



detect and identify

Operating Manual

LB 970 Fluorescence Reader **Twinkle**

Id No.: 34940BA2 Rev. No.: 01



These units are not designed for use in hazardous areas.

The units must not be repaired by any person other than Berthold Technologies service engineers or technicians authorized by Berthold Technologies GmbH & Co.KG.

In case of operation trouble please contact your local service organization or the central service department.

MikroWin Version 4.3x Twinkle Software Version 1.02 Twinkle Driver Version 1.02 July 2007

Berthold Technologies GmbH & Co.KG distributed by



211 bis Avenue Kennedy - BP 1140 03103 Montluçon - France 33 (0) 4 70 03 88 55 Fax 33 (0) 4 70 03 82 60 e-mail interchim@interchim.com Agence Paris - Normandie 33 (0) 1 41 32 34 40 Fax 33 (0) 1 47 91 23 90 e-mail interchim.paris@interchim.com



Contents

1.	PREFATORY COMMENTS	1
2.	SAFETY INSTRUCTIONS AND ENVIRONMENTAL CONSIDERATIONS	1
3.	CONSIGNES DE SÉCURITÉ	5
4.	SICHERHEITSHINWEISE	8
5. 5.1 5.2.1 5.2.2 5.3 5.4 5.4.1 5.4.2 5.5	QUICK REFERENCE GUIDE Getting Started Installation of Mikrowin 2000 Mikrowin 2000 Installation Activation of MikroWin <i>Lite</i> Software Twinkle Driver Software Installation Definition of Measurement Sequence and Parameters Creation of New Measurement Sequence and Definition of Parameters Editing Parameter Files Measurement and Evaluation	13 13 15 15 16 18 21 21 26 28
6. 6.1 6.2.2 6.2.3 6.2.3 6.2.4 6.2.5 6.2.6 6.2.7 6.2.8 6.3 6.3.1 6.3.2	SYSTEM DESCRIPTION Overview Reader Unit Top / Bottom Reading Plate Tray Excitation Filter Slide Emission Filter Slide Photomultiplier Excitation Halogen Lamp Connections PC Requirements Software Structure Brief Explanation of Menus and Functions	29 30 31 32 33 34 35 36 37 37 38 38 38
7. 7.1 7.2 7.3 7.4 7.5 7.5.1 7.6 7.6.1	GETTING STARTED AND FIRST MEASUREMENT Setup Site Space Required Unpacking Connecting Software Installation Mikrowin Settings First Measurements Fluorescence Measurement	40 40 40 41 43 43 47 47
8. 8.1 8.2	TWINKLE SOFTWARE FUNCTIONS Software Structure and Operation Installation	48 48 52



8.2.1	Mikrowin 2000 Installation	52
8.2.2	Driver Installation	52
8.2.3	Driver Setup	53
8.3	Export	66
8.3.1	Manual Data Export	66
8.3.2	Automatic Data Export	68
8.4	Instrument Control and Operation	69
8.4.1	Instrument Load Plate	69
8.4.2	Instrument Unload Plate	69
8.4.3	Instrument Excitation Filter Slide	70
8.4.4	Instrument Emission Filter Slide	70
8.4.5	Instrument Plate Editor Injector	71
8.4.6	Instrument Boot Instrument	71
8.4.7	Excitation Filter - Excitation Filter Slide	72
8.4.8	Emission Filter – Emission Filter Slide	76
8.5	Reading Parameters	80
8.5.1	Overview	80
8.5.2	Open/Save Parameter File	81
8.5.3	Well Selection	83
8.5.4	Definition of Measurement Sequence	86
8.5.5	Operations and their Parameters	90
8.5.6	Definition of Evaluation Parameters in Child Windows	101
8.5.7	Definition of Export Parameters	108
8.5.8	Saving Parameter Files	108
8.6	Measurement and Evaluation	109
9.	MAINTENANCE	112
9.1	Cleaning the Instrument	112
9.2	Fuse Replacement	112
9.3	Changing Halogen Lamp	112
9.4	Preparations for Transport	113
10.	TECHNICAL DATA	115
11.	APPENDIX	117
11.1	Details of selected Basic Parameter Files	117
11.2	Customer reply Form	123
11.3	Confirmation on Decontamination	125
11.4	Index	126



1. Prefatory Comments

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 longer 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			

Note

The MikroWin *Lite* software is supplied as standard version with the Twinkle instrument. This software version does not include all functions described in this user guide and in the MikroWin user guide. The versions *Advanced I* and *Advanced II*, which include additional functions, are also available.

Typographical Conventions

<add formula="">, <ok>, <close></close></ok></add>	Buttons are printed inside angular brackets in boldface type
Menu File , Open dialog box	Menu titles and dialog boxes are printed in bold- face type
File Open, Options Read	Menu items are also printed in boldface type; menu and submenu item are separated by a verti- cal line.



Safety Instructions and Environmental Considera-2. tions



and follow the instruction given in this manual for a safe and proper operation of the instrument.



This User Guide includes information and warnings that have to be observed by the user to ensure safe instrument operation.

Attention! This symbol alerts then user to take special care on the very important issues of the manual. It is madatory to read



Please do always act according to the following safety instructions, before as well as during operation of the system! Before taking the instrument into operation, it is absolutely essential to read the user manual, as otherwise the safety of instrument and operator cannot be ensured.

The Fluorometer LB 970 Twinkle has been manufactured in accordance with the safety requirements for electronic and medical 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 have been tested by the manufacturer and are 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 authorised personnel.
- The installation category is II.
- □ The instruments 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 transport safety devices before you turn on the instrument.
- Use the instrument only for the designated application.
- □ The instrument is designed for indoor use only.
- The instrument may not be operated at altitudes above 2000 m above sea level.
- The instrument is designed to be operated within a temperature range of 15 to 40 °C.



- □ The instrument is designed to be operated at a maximum relative humidity of 80 % for temperatures up to 31 °C decreasing linearly to 50 % relative humidity up to 40 °C.
- 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.
- The mains supply voltage fluctuations must not exceed +/-10
 % of the nominal voltage.
- The instrument is provided with a 3-pole grounded plug. If your wall outlet does not allow connection of a 3-pole plug, have a suitable wall outlet installed by qualified personnel or use an adapter for safe grounding. Please observe the safety specifications of the grounded plug.



- □ The instruments are designed according to the IEC 61010-1 or EN 610 10-1 regulations for electrical measuring systems.
- 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.
- 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.
- Turn instrument off before pulling the power cord.
- If the hood is removed, the safety provisions are no longer complied with. Watch out for moving parts. Inside the instrument temperatures may be present which may cause burns. Some parts of the instrument may still be hot after power off, although this is not immediately obvious.
- The electronic unit of the detector generates high voltage. Do not touch it during operation!
- □ Caution: Risk of explosion if battery is inserted incorrectly. The battery may be replaced only using the same battery type or a type recommended by BERTHOLD TECHNOLOGIES by a person authorized by BERTHOLD TECHNOLOGIES. Spent batteries have to be disposed off in accordance with the manufacturer's instructions.
- The instrument has to be set up such that the mains switch is easily accessible.



- 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 cards.
- 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 tubings) always has to be disposed off 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 patient samples.
- □ The operator is responsible for the use of reagents.
- The instrument should be shipped in its own case. During transport the plate slide has to be secured by a safety screw.
- 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.
- Pollution degree is 2.
- □ The instrument may only be used in romms with a max. pollution degree of 2.

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.



3. Consignes de Sécurité



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 **LB 970 Twinkle** a été fabriqué conformément aux prescriptions de sécurité en vigueur pour les appareils de mesure électroniques et médicaux. 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.
- La catégorie de mise en service est de niveau II.
- 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.



- □ L'appareil est destiné uniquement pour une utilisation à une altitude ne devant pas dépasser 2000 m au dessus du niveau de la mer.
- □ L'appareil est destiné uniquement pour une utilisation dans une température ambiante comprise entre 15 et 40 °C.
- L'appareil est destiné uniquement à une utilisation sous humidité relative ambiante maximum de 80%, pour des températures allant jusqu'à 31 °C et diminue linéairement jusqu'à 50% humidité relative pour une température d'ambiance allant jusqu'à 40 °C.
- 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 V).
- □ L'utilisateur porte la responsabilité de la mise en service de l'appareil selon les prescriptions électriques en vigueur.
- L'instrument est fourni avec une fiches à 3 broches dont une prise de terre. C'est une prescription de sécurité. Il est nécessaire que cette fiche puisse être branchèe sur prise reliée à la terre. Dans le cas contraire, il vous faut alors en avertir un électricien afin d'installer une telle prise. Il ne faut pas négliger cette consigne de sécurité.
- □ Les appareils correspondent aux prescriptions de la norme C.I.E. 61010-1 ou EN 610 10-1 concernant les instruments de mesure électriques.
- 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 pièce 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.
- Attention: Il y a un risque d'explosion si la pile n'est pas insérée correctement. Remplacer la pile uniquement par un pile du même type ou un type de remplacement recommandé par une personne autorisée. Les piles usagées sont à éliminer conformément aux instructions et prescriptions de votre pays.



- 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 prélèvements-échantillons de patients.
- L'utilisateur assume la responsabilité exclusive de l'utilisation des réactifs.
- 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.
- Degré de pollution est de niveau 2.
- L'appareil est destiné uniquement pour une utilisation en intérieur de bâtiments avec degré de pollution max. de niveau 2.
- 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 audelà de celles spécifiées dans ce manuel sont réservées aux techniciens autorisés.



4. Sicherheitshinweise





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 Fluorometer LB 970 Twinkle wurde in Übereinstimmung mit den Sicherheitsanforderungen für elektronische und medizinische 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.
- Installationskategortie ist II.
- Die Geräte dürfen nur von dafür geschultem Personal betrieben werden. Es wird allen Anwendern empfohlen, diese Bedienungsanleitung vor Benutzung zu lesen.
- 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 Geräte dürfen nicht in Höhen von mehr als 2000 m über dem Meeresspiegel betreiben werden.
- Die Geräte sind dafür ausgelegt, innerhalb des Temperaturbereiches von 15 bis 40 °C betrieben zu werden.
- Die Geräte sind dafür ausgelegt, bei einer maximalen relativen Luftfeuchte von 80 % (bis zu 31 °C) betrieben zu werden, die linear auf 50 % (bei 40 °C) absinkt.
- Die Stromversorgung darf nicht mehr als ±10 % des Nominalwertes aufweisen. Maximal sind 253 V erlaubt.
- Es liegt im Verantwortungsbereich des Anwenders, dass die geräte nach den lokalen elektrischen Vorschriften installiert wird.
- Die Geräte sind mit einem 3-poligen Netzkabel ausgestattet. Dies ist eine Sicherheitsausstattung. Wenn die Steckdose keinen 3-poligen Anschluss unterstützt, muss ein Fachelektriker eine passende 3-polige Steckdose installieren oder einen passenden Adapter zur Erdung des Anschlusses bereitstellen. Zerstören Sie niemals die Sicherheitsvorkehrungen des geerdeten Anschusses.
- Die Geräte entsprechen den Vorschriften der IEC 1010-1 und EN 61010-1 f
 ür elektrische Messgeräte.
- Nicht öffnen, wenn das Gerät in Betrieb ist.
- Service- und Reparaturarbeiten d
 ürfen nur von Fachleuten ausgef
 ührt 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.
- Gerät ausschalten, bevor der Stecker gezogen wird.
- 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.
- Alle gelieferten Geräte und Zusatzgeräte sind geerdet ans Netz anzuschließen. Schutzkontaktstecker verwenden!





- Achtung: Die interene Batterie kann bei flaschem Einbau explodieren! Die interne Batterie darf nur mit dem gleichen oder einem empfohlenen Ersatzmodell von einer von Berthold Technologies autorisierten Person ausgetauscht werden. Verbrauchte Batterien müssen entsprechend den Herstelleranweisungen entsorgt werden.
- 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 f
 ür den Umgang mit biologischem Abfall, mit Reagenzien und Patientenproben.



- Die Anwendung der Reagenzien liegt im alleinigen Verantwortungsbereich des Benutzers.
- Das Gerät sollte nur in der eigenen Verpackung transportiert werden. Beim Transport ist der Plattenaufnahmeschlitten und der PMT-Antrieb mit den Transportsicherungen zu sichern.



- Zum Reinigen des Gerätes bitte den entsprechenden Teil dieser Bedienungsanleitungen beachten.
- Ordungsgemäße Funktionalität kann nur bei Verwendung der Originalersatzteile garantiert werden.
- Urschmutzungsgrad ist 2.
- Das Gerät darf nur in Räumlickeiten mit einem maximalen Verschmutzungsgrad von 2 betrieben 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.



Special Spare Parts

These spare parts are essential for safe operation of the instrument: always use original spare parts supplied by the manufacturer or an authorized distributor.

Fuse	2.00A/T 5x20 UL 1986 / CSA C22.2	ID No. 09892
Power supply	JWS-150-24/A, 100-240VAC,24V/6.5A(Lambda)	ID No. 34846
Power entry module v	w/ switch 3V/220mAh type CR2032 (Varta)	ID No. 17391
Heating plate	24V/40W (Horn)	ID No. 34846
Halogen photo optic I	amp	12V/75W Typ
64613 (Tungsten)	ID No. 34051	



5. Quick Reference Guide

This Quick Reference Guide will lead you step by step through the various work processes, i.e. getting started, running measurements and performing evaluations. It provides a quick overview on how to work with the software and the instrument.

Twinkle is an extremely versatile instrument for different types of applications in the field of fluorescence measurement. To meet the needs of different users, this Quick Reference Guide is structured as follows:

- The Quick Reference Guide is divided into separate sections, so you can choose those chapters that are important to you: getting started, software installation, new definition of counting parameters, creating counting parameters on the basis of existing parameter files, measurement and evaluation.
- In each section you are guided through the various procedures step by step. These steps are numbered consecutively in each section. Explanations on the individual steps are added in small type font.
- Explanations on the various types of measurement are highlighted specifically.
- For your convenience, illustrations are placed directly next to the respective text.

5.1 Getting Started

- 1. Unpack and set up Twinkle.
- **2.** Remove transportation protection device for emission filter slide mechanics.
- Unscrew transport safety screw fixing the plate slide and keep transport safety devices in a safe place (see chapter 7.4). Do not take instrument into operation before you have removed the transport safety devices, as otherwise severe damage may occur.
- 4. Insert emission filter slide.
- 5. Insert Excitation filter slide.



Transportation lock

- 6. Check if the power supply is within the permissible range of the operating voltage of the *Twinkle*. Connect instrument only if this is the case!
- 7. Connect *Twinkle* to serial port of PC.
- 8. Connect instrument to power using the power cord supplied.
- 9. Turn instrument on.



5.2 Installation of Mikrowin 2000

5.2.1 Mikrowin 2000 Installation

- **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 installing an Advanced version with a *parallel hard lock* the hard lock has to be plugged into the parallel port during installation. When a *USB hard lock* is used the installation has to be performed without the hard lock plugged in. The USB hardlock has to be put into the PC right after installation.
- **Note:** When operating Twinkle with the Stacker Unit the system will work with *Lite* version with limited functionality (no barcode reader support, no user-selectable data file names, no re-stacking capability, no robot loading support). It is recommended to use *Advanced* version in order to get the full functionality of the Stacker unit.
- **Note:** For stacker operation the Mikrowin 2000 release version must be 4.35 or higher. In case you have already been working with a Mikrowin release version lower than 4.35 update your release version to 4.35 or higher (update supplied with the Stacker unit CD).
- 1. Close all *Windows* applications before you start installing the software.
- 2. Insert **Mikrowin 2000** CD into the CD drive. The installation routine starts automatically.
- **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.

- **13.** Click **<Finish>** to complete setup.
- 14. Attach Mikrowin 2000 USB hardlock .

5.2.2 Activation of MikroWin Lite Software

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



tikroWin 2000 Setup		×
Setup Type		
Select the setup type that best s	uits your needs.	
	Click the type of reliap you prefer TypeCal Program will be included with the next common so under Compact Program will be included with minimum required of Compact Program will be included with minimum required of Courton You may redect the options you want to install. Program	pions. Recommended for most pions. econsended for advanced
InstallStick	Destination Folder C-UProgramme Welscowin 2000 < Back (Back)	Bjowne
tikroWin 2000 Setup		X
Select Features		
Select the teatures setup will inst	tal.	
	Select the leadures pour work to initial, and decided the features Con Parameter Con Parameter Con Parameter Con Parameter Con Log Con Log	poudo not norri to install henciptica fina component includes all motabilité coursell diment
InstallSr/dd	< Back Next>	Cancel
Select Program Folder	Setup will add program icons to the Program I may type a new folder name, or select one fro list. Click Next to continue. Program Folders: MikroWin 2000 Existing Folders: Adubte Acrobat 10 Adubte 1650_1570N Series Caere-Anwendungen Creative Hewlett-Packard HP PhotoSmart MG SafubDU MAX Microsoft Office Tools	Folder listed below. You m the existing Folders
	< Back Next >	Cancel

 This dialog will be displayed when starting a not yet activated MikroWin 2000 Lite software (v 4.28) or a not yet activated Lite software without the instrument switched on (v 4.29 and higher)

It is recommended to switch off the **Twinkle/Twinkle** during software activation.

2. Go to Help | Program Activation.

There are 3 ways to acquire the activation code:

- I) on line via internet connection (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 confirm the activation process.
- 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 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>.

roWin 2000 St	artup Inf	fo					
Dear value	d custo	omer,					
This is a not y	et actival	ted versi	on of M	likroWir	2000.		
Only a succes program witho	sfully ac ut limitati	tivated v ions.	ersion o	of <i>Mikr</i> o	Win 200	00 permits	s you to use the
n order to act ocated in sub necessary info	ivate you menu He ormation	ur produ e ip of the to active	ct, pleas e main r ate your	se use ti nenu. Tř · copy o	ne Prog nis dialo f <i>Mikr</i> o\	gram Acti ig will prov <i>Win 2</i> 000.	<i>vation</i> dialog iide you with the
							Close
Don't show this	s message	e again.					
Don't show this	s message	e again.	_				
Don't show this	s message	again.					ОК
Don't show this ram Activation divotion Methods h order to activate Mili relow to us. There are	s message roWin 2000 k Three possib	a again. or this compu	rier, submit ti I the Program	he Program I m ID Code :	D Code sho	wn	OK Cancel
Don't show this ram Activation divotion Methods h order to activate Mil- felow to us. There are if your computer of to start the eartors activation code th	roWin 2000 k three possib connected to the program of xm now on.	or this compu illies to sence o the Internet, activation. Mi	ter, submitt I the Program please pre kroWin 2000	he Program I m ID Code se the "Auto I is searching	D Code sho Activation" I	wen button in order secarly	OK Cencel Help
Don't show this can Activation checkion Methods order to active the Mil- lefer to us. There are if your computer in to start the actions activetion code in if your submitted the heve submitted the heve submitted the code of the activetion of the heve submitted the activetion of the activetion of the heve submitted the activetion of the activetion of the activetion of the heve submitted the activetion of the	roWin 2000 k three possib isto program r om now on. s to the Intern You can use e information, othe Program	or this compu- illies to send o the internet, activation. Mi et, please se o the "Send e syou will reco	ter, submit ti the Program please pre- kraWin 2000 Mail' button Mail' button yve your Ac dialog to act	he Program I m ID Code ss the "Auto I is searching to start your tweto your Code ived your two	D Code sho Activation" I for the nest ratek de inc email client sinco.	win button in order secary Juding the After you Enter the	OK Cencel Help
Don't show this an Activation divetion Methods ander to active Mil- elow to us. There exist in start the automic activation code in Hyou neve access Program (D Code here submitted the accivation Code in Hyou here en occ	roWin 2000 k three possib connected th three possib connected th the program is on now on. s to the Intern You can use e information o the Program ess to the Int	or this compu- liftes to send o the internet, activation Mi et, please se in the "Send e you will reco m Activation of ernet, please	fler, submit til tithe Program plense pre- kroWin 2000 Mail [®] button Mail [®] button Mail [®] button dialog to ect i send us a f	he Program I m ID Code se the "Auto I to acti@mil- to acti@mil- to start.your to start.your to start.your to start.your to start.your to start.your to start.your to start.your	D Code sho Activation" I for the nest rotek de inc email client vita omail, t ence.	wen button in order secary Juding the After you Enter the vID Code	OK Cancel Help Auto Activation
Don't show this warn Activation fivetion Methods ander to active h Mit device to us. There are in the extorm activation code in Pagean ID Code here submitted the received Code in Pagean Pagean ID Code here submitted the received Code in Pagean ID Code here submitted the Pagean ID Code here submitted the here submi	roWin 2000 k three possib i connected li the program om now on. s to the Inferm You can use e information to the Program Sersion the Infer Form" to view i receive you	or this compu- illies to send o the internet, activation. Mi et, please se the "Send e you will rece m Activation to emet, please w an oppropri ur Activation	ner, submit til tithe Program plense pre- kroWin 2000 mid an eMail Mail" button sve your Ap dialog to act i send us a f uset fas form Code vis fer	he Program I m ID Code ss the "Auto lis searching to acti@mik to start your tiveto Codi tiveto your lic ex including a Atter you he k Please and	D Code sho Activation" Is for the nest ratek de inc email client > via smail. Is ionce. the Program we submitte ar the Active	win button in order secary Juding the After you Enter the ID Code d your ston Code into	CK Cancel Help Auto Activation Send eMail
Don't show this ram Activation divetion Methods sorder to active b Mit divetion to us. There are Hypor computer is activation code in activation code fin Program ID Code here submited the received Code in Rypos here no exc Program Shoe Fac Information, you w the Program Activ	roWin 2000 k three possib i connected to the program on now on. is to the Intern 'You can use e information, to the Pogram if receive you effort dialog t	or this compu- libre to sence to the Internet, activation. Mi et please se the "Send e syou will reco in Activation of emet please w on oppropri ur Activation to activate yo	tier, submit ti the Program please pee- kroWin 2000 mid an eMail" button swe your Ac dialog to a fe late fax form Code via feo un licence.	he Program In D Code Is the "Auto Is searching Is searching to acti@milit to startyour to starty	D Code sho Activation ⁴ I for the ness rotek de inc email client via email t innce. the Program we submitte er the Active	wen button in order secary Juding the After you Enfer the I/D Code it your ston Code into	CK Cancel Help Auto Activation Send eMeil Show Pac Form
Don't show this when Activation divetion Methods order to activate Nil order to activate Nil moder the activate bit and the activate program ID Code Program I	s message adWin 2000 k three possib i connected to the program arow an arow an arom arow an arow an arow an arow an arow an arow an arow an arow an arom arow an arow an arom arow an arom arow an arom arow an arom arow an arom arow an arom arow an arom arom arom arom arom arow an arom	e again. or this compu- libres to serve activation. Mi et, please se sithe "Send e sithe "Send e sithe "Send e source activation of erret, please w an appropri ur Activation to sativate you he measurem	fler, submit til I the Progres plense pee kroWin 2000 mid an eMail Mail" button sve your Ac dislog to act send us a f isole to act send us a f isole to act code vis two ar licence.	he Program I m ID Code sa the "Auto Is searching to start your tweftor Cod tweftor Cod tweftor Your In as moluding Attor you ha k Please ent	D Code sho Activation" I for the nesi rotek de inc email client vite email (tient vite email (tient vite email (tient vite email (tient) the Program the Active his program	wm button in order secary Juding the Ather you Enfer the IID Code aton Code into	OK Cencel Help Auto Activation Siend eMdel Show Fex Form
Don't show this winn Activation trive and the second secon	s message woWn 2000 k three possib i connected to site program i site program i to the Intern You can use e internation to the Program ass to the Intern Prom' to view Il receive yo entit discussion dialog to all number of the	or this compu- illies to send a the Internet, activation. Mi et please se the "Send so you will read you will read you will read out when a papeopp or Activation to a activate you he measurem	fier, submit ti the Program plense pre- kroWin 2000 and an eMail Mail' buttop Anal' buttop Anal' buttop Analy to a con- send us of iside fax form Code via fay ar licence.	he Program m ID Code : as the *Auto is searching to act @mini- to starty your lic to starty your lic to starty your lic as including After you have as including After you have a including After you have a including attraction of the start out o	D Code sho Activation ⁴ I for the nest rotek de inc email client sence. the Program we submitte er the Active his program	wm button in order secary Atteryou Enter the itO Code dyour stion Code into	OK Cencel Help Auto Activation Send eMeli Show Fax Form
Don't show this with Activation diveton Methods order to activate Nil norder to activate Nil order to activate Nil program ID Code Program ID Code Program ID Code Program ID Code	ordWn 2000 k three possib i connected ti sto program on now ca. s to the infermation of the Program Form' to vare e information of the Program for noise you after dialog ti d number of the 666618	or this compu- illies to send o this compu- lifies to send o the internet, activation. Mi et please as the "Send of the "Send of the "Send of the Send of the Send the Send of the Send of the Send of the Send of the Send of the Send of the Send the Send of the Send of the Send the Send of the Send of the Send the Send of the Send of the Send of the Send the Send of the Send of the Send of the Send of the Send the Send of the Send of th	Ner, submit II the Program please pre- kroWin 2000 and an eMail Mall" button pre- your Ap dialog to act when your Ap dialog to act server as the server as t	he Program m ID Code as the "Auto is searching to act @mini- to startyour lic as including Attor you ha c Please ent antrolled by 618E2	D Code sho Activation ⁴ I for the next rotek de inc email client > via email client > via email client ser submitte ar the Active his program	ven button in order secary luding the After you After you After you sito Code into s: 22241	OK Concel Help Auto Activation Send eMiti Show Pax Form

Profilname: Ou	tlook	•	<u>N</u> eu
		1	Contrast of the



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.

To print this order form menu. Fill out the required for Next, please fax this do You will receive your pe	, dick on P m fields an cument to rsonal Acti	rint command in t d enter your Prog Mikrotek (++49: vation Code withir	he File pull-down ram ID Code. 2204 75071). h the next 7 days.
Mikrotek Laborsysteme GmbH	Telefon:	(49)2204 / 7467	'5
Olper Straße 35	Fax:	(49)2204 / 750	71
D-51491 Overath ,	E-Mail: info@mikrotek.de		e
Germany	Internet: http://mikrotek.de		da
		f a s'il	0000
I wish to activate n	ny liceno	required	2000.
I wish to activate n	ny licend	required required	2000.
I wish to activate n Name Company Address	ny liceno	required required	2000.
I wish to activate n Name Company Address Country	ny liceno	e of MikroWin required required	2000.
I wish to activate n Name Company Address Country Phone	ny liceno	e of MikroWin required required	2000.
I wish to activate n Name Company Address Country Phone Fax	ny liceno	e of MikroWin required required required	2000.

5.3 Twinkle Driver Software Installation

 Insert CD containing the *Berthold Technologies* LB 970 Twinkle driver software. The installation routine starts automatically.



2. Choose language and confirm with **<OK>**. The setup assistant is started.



Quick Reference Guide

- Click <Next> in the Welcome dialog box. The driver is automatically installed to the *Mikrowin 2000* folder and the appropriate subdirectory.
- 4. Upon successful driver installation, the **Setup Complete** dialog box appears. To be able to work with the new software you have to restart your computer.
- 5. Choose "Yes, restart computer" if displayed and click on <Finish>.

Your computer may be restarted and all program components will be installed in the *Windows* operating system.

- Click on the program icon to start *Mikrowin 2000*.
- 7. The main window of *Mikrowin 2000* is displayed. The main window with menu bar, tool bar and navigation bar is displayed. It includes all menus and menu items. In addition, all child windows (data, template, calculation, result, statistics, graphics) are displayed; these windows can be minimized or maximized as needed.
- 8. The navigation bar can be deselected in the View menu. See *Mikrowin 2000* software User Guide.
- **9.** Set up driver. Select menu item **Installation | Driver** and verify or define parameters (see chapter 8.2.3).
- **10.** Select menu item **Options | Read**. The **Options Read** dialog box is displayed.
- 11. Open the **Device** drop-down list and select the item **BertholdTech Twinkle**.
- **12.** The respective drivers and pre-defined parameter files are loaded. The other text boxes in this dialog box are filled in automatically.
- **13.** Define excitation and emission filter on the **Instrument** menu and install filters into the instrument accordingly (see chapters 8.4.7 and 8.4.8).











- In order to control the microplate reader Twinkle via the software, you have to set up the respective instrument driver (Reader) correctly. Select the menu item Installation | Driver to open the Installation Driver dialog box with a separate tab for each driver type.
- **15.** Highlight BertholdTech Twinkle and click on **<Driver Setup>**.
- Select the COM port of your computer used and click on <Get Configuration> to download the instrument's features and options from the instrument. COM ports 1 through 9 are supprted by the software.

The instrument must be switched on during this process.

BertholdTech Twinkle, Version	1.01 🔀
ComPort	OK Cancel
Instrument Configuration Fluorescence installed Temperature Control installed Bottom Reading installed	Get Configuration



5.4 Definition of Measurement Sequence and Parameters

There are two alternative ways of defining the measurement sequence and parameters:

- a) Measurement and evaluation parameters are defined completely new (see section 5.4.1).
- b) An existing parameter file is used and the parameters are modified as needed. Either you use the parameter files supplied by *Berthold Technologies*: each includes a typical run for each type of measurement (see section 5.4.2). Or you use parameter files you have created yourself and edit this file accordingly.
- <u>*Please note*</u>: A saved parameter file includes: selected wells, individual operations and evaluation parameters (e.g. calculation formulas).

5.4.1 Creation of New Measurement Sequence and Definition of Parameters

- In the *Mikrowin 2000* main window, select the menu item Edit | Reset. Untitled.par is displayed as temporary file name in the status bar.
- 2. Select Options | Read. The Options Read dialog box is displayed.
- 3. Select **BertholdTech Twinkle** from the **Device** dropdown list.
- Click on <Options>. The Options dialog box appears showing two tabs: Samples and Measurement. Well selection (see chapter 8.5.3)
- On the Samples tab, select plate type, desired wells and measurement order (by rows or by columns).
 Select plate type:

Select the wells for measurement by clicking individual wells or dragging the cursor over an area.

Wells selected for reading:

\square			
	\square	\Box	\square
_			

Options Read		
Driver Selection		ОК
Device:	BertholdTech Twinkle	Cancel
Version:	1.01	Help
Description:	Reader device driver for Berthold Technologies Fluorometer Twinkle	Settings

Options														×
Samples	Measu	urement	1											
PI	ate param	neter -											1	
PI	ate type:		Be	erthold	96						•]		
М	easureme	ent Ord	er: (•	By Ro	ows	С	By Colu	umns						
	1	2	3	4	5	6	7	8	9	10	11	12		
4		\bigcirc												
E	30	\bigcirc	0	\bigcirc	\bigcirc									
C		\bigcirc	\bigcirc	\bigcirc	0	0	\bigcirc	\bigcirc	\bigcirc	0	0	\bigcirc		
	20	Q	Q	Q	Q	Q	Q	Q	\bigcirc	Q	Q	Q		
E	<u>0</u>	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q		
F	: <u>O</u>	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q		
0	<u>ک</u>	Q	Õ	Q	Q	Q	Q	Õ	\bigcirc	Õ	Õ	Q		
ŀ	<u>1</u> 0	\bigcirc												
								ок		Abbr	echen	OE	ernehme	#1



^{1, 6, 12, 24, 48, 60, 72, 96} or 384 wells

Define measurement sequence (see chapter 8.5.4)

- **6.** After you have selected the wells, click on the **Measurement** tab to define the measurement sequence with the individual operations.
- **7.** Copy the respective operations one after the other into the **Operations** column:
 - double-click the desired operation in the left column.
 - enter the desired parameters in the respective parameter box and click <**OK**> to confirm you entries.
- 8. In the Operations column you may change the sequence of selected operations by clicking on the

and **f** buttons.

 ✓ ⑦ Delay ✓ \$ Shake ✓ ₩ Fluor. Label ► K Fluor. Scientic S Fluor. Scanning ■ R Fluor. Repeated 	► 1000000000000000000000000000000000000	7 4	Name Courting Time Lamp Energy Excitation Aperture Emission Filter Courter position Meas. operation Second Measurement	370/690 0.10 5000 370 Normal 690 Top by Plate No
Operation Mode Batch Plate F Temperature Control Temperature	Repeats: 1		Celsius	

9. Select Operation Mode (if needed):

Batch:	In this mode several plates can	be
	read in succession using this pr	oto-
	col.	

- Plate Repeats: The same plate can be read several times in succession. Enter the number of repeats in this box.
- 10. Define temperature, if your instrument includes a temperature control (input option: 15 45 °C). <u>Please note:</u> You can enter a target temperature in the instrument which is <u>at least</u> +5 °C above room temperature, i.e. if the room temperature is 20°C the minimum instrument temperature will be 25 °C.
- **11. Keep Plate After Measurement:** When this option is checked the measured plate will be kept in the instrument and can be re-read without ejecting the plate.
- **12.** Confirm entries with **<OK>**.

Options Samples Measurement Measurement Sequence:	0perations: ★ ★ ↓ ₩ ★ 370/690	Operation Name Counting Time Lamp Energy Excitation Filter Excitation Filter Counter position Meas. operation Second Measurement	x 370/690 0.10 5000 370 Normal 690 Top by Plate No
Operation Mode Batch Plate Ri Temperature Control Temperature	epeats: 2 30 * 0K	Celsius Abbrechen	Übernehmen



Define evaluation parameters

Evaluation parameters are selected using the options on the **navigation bar** or via the **tool buttons** and defined on the respective matrices. Generally, these pre-defined parameters hardly have to be modified for simple luminescence and fluorescence measurements. Check the following matrices anyway and make the required changes.

You can add matrices to each child window or deselect these matrices (**Options | Matrix...**) and change the parameters for measurement and evaluation on each matrix (by well, by row or column or for the entire microplate). The exact procedure is described in the *Mikrowin 2000* User Guide.

- **12.** Select **Data** and enter results, sample identifier, error information and dilution factors on the matrices.
- 13. Select Template.

to change the partition of the sample matrix and define controls and standards. The other matrices are partitioned accordingly.

14. Select Calculation.

to define calculation formulas for the individual matrices.

Note: To be able to visualize the readings in the result window, to use them for calculation and to be able to to export them via the matrix and line export drivers.

For a measurement with a single readout the trem **MEA** is to be used in one of the matrices.

Add Formula		
⊡- Common program variables	~	ОК
MEA Measurement Data		Cancel
In case a measurement contai	ns m	ultiple read-

ings (= labels) the terms **LB1** through **LB5** must be used.

A	dd Formula			
	⊟- Kinetics pro	gram variables	~	ОК
	<mark>LB1</mark>	Measurement Data of Label 1	_	Cancel
	- LB2	Measurement Data of Label 2		Cancor
	LB3	Measurement Data of Label 3		Help
	LB4	Measurement Data of Label 4		
	LB5	Measurement Data of Label 5		

The detector has and **overload detection** function to prevent the PMT from damage by high levels of light. Mikrowin supports this by displaying the expression **Overload** instead of a value. Instead of the the expressions **MEA** or **LB**.. in the calculation matrices one has to use the threshold function: **TRH (MEA)** or **TRH(LB..)** respectively.







The threshold level itself and the expression to be displayed are set in the **Options | Threshold** dialogue (Type exactly 2147483646).

Options Threshold	
Threshold Set 1 Threshold Set 2 Threshold Set 3	
Flag / Color / Threshold	Group
Overload • 2147483646	Group No. 1
#Value	
	Apply to all groups
	Apply to all gloups
OK Abbrechen	Übernehmen Hilfe

15. Select Results.

Results and calculated values are displayed after measurement.

Define export parameters (see chapter 8.3)

16. Prerequisite: Install and set up export driver (see also chapter 8.2.3).

The following functions are file-specific, i.e. they are valid only for the active data or parametera file.

- 17.Manual export: Select Export.
- **18.** In the **File Export** dialog box select the item you want, e.g. **Matrix Export Driver** if you want to create an Excel file.
- **19.** Confirm your selection with **<OK>**. The data of the active data file will be exported.





File Export	
Active Export Driver	OK
Matrix Export Driver	Cancel
Line Export Driver	Help
ODBC Export Driver	
Time_Position_rawdata	Province
Directory : C:\Programme\MikroWin 2000\Transf	er



- **20. Automatic Export:** To export the data automatically after measurement, select **File | Export Setup...** in the main window. The **File Export Setup** dialog box is displayed.
- **21.** Select desired export driver. It has to be the same as the one selected in the **File Export** dialog box.

File Export Setup	
List of Active Export Driver	ОК
List of export driver	Cancel
List of export driver Line Export Driver Matrix Export Driver ODBC Export Driver OleDB Result Export Driver	Help
	Remove

Save parameter file (see chapter 8.5.2)

As soon as the measurement sequence has been generated and all parameters mentioned above have been defined as needed, save the settings to the parameter file.

- 22. Select File | Save As
- 23. Enter file name (extension .PAR) and confirm with <**OK**>.



5.4.2 Editing Parameter Files

In this case, an <u>existing</u> parameter file is modified. Basically, all **Mikrowin 2000** parameter files can be edited.

The basic parameter files supplied by **Berthold Technologies** include typical measurement sequences of some major measurement types. Using this data, you just have to make a few changes to define the measurement sequence for your own requirements. A parameter file includes the selected wells, the pre-defined operations and the evaluation parameters (e.g. calculations, result presentation and export). The type of measurement is indicated in the file name. Depending on your instrument setup, it may happen that not all defaulted parameter files can be utilized to the full extent (see chapter 5.1).

Save the changes to these files under a <u>new name</u> in order to keep the basic parameter files!

- 1. Select File | Open in the main window.
- 2. Select the file type *.par in the Open dialog box.
- 3. Select the desired parameter file and confirm with <OK>.

A parameter file is active until a new one is opened or until you exit the program. The name of the open file appears in the bottom right corner of the screen.

- Select Options | Read ... and in the Options Read dialog box click on the <Options> button. The Options dialog box appears. The wells selected for measurement and injections are displayed on the Samples tab.
- **5.** Change well selection as needed (see page 21; for more details see chapter 8.5.3).



ption	5																	×
Samp	oles	Meas	ureme	nt														
Г	Plate	e para	meter															
	Plate	e type	:		18>	(12 pl	ate							•	•			
			_		_				_									
	Меа	surem	ient Or	der:	•	By R	ows		0 E	ly Co	lumns							
				1	- I				• 1	-			40					
		1			3	4	5		2		8	9	10	11				
	Α	KL.	X	X	\mathcal{D}	$(\mathbb{L}$	X	X		\mathbb{U}	(\mathbb{U})	\mathbb{U}	\mathbb{U}		X			
	в		\mathbb{C}	X	D	\bigcirc		X	D	\mathbb{D}	\bigcirc	\bigcirc	\mathbb{O}		\mathbb{D}			
	С			X	D	\bigcirc		X	D	\mathbb{D}	\bigcirc	\bigcirc	\mathbb{C}		\mathbb{D}			
	D			X	D	\bigcirc		X		\mathbb{D}	\bigcirc	\bigcirc	\bigcirc					
ĺ	Е	Ć)(Č		D	Ĉ	R	X)	Ō	Ō	Ō		Ĉ)		
	F	Ć)(Č	X	Ĵ	Č	Ò	K)	Ō	Ō	Ō	Ō	Ć) (5		
	G	C)(C	X	D	Ć	C	K)	Ō	\bigcirc	Ō	Ō	Ō	K	5		
	н	C	C)(D	С	C	X)(\bigcirc	\bigcirc	\bigcirc			K	5		
Ľ				_														
											OK		Abb	reche	n	Übe	emehr	nen

- 6. Select **Measurement** tab. The pre-defined measurement sequence is displayed.
- **7.** Change measurement sequence as needed (see page 22; for more details see 8.5.4).

For differences in the individual types of measurement see chapter.



Verify evaluation parameters

Evaluation parameters are selected using the options on the **navigation bar** or via **tool buttons** and set on the matrices (see page 23). The name of the open parameter file appears in the bottom right corner of the screen.

For more information please refer to the *Mikrowin 2000* user guide.

- 8. Select Data and edit matrices, if necessary.
- 9. Select Template and edit matrices, if necessary.
- **10.** Select **Calculation** and edit matrices, if necessary.

The calculation matrices of the pre-defined parameter files ususally contain the overload display function and the definition of the individual readings od a measurement to matrices (**MEA** or **LB.**, respectively). Refer to 5.4.1, for details.

For differences between the individual types of measurements see chapter 8.5.4 and appendix.

11. Select Results.

Matrices are displayed depending on the defined calculation matrices. Calculated results are displayed depending on the pre-defined formulas.

- **12.** Define export parameters (see page 24).
- 13. Save parameter file (see page 25)

As soon as the measurement sequence has been created and all parameters have been defined as needed, save the parameter file under a <u>new name</u> to keep the basic parameters.

- Select File | Save As
- Enter file name (extension .par) and confirm with <OK>.





Calculation

Norm.par

Name of parameter file

Untitled.dat

Name of data file





💽 🔶 🖻 🗕

? ×

5.5 Measurement and Evaluation

- 1. The *Twinkle* instrument has to be connected completely and powered on.
- **3.** Open parameter file (*.par) for measurement (see chapter 8.5.2). The matrices associated with this parameter file are displayed (depending on your choice of the menus **Data**, **Template**, **Calculation**, **Results**).

4. Click on the **Read** button. A bar with input fields and buttons appears below the displayed matrix.

The <u>name</u> of the parameter file used is displayed in the bottom row.

Click is to open the parameter file and you can edit the default settings, etc.

File name: enter a file name for the data.

With the Mikrowin 2000 Advanced versions the data file name can be set automatically.

- 6. Click <Start>.
- **7.** The plate tray opens. On the screen appears the prompt to insert the microplate to be read.
- 8. Insert plate (A1 at the rear left) and click <OK>.
- **9.** The plate is moved into the instrument and the predefined measurement sequence is started.
- **10.** During measurement, the data already available and the calculations can be viewed on the matrices of the menu **Results**.
- Upon completion of the measurement the plate stays in the instrument to protect it from external influences. To unload it, chose the command <Unload Plate> on the Instrument menu.
- **12.** If **Batch** has been pre-selected, the system expects the next microplate which is being read using the same parameters. Insert plate and click **<OK**>.
- **13.** Measurement data and calculated **r**esults are presented in the **Data**, **Results** and **Graphics** windows.

BERTHOLD







Suchen in: 🔁 ParaTwinkle

🔜 default.pa

6. System Description

6.1 Overview

	The <i>Twinkle LB 970</i> is a Microplate Fluorometer for various fluorescence measurements on microplates. The strength of Twinkle is its sensitivity due to its optimized fluorescence optics and, therefore, supplies excellent results. Clear device design, simple handling as well as instrument control and data evaluation by the Mikrowin 2000 software make Twinkle an indispensable measuring system in the (research) laboratory.
Fluorescencence top reading	Fields of application high sensitivity due to optimised optics
Fluorescence bottom reading	light guide to re-direct excitation and emission to the well bot- toms
FRET	measurement of the energy transfer between 2 fluorescent molecules
	A low-noise photomultiplier is used for these measurements. Highest sensitivities can be obtained due to using of single pho- ton counting technology.
	The instrument includes separate devices for excitation and emission filter with replaceable single filters.
	Moreover, universal application of the <i>Twinkle</i> is ensured by the following features:
	Free choice of plate formats: 6, 12, 24, 48, 96 and 384 well plate formats
	Shaking functions
	Temperature control (optional)
	external barcode reader (optional)
	Connection to lab automation systems (optional)
	Comprehensive software for instrument control and data evaluation (Mikrowin 2000)
	$\hfill\square$ within the software, basic parameter files are supplied


6.2 Reader Unit

The *Twinkle LB* 970 is a desktop instrument with small footprint. It can be set up on any lab workplace



Figure 6-1: LB 970 Twinkle



To obtain good and consistent results, please keep the following in mind:

- Do not expose instrument to direct sunlight.
- □ Set up instrument in dry rooms.
- Open plate tray only for loading or cleaning to keep light and dust out.
- Open instrument cover only for cleaning. Prior to a measurement, the instrument cover has to be closed light-tight for a longer period of time.
- □ Keep plate tray free from dirt.
- Absorb reagents spilled inside instrument immediately using a clean and dry household tissue and then clean the respective location.



6.2.1 Top / Bottom Reading

The Twinkle LB 970 can be equipped with a Bottom Reading optical system. The user can then select wether the samples in a microplate are read from above (= top reading) or from below (= bottom reading) the wells. The type of counting that will be used has to be selected with the **Counter Position** setting: **Top** or **Bottom**



Figure 6-2: LB 970 Twinkle – lever for top / bottom selection

Note: In addition to the selection in the software, the counter position has to be selected manually prior to the measurement start!

Load the plate carrier by selecting **Load Plate** in the **Instrument** menu.

Open the lid at the front panel and push/pull metal lever until it clicks into place:

Push	change	to top	reading
------	--------	--------	---------

Pull change to bottom reading



6.2.2 Plate Tray

The instrument front panel includes the plate tray. It can be opened and closed under control of the *Mikrowin 2000* software.



Figure 6-3: Microplate Orientation

Select **Instrument | Unload Plate** to open the plate tray; then place the microplate on the tray (during priming or washing a prime plate, if no aspiration pump is installed). Position **A1** of the microplate has to be in the rear left corner. Load the microplate such that it rests completely on the plate tray and is fixed by holders.



Incorrectly loaded microplates may cause damage or lead to false results.

Microplate height must not exceed a height of 22.0 mm. Other wise damages to the optical system may occur. This is valid for an instrument *with* the automatic plate height adjustment.

Select **Instrument | Load Plate** to move the plate slide into the instrument and to close the plate tray light-tight.



6.2.3 Excitation Filter Slide

To the right of the plate tray there is a compartment containing the excitation filter slide (instrument front panel, Figure 6-1). To replace or clean the filter you have to eject the slide via software.

Proceed as follows

- G Select Instrument | Excitation Filter Slide.
- □ In the Excitation Filter Slide dialog box, click on the button <Eject Slide>. The door opens slightly and the slide moves out a bit.
- Open the door all the way by hand and pull the filter holder out at both metal pins (bottom and top at filter holder).
- □ Clean or replace filter.
- If you replace the filter, drag the respective filter into the Excitation Filter Slide dialog box to the appropriate positions. See the explanations in chapter 8.4.7.
- Push in filter holder all the way into the slide.
- □ Click <**OK**> in the **Excitation Filter Slide** dialog box. The slide moves all the way into the instrument and the door is closed again.

Replacing filters

- Using a fine pair of tweezers, pull out the lock washer and take the filter out.
- Place new filter into the filter wheel such that the colored side is facing up and the metallized side is facing down. If the side of the filter contains an arrow mark, the arrow has to point up (towards the light).
- Push in lock washer to fix the filter.
- □ In the **Emission Filter Wheel** dialog box, drag the filter onto the respective positions of the filter wheel name.

Cleaning filters

Filters should be cleaned using a lint-free cloth or, better, a micro fiber cloth, as used for cleaning eye glasses.





6.2.4 Emission Filter Slide

Above the plate tray there is a compartment containing the emission filter slide (instrument front panel, Figure 6-1). To replace or clean the filter you have to eject the slide via software.

Proceed as follows

Select Instrument | Emission Filter Slide.

- In the Emission Filter Slide dialog box, click on the button <Eject Slide>. The door opens slightly and the slide moves out a bit.
- Open the door all the way by hand and pull the filter holder out at the metal pin (front of filter holder).
- □ Clean or replace filter.
- □ If you replace the filter, drag the respective filter into the **Emission Filter Slide** dialog box to the appropriate positions. See the explanations in chapter 8.4.7.
- Push in filter holder all the way into the slide.
- Click <OK> in the Emission Filter Slide dialog box. The slide moves all the way into the instrument and the door is closed again.

Replacing filters

- Using a fine pair of tweezers, pull out the lock washer and take the filter out.
- Place new filter into the filter wheel such that the colored side is facing up and the metallized side is facing down. If the side of the filter contains an arrow mark, the arrow has to point up (towards the light).
- Push in lock washer to fix the filter.
- □ In the **Emission Filter Wheel** dialog box, drag the filter onto the respective positions of the filter wheel name.

Cleaning filters

□ Filters should be cleaned using a lint-free cloth or, better, a micro fiber cloth, as used for cleaning eye glasses.





6.2.5 Photomultiplier

The photomultiplier measures light with high sensitivity and low noise. The spectral sensitivity is within a range that is suitable for fluorescence applications.

The photomultiplier operates as an ultra-fast photon counter. The photo electrons released from the photo cathode by the light quanta are multiplied via the dynode chain; at the anode, these photons trigger a fast pulse with a rise time of a few nanoseconds. These counts are amplified by a very fast amplifier. Low-energy single counts created by the noise of the photomultiplier are suppressed by a threshold discriminator. The single counts are counted digitally; their total number is directly proportional to the emitted quantity of light.

So-called counts (cts) are used as unit of measure for the raw data.

Overload Detection

The detector has an **overload detection** function to prevent the PMT from damage by high levels of light. Mikrowin supports this by displaying the expression **Overload** instead of a value. Instead of the the expressions **MEA** or **LB.** in the clculation matrices one has to use the threshold function: **TRH (MEA)** or **TRH(LB1)**, **TRH(LB2)**, ... respectively.

The threshold level itself and the expression to be displayed are set in the **Options | Threshold** dialogue (Type exactly: **2147483646**).

Options Threshold	
Threshold Set 1 Threshold Set 2 Threshold Set 3 Flag / Color / Threshold Set 2 Threshold Set 3 Pverfoad • • • 1 • • • • 1 • • • • • • • • • • • • • • • • • • •	Group Group No. 1
OK Abbrechen	Übernehmen Hilfe

Figure 6-4: Threshold dialog



6.2.6 Excitation Halogen Lamp

The halogen lamp (75 W) is equipped with a fan for overheating protection purposes. The lamp is located on the right hand side of the instrument and is covered by a blue cover lid.

The fan is located to the right of the lamp). The fan works in continuous service when the instrument is turned on. Twinkle works correctly only if the fan is working properly. If the fan is faulty, the instrument has to be turned off to rule out overheating and the fan has to be replaced.

The excitation halogen lamp can be set via software in 65 535 digital steps between 0 and 75 Watt (see also chapter 8.5.5).



Figure 6-5: Right instrument side: fan and excitation halogen lamp

If the CW- Lamp needs replacing, switch off the power and pull off the cover on the right side of the fluorometer to get access to the lamp. Unscrew the panel holding the lamp and withdraw it through the hole in the body of the fluorometer.

Attention : The metal panel over the lamp and the lamp itself can be hot !

Make sure the instrument is switched off and give the lamp a chance to cool before removing it.

Do not touch the lamp, since you might damage it !



6.2.7 Connections

Connections (PC, power supply), mains switch and instrument fuse are located on the instrument rear:

Serial port

Connect a computer (PC, laptop) for control of the *LB* 970 *Twinkle* to the 9-pole serial port.

Mains plug

Connect the instrument to mains using the cable supplied with the instrument (with grounded conductor).

Mains switch

Push the mains switch to turn the instrument on and off. Turn the instrument on before starting the software, so the program can establish communication with the instrument. If the instrument is turned on after you have started the software, you have to select **Instrument | Boot Instrument** to establish communication with the instrument.

Fuse

The instrument fuse is located next to the mains switch in a black fuse holder. For fuse replacement please see chapter 9 Maintenance.

6.2.8 PC Requirements

Recommended PC configuration

Pentium processor, 500 MHz (or better) 128 MB RAM Graphics card 1024x768 (or better) CD-ROM drive Operating system: Win98, Win2000, Win NT, Win XP Serial port RS232 USB port



6.3 Software

6.3.1 Structure

The software for control of the Multilabel Reader *Twinkle* is an extension of the *Windows* application *Mikrowin 2000*. The illustration below shows the menus and functions of the program. Only the system functions printed in bold are described in this user guide. For information on all other functions please refer to the user guide and the *Mikrowin 2000* online help.

File	Edit	View	Read	Instrument	Options	Installation
New	Undo	Tool Bar		Load Plate	Read	Settings
Open	Сору	Status Bar		Unload Plate	Threshold	Automation
Save	Paste	Navigation Bar		Excit. Filter Slide	Controls	Driver
Save As	Reset	Help Card Bar		Emiss. Filter Wheel	Definitions	
Export				Plate Editor	Matrix	
Export Setup]			Boot Instrument	Result	
Print					Curve Fit	
Print Preview					Kinetics	
Print Setup	J				Scanning	

6.3.2 Brief Explanation of Menus and Functions

File menu	To open, save, export or print files and enter the respective parameters. Select the desired export driver for the active data file via the Export item. Enable an automatic export function for the active parameter file via Export Setup . To do this, select the desired export driver. If this function is enabled, data is exported automatically upon completion of a measurement. See also chapter 8.3.
View menu	To select special views. For example you can show or hide the navigation bar or the tool bar.
Read menu	To start a measurement using the active parameter file.



Instrument menu	Instrument functions are presented on this menu:
Load Plate	To move the plate slide into the instrument below the photomul- tiplier.
Unload Plate	To move the plate slide out of the instrument to unload a mi- croplate and load a new one.
Excitation Filter Slide	To move the excitation filter slide out/in (instrument front panel) and to define the filters used.
Emission Filter Wheel	To change and to define the emission filters.
Plate Editor	To define the dimensions of the microplates to be used.
Boot Instrument	To establish communication between instrument and PC if the <i>Twinkle</i> instrument has been turned on an off again while working with <i>Mikrowin 2000</i> .
Options menu	To define the reading parameters. Select Read to open the Options Read dialog box to define the wells to be read and the reading sequence including individual operations.
Installation menu	To define the settings (directories, password, etc.) in the Instal- lation Settings dialog box. To select and define the drivers for the <i>Twinkle</i> instrument and for the export function in the Installation Driver dialog.



7. Getting Started and First Measurement

7.1 Setup Site

The Fluorometer **Twinkle LB 970** has to be set up in dry, fairly dust-free rooms and protected from exposure to direct sunlight and significant temperature fluctuations. It should not be set up next to a radiator or an air conditioning.

7.2 Space Required

Dimensions of LB 970: 465 x 406 x 280 mm (W x D x H)

Set the instrument up such that the rear panel with the connection ports is easily accessible, so it can be turned on and off easily any time.

Allow for sufficient space on the side for the waste and liquid bottles (reagents, wash solution).

Set up the *Twinkle* instrument close to the PC.

7.3 Unpacking

The cardboard box is reusable and should be used again whenever the instrument has to be transported.

When unpacking the instrument, make sure the shipment is complete and shows no sign of damage. The careful packing usually rules out transport damages. Should the instrument or instrument parts be damaged, anyway, please inform the shipping agent or the BERTHOLD TECHNOLOGIES service department immediately!



Unpack instrument and remove foamed inserts with care!



7.4 Connecting

Check if power supply is within the permissible range of the Twinkle's operating voltage. Connect instrument only if this is the case!

Carefully take instrument out of cardboard box and put it onto your workplace.

Remove transport safety locks

- A red tape reminds you to remove the transport safety locks
- Pull out the transportation lock of the emission filter slide rails and insert emission filter slide.
- Pull out the plate carrier by the transportation lock label as indicated and unscrew the transport lock of the plate carrier.
- □ Keep both locks in a safe place.



Figure 7-1: LB 970 – transportation lock plate carrier

Install the transport safety locks again before transporting the instrument! Please read chapter 9.3 Preparation for transport.



Electrical connections

- Connect hardlock supplied with the instrument to the USB port of your PC in case of a Mikrowin 2000 Advanced version only *after* software installation.
- Connect *Twinkle* instrument (PC port) to serial port of PC or laptop.
- Connect instrument to the wall outlet using the power cord supplied with the instrument.
- □ If you have not already done so, connect the computer to the wall outlet.

Power on

Push mains switch (on instrument rear panel) to turn instrument on.



7.5 Software Installation

- Install *Mikrowin 2000* software on your computer (see chapter 2.2.1)
- □ Install driver software (see chapter 5.3) and select the driver for the Twinkle instrument.
- □ Define excitation and emission filters on the **Instrument** menu and install them in the instrument accordingly (see chapter 8.4.7 and 8.4.8).

7.5.1 Mikrowin Settings

- **1.** To define and ajust the general settings of Mikrowin 2000 go to **Installation | Settings**.
- 2. In the General tab check Use tabulators as delimiter.

Installation Settings	
General Directories Password Font LogSystem Common Settings Display only used wells Use tabulators as delimiters Disable ID's for control positions	
OK Abbrechen Oberne	hmen Hilfe
Installation Settings	×
General Directories Password Font LogSystem	1
Measurement Directory C:\Program Files\MikroWin 2000\	Browse
Template Directory	
C:\Program Files\MikroWin 2000\	Browse
Transfer Directory	
C:\Program Files\MikroWin 2000\Transfer	Browse
OK Abbrechen Überne	hmen Hilfe

3. Directories tab: define the default directories for parameter, data and exported files.

Leaving the boxes blank means that the "**My Documents**" folder of the respective logged on Windows user will be used.



4. The **Password** sytem is available in Advanced version only. Users can be defined who get a username and a password to log on to Mikrowin. Specific rights for operations can be adressed to the users.

Note: the first user to be defined must be a master user!

5. The fonts for the Mikrowin windows can be selected here.

Installation Settings
General Directories Password Font LogSystem
List of authorised users
User name Accessrights
master Master
Add User Edit User Remove User
OK Abbrechen Übernehmen Hilfe
Installation Password Add / Edit
User OK
User Name : master Cancel
Password : Help
Password Confirmation : *****
Expiration Date : never expire
Accessrights
Master Window : Measurement> View : Data
C Extended Window : Measurement> View : Sample ID
○ Normal Window : Template> View : Partition
C Special Window : I emplate> View : Groups
Installation Settings
General Directories Password Font LogSystem
List of views
View Font
Window : Measurement> View : Data MS Sans Sent: Standard, 8 pt Window : Measurement> View : Sample ID MS Sans Sent: Standard, 8 pt
Window : Measurement> View : Error MS Sans Senf: Standard, 8 pt Window : Template> View : Partition MS Sans Senf: Standard, 8 pt
Window : Template> View : Groups MS Sans Serif: Standard, 8 pt Window : Template> View : Overlay MS Sans Serif: Standard, 8 pt
Window : Template> View : Controls MS Sans Senf: Standard, 8 pt Window : Template> View : Standards MS Sans Senf: Standard, 8 pt
Window : Calculation> View : All Views MS Sans Senf: Standard, 8 pt Window : Statistics> View : General Statistics Verdana: Standard, 8 pt
Edit Font
OK Abbrechen Dernehmen Hilfe



Getting Started and First Measurement

6. The Log System is available in the Advanced versions of Mikrowin 2000 only.

Check Activate LogSystem to activate the log system. All operations will be documented.

		L
In addition there are some General tab of the Installa < ALT > and the < S > keys f	e Special Settings. Go to the tion Settings dialog. Hit the or access.	Special Settings List of available Options 001 ShowAWels 002 OptimisePrintout 003 CharactersSmyleID
The recommended settings	s are:	004 CharactersErrorInfo 005 SaveDataReadDnly 006 SaveParaReadDnly
001 ShowAllWells	Yes	007 LogSystemLevel
002 OptimisePrintout	Yes	
003 CharactersSampleID	10	
004 CharctersErrorInfo	10	
005 SaveDataReadOnly	Yes	
	Data files cannot be over- written when Yes is se- lected.	
006 SaveParaReadOnly	Yes	
	Parameter files cannot be overwritten when Yes is se- lected.	
007 LogSystemLevel	1	
	Major operations are logged.	
008 EnableDriverUpdate	Yes	
009 EnableDialoAutoHelp	Yes	
010 EnableAxisShading	Yes	
011 DecimalDelimiter	Operating System Default	

Units

No

Yes Blanks will not automatically

No

Mode1

Yes Only accessible dialogs will

be subtracted.

No

012 DefaultMeasurementUnits

014 DisableAutoBlankSubtract

015 DisableWarningMessages

017 ChangeSelGraphicsColors

018 EnableReducedUserInterface

016 DisablePrintClipping

013 RoundCalculationMatrix

Installation Settings					
General Directories Password Font LogSystem					
I✓ Activate LogSystem Print List					
Show all before Show user Show operation 24/11/2006 Set Set					
Date Time User Performed Task					
Number of - maximum / found / all - Items : Set / / /					
OK Abbrechen Obernehmen Hilfe					

Option Adjustment

Description : Show all Wells, even those not measured during the

Yes

ÖK

Help

•

	be displa	yed.
019 NumberVisibleResultMa	atrices	5 (Lite)
		16 (Advanced)
020 DefaultStartupPlateSize	;	12x8 well
021 ResetSidOnRun	No	
022 ResetDilOnRun	No	
023 EmbedTemplateData	No	
	Yes may stead if d ferred to culations places.	y be selected in- lata are to be trans- gether with the cal- to other work-
024 LinkParameterOnSave	Yes	
025 UseDefaultPrinter	This links to the da files will data file i No	s the parameter file ta file. Parmameter be loaded whenver s launched.



7.6 First Measurements

7.6.1 Fluorescence Measurement

- Start Mikrowin 2000 software
- □ Make sure you have the appropriate excitation and emission filters installed.



- Select File | Open or click Open
- Select the directory
 MikroWin 2000\ParaTwinkle\Fluorescence.
- □ Select file type ***.par** in the **Open** dialog box.
- **Open a basic parameter file e.g. Fluorescein.par**.
- □ Click the <**Read**> button in the main window.
- Enter a file name for the data in the status bar.
- Click the <Start> button. The plate slide moves out and on the screen you see the prompt to load a microplate.
- Place a prepared microplate onto the plate slide; make sure well A1 is located in the rear left corner.
- Click <OK> to confirm the prompt. The plate moves into the instrument below the photomultiplier and the selected wells are read.
- Select the **Results** dialog box to view the results on the matrices.
- Results may be displayed graphically, printed out or exported (e.g. as an Excel file).

If the set lamp energy is out of the suitable region you'll get an error message. Please refer to chapter <u>8.5.5.</u> for details about how to adjust the lamp energy.



8. Twinkle Software Functions

8.1 Software Structure and Operation

In the following section you find information on the structure of the software. For more detailed information please consult the Mikrowin 2000 software online help.

Main window	Upon program start, this main window includes six child win-
Program start	Double-click on the program icon to start the software. The main window is displayed.

dows, i.e. Data, Template, Calculation, Results, Statistics and Graphics. You can work with these windows in accordance with the *Windows Multi Document Port* (MDI) definition. In addition, the navigation bar can be displayed; from this

navigation bar you can select the **child windows** with the respective views.

Menu bar

All program functions can be selected from the **menu bar**.

f File Edit View Read Instrument Options Installation Window Help

Tool bar

The **tool bar** of the main window is located directly below the menu bar, providing quick access to various windows and to the print, edit and file operations simply by clicking on the respective tool button.

1	(1)		Q	B	Q	*	I.	·····	Ø.		3
	New	Open	Read	Print	Export	Data	Template	Calculation	Results	Statistics	Graphics

Status bar The program status bar is located at the bottom of the main window. It contains information on the currently loaded data and parameter file. Context-sensitive help information is available in the left section of the status bar. The name of the active parameter file (*.par) is displayed in the right-hand section.

Data file The raw data of the measurement are saved to a **data file** (extension *.dat). Furthermore, this file may contain the respective sample identifier and dilution information for each cavity. Any errors that may have occurred during measurement or evaluation are also stored. In addition, the data file includes the data from the child window **Statistics** (instrument settings and software versions) as well as further information, such as date and time of measurement, the parameter file used and – if activated – user information.



Parameter fileThe parameter file (extension *.par) includes all test-specific
settings which are required to run and evaluate tests, including
all measurement parameters (well selection, measurement se-
quence, filter selection, etc.), all calculation parameters (data
from the child windows Template and Calculation) and may
also include print and export settings.

See appendix for details of selected parameter files.

Basic parameter files The basic parameter files supplied by **Berthold Technologies** include typical measurement sequences of some major types of measurements. Using this data, you just have to make a few changes to define the measurement sequence for your own requirements. A parameter file includes selected wells, predefined operations and evaluation parameters (e.g. calculations, result presentation and export). The file name indicates the respective type of measurement:





Matrices	The matrix system of the program is largely comparable to a spreadsheet software. However, the number of input fields is preset to 8×12 positions, representing the typical microplate format. Plates with less or more cavities may also be emulated. Using this matrix system, all calculations which are required to evaluate a test can be carried out.
Operation	Operation and handling of the program follow the usual <i>Win-dows</i> ® conventions. Special user instructions will be described whenever required. The following sections of chapter 5 describe only <u>those</u> functions in detail which are relevant for control of the <i>Twinkle</i> instrument. In addition, we will discuss some menu items which are essential for creating parameter files and result output. These functions are printed in boldface type in the menu overview below.



Twinkle Software Functions

Menu overview

File	Edit	View	Read	Instrument	Options	Installation
New	Undo	Tool Bar		Load Plate	Read	Settings
Open	Сору	Status Bar		Unload Plate	Threshold	Automation
Save	Paste	Navigation Bar		Excit. Filter Slide	Controls	Driver
Save As	Reset	Help Card Bar		Emiss. Filter Wheel	Definitions	
Export				Plate Editor	Matrix	
Export Setup				Boot Instrument	Result	
Print					Curve Fit	
Print Preview					Kinetics	
Print Setup					Scanning	

Menu items printed in boldface type are described in the following chapters:

		Chapter
File Open	Open parameter files Open data files	8.5.2 8.6
File Save (As)	Save parameter files Save data files	8.5.2 8.6
File Export (Setup)	Export data files	8.3
Read	Measurement	8.6
Instrument	Instrument control functions	8.4
Options Read	Definition of measurement sequence	8.5.3, 8.5.4 and 8.5.5
Installation Driver	Driver setup	8.2.2



8.2 Installation

8.2.1 Mikrowin 2000 Installation

(see Quick Reference Guide page 15)

8.2.2 Driver Installation

(see Quick Reference Guide page 18)

Installation of additional 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**>.

Driver Rename Dialog	
Please enter the new driver name in the edit option below.	ОК
new name	Cancel

Now you can re-install the driver again by the clicking **<Add Driver>** and browsing to the driver directory you created.

Installation Driver Add				
Driver Disk	OK			
Please insert the appropriate Driver Disk into the drive selected!	Cancel			
C:\MikroWin 2000\Drivers	Browse			

Select the respective driver in the dialogue displayed.



Ir	stallation Driver Add			×
	List of available drivers			
	Description	Version	Library	T
	Line Export Driver	4.15	line1.xdl	-
	Matrix Export Driver	4.09	matrix1.xdl	
	RawData Export Driver	4.10	rawdata1.xdl	
	1			_
	ОК	Car	ncel	

You may repeat this procedure as often as necessary to get an appropriate number of export drivers.

8.2.3 Driver Setup

In order to control the Fluorometer *Twinkle* via the software, you have to set up the respective drivers. To work with *Twinkle* you have to set up the **Reader** and **Export** drivers correctly. Select the menu item **Installation | Driver** to open the **Installation I Driver** dialog box with a separate tab for each driver type.

Installation Driver					
Reader CurveFit Export					
Description	Version	Library	Add Driver		
Null Device BertholdTech Twinkle	4.20 1.01	nulldev 1 xdl Twinkle xdl	Delete Driver		
BertholdTech CENTRO	1.11	Centro xdl	Update Driver		
Berthold Tech Mithras	1.04	Mithras xdl			
Berthold Tech. Apollo 1 LB 911	4.06	berthld6xdl			
BertholdTech TriStar	1.02	TriStar.xdl			
			Driver Setup		
OK Abbrechen Übernehmen Hilfe					

Figure 8-1: Installation driver dialog box



Installation Driver dialog box

List of installed drivers	
	This list includes all currently installed drivers of a special type (Reader , CurveFit , Export and Sampler). Each driver type appears on a separate tab. The left column shows the names of the installed drivers; the center column shows the version number of the driver and the right column includes the names of the library file used for execution of the driver functions.
<add driver=""></add>	Click this button to add a new driver or to update an existing driver. You need a program library, i.e. a file with the extension xdl (e.g. an export driver like Matrix1.xdl). Such a file may be found on a special driver disk, a program CD, the local hard-drive or on the network. Click the <add></add> button to open a dialog box from which you can select the drive and directory of the new library file. Click the <browse></browse> button to search for a special folder. If the directory of the library file has been defined, click <ok></ok> to open another dialog, listing all drivers (library files) available in the selected directory. Select one or several entries from the list and click <ok></ok> to install the selected drivers.
<delete driver=""></delete>	To delete an installed driver, you first have to select one from the list of installed drivers. Click the <delete driver=""></delete> button to remove the selected driver.
<driver setup=""></driver>	Click the <driver setup=""></driver> button to configure an installed driver. Each new driver should be set up prior to its first use. A driver- specific dialog opens in which you can define the required con- figuration. The Reader driver dialog typically includes the com- port settings used as well as information on the injectors in- stalled in the instrument, etc. This information should be verified after first installation. A general description of these dialogs is not possible since each driver has its own configuration dialog.



Twinkle Driver Setup

- □ In the **Installation Driver** dialog box, click on the **Reader** tab and select the instrument driver **BertholdTech Twinkle**.
- □ Then click <**Driver Setup**> to open the dialog box **Ber**tholdTech Twinkle (with version number).

BertholdTech Twinkle, Version	I.01 🛛 🔀
ComPort	OK Cancel
Instrument Configuration Fluorescence installed I Temperature Control installed Bottom Reading installed	Get Configuration

Figure 8-2: Setup of device driver

ComPort

Define the PC port to which the *Twinkle* instrument is connected. You may choose **COM1** through **COM9**.

Instrument Configuration

Define the configuration of your *Twinkle* instrument. A selected item is identified by a checkmark (\checkmark).

Fluorometry installed

This item is always selected (\checkmark).

Temperature Control Installed

Select this item if your instrument includes a temperature control .

Bottom Reading Installed

Select this item if your instrument is equipped with the Bottom Reading option for fluorescenec intensity measurements .



<get configuration=""></get>	Download configuration from instrument.
<0K>	Save configuration.
<cancel></cancel>	Discard new entries.



Export Driver Setup

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:

Instalation Driver			X
Reader CurveFit Export Sample	er		
Description	Version	Library	Add Driver
Matrix Ascii Export Driver Extended Ascii Export Driver Line Ascii Export Driver IMatrix XLS Export Standard Matrix XLS Export Kinetics Dri Matrix XLS Export Configurabl	4.03 4.05 4.11 4.06 4.06 4.06	ascii5.xdl ascii6.xdl ascii7.xdl xls1.xdl XLS1.XDL XLS1.XDL XLS1.XDL	Delete Driver
			Driver Setup
01	<	Cancel Ap	ply Help

Figure 8-3: Selection of export drivers

Matrix Export DriverDriver (template) for export of calculated data with matrix (i.e.
plate lay-out) structure. Only data that are visible on result ma-
trices can be exported. File formats may be EXCEL, Text
(ASCII) and CSV.Line Export DriverDriver (template) for export of calculated data with list (i.e. ta-
ble-type) structure. Only data that are visible on result matrices
can be exported. File formats may be EXCEL, Text (ASCII) and
CSV.RawData Export DriverDriver (template) for export of all raw data. File formats may be
EXCEL, Text (ASCII) and
CSV.RawData Export DriverDriver (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 Expo	rt Driver, Version	4.11	X
Export Layo	ut		
Header :			Add
Matrix :	Matrix Matrix No 1 Matrix No 2 Matrix No 2 Matrix No 4	Selection mode Matrix identified by Number Matrix identified by Number Matrix identified by Number Matrix identified by Number	Add Edit Delete
Footer :			Add
– Operation M	lode		
Export :	always	•	
Add Gene	ral Statistics : Yes	•	
Export Targ	et		
Format :	XLS File	Adjustments	ОК
Directory :	C:\Programme\Mikro	Win 2000\Transfer	ancel telp

Figure 8-4: Matrix export driver setup

Export Layout

Define the file layout.

HeaderText 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 key-
board entry (comma, space, etc.).

Matrix Name	#MX
Date	#DT
Time	#TM
Plate Identifier	#PI
Template Identifier	#TI
Tabulator	#TB

Figure 8-5: Context menu for entering header placeholders

<u>Example:</u>

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 matri- ces. <i>Make sure</i> that the matrices and their numbers specified here is identical with the number of the result matrix in the pa- rameter 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></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.
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.
 - FormatYou may select from Text File, XLS File, CSV File, CommPort
and Clipboard.
 - TargetDefine 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.



Operation

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, Version 4.18		X
Export Layo	put		
Header :			Add
Line :	#01#TB#02#TB#03#TB#04#TB#05#TB		Add
Footer :			Add
Data Select	tion der Plate Partition Control Positions Positions without Sample ID empty Positions of Matrix 1	Operation Mode Export : always Data Sequence : A, E Add General Statistics :	▼ 8, C, D, ▼ Yes ▼
Export Targ Format : Directory :	let XLS File C:\Programme\MikroWin 20	Adjustments	OK Cancel Help

Figure 8-6: 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.).

Matrix Name	#M×
Date	#DT
Time	#TM
Plate Identifier	#PI
Template Identifier	#TI
Tabulator	#TB

Figure 8-7: Context menu for entering header placeholders

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.



Line

Date	#DT
Time	#TM
Position (A01)	#PS
Position (A1)	#PO
Error	#ER
Plate Identifier	#PI
Template Identifier	#TI
Test Name	#TS
Tabulator	#TB
Matrix 1	#01
Matrix 2	#02
Matrix 3	#03
Matrix 4	#04
Matrix 5	#05
Matrix 6	#06
Matrix 7	#07
Matrix 8	#08
Matrix 9	#09
Matrix Name	# <name></name>

Figure 8-8: 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.

- FooterText 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 Mod	le	Define additional options.
Ex	port	The proper setting is Always .
Ad	ld General Stati	stics 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.
Fo	rmat	You may select from Text File, XLS File, CSV File, CommPort and Clipboard.
Ta	rget	Define the directory which the file is to be exported to. You may use the browse <> button to locate an appropriate directory.
Ad	ljustment	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!

RawData Exp	ort Driver, Version 4.16	×
Export Layou	t]
Header :	Add	
RawData :	Kinetic Layout : Time / Position 💌 Time Format : hh:mm:ss	
Footer :	Add	
Operation Mo Add Genera	nde Il Statistics : no	
Export Targe Format : Directory :	t Text File Adjustments C:\Programme\MikroWin 2000\Transfer Help	

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.).



Matrix Name	#MX
Date	#DT
Time	#TM
Plate Identifier	#PI
Template Identifier	#TI
Tabulator	#TB

Figure 8-9: 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.


8.3 Export

Prerequisite for exporting data files is the correct installation and setup of the export driver (see chapter 8.2)

There are two different export functions:

- a) <u>Manual data export</u> (File | Export): you select the export driver for the respective measurement. If you wish to export data after a measurement, click the <Export> button on the tool bar.
- b) <u>Automatic data export</u> after each measurement. Prerequisite is that the export driver has been defined in the menu item File | Export Setup. After storing the parameter file, this setting is specific to this parameter file.

8.3.1 Manual Data Export

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.



Figure 8-10: 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. <u>Please keep in mind</u> 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.



8.3.2 Automatic Data Export

Choose the menu item **File | Export Setup** to select the export driver that is to be loaded <u>automatically</u> 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 8.1) and the export driver has been selected in the menu item **File | Export** (see chapter 8.2.1).

- **D** Open parameter file.
- Select File | Export Setup to open the File Export Setup dialog box.

File Export Setup	
List of Active Export Driver	ОК
List of export driver	Cancel
Matrix XLS Export Standard Driver	Help
	Remove

Figure 8-11: 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.



8.4 Instrument Control and Operation

The *Twinkle* Fluorometer is controlled and operated via the *MikroWin 2000* software. This includes opening and closing of the plate.

8.4.1 Instrument | Load Plate

Select **Instrument | Load Plate** to transport the plate slide into the instrument and to close the plate tray. The instrument is closed light-tight.



Open the plate tray only to load microplates, so that as little light as possible may penetrate the counting chamber. If necessary, allow for a delay between plate loading and start of measurement (programmed in the measurement sequence), since white microplates in particular may be phosphorescent after having been exposed to daylight.

8.4.2 Instrument | Unload Plate

Select **Instrument | Unload Plate** to move the plate slide out of the instrument and to unload a microplate.

Leave plate tray open only for loading microplates.



8.4.3 Instrument | Excitation Filter Slide

Select **Instrument | Excitation Filter Slide** to define new excitation or absorbance filters, to place them into respective positions of the slide and to eject the excitation filter slide for changing the slide or physically mounting filters.



8.4.4 Instrument | Emission Filter Slide

Select **Instrument | Emission Filter Slide** to define new emission filters, to place them into respective positions of the wheel and to access the emission filter wheel for changing the wheel or physically mounting filters.





8.4.5 Instrument | Plate Editor Injector

Select **Instrument | Plate Editor** to add, copy or modify microplates and their dimensions.

Plate Editor	
Name	OK
Copy of Berthold 96	
Parameters Number of rows (1 - 32): 8	New Plate Copy
Number of columns (1 - 48): 12	Apply
Height of the plate in (mm): 14.7	Remove Plate
Stacking height in (mm): 13.2	
Distance from corner and from well to well in (mm)	
Usage I Centro I Mithras I Twinkle	

8.4.6 Instrument | Boot Instrument

If the *Twinkle* instrument has been turned off and on again without having shut down the *Mikrowin 2000* program, you have to select the item **Instrument | Boot Instrument**. The software initializes the instrument again and establishes correct communication between instrument and PC. Otherwise instrument control is not possible.



8.4.7 Excitation Filter - Excitation Filter Slide

Definition and maintenance of the excitation filter used takes place via the menu item **Instrument | Excitation Filter Slide**. The following options are available:

- Enter the name and position of the filter used, which are then available for selection when defining the reading parameters (in the parameter files).
- Move the excitation filter slide in and out to change or clean the filter.

Select the menu item **Instrument | Excitation Filter Slide** to open the **Excitation Filter Slide** dialog box.



Add & Remove filter Eject filter slide Select slide: A, B, C Figure 8-12: Control of excitation filter slide



Entering filter names

Click <Add> to open the Add Filter dialog box.

Figure 8-13: Entering a filter name

- □ Enter name of filter in the **Name** text box.
- Define usage of filter for fluorescence measurement.
- Click <OK> in the Add Filter dialog box. The dialog box is closed and the new filter name is listed in the Unused Filter text box in the Excitation Filter Slide dialog box.
- Delete a filter name: highlight the respective filter name and then click <**Remove**>.



Positioning filters

Depending on the position of the filter on the excitation filter slide, allocate the respective filter names to positions 1 to 5 (see Figure 8-14). In the parameter files only those filter positions are displayed for selection which are occupied here. Only those filters can be used for measurements which have been defined in the software. Therefore make sure that the allocation in the software matches the actual filter positions.

Proceed as follows

- Prerequisite is that filter names are displayed in the Unused Filters text box (see previous page).
- Select the approriate filter slide.
- Drag¹ the filter name from the Unused Filters text box to one of the positions 1 to 5 of the schematic illustration of the excitation filter slide.
- You may also drag a filter name from one position on the slide to another, or from a slide position back to the Unused Filters text box.



Figure 8-14: Placing filter names with drag & drop

¹ <u>*Drag & Drop:*</u> Place mouse pointer over filter name, push left mouse button and drag mouse pointer with mouse button held down to the desired position. The filter name is dragged along. Release left mouse button as soon as the filter name has reached the desired position. You can drop the filter name only in the **Unused Filters** box or on one of the 5 filter positions.



Replacing/Cleaning excitation filters

The compartment with the excitation filter slide is located to the right of the plate tray (instrument front panel).

Proceed as follows

- □ Click <**Eject Slide**> in the **Excitation Filter Slide** dialog box. The door opens slightly and the slide moves out a bit.
- □ Open the door all the way by hand and pull out the filter holder at both metal pins (bottom and top on filter holder).
- □ Clean filter or replace it and insert it again.
- If you replace the filter and insert it again, you have to drag the respective filter in the Excitation Filter Slide dialog box to the respective positions (see previous page).
- Push filter holder into the slide again all the way.
- □ Click <**OK**> in the **Excitation Filter Slide** dialog box. The slide moves into the instrument and the door is closed again.



8.4.8 Emission Filter – Emission Filter Slide

The emission filter used is defined and serviced/managed using the menu item **Instrument | Emission Filter Slide**. The following options are available:

- Enter the name and position of the filter placed in the filter slide, which is then available for selection when defining the reading parameters (in the parameter files).
- □ Move the emission filter slide in and out to change or clean the filter.

Select the menu item **Instrument | Emission Filter Slide** to open the **Emission Filter Slide** dialog box. Drag the filters used to the desired filter wheel position.



Figure 8-15: Emission Filter Wheel dialog box

Up to eight different emission filters can be placed into the two emission filter slides located directly below the photomultiplier. The desired filter is moved to the respective reading position under control of the software.



Entering filter names

□ Click <Add> to open the Add Filter dialog box.

- **□** Enter filter name in the **Name** text box.
- Define usage of filter: for fluorescence or luminescence measurement.
- Click <OK> in the Add Filter dialog box. The dialog box is closed and the new filter name is listed in the Unused Filter text box.

Add Filter	X
Name: new filter Usage I Fluorescence	OK Cancel

Figure 8-16: Entering a filter name

Remove filter name: highlight the respective filter name and click <**Remove**>.



Positioning filters

Depending on the position of the filter on the emission filter slide, allocate the respective filter names to positions 1 to 4 (see Figure 8-14). In the parameter files only those filter positions are displayed for selection which are occupied here. Only those filters can be used for measurements which have been defined in the software. Therefore make sure that the allocation in the software matches the actual filter positions.

Proceed as follows

- Prerequisite is that filter names are displayed in the Unused Filters text box (see previous page).
- □ Select the approriate filter slide.
- Drag² the filter name from the Unused Filters text box to one of the positions 1 to 4 of the schematic illustration of the emission filter slide.
- You may also drag a filter name from one position on the slide to another, or from a slide position back to the Unused Filters text box.



Figure 8-17: Placing filter names with drag & drop

Replacing/Cleaning excitation filters

² <u>*Drag & Drop:*</u> Place mouse pointer over filter name, push left mouse button and drag mouse pointer with mouse button held down to the desired position. The filter name is dragged along. Release left mouse button as soon as the filter name has reached the desired position. You can drop the filter name only in the **Unused Filters** box or on one of the 5 filter positions.



The compartment with the emission filter slide is located above the plate tray (instrument front panel).

Proceed as follows

- □ Click <**Eject Slide**> in the **Emission Filter Slide** dialog box. The door opens slightly and the slide moves out a bit.
- □ Open the door all the way by hand and pull out the filter holder at the metal pin (front of the filter holder).
- □ Clean filter or replace it and insert it again.
- □ If you replace the filter and insert it again, you have to drag the respective filter in the **Emission Filter Slide** dialog box to the respective positions (see previous page).
- Push filter holder into the slide again all the way.
- □ Click <**OK**> in the **Emission Filter Slide** dialog box. The slide moves into the instrument and the door is closed again.

Cleaning filters

□ Filters should be cleaned using a lint-free cloth or, better, a micro fiber cloth, as used for cleaning eye glasses.



8.5 Reading Parameters

8.5.1 Overview

The test-specific configuration required to perform and evaluate tests is saved to parameter files (extension *.par).

The **Twinkle** Fluorometer can perform measurements with different types of settings. Each type of measurement may have its own typical sequence. **Berthold Technologies** supplies special basic parameter files, including typical measurement sequences, which can easily be adapted to each user's specific needs. You do not have to create a completely new measurement sequence, but you may utilize and modify an existing structure.

Basic parameter files are supplied covering the operations and calculations most often used (see chapter **8.1**).



Save the changes to these files under a <u>new name</u> to keep the basic parameter files!

Reading parameters can be defined in two different ways:

- a) Completely new definition of measurement and evaluation parameters.
- b) Using an existing parameter file and modifying the parameters as needed. Either you use the basic parameter files supplied by *Berthold Technologies*: each includes a typical measurement sequence for each type of measurement. Or you use parameter files you have created yourself and edit them.



8.5.2 Open/Save Parameter File

Create new parameter file

□ Select Edit | Reset to create a new parameter file. The file name Untitled.par is displayed in the status bar.

Open parameter file

Select File | Open or click the <Open> button on the tool bar to open an existing parameter file. The Open dialog box is displayed.



Figure 8-18: Open dialog box

- From the Files of type drop-down list box, select the type MikroWin Para File (*.par). The contents of the directory TwinklePara is displayed, showing the available parameter files.
- □ Select the file you want and click <**Open**>.
- □ If the parameter file is located in another directory, open the **Look in** drop-down list box and select the directory you want.

The file name of the opened parameter appears on the status bar in the bottom right corner of the screen.

Please keep in mind:

All changes in the reading parameters refer to the open parameter file. Changes are taken over for good when the parameter file is saved.



Save parameter file

Select File | Save so save the active parameter file.

Select **File | Save As** if you want to save the file under a new name or if you have created a new parameter file (**"Unti-tled.par**"). Enter the file name in the **Save As** dialog box and select the file type **MikroWin Para File** (extension *.par). The directory pre-defined in the **Installation settings** dialog box is defaulted. You can change the directory. Click **<OK>** and the new parameter file is saved to the pre-selected directory.

What does a parameter file contain?

A parameter file includes:

Well selection	Options Read
Measurement sequence	Options Read
Evaluation parameters	Child windows: Template Calculation
Sample parameters	Options menu Parameters for controls, limit values …
Export parameters	Automatic export: File Export Setup



8.5.3 Well Selection

Before defining the measurement sequence, you have to select the wells of the microplate you want to measure and those which are to receive an injection.

- □ To edit an existing parameter file, open this file (File | Open).
- □ To create a new parameter file, select **Edit** | **Reset** in the main window. **Untitled.par** is displayed as temporary file name in the bottom right corner of the screen.
- Select Options | Read to open the Options Read dialog box.

C	ptions Read			
	- Driver Selection		[OK
	Device:	BertholdTech Twinkle		Cancel
	Version:	1.01		Help
	Description:	Reader device driver for Berthold Technologies Fluorometer Twinkle	[Settings

Figure 8-19: Options Read dialog box

Driver Selection Device	From the Device drop-down list, select BertholdTech Twinkle to define the measurement sequence for <i>Twinkle</i> .
Version	Shows the driver version. This entry cannot be edited.
Description	Describes the selected driver.
	□ As soon as you have set up the <i>Twinkle</i> driver, click the < Settings > button to open the Options dialog box with the Samples and Measurement tabs (Figure 8-20).
	On the Samples tab, select the plate type and the wells to be measured. The measurement sequence is defined on the Measurement tab.





Figure 8-20: Options dialog box, Samples tab

Samples tab	
Plate parameter Plate type	Select the desired plate type with 96 or 384 wells. The plate matrix is displayed showing the respective number of wells (*only with option automatic plate height adjustment).
Measurement Order By Row / By columns	Choose if the measurement sequence is to be processed by rows or by.
Plate matrix	A schematic presentation of a microplate is displayed showing the selected microplate type. Select the wells to be measured with the cursor. The grey colour indicates which wells are selected for reading.
	<u> </u>



Well selection

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

 A
 (a)
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0
 0<

Measurement); then select the wells with the cursor.

For well selection, you first have to select the mode (first select

Figure 8-21: Well selection

н

- (a) All wells on a plate Click on the empty button in the top left corner (between A and 1). All wells on the microplate are selected in accordance with the pre-defined mode.
- (b) Selected well area With the mouse button held down, drag the mouse pointer diagonally across the desired well area and then release the mouse button. The wells inside this rectangle are highlighted. You can define several independent rectangular well areas on one plate.
- (c) Single wells Click on the desired well.

(d) *Clear selection*<u>Clear all:</u> Click right mouse button and select **Clear** from the context menu.
<u>Undo function selected last:</u> Select last function once more, i.e. enable the respective **Plate Mode** and click once more on the wells you want to deselect or click on the empty button in the top left corner (between A and 1).



8.5.4 Definition of Measurement Sequence

The measurement sequence is defined in the **Options** dialog box on the **Measurement** tab.

First, select the wells for measurement and injection on the **Samples** tab; then define the measurement sequence with the individual operations. To do this, select the **Measurement** tab.

Delay Shake Shake Shuce. Label K Ruor. Kinetic S Ruor. Scanning R Ruor. Repeated	¥ ↑ ↓ ⊞¥ 485/535	Name Counting Time Lamp Energy Excitation Filter Excitation Aperture Emission Filter Counter position Meas. operation Second Measurement	485/535 0.10 7000 F485 Norma F535 Top by Plate No
Operation Mode Batch Plate Temperature Control	Repeats: 1	Celsius	>

Figure 8-22: Measurement tab

Left column Shows the available operations. Steps that can be selected are identified by a checkmark. You can select only those steps that are supported by the instrument and the pre-selected measurement mode. This ensure that the measurement sequence does not include any operations that cannot be carried out.

Operations column Operations required for the measurement sequence are copied from the left column into the center column and arranged in the desired order. Individual operations can be copied for the measurement sequence and can be moved to any position.



	Click on an operation to show the respective parameters in the Operation column. Double-click on an operation to open the associated parameter dialog box to edit the parameters.
	<u>Copy</u> To copy an operation to the center column, double-click on the respective operation in the left column. This opens the parame- ter window. Enter the required parameters and confirm the en- tries with $< OK >$. The window is closed and the operation is en- tered in the center column behind the last one (chapter 8.5.5).
	Select order In the Operations column the selected operations are first listed in the order of their selection. To change the order, click on the buttons and . Select the operation which you want to move to another position and click on the respective button until you have reached the desired position. The arrows on both buttons indicate whether the operation is moved up or down.
	<u>Clear operation</u> To delete an operation from the measurement sequence, select this operation and then click on the button.
Operation column	This column shows the parameters of an individual operation selected in the center column (see Figure 5-26).
Operation Mode	Select the desired mode:
Batch:	In this mode several plates can be measured one after the other using <u>the same protocol</u> . <u>Measurement sequence</u> : After it has been processed, the first plate is moved out of the instrument. Load the next plate and start measurement with the same parameters.
Repeat:	<i>The same plate</i> can be measured several times in succession. Define the number of repeats here.



Delay		Name Counting Time	Repeated 0.15
Huor. Label K Fluor. Kinetic S Fluor. Scanning R Fluor. Repeated		Excitation Filter Excitation Aperture Emission Filter Counter position Meas. operation Cycle Time Total Time	5000 F355 Normal F460 Top by Well 37.50 300.0
Operation Mode	e Repeats: 9	°Celsius	

Figure 8-23: Defined measurement sequence with display of reading parameters

Temperature Control	Define the desired temperature if your instrument includes a
	temperature control (input option: 15° - 42°C).
	Please note: You can enter a target temperature in the instru-
	ment which is <u>at least</u> +5°C above room temperature.

Keep Plate After Measurement If this option is checked the plate will be kept within th instrument and the user has the possibility to execute a consecutive reading og the same plate without moving the plate out before the 2nd reading.



Proceed as follows to define a new measurement sequence:

- □ Select Edit | Reset. Untitled.par is displayed as temporary file name in the bottom right corner of the status bar.
- Select Options | Read to open the Options Read dialog box.
- Select **BertholdTech Twinkle** from the device drive list.
- □ Click <**Options**> to open the **Options** dialog box with the **Samples** and **Measurement** tab.
- □ Select the plate type on the **Samples** tab.
- □ In the row **Plate Mode**, enable the item **Measurement** and then select the wells for measurement.
- Select injector in the Plate Mode row and then select the wells into which the selected injector is to dispense.
- After selection of the wells, select the Measurement tab and define the measurement sequence with the individual operations.
- Copy these operations one after the other to the **Operations** column: in the left column double-click on the desired operation and enter the parameters in the respective window.
- Confirm your entries with <OK>. The respective operation is entered in the Operations column behind the operation defined last.
- In the Operations column, the selected operations are first displayed in the order of their selection. Click the buttons

★ and ↓ to change the order, click × to delete an operation.

- ☐ The parameters of the operation selected in the **Operations** column are displayed in the **Operation** column.
- □ Select the operation mode.
- Enter the temperature, if the instrument includes temperature control and temperature control is needed for the measurement sequence.
- \Box Confirm entries with <**OK**>.



8.5.5 Operations and their Parameters

	<u>Double-click on an operation in the left column</u> to open the re- spective properties dialog box. Click <ok></ok> to accept the entries and append the respective operation to the last operation de- fined in the Operations column.
	<u>Double-click on an operation in the Operations column</u> to open the properties dialog box and edit the entries. Click <ok< b="">> to accept the entries.</ok<>
By plate / By wells / By strip	The measurement mode By plate or By wells or By Strip has to be selected for the operations.
	By plate means that a defined operation is first performed for all selected wells of the plate, before the next step starts.
	By wells means that all consecutive operations that are set as " by well " are first performed for the 1 st well, then for the 2 nd well, etc.
	By strip means that all consecutive operations that are set as " by strip " are first performed for the 1 st row or column (depend- ing on the selected reading order), then for the 2 nd , etc.
	The ability to choose the mode individually per operation en- ables the user to program complex operation sequences.
Perform on first	
plate repeat only	If you choose Perform on first plate repeat only , the respec- tive operation is performed only once at the start of a series of repeat measurements (see also Repeat).



Delay Delay before an operation

Double-click on Delay to open the **Delay properties** dialog box and define the delay between two operations.

Delay properties		×
Parameters:	(0.1 - 3600 s)	OK Cancel
Operation Mode: — C By plate	By wells	C By strip
Perform on first p	late repeat only	

Figure 8-24: Delay properties dialog box

Duration Define delay between 1 and 3600 seconds.

Measurement Mode

This function can be used in the plate or well or strip mode.

BERTHOLD TECHNOLOGIES

Shake Shake

Double-click on Shake to open the **Shake properties** dialog box and define the parameters for microplate shaking.



Figure 8-25: Shake properties dialog box

Duration	Define shake duration in seconds.
Speed	Define shake speed. You may choose $\ensuremath{\textbf{Slow}}$, $\ensuremath{\textbf{Normal}}$ or $\ensuremath{\textbf{Fast}}$.
Diameter	Define amplitude of shake motion.
Туре	Select shake mode: linear, orbital or double orbital.
Measurement Mode	This function can be used in the plate or well or strip mode.

The combination of spped and amplitude define shaking performace expressed in RPM:

Linear	&	orbital	mode:
--------	---	---------	-------

Speed	Amplitude						
	0.1 mm	2.5 mm	5 mm				
low	438	42	22				
medium	882	90	44				
high	1758	174	88				

Double orbital mode:

Speed	Amplitude						
	0.1 mm	2.5 mm	5 mm				
low	219	21	11				
medium	441	45	22				
high	879	87	44				



Fluorometry Label Fluorescence measurement

Double-click on Fluorometry Label to open the **Fluorometry** dialog box for entry of the parameters for the fluorescence measurement.

Fluorescence		
Parameters: Name: Counting Time: Lamp Energy: Excitation Filter: Emission Filter: Counter position:	Fluor. Labe 0.1 (0.05 · 600 s) 5000 (0 · 65535) F355 (Umbelliferone) · Slot A1 • F460 (Umbelliferone) · Slot A1 • • Ton © Bottom	OK Cancel
Counter position:		
 By plate 	C By wells C By strip	
C Second Measu	rement	
Excitation Filter:	F355 (Umbelliferone) - Slot A1	
Emission Filter:	F460 (Umbelliferone) - Slot A1	

Figure 8-26: Fluorometry dialogue box

Name	Name of measurement.		
Counting Time	Counting time in seconds.		
Lamp Energy	Enter a value between 0 and 65 535. This range corresponds to 0 to 75 Watts and is divided in digital steps.		
	The lamp energy ought to be set in a way that the full dy- namic range (~20 Mio CPS) is used. This setting is depend- ent on various parameters incl. max. concentration of the dye, quantum efficiency of the dye, transmission properties of the filters, etc.		
	The halogen lamp is stabilized by a control circuitry. Dependent on wavelength and selected filtes this can limit the maximum usable lamp energy. If the lamp energy is set too high you will get the error message " <i>Halogen Lamp Stabilisation Error</i> ". The action to take is to decrease the lamp energy settings.		
Excitation Filter	Select the desired excitation filter. The pre-defined filter with name and position in the filter slide is displayed.		



Emission Filter	Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed.
Counter position	Select if a fluorescence measurement should take place with the top or bottom optics.
	Note: You need to manually switch the optics for top or bottom reading prior to measurement!
Operation Mode	This function can be used in the plate or in the well or in the strip mode. It is recommended to use the <i>by plate</i> mode for fast throughput of a plate as the lamp is stabilized only once.
Second Measurement	Check this option if a second measurement immediately after the first one has to be performed.



K Fluorometry Kinetic Fluorescence Kinetics measurement

Double-click on K Fluorometry Kinetic to open the Kinetics properties dialog box.

A kinetics measurement always takes place in the **By wells** mode.

Kinetics propert	ies			
Parameters:				ОК
Name:	Fluor, Kinel	ic		Cancel
Total Time:	1	(1 · 86400 s)		Cancer
Counting Time:	0.1	(0.05 - 600 s)		
Delay:	0	(0 · 600 s)		
Repeats:	11	(1 - 999)		
Lamp Energy:	5000	(0 - 65535)		
Excitation Filter:	F355 (Umb	elliferone) - Slot A1	•	
Emission Filter:	F460 (Umb	elliferone) - Slot A1	•	
Counter position:	Top	C Bottom		

Figure 8-27: Fluorescence Kinetics parameters

Name	Name of kinetics measurement.
TotalTime	Specify the total counting time for each selected well.
Counting Time	Define the single counting time of a kinetics sequence of one well.
Delay	Delay time between individual measurements of a kinetics se- quence of one well.
Repeats	The number of data points per well is automatically calculated from the entered parameters (including the Delay).
Lamp Energy	Enter a value between 0 and 65 535. This range corresponds to 0 to 75 Watts and is divided in digital steps.
	The lamp energy ought to be set in a way that the full dy- namic range (~20 Mio CPS) is used. This setting is depend- ent on various parameters incl. max. concentration of the dye, quantum efficiency of the dye, transmission properties of the filters, etc.
	The halogen lamp is stabilized by a control circuitry. De- pendent on wavelength and selected filtes this can limit the maximum usable lamp energy. If the lamp energy is set too high you will get the error message <i>"Halogen Lamp Stabili-</i>



satio	n Error	". The	action	to	take	is	to	decreas	se the	lamp
energ	ly settir	igs.								
<u> </u>										

- **Excitation Filter** Select the excitation filter. The pre-defined filter with name and position in the filter slide is displayed.
- **Emission Filter** Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed.
- **Counter position** Select if a fluorescence measurement should take place witht the top or bottom optics.

Note: You need to manually switch the optics for top or bottom reading prior to measurement!



S Fluorometry Scanning measurement

Double-click on **S** Fluorometry Scannin to open the **Scan proper-ties** dialog box.

Scanning always takes place in the **By wells** mode.

Scanning operation is useful when cells growing inhomogeneously in the wells are measured.

s	can properties		X
	Parameters:		ОК
	Name:	Fluor, Scanning	Cancel
	Counting Time:	0.1 (0.01 - 600 s)	
	Lamp Energy:	5000 (0 - 65535)	
	Excitation Filter:	F355 (Umbelliferone) - Slot A1 📃	
	Emission Filter:	F460 (Umbelliferone) - Slot A1	
	Counter position:	Top C Bottom	
	Horizontal steps:	10 (1 - 100)	
	Point displacement:	0.56 (0.01 - 50 mm)	
	Scanning mode:	Rectangular O Round	

Figure 8-28: Fluorescence Scanning properties dialog box

Name	Name of scanning operation.
Counting Time	Define individual counting time.
Lamp Energy	Enter a value between 0 and 65 535. This range corresponds to 0 to 75 Watts and is divided in digital steps.
Name Counting Time Lamp Energy Excitation Filter Emission Filter	The lamp energy ought to be set in a way that the full dy- namic range (~20 Mio CPS) is used. This setting is depend- ent on various parameters incl. max. concentration of the dye, quantum efficiency of the dye, transmission properties of the filters, etc.
	The halogen lamp is stabilized by a control circuitry. Dependent on wavelength and selected filtes this can limit the maximum usable lamp energy. If the lamp energy is set too high you will get the error message " <i>Halogen Lamp Stabilisation Error</i> ". The action to take is to decrease the lamp energy settings.
Excitation Filter	Select the excitation filter. The pre-defined filter with name and position in the filter slide is displayed.
Emission Filter	Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed.



Counter position	Select if a fluorescence measurement should take place witht the top or bottom optics.				
	<i>Note:</i> You need to manually switch the optics for top or bottom reading prior to measurement!				
Steps	Number of horizontal and vertical steps: an entry of "10" means the scanning will be performed in a 10 by 10 matrix.				
Point displacement	Step width = distance between data points. Selectable dis- tances: 0.1 to 50 mm.				
Scanning mode:	Rectangular or round can be selected. We recommend to se- lect rectangular .				
	During scanning, one well with up to 10,000 data points can be measured. To measure the well of a 96-well microplate (∅ 6 mm) with 100 data points evenly and completely you have to define the fol- lowing parameters: horizontal and vertical steps: each 10; point displacement : 0.6 mm. However, you may also focus on the center of one well and se- lect smaller distances.				



R Fluorometry Repeat Fluorescence Repeated measurement

Double-click on **R** Fluorometry Repeat to open the **Repeated** dialog box.

The Repeated operation is used for long-term kinetic monitoring when the reaction to be monitored is slow and lasting, e.g. over a couple of mayn minutes or hours as in Phagocytosis.

Re	epeated		
Γ	Parameters:		ОК
	Name:	Fluor. Repeated	Cancel
	TotalTime:	300 (1 · 86400 s)	
	Counting Time:	0.15 (0.15 · 600 s)	
	Cycle Time:	37.5 (37.50 - 6000 s)	
	Repeats:	9 (1 - 999)	
	Lamp Energy:	5000 (0 - 65535)	
	Excitation Filter:	F355 (Umbelliferone) - Slot A1	
	Emission Filter:	F460 (Umbelliferone) - Slot A1	[
	Counter position:	Top C Bottom	
Γ	Operation Mode:		
	O By plate	• By wells	
L			

Figure 8-29: Fluorescence Repeated dialog box

Name of repeated measurement.		
Define the calculated total length of the measurement.		
Define the individual counting time of a repeated measurement per well.		
Define the cycle time.		
Number of repeats per well (automatically calculated from the previous data and the selected number of wells)		
Enter a value between 0 and 65 535. This range corresponds to 0 to 75 Watts and is divided in digital steps.		
The lamp energy ought to be set in a way that the full dy- namic range (~20 Mio CPS) is used. This setting is depend- ent on various parameters incl. max. concentration of the dye, quantum efficiency of the dye, transmission properties of the filters, etc.		
The halogen lamp is stabilized by a control circuitry. De- pendent on wavelength and selected filtes this can limit the		



	maximum usable lamp energy. If the lamp energy is set too high you will get the error message " <i>Halogen Lamp Stabili-</i> <i>sation Error</i> ". The action to take is to decrease the lamp energy settings.			
Excitation Filter	Select the excitation filter. The pre-defined filter with name and position in the filter slide is displayed.			
Emission Filter	Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed.			
Counter position	Select if a fluorescence measurement should take place witht the top or bottom optics.			
	Note: You need to manually switch the optics for top or bottom reading prior to measurement!			
Operation Mode	This function can be used in the plate or in the well or the by strip mode.			
	Repeated operation allows optimized execution of kinetics measurements over longer periods of time.			



8.5.6 Definition of Evaluation Parameters in Child Windows

Evaluation parameters are selected using items on the **navigation bar** or via **tool buttons** and defined on the respective matrices. Check the following matrices anyway and, if necessary, edit the settings.

You may add more matrixes in each menu item (via **Options** | **Matrix...**) and define parameters for each matrix for measurement and evaluation (by well, by row or column or for the entire microplate).

To edit matrix names, double-click on the respective tab. For more information please refer to the *Mikrowin 2000* User Guide.

Child windows

Data child window



The data supplied by the reader unit are entered on the $1^{\rm st}$ matrix named Measurement Data .

Dependent on the measurment type (multiple labels or kinetics) you have options to select the respective set of data to be displayed.

ions	Installation	<u>W</u> indow <u>H</u> e	elp							
Prin	nt Exp	ort Da	ka Tem	plate Calcu	ulation Res	sults St	atistics Gra	3 aphics		
n AC	1:	Unit	Active	Plate : Meas	.#1 at 0	•	Active Lat	pel: mP		-
	2	3	4	5	6	7	8	S Plane (c	(no:	11
7	552	583	570	575	571	580	571	P Plane (c S Plane (r P Plane (r 550	ion) aw] aw] aro	58

Figure 8-30: Data window

You may have a **Sample Identifier** added for each well and for a specific run via a worklist file (in text format) that has to be present in the directory **Program Files\Mikrowin 2000\SampleID**. This function is available in Advanced versions only.

Error Information will be entered automatically in case a calculation error has occurred.

In the **Dilution Factor** tab dilution factors for each individual well may be entered. This function is available in Advanced versions only.


Template child window



Here you may change the partition of the sample matrix and define controls and standards. The result matrices will be partitioned and values can be displayed accordingly.

Any selection and combination of replicates can be assigned to the wells of one plate in the **Partition** tab.

Q Read	Print	Export	ata	[Ter	mplate	Calcula	tion	Results	Statistic	s Gra	3 iphics
late Partitio	on : Six	fold horizontal	•								
#	1 Dup Trip Qua Sixf Eigl Rov Dup Trip	licates horizontal licates horizontal adruplicates horizontal old horizontal htfold horizontal w olicates vertical licates vertical		4		5	6		7	8	9
c											

Figure 8-31: Partition tab

In the **Groups** tab up to 12 groups can be assigned in case multiple standard curves are used, e.g. in IC50 determination assays

The **Overlay** tab enables entering unique names for each of the wells. The names and descriptions of these wells (the overlay) can be put onto a calculation matrix by the formula *OVL*, e.g. for an export of the data together with the sample description.

You can define wells to function as **Controls** which may be used for pure control purposes but also far assay validation and calculations.

ite	1											
N	Instrum	nent Opt	ions <u>I</u> nsta	llation <u>W</u> indow	Help							
	Q Rea) d	Print	Export	🔹 Data	Tem	plate Calc	ulation Res	sults Sta	tistics Gra	3 phics	
1	Contro	ol Type :	💻 Blank	: (BC)	•							
	#	1	Positin Negal	ve Control (PC) tive Control (NC)	^	4	5	6	7	8	9	
	А	Sample	Blank Bo St A Cor	(BC) andard (BO) htrol (AC)	~		Sample	Sample	Sample	Sample	Sample	s
	в		Sample	Sampl	e s	Sample					Sa	mple
	с		San	nple			Sar	nple	Sample	Sample	Sample	s
			1		_		-					

Figure 8-32: Controls tab



The source matrix can be defined individually for each control in the **Options | Controls** dialog box:

Options Controls		X
Control Calculation Valid Control Type	Hation Control History	
	Calculation Type Average	

Figure 8-33: Options | Controls dialog

In the **Standards** tab the concentration of the standards of the standard curve are entered.

ıt	e]										
I	I <u>n</u> strum	nent <u>O</u> ptions	<u>I</u> nstallation	<u>W</u> indow <u>H</u> e	lp						
	(O Rea	d Prir	nt Exp	ort Da	ta Tem	plate Calcu	ulation Re	sults Stat	tistics Gra	3 aphics	
-	Stand	lard Concentra	tion : 1000	•	Dilution F	actor: 1:3	-				
	#	1	2	3	4	5	6	7	8	9	
	A	1000.0000	317.0000	100.0000	32.0000	10.0000	3.2000	1.0000	0.3200	0.1000	- (
	В										

Figure 8-34: Standards tab

The source matrix and the algorithm as well as some fitting options need be defined for the standards in the **Options | Curve-Fit** dialog box:

Options CurveFit	×				
General Intercept View Additions					
Source Matrix					
Average Reader					
Algorithm					
Polynomial Regression Options					
XY-Scale XY-Units					
Y: log. Y: Counts					
X: log. 💌 X: Conc. 💌					
OK Abbrechen Übernehmen Hilfe					

Figure 8-35: Options | CurveFit dialog



Calculation child window

 Similar to spreadsheet software calculation formula can be assigned for individual wells, groups of wells or the entire plate. For each calculation a new matrix must be added. In total 5 matrices (Lite version) or 16 matrices (Advanced versions) may be used.

Pre-set parameter files do already contain calculation matrices but matrices may be erased or additional ones may be added. Any formula may be used for measurement data and results. You can type a formula directly into the calculation formula text box or select it from the **Add Formula** dialog box (see below).

A formula can be defined for individual wells, columns or rows or for the entire plate.

Note: To be able to visualize the readings in the result window, to use them for calculation and to be able to to export them via the matrix and line export drivers you need to assign the readings to respective matrices.

For a measurement with a single readout the trem **MEA** is to be used in one of the matrices.



Figure 8-36: Add Formula: MEA

In case a measurement contains multiple readings (= labels) the terms **LB1** through **LB5** must be used.

Add Formula			
E- Kinetics pr	ogram variables	~	ОК
LB1	Measurement Data of Label 1		Cancel
LB2	Measurement Data of Label 2		
LB3	Measurement Data of Label 3		Help
LB4	Measurement Data of Label 4		
185	Measurement Data of Label 5		

Figure 8-37: Add Formula: LB1 through LB5

The detector has and **overload detection** function to prevent the PMT from damage by high levels of light. Mikrowin supports this by displaying the expression **Overload** instead of a value. Instead of the the expressions **MEA** or **LB.** in the clculation matrices one has to use the threshold function: **TRH (MEA)** or **TRH(LB.)** respectively.

The threshold level itself and the expression to be displayed are set in the **Options | Threshold** dialogue (Type exactly: **2147483646**).



Options Threshold	
Threshold Set 1 Threshold Set 2 Threshold Set 3 Flag / Color / Threshold	Group Group No. 1
OK Abbrechen	Übernehmen Hilfe

Figure 8-38: Threshold dialog box: settings for Overload flagging

Select formula:

Select the desired tab in the Calculation child window and click on Add Formula. The Add Formula dialog box opens.

A	dd Formula			
		^		ок
	■ Kinetics program variables			Cancel
	Common statistic functions using plate partition Statistic functions using normal partition			Help
	Statistic functions using from a partition The Statistic functions using free partition			nop
	E Statistic functions using control overlay			
	Threshold calculation functions			
	E- Curvefit calculation functions			
	Kinetic calculation functions Multiplate calculation functions			
	Agglutination calculation functions			
	Special calculation functions			
	Text output functions Project provide the sections	_		
	Here Basic mathematical runctions Fer Control variables			
	Europeit variables	~		

Figure 8-39: Add Formula dialog box

- Click on the plus sign (+) of the desired formula type to display the list of available formulas.
- □ Select the formula you want from the formula list and click <**OK**>. The formula is entered into the **Calculation formula**



of Position... text box. Please consult the respective chapter in the *Mikrowin 2000* User Guide.

Calculation Formula of Position A02 :	MEA

Figure 8-40: Calculation formula text box

D Define the plate area to which the formula is to be applied:

For the entire plate: Click on button **#**.

For a column or row: Click on the respective **column** or **row button**.

For a single well: First select the well, then select the formula and place the cursor into the formula text box. Then hit the **<Return>** key.



Results child window



The displayed matrices are identical with those defined in the **Calculation** child window. Here, the data and calculated values are displayed. The number of the matrices are used for the selection in the export drivers to create a certain set of data for the export file (see chapter 8.2).

8.5.7 Definition of Export Parameters

You have to define export parameters if you want to export data to an ASCII or Excel file. Prerequisite is that the export driver(s) has/have been installed and set up accordingly (see chapter 8.2).

Please refer to chapter 8.3.2 to define automatic data export after each measurement.

Please see chapter 8.3.1 to perform manual data export.

8.5.8 Saving Parameter Files

Select **File | Save** to save the active parameter file. Select **File | Save As** to save the file under a new name or to create a new parameter file ("**Untitled.par**"). See also chapter 8.5.2.



8.6 Measurement and Evaluation

1. Prepare instrument

The user can then select wether the samples in a microplate are read from above (= top reading) or from below (= bottom reading) the wells. The type of counting that will be used has to be selected with the **Counter Position** setting: **Top** or **Bottom**



Figure 8-41: LB 970 Twinkle – lever for top / bottom selection

Note: In addition to the selection in the software, the counter position has to be selected manually prior to the measurement start!

Load the plate carrier by selecting **Load Plate** in the **Instrument** menu. Open the lid at the front panel and push/pull metal lever until it clicks into place:

Push	change	to top	reading
------	--------	--------	---------

Pull change to bottom reading

2. Open and edit parameter file

- □ Open parameter file (*.par) for measurement (**File | Open**, see chapter 8.5.2.
- Select Options | Read to edit well selection and measurement sequence.
- ☐ You may edit the matrices (Data, Template, Calculation, Results).

3. Start measurement





To start the measurement sequence click on the **Read** button in the main window. A status bar is displayed below the matrix with text boxes and buttons for the measurement:



Figure 8-42: Status bar after start of a measurement

The <u>name</u> of the parameter file used is displayed in the bottom row (right).

Click is to open the parameter file used and edit the default settings, if necessary. The **Options Read** dialog box opens. Click on **<Options>** to open the **Options** dialog box to view and edit the well selection and the measurement sequence.

After clicking the **Start**> button (see below) a prompt will appear whether the changes made are to be saved. Selecting **Yes**> will save the changes to the parameter file. By clicking **No**> the changes will remain temporary only for the follwing measurement.

File name: Enter a name for the data file. It will get the extension ***.dat**. The extension cannot be changed.

The data will be stored in the defaulted directory defined in the **Installation | Settings** dialog. If another directory is preferred

you may click the browse button _____ to select another directory.

Click **<Start>** to start the measurement provided you have entered a file name for the data.

As soon as you have clicked on **Start**> the plate slide moves out of the instrument. On the screen you see the prompt to load a microplate.

Load plate to continue					
ОК	Abbrechen				

Figure 8-43: Loading a microplate for measurement

Load microplate (with A1 at rear left) and click <OK>.

The plate is moved into the instrument and the pre-defined measurement sequence is carried out.

During measurement, you may view the already available data and calculations on the matrices of the **Results** window (Figure 8-44).



Upon completion of the measurement the plate stays within the instrument. To retrieve the plate hit **<Unload Plate>** in the **Instrument** menu.

If you have pre-selected the item **Batch**, the system expects the next microplate to be loaed which will be measured using the same parameters. The data will be stored to the same file.



Figure 8-44: On-line presentation of measured data

Results can also be viewed in the **Graphics** window.



9. Maintenance

9.1 Cleaning the Instrument

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!

Cleaning the inside of the instrument

Before opening the instrument, turn it off and disconnect it from power supply!

Open the screws located on the bottom of the instrument to clean the instrument inside. Then raise the cover.

Always keep the plate slide as well as the inside of the instrument (e.g. below the plate slide) clean. Wipe off any dirt using a damp cloth or optical grade tissue. Use cotton buds for corners. Remove dirt fairly quickly so it does not get dry and may not have any adverse effect on the movement of the plate slide.

9.2 Fuse Replacement

The fuse is located on the instrument rear panel in a black fuse holder to the left of the mains switch.

- **T** Turn instrument off and pull power cord.
- Insert screwdriver or similar tool into fuse holder recess and lift it out by applying slight pressure.
- Take faulty fuses out.
- □ Insert new fuses. Use only the fuse type specified!
- Insert fuse holder again.

9.3 Changing Halogen Lamp

The halogen lamp is located on the right hand side of the instrument.

- □ Turn instrument off and pull power cord.
- Remove lamp cover by pulling.



- Unscrew the panel holding the lamp and withdraw it through the hole in the body of the fluorometer.
- **T**ake out halogen lamp carefully out and disconnect.
- □ Connect replacement lamp. DO NOT TOUCH WITH FINGERS. USE GLOVES.
- Insert new halogen lamp.
- □ Insert the panel holding the lamp.
- □ Attach lamp cover again.



Figure 9-1: Right instrument side: fan and excitation halogen lamp; cover removed

9.4 Preparations for Transport

The **following safety provisions have to be taken** to transport or ship the instrument:

- **I** Turn instrument off and disconnect it from mains.
- □ Remove excitation filter slide.
- **D** Remove emission filter slide.
- Insert transport safety device into the rails of the emission filter compartment.
- Attach the plate carrier transportation lock by tightneing both screws and moving the plate carrier into the instrument as indicated on the label of the lock.





Figure 9-2: Plate carrier with transportation lock

- □ For shipping you must use the original transportation case.
- **D** Put the instrument onto the bottom styrofoam part.
- □ Put the top styrofoam part on top of the instrument.
- □ Tape shipping carton tightly.



10. Technical Data

Operating voltage	100 – 240 V
	Fluctuations must not exceed ± 10 %; max voltage 253 V
Frequency	50 / 60 Hz
Power consumption	150 VA max.
Certifications	CE, UL, CSA
Safety standards	EN 61010-1, EN 61326-1, EN 61000-3-2, EN 61000-3-3 ; EN 61326
	IEC 1010-1
Installation Category	П
Altitude	Max. 2000 m above sea level
Pollution Degree	2
Temperature range	Storage: 0° - 40°C
	Operation: 15° - 40°C
Humidity	10 – 85% no condensation
	Maximum relative humidity of 80 % for temperature up to 31 °C decreasing
	linearily to 50 % at 40 °C
Dimensions	465 x 406 x 280 mm (W x D x H)
Weight	~ 25 kg
Detection unit	Low-noise photomultiplier, single photon counting technology (9780B), 340 –
	650 nm
Reading technologies	Fluorescence (top), fluorescence (bottom), FRET
Excitation source	Halogen lamp, 75 W, 340 to 700 nm
Excitation filters	Ø 15 mm or 12.7 mm with adapter
— • • •	355 nm, 485 nm
Emission filters	Ø 25.4 mm
	460 nm, 535 nm
Performance & Sensi-	Fluorescence: < 1 fmol FITC
	> 6 decades (PMT based detection)
Dynamic range	microplates with 6 to 384 wells (external dimensions: 86.0 x 128.2 mm (W/ x
Fiale Ionnals	L): Petri dish. Terasaki plates, filters: height tolerance: 14.0 – 25.0 mm
Temperature control	Optional: +5°C above room temperature to 42°C
Operation modes	Integral measurement 0.05 – 600 seconds
	Kinetics measurement (total length up to 24 h)
	Repeated measurement (total length up to 7 days)
	Plate repeats (up to 50,000)
	Scanning (up to 10,000 single data points)
	Shaking (3 modes, variable amplitude and speed)
	Dual fluorescence assays: e.g. FRET
	Delay (up to 600 second)
Data evaluation	
Sample parameters	Free plate loading (samples, standards, controls)



	Allocation of sample identifiers
	GLP documentation, e.g. lot number
	Test kit validation formulas
Evaluation	Qualitative cut-off determination with up to 3 categories
	Kinetics
	Linking of different types of evaluation
	Free input of calculation formulas
	Standard curve calculation (only complete version!)
Result display	Presentation in multi-matrix format or list format
	Graphical presentation of measured values
	Integrated statistics functions
Export	Output of all raw data and calculations (manual or automatic) in matrix or list
	format
	Output as a file (network connection possible) or to printer
Robotic Integration	Optional
Stacker	Optional LB 931 with 25 or 50 plate capacity
Interface	RS 232, 9 pole
Operating system	Win98, Win2000, WinNT, Win XP
PC requirements	Pentium processor, 500 MHz (or better), CD ROM drive, display 1024x768
	(or better), serial port, USB port
Software	Mikrowin 2000 Lite
	Optional: Mikrowin 2000 Advanced



11. Appendix

11.1 Details of selected Basic Parameter Files

FRET measurement

Well selection

All wells selected for measurement

Options			181										E
Samples Plate Plate Plate Mea	Measu e param e type: e Mode isureme	neter neter :: ent Ord	t R er: •	arthold Meas By Ro	96 uremer	ıt 🖸	<mark>inj. 1</mark> By Col	Umns	2	<mark>- Inj</mark> Mei	ander	.] Inj. 4	
	1	2	3	4	5	6	7	8	9	10	11	12	1
Α	\oplus	\oplus	\oplus	\oplus	\oplus	\oplus	\oplus	\oplus	\oplus	\oplus	\oplus	\oplus	
В	\oplus	\oplus	\oplus	\bigoplus	\oplus	\bigoplus	\bigoplus	\oplus	\oplus	\oplus		\oplus	
С	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\oplus	\oplus	\oplus	\oplus	
D	\square	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\bigoplus	\oplus	Ê	\square		
E	\square	\square	(\square)	\mathbb{H}	\square	\square	(\square)	\square	\oplus				
F		\square	\mathbb{A}	\mathbb{P}	\mathbb{H}	\mathbb{Z}	\mathbb{A}	\mathbb{H}	(T)	\blacksquare	\blacksquare		
G	X	\square	\mathbb{P}	\mathbb{P}	\mathbb{P}	\mathbb{P}	\mathbb{P}	\mathbb{P}	Ť.	¥.	\mathbb{T}		
н	Ð	U	\square	\square	U	U	\square	\square	U	U	U		
								OK		Abb	recher	Оь	ernehmer

Measurement sequence

2 readings with different emission filters set

ions	
Measurement Measurement Sequence: Measurement Sequence:	Operations: Operation Image: Specific constraints Image: Specific c
Operation Mode Batch Plate F Temperature Control Temperature Keep Plate after Meas	Repeats: 1 25 °Celsius urement
	OK Abbrechen Obernehr



LB 970 Twinkle

Appendix



Calculation

Sample: well position *POS* FRET1: raw data of 1st measurement *TRH(LB1)* FRET2: raw data of 2nd measurement *TRH(LB2)* Ratio: measurement 2/measurement 1 *MA3/MA2*

Fluorescence Scanning measurement

Well selection	All we	ells selected for measurement
Options		X
Samples Measurement		1
Plate parameter Plate type: Costar 24	3524	T
Plate Mode: Measurement	irement 🖸 Inj. 1	1 1 Inj. 4
Measurement Order: (By Ro	ws C By Columns 🥅 Me	leander
	1 1 1	1 1
1 2	3 4 5	6
A (+)(+)	X + X + X +	
в		
C		
	OK Ab	bbrechen Übernehmen

Measurement sequence

6 by 6 scanning, point displacement 3.06 mm (distance between scanning points), round mode (corner points will be skipped)





Calculation

Sample: well position POS

Average: average of all scanning values per well SAVE(MEA) Maximum: maximum value of all scanning values per well SMAX(MEA)

- Minumum: minimum value of all scanning values per well SMIN(MEA)
- Total: total of all scanning values per well SNUM(MEA) * SAVE(MEA)

Graphics

colour display: Red = high signal Double click opens zoomed view Blue = low signal





Appendix

Curve Fitting

Well selection	All wells selected for measurement				
Measurement sequence	none				
Template	Partition: dupli	cates ho	orizontal		
	Overlay:	A1/A2 B1-F2 others	Blank Standard Sample		
	Controls:	A1/A2	Blank (BC)		
	Standards:	B1/B2 C1/C2 D1/D2 E1/E2 F1/F2	10,000 2,000 400 80 16		
MikroWin - [Template]					

New Open	Rea	d Print Ex pl Type : Blank (BC)	port Data Ten	plate Calculation Re	sults Statistics Gr	aphics	
Measurement Data	#	1 2	3 4	5 6	7 8	9 10	11 12
Error Information Dilution Factor Template Partition	A	Blank	Sample	Sample	Sample	Sample	Sample
- Groups - Overlay - Controls - Standards	В	Standard	Sample	Sample	Sample	Sample	Sample
 Calculation Map Reader Average 	С	Standard	Sample	Sample	Sample	Sample	Sample
Blank corr Conc Result Map	D	Standard	Sample	Sample	Sample	Sample	Sample
Reader Average Blank corr Conc	E	Standard	Sample	Sample	Sample	Sample	Sample
Statistics General Statistics User Statistics 1	F	Standard	Sample	Sample	Sample	Sample	Sample
User Statistics 3 Graphics Bar Graphics	G	Sample	Sample	Sample	Sample	Sample	Sample
- Curve Fit - Kinetics - Scanning Sclootion	н	Sample	Sample	Sample	Sample	Sample	Sample



	Q					3	
Data	Standa	rd Concentration : Unused	Dilution F	actor: 1:5 💌	suits statistics Gra	priics	
Measurement Data Sample Identifier	#	1 2	3 4	5 6	7 8	9 10	11 12
Error Information Dilution Factor Template Partition	A	Unused	Unused	Unused	Unused	Unused	Unused
- Groups Overlay - Controls - Standards	в	10000.000	Unused	Unused	Unused	Unused	Unused
Calculation Map Reader Average	с	2000.0000	Unused	Unused	Unused	Unused	Unused
Blank corr Conc Result Mao	D	400.0000	Unused	Unused	Unused	Unused	Unused
- Reader - Average - Blank corr - Conc	E	80.0000	Unused	Unused	Unused	Unused	Unused
Statistics General Statistics User Statistics 1	F	16.0000	Unused	Unused	Unused	Unused	Unused
User Statistics 3 Graphics Bar Graphics	G	Unused	Unused	Unused	Unused	Unused	Unused
Curve Fit	н	Unused	Unused	Unused	Unused	Unused	Unused

Calculation

MAP: entries of overlay **OVL**

Reader: reader values TRH(MEA)
Average: calculation of replicate averages AVE(MA2)
Blank corr: blank corr. of averaged reader values MA3 - BC
Conc: calculation of concentration based on blank corrected values according to standard curve FIT(MA4)

Options

Options Controls
Control Calculation Validation Control History Control Type Blank (BC) Control Description Control Description
Blank Source Matrix Reader
Calculation Type Average
OK Abbrechen Obernehmen Hilfe



Options CurveFit		
General Intercept View Additions		
Source Matrix Blank cor General Setup Algorithm		
Regression		
XY-Scale XY-Units	Curvefit Regression Options	×
Y: log. Y: Counts Y X: log. X: Conc. V OK Abbrechen Obernehmen Hilfe	Regression type OK C lin. X-Data C lin. Y-Data C liog. X-Data C log. Y-Data	

- Note: The curve fitting function can be used with the Advanced versions of Mikrowin 2000 only.
- Note: The blank correction done as indicated (Max BC) does only work if **AutoBlankSubstract** is disabled in the **Special Settings** dialog.



11.2 Customer reply Form

Date:		CPR Number:				
Twinkle LB 970 Problem report						
🗆 Ms / 🗆 Mr	Name:					
	Phone:	Fax:				
	E-mail:					
	Company:					
Twinkle LB 97	70 □ stand alone □ stacker	□ BUTLER □	robot integration			
	Twinkle Embedded Software	e version:				
	Twinkle Driver Software vers	sion:				
	Twinkle serial number:					
	Mikrowin Software version a	nd release version	on:			
	Other equipment attached S	oftware & Hardwa	are version			
Computer type	e (CPU):	Windows vers	sion:			
Approximate t	ime problem occurred (Windo	ws clock):				
Error Message	e:					
Description of	the problem:					



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.



11.3 Confirmation on Decontamination

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 !)

·	., ,	
instru	iment / component:	serial no.:
instru	ment or component has come into con	tact with:
[]	radioactive substances	means of decontamination applied:
	Isotope(s):	
r 1	chemical reagents	means of decontamination applied:
	specify:	
	R and S rules:	
r 1	biological material	means of decontamination applied:
	specifiv:	
r 1	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 m	entioned substances / reagents / agents
[]	I hereby confirm that the instrument or	component specified above was
	decontaminated / cleansed using the a	appropriate method
date:		signature:
nom		addrosa
name		
4:41 o i		
uue.		
pnon	e:	
fax:		7-40.12 – 31531/03



11.4 Index

A

Activation Mikrowin 2000	16
Add formula	106
Additional Export Drivers	52
Aspiration pump	32
AuroBlankSubtract	122
В	
Degie peremeter files	26 40
Dasic parameter mes	20, 49
Batterne	87
Battery	2
Beeps	1
Blank subtraction mode	45
Boot instrument	39
Bottom counting selection	31, 109
Bottom Reading	55
By plate	90
By strip	90
By wells	90
С	
Calculation	105
Child windows	48, 101
Cleaning filters	33, 34, 79
Cleaning the instrument	112
ComPort	55
Connecting	41
Connections	37
Consignes de Sécurité	5
Controls	102
Curve fitting	120
D	120
Data	101
Data export	101
Automatic	68
Manual	66
Data file	18
desimal delimiter setting	48
default directories	43
default massurement unit	43
Delay operation	43
Delay operation	91
Dilution Factor	101
Dimensions	40
	15, 16
Drag&Drop	/4, /8
Driver setup	53
Driver software	18

E

embed parameter file	45
Emission filter	19, 29, 76
Clean	79
Replace	79
Emission filter slide	34
Emission filter slidel	76
Emission filter wheel	39
Error Information	101
Evaluation	28, 109
Evaluation parameters	101
Excel file	24
Excitation filter	19, 29, 72
Clean	75
Replace	75
Excitation filter slide	33, 39
Excitation halogen lamp	36
Export	66
Export driver	24, 57
Export parameters	24
Export Parameters	
Definition	108
Export setup	38
E E	
ſ	
Fan	36
File Export	38, 66
File Export Setup	66, 68
File Open	81
File Save	82
Filter name input	73, 77
Fluorescence Kinetics oper	ation 95
Fluorescence Measurement	47
Fluorescence measurement	operation 93
Fluorescence measurement	s 29
Fluorescence Reading para	meters 93
Fluorescence Repeated ope	ration 99
Fluorescence Scanning mea	asurement 118
Fluorescence Scanning ope	ration 97
FRET	29
FRET measurement	117
Fuse	37
Fuse replacement	112
G	
Catting stants 1	10
Groups	13
Groups	102



Н		Mi
Halogen lamp - changing	112	Mi
hard lock	15, 16, 42	Mi
T		Mı
1		N
IC50	102	Na
Installation Driver	53	No
Installation Mikrowin 2000	15	110
Instrument Boot Instrument	71	0
Instrument Emission Filter Slide	70, 76	On
Instrument Excitation Filter Slide	70, 72	On
Instrument Load Plate	69	On
Instrument Plate Editor	71	Op
Instrument Unload Plate	69	Op
Instrument control	69	Ov
Instrument Driver	20	
Κ		Р
Kinetics	95	Pa
L		
Lamp Energy Fluorescence	93 95 97 99	:
LED	1	1
Line Export Driver	60	Pa
link parameter file	45	Pa
Load plate	39	pas
log system	45	PC
log system level	45	PC
		Per
M		Ph
Main window	48	Pla
Mains plug	37	Pla
Mains switch	37	Pla
Maintenance	112	Pla
Matrix Export Driver	58	PN
Matrix system	50	Po
Measurement	28, 109	Po
Measurement Data	101	Pre
Measurement sequence	22	Pre
Definition	86	Pri
Measurement tab	86	Pro
Menu		Q
File	38	Ou
Installation	39	Qu
Instrument	39	R
Options	39	Ra
Read	38	Ra
View	38	Do

48

38, 51





Menu bar

Menu overview

Repeat mode	87	Start button	110
Repeated	99	Start Measurement	110
replicate definition	102	Status bar	48
Results	108	System description	29
S		Τ	
Safety instructions	1	Technical data	115
Sample description	102	Temperature control	22, 55, 88
Sample ID	101	Template	102
Sample name	102	Tool bar	48
Samples tab	21, 84	Top / Bottom Reading	31
Scanning	97	Top counting selection	31, 109
Scanning measurement	118	Transport safety devices	13
Sensitivity	35	Transport safety locks	41
Serial port	37	Typographical conventions	1
Setup site	40	17	
Shake operation	92	U	
Sicherheitshinweise	8	Unload plate	39
Software		Unpacking	40
Functions	48	W	
Installation	43	· ·	
Structure and operation	48	Well selection	21, 83, 85
Space required	40	wite protection parameter	45
special settings	45	write protection data	45
Special spare parts	12		

