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BioFocus

# Operating Manual

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## Welcome

Dear Customer:

On behalf of Bio-Rad Laboratories, we would like to thank you for investing in the BioFocus Capillary Electrophoresis system. This system combines the best in automation and electrophoresis technologies. We are sure that it will provide you with many years of productive, high-quality performance.

One of the best ways to fully utilize the capabilities of your new BioFocus system is to read this manual. In it, you will learn how to set up the system and operate all hardware and software components. After reading this manual, please keep it close to your system so that you can conveniently refer to it.

This manual covers all BioFocus models. As such, you may find reference to equipment and features that are not present in your particular instrument. Most additional features can be upgraded on existing systems. If you would like to access an optional function, please contact your local Bio-Rad Representative.

Your BioFocus instrument is protected by a comprehensive warranty agreement. Please read this manual thoroughly so that you fully understand the coverage provided and are aware of your rights and responsibilities. One of the responsibilities of system ownership is regular maintenance. Following the maintenance schedules and instructions provided in this manual will help to keep your system functioning optimally and will preserve your investment. Please also keep in mind that Bio-Rad offers a range of comprehensive service agreements that can be tailored to meet your specific needs.

Bio-Rad Laboratories is dedicated to your total satisfaction and would be pleased to answer any questions or concerns.

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Toll-free: 1-(800) 4-BIORAD
Fax: 1-(800) 879-2289

For service or technical assistance, call 1-(800) 424-6723 or send a FAX to Bio-Rad at (510) 741-1047.

For information concerning Bio-Rad Laboratories and its products, visit our Worldwide Web site at http://www.bio-rad.com.

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# Introduction

## About This Manual

### Conventions

This manual uses certain conventions to aid the reader's understanding of text material and to assist operators in using the BioFocus systems.

### Note, Cautions, and Warnings

Notes, cautions, and warnings bring the reader's attention to certain operating procedures.

1. A note indicates a special procedure, an exception to normal operation, or something else of interest to the reader. Notes are located in the left margin, preceded by the word "Note" in italics.

2. A caution precedes an operating step that could damage the equipment or destroy data unless the operator takes certain precautions.



Caution Symbol



Electrical Hazard Warning Symbol

A warning precedes an operating procedure that could cause injury to the operator if not followed correctly.



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## Operating Controls and Menu Selections

*NOTE:* Most computer screen illustrations in this manual were captured using Microsoft Windows 95. If your computer uses Windows 3.1 or 3.11, the screens you see will be slightly different from the ones shown in this manual.

Names of drop-down menus, menu items, and buttons on toolbars in the computer display are set in boldface type, such as "click the **Status** button, or press the **Enter** key."

Throughout this manual the term "click " means pressing and releasing the left mouse button. The term "double-clicking" means pressing and releasing the left mouse button twice rapidly.

## Manual Organization

This manual contains seven major sections and an appendix. Each section describes certain aspects of system operation.

1. **Introduction**. This section contains a brief description of capillary electrophoresis system capabilities. The description covers both absorbance and LIF$^2$ Detector systems. Tables included in this section list specifications for both systems.
2. **Theory of Instrument Operation**. This section discusses the basic theories of capillary electrophoresis, absorbance detection, and laser-induced fluorescence detection. It also describes system components such as capillaries and carousels.
3. **Installation**. Considerations for system installation, unpacking, and connection to auxiliary equipment are described in this section. In addition, there are procedures for installing operating software and integrating software.
4. **Setup**. This section describes system setup, including changing from absorbance to LIF detection, and vice versa. There are also procedures for installing cartridges, filling vials, and inserting and removing vials.
5. **Software Operation**. This section includes descriptions of all elements of the operating software, including system verification and calibration, defining methods, defining configurations, and setting up automation sequences.
6. **Maintenance**. This section includes procedures for performing periodic maintenance. In addition, this section includes procedures for replacing the deuterium UV source and incandescent visible light source.
7. **Troubleshooting**. This section describes systematic ways of detecting and correcting system operating problems. There is also a description of system diagnostics and their use.
8. **Appendix**. This section includes the equipment warranty and provides a glossary of terms used in this manual.



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# Safety

Your safety, and the safety of others, is very important to us. To help you make informed decisions about safety, we have provided comprehensive operating procedures and safety information in this manual on labels affixed to the instruments. This information will alert you to any potential hazards.

You will find important safety information in the following forms:

- Safety labels affixed to your instrument
- Safety messages preceded by a caution or warning symbol.



**CAUTION**

Do not remove covers.

There are no user-serviceable parts inside.

Refer all servicing to qualified Bio-Rad personnel.

The BioFocus is designed and certified to meet EN61010* safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This safety certification does not extend to other chromatography equipment or accessories not EN61010* certified, even when connected to a BioFocus instrument.

The instrument should not be modified or altered in any way. Alteration of this instrument voids the manufacturer's warranty, and creates a potential safety hazard for the user.

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.



**WARNING!**

- The BioFocus instrument weighs a maximum of 64 kg. Do not attempt to lift the instrument without help.
- The LIF2 Detector cabinet weighs a maximum of 30 kg. Do not attempt to lift the cabinet without help.

*EN61010 is an internationally accepted electrical safety standard for laboratory instruments.



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**WARNING!**

- There are hazardous voltages inside the LIF$^2$ Detector and BioFocus instrument cabinets. Do not remove covers when the instrument is connected to AC power.
- Do not defeat any instrument interlocks; they are designed to prevent user injury.

## Laser Safety



**WARNING!** To avoid eye damage, take these precautions:

- Refer to laser safety sections in manuals provided with user-supplied lasers.
- Do not turn on external lasers unless the laser light output has been connected to the LIF$^2$ Detector, the protective cover is installed, and the external interlock is connected.
- Do not look at the end of an optical fiber cable with collecting or magnifying optics unless you are certain that the associated laser is off and cannot be turned on accidentally.
- Observe the limitations for external laser power output as described in "Connecting an External Laser" in the Setup section of this manual.
- Do not run optical fiber cable over sharp edges or make sharp bends in the cable.
- Use of controls, adjustments, or performance of procedures other than those specified herein may result in hazardous laser radiation exposure.



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# BioFocus® Capillary Electrophoresis System Capabilities

BioFocus Capillary Electrophoresis systems have multiple detection capabilities: UV and visible light absorption and optional laser-induced fluorescence. The following paragraphs describe the BioFocus systems and their detection systems' capabilities.

Electrophoresis systems resolve sample components by differences in their respective mass-to-charge ratios, size and chemical interaction. The speed with which a component (analyte) moves in an electric field is called its electrophoretic mobility. Each analyte migrates electrophoretically past a detector, and generates a signal proportional to its concentration. To achieve both good separation and resolution in a short time, voltage field strengths of over a thousand volts per centimeter (30,000 volts total) can be applied, although many routine separations are usually performed at 150-400 V/cm field strength. The voltage can be of either polarity depending on the type of sample analyzed and the methodology used. Capillary electrophoresis combines the high resolution of traditional gel electrophoresis with the sensitivity, ease of use, and high speed of High Performance Liquid Chromatography (HPLC).

Capillary electrophoresis methodologies that can be performed on the BioFocus systems include capillary zone electrophoresis (CZE), isoelectric focusing (IEF), non-gel sieving, and micellar electrokinetic capillary chromatography (MEKC).

The BioFocus family of capillary electrophoresis systems includes two instruments, the BioFocus 2000 and the BioFocus 3000. Both instruments provide high sensitivity, highly reproducible results, and share the following features:

- Fully automated system, with easily-changed, temperature-controlled capillary cartridges and two 32-position carousels.
- In the BioFocus 2000 TC and 3000TC models, the autosampler carousel compartment is temperature-controlled from 4° C to 40° C. This feature is desirable when working with heat-labile samples, when recovering fractions collected from precious samples, or when performing kinetic studies with the BioFocus system. In general, best analytical precision is obtained when both capillary and autosampler are temperature-controlled.
- A high voltage power supply capable of controlling voltages up to 30 kV and currents up to 300 µA with software-reversible polarity.
- PC management of all functions and data with self-checking diagnostics and on-line data integration.



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- Multiple rear panel signal outputs for integrators and PC workstations.

## UV/Visible Light Absorption Capability

The BioFocus 3000 system represents the state of the art in capillary electrophoresis instrumentation and is designed for environments where maximum flexibility and performance are required. In addition to the features listed above, the BioFocus 3000 system includes a high-speed scanning, multiwavelength detector capable of capturing on-the-fly spectral information from 190 to 365 nm (deuterium ($D_2$) light source) or from 366 to 800 nm (tungsten light source). The scanning capability of the BioFocus 3000 can be used for determination of peak identity, peak purity, or to find the optimum detection wavelength of an analyte.

The BioFocus 3000 system is best suited for R&D laboratories where its advanced detection capabilities provide the tools needed for method development and validation.

The BioFocus 2000 retains the high performance features of the BioFocus 3000 system but employs a programmable UV detector capable of single-wavelength detection from 190-365 nm (visible-wavelength detection from 366-800 nm is available with an optional tungsten lamp). The BioFocus 2000TC model includes a temperature-controlled autosampler carousel compartment.

The BioFocus 2000 system is a cost-effective alternative to the BioFocus 3000 system, delivering the same performance in terms of high sensitivity and reproducibility. It is best suited for the routine analytical laboratory where high sample throughput and precision are desired.



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## Laser-Induced Fluorescence System Capabilities

The LIF$^2$ Detector and associated Optics Module add laser-induced fluorescence (LIF) detection capability to the BioFocus 2000 and 3000 Capillary Electrophoresis systems. Together, the BioFocus instrument and LIF$^2$ Detector form a system that provides capability for both laser-induced fluorescence analysis and UV/visible absorption analysis techniques.

LIF detection increases nucleic acid sensitivity by a factor of 1000 compared to standard UV detection. This high sensitivity accommodates many new applications for capillary electrophoresis. Applications include DNA quantification and size determination, DNA size polymorphisms, DNA mutation analysis, synthetic oligonucleotide purity determination, protein/DNA interaction, and carbohydrate analysis.

You can configure each system to meet individual requirements. Systems can have the following laser and detector components:

- One argon ion laser and one PMT detector
- One argon ion laser and two PMT detectors
- One argon ion laser and one helium-neon laser, with two PMT detectors
- One internal laser and one user-supplied laser, with one or two PMT detectors

The standard single laser system contains an argon ion laser with a nominal output of 3.5 mW at a wavelength of 488 nm. The associated photomultiplier (PMT) detector and standard optics have optical bandpass filters that reject the 488 nm excitation light and pass light created by sample fluorescence at 520 nm.

A dual laser system contains an additional helium-neon laser with a nominal output of 1 mW at a wavelength of 594 nm. Because the optical system uses standard 25 mm filters, you can select filters that optimize detection of different fluorescent labels or stains.

BioFocus LIF$^2$ Detectors are fully automated and control one or two lasers and associated detectors through system software, (which identifies the configuration automatically). Systems can collect up to two channels of fluorescence data for processing by the main BioFocus system. It allows users to set parameters that match individual configurations of UV and visible light detectors, laser configuration, and PMT detectors.



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### *Hardware Description*

The LIF detection system consists of five components:

- A stand-alone LIF$^2$ Detector cabinet that houses one or two laser light sources
- An Optics Module that houses an optical assembly, emission filters, and PMT detectors. The Optics Module connects to the LIF$^2$ Detector cabinet by flexible, shielded metal conduits.
- A new fluorescence capillary cartridge that accommodates the new LIF$^2$ detector optics
- A modified cartridge door and an interlock that detects the presence of the Optics Module.
- New software that controls the lasers and PMTs as well as handling data generated during LIF sample analysis.

You can locate the LIF$^2$ Detector cabinet either next to the BioFocus instrument on a bench top or directly below on the floor. The conduits that connect the LIF$^2$ Detector cabinet to the Optics Module contain fiber optics, which transmit laser light output to the capillary cartridge. The conduits also contain electrical connecting cables to provide power and signal connections to the PMT detectors.



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# Specifications

Table 1-1 lists specifications for the basic CE systems. Table 1-2 lists specifications for the for the LIF$^2$ Detector and associated optics. Refer to "Minimum Computer Resources" in the BioFocus System Installation section of this manual for computer and software requirements.



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*Table 1-1 BioFocus Capillary Electrophoresis System Specifications*

| | |
|---|---|
| Operation | Menu-driven programming on IBM PC or 100% compatible computer with Windows 3.1x or Windows 95 software. Up to 198 runs or methods (one automation sequence combined with an optional priority sequence) can be programmed for automated or overnight unattended runs. |
| Automated Sampling & Fraction Collection | Two 32-position removable carousels with capped vial holders for samples, buffers, washes, fractions, and waste (can load up to 31 samples and collect up to 30 fractions). Uses standard 500 µl, 1.5 ml, or 4 ml microcentrifuge tubes or optional large capacity vial holders. |
| Absorbance Detector | BioFocus 2000:<br><br>Programmable single-wavelength UV (190 – 365 nm) with standard deuterium lamp. Optional tungsten lamp available for visible detection in the 366-800 nm range.<br><br>BioFocus 3000:<br><br>Programmable, multi-wavelength UV/Visible (190 to 365 or 366 to 800 nm) allows single wavelength or multiwavelength analysis, or high-speed scanning. Deuterium lamp (190 to 365 nm) and tungsten lamp (366 to 800 nm) light sources with concave holographic grating monochromator. |
| Power Supply | Up to 30 kV constant voltage or 300 µA constant current; software-controlled polarity. History or run voltage and current recorded |
| Injection Modes | Pressure or electromigration |
| User-Assembled Absorbance Cartridge Kits | Consists of temperature-controlled cartridges with coolant ports designed for use with the BioFocus liquid coolant system. Compatible with capillaries between 24 and 100 cm in length, with or without coatings, and 350 to 375 µm OD with any ID. |
| Temperature Control | Programmable for cartridge (15° – 40° C). Cartridge temperature recorded for length of run.<br><br>Optional carousel compartment temperature control from 4° to 40° C available in the BioFocus 2000TC and 3000TC models. |
| Dimensions | 560 mm high by 490 mm wide by 580 mm deep |
| Weight | BioFocus 2000 and 3000: 51 kg. BioFocus 2000TC and 3000TC: 64 kg. |



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*Table 1-1 BioFocus Capillary Electrophoresis System Specifications (continued)*

| | |
|---|---|
| Ambient Temperature | 10° to 40° C |
| Operating Humidity | 0 to 90% relative, non-condensing |
| Other Requirements | Inert compressed gas required for capillary purging and pressure injection of samples. Pressure 100 psi minimum; gas quality equal to or better than HPLC grade. |
| Recorder Output | Four analog output channels each with 10 mV, 100 mV, or 1.0 V full-scale capability. |
| Range Selections | 2.0, 1.0, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, and 0.0005 AUFS. |
| Integrator Output | 1.0 V/AU analog unattenuated signal to external integrator |
| Operating Modes | |
| Constant Voltage | Output 0 to 30,000 VDC, 100 VDC increments, set limit 0.3 to 300 µA |
| Constant Current | Output 0.3 to 300 µA, 0.1 µA increments, set limit 0 to 30,000 VDC |
| Operating Mode Crossover | Automatic upon reaching set limit |
| Available Line Voltages | 100, 120, 220, or 240 VAC, 50-60 Hz |
| Line Voltage Requirements | ±10% of nominal operating voltage (± 3 Hz of nominal operating frequency) |

| Fuses * | 100 to 120 VAC | 220 to 240 VAC |
|---|---|---|
| BioFocus 2000 Detector | 2.5 A (1) | 1.25 A (1) |
| BioFocus 2000 Power Entry | 4 A (2) | 2 A (2) |
| BioFocus 2000TC Detector | 2.5 A (1) | 1.25 (1) |
| BioFocus 2000TC Power Entry | 8 A (2) | 4 A (2) |
| BioFocus 3000 Power Entry | 4 A (1) | 2 A (2) |
| BioFocus 3000TC Power Entry | 8 A (1) | 4 A (2) |

*All fuses are type T, time-lag.



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*Table 1-2 LIF$^2$ Detector Specifications*

| | |
|---|---|
| **Definitions** | |
| Relative Fluorescence Unit (RFU) | Fluorescent signal produced by a 50 picomolar fluorescein concentration in a 75 µm capillary |
| Gain | PMT gain, varied by adjusting PMT voltage, calibrated for each system. |
| **Performance** | |
| *NOTE:* Specifications below are for measurements using a filtered computer output. Analog outputs are comparable except for filtering. | |
| Gain Range | 1X to 1000X |
| Gain setting resolution | <2% at any gain |
| Single laser system, 488 nm | |
| Peak-to-peak noise, ASTM method | 0.1 RFU maximum after 15 minutes warm-up at a gain of 100X or higher, 1 second time constant. |
| Baseline drift | 0.1 RFU maximum per hour after half hour warm-up |
| Two-laser system, 488 nm, 594 nm | |
| 488 nm channel | |
| Peak-to-peak noise, ASTM method | 0.2 RFU maximum after 15 minutes warm-up at a gain of 100X or higher, 1 second time constant |
| Baseline drift | 0.2 RFU maximum per hour after half hour warm-up |
| 594 nm channel | |
| Peak-to-peak noise, ASTM method | 0.3 RFU maximum after 15 minutes warm-up at a gain of 100X or higher, 1 second time constant |
| Baseline drift | 0.2 RFU maximum per hour after half hour warm-up |
| Equivalent capillary slit width (compared to uniformly illuminated slit) | 0.5 mm maximum |



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*Table 1-2 LIF$^2$ Detector Specifications (continued)*

| | |
|---|---|
| **Standard Optical Filters** | |
| Filter holder | Provision for one to three filters, 25 mm diameter |
| 488 nm discrimination filter | 520DF20 |
| Rejection at 488 nm | 10,000:1 |
| Pass, 515 to 545 nm, average | 50% minimum |
| Two-channel system | |
| Beam splitter (dichroic mirror) | 600DRLP02 |
| 594 nm discrimination filter | 630DF30 |
| Optional neutral density filter | Required for use above 50,000 RFU |
| Filters in laser(s) | Laser line notch pass |
| **Electronics** | |
| RS-232 Serial Ports | |
| Connector to computer | 25-pin D female |
| Connector to BioFocus | 9-pin D male |
| Baud rate, both ports | 9600 |
| Port settings | No parity, 8 bits data, 1 stop bit |
| Configuration switch, user-accessible | 4-position; sets laser and PMT configuration |
| Nonvolatile memory on LIF board | 64 words of 16 bits |
| Processor type | Z180, 6 MHz |
| RAM available | 32 K bytes |
| ROM available | 32 K bytes |
| Primary PMT | |
| Module type | Hamamatsu HC120MOD1 |
| Maximum voltage | -1100 VDC |
| Tube type | R5785 |
| Typical response at 420 nm, 1000 VDC | 268 mV/picowatt |
| Typical response at 420 nm, 500 VDC | approximately 0.9 mV/picowatt |
| 50% bandwidth | 190 to 570 nm |
| Secondary PMT | |
| Module type | Hamamatsu HC120MOD2 |
| Maximum voltage | -1100 VDC |
| Tube type | R4457 |
| Typical response at 530 nm, 1000 VDC | 234 mV/picowatt |
| Typical response at 530 nm, 500 VDC | approximately 2 mV/picowatt |
| 50% bandwidth | 220 to 660 nm |



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*Table 1-2 LIF$^2$ Detector Specifications (continued)*

| | |
|---|---|
| Monitor A/D (Laser Power and Current) | |
| Channels | |
| Channel Designations and Range | Four (multiplexed) |
| 1 | Laser 1 power, 0 to 50 mW |
| 2 | Laser 1 current, 0 to 50 A |
| 3 | Laser 2 power, for future expansion |
| 4 | Laser 2 current, for future expansion |
| Bits | 12 |
| Speed | approximately 10 ms per sample |
| Accuracy | 5% (limited by laser circuit accuracy) |
| Linearity | <0.05% |
| Outputs | |
| Integrator channels 1 and 2 | (no autozero offset) |
| Range | 0 – 10 V full scale, fixed |
| Load | 1 kilohm minimum |
| Filtering | Two-pole Bessel, 2 Hz, 1.2 s rise time |
| **Environmental** | |
| Temperature | 10° to 40° C operating<br>0° to 50° C storage |
| Humidity | 0 to 90% relative, noncondensing |
| Line Power Requirements | |
| Voltage | 90 to 132 VAC or<br>200 to 265 VAC |
| Frequency | 50 or 60 Hz |
| Power (LIF$^2$ Detector only) | |
| Single laser system, laser on | 400 to 1000 watts |
| Dual laser system, both on | 440 to 1040 watts |
| **Physical** | |
| Optical and PMT cable length | 2 m maximum, LIF$^2$ Detector cabinet to BioFocus |
| Weight, shipping | less than 39 kg |
| Weight, net | less than 30 kg |
| Dimensions | |
| Module | 432 mm high by 305 mm wide by 610 mm deep |
| Shipping Container | 584 mm high by 457 mm wide by 762 mm deep |



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*Table 1-2 LIF$^2$ Detector Specifications (continued)*

| | |
|---|---|
| **Safety and Regulatory** | |
| CDRH laser safety | Fully meets requirements |
| EN-61010-1 laser safety | Fully meets requirements |
| CE immunity and emissions. | Fully meets requirements |
| **LIF Cartridge Specification** | |
| Capillary outside diameter range | 340 to 370 µm |
| Capillary inside diameter range | 25 to 100 µm |
| Capillary length | 22 to 100 cm |
| Cartridge-to-cartridge emitted light ratio | 0.8 to 1.2X typical, with 75 µm capillaries |
| Unjacketed capillary length | 10 cm |
| **Lifetimes** | |
| Argon ion 488 nm laser | 9,000 hours of use |
| Helium-neon 594 nm laser | 10,000 hours of use |
| Blower | greater than 30,000 hours at 40° C |



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# Theory of Instrument Operation

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# Basic Theory of Capillary Electrophoresis

Capillary Electrophoresis (CE) is an innovative tool for analysts in many areas, including analytical biochemistry, molecular biology, analytical chemistry, medical and other life sciences. Its sensitivity and analytical specificity will provide information on substance identity, quantity, and purity. In all aspects of biotechnology industries, from development of initial concepts in the laboratory to quality control of final products, purification of potentially complex mixtures of labile macromolecules is required, as well as quantitative and qualitative analysis of purified substances. High Performance Liquid Chromatography (HPLC) and electrophoresis are two techniques that have been applied for analysis of biomolecules. CE has properties that make it a superior choice for many of the applications of traditional electrophoresis and HPLC.

The basic CE components are an electrolyte-filled capillary tube with a small vial at each end. The capillary tube is the heart of the system because that is where the separation of sample components occurs. Electrodes connected to a high voltage power supply are immersed in the electrolyte in the vials. Very small amounts of sample are introduced into one end of the capillary and are electrophoretically driven down the lumen toward the opposite electrode. The velocity with which a charged substance moves per unit of electric field is called its electrophoretic mobility. This characteristic is determined by its mass and charge. The degree of separation of various sample components during capillary electrophoresis is usually dependent on their different electrophoretic mobilities.



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# BioFocus Overview

This section gives a description of each of the major components of the system and how they work. For detailed information on the operation of the instrument, please refer to later sections of this manual. Figure 2-1 shows the location of the basic components of BioFocus 2000 and 3000 UV/Visible systems.

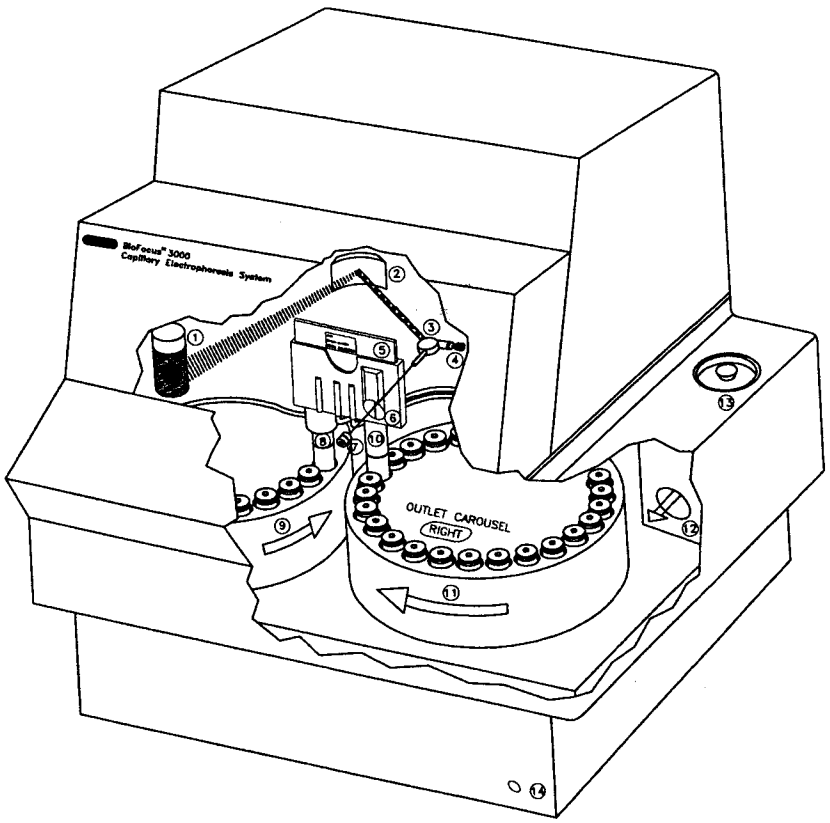



*Figure 2-1 Schematic of the BioFocus Capillary Electrophoresis Systems*

The parts of the system are listed below:

1. UV/Vis lamp
2. Diffraction Grating
3. Beam Splitter
4. Reference Photodiode
5. Temperature-Controlled Cartridge
6. Detector Window
7. Sample Photodiode
8. Capillary Inlet Vial
9. Inlet Carousel
10. Capillary Outlet Vial
11. Outlet Carousel
12. Carousel Cooling Air Duct
13. Cartridge Coolant Reservoir
14. Power-On Indicator Lamp



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# Detectors

## Ultraviolet and Visible Light Detector

Ultraviolet (UV) absorption is a general mode of detection for many substances. An on-line optical detector near the capillary outlet registers the absorbance of light by each sample component as it migrates past it.

For materials with conjugated chromophores, such as aromatic compounds, detection can be in the range of 250 to 300 nm.

The best sensitivity for detecting proteins and peptides, however, is in the range of 190 to 220 nm. Many solvents used in chromatography methods have UV cutoffs that prevent the use of these lower wavelengths. Fortunately, one of the advantages of CE is that UV absorbing solvents are not usually required. This allows greatly enhanced sensitivity. The absorption maxima of chromophores found in peptides is near 200 nm. Shifts of 10 to 18 nm can be caused by close association of multiple chromophores. Some experimentation with wavelength may increase sensitivity.

When set at the proper wavelength, the sensitivity for peptides should be in the picogram (absolute quantity) or parts per million (concentration) range.

Detection of nucleic acids is usually at 260 nm and detection of proteins in isoelectric focusing is usually at 280 nm to compensate for ampholyte interference at 200 nm.

The optical arrangement for the BioFocus detectors is shown in Figure 2-2. Wavelength selection is provided by a concave holographic (diffraction) grating.

### *BioFocus 2000*

The BioFocus 2000 and 2000TC absorbance detector employs essentially the same optical components as the BioFocus 3000 absorbance detector except that the grating is driven by a stepper motor.



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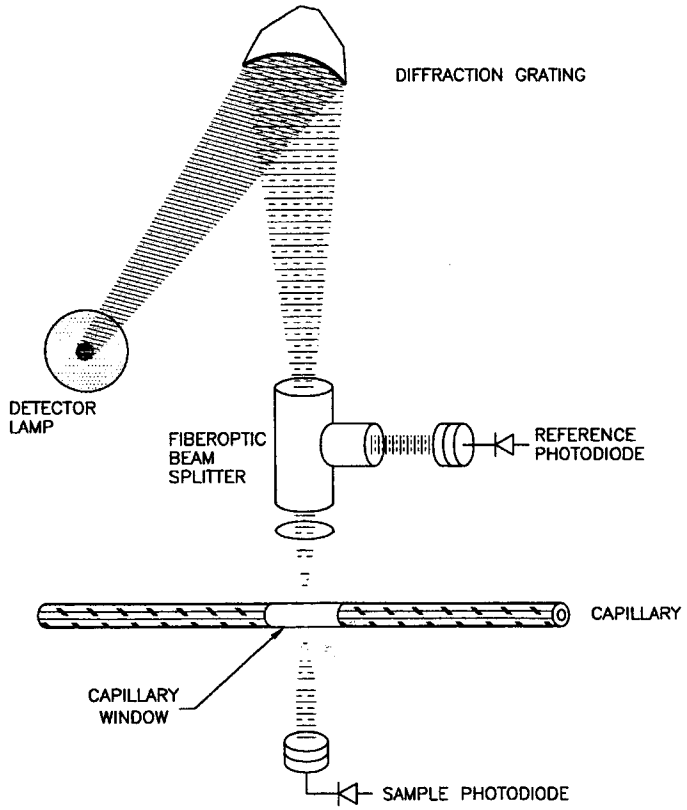



*Figure 2-2 The BioFocus Absorbance Optical System*

There are two detector outputs on the rear panel of the BioFocus 2000 systems, labeled CH 1 and CH 2. Channel 1 is an unattenuated output for interface to an external integrator or data system. The Channel 2 output is range-selectable in the detector section of the BioFocus Method; this channel is meant for interfacing with a recorder. The full scale voltage for each channel can be set to 0.01, 0.1, or 1 V by selecting the appropriate terminal; however, using the 1.0 V F/S terminal for an integrator or data system is recommended.

The following shows the relationship between external output signals and signal displays on the BioFocus computer screen.



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*NOTE:* The ranges, offset, and (for the BioFocus 3000) wavelengths for the real-time display on the BioFocus 3000 monitor screen are set in the Display Parameters section of the Method, and can be changed during a run by clicking **Display** in the **Collect** pull-down menu on the screen.

The BioFocus absorbance detectors are highly sensitive, with a measured noise level of less than 5 x $10^{-5}$ AU peak-to-peak at 200 nm in a 25 µm capillary filled with phosphate buffer at a field strength of 400 V/cm. Maximum sensitivity equals 0.0005 AU, for the path length determined by capillary ID, with a larger ID providing greater sensitivity, but lower resolution. Larger ID capillaries may be used for enhanced sensitivity and greater volume throughput for fraction collection. Detector drift has been measured at less than 2 x $10^{-4}$ AU/hour after 30 minutes warm-up.

In a user-assembled absorbance cartridge, the detector window is a rectangle bounded on the top and bottom by the cartridge aperture and on the sides by two plates which fix the capillary in the optical path. A lens focuses source light from the monochromator on a portion of this rectangle.

Other specifications include: bandwidth = 6.0 nm, wavelength accuracy = ±1.0 nm, wavelength precision = 0.1 nm. Slew rate for the BioFocus 3000 detector is 20 ms from 190 - 800 nm.

A $D_2$ lamp is the light source for wavelengths between 190-365 nm. A tungsten lamp is supplied for wavelengths between 366-800 nm. The tungsten lamp is optional for the BioFocus 2000 system.

True double-beam operation is provided by a fiber optic beam splitter (this is the only area in the detector where fiber optics are used). A reference photodiode continuously monitors the light from one leg of the beam splitter. The other leg is imaged by a lens system through the capillary onto the sample photodiode.

The photodiodes are connected to individual preamplifiers. The preamplifier output voltages are directly proportional to the light intensity at the photodiode active surfaces for a given wavelength.

The outputs of the preamplifiers are sent to a ratiometer. The output of this circuit is a voltage directly proportional to the negative logarithm of the ratio of the sample signal to the reference signal. The signal is filtered with a second order Bessel filter with 10-90% rise times selectable from 0.0 to 9.9 seconds (divide by 2 to convert to an equivalent time constant). This voltage is supplied to the rear panel as the integrator or recorder output.



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## *BioFocus 3000*

The BioFocus 3000 detector collects data at two rates: high-speed scanning collects 96 data points/sec (384 bytes/sec); multi-wavelength and single wavelength detection collect 24 data points/sec (96 bytes/sec). Wavelength selection is provided by a high-speed galvanometric grating drive system. This is called a monochromator (forward optics). This is different from a diode array polychromator (reverse optics), which generally produces less light for detection through a small capillary and cannot be optimized for a single wavelength for maximum sensitivity.

The high-speed scan rate can be roughly estimated after subtracting time for retrace and one extra data point. For example, with a 96 nm scan range and 12 nm intervals, 9 different wavelengths (96/12)+1 would be scanned and the scan rate would be slightly less than 11 scans per sec (96/9); with a 192 nm scan range with 12 nm intervals, 17 different wavelengths would be scanned (192/12)+1 and the scan rate would be slightly less than 6 scans per sec (192/17).

There are five detector outputs on the rear panel of the BioFocus 3000 systems. The first is an unattenuated 1 volt/absorbance unit output to be used for an integrator or data system. The other four are strip chart recorder outputs for which attenuation is set in the BioFocus software's Method section. The output voltage for each of these four channels can be set at 10, 100, or 1000 mV full scale by switches on the rear panel of the BioFocus 3000 system.

*NOTE:* A 100% overrange is allowed; the output will go to 2V when the readings are 0.10 AU or higher.

Full scale refers to the recorder absorption unit (AU) range settings in the method. If channel 1 is set to a range of 0.05 AU and the switches are set to 1000 mV full scale, the output will be 800 mV at a 0.04 AU reading.

In single wavelength mode, these channels can be used to monitor the detector signal at the set wavelength at four different attenuation levels or rise times if desired. In multi-wavelength mode, three of these channels can be used to monitor up to three wavelengths simultaneously on the computer screen; the fourth channel displays the ratio of any two of the monitored wavelengths. In high-speed scanning mode, all analog output channels are disabled. Therefore, "3-dimensional" (isometric) displays may only be viewed in the post-run Spectra software program. An example is shown in Figure 2-3.



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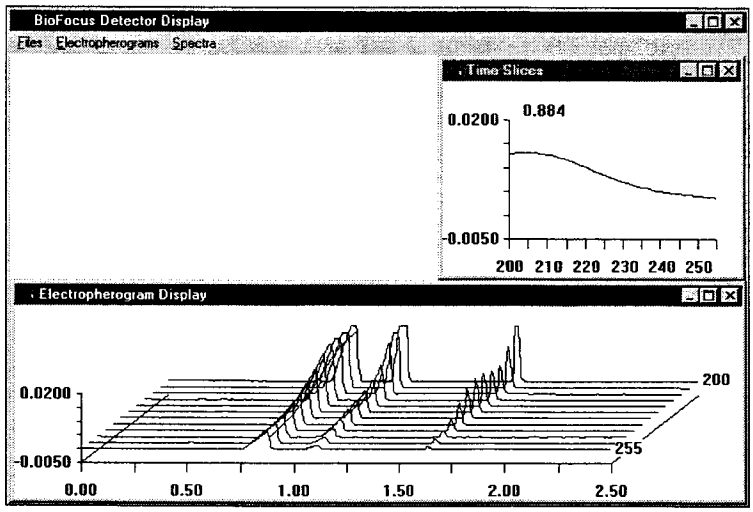



*Figure 2-3 Three-Dimensional Display*

## *LIF² Detector*

Figure 2-4 is a simplified diagram of the light paths in a two-channel LIF2 Detector. Two lasers in the system have their outputs coupled through fiber optics adapters to the cartridge holder.

Light at 488 nm and 594 nm illuminates a section of the capillary detector window near the capillary's outlet. As sample material passes through this section, either native fluorescent samples or the fluorescent tag attached to each sample component fluoresce at a characteristic wavelength longer than the excitation light from the laser. A lens in proximity to the capillary magnifies the image emitted from the sample and transmits it to a mirror mounted at 45 degrees to the light path. Light reflected by the mirror either passes through a beam splitter (dichroic mirror) to the 630 nm PMT detector or is reflected to the 520 nm path. Emission at both wavelengths also passes through from one to three filters to reject stray light and light from the laser excitation sources and then passes to the PMT detector.

As each sample component passes through the capillary's detection window, the PMT generates a signal proportional to the amount of light emitted. The computer normalizes this signal based on each PMT's sensitivity and gain setting. The computer also filters the signal and adds an autozero offset. The computer collects a data base representing signal amplitude versus time during the sample run, which can be seen in real time in an electropherogram displayed on the computer's monitor during the run. After the run, file data may be viewed and further analyzed using Bio-Rad Integration software.



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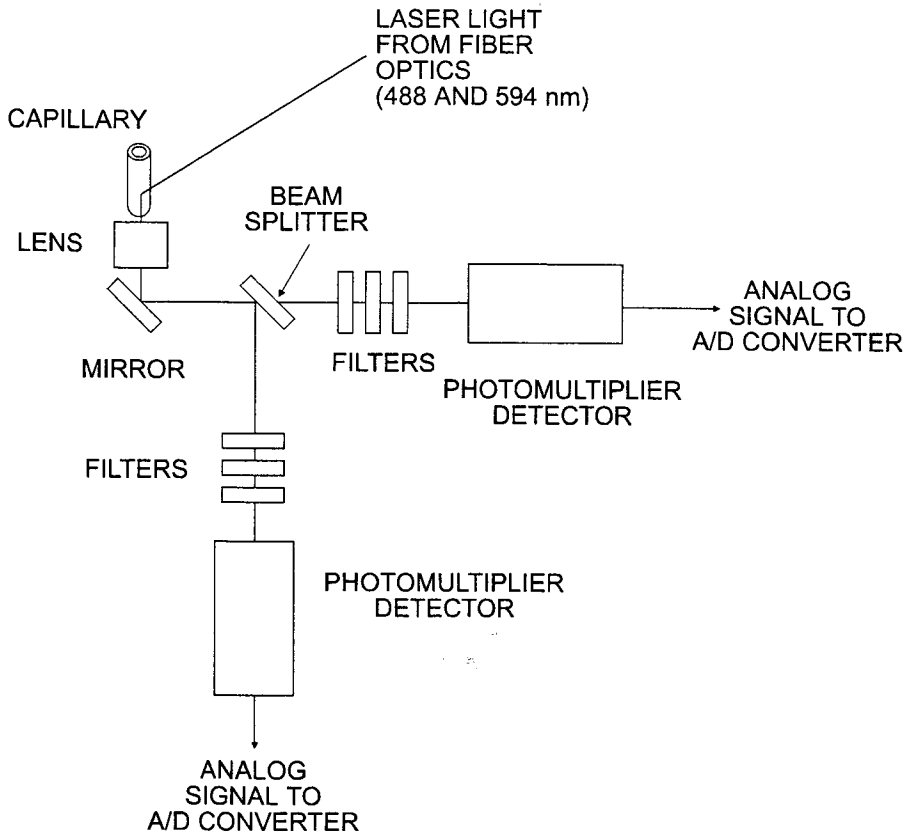



Figure 2-4 Simplified Optics Module Schematic Diagram

# Capillaries

A fused silica capillary tube is used as the separation vessel in capillary electrophoresis. This material is used primarily because of its transparency to UV light, making on-line detection possible. In addition, the technology for fused silica capillaries has been well-developed for gas chromatography, making it readily available for CE adaptation. The capillary is manufactured with an external polymer coating, which allows the capillary to be manipulated without breaking. Without the coating the fused silica would be too fragile for practical application in CE. This external coating must be removed at the point of detection to allow UV light to pass through the walls of the capillary.

The ID of the capillary is key to making the technology work. It must be small enough to prevent convective mixing and to allow efficient dissipation of Joule heat. The smaller the inside diameter, the higher the resolution of the separation and the lower the capillary current and corresponding Joule heating. On the other hand, the lumen must be wide enough to allow the capillary to be filled with various solutions and to provide sufficient path length for the detection of the sample. The path length is the distance through the sample solution at the point of detection and determines the magnitude of the detector signal. The



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shorter the path length, the lower the detector sensitivity. Capillary electrophoresis systems achieve an optimal balance of these competing factors by using capillaries with 25 µm to 75 µm inside diameters.

The inside wall of the fused silica capillary tube becomes negatively charged when filled with buffer above pH 3. This charged surface creates two important effects: electroendosmotic flow of the buffer solution in the capillary, and adsorption of positively-charged sample components to that surface. When sample adsorbs to the surface it covers some of the charged sites and changes the rate of electroendosmotic flow. Adsorption therefore changes the amount of sample available to be detected and the migration time of that sample. Efforts to control or reduce these effects include coating the inside wall of the capillary and putting additives in the buffer.

Bio-Rad provides a patented polymer coating that minimizes negatively-charged sites on the capillary wall, significantly reducing both electroendosmosis and sample adsorption. This permits separations to be performed in short capillaries with low voltages. Run-to-run reproducibility of protein separations is particularly enhanced by use of this capillary coating.

The liquid-cooled User-Assembled Cartridge allows a BioFocus system user to experiment with a greater variety of capillary types and lengths (any length between 24-100 cm). It is designed to accommodate 350-375 µm (OD) capillaries and can be used for all applications with an applied voltage up to 30 kV. If using applied voltages above 30 kV, you must use Fluorinert® for cooling instead of deionized water.

## Electrodes

Two platinum electrodes are suspended beside the ends of the capillary in the cartridge compartment in the BioFocus systems. The inlet electrode is high voltage and the outlet electrode is ground (within one volt). The polarity of the inlet electrode is reversible by a simple software command.

These electrodes cannot be seen easily because they are between the cartridge and carousel compartments. The carousel vial holders will lift microcentrifuge vials up to the point where both electrodes and capillary ends are 1 mm above the bottom of the vial, completing the circuit.

## Carousels

Two carousels for automated sampling and fraction collection are provided with the BioFocus systems. The inlet (left) carousel contains 32 vial holders for samples, run buffers, and washes. The outlet (right) carousel contains 32 vial holders for run buffers, waste, and fractions.



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Two sizes of vial holders are available: 500 µl, and large capacity. The standard vial holder holds a 500 µl microcentrifuge tube with its attached cap (insertion of the cap into the vial holder holds the tube in place during up and down movement of the vial holder). The large capacity vial holder accommodates a 1.5 ml microcentrifuge tube without a cap, or may be used by itself without a microcentrifuge tube.

Each vial holder with its corresponding cap and O-ring forms a sealed chamber around the microcentrifuge tube to control evaporation. The cap is lifted only when the vial is involved with an operation such as sample injection, purging or electrophoresis. See "Inserting and Removing Carousels" in the Setup section of this manual for more details on the carousels. A vial extraction device is provided for removing vials from vial holders.

The interface between the capillary cartridge and elevated inlet and outlet vials is shown in Figure 2-5.

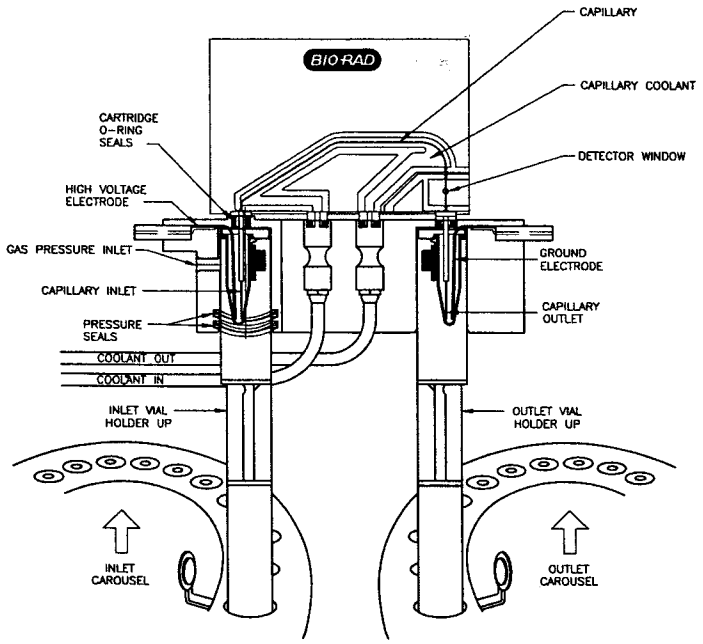



*Figure 2-5 Capillary Cartridge and Vial Holder Interface*



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