Instructions For Use

ProteomeLab XL-A/XL-I

Protein Characterization System

PN LXLAI-IM-10AB
February 2014

Beckman Coulter, Inc.
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BECKMAN COULTER

ProteomeLab XL-A/XL-I
Protein Characterization System
PN LXLAI-IM-10AB (February 2014)

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

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Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate instrument. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

Alerts for Warning, Caution, Important, and Note

⚠ WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the Important adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

Safety During Installation and/or Maintenance

Any servicing of this equipment that requires removal of any covers can expose parts which involve the risk of electric shock or personal injury. Make sure that the power switch is turned off and the instrument is disconnected from the main power source by removing the Mains (power) plug from the outlet receptacle, and refer such servicing to qualified personnel.

Do not replace any instrument components with parts not specified for use on this instrument.

Eye Safety

The ProteomeLab XL-A protein characterization system generates ultraviolet (UV) light; the ProteomeLab XL-I system generates UV light and laser light. During normal operation, neither type of light is accessible to the user.

When the absorbance optical system is used, wear UV-protective safety goggles to prevent eye damage if safety precautions are defeated and UV light becomes available. UV lamps can implode or

explode, so you should wear safety glasses when handling bulbs. The lamp should be serviced by Beckman Coulter Field Service personnel only.

When the interference optical system is used, the laser light source emits light at a power that may be harmful to the eyes. To prevent operator exposure to potentially harmful laser light, an interlock mechanism turns off the laser whenever the chamber door is not closed and latched. If the front panel is removed, a separate interlock mechanism shuts off power to the instrument, including the laser. *If these precautions are defeated and the laser beam becomes accessible, never look into the beam or direct it toward someone else. System interlocks should never be disabled or servicing attempted except by Beckman Coulter Field Service personnel.*

Electrical Safety

To reduce the risk of electrical shock, this equipment uses a three-wire electrical cord and plug to connect this equipment to earth-ground. Make sure that the matching wall outlet receptacle is properly wired and earth-grounded. Check that the line voltage agrees with the voltage listed on the name rating plate affixed to the instrument.

Do not place containers holding liquid on or near the chamber door. If they spill, liquid may get into the instrument and damage electrical or mechanical components.

Any maintenance procedures requiring removal of an instrument panel exposes the operator to the possibility of electrical shock and/or mechanical injury. Such service procedures should be done only by Beckman Coulter Field Service.

Safety Against Risk of Fire

This instrument is not designed for use with materials capable of developing flammable or explosive vapors or hazardous chemical reactions. Do not run such materials (for example, chloroform or ethyl alcohol) in this instrument nor handle or store them near the instrument.

Mechanical Safety

For safe operation of the equipment, observe the following:

- Use only the Beckman Coulter rotors and accessories designed for use in this instrument.
- Do not exceed the maximum rated speed of the rotor or centerpiece(s) in use.
- NEVER attempt to slow or stop a rotor by hand.
- Do not run the instrument unless the leveling feet are in place.
- Do not lift or move the instrument while a rotor is installed.
- NEVER attempt to override the door interlock system while the rotor is spinning.
- Do not lean on the instrument or place items on it while it is operating.

Chemical and Biological Safety

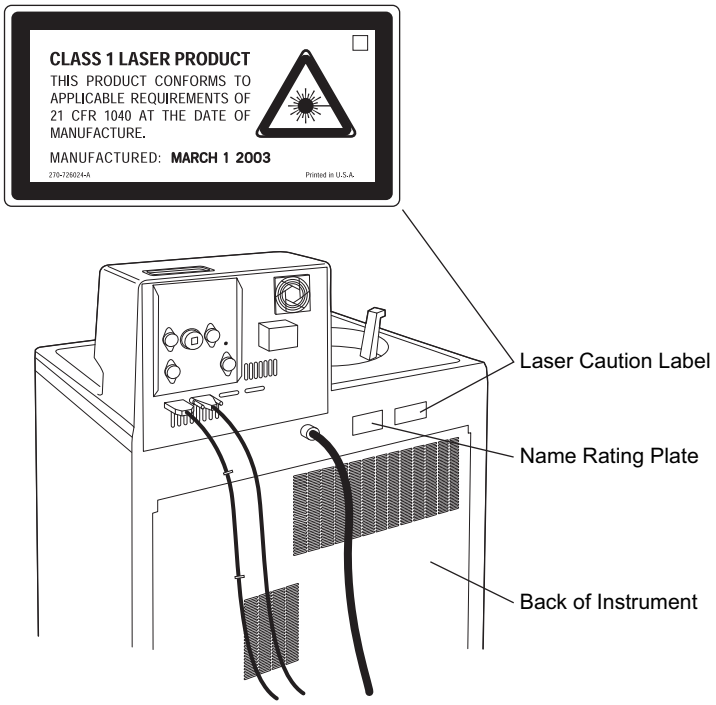
If pathogenic, toxic, or radioactive samples are used in this instrument, it is the responsibility of the user to ensure that all necessary safety regulations, guidelines, precautions, and practices are adhered to accordingly. Ask your laboratory safety officer to advise you about the level of containment required for your application and about proper decontamination or sterilization procedures to follow if fluids escape from containers

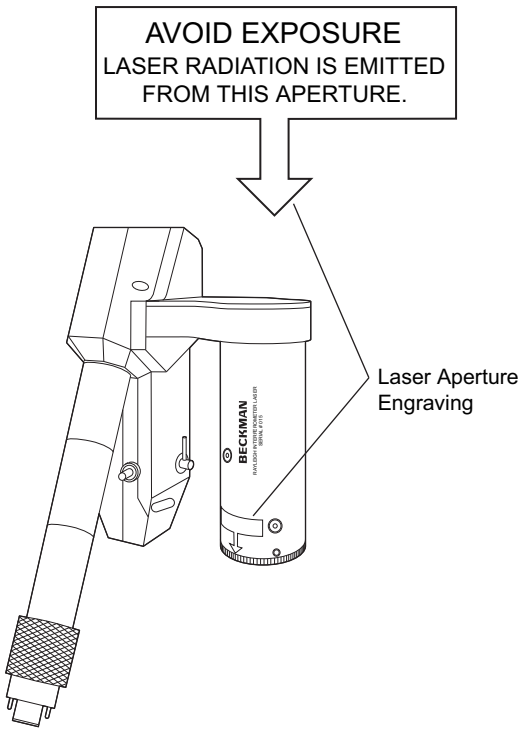
- Observe all cautionary information printed on the original solution containers prior to their use.
- Instrument O-rings have not been designed as bioseals for aerosol or liquid containment.
- Handle body fluids with care because they can transmit disease. No known test offers complete assurance that they are free of micro-organisms. Some of the most virulent—Hepatitis (B and C) and HIV (I–V) viruses, atypical mycobacteria, and certain systemic fungi—further emphasize the need for aerosol protection. Handle other infectious samples according to good laboratory procedures and methods to prevent spread of disease. Because spills may generate aerosols, observe proper safety precautions for aerosol containment. Do not run toxic, pathogenic, or radioactive materials in this rotor without taking appropriate safety precautions. Biosafe containment should be used when Risk Group II materials (as identified in the World Health Organization *Laboratory Biosafety Manual*) are handled; materials of a higher group require more than one level of protection.
- Dispose of all waste solutions according to appropriate environmental health and safety guidelines.

It is your responsibility to decontaminate the instrument and accessories before requesting service by Beckman Coulter Field Service.

Location of Laser Caution Labels on the ProteomeLab XL-I Instrument

A laser caution label and a laser aperture caution label are affixed to the XL-I instrument as shown below.





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Certification

Beckman Coulter ProteomeLab XL-A and XL-I protein characterization systems are manufactured in a facility that maintains certifications to both ISO 9001:2008 and ISO 13485:2003. They have been designed and tested to be compliant (when used with Beckman Coulter rotors) with the laboratory equipment requirements of applicable regulatory agencies. Declarations of conformity and certificates of compliance are available at www.beckmancoulter.com.

Scope of Manual

This manual describes the components of the XL-A and XL-I instruments and how to use the system to collect, display, print, and save data. These instructions should be used with the applicable analytical rotor instruction manual for complete system operation. Beckman Coulter recommends that you read this entire manual, especially the *Safety Notice* and all safety-related information, before operating the instrument or performing instrument maintenance.

- CHAPTER 1, *Specifications* contains system specifications.
- CHAPTER 2, *Hardware Description and Preparation* contains a description of hardware components, including the optical systems, and instructions for preparing the hardware for operation.
- CHAPTER 3, *XL-A/XL-I User Interface Software Description* contains a complete description of the XL-A/XL-I user interface software.
- CHAPTER 4, *System Operation* shows you how to use the XL-A/XL-I user interface software to set up runs, operate the instrument, and view and save data.
- CHAPTER 5, *Troubleshooting and Maintenance* provides care and maintenance instructions and troubleshooting procedures.
- APPENDIX A, *Installation Requirements* contains preinstallation requirements.
- APPENDIX B, *Interference Theory* provides a description of interference theory.
- *Glossary* contains a glossary of terms.

It is assumed that you already know how to use your personal computer and that you are familiar with Microsoft Windows. Computer operating instructions are not included here. Refer to the reference materials that come with the computer if you have questions on computer operation.

You will find information regarding specific protocols and applications in Application Data Sheets (ApDats) available from Beckman Coulter. Specific application and protocol information is not included here. For instructions on using the Beckman Coulter data analysis software, see the instruction manual (publication LXL/A-TB-009) which is available in pdf format on the XL-A/XL-I Windows setup CD.

NOTE If the instrument is used in a manner other than specified in this manual, the safety and performance of this equipment could be impaired. Further, the use of any equipment other than that intended for use by Beckman Coulter has not been evaluated for safety. Use of any equipment not specifically recommended in this manual is the sole responsibility of the user.

Conventions

Certain symbols are used in the product labeling call out safety related and other important information. These international symbols may also be displayed on the centrifuge and are reproduced on the inside back cover of the manual.

Typographic Conventions

Certain typographic conventions are used throughout this guide to distinguish program features and functions.

- Menu commands and commands in dialog boxes appear in bold text. For example, select **File > New**.
- The names of PC keys (for example, **ESCAPE** or **ENTER**) appear in capital letters. The names may be abbreviated or represented differently on your keyboard.
- The names of instrument control panel keys (for example, **VACUUM** or **ENTER**) appear in capital letter and sans serif font.

Recycling Label

This symbol 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:

1. The device was put on the European market after August 13, 2005 and
2. The device is not to be disposed via the municipal waste collection system of any member state of the European Union.

It is very important that customers understand and follow all laws regarding the proper decontamination and safe disposal of electrical equipment. For Beckman Coulter products bearing this label please contact your dealer or local Beckman Coulter office for details on the take back program that will facilitate the proper collection, treatment, recovery, recycling and safe disposal of the device.

Related Information on the Internet

Beckman Coulter has compiled an extensive bibliography of literature on protein characterization. An on-line searchable version of the bibliography is available at the following address:

http://www.beckmancoulter.com/xla

Another Web site that contains related information is the BBRI (Boston Biomedical Research Institute) home page. Access this site at the following address:

http://www.bbri.org

Printer Notice

Due to obsolescence of the onboard printer, it is no longer included with the instrument as of November 2013. For instructions of retrieval of data, please refer to CHAPTER 3, XL-A/XL-I User Interface Software Description.

CHAPTER 1

Specifications

Specifications

Only values with tolerances or limits are guaranteed data. Values without tolerances are informative data, without guarantee.

Physical Data

Description	Specification
Weight	• Instrument only — 465 kg (1025 lb) • IBM computer — approx 9.5 kg (21 lb) • Flat-screen display — approx 7.7 kg (17 lb)
Height	(to top of control head) 120.7 cm (47.5 in.)
Width	94.0 cm (37 in.)
Depth	67.3 cm (26.5 in.)
Ventilation clearances	5.1 cm (2 in.) sides; 15.2 cm (6 in.) rear
Finish (instrument only)	urethane paint on top surface; acrylic baking enamel on metal surfaces; coated polycarbonate finish on control panel
Electrical supply	Class I
Maximum heat dissipation into the room	1.0 kW (3400 Btu/hr)
Humidity restrictions	<95% (noncondensing)
Noise level (measured 0.9 m [3 ft] in front of the instrument)	57 dBa
Installation (overvoltage) category	II
Pollution degree	2[a]

a. Normally only nonconductive pollution occurs; occasionally, however, a temporary conductivity caused by condensation must be expected.

Control Features

Description	Specification
Speed	• Set speed — 1000 to 60,000 in increments of 100 rpm • Speed control — ±20 rpm of the set speed (above 1000 rpm) • Speed display — indicates rotor speed in increments of 10 rpm at speeds below 1000 rpm and 100 rpm at speeds above 1000 rpm
Time	• Set time — up to 999 hours and 59 minutes; HOLD for runs of unspecified length • Time display — indicates time remaining in timed runs, time elapsed in HOLD runs, and estimated time remaining in $\omega^2 t$ runs
Temperature	• Set temperature — 0 to 40°C in 1°C increments • Rotor temperature after equilibration — ±0.5°C of set temperature • Displayed rotor temperature after equilibration — ±0.3°C of set temperature
Ambient temperature range	15 to 30°C
$\omega^2 t$ **integrator**	• Calculate time for $\omega^2 t$ — up to 9.99×10^{14} radians squared per second • $\omega^2 t$ display — shows the accumulated $\omega^2 t$ to three significant digits (in exponential notation)
Analytical acceleration	5 to 400 rpm per second above 500 rpm; 400 is maximum acceleration
Analytical deceleration	5 to 400 rpm per second above 500 rpm; 400 is maximum deceleration
XL acceleration	nine slow acceleration profiles from 0 to 500 rpm, maximum acceleration from 0 rpm to set speed
XL deceleration	nine slow deceleration profiles from set speed to 500 rpm, no brake, maximum deceleration from set speed to 0 rpm
Key switch	used to select normal or locked operation; zonal operation not allowed
Instrument classification	**T**: uses Beckman Coulter analytical rotors only; preparative and zonal operation not allowed

Analytical Specifications

Scanning UV/VIS Absorbance Optical System

Description	Specification
Wavelength range	190 to 800 nm
Photometric display range	0 to 3 absorption units
Scan steps	• Radial scan mode — up to 1650 data points per scan; radial increment step size 0.001 cm minimum, .01 cm maximum • Wavelength scan mode — up to 620 data points per scan; minimum wavelength increment step size 1.0 nm, maximum wavelength increment size 100 nm
Data rate	one absorbance reading every 20 ms (approximately), depending on rotor speed
Replication/data averaging	1 to 99 averages per data point

Interference Optical System

Description	Specification
Wavelength	660 nm
Camera resolution	2048 × 96 pixels
Laser	30-mW Diode
Scan rate	approx every 5 seconds
Interferometer precision	approx ± 0.003 fringe

PC System Controller

Table 1.1 shows the configuration of the IBM PC system controller that Beckman Coulter has specified for use with the ProteomeLab XL-A or XL-I system.

Table 1.1 PC System Controller Configuration

Feature	Beckman Coulter System Configuration
System controller PC	IBM Pentium 4 workstation with PCI bus, mouse, keyboard, DVD-ROM, 40x/12x/40x CD-RW
Processor speed	3.0 GHz Pentium or better
RAM	512 MB minimum
Hard drive configuration	one 40-GB hard drive
Floppy drive configuration	one 3 1/4-inch disk drive
Monitor	one 17-inch LCD flat screen monitor, optimized for 1024 × 768 resolution
Parallel port (for printer, if applicable)	One
Serial port (for RS-232 connection to the ProteomeLab XL-A or XL-I)	One
USB port (for printer, if applicable)	Eight
Microsoft Windows	XP Professional
Printer	HP Deskjet Model 940 or later (optional)
ProteomeLab XL-A/XL-I User Interface Software	Version 5.7 or later
ProteomeLab XL-A/XL-I Data Analysis Software	Version 6.04
Origin Technical Graphics and Data Analysis in Windows	Version 6.0

NOTE Beckman Coulter is not responsible for system integration of computers that do not match the specifications listed in Table 1.1.

Every effort has been made to eliminate operational problems from the ProteomeLab XL-A/XL-I user interface software. Your Beckman Coulter Field Service representative will work with you to solve any computer-related problems; however, Beckman Coulter cannot be responsible for problems resulting from errors in Microsoft Windows code.

CHAPTER 2

Hardware Description and Preparation

Introduction

This section describes the hardware components of the ProteomeLab XL-A and XL-I instruments.

Functional Description

The ProteomeLab XL-A and XL-I protein characterization systems measure solute concentration distributions in one or more sample solutions while they are subjected to the high centrifugal forces. This measurement in turn permits the characterization of many thermodynamic and hydrodynamic properties of macromolecules and their interactions, such as concentration, homogeneity, sedimentation coefficient, diffusion coefficient, and buoyant density, as well as the determination of quantities such as solvation, association and disassociation constants, ligand binding, and stoichiometry. The system can analyze very dilute samples, enabling the use of limited amounts of sample and maximizing solution ideality.

The XL-A and XL-I absorbance optical system contains a UV/VIS spectrophotometer to measure concentration by absorption of light at a chosen wavelength from 190 to 800 nm. The XL-I interference optical system provides a cell image in which the total concentration is determined by measuring the refractive index difference between sample and reference at each radial position as indicated by the vertical displacement of a set of evenly spaced horizontal fringes.

Safety Features

ProteomeLab XL-A and XL-I instruments have been designed and tested to operate safely indoors at altitudes up to 2000 m (6 562 ft). Safety features include the following.

Door

The high-strength structural steel chamber door has a solenoid interlock to prevent operator contact with a spinning rotor. When the door is closed it locks automatically. It can be opened only when the power is on and the rotor is at rest with the chamber at atmospheric pressure. If there is a power failure, the door lock can be manually tripped for sample recovery (see CHAPTER 5, Troubleshooting).

Barrier Ring

A 41-mm (1.63-in.) steel alloy armor ring acts as the primary barrier, surrounded by a 12-mm (0.5-in.) vacuum chamber to provide full protection for the operator.

Overspeed System

The overspeed system is a safety feature designed to ensure that the rotor does not exceed its maximum speed. This system includes a photoelectric device in the rotor chamber next to the drive spindle, and an overspeed disk on the bottom of the rotor. Individual rotor manuals provide information on the correct overspeed disks to be used with each rotor.

The overspeed disk has alternating light and dark sectors. As the rotor spins, the passage of reflecting and nonreflecting sectors over the photoelectric device generates a series of pulses that are detected by the electronic circuitry and software.

After the rotor reaches 1000 rpm, the set speed is checked against the overspeed disk. If the set speed is greater than the maximum speed permitted by the disk, the speed setting is automatically lowered to the disk's maximum speed, but the run continues without interruption. An error message is displayed to alert you to the change.

Rotor Energy System

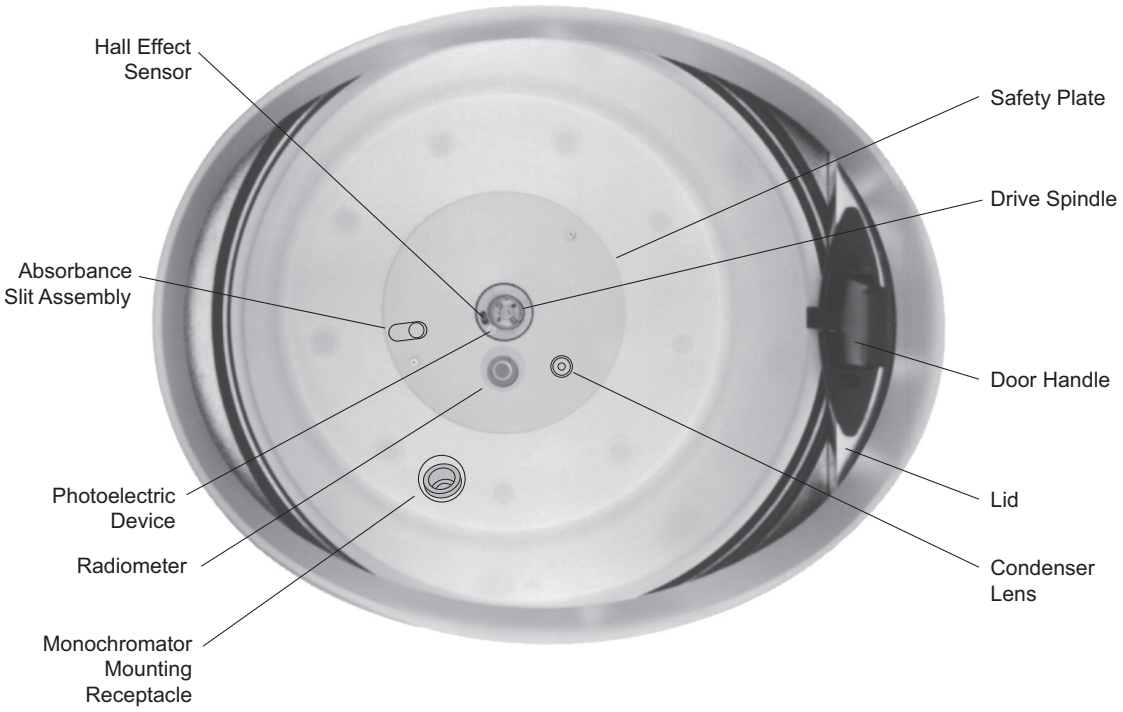
As the rotor accelerates between 15,000 and 20,000 rpm, rotor inertia is measured and the rotor energy is calculated for the speed set by the operator. If the calculated rotor energy is determined to be excessive, the instrument recalculates a permitted set speed and uses this value to avoid possible system damage. A diagnostic message is displayed to indicate the change.

Rotor Chamber

The chamber door is made of high-strength structural steel. A solenoid interlock prevents it from being opened during operation. The door can be opened only if the power is on and the vacuum is off, with the chamber at atmospheric pressure. See CHAPTER 5 for instructions on accessing the chamber to retrieve your sample in case of a power outage.

The rotor chamber is aluminum, coated with a chemical-resistant epoxy finish. The rotor drive spindle and safety plate, along with several components of the optical system, are visible in the bottom of the chamber (see Figure 2.1).

Figure 2.1 The Rotor Chamber



Monochromator

In XL-A systems, the monochromator (see Figure 2.2) contains all components of the scanning UV/VIS absorbance optical system. In XL-I systems, the monochromator (see Figure 2.3) contains components of both the absorbance and interference optical systems. The monochromator is installed into a mounting receptacle at the bottom of the chamber during operation.

Optical Systems

The scanning UV/VIS absorbance optical system contains a spectrophotometer to measure the relative concentration of solute in solution by absorbance of light at a chosen wavelength (from 190 to 800 nm). The XL-I interference optical system measures the total concentration by measuring the refractive index difference between sample and reference at each radial position, represented by the vertical displacement of a set of evenly spaced horizontal fringes.

Absorbance and interference (refractive) optical systems provide complementary ways to determine concentration distributions in the ProteomeLab system. The advantage of the absorbance system is that it can provide sensitive and selective solute detection. For cases in which solutes do not absorb significantly, or when solvents do, refractive detection provides better results. In addition, the interference optics provide greater accuracy at high concentration, higher radial resolution, a greater concentration range, and the ability to trace very steep gradients.

Figure 2.2 The XL-A Monochromator

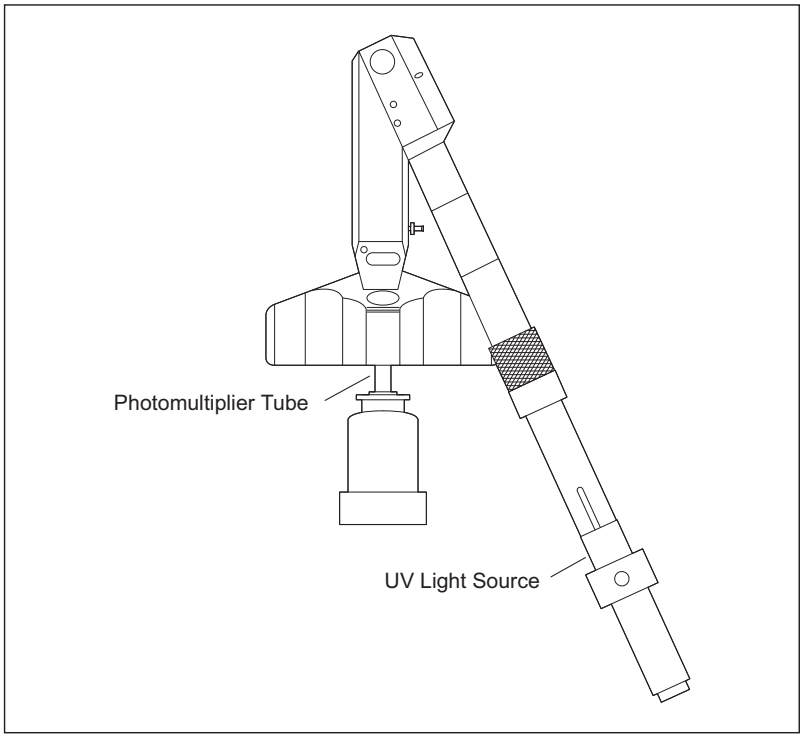
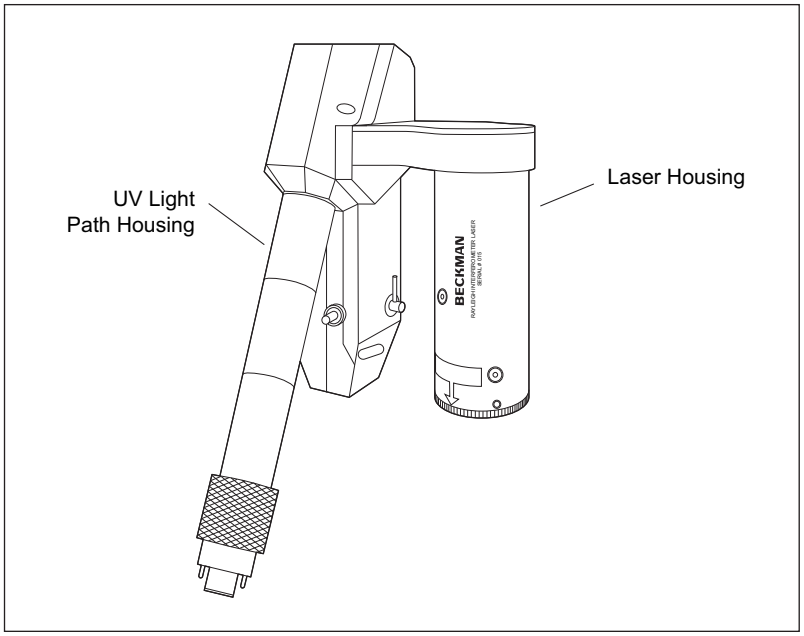


Figure 2.3 The XL-I Monochromator



Absorbance

The absorbance optical system (see Figure 2.4) is completely contained within the instrument chamber. The high-intensity xenon flash lamp provides light in the spectra from 190 to 800 nm. The light passes through the monochromator for wavelength selection. A toroidally curved holographic diffraction grating varies the wavelength and collimates the light. An incident light detector in the monochromator measures light intensity before it passes through the cell, normalizing the data for flash-to-flash variations in lamp intensity.

As the rotor spins, light intensity is first measured through the *reference* sector of the centerpiece (see Figure 2.5) by the light detector (photomultiplier tube) at the end of the measured light path. Readings are obtained by the photomultiplier tube. On the next rotation, the light path is switched from the reference sector to the sample sector and the light intensity is again measured. An absorbance value is then calculated by the system software, based on these two measurements.

The absorbance optical system performs three types of scans: velocity and equilibrium, which are both radial scans in which absorbance is measured as a function of radial distance; and wavelength, in which absorbance is determined as a function of wavelength at preselected radial distances. The interference optical system performs velocity and equilibrium scans in which fringe displacement is measured as a function of radial distance.

Radial scans can be performed in either continuous or step mode. Continuous mode is typically used for velocity scans and step mode is typically used for equilibrium scans. Wavelength scans can be performed only in step mode.

- In continuous mode, data are continuously collected across the cell (the radial detector moves at a continuous rate).
- In step mode, data are collected at selectable steps across the cell. Minimum selectable step size is 0.001 cm; maximum is 0.05 cm.

Orienting the Monochromator Blocking Filter

For scans taken at wavelengths above 400 nm, a 400-nm high-pass filter, located in the monochromator, should be used. This filter blocks the second and third orders of light coming from the diffraction grating which, if not blocked, would appear as stray light and could cause excessive nonlinearity in the data. With the filter in place, data cannot be collected at wavelengths below 400 nm.

A filter lever on the side of the monochromator moves the filter in and out of place. When the lever is parallel to the monochromator stem, the filter is out of the light path and not used. When the lever is perpendicular to the stem, the filter is in place in the light path. Figure 2.6 shows the different orientations of the filter lever.

Figure 2.4 The Scanning UV/VIS Absorbance Optical System

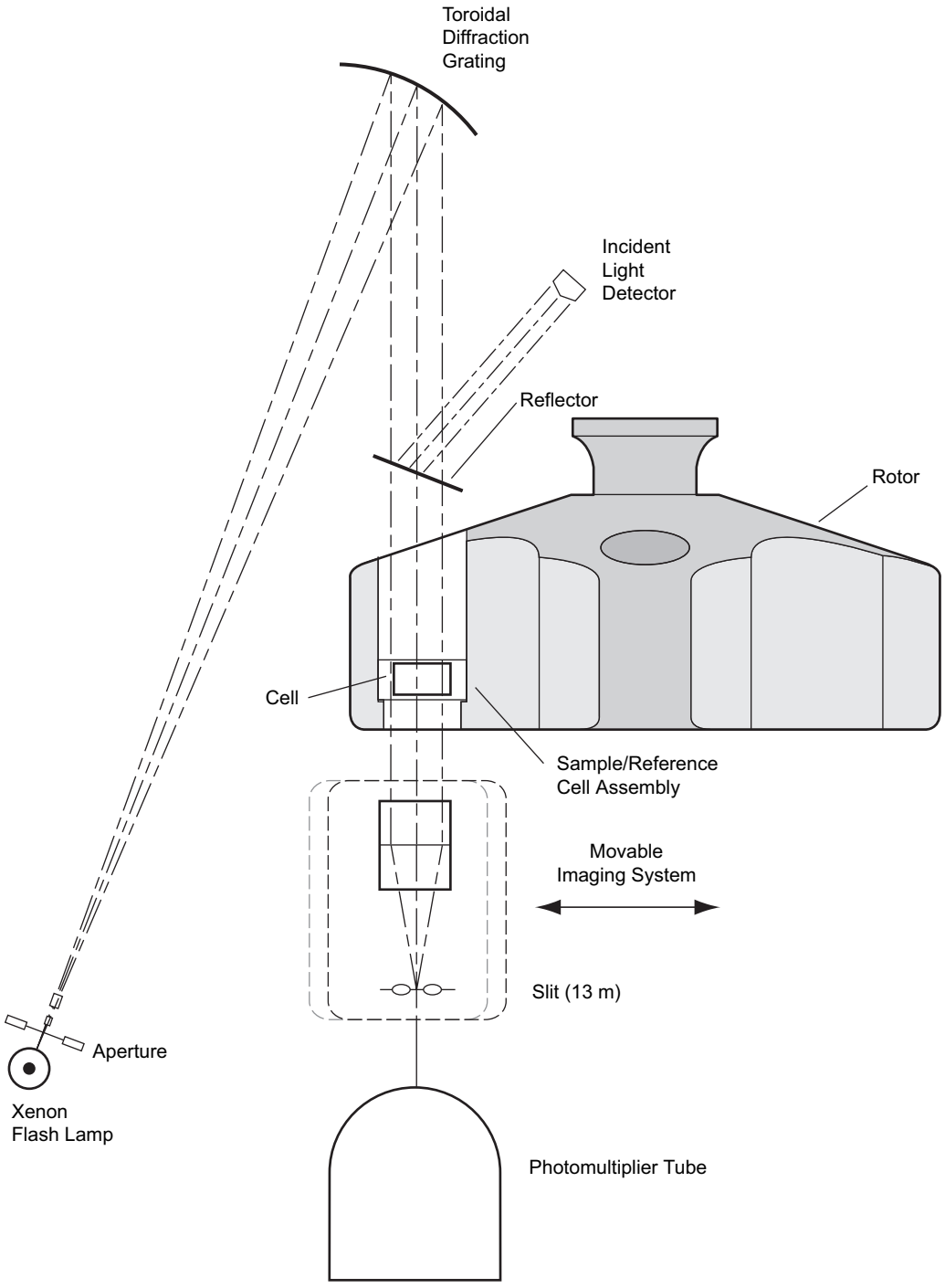


Figure 2.5 Top View of Reference and Sample Sectors in a Two-Channel Cell

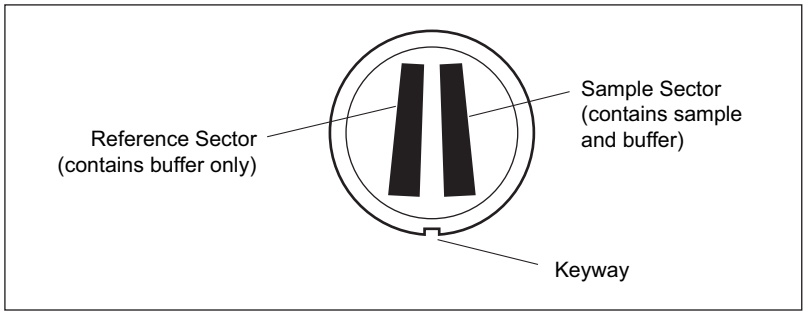
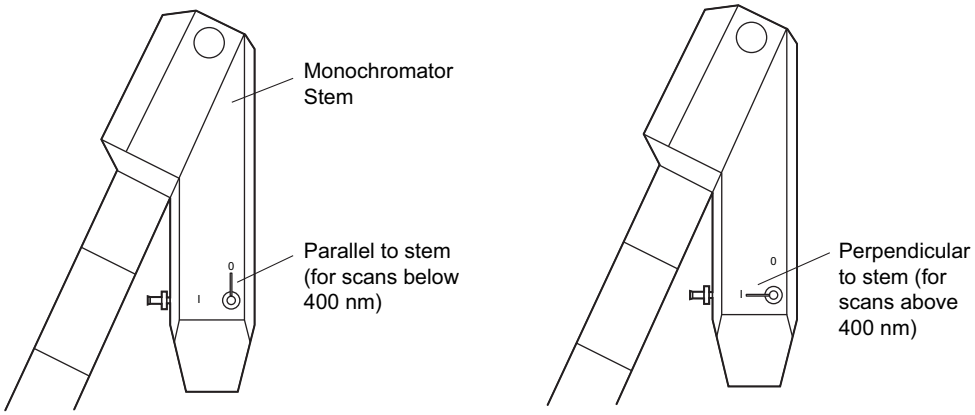


Figure 2.6 Monochromator Filter Lever Positions



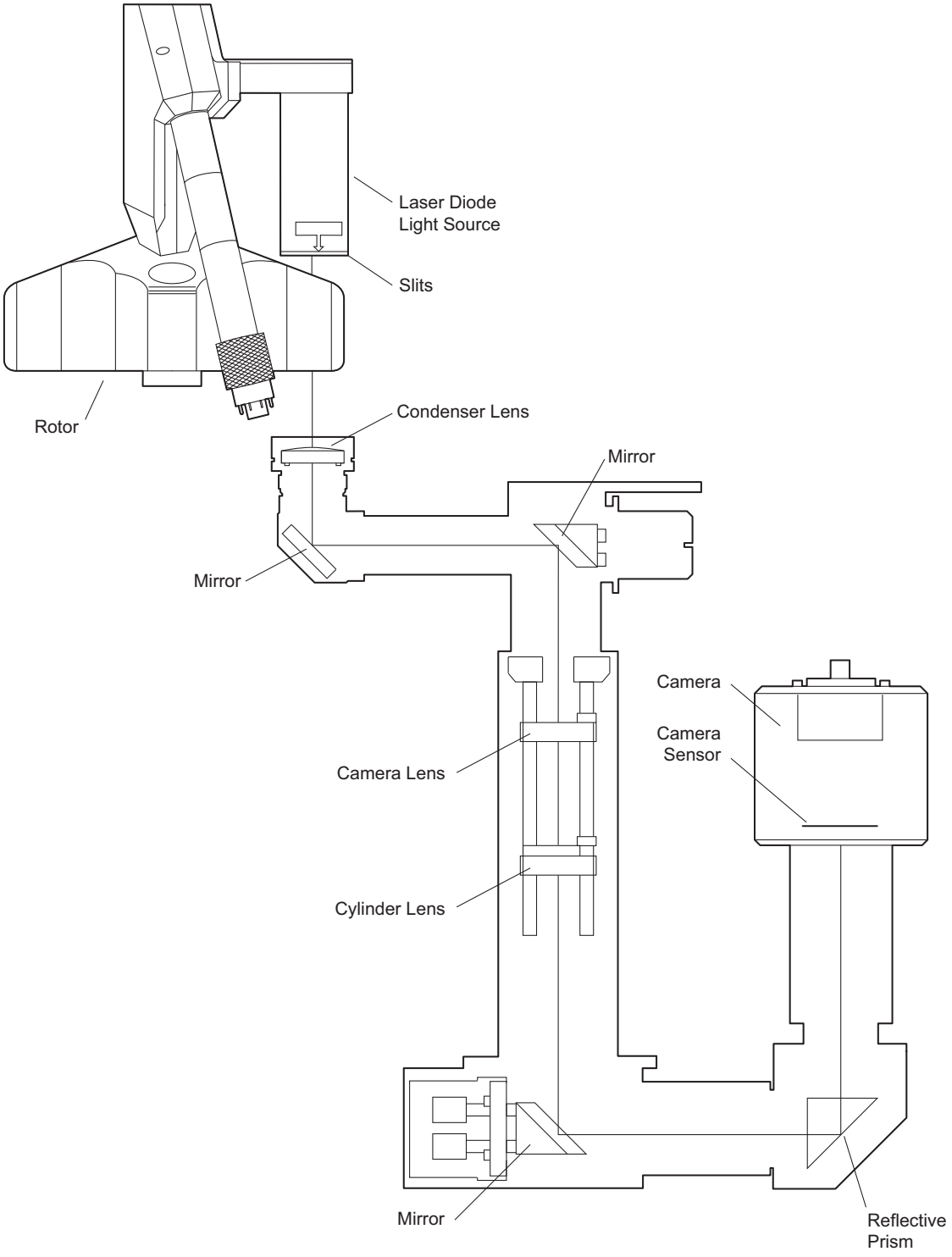
Interference

The XL-I Rayleigh interference optical system consists of a laser diode light source, imaging optics, and a charge-coupled device (CCD) detector. The light source and detector are controlled by system software that synchronizes data acquisition for up to eight rotor cells and performs data reduction.

Figure 2.7 is a diagram of the XL-I interference optical system, which includes the following components:

- *Laser diode light source*, attached to the absorbance system monochromator, located inside the XL-I chamber. The laser produces a single beam of collimated light. The beam passes through two parallel slits in the bottom of the laser housing, just above the rotor, so that two separate beams exit the laser housing.
- *Analytical rotor and cells*. Each cell consists of at least one reference sector and one sample sector. The system is aligned so that one beam passes through the reference sector and the other beam passes through the sample sector in the spinning rotor. Sapphire windows are recommended because they minimize distortion of the light beam at high speeds.
- *Condenser lens*, located in the bottom of the instrument chamber, just below the rotor. This lens keeps light rays within the optical path, including light rays that have been refracted by index of refraction gradients within the cell.

Figure 2.7 The XL-I Interference Optical System

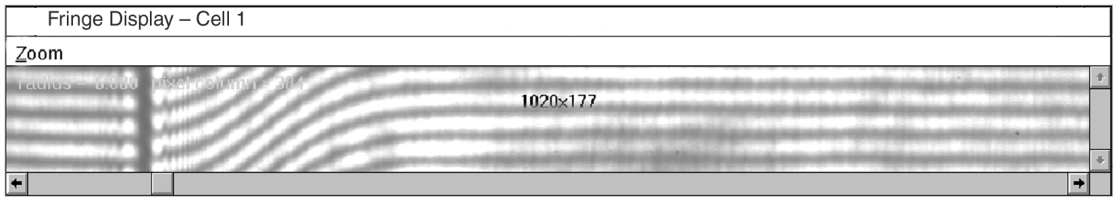


- *Camera lens*, which, together with the condenser lens, focuses the cell on the CCD sensor.
- *Cylinder lens*, which overlaps the two sector images at the camera sensor, creating the interference pattern.

- *Frame Grabber* imaging hardware (installed in PC system controller) and real-time fringe display software (not shown), which work together to acquire the camera image of the cell and display it on the computer screen.
- *IBM PC system controller and XL-A/XL-I user interface software* (not shown), from which the user controls power to the laser in addition to all aspects of run setup and system operation.

If the refractive index gradients of the solute and solvent fall within the measurable limits of the interference system, fringes will form at the focal plane of the condensing lens. These fringes are then imaged on the camera, and displayed in the real-time fringe display on the computer screen (see Figure 2.8).

Figure 2.8 The Fringe Display



The difference in refractive index at various points in the cell will cause equivalent shifts in fringe position at the focal plane. Differences in refractive index between the sample and solvent appear as a shift in the fringe pattern, known as vertical fringe displacement (see Figure 2.8). The boundary region, in which a continual change in refractive index differences exists, is imaged on the camera as a continual fringe shift. This continual fringe shift between the supernatant and the plateau region produces the curvature of the fringes through the boundary.

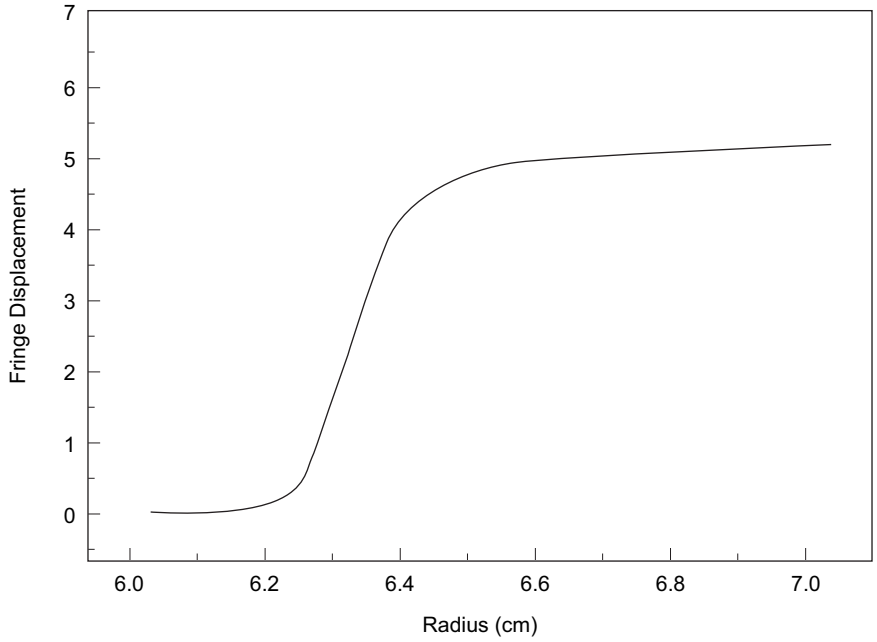
As a scan is being performed, system software performs a fast Fourier transformation (FFT) of the raw data and displays the data on screen as a plot (Figure 2.9) of fringe displacement (Y axis) versus radial position (X axis).

NOTE For a brief description of interference theory, see APPENDIX B.

Vacuum System

The instrument uses a diffusion pump or turbomolecular pump in series with a mechanical vacuum pump to reduce chamber pressure to below 5 microns (0.7 Pa). The system is automatically activated when a scan is started from the PC or when **ENTER** and **START** are pressed, or may be turned on directly by pressing **VACUUM,** if the chamber door is closed. The vacuum system is also automatically activated when precooling or preheating of the rotor is selected. (When the rotor is at rest, the instrument maintains a partial vacuum.) When the vacuum system is on, the chamber pressure is displayed in microns in the lower left corner of the CURRENT VALUES window.

Figure 2.9 Plot of Interference Data Resulting from Fast Fourier Transformation



At the end of a run, the chamber vacuum must be vented (by pressing **VACUUM**) before the door can be opened. (The vacuum *cannot* be turned off when the rotor is spinning faster than 3000 rpm. If the **VACUUM** key is pressed, an error message will appear.) After the door is opened, the chamber returns to approximate room temperature to minimize condensation in the chamber. To help keep the chamber dry and clean, keep the door closed whenever possible.

Temperature Sensing and Control

Rotor temperature is monitored by a radiometer mounted in the bottom of the rotor chamber (see Figure 2.1) when the chamber pressure is below 100 microns. Above 100 microns, *chamber* temperature is measured by a thermistor mounted in the chamber. (If the radiometer fails, a diagnostic message will appear and the system will revert to control by the thermistor; the run will continue.)

The instrument uses a solid state thermoelectric refrigeration and heating system. Neither coolant nor water is needed; cooling is provided by forced air from the fans. The system contains no CFC or CFC substitutes.

When the power is on, the temperature control system activates when the door is closed and the vacuum system is turned on. The displayed rotor temperature is controlled to within ±0.5°C of the set value (or to within ±0.3°C after equilibration).

The run temperature can be set at 0 to 40°C. If no temperature setting is entered, the instrument selects 25°C (the default value) as the operating temperature.

Drive

The frequency-controlled, air-cooled, direct-drive induction motor requires no gears or brushes. In addition, the drive does not require an oil vacuum seal, external oil reservoir, or continuously operating damper. Externally cooled by forced air and internally cooled by oil, the drive delivers ultra-smooth, quiet performance, with high tolerance of rotor imbalance.

Rotor and Cells

Two analytical rotors are available for use in ProteomeLab XL-A and XL-I systems: the An-60 Ti and the An-50 Ti. In the An-60 Ti, up to three cells and a counterbalance can be run at one time. In the An-50 Ti, up to seven cells and a counterbalance can be run. Refer to the rotor manual (LXL/A-TB-003) for a detailed description of both analytical rotors and their accessories.

System Control

The system is controlled by the Microsoft Windows-based XL-A / XL-I user interface software, which runs on a personal computer (PC). Through this user interface, the XL-A or XL-I can be started and stopped, scan settings can be entered, scans started and stopped, and data displayed and saved to disk.

The instrument control panel keys are also active. But, entering a setting on the control panel will overwrite the setting entered via the software, and vice versa. To prevent this situation, you should enter all run settings through the PC. The only instrument control panel key that is regularly used is the **VACUUM** key (see *Activating Chamber Vacuum*, below).

Name Rating Plate

A name rating plate is affixed to the rear of the instrument. Check that the line voltage agrees with the voltage listed on this name rating plate before connecting the instrument. Always mention the serial number and model or system ID number when contacting Beckman Coulter regarding your ProteomeLab XL-A or XL-I system.

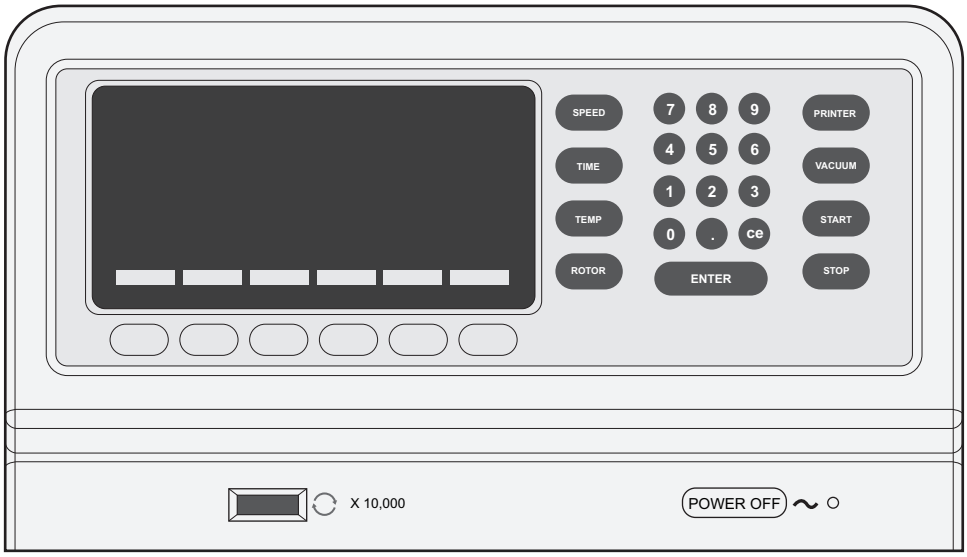
Instrument Controls

Power

A circuit breaker, labeled ON (**I**) and OFF (**O**), is located on the right side panel and controls the electrical power to the instrument. Power may be left on (see Standby Mode, below) except in the case of an emergency or when maintenance is required.

A red POWER OFF button is located on the neck of the control head for your convenience (see Figure 2.10). Pressing the POWER OFF button trips the circuit breaker to the (off) position. To return power to the instrument, the circuit breaker must be returned to the up position.

Figure 2.10 The Control Head



Standby Mode

When the instrument is at rest, it goes into standby mode—a condition that requires only minimal power to maintain a ready state. In standby mode a screen-saver feature allows the screen to go blank until any key on the control panel is pressed, returning the instrument to operating mode.

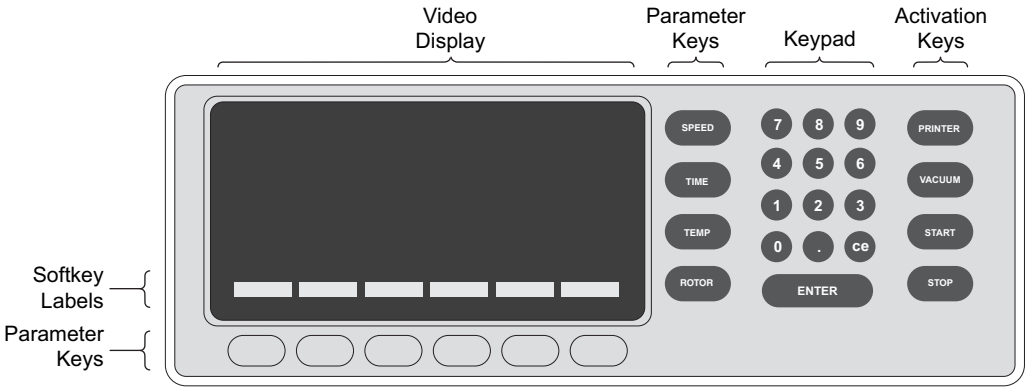
Revolutions Counter

A seven-digit revolutions counter, located to the left of the power switch, displays the accumulated number of rotor revolutions in tens of thousands of revolutions.

Control Panel

Figure 2.11 shows the control panel, which includes a video display and keys for accessing additional instrument features.

Figure 2.11 The Control Panel



Hardkeys

NOTE Because analytical run settings are entered on the PC, the instrument control panel is not typically used (except for the **VACUUM** key). However, the control panel keys are active and their functions are described below.

- Parameter hardkeys—**SPEED**, **TIME, TEMP**, and **ROTOR**—are located to the left of the keypad. (On certain screens, **ROTOR** is also available as a softkey.)
- Activation hardkeys are provided to control specific instrument functions. They are **PRINTER** (if equipped), **VACUUM**, **START**, and **STOP** and are located to the right of the keypad.
- The keypad is used to enter numerical values. It consists of the numbers 0 through 9, a **CE** (clear entry) key, a (.) (decimal) key, and an **ENTER** key.

Softkeys

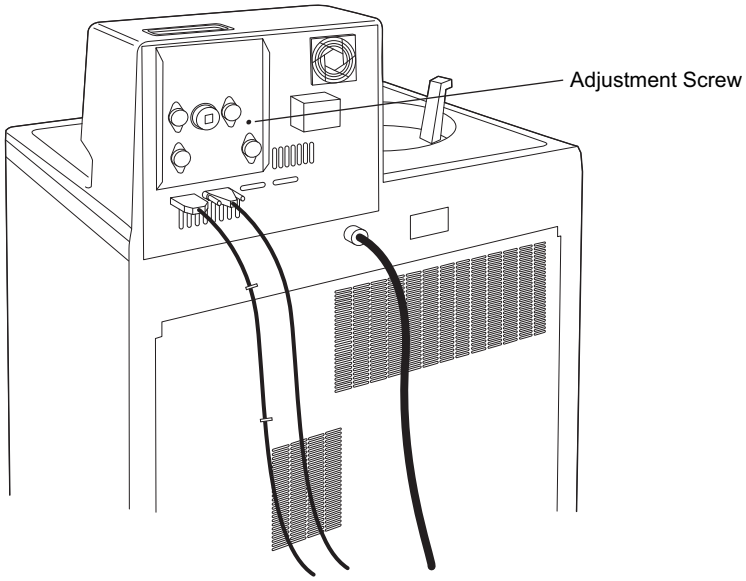
Six unlabeled softkeys are located along the bottom of the control panel, beneath the video display. Labels appear in reverse video, directly above each softkey, and change as required to indicate the current function of the key.

Video Display

A video display is used to show the current values and run settings as well as a variety of run-related functions, which are accessed by pressing the appropriate keys.

A small setscrew on the back of the control head (see Figure 2.12) controls the brightness of the video display (see *Adjusting the Display Screen Brightness* in CHAPTER 5).

Figure 2.12 Location of Adjustment Screw for Control of Video Display Brightness



Printer (If Equipped)

The printer, located within the control head, provides printouts of rotor and run data. A menu of printer options is accessed via the **PRINTER** hardkey. Care of the printer is discussed in CHAPTER 5.

Pre-run Procedures

Preparing a Run

⚠ WARNING

If pathogenic, toxic, or radioactive samples are used, it is the responsibility of the user to ensure that all necessary safety regulations, guidelines, precautions, and practices are followed. Ask your laboratory safety officer to advise you about the level of containment required for your application and about proper decontamination or sterilization procedures to follow if fluids escape from their containers.

1 If the instrument power is off, move the circuit breaker at the right side of the instrument to the up position (ON).

If the instrument is in standby mode, press any key on the PC to make the instrument fully operational.

2 Assemble the rotor cells according to the instructions in the rotor manual.

Precool the assembled rotor if required.

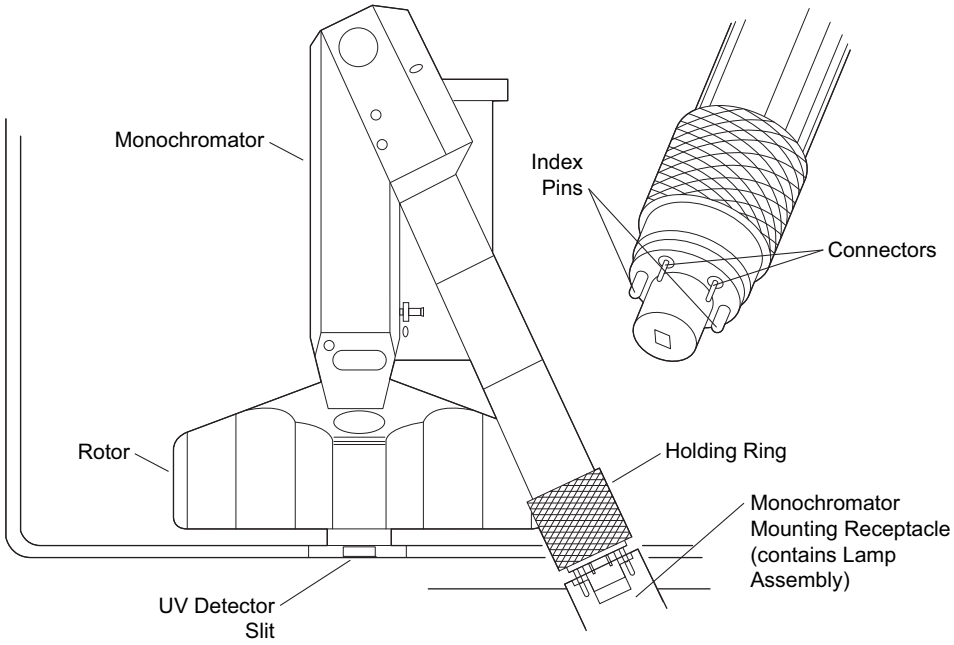
3 Load the rotor into the chamber.

Installing the Monochromator

After the rotor is installed in the chamber, install the monochromator in the chamber as follows.

1 Holding the monochromator at a 30-degree angle to the chamber bottom, align the index pins with the index pin holes and carefully insert the end with the red holding ring into the mounting receptacle (see Figure 2.13).

Figure 2.13 Installing the Monochromator (XL-I monochromator shown)



2 Gently turn the monochromator until you feel the index pins seat in the receptacle.

NOTE To prevent damage to the electrical connectors, the index pins are longer than the connectors and seat first in the mounting receptacle. When the index pins are properly seated, the connectors will be properly positioned.

3 Tighten the red holding ring by hand until snug. *Do not use tools to tighten this ring.*

Activating Chamber Vacuum

When a run begins, the rotor will not accelerate until the chamber pressure drops below 50 µ. If you activate the vacuum system prior to entering the scan settings, the chamber will be evacuated and the rotor can accelerate to set speed when you start the run.

1 After the rotor and monochromator are installed, close the chamber door.

2 Activate the vacuum system by pressing the **VACUUM** key on the instrument control panel.

You can now enter run settings via the PC. See CHAPTER 3 for a description of the XL-A/XL-I user interface software, or CHAPTER 4 for instructions for use of the software.

CHAPTER 3

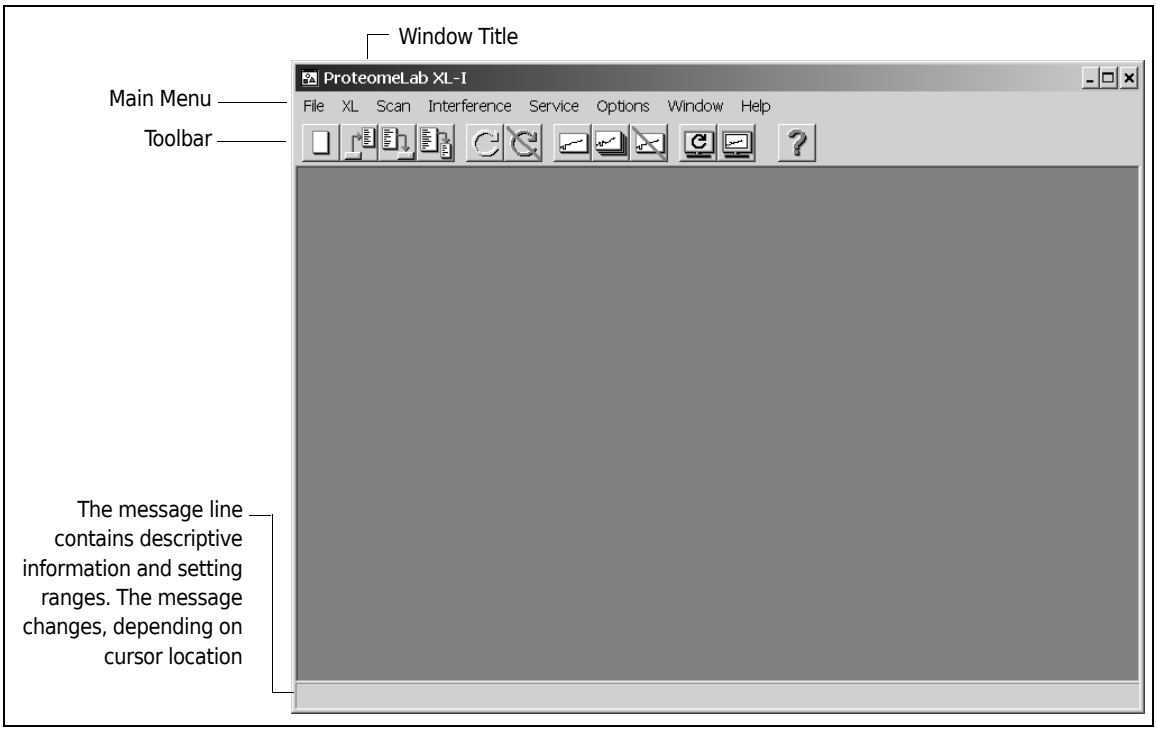
XL-A/XL-I User Interface Software Description

Introduction

This section describes the XL-A and XL-I user interface software menus, commands, and toolbar. For step-by-step instructions on using this software, see CHAPTER 4, System Operation.

This section assumes that a Beckman Coulter Field Service representative has installed the software on your PC. Installation instructions are included in APPENDIX A, Installation Requirements in case you need to reinstall the software.

Main Window



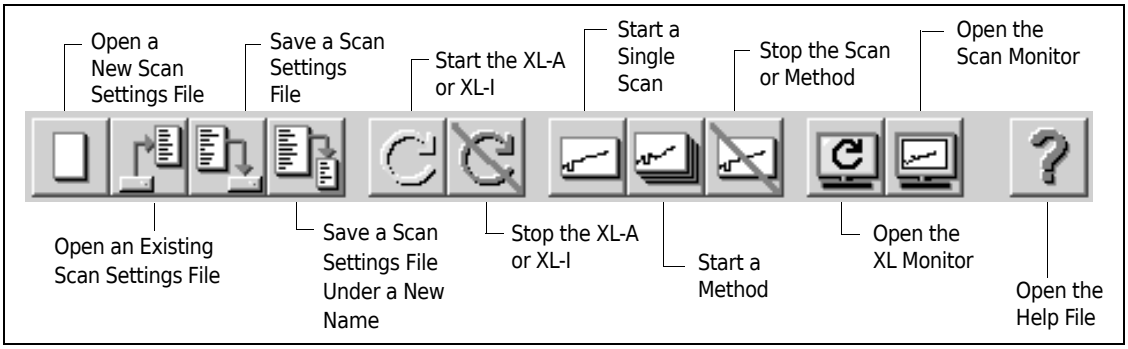
The main window contains the following menus:

- **File**, for opening new and existing scan settings files, saving scan settings files, and exiting the program;
- **XL,** for instrument setup and operation;
- **Scan**, for XL-A or XL-I scan settings file setup and operation;
- **Interference**, for setup of the XL-I interference optical system;
- **Service**, for read-only access to factory-adjusted hardware settings;
- **Options**, for selecting the com port; also for opening the Microsoft Windows Notepad text editor;
- **Windows**, containing standard Microsoft Windows commands for arranging open windows; and
- **Help**, for access to the XL-A and XL-I help system.

Toolbar

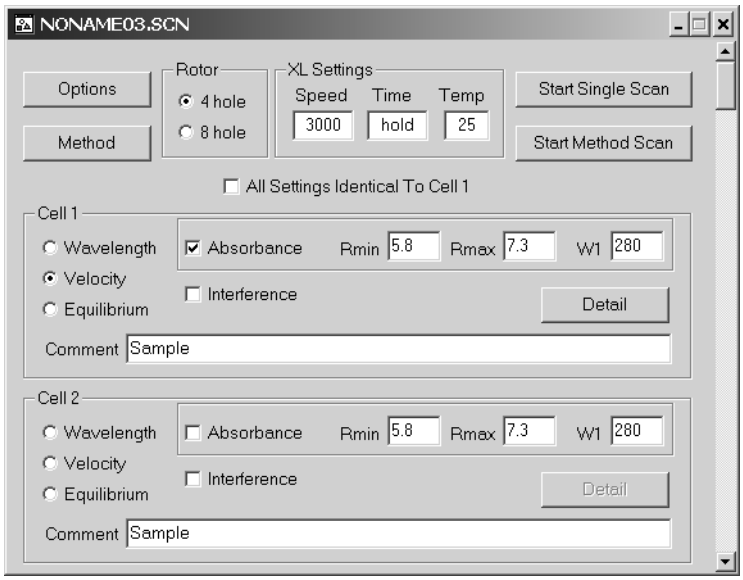
The toolbar offers convenient access to basic procedures such as opening and saving scan settings files, starting and stopping the XL-A or XL-I, starting and stopping scans, displaying scan and status monitor screens, and accessing help.

The toolbar's shortcut command buttons perform the same functions as the corresponding commands in the File, XL, Scan, and Help menus. As the pointer passes over each icon, a description of its function appears in the message line at the bottom of the main window.



File Menu

Choosing **New File** opens a new scan settings file window, shown below. The system automatically assigns a sequence number to the file name (NONAME01, NONAME02, etc.) to prevent files from being overwritten if they are not renamed before being saved



Choosing **Open File** opens an existing scan settings file.

Choosing **Save File** saves the scan settings file to disk. The first time you save a file, the Save As dialog box appears and you can enter a file name. Subsequently, choosing **Save** updates the open file, overwriting all information previously saved.

Choosing **Save File As** duplicates an existing scan pasettings file, letting you save it under a new name or to a new location. The Save As dialog box appears.

NOTE If no scan settings file is open, the **Save File** and **Save File As** commands are inactive.

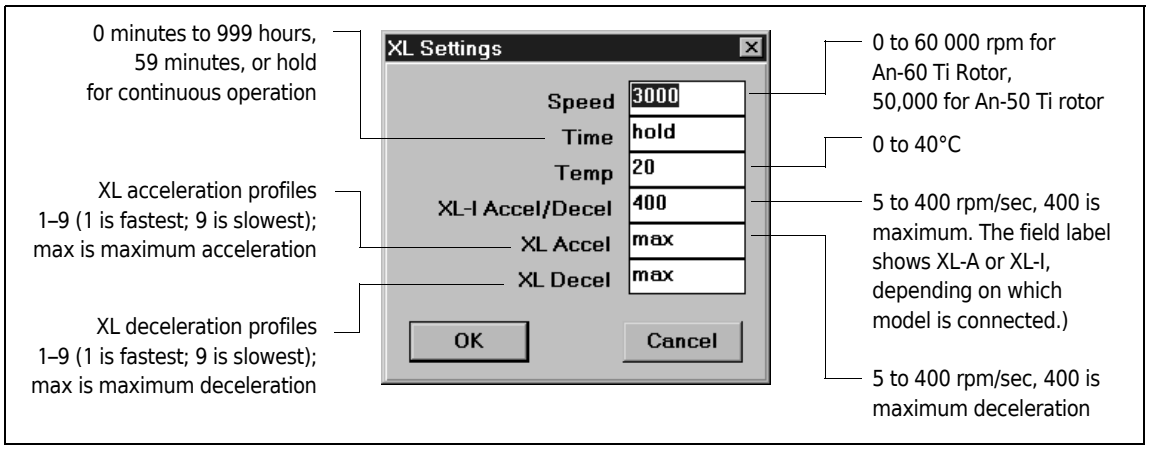
Choosing **Exit** closes the XL-A/XL-I user interface software. If a scan settings file is open and has been changed since the last save, a dialog box appears prompting you to save changes before exiting.

XL MenU

Choose **Start XL** to start the XL-A or XL-I using the settings entered in the XL Settings dialog box, shown below.

Choosing **Stop XL** brings the XL-A or XL-I to 0 rpm.

Choosing **XL Settings** opens the XL Settings dialog box. Default values are shown. The settings in this dialog box are for controlling the instrument when no scan is running. The entries in a scan settings file will overwrite the settings in the XL Settings dialog box when the scan is started.



XL-A or XL-I Accel/Decel

The XL-A or XL-I Accel/Decel field allows (optional) selection of slow acceleration and deceleration above 500 rpm. A setting from 5 rpm/sec to 400 rpm/sec can be entered; 400 rpm/sec, the maximum setting, is the default. The same setting is used for acceleration and deceleration.

For example, if 5 is entered, the system will accelerate from 0 to 500 rpm according to the XL Accel setting (1 through 9, or Max). Then, from 500 rpm to set speed, the system will accelerate at a rate of 5 rpm sec, or 300 rpm/min, requiring about 2 1/2 hours to reach 60,000 rpm. During decel, setting 5 is used from set speed to 500 rpm, and then the XL Decel rate (1 through 9, or Max) is used to 0 rpm. (The maximum setting of 400 is equivalent to the XL Accel and XL Decel Max settings.)

Settings between minimum (5 rpm/sec) and maximum (400 rpm/sec) will result in intermediate acceleration and deceleration rates.

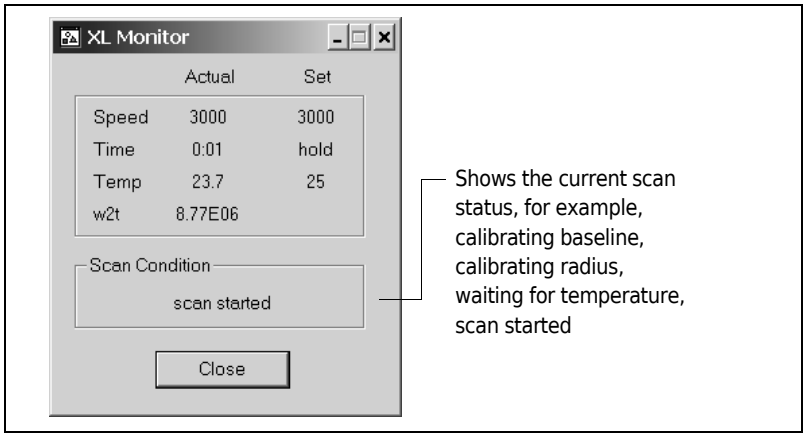
XL Accel and XL Decel

Nine slow acceleration profiles are available (1 is the fastest; 9 is the slowest) to minimize disturbance of the sample. The profile for the selected rotor, along with transition speeds and times, appear on the display when you click in the XL Accel field.

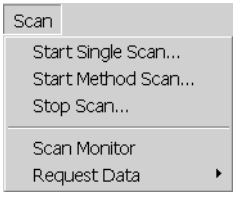
The XL Accel and XL Decel settings are used during acceleration from 0 to 500 rpm and from 500 to 0 rpm. Above 500 rpm, the XL-A or XL-I Accel/Decel setting is used.

Choosing **XL Monitor** opens the following dialog box, which shows the current system status. This dialog box also opens automatically when a scan is started. The speed, time, and temperature settings are taken from the scan settings file, if a scan is running, or from the XL Settings dialog box if no scan is running.

Only the actual $\omega^2 t$ is shown; $\omega^2 t$ cannot be set.



Scan Menu

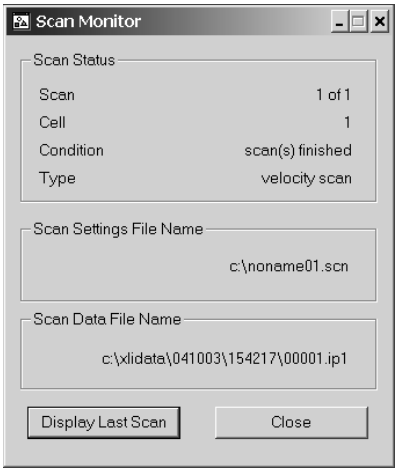


Choosing **Start Single Scan** runs one scan using the settings in the open scan settings file. If no file is open, the File Open dialog box appears, from which an existing file can be opened. The scan will start as soon as the file is selected.

Choosing **Start Method Scan** runs the series of scans specified in the open scan settings file. If no file is open, the File Open dialog box appears, from which an existing method file can be opened. The method will start as soon as the file is selected.

Choosing **Stop Scan** stops the scan or method in progress. If a method is running, no additional scans will be performed.

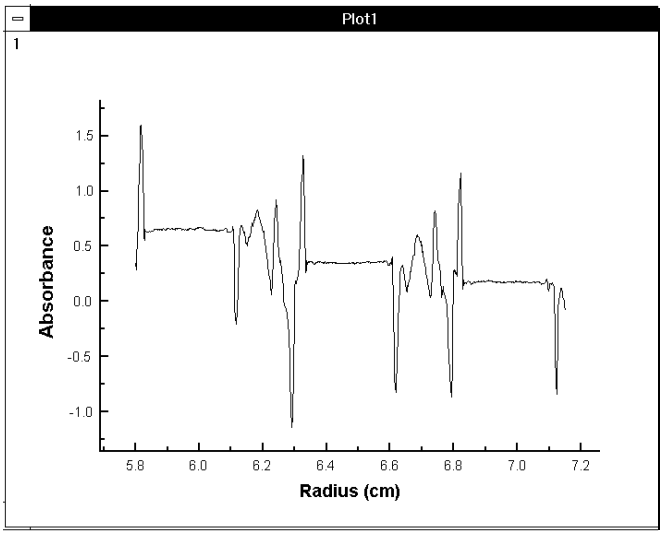
Choosing **Scan Monitor** opens the following window, which shows the status of the scan in progress.



The **Request Data** command (used with absorbance scans only) allows you to view the data from the last scan performed, in absorbance, intensity, or incident format.

During a scan, the system acquires scan data in all three formats and temporarily stores this data on the data acquisition board. However, only the absorbance data is saved automatically to a file on your hard disk. To preserve hard disk space, intensity and incident data are not saved (unless you have selected **Acquire intensity data instead of OD data** in the Scan Options dialog box, in which case intensity data is saved automatically and absorbance data is not). To acquire data in more than one format, you must access the **Request Data** menu when the scan is complete and select the desired data format to view. *Only data from the last scan can be recalled.*

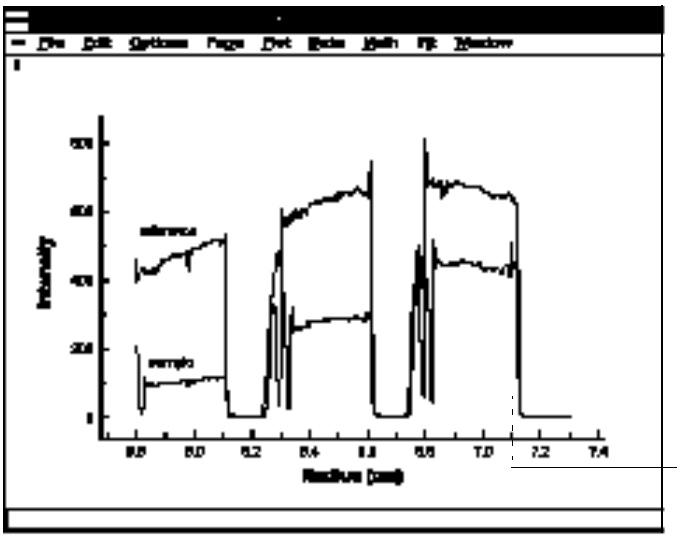
- *Absorbance* format displays the data as a plot of radius versus absorbance.



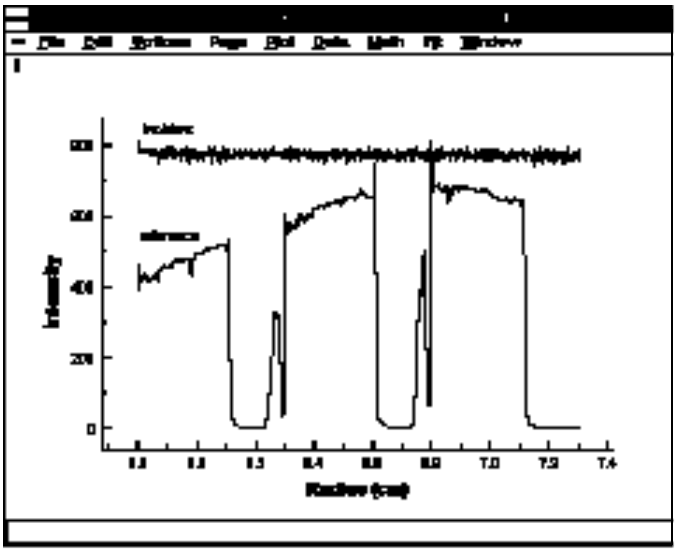
- *Intensities* format shows the raw data collected by the data acquisition board in linear scale.

  Intensity data show the radial position at the bottom of the cell, and thus show which portion of the data will be useful in the analysis. In the example below, of an equilibrium scan taken in a six-channel cell, the bottom of the cell is at about 7.13 cm. In the absorbance data for the same scan, stray light may cause extraneous data beyond 7.13 cm. Including the extraneous data in the analysis will adversely affect the analysis results. After looking at the intensity data and

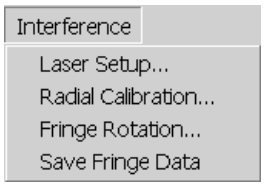
determining the real bottom of the cell, you can select a data subset which deletes all data points beyond 7.13 cm before importing the file for analysis. (See the *XL-A/XL-I Data Analysis Software User's Manual* [LXL/A-TB-009] for instructions on selecting data subsets. A pdf file of this manual is located on the XL-A/XL-I Windows setup CD.)



- An *Incident* plot shows the light present at the incident detector and the intensity of the reference sector. The incident plot line should be straight, although "noisy," indicating good flash-to-flash consistency from the flash lamp. The incident scan is used primarily to verify the efficiency of a questionable flash lamp.

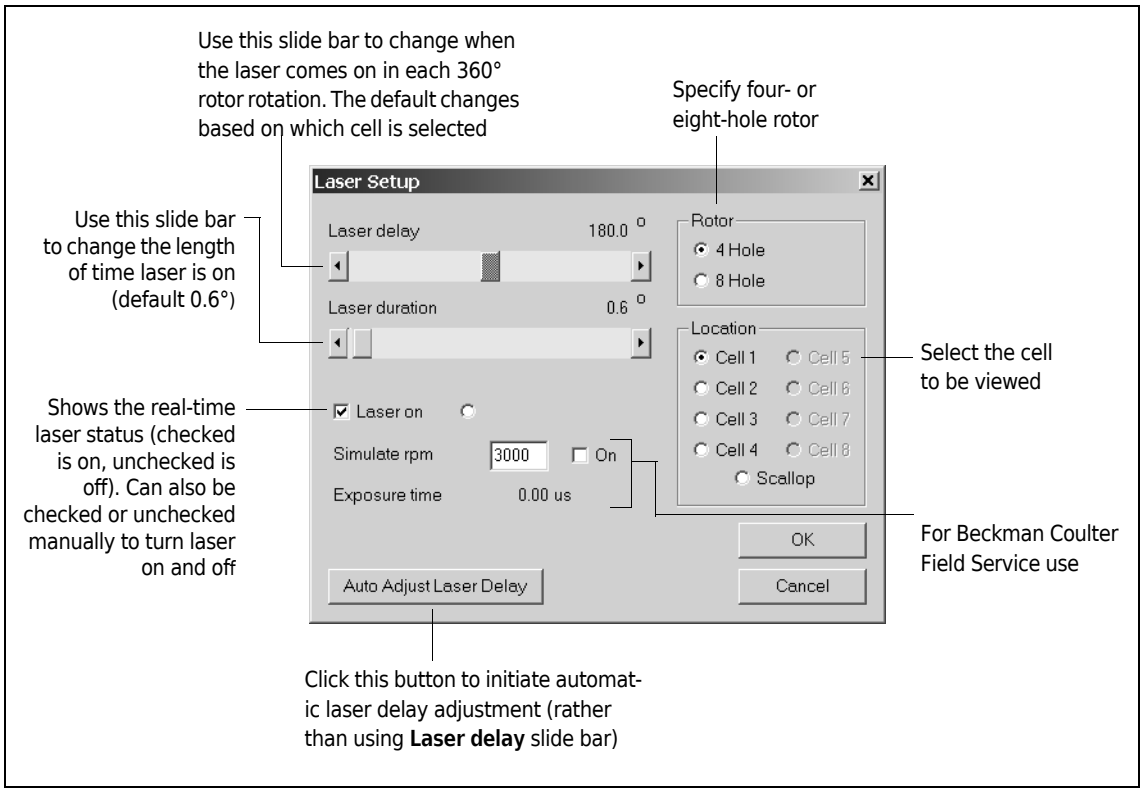


Interference Menu



The Interference menu provides access to XL-I interference optical system controls.

Choosing **Interface > Laser Setup** opens the Laser Setup dialog box.



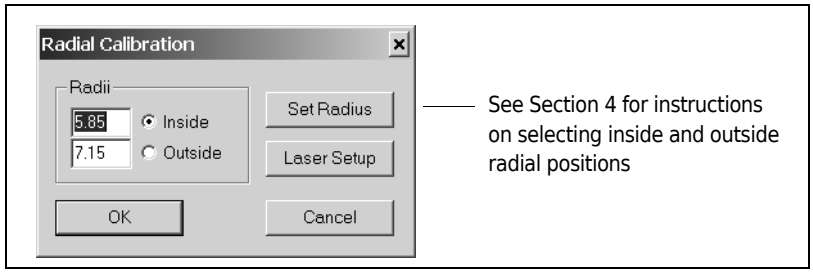
- The laser delay setting is the point at which the laser will come on during each rotor rotation, in degrees of one complete 360° rotation, to illuminate only the specified cell or cells. The setting is different for each cell. If the same rotor is always used, the laser delay setting should not change.
- The laser duration setting is how long the laser will stay on during each rotation, in number of degrees of one complete 360° rotation. The default is 0.6 degrees. Duration settings above the default will typically cause a blurred fringe image.

For the optics to produce an optimal fringe pattern, the timing and duration of each laser beam fire must be adjusted for each cell in use. Clicking the **Auto Adjust Laser Delay** button will automatically adjust the delay setting. This setting can also be manually adjusted using the **Laser delay** slide bar.

It is critical that the laser turn on directly over the specified cell, and stay on just long enough to capture the contents of that cell. A timing system, consisting of magnets in the overspeed disk (on the bottom of the rotor) and a sensor in the instrument chamber, lets the system know when each rotor hole is in the interference optical path. As the rotor spins, cell hole 3 is in the interference optical path when the rotor magnets are directly over the sensor (in a four-place rotor). The system

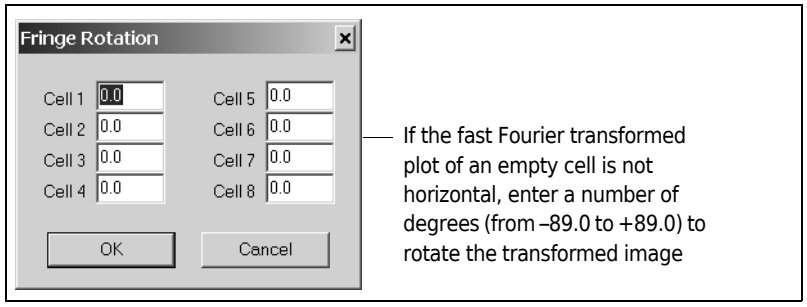
knows the position of the other cell holes in relation to cell 3. One complete rotor rotation is 360 degrees. Cell hole 1 is opposite cell hole 3, at 180 degrees. Cell hole 2 is at 90 degrees, and cell hole 4 is at 270 degrees. (Positions for the eight cell holes in the An-50 Ti rotor are different, but the system uses the same method for locating the cell holes and timing the laser fire.) The user does not need to remember these positions. They appear automatically above the **Laser delay** slide bar after a cell hole is specified, and change as the slide bar is adjusted.

Choosing **Interference > Radial Calibration** opens a dialog box where you can specify the distances from the inner and outer edges of the counterbalance to the center of the rotor. The system uses the counterbalance edge radial positions to determine the radial positions of the other cell holes. The defaults are 5.85 cm for the inside radius and 7.15 cm for the outside radius.



Choosing **Interference > Fringe Rotation** opens a dialog box in which you can rotate the fast Fourier transform plot line to make it flat, with no slope upward or downward. In the box for the appropriate cell, you can enter from –89.0 to +89.0 degrees of rotation. When you enter a value and click **OK**, the plot line will rotate either to the left (if you entered a negative value) or to the right (if you entered a positive value) the specified number of degrees. You can keep entering values until the plot line appears flat.

The goal of rotating the fringes is to achieve a flat baseline in the fast Fourier transform plot. A sloping data plot can adversely affect data analysis.



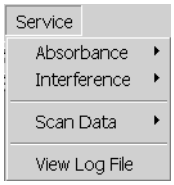
NOTE If your optical system is properly aligned, fringe rotation should not be necessary.

Choosing **Interference > Save Fringe Data** saves the fringe data to a unique ASCII file called fringes.raw. This file is saved to the directory containing the XL-A/XL-I user interface software (typically xliwin). Fringes.raw gets overwritten each time **Save Fringe Data** is selected. Rename the current fringes.raw file if you want to save additional raw fringe data.

The raw fringe file is a pixel-by-pixel numerical representation of the image in the real-time fringe display. The file contains 2048 columns and 96 rows, corresponding with the number of columns

and rows in the real-time fringe display. Each pixel is represented by a value from 0 to 255 (0 being black, 255 being white, and all values between 0 and 255 being gray scale images).

Service Menu



The Service menu provides read-only access to factory-set hardware and calibration settings. If troubleshooting is necessary, you can view the service windows and provide the information to a Beckman Coulter representative. To change service settings, a password is required.

⚠ CAUTION

Changing any of the service settings will affect system operation and could possibly result in system damage. Do not change settings accessed under the Service Menu except under the direction of a Beckman Coulter Field Service Engineer.

Choosing **Service > Absorbance** displays the following submenus:

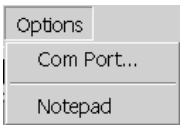
HW Params	Opens a dialog box displaying hardware settings
Cal Params	Opens a dialog box displaying current calibration settings for the radial slit and the monochromator
Scans	A **Delay scan** is a ccessed during a strobe delay calibration check A **Time scan** is accessed to perform a time vs. intensity check A **Wavelength calibration scan** is accessed to perform a system wavelength calibration

Choosing **Service > Interference > Align Laser** provides access to laser settings.

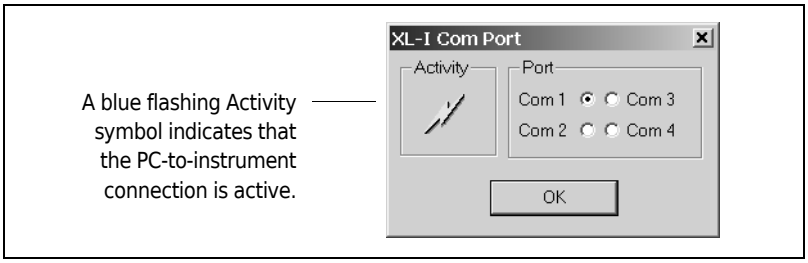
Choosing **Service > Scan Data >Open...** enables selection and display of previously saved scan data files. Selecting **Close All** closes Scan Data files.

Choosing **Service > View Log File** displays the log file from the most recent scans.

Options Menu



Choosing **Options > Com Port** opens a dialog box where you can specify which communication (com) port you want your PC to use for interfacing with the XL-A or XL-I. The default is com port 1. Keeping the default setting will help to avoid system problems. If the com port setting is changed, the PC may have to be rebooted before the change will take effect, depending on the PC's com configuration.



Choosing **Options > Notepad** opens the Windows Notepad program. From Notepad you can open any text file, including the log files (described under **Log Files**, below).

Window Menu

The Window menu contains standard Microsoft Windows commands for arranging and displaying open windows. Use these commands to organize your desktop.

Help Menu

Choosing **Help > About** opens a window containing the XL-A/XL-I user interface software version number and other information.

Choosing **Help > Help** opens the XL-A/XL-I help system.

File Naming and Storage

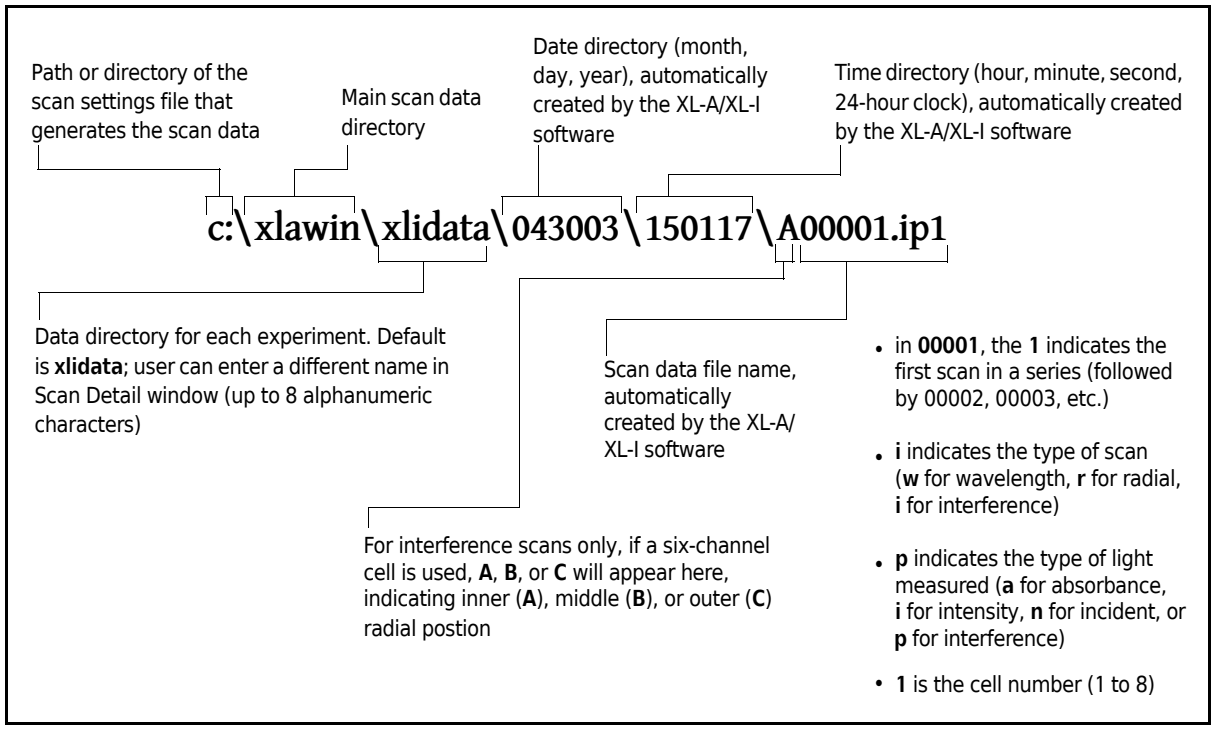
There are two types of files related to XL-A and XL-I operation and data collection: *scan settings files*, which contain the settings to be used for a run, and *scan data files,* which contain the data collected during a run.

Scan SETTINGS Files

Scan settings files contain the user-specified run settings. New scan settings files are opened by selecting **File > New File**. After a scan settings file has been saved, reopen it by selecting **File > Open File**. The extension **.scn** is automatically added when scan settings files are saved. Each scan settings file also has two associated method files with the extensions **.equ** and **.vel,** and a **.int** file containing interference optical system settings if applicable.

Scan Data Files

When a scan or method is started, the instrument collects data and saves the data files according to the directory structure shown below. All scans from one experiment are grouped into the **xlawin** directory, and are further identified by 1) the scan data file directory name (either the default, **xlidata**, or a name entered by the user), 2) the date the scans were taken, and 3) the time the scans were taken. This structure prevents overwriting of previously saved files, because the user interface software creates a new time subdirectory for each scan or series of scans.

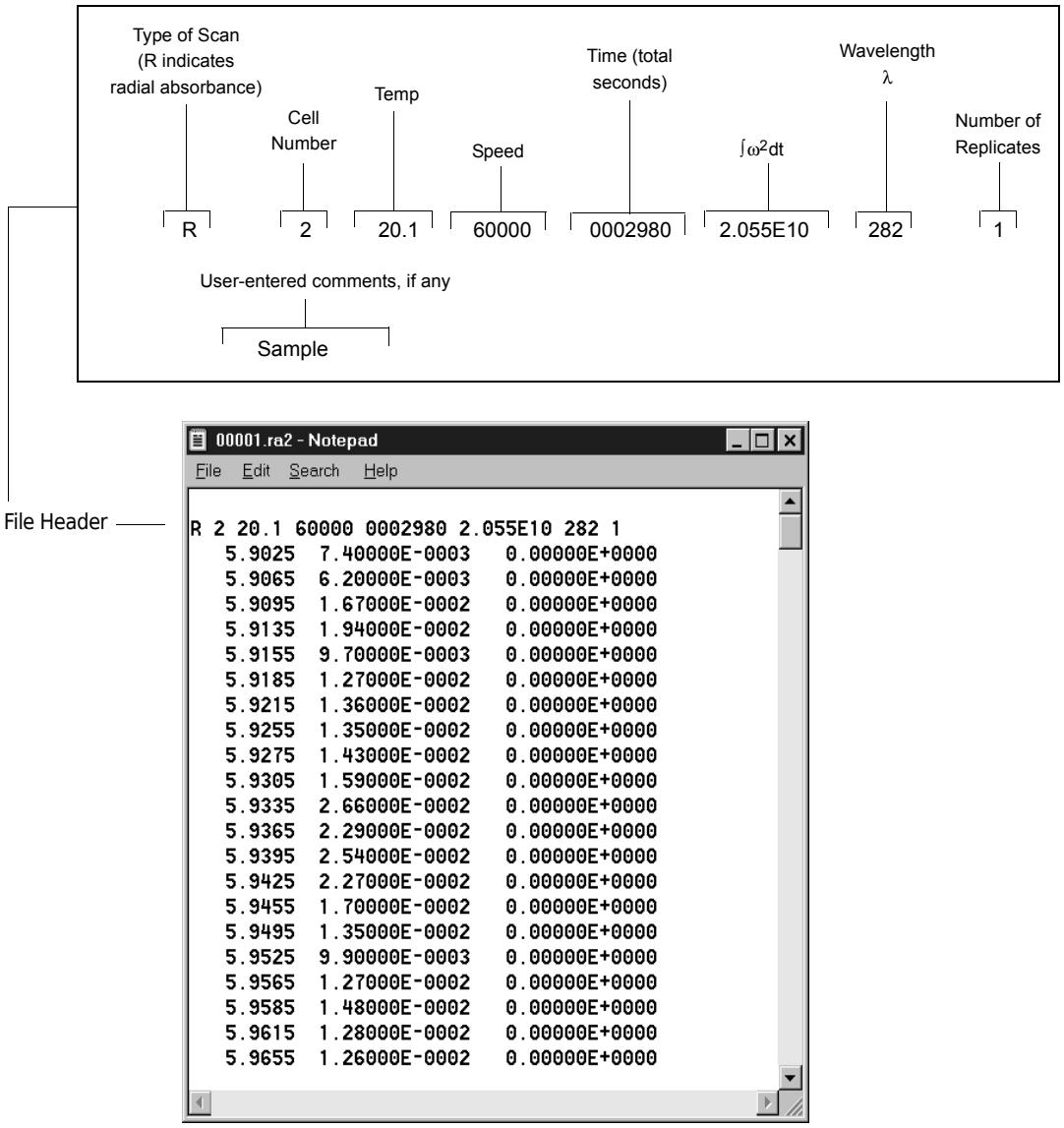


To specify a scan data directory other than **xlidata**, select **File > New File**, then select the scan type and optical system for a cell, and then click the **Detail** button. When the scan or method is started, the scan data files will be saved to the new location.

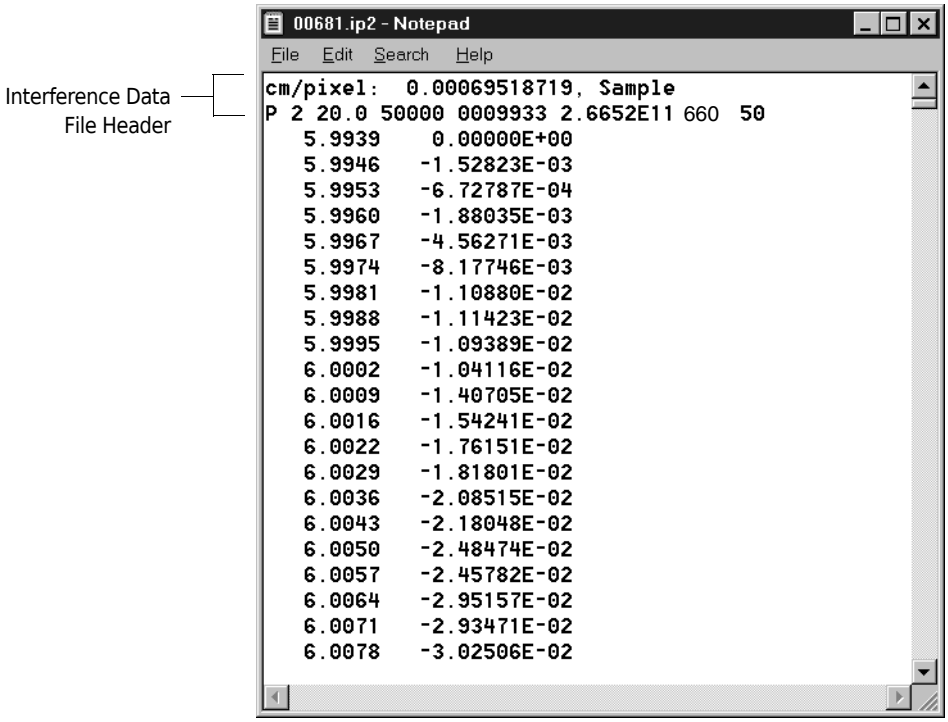
NOTE If you run a method that spans more than 1 day, all of the scan data files for that method are stored in a directory created the day the method was started.

Scan Data

Each scan data file has a two-line header containing information about that file. The header appears at the top of the page when you open the file in a text or word processing program. An example absorbance data file, opened in Windows Notepad, is shown below. (To open Notepad, select **Options > Notepad**.)



An example interference data file is shown below. The top line contains the centimeter-per-pixel setting, along with user-entered comments. The second line contains the same information as in an absorbance file header, except that the wavelength value is the laser diode wavelength, which is always 665 nm.



Log Files

Log files contain a record of all scans performed, scan settings used, and any diagnostics that occurred during a scan or method. The log files are saved to the same directory as the scan data.

The system creates separate log files for interference and absorbance scans. If a method contains both interference and absorbance scans, two log files will be created for the method, and both will be stored in the same scan file directory. Separate log files are created for each cell and, for interference scans, for each centerpiece channel.

- *for interference scans*: the log file name is cell**n**chan**n**.log, where cell **n** is the cell number (1 through 8) and chan **n** is the centerpiece channel (a through c).
- *for absorbance scans:* the log file name is cell**n**abs.log, where cell **n** is the cell number (1 through 8).

The log files are text files that can be opened by Windows Notepad or any word processing program. To open Notepad, select **Options > Notepad**.

Data storage

XL-A and XL-I data files require large amounts of disk space. Before archiving multiple data files to a separate disk or storage device, we recommend compressing the files using any of the commercially available Windows data compression programs. Compressed files must be expanded before they can be reopened by the XL-A/XL-I user interface software, and before they can be imported into the XL-A / XL-I data analysis software.

Organizing Your Files

Use the Windows Explorer to view and organize the files on your computer. See the Windows instruction manual or online help for more details.

README FILE

The XL-A/XL-I Readme file includes a description of any last-minute information that could not be added to this manual before it went to press. It is a text file that can be opened by Windows Notepad or any word processing program.

CHAPTER 4

System Operation

Introduction

This section describes ProteomeLab XL-A and XL-I operating procedures, including

- *preparing the analytical rotor and cells;*
- *setting up and saving scan settings files;*
- *recalling previously saved scan settings files;*
- *displaying data;*
- *starting, stopping, and monitoring the XL-A or XL-I, and*
- *starting, stopping, and monitoring a scan or series of scans.*

Preparing the Rotor and Analytical Cells

Follow these instructions to prepare and install the rotor and analytical cells. (See the applicable analytical rotor manual for complete rotor and cell assembly instructions.)

---

1 Assemble and torque the cell(s).

a. Use two-channel centerpieces for sedimentation velocity experiments; use two-channel or six-channel centerpieces for sedimentation equilibrium experiments.

b. Use quartz windows with the absorbance optical system.

With the interference optical system, use sapphire windows to ensure good fringe patterns at speeds above 30,000 rpm.

See your analytical rotor instruction manual for a list of available centerpieces, windows, and accessories.

---

2 Load the cell(s).

a. *Sedimentation velocity:* using a two-sector centerpiece, place approximately 400 µL total of sample plus solvent in the sample sector and 425 µL of buffer in the buffer sector.

Absorbance optics only: the sample concentration should give an initial absorbance of 0.5 to 1.0 AU.

b. *Sedimentation equilibrium:* using a six-channel centerpiece, place approximately 110 µL total of sample plus solvent in each sample channel and 125 µL of buffer in each buffer channel.

Interference scans require less difference in volume; approximately 5 µL difference is acceptable.

Absorbance optics only: the sample concentration should give an initial absorbance of 0.1 to 0.5 AU.

c. Load the assembled cell with six-channel centerpiece into the rotor so that the channel with the highest concentration of sample is toward the center of the rotor.

3 Adjust the weight of counterbalance to within 0.5 gram of the cell to be placed opposite it in the rotor.

See the analytical rotor manual for instructions.

4 Install the counterbalance in cell hole number 4 (in the An-60 Ti rotor), or in cell hole number 8 (in the An-50 Ti rotor).

5 Load the sample cell(s) into the rotor. If only one cell is run, place it in the hole opposite the counterbalance.

6 Install the rotor by lowering it over the instrument's drive spindle.

7 Install the monochromator (see CHAPTER 2 for instructions).

Absorbance system only: position the monochromator blocking filter lever parallel to the monochromator stem if using wavelengths below 400 nm, or perpendicular to the stem if using wavelengths above 400 nm.

Starting the XL-A/XL-I User Interface Software

1 Turn on the PC system controller.

2 Click the **Start** button, select Programs, and then select ProteomeLab from the list of programs.

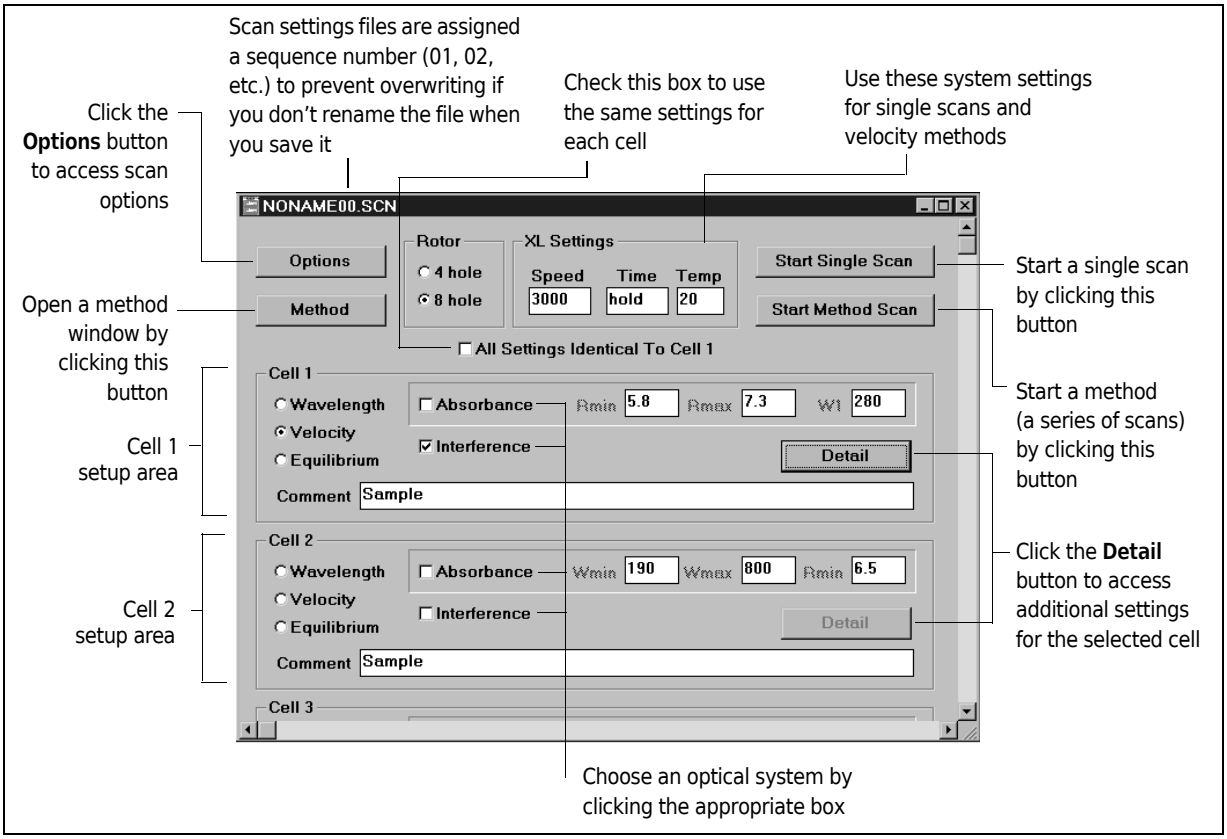
Or, double-click the ProteomeLab icon on your desktop (if a shortcut to the program has been created).

The main window appears.

3 Choose **File > New File.**

Or, click the new file icon (shown to the left).

A blank scan settings file opens (shown below).



4 In the setup area for the cell in use, select the optical system to be used by clicking the **Absorbance** or **Interference** check box.

Both boxes can be checked to enable setup of a method containing both absorbance and interference scans.

If you select **Absorbance**, default values appear in the R min, R max, and Wavelength fields.

If you select **Interference**, the fields are disabled because the values apply to absorbance scans only.

If you select Interference, the wavelength button is disabled.

5 Select the type of scan (wavelength, velocity or equilibrium) by clicking the appropriate button.

Wavelength ScanS (Absorbance Optical System only)

Description

During a wavelength scan, different wavelengths of light (from 190 to 800 nm, user-selectable) are passed through specified radial positions across the cell. The resulting absorbance spectrum shows the absorbance value (λ_{max}) of each solute as a peak on the spectrum plot. If the absorbance values of the solutes are known, and the peaks on the spectrum plot appear at the expected locations and are of good quality, the absorbance optical system is operating properly.

Setting Up Wavelength Scans

1 Choose **File > New File**.

In the scan settings window, click the **Wavelength** button, then the **Absorbance** box for the appropriate cell.

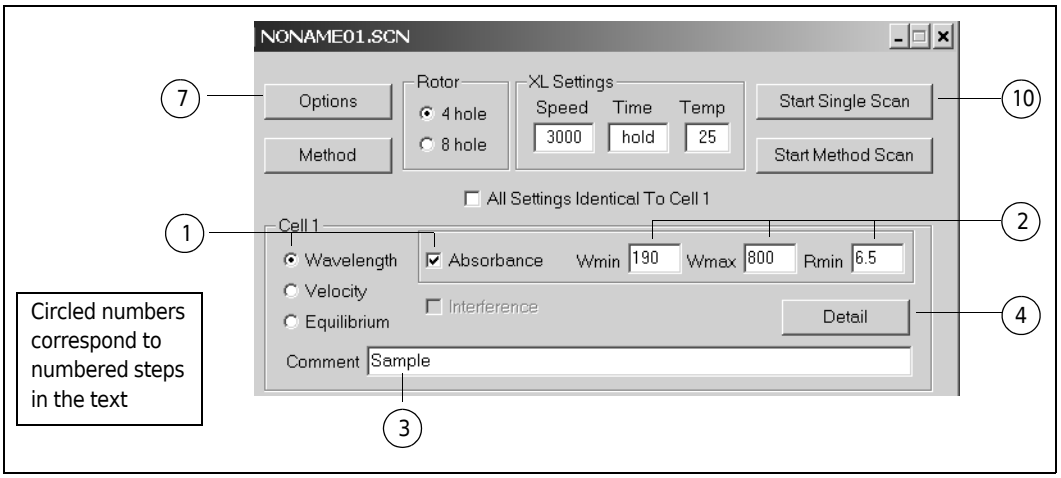
The Wmin, Wmax, and Rmin fields become active.

2 Enter the Wmin (minimum wavelength), Wmax (maximum wavelength) and Rmin (minimum radial distance) values in the appropriate fields.

Or, accept the defaults.

3 Enter a comment in the Comment field (optional).

Comments are saved to the scan data file.

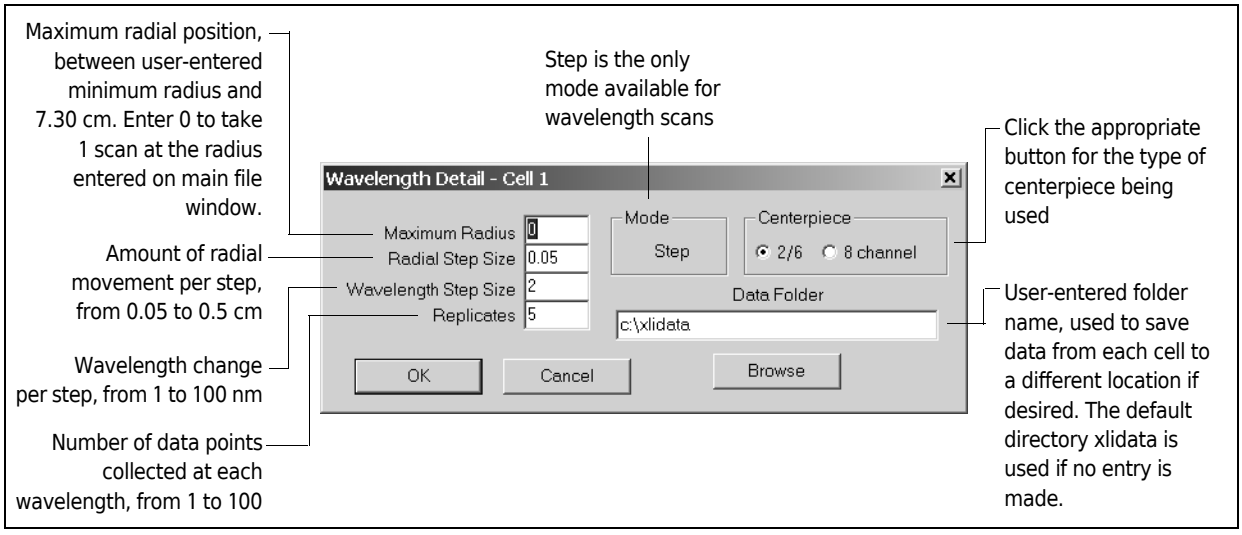


You could start a scan now, using these basic settings.

Additional scan settings are available in the Wavelength Detail dialog box, shown in step 4.

4 Click the **Detail** button.

The Wavelength Detail dialog box appears.



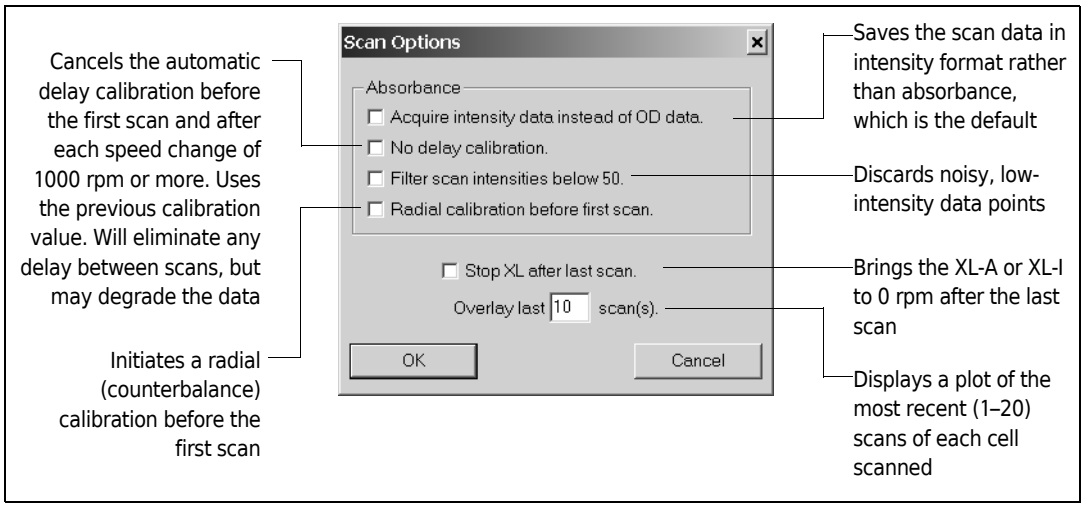
5 Enter a setting or accept the default value for each field.

You should specify five to ten Replicates to obtain a clean spectrum.

6 Click **OK** to accept changes or **Cancel** to cancel changes and use all default values.

7 Click the **Options** button on the scan settings window.

The Scan Options dialog box appears.

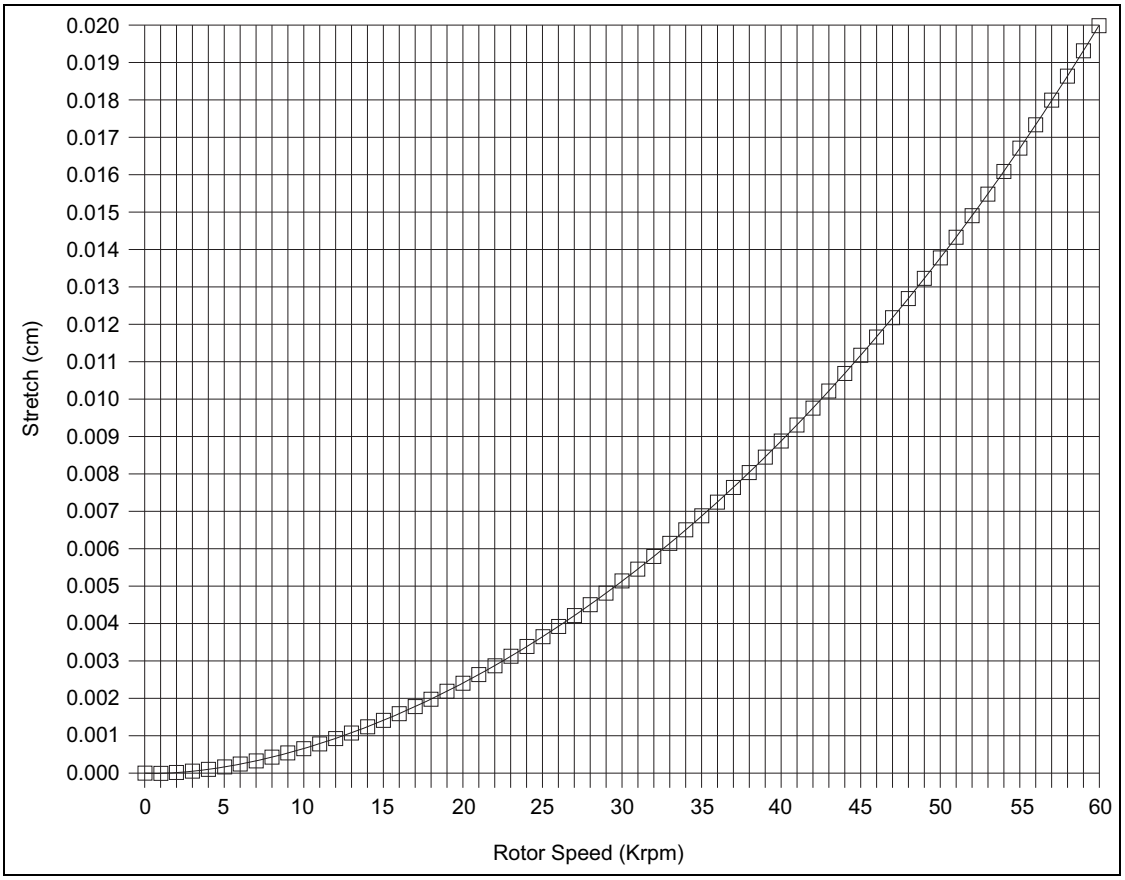


8 Click the box next to each option you wish to activate.

- Do not use the **Filter scan intensities below 50** option with wavelength scans.
- Radial calibration should be done at low speeds (approximately 3000 rpm) for optimum accuracy.
  - As rotor speed increases, a rotor will begin to stretch; this stretch increases at higher speeds (see Figure 4.1).
  - Calibration should be performed at lower speeds, where rotor stretch is negligible.
- The **Overlay last __ scans** option allows display of up to twenty successive scans of each cell.
  - The maximum number of overlays depends on the number of scans requested in the current settings.
  - The total number of data sets plotted can be no more than 150, or system memory may be overloaded, resulting in data loss.

  The system automatically calculates the maximum number of allowable overlays, based on current scan or method settings. When you start the scan or method, if you have requested too many overlays an error message appears listing the maximum allowable number. You must go back to the Scan Options dialog box and enter this number (or fewer) overlays in the **Overlay last __ scans** box.

Figure 4.1 An-60 Ti Rotor Stretch at Outer Cell Wall



9 Choose **OK** to use the selected options; choose **Cancel** if you do not want to activate any of the options.

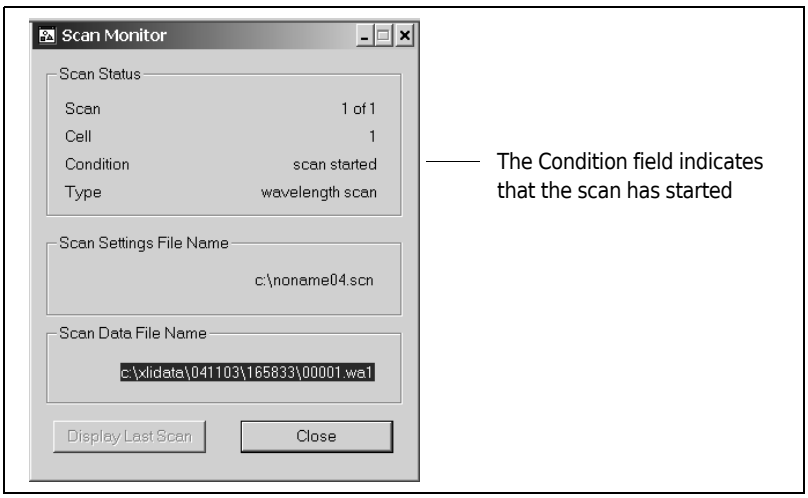
The Scan Options dialog box closes.

10 At this point you can either start the scan, or save the scan settings to a file.

To start the scan,

a. Click the **Start Single Scan** button.

- The scan monitor window opens and displays the status of the instrument and the scan.
- The XL monitor window also opens.



To save the scan settings to a file,

a. Double-click the control menu box at the top left of the NoName.SCN window.

A dialog box appears verifying that you want to save the changes.

b. Click **Yes**.

The Save As dialog box appears.

c. Enter a file name and choose a drive and directory.

Click **OK** when done.

Setting Up Wavelength Methods

A series of wavelength scans, also known as a wavelength method, can be set up as follows.

1 Choose **File > New File**.

2 In the setup area for the cell to be used in the method, click the **Wavelength** button.

The **Method** button at the top left of the window becomes active.

3 Click the **Method** button.

A window appears in which multiple scans can be set up at different speeds and temperatures, with varying amounts of time between scans. This window is used for setting up sedimentation equilibrium methods as well, and is described in detail under *Setting Up Sedimentation Equilibrium Methods*, below.

Sedimentation Velocity Scans

Description

Sedimentation velocity experiments are used to determine the sedimentation coefficient, *s*, diffusion coefficient, D, and other properties of one or more solutes. In a boundary sedimentation velocity experiment, an initially uniform solution is placed in the cell. At a sufficient centrifugal force, the macromolecules move toward the bottom of the cell at a rate proportional to their *s*. This movement produces a depletion of solute near the meniscus and the formation of a sharp boundary between the depleted region and the uniform concentration of sedimenting solute. Although the velocity of individual particles cannot be determined, the rate of movement of this boundary can be measured, traditionally by measuring the rate of movement of the boundary midpoint.*

NOTE The transport method of velocity data analysis requires input of an initial concentration, C_0. If you plan to use the transport method, take one scan at 3000 rpm before proceeding to run speed. The point on the Y-axis corresponding to the plateau region of the data in the initial scan represents a fairly accurate C_0 estimate.

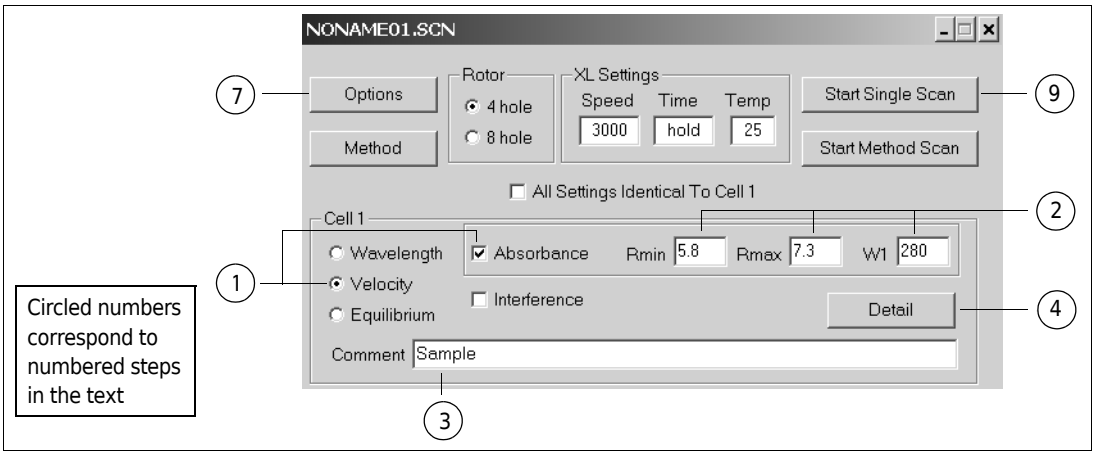
Setting Up Sedimentation Velocity Scans: Absorbance Optical System

1 Choose **File > New File**.

In the scan settings window, click the **Absorbance** box and the **Velocity** button for the appropriate cell(s).

The Rmin, Rmax, and W1 fields become active.

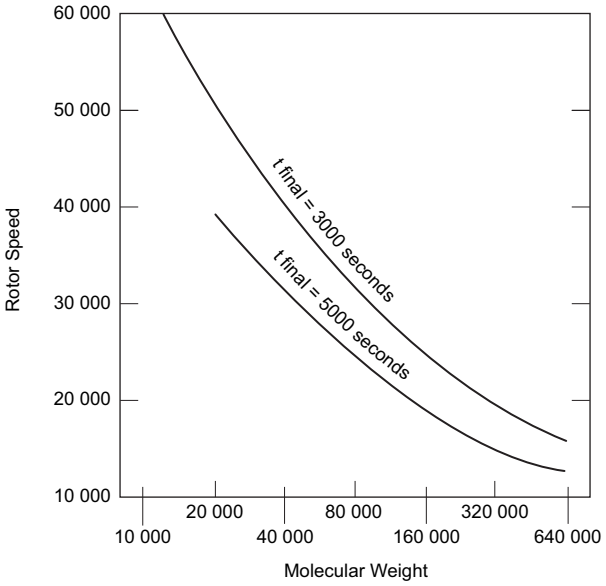
* Ralston, G., *Introduction to Analytical Ultracentrifugation*, Beckman Instruments, 1993, and Hansen, J., Lebowitz, J,. and Demeler, B., Analytical Ultracentrifugation of Complex Macromolecular Systems, *Anal. Biochem.* 33 (1994).



If you want to use the same scan settings for each cell, enter settings for Cell 1 and then click the **All Settings Identical to Cell 1** box.

Figure 4.2 can help you determine the run speed required for your experiment.

Figure 4.2 Plot of Suggested Rotor Speed Versus Molecular Weight for Two Data Collection Times (L. Holladay, 1980)



2 Enter the Rmin (minimum radius), Rmax (maximum radius) and W1 (wavelength) values in the appropriate fields.

Or, make no entries to accept the defaults.

3 Enter a comment in the Comment field (optional).

Comments are saved to the scan data file.

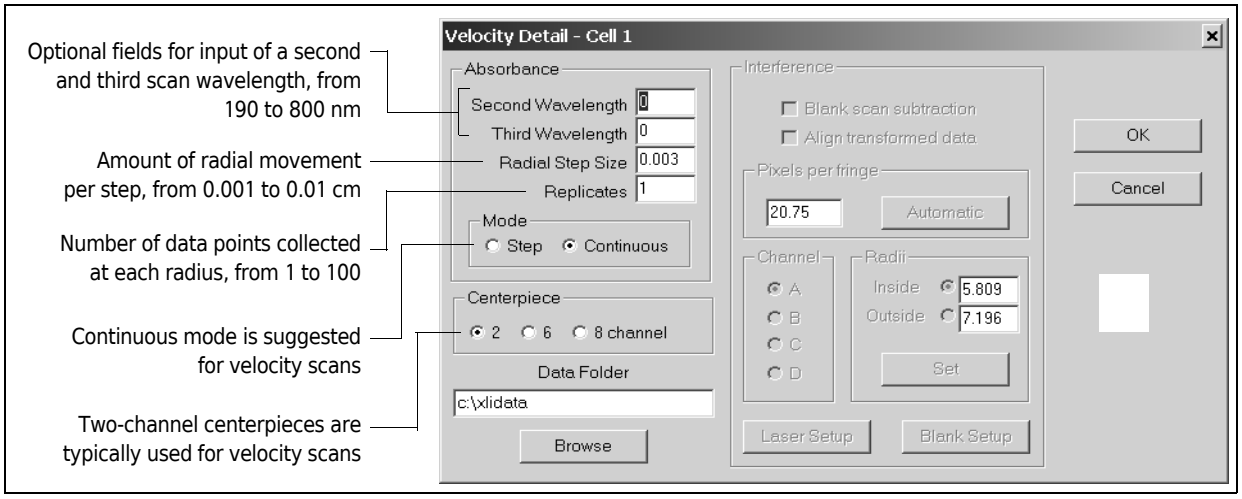
You could start a scan now, using these basic settings.

Additional scan settings are available in the Velocity Detail dialog box, shown in step 4.

4 Click the **Detail** button.

The Velocity Detail dialog box appears.

Settings specific to the absorbance system are in the left half of the window.



5 Enter a setting or accept the default value in each field.

6 Click **OK** to accept changes or **Cancel** to cancel the changes and use all default values.

7 Click the **Options** button on the scan settings window.

The Scan Options dialog box appears.

8 Select an option to initiate it.

We recommend selecting **Radial calibration before first scan** before every scan or method.

NOTE Radial calibration should be performed at a low speed (approximately 3000 rpm). Remember to deselect the radial calibration option following the first run.

9 Click **Start Single Scan**.

The Scan Monitor window opens and displays the status of the instrument and scan.

10 When scan is complete, a plot of the scan appears automatically.

If leakage has occurred, indicated by movement of the meniscus, reload the cell(s) and repeat the scan.

Check and adjust any settings as needed.

Setting Up Sedimentation Velocity and Sedimentation Equilibrium Scans: Interference System

This section contains steps for setting up the interference optical system for equilibrium and velocity scans.

The steps for setting up the laser are shown below, along with recommended settings for cell of 1 of a 4-hole rotor. The recommended settings produce good results in most cases, but may require some adjustment for your system.

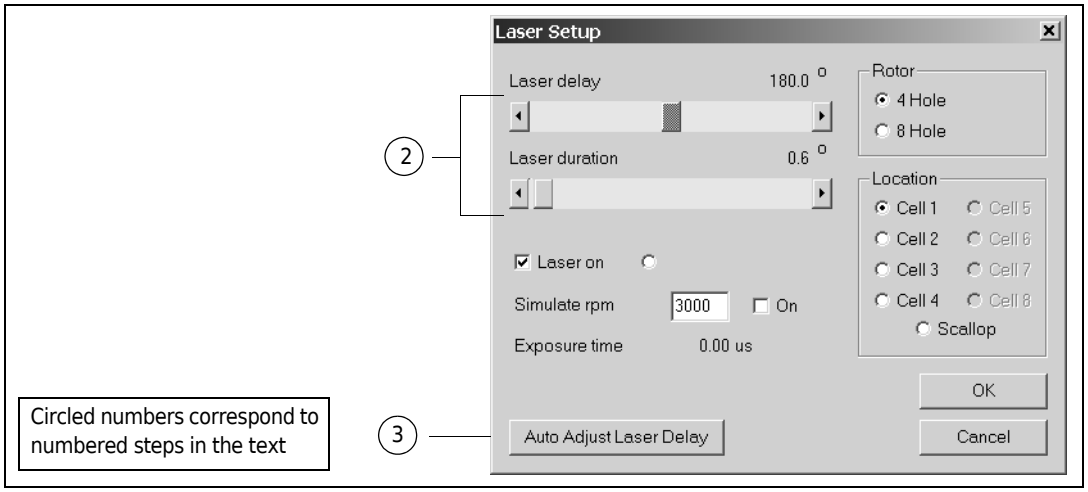
Adjusting the Laser Settings

1 Select **Interference > Laser Setup**.

2 Select the following laser settings by clicking and dragging the sliding box for each setting to the left or right (or by clicking the right and left arrows at the end of the bar).

- Laser delay: 180°
- Laser duration: 0.6

3 Click the **Auto Adjust Laser Delay** button.



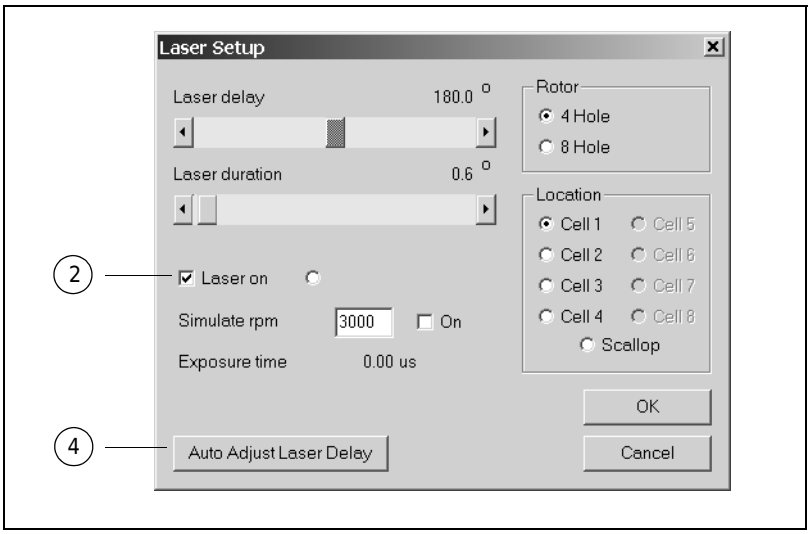
These settings should result in a clear, medium-contrast fringe pattern in the fringe display.

To further adjust the settings *after the recommended settings above have been tried*, follow the steps below.

Additional Adjustment of the Laser Settings

1 Click the **Laser Setup** button.

The Laser Setup window for the selected cell appears.



2 Click the **Laser on** box.

3 Click the mouse once anywhere on the fringe display.

4 When fringes appear in the fringe display, click the **Auto Adjust Laser Delay** button.

A scan begins; during this scan, the system finds the optimum laser setting for the radius you selected in step 3.

Note that there must be some image in the fringe display for the auto adjust function to work (the display cannot be black).

5 When the auto adjust scan is complete, click **OK**.

The Laser Setup window closes.

For a particular XL-I system and rotor/cell combination, once the laser has been set up properly, the laser settings should not need to be changed before each run. After you determine the optimum settings for your system following the steps in **Adjusting the Laser Settings** above, save these settings to a scan settings file called “masterfile” (or the name of your choice. Your Beckman Coulter Field Service engineer may have set up such a file during installation.) Follow the steps below to create a master file.

Master Laser Settings Scan File

1 Select **File > New File**.

2 Select **Interference > Laser Setup**.

3 Adjust the laser settings as described above and click the **Auto Adjust Laser Delay** button.

Alternately, input settings that you (or a Beckman Coulter Field Service engineer) have experimentally determined to be optimum.

Click the **OK** button.

4 Select **File > Save File As.** Name the file "masterfile."

5 Whenever you set up an experiment using the interference optics, open the master file and enter new run and scan settings in that file, but *do not adjust the laser settings*.

When you are done with setup, rename the new file to preserve the master file template.

Correct counterbalance radial settings are essential to proper system operation. You only need to calibrate these settings if you install a different rotor or if your optical system has been adjusted or realigned. If your counterbalance is properly calibrated, go on to the next step, *Enter Scan Settings*.

Set Inside and Outside Radial Positions for the Counterbalance

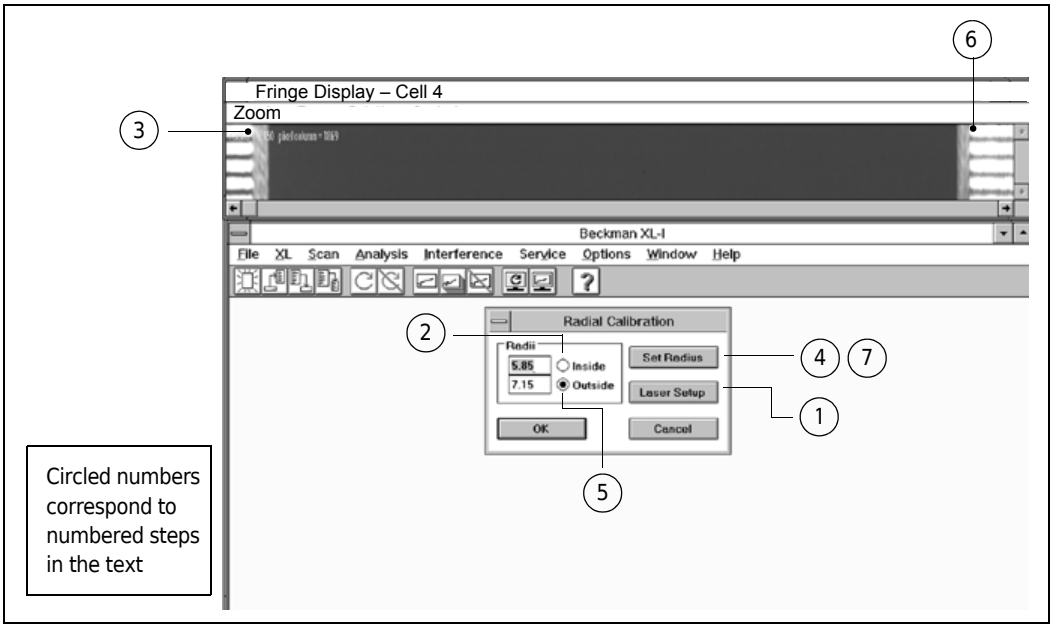
1 With the fringe display open and the instrument running at 3000 rpm, choose **Interference > Radial Calibration**.

The Radial Calibration dialog box appears.

Make sure that fringes from the counterbalance are being displayed.

If you are not sure, click the **Laser Setup** button and, in the Location area, click cell 4 (if you are using a four-hole rotor) or cell 8 (if you are using an eight-hole rotor).

Click **OK** to return to the Radial Calibration dialog box.



2 Click the **Inside** button.

3 Click once on the fringe display at the position representing the inner edge of the counterbalance, approximately 5.85 cm from the axis of rotation.

This position is indicated by a black dot at the upper left of the figure above.

The radial distance of the position you clicked appears in the Inside field.

To change the value, click on another position.

4 When you are done, click the **Set Radius** button to accept this value.

5 Click the **Outside** button.

6 Click once on the fringe display at the position representing the outer edge of the counterbalance, approximately 7.15 cm from the axis of rotation.

This position is indicated by a black dot at the upper right of the figure above.

The radial distance of the position you clicked appears in the Outside field.

To change the value, click on another position.

7 Click the **Set Radius** button to accept this value.

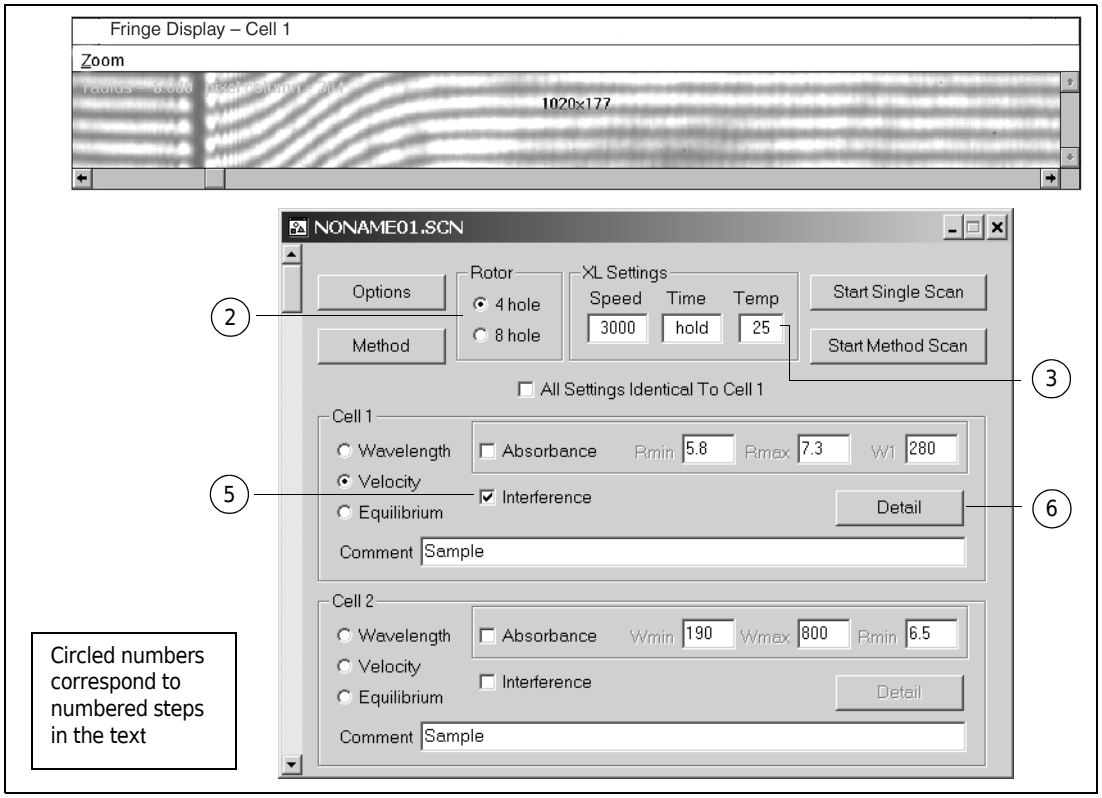
8 Click **OK** to close the dialog box.

Enter Scan Settings

1 Open the master laser settings file by selecting **File > Open File**, and then selecting the appropriate master file name.

To open a new scan settings file, select **File > New File** or click the new file icon in the toolbar (the far left icon).

A scan settings file window opens and the fringe display appears above it.



2 In the Rotor area, indicate the number of cell holes in the rotor in use by clicking either the **4 hole** or the **8 hole** button.

3 In the XL Settings area, leave the speed and time at the default settings (3000 rpm, hold).

Change the default temperature setting if necessary.

4 Start the Instrument

Choose **Start XL** from the XL menu.

Or click the green start icon in the toolbar.

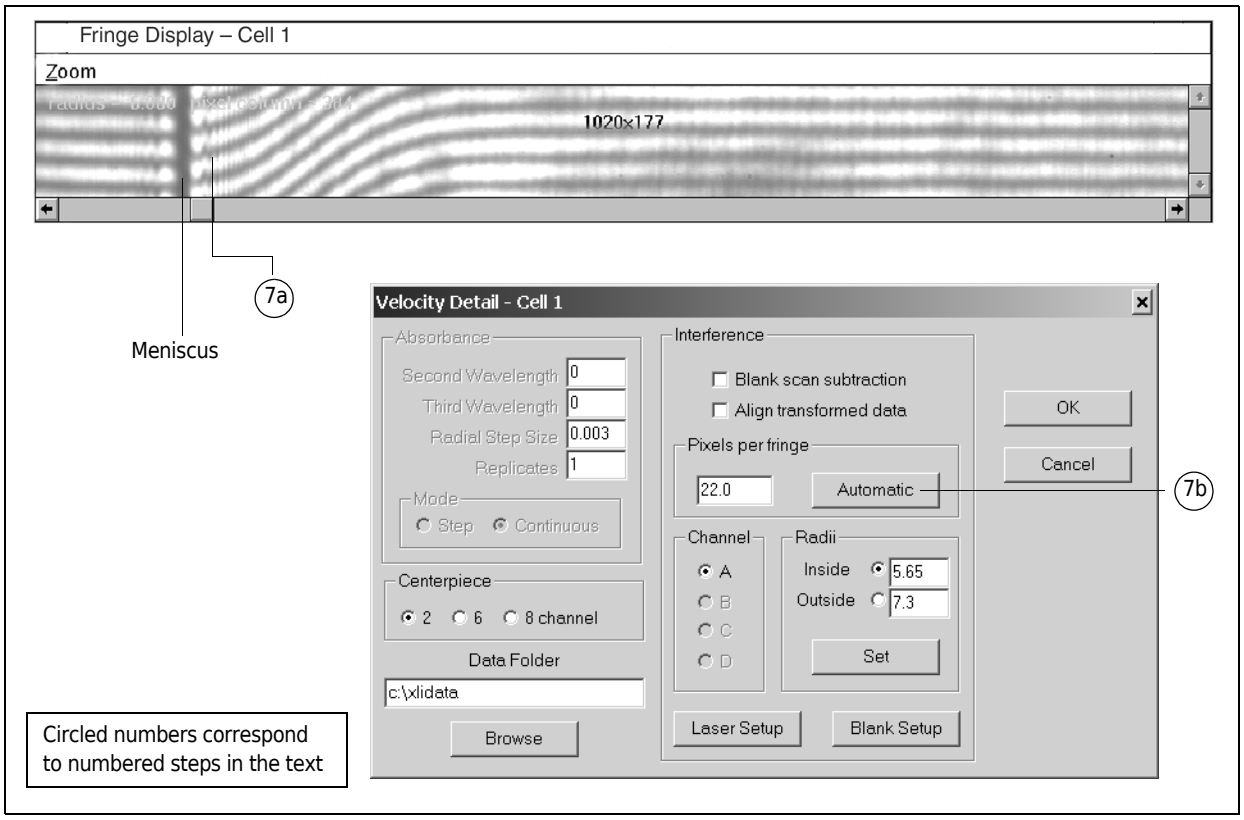
At 3000 rpm, fringes should appear in the fringe display when the laser is turned on.

5 In the new scan settings file, in the setup area for the appropriate cell, click the **Interference** box.

The **Velocity** button will highlight automatically.

6 Click the **Detail** button for the cell being set up; the Velocity Detail window for that cell appears.

Interference settings appear in the right half of the window.



7 Check/Adjust the Pixels Per Fringe Setting

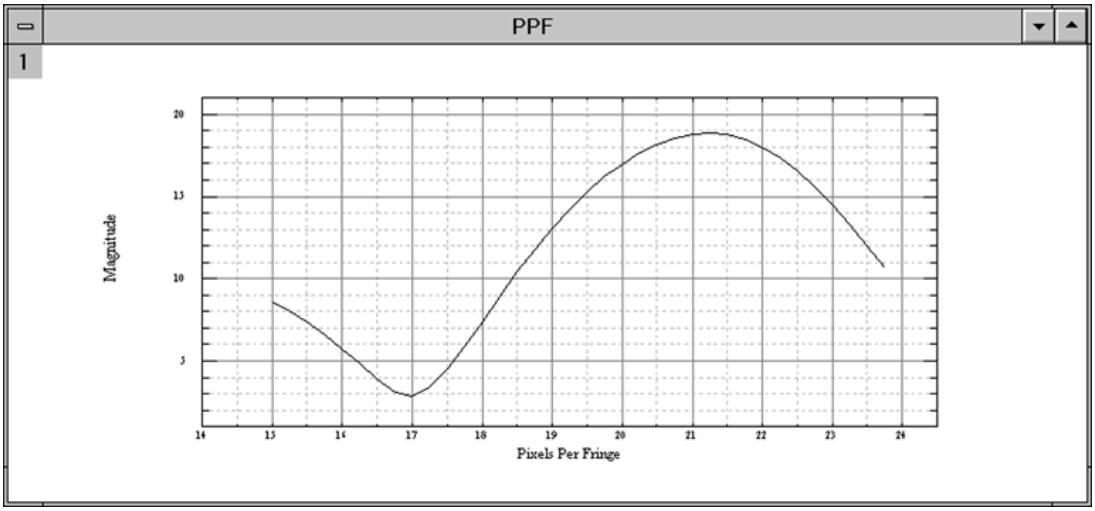
Check the pixels per fringe setting.

a. Click once on the fringe display anywhere to the right of the meniscus.

b. Click the **Automatic** button.

c. Repeat steps 7a and 7b at several radial locations to determine an average pixels per fringe for the whole image.

The system takes a scan and displays a plot of Pixels per Fringe vs. Magnitude. The pixels per fringe setting is optimum when the plot line is a smooth curve, as shown in the example below. The top peak of the curve indicates the maximum magnitude, which is the optimum pixels per fringe setting (approximately 21 in the example).

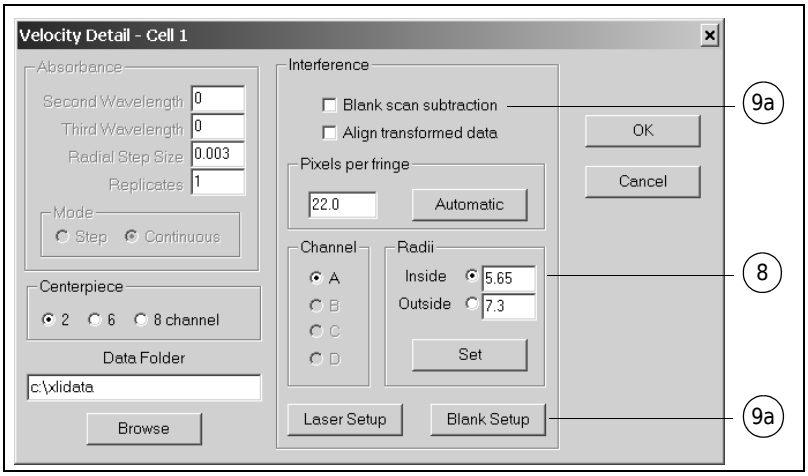


To estimate the pixels per fringe value, count the fringes visible in the fringe display from top to bottom. (One light band plus one dark band constitutes one fringe.) Then divide that number of fringes into 96 (the number of pixels in each column of the fringe display). For example, if there are five fringes, dividing 96 pixels by five fringes gives you a pixels per fringe value of 19.2. This value will vary slightly because the display may contain partial fringes. Extreme difference in the automatically calculated pixels-per-fringe value and the number of visible fringes divided by 96 may indicate that the system is not properly aligned.

8 Set Inside and Outside Radius

Set the inside and outside radius for each cell.

These settings determine the portion of the cell to be included in the scan.



a. In the Radii setup area of the Velocity Detail window, click **Inside**, then click the fringe display once on the left edge of the area to be scanned, typically to the right of the meniscus.

Click the **Set** button.

b. Click **Outside,** then click once near the far right of the fringe display, just to the left view of the bottom of the cell.

Click the **Set** button.

9 Setup Blank Scan Subtraction

The purpose of blank scan subtraction is to reduce the number of extraneous data points in a data file. Subtracting the scan data in one file from the scan data in another file reduces noise in the resulting data by cancelling the effects of anomalies in the XL-I system. For example, if a scratched lens in the optical system (see Figure 2-7) causes an unwanted peak in the sample data, the scratched lens will cause the same peak in the blank scan data. Subtracting the blank scan data from the sample data will cause the peak to disappear. Blank subtraction can also remove the effects of scratches or deformities in the cell windows from the data.

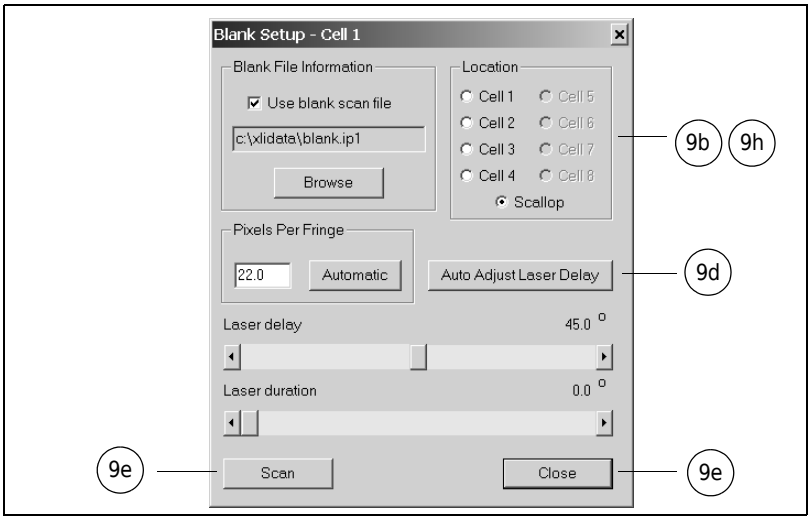
Blank scans are typically taken in an empty cell hole, the scallop (in four-place rotors) or, to reduce the effects of cell window imperfections, can be taken of a regular analytical cell filled with buffer or buffer/sample.

NOTE To obtain optimum blank subtraction correction for a cell (for example, cell 1), use the same laser settings (duration, brightness, and contrast) in both the Laser Setup window and in the Blank setup window for cell 1. Open the Laser Setup window by selecting **Interference** > **Laser Setup**. Open the Blank Setup window by selecting **File** > **New File**, clicking the interference box for cell 1, clicking the **Detail** button for cell 1, and then clicking the **Blank Setup** button.

To set up and use a new blank scan during a run,

a. In the Velocity Detail window, click the **Blank scan subtraction** check box; then click the **Blank Setup** button.

The Blank Setup window for the selected cell appears.



b. In the Location area, choose the cell to be used for the blank scan.

In four-hole rotors, the scalloped edge of the rotor is typically used and is the default location.

For eight-hole rotors, any cell can be chosen; cell 1 is the default location.

c. Click once on the fringe display at the center of the cell (approximately 6.5 cm).

d. When some image is visible in the fringe display, click the **Auto Adjust Laser Delay** button.

e. Click the **Scan** button.

The system performs a scan and saves it to the file path shown under Blank File Information. You can save the blank scan to any location by changing the path in this field. (The default location is the same drive and directory in which the user interface software is located.)

Click the **Close** button when the scan is done.

Up to ten blank scans can be saved per cell. They must follow the naming structure "blank.ipx," where x equals 0 through 9, so that the system will recognize them as blank scans.

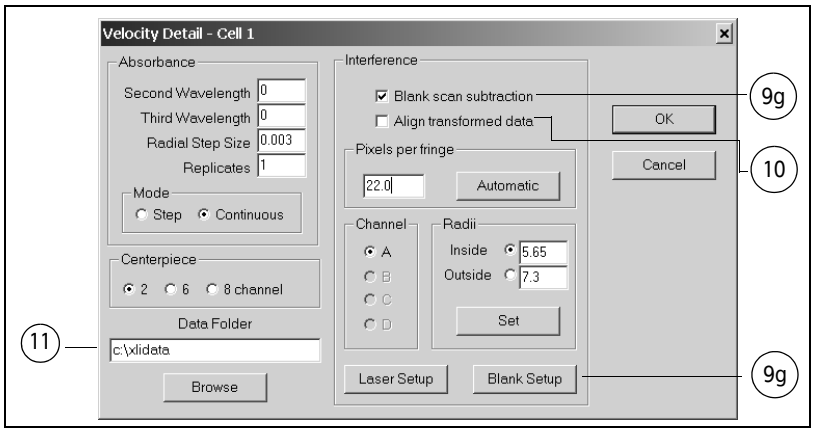
You do not have to save the blank scan now. You can skip step e and go onto step f. When you start the scan in step f, the blank you've set up will automatically be subtracted.

f. On the scan settings file window, click the **Start Single Scan** button.

When the scan is done, the plot will show the results of the blank scan subtraction.

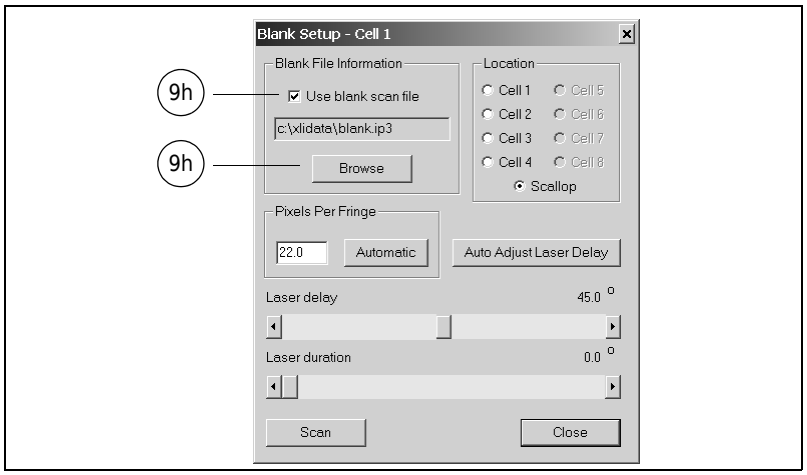
To use a previously saved blank scan,

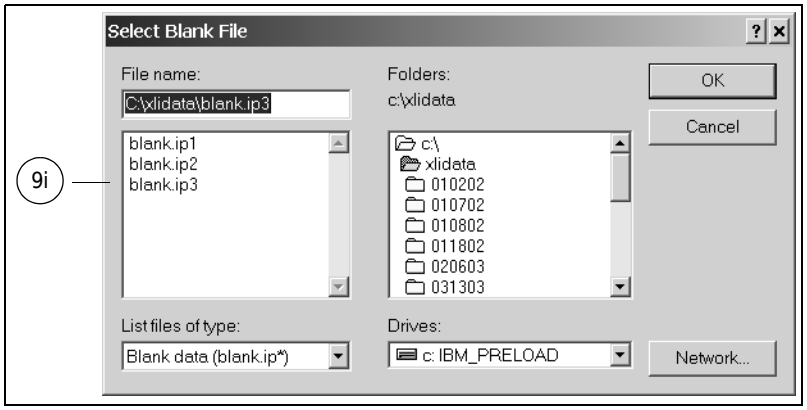
g. In the Velocity Detail window, click the **Blank scan subtraction** box; then click the **Blank Setup** button.



h. In the Blank Setup window, check the **Use blank scan file** box and click the **Browse** button.

The Select Blank File dialog box appears, where you can search for a previously saved blank file to use.





i. Select the blank file to use from the list of files. Navigate to the file if necessary.

Click **OK**. The selected file appears in the Blank File Information field on the Blank Setup window.

j. When you start the scan, the system opens the specified blank scan and automatically subtracts it.

When the scan is done, the plot will show the results of the blank scan subtraction.

10 Align the Transformed Data

If you want the system to align the scan data as it performs the fast Fourier transformation, to ensure that the data plot has a flat baseline, click the **Align transformed data** box.

11 Specify a Data Folder

If desired, change the name of the folder where the data will be stored on your hard disk.

The system defaults to the following path: c:\xliwin\xlidata\today's date\time of scan.

If you enter a new folder name, the files will still be stored in subdirectories named with the date and time.

No overwriting of files can occur unless the computer clock is reset.

12 Click the OK button to close the Velocity Detail window.

13 Start the Scan

At this point, you can either set up and run a single scan or a method from the scan settings window.

a. To do a single scan at a speed higher than 3000 rpm, change the speed in the XL Settings area.

- Click the **Start Single Scan** button.

b. To set up a method, click the **Method** button.

- Enter settings and then click the **Start Method Scan** button.

14 Data Analysis

When all scans are complete, open the XL-A/XL-I Data Analysis Software and import your velocity data files for viewing and calculation of *s*, D, and g(*s**).

See the *Optima XL-A/XL-I Data Analysis Software User's Manual* (publication LXL/A-TB-009) for information on data analysis. A pdf file of this manual is on the XL-A/XL-I Windows setup CD.

Setting Up Sedimentation Velocity Methods

Sedimentation velocity methods can contain up to 999 scans. You can specify a time to wait before beginning the scans, a time between scans, and the total number of scans. The speed, time, and temperature settings used for velocity methods appear in the XL Settings area at the top of the scan settings window.

---

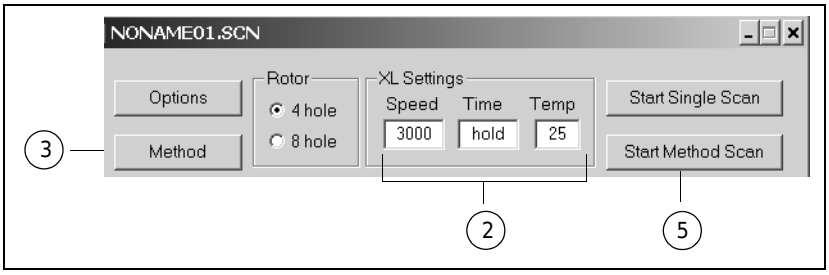
1 Choose **File > New File.**

Or click the new file icon in the toolbar (the far left icon).

---

2 Enter the instrument speed, time, and temperature settings in the XL Settings area.

Use default settings if desired.

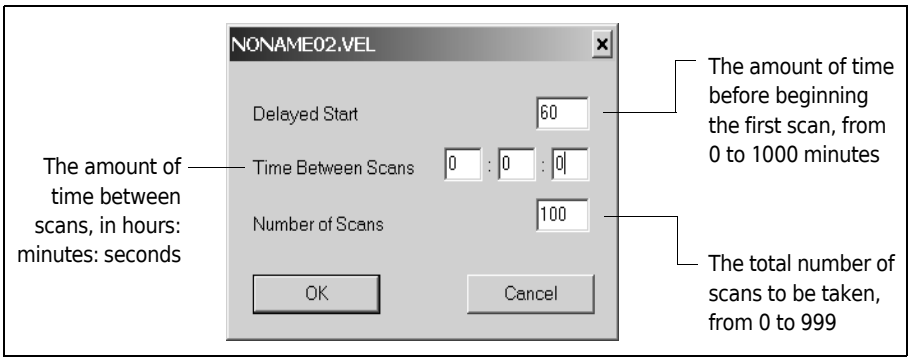


---

3 With the **Velocity** button selected, click the **Method** button.

A method window, called NONAMExx.VEL, appears.

(Method windows have the same name as the scan settings file they are associated with.)



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4 Enter settings as required. Defaults are shown above. Click **OK** when done.

5 To run the method, click the **Start Method Scan** button on the scan settings window.

NOTE To ensure consistent time delays between scans, do not use your computer for other tasks while a method is running. This is especially important with velocity scans, in which the scans must be taken at precisely timed intervals.

Sedimentation Equilibrium Scans

Description

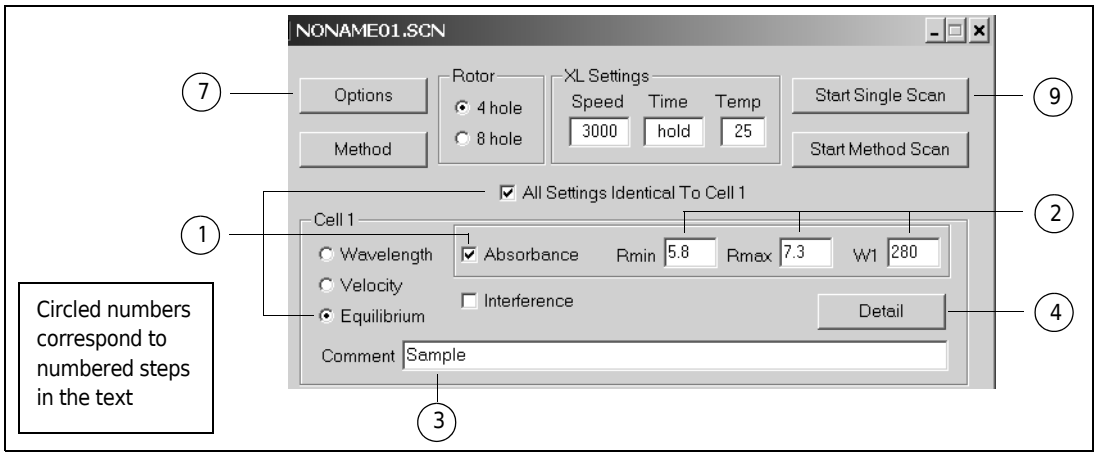
In sedimentation equilibrium experiments, a condition of equilibrium is allowed to develop in the cell. As a solute begins to sediment toward the cell bottom and the concentration at the bottom increases, the process of diffusion opposes the process of sedimentation. After a period of time, the two opposing processes approach equilibrium, and the concentration of the solute increases exponentially toward the cell bottom. At equilibrium, the resulting solute distribution does not change with time. Measurement of the concentration at different points across the cell leads to the determination of the molecular weight of the sedimenting solute. Sedimentation equilibrium is the preferred method for accurate molecular weight determinations and for studies of homogeneity and molecular associations.*

Setting Up Sedimentation Equilibrium Scans: Absorbance system

1 Choose **File > New File**.

In the scan settings window, click the **Absorbance** box and the **Equilibrium** button for the appropriate cell(s).

The Rmin, Rmax, and W1 input boxes become active.



* Ralston, G., *Introduction to Analytical Ultracentrifugation,* Beckman Instruments, Inc., 1993, and Furst, A., *Overview of Sedimentation Velocity for the Optima XL-A Analytical Ultracentrifuge*, Beckman Instruments, Inc., 1991.

If you want to use the same scan settings for each cell, enter settings for Cell 1 and then click the **All Settings Identical to Cell 1** box.

2 Enter the Rmin (minimum radius), Rmax (maximum radius) and W1 (wavelength) values in the appropriate boxes.

Or, make no changes and accept the defaults.

3 Enter a comment in the Comment field (optional).

Comments are saved to the file header data.

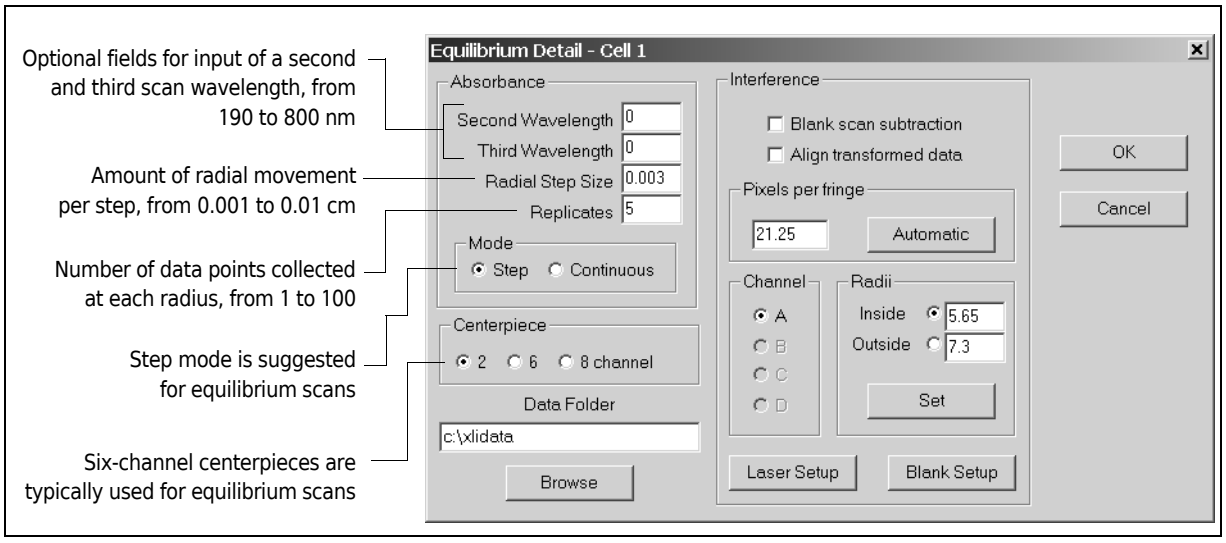
You could start a scan now, using these basic settings.

Additional scan settings are available in the Equilibrium Detail dialog box, shown in step 4.

4 Click the **Detail** button.

The Equilibrium Detail window appears.

Settings specific to the absorbance system are in the left half of the window.



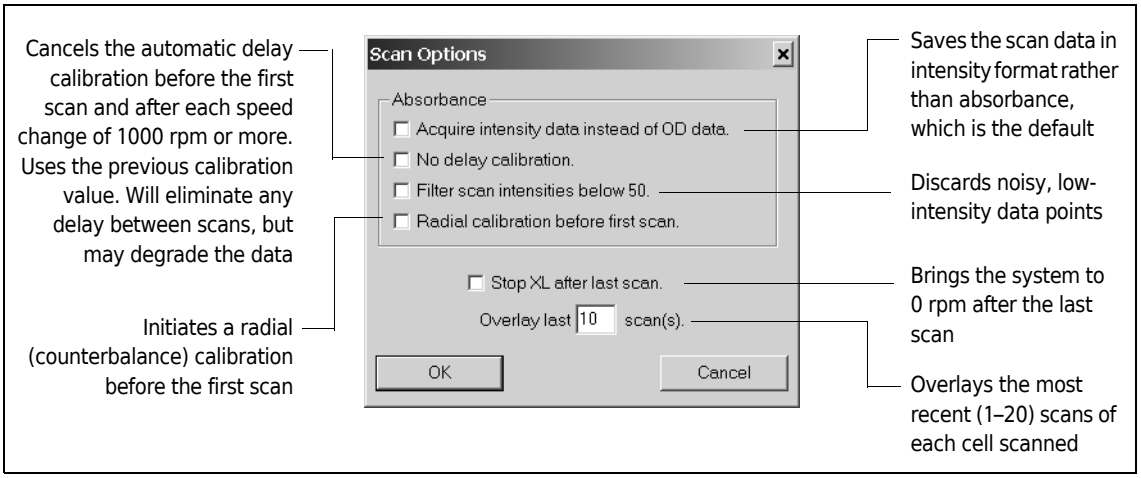
5 Enter a setting or accept the default setting in each field.

6 Click **OK** to accept changes or **Cancel** to cancel the changes and use all default values.

The window closes.

7 Click the **Options** button on the scan settings file window.

The Scan Options dialog box appears.



8 Select an option to initiate it.

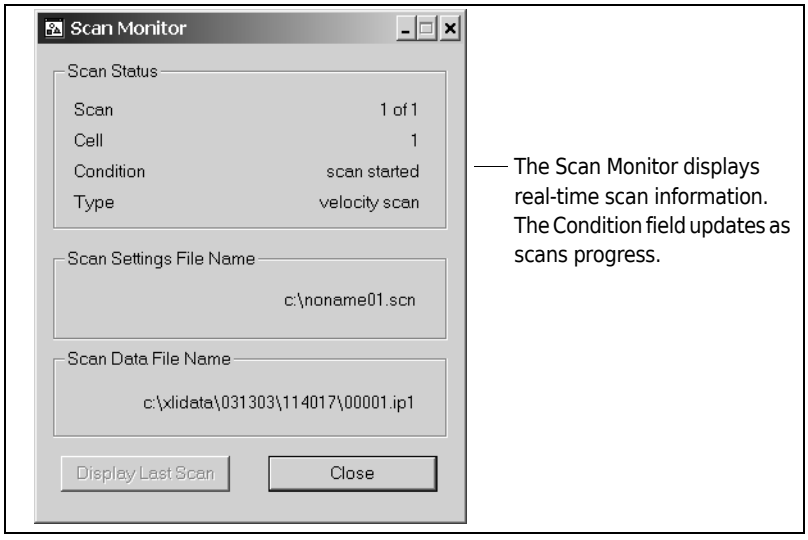
We recommend selecting **Radial calibration before first scan** before every absorbance scan or method.

NOTE Radial calibration should be performed at a low speed (approximately 3000 rpm). Remember to deselect the radial calibration option following the first run.

9 Click **Start Single Scan**.

The Scan Monitor window opens and displays the status of the instrument and scan.

The XL Monitor window also opens.



10 When scan is complete, a plot of the scan appears automatically.

If leakage has occurred, indicated by movement of the meniscus, reload the cell(s) and repeat the scan.

Check and adjust settings as needed.

Speed and Time Settings for Equilibrium Scans

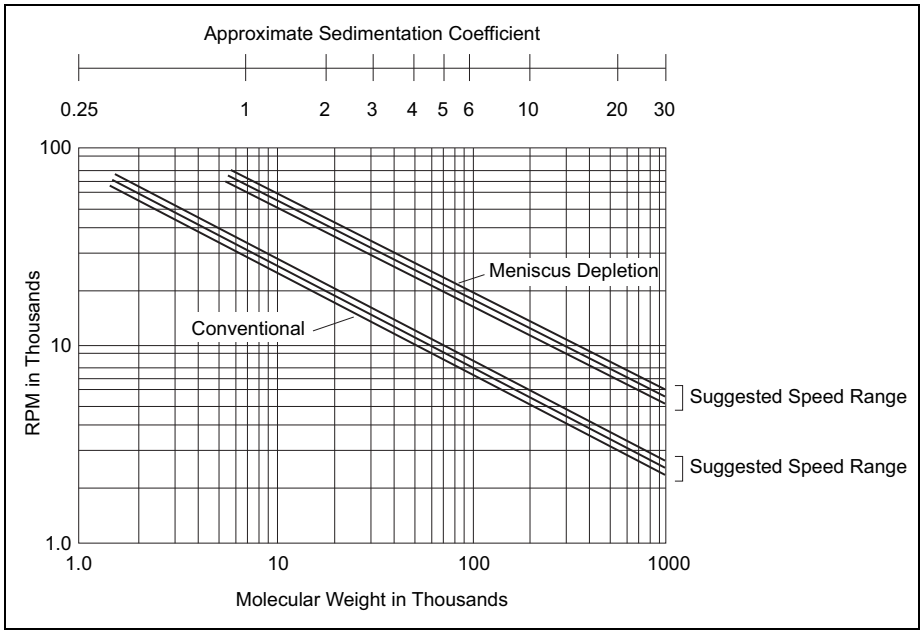
Figure 4.3 shows suggested speed ranges for use when the molecular weight and approximate sedimentation coefficient are known.

The estimated time required to reach equilibrium in seconds is approximately

$$\frac{(\text{solution column height})^2}{D}$$

The solution column height is in cm, and D is in cm^2/s. D can be *estimated* by $3 \times 10^{-5}/M^{1/3}$, where M = the sequence molecular weight.

Figure 4.3 Rotor Speed Selection Chart for Sedimentation Equilibrium Runs



Setting Up Sedimentation Equilibrium Methods

You can include up to 9900 scans in equilibrium and wavelength methods. This section discusses equilibrium methods; however, follow the same steps when setting up wavelength methods.

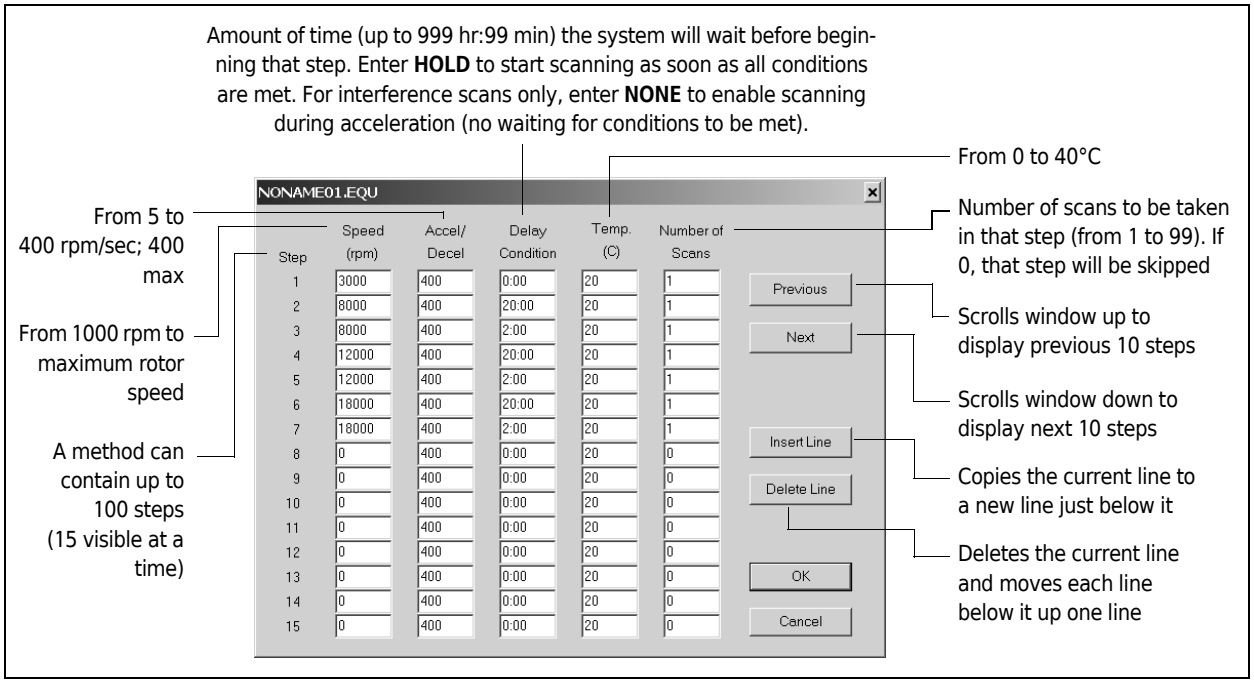
NOTE Although the maximum number of scans in a method is 9900, this number is actually limited by the size of your hard disk. When you start a method, the software estimates the amount of hard disk space the method data will require and compares that estimate with the space available. If there is not enough disk space, a message will alert you and you can either modify the method or proceed at your own risk. The safest action is to modify the method, or to free up space on your hard disk by deleting unnecessary items, or both.

---

1 In the scan settings window, click the **Equilibrium** button, then click the **Method** button.

The method window, called NoName.EQU, appears.

For each step, when speed and temperature conditions are reached, the scan(s) in that step will be initiated. When the scans specified in that step are done, the system will initiate the conditions for the next step (speed, temperature, etc.). When those conditions are reached, the scans specified in the next step will start. At least one scan must be requested in a step, or the step will be skipped.



---

2 Enter the setttings for each step.

---

3 Click **OK** when the setup is complete.

The method window will close.

The method will be saved as part of the open scan settings file when that file is saved.

4 To run the method, click the **Start Method Scan** button.

The method will end when it reaches a step in which the speed setting is 0 rpm and the delay condition is 0:00.

Using Previously Saved Scan Settings

To open and run a previously saved scan settings file, follow the steps below.

NOTE *Interference files only*. Previously saved laser settings and calibration settings should be used only if you are using the same rotor and overspeed disk. If you are using a different rotor, or if you have replaced your rotor's overspeed disk, readjust all laser settings before running saved scan settings.

1 Open the XL-A/XL-I user interface software.

2 Choose **Start Single Scan** or **Start Method Scan** from the Scan Menu.

Or, click the appropriate toolbar icon.

The Open dialog box appears. The currently selected directory will be highlighted. It may be necessary to change directories to find previously saved .scn files.

3 When you find the name of the required .scn file, double-click it.

It will appear in the File Name field.

4 Click **OK**.

The scan or scans start immediately.

The Scan Monitor and the XL Monitor windows open.

5 To stop the scan, choose **Stop Scan** from the Scan menu or click the stop scan toolbar icon (fourth icon from the right).

NOTE After you select **Stop Scan**, there may be a delay before the scan stops, depending on the status of the hardware. If you try to do anything else before the scan has stopped, the Scan in Progress dialog box appears asking if you want to stop the scan. Click **Yes** to reaffirm the **Stop Scan** command, and wait until the scan has stopped.

Ending a Run

Select **File > Stop XL**, or press **STOP** on the instrument control panel, to end a run in the HOLD mode. Timed runs will end automatically when the set time has elapsed. After the run has stopped, press **VACUUM** on the instrument control panel to vent the chamber.

Removing the Monochromator

When the chamber is vented, open the chamber door and remove the monochromator as follows.

1 Unscrew the red holding ring to release the monochromator from the receptacle.

2 Carefully lift the monochromator out of the receptacle.

⚠ CAUTION

Do not twist, bend, or drop the monochromator.

Always store the monochromator in the case provided to protect it when not in use. The monochromator should always be protected to prevent foreign matter from accumulating on the optical components.

Removing the Rotor and Cells

Lift the rotor straight off of the drive spindle. Refer to the applicable analytical rotor instruction manual for instructions on disassembly and care of the cells.

CHAPTER 5

Troubleshooting and Maintenance

Introduction

This section contains information on troubleshooting system malfunctions. It also contains care and maintenance procedures that should be performed routinely. Troubleshooting and maintenance not covered in this manual should be handled by Beckman Coulter Field Service. Refer to the analytical rotor manual (LXL/A-TB-003) and Chemical Resistances *(publication IN-175) for instructions on the care of rotors and their accessories.*

NOTE It is your responsibility to decontaminate the instrument, as well as any rotors and/or accessories, before requesting service by Beckman Coulter Field Service.

Retrieving Your Sample In Case of Power Failure

If a power failure occurs during a run, the rotor begins to decelerate with the brake off. If the rotor is still spinning above 1000 rpm when power is restored, the system will resume operation and the rotor will return to set speed. A diagnostic message will alert you that a power outage occurred.

If the rotor is spinning below 1000 rpm when power is restored and the instrument is in the TIME mode, the system will *automatically reset the TIME to the original set time and restart the run.* In the HOLD mode, it will return to set speed and elapsed time will begin to accumulate *from zero.* A diagnostic message will alert you that a power outage occurred and that the run was restarted automatically.

If a power failure lasts for several hours, it may be necessary to retrieve the sample from the rotor. (A rotor decelerating without the brake may take hours to come to a complete stop.) To gain access to the rotor, it will be necessary to remove the control head cover and front panel to disengage the door lock.

⚠ WARNING

Any maintenance procedure requiring removal of a panel exposes the operator to the possibility of electrical shock and/or physical injury. Therefore, turn the power OFF and disconnect the instrument from the main power source by removing the Mains (power) plug from the outlet receptacle, and refer such maintenance to qualified ervice personnel.

⚠ WARNING

NEVER attempt to slow or stop the rotor by hand.

⚠ WARNING

The following procedure should be performed only when absolutely necessary and only by qualified service personnel.

---

1 If equipped with a printer, tear off any paper extending from the printer before trying to slide the control head cover off the instrument.

---

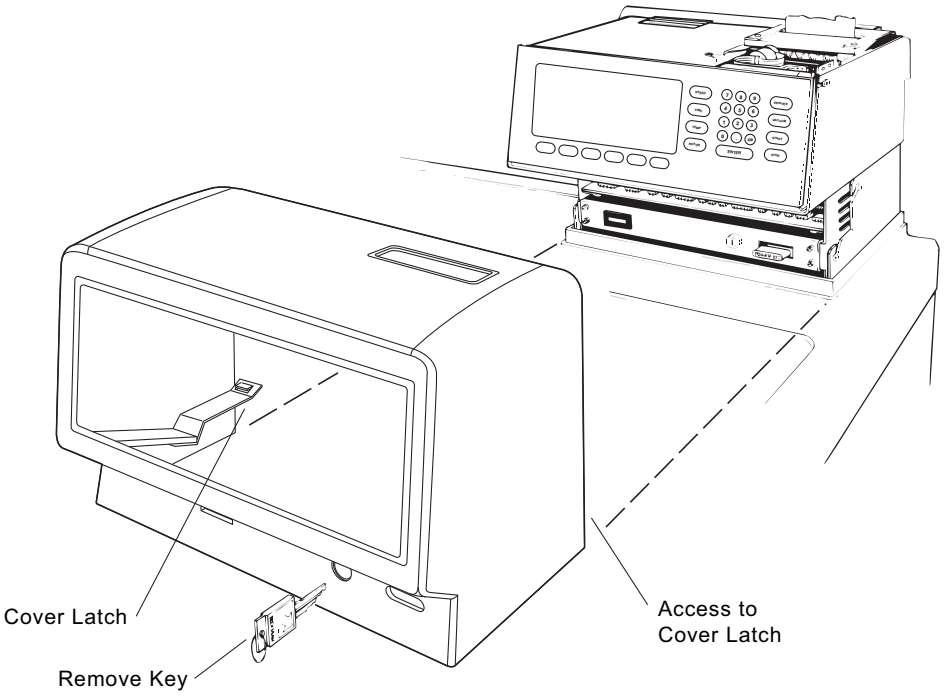
2 Locate the two metal latches (see Figure 5.1) at the lower outside corners on the back of the control head.

Use your fingers to depress the latches to disengage the cover from the control head.

With both hands, lift it slightly and pull it toward you, until it is free.

Set the cover aside in a safe place.

Figure 5.1 Removing the Control Head Cover



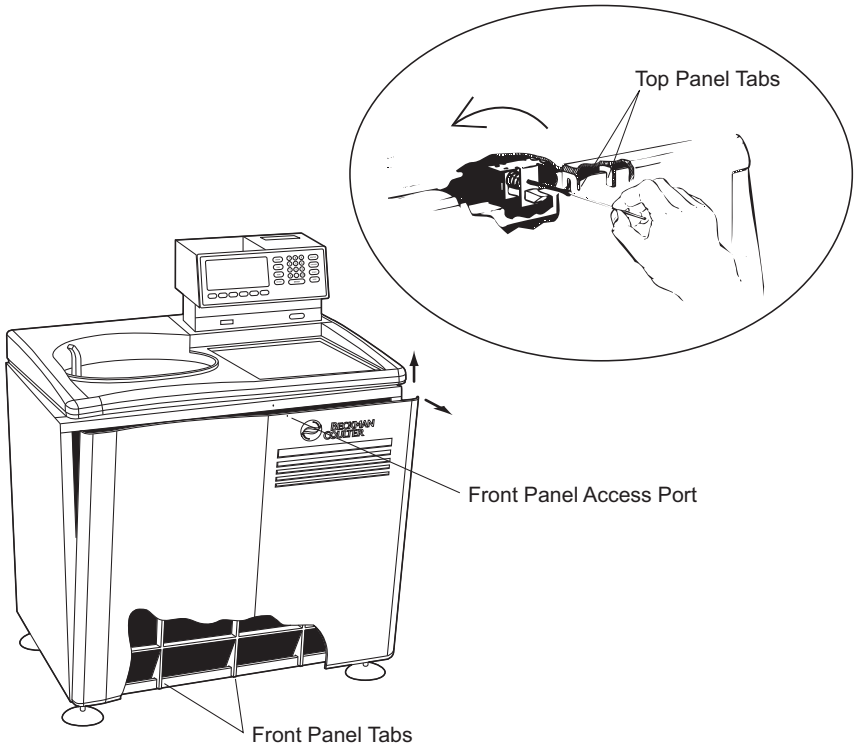
3 To open the top cover, insert a #2 (0.25-inch diameter) Phillips-head screwdriver into the hole located in the front, center of the top cover (see Figure 5.2).

Turn the screwdriver counterclockwise (to the left) until the screw bottoms out.

Then, to release the latch, push the screwdriver inward.

Once the latch is released, lift the top cover.

Figure 5.2 Depressing the Latch to Loosen the Front Panel



4 Now lift the front panel to free the bottom tabs that secure it in place.

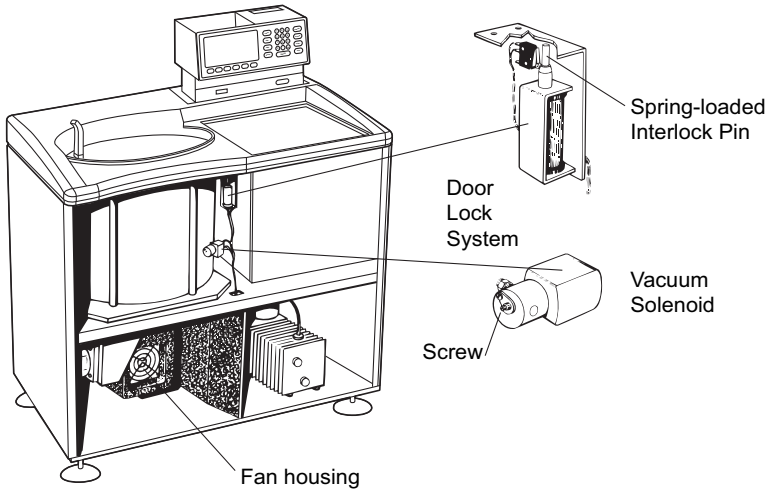
⚠ WARNING

After removing the panel, listen carefully for any sounds coming from the drive. Then touch the fan housing (see Figure 5-3) to feel if it is vibrating and listen again. Do not proceed if any sound or vibration is emitted from the housing.

5 Vent the vacuum chamber by slowly turning the small setscrew on the vacuum solenoid valve to the right (clockwise), as shown in Figure 5.3, until you hear the air released into the chamber.

If you hear a whining noise, close the valve and wait, because the rotor is still spinning.

Figure 5.3 Interior View of Instrument (Panel Removed)



6 About 10 seconds after the hissing noise stops, turn the setscrew to the left (counterclockwise) until it stops.

7 Push down on the interlock pin with your finger (Figure 5.3) while opening the chamber door with your free hand. (If the rotor is still spinning, close the door and wait).

Be very careful not to touch any wires or circuitry

⚠ WARNING

Do not attempt to run the instrument before returning the front panel to its correct position.

8 After retrieving the sample, close the chamber door, then replace the front panel on the instrument.

Insert the tabs at the bottom of the panel onto the lip at the base of the instrument, being sure to align the front panel with the side panels (see Figure 5.4).

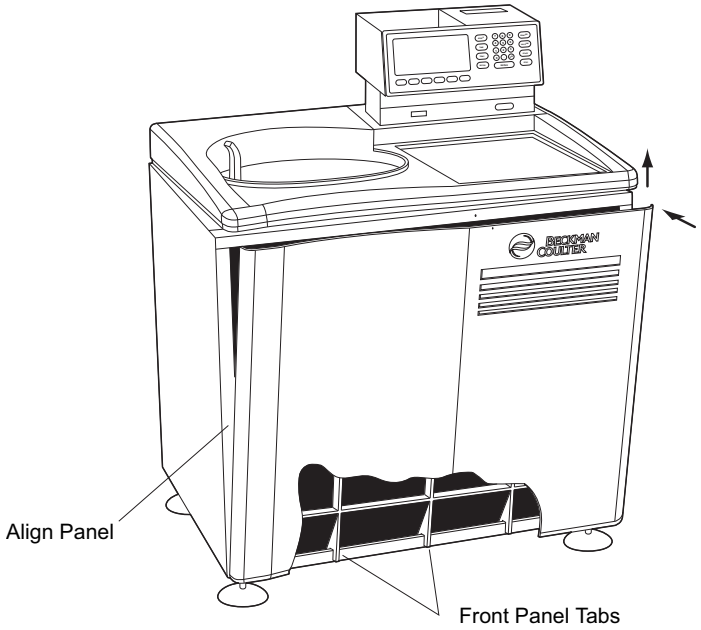
9 Lift the front edge of the top panel a few inches and insert the upper edge of the front panel under it.

Push back gently to engage the tabs, then down to activate the latch.

10 Replace the control head by sliding it along the small ridges provided, making sure to clear the printer (if equipped) on top and the emergency power switch in front.

When the control head cover is correctly positioned, push it until the latches snap shut and the cover is aligned with the rest of the instrument panels.

Figure 5.4 Reinstalling the Front Panel



Error and Diagnostic Messages

Analytical Messages

If the XL-A/XL-I user interface software detects a problem during operation, a dialog box on the PC screen reports diagnostic conditions specific to the analytical hardware, software, and communications between the analytical software and the XL-A or XL-I instrument. Table 5.1 lists each diagnostic message and suggested steps to take to resolve the problem. If the message persists, or there are no suggested actions, call Beckman Coulter Field Service for assistance.

Table 5.1 Diagnostic Messages Appearing on the PC Screen

Error Message	Definition	Operator Action
Wavelength out of range	The wavelength requested is outside the mechanical limit	Call Beckman Coulter Field Service.
Radius out of range	The radius requested is outside the mechanical limit	Call Beckman Coulter Field Service.
Incident ranging failure	The system detected problems with the incident data	Call Beckman Coulter Field Service.
Baseline ranging failure	The system detected problems with the baseline data	Call Beckman Coulter Field Service.
Radial rate failure	Radial detector problem or malfunction	Call Beckman Coulter Field Service.
Wavelength rate failure	Monochromator problem or malfunction	Call Beckman Coulter Field Service.

Table 5.1 Diagnostic Messages Appearing on the PC Screen *(Continued)*

Error Message	Definition	Operator Action
Delay calibration failure	A component or calibration parameter is invalid	Recalibrate the radius by selecting Radial calibration before first scan in the Scan Options dialog box, and then performing one scan. (See Section 4 for scan setup instructions.) If problem persists, call Beckman Coulter Field Service.
Radial calibration failure	Radial calibration failed	Ensure that the correct counterbalance is in cell 4 (An-60 Ti four-hole rotor) or cell 8 (An-50 Ti eight-hole rotor) and properly aligned (refer to the rotor manual), then retry. If problem persists, call Beckman Coulter Field Service.
Radial servo timeout	The radial detector could not reach the requested location	If this diagnostic occurs repeatedly, call Beckman Coulter Field Service.
RAM checksum failure	The XL-A or XL-I RAM failed its integrity test	Call Beckman Coulter Field Service.
PC->DAB checksum error	The XL-A or XL-I DAB (data acquisition board) received a corrupt message from the PC	If any of these errors occur frequently, call Beckman Coulter Field Service. Infrequent occurrences can be ignored.
Illegal PC->DAB command	The XL-A or XL-I DAB (data acquisition board) received an illegal message from the PC	
Input failure or checksum error	The XL-A or XL-I received an invalid input from the PC	
DAB->XL com failure	The XL-A/XL-I user interface software has repeatedly failed to transmit a message to the instrument	Call Beckman Coulter Field Service.
XL com failure	The XL-A/XL-I user interface software did not received a response to its last message from the instrument	
DAB->XL parameter out of range	The XL-A or XL-I hardware sent an out-of-range parameter to the instrument	Call Beckman Coulter Field Service.
DAB->XL checksum error	The XL-A or XL-I hardware sent a corrupt message to the instrument	Call Beckman Coulter Field Service.
Unsupported baud rate	The PC does not support 19200K baud	Call Beckman Coulter Field Service.
Invalid byte size	The PC does not support 8-bit byte size	
Invalid default parameters	The PC does not support the XL-A or XL-I default communication parameters	
Port is locked by another device	The PC com port is being used by another device	
Queue memory allocation failure	Memory problem	Restart Windows. If this doesn't fix the problem, call Beckman Coulter Field Service.

Table 5.1 Diagnostic Messages Appearing on the PC Screen *(Continued)*

Error Message	Definition	Operator Action
Unrecognized PC message	The instrument received a corrupt message from the PC	Check the cable connecting the PC and the instrument for loose connections or damage. Call Beckman Coulter Field Service if either of these messages recurs.
Invalid communications request	The PC created a corrupt message	
Queue index problem	One of the input/output queues on the PC is corrupt	Restart Windows. If this doesn't fix the problem, call Beckman Coulter Field Service.
Wavelength servo timeout	The diffraction grating could not reach the requested wavelength	If this diagnostic occurs repeatedly, call Beckman Coulter Field Service.
ETX not found	Communication problem (message not terminated correctly)	Restart the instrument and restart Windows. If this doesn't fix the problem, call Beckman Coulter Field Service.
Memory allocation failure	The PC could not allocate enough memory for input/output to/from the instrument	Restart Windows. If this doesn't fix the problem, call Beckman Coulter Field Service.
Device is not open	The PC could not open the com port	These com port diagnostics can usually be cleared by restarting Windows. If restarting Windows does not fix the problem, call Beckman Coulter Field Service.
Device is already open	The PC attempted to open a com port which was already open	
Could not configure com port	The PC could not configure the com port	
Com status erro	The PC received a status error from the com port	
No system timer for com	The PC could not create a timer for the receiver	
Could not close com port	The PC could not close the com port when exiting the XL-A/XL-I user interface software	
Could not write to com port	The PC could not write to the com port	
Read com port error	The PC could not read the com port	
A COM error appears on your IBM PC screen after several hours of unattended operation. Any scan or method in progress stops.	The IBM PC goes into a reduced power mode after a period of time with no interaction via the mouse or keyboard. This mode can interfere with com port function, which affects communication with the XL-A or XL-I. Screen savers may also interfere with com port function. *This situation is specific to IBM PCs*.	**1.** Open the autoexec.bat file and look for the POWER.EXE statement. (This statement may be in the config.sys file, if it is not in autoexec.bat). Type REM (for remove) just before POWER.EXE to disable the reduced power mode function (the line should read REM POWER.EXE). Save the file and then reboot the PC. **2.** Turn off any screen saver. Turn off the monitor power switch during extended periods.

Log Files

Diagnostic messages that occur during a run are saved to a log file, which is saved to the same directory as the data files for the current runs. The system creates separate log files for interference data and absorbance data, and separate log files for each cell used in a run. The log file names and descriptions are below.

- *for interference scans*: log file names are cell**n**chan**n**.log, where cell **n** is the cell number (1 through 8) and chan **n** is the centerpiece channel number (a through c).
- *for absorbance scans:* log file names are cell**n**abs.log, where cell n is the cell number (1 through 8).

Log files can be opened by selecting **Options > Notepad**, and then selecting a log file to open.

Control Panel Diagnostics

If the system detects a problem during operation, a diagnostic message will be displayed in reverse video below the CURRENT VALUES window on the instrument control panel. A tone also sounds to alert you to the condition. If more than one problem exists, the most critical one will be identified on the screen. Press **CE** to clear the diagnostic message after correcting the problem. (The message will reappear if the problem still exists.)

Sometimes the message provides cautionary information and the run continues uninterrupted. For example, if the SPEED setting is higher than the speed permitted by the rotor overspeed disk, the speed will be lowered to the maximum speed permitted and a diagnostic message will indicate that this change has been made.

If a serious problem arises, the instrument will shut down with or without brake (or without temperature control), depending on the specific problem. If necessary, the instrument will automatically trip the power. You will be prompted to take corrective action or, if there is no operator solution, to call Beckman Coulter Field Service.

Each diagnostic category has a range of numbers (and letters if necessary) to assist Field Service in determining the nature of the problem. For example, speed-related problems use a diagnostic range beginning with 3 (see Table 5.2). *Be sure to indicate the diagnostic number on the screen* when you contact Beckman Coulter Field Service for assistance.

Table 5.2 lists the diagnostic category, possible cause of the condition, and appropriate user action. If there is no user action or you cannot correct the problem, call Beckman Coulter Field Service.

Table 5.2 Diagnostic Troubleshooting Chart

Diagnostic Category/ Range	Possible Cause	User Action
SPEED 3x	Speed has been set above maximum allowed; or wrong, damaged, or missing overspeed disk Tachometer failure	Check set speed; check for clean, undamaged, and correct overspeed disk (see the analytical rotor manual for replacement instructions). Call Beckman Coulter and wait 4 hours before attempting to open the door if 0 rpm appears immediately after failure.
TEMPERATURE 5x	Temperature control or vacuum system malfunctioning	Check the air inlet (at bottom of the front panel) for obstructions. Call Beckman Coulter Field Service.
DRIVE 6x	Abnormal change in drive speed or overheated drive Erroneous display of 0 RPM immediately after failure, rotor still spinning.	Be sure a rotor is properly installed on the spindle; if power has failed, wait for 5 minutes for drive to cool; check for air inlet obstruction. Call Beckman Coulter Field Service. Wait 4 hours before attempting to open the door.
DRIVE 67	Tachometer failure	Call Beckman Coulter Field Service. Do not attempt to start another run until the instrument is serviced.
VACUUM 4x	Vacuum system failure; or moisture and/or debris may be preventing door from achieving a vacuum seal	Wipe the chamber with a cloth to remove moisture; check rotor cells for possible leakage. Turn the vacuum on for a few hours or overnight. Make sure that door O-ring is clean and in good condition; wipe it with a clean, lint-free cloth or tissue, and coat it with a light film of silicone vacuum grease (335148).
IMBALANCE 7x	Rotor imbalance (at low speeds)	Check for proper rotor loading.
DOOR 8x	Door is open when the **START** key is pressed	Be sure door is closed.
POWER 2x	Loss of power during run	Check TIME display; run may need to be restarted or aborted.
CPU 1x or 9x	Microprocessor malfunction or loss of program memory	No operator action. Call Beckman Coulter Field Service.

In Case Microsoft Windows Locks Up

If the power to the instrument is not turned on, and you start a scan and then exit the XL-A/XL-I user interface software, Microsoft Windows may lock up temporarily. To resolve this condition, press the CTRL-ALT-DEL keys simultaneously and then press the RETURN key. This will close the software and return you to the Windows desktop.

Maintenance

The following procedures should be performed regularly to ensure continued performance and long service life of the instrument.

1 Regularly inspect the rotor chamber for accumulations of sample, water, dirt, or debris.

Clean as required (see *Cleaning*, below).

2 If a cell leaks during a run, wipe the rotor chamber, including the rotor overspeed detector window (the red lamp next to the drive spindle) and the monochromator with a lint-free cloth or tissue.

Do not attempt to clean the UV light detector slit.

If spillage has affected the detector, call Beckman Coulter Field Service.

3 Store the monochromator in the centrifuge chamber with the door closed, or in the wooden storage box provided.

It is important to keep the monochromator clean and dry.

4 Regularly check the air vents for obstructions.

The air intake vent is on the bottom of the instrument, just behind the bottom edge of the front panel.

The air outflow vents are on the back of the instrument.

Keep all vents clean and clear.

Cleaning

Frequent cleaning is recommended. Always clean up spills when they occur to prevent corrosives or contaminants from drying on component surfaces.

NOTE Before using any cleaning or decontamination methods except those recommended by the manufacturer, users should check with the manufacturer that the proposed method will not damage the equipment.

1 Clean instrument surfaces using a cloth dampened with a mild detergent solution such as Solution 555 (339555).

2 Keep the rotor chamber clean and dry by frequent wiping with a cloth or paper towel.

For thorough cleaning, wipe the chamber using a mild detergent such as Solution 555

Dilute the detergent with water (10 parts water to 1 part detergent).

Rinse thoroughly and dry completely.

NOTE Rinse thoroughly and dry completely. Keep the chamber door closed between runs to keep the chamber clean and dry. Wipe the chamber walls with a dry cloth before each run.

3 Clean the control panel by gently wiping it with a cloth dampened with Solution 555,.

Dilute the detergent with water (10 parts water to 1 part detergent).

4 To clean the mesh filter, first remove it by gently pulling the top edge of the bezel towards you to free the filter.

Use a soft brush or lint-free cloth to remove lint and dust.

Wipe off the video screen with a damp, lint-free cloth.

Reinstall the mesh filter by seating the bottom edge of the bezel in place, then pressing the top edge towards the screen until it snaps into place.

5 Clean the chamber door O-ring every few months by wiping it with a tissue.

Replace the O-ring whenever it becomes worn or damaged.

Lightly coat the O-ring with silicone vacuum grease before reinstalling it to ensure an optimum vacuum seal.

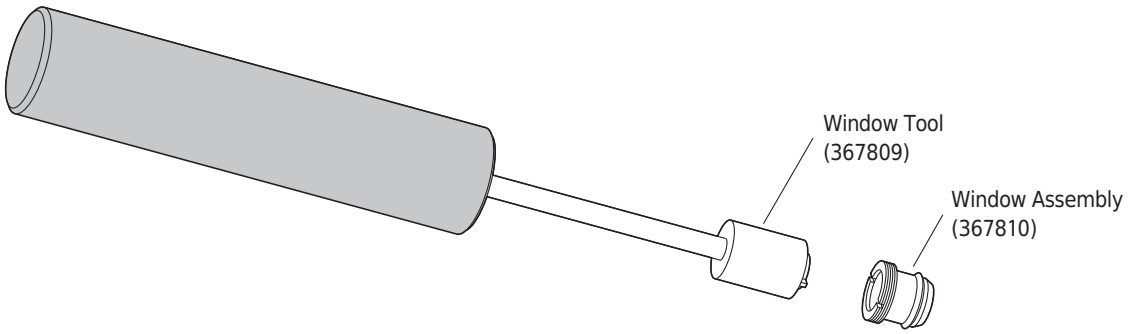
Cleaning the Monochromator Window

A dirty monochromator window affects the quality of scans from the absorbance optical system. The XL-A window kit (368488) contains a lamp assembly (368449) that requires installation by Beckman Coulter Field service. The kit also contains a monochromator window assembly (367810; see Figure 5.5) that can be removed and cleaned by the user, and a tool (367809; see Figure 5.5) for installing and removing the window assembly.

NOTE Kit (368488) is for use only with XL-A and XL-I instruments manufactured after March 1995.

The cleaning procedure below can be performed only after a Beckman Coulter field service representative has installed lamp assembly (368449).

Figure 5.5 Monochromator Window Assembly and Window Installation and Removal Tool



Cleaning Procedure

The window assembly sits just beneath the monochromator in the instrument chamber. To clean the window,

1. Open the chamber door.

   Carefully remove the monochromator by unscrewing the red holding ring and lifting the monochromator out of the chamber.

   Place the monochromator into its protective case.

2. Insert tool (367809) into the window assembly housing.

   Unscrew and remove the window.

3. To clean the window, use a clean, non-abrasive electric pencil eraser on both sides of the window.

   Then rinse with ethanol (90%) on a cotton swab, also on both sides of the window.

   Ethanol (90%) alone can also be used for cleaning if no eraser is available.

⚠ CAUTION

Ethanol is a flammability hazard. Do not use it in or near operating centrifuges.

4. Screw the window assembly housing into the hole in the chamber with your fingers until the threads engage, then insert the window tool and tighten until snug.

5. Reinstall the monochromator following the instructions under *Installing the Monochromator* in CHAPTER 2.

Decontamination

If the instrument, rotor, and/or accessories are contaminated with radioactive or pathogenic solutions, follow appropriate decontamination procedures. Refer to *Chemical Resistances* (publication IN-175) or contact Beckman Coulter Field Service to be sure the decontamination method will not damage any part of the instrument (or accessories).

Sterilization and Disinfection

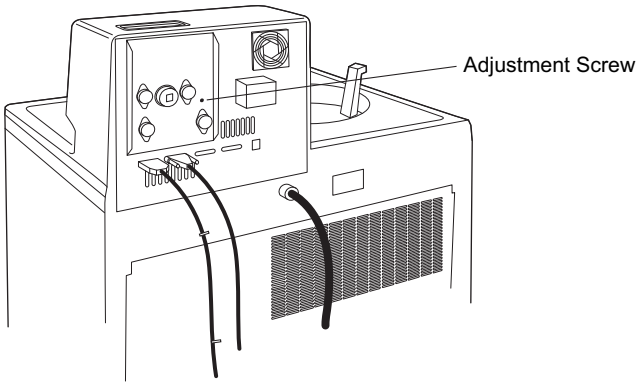
The top surface of the instrument is finished with urethane paint; the sides are finished with acrylic baking enamel. Ethanol (70%) may be used on both these surfaces. See *Chemical Resistances* for more information regarding chemical resistance of instrument and accessory material.

While Beckman Coulter has tested these methods and found that they do not damage the instrument, no guarantee of sterility or disinfection is expressed or implied. When sterilization or disinfection is a concern, consult your laboratory safety officer regarding proper methods to use.

Adjusting the Display Screen Brightness

When your instrument is installed, Beckman Coulter Field Service will check the brightness of the screen and adjust it if necessary. To adjust it at a later time, locate the small screw at the back of the control head (see Figure 5.6). Use a small nonconductive screwdriver to turn the screw to the left or right to adjust the contrast.

Figure 5.6 Location of the Screw that Adjusts Video Brightness

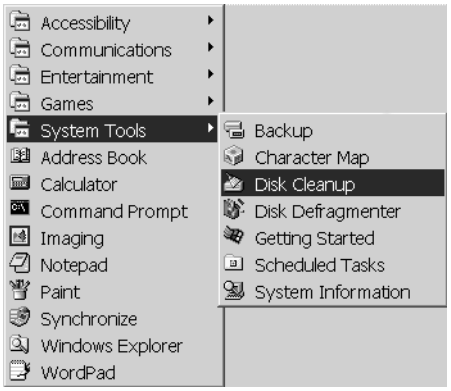


Hard Drive Maintenance

Your PC comes with accessory programs that you can use to keep your hard drive operating efficiently. The Disk Cleanup accessory enables you to delete unnecessary files from your hard drive, and the Disk Defragmenter accessory defragments your hard drive. We recommend that you run both accessory programs often, at least once a month if your system is used frequently.

Running Disk Cleanup

1 Select **Start > Programs > Accessories > System Tools > DiskCleanup.**



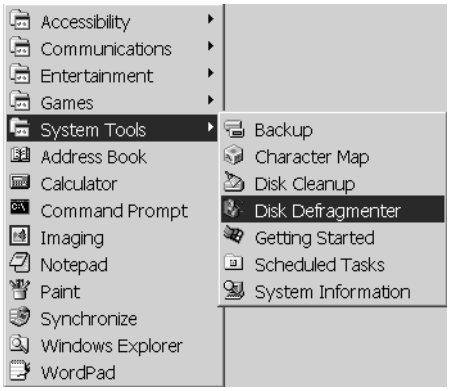
2 Specify that you want the Disk Cleanup to check your hard drive and click the **Start** button.

As the scan progresses, Disk Cleanup will display files for deletion and prompt you to delete the files or choose not to. When the process is complete, Disk Cleanup displays the results.

3 After viewing the results, click the **Close** button to close the dialog box and to exit Disk Cleanup.

Running Disk Defragmenter

1 Select **Start > Programs > Accessories > System Tools > Disk Defragmenter.**



2 Specify that you want to defragment your hard drive and click **OK**.

Disk Defragmenter will check the percentage of drive fragmentation and tell you whether or not the drive needs to be defragmented.

You can then either start defragmentation by clicking the **Start** button, or click **Exit** to exit the program.

Variations Between Results Obtained with Absorbance Optics and Bench-top Spectrophotometers

The XL-A and XL-I absorbance optics may not necessarily give the same numerical value for absorbance of a solution as that from a bench-top spectrophotometer. Either instrument may not give the "true" absorbance of a solution. The number obtained from a spectrophotometer depends on factors such as the emission spectrum of the source, the bandpass of the monochromator, the wavelength response of the detector, the path length, as well as optical and electronic factors and how the instrument is used. Solutes of the macromolecular size commonly studied in the ProteomeLab system tend to scatter light of visible or ultraviolet wavelength to a significant extent. Thus the geometry of the optical arrangement has an effect on the amount of light that reaches the detector.

In spectrophotometry that uses a spinning cell, additional factors enter in. Not only is the light path length through the sample different from the usual cuvettes, but fractions of the sample solution may quickly sediment and be lost to the measurement.

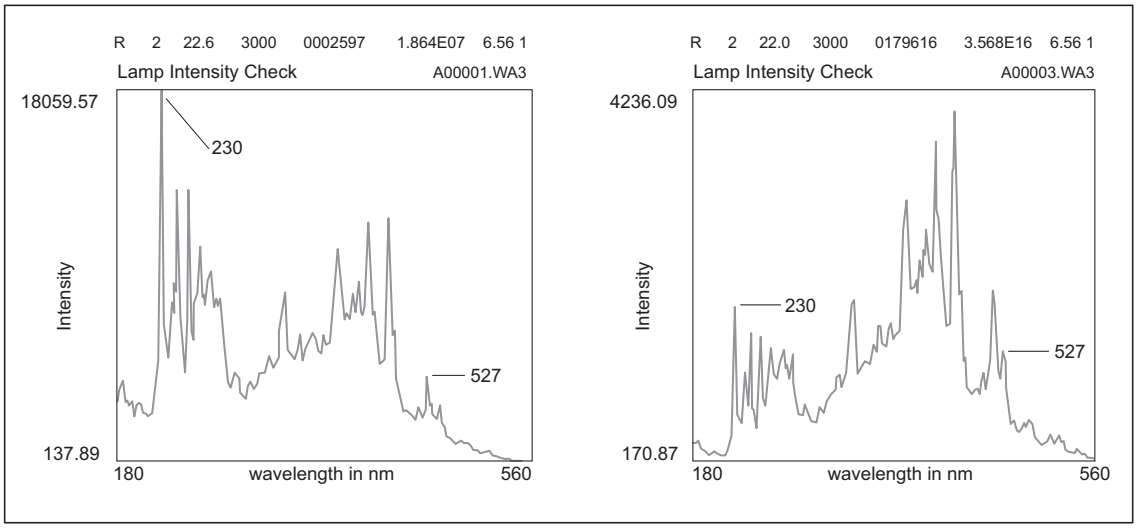
The important factor for both types of instrument is that the readout values at any wavelength be linearly related to the product of concentration and path length. The measurements commonly made in the XL-A or XL-I require only that this relationship hold. Each XL-A and XL-I instrument has been factory tested to ensure that the absorbance values are linear with concentration in the cell.

Wavelength Accuracy Check

The actual wavelength performance of the XL-A or XL-I absorbance optical system was measured at the factory prior to shipment. Figure 5.7 shows two wavelength plots. The first is an ideal wavelength scan of a windowless cell obtained in a calibrated XL-A or XL-I; the second is a wavelength scan indicating that the lamp may need cleaning. Note the difference in intensity values.

Check the performance of your instrument's optical system by setting up and running a wavelength scan as follows. Be sure instrument power is on and the XL-A/XL-I user interface software is running.

Figure 5.7 Wavelength Scans of a Windowless Cell in a Calibrated XL-A or XL-I

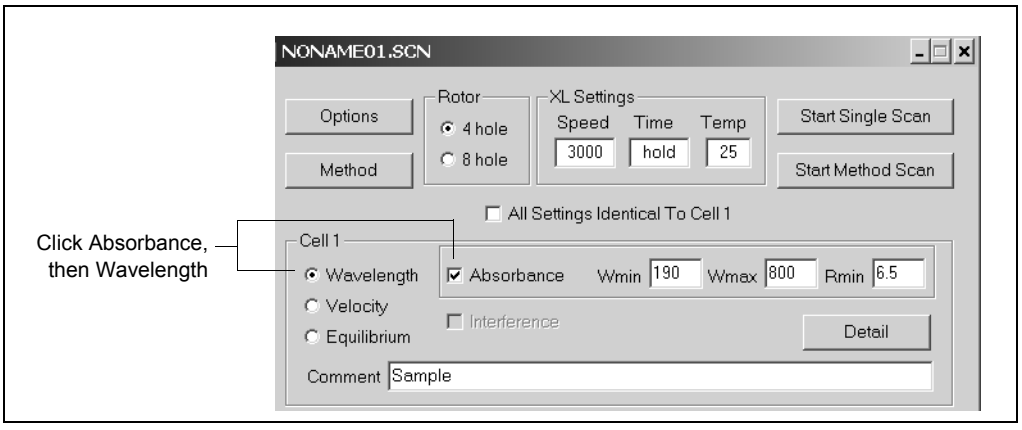


NOTE In Figure 5.7 the scan on the left indicates good lamp output. The "noisy" scan on the right may indicate that the lamp needs cleaning.

1 Place a windowless cell in any empty cell (cell 1 is used in the example).

2 Choose **File > New File.**

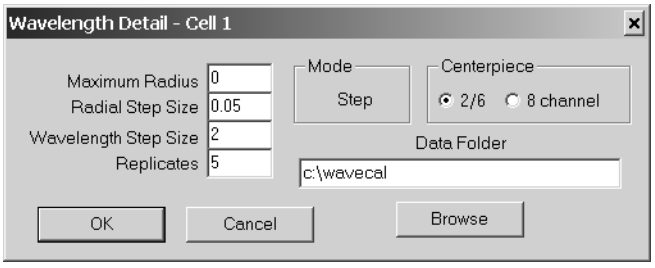
Click the **Absorbance** button and then the **Wavelength** button.



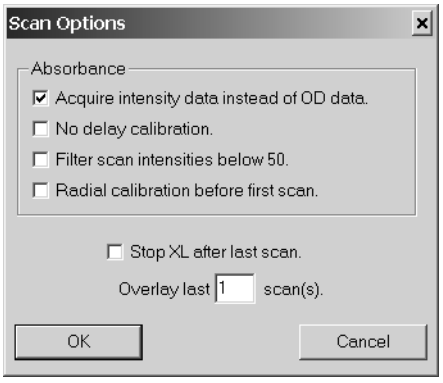
3 Set up a single wavelength scan using the default settings: Wmin 190; Wmax 800; Rmin 6.5.

4 Click the **Detail** button.

Change the default wavelength step size to 1 and the data folder name to Wavecal. Click **OK**.



5 Click the **Options** button. Click the **Acquire intensity data instead of OD data** box.



6 Click the **Start Single Scan** button.

7 When the scan is complete, choose **Edit Data** from the Analysis menu.

8 Click **Import Data** and select the name of the scan completed in step 6.

9 Click on the data reader tool in the Origin toolbox.

Click the data reader on one end of the plot line and drag it to each peak to determine the peak position wavelengths.

10 Compare the scan with the scan shown at the left in Figure 5.1.

There should be a peak at 230 nm and another at 527 nm.

If the intensity of the 230-nm peak is below 6000, the lamp probably needs cleaning.

See *Cleaning the Monochromator Window* earlier in this section.

Printer Maintenance (If Equipped)

To change the ribbon (927506) or replace the paper (927505), you must remove the control head cover to gain access to the printer. Follow the instructions provided under ***Retrieving Your Sample In Case of Power Failure*** earlier in this section. Once the control head cover is removed, locate and review the summary of instructions fastened to the inside of the cover.

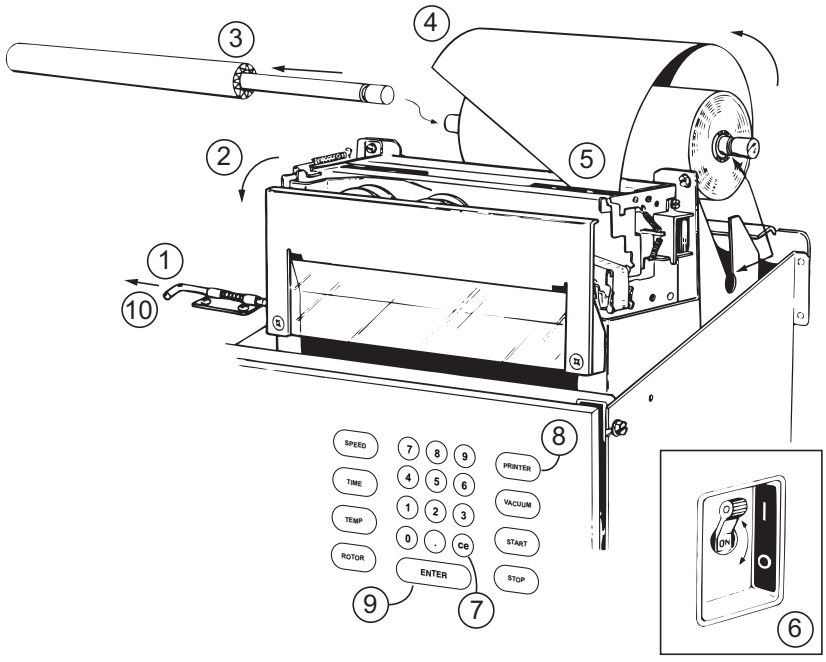
Changing the Printer Paper

1 Pull the latch to the left.

2 Lift the printer assembly up and tilt it towards you.

Release the latch so that it holds the printer in the raised position (see Figure 5.8).

Figure 5.8 Printer Assembly in Raised Position



⚠ WARNING

Be careful NOT to touch the print head immediately after printing as it can get very hot.

3 Lift the grooved rod from the slots to remove the old paper roll.

Discard the old paper roll but keep the rod.

4 Trim the edge of the new paper at an angle, with the pointed edge on the right.

Insert the rod through the new roll of paper, then return the rod and paper to the printer, in the slots provided, making sure that the paper feeds from the bottom.

Make sure that the grooves in the rod are properly fitted into the slots.

5 Insert the edge of the paper into the printer until it touches the feeder roller—you will not be able to insert it any further..

Check that the longer edge of the paper is on the right side (as you face the printer) in order to trip the paper sensor switch

6 Turn the instrument power off for 2 seconds, then on again to cycle the power to trip the paper sensor switch.

7 Press **ce** to clear the diagnostic message.

8 Press the **PRINTER** hardkey to access the printer menu.

9 Choose **ADVANCE PAPER**, then press **ENTER** several times until the paper feeds through the printer and comes out the slot provided.

10 Pull the latch to the left, lower the printer back into place, then release the latch.

Tear off any paper extending from the printer, then replace the control head cover.

Press **ENTER** to advance the paper through the slot in the control head cover.

Changing the Printer Ribbon

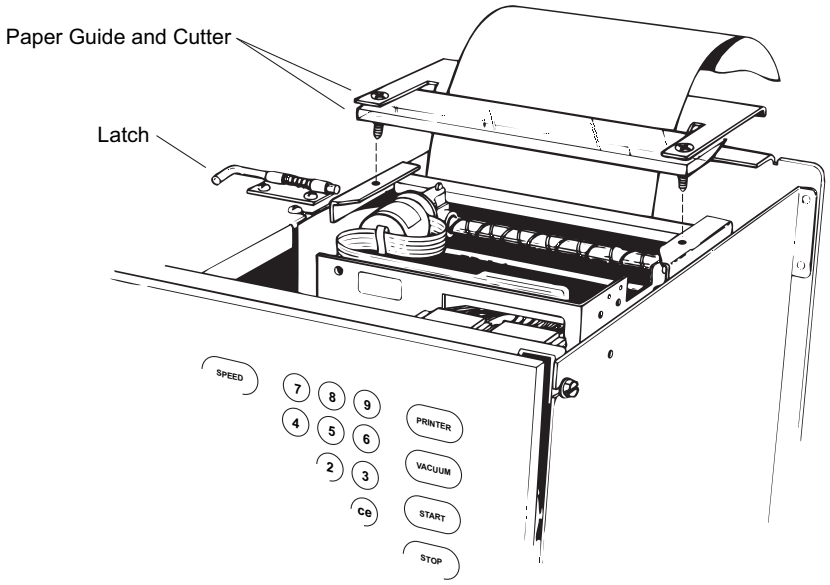
To change the ribbon, you must remove the control head cover to gain access to the printer. Follow the instructions provided under ***Retrieving Your Sample In Case of Power Failure*** earlier in this section. When the control head cover is removed, follow the instructions below.

⚠ WARNING

Be careful NOT to touch the print head immediately after printing as it can get very hot.

1 Remove the paper guide, clear plastic cutter, and two screws as a unit as shown in Figure 5.9.

Figure 5.9 Removing the Paper Guide and Plastic Cutter



2 Pull the latch to the left, lift the printer assembly up, and tilt it towards you.

Release the latch so that it holds the printer in the raised position.

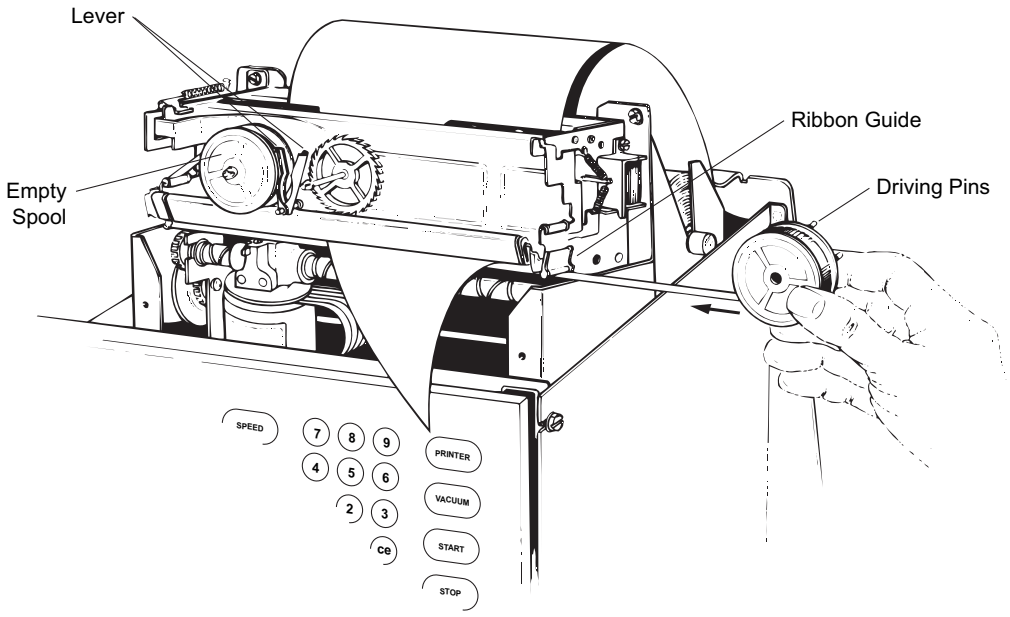
3 Release the lever that contacts the ribbon and lift one spool off the shaft.

Remove the other spool in the same manner, then gently unthread the ribbon from between the platen and print head.

4 To insert a new ribbon, make sure that the empty spool is on the left and that the ribbon travels to the right (clockwise; see Figure 5.10).

Be sure that the driving pins on the spools are facing down.

Figure 5.10 Inserting the New Ribbon



5 Holding the ribbon taut, slide the ribbon between the print head and the platen, wrap it around the black ribbon guide at the right end of the platen, then drop the spool onto the spool shaft.

(You will need to release the lever to engage the spool.)

You should hear a click as the spool falls into position.

6 Install the other spool in the same fashion.

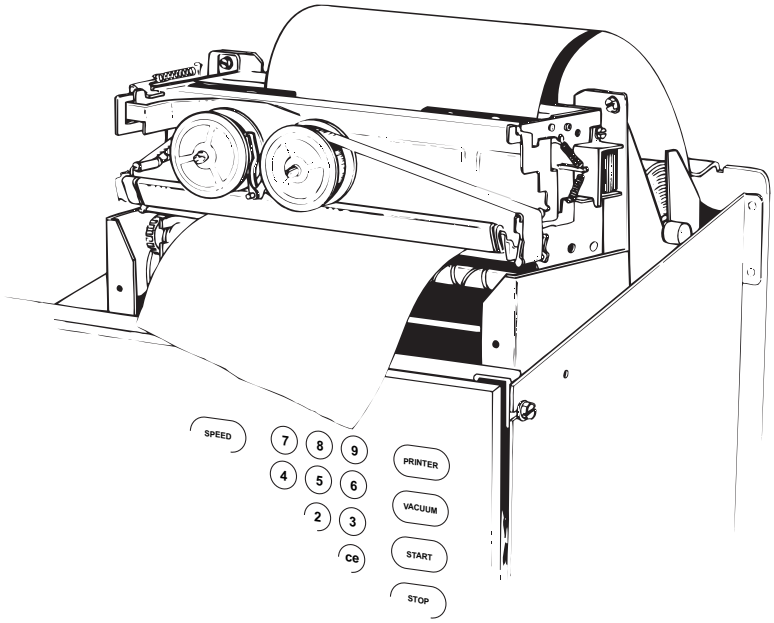
If necessary, turn the left spool to the right (clockwise) to take up the ribbon slack (see Figure 5.11).

7 Release the latch and return the printer assembly to its operating position.

8 Replace the clear plastic cutter and paper guide and tighten the two screws.

9 Reinstall the control head cover as instructed under ***Retrieving Your Sample In Case of Power Failure***.

Figure 5.11 Ribbon Spools Installed



Storage and Transport

Storage

When storing the instrument for an extended period, cover it to protect it from dust and dirt. Temperature and humidity conditions for storage should meet the environmental requirements described under CHAPTER 1, *Specifications*.

Returning an Instrument

Before returning an instrument or accessory for any reason, prior permission (a form) must be obtained from Beckman Coulter. Contact your local Beckman Coulter office to obtain the form and instructions for packaging and shipping.

To protect our personnel, it is the customer's responsibility to ensure that all parts are free from pathogens and/or radioactivity. Sterilization and decontamination must be done before returning the parts.

All parts must be accompanied by a note, plainly visible on the outside of the box or bag, stating that they are safe to handle and that they are not contaminated with pathogens or radioactivity. Failure to attach this notification will result in return or disposal of the items without review of the reported problem.

Supply List

Call Beckman Coulter Sales (1-800-742-2345 in the United States; outside the U.S. contact your local Beckman Coulter office or visit us on the web at www.beckmancoulter.com) for information on ordering parts and supplies. For your convenience, a partial list is given below.

Analytical Rotors and Accessories

NOTE For a complete list of rotor replacement parts, including centerpiece components, see the analytical rotor manual (LXL/A-TB-003).

Description	Part Number
An-50 Ti analytical rotor	363782
An-50 Ti rotor overspeed disk	363784
An-60 Ti analytical rotor	361964
An-60 Ti rotor overspeed disk	360256
Flow-through centerpiece assembly w/quartz windows	392772
Flow-through centerpiece assembly w/sapphire windows	392773

Replacement Parts and Supplies

NOTE For MSDS information, go to the Beckman Coulter website at www.beckmancoulter.com.

Description	Part Number
Chamber O-ring	801778
Detector assembly	360087
Detector cover	356585
Detector cover, wide aperture	360081
Light holder cover	356584
Direct-drive vacuum pump oil (1 L)	341661
Diffusion pump oil (250 cc)	330246
RS-232 nine-conductor cable assembly	372141
Printer paper, one roll (4 1/2 in. wide)	927505[a]
Ink ribbon, black and red (for IBM PPS II Model 2380)	927506[a]

Description	Part Number
Window Cleaning Kit contains window assembly (367810), window tool (367709), and lamp assembly (368449)	368488
Hand cleaner	927741
Silicone vacuum grease (1 oz)	335148
Solution 555 (1 qt)	339555

a. Used on instruments equipped with an onboard printer.

APPENDIX A

Installation Requirements

Requirements for Installation

⚠ CAUTION

Do not attempt to install or turn on power to the ProteomeLab XL-A or XL-I. Its purchase price includes installation by Beckman Coulter personnel. Installation by anyone other than an authorized Beckman Coulter representative invalidates any warranty covering the instrument.

Preinstallation requirements have been sent prior to shipment of the instrument. The following information is provided in case the instrument must be relocated. Contact Beckman Coulter Field Service to adjust and level the instrument if it must be moved. (The pads on each leveling leg are designed to affix the instrument to the floor to prevent instrument rotation in case of a rotor mishap.)

Electrical Requirements

Instrument rating	• 220 to 240 VAC, 50 Hz, 20 A • 200 to 240 VAC, 50/60 Hz, 20 A
Power line range, XL-A or XL-I instrument	180 to 264 VAC, 60 or 50 Hz (single phase), 30 A
Power line range, IBM computer	100 to 120 VAC, 60 Hz, or 220 to 240 VAC, 50 Hz

To reduce the risk of electrical shock, this equipment uses a three-wire electrical cord (1.8 m; 6 ft) and plug to connect the equipment to earth ground (see Figure A.1). In regions where the instrument is supplied with an unterminated cord, a plug that meets local electrical and safety requirements must be supplied. (Contact your local Beckman Coulter office for specific information regarding these requirements.) See Table A.1 for the required wire connections. Make sure that the matching wall outlet receptacle is properly wired and earth grounded.

The optical systems are completely contained within the rotor chamber and instrument housing, and require no additional power source.

Figure A.1 ProteomeLab XL-A or XL-I Electrical Requirements

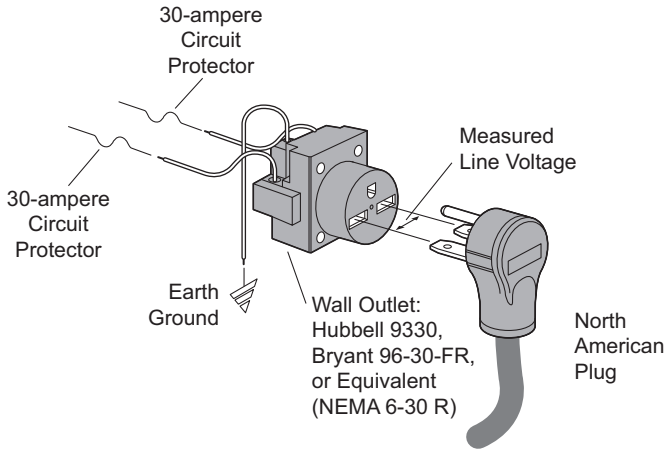


Table A.1 Required Wire Connections

Wire Insulation Color	Terminal	Symbol	
		Harmonized	North American
Green/Yellow	Earth ground	⏚	⏚
Light Blue	Neutral	N	L
Brown	Live or Line	L	L

An uninterruptable power supply outlet is recommended for the PC to avoid loss of data during power interruptions; if this is not available, a multi-outlet power surge protector should be used.

To ensure safety, the instrument should be wired to a remote emergency switch (preferably outside the room where the centrifuge is housed, or adjacent to the exit from that room). In case of a malfunction, the centrifuge can be disconnected from the main power source by removing the Mains (power) plug from the outlet receptacle.

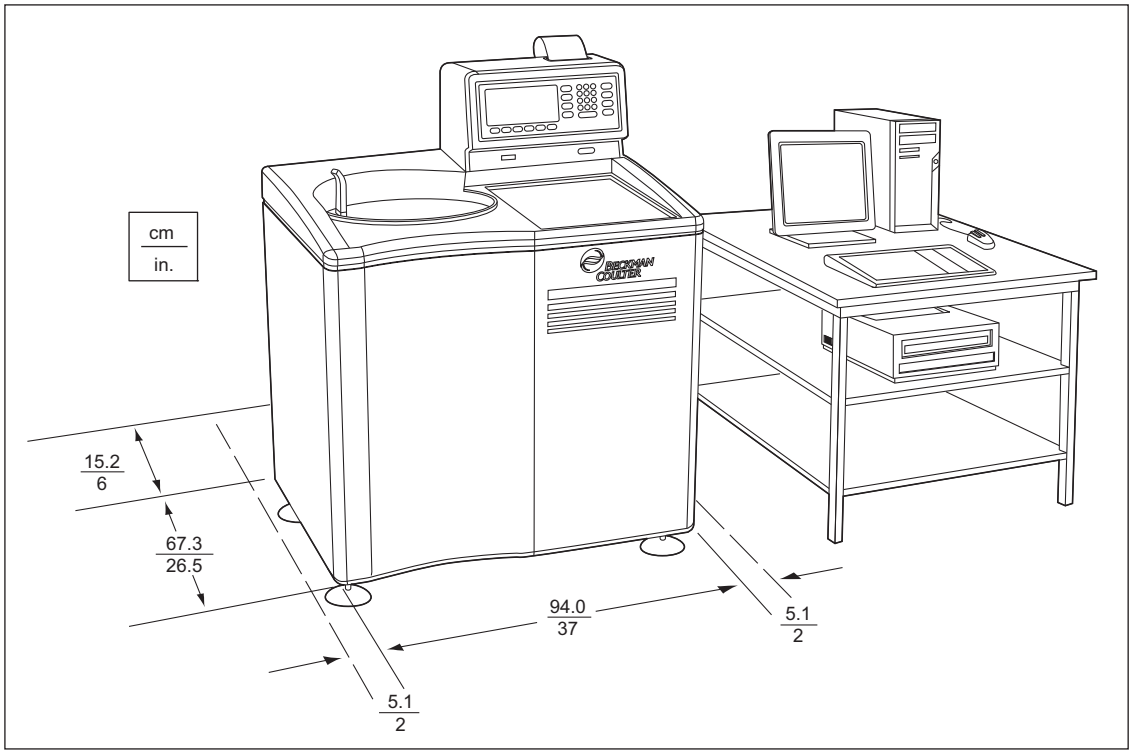
Space Requirements

The space requirements for the ProteomeLab XL-A or XL-I instrument and data system are shown in Figure A.2. A 5.1-cm (2-in.) clearance is required on both sides of the instrument, as the feet extend 5.1 cm (2 in.) beyond the instrument. The 5.1-cm (2-in.) side clearance also enables access to the circuit breaker on the right-side panel. A 152-mm (6-in.) clearance is required at the rear of the instrument for servicing and to ensure sufficient air ventilation. The instrument must have adequate air ventilation to ensure compliance to local requirements for vapors produced during operation.

The Mains (power) plug is the disconnect device and must remain easily accessible. Position the instrument so that it is easy to remove the Mains (power) plug from the outlet receptacle.

The PC and printer can be located on either side of the instrument on a computer stand, or on an approximately 0.9-m (3-ft) wide area on a table or bench top.

Figure A.2 ProteomeLab XL-A or XL-I Instrument and Data System Space Requirements



Approximately one square foot of additional bench space is needed for permanent mounting of the cell torque wrench assembly, which is required for assembling the analytical cells. Follow the instructions provided with the torque wrench assembly (publication E-TB-016), making sure to secure the assembly to the front or corner of the lab bench.

Bio-Safety Level 3 Installation

For laboratories with epoxy aggregate floors, such as BSL-3 labs, a non-invasive installation kit (PN 393862) is available. The kit, which consists of a high-strength adhesive tape, is CSA certified for use on epoxy aggregate floors only.

Installing the ProteomeLab XL-A/XL-I User Interface Software

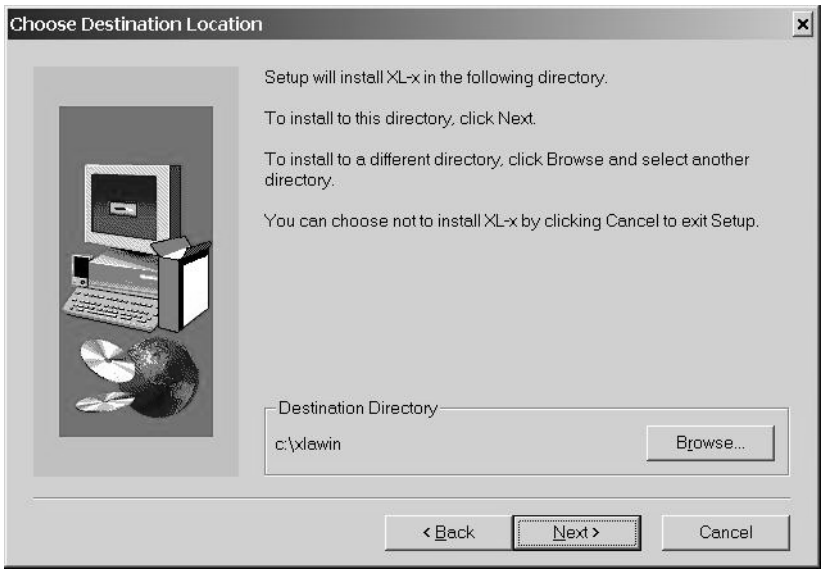
A Beckman Coulter Field Service representative will install the hardware and software required for system operation. The following procedures are provided in case you need to reload the user interface software for any reason.

1 Insert the XL-A/XL-I Windows Setup CD.

2 Run **Setup.exe** by double-clicking the **Setup.exe** file from within Windows Explorer, or by choosing **Setup.exe** from the Add/Remove Programs window on the Control Panel.

3 Follow the instructions in the Install Wizard.

a. When the following window appears, keep the default Destination Directory as specified, and press the **Next** button.



b. Keep the default Program Folder as "**XL-x**", and press the **Next** button.

c. Select the **Next** button, and the installation will start copying the program files.

d. Select **Finish** to complete the setup and reboot the computer.

Adjusting the Screen Resolution

The XL-A/XL-I user interface software was designed to run under Windows XP, with the screen resolution set to 1024 × 768 and large fonts selected. For best viewing, the display should be configured to these settings. To change the screen resolution:

1 Select the Display icon from the Control Panel.

2 Under the Settings tab, set the Screen area to 1024 by 768 pixels.

Click the **Advanced...** button and set the Display font size to large fonts.

Uninstalling the XL-A/XL-I User Interface Software

1 Open the Control Panel and double click the Add/Remove Programs icon.

2 Select "**XL-x**" and press the **Change**/**Remove** button.

3 Select the **Yes** button to confirm file deletion.

4 The Uninstall Wizard will prompt you through the uninstall procedure.

Press the **Yes** button to remove the two BitFlow driver files, BitFlow.sys and BitFlow.gat, when the question appears.

5 Manually remove the **C:\xlawin** directory.

APPENDIX B

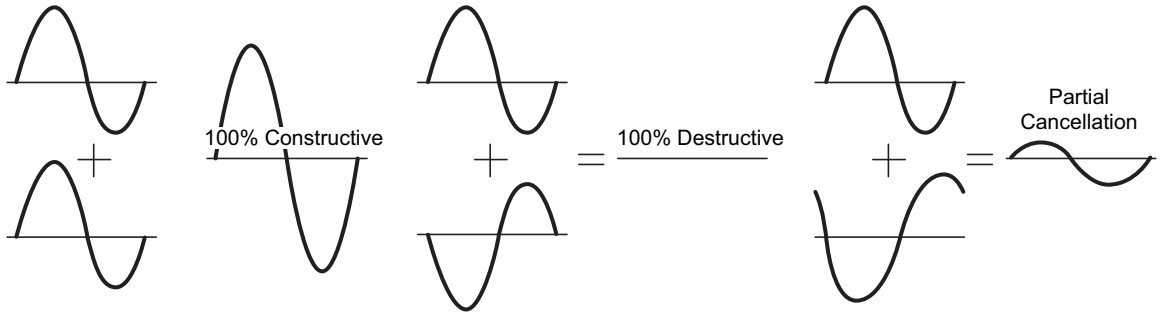
Interference Theory

Description of Interference Theory

Interference is a characteristic of all wave motion, whether they are water, sound, or light waves. It is easily seen in a pool of water, when two objects are dropped into the water near each other. Small waves or ripples spread out in a circle around the spot where each object was dropped. As the waves created by the two objects meet, in some places crests overlap crests, while in other places crests overlap troughs.

When the crest of one wave overlaps the crest of another, the waves are in phase and their individual effects add together. The result is a wave of increased amplitude. This is called *constructive interference*. When the crest of one wave overlaps the trough of another, the waves are out of phase and their individual effects are reduced or canceled. The high part of one wave simply fills in the low part of another. This is called *destructive interference*. Figure B.1 illustrates constructive and destructive interference.

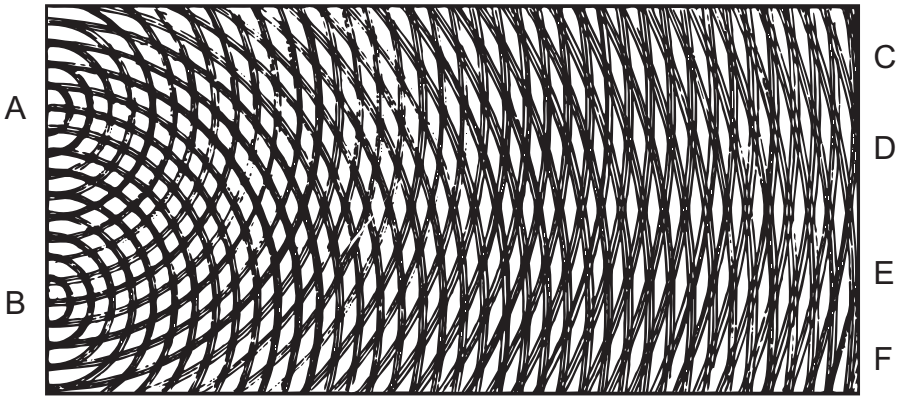
Figure B.1 Wave Interference



The interference of light waves to form a fringe pattern was demonstrated in the classic experiment of Thomas Young (Figure B.2). Young found that light directed through two closely spaced pinholes recombined to produce fringes of brightness and darkness on a screen behind. The bright fringes of light resulted from light waves from the two holes arriving crest to crest, while the dark areas resulted from light waves arriving trough to crest.

In the XL-I interference optical system, monochromatic light is directed through two closely spaced parallel slits to produce a fringe pattern consisting of straight lines (Figure B.3).

Figure B.2 Thomas Young's Original Drawing of a Two-Source Interference Pattern



NOTE Letters C, D, E, and F mark regions of destructive interference in Figure B.2.

Figure B.3 Interference Pattern Created by Two Slits

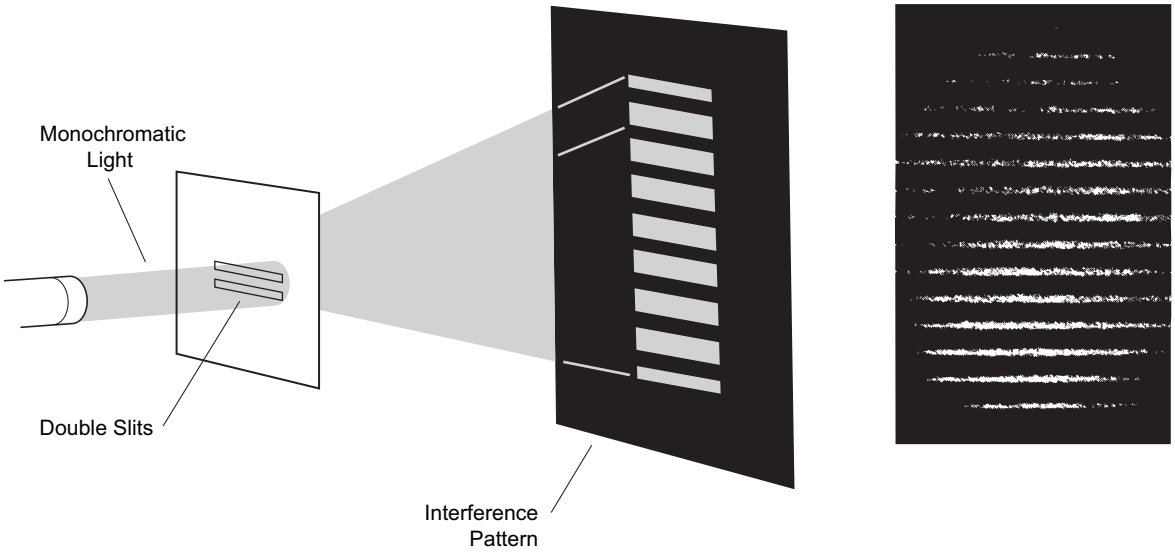


Figure B.4 shows how the series of bright and dark lines results from the different path lengths from the slits to the screen. For the central bright fringe, the paths from each slit are the same length, and the waves arrive in phase and reinforce each other. The dark fringes on either side of the central fringe result from one path being longer (or shorter) by one-half wavelength, where the waves arrive half a wavelength, or 180°, out of phase. The other sets of dark fringes occur where the paths differ by odd multiples of one-half wavelength: 3/2, 5/2, and so on.

In the XL-I, the two beams exiting the slits in the monochromator pass through the analytical cells. One beam passes through the sector containing sample, and the other beam passes through the sector containing solvent (the reference sector). The beams are combined, magnified, and focused onto the detector array of a video camera, and the resulting equally spaced fringes are then displayed on the computer screen. Differences in refractive index between the sample and solvent appear on the computer screen as a change in the fringe pattern, known as vertical fringe displacement (shown in Figure B.5).

Figure B.4 Light from O passes Through Slits M and N and Produces an Interference Pattern on Screen S

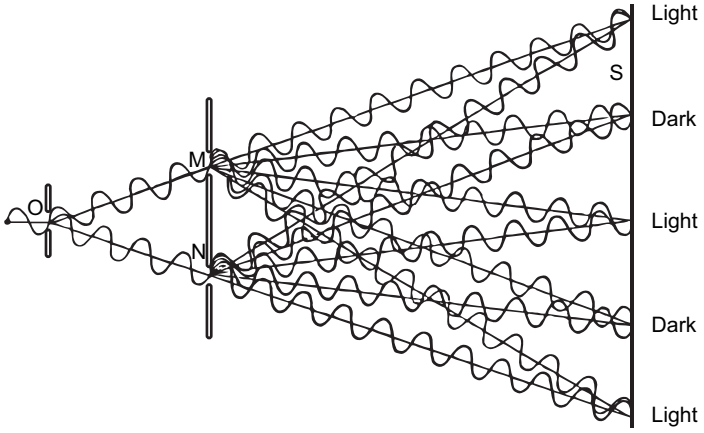
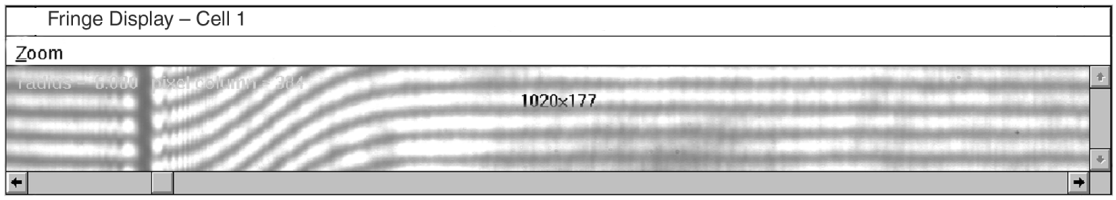


Figure B.5 Vertical Fringe Displacement in the ProteomeLab XL-I Fringe Display



Fringe displacement is a result of the different path lengths, from light source to detector, encountered by the two beams of light. The optical path length depends not only on distance, but also on the index of refraction (amount of material) encountered over the distance. The higher the index of refraction, the longer it will take the light to reach the detector. The apparent speed of light in any material is $c = c_o/n$, where c_o is the velocity in a vacuum and n is the refractive index of the material. Thus the time required for the beams of light to traverse the optics will be different if there is a refractive index difference in the two paths. This refractive index is essentially a measure of a substance's electron density and polarizability. Any material with a refractive index different from the solvent will contribute to the overall refractive index and any difference between the reference and sample light paths will be registered by the interference optics as a fringe displacement.

Because virtually all materials change a solvent's refractive index, the interference optical system can detect almost any material in solution. However, the interferometer cannot discriminate between a solution's components.

Thus, presuming that the difference in refractive index of solute and solvent falls within the measurable limits of the interference system, fringes will form at the focal plane of the condensing lens. The difference in refractive index at various points in the cell will cause equivalent shifts in fringe position at the focal plane. These fringe shifts will be imaged on the plate at positions that correspond to the location in the cell of the refractive index difference the shift represents. And the boundary region, in which a continual change in refractive index differences exists, will be imaged on the plate as a continual fringe shift. This continual fringe shift between the supernatant and the plateau region produces the curvature of the fringes through the boundary.

References

Conceptual Physics, Seventh Edition. Paul G. Hewitt, Harper Collins College Publishers, 1993.

Choosing Which Optical System of the XL-A Analytical Ultracentrifuge to Use (publication A-1821). Thomas M. Laue, 1995.

Glossary

Absorbance, optical density — A measure of the quantity of light absorbed by a solution. It is equal to log o/I, where Io is the intensity of the incident light, and I is the intensity of the transmitted light.

Boundary — The interface between solvent and solution which moves down the cell under centrifugal force during sedimentation.

Buoyant Density — The density of a particle in a specified liquid medium.

Cell Assembly — The assembly that contains the sample and solution in the analytical rotor. The cell assembly comprises a centerpiece, two window assemblies, and a housing to hold the centerpiece and windows.

Centerpiece — The part of a cell assembly that contains the liquid sample. In some centerpieces, the cavities are wedge-shaped to maintain boundary integrity and have edges along radial lines. Multichannel centerpieces with rectangular-shaped cavities have shorter sample column heights and are used for rapid equilibrium runs. In a double-sector cell, one of the sectors contains the solution of sample plus solvent and the other contains only solvent, providing a baseline for the optical system.

Com Port — A computer connection where you plug in the cable for a serial device (such as the ProteomeLab XL-A or XL-I instrument). The XL-A/XL-I user interface software enables selection of Com ports 1, 2, 3, and 4.

Counterbalance — The counterbalance is used to balance the analytical rotor and to provide a reference for calibrating radial distances. The system calibrates the absorption optics system based on the position of the counterbalance. As light passes through pairs of inner and outer reference holes, radial distances in the cell and the distances between reference edges are measured.

Data Acquisition Board — Data system component that controls the strobe trigger, monochromator, and scanner position, and that processes the detected signal.

Default — An option, command, or device that is automatically selected by the program as the most likely option. For example, in most dialog boxes requiring values, the default values will appear and will be automatically chosen if you click **OK** or press **ENTER** without entering different values.

Diffraction — The spreading out of light by passing it through a narrow slit or past the edge of an obstacle.

Diffusion — The net flow of molecules from a region of high concentration to one of low concentration as the result of random movement.

Diffusion Coefficient (D) — Unless otherwise specified, the diffusion coefficient refers to the translational diffusion coefficient, a measure of the translational motion of a particle that depends on the size and shape of the diffusing particle. The value of D is characteristic of a macromolecule and is associated with the spreading of a boundary in sedimentation velocity experiments.

Directory — Part of a structure for organizing your files into convenient groups on a disk. A directory is like a file drawer—it can hold a group of files, or it can contain both files and subdirectories (which can also contain files).

Extension — The period and three letters at the end of a filename, identifying the kind of information the file contains. For example, the extension .scn indicates a scan file and .org indicates an Origin document.

Extinction Coefficient — The proportionality constant e, in Beer's law for light absorption: A = elc., where A is absorbance, l is the length of the light path, and c is the concentration. If concentration is expressed on a molar basis, e becomes the molar absorptivity, molar absorption coefficient, or molar extinction coefficient, that is, e = A/lc, where l is the length of the light path in centimeters, and c is the molar concentration.

Filename — A string of one to eight characters, plus a three-character extension, which the operating system identifies as the file.

Interference — The phenomenon occurring when a beam of light is passed through two parallel slits, creating a pattern of dark and light bands (fringes) on a flat surface behind the slits. If a solution is placed in the light beam ahead of the slits, the solution components will bend the light according to their refractive index, causing a measurable shift in the fringes on the flat surface.

Meniscus — The curved upper surface of a liquid column (concave when the containing walls are wetted by the liquid; convex when it is not).

Parameters — Numeric values that are entered into instrument memory for specific functions. Examples are number of scans, or speed and temperature values.

Photomultiplier — An electron multiplier in which the first stage consists of photoelectric emission from a cathode. In the ProteomeLab XL-A or XL-I, the photomultiplier amplifies the light signal that it receives from the detector.

RAM (Random Access Memory — The computer's memory, in which both a program and data are held ready for processing. Whatever is stored in RAM is lost when the computer is turned off.

Radial Scan — A scan in which absorbance is measured as a function of radial distance.

Sedimentation Equilibrium — One of two types of experiments that can be performed with the ProteomeLab XL-A or XL-I. See the Introduction to Analytical Ultracentrifugation (Ralston, Greg, 1993) included with this manual, for a detailed explanation of sedimentation equilibrium.

Sedimentation Velocity — One of two types of experiments that can be performed with the ProteomeLab XL-A or XL-I. See the *Introduction to Analytical Ultracentrifugation* (Ralston, Greg, 1993), included with this manual, for a detailed explanation of sedimentation velocity.

Solvation — The state or degree of combination of a solute with a solvent or of a dispersed phase with a dispersion medium.

Spectrophotometer — A photometer for measuring the relative intensities of the light in different parts of a spectrum.

UV (Ultraviolet Light) — Electromagnetic radiation of wavelengths just shorter than those of visible light.

Vacuum — In practice, a region in which pressure is considerably less than atmospheric pressure.

Wavelength — The distance between two points having the same phase in two consecutive cycles of a periodic wave, along a line in the direction of propagation.

Wavelength Scan — A scan in which absorbance is determined as a function of wavelength at preselected radial distances. A wavelength scan results in an absorbance spectrum showing the absorbance value (λ_{max}) of each solute.

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Beckman Coulter, Inc. ProteomeLab XL-A and XL-I Protein Characterization Systems Warranty

Subject to the exceptions and upon the conditions specified below, Beckman Coulter, Inc., agrees to correct, either by repair, or, at its election, by replacement, any defects of material or workmanship which develop within one (1) year after delivery of the ProteomeLab Protein Characterization System (the product), to the original Buyer by Beckman Coulter, or by an authorized representative, provided that investigation and factory inspection by Beckman Coulter discloses that such defect developed under normal and proper use.

Some components and accessories by their nature are not intended to and will not function for as long as one (1) year. If any such component or accessory fails to give reasonable service for a reasonable period of time, Beckman Coulter will repair or, at its election, replace such component or accessory. What constitutes either reasonable service and a reasonable period of time shall be determined solely by Beckman Coulter.

Replacement

Any product claimed to be defective must, if requested by Beckman Coulter be returned to the factory, transportation charges prepaid, and will be returned to Buyer with the transportation charges collect unless the product is found to be defective, in which case Beckman Coulter will pay all transportation charges.

Beckman Coulter makes no warranty concerning products or accessories not manufactured by it. In the event of failure of any such product or accessory, Beckman Coulter will give reasonable assistance to the Buyer in obtaining from the respective manufacturer whatever adjustment is reasonable in light of the manufacturer's own warranty.

Damage to the instrument while operating a rotor not of Beckman Coulter manufacture is not covered by warranty or service contract terms. Further, Beckman Coulter shall be released from all obligations under all warranties either expressed or implied, if the product covered hereby is repaired or modified by persons other than its own authorized service personnel, unless such repair is made by others who meet qualifications similar to those required of Beckman Coulter's service personnel, or unless such repair in the sole opinion of Beckman Coulter is minor, or unless such modification is merely the installation of a new Beckman Coulter plug-in component for such product.

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During the instrument warranty period (one year), there will be no charge for drive replacement if the drive unit is installed, serviced, and operated in accordance with the conditions listed below. During the drive's second through tenth year of use there is a prorated drive replacement price based on years of use if the drive unit is installed, serviced, and operated in accordance with the conditions listed below.

Drive replacement price for units not under service contract* = current drive exchange price $\infty \langle \frac{\text{years of use}}{10} \rangle$ + labor and travel.

* For details of drive coverage with a service contract, contact your local Beckman Coulter service representative.

Conditions

1. The drive has been operated only within its rated speed and temperature ranges.
2. The drive unit has not been subjected to unequal loading, improper rotor installation, corrosion from material spilled onto the hub or accumulated in the chamber of the instrument.
3. The drive unit has not been disassembled, modified, or repaired, except by Beckman Coulter personnel.
4. The drive unit was installed by a Beckman Coulter Field Service representative.
5. The instrument in which the drive unit has been used and operated, and its associated rotors, were manufactured by Beckman Coulter and serviced only by Beckman Coulter Field Service representatives.

If the above conditions are not met, the full appropriate exchange price for the drive will be charged.

Disclaimer

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 BECKMAN COULTER, INC. SHALL HAVE NO LIABILITY FOR SPECIAL OR CONSEQUENTIAL DAMAGES OF ANY KIND WHATSOEVER ARISING OUT OF THE MANUFACTURE, USE, SALE, HANDLING, REPAIR, MAINTENANCE, OR REPLACEMENT OF THE PRODUCT.

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	Dangerous voltage Gefährliche electrische Spannung Voltaje peligroso Courant haute tension Pericolo: alta tensione	Tensão perigosa Опасное напряжение тока 危险电压 危険な電圧 위험한 전압
	Caution, consult accompanying documents Vorsicht, konsultieren Begleitdokumente Atención, consulta documentos adjuntos Attention, consultent des documents d'accompagnement Attenzione, consulta i documenti di accompagnamento	Cuidado, ulta documentos adjuntos Внимание, советует с сопроводительными документами 注意, 咨询附属单证 注意, 伴う文書に相談しなさい 주의, 동반 문서를 상담하십시오
	Biohazard Potentiell infektiösem Material Riesgo biológico Risque biologiqu Pericolo biologico	Material infeccioso potencial биологической опасности 可能的传染性物 潜在的な感染性物質 전염하는 물자
	On (power) Ein (Netzverbindung) Encendido Marche (mise sous tension) Acceso (sotto tensione)	Fora (o poder) На (мощности) 开（电源） ン（電源） 에 (힘)
	Off (power) Aus (Netzverbindung) Apagado Arrêt (mise sous tension) Spento (fuori tensione)	Fora de (poder) С (сила) (电源) ン（電源） 떨어져 (힘)
	Protective earth (ground) Schutzleiteranschluß Puesta a tierra de protección Liaison à la terre Collegamento di protezione a terra	Terra de proteção (terra) Защитное заземление (земля) 保护接地 保護アース（接地） 방어적인 지구 (지상)
	Earth (ground) Erde (Masse) La tierra (suelo) Terre (sol) Scarica a terra	Terra Земли 接地 アース（接地） 지구 (지상)
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