applications guide to microplate systems

GETTING THE MOST FROM YOUR MOLECULAR DEVICES MICROPLATE SYSTEMS

typical applications for Molecular Devices microplate readers

	absorbance readers						fluorescence readers	luminescence readers
assay type	SpectraMax® Plus^{384}	SpectraMax® 190	SpectraMax® 340PC^{384}	VersaMax™	VMax®	EMax®	Gemini XS	LMax™
ELISA/IMMUNOASSAYS	●	●	●	●	●	●	●	●
PROTEIN QUANTITATION	●							
UV (280)	●	●						
Bradford, BCA, Lowry	●	●	●	●	●	●		
NanoOrange™, CBQCA							●	
NUCLEIC ACID QUANTITATION								
UV (260)	●	●						
PicoGreen™, OliGreen™, RiboGreen™							●	
DNAQuant™								●
GENOMICS								
SNP							●	
molecular beacons							●	
REPORTER GENES								
alkaline phosphatase, ß-galactosidase	●	●	●	●	●	●		
ß-lactamase							●	
luciferase								●
GFP							●	
CELL VIABILITY/PROLIFERATION/DEATH								
MTT, XTT, crystal violet	●	●	●	●	●	●		
LDH	●	●	●					
alamar blue	●	●	●	●	●	●	●	
calcein AM, CyQuant™ GR							●	
SYTOX Green, ethdium homodimer							●	
CHEMICAL MEASUREMENTS								
ATP								●
reactive oxygen species	●	●	●	●	●	●		●
CELL SIGNALLING								
aequorin Ca^{++}								●
ENZYME ACTIVITY ASSAYS								
proteases	●	●	●	●	●		●	
NADH	●	●	●					
GST	●	●	●	●	●	●		
phosphatases/kinases	●	●	●	●	●	●		

integrated time saving better results cost saving flexible

applications guide to microplate systems

volume 2

Molecular Devices

preface

Molecular Devices Corporation is pleased to publish this new edition of our Applications Guide. This publication represents a sampling of scientific articles published by our customers and our in-house scientists in many areas of life sciences, highlighting microplate assay methods and the use of Molecular Devices' microplate readers. We provide abstracts and brief methods for each of the articles cited in the guide. For full-length copies of Molecular Devices' Application Notes, please visit our web site at: *www.moleculardevices.com/pages/literature.*

Molecular Devices is committed to providing instrumentation and applications to increase microassay capabilities for all laboratories conducting life sciences research.

Please contact Molecular Devices at info@moldev.com with any comments concerning this guide or if you have any papers that you would like to submit to subsequent editions of the guide.

Thank you for your interest in Molecular Devices Corporation. We wish you continued success in your research endeavors.

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Article titles in the Table of Contents are linked to the articles in the guide. The Acrobat Bookmarks palette also contains links to the chapters and major chapter sections. Additionally, Molecular Devices' microplate systems application notes are included on the CD as PDF files. Links to these PDF files have been placed just below the article titles. The links look like this:

FULL TEXT →

If you view the document in Full Screen mode, you must press the escape key to return to displaying Acrobat's bookmarks and navigation buttons.

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cell biology

Bacterial cell growth: absorbance

The ssu locus plays a key role in organosulfur metabolism in *Pseudomonas putida* S-313

J. Bacteriol. 182: 2869–78 (2000).

Antje Kahnert[1], Paul Vermeij[1], Claudia Wietek[1], Peter James[2], Thomas Leisinger[1], and Michael A. Kertesz[1,3].

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[2] Protein Chemistry Laboratory, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich, Switzerland.

[3] School of Biological Sciences, University of Manchester, Manchester M13 9PT, United Kingdom.

Methods: cell growth assays. Cell growth experiments were performed at 30 °C in microtiter plates containing 150 µL of culture, using a SpectraMax Plus microtiter plate reader with SoftMax Pro software (Molecular Devices), as previously described. The turbidity at 650 nm was measured every 5 minutes, and the plate was shaken for a period of 30 seconds before each measurement.

Bacterial cell labeling: fluorescence

Field-scale evaluation of CFDA/SE staining coupled with multiple detection methods for assessing the transport of bacteria *in situ*

FEMS Microbiology Ecology 37: 55–66 (2001).

Mark E. Fuller[1], Brian J. Mailloux[2], Pengfei Zhang[3], Sheryl H. Streger[1], James A. Hall[2], Simon N. Vainberg[1], Andrew J. Beavis[4], William P. Johnson[3], Tullis C. Onstott[2] and Mary F. DeFlaun[1].

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[4] Flow Cytometry Core Facility, Department of Molecular Biology, Princeton University, Princeton NJ 08544.

Background: Previous bacterial transport studies have utilized fluorophores which have been shown to adversely affect the physiology of stained cells. This research was undertaken to identify alternative fluorescent stains that do not adversely affect the transport or viability of bacteria. 5-(and 6-) carboxyfluorescein diacetate, succinimidyl ester (CFDA/SE) efficiently stained DA001 without causing undesirable effects on cell adhesion or viability. Members of many other gram-negative and gram-positive bacterial genera were also effectively stained with CFDA/SE. More than 95% of CFDA/SE stained *Comamonas* sp. strain DA001 cells incubated in artificial groundwater (under no-growth conditions) remained fluorescent for at least twenty-eight days as determined by epifluorescent microscopy and flow cytometry. The bright, yellow-green cells were readily distinguished from autofluorescing sediment particles by epifluorescence microscopy. A high throughput method using microplate spectrofluorometry was developed, which had a detection limit of mid-10^5 CFDA/SE-stained cells/mL; the detection limit for flow cytometry was on the order of 1000 cells/mL. The

results of laboratory-scale bacterial transport experiments performed with intact sediment cores and nondividing DA001 cells revealed good agreement between aqueous cell concentrations determined using the microplate assay and aqueous cell concentrations determined by other enumeration methods. This research indicates that CFDA/SE is very efficient for labeling cells for bacterial transport experiments and that it may be useful for other microbial ecology research as well.

Methods: bacterial cell analysis. Enumeration of CFDA/SE-stained cells was performed using a Molecular Devices Gemini (Molecular Devices Corp.) Thirty-five microliters of sample was pipetted into black OptiPlates (Packard Instrument Company), and the fluorescence (Ex 495 nm, Em 538 nm, cutoff 530 nm) of each sample was measured. A standard curve relating the stained cell population (based on direct counts using epifluorescent microscopy of the stained cells prior to transport to the field) and fluorescence was used to convert sample fluorescence to CFDA/SE-stained cells/mL. The limit of detection in the field was 7 x 10^4 stained cells/mL.

Animal cell growth & viability: absorbance

MTT reduction — a tetrazolium-based colorimetric assay for cell survival and proliferation

FULL TEXT →

Molecular Devices MAXline Application Note #5.

Joan M. Chapdelaine.

Pharmakon Research International, Inc., Waverly, PA, 18471.

Summary. The MTT assay is a quantitative colorimetric assay for mammalian cell survival and cell proliferation. It depends on the reduction of the tetrazolium salt MTT (3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide) by the mitochondrial dehydrogenase of viable cells to form a blue formazan product. The assay measures cell respiration, and the amount of formazan produced is proportional to the number of living cells present in culture. The assay has been shown to be a simple, rapid alternative to counting cells by dye inclusion/ exclusion, monitoring the release of ^{51}Cr from lysed cells, or the incorporation of [^{3}H]-thymidine into cellular DNA. The MTT assay has been used with a growing number of cell types including primary cultured cells as well as established cell lines. This colorimetric microplate assay is cost effective because of the number of tests which can be performed at one time without the problem of radioisotope and contaminated materials disposal.

Methods: MTT assay. The plates were read at 570 nm with a 630 nm reference to negate the effect of cell

debris and precipitated proteins which may be produced by the acidic alcohol addition.

A new quantitative nitroblue tetrazolium reduction assay based on kinetic colorimetry

J. Clin. Lab Anal. 4: 86–89 (1990).

Gabriel Virella, Tab Thompson, and Rebecca Haskill-Strowd.

Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC 29425 USA.

Authors report a new quantitative method to follow neutrophil stimulation. The kinetic, colorimetric assay follows the reduction of nitroblue tetrazolium (NBT) in a microplate. The assay is conducted along standard conditions in a microplate, and the color change corresponding to NBT reduction is monitored for 25 minutes at 490 nm. NBT reduction is shown to be clinically significant. NBT reduction values at 1 x 10^7 cells/mL in normal individuals ranged from 2.59 to 7.41 (4.73 ± 1.89) mOD/min. While patients presenting with symptoms suggestive of chronic granulomatous disease were 0.31 mOD/min. The authors report that this method is considerably simpler than any alternative method for the performance of quantitative NBT assays.

An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT

J. Immunol. Methods 142: 257–265 (1991).

Neal W. Roehm, George H. Rodgers, Stephen M. Hatfield, and Andrew L. Glasebrook.

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 USA.

Cell viability and proliferation by normal activated T cells and several cytokine dependent cell lines were evaluated using a new chromogenic tetrazolium salt, XTT (sodium 3´-[1-[(phenylamino)-carbonyl]-3, 4-tetrazolium]-bis (4-methoxy-6-nitro)benzene-sulfonic acid hydrate). Reduction of XTT by dehydrogenase enzymes of metabolically active cells yields a highly colored, water soluble formazan product obviating the need for crystal solubilization prior to absorbance measurements. The reduction of XTT by the murine cells examined was not particularly efficient. However, addition of electron coupling agents such as phenazine methosulfate (PMS) or menadione (MEN) potentiates the reduction of XTT. The combination of XTT/PMS generates higher formazan absorbance values as compared to values with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). XTT is read at 450 nm versus 570 nm for MTT and both use 650 nm as a reference. Authors report the use of XTT in colorimetric proliferation assays offers significant advantages over MTT, resulting from reduced assay time and sample handling, while offering equivalent sensitivity.

Animal cell death: absorbance

Identification of the discontinuous epitope in human complement protein C9 recognized by anti-melittin antibodies

J. Immunol. 143: 553–7 (1989).

Roney O. Laine and Alfred F. Esser.

Laboratory of Structural Biology, Department of Comparative and Experimental Pathology, and the Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610.

Methods: hemolytic assays. Inhibition of C9-mediated lysis of RBC by the anti-peptides was assayed with EAC1-7 as indicator cells prepared as described. Hemolysis was measured by monitoring turbidity at 650 nm in 96-well microtiter plates (Falcon Microtest III) in a VMax microplate reader (Molecular Devices Corp.) In each sample well 50 µL containing 2.5 x 10^7 EAC1-7 in GVB-M (5 mM Veronal (barbital), 145 mM NaCl, 0.1% (w/v) gelatin plus 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$ pH 7.4) and 70 fmol of purified C8 were incubated together at ~4 °C before addition of C9 or C9 preincubated with anti-peptide. Anti-peptide, anti-melittin or nonimmune IgG (30 nmol) was incubated with 70 pmol C9 for 15 minutes at room temperature before mixing with the EAC1-8 cells and adjusting the total well volume to 190 µL with GVB-M, if necessary. The microtiter plates were then incubated at 37 °C, and A_{650} was measured at predetermined time intervals in the microplate reader. The time required to lyse 50% of the cells (t50%) was used to compare the effects of different antibodies on C9-mediated lysis.

Animal cell proliferation: fluorescence

Endothelial cell surface F1-FO ATP synthase is active in ATP synthesis and is inhibited by angiostatin

Proc. Natl. Acad. Sci. USA 98: 6656–6661 (2001).

Tammy L. Moser[1], Daniel J. Kenan[1], Timothy A. Ashley[1], Julie A. Roy[1], Michael D. Goodman[1], Uma K. Misra[1], Dennis J. Cheek, and Salvatore V. Pizzo[1].

[1] Department of Pathology and Duke University School of Nursing, Duke University Medical Center, Durham, NC 27710.

Methods: cell proliferation assay. Cell density was measured after 24 hours by using the CyQUANT® Cell Proliferation Assay Kit (Molecular Probes) in the Gemini fluorescence microplate reader (Molecular Devices Corp.)

Using the CyQUANT Cell Proliferation Assay Kit in the *f*max microplate fluorometer

FULL TEXT →

Molecular Devices MAXline Application Note #23.

This application note details two methods for using the CyQUANT Cell Proliferation Assay Kit with the *f*max microplate fluorometer. In the first method, cellular proliferation is quantitated using a cell-based standard curve. In the second method, cellular proliferation is quantitated using RNAse treated cell samples and a DNA standard curve. The data in this appli-

cation note were obtained using an excitation wavelength of 485 nm and an emission wavelength of 538 nm. Excitation and emission filters of these wavelengths are included as standard equipment with the *f*max microplate fluorometer. Molecular Probes describes a dynamic range from 50 to at least 50,000 cells for the standard assay in a microplate format. The kit contains sufficient reagent for 1000 assays using a 200 µL-per-well volume. The data presented here are preliminary and do not represent a fully optimized assay.

LIVE/DEAD® Viability/Cytotoxicity Kit assay for animal cells using the Gemini XS fluorescence microplate reader

FULL TEXT →

Molecular Devices MAXline Application Note #43.

Anne T. Ferguson, Ph.D.

Methods: LIVE/DEAD Viability/Cytotoxicity assay. The LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes, cat# L-3224) was used with Chinese Hamster Ovary (CHO) cells in 96-well black microtiter plates with clear bottoms (Costar, cat# 3603). CHO cells were grown in Ham's F12 medium containing 10% FBS. Cells were washed three times with PBS and then treated for 20 minutes with 3 mL 0.526 mM EDTA in PBS. The detached cells were harvested and diluted in PBS. An aliquot was counted using a hemocytometer. The concentration of cells was adjusted to 2.5 x 10^5 cells/mL in PBS, which is equivalent to a starting concentration of 2.5 x 10^4 cells/100 µL. For preparation of dead cells, half of the cells were treated with 1:100 dilution of 10% Triton X-100 for 10 minutes. Both live and dead cells were serially diluted 1:3 in PBS to obtain a range of concentrations from 2.5 x 10^4 to 10^3 cells/100 µL. Cells were plated such that each column of the microplate had 8 replicates of a live or dead cell dilution. The negative control/background was 100 µL of PBS. A solution of 11.4 µM calcein AM (cal AM)/5.7 µM ethidium homodimer-1 (EthD-1) in PBS was made and 100 µL aliquots were added to each well of the microtiter plate, including the control wells. The final concentration of dyes in each well was 5.7 µM cal AM/2.85 µM EthD-1. Cells were incubated with the dyes for 30 minutes at 37 °C in a tissue culture incubator and then analyzed using the Gemini XS. The optimal instrument settings for cal AM-stained live cells were Ex 485 nm/Em 525 nm using a 515 nm emission cutoff filter and for EthD-1 stained dead cells were Ex 525 nm/Em 620 nm using a 590 nm emission cutoff filter.

Measurement of green fluorescent protein in the Gemini XS spectrofluorometer

FULL TEXT →

Simon Lydford[1] and Thomas Giller[2].

Molecular Devices MAXline Application Note #44.

1 Molecular Devices Limited.

2 Axovan Limited.

Methods: GFP assay. Human embryonic kidney 293 cells (HEK-293) were transiently transfected with a wild-type GFP expression plasmid (pS65T, Clontech, discontinued product; cytomegalovirus immediate early promoter drives expression of GFP) using Lipofectamine as per manufacturer's instructions (Gibco/Invitrogen, Cat. No. 18324-012). Cells were grown to confluence in 162 cm^2 flasks under standard cell-culture conditions. Fluorescence microscopy revealed an expression level in excess of 75%. The GFP-expressing cells were removed with trypsin, and the cells were washed with medium (MEM Alpha, Gibco-RBL Cat. No. 22571-020). The appropriate number of cells was seeded in alternate columns of a 96-well microplate (Costar, black walled, clear bottomed Cat. No. 3603) and diluted with mock-transfected cells to maintain a constant cell number of 200,000 cells per well. This resulted in a final number of 6,250 (column 12) to 200,000 (column 2) GFP-expressing cells per well in a volume of 100 µL. These cells were seeded in alternate columns 2, 4, 6, 8, 10, and 12. In contrast, columns 1, 3, 5, 7, 9 and 11 were seeded with 200,000 mock-transfected cells per well. Then the plate was returned to the incubator for 48 hours. Initially, excitation and emission scans were run to select the optimum excitation wavelength and emission wavelength/cutoff filter combination for the maximum signal/background ratio. The parameters selected for all subsequent experiments were: GFP; λEX – 472 nm, λEM – 512 nm with a 495 nm emission cutoff filter.

Lipid docking assay: fluorescence

Close is not enough: SNARE-dependent membrane fusion requires an active mechanism that transduces force to membrane anchors

J. Cell Biol. 150: 105–117 (2000).

James A. McNew[1], Thomas Weber[1], Francesco Parlati[1], Robert J. Johnston[1], Thomas J. Melia[1], Thomas H. Söllner[1], and James E. Rothman[1].

[1] Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021.

Methods: liposome docking assay. Adherence of the docked liposomes was performed for 1 hour at room temperature on a platform. Undocked v-SNARE–containing liposomes were removed by five 300 µL washes with PBS. The remaining docked, v-SNARE–derived rhodamine fluorescence was determined by solubilizing the remaining lipid in 100 µL of 2% SDS and measuring rhodamine fluorescence (excitation 535 nm, emission 590 nm, emission cutoff at 570 nm) in a fluorescent plate reader (Gemini, Molecular Devices Corp.)

Cell adherence: absorbance

2′-Hydroxychalcone inhibits nuclear factor-B and blocks tumor necrosis factor — and lipopolysaccharide-induced adhesion of neutrophils to human umbilical vein endothelial cells

Mol. Pharmacol. 58: 526–34 (2000).

Babita Madan[1], Sanjay Batra[1], and Balaram Ghosh.

[1] Molecular Immunology and Immunogenetics Laboratory, Centre for Biochemical Technology, University of Delhi Campus (North), Delhi, India.

Methods: cell adherence assay. Adhesion of neutrophils to endothelial monolayers was assayed as described previously (Dobrina *et al.*, 1991). Nonadherent neutrophils were removed by washing the wells with PBS thrice. Adherent neutrophils were assayed colorimetrically by adding a substrate solution (100 µL/well) consisting of o-phenylenediamine dihydrochloride (40 mg/100 mL in citrate phosphate buffer, pH 4.5) containing 0.1% cetitrimethyl ammonium bromide as peroxidase solubilizing agent. The interference by the few contaminating eosinophils was abolished by adding a selective eosinophil peroxidase inhibitor, 3-amino-1,2,4 triazole (1 mM) to the substrate solution. After 2 minutes of incubation, 2N H_2SO_4 (50 µL/well) was added to stop the reaction. The absorbance was determined at 490 nm using an automated microplate reader (SpectraMax 190; Molecular Devices Corp).

G-protein coupled receptors: luminescence

Ca^{++}-induced G protein-coupled receptor (GPCR) activation monitored via aequorin luminescence in the LMax™ microplate luminometer

FULL TEXT →

Molecular Devices MAXline Application Note #42 (2000).

Jinfang Liao, M.D., Ph.D. and Evelyn McGown, Ph.D.

This application note demonstrates how to use LMax to detect receptor-activated Ca^{++} signals in cells stably expressing apo-aequorin. In these cell-based assays, we were able to construct agonist concentration-response curves for the activation of a Gq-protein-coupled purinergic receptor expressed endogenously in CHO cells. Thus, the LMax microplate luminometer can be used to study GPCR pharmacology. Furthermore, with a constant agonist concentration in the injector reservoir, the LMax microplate luminometer could potentially be used to run high-thoughput luminescent assays for GPCR antagonist screening.

biochemistry

Adenylate kinase/NADH

The pro-apoptotic proteins, Bid and Bax, cause a limited permeabilization of the mitochondrial outer membrane that is enhanced by cytosol

J. Cell. Biol. 147: 809–22 (1999).

Ruth M. Kluck[1], Mauro Degli Espostie, Guy Perkins[2], Christian Renken[3], Tomomi Kuwana[1], Ella Bossy-Wetzel[1], Martin Goldberg[4], Terry Allen[4], Michael J. Barber[5], Douglas R. Green[1], and Donald D. Newmeyer[1].

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3 Biology Department, San Diego State University, San Diego, California 92182.

4 Paterson Institute, Christie Hospital NHS Trust, Manchester M20 9BX, United Kingdom.

5 Department of Biochemistry and Molecular Biology, University of South Florida, College of Medicine, Tampa, Florida 33612.

Methods: mitochondrial AK activity assay. Mitochondrial AK activity was measured by a modification of the method of Schmidt *et al.* 1984. Extract aliquots (50 µL) containing ~0.05 mg mitochondrial protein were pelleted at 12,000 g for 3 minutes, and the pellet washed twice in 800 µL buffer D (60 mM sucrose, 210 mM mannitol, 10 mM KCl, 0.5 mM DTT, 10 mM succinate, 10 mM HEPES/KOH, pH 7.5, and 5 mM EGTA) to remove contaminating cytosolic AKs. The mitochondrial pellets were lysed with 50 µL of 1% Triton X-100 in buffer D to release remaining AK, and the sample stored at –80 °C. AK activity was measured in a mixture composed of 1 mL of 130 mM KCl, 6 mM $MgSO_4$, 100 mM Tris-HCl pH 7.5, 15 µL 0.1 M NADH, and 5 µL each of 0.1 M ATP, 100 mM phosphoenol pyruvate, 1 mM rotenone, 1.5 mM oligomycin, a mixture of pyruvate kinase and lactate dehydrogenase (80 U/mL each), and 0.15 M AMP. 200 µL of buffer mix was added to 6 µL of sample in a microtiter plate. The absorbance decrease of NADH was measured at 366 nm in a microtiter plate reader (SpectraMax 250, Molecular Devices Corp.) for 10 minutes at 22 °C. The rates were calculated (SoftMax Pro) and calibrated against chicken muscle myokinase (Sigma) activity. Rates obtained in the presence of the inhibitor di-adenosine pentaphosphate (400 µM; Sigma) were minimal and subtracted as background.

Alcohol dehydrogenase, glutathione S-transferase & esterase activity

Quantitative kinetic assays for glutathione S-transferase and general esterase in individual mosquitoes using an EIA reader

Insect Biochem. 19:741–751 (1989).

David F. Grant, Daniel M. Bender, and Bruce D. Hammock.

Departments of Entomology and Environmental Toxicology, University of California, Davis, CA 95616 USA.

Summary. Microassays for alcohol dehydrogenase (ADH), glutathione S-transferase (GST), general esterase (EST), and total protein have been applied to the analysis of insecticide resistance in individual mosquitoes. The microassays were performed in a

VMax kinetic microplate reader and the results were compared to previously established spectrophotometer based protocols. The microassays run in parallel increased the efficiency of screening large numbers of samples. The advantages of running multiple enzyme assays on individual insects is discussed in light of theories on the molecular biology of insecticide resistance.

β-galactosidase

Action of the thiamine antagonist bacimethrin on thiamine biosynthesis

J. Bacteriol. 182: 5606–10 (2000).

Julie L. Zilles, Laura R. Croal, and Diana M. Downs.

Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin 53706.

Methods: β-galactosidase activity. Subcultures were grown to 0.3 to 0.4 A650 before being assayed. β-Galactosidase activity was assayed as previously described, except that volumes were reduced 10-fold, and absorbance was read in a microtiter plate using a SpectraMax Plus microplate reader (Molecular Devices Corp.)

HveA (herpesvirus entry mediator A), a coreceptor for *herpes simplex* virus entry, also participates in virus-induced cell fusion

J. Virol. 72: 5802–10 (1998).

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[3] Center for Oral Health Research, Northwestern University Medical School, Chicago, Illinois 60611.

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Methods: β-galactosidase activity. Cells were reseeded into both 96-well culture dishes (quantitative analysis) and 24-well culture dishes (qualitative analysis) in the absence or presence of various concentrations of anti-HveA serum or pre-immune control serum control. Approximately 24 hours after plating, the 24-well plates were fixed, permeabilized, and overlaid with ferricyanide buffer supplemented with X-Gal (0.5 mg/mL; Gibco BRL), while the 96-well plates were rinsed with PBS and solubilized in PBS-0.5% NP-40 supplemented with o-nitrophenyl-b-D-galactopyranoside (ONPG; Sigma) at 3 mg/mL. Plates were read in a SpectraMax 250 (Molecular Devices Corp.)

β-lactamase

A microtiter-based assay for the determination of ID_{50}s of β-lactamase inhibitors employing reporter substrates detected at UV or visible wavelengths

FULL TEXT →

Molecular Devices MAXline Application Note #20.

David J. Payne and Sarbendra Pradhananaga

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Methods: β-lactamase assays. β-lactamases are either plasmid or chromosomally encoded bacterial enzymes which hydrolyze β-lactam antibiotics. This application note describes a simple procedure to determine the inhibition (ID_{50} value) of β-lactamases by various agents using the chromogenic cephalosporin nitrocefin (λmax = 482 nm) and the carbapenem antibiotic imipenem (λmax = 299 nm) as reporter substrates. Imipenem was used for those carbapenemases which did not have significant hydrolytic activity against nitrocefin. The method described uses the UV capability of the SpectraMax 250 microplate spectrophotometer and can be readily modified to determine ID_{50} values of inhibitors of other enzymes requiring UV/Vis monitoring.

Caspase-3

The pro-apoptotic proteins, Bid and Bax, cause a limited permeabilization of the mitochondrial outer membrane that is enhanced by cytosol

J. Cell Biol. 147: 809–22 (1999).

Ruth M. Kluck *et al.*

Methods: measurement of DEVDase activity. To measure caspase-3-like activation, extract aliquots (2 µL) were incubated with DEVD-pNA (N-acetyl-Asp-Glu-Val-Asp-p-nitroanilide, 40 µM; Biomol) in 200 µL of a buffer (250 mM sucrose, 20 mM HEPES/KOH pH 7.5, 50 mM KCl, 2.5 mM $MgCl_2$, and 1 mM DTT) similar to that used to make the egg extracts. Incubations were kept at 22 °C and A405 development monitored over 30 minutes (SpectraMax 250 microplate spectrophotometer, Molecular Devices Corp.)

Catalase & glutathione S-transferase

Clusterin has chaperone-like activity similar to that of small heat shock proteins

J. Biol. Chem. 274: 6875–6881 (1999).

David T. Humphreys[1], John A. Carver[2], Simon B. Easterbrook-Smith[3], and Mark R. Wilson[1].

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[3] Department of Biochemistry, The University of Sydney, Sydney, New South Wales 2006, Australia.

Summary. Clusterin is a highly conserved protein which is expressed at increased levels by many cell types in response to a broad variety of stress conditions. At physiological concentrations, clusterin potently protected glutathione S-transferase (GST) and catalase from heat-induced precipitation and α-lactalbumin and bovine serum albumin from precipitation induced by reduction with dithiothreitol.

Methods: enzyme assays. Catalase was incubated with 1.0 mL of H_2O_2 substrate solution (0.12% (v/v) H_2O_2 in 50 mM Na_2HPO_4, pH 7.0) at 37 °C for 5 minutes before the reaction was stopped with 150 µL of 4 M NaOH. The absorbance of H_2O_2 was measured at 250 nm on a SpectraMax 250 microplate reader (Molecular Devices Corp.) Enzyme activity was measured as a decrease in absorbance. GST was heated at 37 or 50 °C for 30 minutes. GST was then diluted into substrate solution (1 mM GSH, 2 mM 1-chloro-2,4-dinitrobenzene in 0.1 M phosphate, pH 7.4) to a final concentration of 4.5 µg/mL and incubated at 37 °C for 5 minutes before measuring the absorbance at 350 nm. Enzyme activity was measured as an increase in absorbance, corresponding to the appearance of 1-S-glutathionyl-2,4-dinitrobenzene.

Elastase

Inhibitors directed to binding domains in neutrophil elastase

Biochemistry 29: 9970–77 (1990).

Suresh C. Tyagi and Sanford R. Simon.

Department of Biochemistry and Cell Biology and Department of Pathology, State University of New York at Stony Brook, Stony Brook, New York 11794.

Methods: human neutrophil elastase (HNE) activity. The amidolytic and HNE were assayed with MeOSu-cAAPVpNa as a substrate (Nakajima et al 1979). The release of p-nitroaniline was monitored by recording the absorbance at 405 nm with a VMax microplate reader (Molecular Devices Corp.) operating in the kinetic mode. The buffer used contained 3.3% dimethylformamide and 0.01% Triton X-100. Kinetic data was collected for five minutes, during which reaction rates remained linear.

Esterase

A microassay method for neurotoxic esterase determinations

Fundam. Appl. Toxicol. 16: 110–116 (1991).

Linda Correll, and Marion Ehrich.

Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24061 USA.

Summary. The authors report new microassay methods for measuring the activities of neurotoxic esterase, EC 3.1.1 (NTE, also known as neuropathy target esterase) and acetylcholinesterase EC 3.1.1.7 (AChE) and total protein

determinations in spinal cord regions of hens. NTE activity is reported as the change in phenol released from phenyl valerate as measured at 510 nm following the addition of 4-aminoantipyrine and potassium ferricyanide. Hydrolysis of acetylthiocholine iodide by AChE was followed as the change in absorbance at 412 nm as dithionitrobenzoic acid (DTNB) was reduced by the enzyme product.

The Bradford method read at 595 nm was used to measure total protein. Microassay methods were validated by comparison of NTE and AChE activity in brains of control and experimental hens with more conventional assays. NTE activities in brains of control and experimental hens given DFP (0.5 mg/kg sc) 24 hours before samples were collected produced similar results using both types of NTE analytical procedures. The reported advantages of the microassay include the capability of being used for small regional esterase activity measurements, the number of samples processed was increased while the time needed was reduced, and the total assay volume (0.3 mL) is 1/20 of sample and reagents necessary in more conventional assays.

Glutathione, myeloperoxidase & malondialdehyde assays

Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples

Anal. Biochem. 190: 360–365 (1990).

Margaret A. Baker, George J. Cerniglia, and Aziza Zaman.

Department of Radiation Oncology, University of Pennsylvania, Philadelphia, PA 19104 USA.

Summary. The authors describe a rapid, sensitive, and easy assay for measuring reduced and oxidized glutathione (GSH and GSSG), both intracellular and extracellular in biological samples. Cell and tissue sample preparation for the GSH enzymatic recycling assay is described. The method follows 5,5´-dithio-bis(2-nitrobenzoic acid) (DTNB) reduction at 405 nm in the UVMax microplate reader. The kinetic microplate assay is sensitive to 5 pmol, uses less sample than previous methods, and enables increased sample processing.

Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose) polymerase

Proc. Natl. Acad. Sci. USA 97: 10203-10208 (2000).

Lucas Liaudet[1], Francisco Garcia Soriano[3], Éva Szabó[2], László Virág[2], Jon G. Mabley[2], Andrew L. Salzman[1,2], and Csaba Szabó[2].

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Methods: myeloperoxidase (MPO) assay. Tissues were homogenized (50 mg/mL) in 0.5% hexadecyltrimethylammonium bromide in 10 mM 3-(N-morpholino)propanesulfonic acid (Mops) and centrifuged at 15,000 g for 40 minutes. The suspension was then sonicated three times for 30 seconds. An aliquot of supernatant was mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM hydrogen peroxide. Activity was measured spectrophotometrically as the change in absorbance at 650 nm at 37 °C by using a SpectraMax absorbance microplate reader (Molecular Devices Corp). Results are expressed as milliunits MPO activity per milligram of protein, which were determined with the Bio-Rad assay.

Methods: malondialdehyde (MDA) assay. MDA formation was used to quantify the lipid peroxidation in tissues and measured as thiobarbituric acid-reactive material. Tissues were homogenized (100 mg/mL) in 1.15% KCl buffer. Homogenates (200 µL) were then added to a reaction mixture consisting of 1.5 mL of 0.8% thiobarbituric acid, 200 µL of 8.1% (vol/vol) SDS, 1.5 mL of 20% (vol/vol) acetic acid (pH 3.5), and 600 µL of distilled H_2O and heated at 90 °C for 45 minutes. After cooling to room temperature, the samples were cleared by centrifugation (10,000 g for 10 minutes), and their absorbance was measured at 532 nm by using 1,1,3,3-tetramethoxypropane as an external standard. The level of lipid peroxides was expressed as nanomoles MDA per milligram of protein.

Methods: glutathione (GSH) assay. Tissues were homogenized (100 mg/mL) in 5% (vol/vol) sulfosalicylic acid. The homogenates were centrifuged at 10,000 g for 20 minutes, and an aliquot of the clear supernatant (20 µL) was combined with 0.3 M Na_2HPO_4 (160 µL) and 0.04% 5,5′-dithiobis-(2-nitrobenzoic acid) in 1% sodium citrate (20 µL). After a 10-minute incubation at room temperature, absorbance was read at 405 nm in a SpectraMax absorbance microplate reader. Concentrations of GSH were calculated from a standard curve constructed with known concentrations of reduced GSH.

Lactate dehydrogenase

Metabolic defects caused by mutations in the *isc* gene cluster in *Salmonella enterica Serovar Typhimurium*: implications for thiamine synthesis

J. Bacteriol. 182: 3896–3903 (2000).

Elizabeth Skovran and Diana M. Downs.

Department of Bacteriology, University of Wisconsin Madison, Madison, Wisconsin 53706.

Methods: lactate dehydrogenase assays. The lactate dehydrogenase assay was modified from the method of Oeschger. The reaction mixture contained pyruvate-specific D-lactic acid dehydrogenase (catalog no. L3888; Sigma Chemical Co.) (8.7 U), 10 mM

Tris-HCl (pH 8), 240 mM KCl, 20 mM $MgCl_2$, and 1 mM pyruvate or 10 to 100 µL of spent growth medium. The preassay mixture was incubated at 30 °C for 2 minutes before the reaction was initiated with NADH (final concentration, 25 mM). The oxidation of NADH was monitored as a decrease in the absorbance at 340 nM in a quartz microtiter plate using a SpectraMax Plus microplate reader (Molecular Devices Corp.)

NADPH oxidase/ cytochrome c reduction

Mapping of functional domains in p47phox involved in the activation of NADPH oxidase by "peptide walking"

J. Biol. Chem. 273: 15435–44 (1998).

Igor Morozov, Ofra Lotan, Gili Joseph, Yara Gorzalczany, and Edgar Pick.

The Julius Friedrich Cohnheim-Minerva Center for Phagocyte Research, Department of Human Microbiology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.

Methods: cell-free NADPH oxidase assay. The superoxide generating NADPH oxidase of phagocytes consists, in resting cells, of a membrane-associated electron transporting flavocytochrome (cytochrome b559) and four cytosolic proteins as follows: p47phox, p67phox, p40phox, and the small GTPase, Rac(1 or 2). O_2 production was initiated by the addition of NADPH and quantified by following the rate of cytochrome c reduction, at 550 nm, in a kinetic assay performed in a SpectraMax 340 microplate reader (Molecular Devices Corp.), using SoftMax Pro software.

P450 enzymes

Evidence for peroxisome proliferator-activated receptor (PPAR)-independent peroxisome proliferation: effects of PPAR-specific agonists in PPAR-null mice

Mol. Pharmacol. 58: 470-76 (2000).

John G. DeLuca[2], Thomas W. Doebber[1], Linda J. Kelly[1], Ramon K. Kemp[2], Sylvain Molon-Noblot[2], Soumya P. Sahoo[1], John Ventre[1], Margaret S. Wu[1], Jeffrey M. Peters[3], Frank J. Gonzalez[3], and David E. Moller[1].

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[3] Laboratory of Metabolism, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

Methods: measurement of CYP4A levels. CYP4A protein levels were assessed in whole liver homogenate, and samples were solubilized in 0.5% sodium cholate/Triton X-100, using rabbit anti-rat CYP4A1 as the primary antibody (Gentest Corp., Waltham, MA) and goat anti-rabbit Ig, horseradish peroxidase-conjugated, as the secondary antibody (Pierce Chemical Co., Rockford, IL). Solubilized samples were diluted in 0.05 M carbonate/bicarbonate buffer, pH 9.6, to give approximately 20 ng of protein/50 µL. This was adsorbed to 96-well Maxisorp titer plates (Nalge Nunc

International, Rochester, NY) for 1 hour at 37 °C. Wells were then rinsed four times with 0.05% Tween 20 in Ca^{2+}- and Mg^{2+}-free PBS (rinse/block buffer), and 100 µL of primary antibody diluted 1:4000 in Superblock (Pierce Chemical Co.) containing 0.05% Tween 20 (antibody dilution buffer) was added to each well. After 1 hour of incubation at 37 °C, plates were rinsed as above with rinse/block buffer, 100 µL of secondary antibody diluted 1:2000 in antibody dilution buffer was added, and plates were incubated for 1 hour at 37 °C. After incubation, plates were rinsed as above, 100 µL of ABTS 1 STEP (Pierce Chemical Co.) was added, and plates were incubated at room temperature for 20 minutes. The reaction was stopped by addition of 50 °C 2% SDS containing 0.5 mg/mL Proteinase K, and plates were read immediately in a SpectraMax 340 plate reader (Molecular Devices, Corp.)

Caspase-3

The human DIMINUTO/DWARF1 homolog seladin-1 confers resistance to Alzheimer's disease-associated neurodegeneration and oxidative stress

J. Neuroscience 20: 7345-52 (2000).

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2 Department of Neurology, Clinica Universitaria de Navarra, Pamplona 31008, Navarra, Spain.

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4 Institute for Arteriosclerosis Research, University of Münster, 48149 Münster, Germany.

5 Division of Psychiatry Research, University of Zurich, 8008 Zurich, Switzerland.

Methods: caspase-3 activity. Caspase-3 activity was measured in cell lysates of EGFP clones plated to identical densities by using the caspase-3 assay kit (PharMingen, Becton Dickinson GmbH). After exposure to 200 µM H_2O_2 for 2 and 4 hours or to 25 µM A25-35 for 4 hours, cells were washed briefly in PBS and lysed in 100 µL 10 mM Tris-HCl, pH 7.5, 10 mM NaH_2PO_4/$NaHPO_4$, pH 7.5, 130 mM NaCl, 1% Triton-X-100, 10 nM NaPPi. Lysates (100 µg protein) were incubated in 200 µL HEPES buffer for 1 hour at 37 °C with 5 µg of the caspase-3 fluorogenic substrate Ac-DEVD-AMC or with 5 µg Ac-DEVD-AMC in the presence of 0.5 µg of the caspase-3 aldehyde inhibitor Ac-DEVD-CHO in a 96-multiwell plate. AMC liberated from Ac-DEVD by caspase cleavage was measured on a spectrofluorometer (Gemini, Molecular Devices Corp.) at excitation wavelength of 380 nm and an emission wavelength spectrum from 420 to 460 nm.

Fluorometric protease assays in the Gemini microplate spectrofluorometer: example using caspase-3

FULL TEXT →

Molecular Devices MAXline Application Note #35 (1999).

Evelyn McGown, Ph.D. and Anna Lam, B.S.

Summary. This application note describes how to optimize a protease assay in the Gemini microplate spectrofluorometer. We chose to use the protease caspase-3 and a fluorogenic peptide substrate containing a coumarin derivative. Fluorogenic peptide substrates can interfere with the measurement of their own hydrolysis products. If the emission spectra overlap, the assay may have a high background. If the absorption spectra overlap, or if the absorbance of the substrate overlaps with the emission of the product, the substrate can quench the signal from the product. Therefore, both the excitation and emission wavelengths must be optimized to minimize interference from the substrate, while maintaining sufficiently high product fluorescence. The Gemini microplate spectrofluorometer facilitates the process by having dual monochromators that allow optimal

excitation and emission wavelengths to be easily determined.

Methods: caspase-3 assay. Z–DEVD–AMC substrate was purchased as part of Molecular Probes EnzChek Caspase-3 Assay Kit #1 (catalog #E-13183). Recombinant caspase-3 enzyme was purchased from Calbiochem (catalog #235417), and 7-Amino-4-Methylcoumarin (AMC) from Sigma (catalog #A 9891). Black 96-stripwell plates and white 96-well plates were purchased from Corning Costar. All dilutions of the substrate and enzyme were made with 1X Reaction Buffer (prepared by diluting 200 µL of the 5X Reaction Buffer and 5 µL of DTT, both included in the kit, with 795 µL of deionized water.) Stock Z-DEVD-AMC (10 mM) was prepared by adding 520 µL of DMSO into the vial of Z-DEVD-AMC. Working solutions of Z-DEVD-AMC stock were typically 1:100 dilutions of the stock, i.e. 5 µL of the stock solution into 495 µL of 1X Reaction Buffer. The stock enzyme was frozen in 2 or 4 µL aliquots (to avoid repeated freeze/thaw cycles), and working enzyme solutions were prepared by diluting the stock 1:10 with 1X Reaction Buffer. The stock AMC solution was 2 mM in DMSO. The optimal instrument settings are as follows: Ex 368 nm/Em 467 nm with either a 420 nm or 435 nm emission cutoff filter. The estimated limit of detection using white microplates was ~0.02 ng per well and for black microplates was 0.10 ng per well.

chemistry

Cholesterol & triglycerides

Ultrasensitive enzymatic cholesterol and triglyceride profiles of density gradient lipoprotein fractions

8th International Symposium on Atherosclerosis, Roma, International Atherosclerosis Society (eds), CIC Edizioni Internazionali, (Roma). (1988).

John D. Belcher and Jack O. Egan.

University of Minnesota, Minneapolis, Minnesota USA.

Summary. Microassays were developed for the measurement of total cholesterol (TC) and triglyceride (TG) by enzymatic methods. The assays are linear between 1 and 100 mg/dL with a sensitivity of 1 mg/dL for both TC and TG. The assay uses 30 µL of sample or standard which is added to each well followed by 150 µL of either TC or TG enzymatic reagent. The microplates were incubated at room temperature for 15 minutes and read in a VMax microplate reader at 490 nm with a 650 nm reference. The mean accuracy of the assay was -0.5% and -0.7% for TC and TG, respectively, and the within- and between-assay coefficients of variation were below 3%.

Citrate & glucose consumption

Mechanism of citrate metabolism in *Lactococcus lactis*: resistance against lactate toxicity at low pH

J. Bacteriol. 181: 1451–57 (1999).

Christian Magni[1,2], Diego de Mendoza[2], Wil N. Konings[1], and Juke S. Lolkema[1].

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Methods: measurement of citrate and glucose consumption rates. Cells were harvested and resuspended to an OD_{660} of 6 in 50 mM potassium phosphate buffer (pH 5.5). Citrate utilization was initiated by the addition of 500 µL of cells to 1,500 µL of buffer containing 2 mM citrate. When indicated, glucose and lactate were included at concentrations of 0.5 and 2 mM, respectively. Glucose utilization was initiated by addition of 50 µL of cells to 1,950 µL of buffer containing 0.5 mM glucose and 2 mM citrate when indicated. At the indicated times, 250 µL of the cell suspension was centrifuged for 15 seconds in an Eppendorf tabletop centrifuge operating at maximal speed, and a sample of the supernatant was stored in liquid nitrogen until analysis. The concentrations of citrate, pyruvate, and glucose in the supernatants were measured by commercially available enzyme kits for citrate and D-glucose (Boehringer). The protocol for the measurement of citrate was modified slightly to allow the measurement of pyruvate (and oxaloacetate) at the same time, as described before. Both protocols were modified for use in 96-well plates as follows. For the citrate assay, 100 µL of the

buffer provided by the manufacturer was mixed with 50 µL of water, after which the A_{340} was measured. Subsequently, 50 µL of the supernatant was added and the OD was read again. The difference between the two readings is a measure of the pyruvate (or oxaloacetate) concentration in the sample. After addition of 1 µL of the citrate lyase solution, the absorbance was read again, which provides the data for the calculation of the citrate concentration as indicated in the protocol. For glucose determination, the volumes in the manufacturer's protocol were scaled down to give a total volume of 200 µL. The ODs were read by using a SpectraMax 340 microplate spectrophotometer (Molecular Devices Corp.)

Heavy metals

Cu(II) potentiation of Alzheimer A neurotoxicity: correlation with cell-free hydrogen peroxide production and metal reduction

J. Biol. Chem. 52: 37111–16 (1999).

Xudong Huang, Math P. Cuajungco, Craig S. Atwood, Mariana A. Hartshorn, Joel D. A. Tyndall, Graeme R. Hanson, Karen C. Stokes, Michael Leopold, Gerd Multhaup, Lee E. Goldstein, Richard C. Scarpa, Aleister J. Saunders, James Lim, Robert D. Moir, Charles Glabe, Edmond F. Bowden, Colin L. Masters, David P. Fairlie, Rudolph E. Tanzi, and Ashley I. Bush.

Methods: metal reduction assays. Assays were performed using a 96-well microtiter plate (Costar), based upon a modification of established protocols. Polypeptides (10 µM) or vitamin C (10 µM), Cu(II)-glycine and Cu(I) indicator (250 µM), either bathocuproine disulfonic acid (BC) or bicinchoninic acid (BCA, 4,4′-dicarboxy-2,2′-biquinoline), were coincubated in PBS at 37 °C. Absorbance was measured using a plate reader (SpectraMax Plus, Molecular Devices Corp.) In control samples, both metal ion and indicator were present to determine the background buffer signal. Absorbance of metal ion and peptide present in the absence of indicator were taken to estimate the contribution of light scattering due to turbidity. The net absorbance (A) was obtained by deducting the absorbance from these controls from the absorbance generated by the peptide and metal in the presence of the indicator. Cu(I) concentrations (µM) were calculated as $A \times 10^6/M$, where M is the known molar absorption coefficient ($M^{-1}\ cm^{-1}$). For Cu(I)-BC, M = 12,250 at 483 nm; and for Cu(I)-BCA, M = 7700 at 562 nm.

Dramatic aggregation of Alzheimer A by Cu(II) is induced by conditions representing physiological acidosis

J. Biol. Chem. 273: 12817–26 (1998).

Craig S. Atwood[1], Robert D. Moir[2], Xudong Huang[1], Richard C. Scarpa[1], N. Michael E. Bacarra[1], Donna M. Romano[2], Mariana A. Hartshorn[1], Rudolph E. Tanzi[2], and Ashley I. Bush[3].

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Methods: Cu^{2+}-induced spectral changes in amyloid protein. Cu^{2+}-induced spectral changes in A were monitored by incubating A1-40 or A1-42 (5 µM) in 20 mM ammonium acetate buffer, 150 mM NaCl (pH 7.4), with and without various concentrations of Cu^{2+} for 5 minutes prior to loading onto a quartz microtiter plate. Absorbance was scanned between 200 and 800 nm on a SpectraMax Plus spectrophotometer (Molecular Devices Corp.) Incubation of Cu^{2+} with A induced a change in the absorbance profile of the peptide, which was maximal at 208 nm. However, subsequent analyses were performed at 214 nm where the spectral shift was still large but the background signal was lower.

Hydrogen peroxide

Cu(II) potentiation of Alzheimer A neurotoxicity: correlation with cell-free hydrogen peroxide production and metal reduction

J. Biol. Chem. 274: 37111–16 (1999).

Xudong Huang[1], Math P. Cuajungco[1], Craig S. Atwood[1], Mariana A. Hartshorn[1], Joel D. A. Tyndall[3], Graeme R. Hanson[4], Karen C. Stokes[5], Michael Leopold[5], Gerd Multhaup[6], Lee E. Goldstein[1], Richard C. Scarpa[1], Aleister J. Saunders[1], James Lim[1], Robert D. Moir[7], Charles Glabe[9], Edmond F. Bowden[5], Colin L. Masters[10], David P. Fairlie[3], Rudolph E. Tanzi[7], and Ashley I. Bush[1].

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[10] Department of Pathology, University of Melbourne, and Neuropathology Laboratory, Mental Health Research Institute of Victoria, Parkville, Victoria 3052, Australia.

Methods: hydrogen peroxide assay. The colorimetric H_2O_2 assay was performed in a 96-well microplate (SpectraMax Plus, Molecular Devices Corp.), according to a modification of an existing protocol. Polypeptides (10 µM) or vitamin C (10 µM), Cu(II) (1 µM), and a H_2O_2 scavenging agent, tris(2-carboxyethyl)phosphine hydrochloride (Pierce, 50 µM), were co-incubated in PBS buffer (300 µL), pH 7.4, for 1 hour at 37 °C. Following incubation, the unreacted tris(2-carboxyethyl)-phosphine hydrochloride was detected by 5,5′-dithiobis(2-nitrobenzoic acid) (Sigma, 50 µM). The amount of H_2O_2 produced was quantified based on the formula: H_2O_2 (µM) = A x 10^6/(2 x L x M), where A is the absolute absorbance difference between a sample and catalase-only (Sigma, 100 units/mL) control at 412 nm; L = the vertical pathlength, corrected automatically by the plate reader to 1 cm; M is the molecular absorbance for 2-nitro-5-thiobenzoate (14,150 M^{-1} cm^{-1} at 412 nm).

Inorganic phosphate

Two active forms of UDP-N-acetylglucosamine enolpyruvyl transferase in gram-positive bacteria

J. Bacteriol. 182: 4146–52 (2000).

Wensheng Du, James R. Brown, Daniel R. Sylvester, Jianzhong Huang, Alison F. Chalker, Chi Y. So, David J. Holmes, David J. Payne, and Nicola G. Wallis.

Anti-Infectives Research, SmithKline Beecham Pharmaceuticals, Collegeville, Pennsylvania 19426.

Methods: detection of inorganic phosphate. The activity of MurA1 and MurA2 was assayed by measuring the release of P*i* from the UDPAG and PEP reaction, using a malachite green assay in 50 mM HEPES (pH 7.5) at room temperature. A typical assay of 200 µL contained substrates and enzyme (MurA1 at 200 nM or MurA2 at 50 nM). For kinetic measurements, the assay was performed with a five-by-five array of various substrates on a half-area 96-well microtiter plate (Costar 3696; Corning Inc., Corning, N.Y.) with a SpectraMax Plus plate reader (Molecular Devices Corp.)

Microdetermination of phosphorus using the SpectraMax Plus microplate spectrophotometer: choice of microplate, cuvette or test tube assay formats

FULL TEXT →

Molecular Devices MAXline Application Note #24.

This application note details two methods for measuring inorganic phosphorus using Molecular Devices' SpectraMax Plus microplate spectrophotometer. The first method uses a commercial kit obtained from Sigma Diagnostics, based on the original Fiske-Subbarow chemistry, and offers the convenience of ready-made reagents. The second method is approximately 10 times more sensitive, and thus is useful for samples containing 0.1 to 5.0 µg P*i*/mL. Whichever method is used, the SpectraMax Plus microplate spectrophotometer offers the versatility of making the absorbance measurements in microplates, in standard 1 cm cuvettes, or in 12 x 75 mm test tubes.

Reactive oxygen species

Superoxide anion production from human neutrophils measured with an improved kinetic and endpoint microassay

J. Immunol. Methods 142: 95–104 (1991).

E. Sabrinah Chapman-Kirkland, James S. Wasvary, and Bruce E. Seligmann.

Research Department, Ciba-Geigy Corporation, Summit, NJ 07901 USA.

Summary. Superoxide dismutase (SOD)- inhibitable reduction of cytochrome c is widely used to measure superoxide production by neutrophil cells and cell fragments. The authors report using a dual wavelength measurement and novel modifications to the microplate assay to lessen the perturbations caused by the microplate and improve experimental reproducibility. A new surface modified microplate (Plastek A*) was compared to

untreated microplates. The modified surface prevented the adherence and consequent activation of PMNs. Neutrophils in the two types of plastic microplates were shown to have a statistically significant difference in basal as well as stimulated levels of superoxide production. Absorption measurements were made using dual wavelength measurement at 550 nm (1 nm bandwidth), the absorption maximum of reduced cytochrome c, and 557 nm (1 nm bandwidth), an isosbestic point. The authors observed that the difference values (550–557 nm) significantly increased reproducibility and sensitivity (detection limit) as compared to the single wavelength measurement at 550 nm.

Kinetic microplate assay for superoxide production by neutrophils and other phagocytic cells

Methods in Enzymology 196: 567–575 (1990).

Laura A. Mayo and John T. Curnutte.

Department of Molecular and Experimental Medicine, Research Institute of Scripps Clinic, La Jolla, CA 92037 USA.

Summary. This review describes methods of isolation and use of human neutrophils in assays for superoxide production. The kinetic microplate assay for superoxide follows the reduction of cytochrome c at 37 °C in a Molecular Devices microplate reader fitted with a special narrow (1 nm) bandwidth 550 nm interference filter. Single well illumination and use of AUTOmix in whole cell assay are reported to be valuable. Analysis of nonlinear kinetic data with SoftMax Pro software is presented.

ATP

ATP quantitation in the LMax™ microplate luminometer

FULL TEXT →

Molecular Devices MAXline Application Note #40 (2000).

Evelyn McGown, Ph.D. and Michael Su, M.S.

Summary. The LMax microplate luminometer gives a detection limit of approximately 0.02 fmol ATP/well and a dynamic range of 5 logs with the ENLITEN® ATP assay system from Promega Corp. These results are as good as, if not better than, results obtained on a standard tube luminometer. The LMax offers the advantage of a 96-well format and automated reagent addition for better precision and higher throughput. In addition, SoftMax Pro for LMax provides a powerful and convenient instrument control and data calculation package.

Reactive oxygen species

Measuring oxidative bursts with the photoprotein pholasin

Communications in Clinical Cytometry 46: 324 (2001).

Doug Redelman[1], Qiao Zhong[2], Dorothy Hudig[2], Linda M. Castell[3], Don Roberts[4] and Wayne Y. Ensign[4].

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[2] University of Nevada, Reno NV.

[3] University of Oxford, UK.

[4] Naval Health Research Center, San Diego, CA.

Methods: kinetic assay for superoxide anion. The oxidative burst stimulated by formyl-methionyl-leucyl-phenylalanine (fMLP) was measured in a kinetic chemiluminescence assay (ABEL, Knight Scientific Ltd., Plymouth, UK). The chemiluminescent agent in this system is the photoprotein from the mollusk *Pholas dactylus* (Pd-prot or Pholasin). The 96-well microplate assay kit includes blood dilution medium, Pd-prot, fMLP, phorbal ester (PMA), and Adjuvant-K, a proprietary luminescence-enhancing agent. To perform the assay, whole blood was diluted 1:100 (20 µL + 20 mL) in the provided diluent. The wells of the white opaque microplates had diluent, Pd-prot and the enhancing agent plus 200 µL of 1:100 diluted whole blood in a total volume of 175 µL. The reactions were set up as sets of 8 wells (4 duplicate blood samples) at a time in order to minimize the effects of prolonged standing. The plates were read in a kinetic luminometer (LMax, Molecular Devices Corp.) Two types of readings were used. First, the wells were measured in "slow kinetics" mode in which the reading head moved from well to well to determine the background from each well over a 5-minute period. The stimulated responses were read in "fast kinetics" mode in which the reading head remained positioned over a single well for the duration of the assay (100 seconds). The response was initiated by injecting 25 µL fMLP solution to produce a final concentration of ~1 µM fMLP in the 200 µL total reaction volume. The response was measured once per second and the data are reported as "relative light units" (RLU). SoftMax Pro software was used to process the data.

protein structure & function

protein & cell aggregation

Absorbance

Glycosylation at Asn-184 inhibits the conversion of single-chain to two-chain tissue-type plasminogen activator by plasmin.

Biochemistry 29: 4175–4180 (1990).

Arthur J. Wittwer and Susan C. Howard.

Department of Cell Culture and Biochemistry, Central Research Laboratories, Monsanto Company, St. Louis, MO 63167 USA.

Summary. Tissue-type plasminogen activator (tPA) a glycosylated serine protease is an effective thrombolytic agent. The native single-chain tPA (sc-tPA) is converted by plasmin to a two-chain tPA (tc-tPA). Two glycoforms of native sc- tPA are known. Type I sc-tPA is fully glycosylated, while type II lacks glycosylation at Asn-184. The rates at which type I and type II human melanoma sc-tPA were converted to type I and type II tc-tPA by plasmin were determined by observing the amidolytic activity using a nitroanilide chromogenic substrate and following the rate of change at 405 nm in a Molecular Devices VMax kinetic microplate reader. Results indicate that glycosylation of tPA at Asn-184 hinders conversion of sc-tPA to tc-tPA. Glycosylation of tPA may serve to modulate activity permitting type I sc-tPA to persist in the single-chain form longer than type II sc-tPA under physiological conditions.

Measuring platelet aggregation with a microplate reader. A new technical approach to platelet aggregation studies.

Am. J. Clin. Pathol. 94: 613–617 (1990).

Joseph C. Fratantoni and Betty J. Poindexter.

Division of Blood and Blood Products, Center for Biologics Evaluation and Research, Bethesda, MD 20892 USA.

Summary. Platelet aggregation measurements were performed with the use of a VMax microplate reader (Molecular Devices Corp.) Authors report that many wavelengths were evaluated and found to yield equivalent results. The largest change in optical density is observed at lower wavelengths. The 96-well microplate permits all test and control platelet samples including replicates to be observed simultaneously over the course of a 10 to 15 minute assay. The technique was validated by demonstrating the similarity of dose-response curves to those obtained with a standard aggregometer for the stimulants adenosine diphosphate, thrombin, and arachidonic acid.

Dramatic aggregation of Alzheimer A$^{\beta}$ by Cu(II) is induced by conditions representing physiological acidosis

J. Biol. Chem. 273: 12817–26 (1998).

Craig S. Atwood[1], Robert D. Moir[2], Xudong Huang[1], Richard C. Scarpa[1], N. Michael E. Bacarra[1], Donna M. Romano2, Mariana A. Hartshorn[1], Rudolph E. Tanzi[2], and Ashley I. Bush[3].

[1] Department of Psychiatry and Genetics and Aging Unit.

[2] Department of Neurology and Genetics and Aging Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114.

Methods: protein aggregation. Turbidity measurements, as an assay for aggregation, were performed in a flat-bottomed 96-well microtiter plate (Corning Costar Corp.), and absorbances (405 nm) were measured using a SpectraMax Plus spectrophotometric microplate reader (Molecular Devices, Corp.) Automatic 30-second plate agitation mode was selected for the plate reader to evenly suspend the aggregates in the wells before all readings.

Protein C inhibitor acts as a procoagulant by inhibiting the thrombomodulin-induced activation of protein C in human plasma

Blood 91: 1542–47 (1998).

Marc G.L.M. Elisen, Peter A.Kr. von dem Borne, Bonno N. Bouma, and Joost C.M. Meijers.

Department of Haematology, University Hospital and Institute of Biomembranes, Utrecht University, Utrecht, The Netherlands.

Methods: gel assembly assay. Turbidimetry was used to monitor the TM-mediated thrombin inhibition by PCI. The change in turbidity during fibrin formation was monitored at 405 nm in a microplate reader. An increase in turbidity indicated gel assembly. All experiments were performed in citrated plasma recalcified with $CaCl_2$ (final concentration of 17 mmol/L) resulting in a free Ca^{2+} concentration of 2.3 mmol/L. A mixture of recombinant tissue factor (Innovin, final dilution 3 x 10^4) and calcium necessary for recalcification was added to 67.5 µL of plasma to initiate clotting. The volume was adjusted to 125 µL with HEPES buffer, resulting in a final plasma concentration of 54%. After mixing, 100 µL of the reaction mixture was transferred to a microplate, and turbidity at 405 nm was monitored at 37 °C using a SpectraMax 340 microplate reader (Molecular Devices Corp.)

Clusterin has chaperone-like activity similar to that of small heat shock proteins

J. Biol. Chem. 274: 6875–81 (1999).

David T. Humphreys, John A. Carver, Simon B. Easterbrook-Smith, and Mark R. Wilson.

Methods: protein precipitation assay. Solutions of clusterin were prepared in 0.3 mL of 50 mM sodium phosphate containing 0.1 M NaCl and incubated at 37 °C with or without 20 mM DTT. During this period absorbance readings at 360 nm (measures light scattering) were acquired every 5 minutes for a total of 5 hours in a SpectraMax 250 plate reader (Molecular Devices Corp.)

pharmacology

ADME toxicity & drug screening

Absorbance

New colorimetric cytotoxicity assay for anticancer-drug screening

J. Natl. Cancer Inst. 82: 1107–1112 (1990).

Philip Skehan, Ritsa Storeng, Dominic Scudiero, Anne Monks, James McMahon, David Vistica, Jonathan T. Warren, Heidi Bokesch, Susan Kenney, and Michael R. Boyd.

Division of Cancer Treatment, National Cancer Institute, Frederick, MD 21701 USA.

A rapid, sensitive, simple, and inexpensive method is described for measuring the cellular protein content of adherent and suspension cultures in 96-well microplates. Sulforhodamine B (SRB) is used to stain trichloroacetic acid (TCA) fixed cultures. The optical density of the stain is read in a VMax microplate reader (Molecular Devices Corp.) at 564 nm. The computer-interfaced microplate reader enables error free, high throughput of collected data. The SRB assay is linear with cell number ranging in cell densities from sparse subconfluence to multilayered supraconfluence and is very sensitive (a signal-to-noise ratio of 1.5 with 1000 cells per well). The SRB assay provides a colorimetric endpoint that is nondestructive to the cell architecture for the fixed cells, indefinitely stable, and visible to the naked eye. The method is suitable for routine and very large-scale applications.

In vitro toxicity of various classes of test agents using the neutral red assay on a human three-dimensional physiologic skin model

In Vitro Cell. Dev. Biol. 27A: 239–244 (1991).

Dennis Triglia, Sonia Sherard Braa, Christine Yonan, and Gail K. Naughton.

Marrow-Tech, Inc., La Jolla, California 92037 USA.

Authors describe a new three-dimensional human skin model used to assess in vitro toxicity of test agents from various classes. This model consists of human neonatal foreskin-derived fibroblasts and epidermal keratinocytes grown in layers on a nylon mesh. Cells in this new dermal substrate are metabolically viable and actively dividing. The toxicity test is a modified neutral red viability assay. The endpoint assay was read at 540 nm. Authors obtained dose-dependent toxicity curves for test agents from the following classes: detergents (n = 15), alcohols (n = 5), metal chlorides (n = 10), perfumes and colognes (n = 5), shampoos (n = 4), conditioners (n = 3), moisturizers (n = 3), pesticides (n = 3), and antimicrobial preservatives (n = 4). Comparisons are made between the human skin model toxicity and *in vivo* ocular irritancy data.

Methods: neutral red toxicity assay. 14-mm disks containing fibroblasts and keratinocytes are placed into 24-well plates and treated with 2 mL of various concentrations of test agents overnight at 37 °C/5% CO_2 in a humidified incubator. Media is aspirated and replaced

with 1 mL per well of DMEM containing 50 µg/mL neutral red (NR). The plates are incubated for 3 hours at 37 °C/5% CO_2 after which NR is aspirated and disks are washed twice with 1 mL PBS containing calcium and magnesium for 3–5 minutes followed by another wash for 1–2 minutes with 1 mL of an aqueous solution containing 0.5% formaldehyde and 1% calcium chloride. The formaldehyde is aspirated and the NR absorbed by the cells is solvent-extracted with 2 mL of 1% acetic acid in 50% aqueous ethanol on a shaker platform for 1 hour. Two hundred microliter aliquots of each of the extracted NR solutions are transferred to a 96-well plate, and the optical density at 540 nm (OD_{540}) of each well is determined spectrophotometrically by a VMax microplate reader (Molecular Devices Corp.) making a blank correction to pretreated nylon mesh (without cells) which have been similarly incubated with NR. The mean OD_{540} of the untreated control wells is set to represent 100% viability. Results for each concentration are plotted as percent of untreated controls *vs.* concentration of test agent, and an NR-50 value (concentration of test agent that reduces NR uptake by 50% compared to untreated controls) is determined directly from the graph. The lower the NR-50 value, the more toxic the compound because less is required to achieve 50% killing of the cells on the mesh.

A simple colorimetric assay for phenotyping the major human thermostable phenol sulfotransferase (SULT1A1) using platelet cytosols

Drug Metabolism and Disposition 28: 1063-68 (2000).

Lynn T. Frame[1], Shogo Ozawa[2], Susan A. Nowell[5], Hsien-Chang Chou[2], Robert R. DeLongchamp[3], Daniel R. Doerge[4], Nicholas P. Lang[1,5], and Fred F. Kadlubar[2,5].

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Abstract. A thermostable phenol sulfotransferase, SULT1A1, has been implicated in numerous detoxification and bioactivation pathways; however, little is known regarding its endogenous function or its putative role in mediating risk for human environmental disease. A simple endpoint colorimetric assay is described that can be used for rapid phenotyping of SULT1A1 activity in human populations. The assay utilizes a microtiter-plate format and relatively small amounts of platelet cytosol-derived enzyme. The enzyme catalyzes the synthesis of 2-naphthylsulfate from 2-naphthol and 5′-phosphoadenosine 3′-phosphosulfate (PAPS), whereas addition of p-nitrophenyl sulfate to the assay contributes to an effective PAPS-regenerating system. In contrast to other sulfotransferase assay methods, 3′-phos-

phoadenosine 5´-phosphate (PAP) does not accumulate during the incubation to interfere with enzyme activity, but instead serves as a cofactor to cause the removal of sulfate from p-nitrophenyl sulfate to regenerate PAPS. This reaction concomitantly results in generation of p-nitrophenol that can be quantified colorimetrically at 405 nm (Σ = 18,200 M^{-1}) to give an indirect measure of sulfotransferase activity. Using platelet enzyme preparations from adult human subjects, sulfation rates of two prototypical thermostable phenol sulfotransferase substrates (2-naphthol and p-nitrophenol) and one thermolabile phenol sulfotransferase substrate (dopamine) were determined using standard radiochemical protocols. These data were then compared with results from the colorimetric assay using 2-naphthol as substrate. There was a good correlation between the phenotyping assay and radiochemical assays for both 2-naphthol sulfotransferase and p-nitrophenol sulfotransferase activity (r = 0.85 and 0.69, respectively). However, SULT1A1 activity was approximately 10 to 20 times higher with the colorimetric determination. As anticipated, there was no correlation between SULT1A1 activity and dopamine sulfotransferase activity (r = 0.07) in these human platelet preparations. This inexpensive and rapid method for phenotyping SULT1A1 activity may help investigators assess a role for this enzyme in disease susceptibility.

general methods

Absorbance

A microtiter-based assay for the detection of protein tyrosine kinase activity.

Anal. Biochem. 190: 249–253 (1990).

Jeffrey S. Cleaveland, Peter A. Kiener, David J. Hammond and Bernice Z. Schacter.

Bristol-Myers Squibb, Wallingford, CT 06492 USA.

Summary. A rapid, nonradioactive alternative assay has been developed to screen for protein tyrosine kinase (PTK) inhibitors. This assay uses a substrate polymer adsorbed to a microplate which can be phosphorylated by PTK. The amount of phosphotyrosine produced is determined in an enzyme-linked immunosorbent assay (ELISA) using an antibody to phosphotyrosine. Color development is shown to be dependent upon assay time, enzyme, ATP, and substrate concentrations. Specific PTK inhibitors reduced the total phospho-tyrosine incorporation into the substrate polymer. Results obtained in the ELISA compare with those obtained by direct phosphorylation of the substrate with [^{32}P]ATP.

Pathogenesis of *herpes simplex* virus-induced ocular immunoinflammatory lesions in B-cell-deficient mice

J. Virol. 74: 3517–24 (2000).

Shilpa P. Deshpande, Mei Zheng, Massoud Daheshia, and Barry T. Rouse.

Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37996-0845.

Methods: HSV ELISA. To assess the role of virus replication in lesion development, mice were infected on the cornea and 5 days later were given intravenously 300 µL of anti-HSV serum (36.5 µg/mL; HSV-specific IgG). Sera were collected from HSV-1 re-immunized BALB/c mice and checked for HSV-specific total IgG by standard enzyme-linked immunosorbent assay (ELISA) as described previously. Briefly, the ELISA plates were coated with 100 µL of HSV antigens or anti-mouse IgG (1 µg/mL; PharMingen) as a standard in carbonate buffer (pH 9.8) overnight at 4 °C. Serum samples were diluted 1:200 in phosphate-buffered saline (PBS) and run in triplicate with purified mouse IgG as a standard (PharMingen), followed by horseradish peroxidase-conjugated goat anti-mouse IgG (PharMingen). Quantification was performed with a SpectraMax microplate reader (Molecular Devices Corp.)

Luminescence

High sensitivity, wide dynamic range, horseradish peroxidase (HRP) ELISA using the LMax™ microplate luminometer

FULL TEXT →

Molecular Devices MAXline Application Note #41 (2000).

Evelyn McGown, Ph.D. and Michael Su, M.S.

Summary. Molecular Devices LMax microplate luminometer provides an easy and sensitive means of measuring chemiluminescent ELISAs in a microplate format. This particular work was done with a commercial kit for human TNF-α; however, excellent results would also be expected with other optimized HRP-based ELISA assays using luminol/peroxide substrate.

Methods: HRP assay. Materials included the Quantikine Human TNF-α Immunoassay kit (R&D Systems, Cat #DTA50). The kit contains all reagents and standards needed for the assay, including the polystyrene microplate (12 strips of 8 wells/strip) pre-treated with antibody against TNF-α.The kit instructions were followed for preparation of the reagents and substrate solution. The TNF-α stock standard was reconstituted with 0.5 mL deionized water, gently vortexed and allowed to stand for 15 minutes at ambient temperature. The working standards were prepared by making a serial 1:10 dilution of the stock standard in calibrator diluent (QDP5). The concentrations of the working standards ranged from 7000 pg/mL to 0.07 pg/mL.

After first putting 50 µL assay diluent (QD1-27) into wells of the pre-treated microplate (supplied with the kit), working standards of TNF-α and the blank (QDP5) were pipetted in triplicate (200 µL each). The kit instructions were followed for the subsequent aspiration, washing, incubation with conjugate, aspiration and washing steps. After the final step, the addition of substrate solution, the wells were covered with an adhesive strip and incubated with shaking at ambient temperature for 30 minutes. The adhesive cover was removed and the microplate was placed into the LMax. To ascertain the optimal integration time, the plate was read at 0.1, 1, 5 and 10 seconds per well.

protein quantitation

Absorbance

PathCheck® applied to measurement of protein solutions in the SpectraMax® Plus microplate spectrophotometer

FULL TEXT →

Molecular Devices MAXline Application Note #26.

Protein concentration is commonly estimated by measuring the absorbance at 280 nm (A_{280}) and calculating the concentration using the extinction coefficient of the protein. The procedure is feasible because extinction coefficients are typically obtained using standard cuvettes with a fixed 1 cm pathlength. Extinction-based protein assays can also be done in microplates, but they have not been widely used because of the inconvenience of compensating for variable pathlengths of samples in microplate wells. The introduction of the Molecular Devices' SpectraMax Plus microplate spectrophotometer marks the first time that photometric measurements made in a microplate format can be automatically normalized such that the resultant values are identical to those obtained using the corresponding solutions in a standard 1 cm pathlength cuvette. This application note outlines the use of the pathlength correction feature "PathCheck", compares absorbance results obtained in cuvettes and microplate, and gives an example in which PathCheck is applied to column chromatographic fractions and the elution profile is displayed.

Fluorescence

Using the NanoOrange Protein Quantitation Kit in the *f*Max microplate fluorometer

FULL TEXT →

Molecular Devices MAXline Application Note #22.

Summary. This application note describes how to use the NanoOrange® Protein Quantitation Kit from Molecular Probes in the *f*Max fluorescence microplate reader with SoftMax® Pro software from Molecular Devices Corporation. This assay is much more sensitive than traditional photometric methods such as A_{280}, BCA, Bradford, or Lowry assays in the microplate format. The dynamic range of this assay in microplate format is 100 ng/mL to 10 µg/mL. The assay is read with an excitation wavelength of 485 nm and an emission wavelength of 590 nm. Both the excitation and emission filters are standard on the *f*Max fluorescence microplate reader. The kit from Molecular Probes contains sufficient reagent for 2000 assays using a 200 µL volume. The data presented in this application note confirm the dynamic range and lower limit of detection described by Molecular Probes in their Application Note (MP 6666). The data presented here are preliminary and do not represent a fully optimized assay.

nucleic acid quantitation

Ultraviolet

DNA and RNA measurements in SpectraMax microplate spectrophotometers

FULL TEXT →

Molecular Devices MAXline Application Note #33 (1999).

Evelyn McGown, Ph.D.

Summary. This application note provides guidelines for optimizing DNA/RNA absorbance measurements in SpectraMax microplate spectrophotometers. Topics include recommendations for best quality DNA absorbance values, using Molecular Devices microplate spectrophotometers' PathCheck feature to normalize absorbance values to a 1-cm pathlength, options for eliminating microplate OD in PathCheck calculations, DNA/RNA estimation using absortivity, and using SoftMax® Pro to calculate DNA concentration.

UV absorbance measurements of DNA in microplates

Biotechniques 28: 60-64 (2000).

Evelyn L. McGown.

Molecular Devices Corporation, Sunnyvale, CA USA

Summary. With attention to technique, accurate and reproducible DNA measurements can be made easily in microplates. The lower limits of detection and quantitation are comparable to those obtained with semi-micro cuvettes in conventional spectrophotometers.

Fluorescence

Using the OliGreen® Oligonucleotide Quantitation Reagent in an *f*Max microplate fluorometer

FULL TEXT →

Molecular Devices MAXline Application Note #21.

Summary. This application note describes how to use OliGreen Oligonucleotide Quantitation Reagent from Molecular Probes in an *f*Max fluorescence microplate reader with SoftMax® Pro software (both from Molecular Devices Corporation). Single-stranded DNA and oligonucleotides are traditionally measured at 260 nm. OliGreen has two advantages over the traditional method: greater sensitivity and specificity. Molecular Probes describes a dynamic range of 1 ng/mL to 1 µg/mL and sensitivity of 200 pg/200 µL in their application note on the use of OliGreen (MP 7582 02/08/96). This assay is an endpoint assay read using an excitation wavelength of 485 nm and emission wavelength of 538 nm. A single concentration of the OliGreen dye is used for the entire dynamic range. The OliGreen kit provides enough reagent in a single kit to perform approximately 2000 samples in a volume of 200 µL each.

Luminescence

DNA quantitation in the LMax™ microplate luminometer

FULL TEXT →

Molecular Devices MAXline Application Note #37 (2000).

Evelyn McGown, Ph.D. and Michael Su, M.S.

Summary. The LMax microplate luminometer detects a lower limit of 5 pg DNA/well with the DNAQuant™ DNA quantitation system (Promega Corporation, Product No. K4000). These results are as good as, if not better than, the results obtained using a standard tube luminometer. The LMax microplate luminometer also offers the additional advantage that adding luciferin/luciferase reagent and the subsequent read can be automated so that variability due to differences in timing of the flash luminescence reaction can be eliminated.

SNPs: fluorescence

Measurement of molecular beacons in the Gemini spectrofluorometer

FULL TEXT →

Molecular Devices MAXline Application Note #36 (1999).

Evelyn McGown, Ph.D. and Michael Su, M.S.

Summary. This application note describes measurement of probe/target complexes with the Gemini microplate spectrofluorometer. Samples of molecular beacons and complementary targets were obtained from Research Genetics, Huntsville, Alabama. The microplates used were Nunc brand, black 384-well microplates, surface-treated to minimize binding (cell culture-treated). All samples were prepared in 10 mM Tris buffer, pH 8, containing 1 mM $MgCl_2$, and the final concentrations of beacon and target were 0.3 mM and 1.6 mM, respectively, in a total volume of 54 µL. The reaction mixtures were incubated at least 30 minutes at ambient temperature before measurement in a Gemini microplate spectrofluorometer. With its dual scanning monochromators, it is easy to optimize excitation and emission wavelengths for specific fluorophores and to customize the settings for combinations of probes. Mixtures of 2, 3, and (probably) 4 probes in a single mixture can be resolved.

Luminescence

Dual-luciferase quantitation in the LMax™ microplate luminometer

FULL TEXT →

Molecular Devices MAXline Application Note #39 (2000).

Evelyn McGown, Ph.D. and Michael Su, M.S.

Summary. Colorimetric reporter assays have been widely used, but bioluminescent reporter systems, especially using firefly luciferase, are becoming increasingly popular because of their speed, sensitivity and wide dynamic range. Promega Corporation has recently introduced their Dual-Luciferase® Reporter Assay System which utilizes the firefly (*Photinus pyralis*) and Renilla (*Renilla reniformis*) luciferases. Because the enzymes have different substrate requirements, they can both be measured in a single reaction mixture. The firefly luciferase is typically used as the experimental reporter, and the renilla luciferase serves as the control. Firefly luciferase enzyme catalyzes the oxidation of luciferin with the concomitant release of light. The reaction requires ATP, Mg^{+2} and O_2. Renilla luciferase catalyzes the O_2-dependent oxidation of coelenterate luciferin (coelenterazine) but does not require Mg or ATP. Both reactions can easily be measured in Molecular Devices' LMax microplate luminometer. An example of results is presented in this application note.

instrumentation analysis

Absorbance

Using the SpectraMax® Plus for USP dissolution calibration

FULL TEXT →

Molecular Devices MAXline Application Note #27.

Summary. Calibration of a dissolution apparatus is necessary to produce accurate results for dissolution testing of pharmaceutical dosage forms and to allow comparison between batches and between laboratories. USP requirements for calibration are given in the Apparatus Suitability Test in USP <711> Dissolution. USP <711> specifies that both prednisone and salicylic acid are to be measured by UV spectrophotometry (242 nm and 296 nm) by comparison to a standard solution of known concentration. (Compendial requirements for UV spectrophotometry are given in USP <851> Spectrophotometry and Light Scattering). This application note describes how spectrophotometric analyses for dissolution calibration can be quickly and conveniently carried out in a 96-well format using the SpectraMax Plus microplate spectrophotometer. The PathCheck feature of the SpectraMax Plus automatically corrects the results to a 1 cm pathlength; therefore, samples read in a microplate give the same values that they would if read in a standard cuvette. Accurate pipetting of the samples into the wells is not necessary; indeed, PathCheck corrects for pipetting errors. Using flexible custom formulas and the spreadsheet capability of SoftMax Pro® software, the data are calculated automatically, and final results are displayed as overall PASS/FAIL for each apparatus.

Verifying multichannel pipettor performance with standard dispense solutions in the SpectraMax® Plus

FULL TEXT →

Molecular Devices MAXline Application Note #28.

Summary. Two common methods for checking precision and accuracy of pipettors are gravimetric and spectrophotometric. Ideally, pipettor performance should be checked using the actual dispense reagent as the calibrator solution. However, most dispense reagents are colorless, and an absorbance measurement cannot be obtained in the visible range (400–750 nm) without the addition of a dye. The SpectraMax Plus microplate spectrophotometer uses the near infrared absorbance of water to determine the pathlength in each well, which is proportional to volume. The dispensed volumes are calculated by reference to a pathlength/volume standard curve (prepared separately with a certified pipettor). The standard curve can be prepared once for a given dispense reagent and batch of microplates and stored for subsequent use. This application note describes how to check the performance of multichannel pipettors with the SpectraMax Plus. Pathlengths in the wells are measured directly and volumes are calculated automatically. The performance can be checked using the same buffers or

reagents that are being dispensed by the pipettor, eliminating possible discrepancies in pipettor performance between normal dispense reagent and calibrator solution. Because initial separate calibration of one channel is not necessary, the method is suitable for checking performance of automated pipetting stations, including 96-channel pipettors.

UV absorbance measurements in SpectraMax microplate spectrophotometers

FULL TEXT →

Molecular Devices MAXline Application Note #32 (1999).

Evelyn McGown, Ph.D.

Summary. Despite the huge popularity of microplates, many people experience difficulty when adapting assays to them. One source of confusion is the fact that samples read in a cuvette and in a microplate do not have identical raw absorbance values because of differing optical pathlengths. Also, microplates are susceptible to surface effects including floating particulates, foaming and variable meniscus formation. The susceptibility to particulates has increased in recent years because modern microplate spectrophotometers such as SpectraMax are designed to accommodate microplates with smaller wells and thus have smaller beams than older instruments. All of these factors cause microplates to demand more attention to careful technique to get accurate absorbance results. Especially in the UV spectral range, appropriate procedures should be followed to avoid poor reproducibility and unsatisfactory sensitivity. This application note provides guidelines for optimizing UV measurements in SpectraMax microplate spectrophotometers, particularly measurements of proteins at 280 nm and nucleic acids at 260 nm.

Fluorescence

Selecting excitation and emission wavelengths using the Gemini microplate spectrofluorometer — basic principles

FULL TEXT →

Molecular Devices MAXline Application Note #30.

Summary. This application note gives a basic procedure for optimization of excitation and emission wavelengths of the Gemini microplate spectrophotometer. An example is given for application of the procedure to a fluorophore with a relatively large Stokes' shift (quinine).

The first step in developing fluorescence analysis methodology is to select the excitation wavelength. The next step is to select the optimum combination of emission wavelength and cutoff filter that gives the highest possible signal/background ratio. Generally, samples containing 10^{-8} M to 10^{-6} M fluorophore will give sufficient signal. For optimal signal/background results, data should be acquired with the highest PMT (voltage) setting. Thus, the fluorophore concentration should be low

enough that the emission scans can be done with high PMT without saturating the detector.

Optimizing excitation and emission wavelengths for narrow Stokes' shift fluorophores using the Gemini and SoftMax® Pro

FULL TEXT →

Molecular Devices MAXline Application Note #31.

Summary. When optimizing excitation and emission wavelengths, the easiest case is when the fluorophore has a relatively large Stokes' shift (> 80 nm); the optimal wavelengths are those giving maximal signal, assuming no background interference. If the Stokes' shift is narrow, the selection/optimization process is less straightforward because scattered excitation light interferes with the fluorescent signal. This application note gives details of an optimization procedure for fluorophores with Stokes' shifts less than 80 nm and includes custom SoftMax Pro formulas to assist in the selection process. The fluorophore used in this example is fluorescein.

Sensitivity test for Molecular Devices microplate fluorometers

FULL TEXT →

Molecular Devices MAXline Application Note #45 (2001).

Evelyn McGown, Ph.D. and Michael Su, M.S.

Summary. This application note contains the recommended procedure for verifying the sensitivity of the Gemini, Gemini XS, the FlexStation™ and the *f*Max® in fluorescence mode (top read). If they are functioning normally, and there is no operator error, this test will demonstrate the sensitivity of the instrument in 96-well format. For the purposes of this application note, sensitivity is defined as the amount of fluorescein (in femtomoles/well) giving a signal equal to or greater than 3 standard deviations of the signal from the background wells (i.e., the wells filled with buffer.) The test is done with 0.2 mL/well, so the fmol/well expression can be translated to concentration (picomolar) by multiplying by 5. The calculations are made from fluorescence measurements of 2 microplates, one filled with 100 nM fluorescein and the other filled with buffer.

AquaMax™ 1536 dispenser accuracy and precision testing

FULL TEXT →

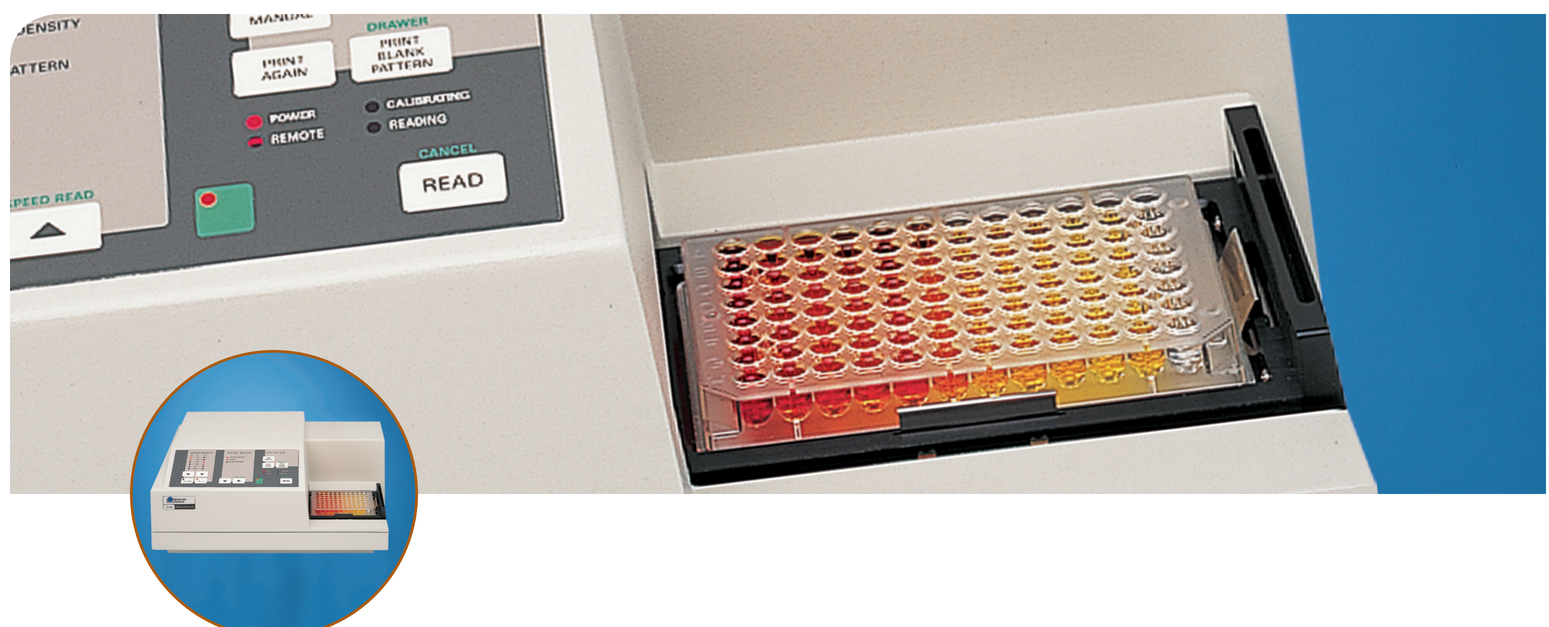
Molecular Devices AquaMax Application Note #1 (2000).

Gayle Teixeira.

Summary. This application note is intended to provide a basic procedure for testing accuracy and precision. The AquaMax 1536 dispenser has been designed to achieve a 1.0 µL volume dispense with an accuracy of ± 5.0% and precision of less than 10% CV.

EMax® and VMax® microplate readers

SIMPLE, RELIABLE ENDPOINT AND KINETIC ANALYSIS



- → READ LOW AND HIGH CONCENTRATIONS
- → RUGGED, RELIABLE, WITH REPRODUCIBLE RESULTS
- → EASY TO USE
- → POWERFUL SOFTMAX PRO SOFTWARE

The EMax and VMax microplate readers are designed for reproducible results and rugged performance. Applications for the EMax microplate reader include endpoint ELISA, total protein assays, platelet aggregation studies, endpoint LAL, cytokine determination and quantitation of cytoproliferation by MTT reduction or by staining with crystal violet. The VMax microplate reader builds on the capabilities of the EMax by adding applications based on kinetic measurements such as enzyme studies and kinetic based ELISA measurements.

PERFORMANCE HIGHLIGHTS

The readers are capable of reading up to six plates a minute using a five-second read time. Single- or dual-wavelength measurements can be made and as many blank wells as required can be located anywhere on the plate. The AUTOMIX feature ensures adequate mixing before and between readings for accurate readings of solid-phase reactions such as ELISA assays.

SoftMax® Pro microplate data analysis software provides integrated instrument control and data analysis for both Windows®- and Macintosh®-based computers. Software validation and FDA 21 CFR Part 11 compliance tools are also available. With SoftMax Pro, data can be visualized as gray scales, kinetic plots or reaction rates. Powerful curve-fitting routines and statistical analysis are included.

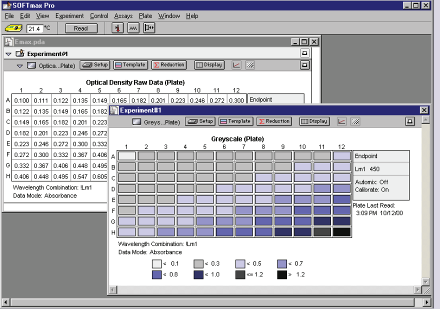
SINGLE-WELL ILLUMINATION

The single-well illumination design of the EMax and VMax readers sequentially illuminates each well with 100% of the available light, resulting in an optimized signal-to-noise ratio. The light-tight reading chamber eliminates stray light for linearity to higher OD readings.

STATIONARY PLATE DESIGN

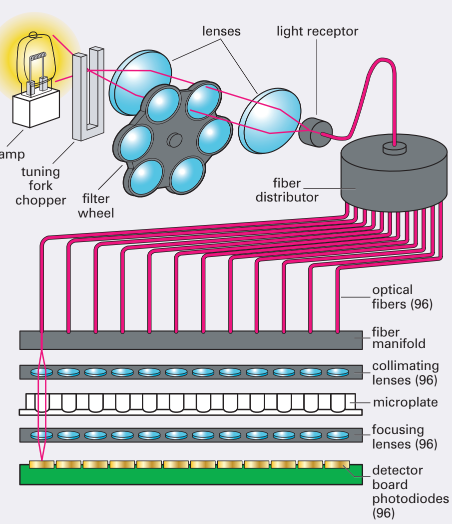
The stationary plate design yields data comparable to high-quality spectrophotometers because the microplate does not move during the read. There is no moving meniscus, so the pathlength is always the same. An additional benefit of stationary plate reading is a reduction in moving parts, contributing to a robust mean time between failure (MTBF) of 20,000 hours.

results at-a-glance



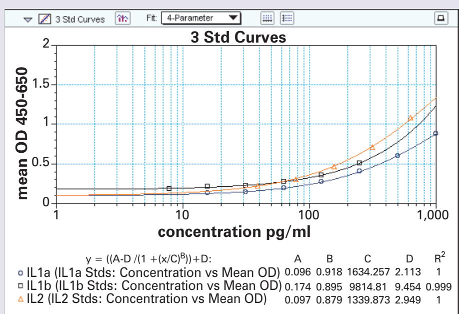
The grayscale option presents raw data in eight shades of gray for a quick visual check of assay results.

sequential illumination optics



The light distribution design illuminates each well sequentially with 100% of the incident light, eliminating cross-talk.

flexible data analysis



Multiple standard curves from different microplates can be plotted on one graph with SoftMax Pro microplate analysis software.

TECHNICAL SPECIFICATIONS

Performance Specifications

Measurement range:	400–750 nm 0–4.0 OD
Photometric resolution:	0.001 OD
Read modes (EMax):	Endpoint
Read modes (VMax):	Endpoint, kinetic
Read times (96-well microplate):	
Endpoint read-only:	5 seconds
Calibration:	5 seconds
Min. kinetic interval:*	5 seconds
AUTOMIX:	VMax only
Cross-talk control:	Single-well sequential illumination of microplate wells eliminates well-to-well cross-talk
Calculated MTBF:	> 20,000 hours
Optical alignment:	No adjustment required during instrument lifetime

* VMax reader only.

General Specifications

Dimensions (in.):	7.38 (H) x 18.25 (W) x 13.38 (D)
Dimensions (cm):	19 (H) x 47 (W) x 34 (D)
Weight:	22 lbs. (10 kg)
Power consumption:	< 35 watts
Power source:	90–250 VAC, 50/60 Hz

ORDERING INFORMATION

EMax Endpoint Microplate Reader
Contact your Molecular Devices sales representative for configuration options.

- → EMax endpoint microplate reader
- → SoftMax Pro microplate analysis software
- → SoftMax Pro validation package
- → IQ/OQ/PQ validation manual

VMax Endpoint/Kinetic Microplate Reader
Contact your Molecular Devices sales representative for configuration options.

- → VMax endpoint/kinetic microplate reader
- → SoftMax Pro microplate analysis software
- → SoftMax Pro validation package
- → IQ/OQ/PQ validation manual

SALES OFFICES

- → USA +1-800-635-5577
- → UK +44-118-944-8000
- → Germany +49-89-9605-880
- → Japan +81-3-5282-5261
- → South Korea +82-2-3471-9531
- → China +86-21-6887-8820

Check our web site for a current listing of our worldwide distributors.
www.moleculardevices.com

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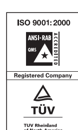
Specifications subject to change without notice.



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Changing the Lamp in Series I MAXline Microplate Readers

Warnings and Cautions

BIOHAZARD: Always wear gloves when performing any type of service, especially if it involves potential contact with spilled fluids or fluid residues of any kind.

WARNING: Always turn the instrument off and remove the power cord from the back prior to any maintenance or installation operation.

WARNING: Use only the tools described to perform the steps defined in the instructions.

WARNING: Never perform any operation on the instrument in an environment where liquids or potentially damaging gases are present.

WARNING: Risk of electrical shock, Refer servicing to qualified personnel.

CAUTION: Use of organic solvents (such as dichloromethane) may cause harm to the fiber optics in the microplate readers. Extreme caution is advised when using organic solvents. Always use a plate lid and avoid placing a plate containing these materials in the microplate reader for prolonged periods of time. Damage caused by the use of incompatible or aggressive solvents is NOT covered by the instrument warranty.

CAUTION: Never touch any of the fiber optic cables or their housing, manifold, or rotor connections. These fibers are extremely delicate and critical to the performance of the instrument.

CAUTION: Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so could cause misalignment and possibly void warranty.

CAUTION: Never touch the surfaces of the interference filters or optical lenses.

Changing the Lamp

Tmax

Instructions for THERMOmax

1) Switch the instrument off. Remove the power cord from the wall socket and then from the instrument's power port.

2) Turn the instrument around so you can reach the back of it easily. Remove the printer cable and/or computer cable (if connected) from the back of the instrument (it isn't essential to do this, but it does make it easier to change the lamp).

3) On the right hand side of the rear panel (as you face the back of the instrument) is a metal cover secured by two spring screws. Underneath the cover is the lamp assembly. Loosen the screws and gently slide the cover to the right, then pull it towards you to disengage it from the instrument (see Figure 1).

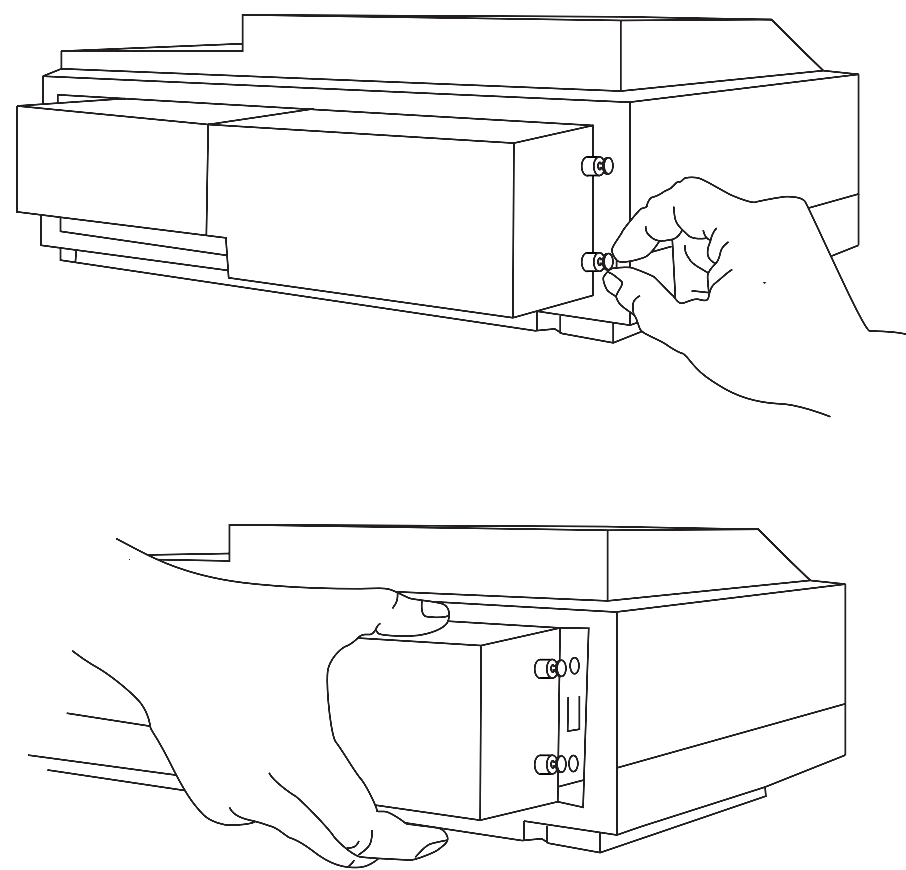

Figure 1: Removing the Lamp Cover

Examine Figure 2. Locate the lamp ejection lever on the right side of the lamp assembly. Gently depress the lever, then grasp the lamp by the white ceramic base as it is ejected.

⚠ **WARNING:** Do not touch the lamp bulb while removing the lamp! The bulb may be hot.

Discard the burned-out lamp.

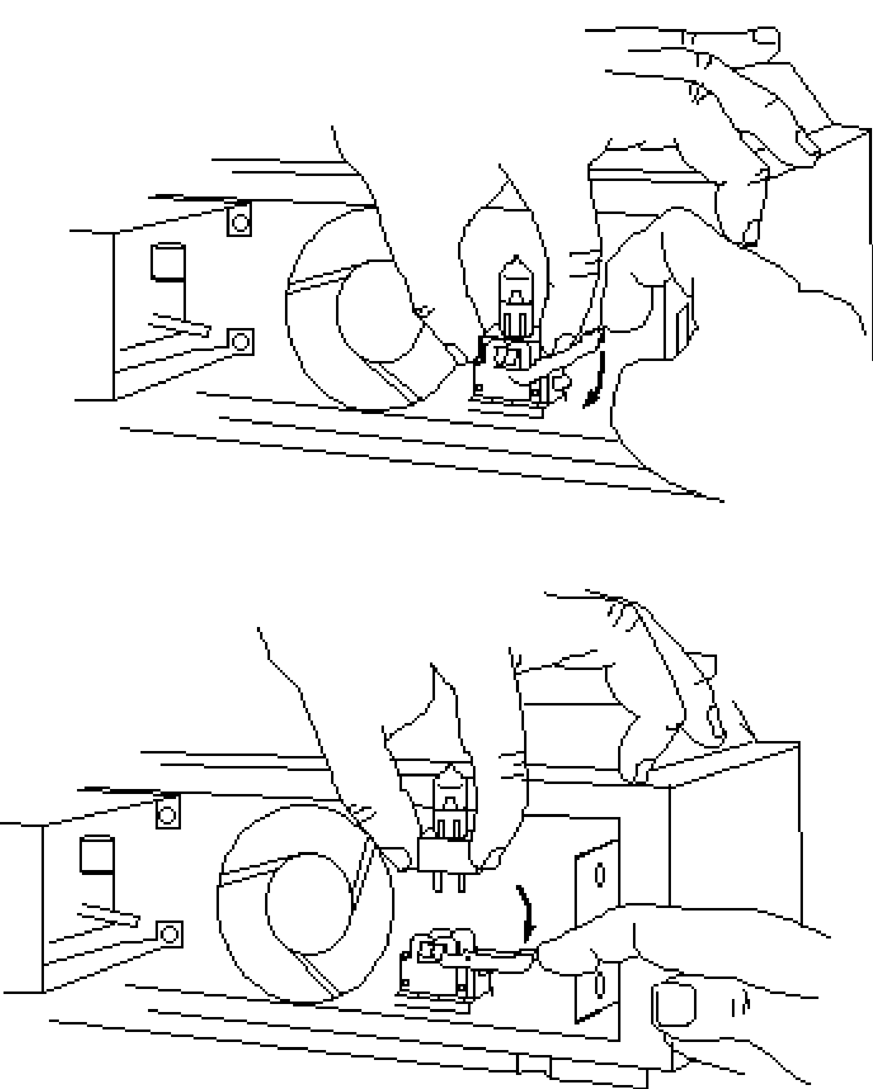

Figure 2: Removing the Burned-Out Lamp

4) Pick up the new lamp by its ceramic base.

⚠ **CAUTION:** Do not touch the glass portion of the new lamp, because fingerprints will degrade the lamp's performance.

5) Note that the two pins that protrude from the base are of different thicknesses, and that the corresponding sockets for the pins are of different diameters. Orient the pins in the correct sockets and gently push the new lamp into place (Figure 3). The ejection lever will automatically move to its original position.

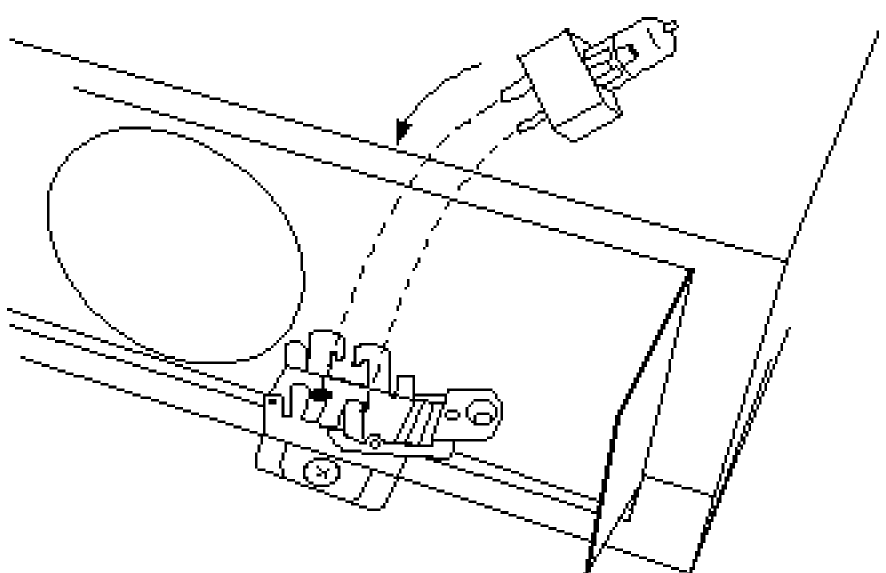

Figure 3: Installing the New Lamp

6) Reposition the metal cover over the lamp, then tighten the spring screws.
7) Reconnect the power cable to the instrument and the wall socket. If using the instrument with a computer/printer, reconnect their cables to the instrument.
8) Turn on the instrument (and computer, if using one), then test the new lamp by performing an endpoint read. Use the lowest available wavelength to ensure the most rigorous test of the new lamp.

Instructions for Emax and Vmax

1) Switch the instrument off. Remove the power cord from the wall socket and then from the instrument's power port.
2) Turn the instrument around so you can reach the back of it easily. Remove the printer cable and/or computer cable (if connected) from the back of the instrument (it isn't essential to do this, but it does make it easier to change the lamp).
3) Turn the instrument around so you can reach the back of it easily.
4) On the right hand side of the rear panel (as you face the back of the instrument) is a metal plate secured by two screws. The plate covers the lamp assembly (see Figure 4). Use a Phillips screwdriver to remove the screws, then remove the plate from the instrument.

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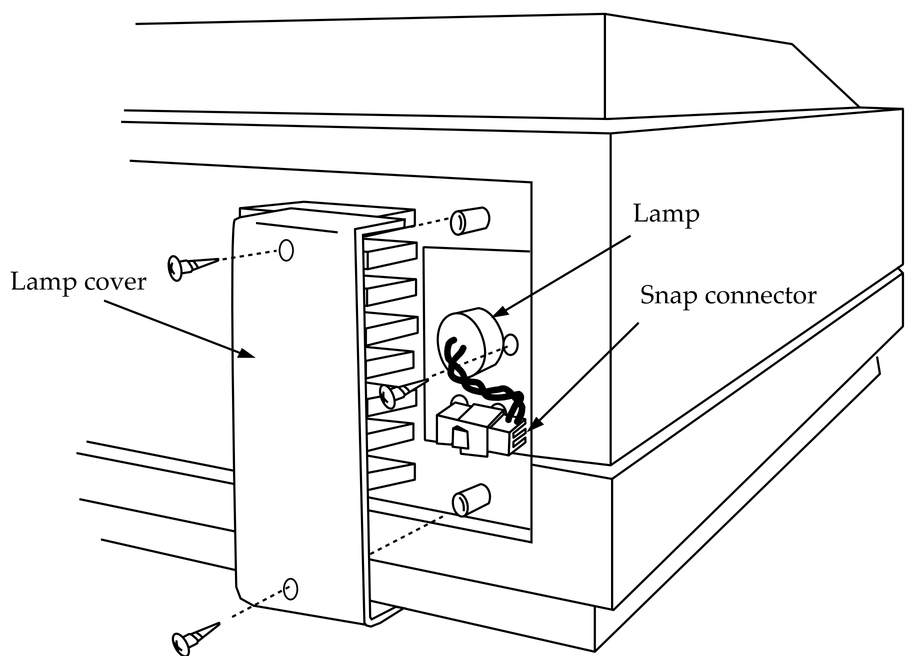

Figure 4: Removing the Lamp Cover (Emax/Vmax)

5) Remove the lamp screw (on the right side of the lamp, refer to Figure 4) then gently pull the connecting wires to remove the lamp from the body of the instrument.

6) Firmly grasp the snap connector beside the snap, then push the snap down with your thumb and pull the male and female halves of the connector apart (see Figure 5.

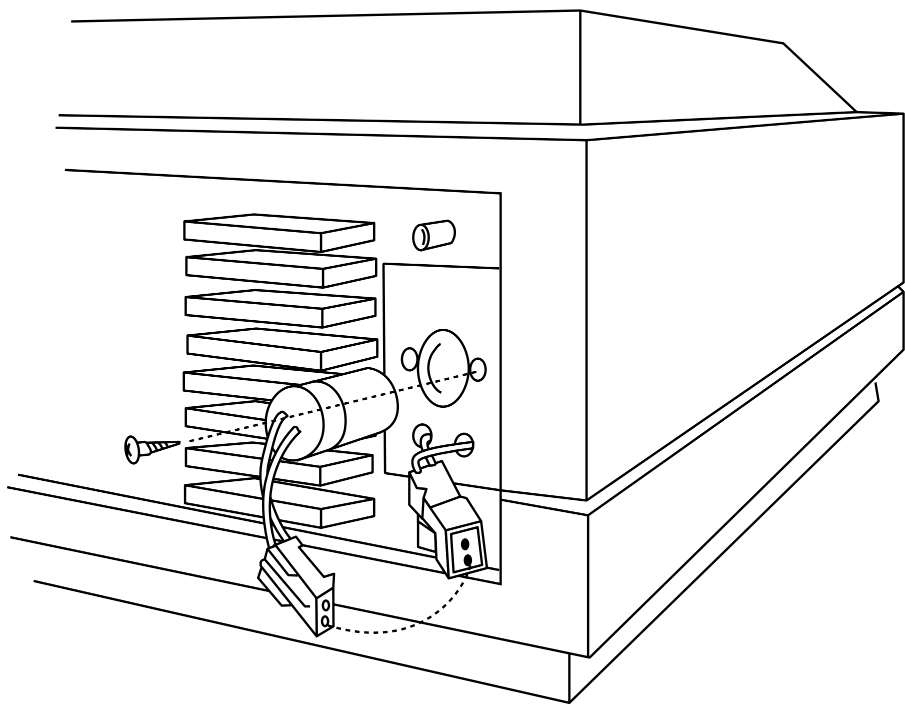

Figure 5: Removing the Burned-Out Lamp (Emax/Vmax)

7) Discard the burned-out lamp, then connect the new lamp to the snap connector.

8) Position the new lamp in the socket in the instrument, then secure it with the lamp screw.

9) Replace the lamp cover and secure it with the two Phillips screws. It's ok to bend the lamp wires to the side so that the cover fits.

10) Reconnect the power cable to the instrument and the wall socket. If using the instrument with a computer/printer, reconnect their cables to the instrument.

11) Turn on the instrument (and the computer, if using one), and then test the new lamp by performing an endpoint read. Use the lowest available wavelength to ensure the most rigorous test of the new lamp.

Changing the Fuse in Series I MAXline Microplate Readers

Warnings and Cautions

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WARNING: Never perform any operation on the instrument in an environment where liquids or potentially damaging gases are present.

WARNING: Risk of electrical shock, Refer servicing to qualified personnel.

CAUTION: Use of organic solvents (such as dichloromethane) may cause harm to the fiber optics in the microplate readers. Extreme caution is advised when using organic solvents. Always use a plate lid and avoid placing a plate containing these materials in the microplate reader for prolonged periods of time. Damage caused by the use of incompatible or aggressive solvents is NOT covered by the instrument warranty.

CAUTION: Never touch any of the fiber optic cables or their housing, manifold, or rotor connections. These fibers are extremely delicate and critical to the performance of the instrument.

CAUTION: Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so could cause misalignment and possibly void warranty.

CAUTION: Never touch the surfaces of the interference filters or optical lenses.

Changing the Fuse

Fuses burn out occasionally and need replacement. If upon switching the instrument on you have no indication that the instrument is getting power (power light and other control panel lights not on), first check to see that the power cord is securely plugged in to a functioning power outlet and to the power port at the rear of the MAXline Microplate Reader. If power failed while the instrument was already on, check to ensure the power cord has not been loosened or disconnected and that power to the power outlet has not been cut off. If these checks fail to remedy the loss of power, follow the steps listed below and, if necessary, replace the fuses. Spare fuses are shipped with the instrument in the original carton. They may be taped to the back of the MAXline Microplate Reader.

NOTE: If a fuse has blown in the instrument, Molecular Devices recommends removing and reinstalling both fuses, not just one, to ensure continued proper operation.

If you no longer have spare fuses, new ones may be obtained from Molecular Devices. For THERMOmax™, order a 3AT 5 mm x 20 mm (SloBlo) fuse; for Emax® and Vmax®, order a 1AT 5 mm x 20 mm (SloBlo) fuse.

1) Switch power to the instrument off and then remove the power cord from the outlet and from the microplate reader power cord receptacle.
2) Remove the printer cable and computer cable (if connected) from the back of the MAXline Microplate Reader.
3) Turn the instrument around for easy access to the rear panel.
4) On the left-hand side of the rear panel (viewed from the back) is the power cord receptacle. As shown in Figure 1, insert a small, flat-bladed screwdriver into the slot above the switch at the top of the black plastic cover. Gently pry the cover open. The cover will hinge down, allowing you to see the fusebox inside.

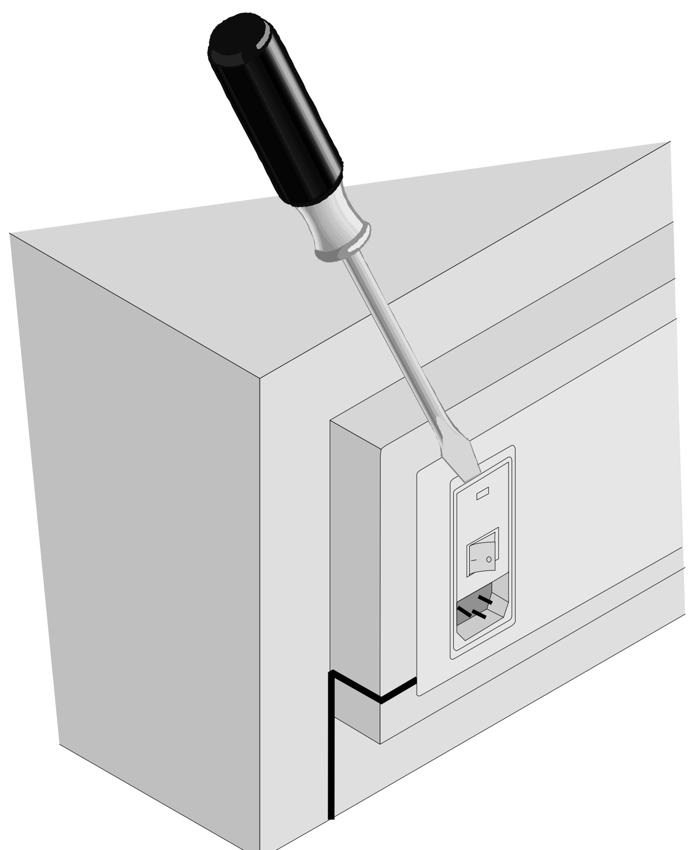

Figure 1 : Pry Open the Fuse Box Cover

5) Insert the blade of the screwdriver into the small slot on the top front of the fuse box. Pull the fusebox toward you, out of the instrument. When removed, the fuse assembly will appear as shown in Figure 2.

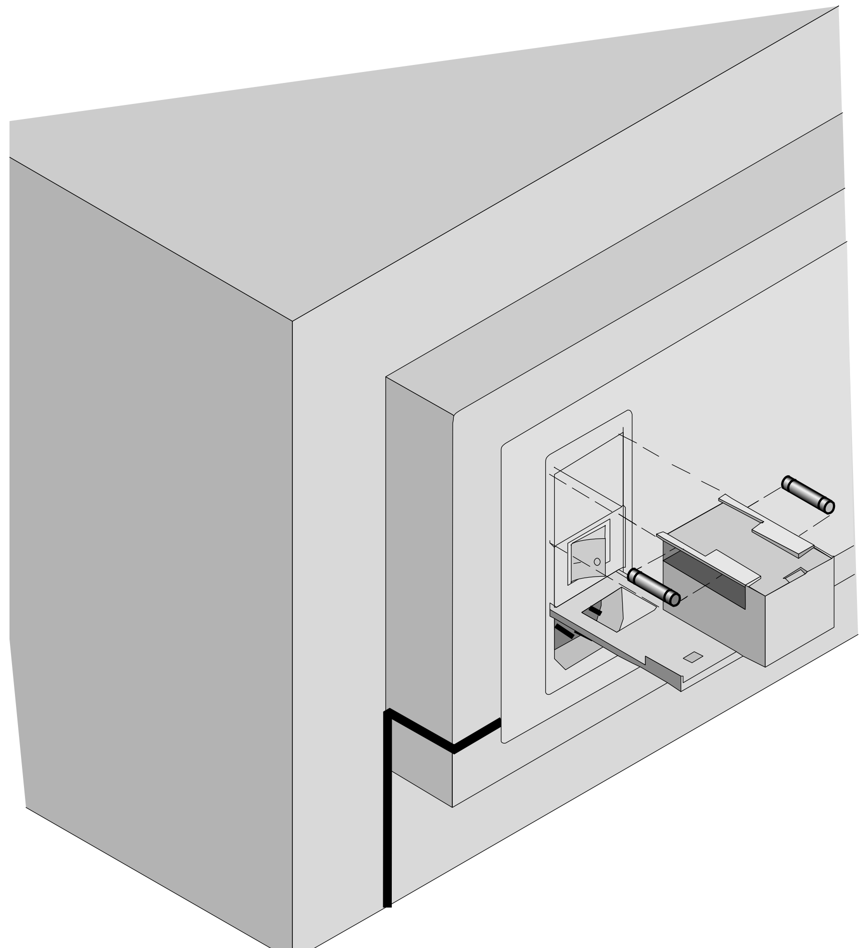

Figure 2 : Removing the Fuse Box

6) The fuses are located on the top sides of the holder. While it is possible that only one fuse may have blown, Molecular Devices recommends that you replace both fuses to ensure continued proper operation. Pull both fuses out of the holder and discard them.

7) Insert new SloBlo-rated fuses into the fuse holder. Either end of the fuse may be forward.

8) Insert the fuse holder into the fuse box, making sure that the fuses are closest to the top of the holder and the removal slot is toward you. Slide the fuse holder all the way into the box.

9) Lift the cover and press it into place, making sure the cover snaps closed.

10) Reconnect the power cord to the instrument and to the wall outlet and reconnect other cables previously disconnected.

Removing the Drawer Subplate

Tmax

The microplate drawer subplate has been designed and developed for the THERMOmax microplate reader to maintain the temperature uniformity of the microplate. Without the subplate in the microplate drawer, the temperature across the microplate may not be uniform. The microplate drawer subplate can be removed if it interferes with the secure placement of a microplate in the drawer or to clean the drawer carriage and the subplate.

Turn the THERMOmax on and the INCUBATOR off. If the drawer is closed, the microplate drawer can be opened by touching the DRAWER key. Place the threaded subplate removal tool in the A1 position of the subplate. That position is marked on the drawer carriage. Turn the subplate removal tool 3 full turns until it holds the subplate securely. With one hand, compress the spring nearest you on the microplate drawer and, with the other hand on the subplate removal tool, pull the subplate out of the drawer.

To return the microplate subplate to the drawer, open the microplate drawer of the THERMOmax. Place the subplate in the microplate drawer matching the notches with the microplate drawer springs. Press the subplate into the drawer.

Installing Optional Optical Filters in Series I MAXline Microplate Readers

Warnings and Cautions

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⚠ **CAUTION:** Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so could cause misalignment and possibly void warranty.

⚠ **CAUTION:** Never touch the surfaces of the interference filters or optical lenses.

Installing Optional Optical Filters

If you need additional optical filters not already installed on your instrument, order these directly from Molecular Devices. Our toll free number is (800) 635-5577. International calls may be placed to (408) 747-1700, or a telefacsimile sent to (408) 747-3602 or (408) 747-3601. Depending on the type of instrument you are using, standard filters having a 10-nm bandwidth can be ordered as follows:

- For THERMOmax™, between 340 nm and 750 nm;
- For Emax® and Vmax®, between 400 nm and 750 nm.

Filters having a 1-nm bandwidth can be special ordered for any wavelength within the overall instrument parameters given above (i.e., between 340 nm and 750 nm for THERMOmax, and between 400 nm and 750 nm for Emax and Vmax). Contact Molecular Devices for more information regarding ordering a special optical filter.

When your new filter(s) has arrived, please carefully review the installation instructions before installing the filter(s).

Tools: You will need a 3/32-inch hex wrench and a medium Phillips-head screwdriver to install your new filter(s).

I. Prepare the Microplate Reader

1) It is necessary that the drawer be empty and fully closed before commencing installation. If it is not, turn on the instrument, and after the drawer is completely out and any microplate has been removed, touch the [SHIFT] key and then the [PRINT BLANK PATTERN] key. This will close the drawer. When the drawer is all the way in, switch the power off so that the drawer remains in.

2) Unplug the power cord from the wall socket and from the rear panel of the instrument. Also, disconnect the printer or computer cable from the rear panel of the microplate reader. Make sure you have space behind the instrument approximately equal to the front to back width of the instrument.

3) Lay out a soft cloth in the space behind the instrument. Gently lift the instrument upward and turn it over onto the soft cloth so it is lying upside-down on the top casing as shown in Figure 7.

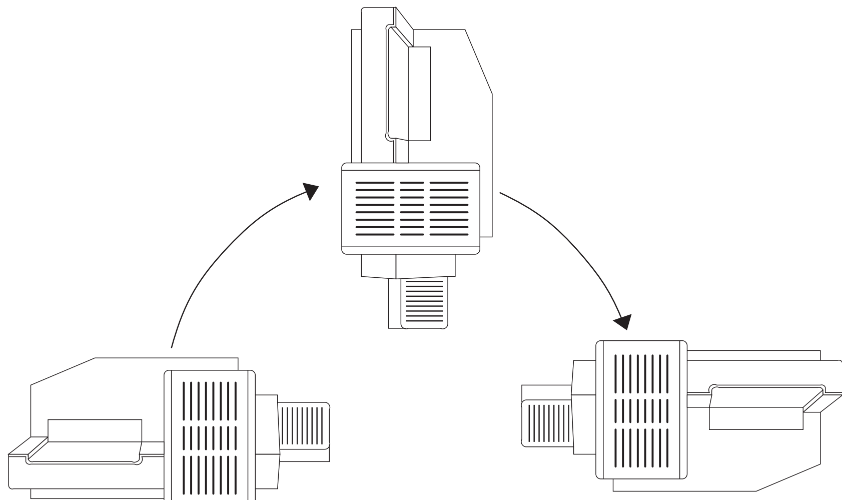

Figure 7: Turning the MAXline Microplate Reader Over (THERMOmax Shown)

4) Locate the seven silver topped screws as shown in Figure 8. Using a Phillips screwdriver, remove these seven screws from their slots on the bottom of the instrument. DO NOT LOOSEN OR REMOVE ANY BLACK TOPPED SCREWS. Note that two of the screws (5 and 6) are shorter than the other five. Put all seven screws in a secure location.

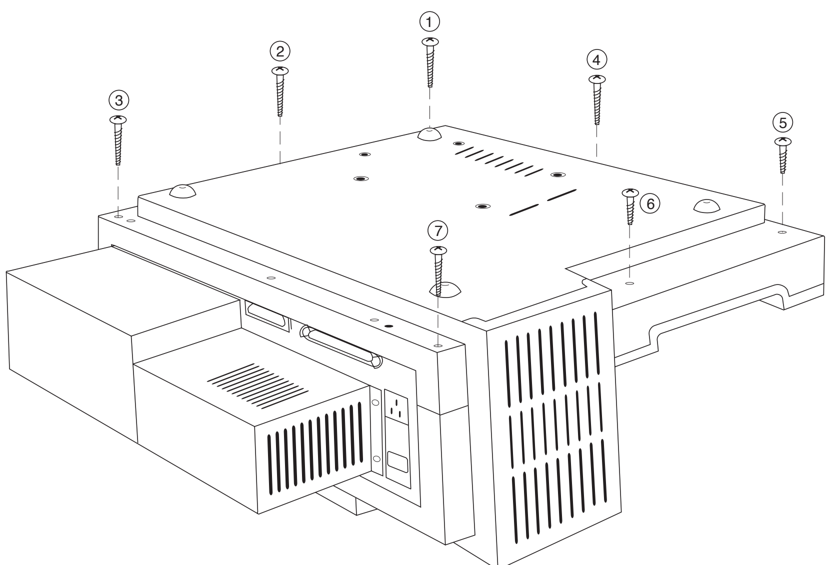

Figure 8: Underside of the Microplate Reader. This illustration shows the location of the seven screws that need to be removed before separating the top and bottom halves of the casing. Note: depending on the model you have, the back of the plate reader may not look exactly like this.

5) Firmly holding the upper and lower portions of the case together, turn the instrument back over to the normal operating position.

6) Examine Figure 9 before performing this step. Carefully lift the upper portion of the MAXline Microplate Reader about eight inches straight up over the lower portion. Then move the upper portion straight back behind the lower portion and place it down.

⚠ **CAUTION:** Do not attempt to separate the two casing halves by more than 8 inches. A flat ribbon cable connects the lower portion of the instrument to the control panel. Do not attempt to disconnect this ribbon cable.

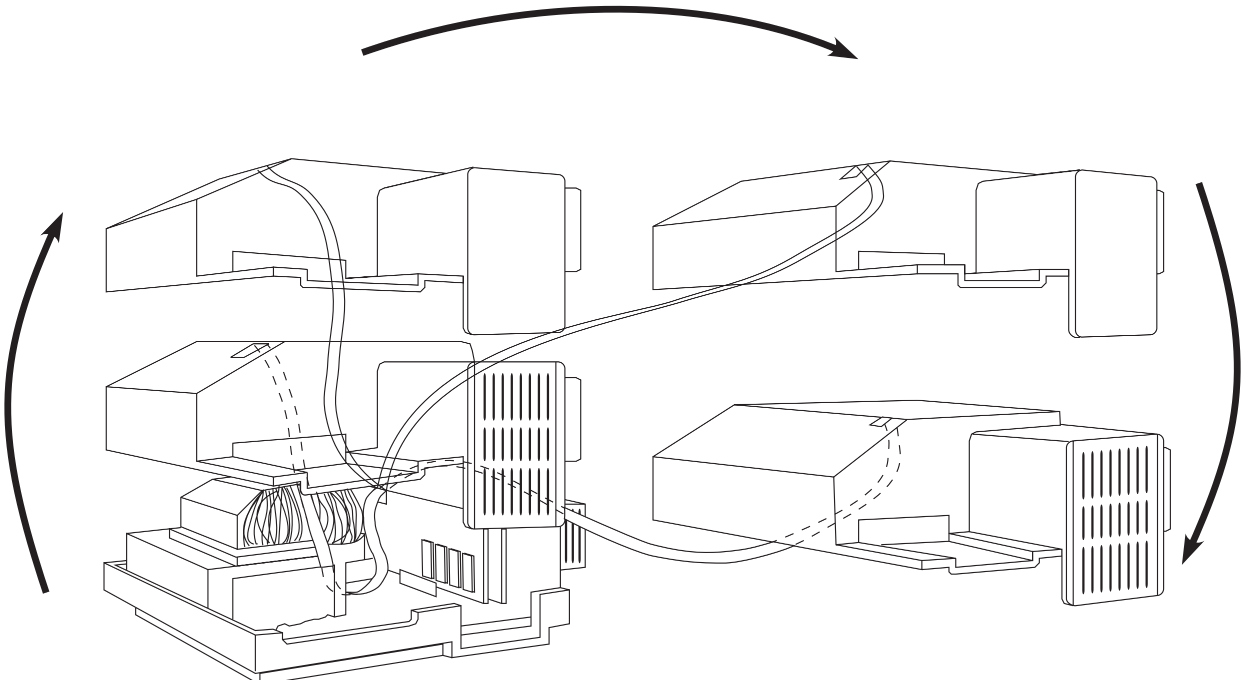

Figure 9: Separating the MAXline Microplate Reader Casing. Note the attachment of the ribbon cable and hence the importance of keeping the casing halves close together.

7) Locate the light source assembly at the rear left-hand side of the instrument as viewed from the front of the microplate reader.

NOTE: If installing a filter into an Emax or Vmax, the filter wheel housing will be clearly visible—you can skip to step 1 of the next section (Section II). If installing the filter into a THERMOmax, please follow steps 8 and 9, below.

Tmax

8) For the THERMOmax, unfasten the spring-loaded holding clasps on the top and left side of the light chamber assembly as shown in Figure 10.

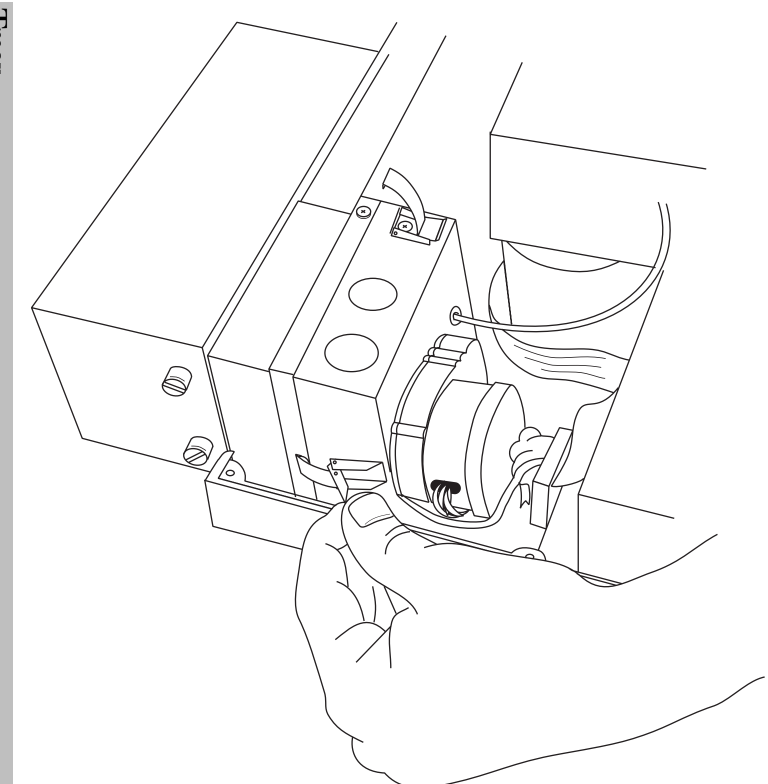

Figure 10: Light Source Assembly Spring Clamps

9) Grasp the front portion (nearest you) of the light source and gently slide it about two inches toward the front of the instrument as shown in Figure 11.

NOTE: The metal block of the light source is on a rail that curves inward slightly. This is normal.

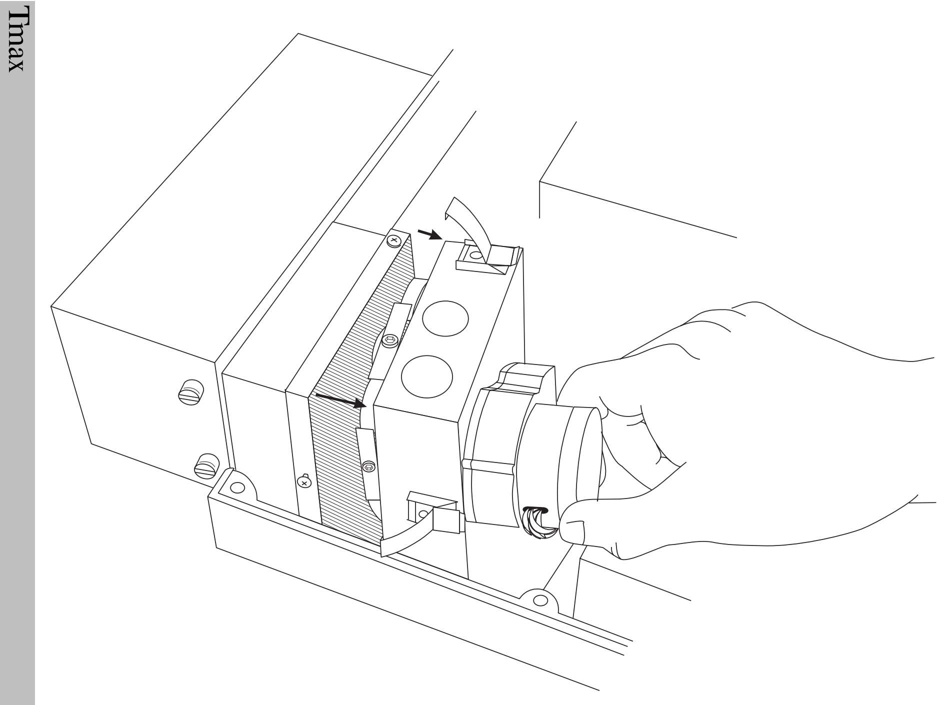

Figure 11: Opening the Light Source Front Cover

II. Install the Filter

1) The filter wheel housing and the filter wheel will be clearly visible. Do not touch the surfaces of the filters. Touching only the perimeter of the filter wheel, turn the wheel so that the filter slot with the black plastic disk (or the filter you are replacing) is in the top or highest position of the filter wheel.

2) Using a 3/32-inch hex wrench, LOOSEN the hex-head screw securing the bracket over the appropriate filter slot (about two full turns). Refer to Figure 12.

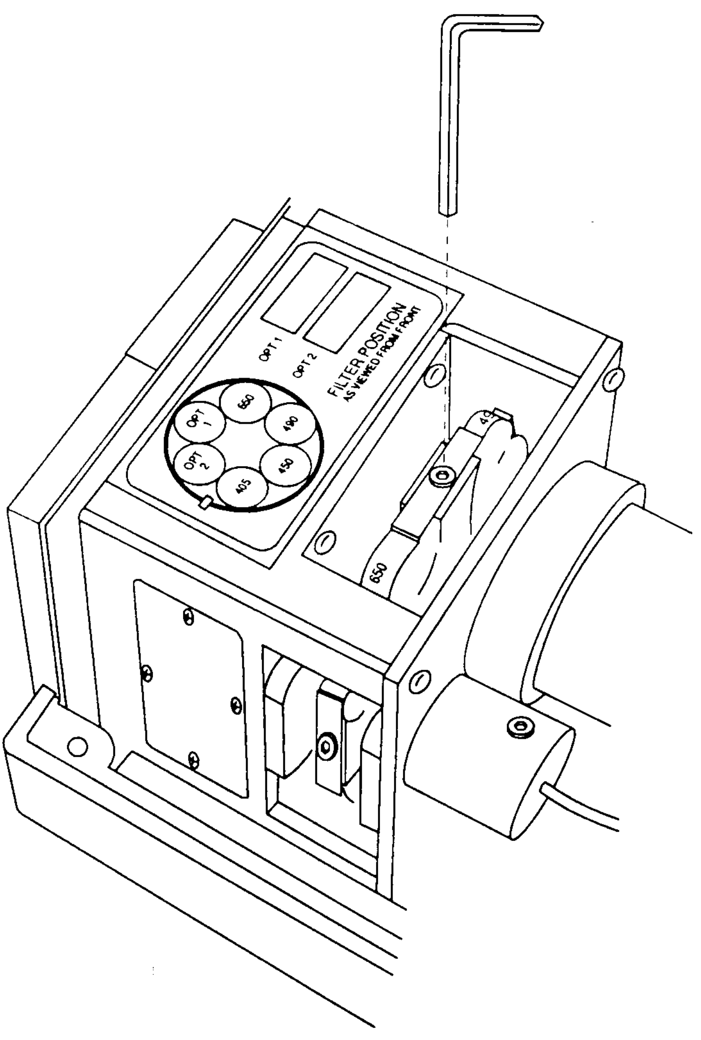

Figure 12: Filter Wheel Housing

3) DO NOT REMOVE THIS SCREW OR THE BRACKET(S). Turn the bracket sideways as shown in Figure 13.

NOTE: The home position (or Filter position #1) has only one bracket: on the right side of it is a vertical flag.

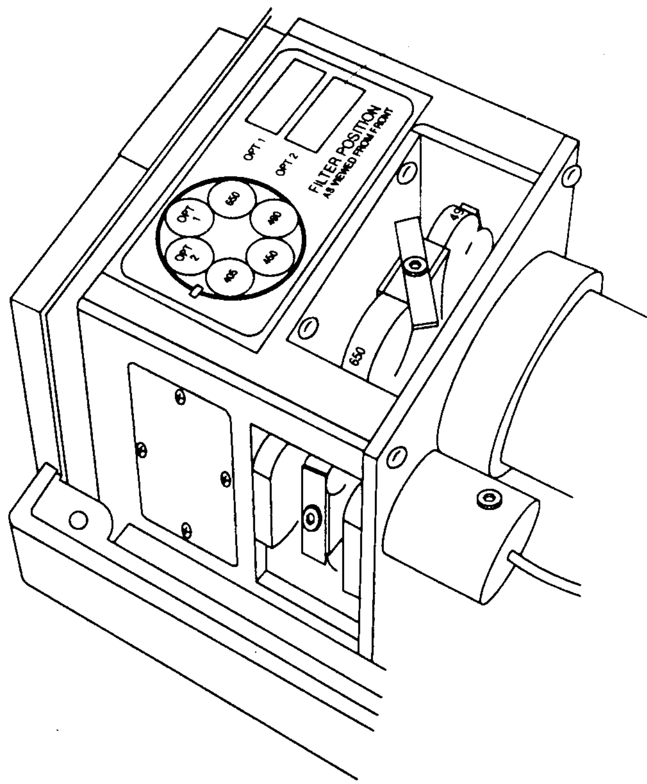

Figure 13: Filter Wheel Housing with Bracket Turned Sideways

4) Make a note of the position where you are installing the filter. You will need this information to update SOFTmax or SOFTmax PRO software and the instrument keypad. When counting the filters, the filter to the left of the vertical flag is #1 and the filter positions are then counted clockwise from 1 to 6.

5) Unpack the filter to be installed, being careful not to touch either optical surface. Inspect the filter: both surfaces should appear flat and monocolored. Identify the recessed side and the flat side of the filter. Also, note the wavelength in nanometers printed on the outer face of the metal ring around the filter. These features help orient the filter for installation.

6) Turn the filter so that it is parallel to the appropriate open slot on the filter wheel and so that the recessed side faces the front of the microplate reader while the flat side faces the reader. When properly oriented, the wavelength printed on the perimeter can be read from the front of the microplate reader. Carefully insert the filter into the open slot in the filter wheel (see Figure 14).

NOTE: For the 340nm filter, both sides of the filter are recessed.

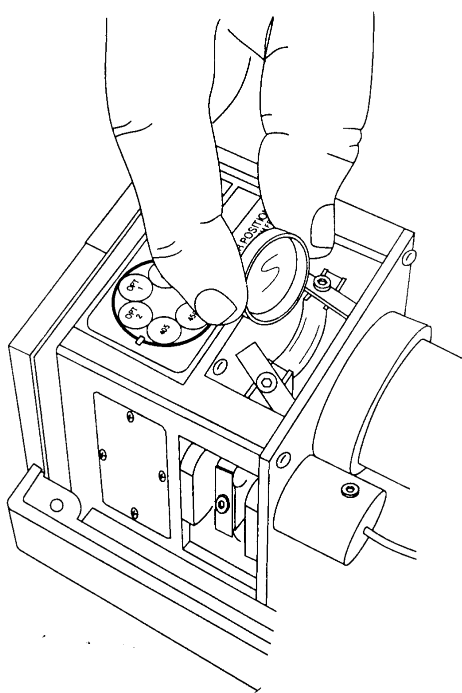

Figure 14: Installing the New Filter into the Filter Wheel

7) Turn the bracket back to the original position over the new filter and use the hex wrench to tighten the screw which holds the bracket. Repeat for all filters installed.
8) Gently spin the filter wheel to make sure it moves freely and to ensure that the optional filter has been seated correctly in the filter slot.

III. Reassemble the Microplate Reader

Tmax

1) Holding the clasps, gently slide the light source front cover on its rail to the point where it fits into the matching half.
2) Replace the spring-loaded holding clasps on the front and side of the chamber assembly.
3) Reexamine Figure 9 prior to this step. Pick up the top cover and carefully position it directly over the lower portion of the microplate reader without putting any stress on the ribbon cable. Be sure to align the two halves carefully on all edges before lowering the top cover into place.
4) Holding the upper and lower portions of the microplate reader firmly together, turn the instrument upside down.
5) Insert and tighten all seven screws in their slots. Recall that two of the screws are shorter, see Figure 8 for proper placement.
6) Turn the microplate reader right-side up and reconnect the power cable and printer or computer cable.

IV. Update the Instrument Keypad

It is very important that the correct position of the filter installed be indicated on the instrument keypad.

1) Note where the filter is in the filter wheel (determined in Section II, Step 3, above).

2) Place the sticker that came with the filter or a piece of tape with the wavelength written on it next to the appropriate number on the front pad of the microplate reader.

V. Update the Software

If you are using the instrument in stand-alone mode, you are done with filter installation. If you are controlling the instrument with SOFTmax® software, please read the following section. It is very important that the filter you have installed in the microplate reader be called out in the software in the correct manner. The filter values in the software must match the positions of the filters in the filter wheel.

Instructions for SOFTmax for Windows or Macintosh Software

1) Launch the SOFTmax program.

2) Select *Filters* under the CONTROL menu.

3) Type the wavelength of the filter you have just installed into the appropriate position on the filter list and click OK. The position in this list MUST match the position of the filter on the filter wheel as noted in Section III, step 3, above.

4) Select *Instrument* under the SETUP menu. Your new filter should be available under the wavelength drop menu.

Instructions for SOFTmax PRO Software (Windows or Macintosh)

1) Launch the SOFTmax PRO program.

2) Select *Preferences* under the EDIT menu. Click on the `Filter` button.

3) Type the wavelength of the filter you have just installed into the appropriate position on the filter list. The position in this list MUST match the position of the filter on the filter wheel as noted in Section III, step 3, above.

4) Select *Instrument Setup* from the CONTROL menu, or press the `Instrument Setup` button on the Plate Section. Your new filter should now be available in the WAVELENGTH menu.

NOTE: Filter wavelengths in this menu appear in numerical order, not the order in which they are installed in the filter wheel.

SPECTROSOFT

Users Guide

Version 6.0

Revision 1.2

SPECTROSOFT

Users Guide

Revision 2.0

Technical Support **703-821-3948**

Http:// www.mtxlsi.com

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Comment:

Chapter 1 System Overview

Introduction

Spectrosoft is a micro-plate based reader control and data reduction program used in EIA/FIA applications. This control and data acquisition program was designed by engineers to fill the need for a universal computerized instrument control program to run various types of readers from different manufacturers. Spectrosoft has the ability to provide researchers and users greater flexibility and uniformity for greater utilization and compatibility of data, control algorithms, screen displays and printouts, whatever instrument you may be using.

Spectrosoft displays data; printouts and reports are in identical formats no matter which type of instrument is being used. These similarities provide uniformity between organizations.

Spectrosofts edge over other instrument control programs is due to the ease of operation through the Windows interface, the need to learn only one program to retrieve data from various manufacturers of readers and the familiarity of same screen viewing.

This standardization of program and assay displays saves numerous hours of learning new programs and or reformatting data to achieve compatibility. The elimination of the need to "boil down" data input from different sources for analysis and sharing is one of Spectrosofts many strong features.

Once you become familiar with all of Spectrosofts capabilities and operations, you can command any reader anywhere with confidence and be sure that you are using the various kinds of instruments correctly. Spectrosoft helps eliminate those variables encountered when operating an unknown instrument in a different lab for example.

Spectrosoft includes the built in, internal command and control codes for most every type of microplate reader commonly available on the market today. Spectrosoft is laid out in a logical format so you can easily learn its powerful features.

This guide follows the displayed conventions at the top of your windows display screen. Retrieving data and reading plates is usually achieved within minutes of installing Spectrosoft.

Spectrosoft was designed to eliminate operator involvement in sequencing and controlling. Since all of the selected instruments features can be invoked by clicking on the desired feature, the operator need only to know what features to select. Spectrosoft does the rest. No manual programming or set up is needed as in other "Data Collection" programs, the correct features to operate each individual instrument is already built in. Lab time is valuable, and Spectrosoft has all the necessary built-in instructions needed to produce whatever sequencing the user may wish to conduct.

Purpose

The purpose of the ***"SPECTROSOFT"*** system is to:

1. Acquire microplate absorbance, fluorescence and luminescence values from EIA/FIA microplate readers, controlling plate motions, kinetic timings, shaking and or incubating.
2. Templating, controls and blanking options for wells, rows or columns along with averaging options.
3. Reads only the area of interest you select (block read).
4. Kinetically controls readers with ***"Auto-read"*** . Displays elapsed time, number of plates read, monitors real-time kinetic results.
5. ***"Auto-baud"*** connect feature determines the instruments' communication setting automatically to eliminate switch guessing.
6. Real-time graphic display of individual well developing during kinetic reads. Display graphics can be saved, printed and exported.
7. Automatic storing of data (in database in text and/or ASCII format) after every kinetic or manual read.
8. Instrument control panel to select individual instrument commands (filters, mixing, plate motions, etc.).
9. Built-in database to store readings with time stamps, test no., operator ID, batch number, filter setting, instrument type, etc.
10. Data base search and sort read data on time, test number, operator ID, batch number, session ID, etc.;
11. Print graphs, plate data and analysis reports, in color or black and white using ***"Spectrosofts"*** built in printer control panel.

System Users

The primary facilities of Spectrosoft are in production, clinical and research areas and in assisting technicians who are responsible for performing microplate assays.

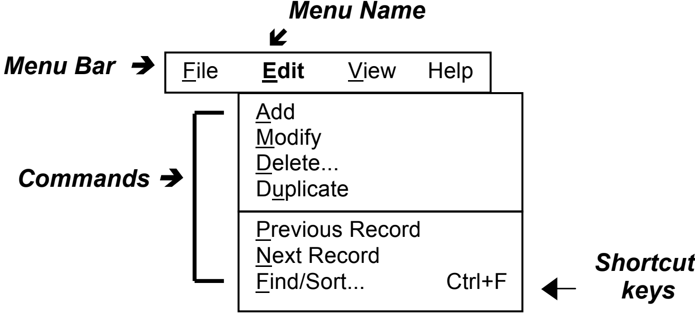
Chapter 2 System Navigation

WINDOW APPLICATION TERMINOLOGY

Introduction

Specific terms will be used throughout this manual to describe the areas of a window. These terms will be defined or illustrated on the next few pages.

Menu Terminology



Menu Terminology Definitions

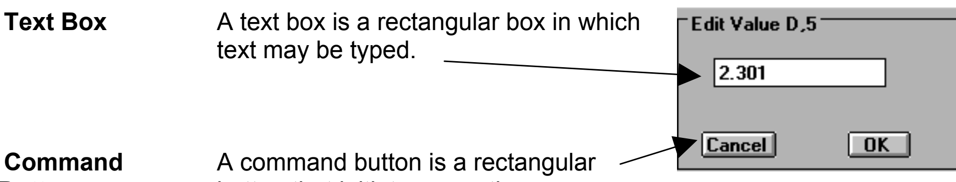
A **command** is an instruction that tells Spectrosoft to do something. Commands are grouped in menus.

A **menu bar** groups several menu names.

A **menu name** is the identifier given to a group of commands.

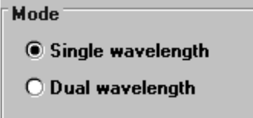
A **shortcut key** is another way of choosing a command. Keystrokes are used instead of clicking the command.

Text Box A text box is a rectangular box in which text may be typed.

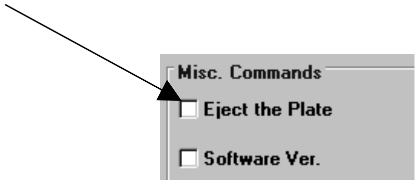


Command Button A command button is a rectangular button that initiates an action.

Radio Button A radio button is a small circle that is selected or cleared to turn an option on or off. Only one radio button can be selected at one time within a group of radio buttons.



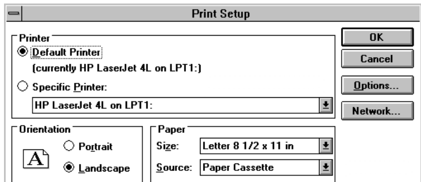
Check Box A check box is a small box that is selected or cleared to turn an option on or off.



Selecting List Items One way to indicate which item in a list box is to be selected for adding, deleting, etc. is to highlight it. This is done by clicking the item of choice.

Dialog Boxes

Dialog boxes contain command buttons and various options through which a particular command or task may be accomplished. The Print Setup dialog box is shown at the right.



Pointer

A pointer is the object that can be seen moving on the screen when the mouse is moved. The shape of the pointer depends on your system.

A commonly used pointer is an arrow ( ).

SHORTCUT KEYS

Introduction

SPECTROSOFT, like every Windows application, contains at least two methods to access screens or perform various functions: the mouse, or keyboard equivalents to mouse actions.

Menu Bar and Menus

You may use the following key presses to navigate through the menu bar and menus.

Key press	Result
<Alt> *or* <F10> *or* <Alt> + the underscored letter	Activate the menu bar (i.e. pull down menu commands).
<Alt> *or* <F10>	Deactivate the menu bar or return to the menu bar from a drop-down menu.
Left or Right Arrow	Move between menu bar options.
Underscored letter or number	Activate a menu command in a drop-down menu or activate a menu bar option with an exclamation point.
Up or Down Arrow	Move between (highlight) menu options in a drop-down menu.
<Enter>	Activate the selected (highlighted) menu option.
<Sec>	Cancel the selected menu.

Window You may use the following key presses when working within a window.

Key Press	Result
<Tab>	Move from option to option (left to right and top to bottom).
<Shift> + <Tab>	Move from option to option in reverse order.
<Alt> + underscored letter of option	Move to or activate the option.
Any Arrow key	Move the selection cursor from option to option within a group of options.
<Ctrl> + <Home>	Move to the first item or character in a list or text box.
<Ctrl> + <End>	Move to the last item or character in a list or text box.
<Page Up> or <Page Down>	Scroll up or down in a list, one screen at a time.
A letter key	Scroll to the next option in a list starting with the selected letter.
<Alt> + Down Arrow	Open a list.
<Spacebar>	Select an item or cancel a selection in a list. Select or clear a check box.
<Ctrl> + Slash (/)	Select all the items in a list (when multiple selection is possible).
<Ctrl> + Backslash (\)	Cancel all selections except the current selection.
<Shift> + Any Arrow key	Extend or cancel the selection in a text box one character at a time.
<Shift> + <Home>	Extend or cancel the selection to the first character in a text box.
<Shift> + <End>	Extend or cancel the selection to the last character in a text box.
<Enter>	Carry out a command. Choose the selected item in a list, and then carry out the command.

USING THIS GUIDE

Assumptions The Spectrosoft User Guide assumes users have some prior knowledge of Windows.

Conventions There are certain conventions that are followed throughout this User Guide. These conventions are described below.

Click. When the directions say “Click”, click the left mouse button.

Window Titles. All window titles in the text are italicized.

Steps. Specific steps that require interaction with the computer are preceded with brackets [ ]. The brackets will contain a number [1], [2], etc.

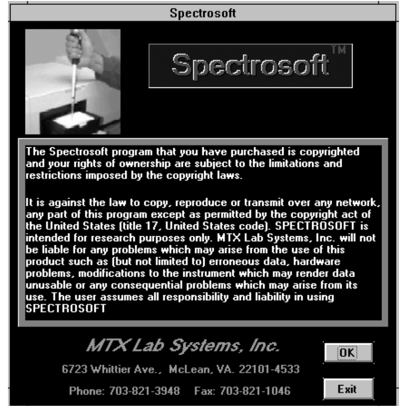
Chapter 3 Operations

Accessing

Spectrosoft is accessed by clicking the Spectrosoft Icon located in the Spectrosoft group form.

Liability

This screen displays the Spectrosoft user responsibility and liability issues.



Registration

After SPECTROSOFT is installed, the user is asked to fill in the registration document and return the registration form to MTX Lab Systems for future updates and assistance.

Features

There are eight tool bar screen features associated with Spectrosoft:

- Instruments- Selects instrument to be used.
- Plate - Displays the read data in microplate format.
- Database - Allows access to the stored plate information.
- Autoread - (Kinetic) Controls Kinetic readings.
- Communication – Allows user manipulation of comm settings.
- Options - Displays print, save and file options.
- About – Version of Spectrosoft
- Exit

Each of these features is discussed in this chapter of the User Guide.

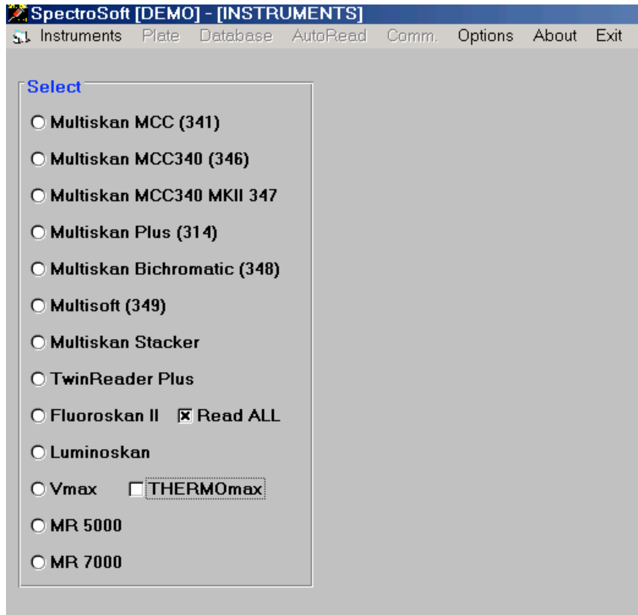
INSTRUMENT SCREEN

Purpose

The “Instrument” screen is used to select which instrument is to be used with Spectrosoft. Additionally, the specific instrument commands such as filter selection, incubation and or plate movement are performed on this screen after the instrument is selected.

Operations

The “Instrument” screen shows the currently supported microplate readers. Simply click on the reader to be connected to and Spectrosoft will begin the connection process from the computer to the instrument. Additional instruments will be added in the future as demand requires.



After selecting the instrument, Spectrosoft will try to establish communication with the instrument by using the default communication settings. There will be a slight delay in establishing the connection if this is the first time that Spectrosoft is being used to connect to an instrument.

Most instruments have multiple switchable baud rates, control and transmit/receive settings so it may take a few try’s to get it to work the first time. However, once the connection is successfully made, Spectrosoft will re-connect correctly each successive time the computer is restarted without any delay. Spectrosoft remembers all final settings during normal exiting and shut down of the computer.

Once the connection is successful, Spectrosoft will automatically switch to the “Plate” screen. At this point, the user may begin to set up the operating parameters and begin reading.

If Spectrosoft is unsuccessful in making a connection to the instrument, it will display the reason for the failure.

Click “OK” and follow the on screen prompts for suggestions to correct the problem. Specific problems include but are not limited to; cable connected to the wrong port connector of the computer, cable connected to the wrong port of the instrument, incorrect port settings in the computers control panel, contention with an internal modem, network blocking of the port, lamp failure inside the instrument, I/O port failure of the instrument, I/O port failure of the computer, or wrong instrument selected.

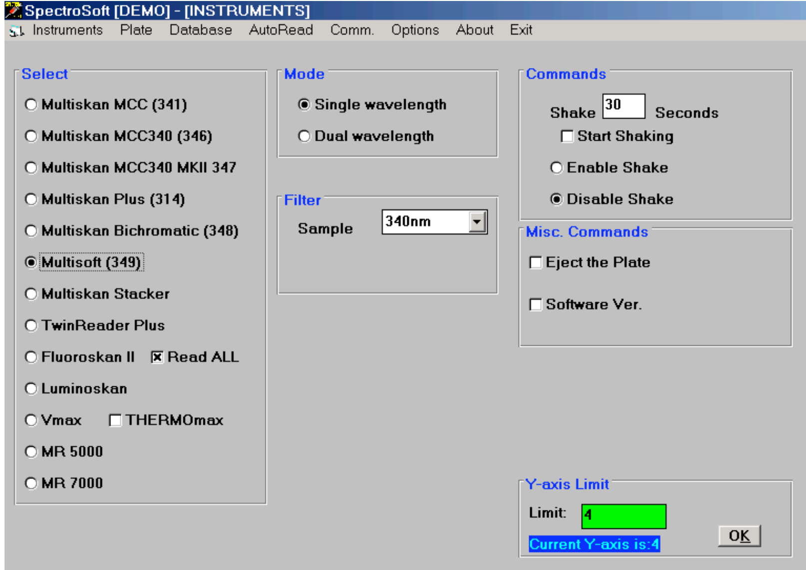
INSTRUMENT COMMANDS

Each type of instrument has specific commands unique to its particular operation, such as filter selection, plate shaking, incubating, single and dual reads, etc. Once a specific instrument has been selected and the comm port has properly responded, the user may switch back to the Instrument Screen to enable any specific commands.

This instrument screen automatically displays the individual commands unique to the instrument selected. Filters of varying wavelength and reading modes may be selected at this time. Spectrosoft preferences file (Spectro.ini) incorporates a listing of the standard filters normally available on each instrument in its memory so the user may instantly select from the menu, whichever filter is required. Single or Dual wavelength may be selected and data calculations are enabled at this time. Incubation, shaking or specific plate movement can also be controlled from this screen.

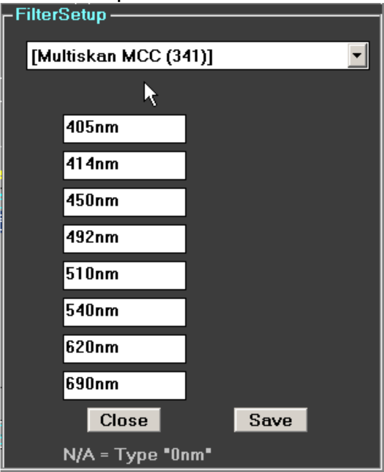
Additionally, during kinetic reads, depending on the instrument selected the user can select the screen display “Y” axis graph to properly show the developing cure after each read. For example, if a Multiskan MK II is being used for plate reading, the user knows that the read outs maximum

values will be no higher than 4.0 O.D. therefore the "Y" axis limit should be set appropriately. When using Fluorescence readers, the "Y" limit could be as high as 99999.



NON-STANDARD FILTER SETTINGS

If you wish to use non-standard filter settings in a particular instrument, it recommended that you use the following procedure. Click Filter Setup form the Option screen.



Select the appropriate instrument and enter the settings. This tells Spectrosoft which filters are located within your instrument and what position they are inserted in. Selecting which filter to read with comes from your selection on the Instrument Screen.

When making changes to the filter configuration i.e. Filter Wheel, Filter Strip, etc., it is recommended that you check each filter for integrity. Check that the filters are installed correctly (mirrored surface facing the light source) and verify that the filter wavelength matches the set configuration. It is recommended that you write these numbers down in sequence. Cleaning the filters at this time is also highly recommended since some instruments will not pass their initial power up and calibration check if the filters and optics are dirty.

Next, you can change the filter setting by clicking the "Filter Setup" button in the options screen. Make sure to restart the Spectrosoft in order for the setting to take effect.

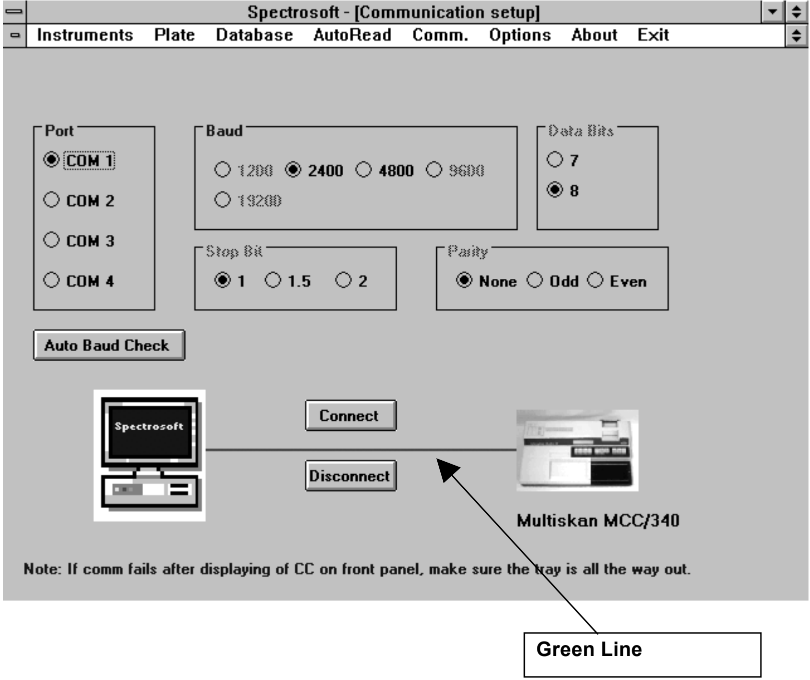
Last but not least, most instruments require that any new filter settings also be programmed in to the instruments internal microprogram memory. Refer to your specific operation manual for instructions to change your reader settings.

CAUTION !!! Changing filters in an instrument should be followed by an in depth quality control inspection, as well as a test and verification of the instruments' filter configuration.

Spectrosofts SPECTRO.INI file, and a "Live" test of the newly configured filter listing to be sure that the correct filter, wavelength settings and results are displayed. Under no circumstances will MTX Lab Systems, Inc. be liable for improper results due to operator or other user failure to properly match the settings with the instruments' filter positioning.

COMMUNICATION SCREEN

Purpose This screen allows the user to establish a connection between the computer and an instrument.



Connection

If this is a first time installation and the green connect line does not light up, don't worry; it is not usual that the instrument, cable and program all would have the identical settings right out of the box the first time it is used.

All instruments have multiple baud rate settings, and some also have different transmit and receive settings on the instrument's comm port as well. Taking into account all of the possible variables between those three items, it is not unusual that some checking and or adjusting may need to be performed.

The only time you normally need to view and manipulate the controls on this screen is during start up for a new installation or if for some reason your instrument has failed, i.e. lamp failure, and you need to reconnect after repair.

If you arrived at this screen from the "Fail to Connect" dialog box on start up for first time operations, then Spectrosoft provides an Auto Baud Check button to automatically connect to the instrument via the "Auto Baud" routine. Spectrosoft eliminates the Baud Rate setting variable between the computer and instrument by incorporating an automatic polling routine from the computers I/O port to the instrument. The computer will send out the wake up command to the attached instrument at all available baud rates and bit combination settings until the correct response is received from the instrument.

We recommend that the **Auto Baud** feature be selected anytime an instrument is newly installed and after the dialog box says the instrument did not respond. The computer will automatically send the connect commands at all the different baud rates available on the instrument until it connects.

When using the **Auto Baud** for the first time, select all four of the computers I/O ports and let Spectrosoft find the instrument on its own.

If the instrument fails to connect after this procedure has run a couple of times, then most likely the problem would be the cable type or switch settings. Spectrosoft is shipped with a tested and verified I/O cable so the recommendation for the transmit and receive switch settings on the reader is displayed on the bottom of the comm screen.

This time-saving feature automatically configures Spectrosoft so that the next time the program is started the instrument will connect instantly without further intervention. Spectrosoft remembers the last configuration for the selected instrument so future connections occur immediately

Once the green connect line is displayed, your instrument should display the proper response, i.e., "CC" (computer control) or "Out", etc. Each manufacturer's instrument has

its own display response to indicate it is in computer control mode. If you experience any difficulties, check the operation manual for your instrument for suggestions on their unique control commands and/or set up.

Note: Some older instruments require that they be manually put into computer control from the instruments operator panel prior to access by Spectrosoft. It is also recommended that the user De-select the DSR enable switch on the com port switches on each reader. This is done on most instruments by turning off switch 4 on the back of the reader.

Operations If you are doing some specific testing and troubleshooting and wish to manually select your port and com settings you may do so using this screen. The operator may at any time manually select the appropriate timing and com port settings below by clicking on the desired buttons as described. This is especially helpful when multiple instruments are attached to multiple com ports and only one instrument is to be run.

[1] To select the computers **Ports**, click on the appropriate radio button.

Caution should be exercised here due to the fact that many computers have multiple ports. Some ports are dedicated to a modem, I/O port mouse, or other comm equipment. Having a port configuration diagram of your computer system and its associated peripherals will assist in bringing up Spectrosoft more efficiently. If you experience port contention problems with a modem installed on a port before the instrument you may wish to skip the autobaud checking of that particular port. This is especially important where networked computers and modems are employed.

[2] To select the **baud rate**, simply click the appropriate radio button. If you already know the instrument setting, simply click on the desired baud rate.

[3] To select the **data bits configuration**, click the appropriate radio button. Data bits are hard wired by the instrument manufacturer. Normally it is not possible to change the data bit stream of the instrument. Leaving Spectrosoft to select this variable is suggested.

[4] To select the **stop bit**, click the appropriate radio button. Hard wired by the instrument manufacturer.

[5] To select the **parity**, click the appropriate radio button. Some instruments do not use the parity bit. Leave this setting to Spectrosoft.

[6] Click the **connect** button. If the settings match, the system will make the connection with the user selected settings. The system will display a dialog box, “Initializing” on screen. When the connection is made, the system will display a green line between the instrument and the computer.

[7] Click the **disconnect** button. Use the disconnect button prior to shutting down the reader and computer so Spectrosoft can “Remember” the last operational settings. The system will break the connection with the instrument by sending the disconnect command. The instrument will respond by disconnecting and resetting its display screen to its normal mode when the instrument is first turned on. Control of the instrument is returned to the operator panel and may be operated manually at this time.

Troubleshooting Operational Problems

There are virtually countless settings as well as numerous manufacturers and models of computers, drivers, and software that we could not possibly tell you what wrong setting is present in your system. It is not uncommon for a well-established computer system to run many applications perfectly and then completely crash on one particular application. It is recommended that you contact our technical support office with a detailed fax regarding any problems you may be experiencing, along with the computers make, model, installed options, port configuration, memory and drive configurations along with any other abnormal operational problems. Our technical and will record and diligently strive to solve, in time, any unusual problem customers may experience.

It is strongly recommended that you list and record your systems configuration from each operational screen available on your computer. This will not only help to fix any Spectrosoft operational problems, but can also serve as an aid for future system problem resolution.

MTX Technical Support can only recommend items to check. Because of the variations and complexity of PCs and the wide variations of port and control panel settings along with the varied drivers of numerous versions, it is impossible for MTX to troubleshoot every computer which does not work well with Spectrosoft. Additionally, Spectrosoft will display a red line between the instrument and the computer confirming the successful disconnect. At this time you can still access other features of Spectrosoft, such as displaying the “Autoread” data file, database searches, printing, data reductions, calculations, etc., without having some Be aware that the instrument connected and on-line.

AUTOBAUD SCREEN

Purpose

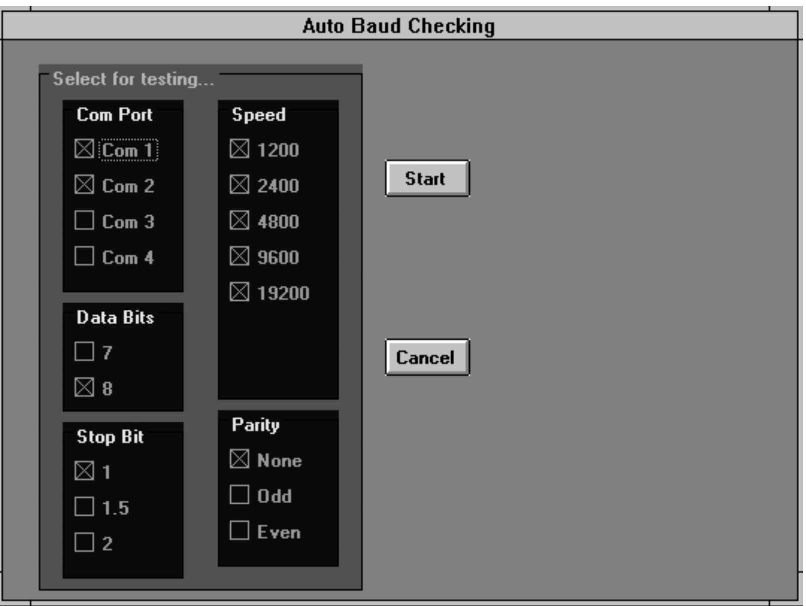
The Auto Baud Checking screen displays the status of the computer I/O port's progress as it sequences through the various settings while trying to establish a connection to the instrument.

If this is a first time installation and you do not get the green connection line right away, don't worry, it is highly unlikely that the instrument, cable and program all should have the correct settings right out of the box the first time it is used. All instruments have multiple baud rate settings, and some have different transmit and receive settings on their respective comm port as well.

Taking into account all of the possible variables between the various units, it is normal that some checking or adjusting must first be performed during the first use. Spectrosoft eliminates the Baud Rate setting variable between the computer and instrument by incorporating an automatic polling routine from the computer to the instrument. The computer sends out a unique wake up command to the selected instrument, at all available baud rate settings until the correct response is received back from the instrument.

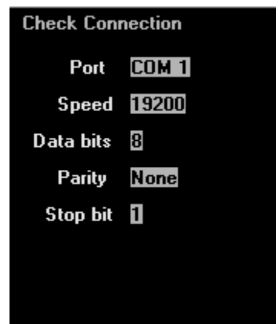
We recommend that the **Auto Baud** feature be selected anytime an instrument is first installed and after the dialog box says "No response from instrument". This time saving feature eliminates excessive operator intervention by configuring each instrument's baud rate automatically and then will automatically configure the Spectrosoft program for the next usage. Spectrosoft remembers the last successful setting and assumes the user will be replicating the operation the next time Spectrosoft is brought up The instrument will connect instantly each time the system is brought up thereafter.

Once the green connect line is visible, the instrument you are using should display the proper response, i.e., "CC" (computer control) or "Out Ready" etc. Each particular instrument has its own display response. If you experience difficulty, check with the operation manuals for each instrument for suggestions for their unique control commands and/or set up.



Operations

If the user wishes to modify or intervene in any of the various port settings, simply click the mouse to the desired setting and apply.



[1] Set the **Comm ports** check box to ‘on’

[2] Set the **Stop** bit check box to ‘on’

[3] Set the **Speed** check box to ‘on’

[4] Set the **Parity** check box to ‘on’

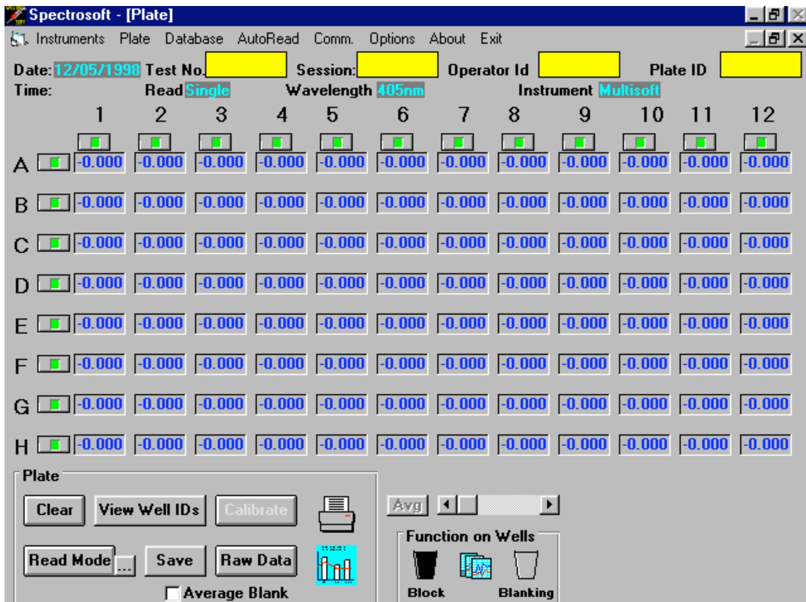
[5] Click the **Start** button

The computer displays the variable output settings as it scrolls through the different combinations and automatically sends the connect command at all the different settings until it connects. Once the connection is made, the system will display the green connect line showing the connection was successful. The user can cancel the operation at any time by clicking on the **cancel** button.

PLATE SCREEN

Purpose

The Plate Screen is the user's primary display and control screen for reading and viewing the microplates, verifying the instruments operating modes, filter settings, displaying captured data, formatting plate layouts, saving data to the spreadsheet or database, retrieving data and identifying and printing the captured microplate data. Other features include blanking, blocking, well identifications, templating, calculations, averaging, graphing and time and date stamp for each plate read.



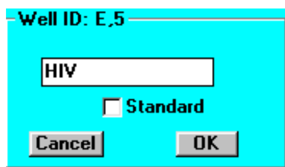
Operations

Reading a plate

(After successful computer control is established)

[1] To initiate reading of a microplate, simply click the Read Mode button on the bottom of the screen. The "Read" button commands the instrument to draw in the plate to the read area, collect the absorbance (Fluorescence) values and display them on the screen. Once a microplate is read, the values are displayed in the associated wells as raw data values.

The following describes Spectrosofts unique control and manipulation features for the various wells, data displays, blanking, blocking, and additional optional plate screen features.



Setting identifiers for Wells

(Well I.D.'s)

Setting markers (names) for certain wells helps the user identify various wells for their unique characteristics:

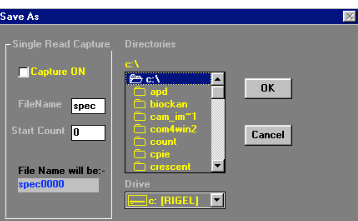
[1] If a user wants to identify certain wells as "Serum" or "Reagent", or a "Patient" for example, **double-click** on the desired well and Spectrosoft displays the Well ID change dialog box at the bottom of the screen.

[2] Simply type in the desired label, name, or standard and then click on OK.

Templates
[3] Once a plate is laid out with it's various characteristic well identifiers, blanks, standards, etc., it can then be saved as a "Template" by saving it in the database by some assigned test number and or name. Further usage of the saved "Master Template" can then be retrieved, read and then saved as another test number and name, saving the original "Clean" template for further use and or replication.

A word of caution on saving data; since in a very short period of time, a user can amass an extremely large base of data, a user or lab manager should create a management naming scheme and or a log to sort and retrieve data to eliminate time wasted searching and manual verifying. Just like word processing, spreadsheets or other database programs, users save data in unique "Directories" specific to their data such as "C:\Letters, Fax's, etc. to sort the various data as to its use. This is most helpful when data collection expands into several types of assays or projects.

Automatic Saving and Printing Options

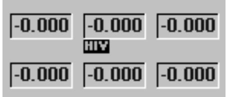


If you wish to Automatically save data in a sequential naming convention, without having to name and save each plate you read, Spectrosoft will do this automatically if you click on the optional three dot button (next to the read button) Read Mode ... and select the "Capture on" feature. Select a name your sequential plate reads or use the default suggested name and click OK. Each plate read from this point on will be automatically named the selected name plus 1. i.e. First plate is named Larry0000, the next plate read is automatically saved as Larry0001, etc.
To disable this feature, simply click "Capture Off" and begin manually naming your plates as desired.

Autoprint Feature

Spectrosoft can automatically print the data read from each plate immediately after reading the plate. This feature is useful if a user just wants an automatic printout for later analysis. Some Clinical Laboratories require immediate printout for GMP processing and compliance. Select this "Autoprint" feature on the "Options" screen. Be sure your printer is ready, on line and has sufficient paper. Printing in this manner during a kinetic read can effectively print an entire ream of paper in a very short time.

View identifier



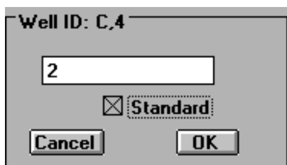
[1] Click **View Well ID's** button to view the various well identifiers on the plate. The screen will display the unique well ID just below each labeled well.

After clicking on the **View Well ID's** button, the View Well Ids button will change to Hide

Well IDs.

[2] Click on **Hide Well ID's** button, and the system will hide the Well ID's and change the button to **View Well ID's**.

Set Standard on a Well



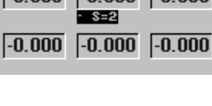
To identify a particular well as a standard:

[1] Double-**click** the desired well. The system will display the Well ID change dialog box

[2] Enter the standard or "known" value in the **text field.**

[3] Set the **Standard** check box to 'on' and press **OK** button.

View Standard



[1] Click **View Well ID's** button to view the identifier on the plate. The system will show the standard below the wells and the View Well ID's button will change to "Hide Well ID's". Viewing the expected standard value just below the actual read value is helpful in spotting errors quickly. Any large variations against what was expected could indicate reader problems, pipetting inaccuracies, plate problems, degradation of reagents and chemistry, lab or operator errors.

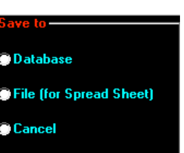
[2] Click on the **Hide Well ID's** button, and the system will hide well ID standard values.

Clearing a plate

To clear a current plate or all plates:

[1] Click **clear** button, and the system will display the clear dialog screen.

[2] Select the appropriate radio button, and the system will clear all values.



Saving a plate

To save the data in a database or in Spreadsheet (Excel, 123, etc.), format, click on the desired destination format:

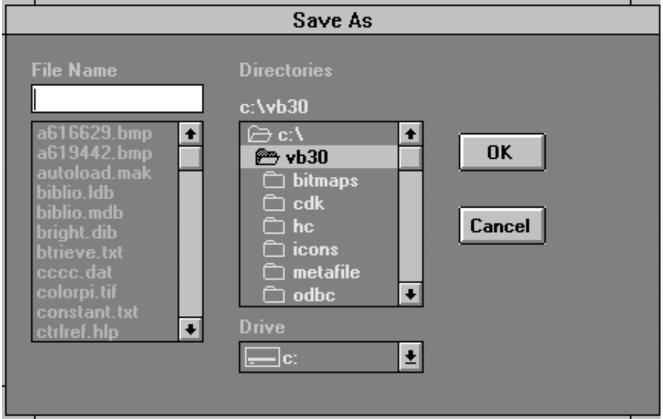
[1] Click the **Save** button, and the system will display the save dialog screen.

[2] Select the **File** radio button, and the system will save the current plate values.

Note: Make sure the Test No. Field is filled out or the system will prompt you to enter the field. Additionally, it is helpful to create a database management directory to properly organize your saved data so as not to cause confusion for other users when trying to retrieve data. Just as in other program files (MSWORD, WordPerfect, etc.), data is saved in an organized file extension for various types of letters, memos, faxes, etc.

Names for Spectrosoft data files should follow some organized convention to facilitate ease of retrieval. Extensions should not be used when calling or saving. The user assigns its own extension in the option screen.

[3] Select the **Spreadsheet** radio button, and the system will display the file save dialog screen shown below.



[4] Enter a **File name** in the text box.

[5] Select the directory path for your current

spreadsheet. For ease of conversions to various (exported) programs, it is recommended that you select the .TXT.

[6] Click **OK** button.

[7] The Spectrosoft saved data is now properly formatted and available for your spreadsheet import function. When importing your saved data in a spreadsheet, remember to select the desired format, I.E. microplate or flat file.

Printing a plate

To print the current plate:

[1] Click the **printer** Icon, the system will display with print option.

[2] Click **yes** button if you want to print values with identifiers.

Note: See the “Options” screen for more about print setup and controls.

View Raw data

To view all wells with raw O.D. values:

[1] Click the Raw Data button; the system will display all values as received from the instrument without any blank subtractions.

View Calibrated data

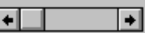
To view all wells with calibrated data, I.E. wells with the blank well value subtracted:

[1] Click the Calibrate button, the system will display all values based on the blanking well, wells or multiple well averages.

[2] You may toggle between Raw and Calibrated data at will. You may also save and print the data as displayed on the screen as you wish.

View multiple plates

When using the “Autoread” feature, the data is captured and stored in memory available for viewing during and after the read process. The system can logically store a maximum of 400 plates of data on a long-term kinetic read.



To view each kinetically read plate, the user manipulates the plate view control bar above the function on wells display box. This plate view bar operates similar to a CD-ROM or player.

[1] Clicking on the right arrow displays the next successively read plate. Clicking the right double arrow as far right as possible displays the last plate read.

[2] Clicking on the left arrow displays the previously read plate. Clicking the left double arrow as far left as possible displays the first plate read.

Blank Modes

Note: All single wavelength non-averaged plate reads are initially displayed on the plate screen as raw O.D.'s (or raw Fluorescence units). In order to view the calibrated (Blanked) values of the raw well data, select one of the desired blanking modes described below.

Single well Blank

Drag and drop the blanking cup over the desired blank well. The background color will turn orange. Click the Calibrate button. Subsequent well values will be subtracted by the value of the read blank value of the blank well.

To remove the blank function from a single well

Drag and drop the blanking cup back over the blank well, and the background color will turns back to gray. Any subtracted values must be re-calculated prior to un-blanking in order to view to the original raw data if the blank is removed.

Row Blanking

Drag and drop the blanking cup over the desired row letter **A** through **H .** The background color of the selected row will change to orange. All subsequent vertical column readings (above and or below the selected blank row) will be subtracted from the read blank value of the associated column blank value. The calibrate button must be activated after the read in order to view the subtracted values.

To remove blank from a row of wells

Drag the blanking cup back over the row numbered **A** through **H**, and the background color will turn back to gray.

Column Blanking,

Drag the blanking cup over the column numbered **1** through **12**, and the background color will change to orange. All subsequent readings will be subtracted from the associated horizontal blank read value.

To remove blank from a column of wells,

Drag the blanking cup back over the column numbered **1** through **12**, and the background color will turn to gray.

Multiwell Blanking with Averaging

To obtain an average blank value using multiple blank wells:

[1] Click the ☐ Average Blank check box at the bottom of the plate screen.

[2] Drag and drop the blanking cup to the desired wells to be used as the blank values.

[3] Read the plate. Spectrosoft automatically averages the raw blank readings from the selected blank wells, after the plate read values are displayed, and then force sets the blank wells to the new “Average” calculated value. Additionally, Spectrosoft automatically calibrates (Blanks) the remaining well values and displays them.

[4] The operator may then click the “Calibrate” or the “Raw” data buttons to alternate the display of the values of the wells against the subtracted “Averaged” blank value.

Column Mean Blank

To calculate well values against a mean blank of values read from a designated column of blanks:

[1] Click the ☐ Average Blank check box and drag the cup to the desired blank column heading (1-12).

[2] Spectrosoft automatically averages the read values from the designated column and then sets them to the calculated mean for final calculating of the remaining wells read. Clicking on "Calibrate" displays the results of the subtracted mean value from the raw values.

Row Mean Blank

To calculate the well values against a mean blank of values read from a designated row of blanks:
[1] Click the ☐ Average Blank box and drag the blank cup to the desired blank row.

[2] Spectrosoft automatically averages the blank values read from the designated row and then sets them to the calculated mean for final calculating of the remaining wells read. Clicking on "Calibrate" displays the results of the subtracted mean value from the raw values.

Blocking

Blocking is a useful option to block out unneeded wells from view.

To Block out a single well

Drag and drop the Block cup over the desired well to be blocked out, The foreground color turns gray.

To remove a Block from a single well

Drag the Block cup over the well, and the foreground color turns blue.

To Block a row of wells

Simply point the mouse on the desired rows green button box (preceding each row) and click. The selected row (lettered **A** through **H)** will turn to an empty blank background.

To remove Block from a row of wells

Click on the red box preceding the desired blocked out row. The row (numbered **A** through **H)** will be restored to normal.

To Block a column of wells

Drag the mouse to the green box preceding the column numbers (**1** through **12)**, the display will blank out and the box will turn red.

To remove Block from a column of wells

Drag the mouse to the red box preceding the selected column numbered (**1** through **12)**, and click. The foreground color returns to normal.

Real-time Graphic Display of Kinetic Data Points

Spectrosoft provides the user with real-time graphic representation of well

values, (data points), as they are read in kinetic mode. Up to 12 individual well graphs can be selected for viewing as the kinetic read progresses. The “Y” axis value can be adjusted to provide close up viewing of the data points as they develop. Additionally, the graph can be viewed in various graphic configurations such as 3D, Line (point to point), and bar.

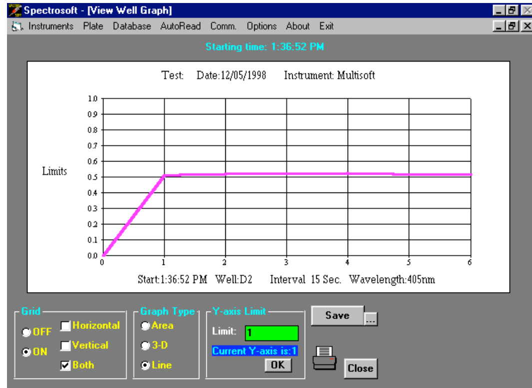
To display a graph of a single well

Drag the graph icon to the desired well. A miniature display graph replaces the current well values. To expand the graph for detailed viewing, simply click on the selected well graph icon. The display now allows further detailed studies of values, types of display such as 3D, bar, line and fill.

To remove graph from a single well

Move the mouse over the graph and click the right mouse button.

To Zoom the Graph



Move the mouse over the Icon and click the left mouse button.

Note: The graph will only be displayed if the well contains more then one kinetic read.

Saving the Graph

The graph may be saved by clicking on the button. The user must provide the file name for saving the graph by clicking the button located next to the save button.

The Graph may also be viewed by other kinds of .BMP file viewers as used in expanded displays.

The graph may be printed
by clicking the
button.

ANALYZED DATA OPTIONS

Spectrosoft provides the user several calculation features. First, clicking on Calibrate causes the blank value from the blank well to be subtracted from the read unknowns, next clicking on the Graph Icon displays the following,

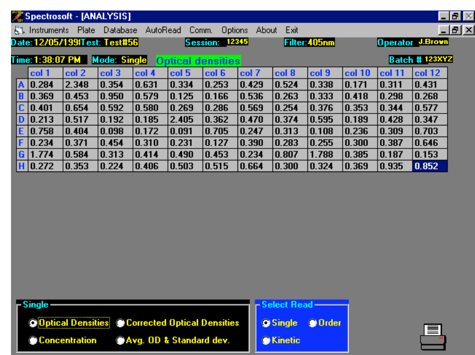
On a Singles read:

1. Optical densities
2. Concentration
3. Corrected optical densities
4. Avg. OD and standard deviation

On Kinetic read:

1. Avg. OD and standard deviation

Optical densities

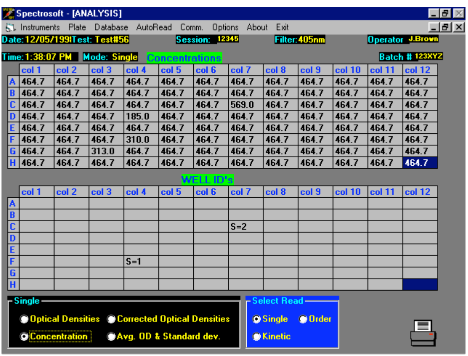


[1] Click from plate screen and the Analysis screen will be displayed.

[2] Click on **Optical densities** radio button to view analysis data.

[3] Click on to print the data on printer.

Concentration

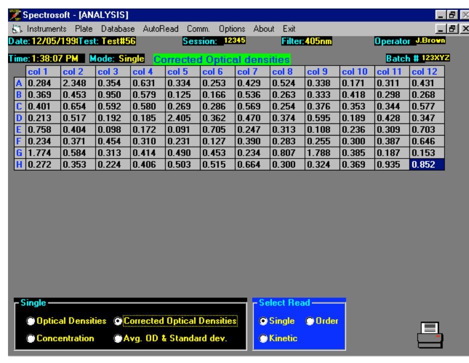


[1] Click from plate screen and the Analysis screen will be displayed.

[2] Click on **Concentration** radio button to view analysis data.

3] Click on to print the data on printer.

Corrected Optical densities

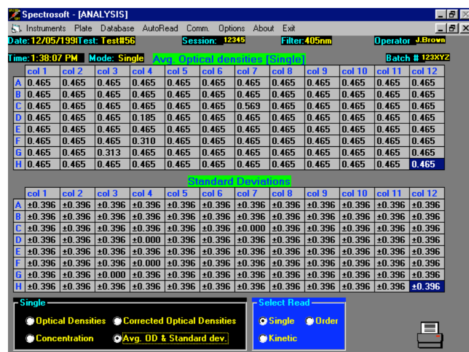


[1] Click from plate screen and the Analysis screen will be displayed.

[2] Click on **Corrected Optical densities** radio button to view analysis data.

3] Click on to print the data on printer.

Average OD & standard deviation (single read)

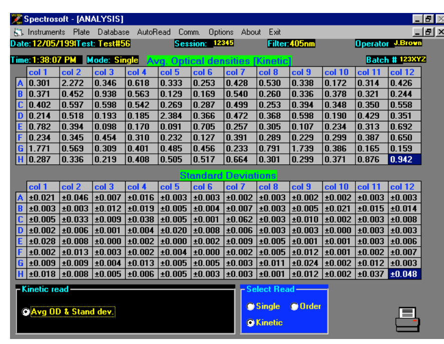


[1] Click from plate screen and the Analysis screen will be displayed.

[2] Click on **Avg. OD & standard deviation** Radio button to view analysis data.

3] Click on to print the data on printer.

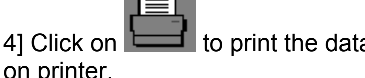
Average OD & standard deviation (Kinetic read)



[1] Click from plate screen and the Analysis screen will be displayed.

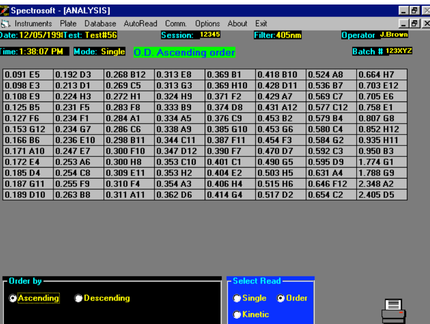
[2] Click on Kinetic radio button in the select read option box.

[3] Click on **Avg. OD & standard deviation**
Radio button to view analysis data.



4] Click on to print the data on printer.

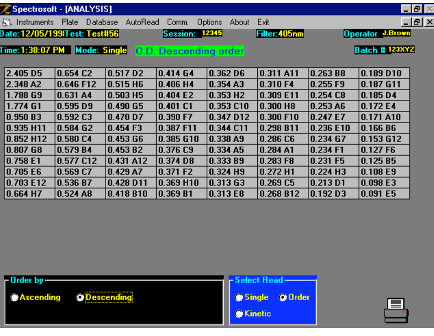
Sort and Display Well Values



[1] To sort well values, click on the graph from the plate screen. To sort and display ascending values click **"Order"** then select ascending or descending. Spectrosoft will then display the read values in either the lowest to highest or vice versa along with the associated wells.

Sorting Well Data by Values

Spectrosoft can sort and display your plate data in an ascending or descending value mode. Simply click on the Sort Icon and select whether you wish to view the data in ascending or descending order. Spectrosoft automatically displays the data in the selected format.

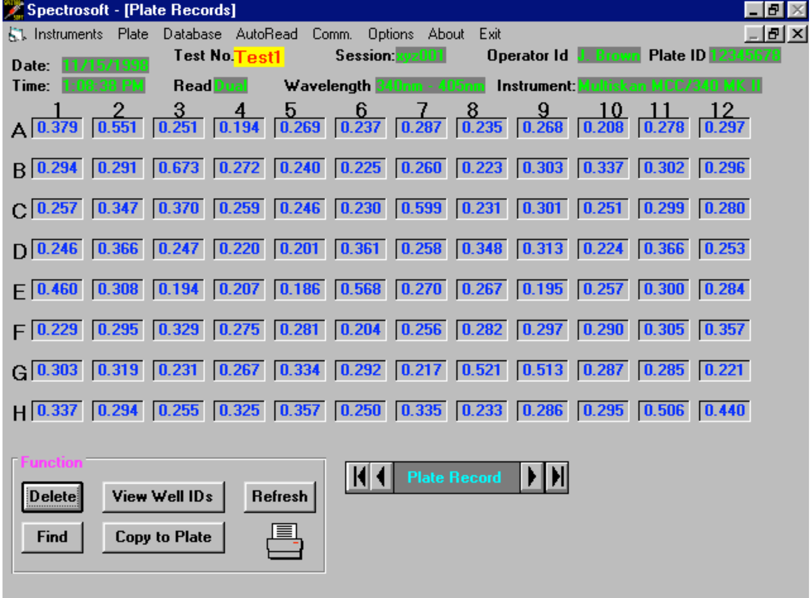


DATABASE SCREEN

Purpose

Microplate data can be stored in Spectrosofts own built in database and can be retrieved at any time for further analysis. On this screen, the user cannot alter any field. Once data is stored and then retrieved to this screen, it can only be copied to the plate screen then modified and saved. It can also be saved by another name thereby creating another plate.

The previously saved data is never changed or destroyed unless the user invokes the delete command.



Operations

Viewing a plate

The stored records in the Spectrosoft database can be viewed in two ways. First by calling (name search), or by clicking on the Plate Record Bar.

The sequential plate record search is performed using the Plate Record bar. This is done two ways, by clicking the buttons below:

[1] Click the ▶ button to view the next record.

[2] Click the button to view the previous record.

[3] Click the button to view the first record.

[4] Click the button to view the last record.

Deleting Records

To delete the currently displayed record;

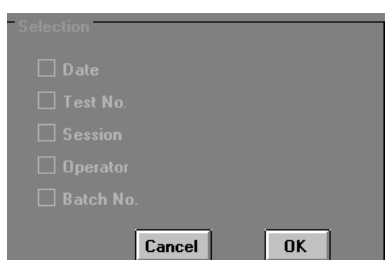
[1] Click the Delete button and the delete message box will appear.

[2] Click the **Yes** button to delete the record.

[3] Click the **No** button to cancel the delete operation.

Find

To find a record from the database, click the Find button, a selection dialog box appears.



[1] Make your selection by clicking the appropriate square box; a text field will appear.

To de-select, click the appropriate square box.

[2] Enter the search criteria and click OK button.

The system will show the first record which matches the criteria and the Find dialog box appears on the button right hand corner of the screen.

[3] The remaining records matching the search will be found and displayed by clicking the **next** button.

[4] Click the **previous** button to view the

previous record which matches the criteria.

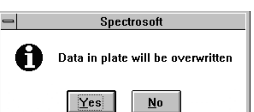
Print

The user can print the currently displayed plate data by clicking the button.

Copy to plate-Templates

The user can replicate a whole plate with its OD, tag ID's, standard setting, etc. to a new plate name in the database. This can be used as a template for subsequent readings.

[1] Click the button, a verify dialog box will appear,



[2] Click OK button to save the data,

After the data has been moved, the system will display the plate read screen. At this point the data can be modified, or this new "Template" can be used to get new data using the previously stored plate parameters such as blanks, standards, well I.D.'s, etc. This templating feature is a great time saver and ensures exact replication of stored templates for critical plate processing.

AUTOREAD SCREEN

Purpose

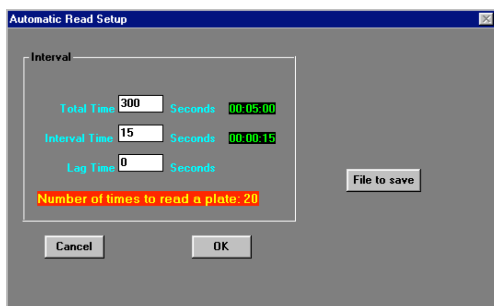
The purpose of "Autoread" is to record kinetic readings on a plate over a period of N times. The purpose of kinetically reading a microplate can be to record enzyme activity over a specified period of time, or to measure and count bacteria growth. These timed readings can give users a picture over time of the aggressiveness of certain bacteria or other growth or enzyme activity. The data provided by the Autoread process is placed in a "Flat file". This contiguous data is one long file. Spectrosoft can record thousands of reads on a single command. Long term reliability and accuracy is another of Spectrosofts strong points.

Spectrosofts accurate timing display records a "Snap Shot" of microplate activity over short as well as long term assays. Instruments can be placed in incubators to stimulate growth of bacteria and the instrument can be left unattended for weeks at a time.

It is recommended that if the user intends to use the instrument for long term assays, that the use of a UPS or other backup power system be employed.

This screen is invoked by clicking "AUTOREAD" from the tool bar. The following screen will appear.

Operation



<u>Total Time</u> User specifies the Total time in seconds for a particular read session. As you enter the numbers, Spectrosoft instantly calculates and displays the total time in Hours, Minutes and Seconds

<u>Interval Time</u> The wait time between each read, in seconds.

Note: Each instrument has a different interval time due to mechanical delay. See below for specified interval times. Spectrosoft knows the interval time of the instruments it controls and will advice you at the moment you are entering the timing

parameters.

Note: If the shake option is enabled before every read, then the shake time will be added to the interval time.

Lag Time Wait time before the first plate is read.

[1] Fill in the time fields.

INTERVAL TIME (Seconds)

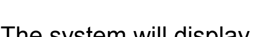
Instrument	Single Filter	Dual Filter
Multiskan MCC/340	45	90
Multiskan MCC/340 MK II	15	30
Multiskan MCC/340 Plus MK II	15	N/A
Multiskan Bichromatic	15	N/A
Multisoft	15	30
Fluoroskan II	90	N/A
Luminoskan	90	N/A
VMAX	25	50
MR 5000	30	N/A
MR 7000	30	N/A

To save your kinetic readings, click the File to save button. The save file dialog will appear.

[2] Enter a file name of your choice, where the data will be stored and later used by the EXCEL, 123, Quatro Pro, or other spreadsheet program.

[3] Select the directory (from the directory list) where the file will be stored.

[4] Click button.



The system will display

File Save as:- c:\test.dat Just below the cancel button. It is just a reminder that the data will be stored in a file

[5] If the user does not wish to save the data in a file, click the Cancel button.

[6] Click the OK button and a progress dialog box will appear on the bottom right side of the screen.

This dialog box shows

1. Interval time selected
2. The lag time
3. How many plates have been read
4. Time stamp of the last plate
5. The file name where the data is being saved

[7] To cancel the kinetic read at any time, simply click the **abort** button, and control will revert back to the user plate screen.

Exporting Data

Loading a file to EXCEL

[1] Double-click the icon.

[2] Open the file saved by Spectrosoft.

[3] Select comma as delimiter.

[4] Proceed with the Excel commands to process your data.

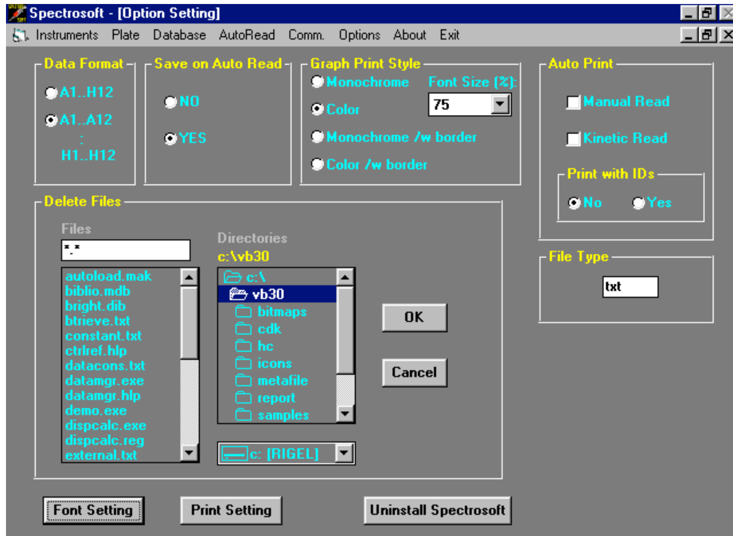
OPTIONS SCREEN

Purpose

This screen allows the user to perform various system settings such as the format of the data stored and the way in which it should be exported to spreadsheets. This screen also allows the user to modify the print settings, fonts, colors, etc., beyond what windows formats the print data for. This gives users flexibility with out having to exit Spectrosoft and invoke the windows main and control panel screen. This screen only applies to the way that Spectrosoft prints and does not modify the windows settings.

This screen is invoked by clicking the OPTION from the tool bar and the following screen will appear:

Operations



Data Format

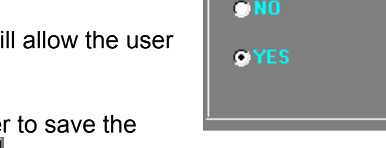
There are two data format possibilities for the Kinetic read data from Spectrosoft prior to exporting. The system stores the plate data in a flat file (used by EXCEL and is compatible with other spreadsheet programs) based on the format selected.

Selecting A1..H12 will store all 96 wells in one row.

Selecting A1..A12 : H1..H12 will store in an 8 by 12 array as required to view the 96 well plate format correctly in the EXCEL spreadsheet.

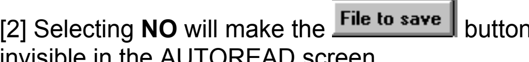
Most calculation programs previously created by users already use this format and are compatible with other calculator programs.

Save on Auto Read



Based on this option, the system will allow the user to save the kinetic read in a file.

[1] Selecting **YES** will allow the user to save the data in a file by making File to save button visible in the AUTOREAD screen.

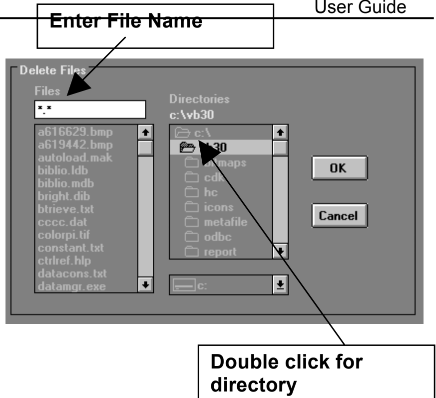


[2] Selecting **NO** will make the File to save button invisible in the AUTOREAD screen.

Delete File

If disk space should ever become a problem, the user may need to delete the kinetic data from the hard drive to free up some space. This option allows the user to select the directory and file name for deletion.

Note: Spectrosoft data storage capabilities are limited only by the size of the memory of the users hard drive. Today's Pentium processors with well over 10 Gigabyte storage capacity will give users, millions of reads of plate data storage.



Font setting

Various printers format data in different ways. This option allows the user to set the font, font style, size of font, etc. for printing. Clicking on the Font Setting Button takes you to the Windows Font Selection and setup. From this display you can manipulate your printed data with the available fonts and sizes installed as standard in your system.

Print setting

Spectrosoft contains its own printer control panel similar the one used in the Windows operating system. There is no need to switch out of Spectrosoft to control any special items. This screen allows the user to set the printer, orientation, page size, colors, etc. for printing.

Some older dot matrix printers may not be able to print the entire row of data on one print line. In this case, the user can select the landscape mode instead of the portrait mode to print the data sideways on the paper. Clicking on the Print Setting button calls up the specific print options for your current default printer. From this screen you may change the format of the data, its color, how it is presented on the page, etc. Different printers have different options. You should refer to your specific printers operations manual for any detailed print options you wish to enable. It is also a standard feature for Spectrosoft to print to any Networked printer as long as it is selected and on line and ready.

ABOUT SCREEN

Purpose This screen shows the software version of Spectrosoft program. Also provides the user with a phone number for assistance.

EXIT

Purpose Used to abort the Spectrosoft software.
This is the preferred method to shut down Spectrosoft. Using the EXIT button causes Spectrosoft to remember the last settings.

Chapter 4

SYSTEM COMPONENTS

Hardware Requirements

The minimum hardware requirements for Spectrosoft are as follows:

- 3 MB of hard disk space for Spectrosoft
- 1 MB of hard disk space for creating temporary files
- 16 MB of RAM (minimum). 32 MB or higher RAM is recommended
- Intel 486 CPU (minimum). An Intel Pentium is recommended.
- Desk Top, Lap Top, Tower, U.S. and European Versions included.

Software Requirements

The software requirements for Spectrosoft are as follows:

- Windows 95/98/2000/XP/ME

Chapter 5

SYSTEM INSTALLATION AND SETUP

Installing Spectrosoft

To install Spectrosoft:

[1] If you purchased the Software, install the hardware authorization Dongle on to the connector of the Computer's Printer Parallel port prior to installation the software. **To install Spectrosoft requires Administrative privileges.**

[2] Place the Spectrosoft program CD in the disk drive. Wait for the Autorun sequence to begin. If the Autorun does not start then follow the next instructions.

[3] Access Windows Program Manager.

[4] Click **File** on the Program Manager menu bar.

[5] Click the **Run** command.

[6] Type **[CD Drive*]:SETUP.EXE** in the text box. Click **OK**.

*Use the appropriate CD Drive letter

The following dialog box is displayed:

[6] Click the **Continue** command button to select the default install directory (C:\SPECTRO).

Register Today !

Now would be a great time to fill out your registration card and send to us.
Registering entitles you to:

* Customer Support
* Updates on product upgrades and fixes
* Information about new MTX products and services

[7] If an old database file is present in the install directory, the install program will give user the choice of keeping it or overriding with an empty database file.

[8] Click **OK**.

Accessing Spectrosoft

[1] Double-click the Spectrosoft icon in the Spectrosoft group form;

OR

[1] Access Windows Program Manager.

[2] Click **File** on the Program Manager menu bar.

[3] Click the **Run** command.

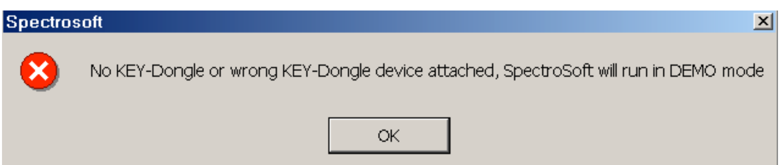
[4] Enter the path (must include SPECTRO.EXE).

To run the Spectrosoft application, simply double click the

icon.

Spectrosoft fails to detect the Dongle

If Spectrosoft fails the detect the Dongle you will get the following message:



Make sure the Dongle is attached to the printer port and try to start the Spectrosoft. If you still get the above message, then run the install.exe program from CD. System will prompt for a success/fail operation. If operation is unsuccessful, please contact Tech. Support for additional assistance.

Dongle Installation

Install the hardware authorization Dongle on to the Connector of the Computer's Printer Parallel port. This authorizes Spectrosoft to run the full compliment of options such as viewing the full plates data, saving, printing, etc. Without the hardware dongle, Spectrosoft will run in Demo mode and prevent users from accessing all the features of the software. Make sure to move the dongle when ever changing computers! The dongle represents the **full cost** of Spectrosoft.

Printer Port Verification

If your Spectrosoft program runs in demo mode after installing the Dongle on the printer port of your computer, check the Windows Control Panel to verify the presence of a printer port. The Printer port of most computers is a 25 pin Male connector located adjacent to your serial ports. Since most newer computer printers use the new USB port, your printer port should be free to install your dongle, however if you are using a printer parallel port for your printer, it is a simple operation to install the Dongle between the printer cable and your port.. Don't confuse the parallel port with other serial ports which can also be 25 pin connectors in some older computers.

Additional Authorizations and licences

Since the value of the Spectrosoft program is contained in the Dongle, you can simply load your CD-rom on any other computer and copy the CD-rom if you wish. In order to use the program in full mode you simply need to purchase another dongle for as many computers as you wish. Lost Dongles are not replaced free of charge. They must be ordered from MTX customer service. There is however a small discount for second licenses.

Chapter 6

Mtx Lab Systems, Inc.
Comment:

ACCESSING SPECIAL FUNCTIONS AND INSTRUMENT CONTROLS

General Instrument Operations and User Tips

This section of the Spectrosoft manual provides specific user information regarding the individual instruments and their controls. This section also provides users with some insight to various instrument anomalies and other useful operational hints and other information not specifically mentioned in instrument manuals.

Each instrument has a unique and different way of setting up and operating. Even though the hardware for each instrument looks different, each manufacturer has paid special attention to designing its instrument to provide their product a special advantage to make its instrument more useful.

Most instruments provide nearly equal readings despite some small differences in filter bandwidths, lamp intensities, optical paths and methods of reading. Spectrosofts power is in its ability to control and acquire the plate data from many different manufacturers instruments and present it to the user in identical screen or printed format.

Using a Multiskan to read a plate will give nearly the exact same readings on a VMAX or MR5000. Only the commands and controls for each instrument vary and Spectrosoft is the only known product that contains the complete repertoire of instrument commands for each instrument shown on the instrument screen. MTX does regularly add new instruments to its repertoire, and at the request of users, can add special or unique instruments to the instrument list.

Following is a series of discussions regarding the various instruments that Spectrosoft can work with. The information below has been gleaned from years of expertise in sales and service of these instruments and knowing what users experience when using them.

It should be noted here that MTX manufactures a specific cable for each instrument. The years of technical support providing customers with help using their own cable has led to frustration, down time, lost productivity and a bad taste all because the cable was not the correct type. Once an instrument is working with a correct cable and correctly functioning computer/interface, the system works extremely reliably. In fact, customers who perform long term growth assays, use Spectrosoft with ELISA readers for kinetic readings lasting over a month. This kind of nonstop reliability is just one of Spectrosofts desirable features.

Hardware problems with instruments can easily be overcome without great expense. MTX has many years experience working with all types of readers and can solve most any kind of problem. It is not necessary to live with an intermittent instrument when a small investment will provide many more years of quality reliable service.

It is not possible here to provide users with all possible operational and or environmental cautions. It is however strongly advised that the user take all precautions to ensure they are protected against problems caused by static shock, over heating, power fluctuations, etc.

There are many products available on the market today that protect computers and the instruments they share, such as UPS systems to prevent brown outs and power problems.

Extremely dry labs especially in northern climates, experience static shock which can destroy the logic circuits of an instrument. Providing humidified air not only helps keep the instrument from failure due to static shock, it aids in reducing evaporative losses from uncovered microplates.

Keeping an instrument out of the weather is very important. Although there are some instruments which are very rugged, most require the normal room temperature and humidity of a comfortable lab. Keep your instruments away from direct sun light or windows, keep them off of radiators or any heat generating equipment such as incubators. Keep the unit plugged into its own wall socket and not shared with other high current drawing devices such as a Laser or MRI.

Keep your instrument clean and dust free. Always use a dust cover, (make one if necessary). Clean up any liquid spills on the instrument as they soon migrate to the optics and damage over time will occur.

Institute a quality program to monitor your instruments performance. Acquire a known standard plate, either a known test plate of your assay standards, or MTX supplies stable, long term glass neutral density plates with traceable standards for exacting measurements. The traceable plate is very convenient to use and requires recalibration every two years. It is acceptable for traceable readings for certified labs.

If your assay readings ever begin to vary, many factors such as lamp life, filter transmission levels, dirt or contamination begin to show up. Even microplates can vary from batch to batch as can calibration of and dispensing variabilities of single and multichannel pipettes and their associated disposable tips.

Use only flat bottom clear microplates when performing elisa readings on a vertical light path spectrophotometer. Using round bottom, vee bottom or pvc plates will diffract and absorb the light and cause variable readings. One solution however to this dilemma is the optional agglutination reader of the Multiscan family. If it is necessary to use those types of plates, better results may be obtained with a Multisoft reader. Depending on how accurate you require your readings must be, the non standard plates may be acceptable.

Only an experienced multidiscipline service provider such as MTX, can quickly and efficiently diagnose your problem. Calling our technical support department can help you determine your next course of action.

MTX provides a complete host of services to assist users in performing microplate assays with confidence. From traceable and certified pipette calibration (with documentation), to clear high quality microplates, New, as well as remanufacturered, instruments, all instrument repair and spare parts, remanufacturing of your old instrument as well as certified traceable calibration and documentation for any microplate reader.

Luminoskan Operations

Accessing Luminoskan Features

Mtx Lab Systems, Inc.
Comment:

Spectrosoft provides a unique control panel to allow the user to enable the special operating commands of the Luminoskan. (These features are available only on newer models of the Luminoskan.) Spectrosoft will however command the older models of the Luminoskan to read but it is not able to directly control the shaking, incubating or dispensing from the computer port. Some older versions of the Luminoskan can be modified to work with the Spectrosoft so contact MTX for details.

To access the Luminoskan features, click the accessory button “Lumi” just below the “Read Mode” button on the bottom of the “Plate” screen of Spectrosoft. This button is available only after selecting the Luminoskan (from the instrument screen) when bringing up Spectrosoft.

It is highly recommended that the user, at this point, refer to the Luminoskan Operators manual for specific instructions and in depth explanations regarding the features accessed here. The controls for the Luminoskan can be very complex, and so changing the read mode and or read timing can have a significant effect on the results of the data read from a plate.

An example of significant reading differences can occur if the user has prepared a plate to do a flash reaction, usually after injection of a chemi-luminescent tag, the Luminoskan should be set up to read in Peak mode to allow it to capture the highest reading. If the reader was set to single mode instead, the data read could be much lower than expected by several orders of magnitude due to the fact that the shutter takes time to open and the read logic is not prepared to read just after the injection, thereby missing the most important data. Conversely, setting the reader to Peak when expecting only a low level reading can blind the phomultiplier by keeping it open to long. Since the Luminoskan can be programmed to operate in so many different modes and to operate virtually endlessly, such as in kinetic readings with timing, the user must be familiar with the operating characteristics of the Luminoskan to be able to take full advantage of Spectrosofts features and to program the unit to get optimal results from the reader.

Luminoskan Setup and Control Screen

Once the “Lumi” button is pushed, Spectrosoft displays the Luminoskan “Setup” screen. This screen gives the operator access to controlling all the optional features of the Luminoskan. The display is self-explanatory. Clicking on any of the features of the “Setup” screen here control the reader in this mode until the program is closed.

Dispensing

If you are using the Dispenser pumps to inject into the plate, you must first prime the pump tubing. The Luminoskan will prevent you from dispensing until the prime function has been

Completed. Holding this screen button down will dispense liquid until the tube is clear of all air. Simply hold the end of the dispenser into the waste collection reservoir until the liquid begins to come out. Install the injector tube into the injector head and you are ready to dispense and read.

Emptying the Pump tubing

After reading and dispensing, the tubes should be evacuated of all liquid residues by pushing the appropriate pump button and clicking the Empty dispenser check box. Further, if you wish to purge the tubing of any residue, it is highly recommended to flush the tubes by priming with DI water, or other cleaning agent, until clean.

Plate Shaking

The plate shaking feature on the Luminoskan can be controlled by clicking the Continuous On click box. The shaking can be cycled on and off depending on the assay requirements by selecting the desired on and off time. Remember to turn off the Shaking after reading for the day.

Gain Setting

Photomultiplier gain control is selected using the High or Low setting of the Gain Control function. If you experience typically low levels of readings due to small reactions, you can increase your separation by selecting the High gain feature. This “Gain” causes the high voltage of the P.M. tube to increase causing the reader to sense extremely low levels of reactions that might be missed other wise. Caution on using the High Gain, if there are high levels of reaction in some wells, the P.M. tube may be blinded and the reader will stop reading. It can take several minutes for the P.M. tube to “Cool” down to recover before reading can resume.

Miscellaneous Controls

When enabling the Luminoskan to begin reading, you must first insert a microplate into the plate carrier. Since the Luminoskan keeps the carriage inside during power on and autocalibration you must click on “Plate Out” to get access to the plate carriage. Once the plate is installed, set the temperature as desired and wait until the temperature is reached before initiating the read function. Spectrosoft displays the dynamic temperature reading.

Measurement and Read Mode Settings

Once all the basic controls for the Luminoskan have been selected, the last function is to select the “Read Mode”. The Read Mode is the single most important control of the Luminoskan. Incorrect selection of this setting can have detrimental effects on the readings. Be sure to consult the operations manual for the Luminoskan to correctly select the desired setting for the assay being performed.

Single Mode setting is the most common reading performed by the Luminoskan. It serves as the basic mode of reading for many forms of assays for quick scanning. This setting used in conjunction with the “AutoRead” feature of Spectrosoft allows the reader to Kinetically read a plate an almost unlimited number of times, creating a Flat File of read data which can be exported into sophisticated analysis programs or simply analyzed using the features installed within Spectrosoft. Refer to the various calculation and blanking modes above. It is possible to read a plate thousands of times collecting millions of bytes of data from just one microplate using the AutoRead feature in single mode.

Peak Mode

The Peak Mode allows the user to take advantage of reactions which may be unstable or when it is not known what time period the peak reading will occur. The user must select a lag time, and a total time to read for this reading to be validated. Use this setting whenever the assay calls. Experiment with this setting to optimize your readings.

Integral Mode

This is the instrument power on default mode. This is used when dispensing is not critical with read times. Integrates readings taken over the selected time period with possible lag times.

Slope

Measures the difference in the signal between the start and the end of the selected time period. Total time and lag time must be selected.

Measure a Well (N Sample)

This mode is selected as an alternative to the built in AutoRead function of Spectrosoft. This mode allows a multiple read of a well as in scanning a well, and monitoring for changes. The Lag time and Total time just coincide with the reader’s ability to read a whole plate in the allotted total of the lab time and total time. Using block read for partially read plates are suggested to avoid reading all 96 wells if they are not being used.

Read

Once the Luminoskan settings have been selected, Clicking on the read button will command the reader to begin reading until the end of any selected time periods have completed. Once the data has been displayed on the “Plate” screen, the user may comence with any other calculation, store, and print functions. The settings will remain in place as long as Spectrosoft is exited normally and the computer is shut down correctly. Power fails and other anomalies such as system hangs can prevent the settings from being saved.

Multiskan Operations

Prior to connecting to any Multiskan reader, it is recommended that you first read a plate using the operator control panel keys, to verify that the reader is functioning correctly in stand alone mode. There is nothing more frustrating than trying to connect to an instrument that is not working correctly in the first place.

After powering on the instrument you will observe the instrument performing its Autocalibration sequence. This self test checks the filter transmission levels, lamp intensity, optics and detectors. If there are no blank errors, and the plate carriage moves in and out smoothly, there is a good chance the unit will connect to the computer with little difficulty. Be sure to first check the instruments defaults and filter settings.

On the Multiskan MKII type 347 readers, the filter settings for the instrument are stored in the readers microprocessor memory and are entered by the operator when first used. Unless some changes have occurred since the instruments manufacture they should be set in the factory settings. Refer to the operator manual for your specific reader but check them any way before doing any critical work. Normally to access the filter setup in the instruments memory, press the Parameter button and scroll in either direction using the arrow keys until you observe the Filter Setting menu. Enter and scroll once again to verify the settings. If they match the standard settings stated in the operator manual then simply press the enter key once again to escape the setup menu.

It is of utmost importance to remove the geared filter wheel containing the filters, from the inside of the instrument, and verify that each wavelength filter is in its correct position and that it is facing in the correct direction. Simply unscrew the small Phillip style screw and the “U” shaped holder spring and drop the filter out. The wavelength is printed on the side of the filter band. This is also a good opportunity to observe the filters condition. If you see any spots or “Hallowing” it is a good bet that the filter needs to be replaced. (MTX stocks all available wavelengths for every Multiskan reader manufacturered).

Most filters have arrows pointing the direction that light is flowing, but some don’t. Depending on the wavelength, most filters have a colored side and a mirrored side, but some look as mirrored on both sides. The most mirrored side faces the light source to block out undesirable energy. Be sure to install them correctly in the wheel, and also observe that the wheel has a magnetic slug which contacts a similar magnetic slug in the receptacle of the filter wheel module, to ensure that the wheel is facing the correct direction. If the filter wheel springs back up when inserting the wheel in to the holder, you must turn the wheel around.

Once you have verified the filter configuration and the reader manually reads a plate (or empty plate carriage), you may proceed to connect the reader to the computer.

One important note when connecting the Multiskan type 347 reader to the computer, some Multiskan models have two identical RS232, DB25, and type connector ports on the rear of the instrument chassis. Be sure to connect your cable to the top one (part of the top cover assy) only! The lower connector is only for the parallel printer port and is enabled from the front operator panel using the parameter button.

Some Multiskan type 347's require the user to plug a cable inside the instrument to enable the parallel port in addition to the serial port. Note the switch setting decal affixed to the rear cover. It is important that you regard these settings as you observe them from the rear as the instrument sits in its normal operating position. (Avoid trying to read too much into the switch setting configuration, read them as the instrument sits on the bench, don't try to turn the reader upside down, the switches have numbers printed on them only for manufacturing purposes). Also note that one bank of switches should all be in the down position.

One very important note on the switch settings for Multiskan family of readers. Switch 5,6,7 and 8 are used to adapt to and control the cable configuration. If you are not sure what type of cable you are using (MTX provides the correct cable and switch setting for each instrument from our service center), then the switches can assist you in setting your instrument to the correct configuration.

These four switches control the transmit and receive signals. They are mutually exclusive from each other in pairs. I.E. If 5 and 6 are up, then 7 and 8 must be down. In this setting, the pin two on the cable transmits commands and pin three receives commands. If the pair is set opposite, then 5 and 6 are down and 7 and 8 are up. Then pin two on the cable receives commands and pin three of the cable sends. Switch four is always down to prevent confusion. Switch 1,2, and 3 are used to set the baud rate setting of the instrument and normally doesn't need to be set because Spectrosoft does an automatic connect using the Autobaud feature described in the Communication Screen dialog in chapter three. Once you have a successful connection, Spectrosoft remembers the settings and will automatically connect the next time you use the instrument.

Turn on the instrument, verify correct Autocalibration and disable the built in printer if you want to save paper. This will save paper and wear on the printer if you ever wish to use the reader stand-alone. Refer to the operator manual for specific instructions for each type of reader.

Once Spectrosoft is brought up on the computer, you will click on the desired instrument and wait until the successful connection has been made. The Windows hourglass on the plate screen will appear for a few seconds until the connection is made. If there are connection problems switch to the comm screen and activate the autobaud button to select the different baud rates. If there still are problems then install a null modem adapter or switch the transmit and receive switches on the back of the reader to the opposite direction. More specific and detailed troubleshooting procedures are located in the Comm screen description earlier in this manual.

Because of the many different computer styles and makes available on the market today, it is not possible for MTX to evaluate and or advise on each one. So far, Spectrosoft works on every customer application to date. Your particular computer may or may not have individual

comm ports, and further with the proliferation of Palm organizers taking control of I/O ports, it can become exceedingly difficult to make your instrument work especially if your computer has been used for many different applications, or has had many different types of software installed and or deleted. All it takes is one accidental deletion of a program or an important DLL to be missing and nothing will work. In cases like these, it is better for the user to reinstall the windows operating system, after backing up all your important files.

Spectrosoft allows users to connect instruments and port switch boxes on up to four different computer I/O ports. This can be compounded using switch boxes leading to many different instruments (those shown on the Instrument screen). You can have instruments/switch boxes on four ports and branching out to many different instruments no matter which manufacturer.

To switch between instruments, simply go to the Com Screen and disconnect the currently connected reader, make your changes, then select another instrument from the instrument screen.

Different Multiskan models react differently when connecting for the first time. The Multiskan family of readers share a similar chassis and cover making them look very similar. However the internal electronics and mechanical design is different and vastly improved with each successive model type. The type 341 MCC or MCC/340 has a step mode plate carriage mechanism. When connecting this model instrument for the first time, the plate carriage should be all the way out to the right. When the CC (computer control response) displays on the control panel LED, the plate carriage moves in partially, stops, blanks on air, then moves the rest of the way in, selects the filter, comes out and is then ready.

The type 347 MCC340 MKII automatically parks the plate carrier in the out position on power on. It also automatically blanks, filter selects and reads on the fly in one sweeping motion.

However, the Multiskan type 347 sends out raw absorbance data to Spectrosoft for further analysis.

In the case of Dual wavelength readings, the reader sends raw data for each filter reading and Spectrosoft displays the difference of the raw readings. The user may then subtract the blank values by dragging and dropping the blanking cup on to the desired blank wells. Further, the user may select several blank wells and an average on those may be obtained by clicking the average blank check box on the bottom of the plate screen.

Multisoft Agglutination Operations

The Multisoft reader has two different modes of operation that can be controlled from Spectrosoft. In the EIA mode, the Multisoft reader acts just like a Multiskan 340 in the previous operations description. Refer to the Multiskan operations discussion above for further information on the use of the Multisoft reader in EIA mode.

The addition of the Agglutination mode provides users with fast data acquisition and processing of agglutination samples in microplate format. In agglutination mode the reader switches its shutter over the detector and effectively takes up to 20 snap shots of the microplate well as it passes between the shutter and the detector.

Spectrosoft collects all twenty snapshots of each of the 96-microplate wells, and formats them for display on the plate screen in successive mode.

To enable the agglutination mode after powering on the instrument, remove any microplates from the plate carrier, connect to Spectrosoft. While on the Plate screen, click on the AGG button adjacent to the Read Mode button. The reader will switch on the shutter and begin its Autocalibration sequence. This takes about 30 seconds. Once in agglutination mode, the reader will not allow the 340nm filter to be selected. The reader automatically selects the 405 filter for the autocalibration.

Once a successful autocalibration sequence has been completed, insert your subject microplate, click on the read mode button, and observe the instrument reading the plate.

To view a particular wells graphical display of absorbances, drag the Graphic icon from the bottom of the screen and deposit it on the well of interest. You may then, print, save or change the displays “Y” axis as desired.

The Read data is available for saving automatically, or viewing on the plate screen using the Bi-Directional arrow buttons. Each successive click on the right arrow button displays the next read plates data.

Spectrosoft provides many options for printing, autoprinting after read, autosaving after read or saving to database or spreadsheet. The spreadsheet save automatically saves the data in .TXT format to allow it to be imported by 123, Access, Excel, Quattro Pro, etc. Without having to do any manual parsing. The exported data from Spectrosoft will be displayed in the spreadsheet display screen in 96 well microplate format. From there, a user may add calculations, formulae, etc.

Fluoroskan II operations

Fluoroskan II connection and operation.

Before connecting the instrument using Spectrosoft, you must first, power on the Fluoroskan II reader and allow the unit to warm up until the “Wait” display goes off on the Fluoroskans front panel. This warm up time is required for the lamp to reach temperature for correct calibrated readings. (If at any time the lamp fail message comes on, call MTX for replacement information.)

Once the reader becomes ready, I.E. displays Out Ready, you may proceed to connect and perform readings. If this is a first time installation, refer to the above connection dialog in the com screen section of this manual in chapter three. Connection to the Fluoroskan II is virtually the same as all other instruments.

There are multiple switches, cable configurations, and Spectrosoft control settings used to get the Fluoroskan II to connect properly so use this manuals “Com” screen instructions to get the correct settings for computer control. Note: Be sure the cable is connected to the RS232 serial port DB25 connector located in the rear center of the reader. The right most end connector is either for a serial or parallel printer, depending on which model Fluoroskan II is being used.

The operator manual for the Fluoroskan II describes, in depth, the switch positions for each switch and serial port connector. If this is a first time connection it is recommended that you set the baud rate switch to 9600. This is switch 1,2, and 3. Switch 4 should be down. Switch 5/6 and 7/8 should be set in pairs as opposites. If 5/6 is up then 7/8 should be down and vice versa. These switches control the type of cable being used. If you are not sure if you have a straight cable or a null modem cable, use the one supplied with Spectrosoft and set the switches to 5/6 up and 7/8 down.

Switch to the comm screen and let the Autobaud feature select the instrument. Once you have an initial successful connection with the reader, Spectrosoft remembers the settings on shut down. The next time you bring up Spectrosoft, it will connect immediately. Just remember the time out of the warm up period.

Once the reader is connected (after the warm up period), it responds with the message, "OUT" on the display panel and performs an initial Autocalibration check. If there are any problems with the reader such as low lamp, bad filters, or incorrect levels of the autocalibration, the reader will display an instrument failure message both on the reader and the computer. At this point, service is the only remedy. It is not possible for the Fluoroskan to read a plate with this kind of malfunction. Once the Autocalibration sequence is successful, the reader is ready for any Spectrosoft command. If you wish to incubate the microplate at this time, then switch to the instrument screen for a display of the instruments individual commands. Click on Temp, 37 Do not disturb the readers settings or try to set any controls using the operator panel on the instrument. Any interruption of the computer control will knock it off line and will therefore not respond to Spectrosofts read command. Any time you wish to manually control the instrument, simply go to the com screen, click on disconnect, wait for the red line to display, and then exit Spectrosoft. This proper disconnecting and exiting of Spectrosoft allows the program to remember the last settings used, such as the baud rate, filter settings, comm settings, etc. So the next time you use the instrument, you will connect immediately, and resume the same protocol you had the last time the reader was used. This time saving feature promotes compatibility with all instruments. No matter what instrument you connect to next, you can bring it up instantly and with out the hassle of re-setting the controls.

The Fluoroskan II reads microplates in a column wise then row wise direction. It is advised the user try to use as many wells in a microplate as possible due to the fact that the instruments microprogram can not be changed to alter the way it reads the plate and the number of wells read. Once the Fluoroskan receives the read command, it reads the whole plate and cannot be stopped. The data received by Spectrosoft must be all 96 wells whether or not the user has filled them. Spectrosoft does however allow the user to block out the unused rows and columns on the plate screen by clicking on the red boxes adjacent to the row/column identifier.