MULTI

PARADIGM™ Multimode Detection Cartridge

User's Manual

Beckman Coulter PN A48438
Revision AB
August 2007

Beckman Coulter, Inc.
4300 N. Harbor Blvd.
Fullerton, CA 92835

BECKMAN COULTER®

Except as provided in writing signed by an officer to Beckman Coulter, Inc., this system and any related documentation are provided "as is" without warranty of any kind, expressed or implied, including that the system is "error free." This information is presented in good faith, but Beckman Coulter, Inc. does not warrant, guarantee, or make any representations regarding the use or the results of the use of this system and related documentation in terms of correctness, accuracy, reliability, currentness, omissions, or otherwise. The entire risk as to the use, results, and performance of this system and related documentation is assumed by the user.

Except as expressly provided herein, Beckman Coulter, Inc. makes no other warranty, whether oral or written, expressed or implied, as to any matter whatsoever, including but not limited to those concerning merchantability and fitness for a particular purpose, nor is freedom from any patent owned by Beckman Coulter, Inc. or by others to be inferred.

LIMITATIONS OF LIABILITY

Beckman Coulter, Inc. shall not be liable, to any extent whatsoever, for any damages resulting from or arising out of the use or performance of this system and related documentation or the procedures specified in this manual, regardless of foreseeability or the form of action, whether in contract, tort (including negligence), breach of warranty, strict liability or otherwise, and including but not limited to damages resulting from loss of data, loss of anticipated profits, or any special, indirect, incidental or consequential damages. In no event shall Beckman Coulter, Inc.'s liability to the user exceed the amount paid by the user to Beckman Coulter, Inc. hereunder. The user assumes full responsibility for the results obtained from the use of this system and related documentation and for application of such results.

Beckman Coulter, Inc.
4300 N. Harbor Boulevard
Fullerton, CA 92834-3100
Website: www.beckmancoulter.com

Biomek is a registered trademark and PARADIGM and SAMI are trademarks of Beckman Coulter, Inc.

HTRF is a registered trademark of Cisbio International.

Microsoft, Windows, and Windows NT are either registered trademarks or trademarks of Microsoft Corporation in the United States and/or other countries. IBM is a trademark of International Business Machines Corporation in the United States, other countries, or both. Pentium is a registered trademark of Intel Corporation. All other trademarks appearing in this manual are the property of their respective owners.

Copyright © 2007 by Beckman Coulter, Inc. All rights reserved. No part of this publication may be reproduced, distributed, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, or stored in a database or retrieval system, without the prior written permission of Beckman Coulter, Inc.

Warranty and Returned Goods Requirements

All standard Beckman Coulter, Inc. policies governing returned goods apply to this product. Subject to the exceptions and upon the conditions stated below, the Company warrants that the products sold under this sales agreement shall be free from defects in workmanship and materials for one year after delivery of the products to the original Purchaser by the Company, and if any such product should prove to be defective within such one year period, the Company agrees, at its option, either (1) to correct by repair or at the Company's election by replacement, any such defective product provided that investigation and factory inspection discloses that such defect developed under normal and proper use, or (2) to refund the purchase price. The exceptions and conditions mentioned above are as follows:

- Components or accessories manufactured by the Company which by their nature are not intended to and will not function for one year are warranted only to reasonable service for a reasonable time. What constitutes a reasonable time and a reasonable service shall be determined solely by the Company. A complete list of such components and accessories is maintained at the factory.
- The Company makes no warranty with respect to components or accessories not manufactured by it. In the event of defect in any such component or accessory, the Company will give reasonable assistance to Purchaser in obtaining from the manufacturer's own warranty.
- Any product claimed to be defective must, if required by the Company, be returned to the factory, transportation charges prepaid, and will be returned to Purchaser with transportation charges collect unless the product is found to be defective, in which case the product must be properly decontaminated of any chemical, biological, or radioactive hazardous material.
- The Company shall be released from all obligations under all warranties, either expressed or implied, if any product covered hereby is repaired or modified by persons other than its own authorized service personnel, unless such repair by others is made with the written consent of the Company.
- If the product is a reagent or the like, it is warranted only to conform to the quantity and content and for the period (but not in excess of one year) stated on the label at the time of delivery.

It is expressly agreed that the above warranty shall be in lieu of all warranties of fitness and of the warranty of merchantability, and that the company shall have no liability for special or consequential damages of any kind or from any cause whatsoever arising out of the manufacture, use, sale, handling, repair, maintenance, or replacement of any of the products sold under the sales agreement.

Representatives and warranties made by any person, including dealers and representatives of the Company, which are consistent or in conflict with the terms of this warranty, shall not be binding upon the Company unless reduced in writing and approved by an expressly authorized officer of the Company.

Parts replaced during the warranty period are warranted to the end of the instrument warranty.

NOTE

Performance characteristics and specifications are only warranted when Beckman Coulter, Inc. replacement parts are used.

Safety Information

WARNING

If the equipment is used in a manner not specified by Beckman Coulter, Inc., the protection provided by the equipment may be impaired.

Warning and Caution Definitions

All Warnings and Cautions in this document include an exclamation point, a lightning bolt, or a light burst symbol framed within a triangle.

The exclamation point symbol is an international symbol which serves as a reminder that all safety instructions should be read and understood before installation, use, maintenance, and servicing is attempted.

WARNING

A WARNING calls attention to a condition or possible situation that could cause injury to the operator.

CAUTION

A CAUTION calls attention to a condition or possible situation that could damage or destroy the product or the operator's work.

When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Electrical Safety

To prevent electrically related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies. Contact a service engineer for any servicing of equipment requiring the removal of covers or panels.

High Voltage

This symbol indicates the potential of an electrical shock hazard existing from a high voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-holed) wall outlets. Use only multiplug power strips provided by the manufacturer.

Disposal of Electronic Equipment

It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that:

- the device was put on the European Market after August 13, 2005.
- the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

Laser Light

This symbol indicates that a potential hazard to personal safety exists from a laser source. When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Laser Specifications

The Paradigm Multimode Detection Platform is rated a Class 1 Laser Product, because it houses a laser module. During normal operation, the laser light is not accessible to the user.

Figure 1.1 Label on rear of instrument

The embedded laser module has the following specifications:

Laser type:	diode laser
Wavelength:	650nm
Max. output power:	2 mW, cw
Laser Class:	Class 2M (IEC60825-1, ed. 1.2: 2001)
Fan angle:	55°±5°

The embedded laser module is ON, only if the microplate chamber flap is closed (hardware interlock). The user or the service engineer is not exposed to any radiation from the embedded laser module during operation, maintenance, or service. The closed microplate chamber acts as the protective housing.

Chemical and Biological Safety

Normal operation of the instrument may involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When using such materials, observe the following precautions:

- Handle infectious samples according to good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original solutions containers prior to their use.
- Dispose of all waste solutions according to your facility's waste disposal procedures.
- Operate the instrument in accordance with the instructions outlined in this manual, and take all the necessary precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids may occur; therefore, take appropriate safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Use an appropriately contained environment when using hazardous materials.
- Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the appropriate cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.

NOTE

Observe all warnings and cautions listed for any external devices attached or used during operation of the instrument. Refer to applicable external device user's manuals for operating procedures of that device.

Moving Parts

To avoid injury due to moving parts, observe the following:

- Never attempt to exchange labware, reagents, or tools while the instrument is operating.
- Never attempt to physically restrict any of the moving components of the instrument.
- Keep the instrument work area clear to prevent obstruction of the movement.

Cleaning

Observe the cleaning procedures outlined in this user's manual for the instrument. Prior to cleaning equipment that has been exposed to hazardous material:

- Appropriate Chemical and Biological Safety personnel should be contacted.
- The Chemical and Biological Safety information contained in this user's manual should be reviewed.

Maintenance

Perform only the maintenance described in this manual. Maintenance other than that specified in this manual should be performed only by service engineers.

IMPORTANT

It is your responsibility to decontaminate components of the instrument before requesting service by a service engineer or returning parts to Beckman Coulter for repair. Beckman Coulter will NOT accept any items which have not been decontaminated where it is appropriate to do so. If any parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

Table of Contents

Warranty and Returned Goods Requirements, iii

Safety Information, v

Warning and Caution Definitions, v

Electrical Safety, vi

High Voltage, vi

Disposal of Electronic Equipment, vi

Laser Light, vii

Laser Specifications, vii

Chemical and Biological Safety, viii

Moving Parts, ix

Cleaning, ix

Maintenance, x

Multimode Detection Cartridge, 1

Overview, 1

About Fluorescence Intensity, 3

Applications of Fluorescence Intensity, 3

Analyzing Fluorescence Intensity Data, 3

About Time-Resolved Fluorescence, 8

Applications of Time-Resolved Fluorescence, 9

Analyzing Time-Resolved Fluorescence Data, 9

About Glow Luminescence, 15

Applications of Glow Luminescence, 15

Analyzing Glow Luminescence Data, 16

Performing Measurements, 19
Microplate Recommendations, 21
Measurement Specifications, 22
Preventive Maintenance and Troubleshooting, 24
Verifying Performance with Check Plate, 25
Troubleshooting, 26

Multimode Detection Cartridge

Overview

The Multimode Detection Cartridge is a detection cartridge for use with the PARADIGM™ Detection Platform. Installing the Multimode Detection Cartridge enables several detection methods, including:

- fluorescence intensity (FI) for coumarin, fluorescein, rhodamine, and Texas Red.
- time-resolved fluorescence (TRF) for europium chelate.
- glow luminescence.

A detection cartridge contains the light source, optics, and electrical components needed to perform specific measurement modes or for specific applications. The PARADIGM Detection Platform requires these detection cartridges to enable different measurement modes.

The PARADIGM Detection Platform is a modular multimode microplate detector. User-installable and removeable detection cartridges allow the detector to be configured for specific applications and easily expand the capabilities of the detector at any time.

The Multimode Detection Cartridge occupies three (3) slots. It may be installed in either the top read detection cartridge transport for top reading, or in the bottom read detection cartridge transport for bottom reading. Refer to *Installing Detection Cartridges* on page 1-18 of the *PARADIGM™ Detection Platform User's Manual*, for installation instructions.

NOTE

It is recommended to install the Multimode Detection Cartridge the top read detection cartridge transport when running time-resolved fluorescence or glow luminescence detection methods.

About Fluorescence Intensity

In a fluorescence intensity method, the source light is directed through an excitation filter and then focused by an objective lens from either above or below the plate, depending on whether the cartridge is installed in the top or bottom read detection cartridge transport. The filter passes only the wavelength necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by one or two photo multiplier tubes.

The Multimode Detection Cartridge contains filter sets for measuring fluorescence intensity of coumarin, fluorescein, rhodamine, and Texas Red. The Multimode Detection Cartridge uses an high power LEDs and optimized filter sets together with the PARADIGM Detection Platform's photon counting detection, resulting in excellent detection limits and linear dynamic range at short read times.

Applications of Fluorescence Intensity

Some assays use a fluorescent label to selectively attach to certain compounds. The amount or concentration of the compound can then be quantified by measuring the fluorescence intensity of the label, which is attached to the compound. Such methods are often used to quantify low concentrations of DNA or RNA, for example.

Analyzing Fluorescence Intensity Data

Fluorescence intensity data is dependent on a number of variables. As a result, raw data from the same plate may appear significantly different from one instrument to the next (for example, a PARADIGM Detection Platform versus a DTX Multimode Detector). Raw data is compared to a standard curve with known concentrations of a reference label.

A standard curve consists of, at a minimum, a blank sample and a reference standard sample of known concentration. The raw data can then be expressed in equivalent concentration of the reference label.

The raw data is normalized to counts per second by dividing the number of counts by the read time per well. When selecting a fast read mode, the raw data becomes slightly lower because some signal during continuous plate movement is collected under non-optimum focusing conditions.

Analyzing and validating fluorescence intensity data typically consists of the following:

- *Blank, and Background Correction* (page 1-4)
- *Detection Limit* (page 1-6)
- *Linearity and the Linear Dynamic Range* (page 1-7)

Blank, and Background Correction

A blank is a well that contains everything used with the sample wells except for the label and sample-specific compounds which may yield unwanted interference. Using assay buffer only or an empty well may be some approximation of a blank, but should be verified for each assay.

The blank sample reveals the offset underlying each raw data sample. This offset does not carry information on the label and is therefore subtracted before any data reduction is performed. The blanked raw data are proportional to the amount of label in a sample such that the label concentration is quantified by (equation 1).

$$conc_{label} = (sample - blank) / (standard - blank) \times conc_{std} \qquad \text{(equation 1)}$$

where $conc_{std}$ is the concentration of the standard, and sample, blank and standard are average values of any replicates for the sample, blank and standard wells. In the general case where the standard curve covers a concentration range of more than a few linear logs,

(standard - blank) / $conc_{std}$ is equivalent to the slope of the standard curve, and the concentration of the label is determined by (sample - blank) / (slope of standard curve).

Background is caused by multiple effects of the assay, microplate, and detector optics. Low background is an indicator for a suitable plate type and match between cartridge (filter set) and fluorophore. Background is quantified using the blank and standard, and can be expressed by the following equation:

$$\text{background} = (\text{blank}) / (\text{standard} - \text{blank}) \times conc_{std} \qquad \text{(equation 2)}$$

where $conc_{std}$ is the concentration of the standard, and blank and standard are average values of any replicates for the blank and standard wells.

For improved results, it is recommended to run replicates for all blanks, controls, and samples. In this case, the blank value subtracted is the average value of all blanks. Multimode Software performs automatic blank averaging and reduction if blanks are defined on the plate layout in the protocol (refer to *Creating Protocols* on page 7-137 of the *Multimode Analysis Software User's Manual).*

Detection Limit

After blank subtraction from the raw data, some wells may be unexpectedly lower (or higher) than zero due to noise, blank contamination, blank replicate non-uniformity, or an unsuitable blank. Because of this, there is a lower limit on the concentration that may be quantified. Technically, this detection limit is defined by the non-uniformity of blank replicates (standard deviation), quantified by (equation 3), which is analagous to (equation 1):

$$\text{det limit} = 3 \times (\text{stdev(blank)}) / (\text{standard} - \text{blank}) \times \text{conc}_{\text{std}} \qquad \text{(equation 3)}$$

where conc_{std} is the concentration of the standard, stdev(blank) is the standard deviation of the blank replicates, and blank and standard are average values of the replicates for the blank and standard wells.

Determining the detection limit for an assay requires multiple blanks and the calculation of standard deviation. Due to the invocation of noise (by the use of the standard deviation), the detection limit typically depends on read time, and is thus a measure for sensitivity at a given read time.

Linearity and the Linear Dynamic Range

Within a wide range at not too high concentrations, blanked raw data is proportional to the amount of label in a sample. When the standard curve after blank reduction is not linear in concentration at the lower end, there may be an inappropriate or contaminated blank.

At very high concentrations, saturation (that is, when the relationship between concentration and raw data is no longer linear) may be seen in raw data from products other than PARADIGM Detection Platform. The PARADIGM Detection Platform and the Multimode Detection Cartridge prevent saturation by adapting the excitation light intensity to each sample and scaling the raw data accordingly, resulting in a larger linear dynamic range.

The linear dynamic range (LDR) is defined by:

$$LDR = \log_{10}[(\text{max conc lin}) / (\text{detection limit})] \qquad \text{(equation 4)}$$

where max conc lin is the highest concentration in the linear range that may be quantified.

About Time-Resolved Fluorescence

Time-resolved fluorescence (TRF) is a measurement technique that depends on three characteristics which lead to better discrimination between the specific signal (proportional to the amount of label) and the unspecific fluorescence resulting from background and compound interference:

- pulsed excitation light sources.
- time-gated electronics faster than the fluorescence lifetime (LT).
- labels with prolonged fluorescence LT.

The time-gating electronics introduce a delay between the light pulses cut-off and the start of signal accumulation, as shown in Figure 1.1 on page 12. During the delay (t_2), the unspecific fluorescence caused by test compounds, assay reagents, and the microplate vanishes while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains under the decay curve during period $t_3$with the benefit of reduced background.

The Multimode Detection Cartridge and PARADIGM Detection Platform provide the pulsed excitation light sources and time-gated electronics; the label is an assay characteristic, and can often be found in dyes based on lanthanides (rare earth metals) such as europium (LT of about 700 µs), samarium (LT of about 70 µs), or terbium (LT of about 1000 µs).

The Multimode Detection Cartridge utilizes an ultraviolet LED that emits in the range between 350-380 nm for excitation of europium chelates, and comes equipped with emission filters for europium. This enables single label europium assays to be run.

NOTE

Europium cryptate and terbium require excitation wavelengths below 330 nm and cannot be measured with this Multimode Detection Cartridge.

Applications of Time-Resolved Fluorescence

Time-resolved fluorescence (TRF) is useful in some fluorescence immunoassays, such as DELFIA (dissociation-enhanced enzyme linked fluorescence immunoassay). TRF is also useful in some assay variants of TR-FRET (time-resolved fluorescence resonance energy transfer) in which the FRET acceptor label acts as a quencher only and does not emit fluorescence. The proximity between donor label and acceptor (quencher) is then quantified by the intensity decrease of the donor label.

DELFIA requires washing steps as in an ELISA, but the TR-FRET assay involving quenching is a homogeneous microplate assay technique and requires only mixing and measuring—no wash steps are required. It can also be miniaturized, which makes it useful for high-throughput screening applications.

Analyzing Time-Resolved Fluorescence Data

A time-resolved fluorescence (TRF) measurement includes a number of pulses. Each pulse consists of turning on the LED, turning off the LED, pausing for a specified length of time, and measuring the fluorescence intensity of the sample. These pulses are repeated several times, as specified in the measurement protocol timing parameters (see Table 1.1 for suggested timing parameters).

Analyzing and interpreting TRF data typically consists of the following:

- *Blank Correction* (page 1-10)
- *Data Normalization* (page 1-10)
- *Data Optimization* (page 1-11)

Blank Correction

Although background is significantly lower than with fluorescence intensity measurements, it is recommended to use blank or assay controls.

A blank is a well that contains everything used with the sample wells except for the label and sample-specific compounds. Using assay buffer only or an empty well may be some approximation of a blank, but should be verified for each assay.

The blank sample reveals the offset underlying each raw data sample. This offset does not carry information on the label and is therefore subtracted before any data reduction is performed.

For improved results, it is recommended to run replicates for all blanks, controls, and samples. In this case, the blank value subtracted is the average value of all blanks. Multimode Software performs automatic blank averaging and reduction if blanks are defined on the plate layout in the protocol refer to *Creating Protocols* on page 7-137 of the *Multimode Analysis Software User's Manual.*

Data Normalization

TRF raw data changes in magnitude when the timing parameters are changed. However, TRF data are normalized for a number of 1000 pulses. This means that the sample raw data does not change when only the number of pulses is changed.

When selecting a fast read mode, the raw data becomes slightly lower because some signal during continuous plate movement is collected under non-optimum focusing conditions.

Data Optimization

There are two timing parameters which may be optimized to adjust the performance of the measurement as desired: time per plate (or time per well) and integration time per cycle.

The measurement noise is dependent on the read time per sample (time per plate or time per well). In particular, the detection limit improves when the read time is increased. For TRF, the read time per well increases with the selected number of pulses. The time between pulses (see Table 1.1), however, may be different on various systems. Therefore, it is important to specify the read time when comparing measurements.

All low light level detection devices have some measurement noise in common. In order to average out the measurement noise, optimization of the time per plate (or time per well) shall target for accumulating as many counts as possible. Within some range, the reduction of noise (CVs, detection limit) can be accomplished by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations.

Further optimizing measurement results is done by optimizing the timing parameters. Table 1.1 may be used as a guideline for the selection of timing parameters.

Table 1.1 Time-resolved fluorescence timing parameters

Parameter	Value	Comment
Pulse length	0.100 ms	suggested value for Multi cartridge; period for excitation of sample; t_1 in Figure 1.1
Measurement delay	0.010 ms	suggested value for Multi cartridge; delay to ensure excitation pulse has vanished; t_2 in Figure 1.1
Integration ime per cycle (pulse)	0.890 ms	suggested value for Multi cartridge; time window accumulating signal; t_3 in Figure 1.1

Figure 1.1 Timing parameters for time-resolved fluorescence

9018923.AI

When neglecting the time delay compared to the integration time window, the accumulated signal A can be approximated by the following equation:

$$A/A_{max} = (1-\exp(-M)) \times 100\% \qquad \text{(equation 5)}$$

In the equation above, M is the size of the time window (or integration time) divided by the exponential decay time constant (or the fluorescence LT of the label).

$$M = (\text{integration time})/(\text{fluorescence LT}) \qquad \text{(equation 6)}$$

For example, using Europium, which has a fluorescence lifetime of 700 µs, and the suggested Integration time per cycle from Table 1.1 of 0.890 ms (or 890 µs), M = 890/700 = 1.3. Inserting this value of M into (equation 5) yields A/A_{max} = 71%.

Table 1.2 displays the achievable accumulated signal percentage for selected values of M. To optimize the integration time per cycle (pulse), the integration time should be set such that the value of M enables the desired signal to be achieved. For example, to achieve 86% signal, select an integration time such that M is equal to 2.0. Using the Europium example and solving (equation 6) for the integration time, the integration time may be set to M (2.0) times the fluorescence lifetime (700 µs), or 1400 µs (1.4 ms).

Table 1.2 Achievable accumulated signal percentage versus M

M	0.25	0.5	0.75	1.00	1.25	1.50	2.00	3.00
A/A_{max} [%]	22	39	53	63	71	78	86	95

About Glow Luminescence

In a glow luminescence method, the intensity at which light is emitted from a chemiluminescent or bioluminescent reaction is measured. The light output is measured as the rate of photons per time and is expressed as counts per second. In glow luminescence reactions, the light output decays slowly with time.

The Multimode Detection Cartridge contains the components for measuring the light intensity from luminescence. Since the light is emitted as a result of a chemical reaction, no excitation light and no excitation filters are required to measure luminescence.

NOTE

The Multimode Detection Cartridge is not suitable for measurement of flash luminescence reactions, which require injectors.

Applications of Glow Luminescence

Chemiluminescent reactions may be induced to measure the quantity of a particular compound in a sample. Examples of luminescent assays include:

- Reporter gene assays (the measurement of luciferase gene expression)
- Quantitation of adenosine triphosphate (ATP) as an indication of cell counts with cell-proliferation, cytotoxicity, and biomass assays.
- Enzyme measurements with luminescent substrates, such as immunoassays.

Analyzing Glow Luminescence Data

The conversion rate of photons to counts is individual for each detector. Therefore, raw data from the same plate may appear significantly different from one instrument to the next. In addition, the data format used by other manufacturers may not be counts/s and may be different several orders of magnitude. It is important to know that the number of counts and the size of figures is in no way an indication of sensitivity (see *Detection Limit* on page 1-17) .

Concentrations or qualitative results are derived from raw data by means of a standard curve or comparison with reference controls.

A standard curve consists of, at a minimum, a blank sample and a reference standard sample of known concentration. The raw data can then be expressed in equivalent concentration of a reference label. The raw data is normalized to counts per second by dividing the number of counts by the read time per well.

Analyzing and validating luminescence intensity data typically consists of the following:

- *Background Correction* (page 1-17)
- *Detection Limit* (page 1-17)
- *Sample Volumes and Concentration of Reactants* (page 1-18)

Background Correction

The light detected in a luminescent measurement typically has two components: specific light from the luminescent reaction; and an approximately constant level of background light caused by various factors, including the plate material and impurities in the reagents. The background can be effectively measured using blank replicates. Blanks should include the luminescent substrate (chemical energy source) but not the luminescence agent (typically an enzymatic group which makes the substrate glow).

For improved results, it is recommended to run replicates for all blanks, controls, and samples. In this case, the blank value subtracted is the average value of all blanks. Multimode Software performs automatic blank averaging and reduction if blanks are defined on the plate layout in the protocol refer to *Creating Protocols* on page 7-137 of the *Multimode Analysis Software User's Manual*.

Detection Limit

The detection limit is the smallest measurable signal above the background. The background can be determined as described above, taking a number of blank measurements and calculating an average value and standard deviation for the blanks. The detection limit can then be determined as the average blank plus three standard deviations. If the average sample value measures above the detection limit, the sample can be detected at a statisically significant level.

The detection limit can be described by the following equation:

$$\text{det limit} = 3 \times \text{stdev(blank)} / (\text{standard-blank}) \times \text{conc}_{\text{std}} \qquad \text{(equation 7)}$$

where standard is the signal of a standard, blank is the mean of blanks, stdev(blank) is the standard deviation of the blanks, and conc_{std} is the concentration of the standard.

Sample Volumes and Concentration of Reactants

The concentration of the luminescent agent impacts the amount of light output in a luminescent reaction. Light is emitted as a result of a reaction between two or more compounds; therefore, the amount of light output is proportional to the amount of the limiting reagent in the sample.

For example, in an ATP/luciferin-luciferase system, when total volume is held constant and ATP is the limiting reagent, the blanked light output is proportional to the concentration of ATP in the sample. At very high concentrations of ATP, the excess requirement for the availability of substrate may become violated because all the substrate is used up, resulting in earlier decay of the strongest samples. In effect, it may appear to be a non-linearity of the detector, while it actually is an effect of the assay.

Performing Measurements

When the Multimode Detection Cartridge is installed in the PARADIGM Detection Platform, detection methods and protocols to perform fluorescence intensity measurements are enabled in Multimode Software.

When installed in the top read detection cartridge transport, the Multimode Detection Cartridge enables the following detection methods:

- Fluorescence Intensity Top
- Time-Resolved Fluorescence
- Glow Luminescence

When installed in the bottom read detection cartridge transport, the Multimode Detection Cartridge enables the following detection methods:

- Fluorescence Intensity Bottom

The Multimode Detection Cartridge enables several default and sample measurement protocols in Multimode Software for different detection modes, plate formats, and read times, which may be used as configured or as examples to create new protocols.

Additional detection methods and protocols using the Multimode Detection Cartridge may be created using Multimode Software.

- For information on creating detection methods using the Multimode Detection Cartridge, refer to *Creating and Editing Detection Methods* on page 5-77 of the *Multimode Analysis Software User's Manual.*
- For information on creating and running protocols using the Multimode Detection Cartridge, refer to *Creating and Running Protocols* on page 7-135 of the *Multimode Analysis Software User's Manual.*

NOTE

Measurement protocols that use multiple cartridges may also be created and run on the PARADIGM Detection Platform.

Microplate Recommendations

The type of microplate and the way it is handled can affect the measurement performance of the instrument. Plate height may not exceed 25 mm. Select a plate type with properties suited for the application and for use with multimode detectors. Table 1.3 lists microplate selection guidelines for each supported measurement type.

NOTE

For each type of assay (defined by plate type and sample volume), it is recommended to optimize the labware and reading height (refer to *Creating and Editing Labware* on page 6-115 of the *Multimode Analysis Software User's Manual)*.

Plate handling guidelines include:

- Keep unused plates clean and dry.
- Make sure the strips on strip plates are inserted correctly and level with the frame.

Table 1.3 Microplate selection guidelines

Measurement Technique	Microplate Type	Additional Considerations
Fluorescence Intensity Top Read	solid black	When an application specifies a surface treatment, only use plates with the correct treatment.
Time-Resolved Fluorescence	solid white	When an application specifies a surface treatment, only use plates with the correct treatment.
Glow Luminescence	solid white	When an application specifies a surface treatment, only use plates with the correct treatment.
Fluorescence Intensity Bottom Read	solid black with clear bottom	When an application specifies a surface treatment, only use plates with the correct treatment.

Measurement Specifications

The specifications for measurements using the Multimode Detection Cartridge are shown in Table 1.4.

Table 1.4 Measurement specifications

Parameter	Specification
Detection Cartridge Name	PARADIGM™ Multimode Detection Cartridge
Short Name	MULTI
Part Number	A41576
Weight	3.1 lbs (1.4 kg)
Detection Techniques	Fluorescence Intensity Time-Resolved Fluorescence Glow Luminescence
Type	Single emission
Number of Slots	3
Top/Bottom	Top or Bottom
Light Source	high power LEDs TRF: pulse period = 10 X pulse length
Filter Set	**Coumarin:** EX: 360-35; EM: 465-35 **Fluorescein:** EX: 485-20; EM: 535-25 **Rhodamine:** EX: 535-35; EM: 595-35 **Texas Red:** EX: 585-10; EM: 635-35 **Europium chelate (TRF):** EX: 370-80; EM: 616-10
Labels	**Fluorescence Intensity:** Coumarin, Fluorescein, Rhodamine, Texas Red **Time-Resolved Fluorescence:** Europium Chelate

Table 1.4 Measurement specifications

Parameter	Specification
Detection Limit Top and Plate Read Times	**Fluorescence Intensity (Fluorescein):** 96-well (200 µL): 0.4 fmol/well (2.5 min read) 384-well (75 µL): 0.3 fmol/well (4 min read) **Time-Resolved Fluorescence (Europium):** 96-well (200 µL): 25 amol/well (2.5 min read) 384-well (75 µL): 15 amol/well (4 min read) **Glow Luminescence (ATP):** 96-well (200 µL): 2 fmol/well (2.5 min read) 384-well (75 µL): 2 fmol/well (4 min read)
Linear Dynamic Range Top	Fluorescein: 5 logs in a single plate read
Check Plate Tests	test names ending with -MULTI

Preventive Maintenance and Troubleshooting

To ensure optimum operation of the instrument, perform the following preventive maintenance procedures as necessary:

- Open the detection cartridge transport only when removing or installing detection cartridges.
- When the detection cartridge is not in use, always keep the red cap in place and store in the detection cartridge box.
- Follow appropriate decontamination procedures as instructed by the laboratory safety officer.
- Contact a Beckman Coulter service engineer to inspect the instrument every two years.
- Use a Multimode Check Plate to regularly verify the performance of the cartridge. Contact Beckman Coulter sales or service for more details.
- Respond appropriately to any error messages displayed by Multimode Software.

Verifying Performance with Check Plate

The Multimode Check Plate is used to verify the performance of absorbance, fluorescence, and luminescence measurements performed on the PARADIGM Detection Platform. Verification runs performed with the check plate only verify detection cartridge performance; factory- and user-defined settings are not changed. The tests which need to be performed to verify performance differ between detection cartridges.

To verify the measurement performance of the Multimode Detection Cartridge, run all check plate test suites with the detection cartridge short name MULTI appended to the end of the test name.

NOTE

Refer to the *Multimode Check Plate User's Manual* for information on running tests with the check plate.

Troubleshooting

WARNING

Only officially trained service engineers may perform service procedures on the instrument. Contact a Beckman Coulter service engineer when service is required.

Perform the following troubleshooting techniques when necessary. Also refer to *Performing Basic Maintenance and Troubleshooting* on page 2-1 of the *PARADIGM™ Detection Platform User's Manual*.

Table 1.5 Troubleshooting the PARADIGM Detection Platform

If	Then
Functional failure	Install the cartridge in a different detection cartridge slot. Contact Beckman Coulter Customer Technical Support.
Detection cartridge fails verification tests with check plate	Refer to the *Multimode Check Plate User's Manual*. Contact Beckman Coulter Customer Technical Support.

PARADIGM™ Multimode Detection Cartridge User's Manual
PN A48438 AB (August 2007)

Copyright © 2007 Beckman Coulter, Inc.

All rights reserved. No part of this document may be reproduced or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without prior written permission from Beckman Coulter, Inc.

Find us on the World Wide Web at:
www.beckmancoulter.com

EC REP
Beckman Coulter Ireland Inc.
Mervue Business Park,
Mervue, Galway,
Ireland (353 91 774068)

Beckman Coulter do Brasil Com e Imp de Prod de Lab L
Estr dos Romeiros, 220 - Galpao G3 - Km 38.5
zip code 06501-001 - Sao Paulo - SP - Brasil
CNPJ: 42.160.812/0001-44

製造販売元：ベックマン･コールター株式会社
東京都江東区有明二丁目 5 番 7 号

生产商：
贝克曼库尔特有限公司，美国加利福尼亚州富勒顿
邮编：92835，电话：（001）714-871-4848

www.beckmancoulter.com

BECKMAN COULTER®