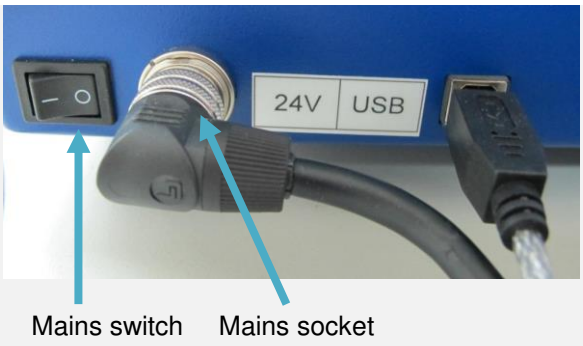
4. Open the big front flap and **remove transportation safety device**

5. Connect USB cable to USB port of instrument

USB port

6. Connect power cord to the respective socket of the instrument

7. Verify the mains switch is in **OFF** position



Mains switch Mains socket

8. Check if the power supply is within the permissible range of the operating voltage (**110 – 240 VAC, 50/60 Hz**)

   Connect instrument only if it is matching!

9. Put the jack of the external power supply into the wall outlet

10. For the consecutive software installation the instrument should remain **turned off**.

5.2 Software Installation

The instrument can be run with either ICE or Mikrowin software. Dependent on your software configuration follow either the instructions for ICE software or Mikrowin software installation respectively.

5.2.1 Installation of ICE operating software

Note: The software requires a computer with Windows operating system (Windows 2000, Windows XP, Windows Vista, Windows 7) . For installation local administrator level is recommended but not necessary.

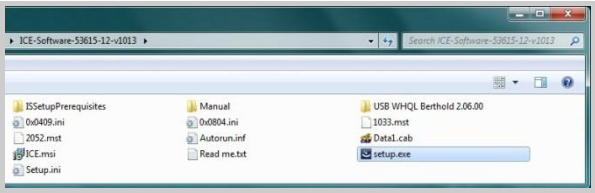
Note: As the software requires some additional resources for proper operation the set up wizard will check for the presence of these resources (**.NET Framework 2.0** and **Crystal Reports for .NET Framework**) on the computer. If the resources are found the installation of **I**nstrument **C**ontrol and **E**valuation (**ICE**) software is started.

In case these resources are not available on the computer the set up wizard will start with the installation of these resources.

1. Close all Windows applications before you start installing the software

2. **Insert software CD** into CD drive
   The set up routine starts automatically

   In case the installation does not start automatically browse to the CD´s root directory and double click **Setup.exe**



3. Click **<Run>** when the Security Warning dialogue appears

   This or similar dialogues may appear during consecutive steps of the installation due to Windows security settings. Always confirm the messages to continue the installation.

4. Choose a **language**
5. Click **<OK>**

6. InstallShield will configure itself

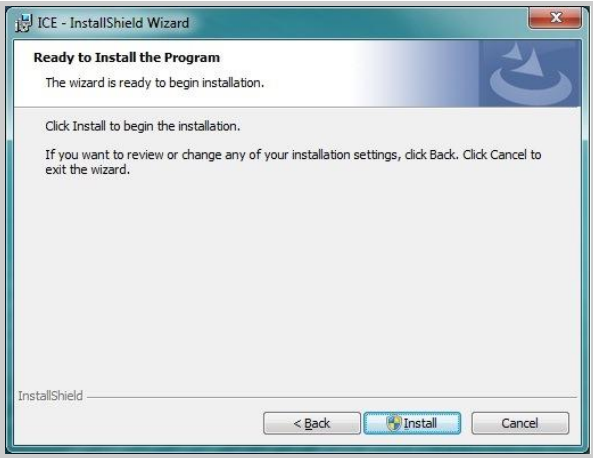
7. Click **<Next>** to start installation procedure

8. Choose an **installation directory**

   Due to support issues it is recommended to use the defaulted one

9. Click **<Next>**

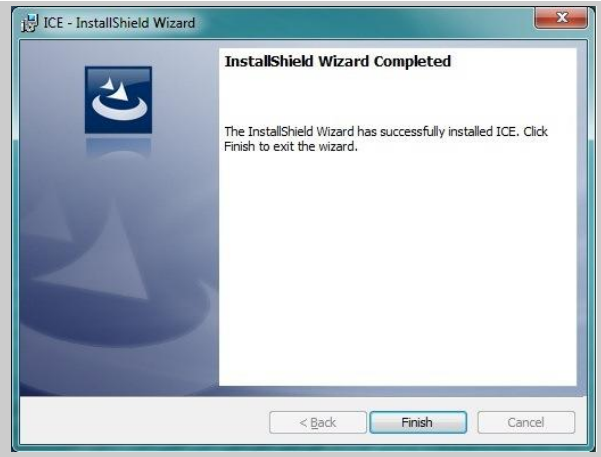
10. Click **<Install>**



11. The installation procedure will be executed automatically

    Click **<Accept>** or **<Run>** should any warning messages appear

12. Click **<Finish>**



13. After a successful installation an **ICE icon** will be visible on the desktop

5.2.2 Installation of Mikrowin 2000 operating software

Note: The software requires a computer with Windows operating system (Windows 2000, Windows XP, Windows Vista, Windows 7) . For installation local administrator level is recommended but not necessary.

Note: For the installation of MikroWin and driver software as well as for any updates and upgrades of the respective software the user has to have ***local Administrator rights*** for the computer.

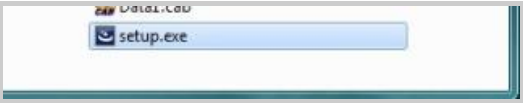
Note: ***Advanced*** versions are delivered with a hard lock (parallel or USB) for copy protection. The hard lock is matched with the installation CD. The hardlock needs to be attached during all operations with MikroWin. The ***Lite*** version needs to get activated with an activation code during or after installation.

Note: When a ***USB hard lock*** is used the installation has to be performed without the hard lock plugged in. The USB hard lock has to be put into the PC right after installation.

1. Close all Windows applications before you start installing the software

2. Insert software CD into CD drive
   The set up routine starts automatically

   In case the installation does not start automatically browse to the CD´s root directory and double click **Setup.exe**

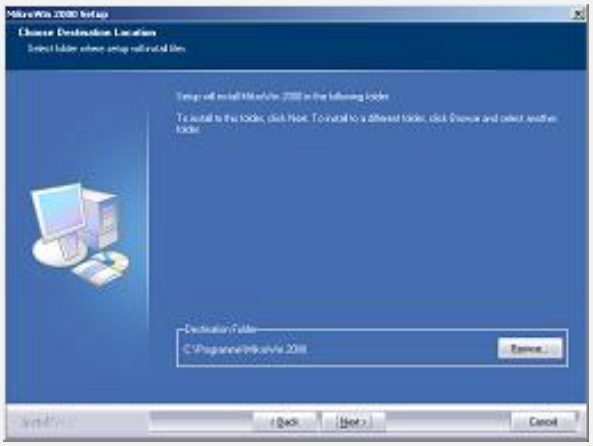


3. Select language and confirm with **<OK>**. The setup assistant is started
4. Enter name and company and click **<Next>**
5. Choose **destination location** (see screen shot to the right).

   The following path is defaulted
   C:\Program Files\Mikrowin 2000

   For support reasons it is recommended to keep the default settings

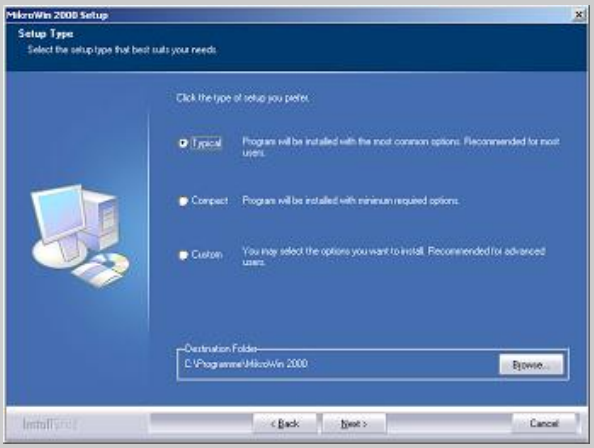
   If you wish to install the program to another folder, click **<Browse>** and select another folder
6. Click **<Next>**



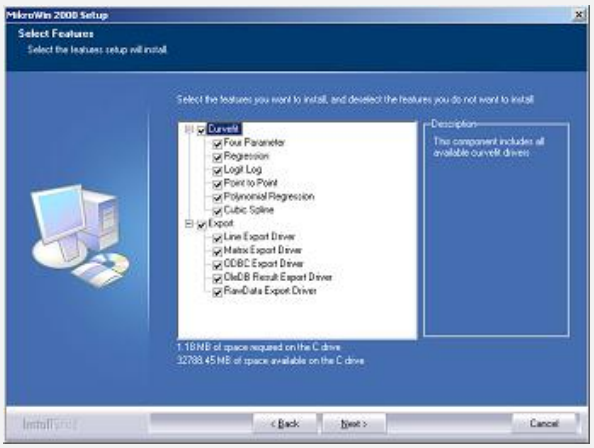
7. Select the **setup type**

   We recommend that you choose **Typical** for your first installation to ensure that all program components are installed.

   If you are familiar with the system, you may choose **Custom** to select the components you need for your application. You may especially **not** want to install the instrument drivers LB96V and Null Device.
8. Click **<Next>**



9. Select the desired components or deselect those components you don't want to install.
10. Click **<Next>**



11. Add program icon to the Mikrowin 2000 program folder.
12. Click **<Next>**.

    Installation is carried out and successful completion is indicated.

11. Click **<Finish>** to complete setup

12. Attach Mikrowin 2000 **USB hard lock** for ***Advanced*** versions

    or

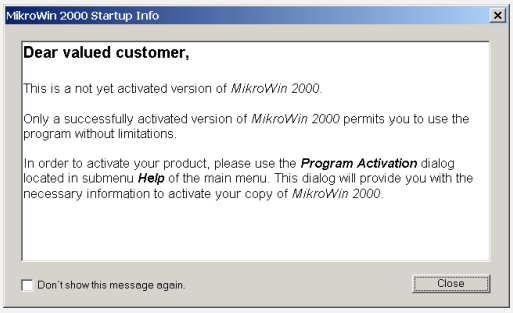
    Run the **Activation** procedure for ***Lite*** versions

5.2.3 Activation of MikroWin *Lite* Software

The Activation prodedure needs to be executed only when a new installation of Mikrowin 2000 has been performed.

1. This dialog will be displayed when starting a not yet activated **MikroWin 2000** ***Lite*** software without the instrument switched on (v 4.29 and higher)

   It is recommended to switch off and disconnect the **instrument** during software activation.

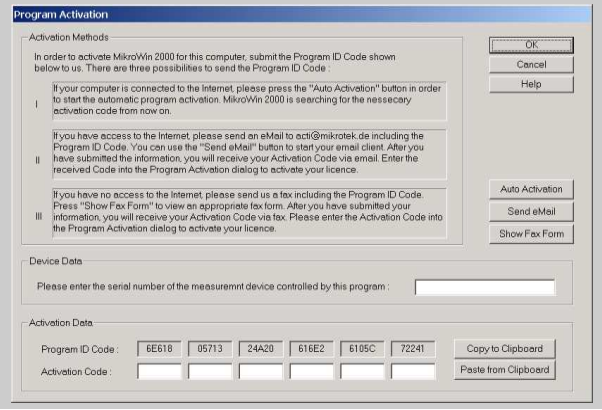


2. Go to **Help** | **Program Activation**

   There are 3 ways to acquire the activation code:
   I) on line via internet (proceed with step **3**)
   II) via email (proceed with step **8**)
   III) via fax (proceed with step **16**)

   Activation via internet:

3. Enter serial number of instrument
4. Click <**Auto Activation**>
5. Click <**OK**> on the next screen displayed to con-



firm the activation process

6. Code will be transferred online and will be automatically entered into the respective boxes

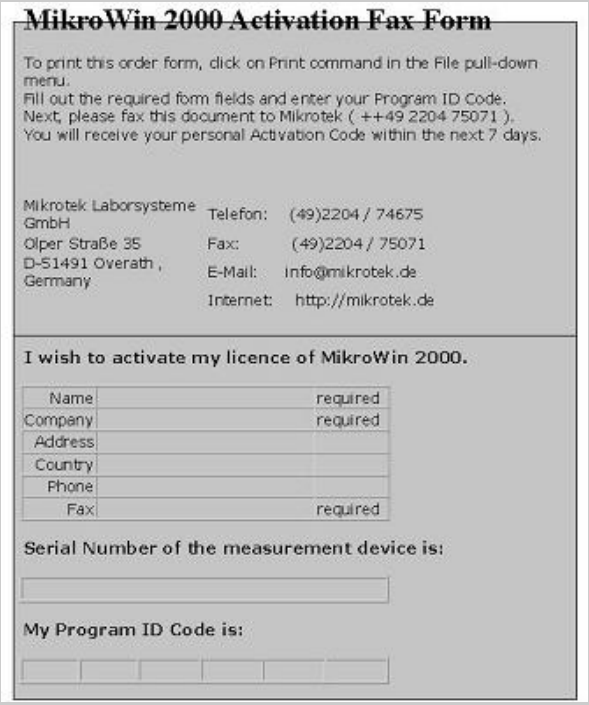
   Activation code will be returned within German office hours only
7. Once code is entered in respective fields click <**OK**>

Activation via email:

8. Click <**Copy to Clipboard**>
9. Click <**Send eMail**>
10. Select suitable email profile
11. use "***MikroWin Program Activation***" as subject and provide these details of your system: ***Program ID Code***, ***Device Serial Number*** and ***Program Licence Code***
12. Email with respective activation code will be returned within 24 h
13. Copy code to clipboard.
14. Re-access the **Program activation** menu and click <**Paste from Clipboard**>
15. Click <**OK**>

Activation via fax:

16. Click <**Copy to Clipboard**>.
17. Click <**Show Fax Form**>.
18. Paste Program ID Code into respective fields and enter additional required information.


MikroWin 2000 Activation Fax Form

To print this order form, click on Print command in the File pull-down menu.
Fill out the required form fields and enter your Program ID Code.
Next, please fax this document to Mikrotek (++49 2204 75071).
You will receive your personal Activation Code within the next 7 days.

Mikrotek Laborsysteme GmbH
Olper Straße 35
D-51491 Overath ,
Germany

Telefon: (49)2204 / 74675
Fax: (49)2204 / 75071
E-Mail: info@mikrotek.de
Internet: http://mikrotek.de

I wish to activate my licence of MikroWin 2000.

Name	required
Company	required
Address	
Country	
Phone	
Fax	required

Serial Number of the measurement device is:

My Program ID Code is:

5.2.4 Installation of TriStar² driver

In order to be able to communicate with the instrument via the USB port (executing operations and receiving data) the driver software needs to be installed and set up.

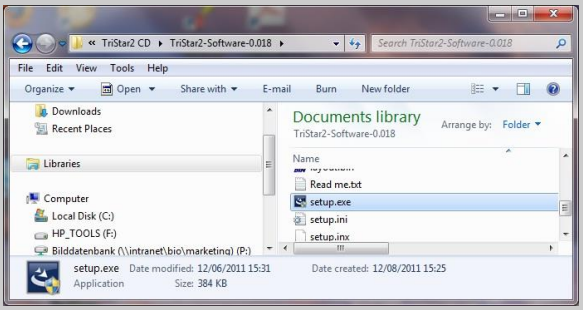
The instrument needs to be **switched off** during this process.

1. Close all Windows applications before you start installing the software
2. Make sure the instrument´s power switch is in **OFF** position

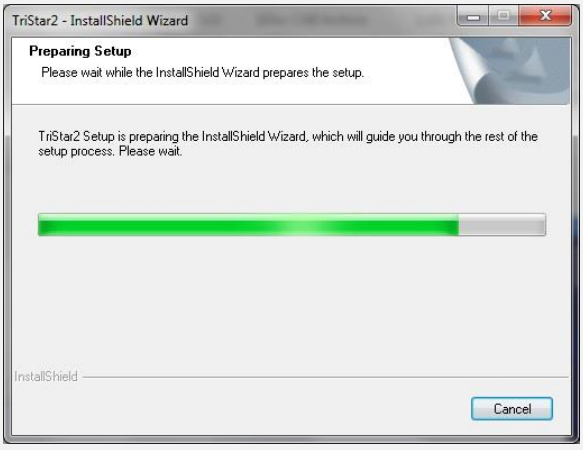
3. **Insert software CD** into CD drive
   The set up routine starts automatically

   In case the installation does not start automatically browse to the CD´s root directory and double click **Setup.exe**

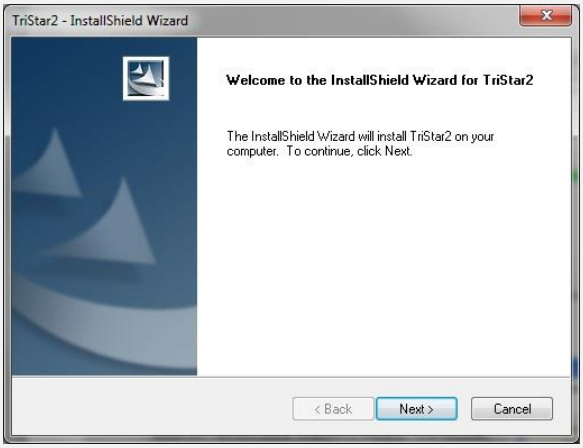
   Click **<Yes>** or **<Accept>** or **<Run>** should any warning message appear on your screen



4. Install Shield will prepare the installation

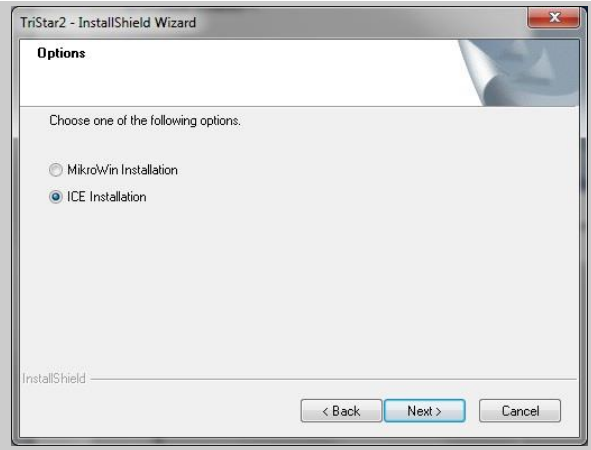


5. Click **<Next>**



6. Select the installation type for or **ICE** (or **MikroWin** depending on which kind of evaluation software you are using and have installed prior)

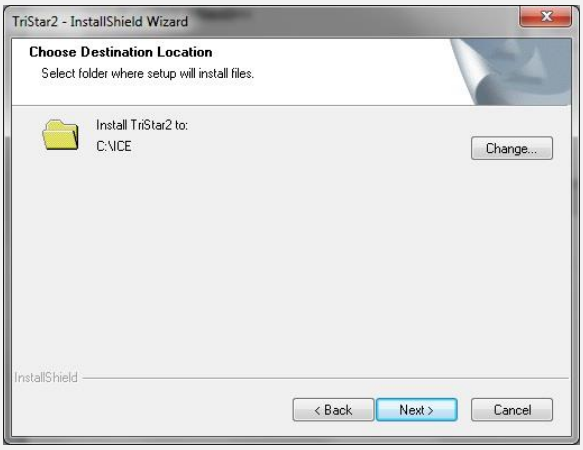
7. Click **<Next>**



8. Choose an **installation directory**

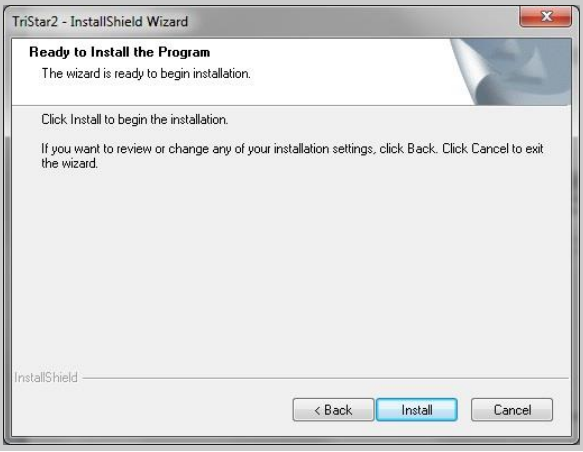
   Due to support issues it is recommended to use the defaulted one

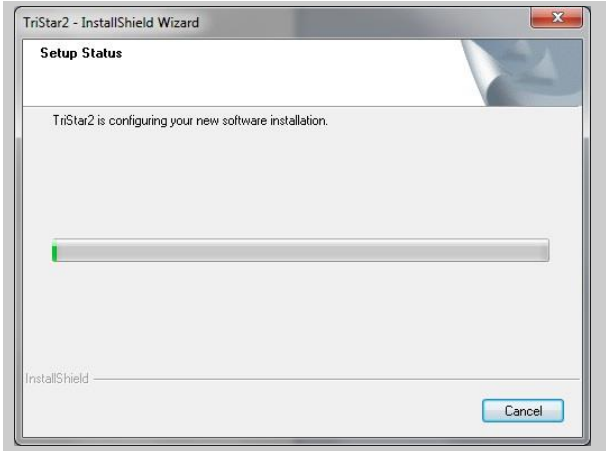
9. Click **<Next>**



10. Click **<Install>**

    Click **<Accept>** or **<Run>** should any warning messages appear

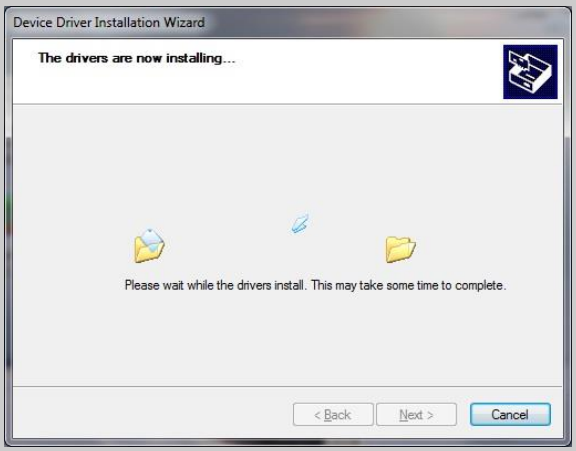




11. Click **<Next>**

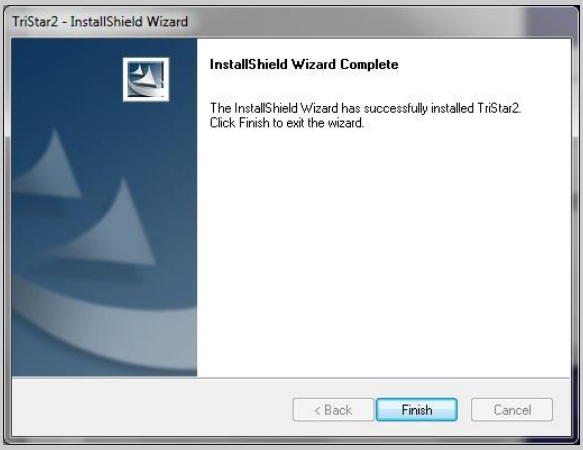
12. Wait for the installation procedure to be finished

    Note: Certain Windows versions/configurations will show security dialog boxes such as "Do you want to allow the following program from an unknown publisher to make changes to this computer". In this instance click **<Yes>**, **<OK>** or **<Run>**



13. Click **<Finish>**

14. Click **<Finish>**



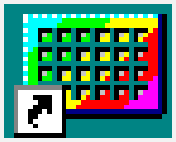
15. Connect the USB cable to a USB port of the computer

16. A message will be shown in the task bar during the USB driver installation

17. After a few minutes a message confirming the successful installation will be displayed in the task bar

18. Turn instrument on by putting mains switch into **ON** position

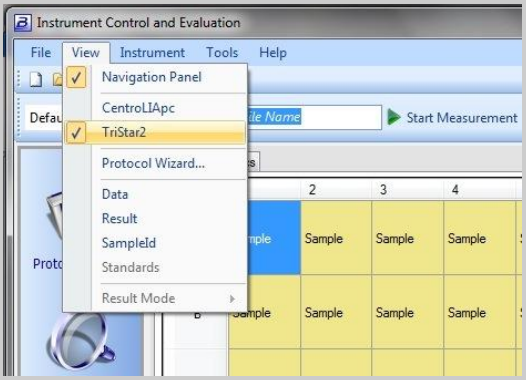
19. Open **ICE** software or **Mikrowin** software dependent on which kind of installation you have done prior



20. Select **TriStar²** in **View** menu ***(ICE)***

    or

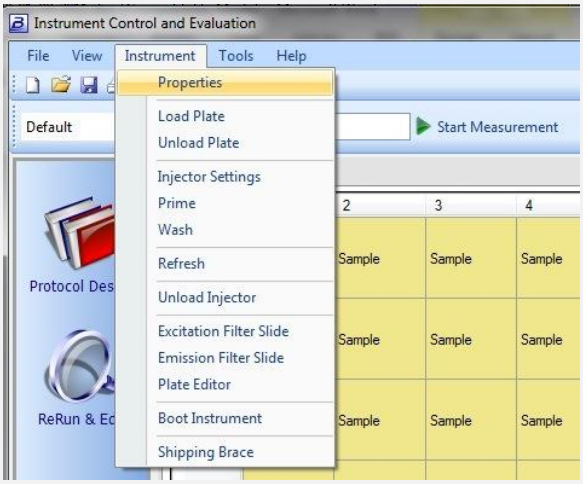
    Select the menu item **Installation** | **Driver** ***(Mikrowin)*** to open the Installation Driver dialog box with a separate tab for each driver type.



21. Go to **Instrument** menu and select **Properties** ***(ICE)***

    or

    Highlight **BertholdTech TriStar2** and click on **<Driver Setup>** ***(Mikrowin)***



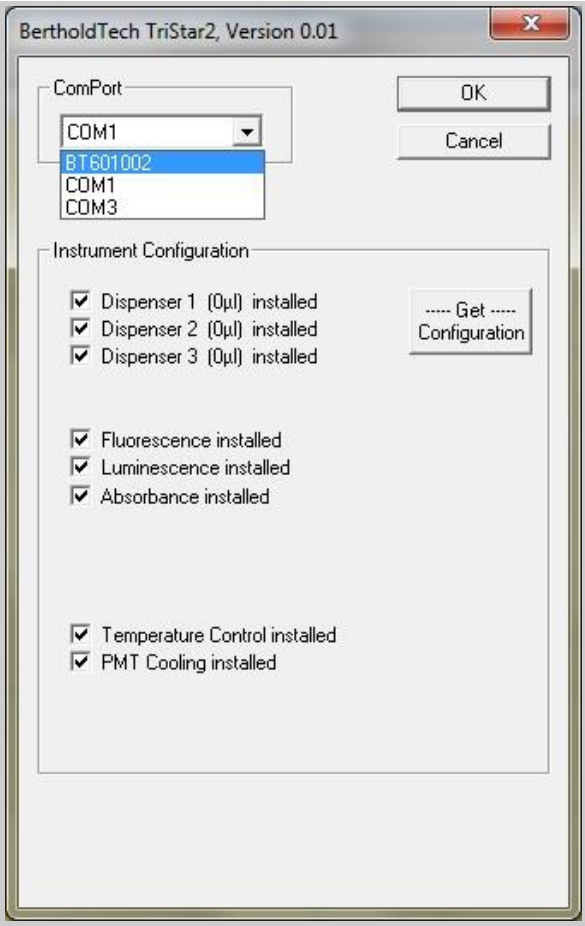
22. Select the entry starting with **BT60….** (e.g. BT601004) in the **ComPort** section

    The raw data are usually displayed as RLU representing the total amount of counts acquired during the reading time per well
    By checking **Calculate RLU/s** the total amount of counts will be divided by the respective reading time

23. Click <**Get Configuration**>
    the available injectors (with their volume) of the instrument will be automatically checked as well as Temperature Control and PMT Cooling when installed

24. Click **<OK>**

25. ***Mikrowin only:*** Click **<OK>** to close the **Installation** | **Driver** dialogue



26. The instrument is now ready to use

5.3 Installing Filters

The instrument comes with an excitation and an emission filter slide, each of capable of holding up to 5 filters.

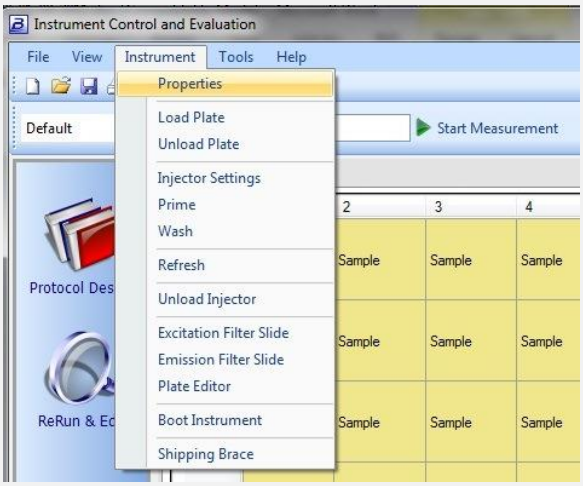
If the instrument is equipped with absorbance reading technology a 450 nm absorbance filter is included.

If the instrument is equipped with fluorescence reading technology a 485/14 nm excitation filter and a 535/25 nm emission filter are included.

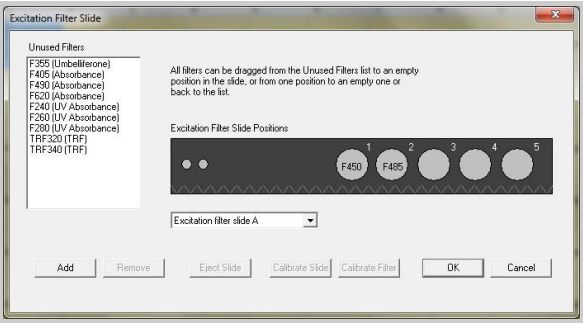
In case additional filter are required they can be ordered individually and can easily be installed both physically and in the software.

5.3.1 Excitation filters

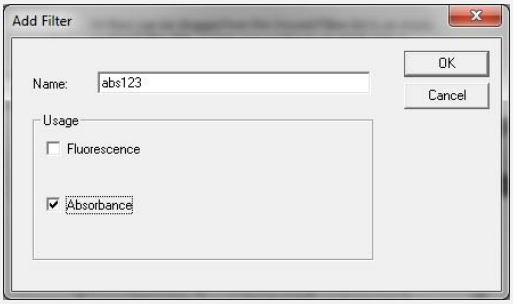
1. Select **Excitation Filter Slide** in the **Instrument** menu



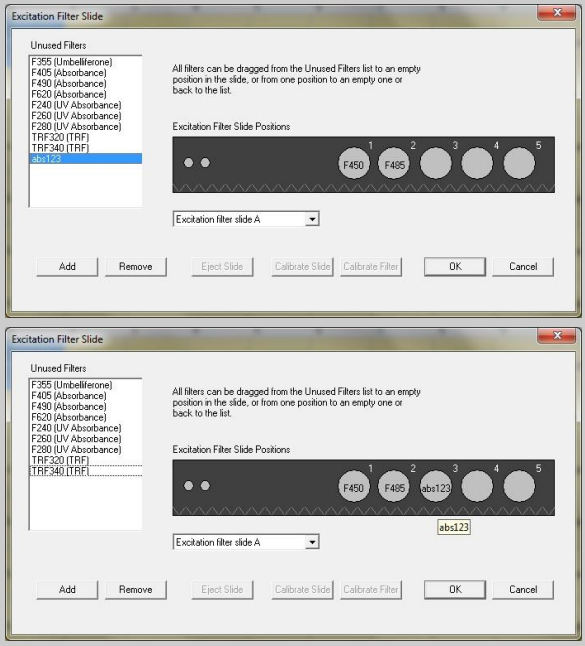
2. Click **<Add>**



3. Define a (descriptive) **Name** for the new filter
4. Check the **Usage**
5. Click **<OK>**



6. Highlight the new filter in the Unused Filters list and drag it into an empty position of the filter slide



7. Open the big flap at the front
8. Click **<Eject Slide>**
9. Remove excitation filter slide from the instrument

10. Mount the filter(s) into the position(s) defined in the software

    for excitation and absorbance filters with diameters of 12.7 mm (½ inch), 15 mm or 25 mm (1 inch) can be used

    12.7 mm filters need to be mounted with a matching adapter (**ID 57194-005**) and a matching clamp ring (**ID 57195-005**)

    15 mm filters need to mounted with a matching adapter (**ID 54666-005**) and a matching clamp ring (**ID 34767-005**) as well

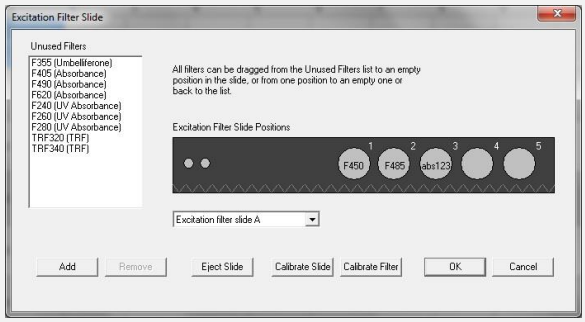
excitation filters mounted

11. Insert the slide again until the front of the slide is aligned with the front of the instrument
12. Click **<OK>**
13. Close the front flap

14. Click **Calibrate Slide** when more than filter have been installed or the instrument has been set up for the first time
15. Highlight the single filter by right-clicking and click **Calibrate Filter** when only a single new filter has been added

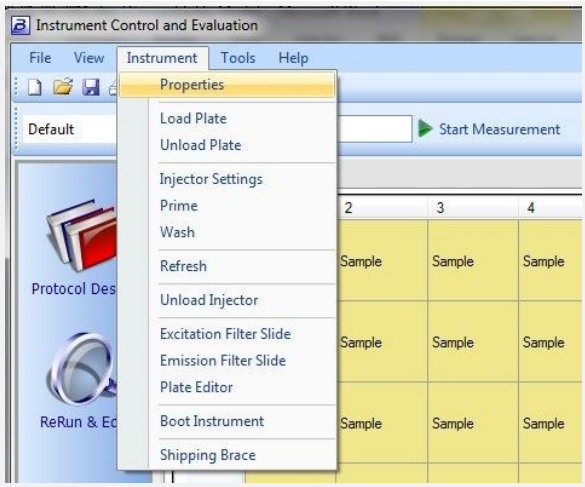
16. The instrument will test and finally store the optimized lamp energy for each filter according to its transmission specifications; this may take a few minutes

    Do not switch off the instrument during this procedure!
17. Click **<OK>** (once the dialog is black and active again)

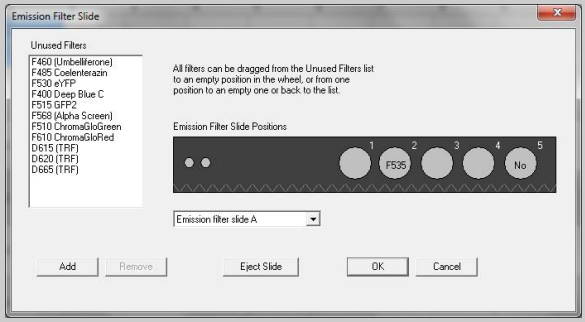


5.3.2 Emission filters

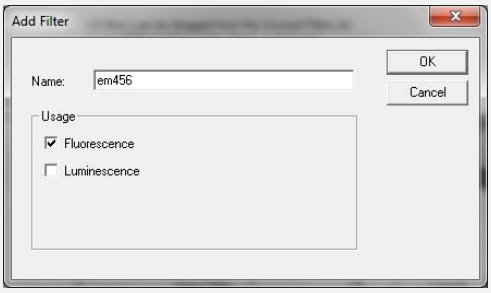
1. Select **Emission Filter Slide** in the **Instrument** menu



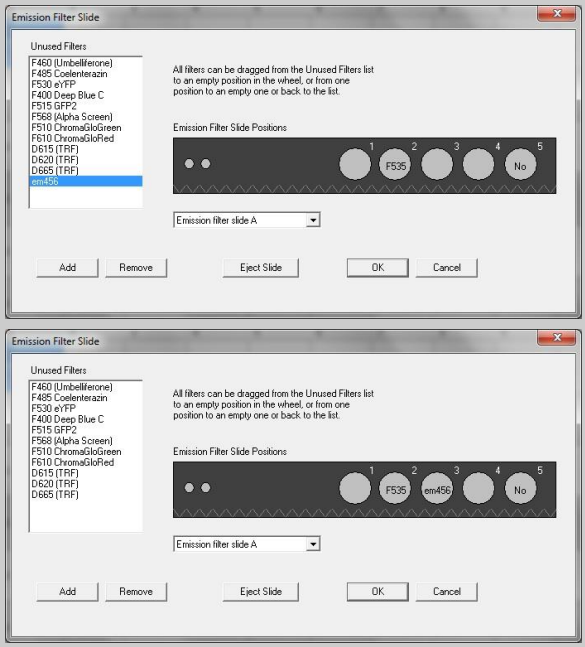
2. Click **<Add>**



3. Define a (descriptive) **Name** for the new filter
4. Check the **Usage**
5. Click **<OK>**



6. Highlight the new filter in the Unused Filters list and drag it into an empty position of the filter slide



7. Open the big flap at the front
8. Click **<Eject Slide>**
9. Remove emission filter slide from the instrument

emission filters excitation filters

10. Mount the filter(s) into the position(s) defined in the software

    25 mm filters are recommended for emission as they are ideally matching the emission light path

    filters with diameters of 12.7 mm (½ inch) and 15 mm may be used but are not recommended as sensitivity will be compromised

    12.7 mm filters need to be mounted with a matching adapter (**ID 57194-005**) and a matching clamp ring (**ID 57195-005**)

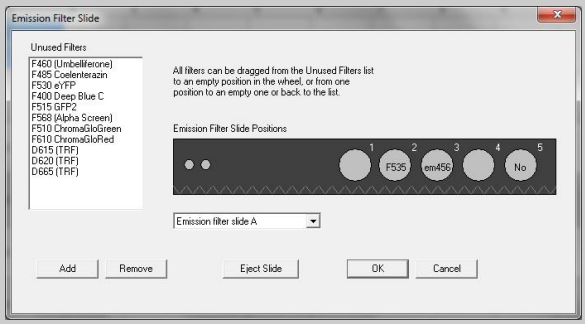
    15 mm filters need to mounted with a matching adapter (**ID 54666-005**) and a matching clamp ring (**ID 34767-005**) as well

Emission filters mounted

Position 5 is reserved for luminescence readings

11. Insert the slide again until the front of the slide is aligned with the front of the instrument
12. Click **<OK>**
13. Close the front flap

14. Click **<OK**



6. Instrument Control and Evaluation Software

6.1 ICE Directories and Files

The directories for data and parameter files are defaulted as described below. Any accessible directory on the computer and the local network can be selected though when saving data and parameter files using the "**Save … File As…**" command.

Default directories

- Data files My Documents\ICE\DataTriStar2
- Protocol files My Documents\ICE\ParaTriStar2

In consequence each Windows user has own directories containing his data and protocol files.

File Names

There is no limitation in naming data and protocol files other than the Windows conventions.

Data file names are to be defined prior to measurement start. Renaming is possible using the "**Save Data File As…**" command producing a copy of the data file with a new name.

Protocol file names are to be defined at the end of creating a protocol. Renaming is possible using the "**Save Protocol File As…**" command producing a copy of the protocol file with a new name.

File Types

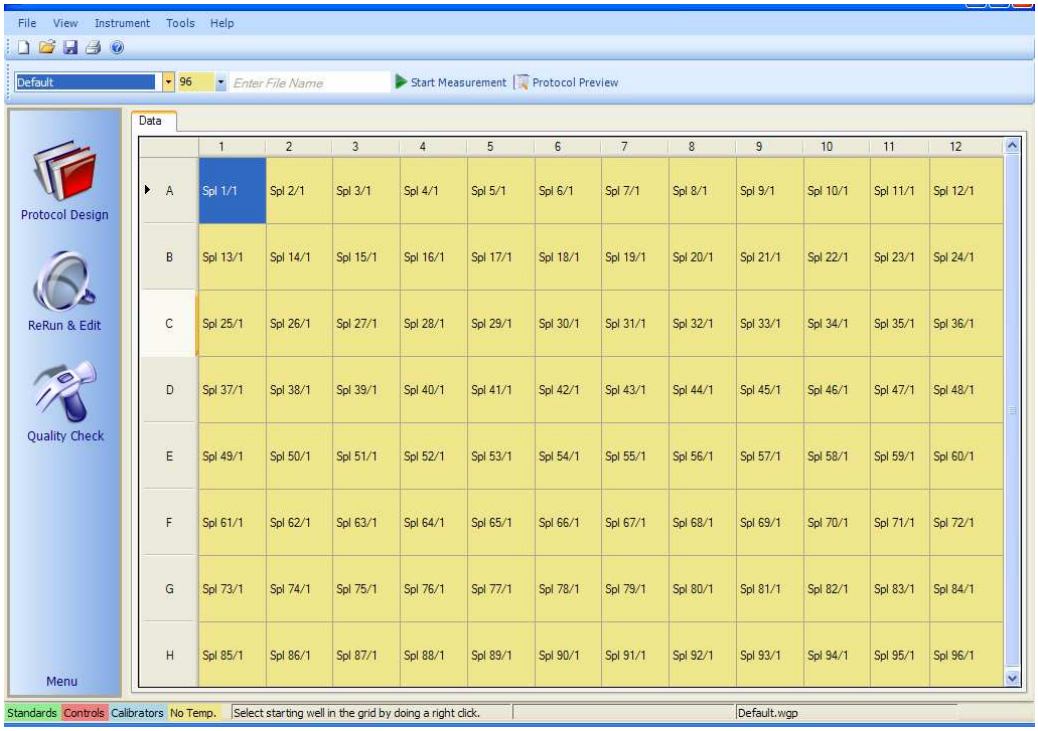
ICE works with 5 file types indicated by the respective file name extensions.

- Protocol files have the extension **.wgp**
- Data files have the extension **.wgd**
- Standard curve files have the extension **.wgs** (to be used as reference curves)
- Multiple Analyte profiles have the extension **.wgm**
- Customized prime sequences have the extension **.wge**

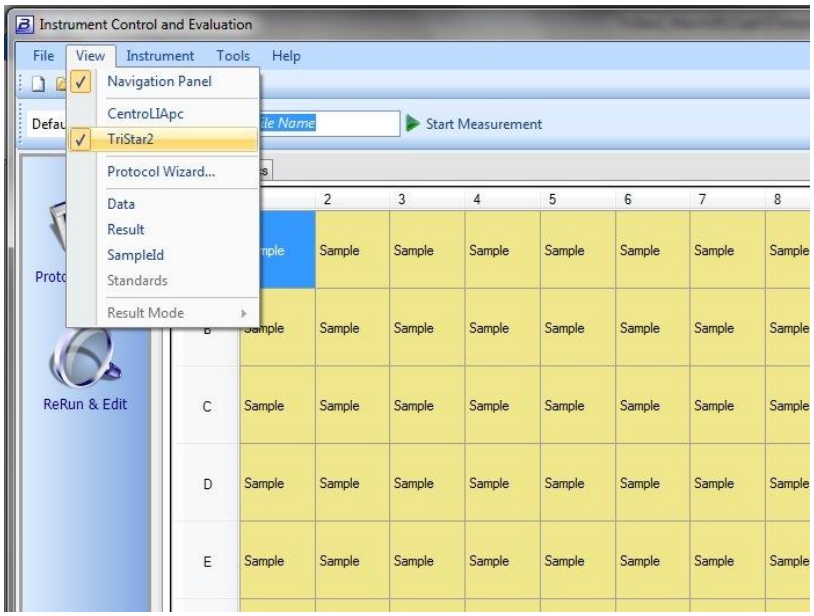
6.2 ICE User Interface

6.2.1 ICE default set up

The next figure shows the default start-up screen of ICE.

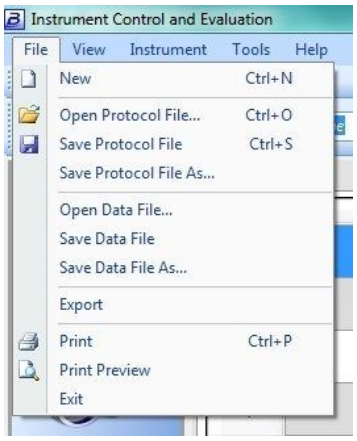


To return to the default layout after any changes may have been made check **Navigation Panel** and **TriStar²** in the **View** menu.



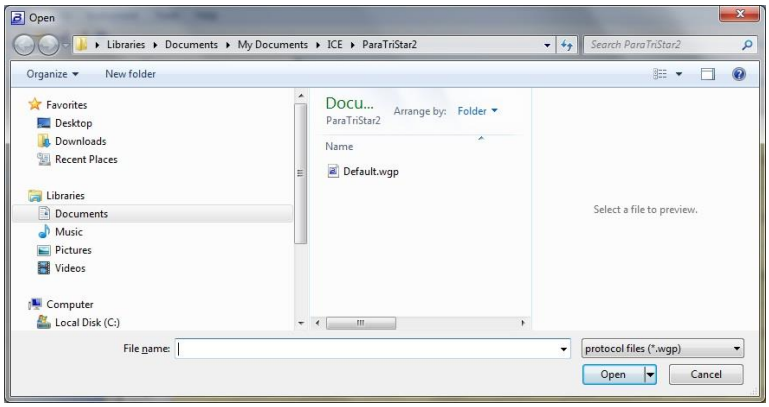
6.2.2 File menu

The **File** menu contains commands to open and save data and protocol files.



New erases data display to start a new measurement

Open Protocol File… opens an existing protocol



Save Protocol File saves loaded protocol file

Save Protocol File As… saves loaded parameter settings with a new name

Open Data File opens an existing measurement

Save Data File saves displayed data

Save Data File As… saves displayed data with a new name

Export exports the data set as EXCEL file according to the settings made in the protocol

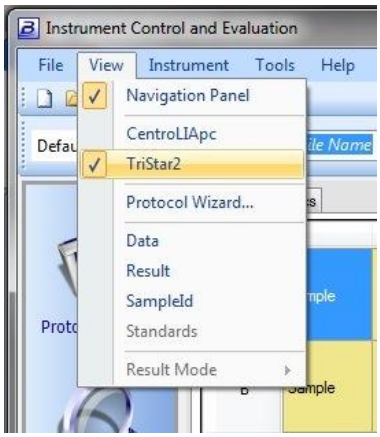
Print prints the selected data set shown on the screen

Print Preview displays a preview of the print-out

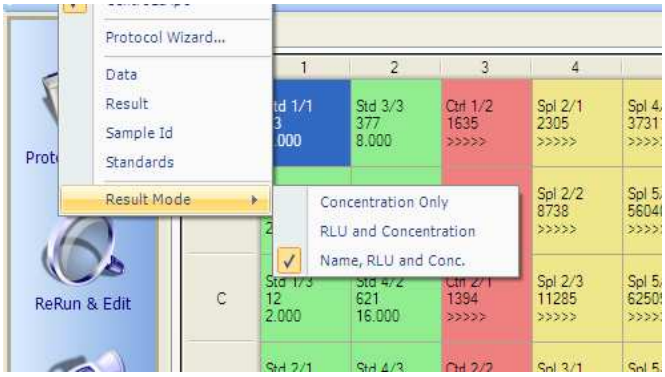
Exit closes ICE software

6.2.3 View menu

The **View** menu defines how the user interface and data are displayed.

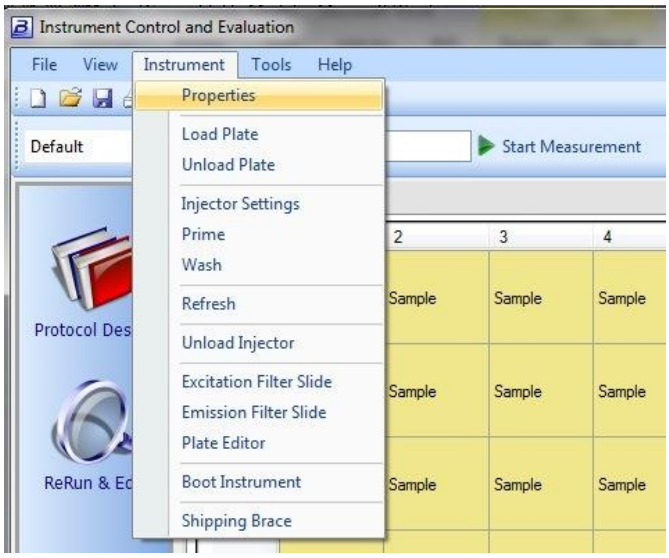


Navigation Panel	shows/hides navigation panel on the left
TriStar²	adjusts user interface for TriStar²
Protocol Wizard	starts wizard for protocol creation
Data	displays raw data (RLU or RLU/s)
Result	displays calculated data
Sample ID	displays sample IDs
Standards	displays standard concentrations
Result Mode	to select the content of the result display

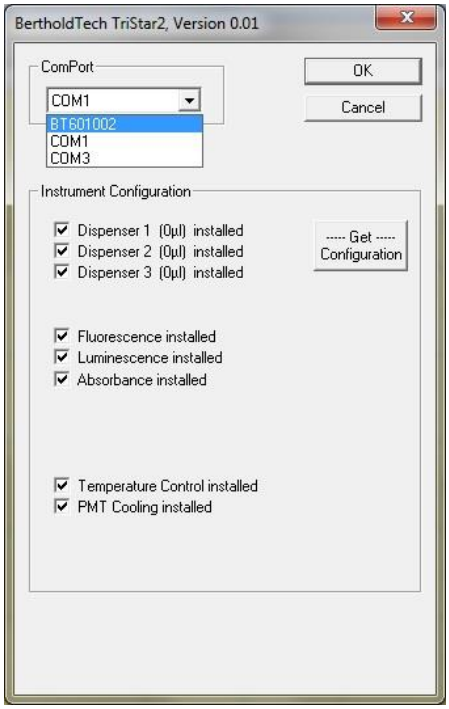


6.2.4 Instrument menu

In the **Instrument** menu basic instrument settings and communication may be accessed.



Properties instrument driver settings



Load Plate moves plate into the instrument

Unload Plate moves plate out of the instrument

Injector Settings general setting for wash and prime sequences

Prime starts the priming sequence (filling the lines)

Wash starts the washing sequence (cleaning the lines)

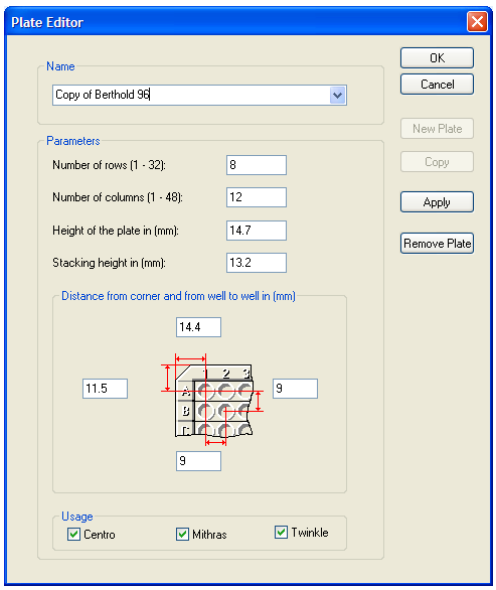
Refresh injects once to fill the tip (e.g. after longer periods of idleness)

Unload Injector starts the unloading sequence (recovering reagents back into the reservoir)

Excitation Filter Slide dialogue for definition and positioning of excitation filters

Emission Filter Slide dialogue for definition and positioning of emission filters

Plate Editor dialogue for definition of microplate dimensions



Note: only 6 to 384 well plates are supported in the TriStar²

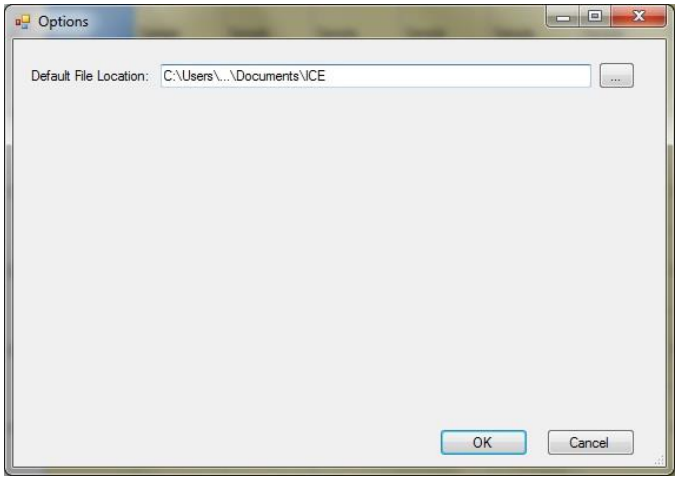
Note: only plate heights of up to 21 mm are supported in the TriStar²

Boot Instrument establishes communication and boots instrument

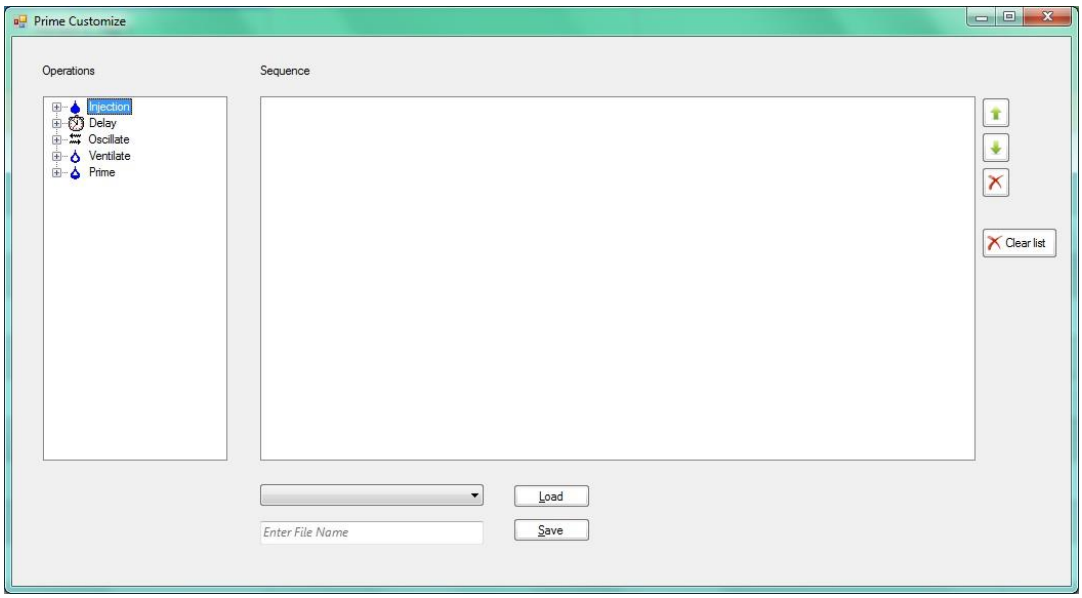
Shipping Brace moves XY table to a position enabling the insertion of the transportation lock

6.2.5 Tools menu

In the **Tools** | **Options** menu you can define the default root directory for the protocol (***ParaTriStar2***) and data (***DataTriStar2***) folders.



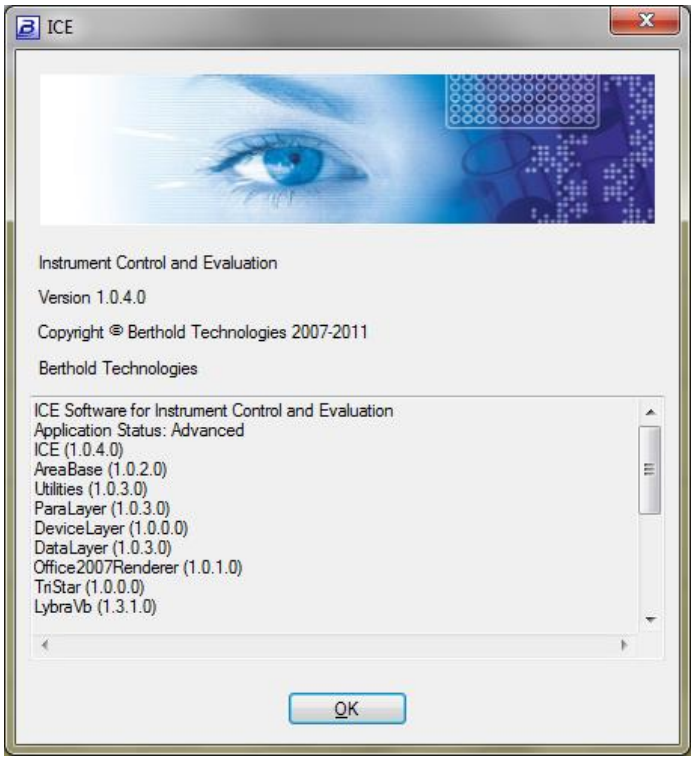
The **Tools** | **Prime Customize** menu user and/or application specific prime procedures can be defined.



For the setting and options please read chapter "Priming Tubings"

6.2.6 Help menu

The **Help** menu allows you to view basic software information.



7. Operation with ICE

Running measurements on the TriStar² is straight forward. The procedure is the same for all types of assay types, e.g. Raw Data, Dual Label, Kinetic, Repeated and Scanning. A measurement can be carried out immediately after a stored protocol is selected. At the end of each measurement the results are stored and may be printed or exported.

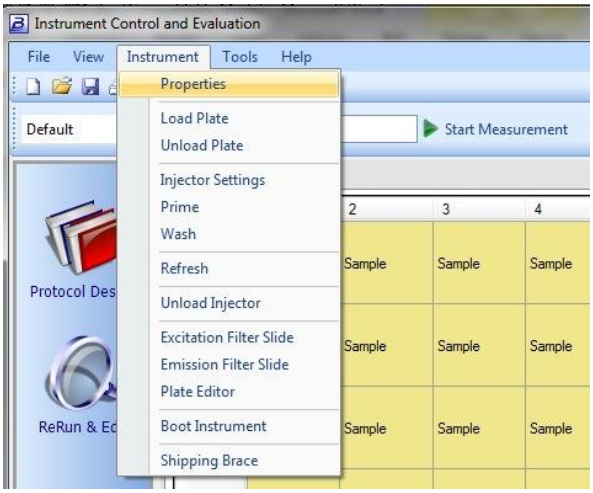
Result file names can be given without limitation. The extension is fixed, though. This is valid for measurement protocols as well.

7.1 Adding and Editing Microplate Dimensions

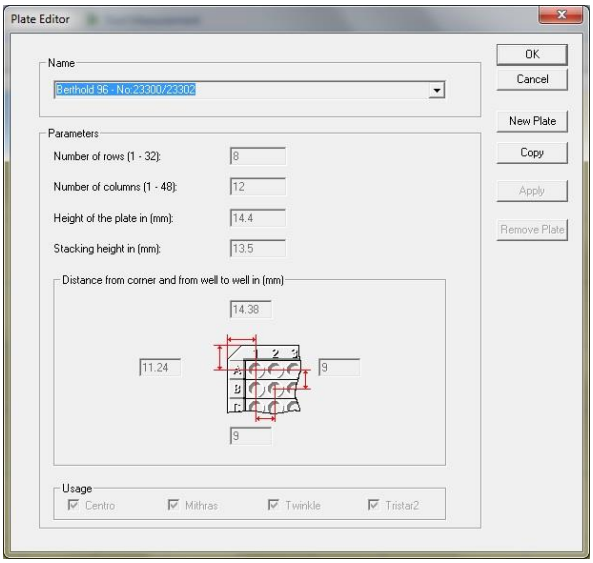
Microplates can differ in their dimensions dependent on brand and type. Please refer to the manufacturer´s most recent information for exact dimensions of the microplates.

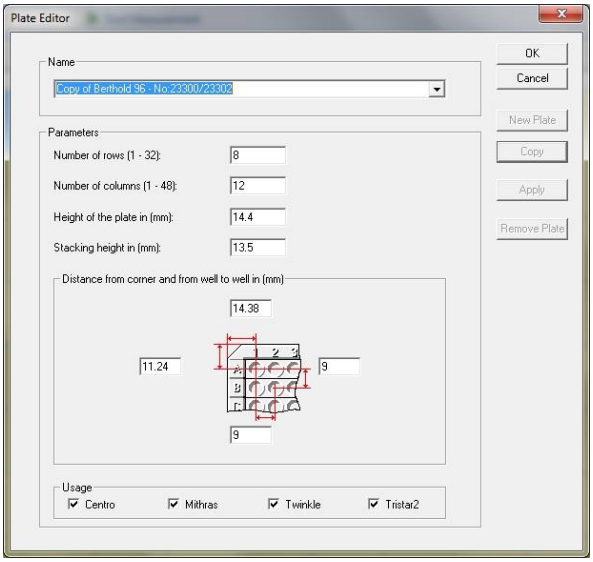
Microplates must be defined in the plate editor prior to defining a measurement protocol.

1. Click **Plate Editor** in the **Instrument** menu



2. Click **<New Plate>** or select a plate with matching well format and click **<Copy>**





3. Assign a (descriptive) **Name**
4. Insert the **Number of rows**, e.g. ***8*** for a 96 well plate
5. Insert the Number of columns, e.g. ***12*** for a 96 well plate
6. Insert the total **Height** of the microplate

   most 96 and 384 well plates are between 14 and 15.5 mm
7. Insert the **Stacking height** of the microplate

   the stacking height is the resulting height (the visible part) when plates are put on top of each other (e.g. in a plate stacker)

   in case this information is not available from the plate manufacturer the stacking height can be derived by stacking 2 plates and measuring the total height; by subtracting the regular height of one of the plates the resulting value will be the stacking height
8. Insert the distance between the left outer edge of the plate and the center of well A1
9. Insert the distance between to upper outer edge of the plate and the center of well A1
10. Insert the distance between the well centers of consecutive rows (vertical well distance)
11. Insert the distance between the well centers of consecutive columns (horizontal well distance)
12. Check the usage ***TriStar2***

    you may check additional instruments in case you have multiple instruments in operation
13. Click **<Apply>**
14. Click **<OK>**

15. The plate can now be used in the protocol files

7.2 Single Raw Data measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine ATP content, single reporter gene expression, activities of caspases, kinases and many other enzymes.

7.2.1 Defining a Single Endpoint protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Click icon **Protocol Design** in the left-hand **Navigation** bar

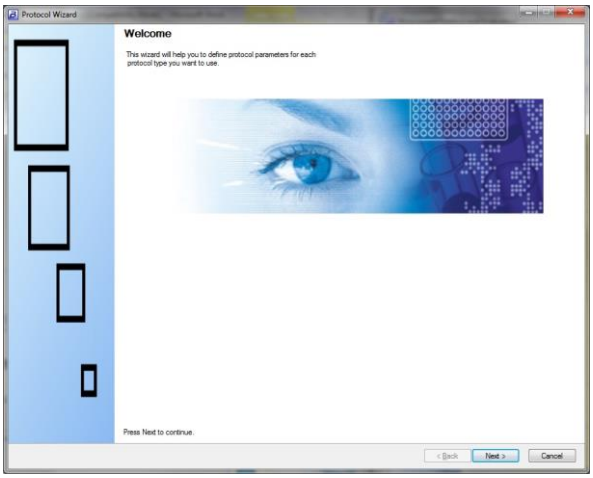
    the navigation bar will appear in a new design

17. Click the **new** icon in the left-hand **Navigation** bar

    again, the navigation bar will appear in a new design

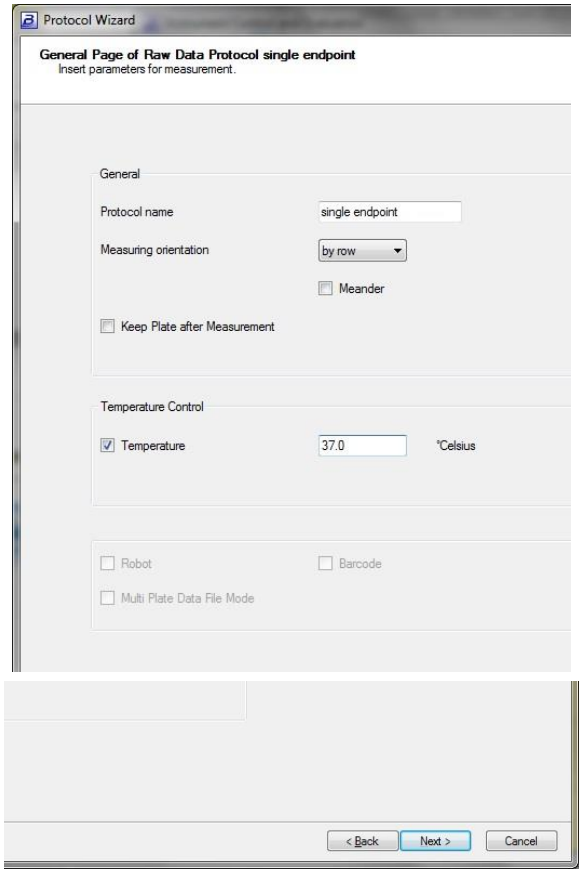
    for editing an existing protocol use the **edit** icon

18. The start up screen of the protocol wizard will show up
    Click **<Next>**



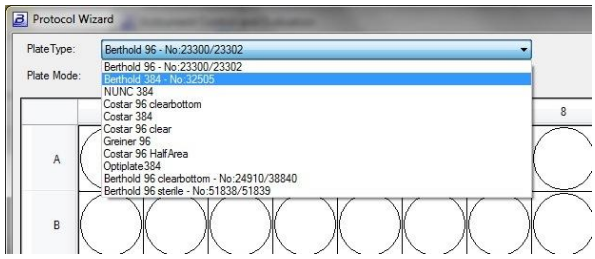
19. Enter a (descriptive) **Name** for your protocol
20. Define the **reading orientation**:
    by column or by row
21. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
22. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
23. Check **Temperature** to activate the temperature control for this protocol
24. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active
25. Click **<Next>**



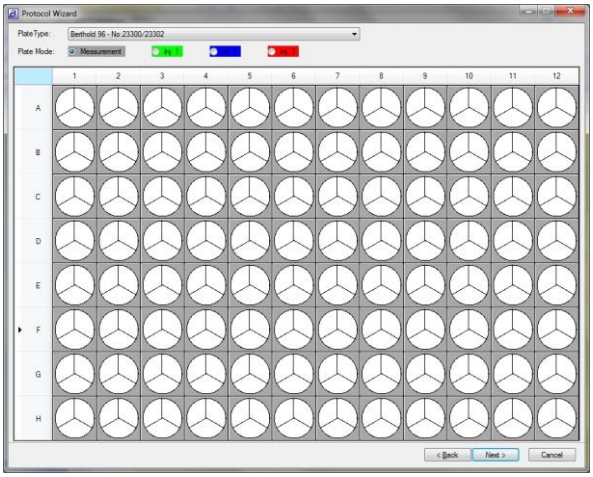
26. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



27. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

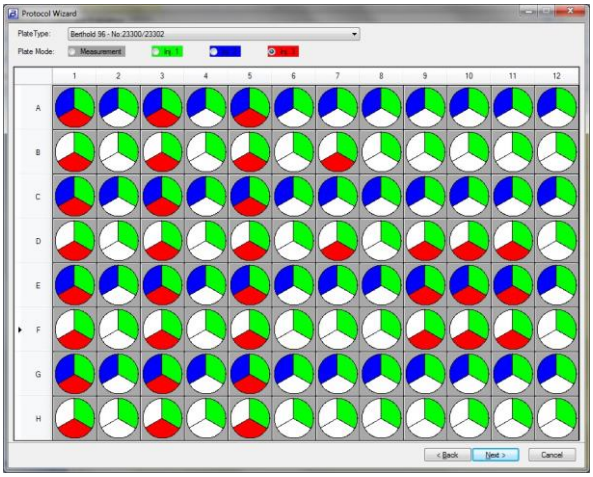
    Wells with a gray outside area are selected for measurement



28. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

    Wells coloured in the respective colour are injected into

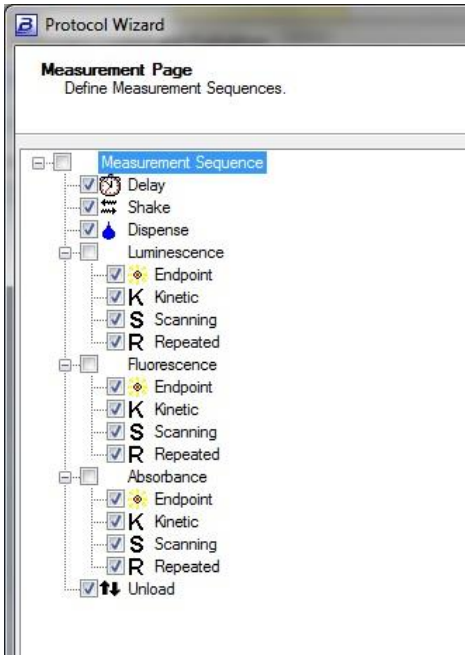
    Note: only wells to be measured can be injected into



29. Click **<Next>**

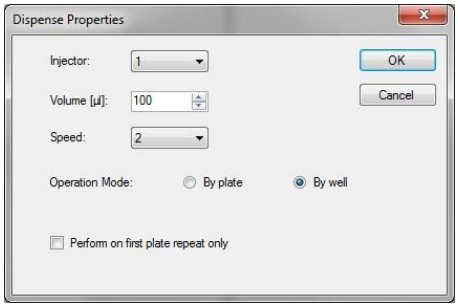
Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking **<OK>** selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well



30. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well



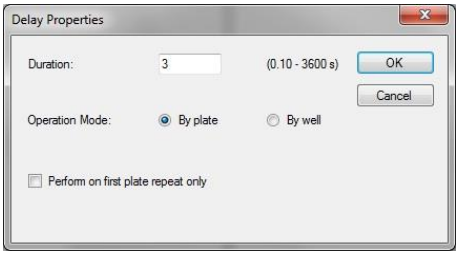
31. Click **<OK>**

    In case additional reagent additions are required repeat this procedure for the other injector(s)

32. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

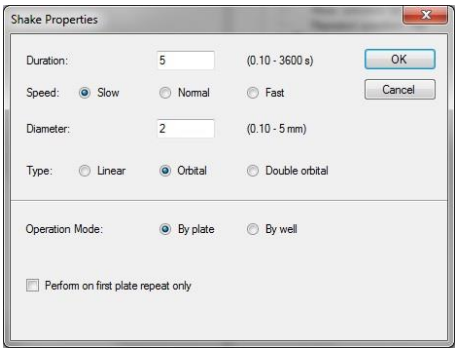
33. Click **<OK>**



34. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

35. Click **<OK>**



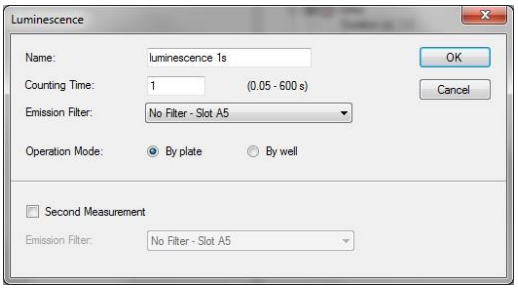
36. Double-click ***Endpoint*** in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter

Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well

37. Click **<OK>**



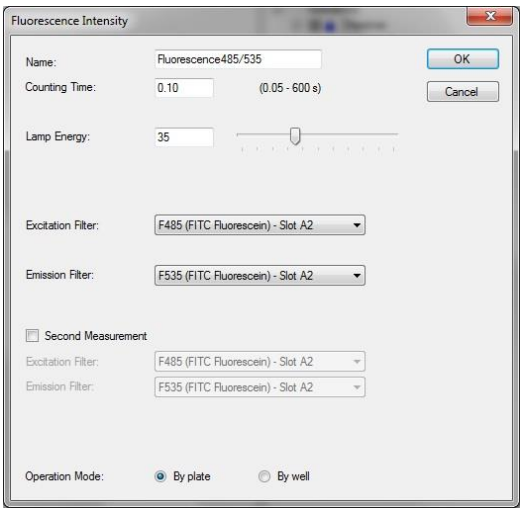
38. Double-click ***Endpoint*** in the Fluorescence section for a fluorescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

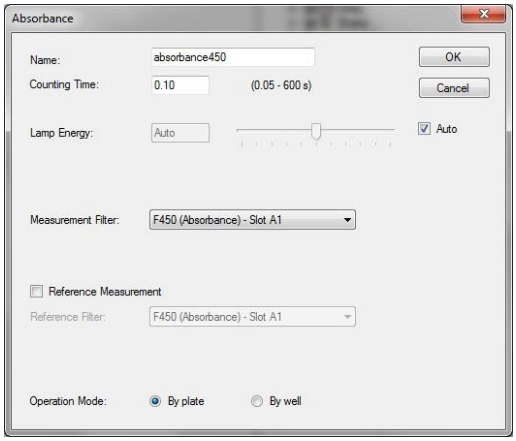
Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well

39. Click **<OK>**



40. Double-click ***Endpoint*** in the Absorbance section for an absorbance reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Excitation Filter	select from the list

Note: filters must be defined prior in the Instrument menu

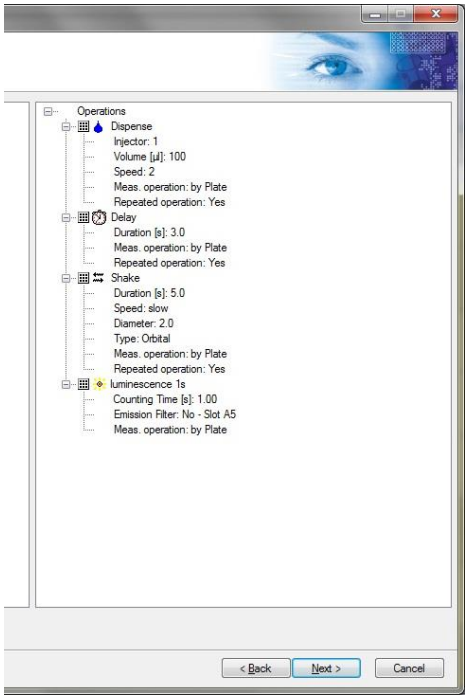
Reference Measurement

Note: the values derived with this filter will be automatically subtracted from the measurement value per well

Reference Filter	select from the list
Operation Mode	by plate or by well

41. Click **<OK>**

42. The **sequence of selected operations** will be displayed on the right-hand side

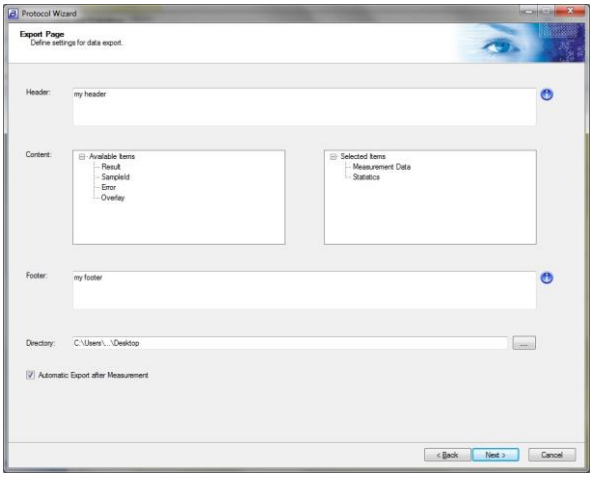


Operations can be moved up or down by clicking on the operation and dragging them to the respective position

Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

43. Click **<Next>**

44. Define **Export** settings



Type your **Header** specific for this protocol

Select the data set by dragging from left to right

Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

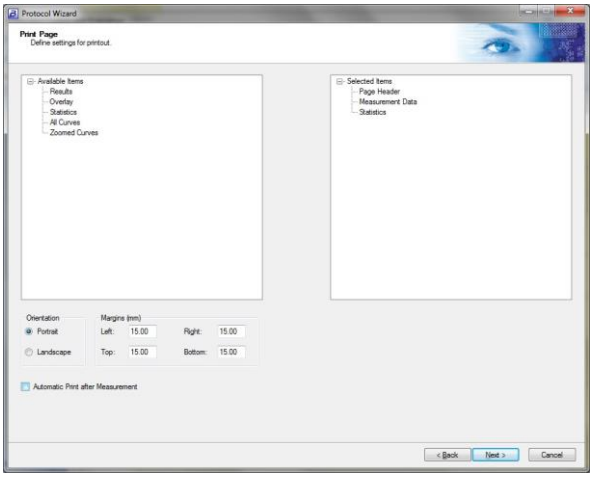
Type your **Footer** specific for this protocol

Define **Directory** for the export file

Check if **Automatic Export** is required

45. Click **<Next>**

46. Define **Print** settings



Select the data set by dragging from left to right

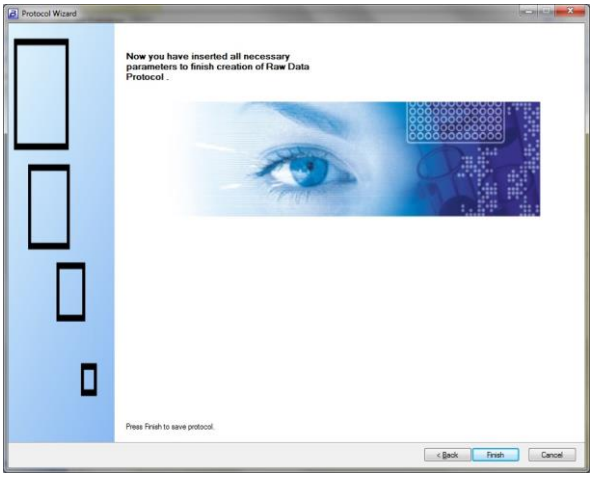
Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

Define **page orientation** and **margins**

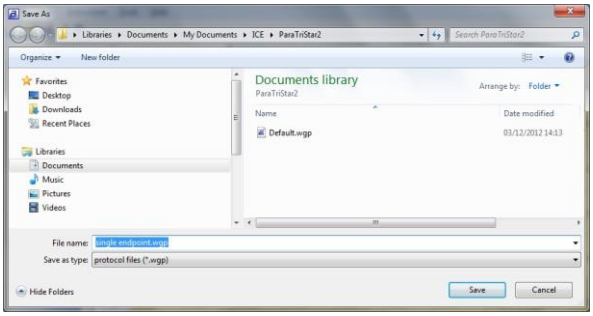
Check if **Automatic Print-out** is required

47. Click **<Next>**

48. Click **<Finish>**



49. Define the protocol **file name**
50. Click **<Save>**



7.2.2 Measurement with a Single Endpoint protocol

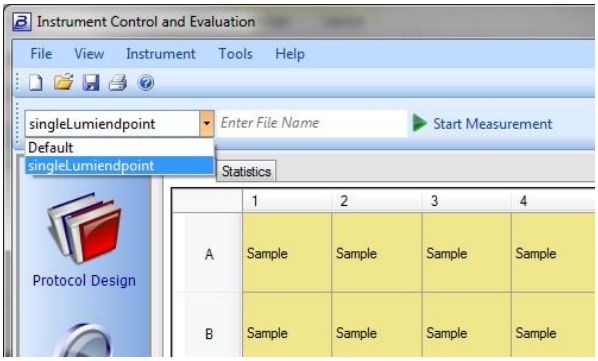
The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

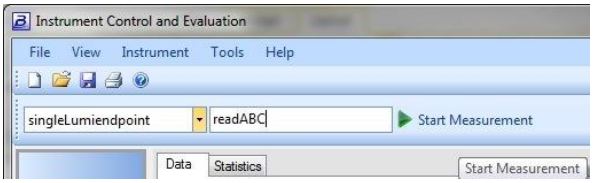
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

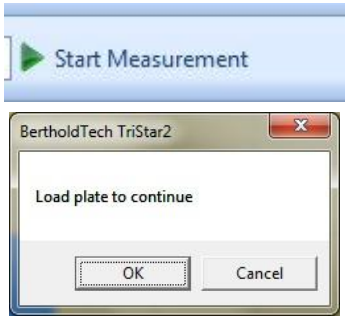
1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**

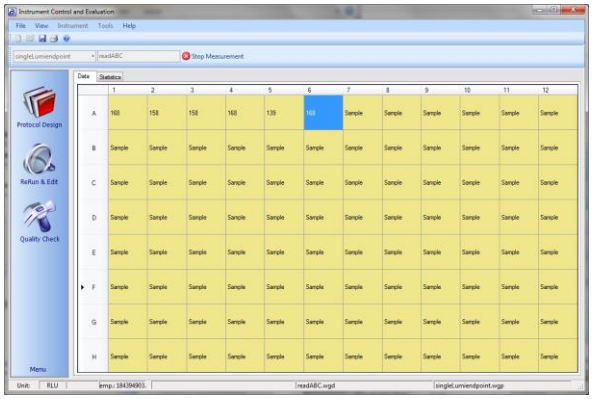


4. Insert the **microplate** with your samples:
   well A1 facing the rear and left

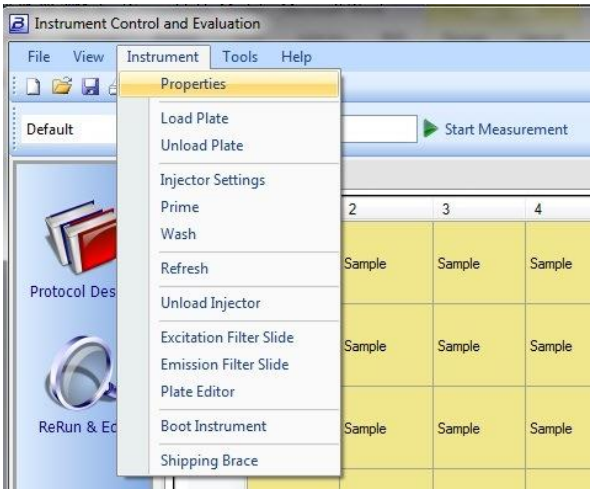
   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates
5. Click **<OK>**

6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed



7. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.3 Dual Label Assay measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine dual reporter gene expression.

7.3.1 Defining a Dual Label protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

1. Click icon **Protocol Design** in the left-hand **Navigation** bar

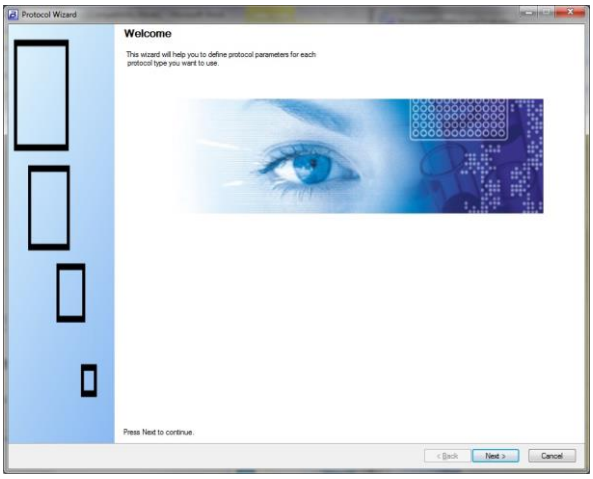
   the navigation bar will appear in a new design

2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

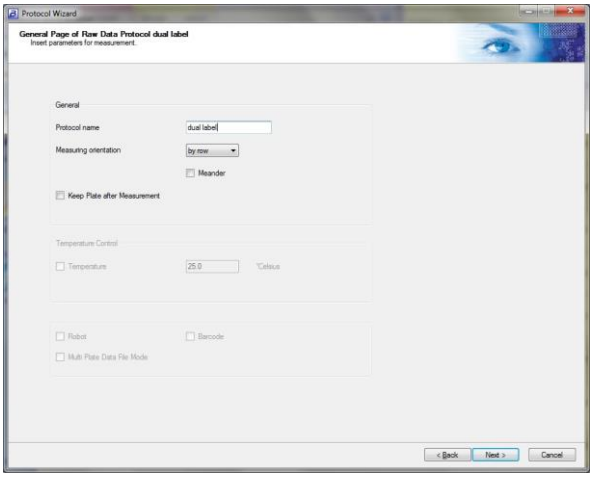
   for editing an existing protocol use the **edit** icon

3. The start up screen of the protocol wizard will show up
   Click **<Next>**



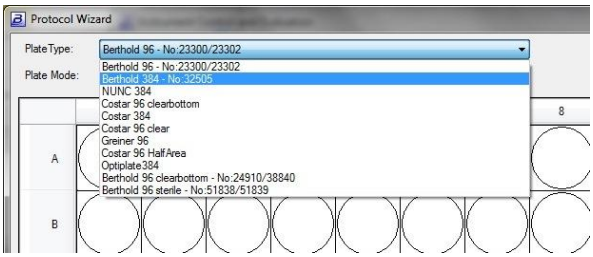
4. Enter a (descriptive) N**ame** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



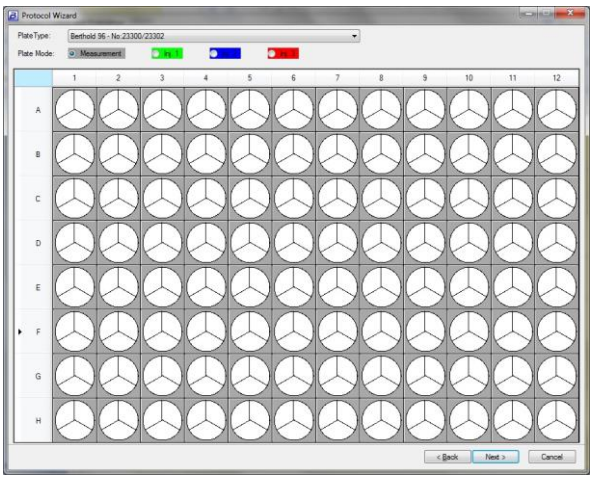
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

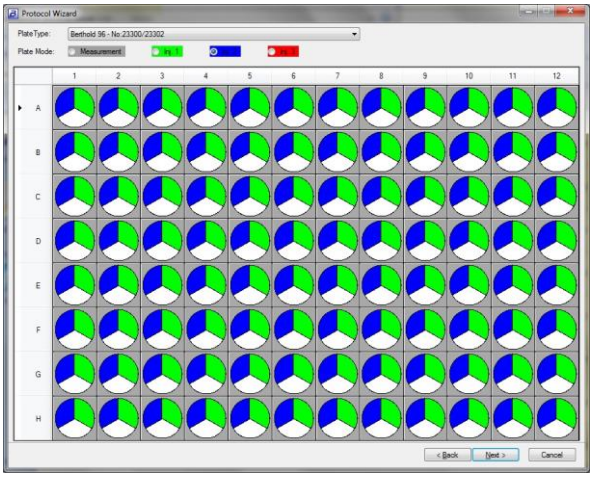
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

    Wells coloured in the respective colour are injected into

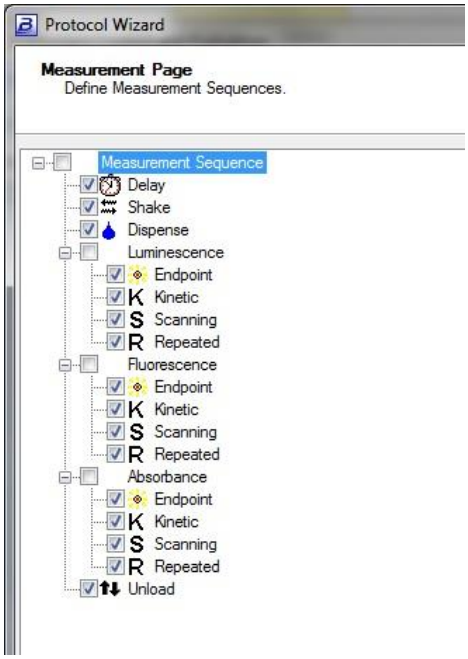
    Note: only wells to be measured can be injected into



14. Click **<Next>**

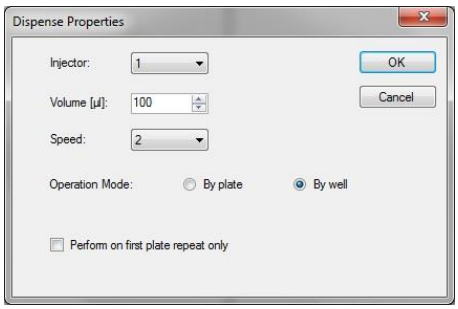
Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well



15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well



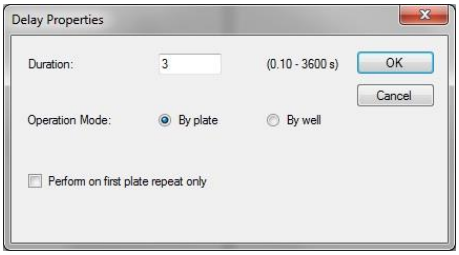
16. Click **<OK>**

    In case additional reagent additions are required repeat this procedure for the other injector(s)

17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

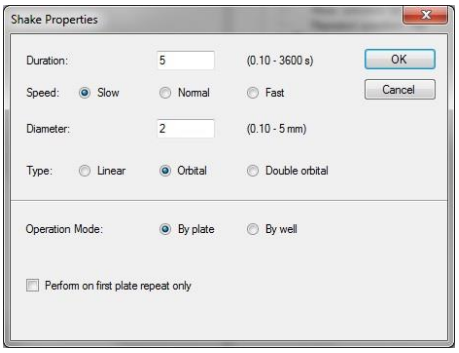
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



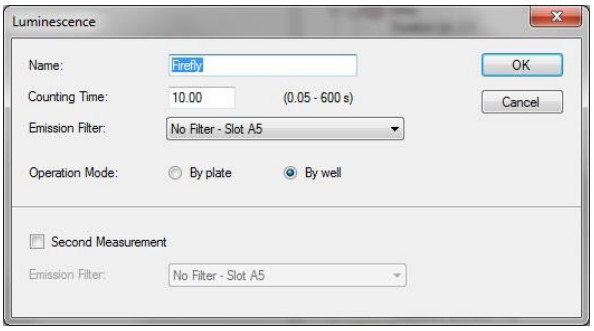
21. Double-click ***Endpoint***, e.g. in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter

    Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well

22. Click **<OK>**



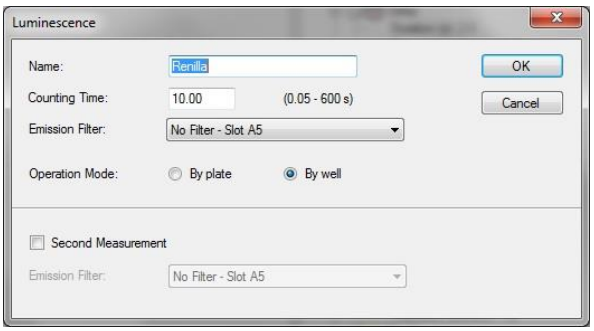
23. Once more double-click ***Endpoint*** , e.g. in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter

    Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well

24. Click **<OK>**

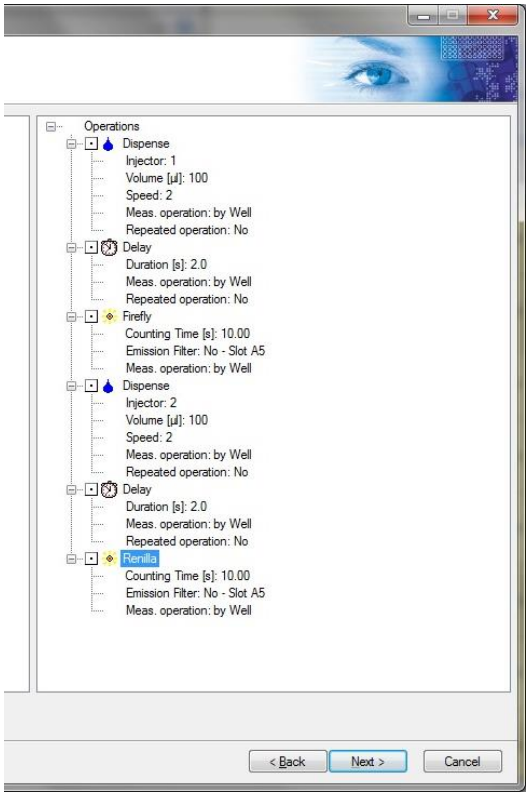


25. The **sequence of selected operations** will be displayed on the right-hand side

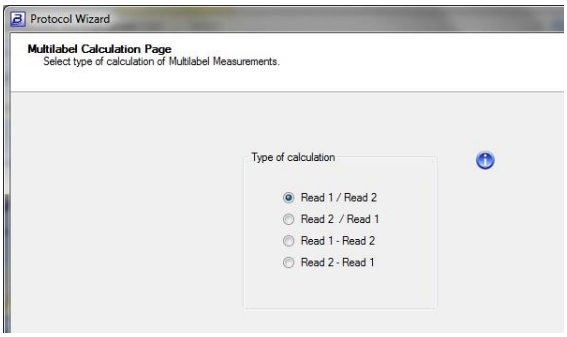
    Operations can be moved up or down by clicking on the operation and dragging them to the respective position

    Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

26. Click **<Next>**



27. Select the calculation to perform with the 2 measurements
28. Click **<Next>**



29. Define **Export** settings

    Type your **Header** specific for this protocol

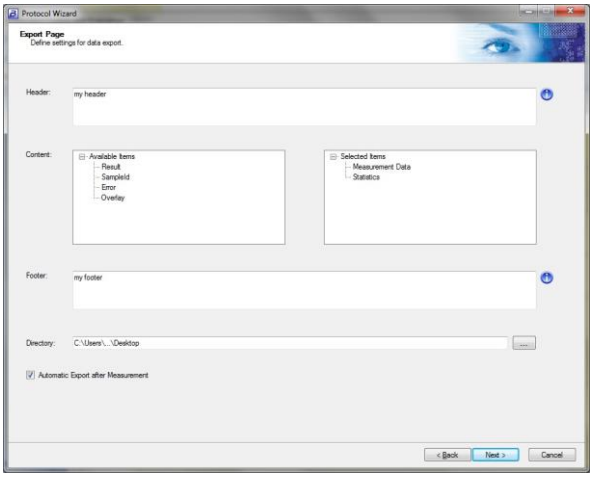
    Select the data set by dragging from left to right

Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required



30. Click **<Next>**

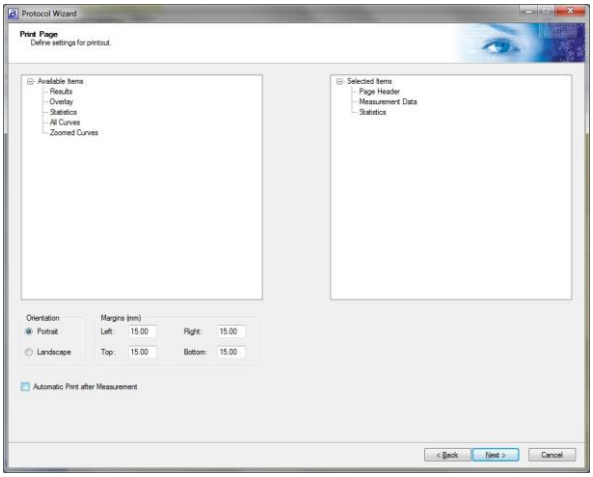
31. Define **Print** settings

    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

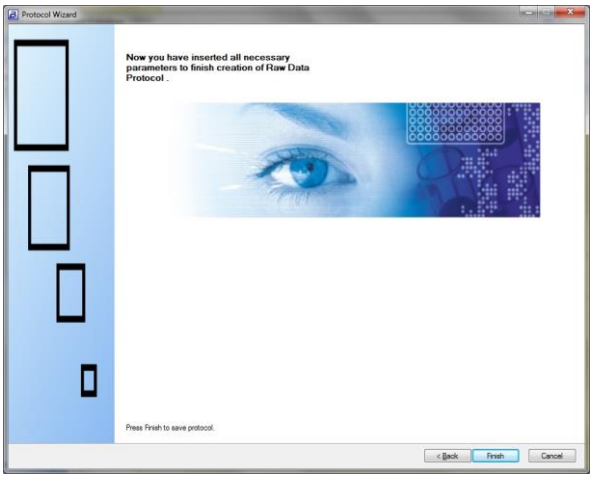
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

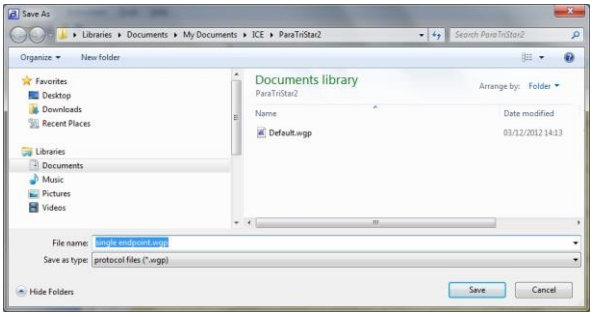


32. Click **<Next>**

33. Click **<Finish>**



34. Define the protocol **file name**
35. Click **<Save>**



7.3.2 Measurement with a Dual Label Assay protocol

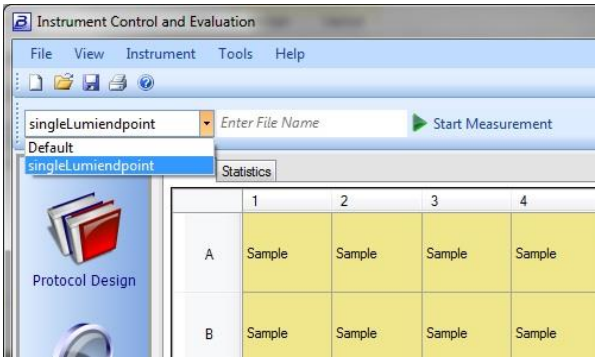
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

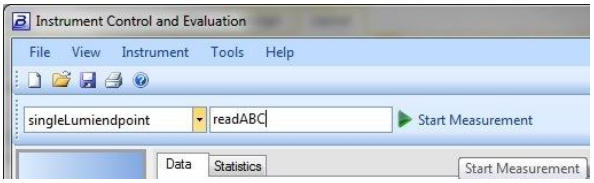
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**

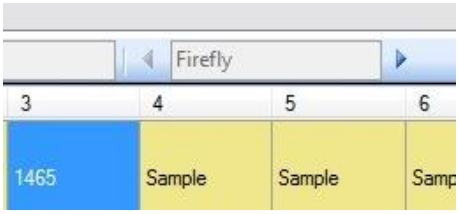
4. Insert the **microplate** with your samples:
   well A1 facing the rear and left

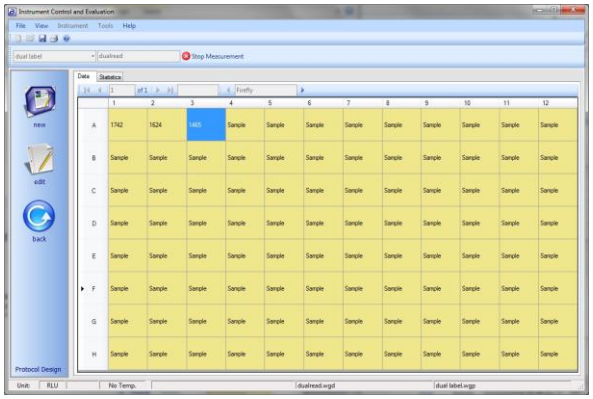
   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates
5. Click **<OK>**

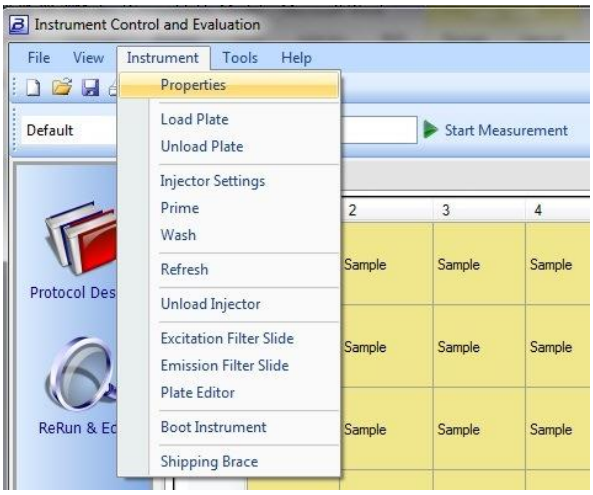
6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

   You may switch between the two readings by clicking on the arrows





7. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.4 Kinetic Measurement

A kinetic measurement mode is appropriate for fast kinetics assays lasting over several seconds up to minutes, e.g. enzyme kinetics and Calcium influx

7.4.1 Defining a protocol for a kinetic measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

1. Click icon **Protocol Design** in the left-hand **Navigation** bar

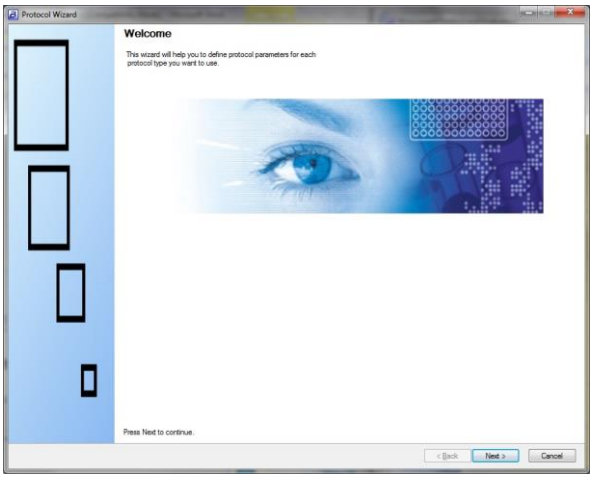
   the navigation bar will appear in a new design

2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

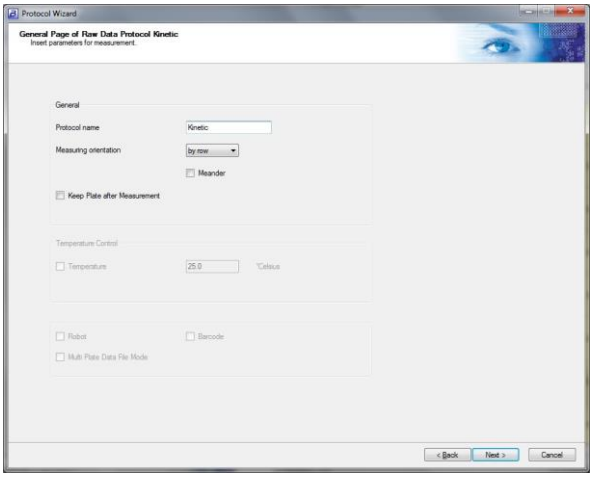
   for editing an existing protocol use the **edit** icon

3. The start up screen of the protocol wizard will show up
   Click **<Next>**



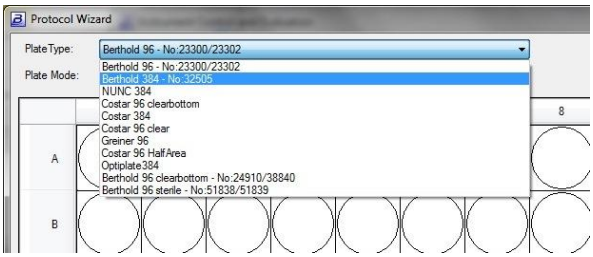
4. Enter a (descriptive) **Name** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



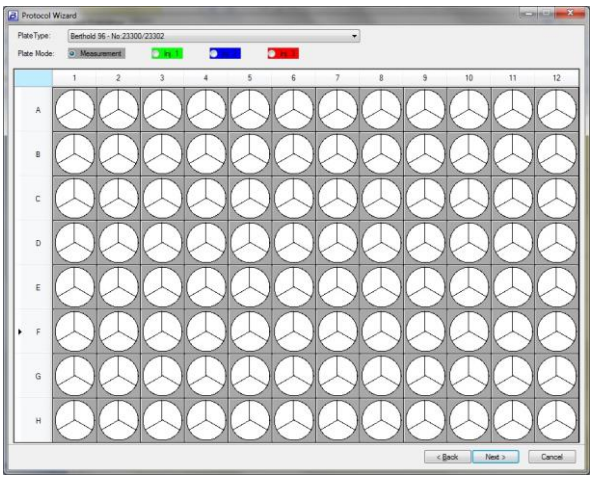
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

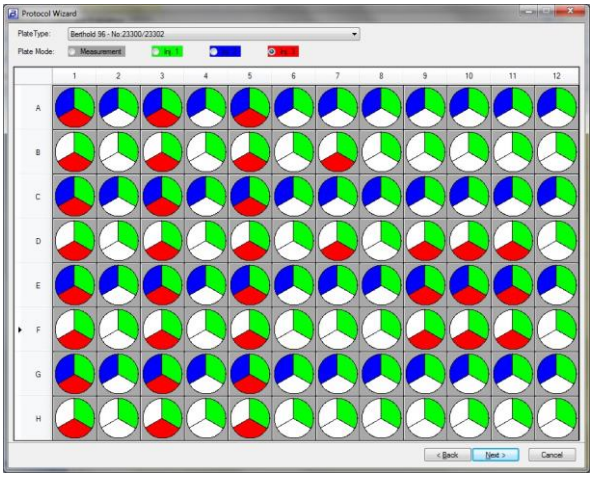
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

    Wells coloured in the respective colour are injected into

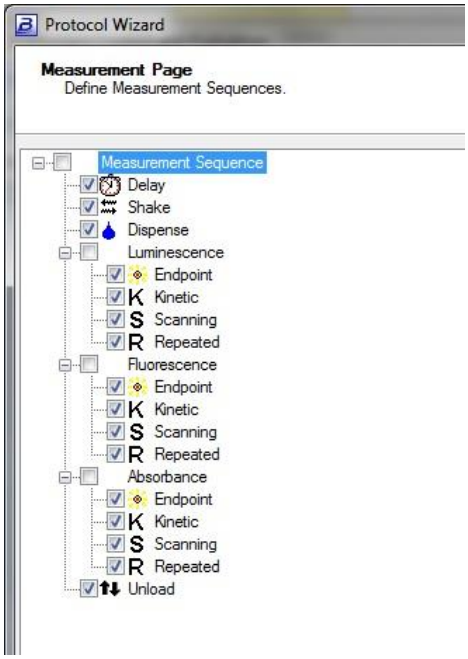
    Note: only wells to be measured can be injected into



14. Click **<Next>**

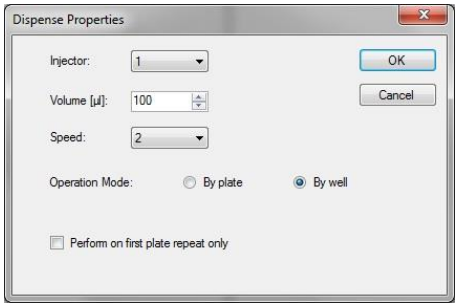
Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well



15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well



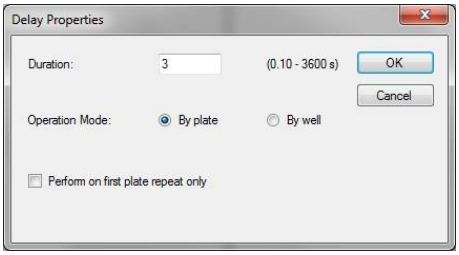
16. Click **<OK>**

    In case additional reagent additions are required repeat this procedure for the other injector(s)

17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

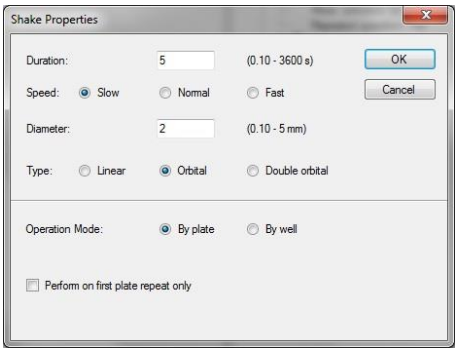
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Kinetic*** in the Luminescence section for a luminescence kinetic reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s

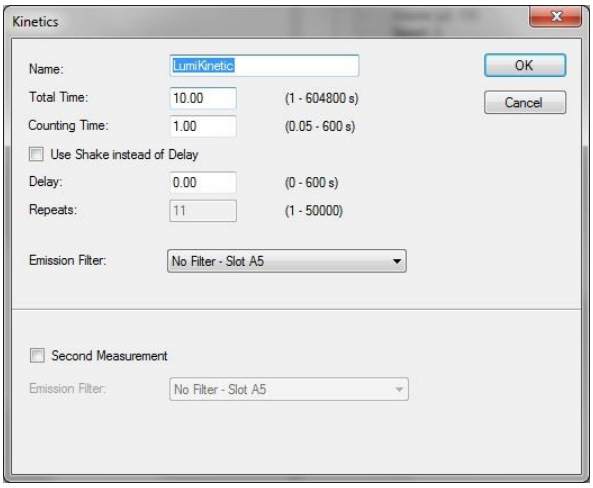
    Check Use Shake instead of Delay if needed

Delay	0 to 600 sec
Repeats	(are calculated)
Emission Filter	usually: No Filter

    Note: filters must be defined prior in the Instrument menu

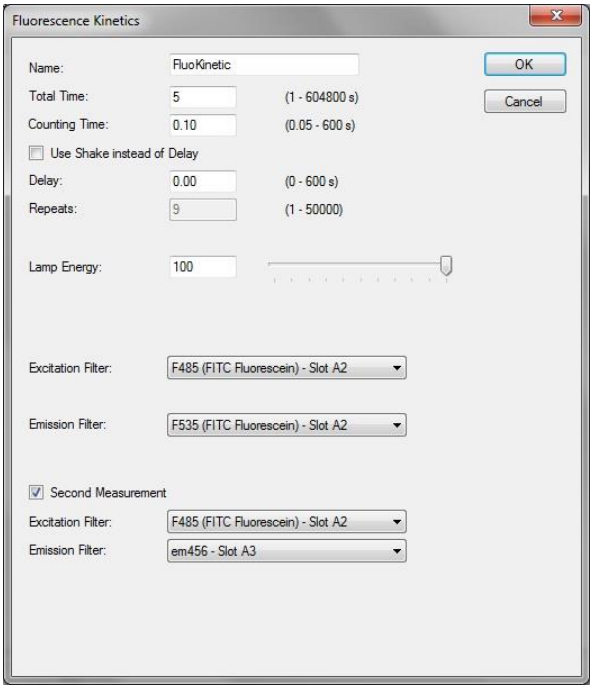
    Second Measurement may be checked in case of ratiometric kinetics, e.g. in BRET applications

22. Click **<OK>**



a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

23. Double-click ***Kinetic*** in the Fluorescence section for a fluorescence kinetic reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake instead of Delay if needed	
Delay	0 to 600 s
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

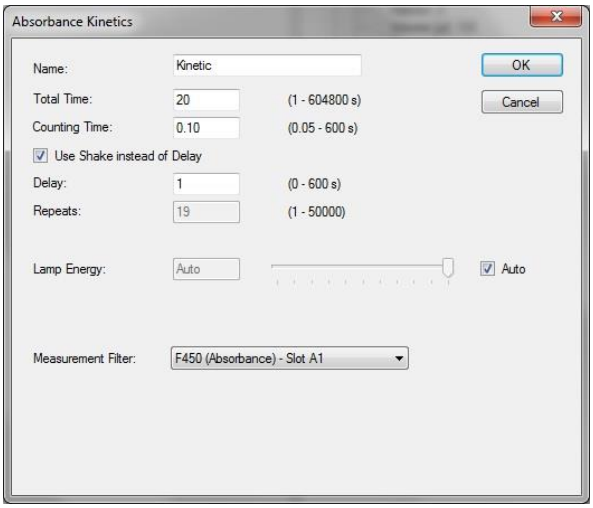
Operation Mode	by plate or by well

Second Measurement may be checked in case of ratiometric kinetics, e.g. in Calcium applications

24. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

25. Double-click ***Kinetic*** in the Absorbance section for an absorbance kinetic reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake instead of Delay if needed	
Delay	0 to 600 s
Repeats	(are calculated)
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter	select from the list

Note: filters must be defined prior in the Instrument menu

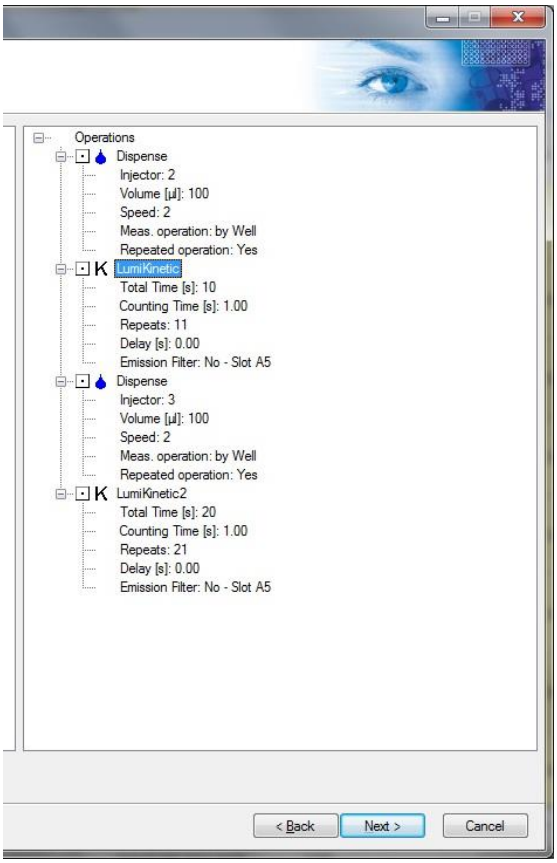
26. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

27. The **sequence of selected operations** will be displayed on the right-hand side

    Operations can be moved up or down by clicking on the operation and dragging them to the respective position

    Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left



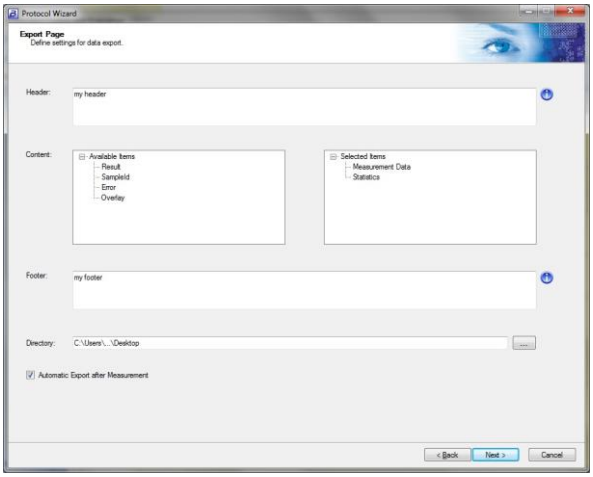
28. Click **<Next>**

29. Define **Export** settings

    Type your **Header** specific for this protocol

    Select the data set by dragging from left to right

Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings



    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required

30. Click **<Next>**

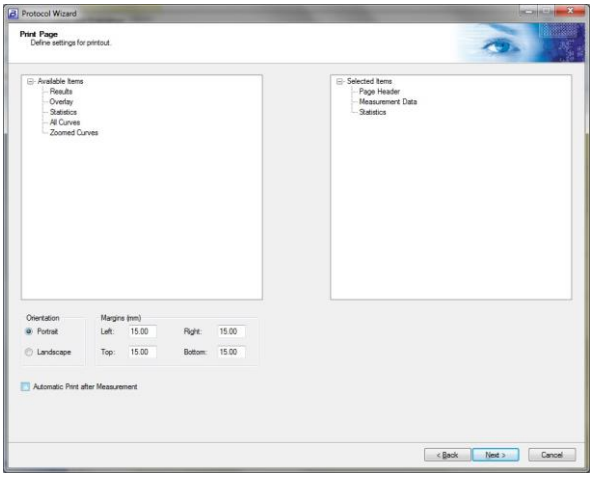
31. Define **Print** settings

    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

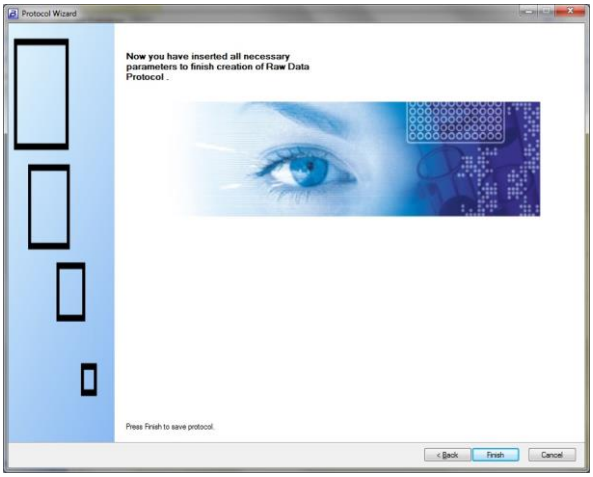
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

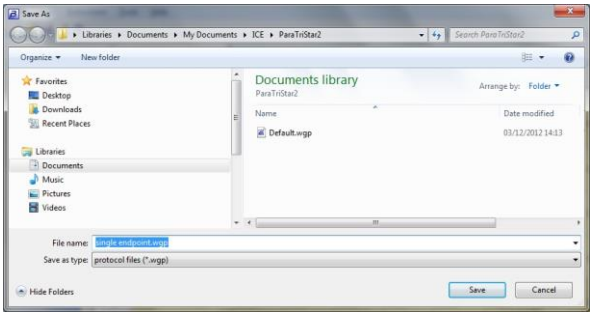


32. Click **<Next>**

33. Click **<Finish>**



34. Define the protocol **file name**
35. Click **<Save>**



7.4.2 Kinetic measurement

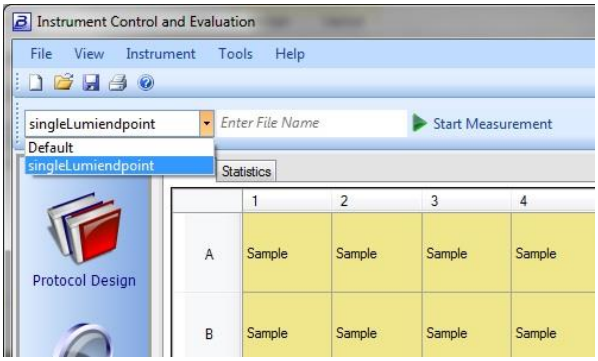
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

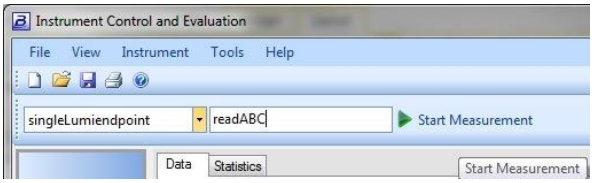
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

8. Select the **protocol** to be used



9. Enter a **file name** under which the measurement is to be stored



10. Click **<Start Measurement>**

11. Insert the **microplate** with your samples:
well A1 facing the rear and left

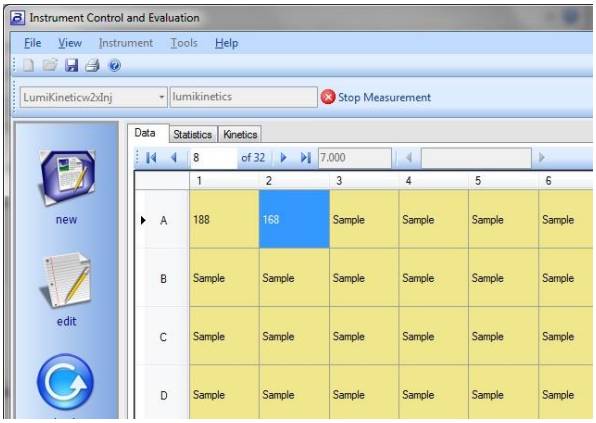
    Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

    Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

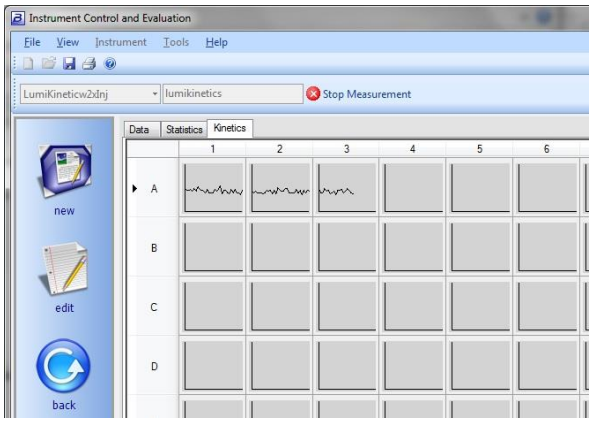
12. Click **<OK>**

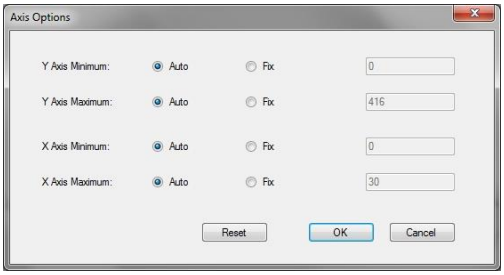
13. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

    You may switch between the individual readings by clicking on the arrows

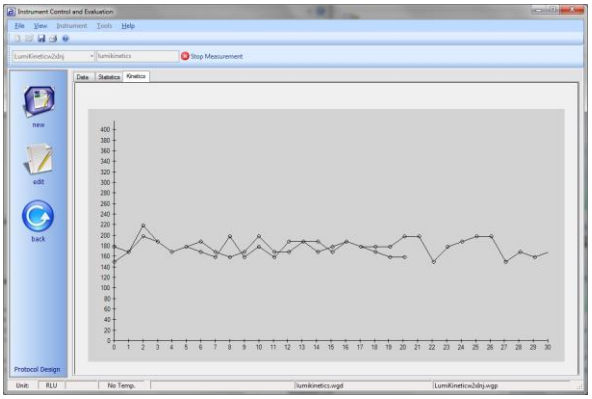


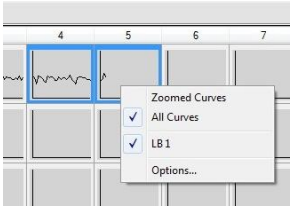
14. You also choose to view the curves by clicking the ***Kinetics*** tab
15. The scale of the axes can be changed by right-clicking into the curves and selecting **Options…**



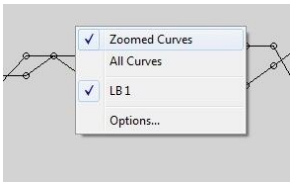


16. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Curves**

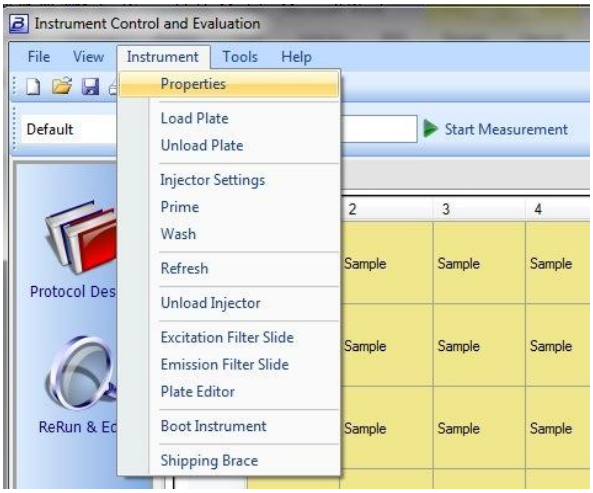




To un-zoom right-click into the zoomed view and select All Curves



17. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.5 Repeated Measurement

A repeated measurement mode is appropriate for long-term kinetic assays lasting over multiple minutes up to several days, e.g. cellular luminescence, slow enzyme kinetics, long-term gene expression or growth monitoring

7.5.1 Defining a protocol for a repeated measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

1. Click icon **Protocol Design** in the left-hand **Navigation** bar

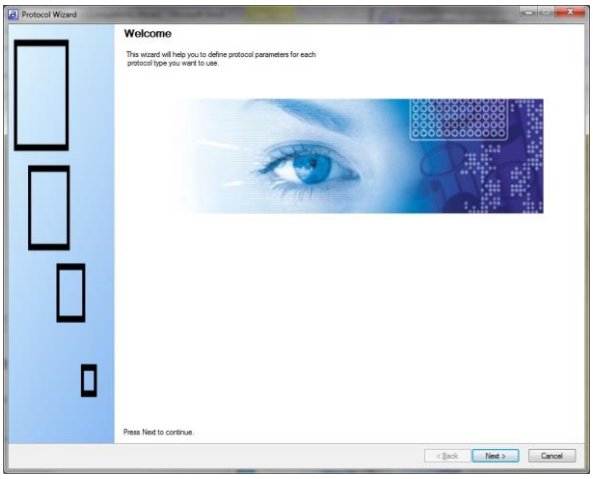
   the navigation bar will appear in a new design

2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

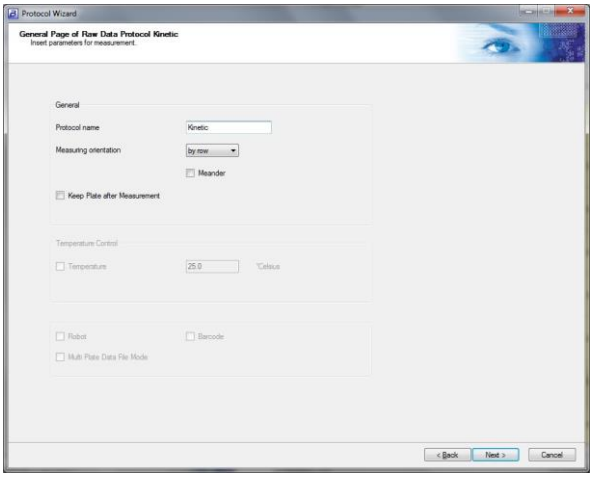
   for editing an existing protocol use the **edit** icon

3. The start up screen of the protocol wizard will show up
   Click **<Next>**



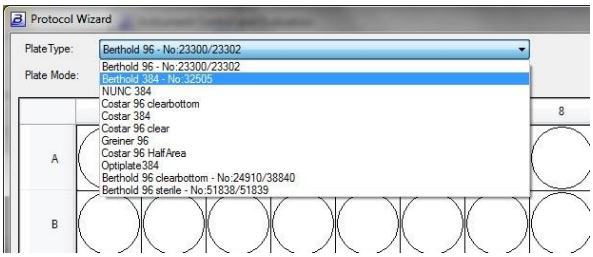
4. Enter a (descriptive) **Name** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



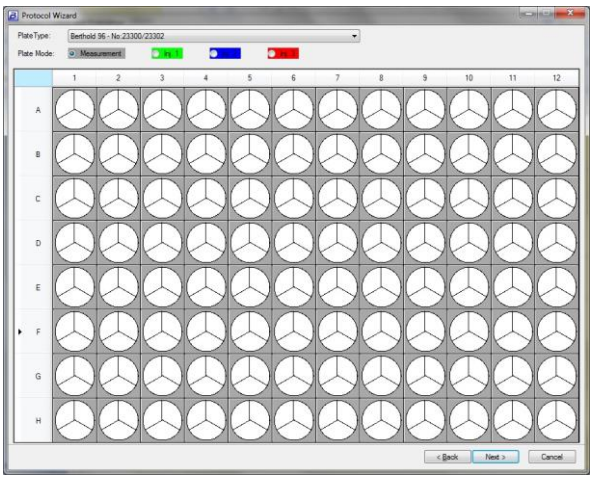
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

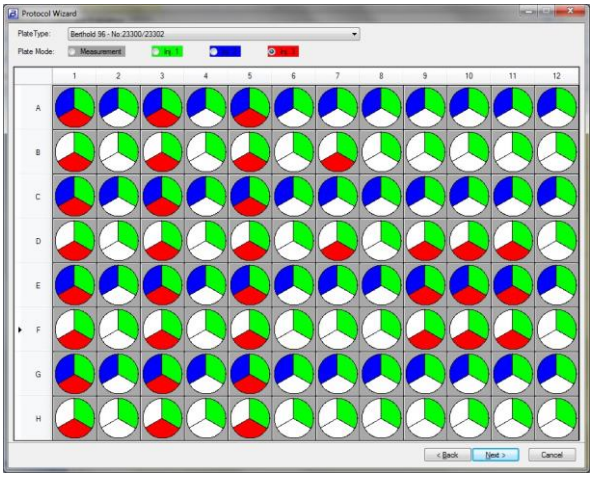
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

    Wells coloured in the respective colour are injected into

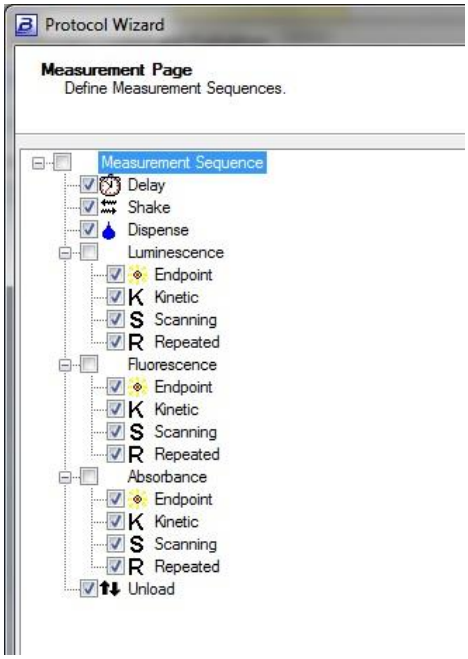
    Note: only wells to be measured can be injected into



14. Click **<Next>**

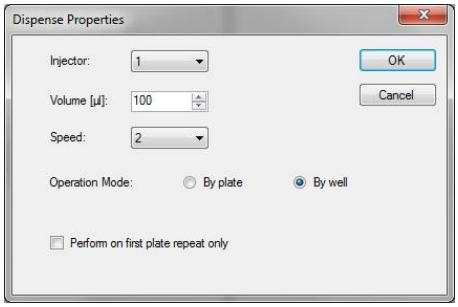
Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well



15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well



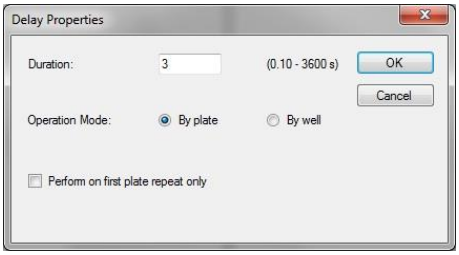
16. Click **<OK>**

    In case additional reagent additions are required repeat this procedure for the other injector(s)

17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

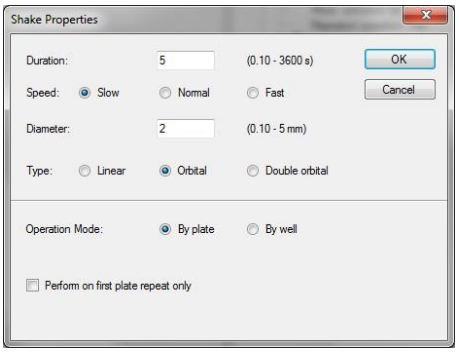
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Repeated*** in the Luminescence section for a luminescence repeated reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Emission Filter	usually: No Filter

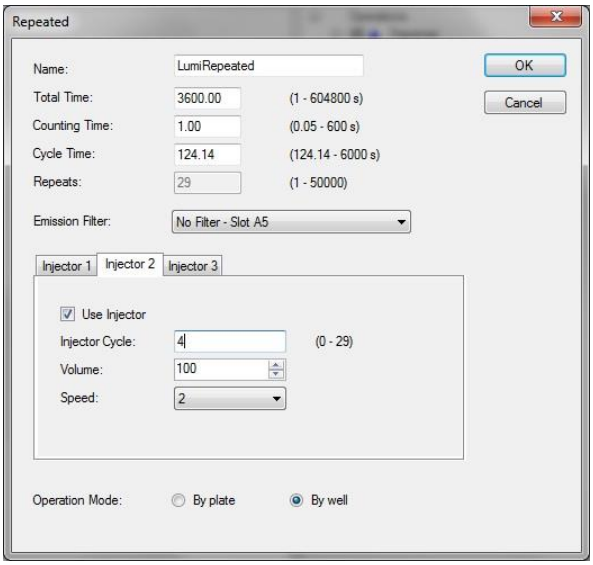
Note: filters must be defined prior in the Instrument menu

Injector 1, …2, …3

Check Use Injector for an injection within the repeated cycle

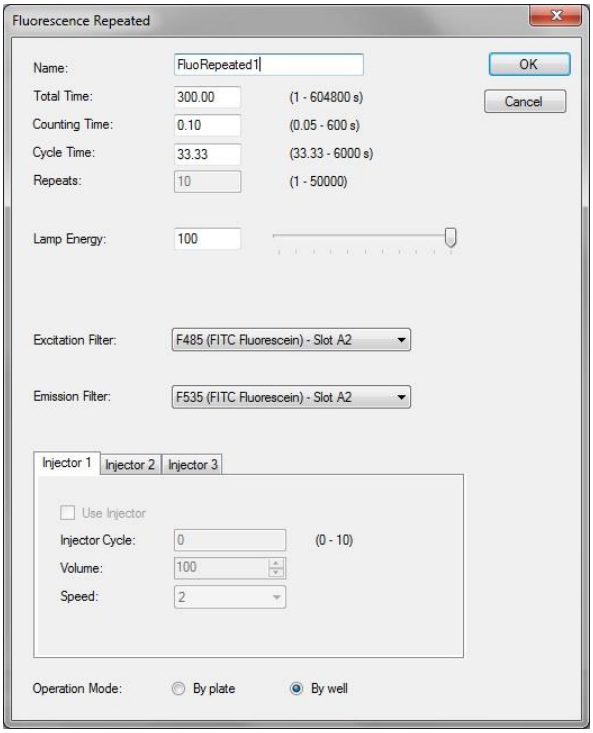
Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

22. Click **<OK>**



a second repeated operation may be added, e.g. for ratiometric applications (BRET)

23. Double-click ***Repeated*** in the Fluorescence section for a fluorescence repeated reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

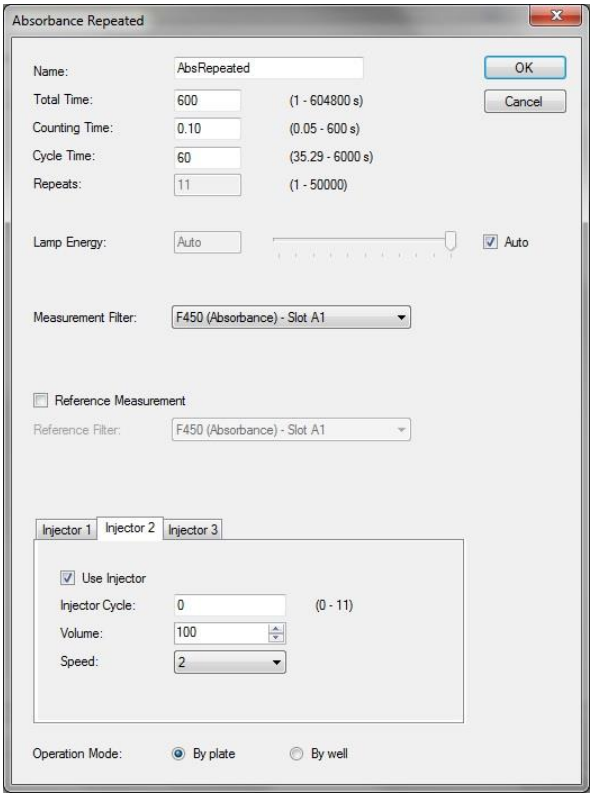
Injector 1, …2, …3

Check Use Injector	for an injection within the repeated cycle
Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

24. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications (FRET)

25. Double-click ***Repeated*** in the Absorbance section for a absorbance repeated reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter	select from the list

Check Reference Measurement if needed

Reference Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Injector 1, …2, …3

Check Use Injector for an injection within the repeated cycle

Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

26. Click **<OK>**

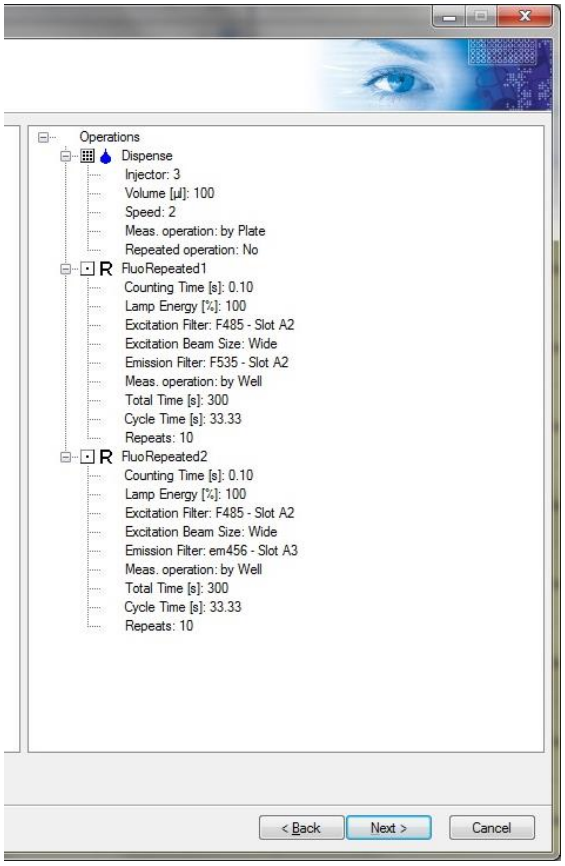
a second repeated operation may be added, e.g. for ratiometric applications

27. The **sequence of selected operations** will be displayed on the right-hand side

    Operations can be moved up or down by clicking on the operation and dragging them to the respective position

    Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

28. Click **<Next>**



29. Define **Export** settings

    Type your **Header** specific for this protocol

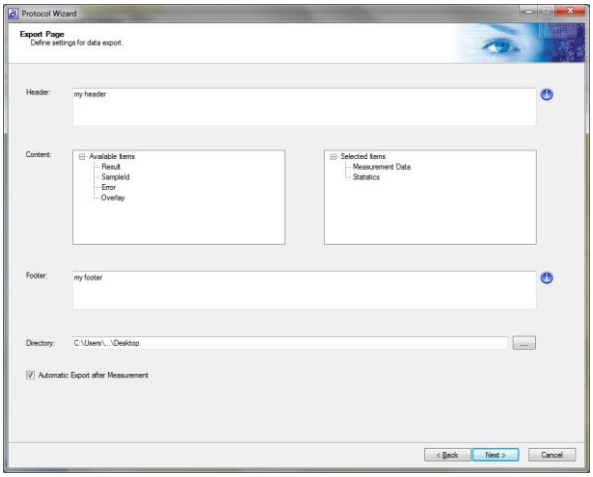
    Select the data set by dragging from left to right

Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required



30. Click **<Next>**

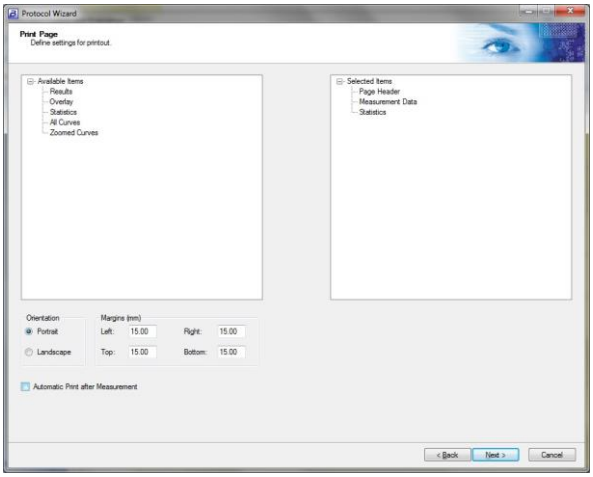
31. Define **Print** settings

    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

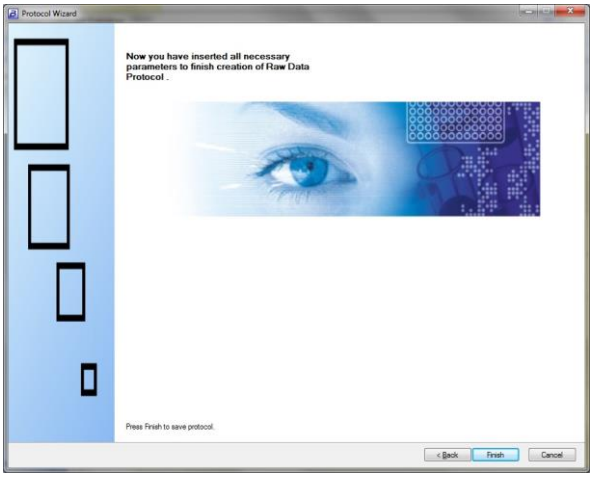
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

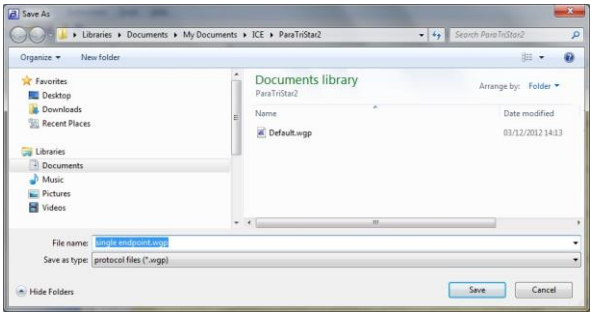


32. Click **<Next>**

33. Click **<Finish>**



34. Define the protocol **file name**
35. Click **<Save>**



7.5.2 Repeated measurement

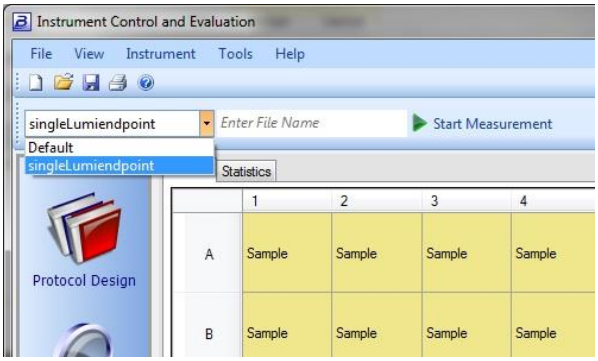
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

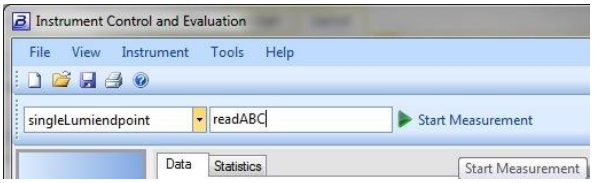
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**

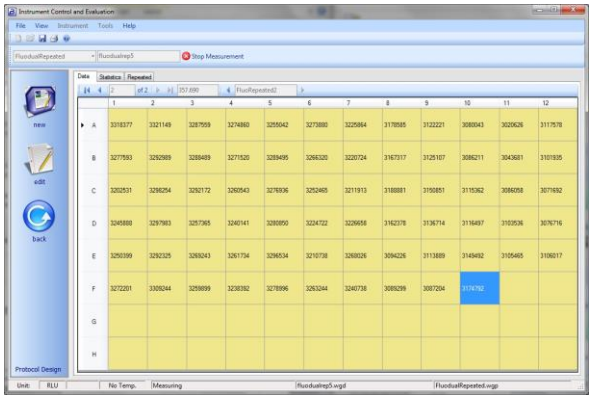
4. Insert the **microplate** with your samples:
   well A1 facing the rear and left

   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

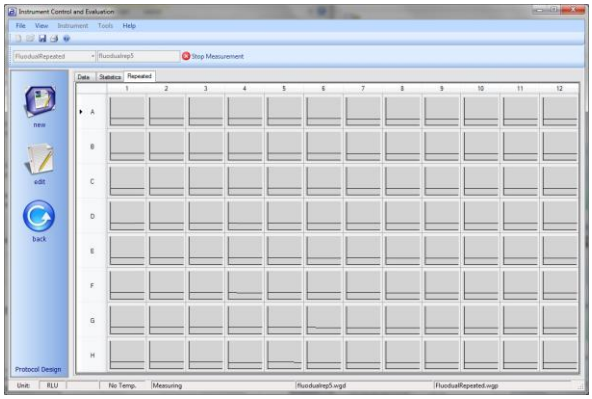
   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates
5. Click **<OK>**

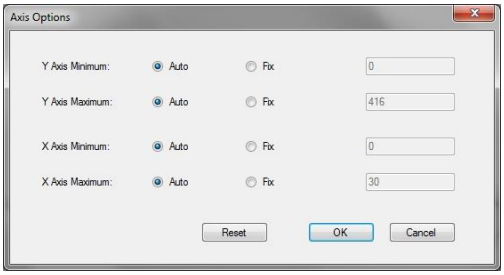
6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

   You may switch between the individual readings by clicking on the arrows

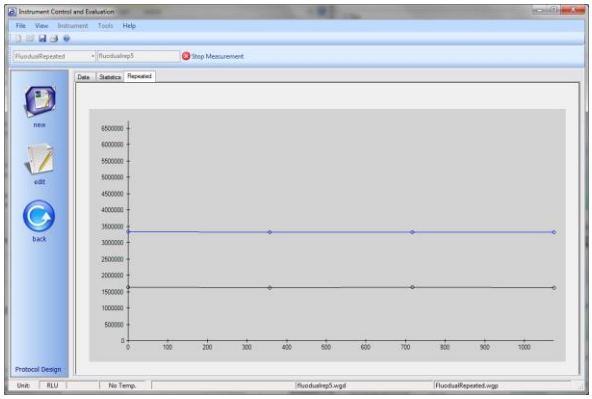


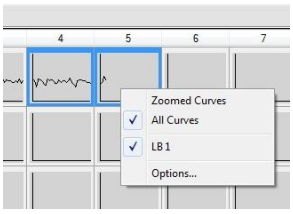
7. You also choose to view the curves by clicking the ***Repeated*** tab
8. The scale of the axes can be changed by right-clicking into the curves and selecting **Options…**



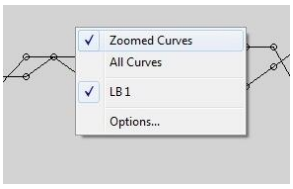


9. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Curves**

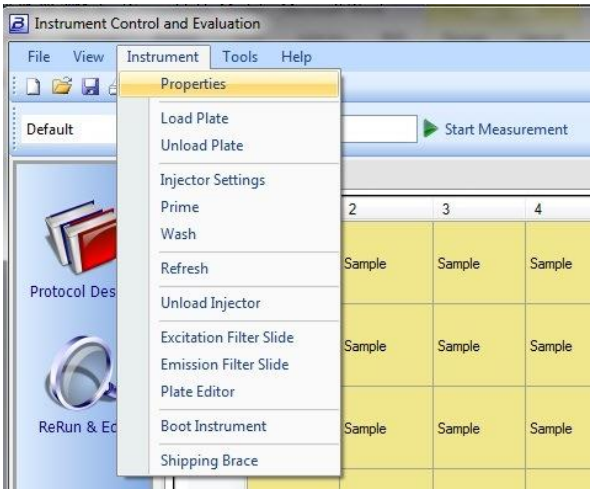




To un-zoom right-click into the zoomed view and select All Curves



10. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.6 Scanning Measurement

A scanning measurement mode is appropriate for assays with heterogeneous distribution of signal, e.g. cellular assays

7.6.1 Defining a protocol for a scanning measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

1. Click icon **Protocol Design** in the left-hand **Navigation** bar

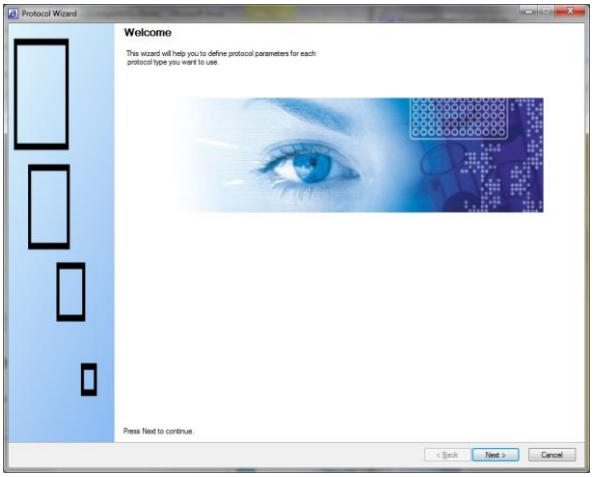
   the navigation bar will appear in a new design

2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

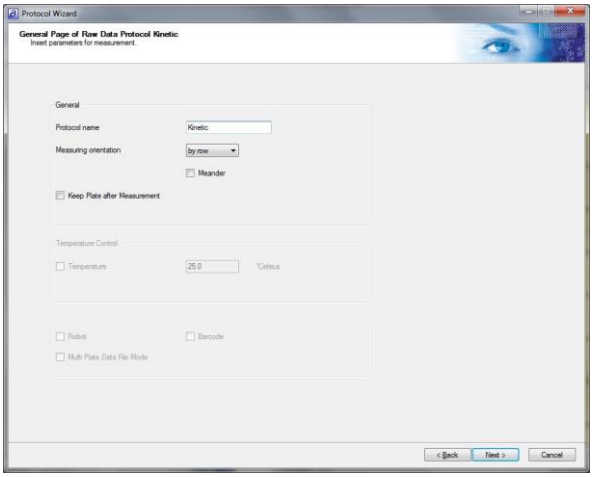
   for editing an existing protocol use the **edit** icon

3. The start up screen of the protocol wizard will show up
   Click **<Next>**



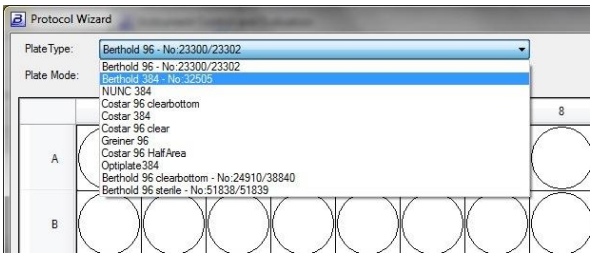
4. Enter a (descriptive) **Name** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



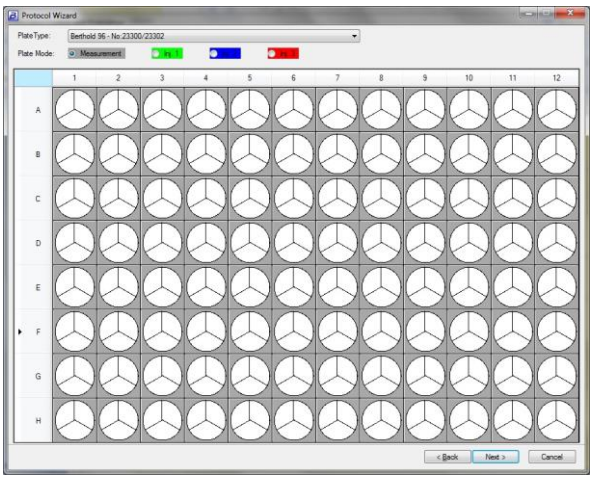
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

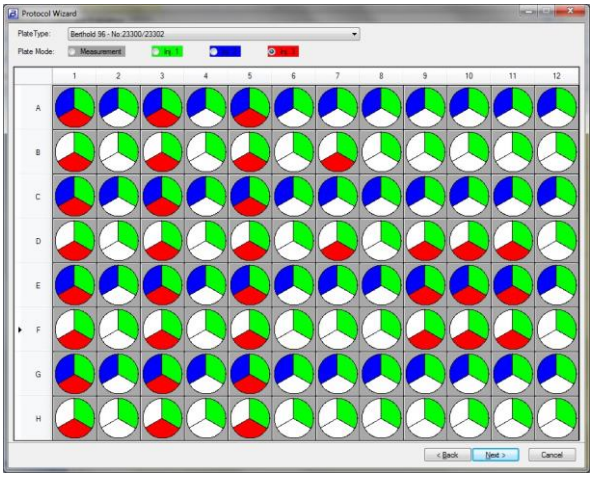
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

    Wells coloured in the respective colour are injected into

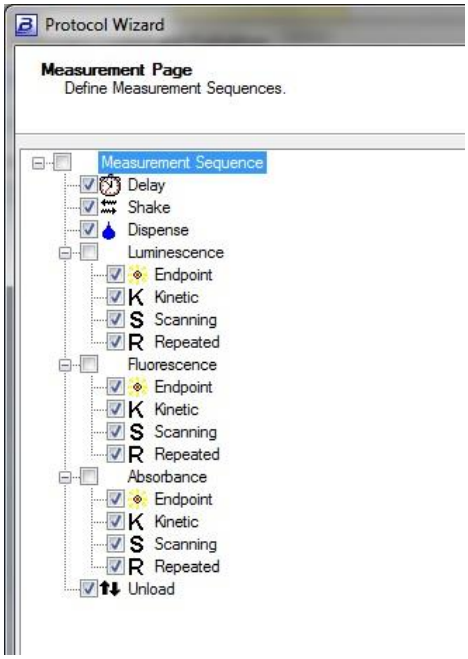
    Note: only wells to be measured can be injected into



14. Click **<Next>**

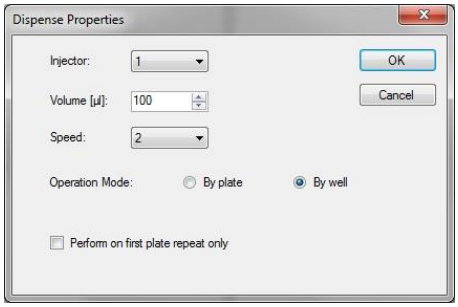
Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well



15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well



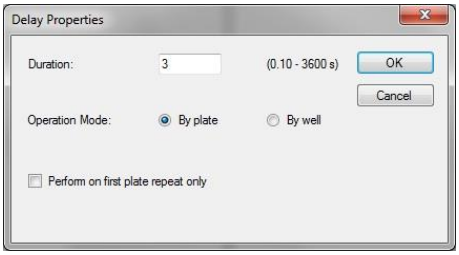
16. Click **<OK>**

    In case additional reagent additions are required repeat this procedure for the other injector(s)

17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

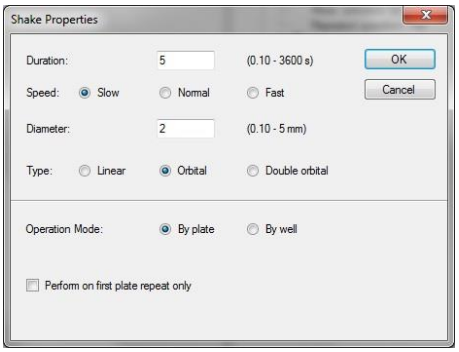
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Scanning*** in the Fluorescence section for a fluorescence scanning reading

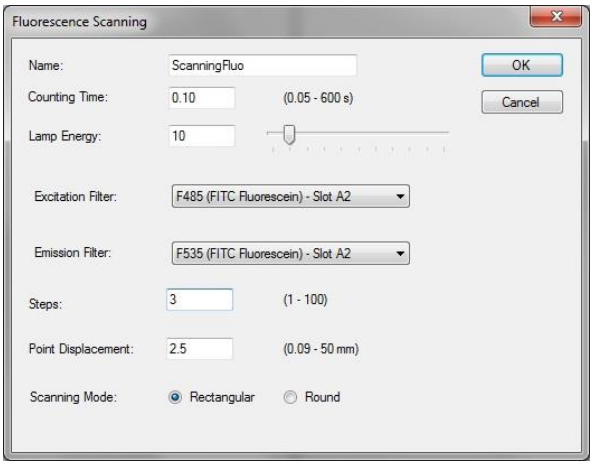
Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

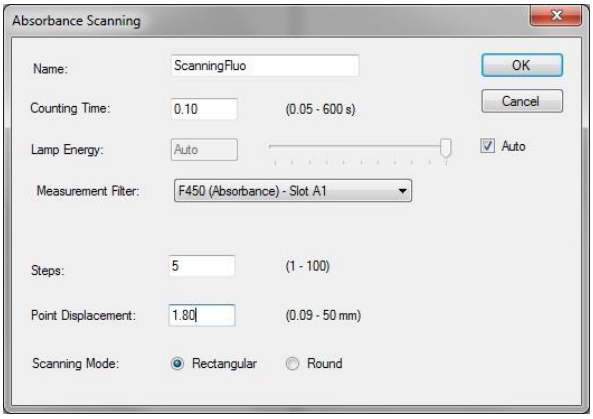
Steps	1 to 100 scanning points in one direction, the other direction will have the same amount of points
Point Displacement	distance between points

Select rectangular or round matrix

22. Click **<OK>**



23. Double-click ***Scanning*** in the Absorbance section for a absorbance scanning reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter	select from the list

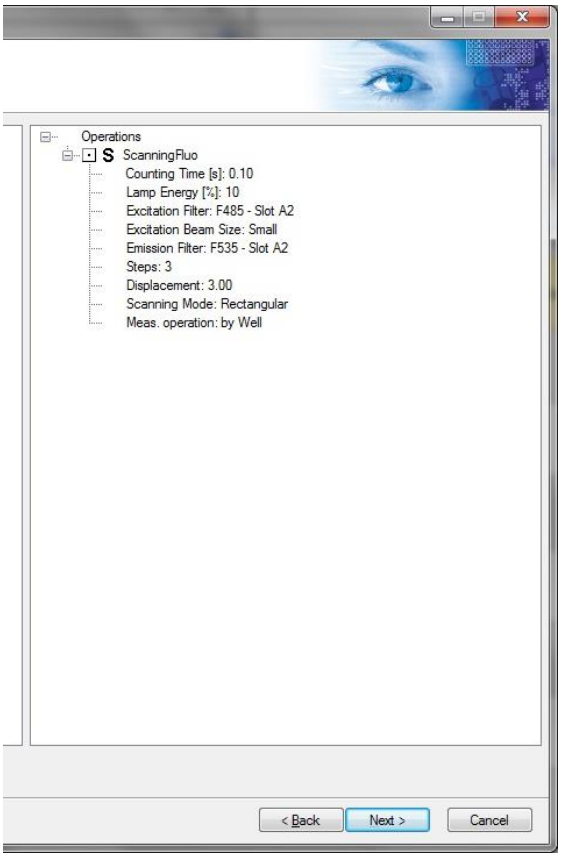
Note: filters must be defined prior in the Instrument menu

Steps	1 to 100 scanning points in one direction, the other direction will have the same amount of points
Point Displacement	distance between points

Select rectangular or round matrix

24. Click **<OK>**

25. The **sequence of selected operations** will be displayed on the right-hand side



Operations can be moved up or down by clicking on the operation and dragging them to the respective position

Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

26. Click **<Next>**

27. Define **Export** settings

    Type your **Header** specific for this protocol

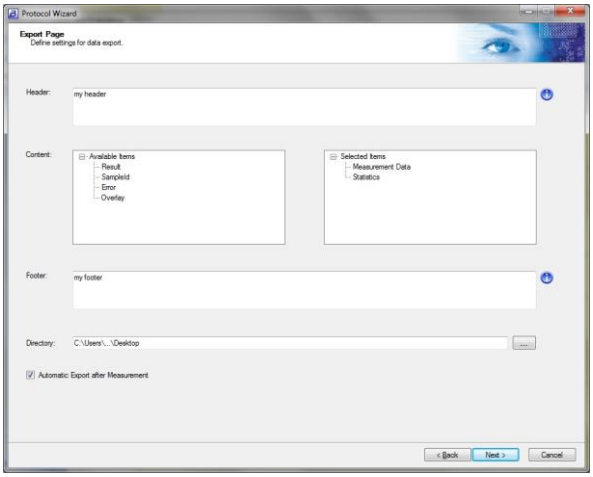
    Select the data set by dragging from left to right

Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required



28. Click **<Next>**

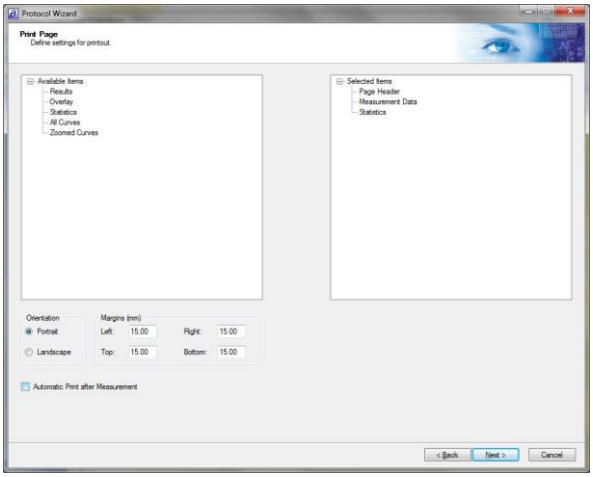
29. Define **Print** settings

    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

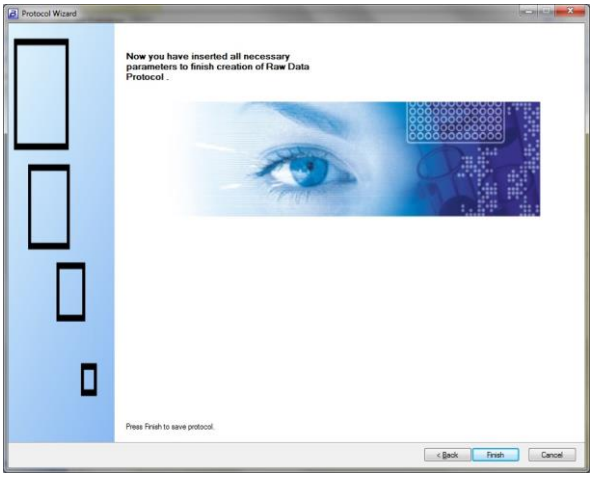
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

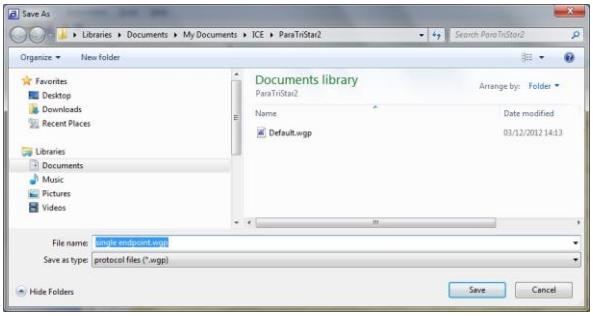


30. Click **<Next>**

31. Click **<Finish>**



32. Define the protocol **file name**
33. Click **<Save>**



7.6.2 Scanning measurement

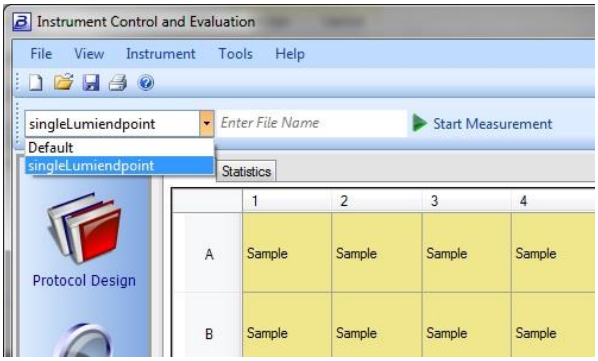
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

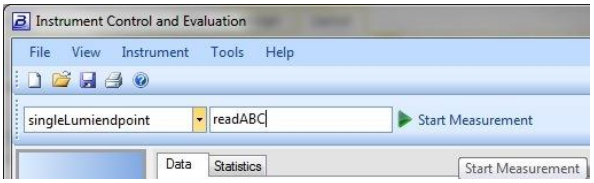
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

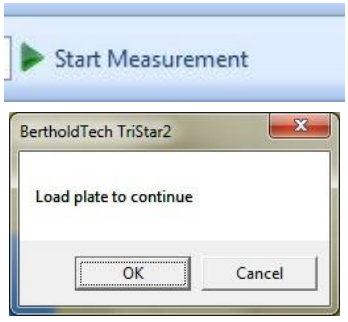
1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**



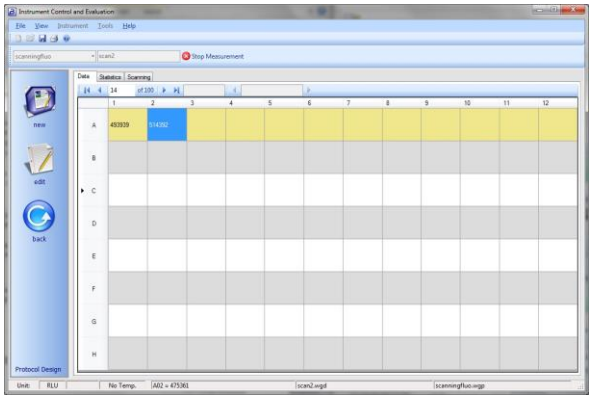
4. Insert the **microplate** with your samples:
   well A1 facing the rear and left

   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

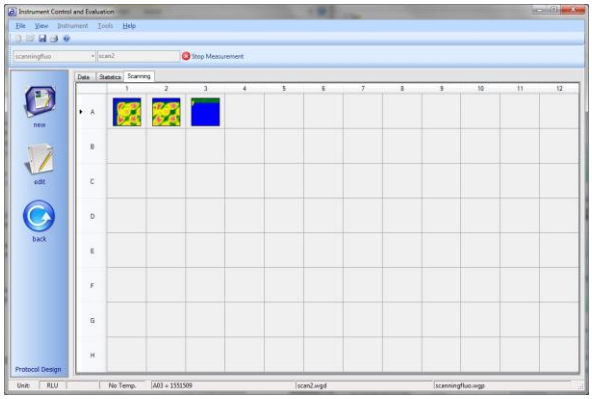
   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates
5. Click **<OK>**

6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

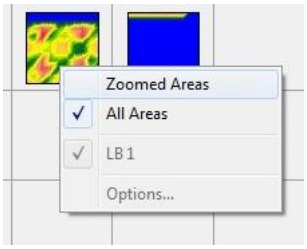
   You may switch between the individual readings by clicking on the arrows

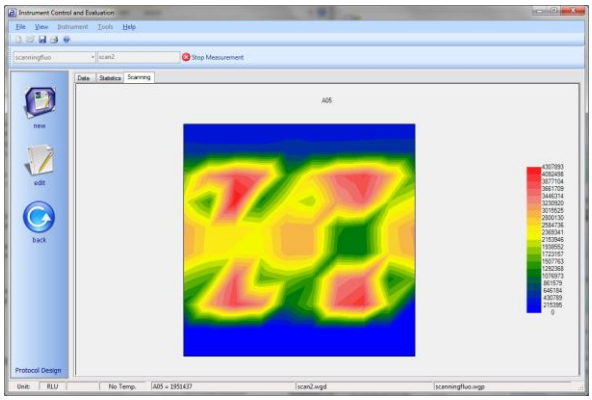


7. You also choose to view a graphical display by clicking the ***Scanning*** tab

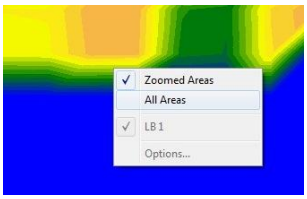


8. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Areas**

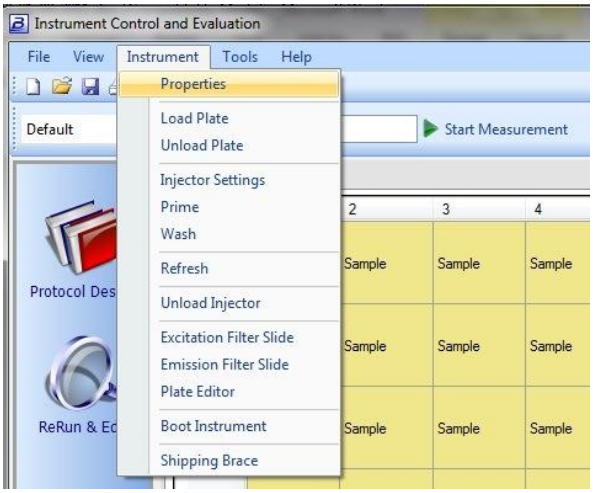




   To un-zoom right-click into the zoomed view and select All Curves



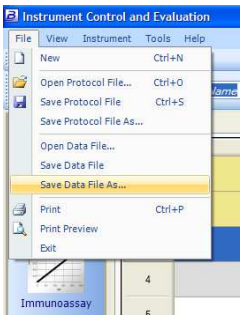
9. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



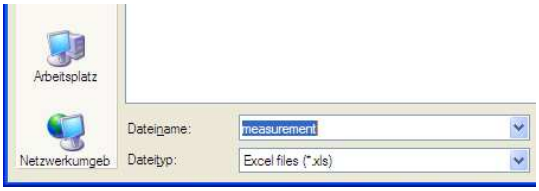
7.7 Data export and print-out

7.7.1 Direct data export

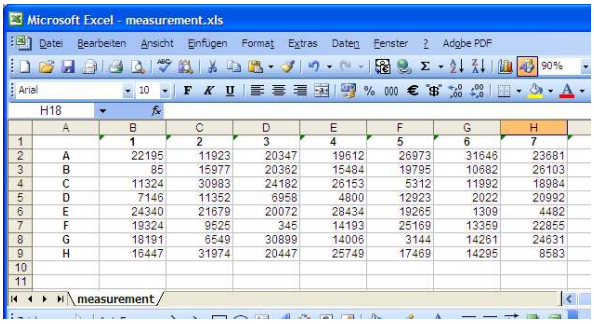
1. Click on **Save Data File As…** in the File menu



2. Select the file format, e.g. **Excel files (*.xls)**
3. Define the file name **without extension**
4. Select the appropriate folder
5. Click **<Save>**



6. Open the *.xls file

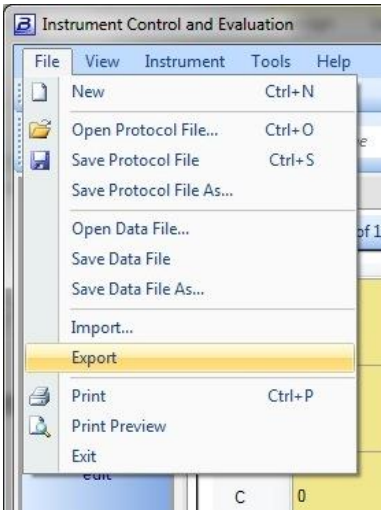


	A	B	C	D	E	F	G	H
1		1	2	3	4	5	6	7
2	A	22195	11923	20347	19612	26973	31646	23681
3	B	85	15977	20362	15484	19795	10682	26103
4	C	11324	30983	24182	26153	5312	11992	18984
5	D	7146	11352	6958	4800	12923	2022	20992
6	E	24340	21679	20072	28434	19265	1309	4482
7	F	19324	9525	345	14193	25169	13359	22855
8	G	18191	6549	30899	14006	3144	14261	24631
9	H	16447	31974	20447	25749	17469	14295	8583
10								
11								

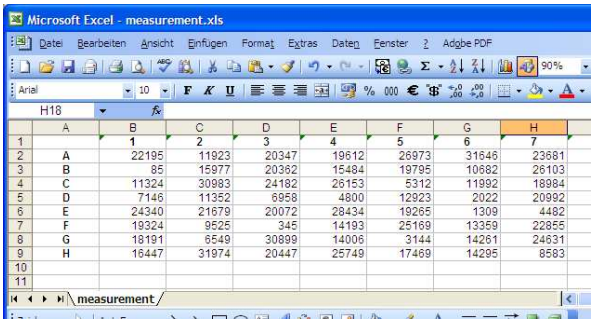
The export will be executed automatically if selected in the respective protocol file.

7.7.2 Data export via Export

1. Click on **Export** in the File menu
2. An EXCEL file will be created with file name resembling that of the data file



3. Open the *.xls file

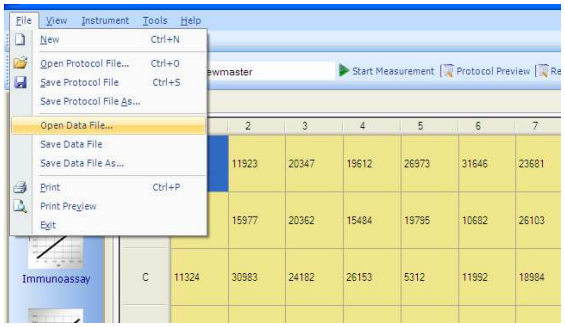


	A	B	C	D	E	F	G	H
1		1	2	3	4	5	6	7
2	A	22195	11923	20347	19612	26973	31546	23681
3	B	85	15977	20362	15484	19795	10682	26103
4	C	11324	30983	24182	26153	5312	11992	18984
5	D	7146	11352	6958	4800	12923	2022	20992
6	E	24340	21679	20072	28434	19265	1309	4482
7	F	19324	9525	345	14193	25169	13359	22855
8	G	18191	6549	30899	14006	3144	14261	24631
9	H	16447	31974	20447	25749	17469	14295	8583
10								
11								

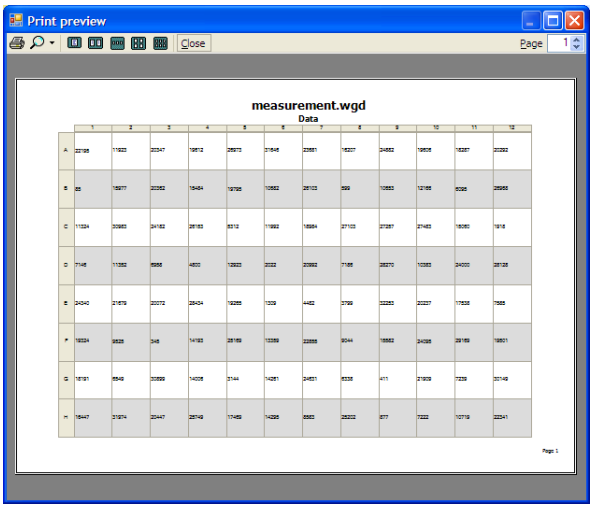
The export will be executed automatically if selected in the respective protocol file.

7.7.3 Direct data print-out

1. If not opened already open the respective data file by selecting **Open Data File** in the **File** menu



2. Select **Print Preview** in the **File** menu to get a preview of the print-out
3. Select **Print** in the **File** menu to start printing the data



The print-out will be executed automatically if selected in the respective protocol file.

8. Operation with Mikrowin 2000

Running measurements on the TriStar² is straight forward. The procedure is the same for all types of assay types, e.g. Raw Data, Dual Label, Kinetic, Repeated and Scanning. A measurement can be carried out immediately after a stored protocol is selected. At the end of each measurement the results are stored and may be printed or exported.

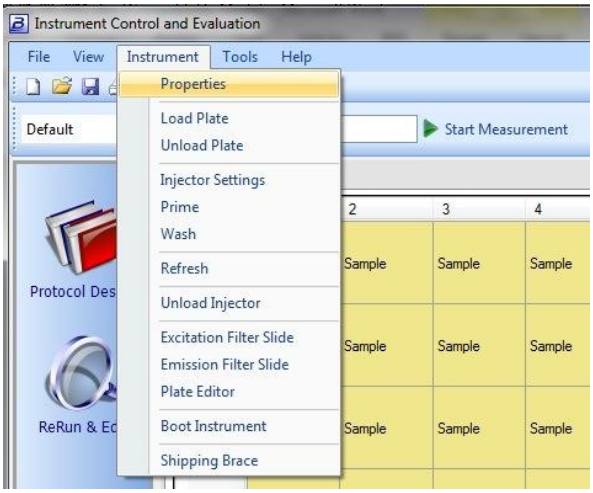
Result file names can be given without limitation. The extension is fixed, though. This is valid for measurement protocols as well.

8.1 Adding and Editing Microplate Dimensions

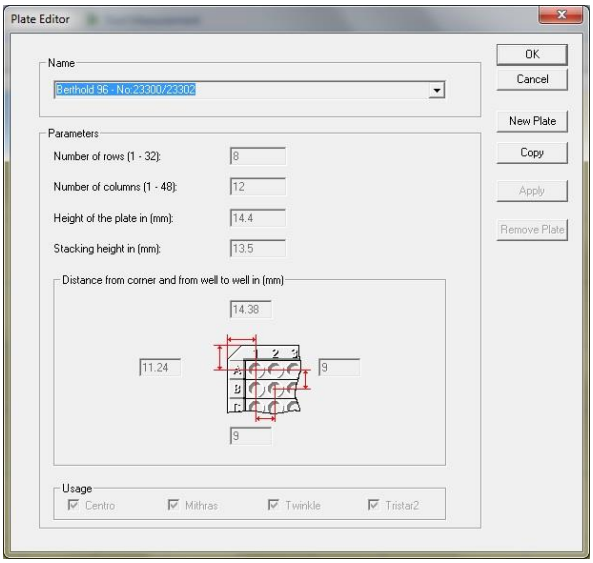
Microplates can differ in their dimensions dependent on brand and type. Please refer to the manufacturer´s most recent information for exact dimensions of the microplates.

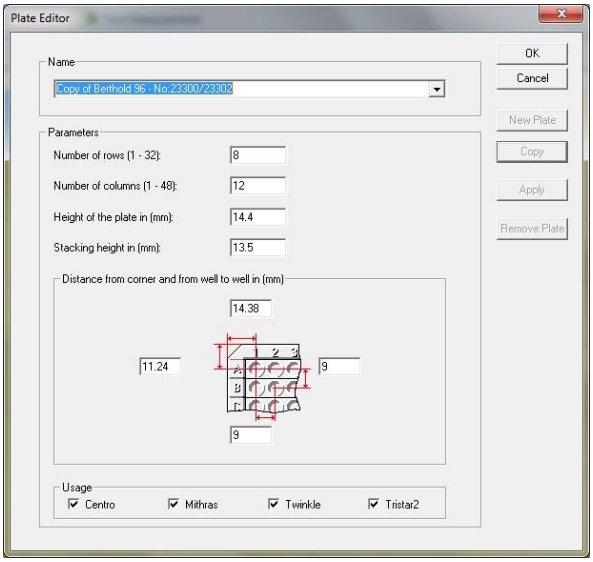
Microplates must be defined in the plate editor prior to defining a measurement protocol.

51. Click **Plate Editor** in the **Instrument** menu



52. Click **<New Plate>** or select a plate with matching well format and click **<Copy>**





53. Assign a (descriptive) **Name**
54. Insert the **Number of rows**, e.g. ***8*** for a 96 well plate
55. Insert the Number of columns, e.g. ***12*** for a 96 well plate
56. Insert the total **Height** of the microplate

    most 96 and 384 well plates are between 14 and 15.5 mm
57. Insert the **Stacking height** of the microplate

    the stacking height is the resulting height (the visible part) when plates are put on top of each other (e.g. in a plate stacker)

    in case this information is not available from the plate manufacturer the stacking height can be derived by stacking 2 plates and measuring the total height; by subtracting the regular height of one of the plates the resulting value will be the stacking height
58. Insert the distance between the left outer edge of the plate and the center of well A1
59. Insert the distance between to upper outer edge of the plate and the center of well A1
60. Insert the distance between the well centers of consecutive rows (vertical well distance)
61. Insert the distance between the well centers of consecutive columns (horizontal well distance)
62. Check the usage ***TriStar2***

    you may check additional instruments in case you have multiple instruments in operation
63. Click **<Apply>**
64. Click **<OK>**

65. The plate can now be used in the protocol files

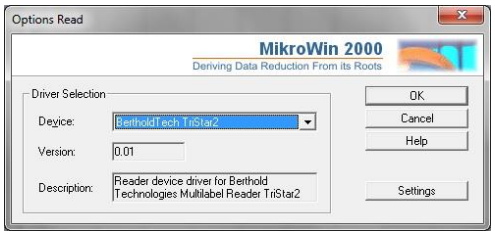
8.2 Single Raw Data measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine ATP content, single reporter gene expression, activities of caspases, kinases and many other enzymes.

8.2.1 Defining a Single Endpoint protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

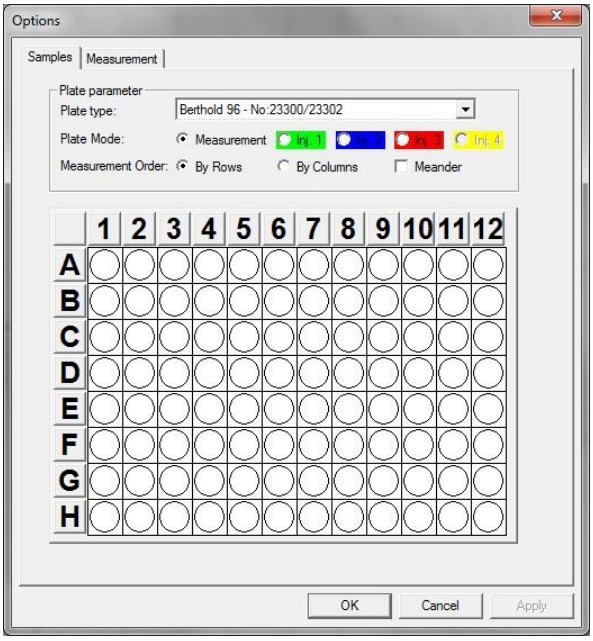
1. Click **Read** in the **Options** menu



2. Select **BertholdTech TriStar2**
3. Click **<Settings>**

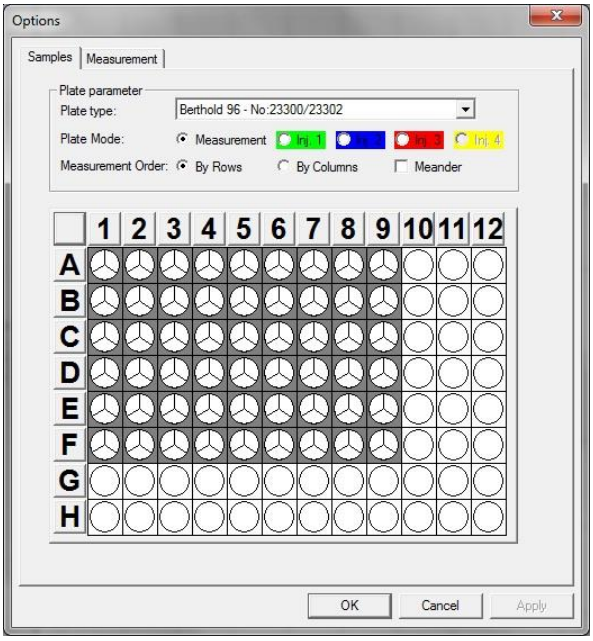
4. Select the **Plate Type** (microplate format)

   Note: the microplate has to be defined in the Plate Editor prior to defining a protocol
5. Define the **reading orientation**:
   by columns or by rows
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top



7. Select the wells to be measured by clicking the Measurement radio button
   - for the whole plate, click the top left corner
   - for a row, click the respective character
   - for a column, click the respective number
   - for an area, click and drag the mouse
   - for an individual well, click into it

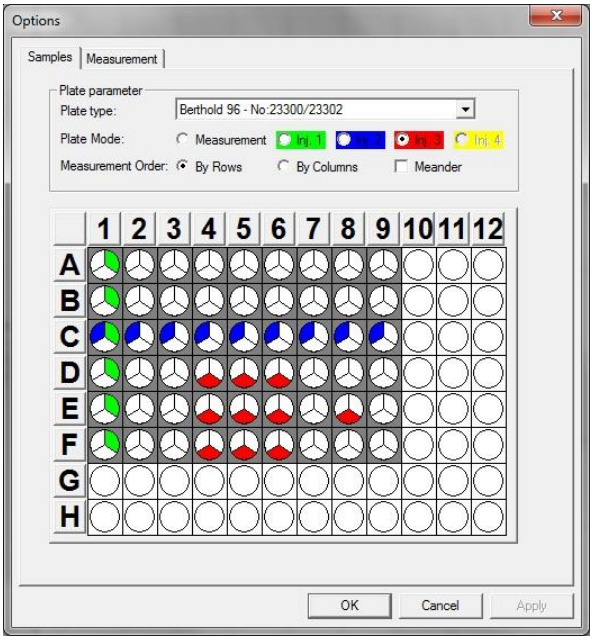
   Wells with a gray outside area are selected for measurement



8. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
   - for the whole plate, click the top left corner
   - for a row, click the respective character
   - for a column, click the respective number
   - for an area, click and drag the mouse
   - for an individual well, click into it

   Wells coloured in the respective colour are injected into

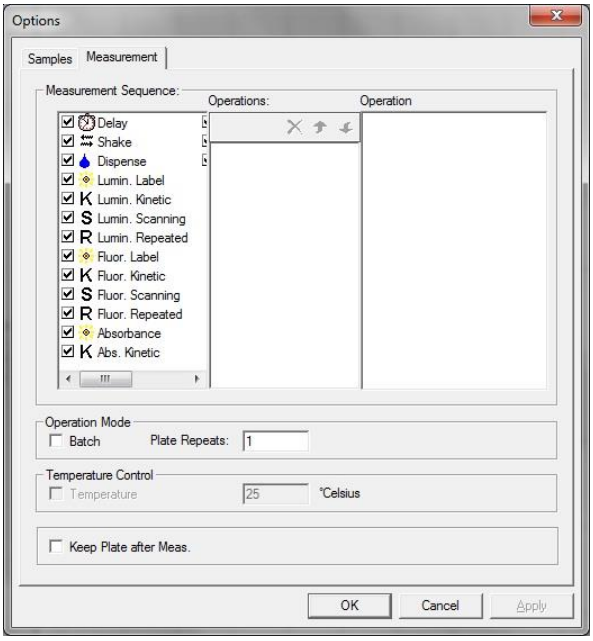
   Note: only wells to be measured can be injected into



9. Click onto the **Measurement** tab

   Define the Measurement Operations

   - Available operations are shown on the left-hand area
   - Allowed operations are indicated by a check mark
   - Double-clicking an operation open the specific properties dialogue
   - Confirming the settings by clicking **<OK>** selects the operation and adds it to the operation sequence visible in the right-hand area
   - The operation may be executed
     by plate the operation will be executed for all selected before the consecutive operation is started
     by well all consecutive by well operations will be executed for a well before moving on to the next well

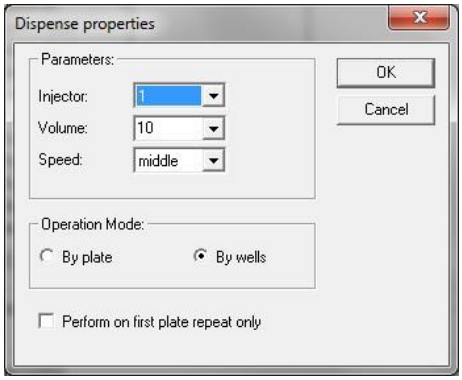


10. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	low – middle - high
Operation Mode	by plate or by well

11. Click **<OK>**

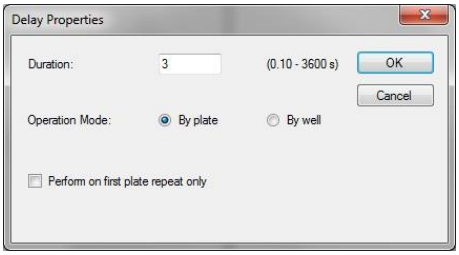
    In case additional reagent additions are required repeat this procedure for the other injector(s)



12. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

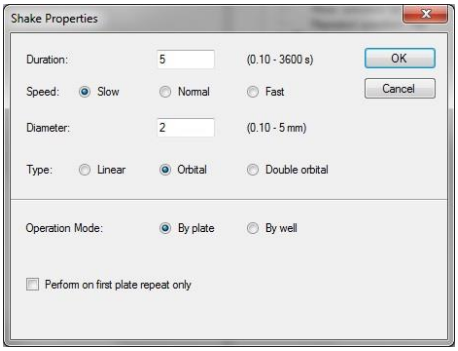
13. Click **<OK>**



14. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

15. Click **<OK>**



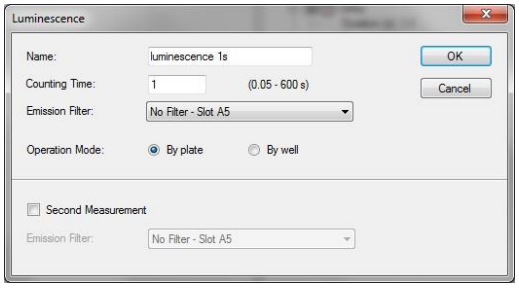
16. Double-click ***Lumin.Label*** for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter

Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well

17. Click **<OK>**



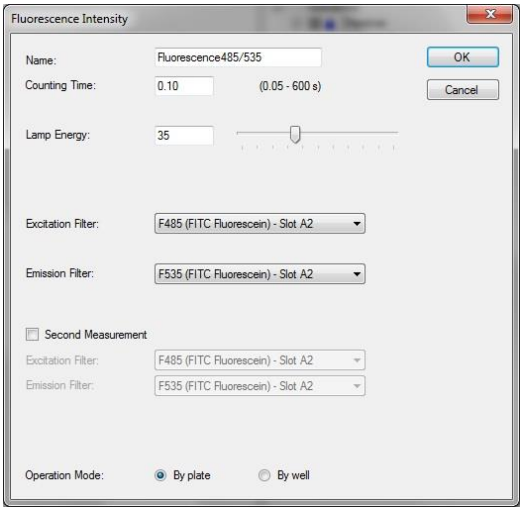
18. Double-click ***Fluor. Label*** for a fluorescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well

19. Click **<OK>**



20. Double-click ***Absorbance*** for a absorbance reading

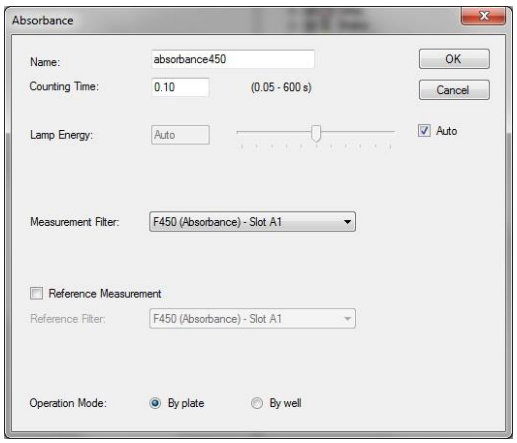
Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Excitation Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Reference Measurement



Note: the values derived with this filter will be automatically subtracted from the measurement value per well

Reference Filter select from the list

Operation Mode by plate or by well

21. Click **<OK>**

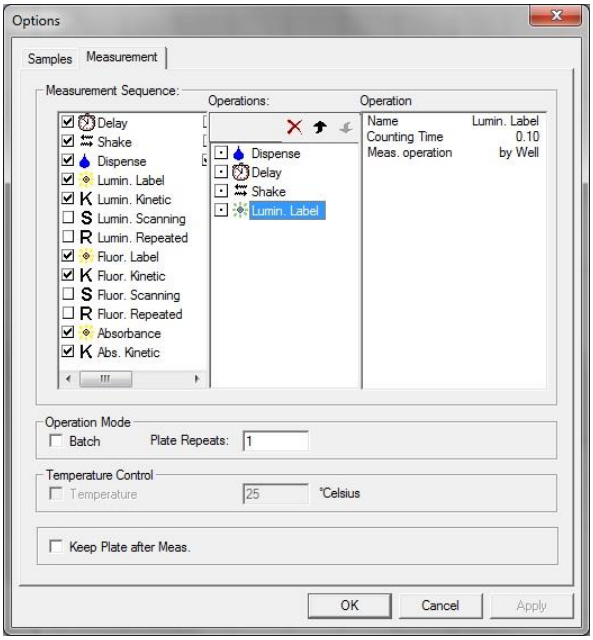
22. The **sequence of selected operations** will be displayed in the center column

    Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

    Operations can be deleted by highlighting and clicking the cross

    Details of the operation highlighted can be viewed on the right column

    An operation can be edited by double-clicking on it in the center column



23. Check **Batch** and define the number of plates in **Plate Repeats** in case you want a number of plates to be stored into a single data file

    Note: this setting can only be used in single endpoint measurements

24. Define a number in **Plate Repeats** only in case you want the selected operations to be repeatedly executed

25. Check **Temperature** to activate the temperature control for this protocol

26. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active

27. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished

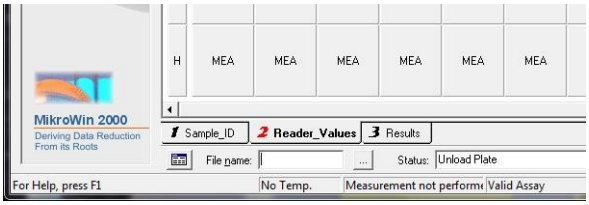
28. Click **<OK>**

29. Click **<OK>** once more

30. By default the plain measurement data will be located on **Result matrix 2 "Reader_Values"**

    For export und print you have to refer to this matrix.

    Should you wish to define any additional calculations please refer to the Mikrowin manual.



31. To activate automatic export click **Export Set-up…** in the **File** menu
32. Select the appropriate and pre-defined export driver

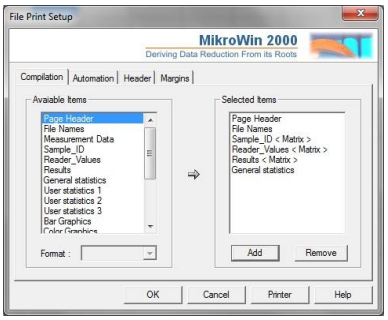
    The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

33. Click **<OK>**

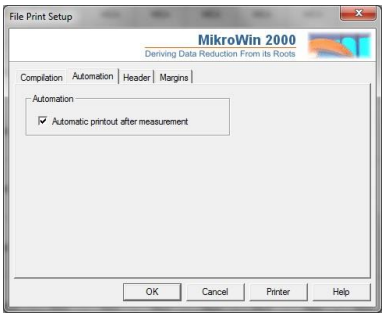
34. Click **Print Setup…** in the File menu
35. Select the data set by highlighting and clicking **<Add>**

Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
Reader_Values	measured data (matrix 2)*
Results	Averages (matrix 3)*
Gen. Statistics	measurement settings

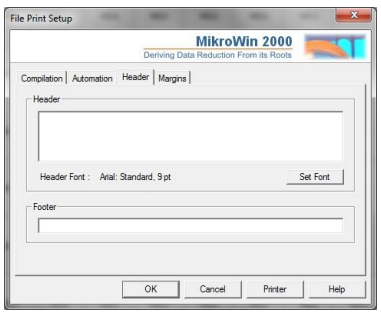
    * the selection and content depends on the matrix definition done in the Calculation section



36. Check if **Automatic Print-out** is required

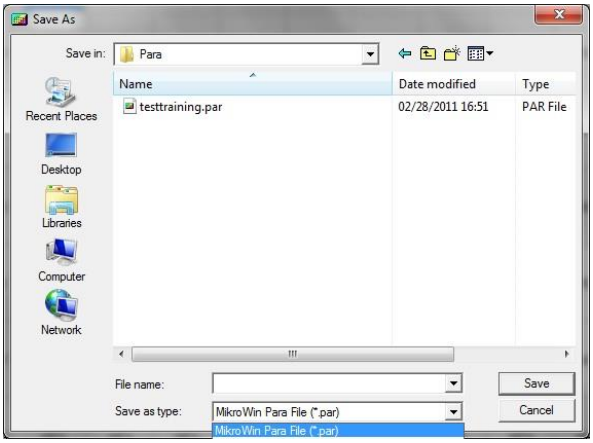


37. Type a **header** and/or **footer**



38. Click **<OK>**

39. Go to **File | Save As…**
40. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2***
41. Select the file type **Mikrowin Para File (*.par)**
42. Type a meaningful **file name**
43. Click **<Save>**



8.2.2 Measurement with a Single Endpoint protocol

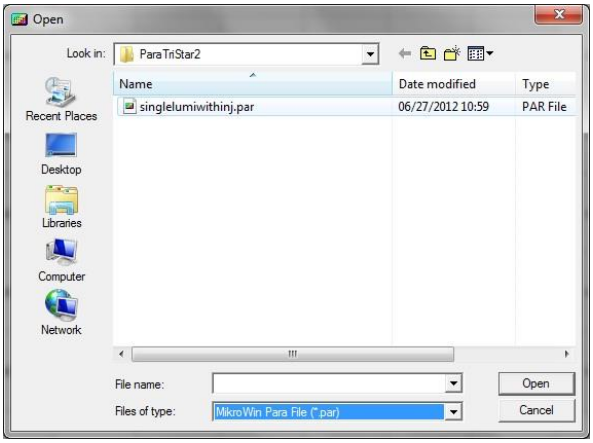
The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

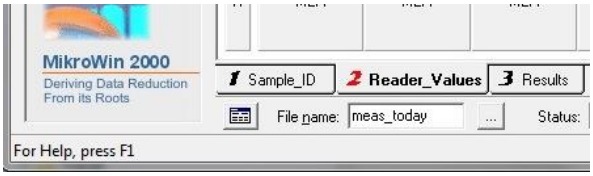
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Click **Open** in the **File** menu
2. Select **File of type**: Mikrowin Para File
3. Select the appropriate file
4. Click **<Open>**



5. Enter a **file name** under which the measurement is to be stored



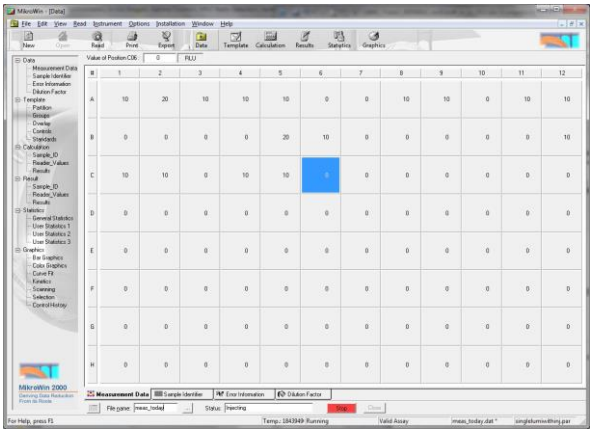
6. Click **<Start>**

7. Insert the **microplate** with your samples:
   well A1 facing the rear and left

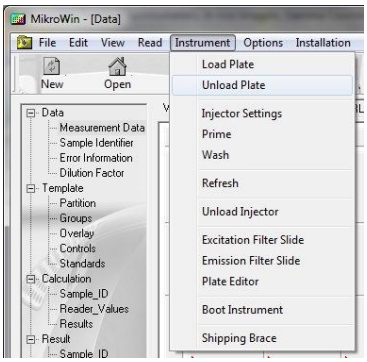
   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates
8. Click **<OK>**

9. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed



10. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



8.3 Dual Label Assay measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine dual reporter gene expression.

8.3.1 Defining a Dual Label protocol

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click ***Lumin.Label*** for a luminescence reading

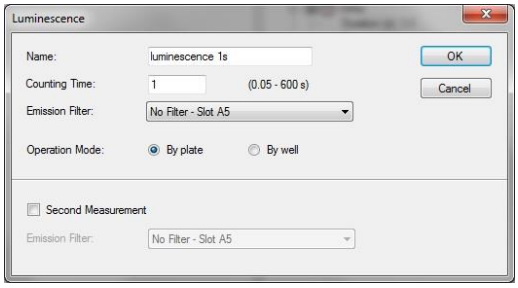
    Name — give a (descriptive) name

    Counting Time — 0.05 to 600 s

    Emission Filter — usually: No Filter

    Note: filters must be defined prior in the Instrument menu

    Operation Mode — by plate or by well



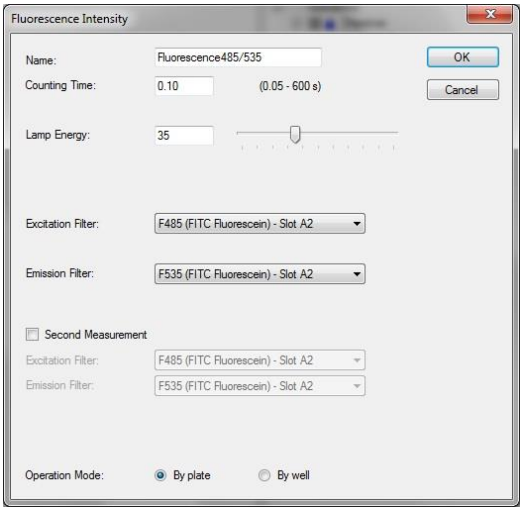
17. Double-click ***Lumin.Label*** once more and define the settings for the second reading

    For very fast switch between the first and the second reading, e.g. for fast BRET kinetics or for ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

    You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

18. Click **<OK>**

19. Double-click ***Fluor. Label*** for a fluorescence reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

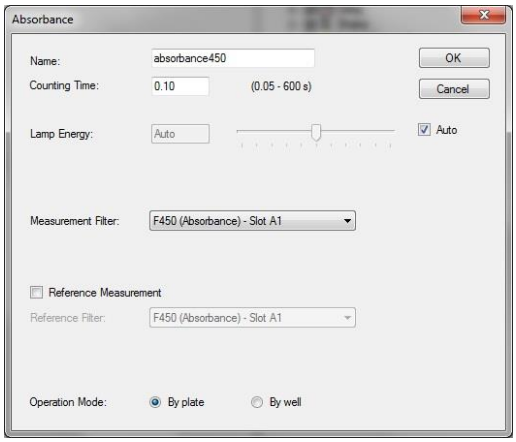
Operation Mode	by plate or by well

20. Double-click ***Fluor.Label*** once more and define the settings for the second reading

    For very fast switch between the first and the second reading, e.g. for fast BRET kinetics or for ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

    You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

21. Click **<OK>**
22. Double-click ***Absorbance*** for an absorbance reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Excitation Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Reference Measurement

Note: the values derived with this filter will be automatically subtracted from the measurement value per well

Reference Filter	select from the list
Operation Mode	by plate or by well

23. Double-click ***Absorbance*** once more and define the settings for the second reading

    For very fast switch between the first and the second reading, e.g. for fast BRET kinetics or for

ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

24. Click **<OK>**

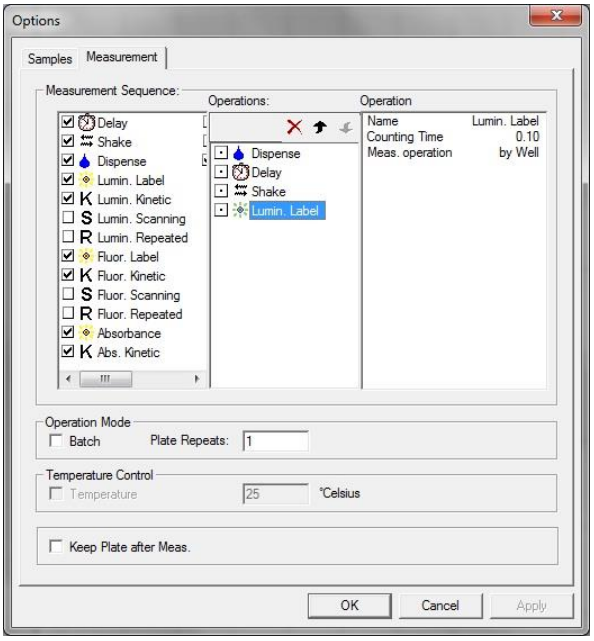
25. The **sequence of selected operations** will be displayed in the center column

    Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

    Operations can be deleted by highlighting and clicking the cross

    Details of the operation highlighted can be viewed on the right column

    An operation can be edited by double-clicking on it in the center column



26. Do not check **Batch**

    Note: this setting can only be used in single endpoint measurements

27. Define a number in **Plate Repeats** only in case you want the selected operations to be repeatedly executed

28. Check **Temperature** to activate the temperature control for this protocol
29. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active

30. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished

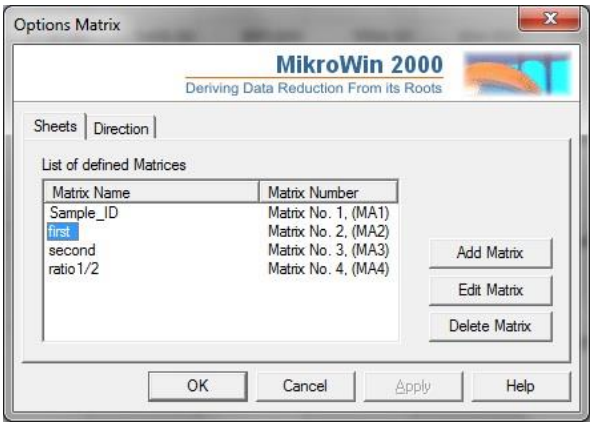
31. Click **<OK>**

32. Click **<OK>** once more

For further calculations of the measurements follow the next steps:

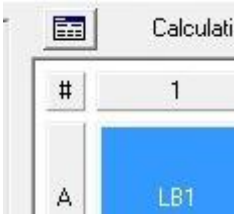
33. Go to **Options** | **Matrix** and click **<Add Matrix>**
34. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **first reading**

    Do the same for Matrix No. 3 (rename to e.g. **second reading**) and Matrix No. 4 (rename to e.g. **ratio**)
35. Click **<OK>**



For export und print you have to refer to this matrices.

36. Change the view to the **Calculation** section
37. Click on the ***2*** **first reading** tab
38. Type **LB1** into the Calculation Formula: LB1 = Label 1 = first of readings
39. Click the double-cross to assign for all wells

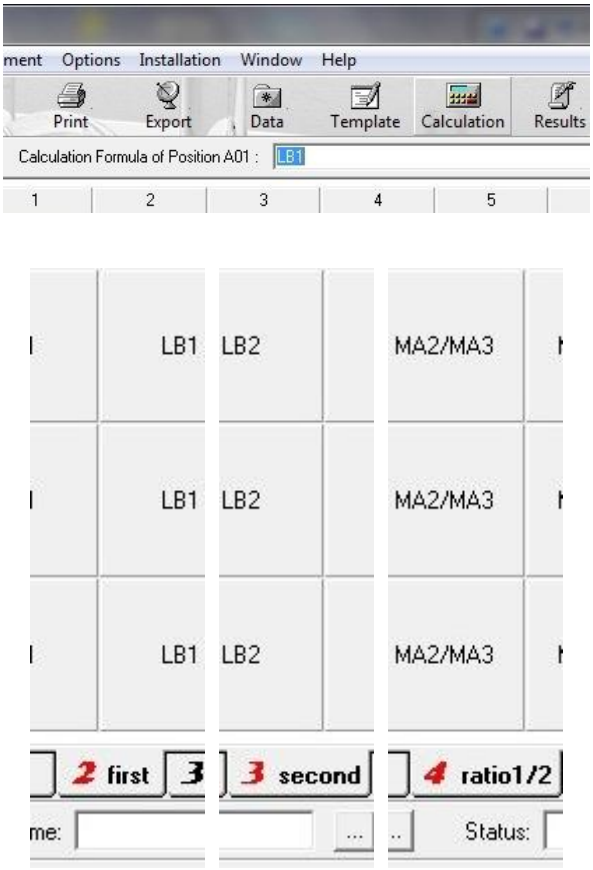


40. Proceed with the two other matrices alike:

    3 **second reading LB2**
    LB 2 = Label 2 = second of readings

    4 **ratio MA2/MA3**
    MA2 = Matrix 2, MA3 = Matrix 3, MA2/MA3 = values of Matrix 2 divided by values of Matrix 3

Should you wish to define any additional calculations please refer to the Mikrowin manual.



41. To activate automatic export click **Export Set-up…** in the **File** menu
42. Select the appropriate and pre-defined export driver

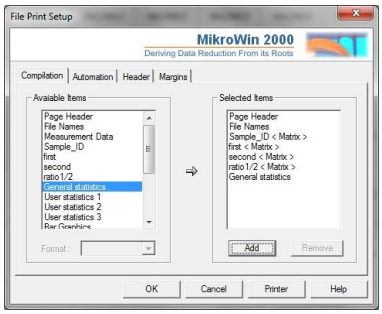
The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

43. Click **<OK>**

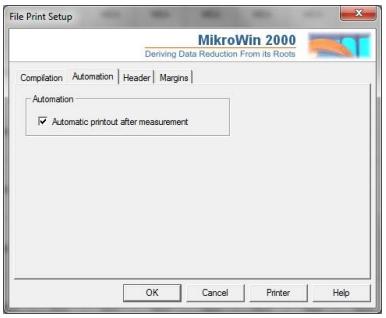
44. Click **Print Setup…** in the File menu
45. Select the data set by highlighting and clicking **<Add>**

Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
first	measured data (matrix 2)*
second	measured data (matrix 3)*
ratio	ratio of readings (matrix 3)*
Gen. Statistics	measurement settings

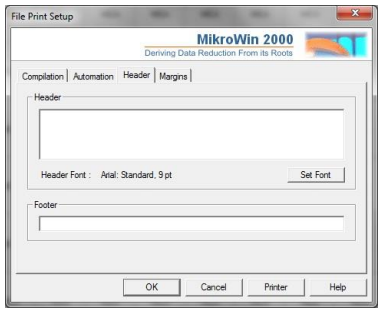
* the selection and content depends on the matrix definition done in the Calculation section



46. Check if **Automatic Print-out** is required

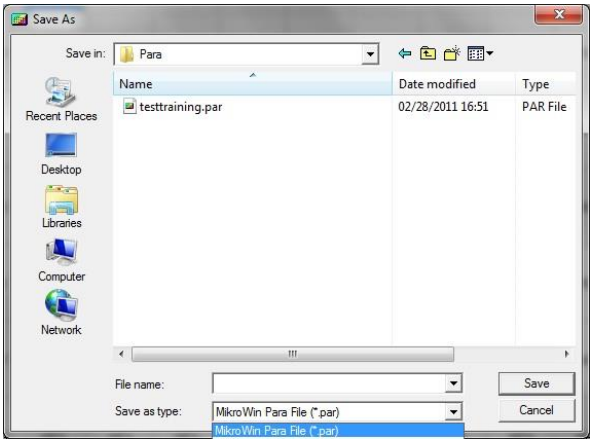


47. Type a **header** and/or **footer**



48. Click **<OK>**

49. Go to **File | Save As…**
50. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2***
51. Select the file type **Mikrowin Para File (*.par)**
52. Type a meaningful **file name**
53. Click **<Save>**



8.3.2 Measurement with a Dual Label Assay protocol

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2 "Measurement with a single endpoint rotocol"**.

8.4 Kinetic Measurement

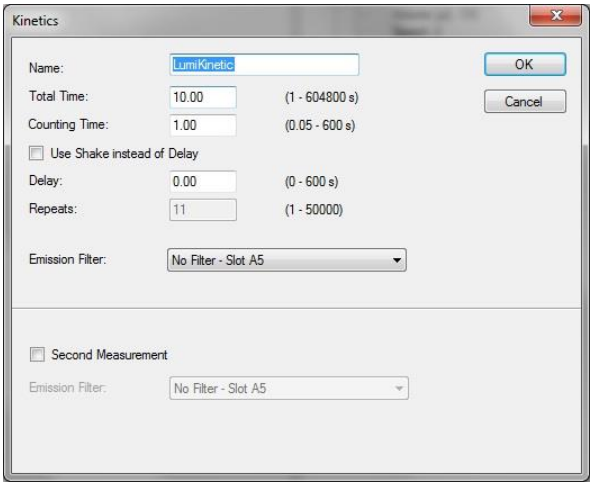
A kinetic measurement mode is appropriate for fast kinetics assays lasting over several seconds up to minutes, e.g. enzyme kinetics and Calcium influx

8.4.1 Defining a protocol for a kinetic measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click ***Lumin. Kinetic*** for a luminescence kinetic reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s

Check Use Shake instead of Delay if needed

Delay	0 to 600 sec
Repeats	(are calculated)
Emission Filter	usually: No Filter

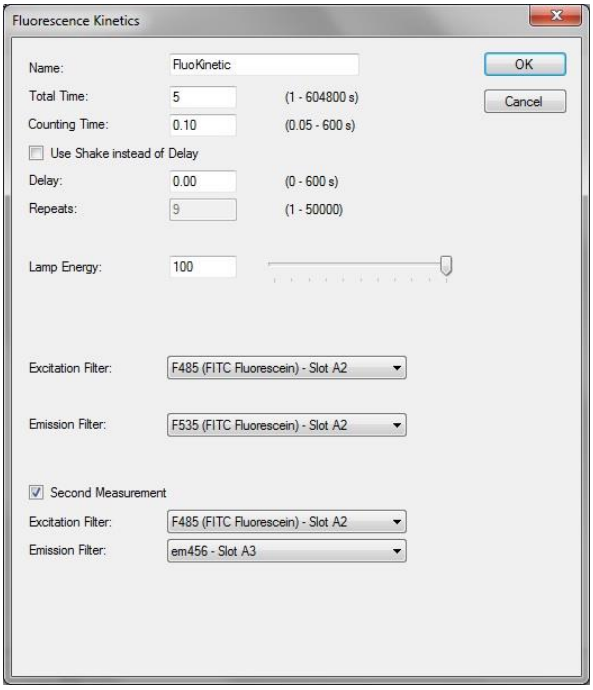
Note: filters must be defined prior in the Instrument menu

Second Measurement may be checked in case of ratiometric kinetics, e.g. in BRET applications

17. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

18. Double-click ***Fluor. Kinetic*** for a fluorescence kinetic reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake instead of Delay if needed	
Delay	0 to 600 s
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

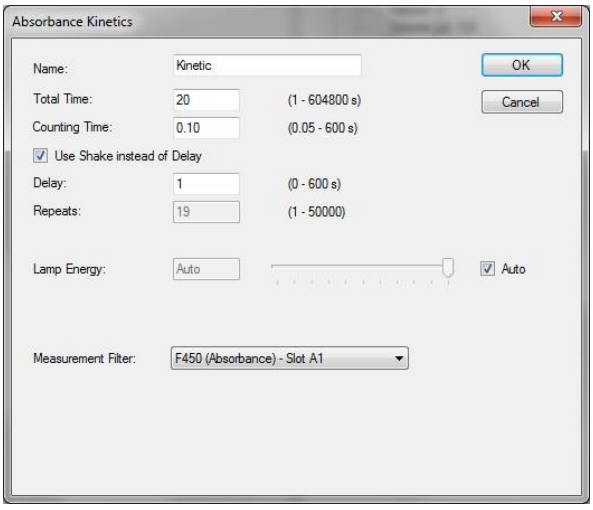
Operation Mode	by plate or by well

Second Measurement may be checked in case of ratiometric kinetics, e.g. in Calcium applications

19. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

20. Double-click ***Abs. Kinetic*** for an absorbance kinetic reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake instead of Delay if needed	
Delay	0 to 600 s
Repeats	(are calculated)
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter	select from the list

Note: filters must be defined prior in the Instrument menu

21. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

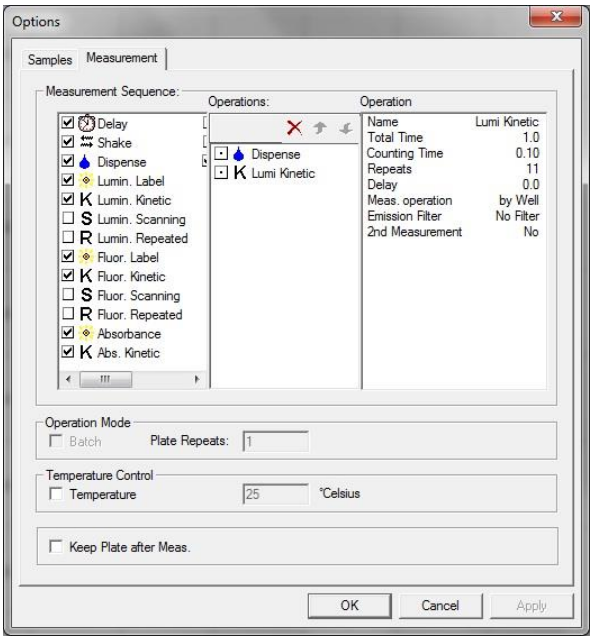
22. The **sequence of selected operations** will be displayed in the center column

    Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

    Operations can be deleted by highlighting and clicking the cross

    Details of the operation highlighted can be viewed on the right column

    An operation can be edited by double-clicking on it in the center column



23. Check **Temperature** to activate the temperature control for this protocol
24. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active

25. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished

26. Click **<OK>**

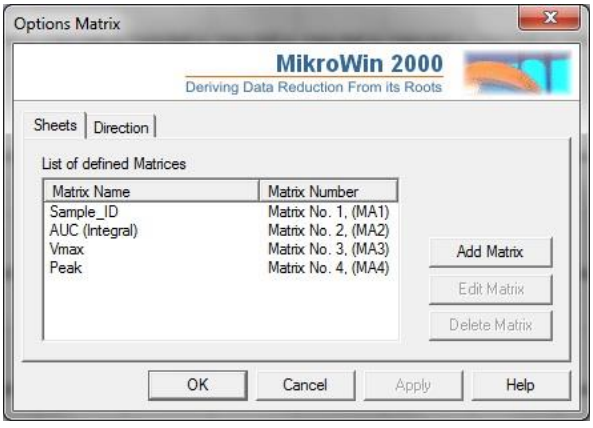
27. Click **<OK>** once more

    For further calculations of the measurements follow the next steps:

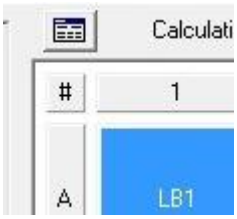
28. Go to **Options** | **Matrix** and click **<Add Matrix>**
29. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **AUC (Integral)**

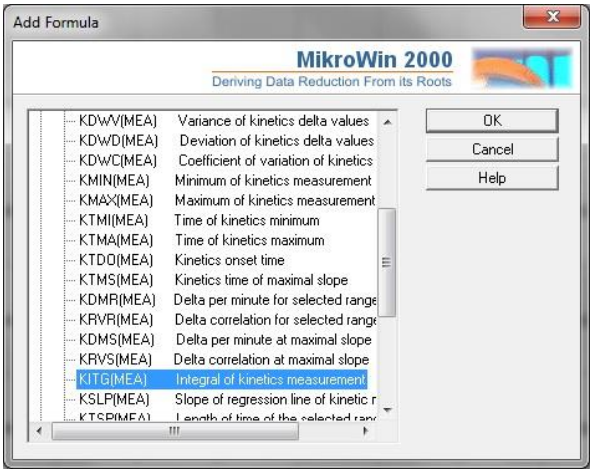
    Do the same for Matrix No. 3 (rename to e.g. **Vmax**) and Matrix No. 4 (rename to e.g. **Peak**)
30. Click **<OK>**

    For export und print you have to refer to these matrices.



31. Change the view to the **Calculation** section
32. Click on the ***2*** **AUC (Integral)** tab
33. Click **<Add Formula>** and expand **Kinetic calculation functions**
34. Select **KITG(MEA)** and click **<OK>**
35. Click the double-cross to assign for all wells





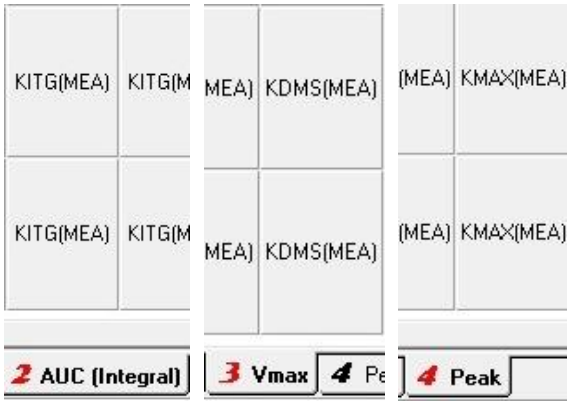
36. Proceed with the two other matrices alike:

3 **Vmax** **KDMS(MEA)**

4 **Peak** **KMAX(MEA)**

When 2nd measurement has been checked MEA need to be replaced by LB1 and LB2 respectively and additional matrices may need to be created

Should you wish to define any additional calculations please refer to the Mikrowin manual.



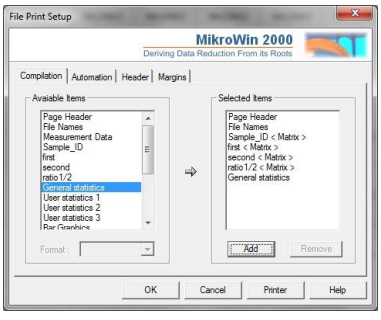
37. To activate automatic export click **Export Set-up…** in the **File** menu
38. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

39. Click **<OK>**

40. Click **Print Setup…** in the File menu
41. Select the data set by highlighting and clicking **<Add>**

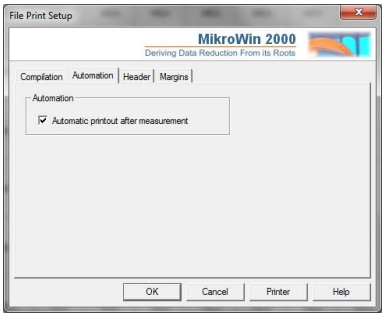
Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
AUC	area under curve (matrix 2)*
Vmax	delta @ max slope (ma. 3)*
Peak	peak value (matrix 4)*



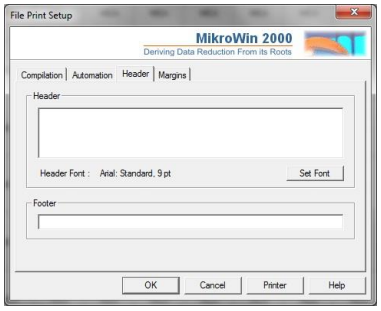
Gen. Statistics measurement settings
Kinetics Graphics curves

* the selection and content depends on the matrix definition done in the Calculation section

42. Check if **Automatic Print-out** is required

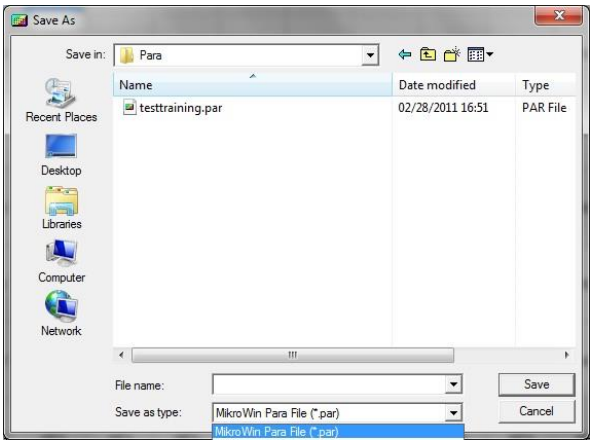


43. Type a **header** and/or **footer**



44. Click **<OK>**

45. Go to **File | Save As…**
46. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2***
47. Select the file type **Mikrowin Para File (*.par)**
48. Type a meaningful **file name**
49. Click **<Save>**



8.4.2 Kinetic measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2 "Measurement with a single endpoint rotocol"**.

8.5 Repeated Measurement

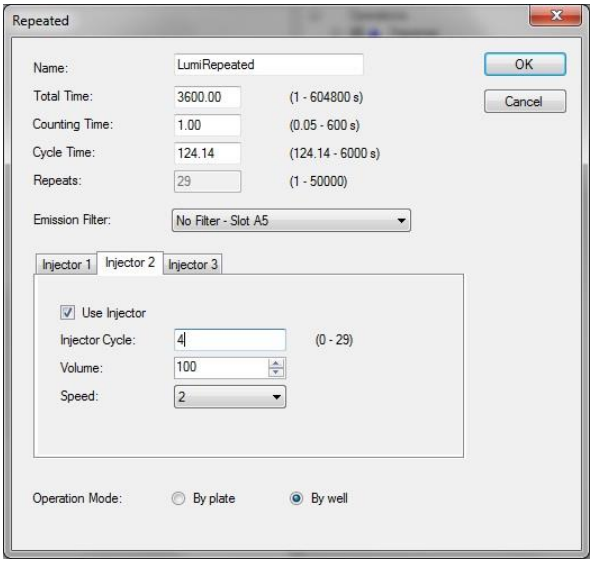
A repeated measurement mode is appropriate for long-term kinetic assays lasting over multiple minutes up to several days, e.g. cellular luminescence, slow enzyme kinetics, long-term gene expression or growth monitoring

8.5.1 Defining a protocol for a repeated measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click ***Lumin. Repeated*** for a luminescence repeated reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Emission Filter	usually: No Filter

Note: filters must be defined prior in the Instrument menu

Injector 1, …2, …3

Check Use Injector for an injection within the repeated cycle

Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

17. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications (BRET)

18. Double-click ***Fluor. Repeated*** in the Fluorescence section for a fluorescence repeated reading

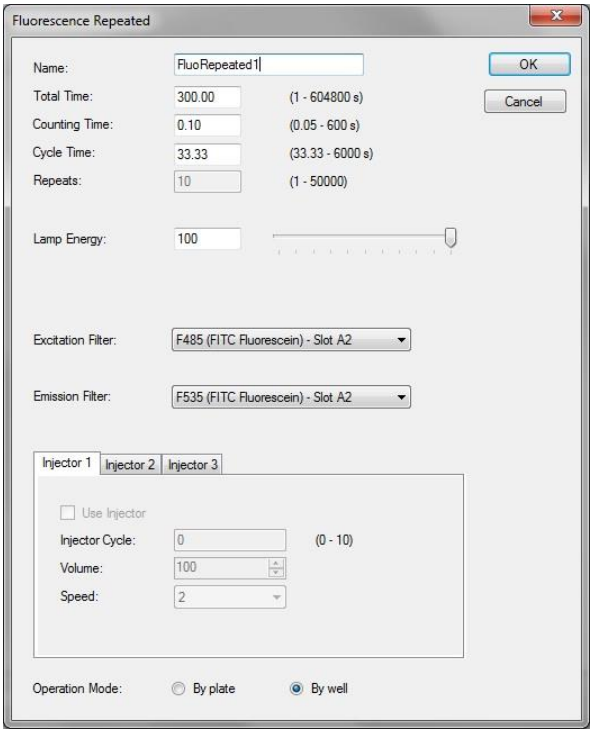
Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Injector 1, …2, …3

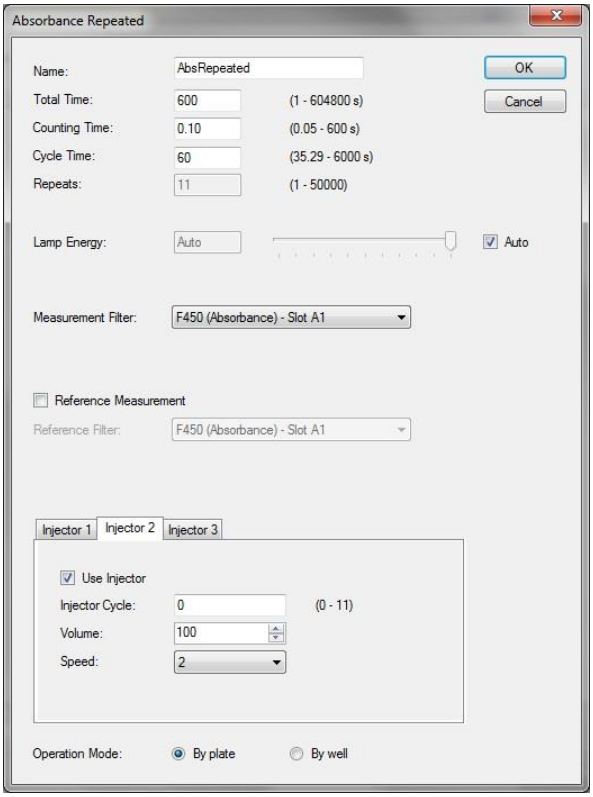
Check Use Injector for an injection within the repeated cycle	
Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

19. Click **<OK>**



a second repeated operation may be added, e.g. for ratiometric applications (FRET)

20. Double-click ***Abs. Repeated*** in the Absorbance section for a absorbance repeated reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter	select from the list

Check Reference Measurement if needed

Reference Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Injector 1, …2, …3

Check Use Injector for an injection within the repeated cycle

Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

21. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications

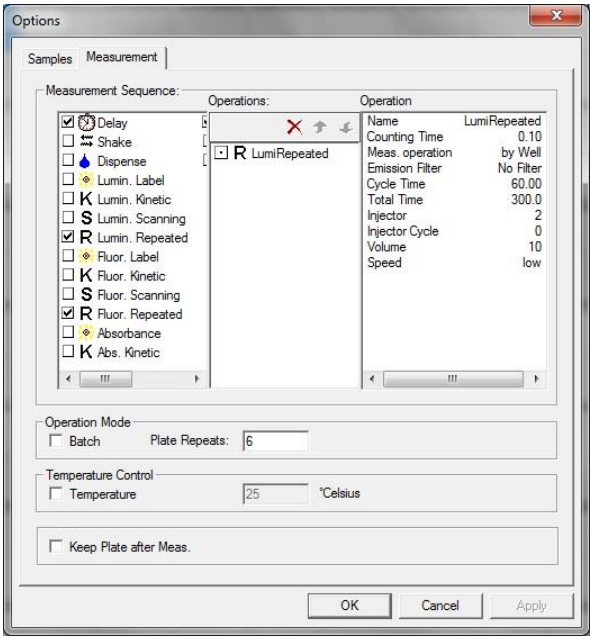
22. The **sequence of selected operations** will be displayed in the center column

    Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

    Operations can be deleted by highlighting and clicking the cross

    Details of the operation highlighted can be viewed on the right column

    An operation can be edited by double-clicking on it in the center column



23. Check **Temperature** to activate the temperature control for this protocol
24. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active

25. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished

26. Click **<OK>**

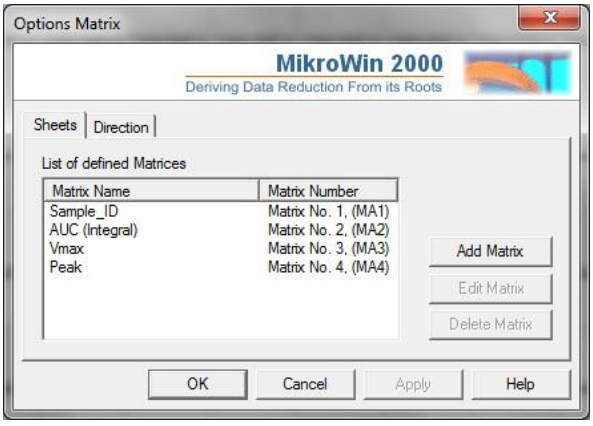
27. Click **<OK>** once more

    For further calculations of the measurements follow the next steps:

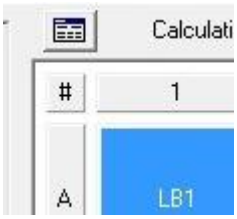
28. Go to **Options** | **Matrix** and click **<Add Matrix>**
29. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **AUC (Integral)**

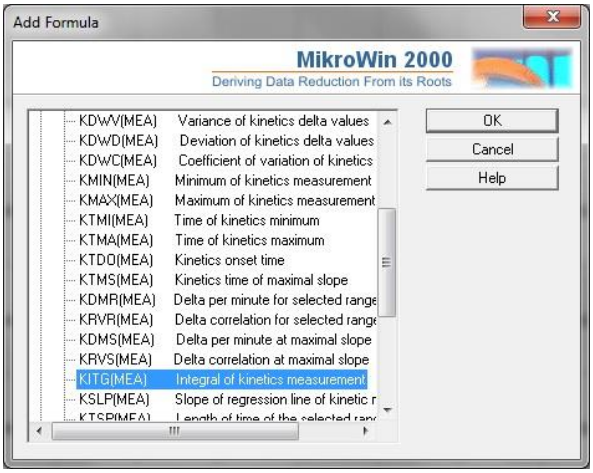
    Do the same for Matrix No. 3 (rename to e.g. **Vmax**) and Matrix No. 4 (rename to e.g. **Peak**)
30. Click **<OK>**

    For export und print you have to refer to these matrices.



31. Change the view to the **Calculation** section
32. Click on the ***2*** **AUC (Integral)** tab
33. Click **<Add Formula>** and expand **Kinetic calculation functions**
34. Select **KITG(MEA)** and click **<OK>**
35. Click the double-cross to assign for all wells





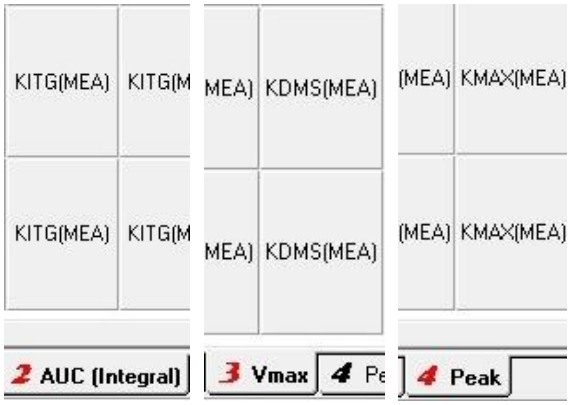
36. Proceed with the two other matrices alike:

3 **Vmax** **KDMS(MEA)**

4 **Peak** **KMAX(MEA)**

When 2nd measurement has been checked MEA need to be replaced by LB1 and LB2 respectively and additional matrices may need to be created

Should you wish to define any additional calculations please refer to the Mikrowin manual.



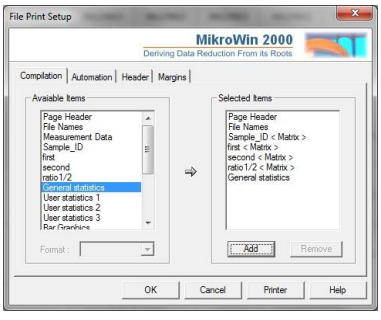
37. To activate automatic export click **Export Set-up…** in the **File** menu
38. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

39. Click **<OK>**

40. Click **Print Setup…** in the File menu
41. Select the data set by highlighting and clicking **<Add>**

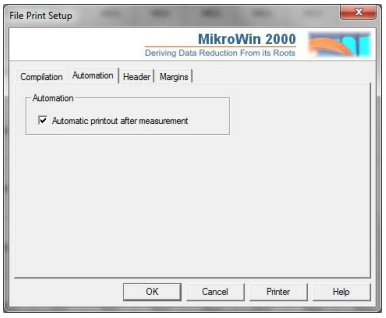
Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
AUC	area under curve (matrix 2)*
Vmax	delta @ max slope (ma. 3)*
Peak	peak value (matrix 4)*



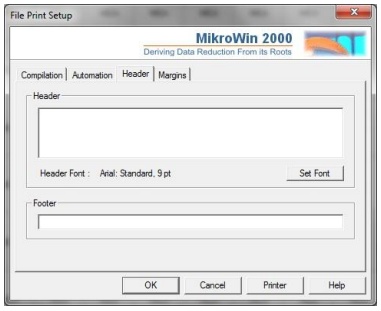
Gen. Statistics measurement settings
Kinetics Graphics curves

* the selection and content depends on the matrix definition done in the Calculation section

42. Check if **Automatic Print-out** is required

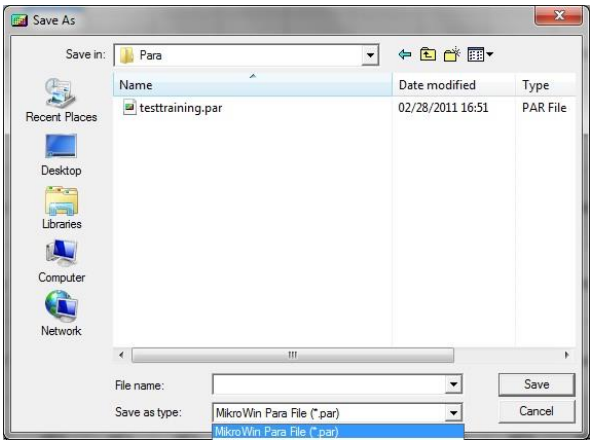


43. Type a **header** and/or **footer**



44. Click **<OK>**

45. Go to **File | Save As…**
46. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2***
47. Select the file type **Mikrowin Para File (*.par)**
48. Type a meaningful **file name**
49. Click **<Save>**



8.5.2 Repeated measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2 “Measurement with a single endpoint rotocol”**.

8.6 Scanning Measurement

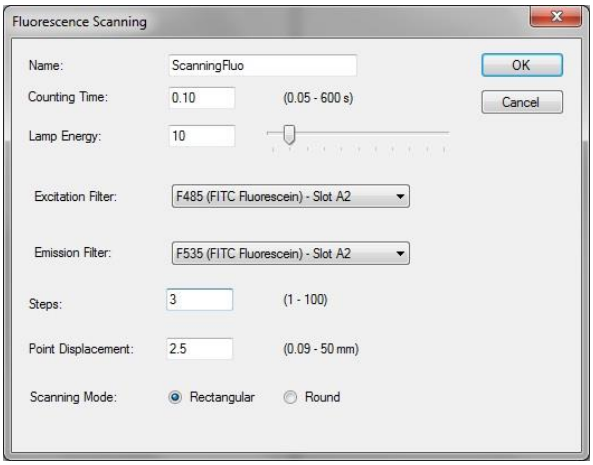
A scanning measurement mode is appropriate for assays with heterogeneous distribution of signal, e.g. cellular assays

8.6.1 Defining a protocol for a scanning measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click ***Fluor. Scanning*** for a fluorescence scanning reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list

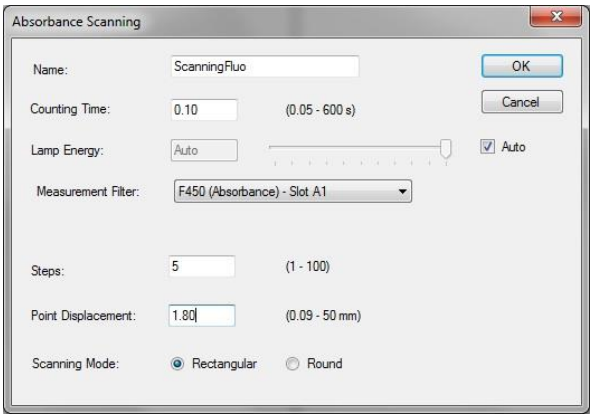
Note: filters must be defined prior in the Instrument menu

Steps	1 to 100 scanning points in one direction, the other direction will have the same amount of points
Point Displacement	distance between points

Select rectangular or round matrix

17. Click **<OK>**

18. Double-click ***Abs. Scanning*** for a absorbance scanning reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Steps	1 to 100 scanning points in one direction, the other direction will have the same amount of points
Point Displacement	distance between points

Select rectangular or round matrix

19. Click **<OK>**

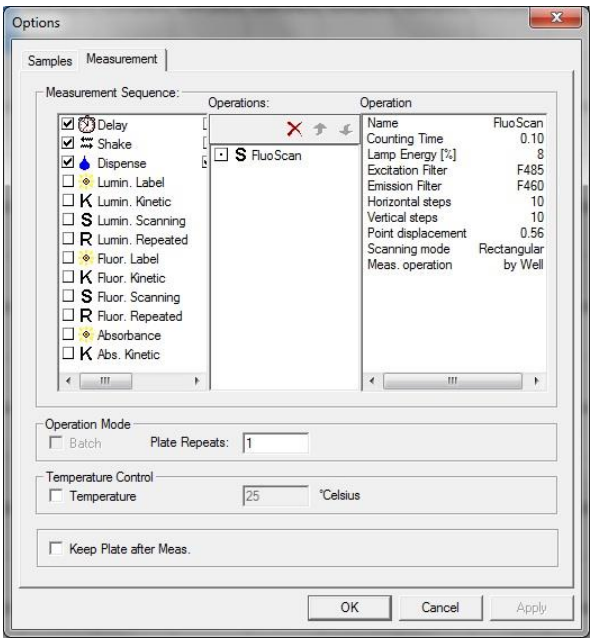
20. The **sequence of selected operations** will be displayed in the center column

    Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

    Operations can be deleted by highlighting and clicking the cross

    Details of the operation highlighted can be viewed on the right column

    An operation can be edited by double-clicking on it in the center column



21. Check **Temperature** to activate the temperature control for this protocol

22. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

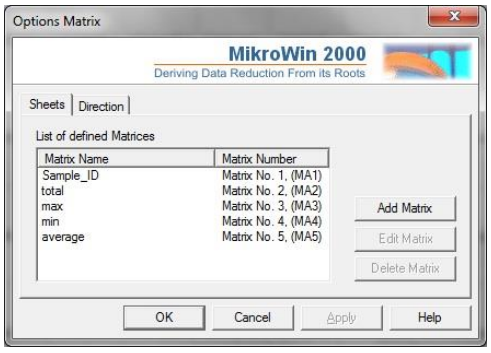
    Robot, Barcode and Multi Plate Data File Mode are currently not active

23. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished

24. Click **<OK>**

25. Click **<OK>** once more

    For further calculations of the measurements follow the next steps:



26. Go to **Options** | **Matrix** and click **<Add Matrix>,** click **<Add Matrix>** again

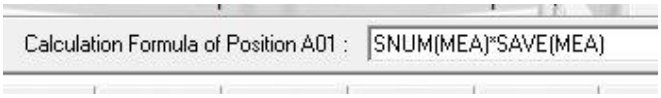
27. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **total**

    Do the same for Matrix No. 3 (rename to e.g. **max**), Matrix No. 4 (rename to e.g. **min**) and Matrix No. 5 (rename to e.g. **average**)

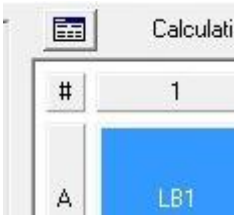
28. Click **<OK>**

    For export und print you have to refer to these matrices.

29. Change the view to the **Calculation** section
30. Click on the ***2*** **total** tab
31. Click **<Add Formula>** and expand **Area Scan functions**
32. Select **SNUM(MEA)** and click **<OK>**
33. Type an **asterisk (*)**
34. Click **<Add Formula>** and expand **Area Scan functions**
35. Select **SAVE(MEA)** and click **<OK>**



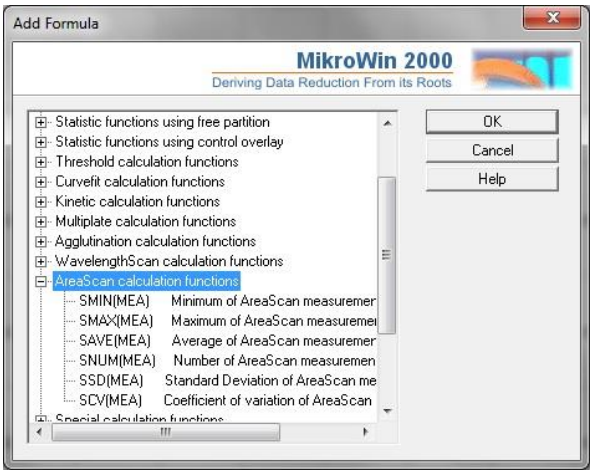
36. Click the double-cross to assign for all wells

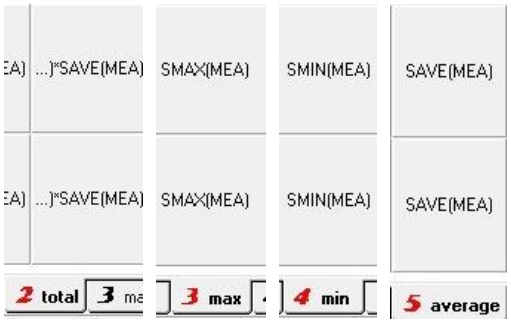


37. Proceed with the three other matrices alike:

3 **max**	SMAX(MEA)
4 **min**	SMIN(MEA)
5 **average**	SAVE(MEA)

Should you wish to define any additional calculations please refer to the Mikrowin manual.





38. To activate automatic export click **Export Set-up…** in the **File** menu
39. Select the appropriate and pre-defined export driver

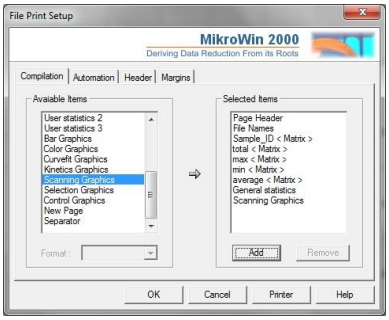
The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

40. Click **<OK>**

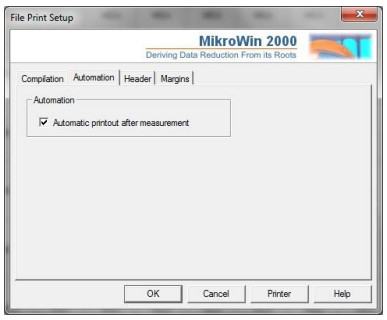
41. Click **Print Setup…** in the **File** menu
42. Select the data set by highlighting and clicking **<Add>**

Page Header	header part
File Names	par and dat files names
Sample ID	sample info (matrix1)*
total	sum of readings (matrix 2)*
max	max. reading (matrix 3)*
min	min. reading (matrix 4)*
average	average of read. (matrix 5)*
Gen. Statistics	measurement settings
Scanning Graphics	blue-to-red map

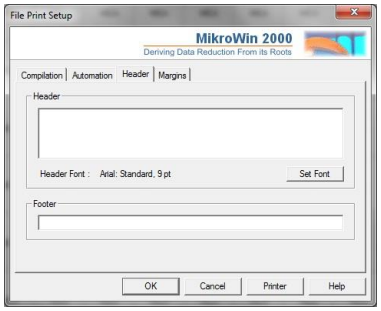
* the selection and content depends on the matrix definition done in the Calculation section



43. Check if **Automatic Print-out** is required

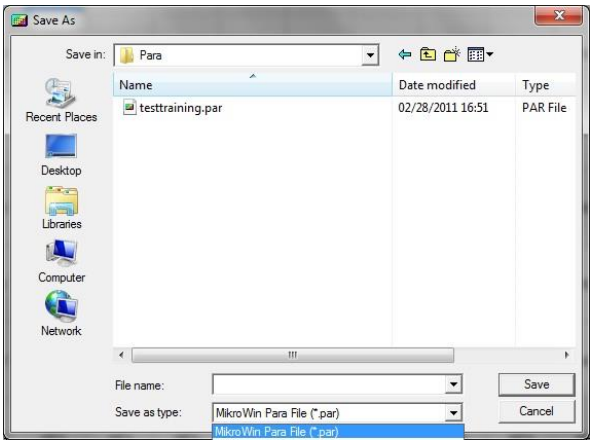


44. Type a **header** and/or **footer**



45. Click **<OK>**

46. Go to **File | Save As…**
47. Create and/or Select an appropriate directory, e.g. **ParaTriStar2**
48. Select the file type **Mikrowin Para File (*.par)**
49. Type a meaningful file name
50. Click **<Save>**



8.6.2 Scanning measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

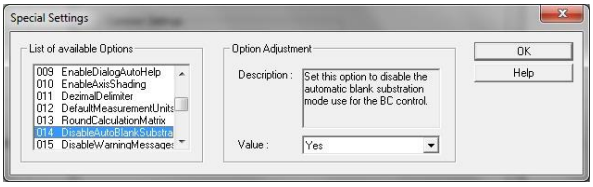
Follow the steps as described in paragraph **8.2.2 “Measurement with a single endpoint rotocol”**.

8.7 Measurement with curve fitting

A raw data measurement that contains standards with known concentrations which are used to determine unknown concentrations of the samples.

8.7.1 Defining a protocol with curve fitting

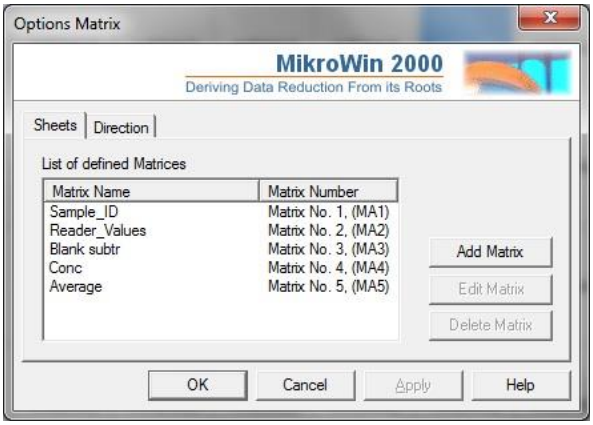
When working with Blank subtraction it is recommended to change a global setting in Mikrowin first.



1. Go to **Installation** | **Settings** and hit the **ALT** and the **S** keys
2. Scroll to **014 DisableAutoBlankSubtraction**
3. Select the value **Yes**
4. Click **<OK>**
5. Click **<OK>** once more

Next, follow the instructions until step 29 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

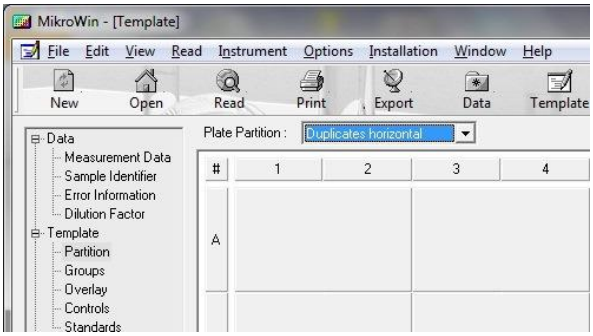


For further calculations of the measurements follow the next steps:

30. Go to **Options** | **Matrix** and click **<Add Matrix>,** click **<Add Matrix>** again
31. Highlight Matrix No. 3, click **<Edit Matrix>** and rename to e.g. **Blank subtr**

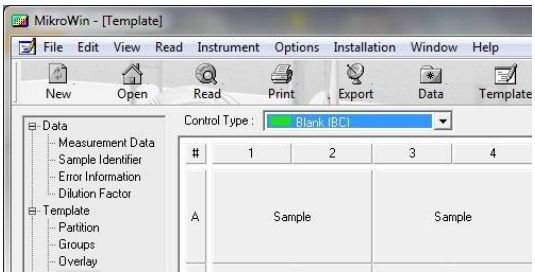
    Do the same for Matrix No. 4 (rename to e.g. **Conc**) and Matrix No. 5 (rename to e.g. **Average**)
32. Click **<OK>**

For export und print you have to refer to this matrices.

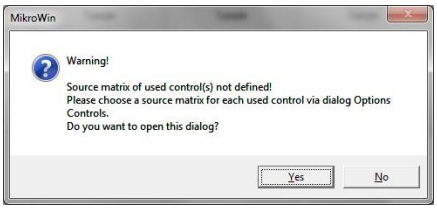


33. Change the view to the **Template** section
34. Click on the **Partition** tab and select a pattern matching your replicates in the **Plate Partition** drop box
35. Click the double-cross to assign for all wells

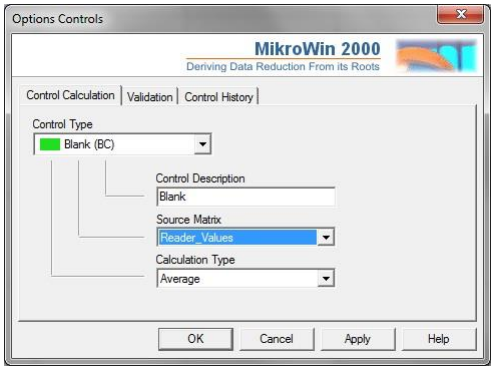
36. Click on the **Controls** tab
37. Select **Blank (BC)** in the **Control Type** drop box
38. Click into wells **A1**/**A2** to assign the blank to these wells



39. Click **<Yes>** in ***Warning! Source matrix***… dialogue



40. Select **Reader_Values** in **Source Matrix** drop box

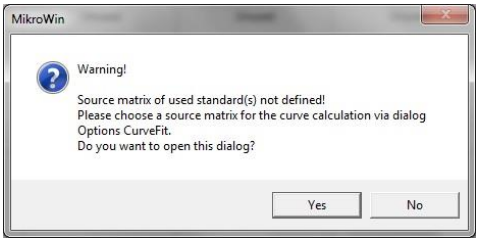


41. Click on the **Standards** tab
42. Click into **Standard Concentration** field and enter the concentration of the first standard
43. Select the matching dilution in the **Dilution Factor** drop box
44. Click into B1/B2 (resp. the set of wells containing the **first standard concentration**) and drag the mouse to the set of wells with the last standard concentration

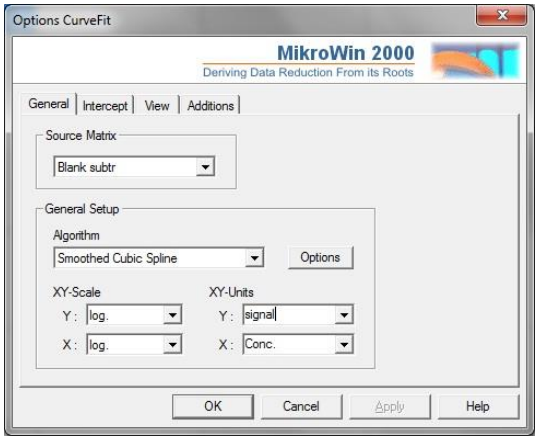
    In case you work with non-regular concentration series, click into the first set of wells, enter the concentration and hit the **ENTER** key

    Go ahead until the last concentration is being entered

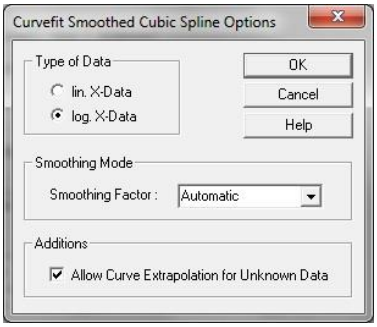
45. Click **<Yes>** in ***Warning! Source matrix***… dialogue



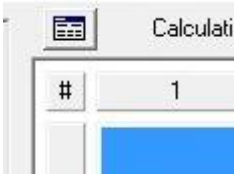
46. Select the **Source Matrix**, e.g. **Blank Subtr**
47. Select the curve fit **Algorithm**, e.g. **Smoothed Cubic Spline**
48. Define the **X and Y axis scales**, e.g. log for both
49. Type or select the axes **Units**
50. Click on **<Options>**

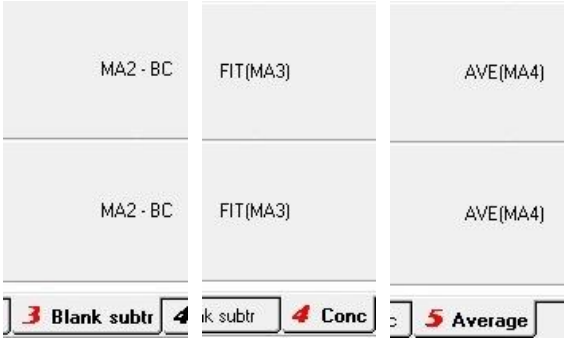


51. Select the **Type of Data**, whether they span a linear or a logarithmic range
52. **Smoothing Factor** can be kept as Automatic
53. **Curve Extrapolation** may be checked
54. Click **<OK>**
55. Click **<OK>** once more



56. Change the view to the **Calculation** section
57. Click on the ***3*** **Blank subtr** tab
58. Type **MA2 - BC** into the Calculation Formula:
    MA2 = matrix 2 = contains the reader values
    BC = Blank Control
59. Click the double-cross to assign for all wells





60. Proceed with the two other matrices alike:

    4 **Conc** **FIT(MA3)**

    FIT = curve fitting

    MA3 = matrix 3 = the fit concentration calculation is applied to the values of matrix 3

    5 **Average** **AVE(MA4)**

    AVE = calculation of mean value

MA4 = matrix 2 = calculation is done on the values of matrix 4 (in this case the concentrations)

Should you wish to define any additional calculations please refer to the Mikrowin manual.

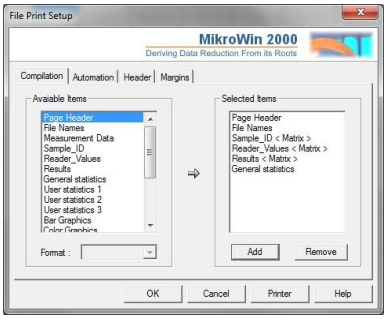
44. To activate automatic export click **Export Set-up…** in the **File** menu
45. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

46. Click **<OK>**

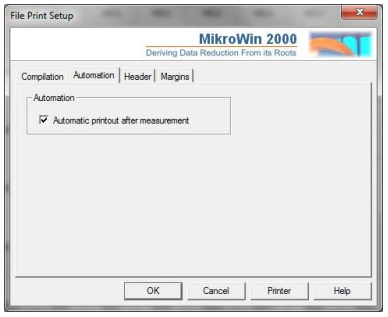
47. Click **Print Setup…** in the File menu
48. Select the data set by highlighting and clicking **<Add>**

Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
Reader_Values	measured data (matrix 2)*
Blank subtr	blank corrected (matrix 3)*
Conc	calc. conc. (matrix 4)*
Average	Averages (matrix 5)*
Gen. Statistics	measurement settings
Curvefit Graphics	standard curve

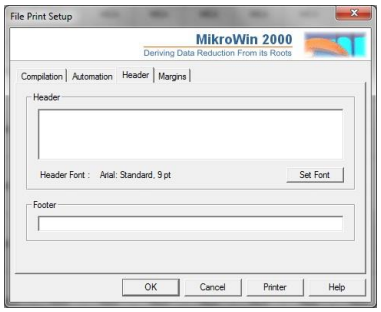


* the selection and content depends on the matrix definition done in the Calculation section

49. Check if **Automatic Print-out** is required

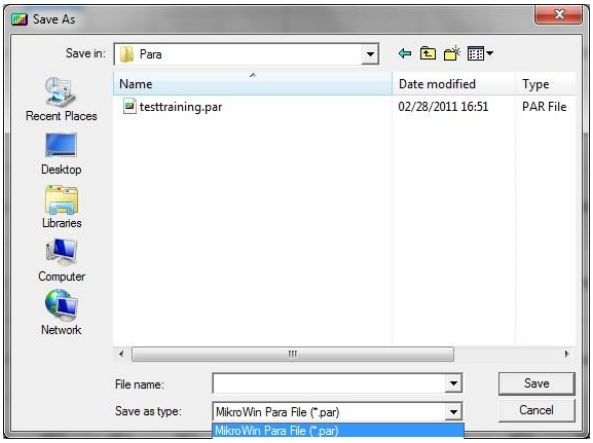


50. Type a **header** and/or **footer**



51. Click **<OK>**

52. Go to **File | Save As…**
53. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2***
54. Select the file type **Mikrowin Para File (*.par)**
55. Type a meaningful **file name**
56. Click **<Save>**



8.7.2 Measurement with a Curvefit parameter file

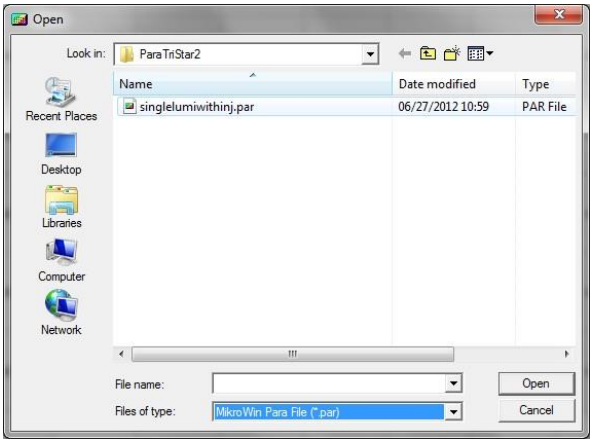
The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

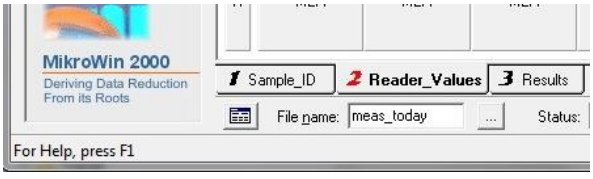
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

11. Click **Open** in the **File** menu
12. Select **File of type**: Mikrowin Para File
13. Select the appropriate file
14. Click **<Open>**



15. Enter a **file name** under which the measurement is to be stored



16. Click **<Start>**

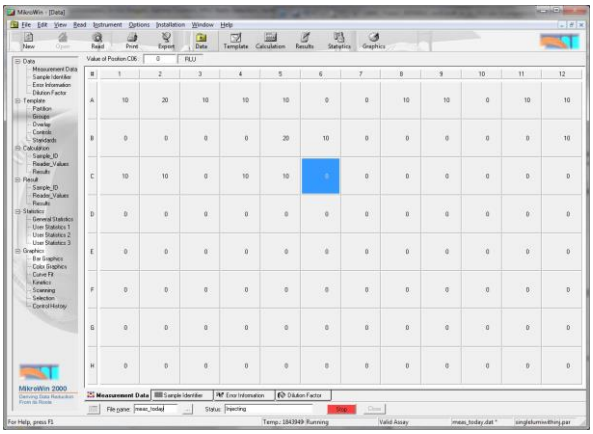
17. Insert the **microplate** with your samples:
well A1 facing the rear and left

    Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

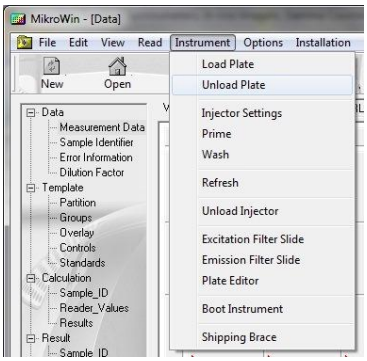
    Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

18. Click **<OK>**

19. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed



20. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument

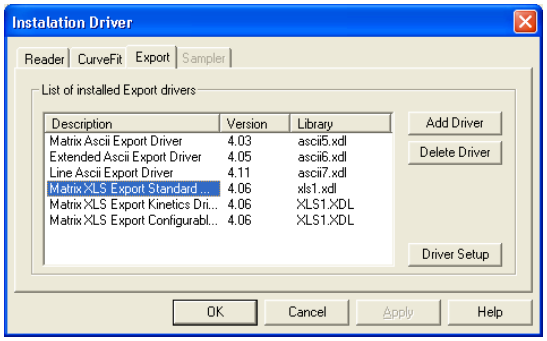


8.8 Export and print-out in Mikrowin

The export of (calculated) results and raw (measurement) data is carried out via export drivers. The export drivers have to be installed and configured. Data can be exported in XLS and TXT file formats.

8.8.1 Export Driver Configuration

Export drivers have to be installed if you want to export data. In addition, you have to set up the export driver and you have to specify data structure, data matrices as well as header and footer. Data is exported depending on the driver selected and configured in this dialog box. To use another data format, you can select another driver before running a measurement or set up the selected driver new.
In the **Installation Driver** dialog box, select the **Export** tab to view the available drivers. You may choose:



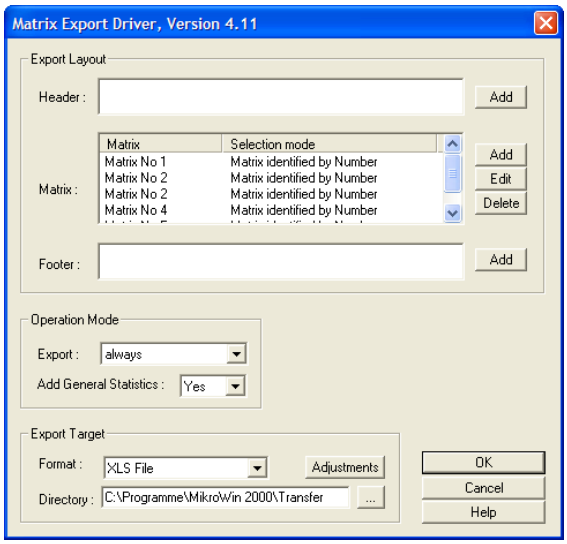
Selection of export drivers

Matrix Export Driver Driver (template) for export of calculated data with matrix (i.e. plate lay-out) structure. Only data that are visible on result matrices can be exported. File formats may be EXCEL, Text (ASCII) and CSV.

Line Export Driver Driver (template) for export of calculated data with list (i.e. table-type) structure. Only data that are visible on result matrices can be exported. File formats may be EXCEL, Text (ASCII) and CSV.

RawData Export Driver Driver (template) for export of all raw data. File formats may be EXCEL, Text (ASCII) and CSV. Whether data in the export file are presented in list or matrix format depends on the settings and data origin.

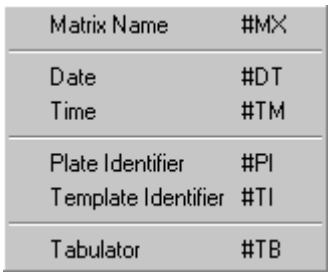
Matrix Export Driver If you select the Matrix export driver, you have to define the following configuration:



Matrix export driver setup

Export Layout Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).



Context menu for entering header placeholders

Example:

Header with date, time and plate identification, separated by tab characters: **#DT#TB#TM#TB#PI**

Matrix In this text box you enter the matrices whose data you wish to export. In general, one exports only data from the result matrices. ***Make sure*** that the matrices and their numbers specified here is identical with the number of the result matrix in the parameter file.

Click <**Add**> to open the context menu and select the matrix number (1 – 15) or define the matrix name. Several matrices can be selected one after the other. They are entered in the matrix list.

Click <**Delete**> to delete the selected matrix from the matrix list.

Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.). This context menu includes the same options as the header context menu.

Operation Mode Define additional options.

Export The proper setting is **Always**.

Add General Statistics Options are **Yes** or **No**.

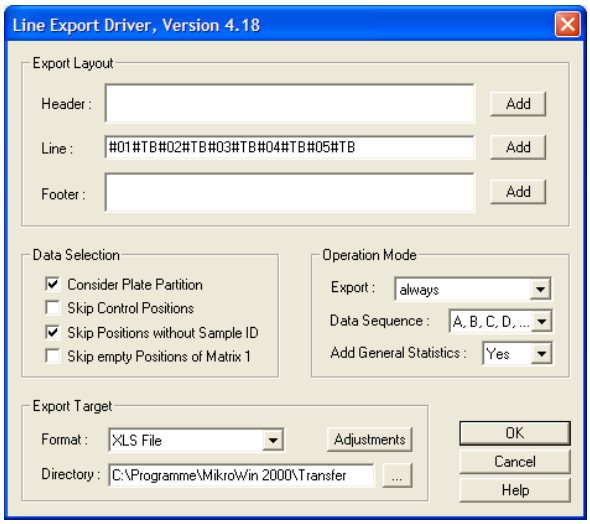
Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from **Text File, XLS File, CSV File, CommPort and Clipboard**.

Target Define the directory which the file is to be exported to. You may use the browse <**...**> button to locate an appropriate directory.

Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

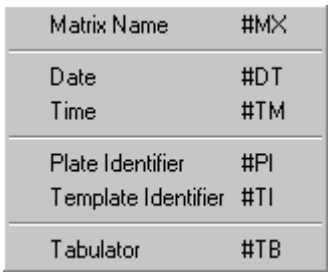
Line Export Driver Select this export driver to define a table-type file. Parameters (header, matrix and footer as well as the target directory for data storage) are entered in the same manner as for an matrix-type file (see previous section).



Line export driver setup

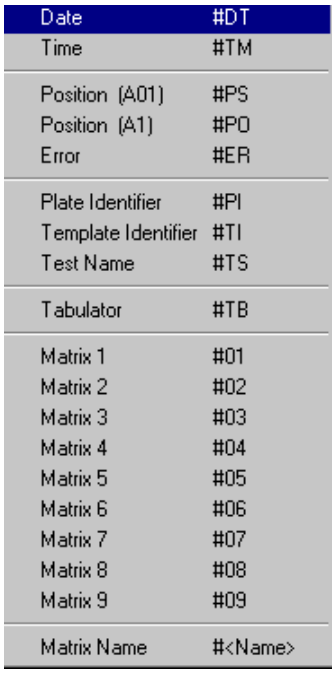
Export Layout Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).



Context menu for entering header placeholders

Line In this text box you enter the matrices whose data you wish to export. In general, one exports only data from the result matrices. ***Make sure*** that the matrices and their numbers specified here is identical with the number of the result matrix in the parameter file.



*Context menu **Line Export Driver***

Click <**Add**> to open the context menu and select the matrix number (1 – 15) or define the matrix name. Several matrices can be selected one after the other. They are entered in the matrix list.
Click <**Delete**> to delete the selected matrix from the matrix list.

Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.). This context menu includes the same options as the header context menu.

Data Selection Define additional options regarding data sources and positioning.

Consider Plate Partition This option should be checked when replicates are used and they are to be exported next to each other.

Skip Control Positions This option may be checked if values of Controls are not supposed to be exported.

Skip Positions without Sample ID Check if only samples with sample IDs are to be exported.

Skip Empty Positions of Matrix 1 This option may be used if the values of unused wells are not to be exported. Matrix 1 must contain an appropriate variable like **MEA** or **LB 1**.

Operation Mode Define additional options.

Export The proper setting is **Always**.

Add General Statistics Options are **Yes** or **No**.

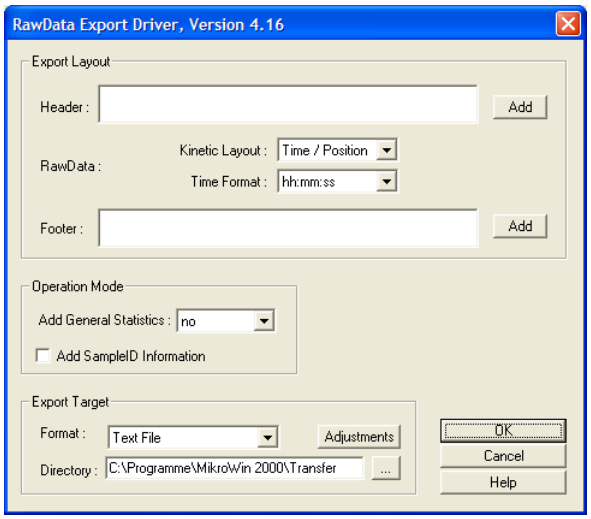
Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from **Text File, XLS File, CSV File, CommPort and Clipboard**.

Target Define the directory which the file is to be exported to. You may use the browse <**…**> button to locate an appropriate directory.

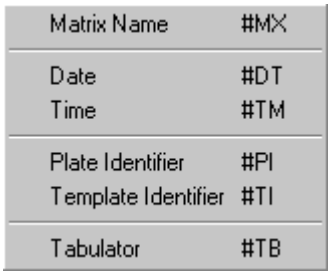
Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

RawData Export Driver With this export driver a file containing all raw data will be created.
For the export of kinetic data the kinetic layout can be selected (see below).
When the Rawdata Export driver is used for values derived from scanning operations each well is displayed in a separate area with the individual reading points displayed in an X-Y matrix representing the scanning positions.
With data coming from multilabel measurements (e.g. BRET) with a single reading per wavelength the data are exported in a respective amount of matrices representing the plate layout.
Data from multi-plate readings (Batch mode) are also exported in a matrix orientation. ***Note:*** only single readings are supported!



Export Layout Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).



Context menu for entering header placeholders

RawData Kinetik Layout
The selection ***Position/Time*** has a column adressed to each well position (left to right) and the consecutive readings are entered in lines (down).
The selection ***Time/Position*** has a line adressed to each well position (down) and the consecutive readings are entered in columns (left to right).

Note: Keep in mind that EXCEL supports a maximum of 256 columns.

Time Output Format
Select the time format a kinetic reading. Choices are: ***hh:mm:ss*** or ***sec.msec***.

Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma,

space, etc.). This context menu includes the same options as the header context menu.

Operation Mode Define additional options.

Add General Statistics Options are **Yes** or **No**.

Add Sample ID information Check if you want that information added to each value.

Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from **Text File, XLS File, CSV File, CommPort and Clipboard**.

Target Define the directory which the file is to be exported to. You may use the browse <**...**> button to locate an appropriate directory.

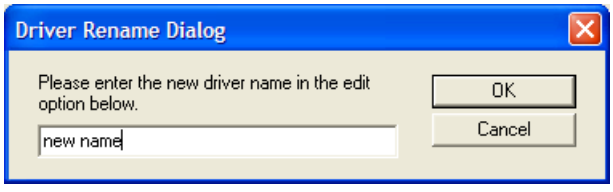
Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

8.8.2 Adding additional / replicating export drivers

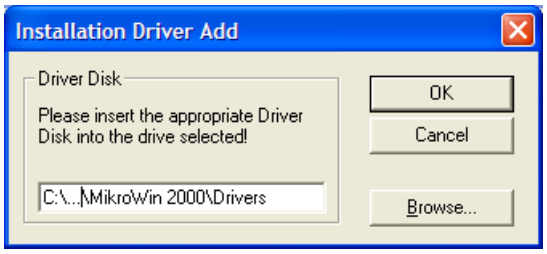
Especially in multi-user environment the individual users will have their own demands for export driver setups. To support this multiple copies of the export driver can be installed and each of the copies can be individually set up.

It is recommended for convenience and security to create a new directory within the ***Mikrowin 2000*** directory (e.g. called "**Drivers**") and copy the original export drivers ***matrix1.xdl***, ***line1.xdl*** and ***rawdata1.xdl*** to it.

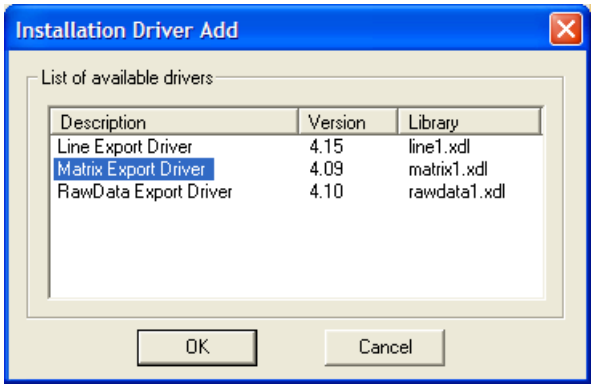
First, rename the export driver that is to be installed a second time by highlighting it in the **Installation** | **Driver** | **Export** menu. Hit the **ALT** and the **R** keys simultaneously. You can enter a new name for the driver. Confirm with <**OK**>.



Now you can re-install the driver again by the clicking <**Add Driver**> and browsing to the driver directory you created.



Select the respective driver in the dialogue displayed.



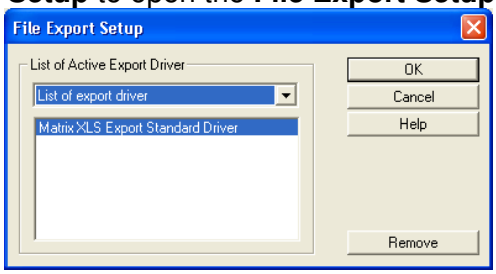
You may repeat this procedure as often as necessary to get an appropriate number of export drivers.

8.8.3 Automatic export

Choose the menu item **File** | **Export Setup** to select the export driver that is to be loaded automatically upon successful completion of a reader run. If a driver has been selected for the active parameter file, data evaluation is performed after completion of the respective measurement and data export is carried out in accordance with the selected driver.

Please keep in mind:
This function is only valid for the active parameter file.
Prerequisite for automatic data export is that the respective export driver has been installed and set up in the menu item **Installation** | **Driver** (see chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**) and the export driver has been selected in the menu item **File** | **Export** (see chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**).

Open parameter file.
Select **File** | **Export Setup** to open the **File Export Setup** dialog box.



File Export Setup dialog box

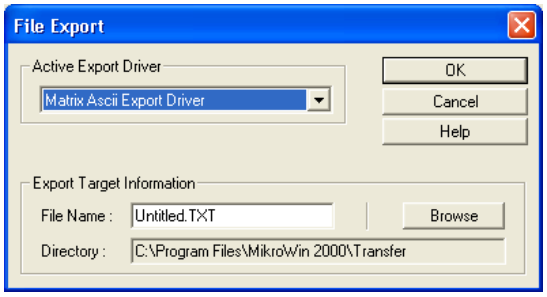
List of Active Export Driver
Select the export driver you want to use for automatic data export upon successful completion of a reader run. Click on the arrow button to open the list showing the available drivers and select the driver you want. The selected drivers appear in the text box directly below the drop-down list box.
To delete a driver from the list, select this driver and then click <**Remove**>.

Click <**OK**> to accept your selection.

You must save the parameter file after the export setup !

8.8.4 Export on demand

The following dialog supports manual export of program data. The data to be exported, the format as well as the export destination depend on the selected driver and its configuration. The actual data export is carried out by an export driver if you click on the <**Export**> button after a measurement.
Open the parameter file you need.
Select **File** | **Export** to open the **File Export** dialog box.



File Export dialog box with open driver list

Active Export Driver Select the export driver you want to use for data export. Click on the arrow button to open the list showing the available drivers and select the driver you want. ***Please keep in mind*** that you have set up the driver you have selected here in the menu **Installation** | **Driver** | **Export**. Otherwise, no data will be transferred!

Export Target Information **File Name**
Shows the file name of the active parameter file. An extension identifying the selected driver is appended (XLS for Excel files and TXT for ASCII files). The file name can be edited.

Directory
The target directory has been defined by the selected export driver during installation. Click the <**Browse**> button to select another target directory.

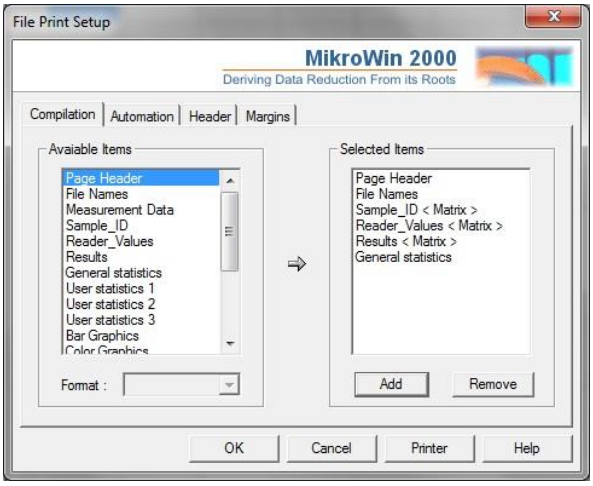
Click <**OK**> to accept your selection.

The export will be executed automatically if selected in the respective protocol file.

8.8.5 Data Print-out

Data and results can be printed automatically whenever a measurement with a parameter file has been done - ***Automatic Print-out*** has to be checked as described in the previous chapters – or on demand for the currently loaded data file.

1. Go to **Print Setup…** in the **File** menu
2. Select the appropriate items by highlighting and clicking **<Add>**



3. You may check the settings and layout by selecting **Print Preview** in the **File** menu to get a preview of the print-out
4. Select **Print** in the **File** menu to start printing the data

The print-out will be executed automatically if checked when created in the respective parameter file.

8.8.6 Print-out of parameter file settings

All settings including the calculations can be printed by clicking **Template Print-out** in the **File** menu. The information will be presented as a HTML file in the web browser from where you can print the content.

9. Maintenance

9.1 Cleaning the Instrument

9.1.1 Cleaning the instrument surface

The **surface** of the instrument is protected by a washable finish. Dirty or dusty surfaces should be cleaned using a damp cloth or optical grade tissue. If necessary, use a mild detergent or diluted EtOH.

Do not use a scouring agent!

9.1.2 Cleaning the inside of the instrument

The inside of the instrument does not need to be cleaned regularly. Only in case liquid spillage it may be necessary to clean the inside.

Do not open the instrument by yourself! Call a Berthold Technologies technical support person.

Before opening the instrument, turn it off and disconnect it from power supply!

Open the screws on the instrument cover to clean the instrument inside. Then detach the cover by moving or lifting it carefully.

Always keep the sample holders and the entire inside of the instrument clean. Wipe off any dirt using a damp cloth or optical grade tissue. Use cotton buds for corners. Remove dirt quickly so it does not get fry and may not have any adverse effect on moving parts.

9.2 Cleaning Tubing

Injector tubing have to be washed

- ❒ before starting work
- ❒ before changing reagents
- ❒ at the end of each work session before turning off the instrument
- ❒ after longer periods of inactivity

Use solutions recommended by the kit manufacturer.

Other recommended cleaning reagents are
- *o deionised water*
- *o diluted alcohol: 70 % Ethanol, Propanol*
- *o 2 - 5 % hypochlorite solution (“bleach”)*
- *o 0.5 – 1 M Chloric acid (HCl)*
- *o 0.5 – 1 M Sodium hydroxide (NaOH)*
- *o 0.1 % SDS*

o *Non-foaming detergent (up to 10 %)*

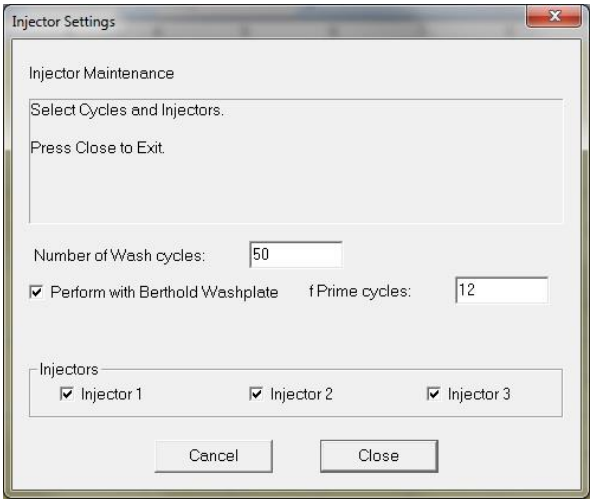
Some of these reagents may be hazardous. Please refer to the respective safety instructions (e.g. R and S codes) of the supplier.

Berthold Technologies´ cleaning solution CLEANIT Standard* (product code 45218) *is an efficient and proven cleaning solution for most of the common reagents in use. It is recommended to use this solution at least once a weak to ensure a long lifetime of the injectors!

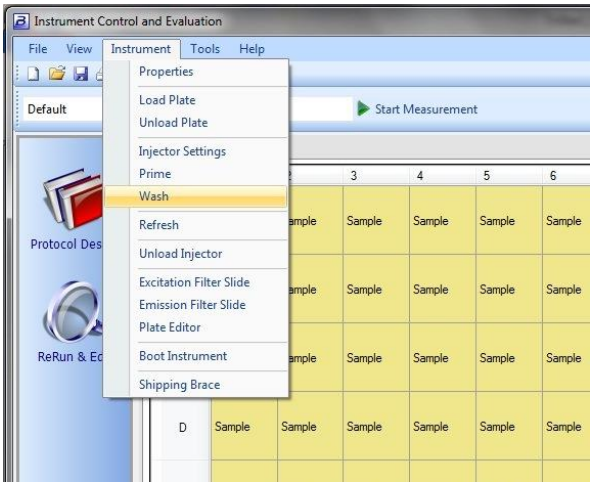
Injector tubing have to be primed

❒ prior to each measurement using the respective reagents.

1. Click **Injector Settings** in the **Instrument** menu
2. Define the **default number of wash cycles** – 50 is recommended
3. check the **use of the Berthold Technologies Washplate** (when available)



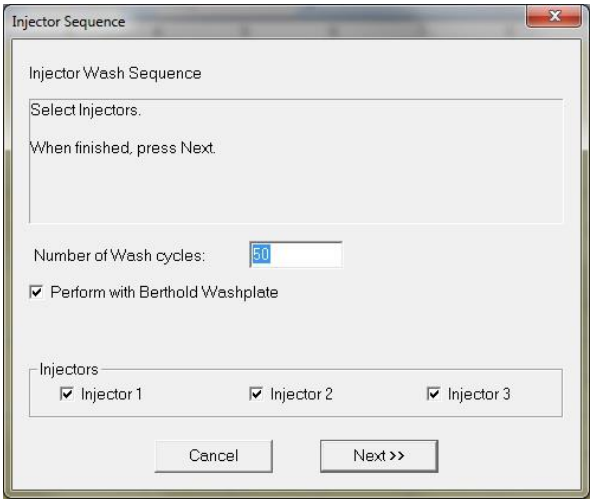
4. Click **Wash** in the **Instrument** menu



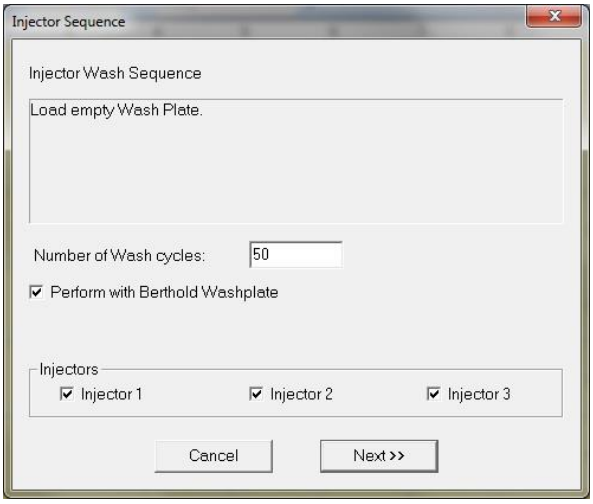
5. Define the number of **Wash Cycles**

   Make sure the total Wash volume does not exceed the volume of the plate being used!

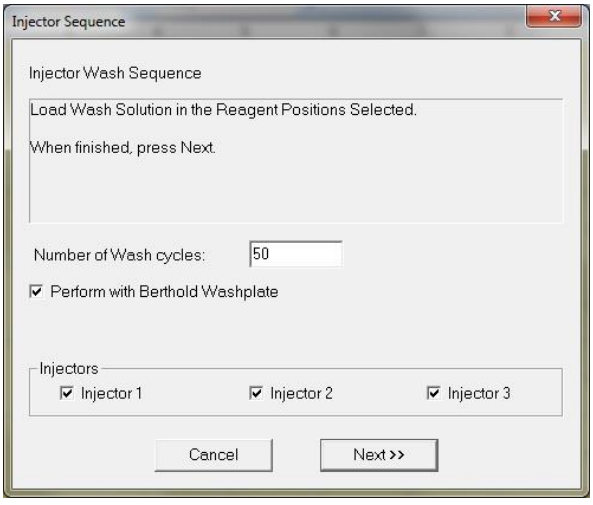
6. Select the respective **injector(s)**
7. Click **<Next>**



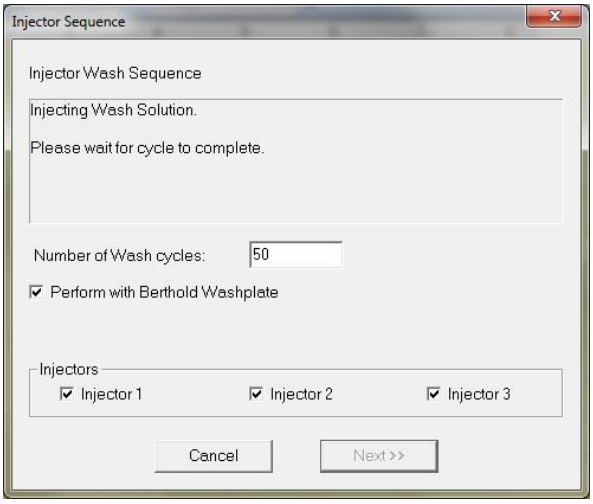
8. Load a **Wash plate**
9. Click **<Next>**



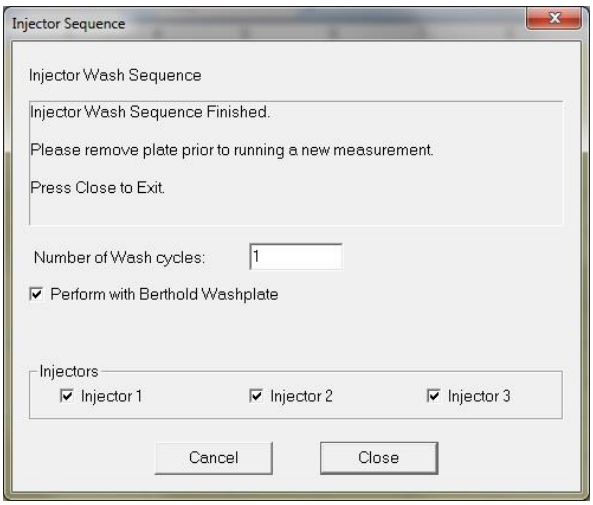
10. Attach the reservoir containing the appropriate-**Wash Solution** (see above)
11. Click **<Next>**



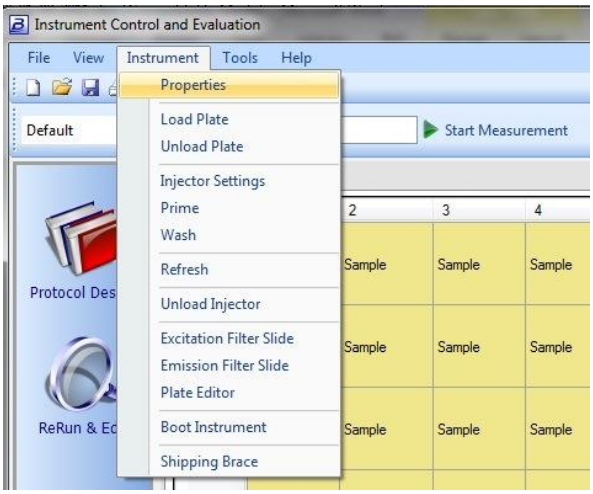
12. Wait until the wash cycles are completed



13. Click **<Close>**



14. **Remove Wash plate** by clicking **Unload Plate** in the Instrument menu



Note: It is recommended to leave deionised water in the injection lines during idle periods of hours up to a few days.

Only in case the instrument stays idle for multiple days up to weeks it is recommended to empty the lines by starting the Wash procedure without a wash solution.

9.3 Priming Tubing

9.3.1 Priming before Measurement

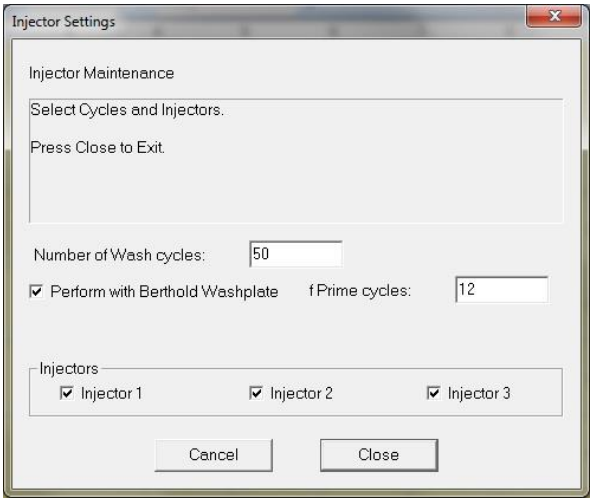
Injection lines have to be primed (filled) prior to measurements which require the use of injectors for reagent addition.

Note: It is strongly recommended to perform the priming with deionized water first and leaving the lines filled with deionized water before priming with reagents.

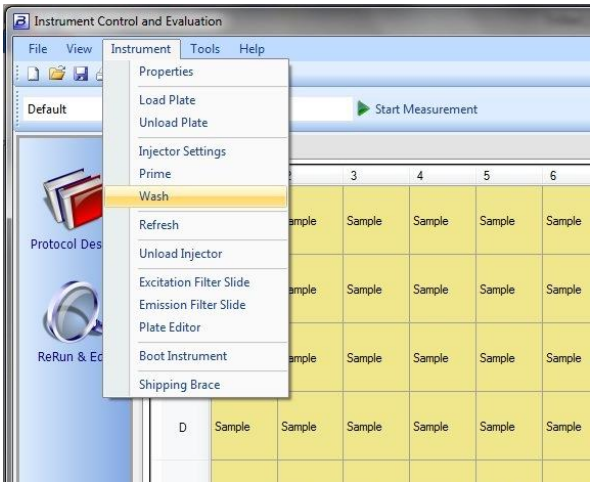
This procedure avoids reagents aerosol splashes at the injector tips and thus contamination of the instrument.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

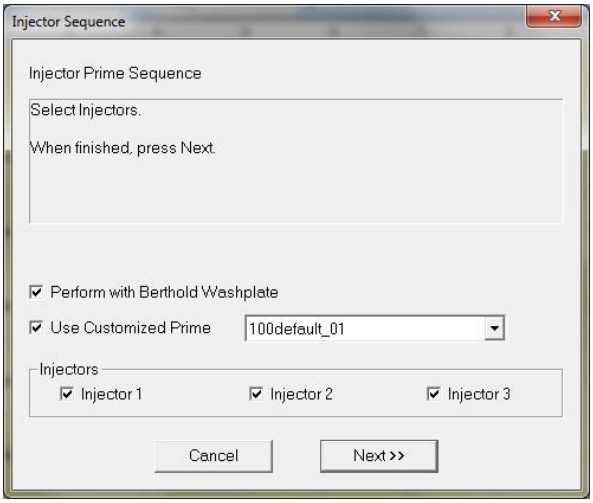
1. Click **Injector Settings** in the **Instrument** menu
2. Define the **default number of prime cycles** – 12 is recommended – which will be used for the default priming and check the **use of the Berthold Technologies Washplate**



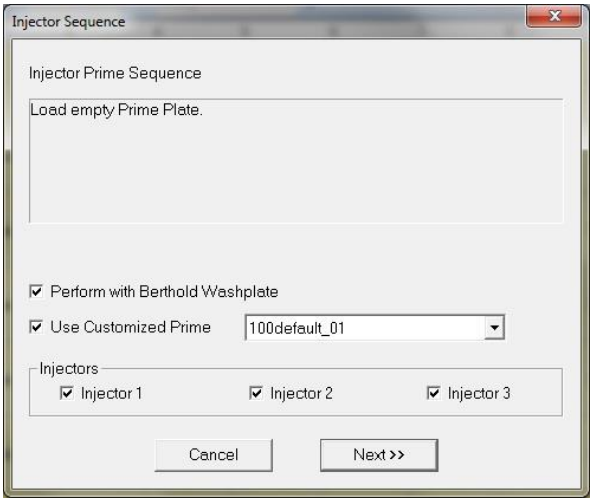
3. Click **Prime** in the **Instrument** menu



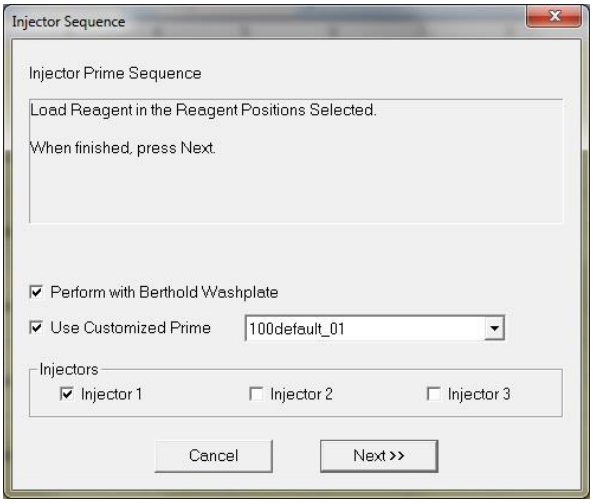
4. Check Berthold **Washplate** (when available)
5. Select the prime mode
   a. Check **Use Customized Prime** to select a user defined method (see next chapter)
   b. Uncheck Use Customized Prime to use the default prime mode
6. Select the respective **injector(s)**
7. Click **<Next>**



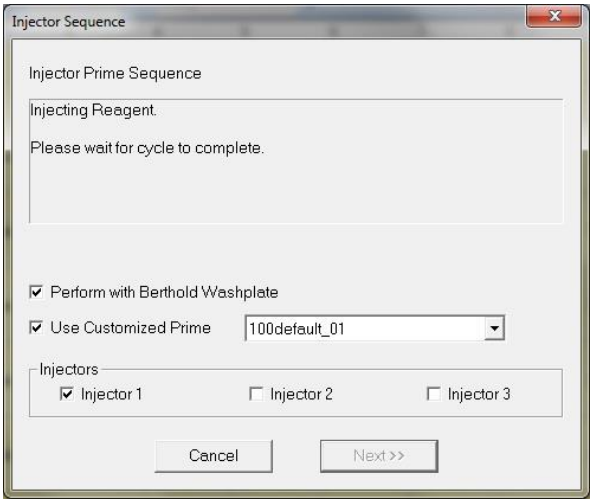
8. Load a prime plate / wash plate
9. Click **<Next>**



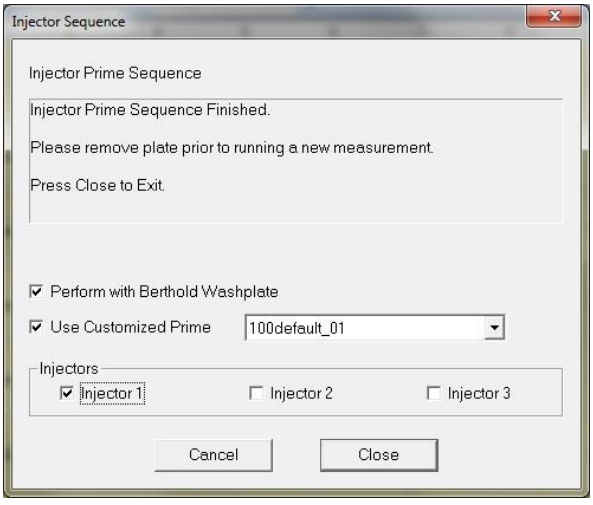
10. Attach the reservoir(s) containing the appropriate **Assay Reagents** (or deionized water; see above)
11. Click **<Next>**



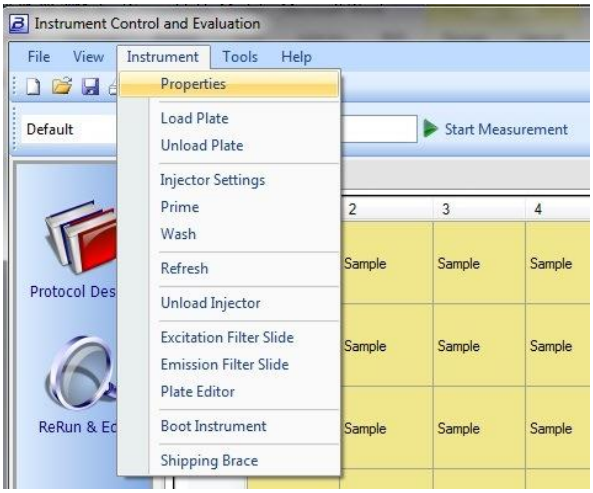
12. Wait for the Prime procedure to be finished for one injector



13. Click **<Close>**



14. **Remove prime** / **wash plate** by clicking **Unload Plate** in the Instrument menu

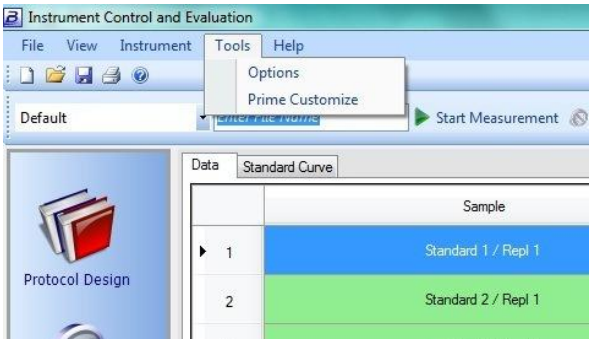


15. The instrument is now ready for use

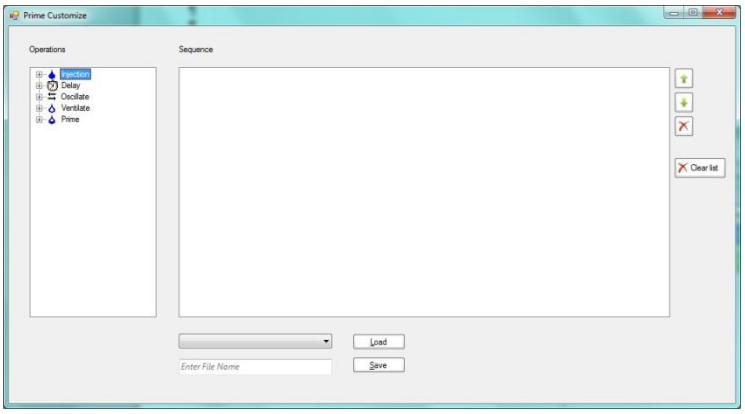
9.3.2 Customizing the Priming Sequence

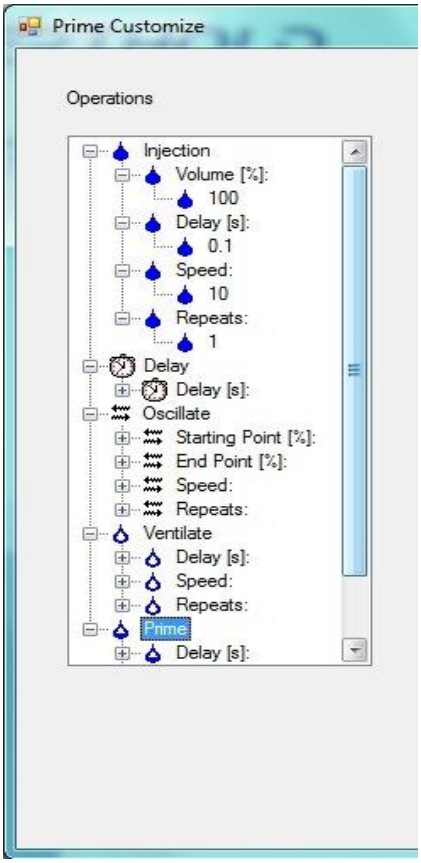
Some reagents (e.g. high viscosity) or solutions (e.g. cells) require special priming procedures which can be defined individually.

1. Click **Prime Customize** in the **Tools** menu



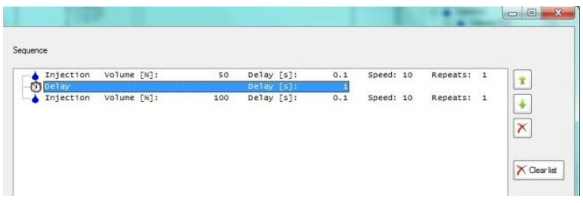
2. The Prime Customize dialog will be displayed





clicking on ⊞ in the Sequence window will expand the respective folders and display the settings

3. The respective operation can be selected for the prime sequence by dragging it from the left column to the right column (**Sequence**)



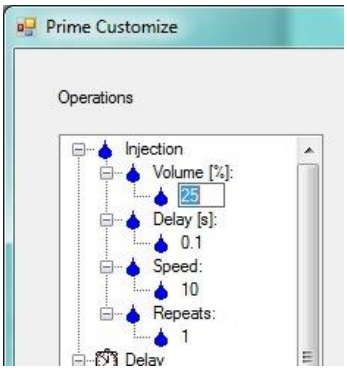
to change the sequence the arrow buttons can be used

to remove an operation the button can be used

The operations and their settings:

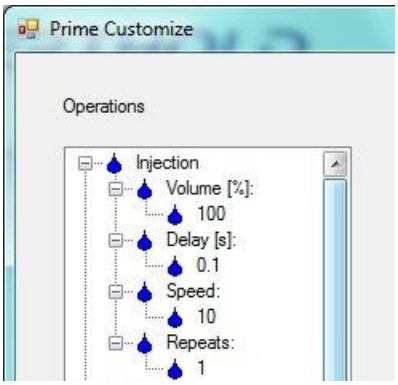
To change the settings

- expand the operation
- expand the setting
- click onto the number
- click onto the number a 2nd time
- type the appropriate number
- confirm with the **ENTER** key



a. **Injection**
the injector is filled with the max. injection volume from the reagent reservoir and injects the set volume

Volume	percentage of the max. inj. vol.
Delay	delay before the operation in sec
Speed	1 … 10
Repeats	number of repeats

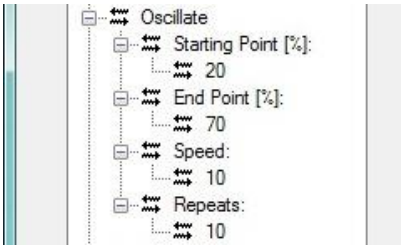


b. **Delay**
a delay time that elapses between operations, e.g. to mimic the injection timing of the assay (this can be important with a cell suspension)

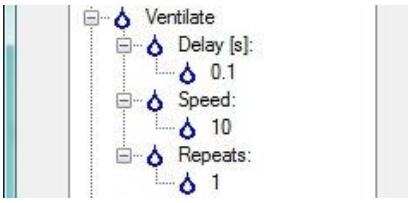
Delay	elapsing time in sec

c. **Oscillate**
the injector is (partly) filled and oscillates between the set positions (back into the reagent reservoir)

Start. Point	percentage of the max. inj. vol.
End Point	percentage of the max. inj. vol.
Speed	1 … 10
Repeats	number of repeats

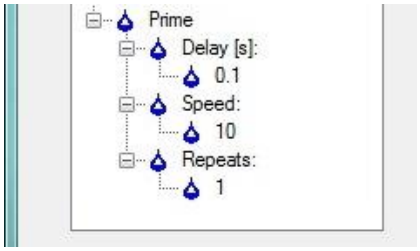


d. **Ventilate**
the injector is completely filled (beyond the max. injection volume) from the reagent reservoir and injects the total volume of the bellow



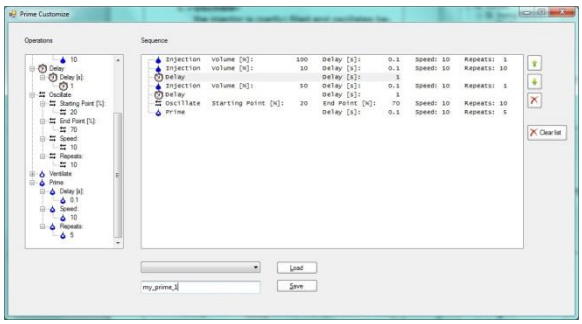
Delay	delay before the operation in sec
Speed	1 … 10
Repeats	number of repeats

e. **Prime**
the injector is filled with the max. injection volume from the reagent reservoir and injects the full volume



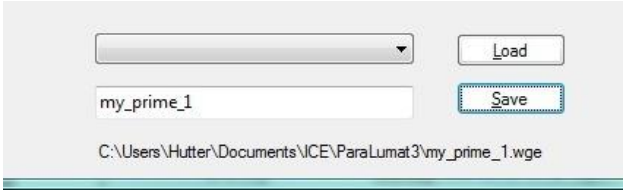
Delay	delay before the operation in sec
Speed	1 … 10
Repeats	number of repeats

4. After the sequence is completed enter a **name** for this priming sequence and click **<Save>**



the file will get the extension .wpe

the respective directory will be displayed



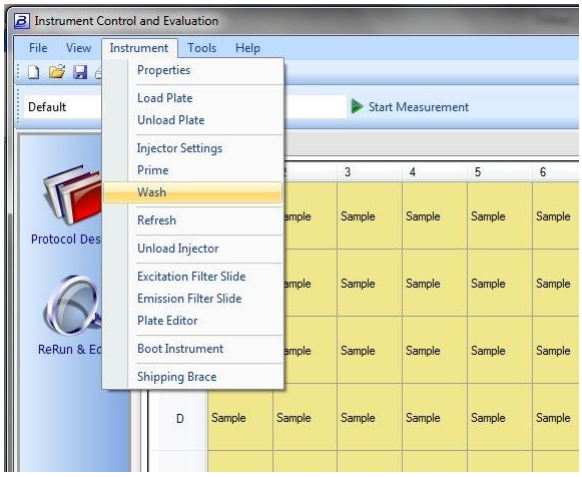
5. Close the dialog by clicking

9.4 Emptying Tubing

This operation can be used to empty the injection lines after the measurement and re-collect valuable reagents in the reagent reservoirs.

Note: Make sure the reagent reservoir are connected to the injection tubings !

1. Click **Unload Injector** in the **Instrument** menu

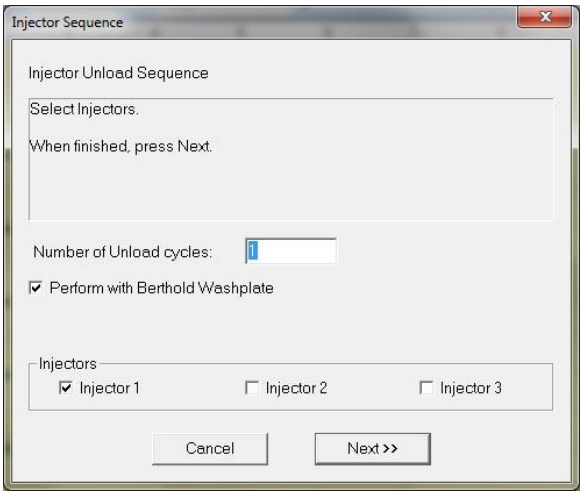


2. Define the **Number of Unload cycles**

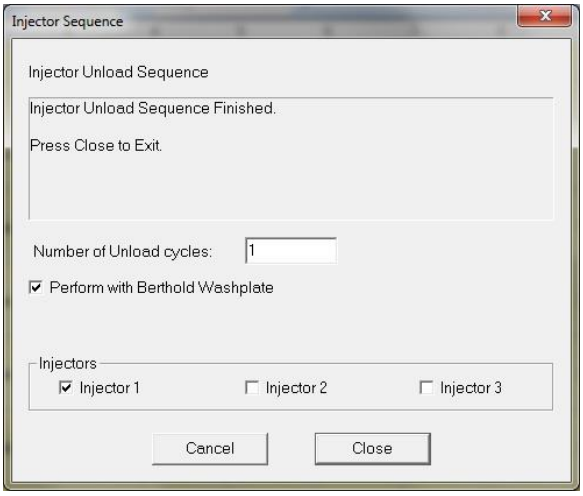
   each cycle is equivalent to the max. injection volume of the injector installed

   a minimum of 10 is recommended

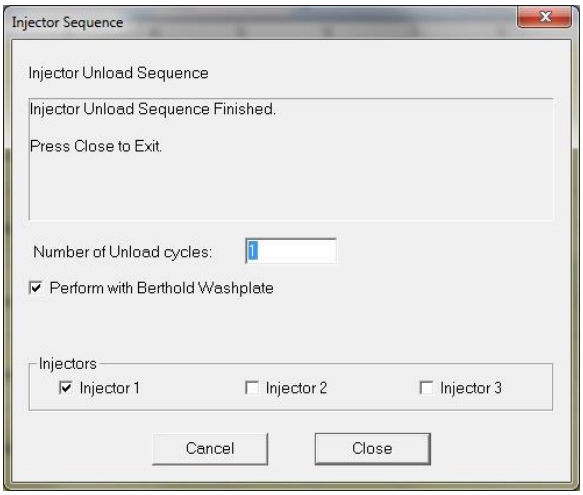
3. Define whether **Injector 1** or **Injector 2** or **Injector 3** or any combination are to be emptied
4. Click **<Next>**



5. Wait for the unload operation to be finished



6. Click **<Close>**



9.5 Preparations for transport

The **following safety provisions have to be taken** to transport or ship the instrument:

- Turn instrument off and **disconnect it from mains**
- Insert transport safety device(s)

- For shipping you **must** use the **original transportation case**
- Encase the instrument with the styrofoam parts
- **Tape shipping carton tightly**
- Have a filled in **Declaration on Decontamination** accompany the instrument when shipping back to Berthold Technologies or one of its representatives

10. Trouble shooting

Symptom	*Possible cause*	*Solution*
LED flashes red accompanied by 2 beeps	CAN module not correctly installed	1) switch instrument off and on again 2) call service
LED stays orange	Cable between instrument and computer is not connected Wrong COM assigned	1) attach cable properly 2) use service software and run “Scan COM ports” command
Instrument does not respond to software commands (status “Timeout Error”)	Cable between instrument and computer is not connected Wrong COM assigned	1) attach cable properly 2) use service software and run “Scan COM ports” command
LED stays dark	Instrument not switched on Mains not plugged in mains supply deactivated mains plug defective	1) switch instrument on 2) plug in mains 3) check with local house electrician 4) call service
Lower signal than expected	Pipetting/preparation error substrate consumed	1) verify by checking replicate and other samples / controls / standards and prepare faulty sample again 2) prepare new plate and read immediately after adding substrate
Signal not above background readings	No sample No reagents added	1) check sample preparation 2) add reagents
No signal at all	Faulty PMT	Call service
Plate is not moved to measurement position	Plate not correctly inserted Wrong frame Plate too high	1) insert plate correctly 2) change frame 3) use another plate with a total max. height of 16 or 21 mm respectively

Error message no plate	No plate Wrong frame	1) insert plate 2) insert black frame for 15 mm plates
High background signal	Reagents not prepared properly Reagents contaminated Plate contaminated	3) prepare reagents properly 4) prepare fresh reagents 5) use another clean plate 6) call service
Standard curve cannot be calculated	Standards pipetted in wrong order	1) prepare new plate with correct layout of standards 2) use the edit function in the standard curve tab
Excel Files cannot be opened	Excel is not installed	Install Excel
Adobe PDF files cannot be opened	Adobe Acrobat Reader is not installed	Install Adobe Acrobat

11. Technical Data

Mains Supply	100 – 240 VAC Fluctuations must not exceed ± 10 %; max. voltage 253 VAC 50 / 60 Hz
Operating voltage	24 VDC
Power consumption	220 VA
Certifications	CE, UL, CSA
Safety standards	EN 61010-1, EN 61326-1, EN 61000-3-2, EN 61000-3-3
Installation category	II
Temperature range	storage: 0° - 40°C operation: 15° - 35°C
Humidity	10 – 80%, not condensing maximum relative humidity of 80 % for temperatures up to 31 °C decreasing linearly to 50 % relative humidity up to 40 °C
Altitude	< 2000 m (above sea level)
Pollution degree	2
Dimensions	391 x 470 x 344 mm (W x D x H)
Weight	21 kg
Plate formats	6 to 384 well, solid and strip Dimensions 128 x 86 mm (L x W), height 14.0 – 21.0 mm (adapters necessary Petri dishes 35 and 60 mm
Measurement technology	Luminescence Fluorescence Absorbance
Operation modes	Integral measurement: 0.1 – 200 s Dual integral measurement: 0.1 – 200 s each Kinetic Repeated Scanning Delay: 0 – 600 s Shaking Injection
Detector	Photomultiplier operated in single photon counting technology
Sensitivity	Luminescence: ATP: 6 amol/well (96) Fluorescence: FITC: 0.3 fmol/well (384) Absorbance: Accuracy better 2 %, precision better 0.6 % (96 well, 2 OD)
Dynamic Range	6 orders of magnitude
Crosstalk	10^{-6} (black plates)
Interface(s)	USB
Operating system	Win Vista, Win 7

PC requirements	Pentium, 500 MHz (or better), CD ROM drive, USB port
Software	wizard support for parameter entries input of plate format selection of wells raw data assays (reporter genes, caspases, etc) dual raw data assays (e.g. dual reporter genes) kinetic repeated scanning ratio calculation or subtraction data export: EXCEL

12. Appendix

a. Customer Reply Form

Send Customer Reply Form to:

Berthold Technologies GmbH & Co KG
Technical Support
Calmbacher Str. 22
75323 Bad Wildbad
Germany
Phone: +49 7081 177 114
Fax: +49 7081 177 301
Email: service@berthold.com

or **your local representative**.

A blank Customer Reply Form can be found overleaf.

Customer Reply Form

Date: Customer no.:

Name:

Company:

Department:

Address:

Address:

Phone: Fax:

Email:

Instrument:

ID no.:

Serial no.:

Embedded software version:

Instrument driver software version:

Accessory instruments:

PC Software: PC software version:

Windows version:

Computer type: CPU type:

Other installed software:

Time when problem occurred (Windows clock):

Error message(s):

Description of the problem:

b. Confirmation on Decontamination Form

Confirmation on Decontamination

If you return an instrument to BERTHOLD TECHNOLOGIES for servicing purposes which is not properly decontaminated, there will be a health risk for BERTHOLD TECHNOLOGIES employees. We therefore need your confirmation that the instrument was decontaminated and cleaned properly before shipping. If the form below is not filled in accordingly and completely, we are forced to reject the instrument. Please understand that this is intended to protect our employees from any hazards.

Please put one copy into the shipping box and a duplicate into an envelope attached to the outside.

(**Please use capital letters !**)

instrument / component:	serial no.:
instrument or component has come into contact with:	
[ ] radioactive substances	means of decontamination applied:
Isotope(s):	
[ ] chemical reagents	means of decontamination applied:
specify:	
R and S rules:	
[ ] biological material	means of decontamination applied:
specifiy:	
[ ] contagious agents	means of decontamination applied:
specify:	
indicate security level of the laboratory the instrument has been used in	
[ ] S1 [ ] S2 [ ] S3 [ ] S4	
[ ] I hereby confirm that the instrument or component specified above was not contaminated with any of the above mentioned substances / reagents / agents	
[ ] I hereby confirm that the instrument or component specified above was decontaminated / cleansed using the appropriate method	
date:	signature:
name:	address:
title:	
phone:	
fax:	

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