

BioFocus System Installation

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Operating Requirements

System Location

NOTE: The procedures in this section pertain to BioFocus system installation. Refer to “LIF2 Detector Installation” on page 3-10 for LIF Module installation.

BioFocus systems should be placed on a level benchtop or a sturdy cart. The instruments weight approximately 60 kg. The width of the instrument is 50 cm. A bench depth of 58 cm and a height clearance of 58 cm are also required. Operation of the system in direct sunlight may cause excessive drift or unexpected changes in the baseline. We recommend that you find a location where changes in ambient temperature are minimized. Ambient temperature during operation should not exceed 40° C for proper performance. The relative humidity should not exceed 90%, noncondensing.

AC Power Requirements

NOTE: The BioFocus 2000 and BioFocus 3000 systems are delivered configured for 120 VAC operation. To configure a system to operate at voltages other than 120 VAC, follow the directions in the Maintenance section of this manual prior to applying system power.

An isolated or filtered power line is highly desirable to minimize electrical noise which might interfere with computer or instrument function. Use of a surge suppressor/line filter with a 10 A or higher rating is recommended as a minimum precaution. All pieces of equipment that are used with the BioFocus such as the LIF Module, computer, integrators, or recorders should be plugged into the same power strip.

Items Not Included with the BioFocus System

- Commercial grade nitrogen or helium source regulated at a constant pressure between 75 and 100 psi (520 to 700 kPa)
- Distilled or deionized water (35 ml)
- Methanol (5 ml per week)
- Adapters for any gas supply other than 1/8 in. or 1/4 in. female NPT

Unpacking

Shipping Check

Check that there are three shipping containers for the BioFocus system: the instrument, an accessories package, and a package containing the carousels.

Inspect the shipping containers for damage. If more than minor damage is noted, contact the shipping company.

Accessories Check

Open the accessories package and check its contents versus the packing list. This package has items that will enable the instrument to interface with other system components.

Unpacking the System

Next, unpack the instrument by following the steps below.

1. Cut the nylon strap on the instrument packaging (largest box)
2. Slide the cardboard cover off vertically.
3. Open the two flaps on the cardboard apron around the base.
4. Remove the front and rear packaging.



WARNING! Get a helper; one person should not attempt to lift the instrument.

CAUTION! Do not lift the instrument by the front door. The weight of the instrument may bend the door hinge and cause door misalignment.

5. Grip the instrument under the overhanging regions on the sides, lift to clear the packaging, then rotate the instrument 45 degrees and set it down again on the packaging base. Do not attempt to lift the instrument onto a bench or carry the instrument using the sides for gripping, because these are not secure gripping points.
6. Place fingers under the instrument for further movement. Lift with one person on each side (not front and back). Always lift heavy objects using bent knees, not with body bent over, to avoid back injury.
7. Remove the “Do Not Lift” Label from the Sample Area Door on the BioFocus 3000. If possible, save the packaging for future shipments.

System Connections and Setup

NOTE: The power connection, gas connection, and computer connections are on the back of the instrument. If the instrument is going to reside in a tight spot where access to the rear of the instrument is limited, it is best to make connections before positioning the instrument.

Power

Check to see that the power entry module, on the rear panel of the instrument (near the power switch), is set to the correct voltage for your country. The options are 100, 120, 220 and 240 VAC, 50 or 60 Hz.

If the power entry module is not configured correctly, see “Configuring Voltage and Replacing Fuses” in the Maintenance section of this manual.

After checking the voltage setting, remove the warning label covering the AC power receptacle and insert the female end of the power cord.

Gas Line and Regulator

Connect the instrument to the gas supply. See Figure 3-1.

CAUTION! The use of plant-supplied compressed air can damage the pneumatic control system due to condensation or contaminants. Commercial filters and traps do not clean the air adequately. Use only commercial grade compressed nitrogen or helium with the instrument.

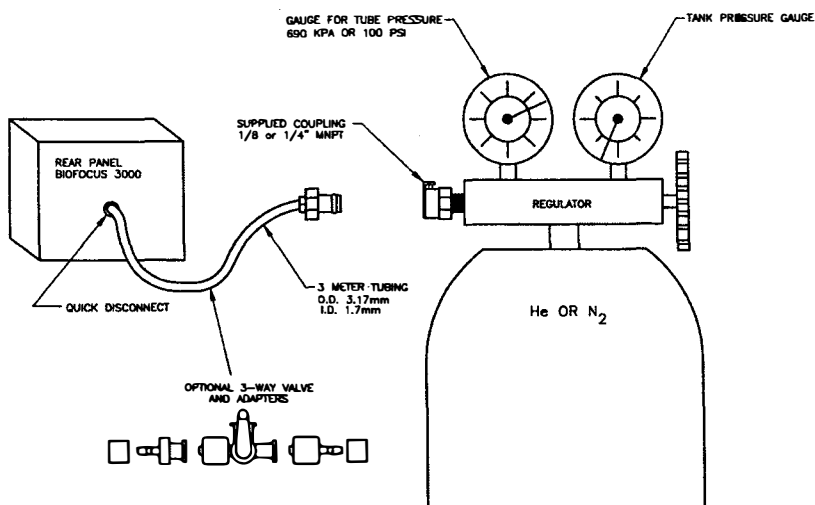


Figure 3-1 BioFocus System Interface with an External Compressed Gas Source

There are two couplings that provide connection to 1/4 in. and 1/8 in. NPT female threads such as those found on pressure regulators. If one of these is used, be sure to use two wraps of PTFE plumbing tape around the male pipe threads before installation. Pipe threads must be installed with sufficient torque to provide sealing. The accessories provided accommodate most interconnections, but there may be others that need to

be provided by the equipment user. You may dismantle the tubing assembly supplied in the accessory kit or cut tubing because these parts are not used for anything other than gas supply connection. The three-way valve and other fittings supplied may also be inserted in the pneumatic tubing assembly to facilitate hook-up or disconnection of the BioFocus instrument.

Connect the quick connect fitting on the tubing kit to the inlet gas fitting denoted by a label on the rear of the instrument.

Serial Cables

Install serial communications cables from instrument to the computer.

Place the COM1 and COM2 labels on the serial cables. The BioFocus 2000 COM1 connection uses a cable which is terminated on one end by an RJ45 connector. This connector resembles one used for telephones. Reversing the connection of these cables will *not* damage the instrument *but* will result in an error message during power up.

Connect the communications cables to the rear of the instrument and to the computer using the adapter provided if necessary. Securing the connectors using the attached screw fasteners will prevent problems due to the communications cables from becoming loose later.

Connecting Power

Plug the BioFocus instrument into the AC power source.

The computer should also be plugged in at this time also. It is best to plug the BioFocus instrument and the computer into the same surge suppressor/line filter or receptacle to reduce electrical grounding loop problems.

Coolant

Fill the reservoir located on the right hand side of the instrument with coolant.

In general, use Fluorinert if mobilizing voltages over 20 kV are planned. If not, use a mixture of deionized water and 5 ml of methanol. The system capacity is 60 ml and the liquid level should always be maintained between the two grooves on the inside of the reservoir.

NOTE: If using water as a coolant, maintain 20% methanol in the water to control the growth of microorganisms.

Condensation Tray

Slide the Condensation Tray from the Accessories Kit under the instrument with the high side toward the front, drain plug to the rear. In high humidity environments the tray should be emptied every day. Use a small (0.5 l) container to transport the water for disposal. Slide the tray out part way then depress one corner to allow the water to drain into the container.

Auxiliary Connections

BioFocus 2000

Connectors are on the upper rear portion of the instrument. Refer to Figure 3-2.

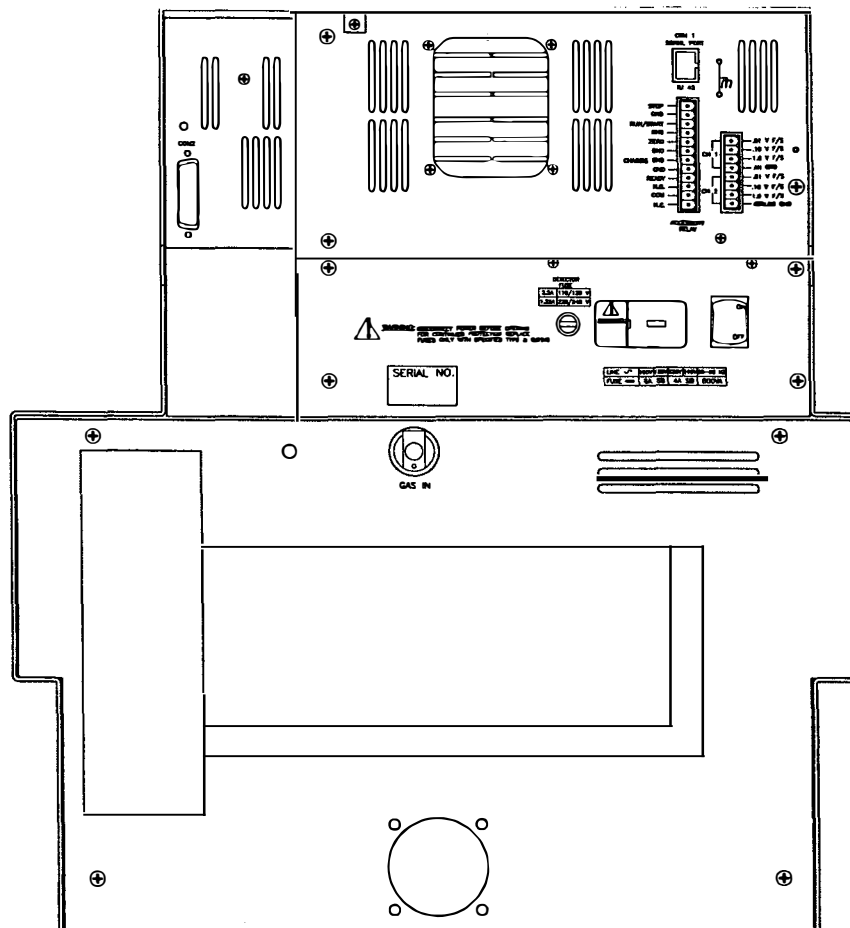


Figure 3-2 *BioFocus 2000 Rear Panel*

Peak Area Integrator or LIM Connection

1. Connect Integrator Start to Accessory Relay: N.O. and COM
2. Connect the Input for the integrator:
3. Connect the + connector on the Integrator to Channel 1 1.0 V F/S on BioFocus
4. Connect the - connector on the Integrator to Channel 1 AN GND on BioFocus
5. *Do not* connect the remaining connector to the instrument unless the cable shield needs grounding and a ground is not provided by the Integrator or LIM.

Strip Chart Recorder Connection

NOTE: Channel 2 output varies with the Detector full scale setting in the methods you define.

1. Connect the + connector on the recorder to Channel 2 on the BioFocus; 1.0, 0.1 or 0.01 V full scale.
2. Connect the - connector on the recorder to Channel 2 on the BioFocus; analog ground.

BioFocus 3000

Connectors are located on the upper rear portion of the instrument. See Figure 3-3.

Peak Area Integrator or LIM Connection

1. Connect Integrator Start cables across Remote Start Out.
2. Connect the Data Input for the integrator:
3. Connect the + connector on the integrator to Channel 1 1.0 V full scale on the BioFocus
4. Connect the - connector on the integrator to Channel 1, AN GND on the BioFocus
5. *Do not* connect the remaining connector to the instrument unless the cable shield needs grounding and ground is not provided by the integrator or LIM.

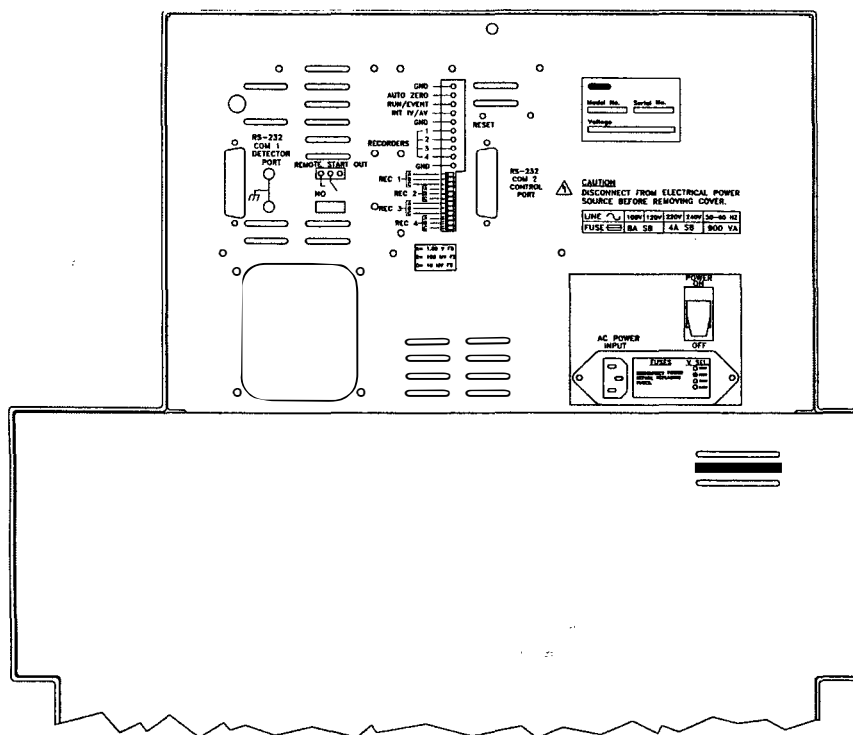


Figure 3-3 BioFocus 3000 Rear Panel

Strip Chart Recorder Connection

The instrument can accommodate up to four strip chart recorder channels connected to the BioFocus for monitoring different wavelengths. A fifth can be connected to the Integrator output. The switches below the connections are used for setting the output range of the port. The switches are on to the left when facing the rear of the instrument. Only one switch should be in the on position for each port. The A, B and C correspond to 1 V, 100 mV and 10 mV full scale respectively. (This means plus and minus; total full scale is double the value given.) The AU range of the outputs corresponds to the range selected for the detector in the method that is controlling operation.

Use the connector provided to connect:

- (+) on the recorder to 1, 2, 3 or 4 on the BioFocus
- (-) on the recorder to GND on the BioFocus

Other outputs and inputs listed by the connectors on the rear panel are for standalone operation of the detector and should not be used with the BioFocus Instrument.

LIF² Detector Installation

Operating Requirements

Environment

The LIF² Detector requires the same operating environment as the BioFocus instrument. Refer to “System Location” on page 3-3.

System Location

You can locate the LIF² Detector cabinet either to the right of, or directly below, the BioFocus instrument. The conduits that connect the cabinet to the Optical Head are 2 m long, which determines the maximum distance between the two units. You should place the units on a bench or cart that is capable of supporting 30 kg.

The LIF² Detector cabinet is 305 mm wide, 610 mm deep, and 432 mm high. See Figure 3-4. There must be sufficient clearance on the left side for cooling air intake and at the top rear for cooling air exhaust.

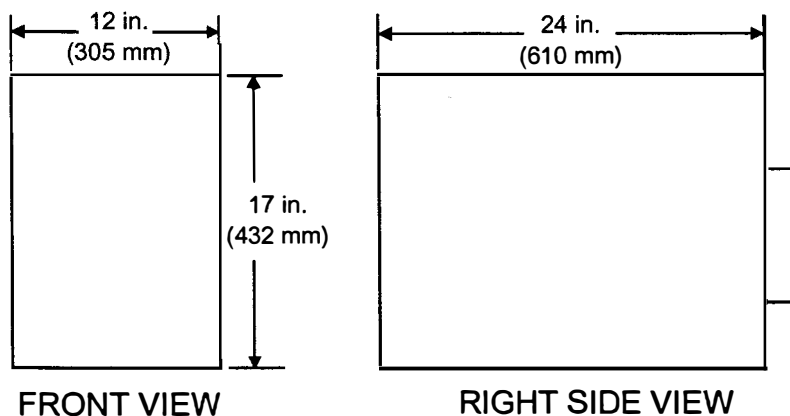


Figure 3-4 LIF Module Cabinet Dimensions

AC Power Requirements



WARNING! To avoid electrical shock, ground the LIF Detector by connecting the power cord to a three-contact grounded power outlet.

You should connect the LIF² Detector to an isolated or filtered AC power source to minimize line noise that might interfere with instrument operation. Use a surge suppressor and line filter with a 10 ampere rating as a minimum.

To avoid noise on the RS-232 data lines, ground connections between the BioFocus instrument and LIF Modules should be as short as possible. If a single circuit provides enough current for both units, connect them to the same circuit. If not, choose two circuits wired to the same multiple outlet box.

The system operates on line voltages from 90 to 132 VAC or 200 to 265 VAC, 50 to 60 Hz. Maximum power consumption is as follows:

Single laser system, laser on	400 watts
Dual laser system, both on	550 watts

Unpacking

Shipping Check

Inspect the shipping container for damage. If more than minor damage is noted, contact the shipping company.

Unpacking the System

Next, unpack the instrument by following the steps below.

1. Cut the nylon straps on the instrument packaging.
2. Slide the cardboard cover off vertically.

WARNING! Get a helper; one person should not attempt to lift the instrument.



3. Grip the instrument under the cabinet on the sides, and lift to clear the packaging. Always lift heavy objects using bent knees, not with body bent over, to avoid back injury.

Serial Cables to BioFocus

The LIF² Detector has one connection to the computer and one to the BioFocus instrument chassis:

- Connect J1 (25-pin connector) on the LIF Module to COM 2 on the computer.
- Connect J2 (9-pin connector) on the LIF Module to COM 2 on the BioFocus instrument.

Rear-Panel LIF² Detector Outputs

Terminal Strip TB1 on the LIF² Detector cabinet's rear panel has analog output connections for a strip chart recorder or external integrator, as well as an interlock output for external laser control. The table below lists connections.

Pin Number	Connection
1	Channel 1 Analog Output (0 - 10 V Full Scale)
2	Channel 1 Ground
3	Channel 2 Analog Output (0 - 10 V Full Scale)
4	Channel 2 Ground
5 - 6	Interlock Contact Closure (Normally Closed)
7 - 8	Spare

NOTE: The mating connector for TB1 may be unplugged so that you can make connections easily. Insert wires in the connector and use a flat-bladed screwdriver to secure the wire.

Minimum Computer Resources

NOTE: The computer used to control the BioFocus instrument should be dedicated to this use. You should not have an active network connection or any application that could interrupt system control or data collection.

The following is a list of minimum computer resources required to run BioFocus 2000 or 3000 operating software Version 6.00.

Minimum Requirements

IBM or 100% compatible computer with:

- MS-DOS version 6.2x
- Windows version 3.1(x) or Windows for Workgroups version 3.11
- 66 MHz 486DX/2 processor
- 8 megabytes of memory
- 500 megabyte hard disk
- 3-1/4 inch, 1.44 megabyte floppy disk drive
- Microsoft (or 100% compatible) bus mouse or PS/2 compatible mouse
- 8 megabyte permanent swap file
- VGA (640 by 480 pixels or greater), 256-color graphics or better
- Color monitor, 15-inch or larger screen
- Two 16550 FIFO buffer capable RS-232 serial ports

Strongly Recommended Options:

- Parallel printer port
- Centronics-compliant parallel printer, with printer memory sufficient to process full page graphics at 300 dpi resolution or better

Recommended Configuration

IBM or 100% compatible computer with:

- 100 MHz Pentium processor or equivalent
- Windows 95
- 16 megabytes of memory
- 1 gigabyte hard disk
- Microsoft (or 100% compatible) bus mouse or PS/2 compatible mouse
- Windows 95 automatic swap file management enabled
- VGA (640 by 480 pixels or greater), 256-color graphics or better
- Color monitor, 15-inch or larger screen
- Two 16550 FIFO buffer capable RS-232 serial ports

Strongly Recommended Options:

- Parallel printer port
- Centronics-compliant parallel printer, with printer memory sufficient to process full page graphics at 300 dpi resolution or better

Software Installation

Operating Software

BioFocus software must be installed on an IBM-compatible computer running Windows® version 3.1, 3.11, or 95. Computer resources must be the minimum as listed above. Use the procedure listed below to install operating software.

Windows Versions 3.1x Installation

1. Turn on the computer and wait until Windows comes up and the Program Manager window or icon appears. If the Program Manager icon appears, double-click it to open the Program Manager window.
2. Insert installation disk 1 in drive A: (if the 3.5-inch floppy drive is drive B:, substitute B: for A: below).
3. Click **File** to drop down the File menu.
4. Click **Run** to open the dialog box.
5. Type A:\Install and press **Enter**.
6. Follow the instructions that appear on the computer's monitor.

Windows 95 Installation

1. Turn on the computer and wait until the main Windows 95 screen appears.
2. Insert installation disk 1 in drive A: (if the 3.5-inch floppy drive is drive B:, substitute B: for A: below).
3. Click **Start**.
4. Click **Run**
5. Type A:\Install and press **Enter**.
6. Follow the instructions that appear on the computer's monitor.

Integrator Software

Use the procedure as detailed for operating software installation, with the following exception: type `A:\Setup` instead of `A:\Install`.

The installation program's default is to install the program in `C:\CE3000` or `C:\CE2000` subdirectories. However, it provides the user with the opportunity to designate another installation directory. After completing installation the installation program will create or update a Bio-Rad BioFocus Program Group including program icons for BioFocus 3000 and Spectra or BioFocus 2000 and View Electropherogram.

Starting the Software

The main program file is `CE_3000.EXE` (or `CE_2000.EXE`), and it will reside in the `CE3000` (or `CE2000`) subdirectory of the C drive. To start it, you must have Windows 3.1 or higher installed (consult the Windows User's Guide). We also recommend that your PC be dedicated to BioFocus system operation and data handling. Any unnecessary applications should be deactivated, including screen savers and other terminate and stay resident (TSR) programs that may interfere with some BioFocus Diagnostics screens.

You may also start the program from DOS by going to the `CE3000` (or `CE2000`) subdirectory and typing:

```
win ce_3000 (or win ce_2000)
```

and then pressing the **Enter** key.

Creating a program icon and starting the program from the Windows desktop is an alternative start procedure and strongly recommended. If you did not use this feature of the installation program, the procedure may be performed manually.

BioFocus Software Upgrades

Periodically, Bio-Rad makes software upgrades available for both BioFocus CE systems. Before installing an upgrade disk, open your current version of the BioFocus program and click "**Clean up the Database**" under **Commands**, then exit the BioFocus and Windows program.

NOTE: After your database has been converted, it cannot be returned to its original state. If you think you may want to reuse it with the original software, be sure to copy it to a floppy disk prior to conversion.

When upgrading BioFocus operating software with the installation program, a screen appears with three choices:

1. Do not overwrite current database. This choice retains your previously programmed methods, automation sequences, etc.
2. Overwrite current database, or
3. Cancel installation

Make your selection by clicking the appropriate button and then click OK to continue (or cancel) software installation.

When software changes require a database conversion, a utility program will be provided on the upgrade disk that will allow you to retain your earlier databases. You will need to use this utility only if an “Incorrect Database Version” message immediately appears after you have installed the new software and opened the BioFocus program.

If you see this message, click “OK”. The BioFocus program will now automatically close. You should exit Windows and go into the CE3000 (or CE2000) subdirectory. With your upgrade disk in Drive A (or B), at the CE3000 > (or C:\CE2000 >) prompt, type: a : dbconvrt (or b : dbconvrt)

then press the **Enter** key.

Then follow the directions on the screen. You may also type a:dbconvrt ? for help.

It is important to upgrade your system software *each time* you receive an upgrade disk. Each upgrade disk has a database ID. Before performing any database conversion, a utility program checks the ID of the database that is currently residing in the CE3000 (or CE2000) subdirectory. If it is not the version ID which immediately preceded the upgrade version ID, you will not be able to make the conversion.

NOTE: This command only overwrites old methods, cartridges, reagents, etc. It does *not* delete detector (BFF) files which will remain in the CE3000 (or CE2000) subdirectory. You may also type `a:newverdb ?` for help.

You then have three options:

1. You can abandon (delete) your current database and install the new upgrade disk with a different command by typing:
`a:newverdb` (or `b:newverdb`)
and then pressing the **Enter** key.
2. You can avoid losing your current database by sequentially installing all previous upgrade disks (you must bring up the BioFocus program after each disk and convert the database if indicated).
3. You can reinstall the older version disk which is compatible with your current database structure. You may now resume operation of your BioFocus system, but no software upgrades for your BioFocus system will be possible until you exercise option 1 or 2 above.

Disk Space Warnings

There is a safeguard built into the software to warn the user that there may not be enough disk space to save the data generated by running an automation sequence. When the operator starts an automation sequence, the operating software estimates disk space requirements for the expected data. If there is not enough disk space available, the software presents the user with options for proceeding.

NOTE: The software will not prevent the user from running a sequence with automatic integration and/or AIA conversion, even if it estimates that there may not be enough disk space. It is the user's responsibility to make this determination.

Initiation of an automation sequence can generate three categories of files that make demands on disk space. The categories are:

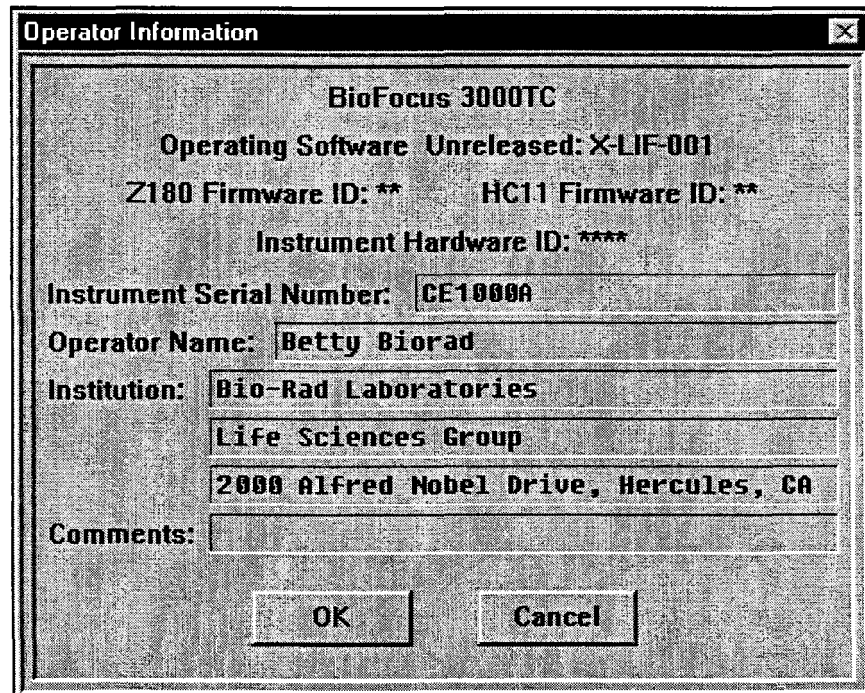
- Data files (.BFF extension) and associated log files
- Integration files, and
- AIA files (.CDF extension)

Data files and associated log files are generated by the automation sequence, while integration and AIA files are optional.

If the operating software indicates that there is not enough disk space for the data files and associated log files, it will not allow the sequence to proceed until the user makes enough space available. If there is not enough space for optional integration and AIA files, the software warns of this condition and advises the user to make more space available or ensure that integration and AIA conversion are turned off.

Operator Information

There are fields for operator information stored in operating software files. The software requests this information upon installation of the operating software and on each subsequent program startup if the serial number field remains empty. The information provided is included, along with operating parameters, in the data files (*.BFF) when the files are created. Operator information is printed along with electropherograms on the Verification Results Form. Operator information can be displayed and edited by selecting **Operator Information** in the command menu. The instrument's serial number is located on the upper part of the rear panel. Figure 3-5 shows a sample information screen.



The screenshot shows a dialog box titled "Operator Information" with a close button in the top right corner. The text inside the dialog is as follows:

BioFocus 3000TC
Operating Software Unreleased: X-LIF-001
Z180 Firmware ID: ** HC11 Firmware ID: **
Instrument Hardware ID: ****

Instrument Serial Number: CE1000A

Operator Name: Betty Biorad

Institution: Bio-Rad Laboratories
Life Sciences Group
2000 Alfred Nobel Drive, Hercules, CA

Comments:

At the bottom of the dialog are two buttons: "OK" and "Cancel".

Figure 3-5 Operator Information Screen

Set-up

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General Precautions

BioFocus systems do not pose a safety hazard during normal operation. There are safety interlocks on user access doors to prevent the possibility of injury.



WARNING! Do not attempt to bypass safety interlocks on the doors.

When opened, the cartridge compartment door (upper door) interrupts instrument operation, turns off the high voltage, and in the case of an LIF system, turns off the lasers. On the left side of the cartridge compartment is a UV (and optional visible) detector lamp under a black cover. The lamps are not deactivated by opening the cartridge area door.



WARNING! Exposure to intense UV light can injure your eyes. Never remove the lamp cover unless the instrument is turned off. To avoid skin burns, always allow sufficient time for the lamp to cool before removing it.

The carousel compartment door (central door with window) also interrupts instrument operation and turns off the high voltage when opened.

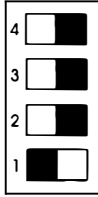
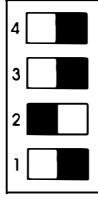
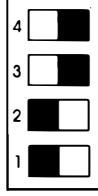

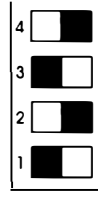
There are some areas with exposed high voltage inside the BioFocus system. Do not remove instrument covers.

Modification of any BioFocus system internal parts may void the warranty.

Turn off gas pressure before disconnecting the gas line from the instrument.

Setting Configuration Switches

Before beginning operation, you must check settings of the switch mounted just inside the LIF² Detector cabinet rear panel. For proper operation, make settings on the switch to match the laser and PMT configuration of your system. The table below shows permitted configurations and settings for each.

Configuration	Switch Settings
One internal argon ion laser, one PMT	
One internal argon ion laser, two PMTs	
One internal argon ion laser, one internal helium-neon laser, two PMTs	
One internal argon ion laser, one external laser, one PMT	
One internal argon ion laser, one external laser, two PMTs	

Changing From UV/Vis Absorbance to LIF² Operation

You may change from UV/Vis absorbance operation to LIF operation at any time. It's a simple procedure that takes about 10 minutes. If you wish to change your detection method from UV or visible absorbance to LIF, follow this procedure.

1. Turn off power to the BioFocus instrument and the LIF module.
2. Turn off power to any external lasers connected to the LIF module.
3. Open the cartridge compartment access door.
4. Remove the capillary cartridge from the cartridge holder.
5. Unscrew the knurled nuts holding the cartridge and detector assembly in place.
6. Remove the cartridge and detector assembly and place it in the compartment to the right of the cartridge holder.
7. Place the LIF cartridge holder and detector assembly in the cartridge compartment as shown in Figure 4-1. Place the flexible conduit in the notch on the right side of the cartridge compartment.
8. Install the LIF cartridge holder by sliding it over the two threaded studs that held the UV/visible cartridge holder.
9. Install the knurled nuts and tighten them finger tight.
10. Rotate the Optics Assembly positioning lever clockwise to retract the lens and allow a capillary cartridge to be inserted.
11. Unscrew the knurled nut holding the filter compartment cover. Remove and install filters as required.
12. Install an LIF capillary cartridge as required.
13. Rotate the Optics Assembly positioning lever counterclockwise until it locks in place past the spring latch. This moves the lens next to the window in the capillary cartridge.
14. Close the cartridge compartment access door.
15. If necessary for first-time installation, connect cables from the computer to the LIF² Detector and from the LIF² Detector to the BioFocus instrument. Connect COM 2 on the computer to COM 2 on the BioFocus instrument.

16. Connect COM 1 on the computer to COM 1 on the BioFocus instrument.

17. The system is now ready for use.

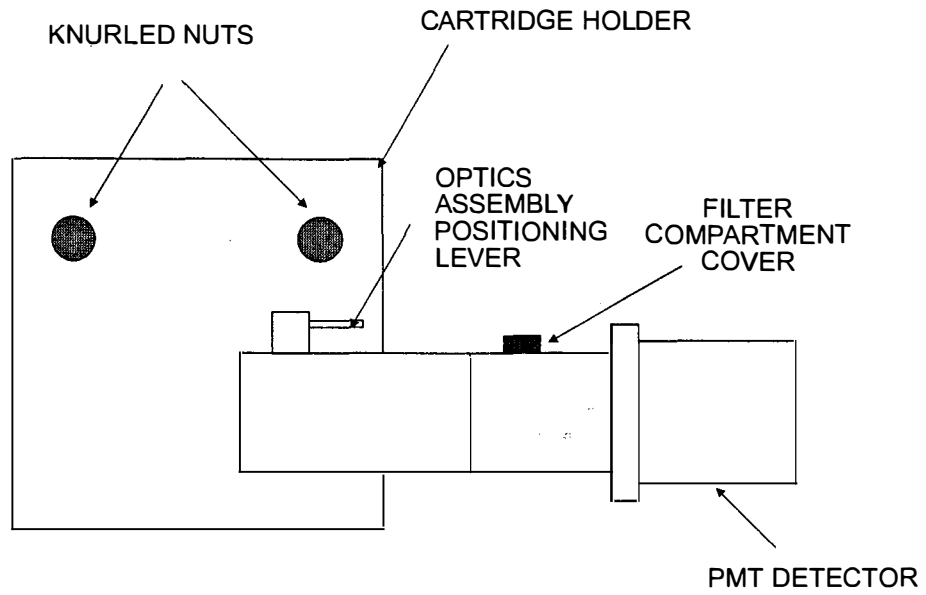


Figure 4-1 LIF Optics Module

Changing From LIF Operation to UV/Vis Absorbance

NOTE: You must have LIF² Detector power turned on whenever operating the BioFocus system in UV/Vis absorbance mode.

This procedure is essentially the same as changing from absorbance operation to LIF operation. Use the procedure below.

1. Turn off power to the BioFocus instrument and the LIF² Detector.
2. Turn off power to any external lasers connected to the LIF² Detector.
3. Open the cartridge compartment access door.
4. Rotate the Optics Assembly positioning lever clockwise until it stops.
5. Remove the capillary cartridge from the cartridge holder.
6. Unscrew the knurled nuts holding the cartridge and detector assembly in place.
7. Remove the cartridge and detector assembly and place it in the compartment on the left side of the LIF² Detector cabinet.
8. Place the absorbance cartridge holder and detector assembly in the cartridge compartment.
9. Install the absorbance cartridge holder by sliding it over the two threaded studs that held the LIF cartridge holder.
10. Install the knurled nuts and tighten them finger tight.
11. Install an absorbance capillary cartridge as required.
12. Close the cartridge compartment access door.
13. The system is now ready for use.

Connecting an External Laser

The LIF² Detector provides a connector for a fiber optic cable from an external, user-supplied laser. The following paragraphs list general considerations and provide a check list for external laser connection.

NOTE: Your system must be factory-configured for external laser connection. If it is not, contact your Bio-Rad representative for details about modifying your system to accept an external laser connection.



WARNING! The LIF² Detector is a Class I laser product. To maintain this class, the limits specified below must be adhered to.

Maximum Power Level Limitations

The maximum level for external lasers is 5 mW emitted into the fiber optics cable in the visible spectrum. The maximum IR level is 400 μ W at 750 nm, 720 μ W at 780 nm, and 1.2 mW at 850 nm. The maximum UV level is 4 μ W at wavelengths between 315 and 400 nm.

Connecting External Laser Fiber Optics to the LIF² Detector

There is an SMA connector for external laser connection located inside the LIF module's right side compartment. Use this procedure to make the connection:

1. Open the storage compartment access lid on the top right side of the LIF² Detector cabinet.
2. Using a screwdriver, remove the safety cover shown in Figure 4-2 to expose the SMA connector mounted in the cabinet's storage compartment.
3. Connect the fiber optics cable from the external laser to the SMA connector.
4. Replace the cover, ensuring that the fiber optics cable does not make a sharp bend.

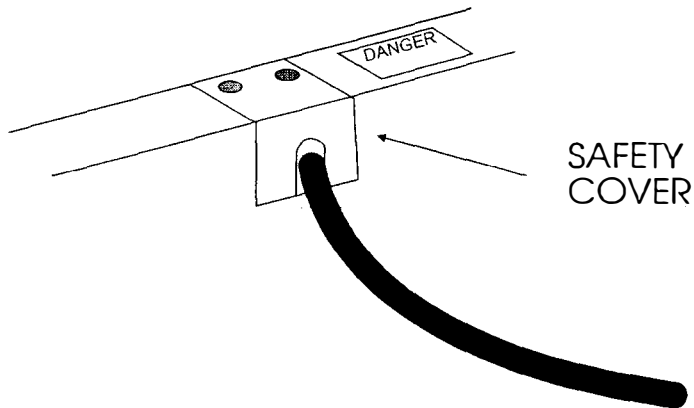


Figure 4-2 External Laser Connection

Setting the Configuration Switches

Set the configuration switches located at the rear of the LIF² Detector cabinet to match your configuration. See “Setting Configuration Switches” in this section.

Connecting the LIF Module Interlock

The LIF² Detector provides an interlock for the external laser. There is a terminal strip on the rear of the LIF² Detector cabinet. Connect the interlock to pins 5 and 6. This connection is a normally closed contact for operation; an open contact indicates that the optics module interlock is open. The external interlock circuit must turn off power to the external laser.



WARNING! Do not defeat the external laser interlock. You may be exposed to hazardous radiation.

Laser Safety Considerations

To ensure that external lasers operate safely, consult the laser manufacturer's manual for safety considerations. Other considerations include:

- Do not look at the end of a fiber optics cable that has collecting or magnifying optics unless you are certain that laser power is off and cannot be turned on accidentally.
- Ensure that the laser beam path is enclosed at the interface between the laser head and the fiber optics cable that leads to the LIF² Detector cabinet.

- Ensure that any non-interlocked connectors or covers that allow access to laser energy require a tool for opening or removing covers and connectors.

Caring for Fiber Optics Cable

You should observe these precautions when connecting fiber optics cable from an external laser to the LIF² Detector:

- Do not bend the cable at a sharp angle
- Do not run the cable over sharp metal edges

Connecting External Equipment to the Terminal Strip

The 8-pin rear-panel terminal strip provides analog signal output and ground as follows:

Pin	Function
1 (bottom pin)	Channel 1 analog signal output, 0 to +10 volts
2	Signal ground
3	Channel 2 analog signal output, 0 to +10 volts
4	Signal ground
5 - 6	External laser interlock (contact closure, normally closed)
7 - 8	Not used in this application

NOTE: The 8-pin connector can be unplugged to allow easy signal and ground connections. Pull the connector straight out of the panel. Use a small flat-bladed screwdriver to tighten screws securing wires once you insert them into the connector. The connector is keyed so that it cannot be re-inserted incorrectly.

Installing the User-Assembled Absorbance Cartridge

To insert the User-Assembled Absorbance Cartridge (see Figure 4-3) open the cartridge area access door and swing the levers on the cartridge holder to the sides.

On the BioFocus 3000 system, we recommend inserting the User-Assembled cartridge with protective sheaths installed over the capillary ends to prevent capillary breakage. Protective sheaths are not necessary when installing the User-Assembled cartridge in the BioFocus 2000 system.

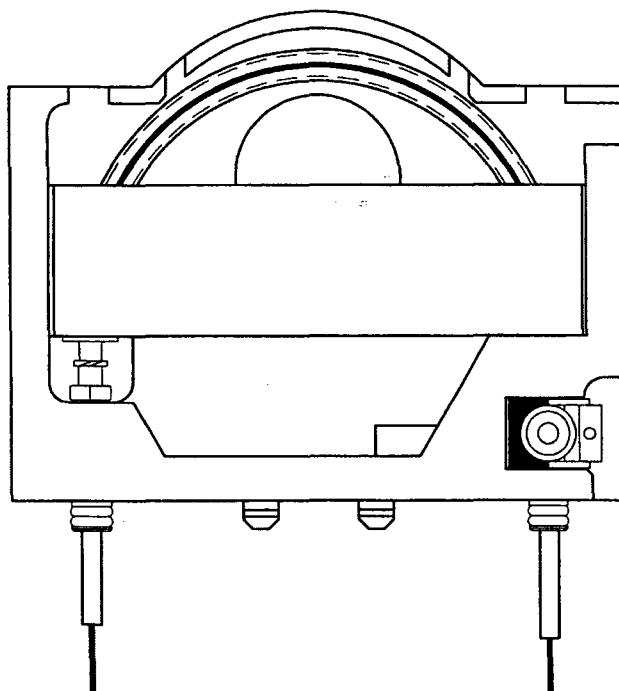


Figure 4-3 User-Assembled Absorbance Cartridge

1. Insert the cartridge into the slot in the top of the Cartridge Holder with the circular boss which contains the capillary window to the right. Push the cartridge down until it stops.
2. Apply one last firm push to seat the seals.
3. Swing both levers into the downward position until they stop.
4. Close the cartridge area access door.
5. Open the sample area door. The sheaths that protect the capillary will be visible in the holes in the bottom of the cartridge holder. Use the Vial Extraction Tool (tweezers) to grip the ends of sheaths. Remove the sheaths by pulling straight down.

6. Close the sample area door.



CAUTION! Absence of a cartridge during a Single Run Group, an Automation Sequence, or an Electrical Diagnostics test may result in damage to the Pressure Chamber/Inlet Electrode.

Installing the User-Assembled LIF Cartridge

To insert the User-Assembled LIF Cartridge, open the cartridge area access door.

1. Rotate the Optics Assembly positioning lever clockwise until it stops.
2. Insert the cartridge into the slot in the top of the Cartridge Holder with the capillary window to the right. Push the cartridge down until it stops.
3. Apply one last firm push to seat the seals.
4. Rotate the Optics Assembly positioning lever counterclockwise until it locks in place past the spring latch.
5. Close the cartridge area access door.
6. Close the sample area door.



CAUTION! Absence of a cartridge during a Single Run Group, Automation Sequence, or an Electrical Diagnostics test may result in damage to the Pressure Chamber/Inlet Electrode.

Removing the Cartridge

NOTE: Many types of capillaries should be purged before storage. See recommendations for the particular capillary.

1. Open the cartridge area access door. The coolant inside the cartridge will be automatically emptied in 15 to 30 seconds.
2. If you are using an LIF cartridge, rotate the Optics Assembly positioning lever clockwise until it stops.
3. Rotate the levers upward 90 degrees.
4. Press the release button located at the bottom center of the Cartridge Holder; this releases the seals.
4. Grasp the upper edge of the cartridge in the center and pull up.

Inserting and Removing Buffer and Sample Vials

The Carousels come configured to hold 0.5 ml vials only. Capped or capless vials may be used. Additional vial holders are provided in the Accessory Kit for holding capless 1.5 ml vials (see “Carousel Vials and Vial Cap Replacement” in the Maintenance section of this manual for installation).

The vials may contain samples or buffers. This is dictated by the Configuration created in the BioFocus software (see “Configurations” in the Software Operation section of this manual).

The vials should be inserted into the vial holders as shown in Figure 4-4. When inserting captive-capped vials, the cap should slide completely into its retaining slot in the vial holder.



CAUTION! Be sure that the vials are pushed all the way down. Capillary breakage or electrode damage may result from vials that are not seated properly in the vial holders.

To remove vials, use the Vial Extraction Tool supplied with the instrument as shown in Figure 4-4.

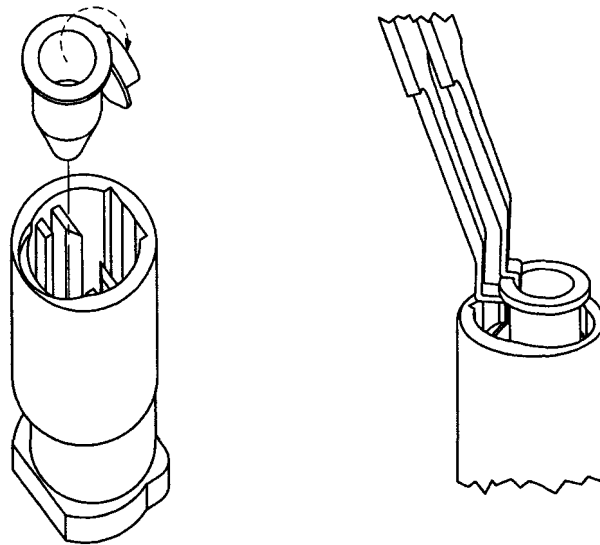


Figure 4-4 Vial Insertion and Removal

Filling Vials

1. To minimize evaporation of samples and buffers, add 0.5 ml of deionized water to the vial holder before inserting the vial.
2. Fill vials with reagents and centrifuge them for 2 minutes before inserting them into the needed positions in the Carousels.
3. When adding liquids to the vials, hold the pipette against the side of the vial to avoid creating air bubbles.
4. To minimize siphoning effects during operation, it is desirable to put the same amount of buffer in the inlet vial as in the outlet vial. We recommend 500 μ l for buffers using 500 μ l vials and 1.3 ml for 1.5 ml vials. Do not fill vials completely to the top; this may cause contamination.
5. We recommend a minimum sample volume of 50 μ l. The minimum volume that can be sampled is 15 μ l.
6. Place 50 μ l of deionized water in the outlet waste vial. This will prevent droplet formation of purge reagents at the tip of the capillary, which may be carried to other reagents.

Inserting and Removing Carousels

If you intend to set the Sample Area temperature below 10° C, then we advise storing the Carousels in a refrigerator prior to use.

1. Open the Sample Area Door.
2. Slide the Carousel into the Sample area with the arrow on the top pointing toward the interior of the instrument. Be careful not to jam the evaporation control caps against the ceiling of the sample area.
3. Push the carousel inward until it stops.
4. Push the Carousel firmly near the top with a slight downward thrust. The Carousel should snap into place on the Turntable.
5. To remove the Carousel, vial position 1 should be positioned over the Lifter. To bring the carousels to “home” position, close the sample door and select **Reset Carousels** under the **Commands** menu. Place fingers or thumb on the top over the evaporation control caps and the remaining finger or fingers under the carousel. Pull the Carousel firmly outward to disengage the detent mechanism. Slide the Carousel out of the instrument.

System Verification

Absorbance Mode

BioFocus CE System performance should be checked at installation and then periodically for proper operation. Bio-Rad provides an absorbance mode verification test kit (part number 148-4170) and a cartridge (part number 148-3053) with all new instruments for this purpose. Features resident in BioFocus software Version 5.20 or higher facilitate the use of the kit and issue a verification report.

Verification Test Kit

The verification test kit uses a single compound sample that is loaded into the capillary using the pressure inject system. The sample is then mobilized through the capillary using the high voltage system. The reproducibility of migration time and peak area are checked over ten instances. Reproducibility within the specifications verifies that the instrument's systems are all functioning consistently.

The kit uses a solution of benzene sulfonic acid as the sample and sodium phosphate as the run buffer. Both solutions are extremely stable even at ambient laboratory temperatures, and have low toxicity. The low pH (2.5) of the buffer minimizes the effects of EOF (ElectroOsmotic Flow), enabling the use of an uncoated fused silica capillary. The sample absorbs in the low UV range (195-220 nm wavelength).

Verification Test Software

BioFocus operating software version 6.00 contains the Reagents, Configuration, and Methods on unchangeable templates that are used to perform the verification test. These templates can be used to create new entities, but cannot be changed or deleted themselves. There is an automation sequence named “Verify” that is pre-programmed. Refer to Figure 4-5. Use the following procedure to run the Verification method:

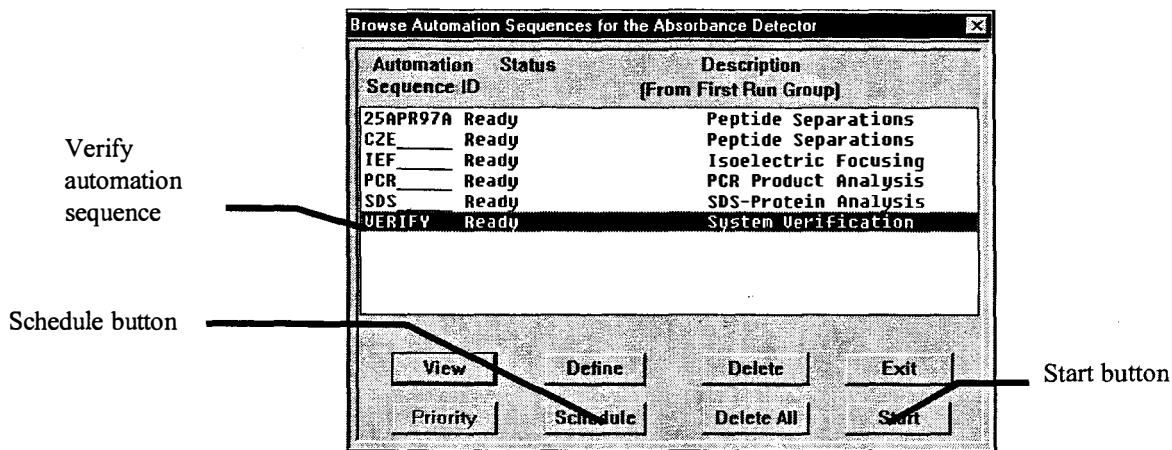


Figure 4-5 Verification Automation Sequence Screen

1. From the main menu, click the **Auto Seqs** button. Click the Verify automation sequence to highlight it.
2. View the configuration by clicking the **View** button and set up both carousels with reagents and sample as listed on the screen and as detailed below.

Inlet Carousel Vial

- 1 Reagent PO4_PREP
- 2 Reagent PO4_RUN
- 3 Verification Test Sample
- 4 Reagent WATER deionized
- 5 Reagent NITROGEN gas purge

Outlet Carousel Vial

- 1 Waste
- 2 Reagent PO4_RUN
- 3 Reagent WATER deionized

3. Install the cartridge supplied with the instrument (catalog number 148-3053) or any BioFocus User-Assembled cartridge with 24 cm of 50 µm inside diameter uncoated capillary installed.
4. From the Autosequences browser, click the **Schedule** button and then click the **Start** button to run the autosequence.

- After completion of the autosequence, open Integrator software and click the **BioFocus System Verification** button. See Figure 4-6.

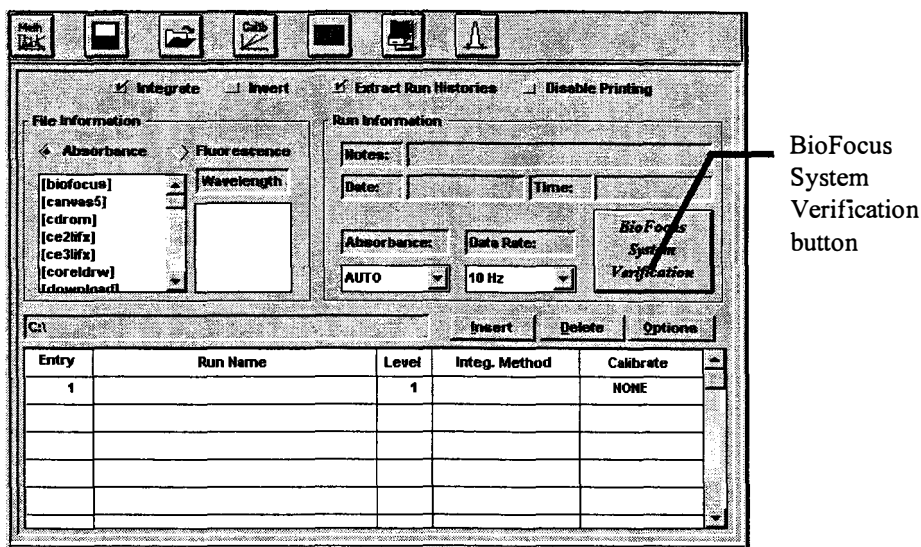


Figure 4-6 System Verification Button

The “Verify” automation sequence begins with a method named “Equilibrate” that is used to stabilize system temperatures. This method is followed by 10 runs of the “Verify” method. The output files of the “Verify” method are stored in a subdirectory called Verify in the root directory on the controlling computer’s hard disk for later evaluation. The “Final” method purges the capillary with water and nitrogen, then turns off the deuterium lamp. Execution of the Verify automation sequence requires approximately 90 minutes.

Integrator software Version 6.00 contains a feature that retrieves the most recent ten files that begin with the VER prefix from the “Verify” directory. A predefined integration method named Verify is used to find the migration time and area of the sample peak in each run. The mean and relative standard deviation are calculated using all 10 runs. The data are tabulated and printed together with operator information on a form that can be used as a record. See Figure 4-7. The form is also displayed on the screen after a printing option has been selected. A text file is created and saved in the Verify directory containing the information displayed on the form. Its title has the form {DDMMYYa.txt} where “a” is an index character that increments with each subsequent file creation of each day. The analysis and reporting routine can be activated by clicking the BioFocus System Verification button in the Integrator software’s File Open window. If there are fewer than 10 verification files, the software gives you an error message.

BioFocus System VERIFICATION REPORT

Report Date Fri Mar 01 16:24:03 1996
 Instrument Model BioFocus 3000TC
 Instrument Serial # 951BR0038
 Instrument Hardware ID 0101
 Z180 Firmware Rev J
 HC11 Firmware Rev F
 Operating Software Version 5.20

Operator Name Tim Wehr
 Institution CE Applications Lab
 Bio-Rad Laboratories
 2000 Alfred Nobel Dr
 Hercules, CA

Comments

OPERATING PARAMETERS

Capillary 24 cm x 50 um coated
 Capillary Temp 22 deg C
 Sample verification test sample
 Inlet 148-5010 phosphate, 0.1M
 Outlet 148-5010 phosphate, 0.1M
 Injection 8 psi*sec
 Run Voltage 9.00 kV - to +
 Detector Single wavelength at 200 nm
 Carousel Temp 20 deg C

TEST DATA

File Name	Migration Time	Peak Area	Date/Time
VER_044	3.17	358075	Fri Mar 01 12:15:34 1996
VER_045	3.18	346201	Fri Mar 01 12:24:10 1996
VER_046	3.18	349941	Fri Mar 01 12:32:26 1996
VER_047	3.18	435425	Fri Mar 01 12:40:40 1996
VER_048	3.19	359233	Fri Mar 01 12:48:56 1996
VER_049	3.18	360125	Fri Mar 01 12:57:10 1996
VER_050	3.18	352467	Fri Mar 01 13:05:24 1996
VER_051	3.19	354192	Fri Mar 01 13:13:38 1996
VER_052	3.18	343057	Fri Mar 01 13:21:54 1996
VER_053	3.17	351593	Fri Mar 01 13:30:08 1996
MEAN	3.178	352030	
STD DEV	0.008	5976	
RELATIVE STD DEV	0.24%	1.70%	
ACCEPTABLE RSD	1.50%	2.50%	

RESULTS PASS PASS

Signature: _____ Date: _____

Figure 4-7 Absorbance Mode Test Form