Instruction For Use

LS 13 320 Laser Diffraction Particle Size Analyzer

PN B05577AB
October 2011

Beckman Coulter, Inc.
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Brea, CA 92821

BECKMAN COULTER

LS 13 320 Laser Diffraction
Particle Size Analyzer
Instructions For Use
PN B05577AB (October 2011)

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

Revision History

First Revision, B05577AB, October 2011
Changes:

- Updated corporate address
- Revised Figure 1.2 Certification Label (Instrument Back Cover)
- Other minor edits and deletions
- Revision page added
- Added the Beckman Coulter, Inc. Customer End User License Agreement
- Trademarks page added

Initial Issue, B05577AA, 2009
Document placed into the BCI “structured” FrameMaker format

Safety

Symbols Used in this Manual

⚠ WARNING

Warning - Indicates a situation or procedure that if ignored can result in personal injury.

⚠ CAUTION

Caution - Indicates a situation or procedure that if ignored may result in damage to the instrument.

IMPORTANT Important – Indicates a situation or procedure that if ignored can result in erroneous test results.

NOTE (Help note) contains hints and useful information to help optimize results quality and ease of use.

Conventions

- The optical bench and sample modules are referred to as the "Analytical Module". The PC, keyboard, monitor and printer, etc., are all referred to collectively as "the computer".
- Bold type letters like this **Enter Sample ID** represent menu or button text appearing on the screen of the computer that can be selected with the mouse or by keystrokes.
-  means **click the mouse button**. Unless the mouse has been inverted for left-handed users, this will be the left mouse button.
- The ☞ symbol **points** you to where additional information on a subject can be found.

Laser Precautions

The LS 13 320 contains a 5 mW diode laser. The instrument therefore may pose certain hazards associated with low-power lasers if misused. You should be aware of these possible hazards as described in the next paragraph. Additionally, misuse of diluents for sample dispersion can also create hazardous situations.

Laser Safety Precautions

IMPORTANT Diode laser power of up to 5 mW at 750 nm or 780 nm could be accessible in the interior if the safety interlocks are defeated.

To comply with Federal Regulations (21CFR Subchapter J) as administered by the Food and Drug Administration's (FDA) Center for Devices and Radiological Health (CDRH), defeatable microswitches are located on the right and left of the door panel. Because the system contains a laser, it should be isolated from non-laser instruments. Users are advised to keep a copy of ANSI standard 2136.1, SAFE USE OF LASERS, near the instrument for ready reference. Copies are available from:

American National Standards Institute
1430 Broadway
New York, NY 10018

General Laser Precautions

IMPORTANT The laser beam can cause eye damage if viewed either directly or indirectly from reflective surfaces (such as a mirror or shiny metal surface). Avoid direct exposure to beam. Do not view directly or with optical instruments.

A laser is a unique light source that exhibits characteristics different from conventional light sources. The safe use of any laser depends upon familiarity with the instrument and the properties of coherent, intense beams of light. The beam can cause eye damage. The beam might cause damage if viewed indirectly from reflective surfaces such as a mirror or shiny metal surface.

IMPORTANT Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Follow these precautions:

- Advise all those using the instrument of these precautions.
- Limit access to the instrument to those trained in the use of the equipment.
- Post warning signs in the area of the laser beam to alert those present.
- Never look directly into the laser light source or at scattered laser light from any reflective surface.

- Stop viewing if:
  - you see an unusually bright spot that makes you uncomfortable (similar to looking at the sun).
  - you get a headache (a symptom of overexposure).
- Do not tamper with or attempt to defeat the safety interlock switches.

LS 13 320 Specific Precautions

- Never place a mirror or optical surface (other than the sample cell assembly) into the optical axis of the system.
- Do not place your hands in the pathway of the door as it closes.
- Do not place your hands or any object inside the optical bench as the module is docking.

Radiation Hazards

In the design and manufacture of the BECKMAN COULTER LS 13 320, Beckman Coulter Inc., Particle Characterization Group, has complied with the requirements governing the use and application of a laser as stipulated in regulatory documents issued by the U.S. Department of Health and Human Services and the Center for Devices and Radiological Health (CDRH). In compliance with these regulatory documents, every measure has been taken to ensure the health and safety of users and laboratory personnel from the possible dangers of laser use. CDRH-required labels are placed near or on those covers that, when removed, might expose laser radiation. See figure 1.1 for the warning labels and their locations.

Figure 1.1 Warning labels - Instrument Front Cover And Laser Cover

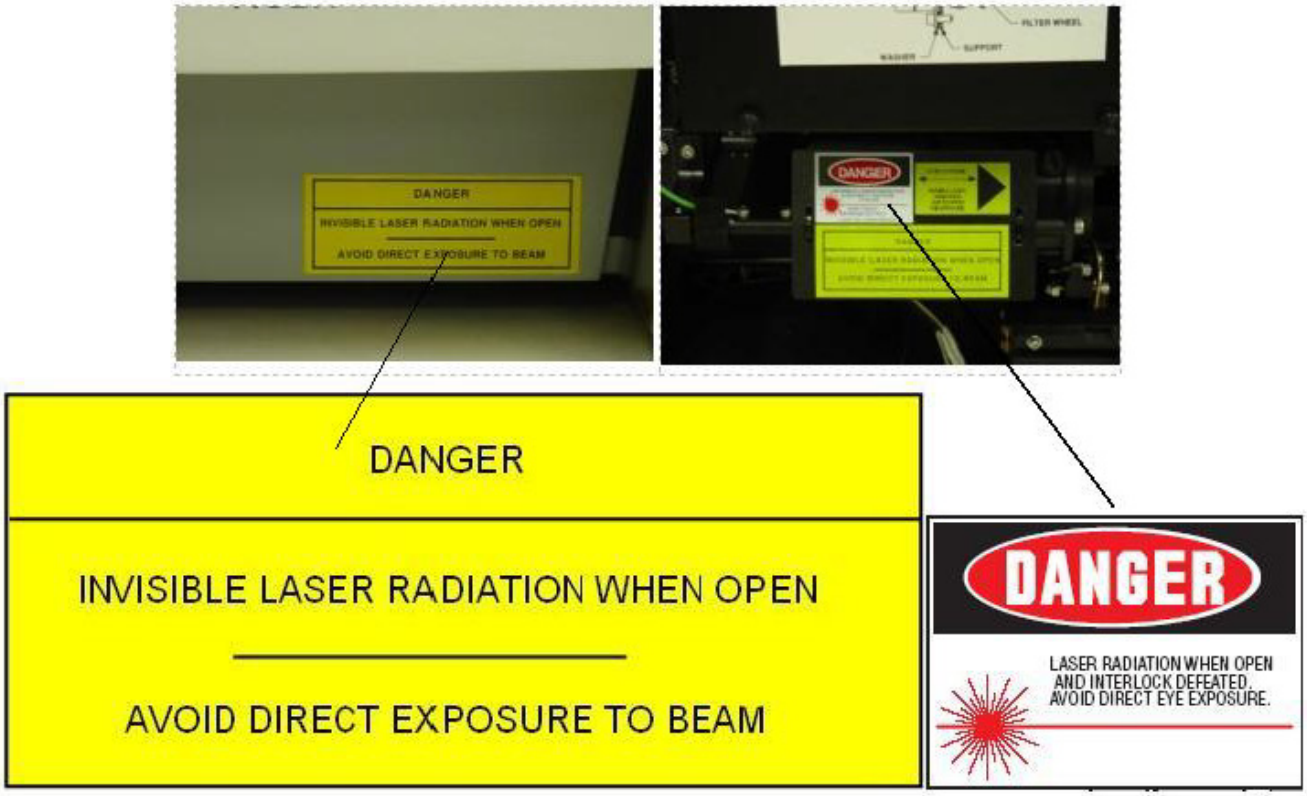


Figure 1.2 Certification Label (Instrument Back Cover)

Other Precautions

Warnings

Mechanical

- Do not place fingers inside bench as automatic sliding door closes.
- Do not place fingers inside bench as module docks into bench.
- Do not interfere with or attempt to disable the interlocks incorporated in the left and right doors.

Electrical

- High voltages are present inside the instrument. Always disconnect the instrument from the main power supply before removing any cover.
- The instrument must be grounded correctly.

Chemical

- Do not use any diluents that are not compatible with the specific wetted surface of the sample module. Consult Beckman Coulter or its local representative before using any chemicals not listed in this manual.
- Proper handling procedures for diluents and reagents used in particle analysis should be adhered to at all times. Consult appropriate safety manuals and Material Safety Data Sheets for all samples, diluents and reagents used.
- Care should be taken when mixing or exchanging diluents. Reactions can occur between incompatible solvents that may be violent.
- Flammable solutions should be prepared for use in an appropriate environment and brought to the instrument only when required for analysis.

Fire

Many non-aqueous solutions are flammable. Where possible choose less flammable alternatives.

Cautions

Chemical

- Take care disconnecting diluent lines. Open-ended tubing may allow liquid to spill out of the vessel.
- Never place containers of liquids on top of the LS 13 320. Repair of instruments damaged or affected by spilled liquids will not be covered by any warranty.

Warming up

As with all sensitive electronic instruments, the LS 13 320 components achieve best performance once they have reached a steady working temperature. This may typically take 15 minutes from power up.

Sources of Error

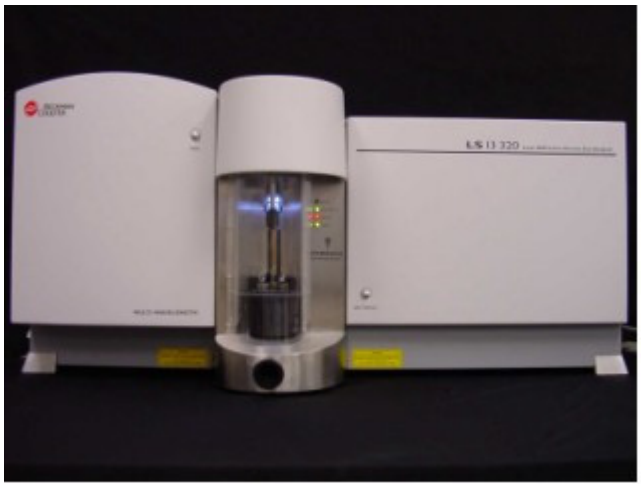
- Sample dispersion
- Air bubbles
- Misalignment
- Incorrect obscuration
- External mechanical vibration, if present, may cause misalignment of the laser. (Automatic alignment should be performed more often.)
- Electrical interference. (Use of an uninterruptible power supply (UPS) is recommended.)
- Sudden changes in temperature can cause misalignment as well as changes to the measured electrical offsets.

Contents

LS 13 320 Introduction

The Beckman Coulter LS 13 320, Figure 1, measures the size distribution of particles suspended either in a liquid or in dry powder form by using the principles of light scattering. This particle size analyzer provides reliable and reproducible results for researchers, quality control laboratories, product and process control departments, or anyone with the need to measure particle size distributions.

Figure 1 LS 13 320 With The Tornado DPS



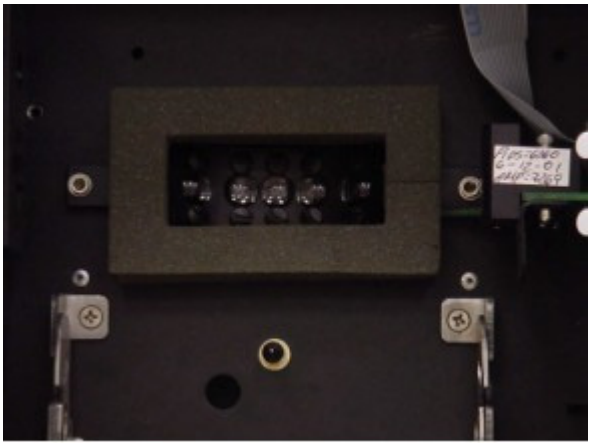
The LS 13 320 consists of an optical bench and five different sample handling modules:

- Universal Liquid Module (ULM)
- Aqueous Liquid Module (ALM)
- Tornado Dry Powder System (DPS)
- Micro Liquid Module (MLM)

In addition an AutoPrep Station can be used in conjunction with the ALM.

The LS 13 320 incorporates Beckman Coulter's patented PIDS (Polarization Intensity Differential Scattering) technology to provide a dynamic range of 0.017 µm to 2000 µm. Figure 2 shows the PIDS detectors.

Figure 2 PIDS Detectors



The LS 13 320 is designed from conception to be fully compliant with the ISO standard covering particle sizing by the laser scattering method (ISO 13320-1 Particle size analysis - Laser scattering methods - Part 1: General principles).

The LS 13 320 includes a sophisticated software package with a multi-component Standard Operating Procedure (SOP). This multi-component Standard Operating Procedure (SOP) will assist operators by ensuring that analyses are the same run-after-run.

Every element of the analysis from method set-up to the final printout can be locked into a user-definable SOP. The SOP routine is divided into two distinct components; an SOM (Standard Operating Method) and a Preference (.prf) file. The SOM includes all aspects of the analysis relating to the instrument settings. The Preference file (.prf) includes choices relating to data presentation and output formats.

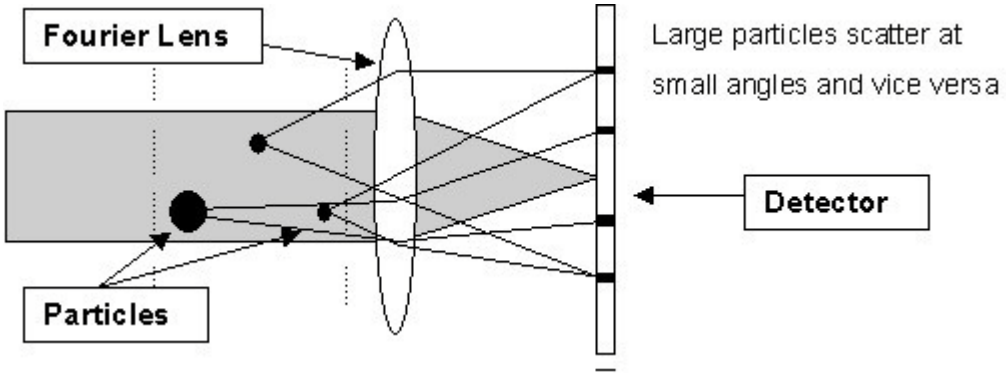
Fourier Optics

Measuring Moving Particles

The LS 13 320 measures particle size distributions by measuring the pattern of light scattered by the particles in the sample. This pattern of scattered light is often called a scattering pattern or scattering function. More specifically, a scattering pattern is formed by light intensity as a function of scattering angle. Each particle's scattering pattern is characteristic of its size. The pattern measured by the LS 13 320 is the sum of the patterns scattered by each constituent particle in the sample.

An important component of making this measurement in an LS 13 320 instrument is the Fourier lens (Figure 3). A Fourier lens serves two functions: it focuses the incident beam so it will not interfere with the scattered light, and it also transforms the angularly scattered light into a function of location on the detection plane. The most important feature of Fourier optics is that the scattered light of any particle at a specific angle will be refracted by the lens so as to fall onto a particular detector, regardless of the particle's position in the beam.

Figure 3 Fourier Optics



The result is that the Fourier lens forms an image of the composite scattering pattern of all particles, the pattern being centered at a fixed point in the Fourier plane. This pattern is centered at the same fixed point regardless of the position or velocity of the particle in the sensing zone.

The individual scattering patterns from the many moving particles in the sample cell are superimposed, creating a single composite scattering pattern that represents the contributions from all the particles in the sample cell. Detectors placed in the Fourier plane record this composite scattering pattern. Over the course of a measurement, a running average is created from the changing flux patterns. When the duration of the measurement is long enough that the flux pattern accurately represents the contributions from all particles, an analysis of the resulting pattern will yield the true particle size distribution of the sample.

Determining PSD

The composite scattering pattern is measured by 126 detectors placed at angles up to approximately 35 degrees from the optical axis. When you view intensity in flux units (light intensity per unit area), you are looking at the scattering pattern.

In order to compute the size distribution, the composite scattering pattern is deconvolved into a set of individual number, one for each size classification, and the relative amplitude of each number is a measure of the relative volume of equivalent spherical particles of that size. This deconvolution is based on either the Fraunhofer or Mie theories of light scattering.

System Components

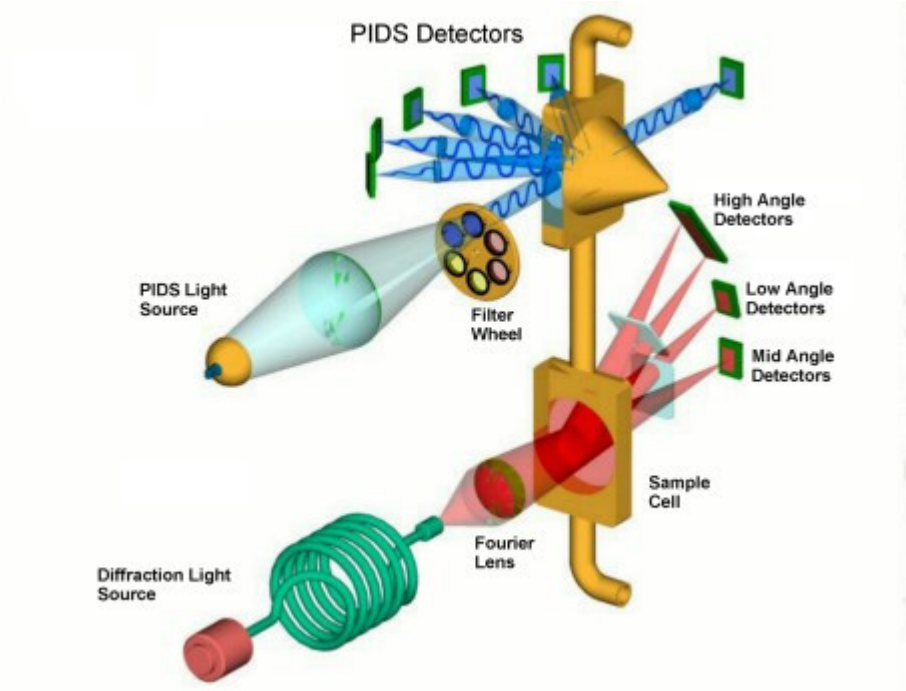
The LS 13 320 Optical Bench Overview

The LS 13 320 optical system is comprised of a source of illumination, a sample chamber in which the sample interacts with the illuminating beam, a Fourier lens system used to focus the scattered light, and an array of photodetectors that record the scattered light intensity patterns.

The laser's radiation passes through a spatial filter and projection lens to form a beam of light.

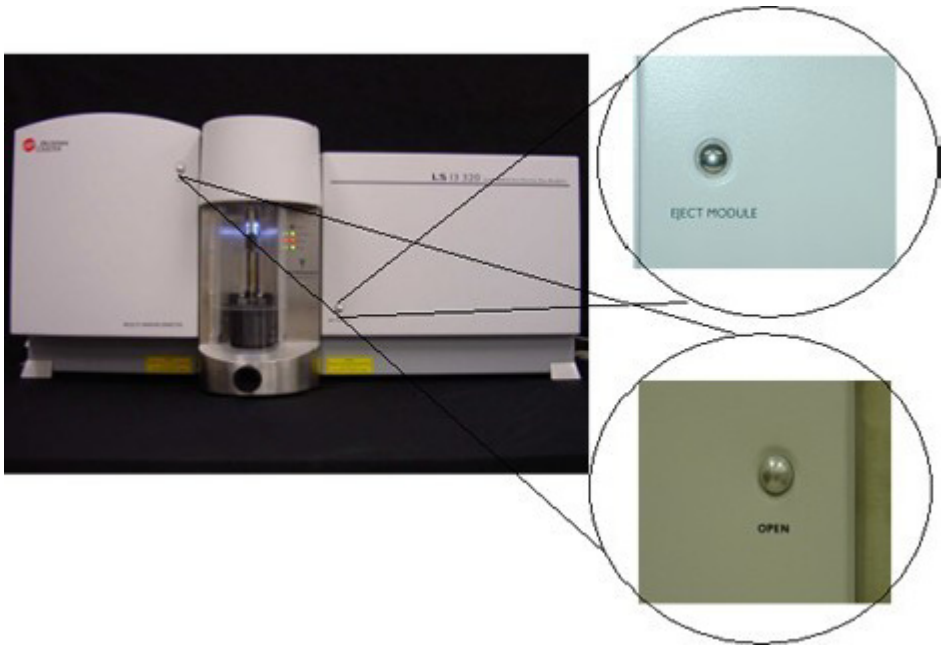
The beam passes through the sample cell where particles suspended in liquid or air scatter the incident light in characteristic patterns according to their size. Fourier optics collect the diffracted light and focuses it onto three sets of detectors, one for the low-angle scattering, the second for mid-angle scattering, and the third for high angle scattering. A block diagram of the LS 13 320 optical system is presented in Figure 4.

Figure 4 Optical System of the LS 13 320



The sample modules are attached to the optical bench by an automatic docking system that can be activated by the push of a button (Figure 5) or via software commands.

Figure 5 Module Eject and Door Open Buttons



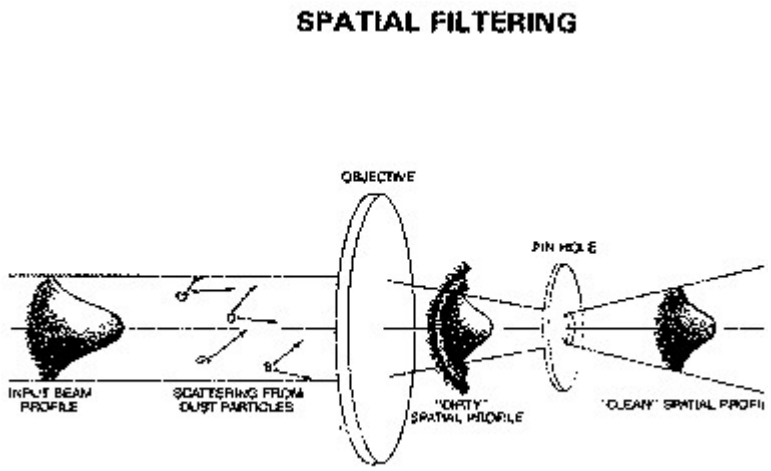
Before a module can be docked the sample cell chamber door must be retracted. This is easily done by pressing the **Open** button found on the left side of the optical bench (Figure 5).

Light Source

The LS 13 320 uses a 5 mW laser diode with a wavelength of 750 nm (or 780 nm) as the main illumination source. It also has a secondary tungsten-halogen light source for the PIDS system. The light from the tungsten-halogen lamp is projected through a set of filters which transmit three wavelengths (450 nm, 600 nm, and 900 nm) through two orthogonally oriented polarizers at each wavelength.

Light from a laser diode is monochromatic- a requirement of the theoretical models that describe a particle's scattering function. However, as opposed to gas or liquid lasers, the light from a laser diode is not “focused”. The monochromatic light from a laser diode must also be “treated” to produce a “clean” beam. The device that is most often used to condition an illuminating source is commonly known as a spatial filter. Most spatial filters consist of a set of optical elements - components such as lenses, pinholes, apertures, etc. - designed to refine the beam to the desired quality. Figure 6 depicts a mechanical spatial filter. One feature of the LS 13 320 is that it incorporates a (patented) fiber optic spatial filter.

Figure 6 Mechanical Spatial Filter



Sample Modules

The main function of the sample-handling module is to deliver particles in the sample, without discrimination to their sizes, to the sensing zone while avoiding the introduction of any undesirable effects such as air bubbles and/or thermal turbulence. The sample module is typically composed of the sample cell and a delivery system. The delivery system may include certain features such as a circulation pump, an ultrasonic probe, or stirring bars to help better disperse and circulate the particles. The LS 13 320 operates with sample cells designed for particles suspended in liquids or in dry powder form. These modules are:

- Tornado Dry Powder System (DPS)
- Universal Liquid Module (ULM)
- Aqueous Liquid Module (ALM)
- Micro Liquid Module (MLM)

In addition the ALM can be used in conjunction with the AutoPrep Station.

Computer System

The operation of the LS 13 320 requires a personal computer (PC) and the PC-based control and analysis software. If you are not using a Beckman Coulter-supplied PC, you must provide one that meets the minimum configuration requirements shown in Table 1, and preferably one that meets the recommended configuration requirements (shown in the same table).

Table 1 Minimum and Recommended Computer Configurations

Item	Minimum Configuration	Recommended Configuration
Microprocessor	800 MHz	1 GHZ Intel or equivalent
RAM	512 Mb	1 Gb or better
Hard Drive	20 Gb	35 Gb or better

Table 1 Minimum and Recommended Computer Configurations *(Continued)*

Item	Minimum Configuration	Recommended Configuration
Monitor	800X600	1024X768
Keyboard	Enhanced 101/102	Enhanced 101/102
Mouse	2 Button	3 Button
MS Windows Version	Win 98 or better	Win Vista

Software

The Microsoft Windows-based LS 13 320 control program provides both hardware control and data management. Among other functions, the program allows you to:

- Display, print, store, and export data.
- Customize (user interface) on-screen and printed reports.
- Define analysis profiles to automate your most frequently used analysis protocols.
- Use the built-in security features (supervisor modes, automatic 21 CFR Part 11 compliance function use with the compliant version of software only).

CHAPTER 1

Theory

The scattering of light is one of the most widely used techniques for measuring the size distribution of particles. In practice, the technique is fast and flexible, offering precise measurements that can be easily adapted to samples presented to the analyzer in various forms. The method involves the analysis (deconvolution) of the patterns of scattered light produced when particles of different sizes are exposed to a beam of light. The LS 13 320 series of instruments takes advantage of these principle to rapidly provide precise and reproducible particle size distributions.

Theoretical Background

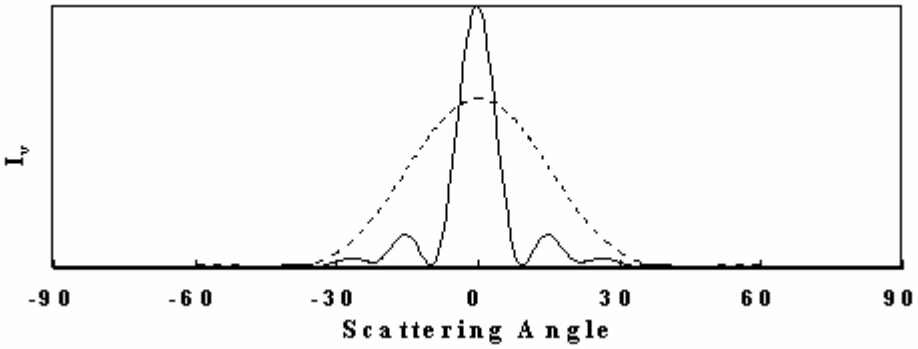
Light Scattering

When light illuminates a particle having a dielectric constant different from that of the medium, depending on the wavelength of the light and the optical properties of the particle, light will be scattered in a unique way. We commonly describe scattering phenomena in terms of diffraction, reflection, refraction, and absorption. When light interacts with the electrons bound in the material that re-radiate light, scattering is observed. Because most materials exhibit strong absorption in the infrared and ultraviolet regions which greatly reduces scattering intensity, most light scattering measurements are performed using visible light of wavelengths from 350 nm to 900 nm. The scattering intensity from a unit volume that is illuminated by a unit flux of light is a function of the complex refractive index ratio between the material and its surrounding medium. This intensity falls within the regime of Rayleigh scattering and is inversely proportional to the fourth order of the light wavelength, i.e., the shorter the wavelength, the stronger the scattering. The reason that the sky is blue at midday and red at sunrise or sunset is that one sees the scattered sunlight during daytime and sees the transmitted sunlight during dawn and dusk. Utilizing this wavelength dependence, we use red as the color for the stoplight and for all traffic control warning signs because red light has the least scattering power in the visible light spectrum. This allows the transmitted light to go through fog, rain, and dust particles and reach the intended detector: in this case the human eye.

Several technologies make use of light scattering to obtain information about materials. Among these technologies elastic light scattering (ELS) is the main method for the characterization of particles of sizes ranging from microns to millimeters. In ELS the scattered light has the same frequency as the incident light, and the intensity of scattered light is a function of the particle's optical properties and dimensions. In general, the scattered light intensity of a particle is a function of the following variables: particle dimension, particle refractive index, medium refractive index,

light wavelength, polarization, and scattering angle. The scattered intensity from a particulate sample is, in addition to the above variables, a function of particle concentration and particle-particle interaction. Some of the variables are constants in a particular experimental setup, such as light wavelength and the particle refractive index. In characterizing particle size using light scattering, one optimizes sample concentration to a proper range so that the sample will scatter enough intensity to enable the measurement to be completed with a desired signal to noise ratio, but not to scatter so much as to saturate the detecting system. Sample concentration is also optimized for minimal particle-particle interaction and minimal multiple scattering so that the measurement is performed based on elastic single particle scattering. In addition, in a light scattering measurement one has to assume that the refractive index and density of particles in the sample are uniform, which is true for most particulate systems. Thus, the scattered intensity is only a function of scattering angle, particle shape, and particle size. If the relations between scattering intensity, scattering angle, particle shape and particle size are known, one is able to resolve size distribution for particles of a particular shape from the measured angular scattering intensity pattern. Theories have been developed to aid in the extraction of the information needed for the determination of particle size from light scattering measurements.

Figure 1.1 Scattering Patterns for Spheres



Mie Theory

The Mie theory describes the interaction of light with a particle of arbitrary size as a function of angle, given that the wavelength and polarization of the light are known and that the particle is smooth, spherical, homogeneous, and of known refractive index. This theory is more complex than the theory set forth by Fraunhofer, in that it accounts for all possible interactions between particles and light, yet it's only applicable to spheres.

Spheres produce light scattering patterns that are characterized by the presence of scattering minima and maxima at different locations (Figure 1.1) depending on the properties of the particles. At small angles (typically smaller than 10 degrees) the scattering pattern for spheres is centrally symmetric instead of axially symmetric, i.e., it displays concentric rings in the direction of the incident light. Therefore, large particles produce scattering intensities that are concentrated at small angles and due mainly to diffraction effects from the edge of the particle.

3.1.2 Fraunhofer Theory

When the particle size is much larger than the wavelength of light or the materials are highly absorptive, the edge effect of the particles contributes more to the total scattered intensity. Interference effects are now due to the bending of light at the particle's boundary (diffraction). In a light scattering measurement, because the light source is far away from scatterers and the optics are usually designed so that the incident beam illuminating the scatterers is homogeneously parallel, only Fraunhofer diffraction will take place. For these spheres, the Fraunhofer diffraction is just a simplified form of the Mie theory with the limiting condition that d >> l.

Fraunhofer theory can only be used for particles that are 1) much larger than the wavelength of the light (typically > 30 mm) and non-transparent, i.e., the particles have different refractive index values than that of the medium (typically with the relative refractive index being larger than 1.2), or 2) highly absorptive (typically with absorption coefficients higher than 0.5). In Fraunhofer theory the refractive index of the material is irrelevant because for large particles the scattering intensity is concentrated in the forward direction -- typically at angles smaller than 10 degrees. For this reason Fraunhofer diffraction is also known as forward scattering. The angle for the first minimum of scattering intensity is simply related to the size by EQ 1.

EQ 1

$$\sin\theta = \frac{1.22 * \lambda}{d}$$

Most of the scattering intensity is concentrated in a very sharp central lobe, which provides a much simpler solution applicable to sizing large particles in a light scattering measurement.

Light scattering is an absolute measurement technology only in the sense that once the experimental setup is correct, calibration or scaling are not necessary in order to obtain the volume (or weight) percentage of each component. In addition, choosing a correct optical model is often the key step in obtaining the correct results.

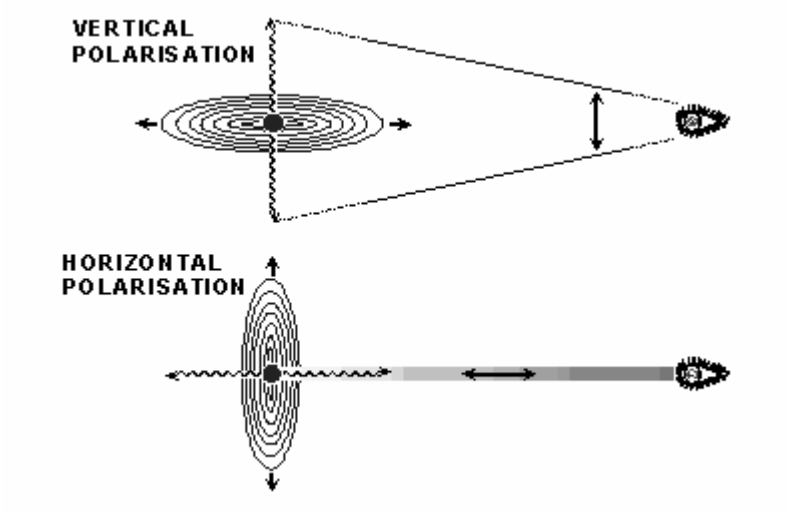
PIDS

Many samples have particle sizes that extend into the submicron range creating a wider size distribution range. However, as a particle size gets smaller, the ratio of particle dimension to light wavelength (d/l) is reduced. Interference effects are thus reduced and the scattering pattern becomes smoother and less angular dependent. At this smaller size range, the sensitivity of particle size to scattering intensity pattern is greatly reduced causing it to be more and more difficult to obtain correct size values. Obviously, if light of a short wavelength is used, the ratio will be great, and so the lower size limit will be effectively extended. Combining the polarization effects of light scattering with the wavelength dependence at high angles, we can extend the lower size limit to as low as 40 nm, almost reaching the theoretical limit. This is the patented Polarization Intensity Differential Scattering (PIDS) technology.

The origin of the polarization effect can be understood in the following way. If a small particle, much smaller than the light wavelength (d << l), is located in a light beam, the oscillating electric field of the light induces an oscillating dipole moment in the particle, i.e., the electrons in the atoms comprising the particle move back and forth relative to the stationary particle. The induced motion of the electrons will be in the direction of oscillation of the electric field, and therefore

perpendicular to the direction of propagation of the light beam. As a result of the transverse nature of light, the oscillating dipole radiates light in all directions except in the direction of oscillation as visualized in Figure 1.2. Thus, if a detector is facing the direction of oscillation it will receive no scattering from single dipoles. When the light beam is polarized in either the vertical or the horizontal direction, the detected scattering intensity Iv and Ih at a given angle will be different. The difference between I and Ih (I-Ih) is termed the PIDS signal. In addition, because the PIDS signal varies at different wavelengths, measurements of the PIDS signal at several wavelengths will provide additional information that can be used to further refine the size retrieval process.

Figure 1.2 Scattering From Different Polarizations



Since the PIDS signal is dependent on particle size relative to the light wavelength, valuable information about the particle size distribution can be obtained by measuring the PIDS signal at a variety of wavelengths.

3.1.4 Particle Non-Sphericity & Light Scattering

Laser Diffraction Measurement for Non-spherical Particles

Many industrial particles, such as soils, sands and mineral powders are irregularly shaped and can have either a smooth or a rough surface. The analysis strategy that all commercial instruments employ in the measurement of randomly oriented particles is to use a spherical approximation and treat every particle as a sphere regardless of the real shape. This method produces a size distribution with one variable, diameter, which is easily traceable and comparable to the results obtained from other technologies. This method sometimes works well for many irregularly shaped particles. Because the finite detector area integrates and smoothes out the intensity fluctuation caused by any surface roughness, and additionally because there is a smoothing effect from the rotation of the particles, the scattering patterns approximate those created by spheres. However, for many particles, because of their deviation from perfect sphericity, the size distribution obtained is only apparent or nominal, and will be biased. In certain extreme cases the results of using a spherical model on non-spherical particles will be very different than from the truth. This type of systematic bias often shows up when comparing laser diffraction results with those obtained from other methods (see Particle Characterization: Light Scattering Methods, R. Xu, Kluwer Academic

Publishers, 2000, and Comparison of sizing small particles using different technologies, R. Xu, O. Diguida, Powder Technology, 2003). As it has been summarized in the international standard ISO 13320-1:1999 Particle Size Analysis – Laser Diffraction Methods- Part 1: General Principles the deviation of particle shape from a sphere can be one of the many sources of bias:

"6.6.3. Another main source for bias arises from the departure from the theoretical assumption for the particulate material. Again, the errors can come from different sources.

- *Firstly, most particles in real life do not fulfill the assumption of sphericity. Non-sphericity of particles leads to different cross-sections in different orientations. Since particles are generally measured in all possible orientations, this leads to some broadening of the particle size distribution as compared to the equivalent volume distribution. Moreover, the median and mean diameter may be shifted, often to a larger size.*
- *Secondly, the particle surface may be rough instead of smooth. This causes diffuse light scattering at the boundary, which often has a similar influence as absorption of light within the particle.*
- *Thirdly, the particles may be optically heterogeneous, as is the case for porous particles. This may lead to an apparent presence of significant amounts of very small particles, which are non-existent."*

PIDS Sizing

As mentioned above, with its patented PIDS assembly the LS 13 320 can provide information about particles within the range of 0.017 microns. Particles in this range offer limited information in diffraction patterns, so the PIDS technology is required to accurately measure these small particles.

Components

The PIDS assembly consists of:

- An incandescent light source and polarizing and bandpass filters
- PIDS sample cell
- An additional seven photodiode detectors, six to measure scattered light plus one to monitor the beam strength

Functions

The PIDS assembly functions are:

- To illuminate sample particles sequentially with beams of light centered at three different wavelengths polarized vertically and then horizontally (relative to the plane containing the light source, scattering region, and detectors) at each wavelength.
- To determine the difference in scattered intensity between the vertical and horizontal polarizations as a function of angle for the three wavelengths of light.

Description

The PIDS assembly provides the primary size information for particles in the 0.017 µm to 0.4 µm range. It also enhances the resolution of the particle size distributions up to 0.8 µm. This additional measurement is needed because it is very difficult to distinguish particles of different sizes by diffraction patterns alone when the particles are smaller than 0.4 µm in diameter.

The PIDS assembly in the Optical Module uses an incandescent tungsten-halogen lamp and three sets of vertically and horizontally polarized filters to provide polarized monochromatic light at three different wavelengths: 450 nm (blue), 600 nm (orange), and 900 nm (near-infrared, invisible). The light is focused through a slit and is formed into a narrow, slightly diverging beam that is projected through the PIDS sample cell.

The PIDS assembly measures the pattern of the difference in scattering of vertically and horizontally polarized light. To obtain as much information as possible about the small particles measured by the PIDS assembly, it illuminates the particles sequentially with vertically and horizontally polarized light of the three different wavelengths (colors). The three wavelengths of light permit three independent measurements of the PIDS effect at different ratios of particle sizes to light wavelength.

For each of the three wavelengths, the differential scattering pattern (PIDS pattern) is observed at six detectors centered at a scattering angle of about 90°. In total, the PIDS assembly makes 36 measurements of the light scattered from the sample: scattered light of two polarizations at three wavelengths and six scattering angles (2 x 3 x 6 = 36). A seventh detector on the opposite side of the cell from the light source measures the intensity of the unscattered light to determine the amount of obscuration. The 36 measurements are then reduced to 18: the difference in scattering between the two polarizations measured at each of the three wavelengths and five scattering angles. These 18 measurements form a pattern that varies sensitively with particle size.

The PIDS measurements are added to the same deconvolution matrix that is used for diffraction sizing. The relative volume of particles in each size channel is determined by a solution for this matrix. The analysis is completely integrated, so although two methods are used, a single solution is obtained.

CHAPTER 2

Installation

⚠ CAUTION

Risk of possible damage to instrument. Unpacking the LS 13 320 without full installation knowledge of the instrument could result in damage or not properly identifying damage during shipment. Do NOT unpack the instrument. An authorized Beckman Coulter Service Representative is responsible for unpacking, installing, and setting the instrument for you.

Each LS 13 320 is tested before shipping. International symbols and special handling instructions are printed on the shipping cartons to inform the carrier of the precautions and care applicable to electronic instruments.

Installation Requirements

Environment

For best performance of the LS 13 320, find a site that is:

- Clean and free of dust
- Well ventilated
- Smoke free
- Isolated from external vibration sources
- Temperature controlled as large or frequent variations in temperature can change the alignment of the laser beam

The system requires a flat, stable surface at least 0.7 m (2.5 ft) wide and 1.8 m (6 ft) in length. A height clearance of at least 0.7 m (2.5 ft) is recommended.

Power Requirements

⚠ CAUTION

If you plan to use a power strip other than one recommended by Beckman Coulter, please call your local Beckman Coulter representative to be sure that your power strip is compatible with your instrument.

Make sure the instrument is close enough to a power receptacle so that the AC power cable reaches it without stress. Power requirements are shown in Table 2.1 and Figure 2.1 shows the electrical requirement labels located in the rear lower right corner of the bench.

Table 2.1 Table 4.1 LS 13 320 Power Requirements

Power Category	Requirement
Input Voltage (V)	100, 120, 220, 240
Input Frequency (Hz)	50/60
Power Consumption (W)	720 Maximum

It is recommended that a surge protector be used to ensure correct operation in the presence of electrical transients and noise. At least three 3-wire service outlets are needed for the instrument with its computer, monitor and printer.

Figure 2.1 Electrical Specification Labels

Temperature and Humidity Requirements

Temperature: 10 to 35° C
Relative Humidity: Zero to 85% without condensation

Sample Modules Specific Requirements

Suspension Fluid for Aqueous and Universal Liquid Modules

IMPORTANT Be sure that the material being sized and the suspension fluid do not violate any hazard waste or clean water discharge regulations.

The Aqueous Liquid Module requires clean, bubble-free liquid as its suspension fluid. Regular tap water that is filtered and degassed can be used. We recommend that you use a 0.2 µm or smaller pre-filter.

Drain for Aqueous Liquid Module

Connect the drain hose to a wastewater drain located near the instrument.

Drain for Universal Liquid Module

If a suspension fluid other than water is used, connect the drain hose to a dedicated waste disposal container. It is the users responsibility to properly dispose of any hazardous samples and suspension fluids used in the instrument.

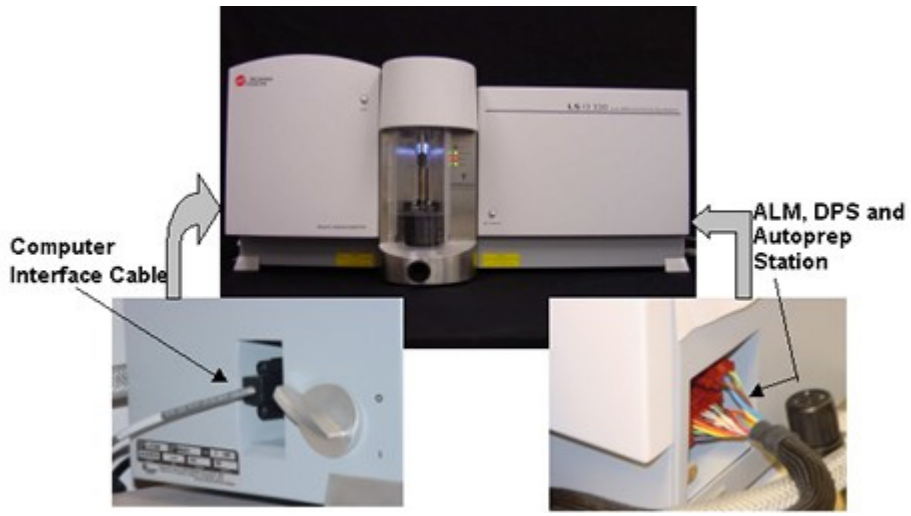
Vacuum for Tornado Dry Powder System

The Dry Powder System requires a vacuum source (optionally supplied by Beckman Coulter) for operation. If you choose to supply one, you will need a unit capable of sustaining rates up to 420 L/minute at 740 to 750 torr (15 cfm at 5 to 10 inches of water below atmospheric pressure). A remote power switch and hoses are also needed to connect your vacuum source to the Dry Powder System. Your Beckman Coulter Representative can recommend a vacuum source for your system.

Hardware Connections

See Figure 2.2 for the location of interunit connections as well as the on/off switch on the Optical Bench. The location of ports on the computer, monitor and printer may vary according to the hardware you purchase with your unit. Check the appropriate manufacturer's manual.

Figure 2.2 Location of Interunit Connections



Software Installation

Installing and Starting the Software

The installation of the software is carried out automatically when you insert the CD into your computer CD drive. Just follow the steps as you're prompted to complete the installation.

Configuring Software Parameters

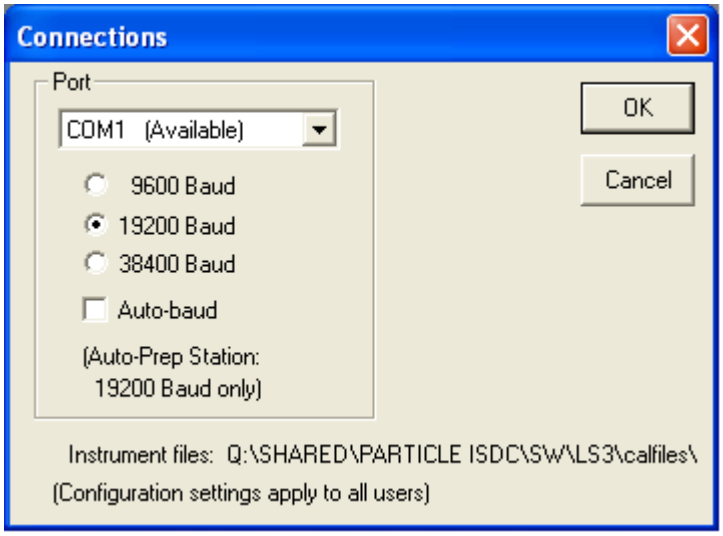
To configure the connection parameters for your LS 13 320 and PC:

- From the LS 13 320 software program select **Security**.
- From the security dialog box, select **Enter Supervisor Mode**.
- Select **Connections**.
- The dialog box with the connection settings appears (Figure 2.3).

In the Connections Parameters box:

- **Port** - accept the current setting or check the radio button, ⊙, to select a different communications port.
- **Baud Rate** – verify that the speed is set to 19200 Baud. If another speed is displayed, check the 19200 Baud radio button.

Figure 2.3 Connections Dialog Box



Instrument Verification

Post-Installation Verification

To verify the performance of your LS 13 320, a Beckman Coulter control sample must be analyzed. The acceptable limits are listed on the assay sheets enclosed with the control samples.

See Getting Started

Daily Verification

We recommend that a control sample be run daily to verify your instrument's performance. Calibration is determined by the optical design. Therefore, no calibration is required. Measuring electrical offsets and aligning the laser beam makes all necessary adjustments.

See Getting Started

Beckman Coulter offers a series of control samples to check the operation of your instrument. A quality control program is also available, which is a convenient way to have the performance of your instrument tracked and any problems identified by qualified Beckman Coulter personnel.

Getting Started

The PC software has been designed to control all the operations of the LS 13 320. Once the instrument and its corresponding module have been set up and the software installed, click on the program icon to launch the program. When starting up the instrument after it has been shut down, allow the instrument to warm up for approximately 20 minutes.

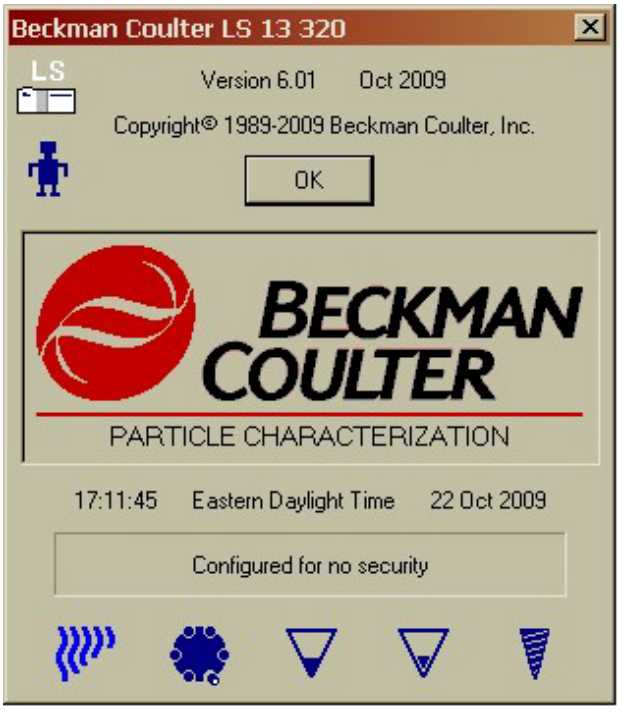
Starting the Instrument and its Control Program

When the program is first launched, the first view presented by the program is the copyright acknowledgement box (Table 2.2).

OK launches the control program. Table 2.2 shows the status screen after selecting **OK** from the above screen.

Table 2.2 Initial Dialog Box After Software Launch

Non-Compliant Software



Compliant Software

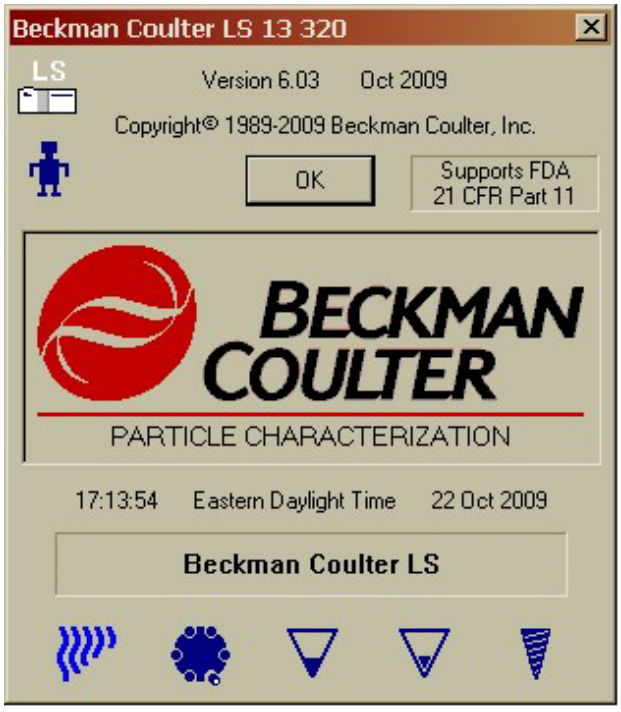
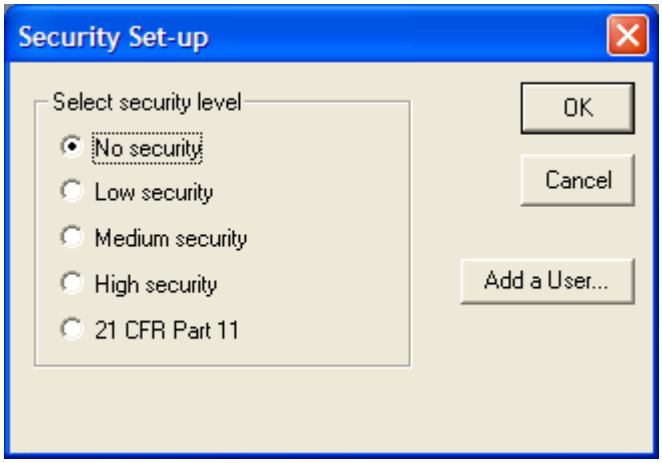
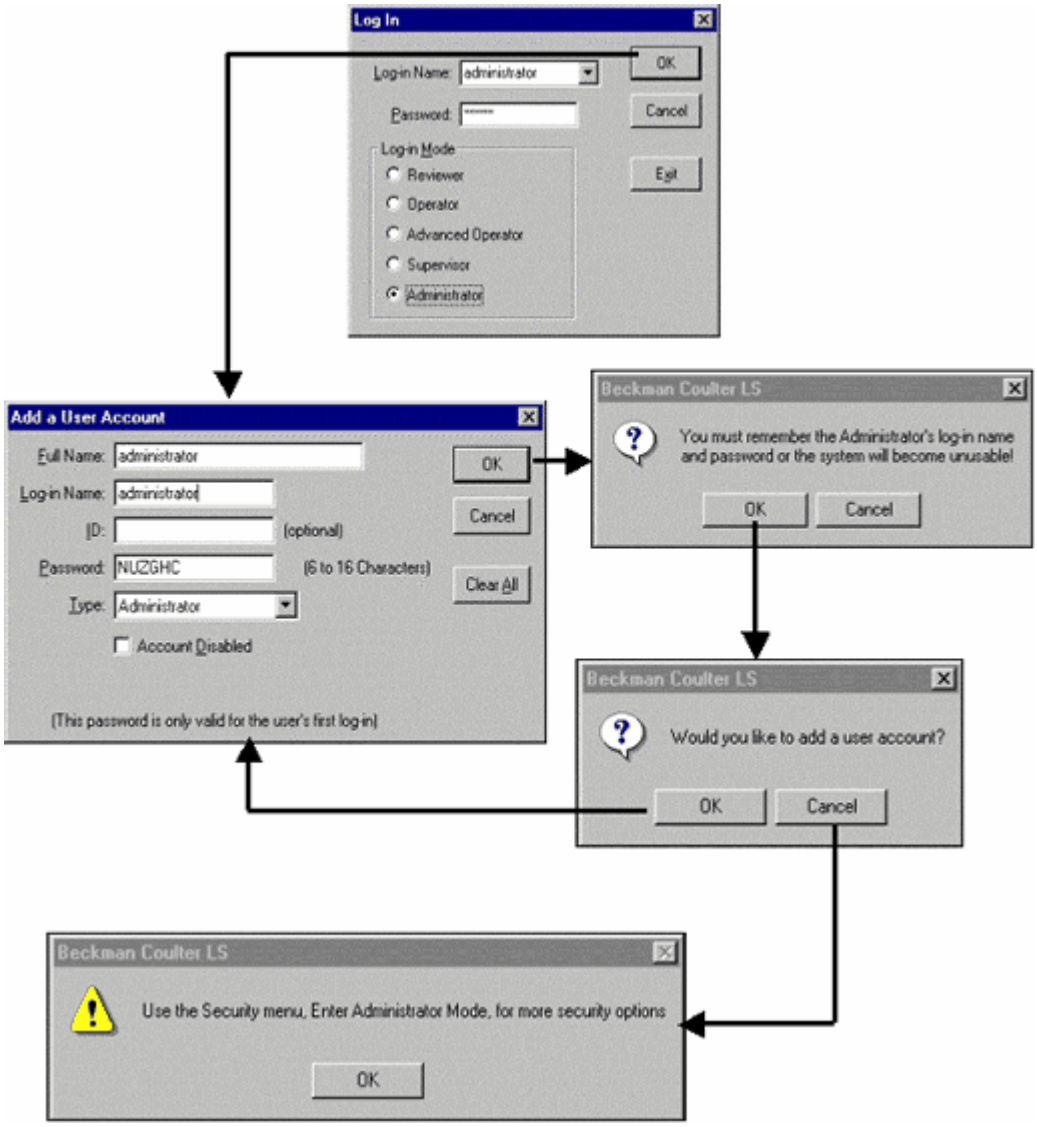


Figure 2.4 Security Level Options



The status screen shown in Figure 2.4 allows you to select the security level you wish to use. For more on security levels refer to Security Options. Once a security level has been selected, the dialog screen shown in Figure 2.4 will not be displayed again. Figure 2.5 shows a diagram of the steps and dialog screens to follow after selecting a security level.

Figure 2.5 User Set-up Flowchart



Selecting a Module

The optical module is automatically recognized by the optical bench when the module is docked into the optical bench.

Run from the menu bar (Figure 2.6).

Use Optical Module (COM 1). If there is no module connected to the LS 13 320 or there is a problem with communication between the computer and the LS 13 320 bench, an error message will be displayed, as shown in Figure 2.7.

Figure 2.6 Selecting An Optical Module

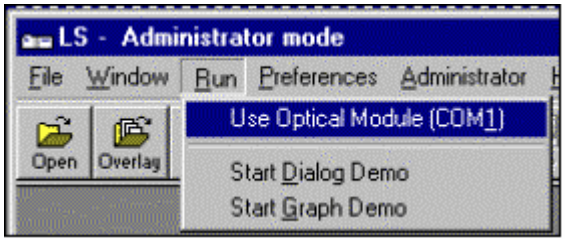
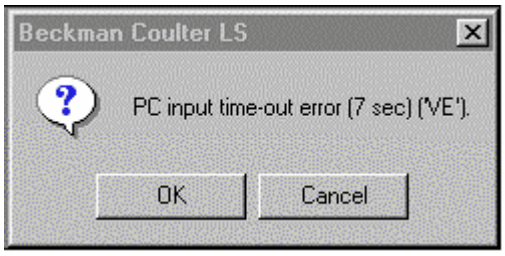


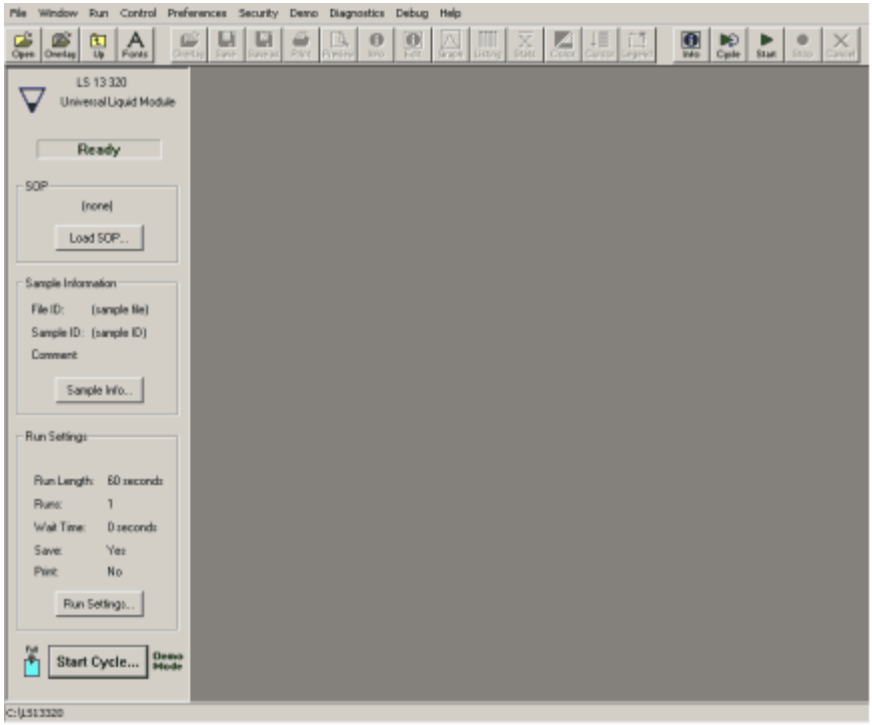
Figure 2.7 Message When An Optical Module Is Not Found



IMPORTANT If this occurs, ensure that an optical module is connected to the LS 13 320 bench. Make sure that the module is docking properly; otherwise contact your Beckman Coulter Service Representative. Also, check the connections between your computer and the LS 13 320 bench.

Once a module has been identified the screen shown in Figure 2.8 will be displayed.

Figure 2.8 Ready Screen



Making Measurements

In order to obtain the light intensity patterns (diffraction or PIDS) described in previous chapters, from which the size distribution is calculated, a number of functions have to be performed by the instrument. These are explained below in the order they are completed during a complete run cycle.

Measuring Offsets

With the laser off, the offset or bias voltages of the amplifier circuit are measured in order to 'zero out' (or establish a baseline of any electrical noise) at a detector channel. This is necessary because the signal at a detector channel (a detector channel consists of a light sensitive detector surface and the associated amplifying electronics) does not necessarily go to zero in the absence of light. Therefore, to accurately measure the light intensity the offset voltage must be measured and subtracted from the scattering signal from the sample.

Alignment

During this step the laser beam is automatically aligned at the center of the detector array. In order to accurately measure the light intensity as a function of scattering angle, the laser beam must be precisely positioned. In the LS 13 320, the laser beam is aligned within 1-2 microns of the center of the detector array on the horizontal and vertical axes.

Measure Background

Here the light intensity levels received by the detectors are measured with no sample in the system, and then subtracted out. This procedure eliminates the signal due to the outer edges of the Gaussian laser beam, in addition to any light leakage or scattering from dust on the lenses or any other particulate present in the optical path. The background should always be measured prior to adding sample to the system.

Measure Loading

This function measures the amount of light scattered out of the beam by the particles so as to determine an appropriate concentration of sample. Enough sample is needed to provide an acceptable signal-to-noise level in the detector channels, but if too many particles are present, light already scattered from one particle will likely be scattered from another, blurring the light intensity pattern. The obscuration reported on the title bar of the run window, or on the status bar, is the percentage of light scattered out of the beam by the particles. When sizing particles without using PIDS an obscuration level of 8% to 12% is appropriate, except when using the Dry Powder Module in which case an obscuration of 4% to 7% is sufficient. When PIDS is used, a PIDS obscuration of 40% to 60% is recommended.

IMPORTANT When analyzing materials with wide size distributions requiring the use of PIDS and diffraction to detect small amounts of large particles. It is recommended that diffraction obscuration is increased regardless of whether the PIDS obscuration is above the maximum recommended value.

Running a Control

After performing the installation functions, the instrument is ready to measure the light intensity patterns and calculate the particle size distribution. The above steps are recommended when analyzing a sample in order to obtain consistent and accurate results. The selection of the controls (provided by Beckman Coulter) to be used will depend on the sample module being used and are listed in Table 2.3. The following section describes the procedure to follow in order to verify the operation of the instrument by running a control sample.

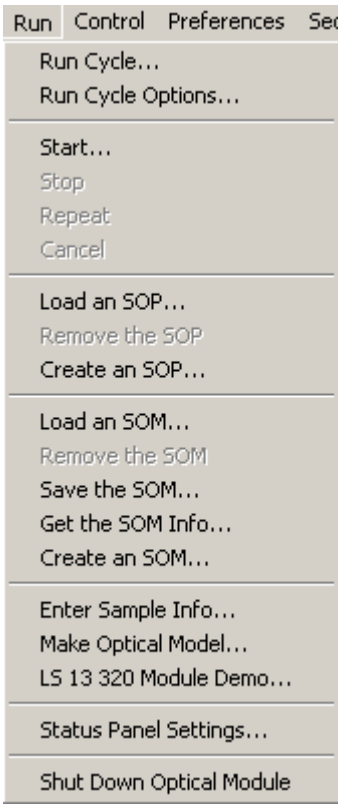
Table 2.3 Controls Used With Specific Sample Modules

Sample Module	Control
Universal Liquid Module (ULM)	Latron 300LS, G15, GB 500
Aqueous Liquid Module (ALM)	Latron 300LS, G15, GB 500
Tornado Dry Powder	G35, GB 500
Micro Liquid Module	G15

1 **Run.**

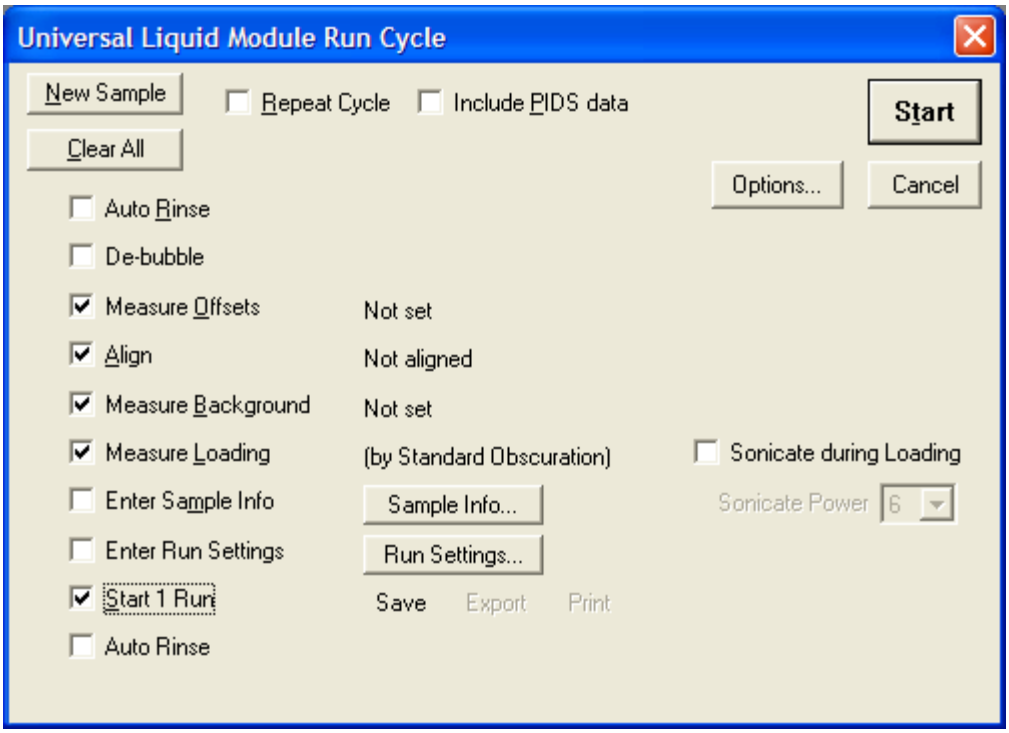
2 **Run Cycle** (as shown in Figure 2.9).

Figure 2.9 Run Menu



3 **New Sample** and select the **Include PIDS** check box (see Figure 2.10) if your sample contains particles below 0.4 µm (ULM and ALM only).

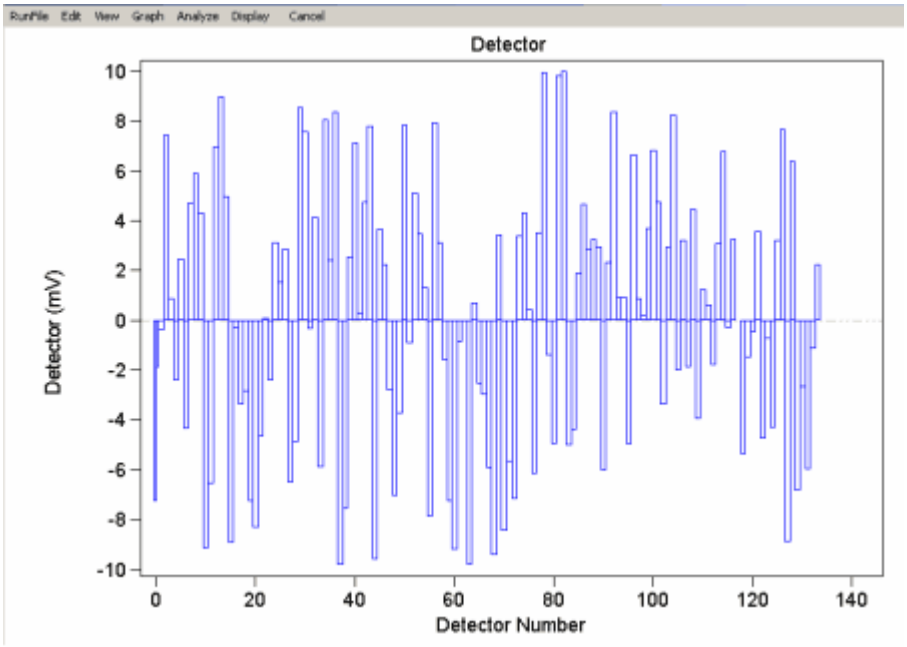
Figure 2.10 Run Menu



4 **Start**. A sequence of steps will automatically follow after selecting **Start** as indicated below.

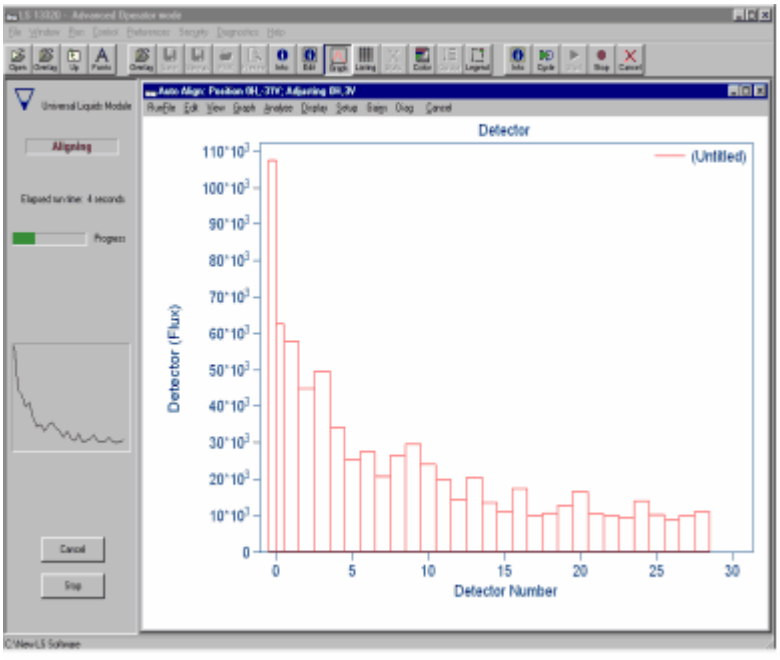
a. Measuring offsets

Figure 2.11 Measuring Offsets Screen



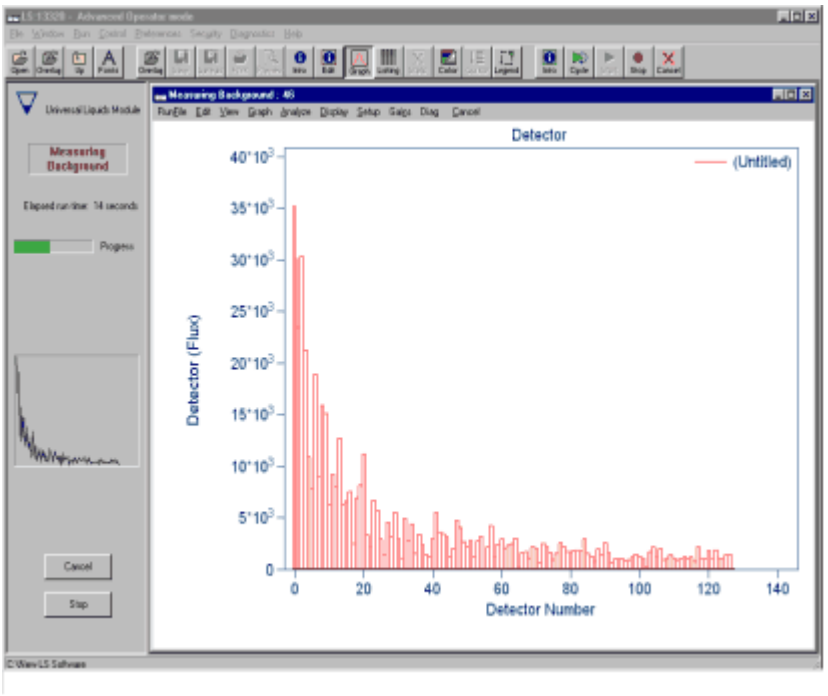
b. Alignment

Figure 2.12 Auto Align Screen



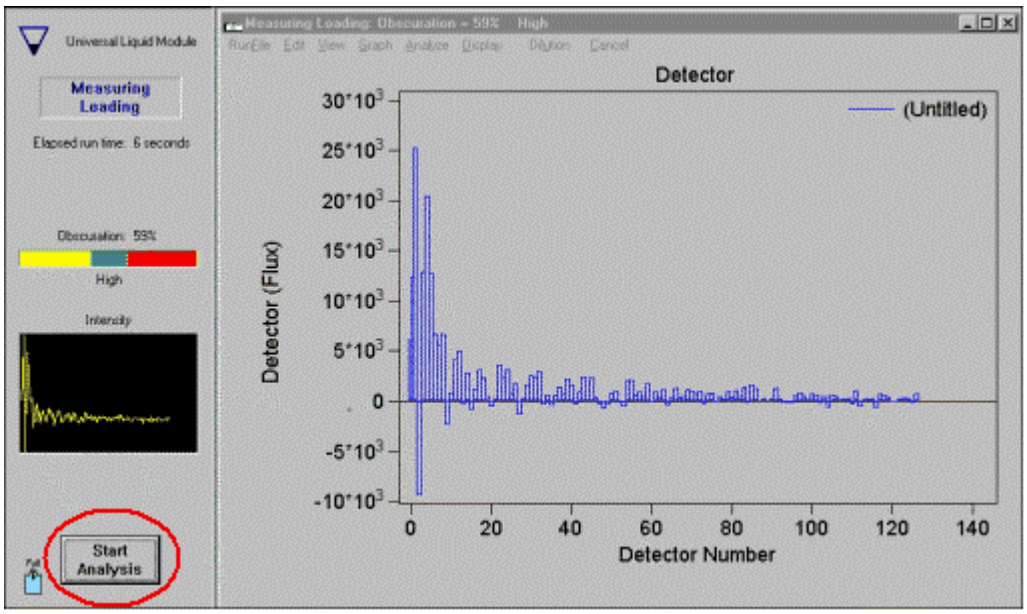
c. Background measurement

Figure 2.13 Measuring Offsets Screen



d. Measure loading

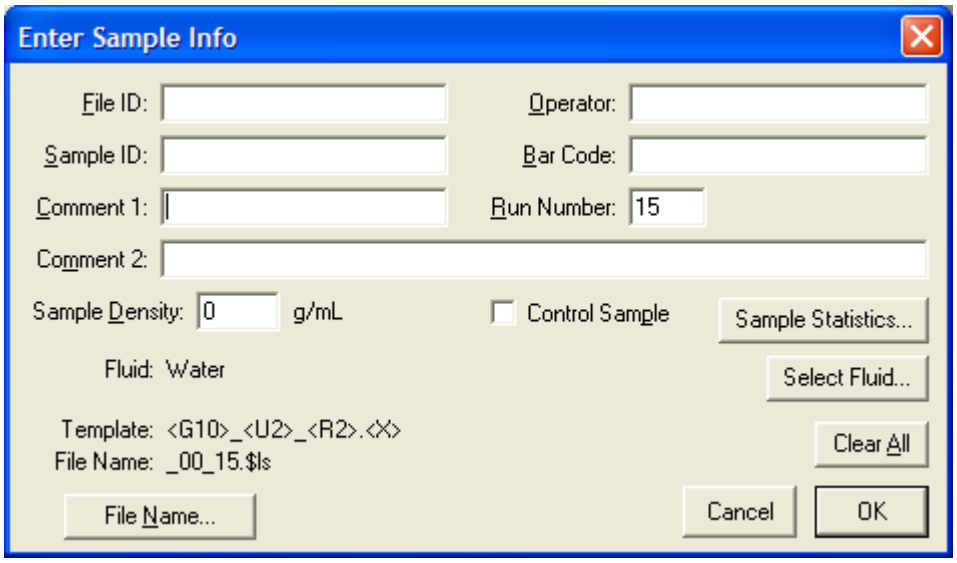
Figure 2.14 Measuring Obscuration (Loading) Screen



Once the correct obscuration is obtained, click on the **Start Analysis** button to continue to the next step.

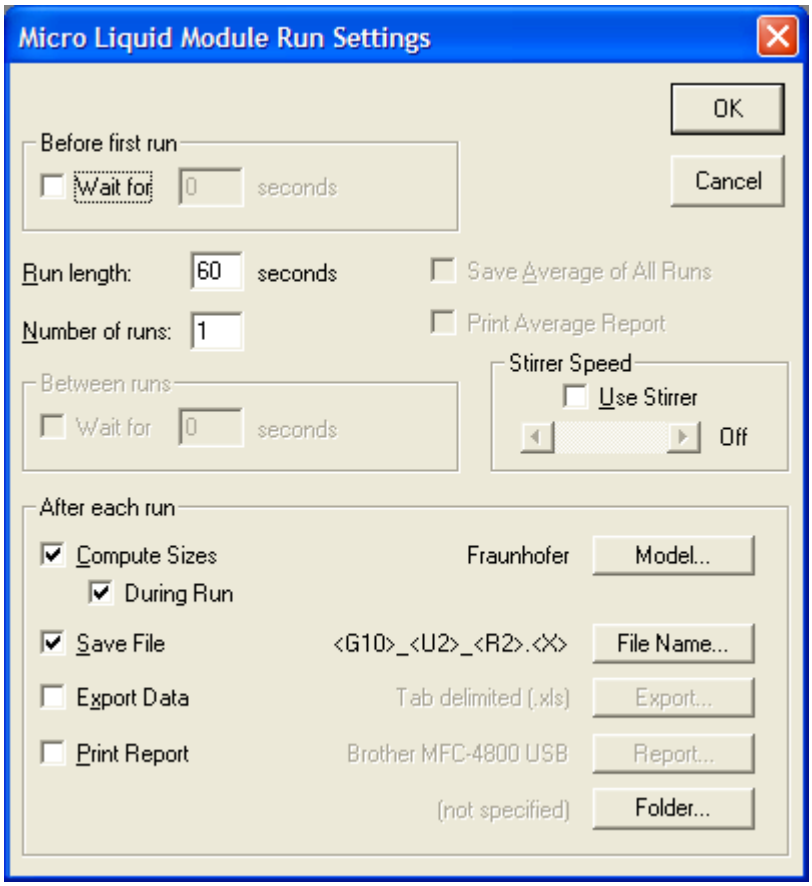
e. Sample information

Figure 2.15 Sample Info Dialog



f. Run information

Figure 2.16 Run Settings Dialog



5 Complete the sample and run information dialog boxes with the pertinent information.

The above procedures can be greatly simplified by using either the Standard Operating Method and/or Standard Operating Procedure features. For more on these procedures see the sample module section.

Running a Sample

Once the performance of the instrument has been confirmed to be correct by an analysis with a control, a sample can then be analyzed. The validity of the results from an unknown sample is based on the good performance of the instrument, as supported by the control analysis, and by the correct preparation of the sample in question. See APPENDIX B, *Sample Handling* for more information on sample dispersion and preparation. The steps to follow to analyze a sample are similar to those used to analyze a control sample. SOPs can also be created to set up the analysis of a particular sample.

1 **Run > Run Cycle** – Figure 2.9.

2 Under **Run Cycle** select **New Sample**. If PIDS is to be used select the **Include PIDS data** check box to activate this option. Select **Run Settings** – Figure 2.10.

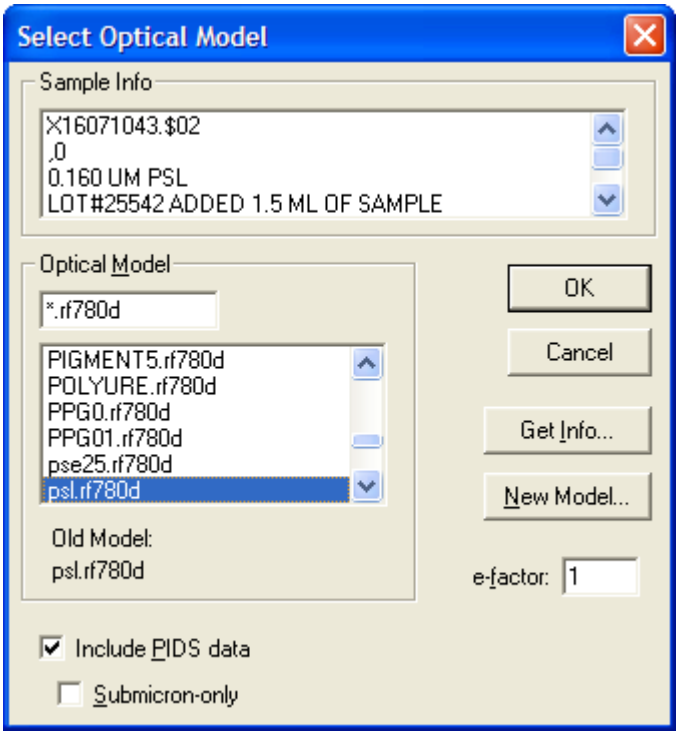
3 In the Run Settings screen (Figure 2.16) choose 60 seconds for the **Run Length**. When using PIDS select 90 seconds for the **Run Length**. Under **After each run** click on the **Compute Sizes** and **Save File** check boxes. Select the **Model** option (Figure 2.17) and choose the correct optical model. If the optical model you need is not found in the list of optical models, you may create a new one that will suit your particular sample (See APPENDIX C, *Optical Models*).

4 **OK**.

5 **Start**.

6 From this point on the sequence of steps is the same as described in the previous section Running a Control.

Figure 2.17 Optical Model Dialog Screen



As with controls, the sample analysis steps can be simplified through the use of Standard Operating Methods and Procedures (see your specific sample module manual for more information).

Reference Background

A reference background can be created for each particular sample module. The reference background can be subsequently used to compare how the module is operating. The reference background must be created with the best background obtained during a run that indicates that the module windows are clean and, if using the ULM, MLM or ALM, the diluent is particle free. When comparing backgrounds from different analysis runs with the reference background, a deviation from the reference background is an indication that the sample module should be inspected (and cleaned if necessary) or the system needs servicing. See the individual sample module section for maintenance procedures.

To create a reference background:

1. Perform a sample analysis. If the background is within the recommended value (less than 2×10^6) you may select this file as your reference background.

2. From the **Run** menu select **Run Cycle Options** (Figure 2.18).

3. Select **New Reference Background**.

4. From the dialog box select the file you want to use as the reference background file. Click **Open**. The background from the file you select will then be saved in the calibration files folder using the following filename format: ZXBYYYY.$ls, where:

   X = L for the ALM
   U for the ULM
   M for the MLM
   P for the Tornado DPS

   Y = the last four digits of the optical LS 13 320 bench serial number

Figure 2.18 Run Cycle Options Dialog

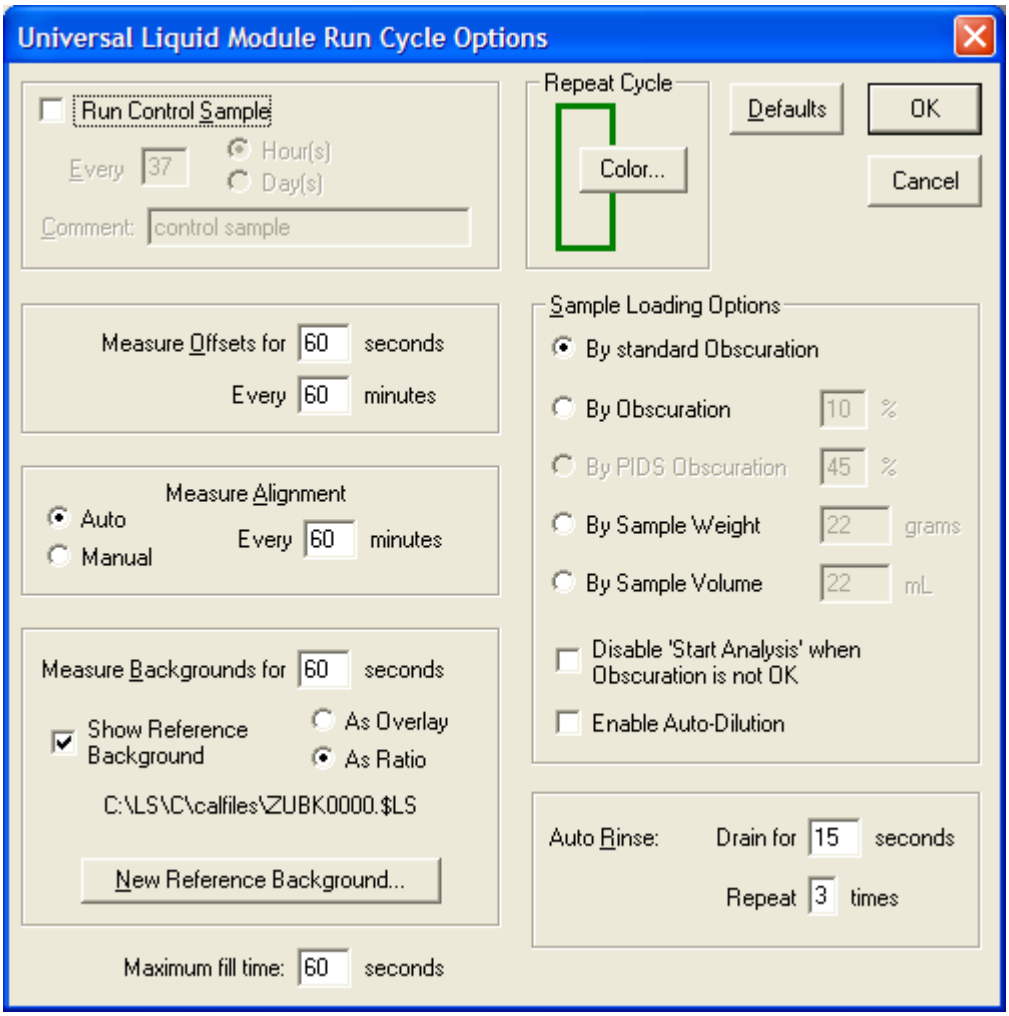
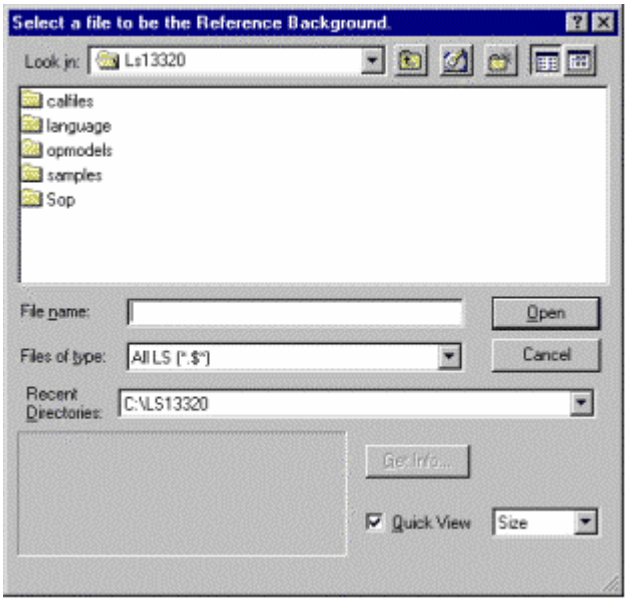


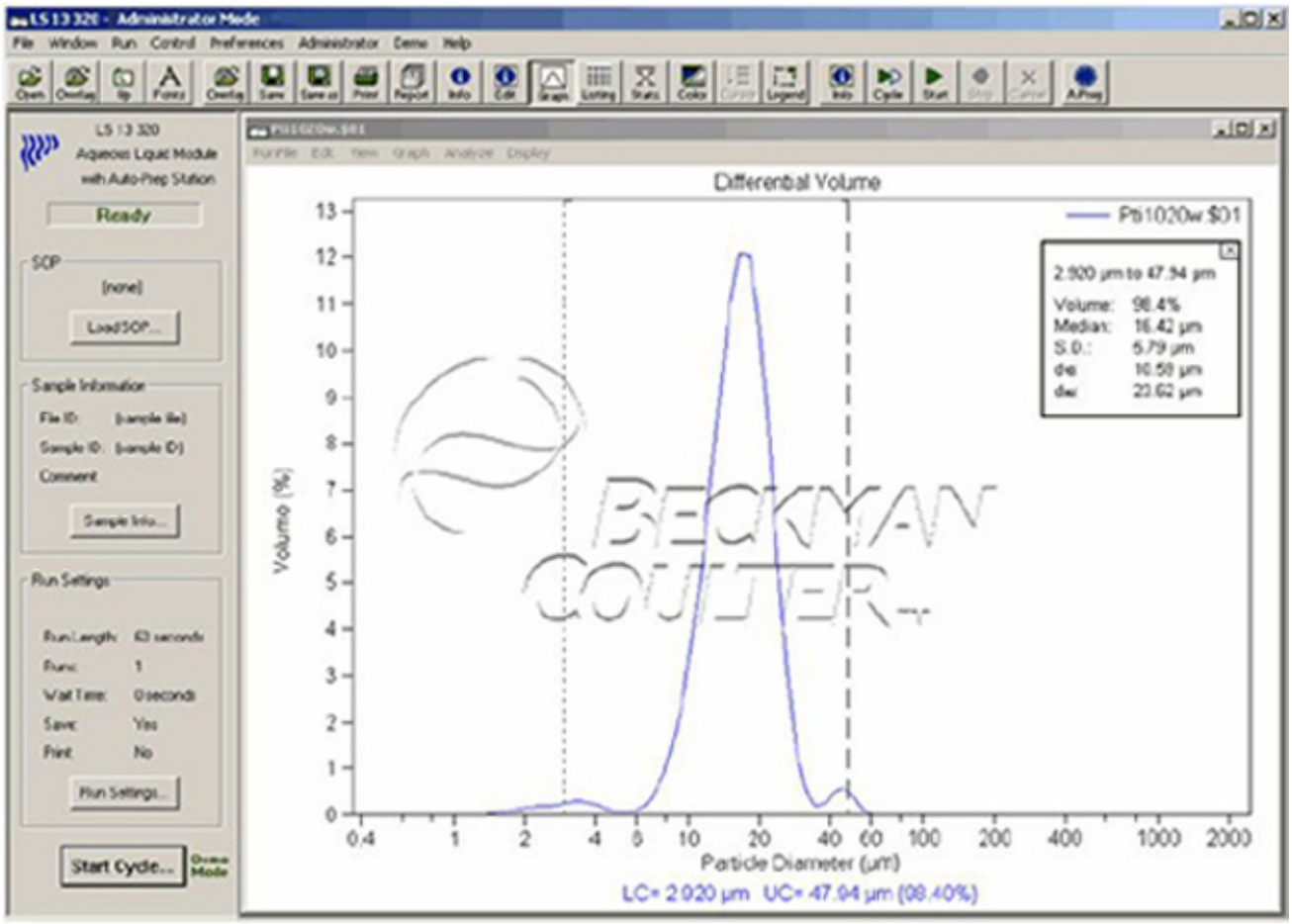
Figure 2.19 Reference Background File Selection



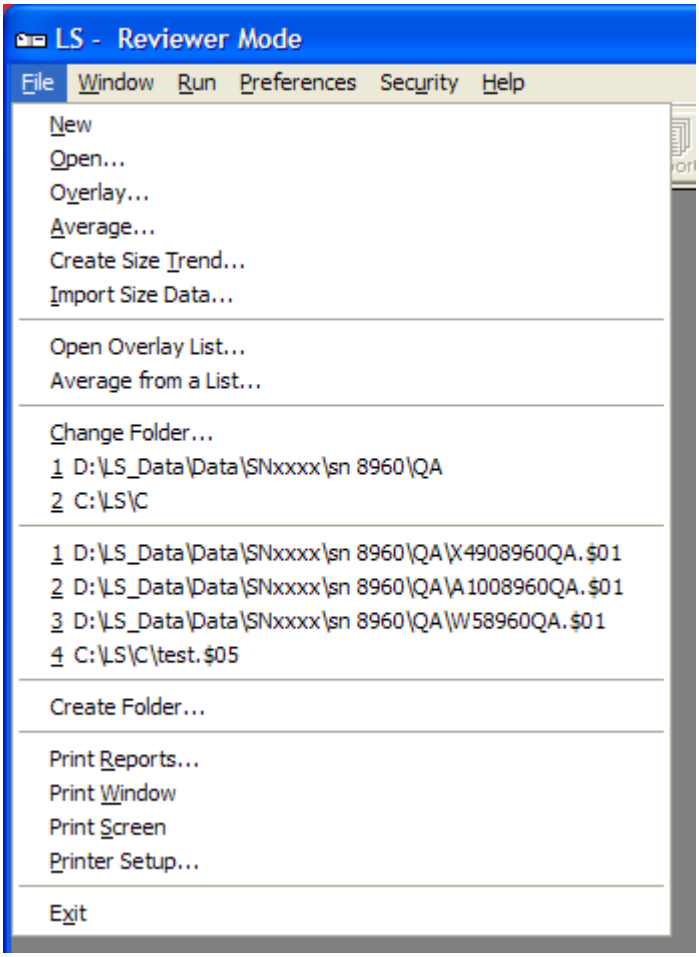
CHAPTER 3

LS 13 320 Software

Figure 3.1 LS 13 320 Software



File Menu Options



New...

The **New** command opens a blank window labeled "Untitled". Use the **New** command to manually enter channel data for a new data file. This function is often useful in the creation of "Standard" or "Reference" data sets.

Open...

The **Open** command displays a dialog containing a list of stored files. Select the file you wish to open either by highlighting the file and using the **Open** button, or by double-clicking on the filename in the list.

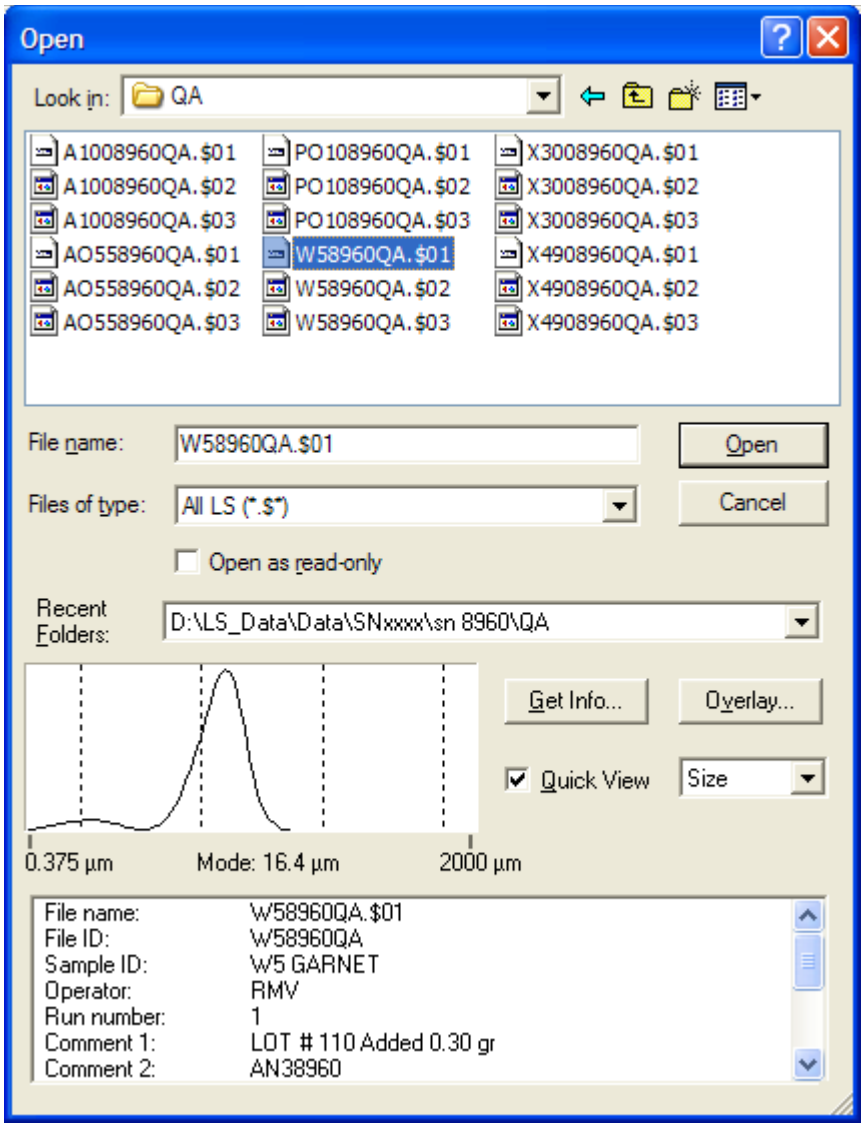
NOTE n order for a file to be displayed by default in the dialog box, whether new, imported, or acquired directly from the LS 13 320, it must have an extension that matches what is shown in the **Files of type**: field.

The **Open** button found on the tool bar may also be used to fulfill this function.

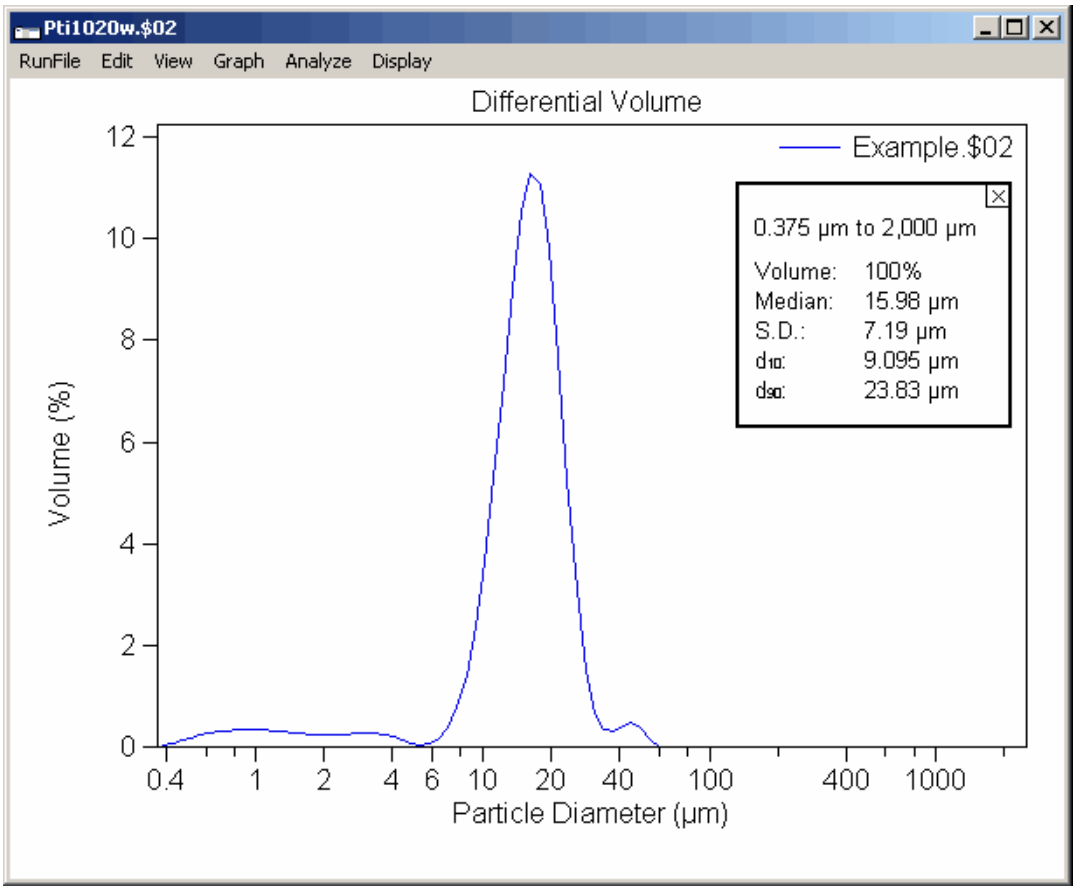
To open a file:

1 **File > Open** or click on the **Open** icon.

2 Select the file you want to open and click **Open**.

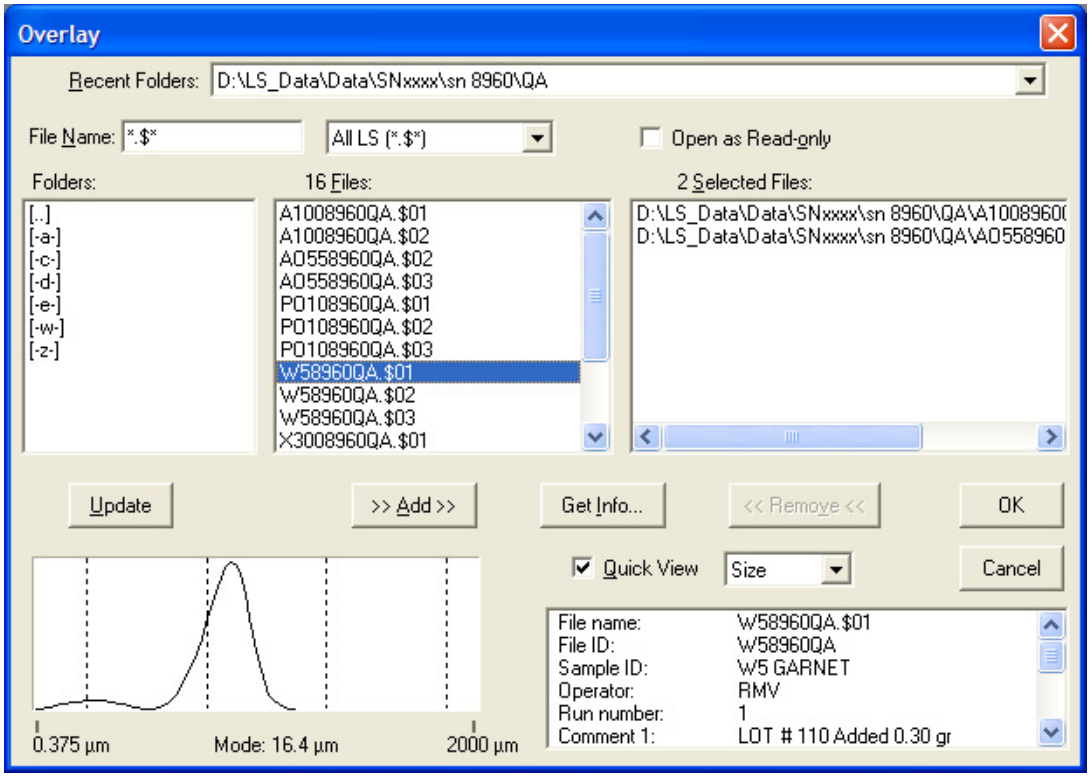


The results screen is displayed along with a box that displays the statistics as shown below. If the statistics box is not displayed, you may access it by selecting **Analyze > Show Statistics on Graph**.



Overlay...

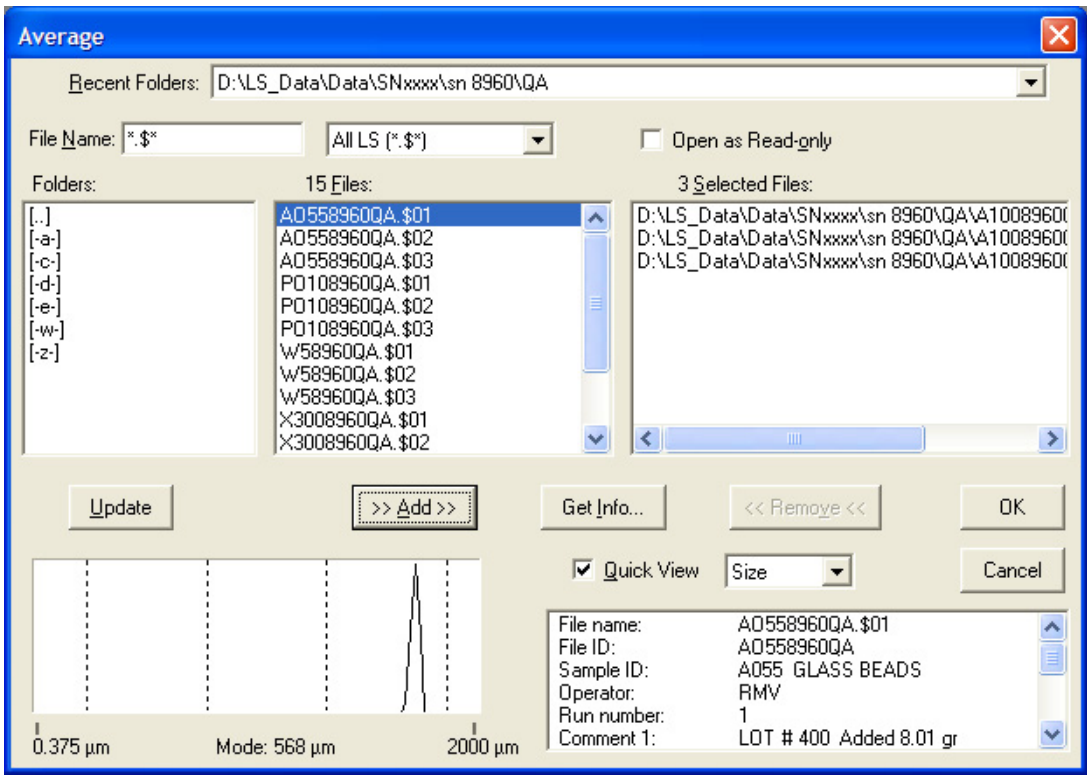
Overlay is analogous to Open, but allows you to open a total of 30 files and display them as an overlay in one graph. Saving an overlay as a .ovr file only saves a list of the names of the files in the overlay. In order to open an .ovr file, all the files named in the list must be present in their original locations



Average...

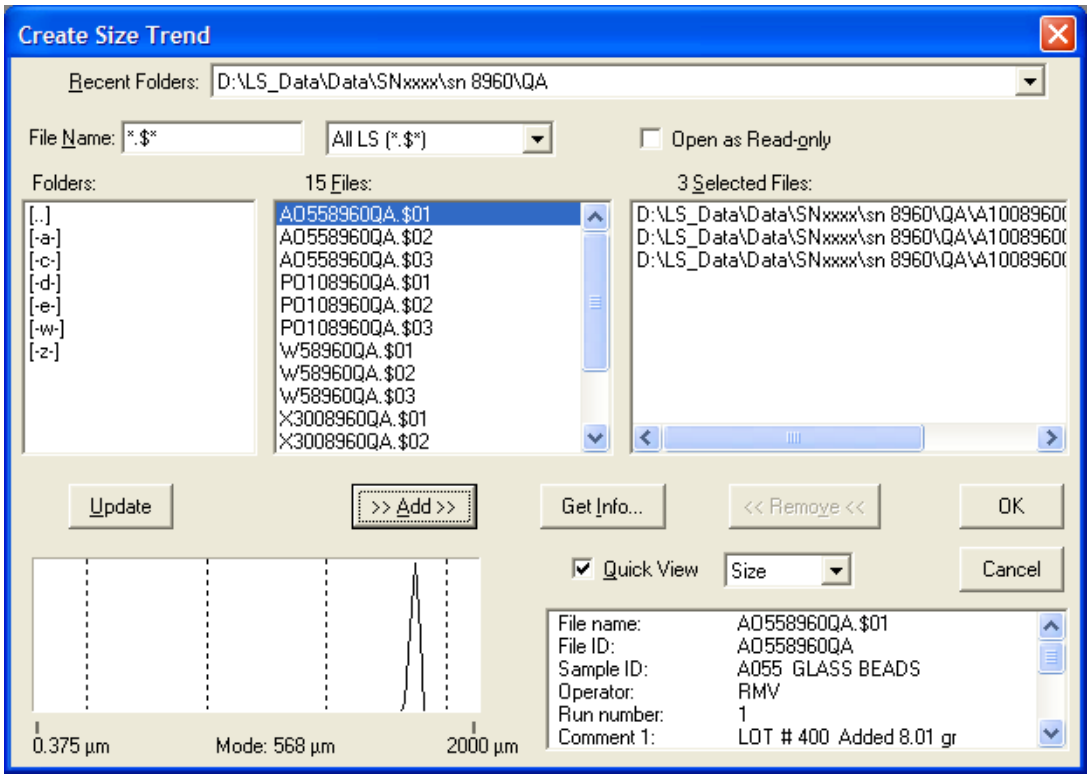
The **Average** command allows you to create a single file produced by averaging the data from up to 150 files on a channel-by-channel basis. Some information (especially header information not common to all files) is not included in the composite file, but the individual runfiles themselves are not altered by the **Average** function.

Averaged files may be printed, edited and saved (with a "*.$av" extension) using the **Save As** command under the **RunFile** menu. You may also save the file by selecting **Save List of Averaged Files...** from the **RunFile** menu. The saved file will be assigned the extension .avg. This file can later be accessed from the **Average From A List...** option in the **File** menu.



Create Size Trends...

The **Create Size Trend** command allows you to view and print sequences or groups of analyses in a single graph. It provides a graph/listing of a chosen sample statistics across sample files. For example, the **Create Size Trend** command allows you to view changes in mean particle size over time by selecting a group of sample files and plotting the mean particle diameter for each run on the same graph. The display of the Trend Graph and/or Listings will depend on the settings selected under the **Preferences** menu.



Using Size Trend

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1 **File – Create Size Trend.** The Create Size Trend dialog box (see figure above) is displayed.

---

2 Select the files to include in the Size Trend Analysis.

---

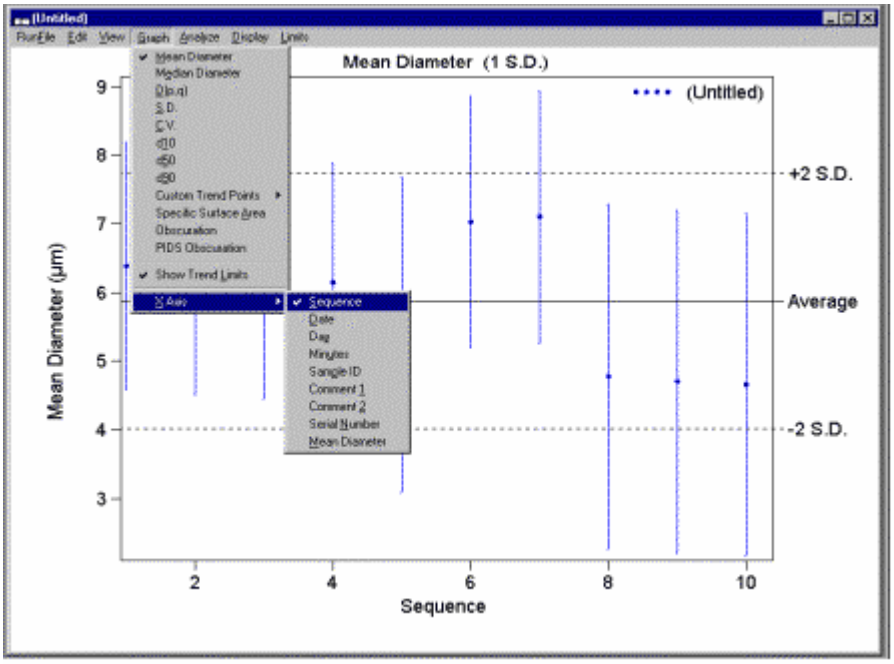
3 **OK.**

---

4 To select the variables to be plotted, **Graph** on the **RunFile** menu bar. The options in the top section of the menu are the y-axis choices. The x-axis choices are provided by the **X Axis** fold out menu.

5 To save a trend file | **RunFile** select **Save**. The file should be saved using the "$tr" extension.

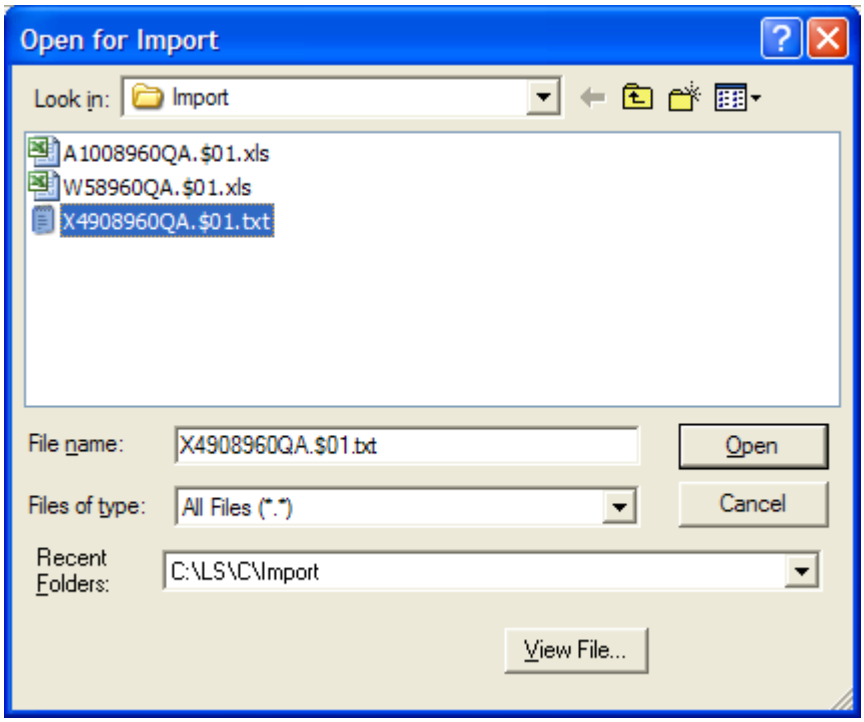
6 To add a new file to an already saved trend file, open the trend file | **RunFile**. Select **Open and Add to Trend**. Select the file to be added. | **OK**.



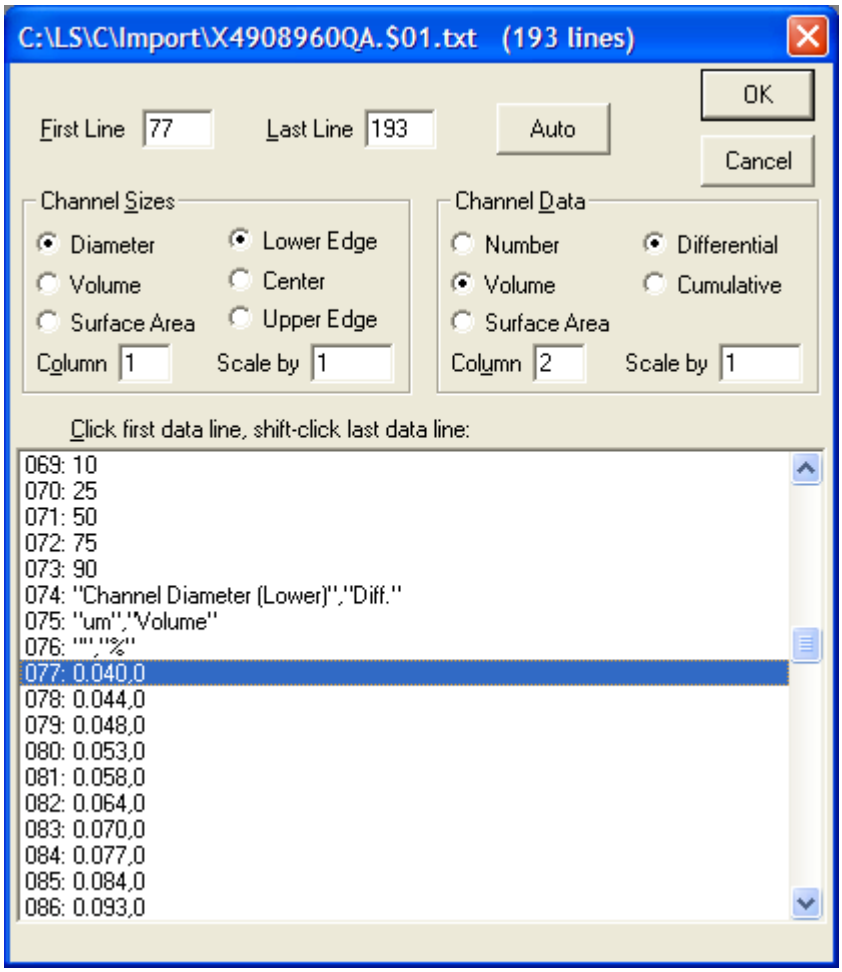
In the Size Trend graph, limit lines and labels may be included to represent the mean or nominal value, and upper and lower limits, and enter labels for these lines. This function is located in the Edit menu.

Import Size Data...

Select **Import Size Data** to import data generated by the software that is in file format of types .txt or .xls. To convert these types of data files, click on the File menu and then click on **Import Size Data**. Next, type the file name in the space provided or select the file of interest in the Open for Import dialog box. Using file names with a 3-character extension beginning with the symbol '$' is recommended when saving imported data. Files that have an extension beginning with "$" will be automatically recognized by the LS 13 320 program.



In the dialog box (see next figure) select the first and last lines that include the data of interest followed by the channel sizes and data.



Open Overlay List...
Opens a saved overlay file (.ovr).

Average From List...
Opens files with the extension .avg.

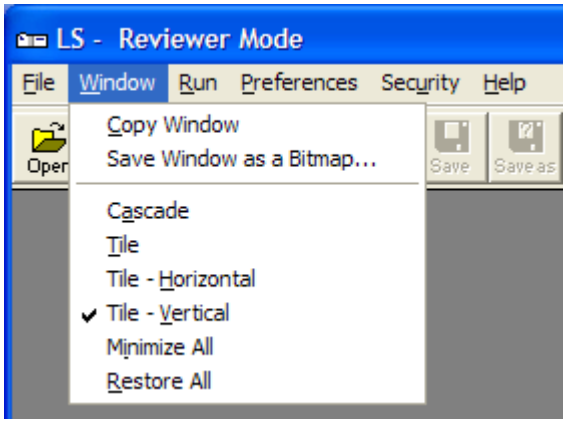
Change Folder...
The Change Folder command can be used to locate data files stored elsewhere on your computer.

Create Folder...
The Create Folder command displays a dialog box that allows you to create a blank folder/directory for the storage of new or renamed data files.

Print Reports...
The Print Reports command allows a separate report to be generated for each file that is selected from a list of files that appears in the Print Reports dialog box. The form and content of these reports is defined by selections made in the Reports section of the Preferences pull-down menu.

Print Window & Print Screen...
Print Window and Print Screen create bitmap images of either the result window or both the main and result windows (as well as anything else visible on the Windows desktop).

Window Menu Options



Copy Window...
The **Copy Window** command creates a copy of the current active window and pastes it into the Windows buffer, where it can then be pasted into other applications.

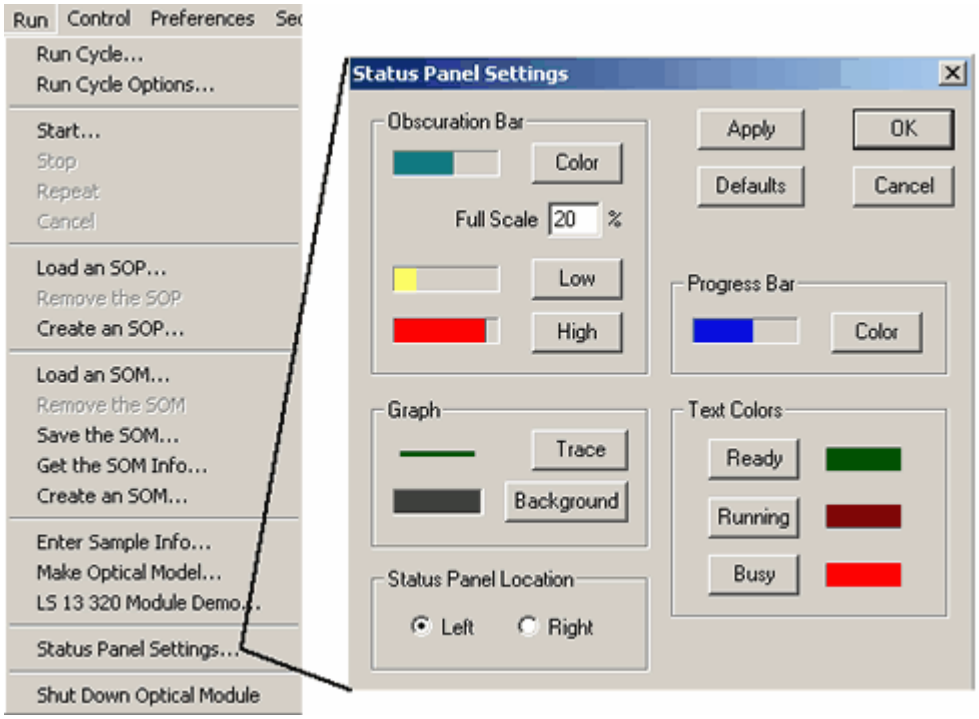
Cascade...
With the Cascade function, up to 16 result windows (e.g., graphs or listings) containing different data can be displayed on the screen simultaneously, overlapping each other so that the upper left corner of each graph window is visible. Open result windows are displayed one behind the other.

Tile-Horizontal...
Using the **Tile-Horizontal** display function, up to 16 open result windows containing different sample data runs can be displayed on the screen simultaneously, with the major axis running horizontally. Tile-Vertical... Using the Tile-Vertical display function, up to 16 open result windows containing different sample data can be displayed on the screen simultaneously, with the major axis running vertically.

Minimize All...
When **Minimize All** is selected, all windows are minimized (i.e., represented only by title bars).

Restore All...
Selecting **Restore All** restores all minimized windows to their original window configurations.

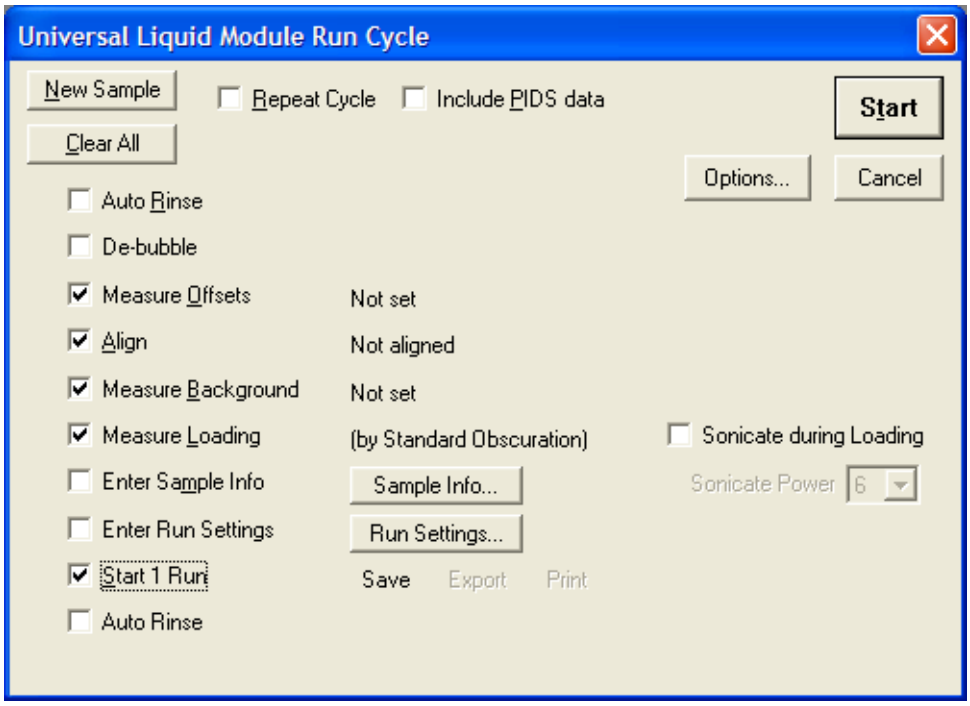
Run Menu Options



IMPORTANT The **Run** menu shown above will become active once the optical module is connected to the PC.

Run Cycle...

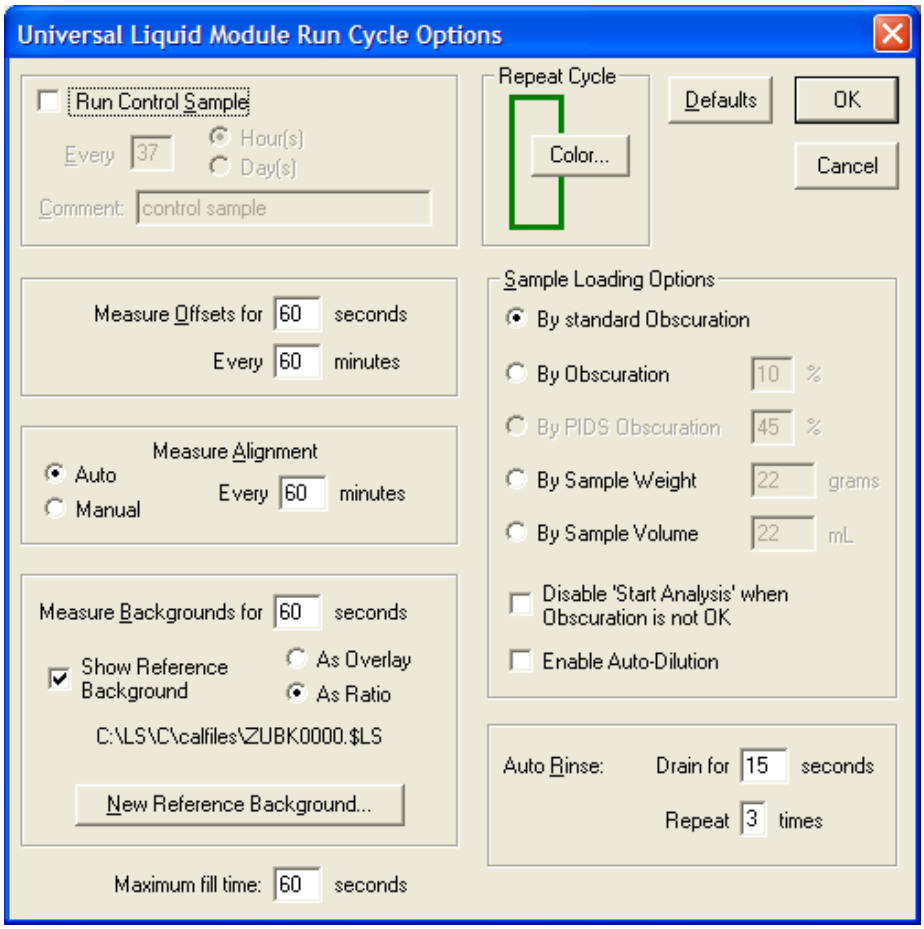
The **Run Cycle** menu allows you to set all the parameters necessary to perform an analysis on a sample. This menu allows the selection of the **PIDS** data option is found as well as **Measure Offsets**, **Align**, **Measure Background**, and **Measure Loading**. These options are customized for each sample module and are explained in more detail in CHAPTER 2, *Installation*, Making Measurements.



See Running a Control Running a Control for more information on the options under the **Run Cycle** menu.

Run Cycle Options...

This option allows the user to set up parameters for various parts... the offset, alignment, and background measurements that will be used under the **Run Cycle** menu described above.



Loading of the sample can be controlled either by obscuration, sample weight, or sample volume. Any one of these options is selected by checking the corresponding check box under **Sample Loading Options**.

Create SOP... Load SOP...

SOPs can be created to provide consistency in the way the analyses will be done. The SOPs can be secured by a supervisor and/or administrator in order to keep any user from changing any of the settings. SOPs are comprised of SOMs and Preference files. Refer to a specific Sample Module Section to learn more about how to create, load, and use an SOM, and to the Preferences section to set up particular preferences.

Create SOM...

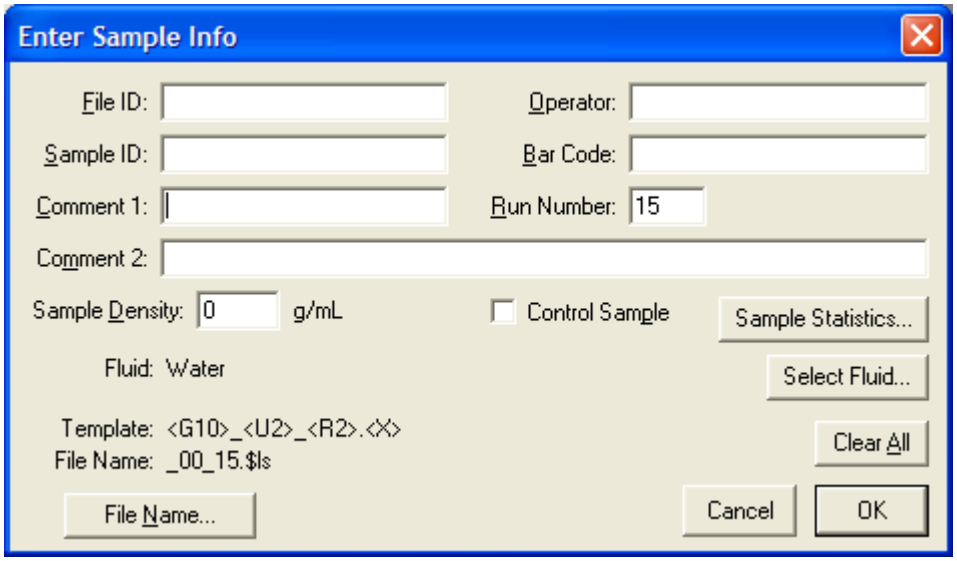
SOMs allow you to set all the analysis parameters that will be needed to perform the analyses on your samples. The SOMs can be used in conjunction with SOP's or by themselves. **Create an SOM** consists of a sequence of six screens which provide a range of options to select from.

To create, load or use an SOM refer to your specific Sample Module Section in this manual for further instructions.

Enter Sample Info...

This dialog box allows you to enter all the pertinent information about the sample to be analyzed. The sample statistics, fluid (diluent), and file name format can also be edited from this dialog box.

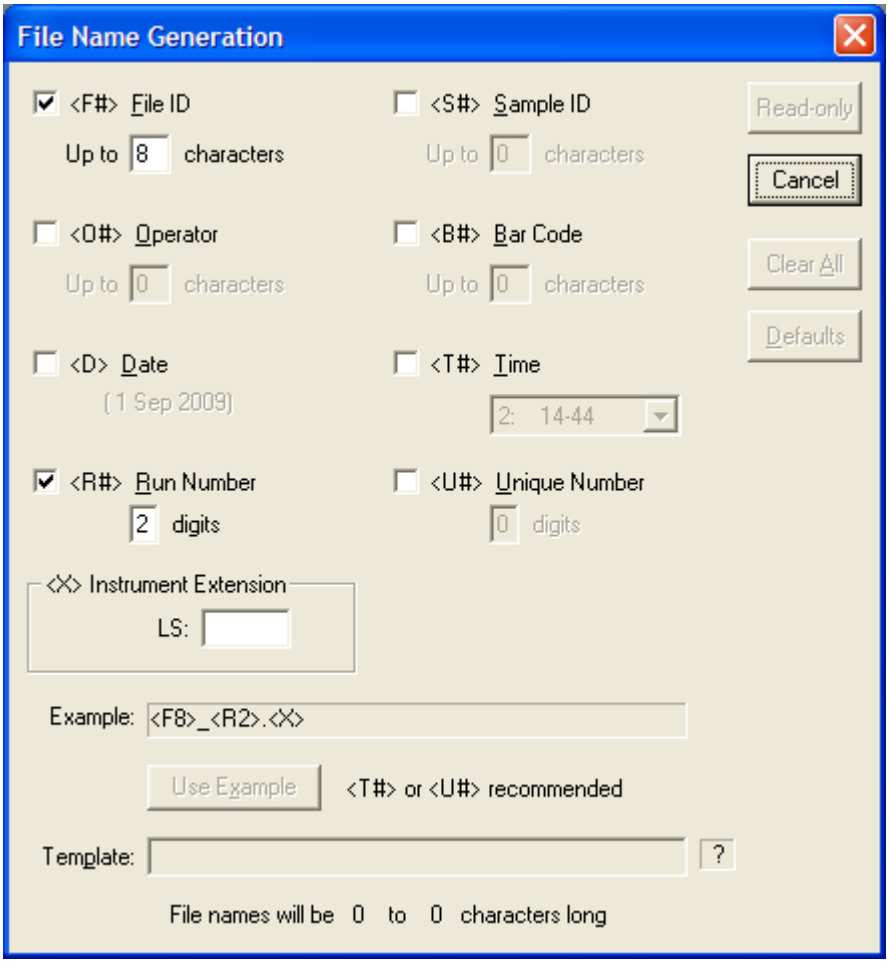
Refer to your specific Sample Module section in this manual for further instructions.



Make Optical Model...
Making optical models is necessary to obtain the correct size distribution on your particular sample. For more on optical models refer to APPENDIX C, *Optical Models*.

File Name Generation...

The file name generation dialog is used to determine the format of new file names. Existing file names cannot be edited using this option.

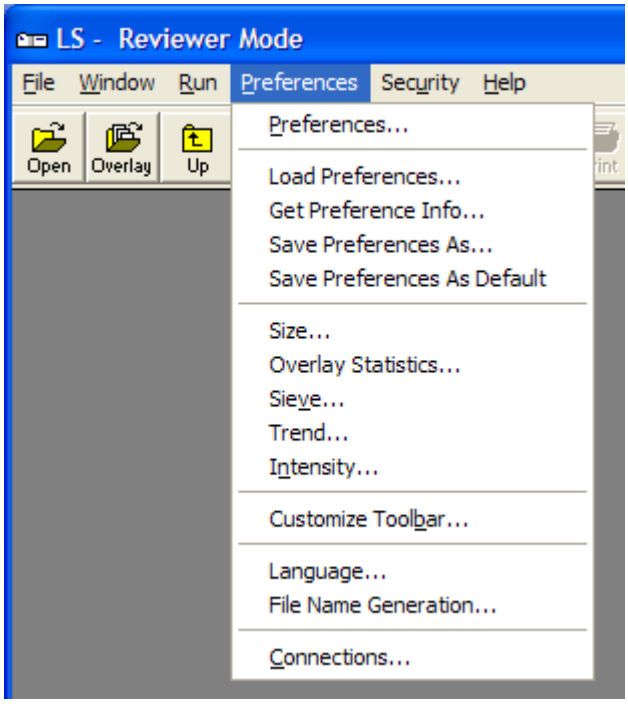


Selecting different options from the "File Name Generation" dialog box will allow you to give your analysis a unique identification with up to 271 characters plus an extension. The maximum number of characters for each option is:

- **<F#>** File ID: 60. Will use up to the first 60 characters of the name selected under **File ID** in the **Enter Sample Info** dialog box.
- **<O#>** Operator: 60. Will use up to the first 60 characters of the name selected under **Operator** in the **Enter Sample Info** dialog box.
- **<D>** Date: 16. Will use date format of your computer.
- **<R#>** Run Number: 5. Will use the run number from the **Enter Sample Info** under **Run Number**. (**Run Number** is also found under the **Development Wizard** dialog box when creating an SOM.)
- **<S#>** Sample ID: 60. Will use the selection under **Sample ID** in the **Enter Sample Info** dialog box.
- **<B#>** Bar Code: 60. Will use the selection under **Bar Code** in the **Enter Sample Info** dialog box.

Preference Options

Preference files define the settings for data presentation, the appearance of the program window, the format for reports, etc. At any time, you may change any of the settings in the dialog boxes accessed by commands in the Preferences menu. Some of these changes will take effect immediately, others only when a result file is re-opened. If the current preferences are saved, all the changes made will become part of that particular preferences file and will take effect any time that the particular preference file is loaded.

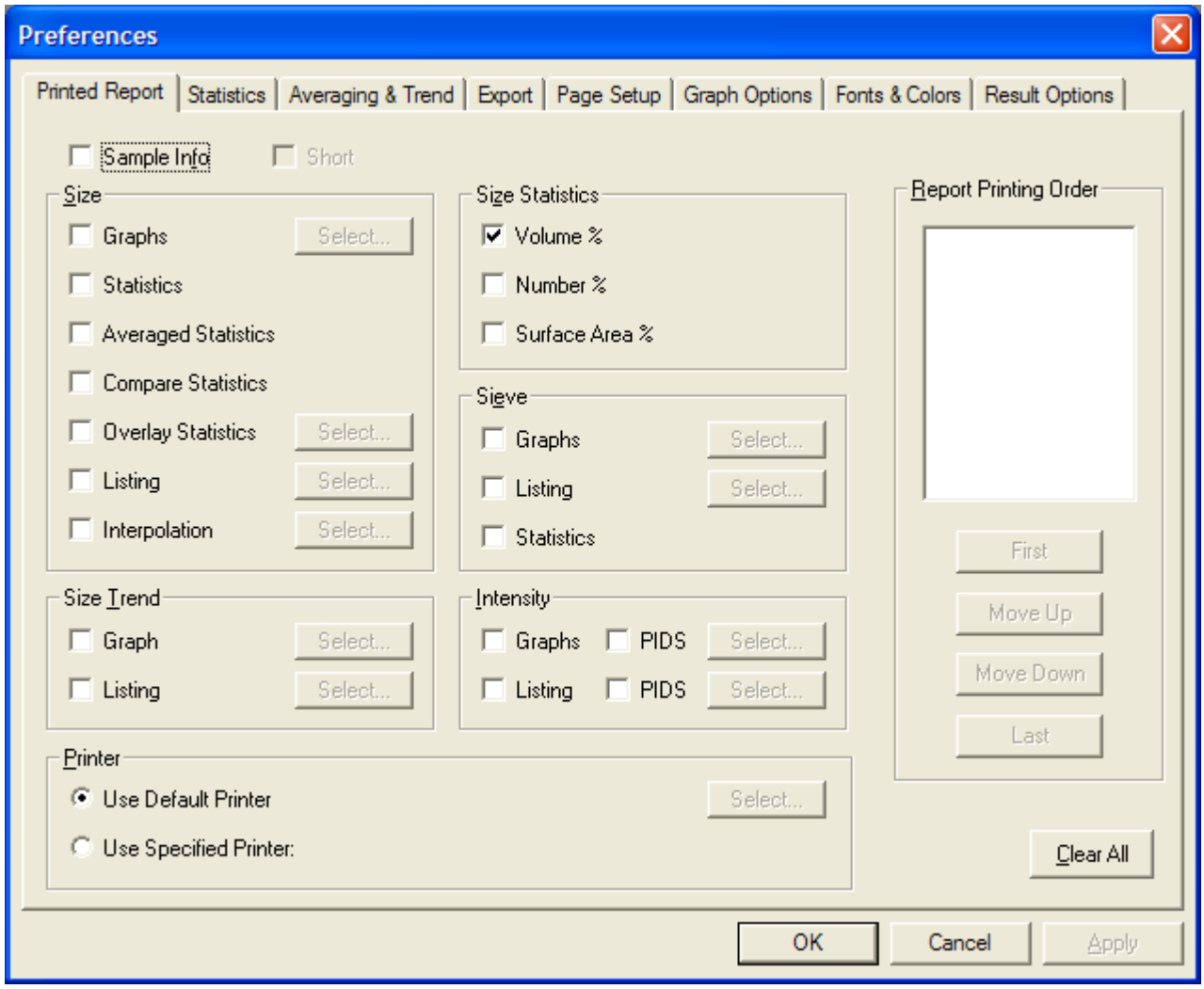


Printed Report

The **Printed Report** tab displays a dialog box that specifies the content of printed reports. This function allows you to choose any combination of the following report elements to be included in your printout:

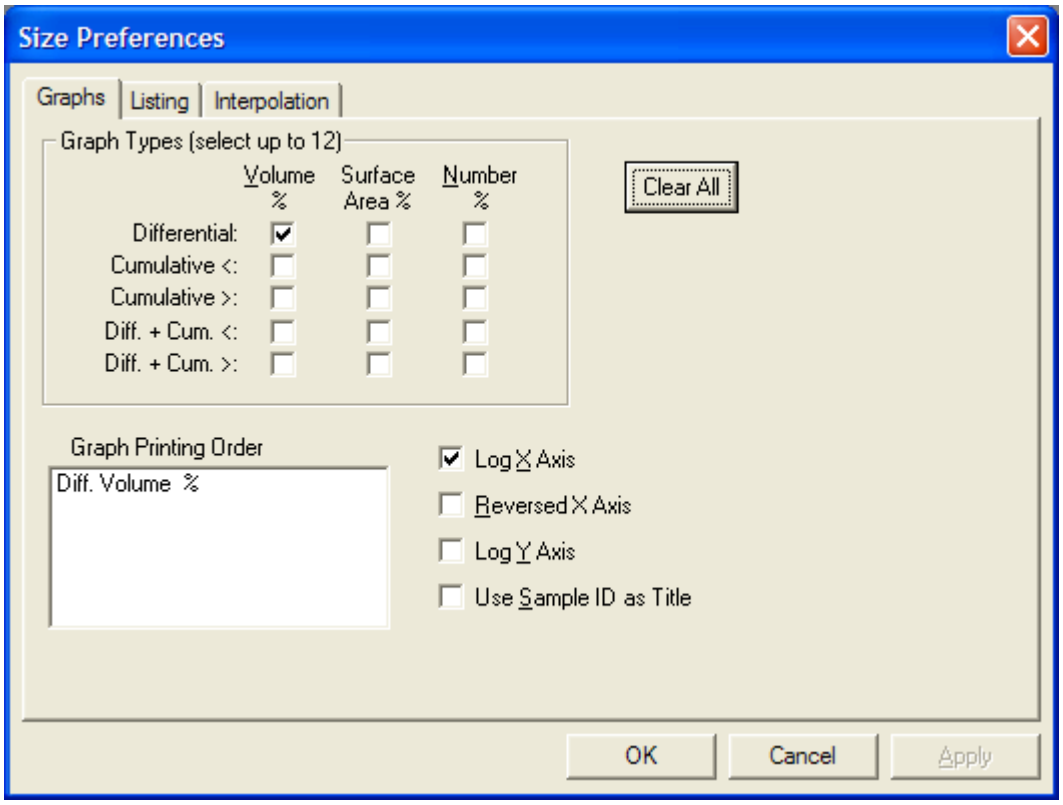
- **Sample Info:** Comments, sample collection parameters, and other file-specific data
- **Short:** Sample info content provided in a shortened format
- **Size Graphs:** Provides a graphical display output selected from the Size Graphs option.
- **Size Statistics:** Selected in the Size Statistics option or by clicking the corresponding dialog box under "Size Statistics"
- **Averaged Statistics:** selected statistics computed by the file averaging function Compare
- **Statistics:** comparison with a previously defined standard data file
- **Overlay Statistics:** selected statistics from a saved Overlay file
- **Listing:** data presented in a tabular format

- **Interpolation:** analysis using user-selectable channel boundaries
- **Size Trend Graph:** trend data graphical and tabular format options
- **Trend Listing:** trend data in a tabular format
- **Sieve:** presents size data (tabular or graphical) using sieve format by mesh size or mesh number



Size Graphs

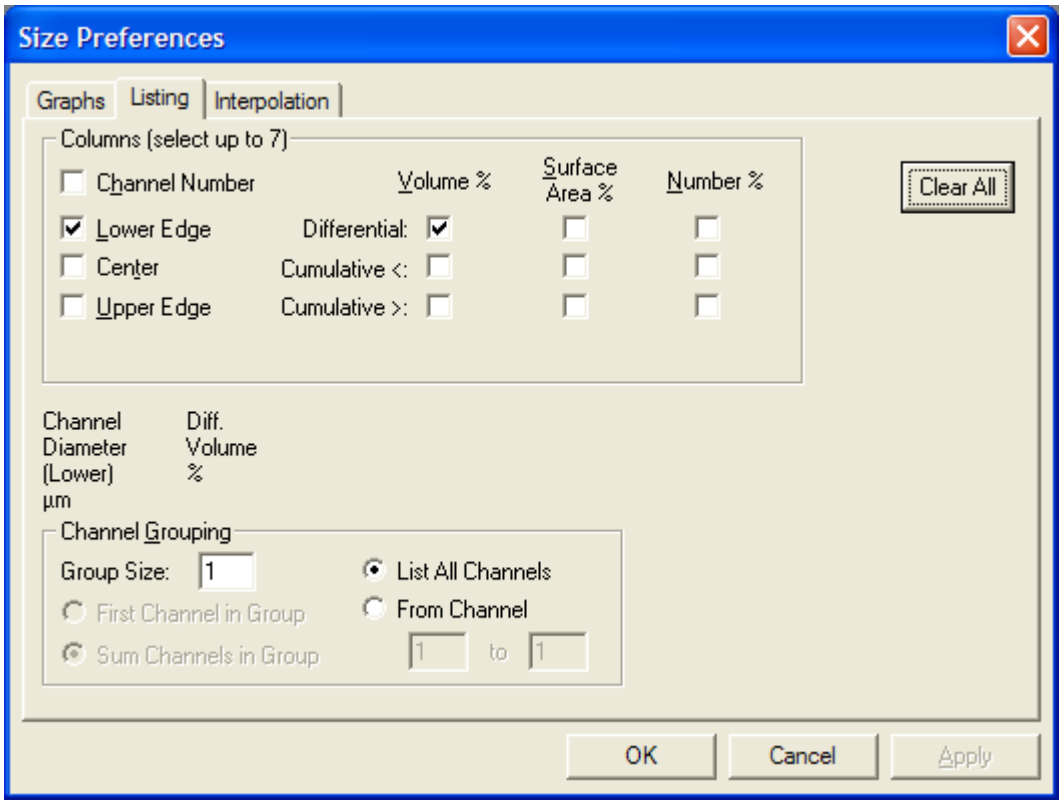
This option displays the **Size Graphs** tab box. Your first selection determines the default format for the graph display format. Additional selections determine the other types of graphical presentations to be included in your report. Up to 12 distribution types may be selected in the desired printing order. Both the x-axis and y-axis can be printed using a log or a linear scale.



Size Listings...

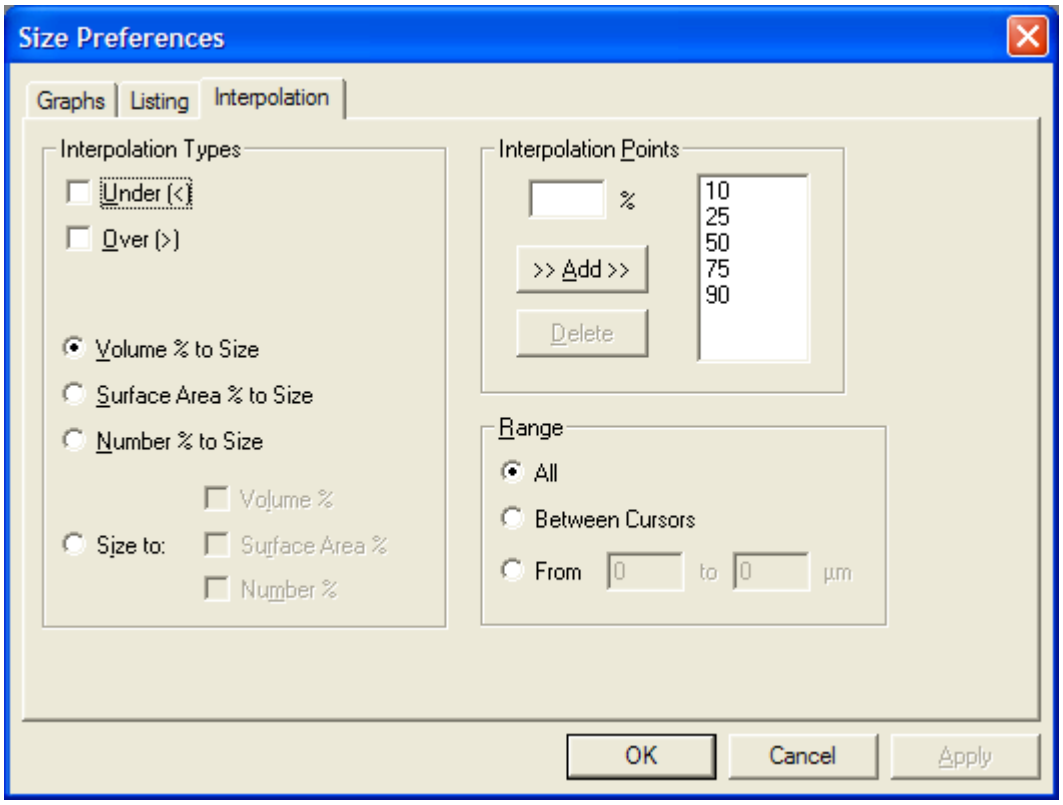
The **Size Listing Preferences** tab allows you to customize tabular data presentations by selecting specific listing information and designating column sequence for quick output to the printer.

Make your selections by clicking the desired option boxes; click again to deselect. Up to seven categories of information may be included in tabular data output.



Size Interpolation

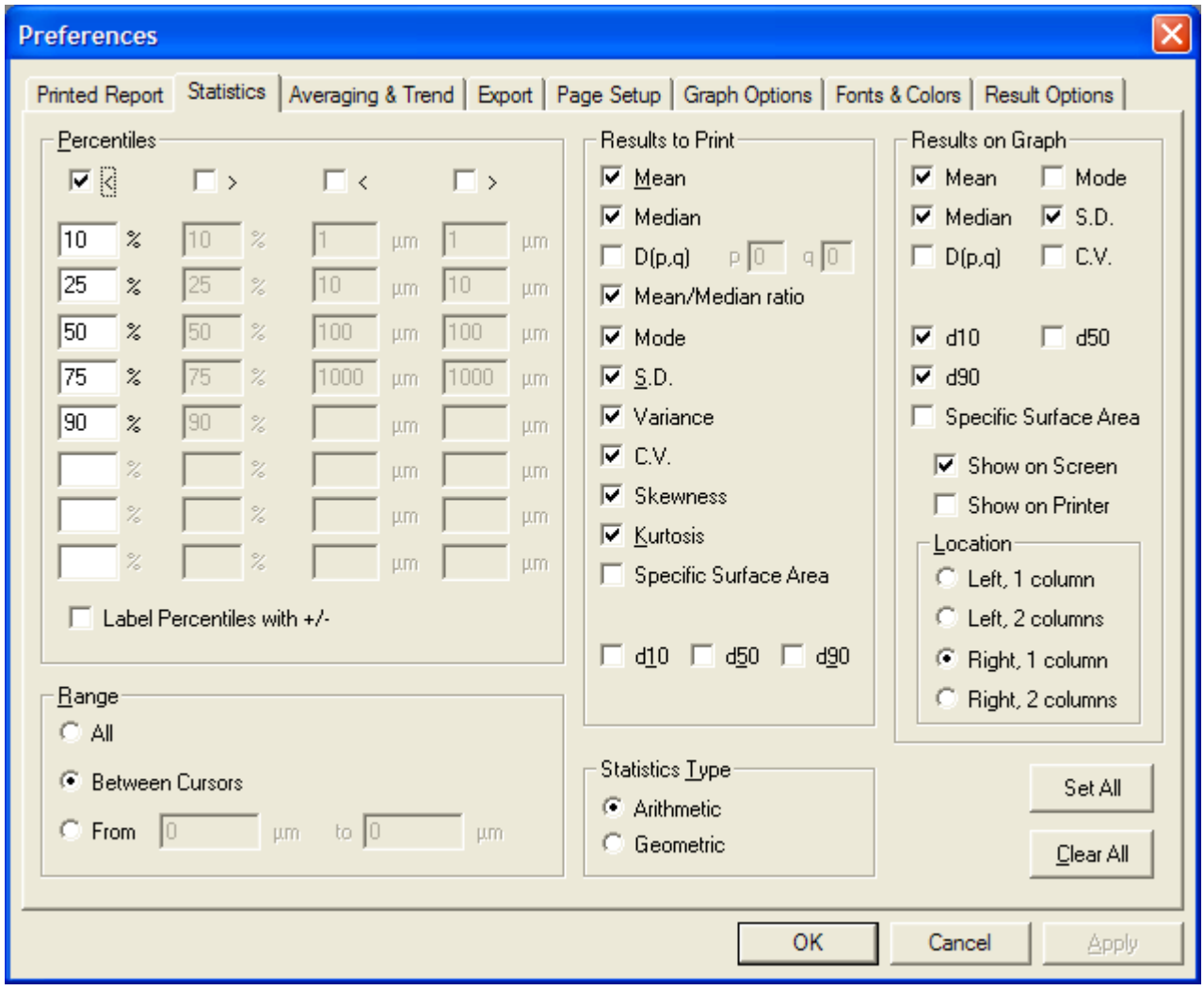
The **Size Interpolation** option software utilizes a reduced number of user-specified size classes to categorize sample data. Use the Interpolation dialog box to enter up to 150 interpolation points. In the group box labeled Interpolation Type, select the distribution % type to size that you want printed. Selecting the "**Size to**:" distribution type will activate the **Volume%**, **Surface Area%**, and **Number%** check boxes. This section also determines whether data are displayed as a cumulative percentage of values either above or below user-defined percentile values.



Statistics

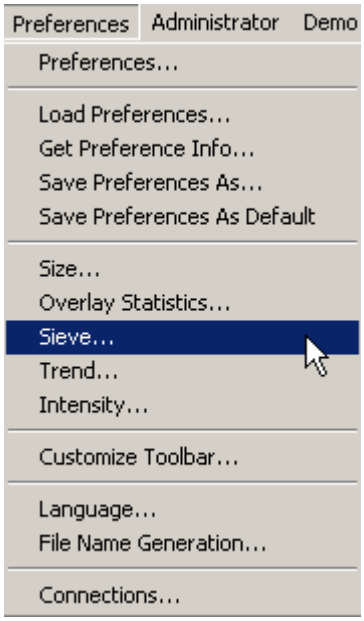
The **Statistics** option is used to define the distribution and statistic type used. It is also useful in defining which percentiles and statistics appear on the size statistics report and when selecting **Analyze > Statistics** option from the individual run file menu.

See APPENDIX D, *Troubleshooting* for more information on the statistics used in particle characterization.

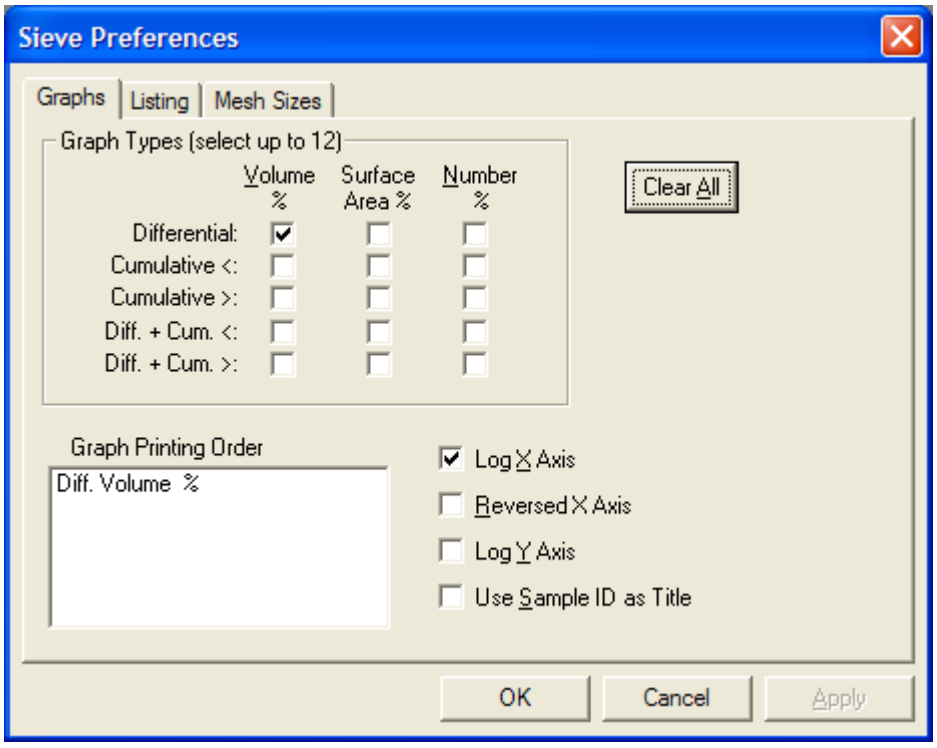

Preferences
Printed Report
Statistics
Averaging & Trend
Export
Page Setup
Graph Options
Fonts & Colors
Result Options
Percentiles
Label Percentiles with +/-
Range
All
Between Cursors
From
to
Results to Print
Mean
Median
D(p,q)
Mean/Median ratio
Mode
S.D.
Variance
C.V.
Skewness
Kurtosis
Specific Surface Area
d10
d50
d90
Statistics Type
Arithmetic
Geometric
Results on Graph
Mean
Mode
Median
S.D.
D(p,q)
C.V.
d10
d50
d90
Specific Surface Area
Show on Screen
Show on Printer
Location
Left, 1 column
Left, 2 columns
Right, 1 column
Right, 2 columns
Set All
Clear All
OK
Cancel
Apply

Sieve...

The **Sieve** dialog offers the option of presetting the size data in a format that allows comparison of data obtained with sieves.



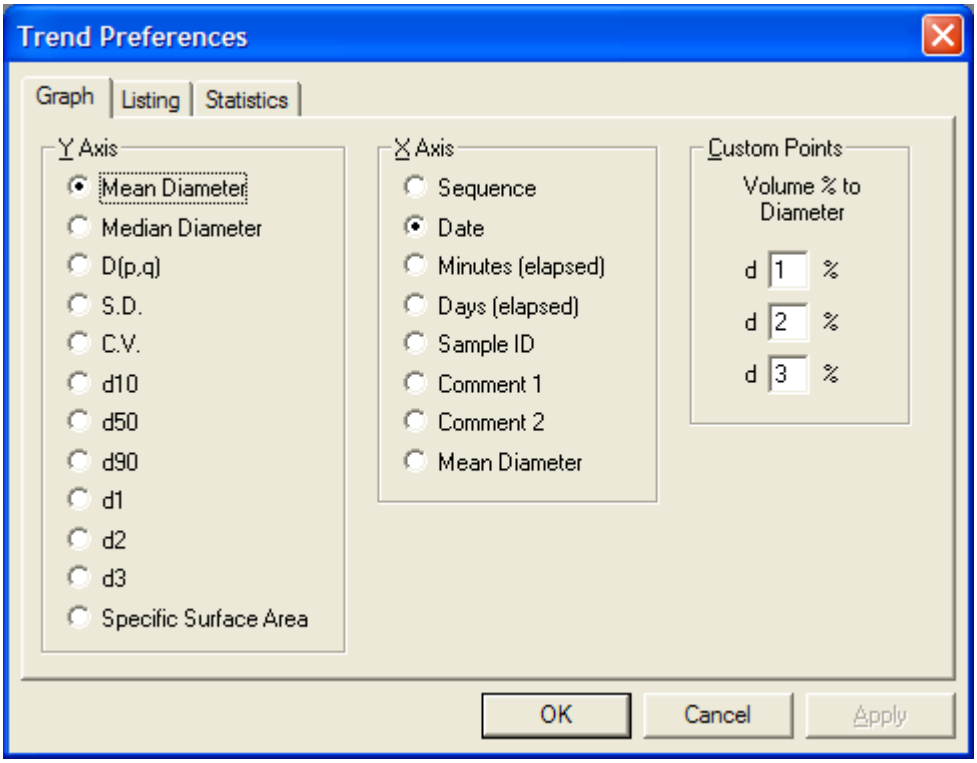
The sieve option allows you to show a **Sieve Graph**, **Sieve Listing**, as well as **Define Mesh Sizes**. The **Sieve Graph** option allows you to select up to 12 different graphs by distribution type. Use this function to define which sieve graphs are printed and their printing order. The first graph type appearing in the "**Graph Printed Order**" list box defines the order of the sieve data when it is printed.



"Sieve Listings" will print up to seven distribution listings including Mesh Size

Trend...

Size Trend Analysis allows you to view and print sequences or groups of analyses in a single graph. A statistical quantity, such as the mean, can be plotted versus time of analysis, date of analysis, or several other choices. Two options in the Preferences menu determine what information is included in the trend reports and listings. The Graph option allows you to select the variables to be displayed on the X and Y axes.

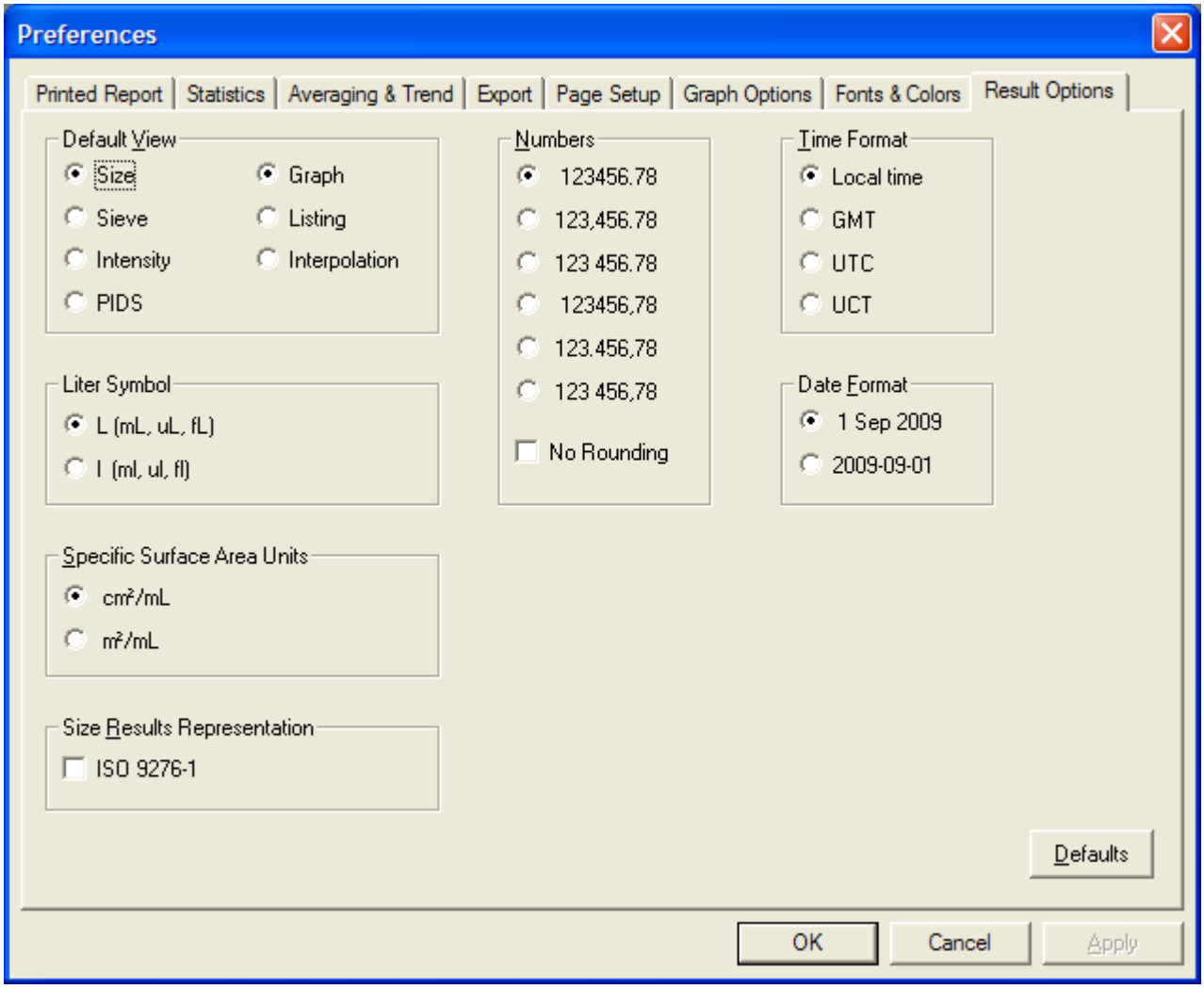


Custom Points lets you select three additional interpolation points

The **Listing** option allows you to select the column headings for the **Trend Listing** report.

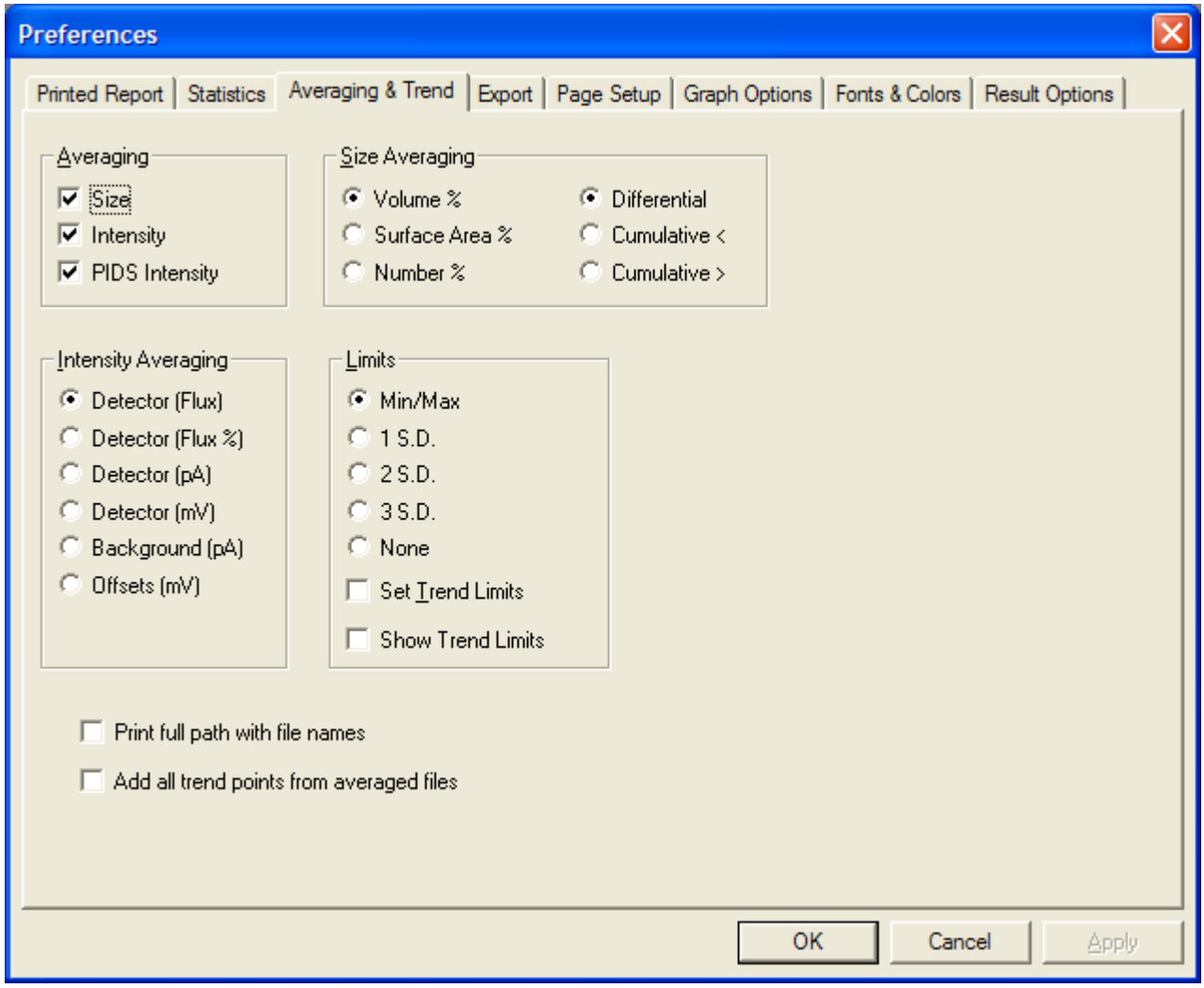
Result Options

Result Options will allow you to select the units of measure in which the data will be reported, i.e. mm3 for volume. The Default View option under this menu lets you choose the display format that will appear at the end of an analysis.



Averaging and Trend

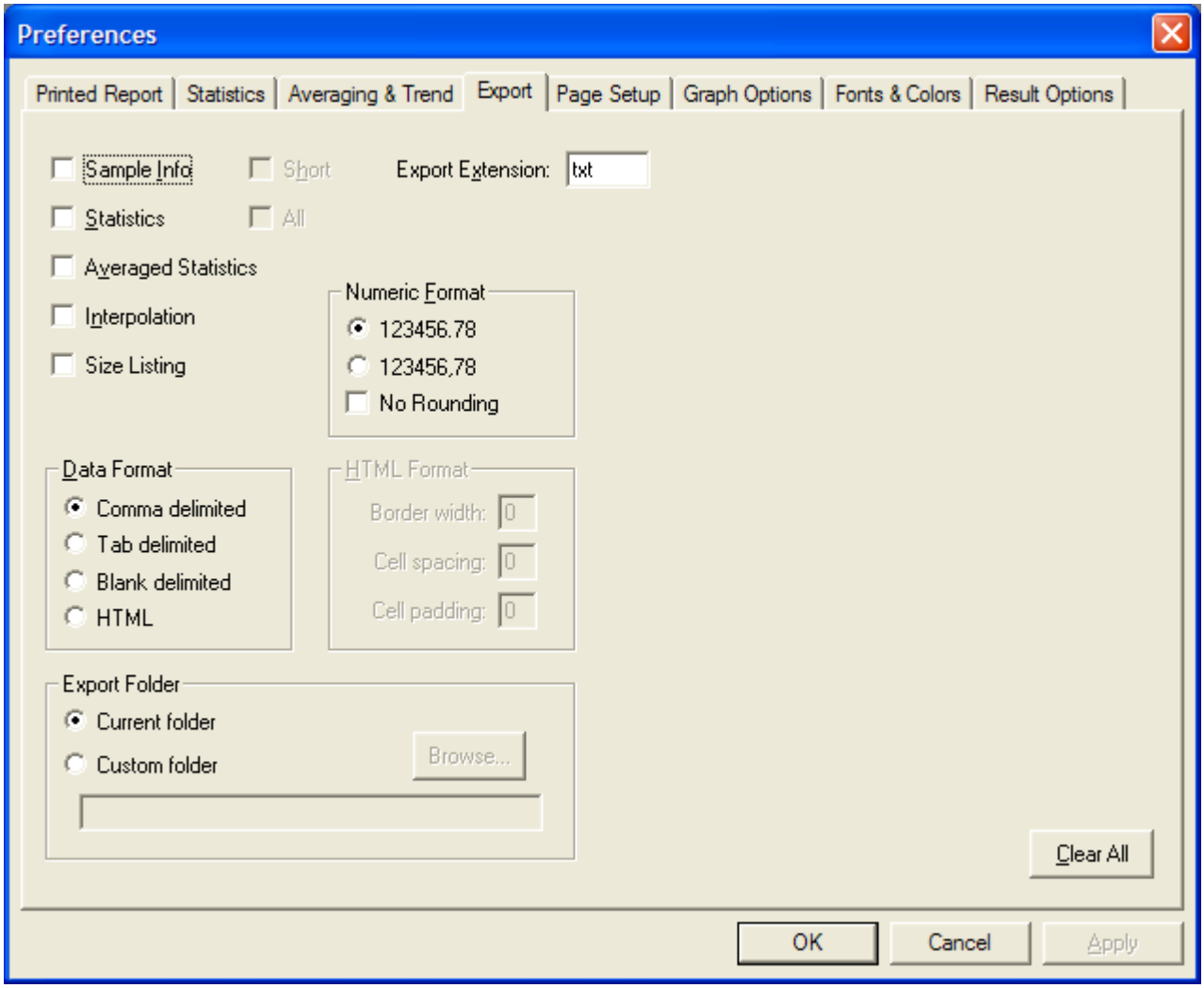
Displays the **Averaging and Size Trend** dialog box, which may be used to select the type of measure to use, distribution type, and trend limits type. It allows file names printed with their full path, and an option to add data from other averaged or trend files.



Data Export

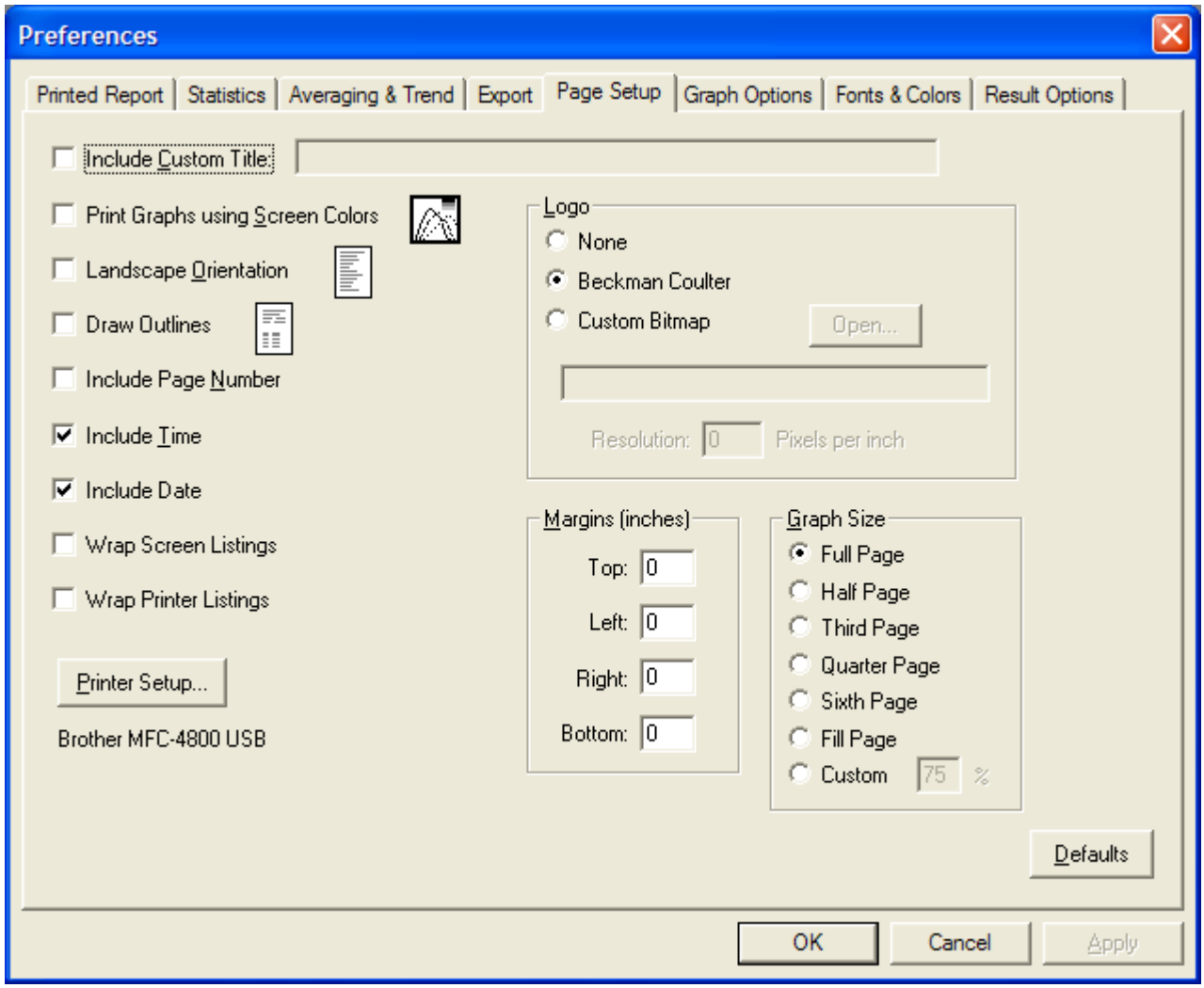
Choices in the **Data Export** dialog allow the user to specify which subsections of the runfile (e.g., the sample info, statistics, etc.) will be exported. An extension, such as.xls, may be entered so that another application will recognize the exported data.

Numeric formatting options are also included in the **Data Export** dialog.



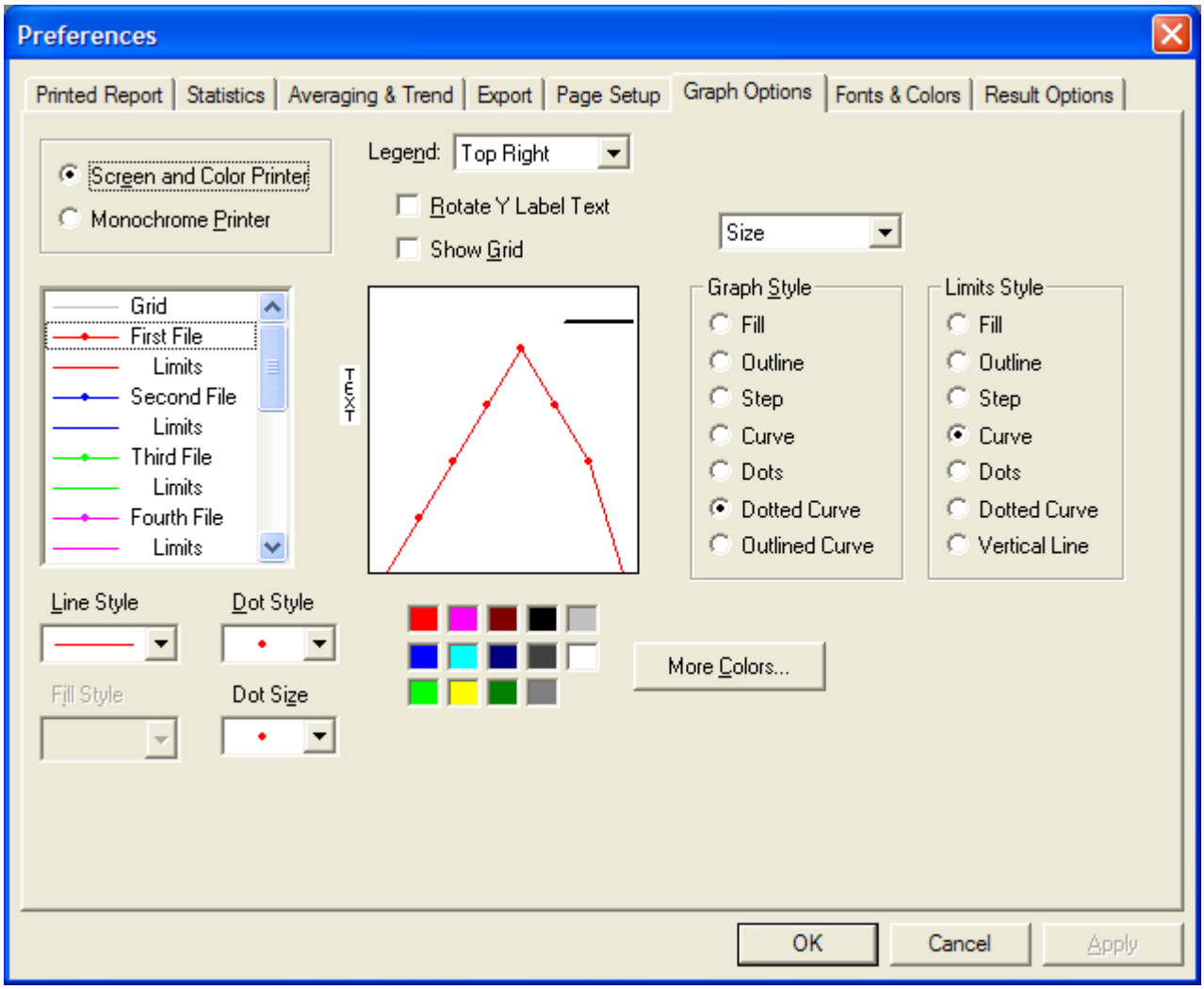
Page Setup

The **Page Setup** tab provides all the options necessary to set up how the report will be printed. By checking the radio button for **Custom Bitmap** under the Logo section, you can use your company logo on all the reports. Just input the folder name followed by the logo file name (must be a bitmap file) in the dialog box next to the **Custom Bitmap** option.


Preferences
Printed Report
Statistics
Averaging & Trend
Export
Page Setup
Graph Options
Fonts & Colors
Result Options
Include Custom Title
Print Graphs using Screen Colors
Landscape Orientation
Draw Outlines
Include Page Number
Include Time
Include Date
Wrap Screen Listings
Wrap Printer Listings
Printer Setup...
Brother MFC-4800 USB
Logo
None
Beckman Coulter
Custom Bitmap
Open...
Resolution: 0 Pixels per inch
Margins (inches)
Top: 0
Left: 0
Right: 0
Bottom: 0
Graph Size
Full Page
Half Page
Third Page
Quarter Page
Sixth Page
Fill Page
Custom 75 %
Defaults
OK
Cancel
Apply

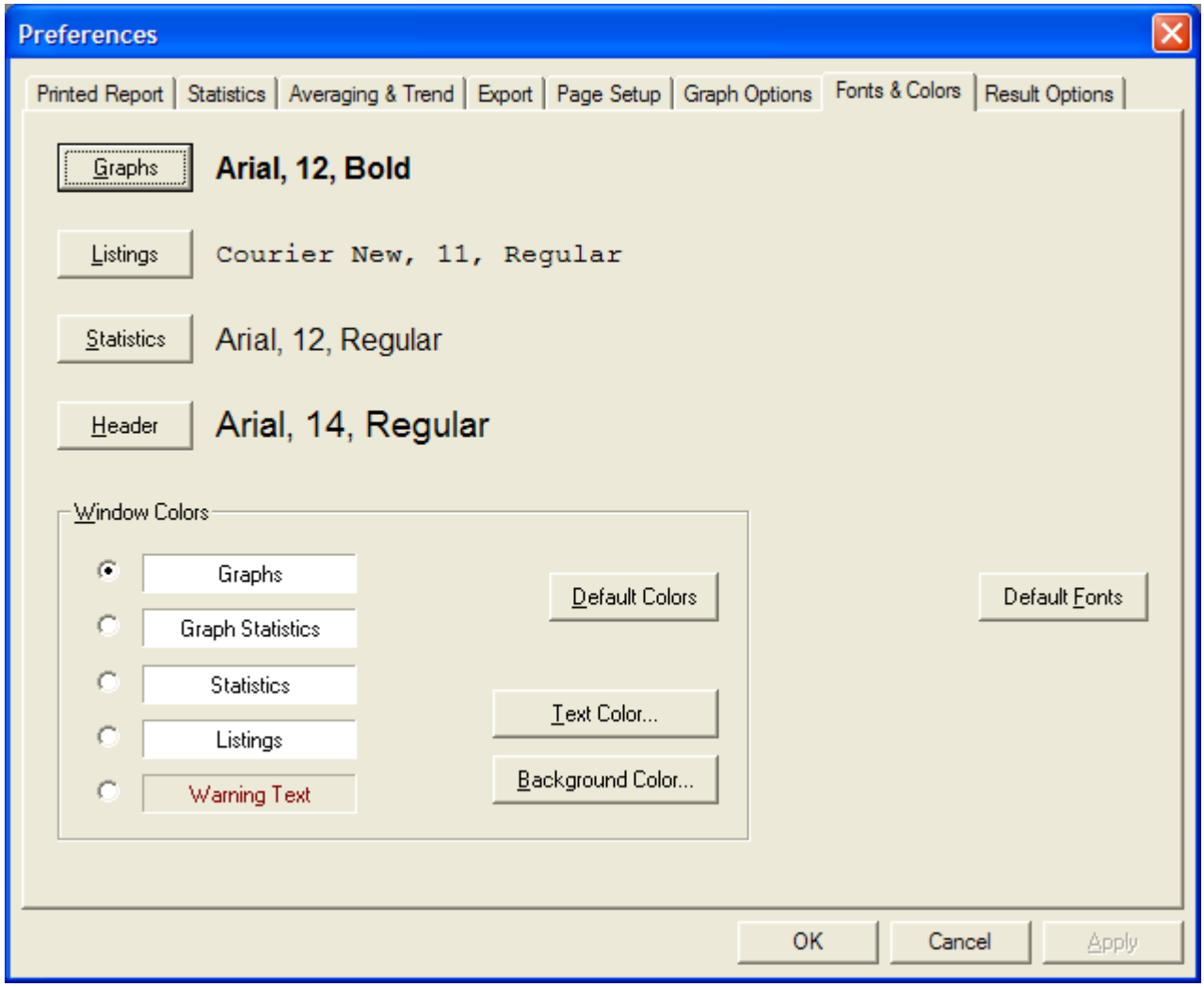
Graph Options

Under this tab you will find all the option to set up the way graphs will be printed in a report.



Fonts & Colors

This option allows you to set different font types and sizes as well as colors for headings, listings, graphs and statistics for display and for the printed reports.



Preference Files...

Load Preferences brings up a dialog box with the names of stored preference files. Selecting one of these files will alter the appearance and function of the software as defined by all of the choices made in previous parts of this section. (The "default.prf" file loads automatically each time the program is launched.)

Edit Preferences Info allows the user to change the name and associated comment of a stored preference file.

Save Preferences writes the choices made into a file which may be associated with a particular sample or operator.

Saving a preference file:

1 Select **Preferences** > **Preference Files**.

2 Select **Save Preference As...** and enter a name for the preference file under the **File Name** field. Make sure the file is saved under the correct folder. If you want this preference to be your default preference, use the **Save Preference As Default** option.

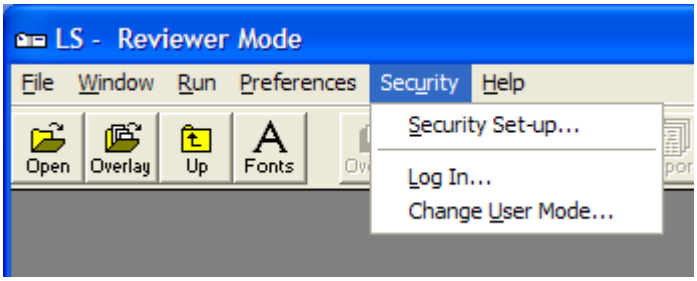
Loading a saved preference file:

1 Select **Preferences** > **Preference Files**.

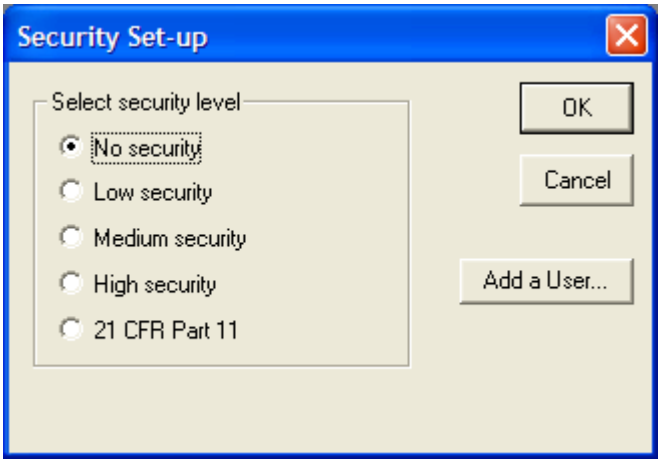
2 Select **Load Preference**.

3 Choose the preference file from the corresponding folder.

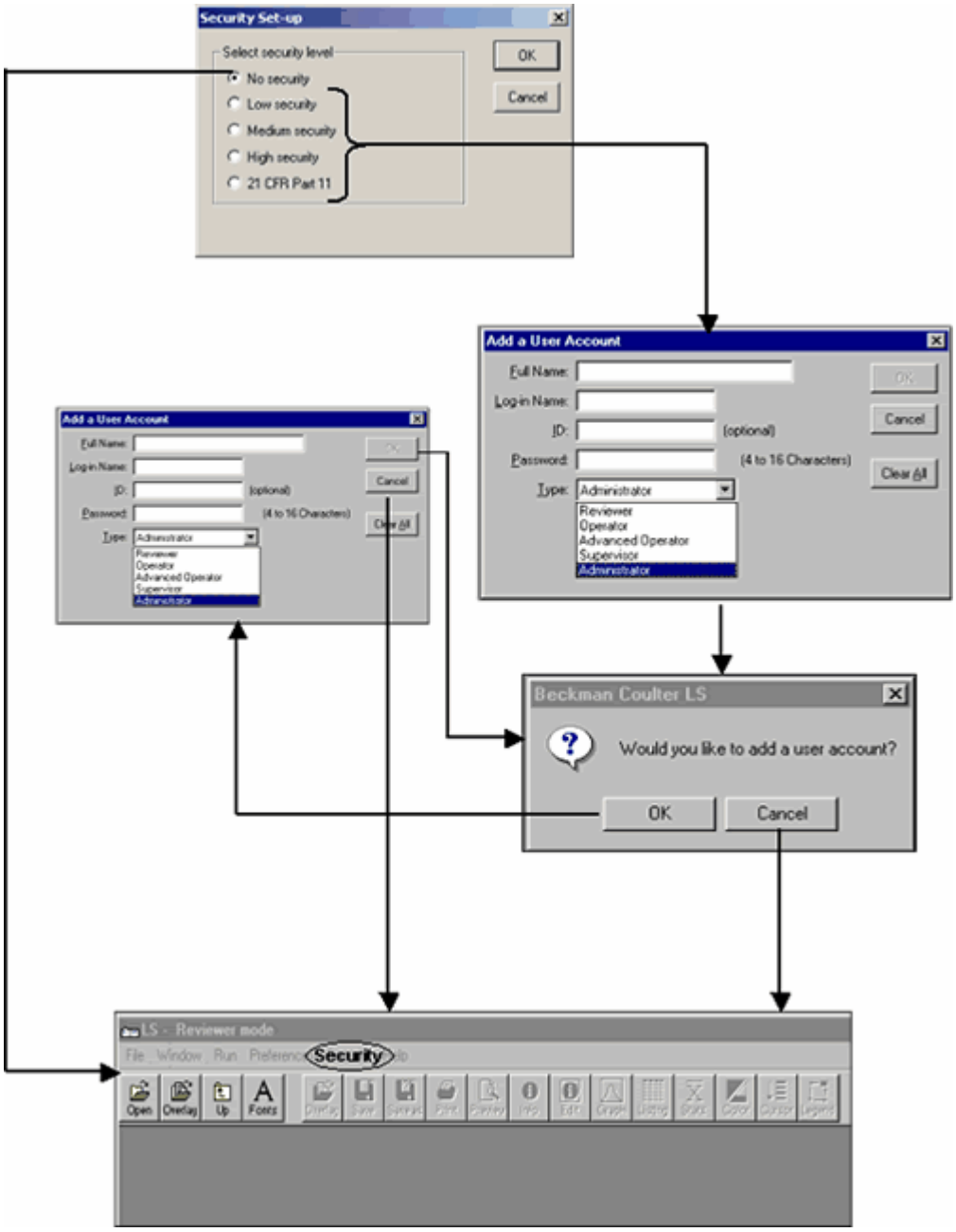
Security Options



The Security options allow the software to be configured for 21 CFR part 11 (for the compliant version of software only) compliance. This option also permits the operation of the software with some degree of security to be able to keep the integrity of the data being acquired by the instrument and stored in electronic form. This is done by selecting different levels of security, from No Security to High Security, from the Security Set-up dialog box shown below.

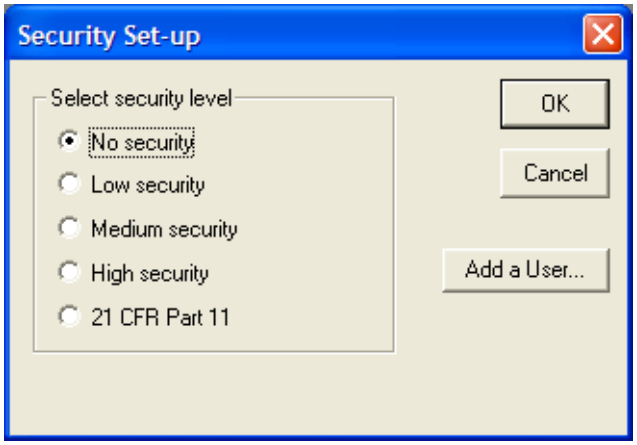


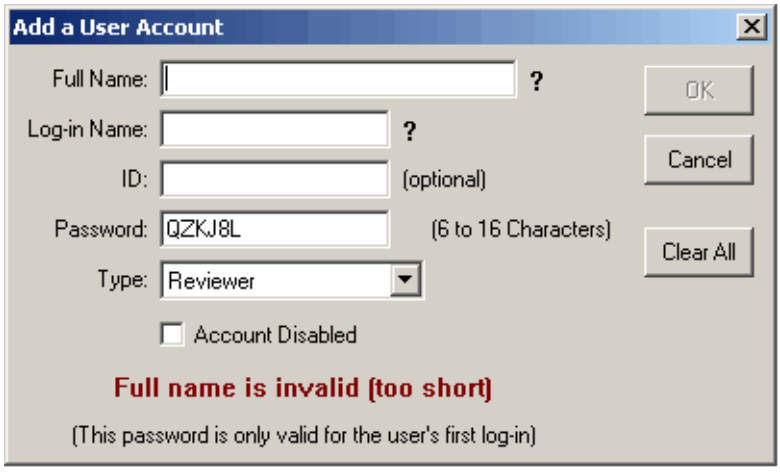
This diagram summarizes the steps when setting up a security level:



Security Setup

The Security Set-up box is accessible when the program is first started after installation. Once a security level is set-up it cannot be changed to a different level. Even though the level cannot be changed, different settings are accessible through the User Privileges options which is accessible only by the administrator. These security options can also be selected at a later time. If you want to set up the security levels at a later time refer to Regulatory Compliance for more information.





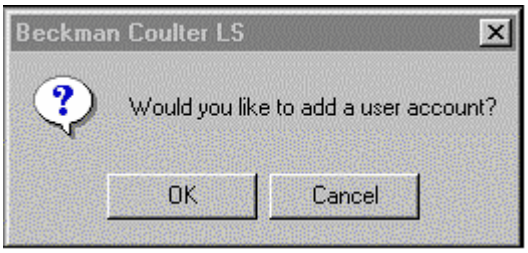
1 Select a level of security

2 **Add a User...**

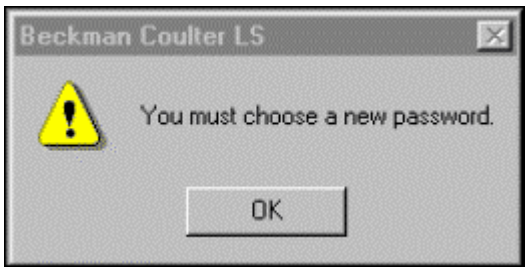
3 The **Add a User Account** dialog box is displayed. The **Full Name**, **Log-in Name**, and **Password** are required fields. Select the **Type** of user.

4 After completing the required information and selecting **OK**, you will be prompted to add another user.

5 Select **OK** if another user is to be added. Repeat step 3. If not select **Cancel**.



After an account has been set up, the user will be prompted to change his/her password.



For any security level selected, other than No Security, an administrator is required to log in. The administrator will set up accounts for each user who will in turn be required to sign-in by use of a Login ID and a password.

NOTE The Log-in dialog box will automatically close after 60 seconds of inactivity.

5.5.2 Types of Users

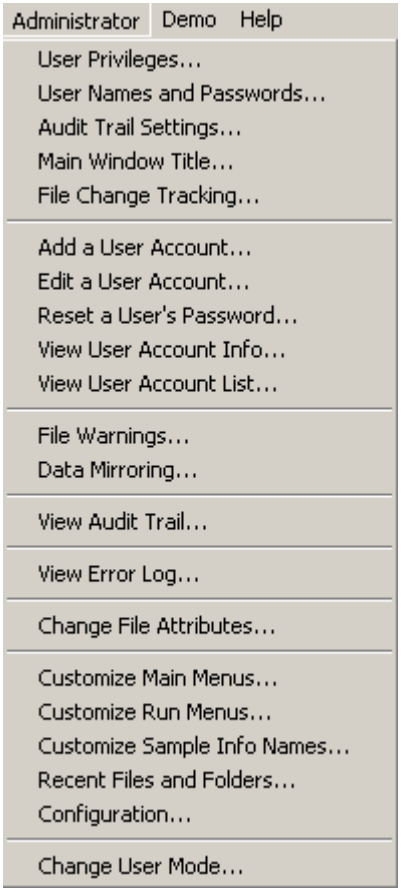
Under the security options there are different types of users with different levels of privileges. Depending on the user type, menu screens will change providing each user with a range of menu options that can be used to set the operation and security of the software. The user types are:

- Administrator
- Supervisor

5.5.2.1 Administrator

The administrator has control over every aspect of the settings of the software and controls and sets the privileges for each user. Only the administrator can set up accounts as well as user names and

passwords. The figure below shows the menus accessible by the administrator when logged-in in **Administrator Mode**.



To access this screen:

1 Select **Security**.

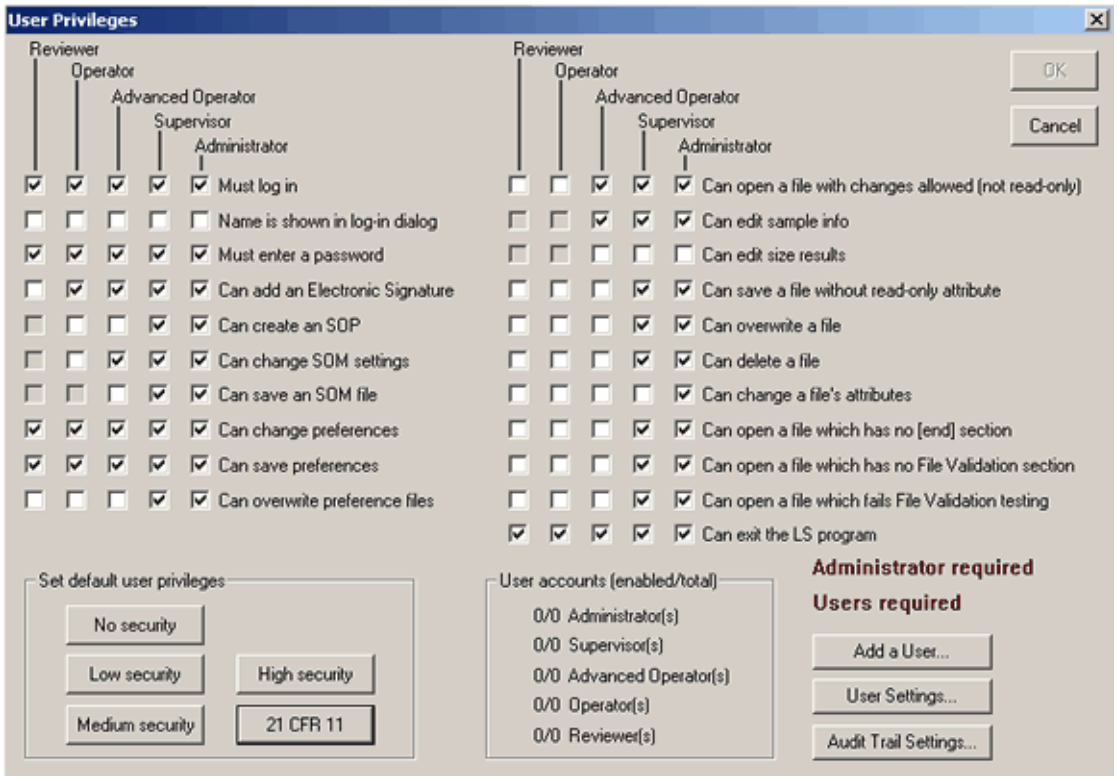
2 Select **Enter Administrator Mode**.

3 If prompted, enter your **Log-in ID** and **Password**.

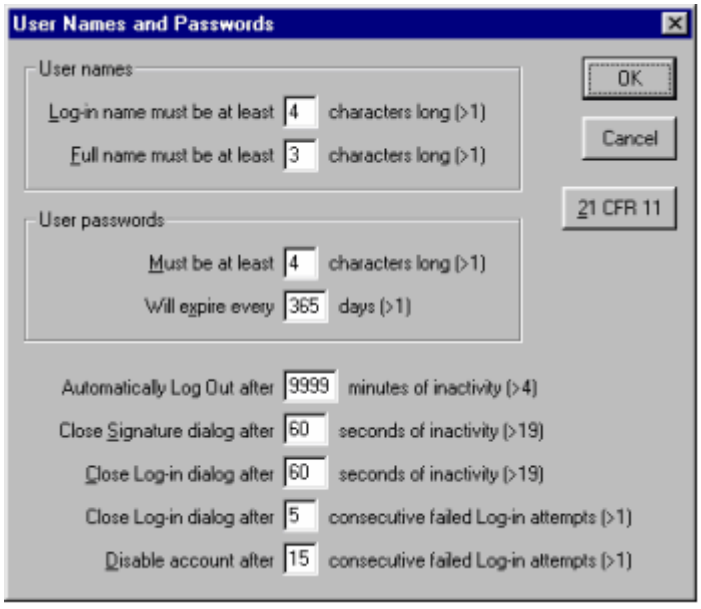
Selecting **User Privileges** displays. a dialog box containing all the options needed to set up the different privileges for each user. The options on the left hand column deal with the creation and saving of methods (SOMs, SOPs) as well as changing and saving preferences. The right hand column deals with file operations.

Under the **User Privileges** dialog box you may also select the **User Settings** button to access the **User Names and Passwords** dialog box. The entire login process is controlled via the user names and passwords dialog. This ensures that the system login is controlled via time dependent metrics, such

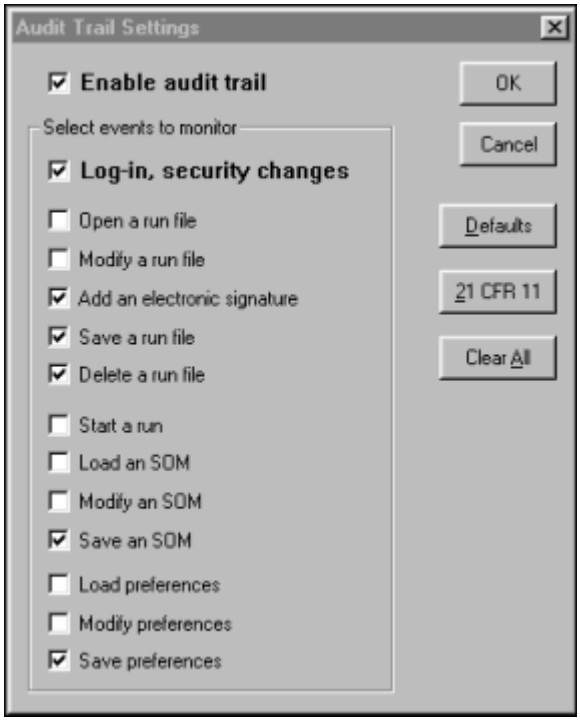
as password expiry and dialog inactivity, and has the required password lengths. Select the options in the dialog box and click **OK**.



NOTE The dialog has a button marked **21 CFR 11** (for the compliant version of software only). When activated this ensures all options are set so that the settings meet with the rules as applicable to 21 CFR part 11. Refer to Regulatory Compliance - 21 CFR Part 11 for more information.



Audit Trail Settings is also accessed from the User Privileges screen or Administrator drop down menus. Audit trail settings allows you to select the events to monitor. These events relate to SOMs, SOPs as well as analysis runs, electronic signatures and preference files. For more information on audit trailing see Audit Trail.



Add a User Account

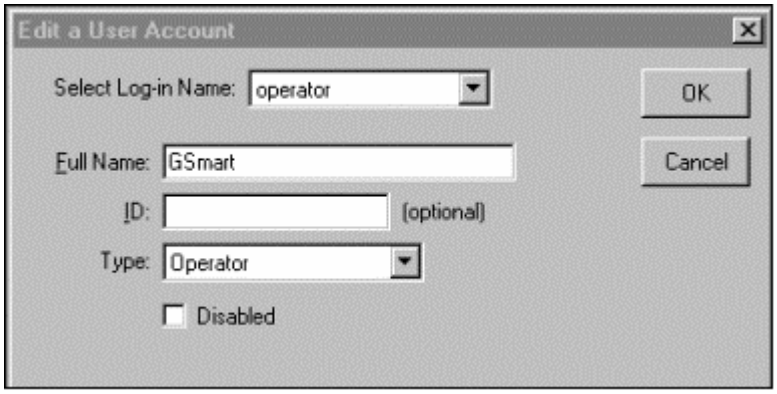
This option enables the Administrator to add additional users.

1 **Add a User Account.**

2 Follow the steps under **Setting Up For Compliance.**

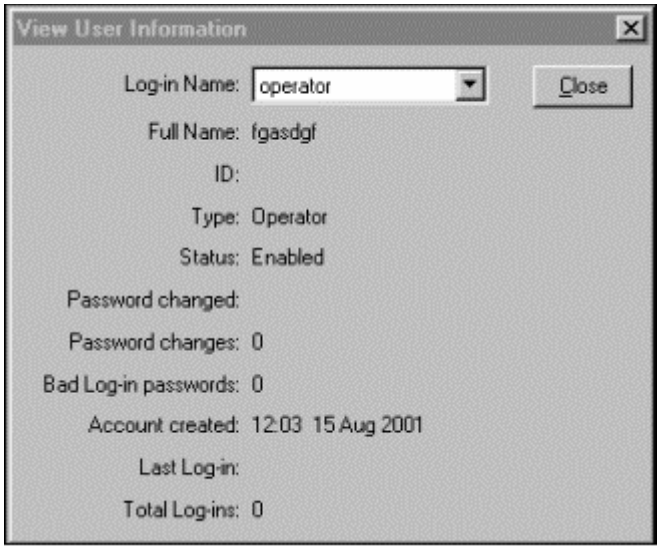
Edit a User Account

Under this option, a current user's account can be edited. Note that only the Type of user, Log-in Name, Full Name, and ID can be edited.



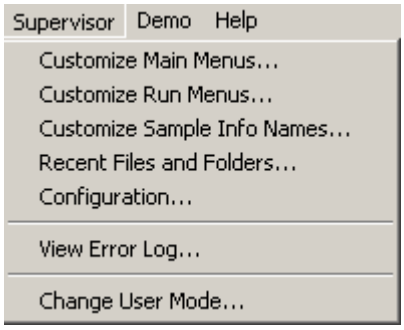
View User Account Info

This option keeps track of any changes done to a user's account as well as the number of log-ins done by the user.



For all other options under the Administrator menus, see Regulatory Compliance - 21 CFR Part 11.

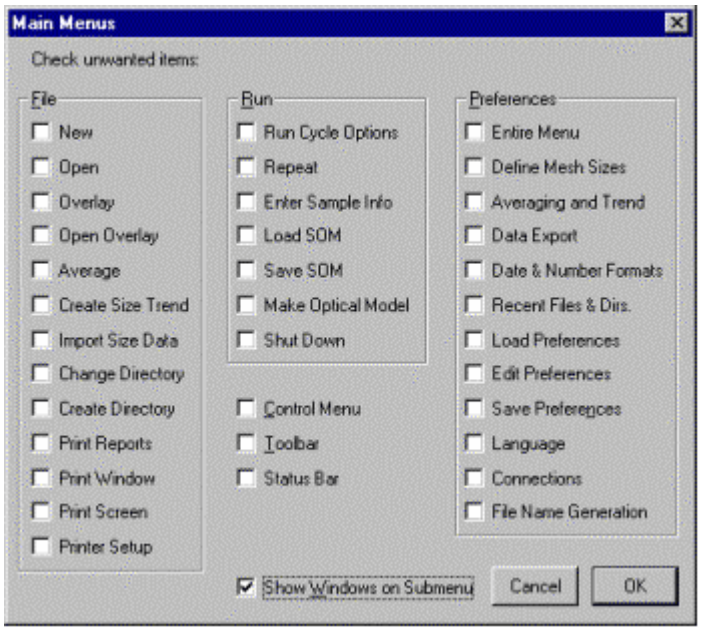
Supervisor



Supervisor is the second highest ranking user after Administrator. To access this mode you must follow the log-in steps as long as there is an account set up for a supervisor. Both the administrator and supervisor have access to the menu items shown in the figure above.

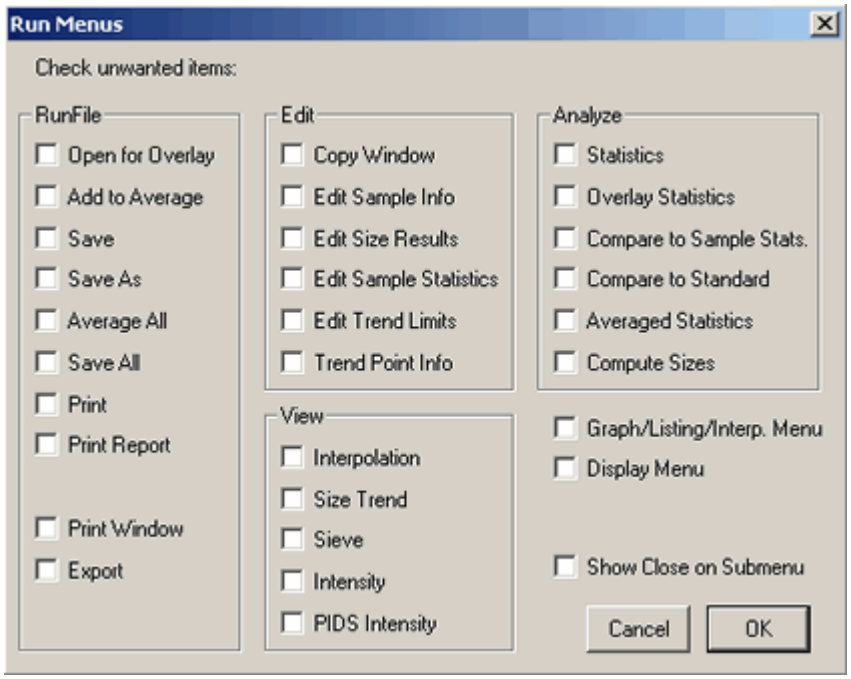
Customize Main Menu

Choices made in this dialog determine the menus that will NOT be available in the main window when setting up **Files**, **Runs**, and **Preferences.** Clicking on any one of these options will deactivate it.



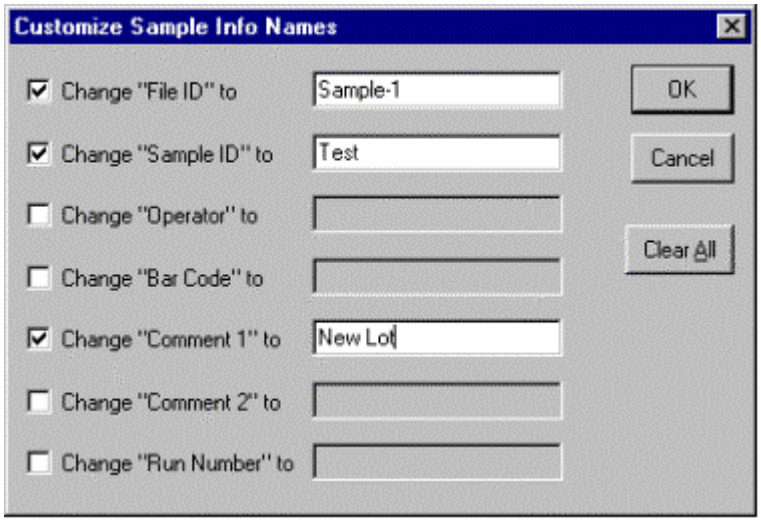
Customize Run Menus

Choices made in this dialog determine the menus that will NOT be available when setting up **RunFile**, **Edit**, and **View**, and **Analyze**. Clicking on any one of these options will deactivate it.



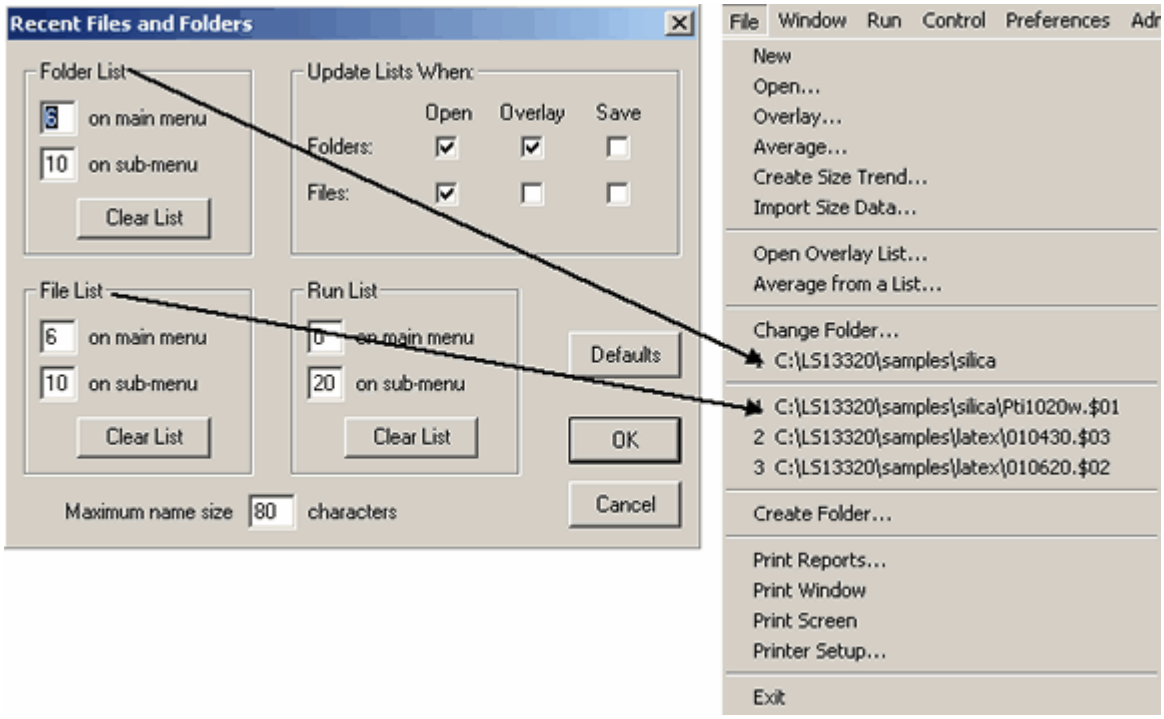
Customize Sample Info Names

These options will change the default field name in the Sample Info dialog box. For example: File ID is the default name for this particular field. Using Sample-1 (see figure below) will change “File ID” to “Sample-1”.



Recent Files and Directories

This dialog box controls the number of files and folders shown in the main File menu. It is accessed by the Administrator from the Administrator menu.

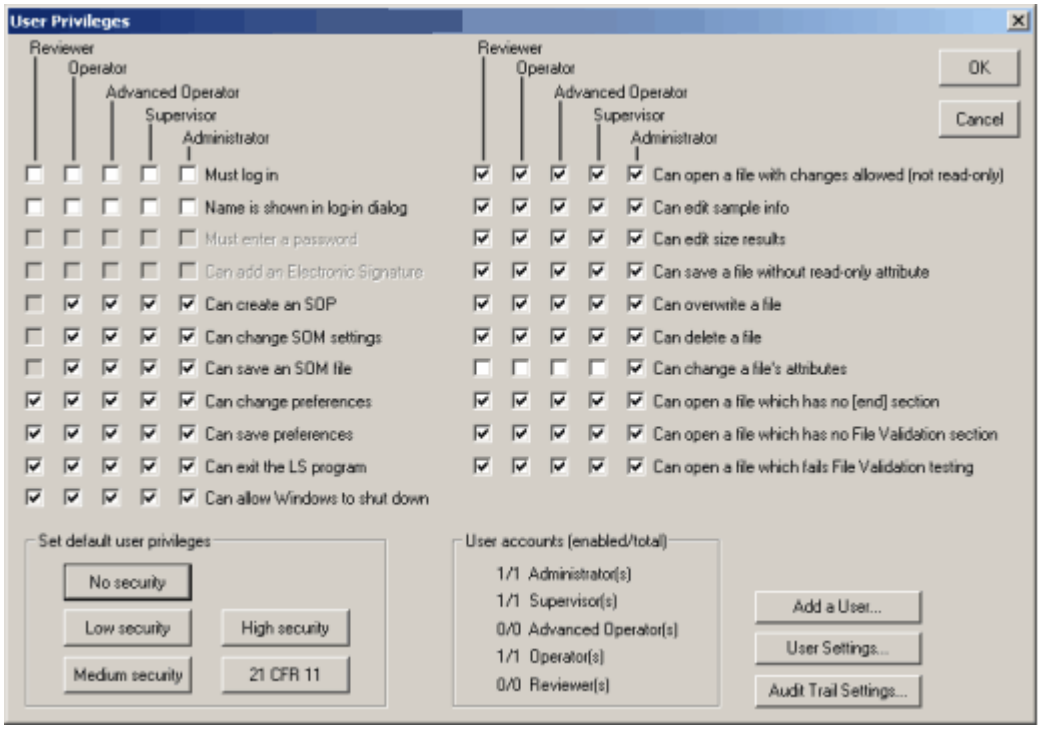


No Security

By selecting **No Security**, there is no need for log-in ID's or passwords. Any user can make changes to data files without the need for signatures. The operator may also create Standard Operating Methods (SOMs) and Standard Operating Procedures (SOPs).

Access to all the administrator options are also available where changes can be made to any of the options provided, i.e., **Audit Trail Settings**. The editing of files will still be tracked with the Audit Trail and reported in the File History.

Figure 3.2 User Privileges Dialog: No Security



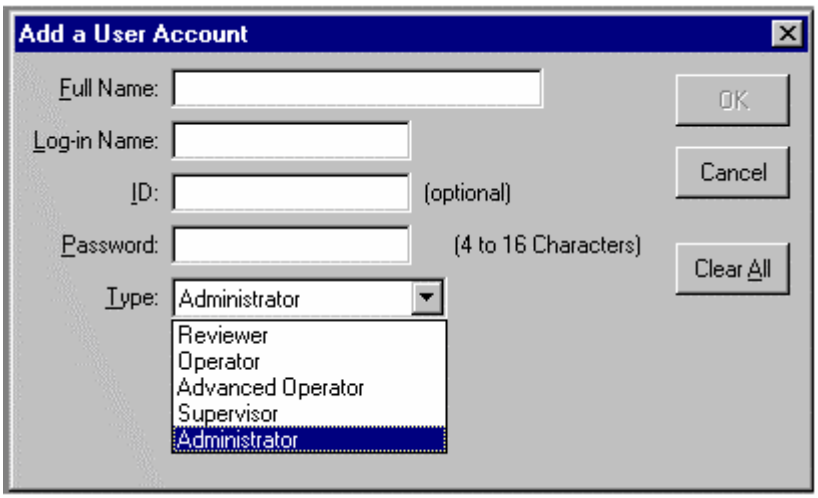
Low Security

Low security requires an administrator to set up accounts for other users. A full name, log-in name and password are required as well as the type of user.

1 Select **Security**.

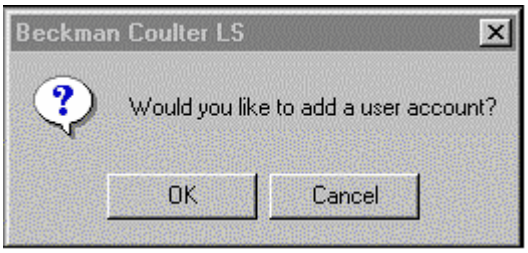
2 From the Security Set Up screen select **Low Security**.

3 The Add a User Account dialog box is displayed.



4 Fill in the dialog boxes with the proper information and choose the type of user from the drop down list box.

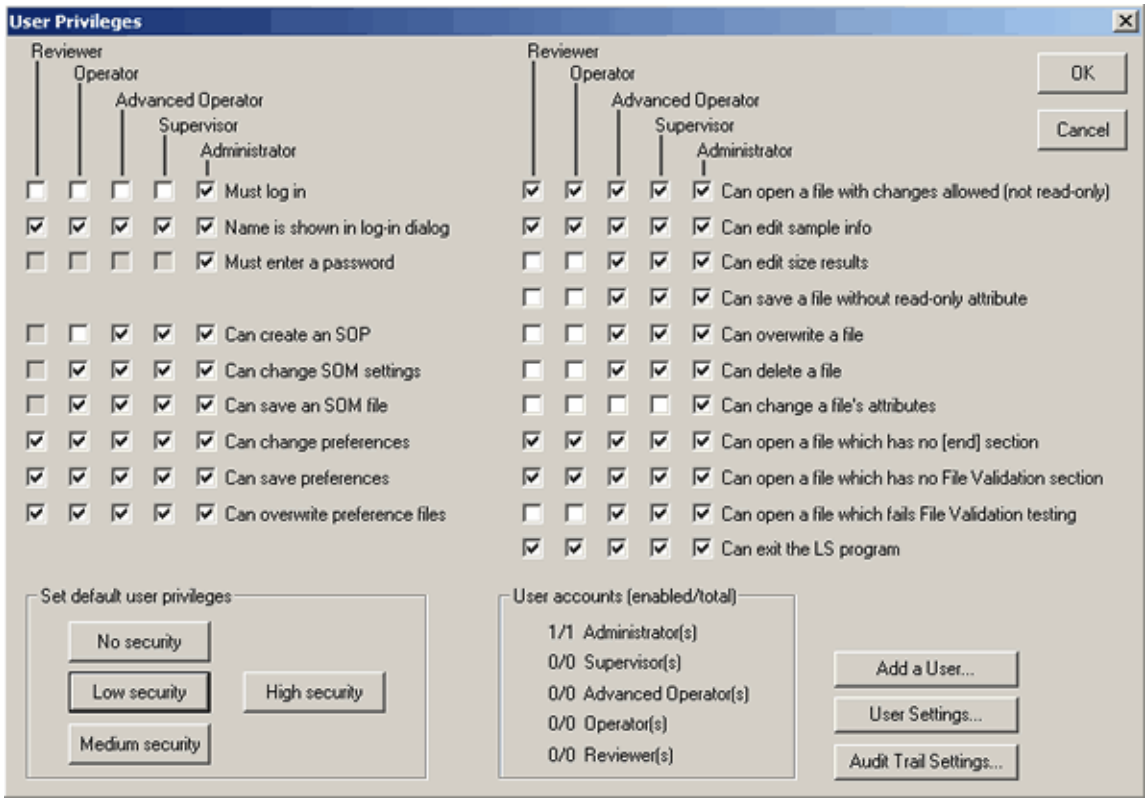
After filling in the dialog boxes with the proper information and selecting **OK**, you will be prompted to add a user account. If **OK** is selected, the Add a User Account screen is displayed again.



Entering **Administrator Mode** and selecting **User Privileges** will show the default options for this level of security.

Refer to Regulatory Compliance for more information on High Security level and 21 CFR Part 11.

Figure 3.3 User Privileges Dialog: Low Security



NOTE Under this security level only the Administrator is required to log in.

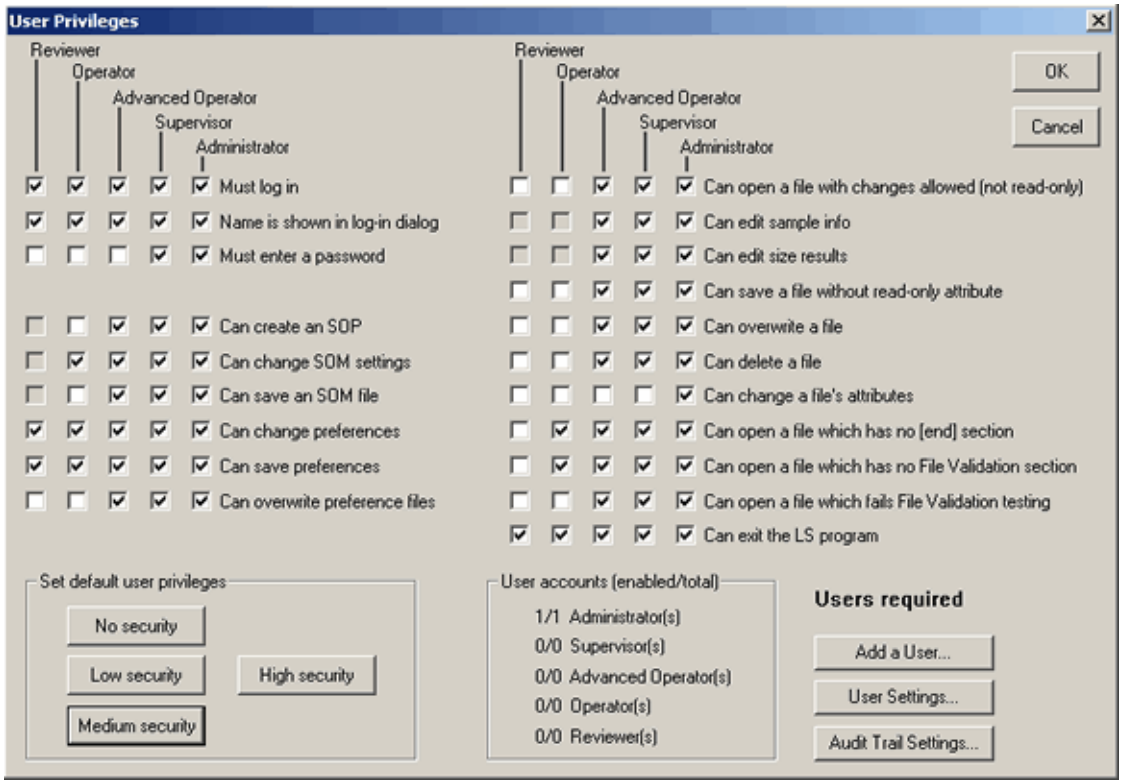
Medium Security

As with low security, **Medium Security** requires the Administrator to set up accounts for other users.

The User Privileges dialog screen default in the medium security setting is set so that all users are required to log in, but only the administrator and supervisor need a password.

These options can be changed by selecting the option boxes for each user under the User Privileges dialog screen shown below.

Figure 3.4 User Privileges Dialog: Medium Security

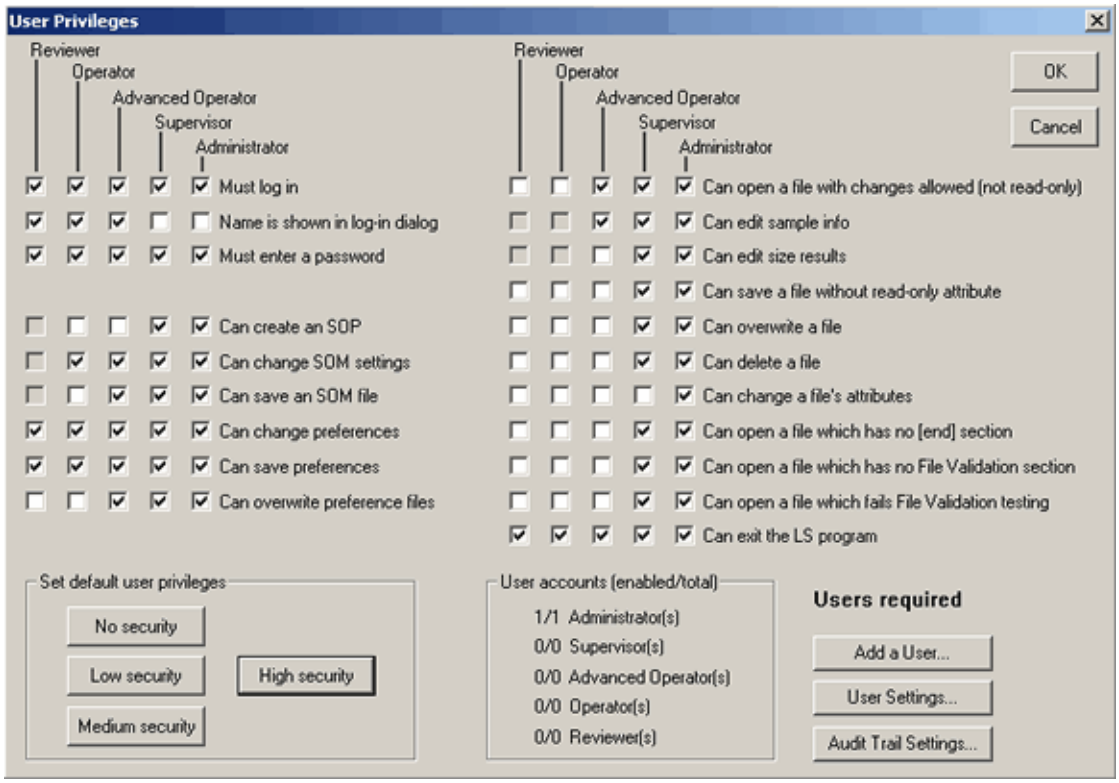


Under this security level all users need to log-in, but only the administrator and supervisor are required to provide a password. The option to add an electronic signature is left open only for the Administrator and Supervisor.

High Security

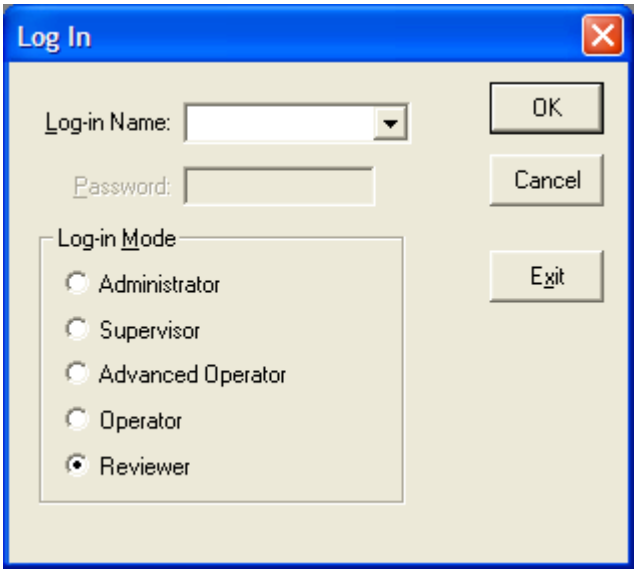
High Security requires the Administrator to set up accounts for other users. The User Privileges dialog screen default in the high security setting is set so that all users are required to log in and to provide a password. These options can be changed by selecting the option boxes for each user under the User Privileges dialog screen shown below.

Figure 3.5 User Privileges Dialog: High Security



Logging Out

As shown under Administrator and User Names and Passwords, a user is logged out after a certain period of time set by the Administrator. The user can also log out by selecting Log-Out from user dialog box. When a user logs out the software will go into a stand-by mode showing the log-in dialog box as shown in the next figure.



Selecting **Exit** from this dialog box will exit the program.

CHAPTER 4

Regulatory Compliance - 21 CFR Part 11

(This chapter applies to the compliant version of the LS 13 320 software only.)

The Electronic Records and Electronic Signatures Rule (21 CFR Part 11) was established by the FDA to define the requirements for submitting documentation in electronic form and the criteria for approved electronic signatures. This rule, which has been in effect since August 20, 1997, does not stand in isolation; it defines the standards by which an organization can use electronic records to meet its record-keeping requirements. Organizations that choose to use electronic records must comply with 21 CFR Part 11. It is intended to improve an organization's quality control while preserving the FDA's charter to protect the public. Since analytical instrument systems such as the LS 13 320 generate electronic records, these systems must comply with the Electronic Records and Electronic Signatures Rule.

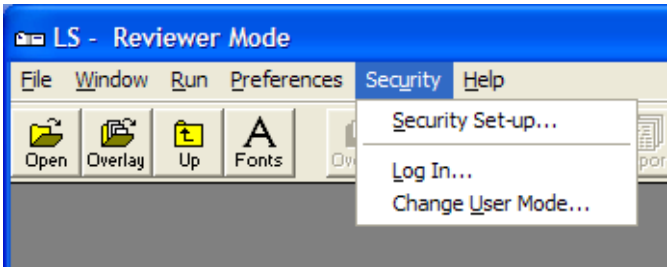
Electronic Record Control - 21 CFR Part 11

The LS 13 320 software employs a system of user names and passwords, consistent with the specifications of Subpart C, Section 11.300. This allows for authorized individuals to use the system, electronically sign a record, access the operation or computer system input or output devices, and perform the operation at hand.

Setting Up For Compliance

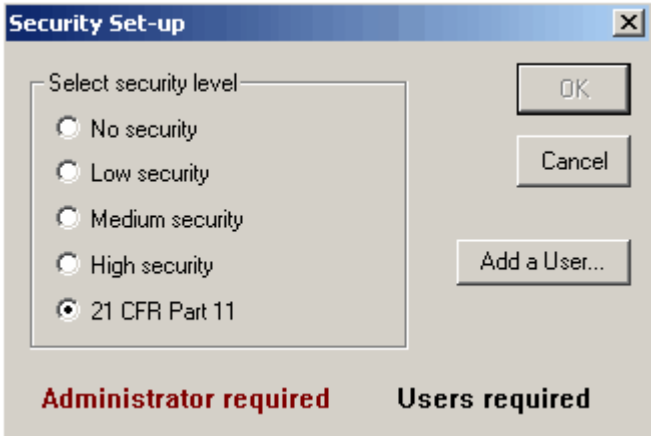
If you selected the security option as described in Security Options, a dialog box appears requiring you to enter a user name and password. If the security option was not selected it can be selected at this time. The following procedure must be followed when setting up security the first time the software is installed. The administrator must complete the high security set up.

1 From the Main Menus select **Security**.



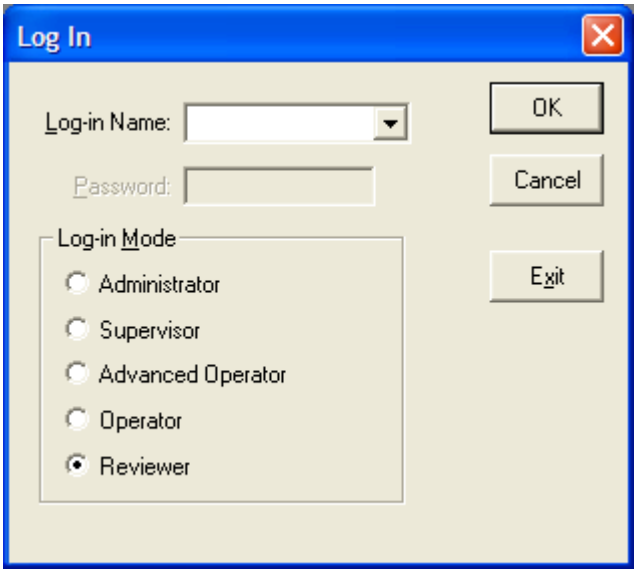
The security level option dialog box is displayed.

2 21 CFR Part 11 to display the following screen.

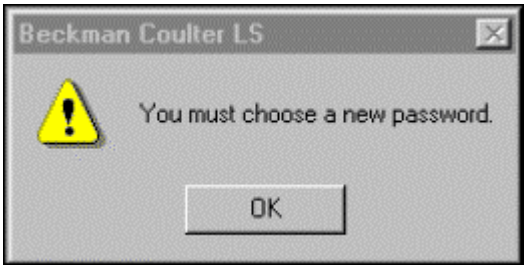


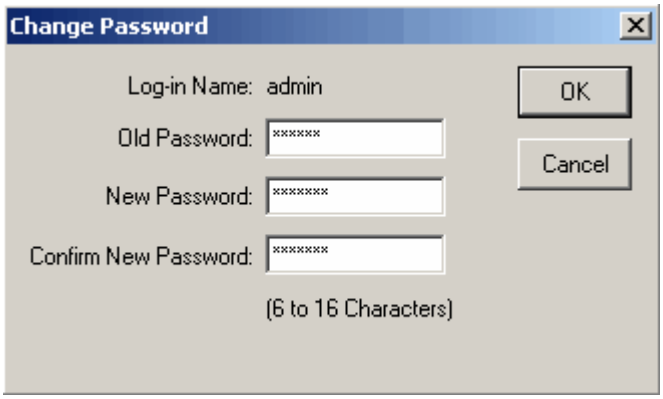
3 Click on the **Add a User...** to display the Add a User Account dialog box. The **Full Name**, **Login Name**, and **Password** are required fields. Select the **Administrator** as the user.

4 After completing the required information and selecting **OK**, another user may be added at this point. After all users have been added, select Cancel and the following dialog screen will be displayed.



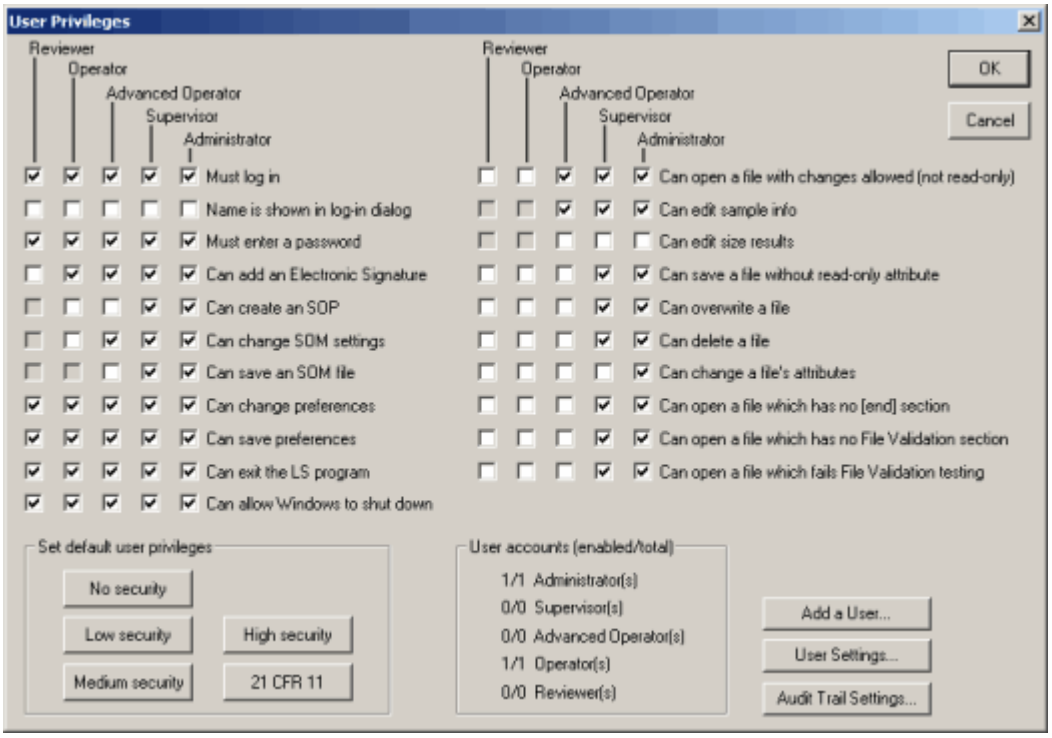
5 You will be prompted to choose a new password.





6 Select **User Privileges**. If using the compliant version of the LS 13 320 software (Version 5.03), the User Privileges dialog box is already configured for 21 CFR Part 11 compliance. This dialog box is covered in more detail in Security Options.

User Privileges Dialog: 21 CFR Part 11 Security



File History

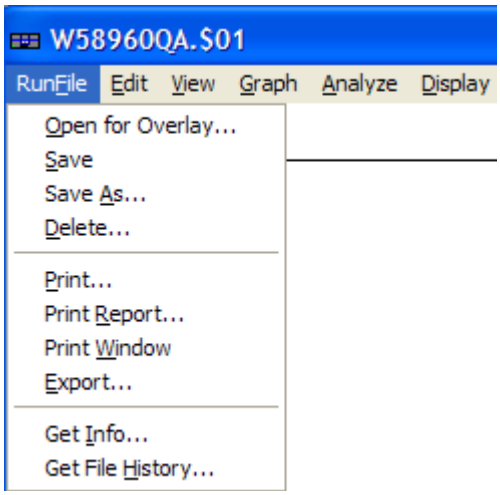
The LS 13 320 software also performs data input and “operational checks”, to ensure that, valid data is entered into the system, and all required steps have been completed to perform the task at hand. The purpose of all such data checking and validation allows for strict procedural enforcement within the LS 13 320 software system, recording all changes that are made to data generated from within LS 13 320 software.

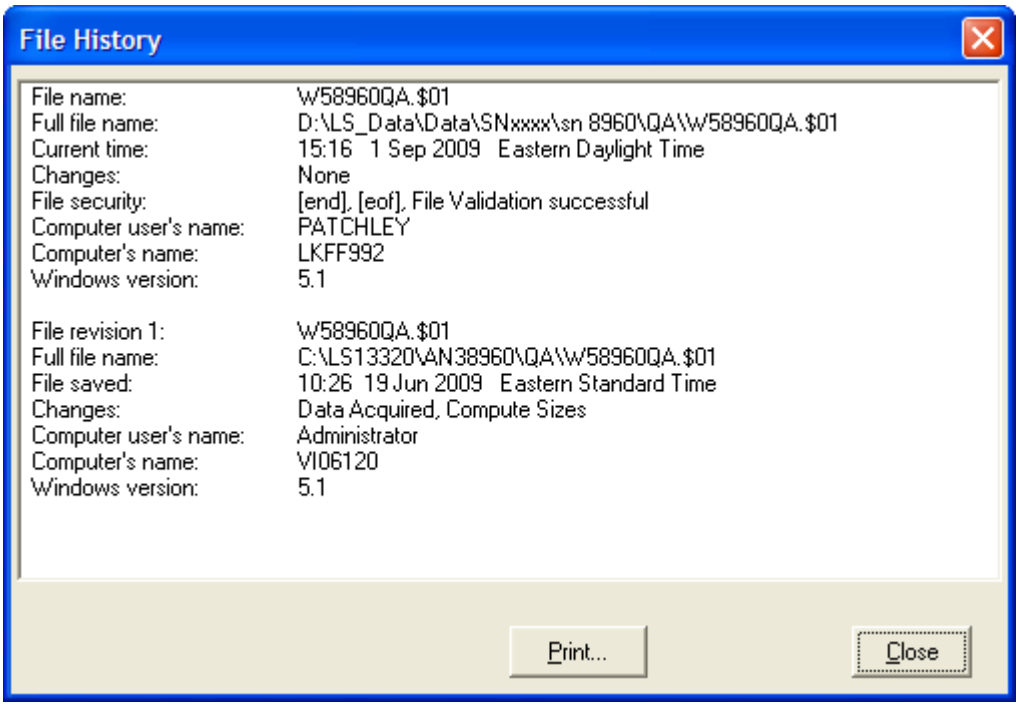
Any file created by the LS 13 320 software can have a 'File history' or auditing enabled. Once auditing has been enabled for a file, it cannot be disabled, nor can it be bypassed. Under these conditions, all changes made to a file are automatically recorded. These changes consist of “computer-generated, time-stamped audit trails to independently record the date and time of operator entries and actions that create, modify, or delete electronic records”. When a change to a file is detected, the LS 13 320 software automatically records the identity of the user making the change, the date and timestamp of the change, the parameter that has been changed, the old value, and the new value. The user is also required to 're-sign' the record electronically and enter a reason for the change, from a pre-defined list or as free text.

This audit trail is then stored as a File History within the file itself, such that '"record changes shall not obscure previously recorded information", and in a "form suitable for inspection, review, and copying by the agency". This ensures that a complete and continuous record of all changes to the file is maintained. Through the file protection and archiving capabilities of the LS 13 320 software, it can be ensured that, audit trail documentation is retained for a period at least as long as that required to maintain records.

To access the File History information:

1 **Run File** and select **Get File History**.



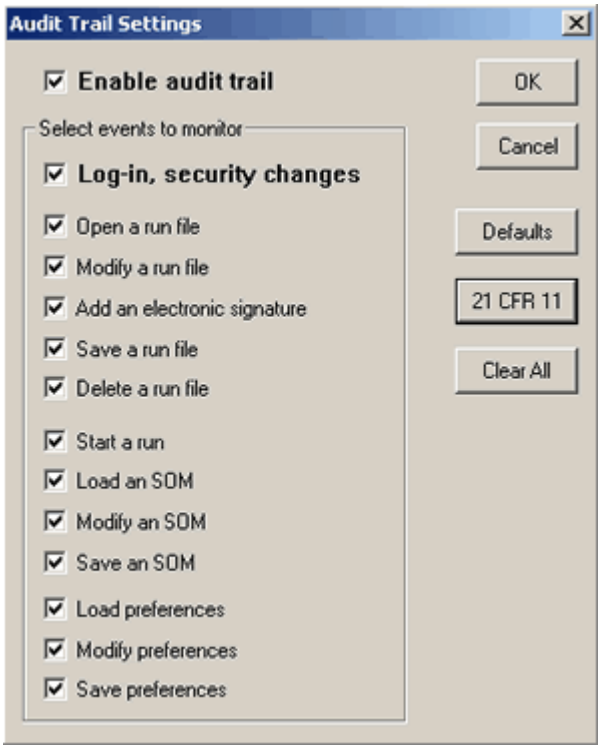


Audit Trail

In addition to the auditing associated with the electronic file itself the LS 13 320 software offers two additional levels of auditing. First, a general system trails all records and stores information at a system level, for example who logged in when, when users were added to the system, etc.

Second, the LS 13 320 software generates an error log that stores and records errors associated with the system e.g., missing files, communication errors, etc.

From the Administrator menu drop down menu, select **Audit Trail Settings**.



Select the options in the dialog box and press **OK**.

NOTE The dialog has a button marked **21 CFR 11**. When activated this ensures that options are set to facilitate compliance with the regulations set forth in part 11 of the CFR volume 21.

Electronic Signatures

An electronic signature can be defined as “A computer data compilation of any symbol or series of symbols executed, adopted, or authorized by an individual to be the legally binding equivalent of the individual's handwritten signature”. This means that an electronic signature is some computer representation of a user's identity, developed to insure the distinct and unique identity of that user. The use of electronic signatures as the “legally binding equivalent of traditional handwritten signatures” then must be “certified” to the agency in writing.

Biometric and non-biometric forms of electronic signature are methods of verifying an individual's identity based on an individual's physical feature(s) or repeatable action(s). Biometrics are generally regarded as techniques such as fingerprints or retinal scans, which are considered to be totally unique to each individual and require specific forms of scanning devices to read and interpret. Non-biometric signatures are those that are computer generated and employ at least two distinct identification components such as an identification code and password. It is this form of electronic signature that is supported by the LS 13 320 software.

Generating Electronic Signatures

The LS 13 320 software employs User ID's and passwords to verify the identification of each user logging into the system. When using this technique, requires maintaining the uniqueness of each combined identification code and password, ensuring that no two individuals have the same combination of identification code and password. This section also requires that identification code and password issuance are periodically checked, recalled, or revised. The LS 13 320 software satisfies both of these requirements.

The administration of the system requires that individuals are added to the list of valid LS 13 320 users via the Add a User Account dialog screen. The identification code or user name of each LS 13 320 user must be unique. No two users on the same LS 13 320 system can have the same user name. It is also required that users, supply a password to access the LS 13 320 software, thus satisfying the requirement of employing at least two distinct identification components, identification code and password. Passwords can be controlled to prohibit the use of duplicates and to force the selection of new passwords after a prescribed period of time.

By the implementation of these features, the LS 13 320 software can satisfy the requirement that, identification code and password issuance are periodically checked, recalled, or revised.

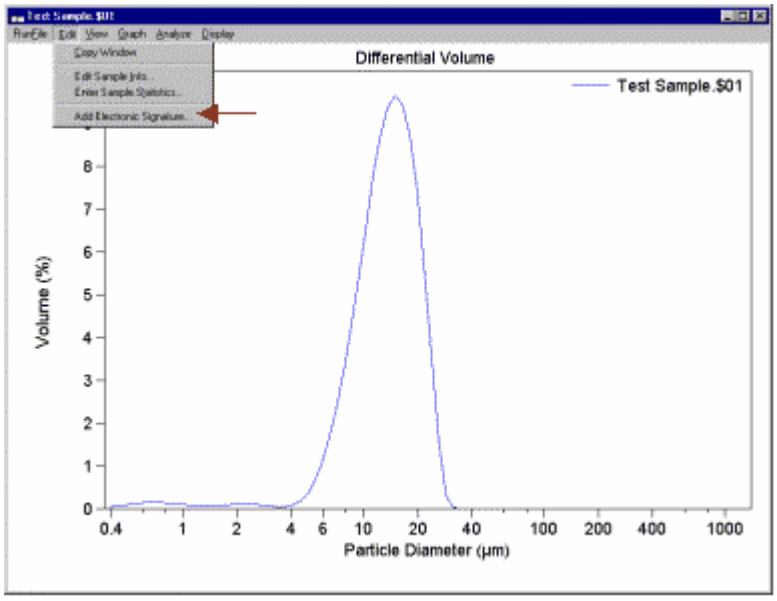
Users may be authorized to generate electronic signatures by the administrator. This authorization is granted in the User Privileges dialog box. When a user has been authorized, he/she can electronically sign a file.

To generate the electronic signature:

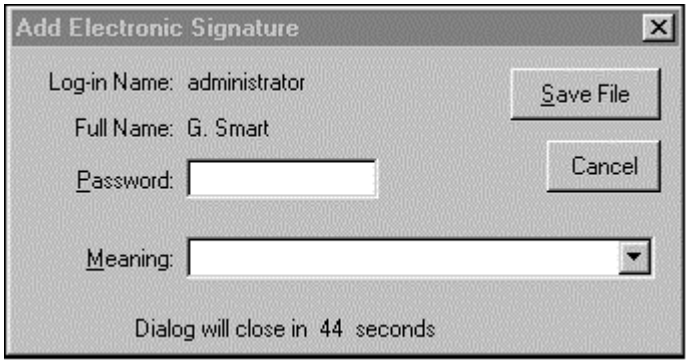
1 Open a file.

2 **Edit**.

3 Select **Add Electronic Signature**.



The following screen is displayed. Provide your password and select a meaning for the electronic signature from the pull down options or type a new meaning.



The Electronic Signature Meanings can be created from the User Privileges dialog box.

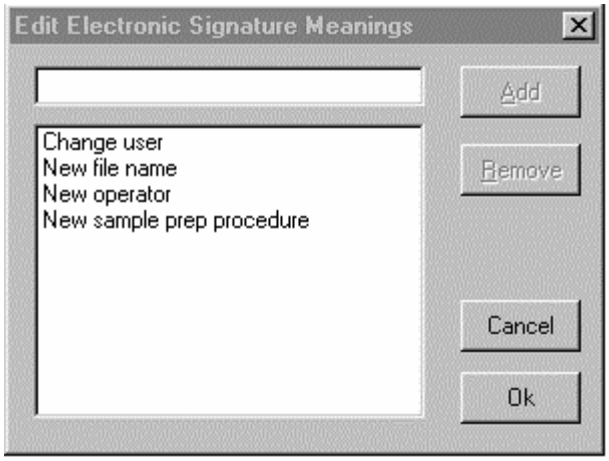
1 From the dialog User Privileges box select Edit Electronic Signature Meanings.

2 Type the meaning in the space provided.

3 **Add.**

4 Selecting **Remove** will remove any meaning you highlight.

5 **OK** when done.



Applying Electronic Signatures

Through the application of LS 13 320 user and password configuration procedures, the system can be configured to ensure that inappropriate use of these identifiers can be performed only by the intentional divulgence of security information.

The LS 13 320 software uses the application of the user name and password to authenticate the user making and saving the changes, in conjunction with file history and audit trailing, to independently record the date and time of operator entries and actions that create, modify, or delete electronic records.

Additional Security Features

The LS 13 320 software offers two important additional levels of security, that while not specifically called for by the regulations, make defining and implementing system policies easier.

Data Mirroring

The administrator has the option to securely store files in a separate location and in addition to that specified by a user. This is done by enabling the data mirroring option, which can only be accessed under administrator mode.

1 From the Administrator mode, select **User Privileges**.

2 Select **Data Mirroring**.

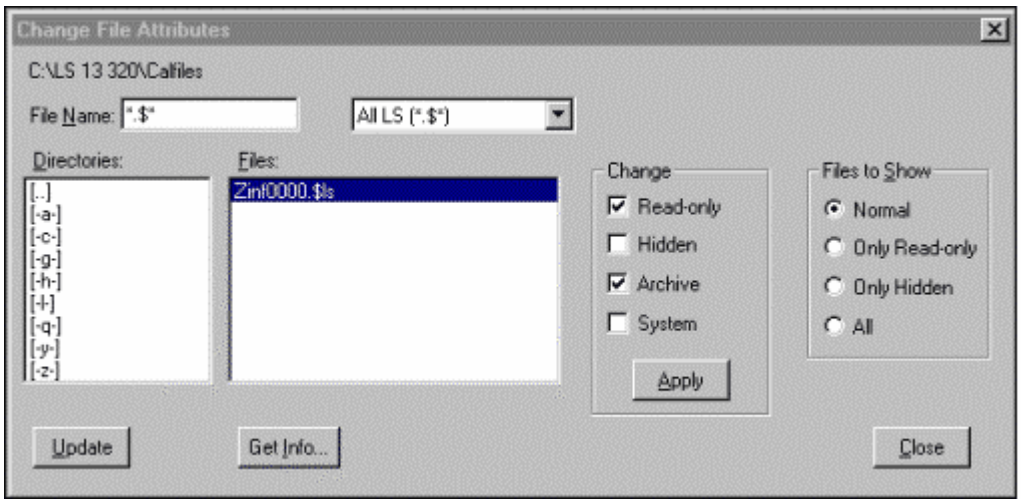
3 Check **Enable Data Mirroring**.

4 Select the drives in which you wish the data to be saved.

IMPORTANT It is important that drives you are mirroring to are part of your network. The computer must be mapped to the network being used. Contact your network server administrator for help on how to map your computer to the network.

File Attributes

Files can be protected to varying degrees using the File Attributes options.



The **File Attributes** allow you to select a specific file and make it a read-only file, hide it, etc. You can also choose to mark it as a normal file or as only a hidden file.

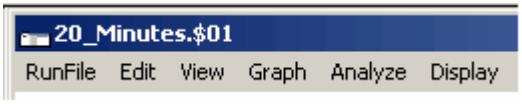
Starting Security-Enabled Software

After all settings for 21 CFR Part 11 security have been configured, every time the LS 13 320 software is started, the user is prompted to enter a user name and password. The software will automatically configure according to the privileges granted to the user by the administrator.

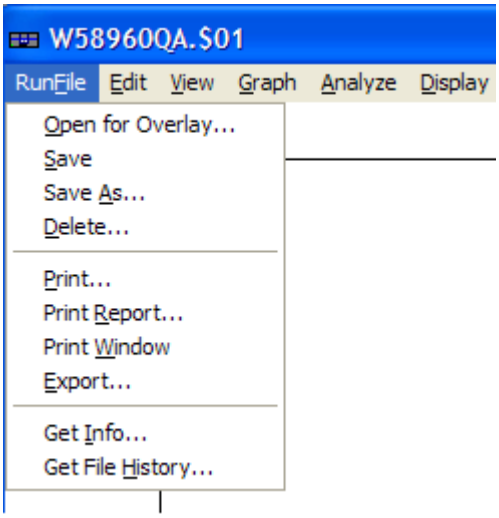
CHAPTER 5

Data File Menus

This section will guide you through the menus that deal with acquired data.



RunFile Menu



The RunFile menu is used to handle data saving and printing as well as to obtain information about the file and the history of changes made to the file.

Open for Overlay

The LS 13 320 software allows you to view more than one data file on the same graph.

This is useful when you want to compare data from different files.

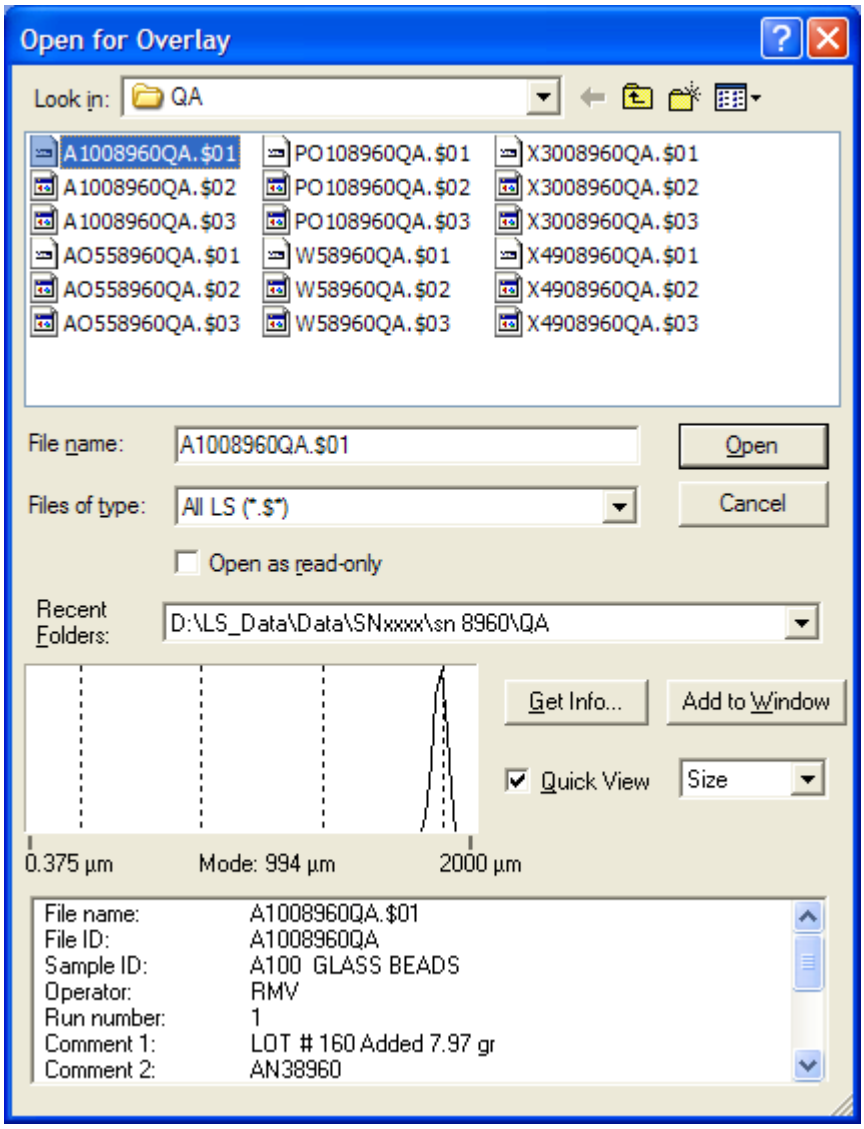
To open new data files for overlay.

1 **RunFile.**

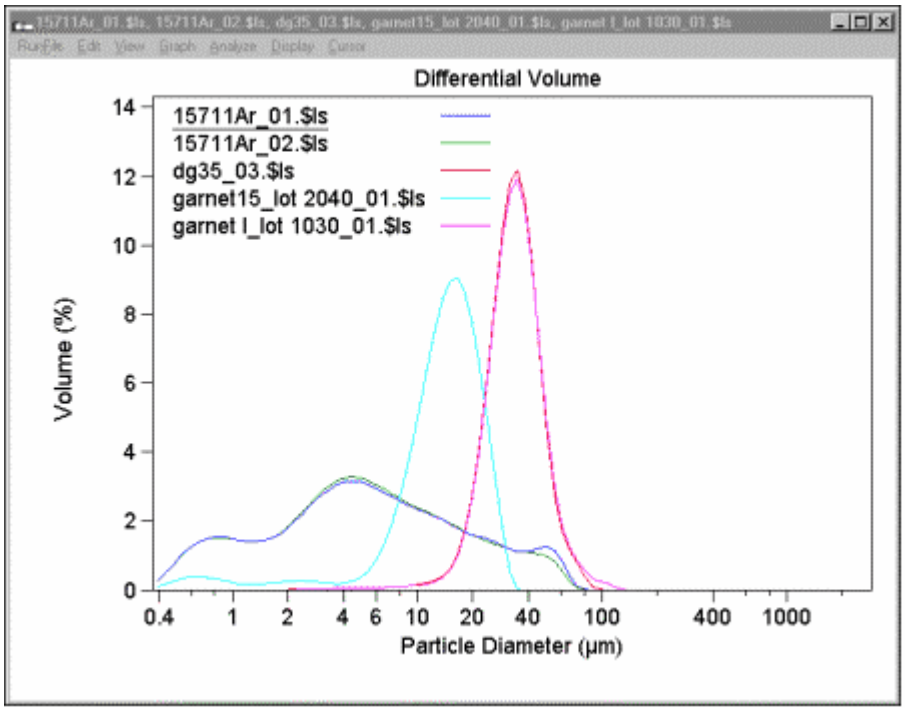
2 **Open for Overlay.**

3 Select the file you wish to overlay with the current opened file.

4 **Open.**



Unlike the **Overlay** command, the **Open for Overlay** command will allow you to open one file at a time, but you can still overlay up to 30 files.



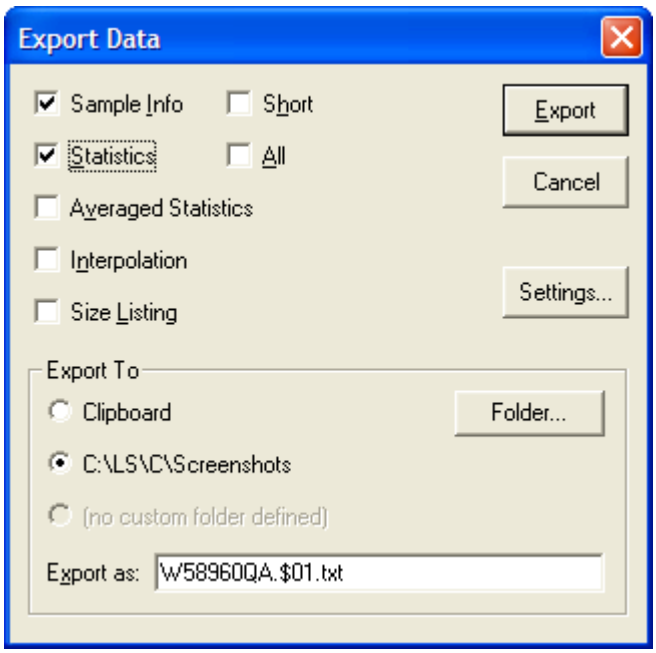
Save

If a file has been modified (edited) it has to be saved in order for the changes to take effect. These changes will be recorded in the File History. When working under a high security level, a reason for any change must be provided. After selecting Save, the following dialog box is displayed:

Make an entry in the **Reason** dialog field and click **OK**.

Export

The Export option allows you to send selected information about a file to, for example, a spreadsheet, using the file name with a .csv (comma separated value) or other extension.



1 **RunFile.**

2 **Export.**

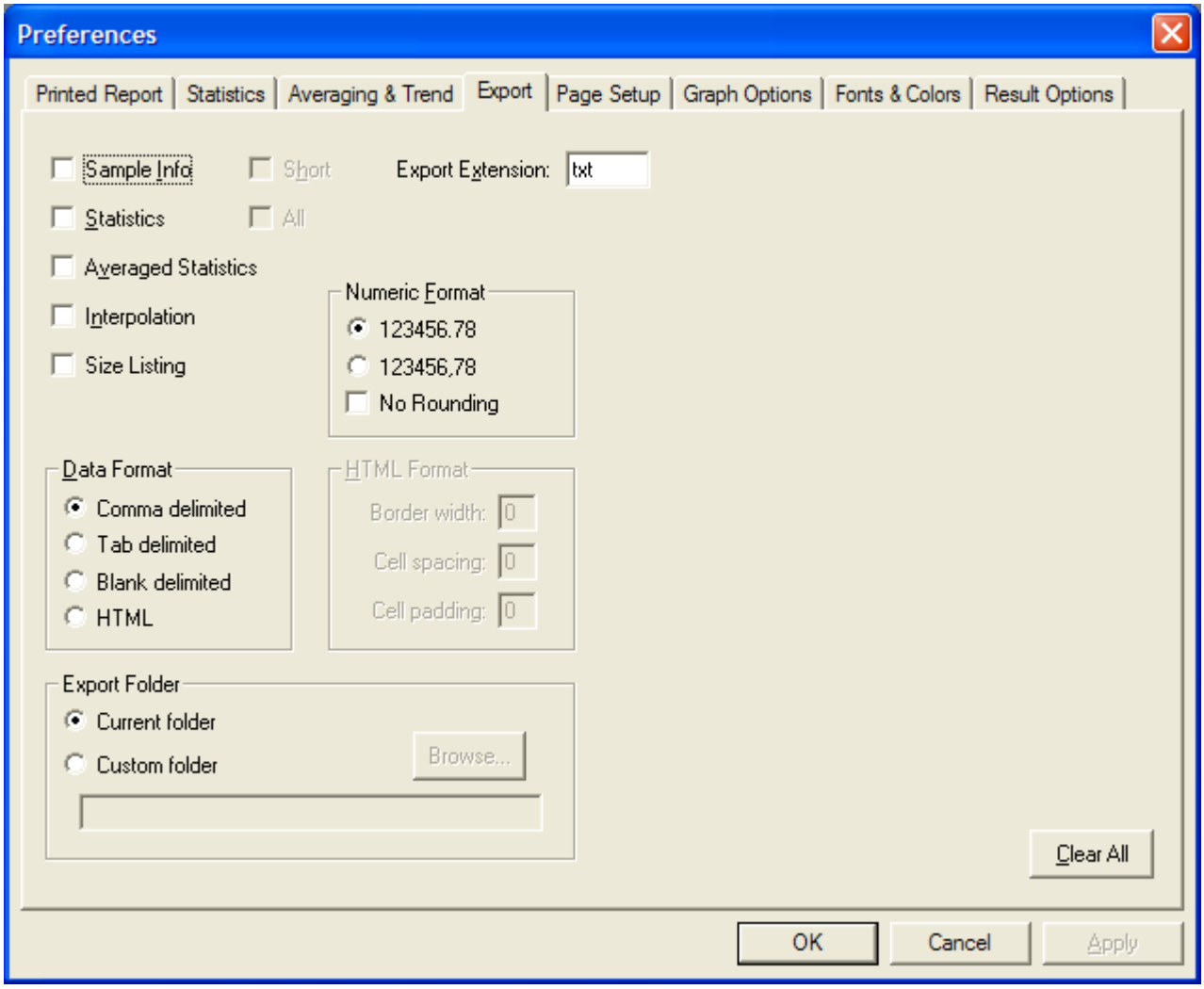
3 Select the options you want to export.

4 Select an option from the Export to dialog. By default files are exported to a Microsoft® Excel spreadsheet file.

5 **Export.**

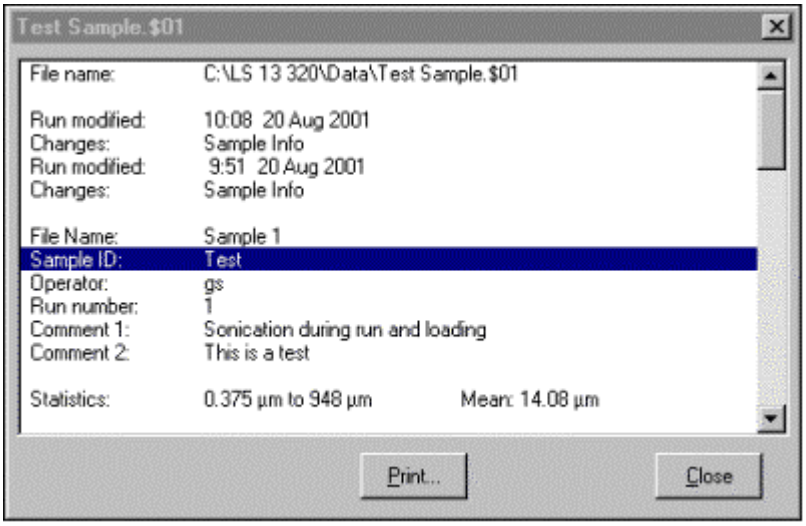
6 Select **Settings** for additional options as shown in the figure below.

After selecting Settings the following dialog box is displayed.



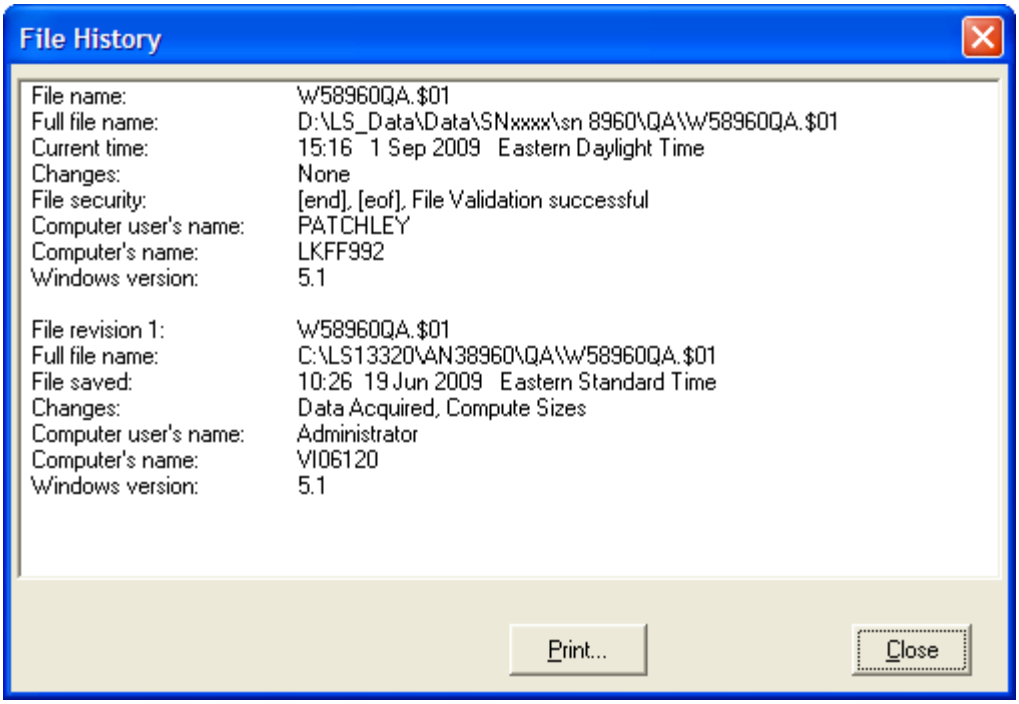
Get Info

Information about the run parameters and sample info are contained in this dialog.



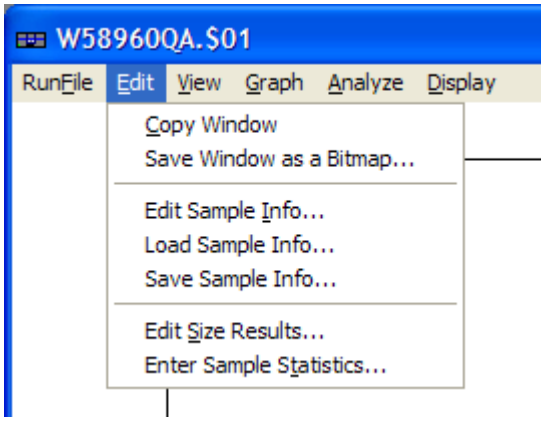
Get File History

The file history is linked to the Audit Trail. This screen records any changes that have been made to a file.



Edit Menu

Figure 5.1 No Security



Set Up With Security

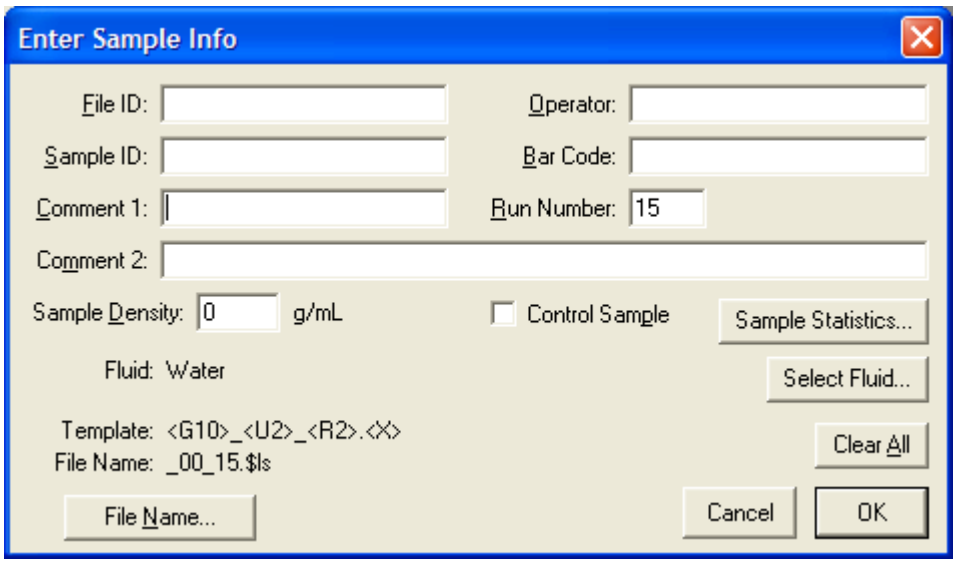
A setup with security selection for the Edit menu would have "Add Electronic Signature" displayed.

Copy Window

This command will place the information displayed in the LS 13 320 window onto the clipboard.

Edit Sample Info

Information about the sample can be edited through this option. Under all levels of security, except No Security, any changes made during the editing process will be recorded in the File History.



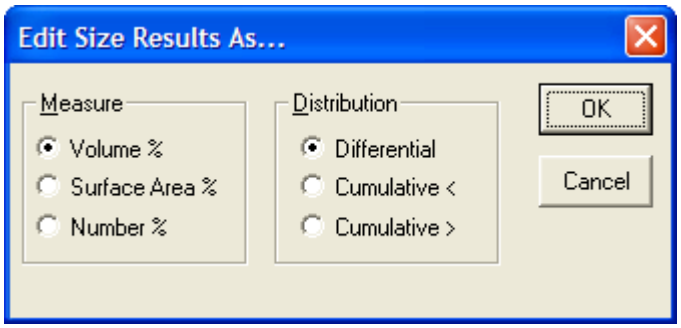
NOTE Expect that all edit options change depending on the level of security.

Under No Security any user can edit the data file. For Low Security the operator and reviewer may edit data only if the administrator selects the options under the user privileges dialog. Medium Security does not allow either the operator or reviewer to edit data. For High Security see Security Options.

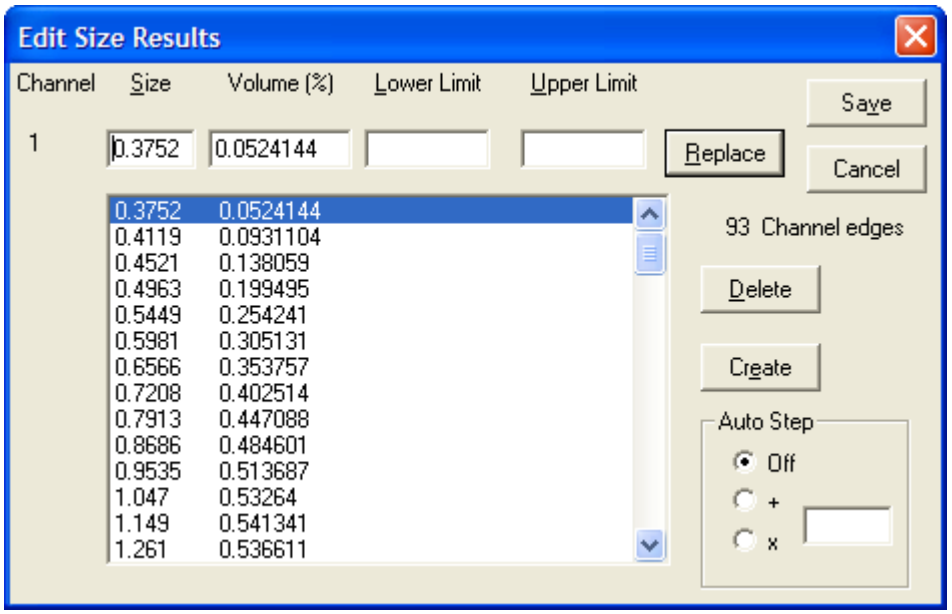
Edit Size Results

Edit size results allows you to actually add and/or delete data values from your size distribution. Under No Security any user has access to this option. For Low and Medium Security the operator and reviewer are not allowed to edit the size data. High Security does not allow any user to access the size data.

Selecting **Edit Size Results** will lead to the following dialog screen:



Select the options you wish to use during the editing and click **OK**. The following screen is displayed:



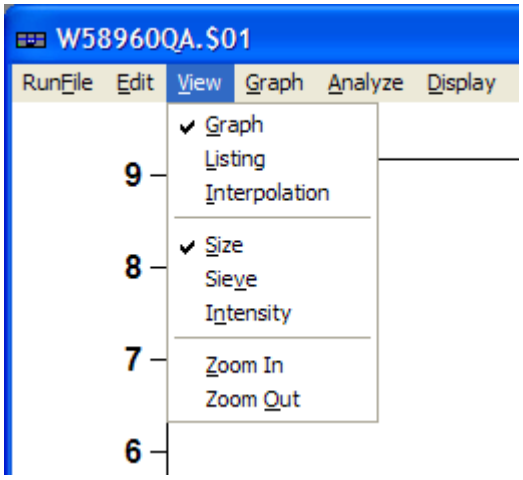
The **Edit Size Results** option allows you to add or delete data points from the distribution. The **Auto Step** option allows you to create size classes by either adding or multiplying a value entered in the **Auto Step** dialog field.

Add an Electronic Signature

(For compliant version of software only)

The addition of an electronic signature is controlled by the security level being used. Under No Security electronic signatures are not required. Low Security allows the administrator to add an electronic signature. Medium Security permits the administrator and supervisor to enter an electronic signature. Under High Security every user, except for the reviewer, is expected to add an electronic signature whenever a file is edited. See Electronic Signatures for more information.

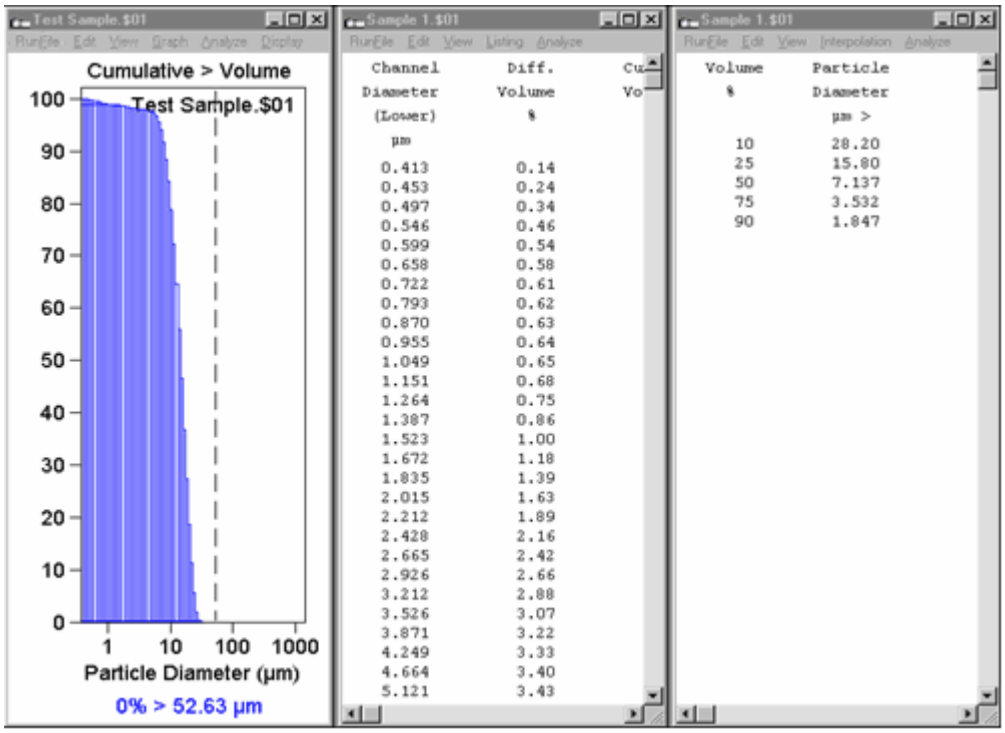
View Menu



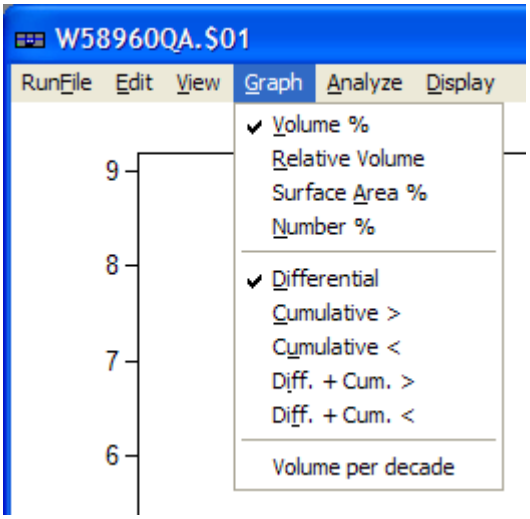
The View menu allows you to choose the way the data is presented on your computer screen. This format can be printed as it appears on the screen using the RunFile, Print command. The data can be displayed in graphical form, by listings, or by showing a set of interpolation points.

Graphs

Graphs of data can be displayed in different formats. The figures below are examples of one type of graph format, a listing, and an interpolation.



The graph options are selected from the Graph menu as shown in the next figure.



Once a graph is displayed there are several operations that can be executed. These are covered in the next sections.

Display Values on One Channel

Point the arrow to a channel and press the left button of the mouse to display a cursor and the channel's value.

Display Multiple Channel Values

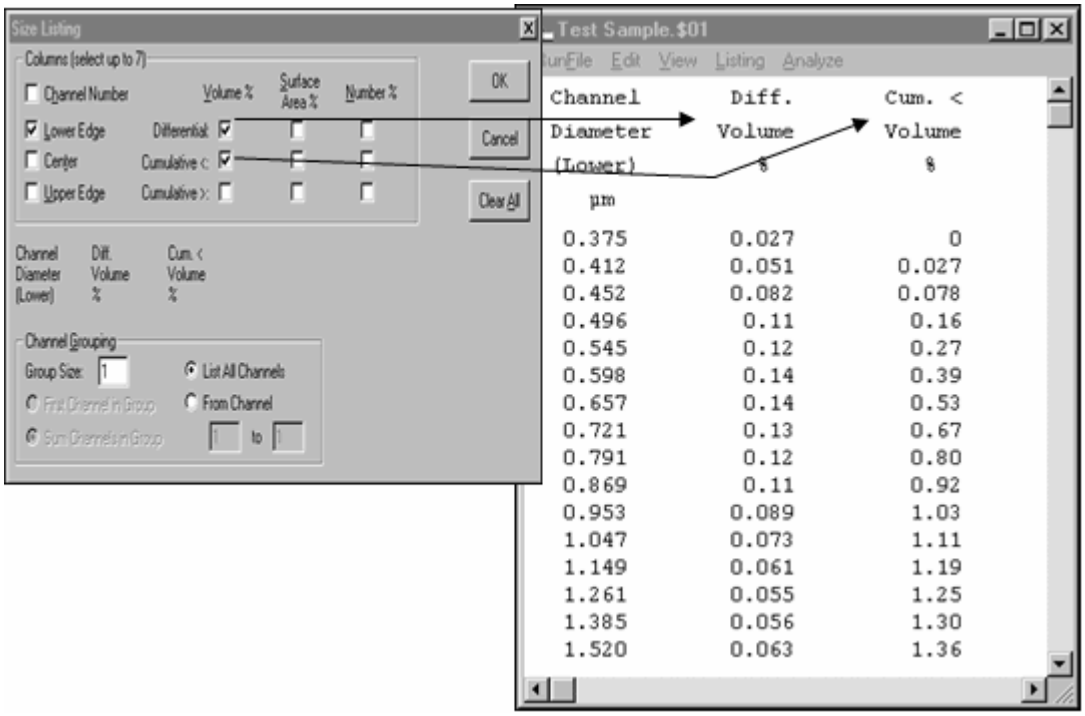
Left click on one channel and drag to a second channel to display the cumulative values for the area they delimit. The channel values for the lower cursor (LC) and upper cursor (UC) also appear.

Expand Part of a Graph

1. Add two cursors to the graph.
2. Point the arrow between two cursors and press the right mouse button.
3. Press the right button again to restore the graph.

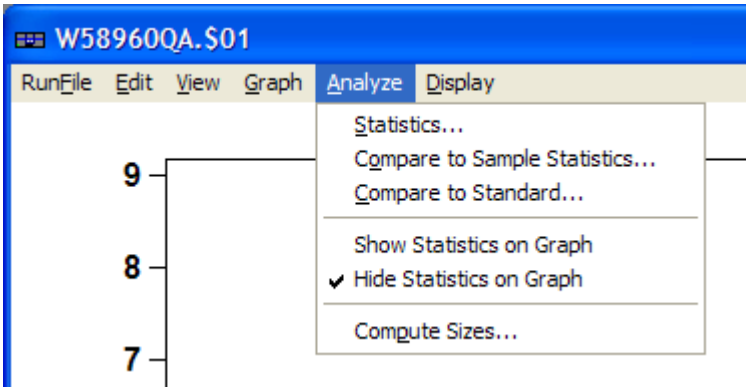
Listing

The listing options are shown in the following figure.



The options selected will be displayed in tabular form with the first column being the first option selected.

Analyze Menu



Statistics

See APPENDIX C, *Optical Models* Statistics, for definitions of statistics used in the LS 13 320 program.

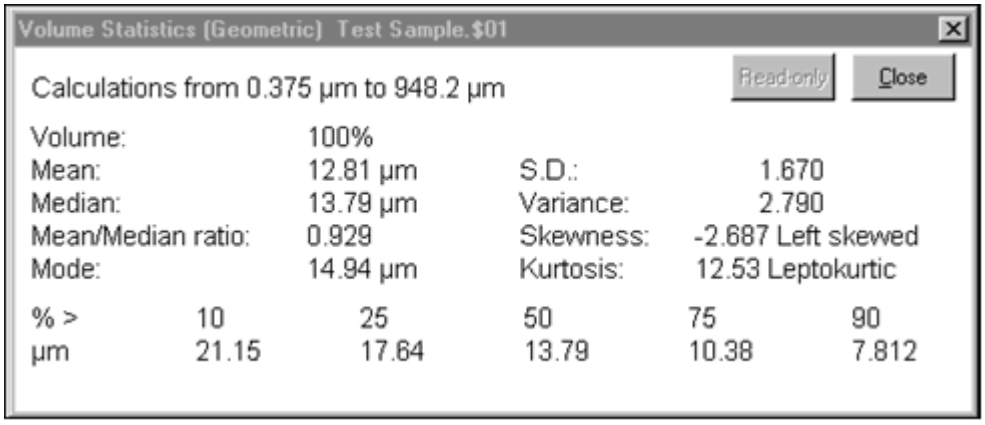
To view the statistics for the active run file:

1 Select **Analyze**, **Statistics**.

2 The Statistics page is displayed.

3 If working in No Security or Low Security modes, select **Save** or **Close**. Medium and High Security modes will not allow you to save the Statistics, because these are displayed as read-only.

4 To view the statistics for only part of the size distribution, add cursors around the desired portion of the distribution and repeat steps 1 to 3.



Compare to Standard

Files can be compared to other files chosen to be standards. These standard files can be an actual instrument control sample or a sample that is used as a standard control for other similar samples.

Compute Sizes

Use this function to analyze raw data with an optical model or to reanalyze size data with a different optical model than the one used when the sample was run. See also heading 14.4, Make an Optical Model.

To select an optical model:

1 Select **Analyze**, **Compute Sizes**.

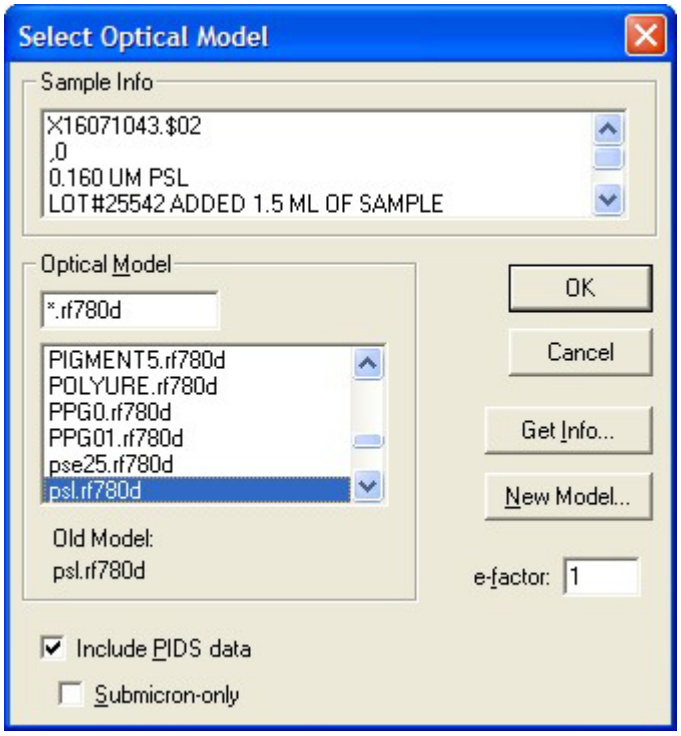
2 Select the optical model needed. If the optical model is not shown in the list, select New Model. For more on optical models see APPENDIX C, *Optical Models*.

3 Select **OK**.

4 Wait while the optical model is loaded and the sizes are computed.

5 Repeat steps 1 to 4 to view results using another optical model or to change back to results from the original one. Only results from the last optical model used for analysis are stored with the run file.

Figure 5.2 Optical Model selection under the Compute Sizes dialog box



e-factor - This factor, also known as the shape factor, helps correlate results obtained from the LS 13 320 to those obtained by other methods, such as the Coulter Principle, SEM, etc.

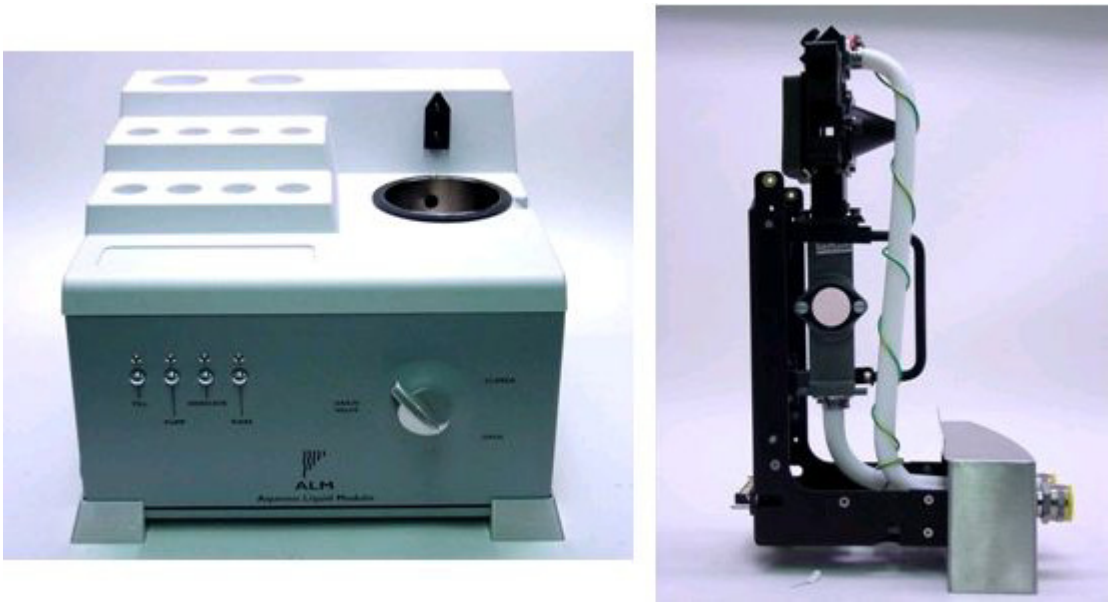
CHAPTER 6

Sample Modules

Aqueous Liquid Module

The Aqueous Liquid Module (ALM) is intended for use with the LS 13 320 Optical Bench. It is capable of suspending samples in the size range of 0.017 µm to 2000 µm.

Figure 6.1 Aqueous Liquid Module (ALM) with Sample Cell



The ALM presents the entire sample to the instrument by re-circulating the sample. The amount of sample needed depends on its size and concentration. Prior sample preparation is often needed to achieve proper dispersion (see APPENDIX B, *Sample Handling* for more information on how to disperse samples) in order to obtain accurate and valid results.

ALM Description

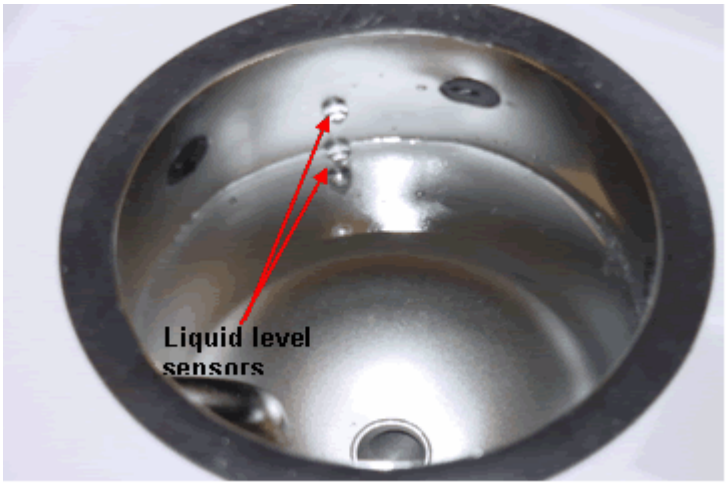
System Description

The ALM system consists of:

- A sample vessel containing the suspension fluid and dispersed sample particles.
- A sample cell.
- A sonicator that aids in the dispersion of the sample.
- A variable speed circulation pump to circulate the particles through the sample cell.
- Inlet and drain hoses.
- Hoses to transfer fluid from the vessel to the sample cell.
- Liquid level sensors.

The ALM is intended to be used with water only and holds a total volume of 1250 mL. Using the automatic fill mode, the system only uses 1100 mL. The ALM has the capability of auto-filling and auto-rinsing using a self-contained internal pump that is operated through software commands. The pump fills the vessel to a set level controlled by liquid level sensors (figure 1). If the vessel is manually filled, an alarm will sound to indicate when the liquid level has reached the overflow sensor, this is intended to avoid fluid overflow.

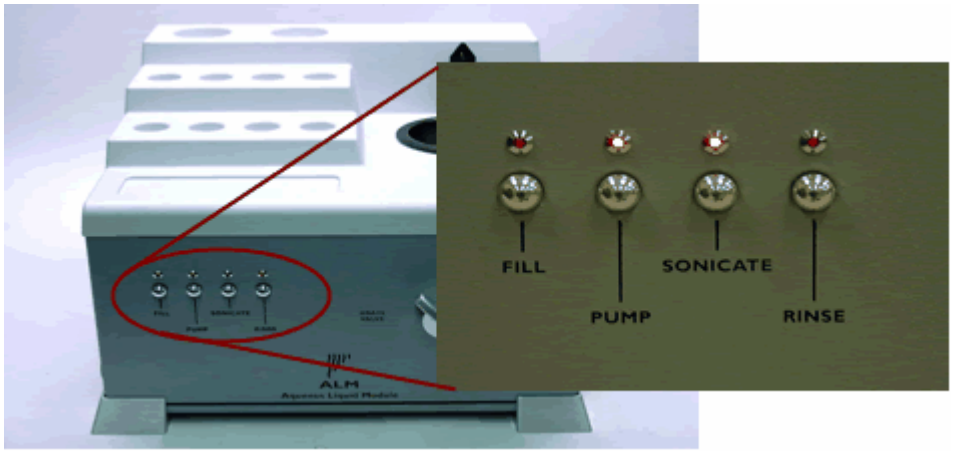
Figure 6.2 Liquid Level Sensors



Indicators

A set of LEDs placed in the front panel of the module is used to provide visual information about the status of the system when it is operated in manual mode. Figure 2 shows these LEDs and their functions. The indicators are active only in manual mode though the functions indicated can also be accessed through the software.

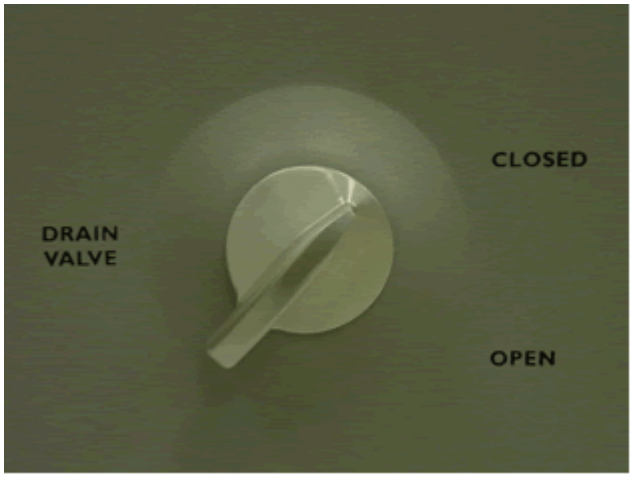
Figure 6.3 Indicators



- **Fill** – opens the inlet valve. Pressing this button will open the inlet and turn the LED ON to indicate the vessel is being filled.
- **Pump** – indicates that the pump is ON.
- **Sonicate** – turns the sonicator ON, the LED is a visual indicator for this state.
- **Rinse** – activates the rinse ring. This feature washes the walls of the vessel.

Figure 6.4 shows the Open/Close knob. This knob can be used to manually open or close the drain valve. The software can also be used to control this valve.

Figure 6.4 Drain Valve Knob

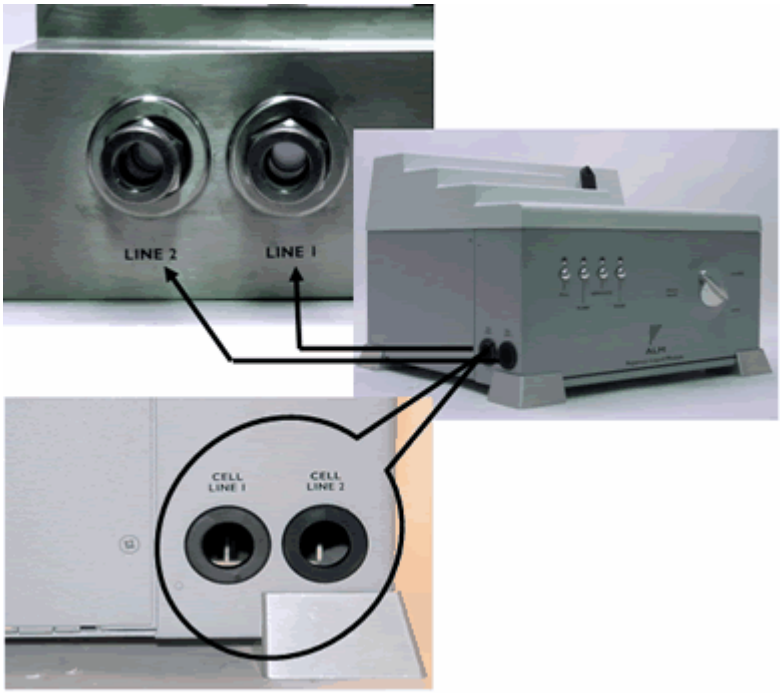


Connecting the ALM

Recirculation Lines

The module is connected to the sample cell by two tubes (“lines”) as shown in figure 4. Line 1 carries fluid from the module to the sample cell, line 2 returns the fluid to the module, making it a closed-loop system.

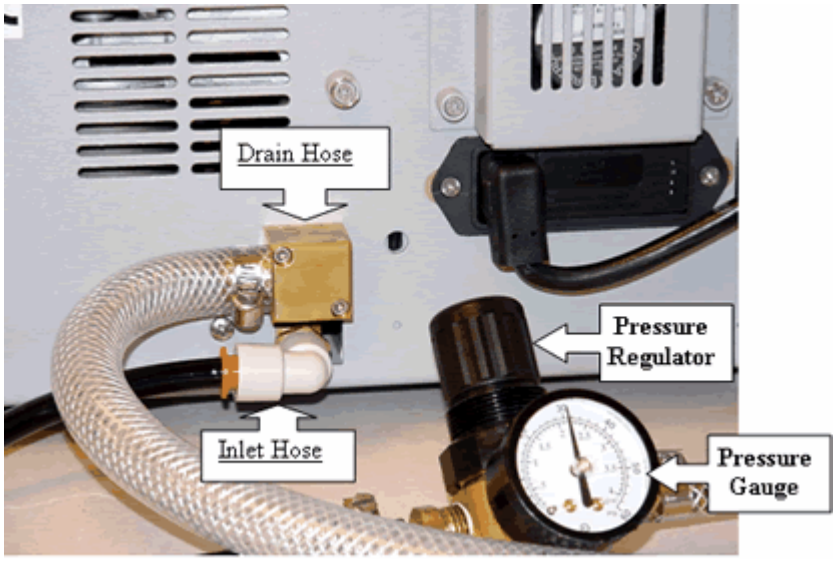
Figure 6.5 Figure 4 Line Locations and Connections



Inlet and Drain Hoses

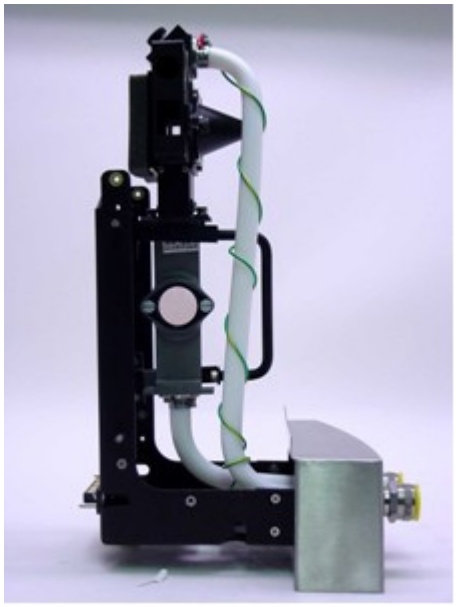
Two hoses are used to fill and drain the system. These hoses connect to the back of the instrument (Figure 6.6). The inlet hose is also connected to a pressure regulator and gauge used to maintain the appropriate water pressure (see specifications for the operating pressures).

Figure 6.6 Inlet and Drain Lines



Loading the Sample Cell Into the Optical Bench

Figure 6.7 Sample Cell



1 Open the LS 13 320 door to load the sample cell by undocking the auto-docking tray. Press the **OPEN** button on the instrument and the sliding door moves to the left. When it is open, press the **EJECT MODULE** button to extend the tray toward the user.

2 In the auto-dock tray, you will notice stainless steel pins extending into the center tray area both at the back of the tray and in the front of the tray. These pins fit into slots on the ALM Sample Cell. The back pins prevent the module from tipping out and the forward pins locate the module in the tray.

3 Hold the ALM Sample Cell by the handle and place it in the auto-dock tray by tipping the Sample Cell to the rear at an approximately 30 degree angle into the auto-dock tray. This is done to clear the forward pins and engage the back pins first.

4 When these pins have engaged into the slots on the back of the Sample Cell, tip the Sample Cell up to its vertical position. The Sample Cell should now be resting on the auto-dock tray.

5 Press the **EJECT MODULE** button and the Sample Cell will be drawn into the LS 13 320.

⚠ CAUTION

Do not place fingers inside bench as the sample cell docks into bench.

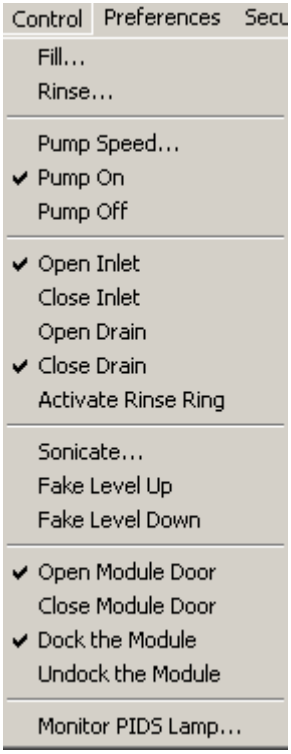
⚠ CAUTION

Do not place fingers inside bench as automatic sliding door closes.

Software

This section covers the controls found in the software program to operate the ALM. It also covers the parts of the Wizards (Standard Operating Methods (SOMs)) that deal with the ALM.

8.1.3.1 Control Menu



The **Aqueous Liquid Module** system can be operated through a series of manual controls accessed through software commands. These controls offer flexibility when there is a need to operate the system manually. To access these control commands:

Control and select the function you want to activate.

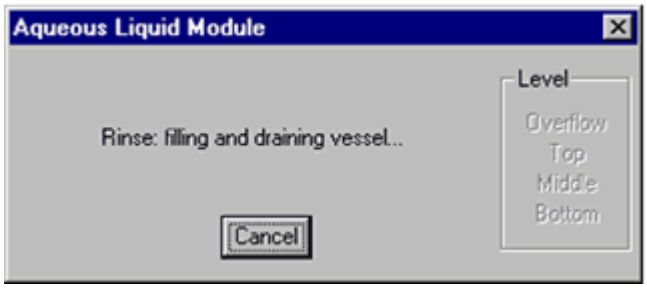
Each function is explained in detail below.

Fill

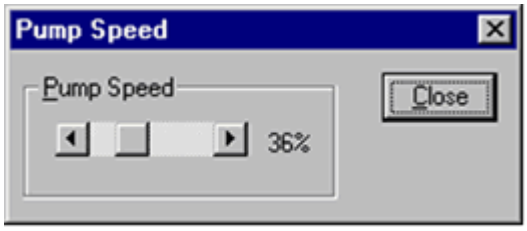
This option fills the vessel up to the third (top) level sensor. Note, if the drain valve is open, it will remain open during the filling process.

Rinse

Selecting this option will rinse the vessel by adding, draining and recirculating fluid throughout the entire module system. During this process the drain valve knob will automatically open and close. Do not attempt to stop this action manually. To stop the system from rinsing select Cancel.



Pump Speed
This option opens a dialog box that allows you to set the speed of the pump. Click on the arrows or drag the indicator to increase or decrease the speed.



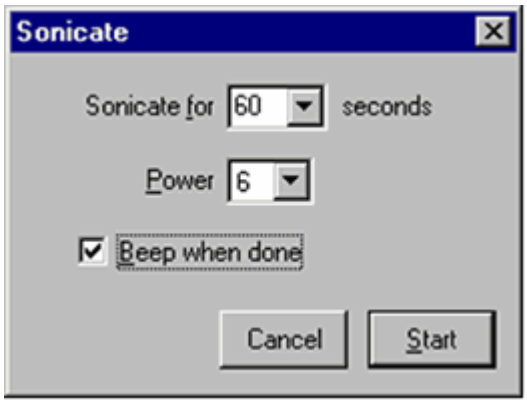
Pump On – Pump Off
Selecting one of these options turns the pump ON or OFF.

Open Inlet
Opens the inlet. If the pump is OFF, selecting Open Inlet will turn it ON. If the vessel is already full, fluid will not be allowed into the vessel.

Close Inlet
Closes the inlet valve regardless of how much fluid is currently in the vessel.

Open Drain
Opens the drain valve. Once the fluid drops below the bottom level sensor, if the pump is ON, the pump will be turned OFF.

Sonicate
Applies sonication to aid in the dispersion of the sample. Maximum allowable time is 999 seconds. The maximum output power by the probe is 73 W.



8.1.3.2 Standard Operating Methods (SOM)

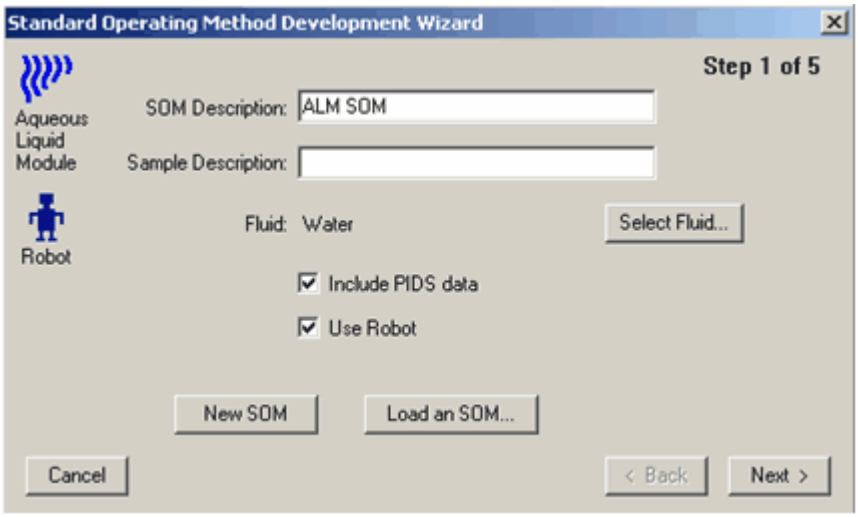
The Standard Operating Methods are used in part to assure consistency in the way analyses are performed. The options available when creating SOMs are similar to those available when using the Run Cycle.

Creating SOMs
To create an SOM:

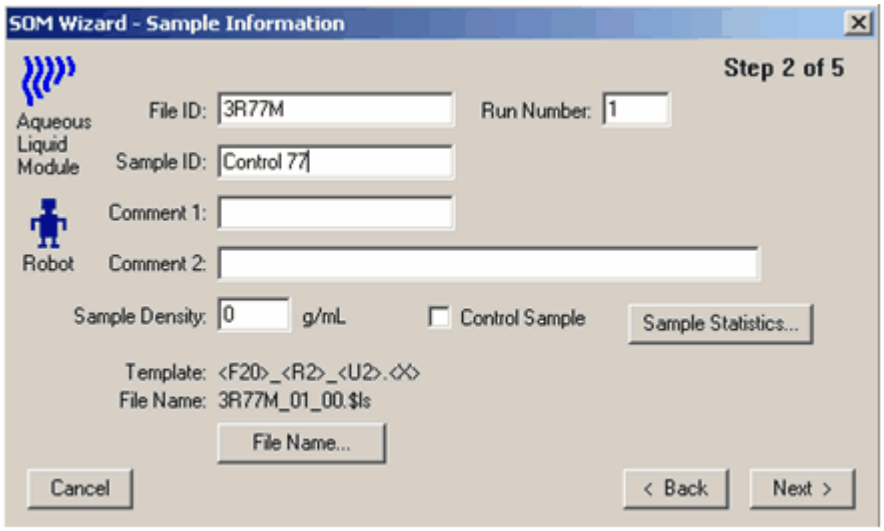
1 **Run.**

2 Select **Create an SOM**. A sequence of screens will guide you through the SOM creation.

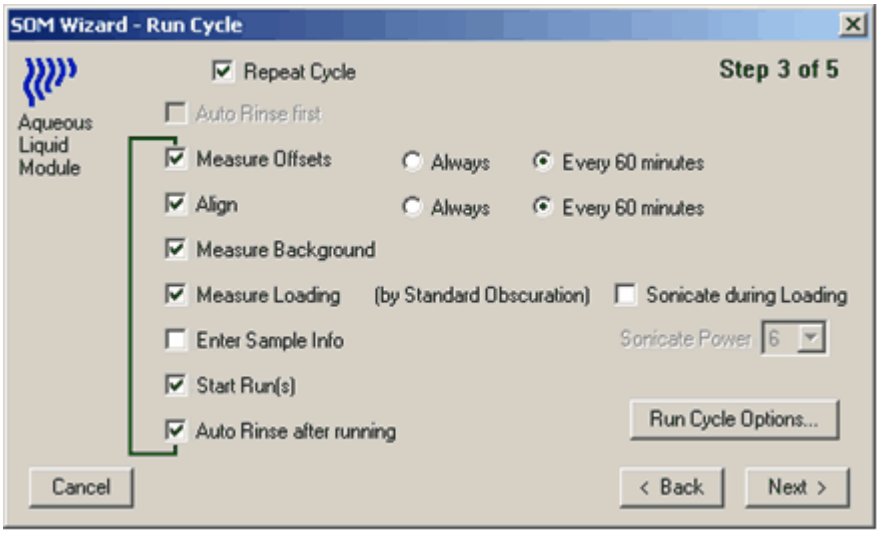
3 Step 1 prompts you to input a description of both the sample and the SOM being created for this particular sample. You can also select the PIDS option if your analysis will include particles in the sub-micron region (smaller that 0.4 µm). If you are using the robot arm, select the Robot check box.

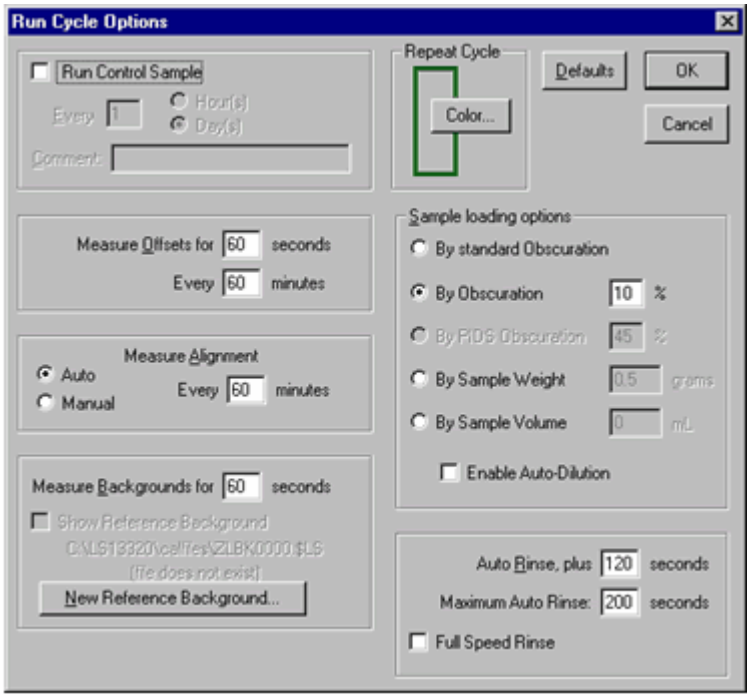


4 Step 2 allows you to enter specific information about your sample as well as the (file name format) entered.

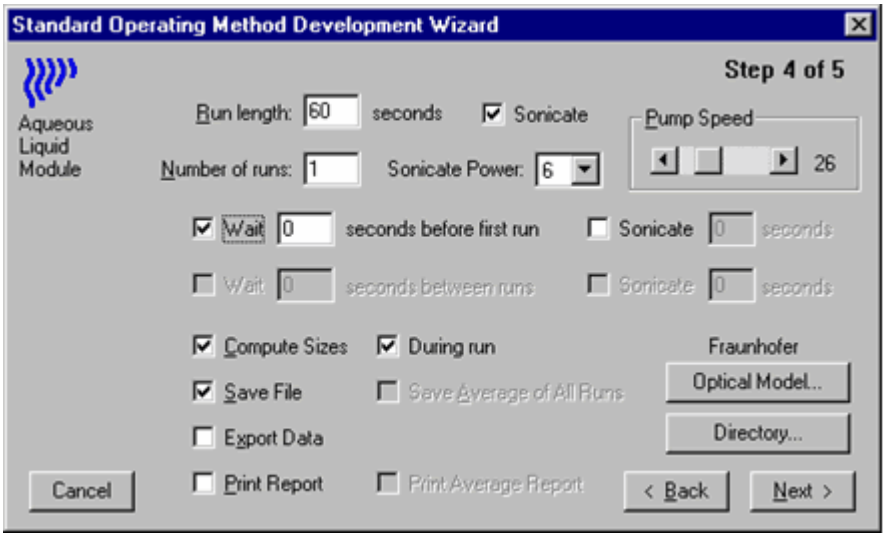


5 Step 3 allows you to set Run Cycle options such as offsets, alignment and background. The **Run Cycle Options** button will take you to the Run Cycle Options dialog box, where the measurement times of the offsets, background, and alignment are set. Sonication is also set within this screen.

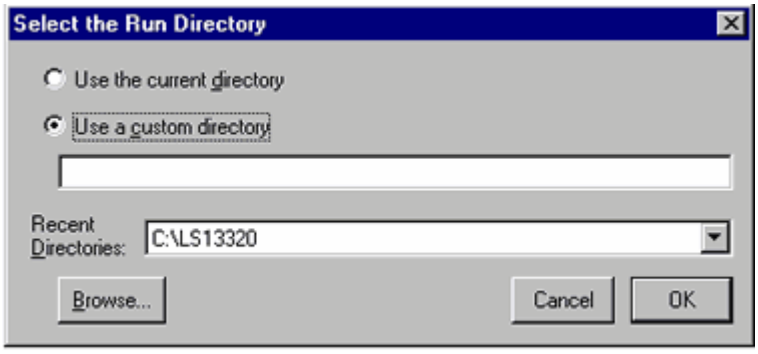




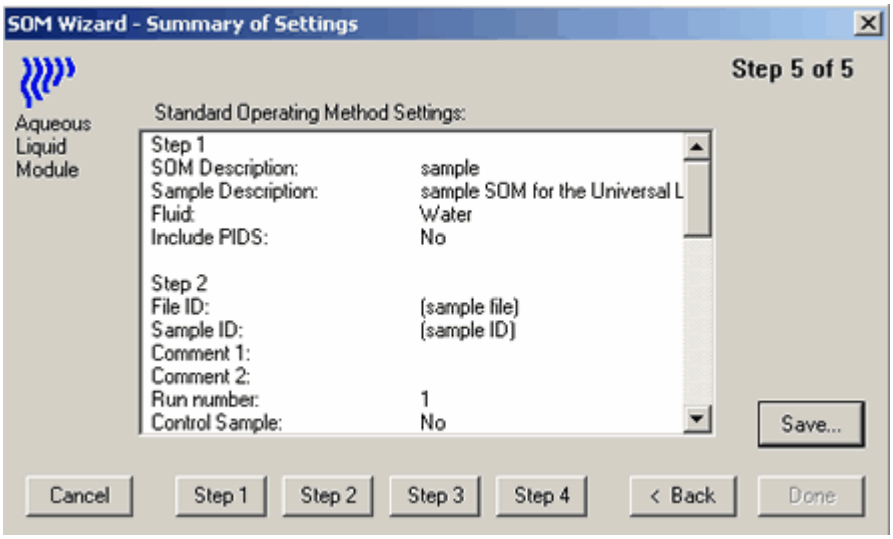
6 In Step 4 the run length is chosen. For analyses that require collection of PIDS data, a run length of at least 90 seconds is recommended. For analyses without PIDS, 60 seconds is the recommended time for the analysis, though analyses may be as short as 10 seconds. The optical model can be selected during this step. For more on optical models see APPENDIX C, *Optical Models*. The **Pump Speed** can be adjusted in this dialog. It is recommended that samples requiring some type of surfactant, that pump speeds be kept low, i.e. 30%, to prevent the formation of air bubbles. Should bubbles be present, alternating high pump speed with low pump speed is useful when trying to de-bubble the suspending fluid. If the pump speed is increased to its maximum (100%) and then reduced to an approximate 50%, air bubbles can be eliminated. This procedure may need to be repeated two to three times to eliminate the bubbles. High pump speeds are also useful when measuring large, dense particles. You can access the manual speed control from the Control menu by selecting **Pump Speed**.



The **Directory** option allows the user to select a custom directory to save the data under or to simply utilize the current directory for saving data.



7 Step 5 provides the user with a summary of the options selected in the previous steps. The SOM is saved during this step.



IMPORTANT SOM files, by default, are saved under the SOP folder, but they can be saved to the folder of your choice as well.

Under high security (21 CFR Part 11, for compliant version of software only), SOMs can only be saved by the Administrator. Under Operator mode, the **Save** button in step 5 of the SOM creation is not displayed.

8.1.3.3 Loading an SOM

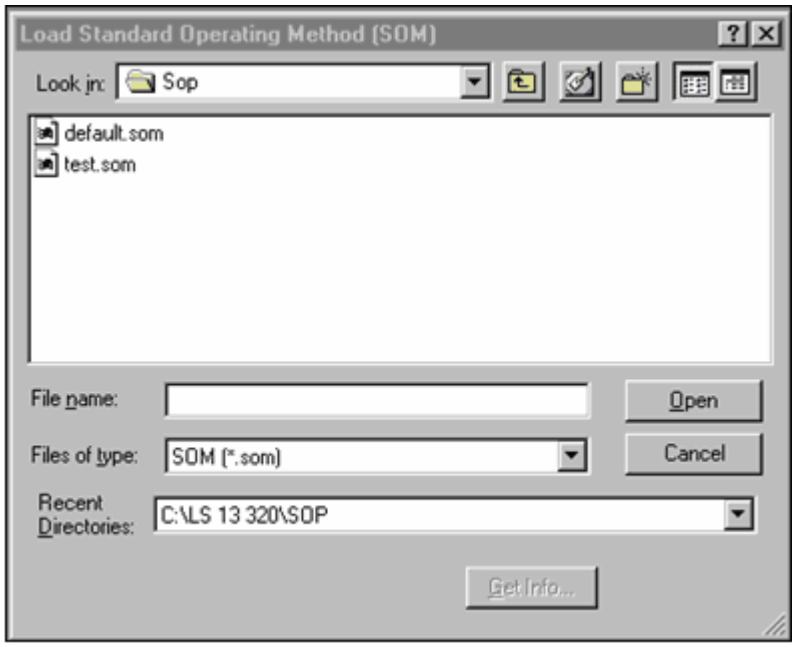
To load an SOM:

1 **Run**.

2 Select **Load an SOM**.

3 Select an SOM file from the list.

When an SOM is loaded, the status panel will display information about the loaded SOM. The **Start Cycle** button will change to **Start SOM**. Clicking on this button will start an analysis using all the settings unique to that SOM. Clicking on the **Remove SOM** button will bring back the **Start Cycle** option.



8.1.3.4 Standard Operating Procedures (SOP)

Creating an SOP

Standard Operating Procedures (SOPs) are the last step in setting all the parameters necessary to fully define restricted operation of the instrument.

To create an SOP:

1 **Run**.

2 Select **Create an SOP**.

3 From the Create an SOP dialog box, select an SOM and Preference file.

4 Select **Save**. By default, the SOP file will be saved under the SOP folder.

Loading an SOP

To load an SOP:

1 **Run**.

2 Select **Load an SOP**. SOPs can also be loaded from the status bar by clicking on the **Load SOP** button.

3 Select the desired SOP file from the SOP subfolder or the subfolder where the file was last saved.

Suspension Fluids

IMPORTANT Be sure the sample being sized and the suspension fluid do not violate any hazardous waste or clean water discharge regulations.

In normal operation, the materials inside the system that come into contact with the suspending fluid and the test particles are as follows:

304 stainless steel	316 stainless steel
borosilicate glass	Teflon® tubing
Tygon® polyvinylchloride tubing	Tygothane® polyurethane tubing
Delrin® (acetal homopolymer)	Buna-N rubber
Viton® O-rings	Kal-rez® O-rings
Brass	Carbon
Kel-F® diaphragm Titanium	Polycarbonate

The ALM requires clean, bubble-free liquid for its suspension fluid. Regular tap water that is filtered and degassed can be used. We recommend that you use a 0.2 µm, or smaller, pre-filter. Only use suspension fluids in the ALM that have flash points greater than 43.3°C (110°F) and meet the requirements listed below. Examples of fluids that can be used in the ALM are water, weak aqueous-based acids or bases, mineral oils, ethylene glycol, etc. The suspension fluid used depends on the type of sample to be analyzed.

Suspension fluid requirements:

- **Chemical Compatibility** - does not adversely react with the ALM components mentioned above.
- **Transparency** - transparent in the 450 to 900 nm range
- **Clarity** - no contamination or extraneous particles
- **Consistency** - no bubbles
- **Density** - lighter than the sample particles
- **Wettability** - able to deflocculate the sample particles
- **Fluidity** - low viscosity for rapid pumping
- **Insolubility** - no dissolution of particles
- **Chemical neutrality** - neutral to the sample and the ALM sample system

Cleaning the ALM

It is important for all glass surfaces in your sample system to be clean. Dusty or coated optical surfaces can cause erroneous results. Some samples or suspension fluids may coat the inside of the sample cell windows. If the instrument is in a dusty or smoky environment, or if vapors are present, the lenses and outer surfaces of the windows may become coated. Check the optical surfaces for cleanliness at least every three months, and clean if needed. Depending on the environment cleaning may be required more frequently.

Cleaning Fluids

Beckman Coulter recommends cleaning solution supplied with the module for cleaning the inner surfaces of the ALM sample system. Beckman Coulter recommends lens cleaning paper for cleaning the lenses and sample cell windows.

Cleaning the Cell

Clean the inner surfaces of the sample system (pump, hoses and within the sample cells) whenever they become coated.

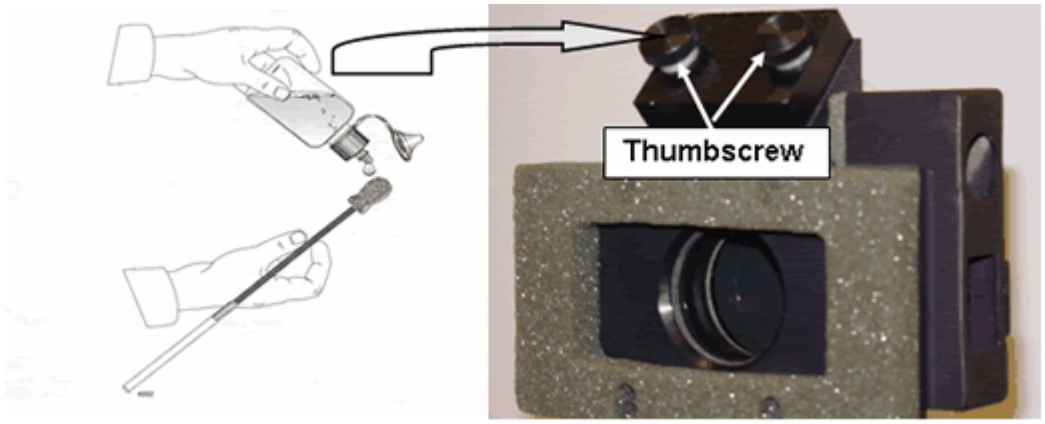
To clean the sample system:

1. Select **Control**.
2. Select **Rinse.** Rinse for approximately five minutes with water. Select **Cancel** to complete the rinsing routine.
3. Select **Control** again.
4. Select **Open Drain**.
5. Select **Close Drain**.
6. Prepare the cleaning solution.
7. Fill the vessel with cleaning solution.
8. Let the cleaning solution circulate for 15 minutes to 1 hour, depending on how dirty the cell is.
9. Select **Open Drain** > **Rinse**.
10. The windows can be cleaned by removing the thumbscrews that hold the cover shown in Figure 6.8.

11 After removing the cover you will have access to the inside of the cell. This will allow you to insert a swab with an extension (provided with your accessory kit) to clean the inside of the cell windows.

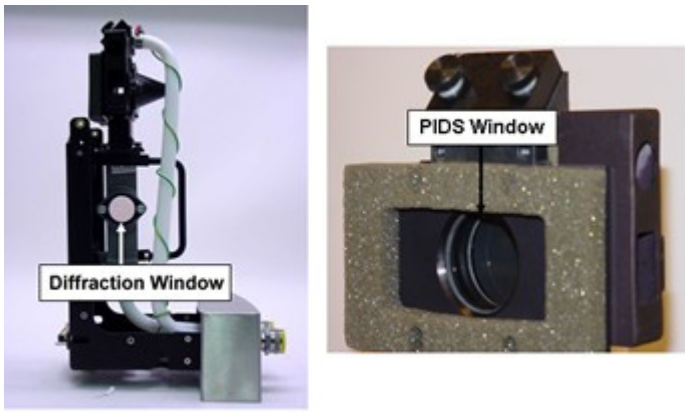
12 Use the same solution prepared in step 6. to swab the windows clean.

Figure 6.8 PIDS Windows Thumbscrews



Make sure you clean both the PIDS and diffraction windows, shown in figure 7.

Figure 6.9 Diffraction and PIDS Windows



Replacing the Diffraction Cell Window

Equipment Needed

- lens tissues
- lens cleaning solution
- window sealing tool

Procedure

Use this procedure to replace a diffraction sample cell window that is scratched or damaged.

To replace a diffraction sample cell window:

---

1 Drain liquid from module.

⚠ CAUTION

Wear appropriate safety equipment for the suspension fluids being handled.

---

2 Open the Optical Bench door and remove the sample cell.

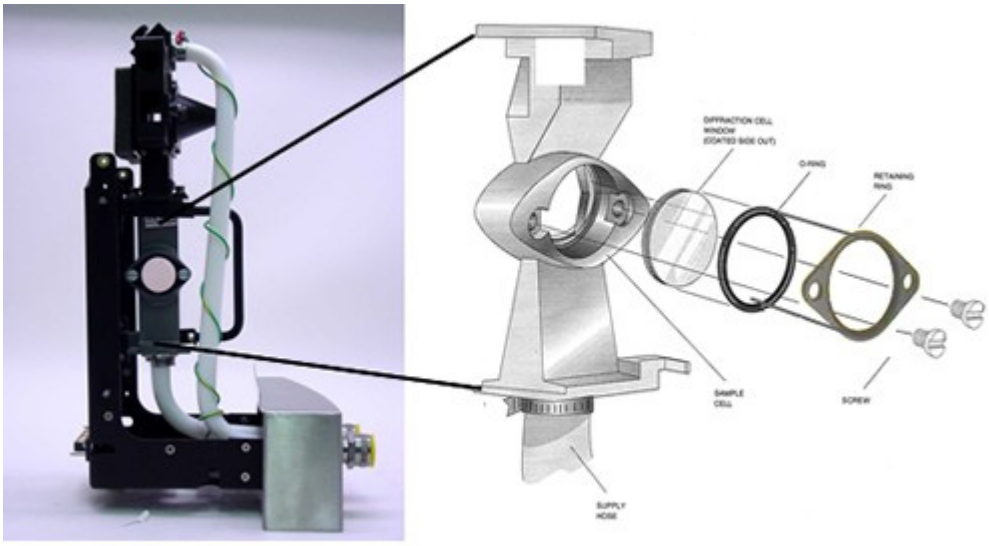
---

3 Drain remaining liquid in the cell by disconnecting the two hoses from the line 1 and line 2 ports on the sample cell front.

---

4 Loosen the two nylon screws to remove each window (Figure 6.10).

Figure 6.10 Diffraction Window



---

5 Cup your hand over the loose retaining ring and tilt the sample cell stand until the retaining ring falls into your hand.

NOTE You can now lay down the sample stand so the window to be replaced is facing up.

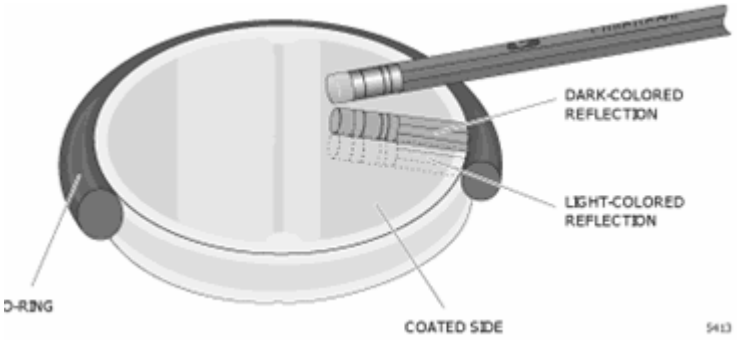
---

6 Stick a piece of masking tape on the window and lift the tape up to remove the O-ring and window. This may take several tries.

7 Discard the old O-ring, window and nylon screws.

8 Hold the new window by its edge to determine the coated side. Refer to the figure below to determine which side is the correct one.

Figure 6.11 Pencil Reflections Test



a. Take the eraser end of a pencil and hold it close to the window, near its edge. Note the two reflections, dark and light colored, of the pencil.

b. Turn the window over and repeat step (a).

c. The side that reflected a dark-colored reflection is the coated side.

⚠ CAUTION

Use lens tissues only once, then discard. Never use silicone-coated, eyeglass lens tissues to clean lenses or sample cell windows, as this type of tissue may leave a film. Wash your hands thoroughly before cleaning any lens or sample cell window. Do not touch optical surfaces with your fingers or skin. Body oils are difficult to remove without harming the anti-reflective coating.

9 Lay the window, coated side down, on a piece of lens tissue.

10 Clean the window using lens tissue wetted with lens cleaner.

11 Gently place the window, coated side to the outside of the sample cell, into the diffraction window opening. It must be flat to avoid scratching the other window.

12 Lubricate the O-ring with lens cleaning solution.

13 Carefully place the new O-ring on top of the window.

14 Insert the grooved end of the window-sealing tool into the sample cell. Push in gently and twist from side to side to seat the O-ring.

15 Remove the tool and check if the O-ring is completely seated down and around the window.

16 Clean the retaining ring, then gently place it on top of the window.

17 Replace the two new nylon screws that hold the retaining ring.

18 Clean the outer surface of the diffraction window as described in step 10.

19 Replace the window on the other side if needed using steps 4 through 18.

20 Insert both hoses into the bottom of the sample cell stand. Make sure they are tight.

21 Load the ALM sample cell into the optical bench and dock it.

22 Perform a leak test.

- **a.** Select **Control**, **Fill**.
- **b.** After the sample vessel is filled, select **Control**, **Pump On**.
- **c.** If any leaks are noticed after 2 to 3 minutes, remove the retaining ring and check that the window and O-ring are seated correctly, then repeat the leak test.

ALM Troubleshooting

Problem	Possible Cause/Solution
No communication with optical bench	• Module docked incorrectly. • Undock module and re-dock.
Module won't fill	• Check to see if it is already full. • Check that connections are air-tight. • Make sure there is enough inlet pressure.
Module won't drain	• Check that drain hose is clear of obstructions.
Pump will not work	• Module is not completely filled with fluid. • Check power to the system. • Sample cell not fully docked.
Module leaking	• Check fittings. • If leakage is inside, contact Beckman Coulter service.

ALM Specifications

Item	Specification		
Power	The ALM derives its power directly from the optical bench < 50 W peak-power		
Temperature	10° to 40°C (50° to 104°F)		
Humidity	0 to 90% without condensation		
Dimensions	Height	Width	Depth
	29.85 cm (11.75 in)	38.7 cm (15.25 in)	34.9 cm (13.75 in)
Weight	15.4 kg (34.0 lb)		

Auto-Prep Station

The Auto-Prep Station (APS) is intended to be used with the LS 13 320 optical bench and the Aqueous Liquid Module (ALM). It is capable of adding dispersant as well as sonicating a total of thirty samples. It is intended for use with aqueous samples only.

Figure 6.12 Auto-Prep Station with Aqueous Liquid Module

The APS adds the entire amount of sample to the ALM vessel by rinsing the sample tube into the ALM vessel with water for a preset, user-selectable time.

APS Description

The APS consists of:

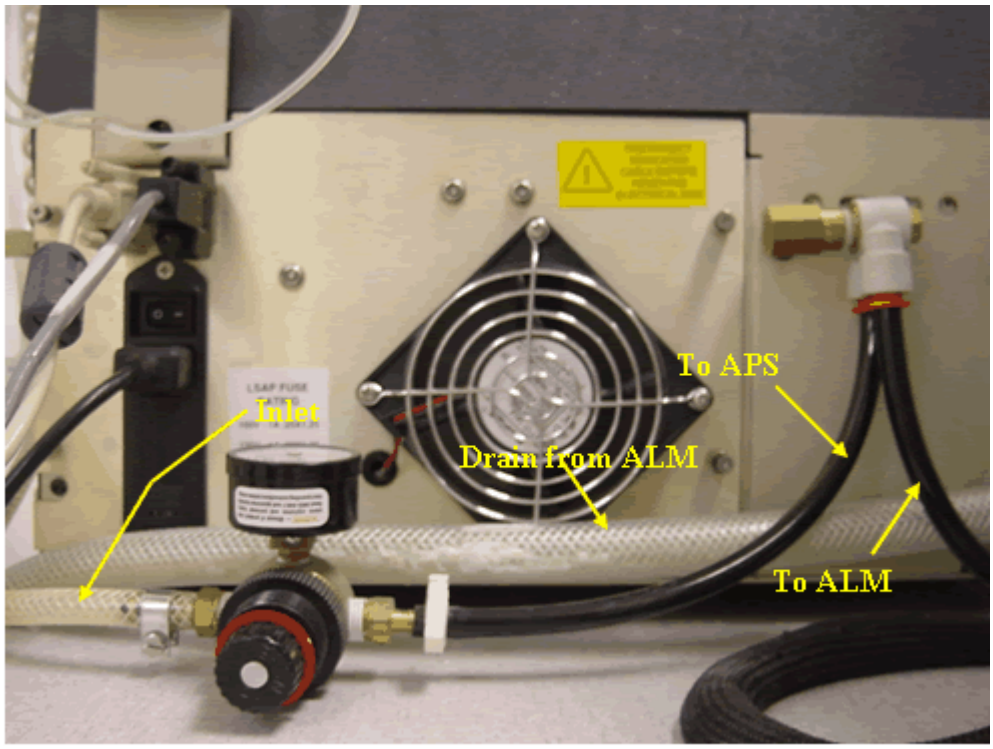
- a carrousel with 30 sample tube slots
- a set of three dispersant bottles
- a sonicator to aid in the dispersion of samples
- a tray to dispose of used sample tubes
- tubing and hoses necessary for the transfer of liquids, i.e. water and dispersants

Each sample tube will hold a maximum of 13 mL of liquid. The system is fully automatic and controlled via software commands.

Connecting the APS

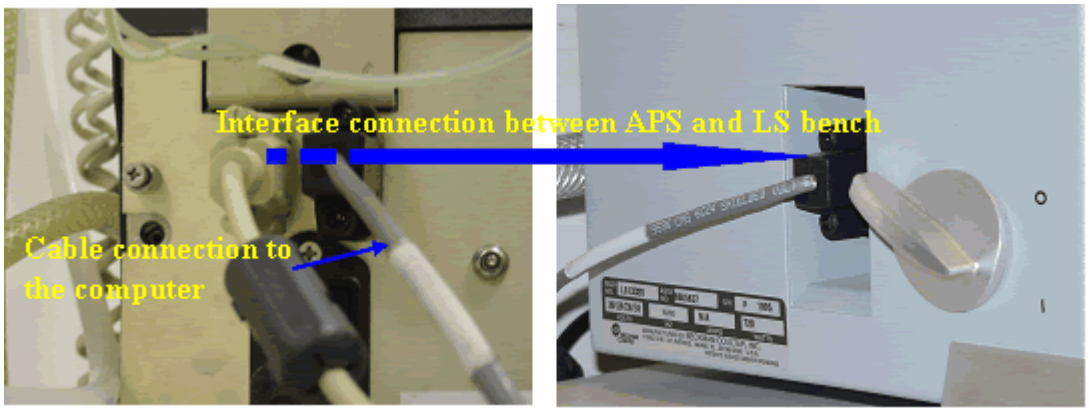
Hoses

The Auto-Prep Station is connected to the Aqueous Liquid Module through a set of hoses included with the module. These are the water inlet line to the APS, a connecting line from the APS to the ALM, and a drain line from the ALM. See the figure below.



Electrical and Communications

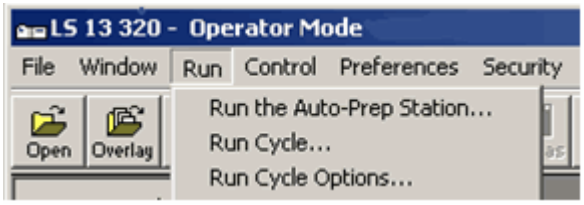
The electrical and communications connections are made through two separate cables that connect from the APS to the right side of the LS bench, as shown in the figure above, and from the APS to the computer. See the figure below.



Software

This section covers the software commands used to operate the Auto-Prep Station as well as how to use SOM wizards to set the APS for consistent results.

Run Menu



The APS is set for operation through a series of options found under the Run menu. Using these options provides flexibility in the way the APS is set up to perform analyses.

Run > Run the Auto-Prep Station... to define which carrousel slots you wish to use.

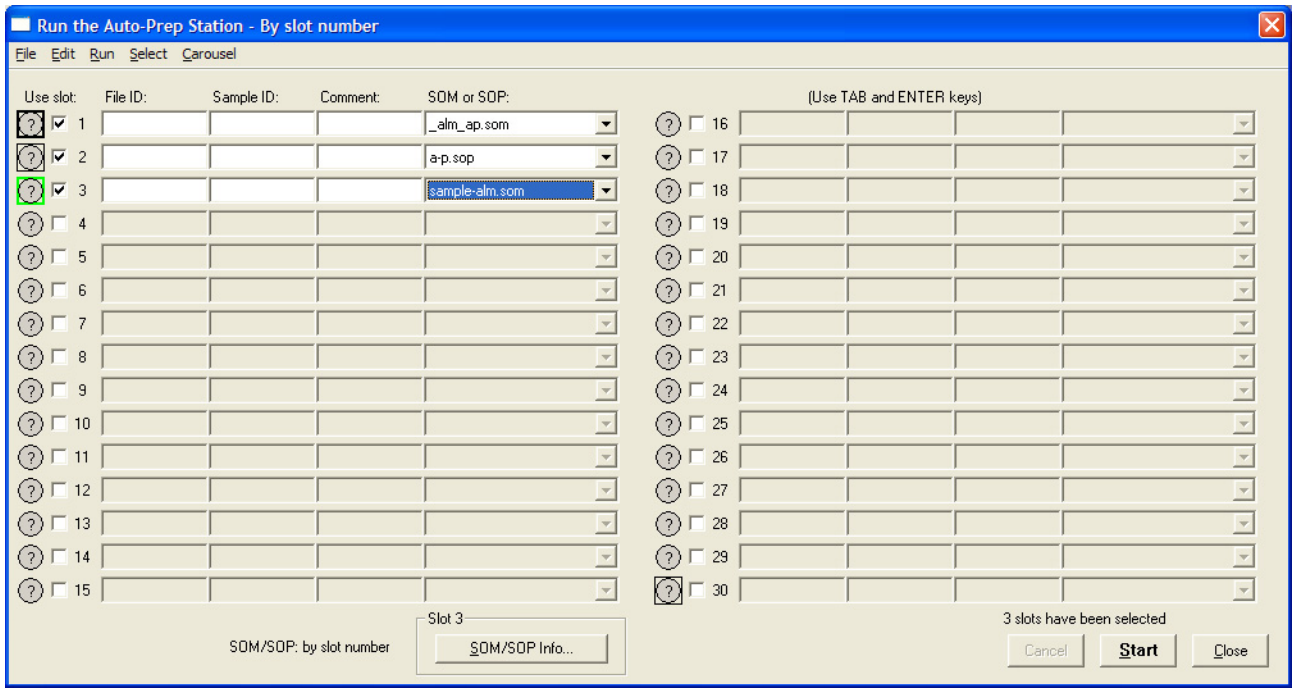
You may also accomplish this by using the carrousel icon 

.

Run Auto-Prep Station

Selection of this option will display the Run the Auto-Prep Station dialog screen. This set-up screen allows you to select the sample tube slots that will be used during the analysis as well as providing options for selection of SOMs, Auto-Prep settings, and operation of the carrousel. Each slot can be selected individually by clicking on the square next to the slot number. This activates all the other options. The slots can also be selected as explained in the following sections. The fields on this screen are:

- **File ID** – use this field to input the sample file name
- **Sample ID** – used for information about the sample
- **Comment** – add any type of comments up to 69 characters long
- **SOM** – allows you to select a previously saved SOM



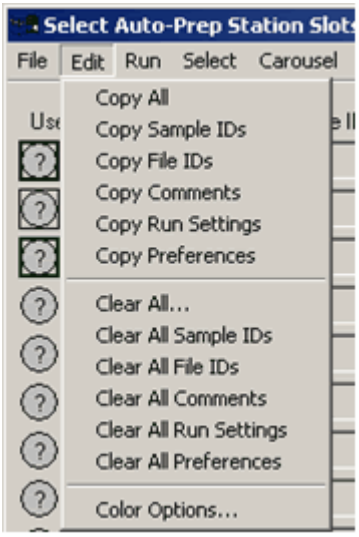
The following section covers each menu of the Run the Auto-Prep Station dialog screen.

File Menu

- **Load All Slots** – allows you to load slots that have been previously saved in a file in the same order in which they were saved.
- **Load Slot 1 and Up** – loads slots that have been saved in a file but starting from slot 1 and increasing sequentially.
- **Save Slot Settings** – saves selected slots into a file of type *.aps.
- **File Name Generation** – see File Name Generation for more information.

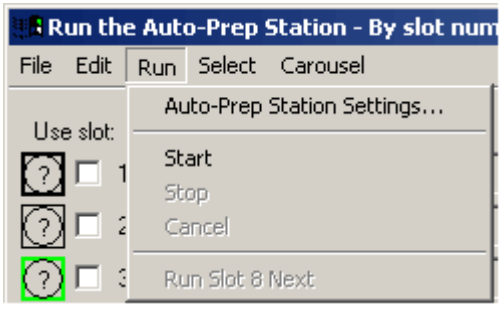
Edit Menu

- **Copy All** – copies information from one slot into all other slots. This is done by:
  - — **Select > Select All Slots.**
  - — **Edit > Copy All.**



- **Copy Sample IDs** – copies the sample ID from one completed slot into all other slots.
- **Copy File IDs** - copies the file ID from one completed slot into all other slots.
- **Copy Comments** - copies the comments from one completed slot into all other slots.
- **Copy Run Settings** - copies the SOM from one completed slot into all other slots.
- **Copy preferences** - copies the preference from one completed slot into all other slots.
- **Color Options** – sets the colors to be used with the icons on the Select Auto-Prep Slots dialog screen.

8.2.3.1 APS Run Menu

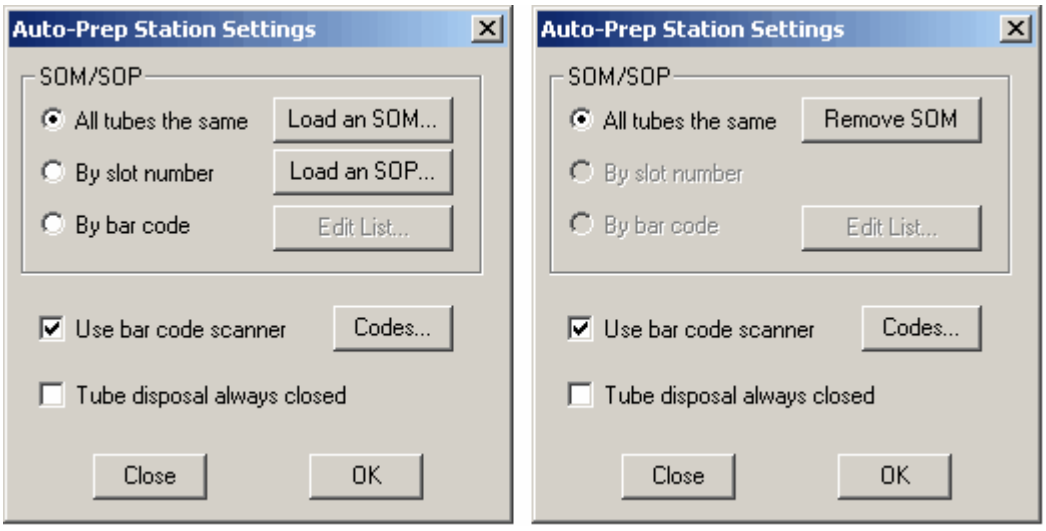


The **Run** Menu is used to select the APS settings, which include the selection of sample tubes, SOMs, and the bar code reader options.

Auto-Prep Station Settings

The **Auto-Prep Station Settings** option helps you select the way in which the sample tubes will be set up for analysis. Three options are provided and each option has a different set up.

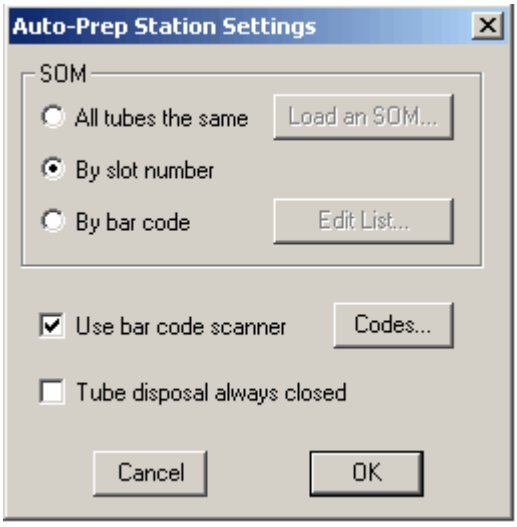
- **All tubes the same** – selecting this radio button sets all slots to use the same SOM, which is selected by clicking on the **Load an SOM** button



Once an SOM is selected it can be removed from all slots by clicking the **Remove SOM** button as shown in the figure above.

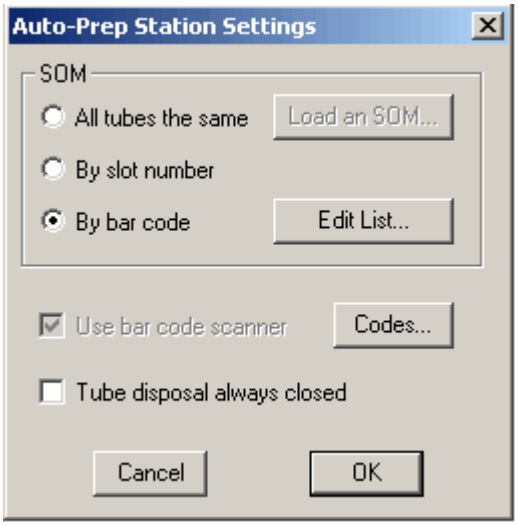
- **By slot number**

  Only the slots that have been selected and defined will be used during an analysis when this option is selected.

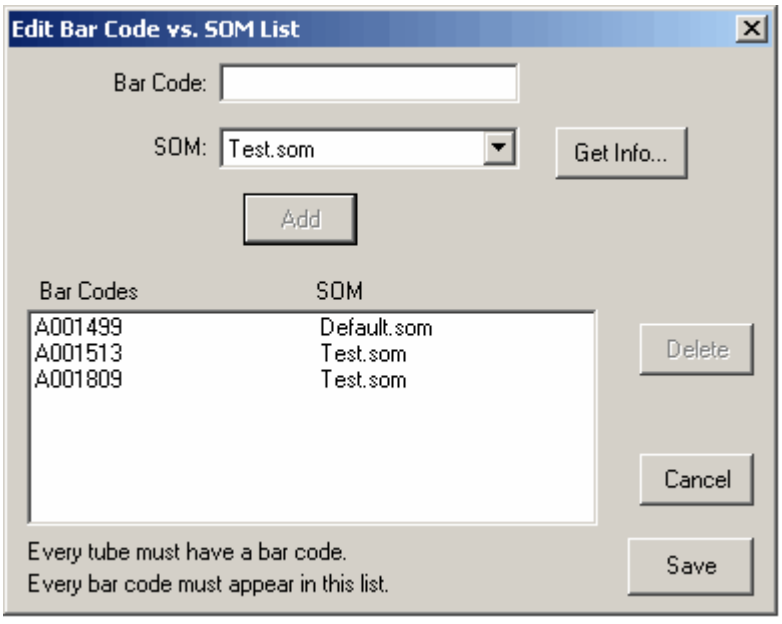


- **By bar code**

  Each tube provided with the Auto-Prep Station comes with a bar code for easy identification when setting samples for analysis.

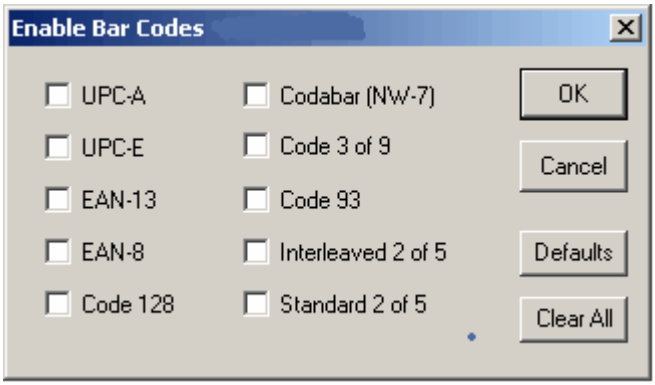


  This selection provides other options to help optimize your analysis. Clicking on the **Edit List** button displays the following dialog box:



  This dialog is employed to create a list of bar codes that will be used to read the sample tube. Bar codes are machine-readable symbols made of patterns of black and white bars that encode bits of information. The list associates a specific tube to an SOM. The bar code is the digital number on the bar code label of each tube.

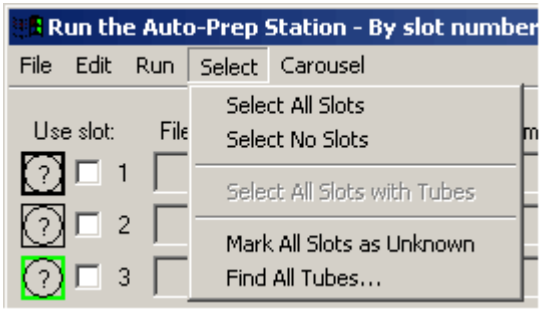
Bar code type selection is done through the following screen. In order for any of these bar codes to work properly, the sample tube must have the selected code.



The following are short descriptions of each of the code types. Note that the default type is Code 128.

- **UPC-A** is 12-digit product code used in the US.
- **UPC-E** is a smaller version of the UPC-A that uses six digits plus a check digit.
- **EAN-13** is used throughout Europe. It is a numeric only scheme using 13 digits.
- **EAN-8** is a shorter version of the EAN-13 code using only 8 digits.
- **Code 128** is a high-density alpha-numeric coding scheme that uses all 128 ASCII characters. It also allows for the inclusion of special characters not present on a keyboard.
- **Codabar (NW-7)** is a numeric only symbol used mostly by libraries and blood banks.
- **Code 3 of 9** or code 39 is the most popular for ID, inventory, and tracking purposes that supports alpha-numeric strings.
- **Code 93** encodes numbers and upper case letters.
- Interleaved 2 of 5 is a high density, continuous number symbol that uses digit pairs encoded in both the bars and the spaces.
- **Standard 2 of 5** is a numeric only coding scheme.

8.2.3.2 APS Select Menu



The Select option allows you to select all the slots, except for the three slots used for set up. It will also allow you to select just those slots that have tubes in them. Mark All Slots as Unknown puts a

question (?) mark next to each tube slot. Find All Tubes advances the carrousel to find all tubes in the carrousel.

8.2.4 APS Troubleshooting

Problem	Possible Cause/Solution
APS icon is not displayed Menus are not displayed	• Cables may be improperly connected. • Check connections (refer to section 2.2 for more information on electrical and communication connections)
Time out error “no communication with module”	• Power cable to ASP not plugged-in
Does not perform according to selected SOM	• Wrong SOM type. • Make sure the SOM selected is for the ALM + APS
Time out error “Auto-Prep Station tube pusher motor timed-out”	• Tube may be stuck in disposal slot. Check the disposal slot. • Bar code label causes tube to get stuck. Check bar code label for looseness.
Time out error “Auto-Prep Station tilt arm motor timed-out”	• Arm may be jammed. Check that modules are aligned and the tubing is not caught on the side
Time out error “Auto-Prep Station sonicator motor timed-out”	• Call service
Time out error “Auto-Prep Station carousel motor timed-out”	• Tube may be stuck in disposal slot. Check the disposal slot. • Bar code label causes tube to get stuck. • Check bar code label for looseness.

Micro Liquid Module

The LS 13 320 Micro Liquid Module (MLM) measures size distributions of particles suspended in liquids from 0.4 microns to 2000 microns. It provides reliable results for researchers, quality control laboratories, product or process control departments, or anyone needing to measure particle size distributions.

Figure 6.13 Micro Liquid Module

The MLM measures the entire sample in the instrument by circulating the sample with a stir bar. The amount of sample needed depends on its size and concentration. Prior sample preparation is often needed to achieve proper dispersion (see APPENDIX B, *Sample Handling* for more information on how to disperse samples) in order to obtain accurate and valid results.

MLM Description

The Micro Liquid Module is a chemically resistant, integrated system consisting of:

- A sample vessel (Micro Liquid Cell) with stirrer to introduce the sample to the system
- Main housing consisting of stirrer control and magnetic stirrer motor

The system is designed to work with small quantities of suspension fluid (12 ml), both aqueous and non-aqueous.

Figure 6.14 Micro Liquid Module

Loading the MLM

To install the MLM:

1. Open the LS 13 320 door to load the sample cell by undocking the auto-docking tray. Press the **OPEN** button on the instrument and the sliding door moves to the left. When it is open, press the **EJECT MODULE** button to extend the tray toward the user.

2. In the auto-dock tray, you will notice stainless steel pins extending into the center tray area both at the back of the tray and in the front of the tray. These pins fit into slots on the MLM. The back pins prevent the module from tipping out and the forward pins secure the module in the tray.

3. Hold the MLM by the top with one hand and on the bottom with the other hand. Place it in the auto-dock tray by tipping the Sample Cell to the rear at an approximately 30 degree angle into the auto-dock tray. This is done to clear the forward pins and engage the back pins first.

4. When these pins have engaged into the slots on the back of the MLM, tip the Sample Cell up to its vertical position. The MLM should now be resting on the auto-dock tray.

5. Press the **EJECT MODULE** button and the MLM will be drawn into the LS 13 320.

⚠ CAUTION

Do not place fingers inside bench as module docks into bench.

⚠ CAUTION

Do not place fingers inside bench as automatic sliding door closes.

Software

This section covers the Wizards (Standard Operating Methods (SOMs)) and controls found in the software program to operate the Micro Liquid Module.

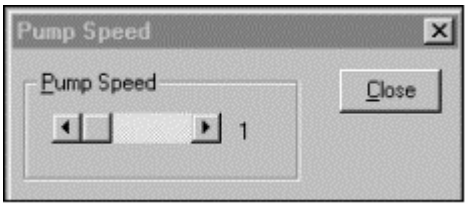
Control Menu

The MLM system can be operated through a series of manual controls accessed through the software.

Figure 6.15 Figure 1 Control Menu

Stirrer Speed

Figure 6.16 Figure 2 Stirrer Speed



This options opens a dialog box that allows you to adjust the speed of the stirrer. Click on the arrows or drag the indicator to increase or decrease the speed. The stirrer keeps the sample suspended without creating bubbles.

Stirrer On/Off

Will turn the stirrer ON and OFF when manual control is needed.

Standard Operating Methods (SOM)

The Standard Operating Methods are used in part to assure consistency in the way analyses are performed. The options available when creating SOMs are similar to those available when using the Run Cycle.

Creating SOMs

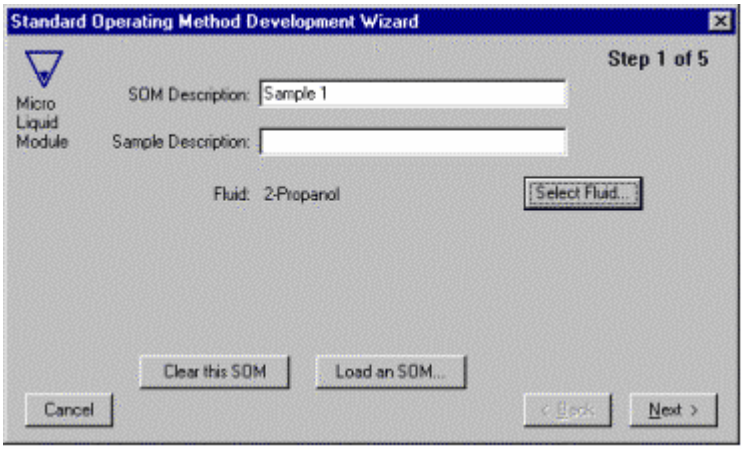
To create an SOM:

1 **Run**.

2 Select **Create an SOM**. A sequence of screens will guide you through the SOM creation.

3 Step 1 (Figure 6.17) prompts you to input a description of both your sample and the SOM you're creating for this particular sample.

Figure 6.17 Figure 3 Step 1 of SOM development



a. **Select Fluid** to select or edit the suspension fluid to be used (figure 4). This screen is a database of common fluids plus any other fluids added by the operator.

b. **Edit List...** to edit or add fluids (see figure 5 for Edit Fluid List dialog screen).

Figure 6.18 Figure 4 Select Fluid Dialog

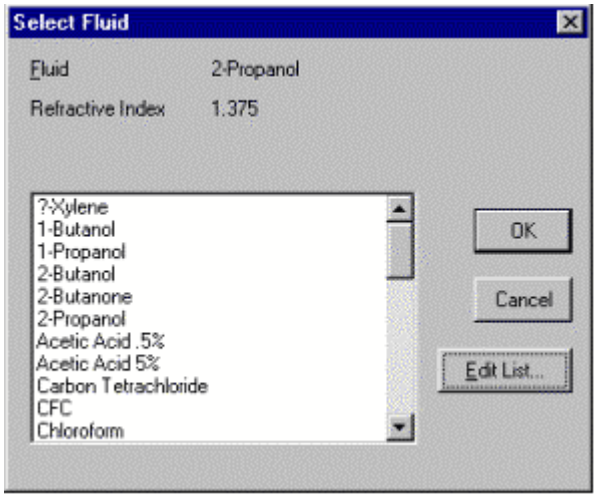
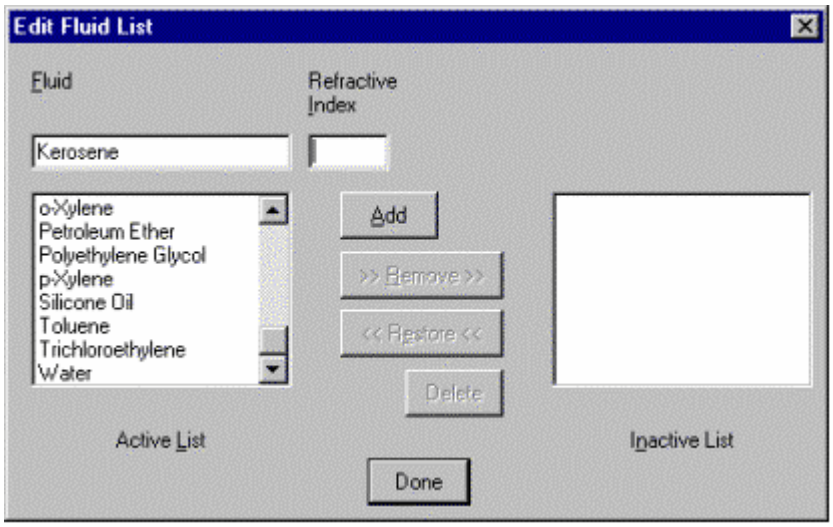
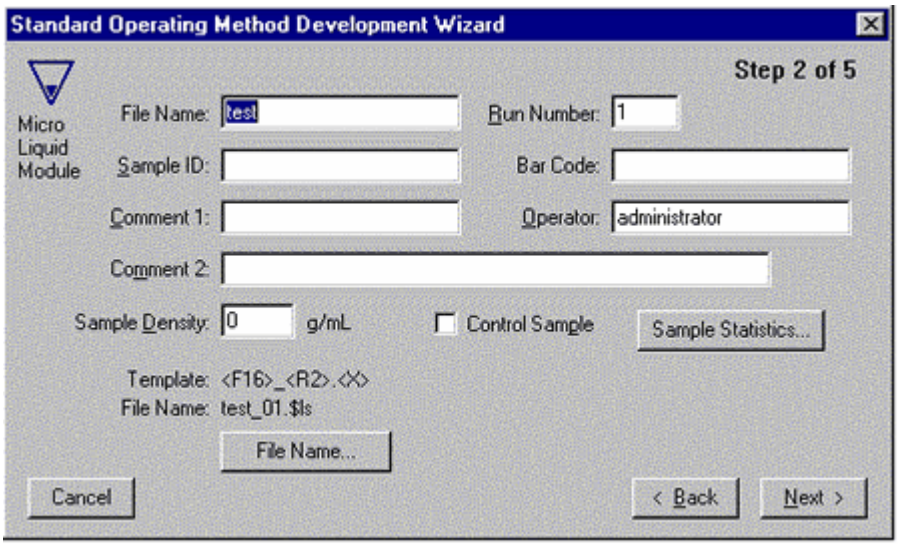


Figure 6.19 Figure 5 Edit Fluid List Dialog



4 In Step 2 (Figure 6.20) information about the sample as well as the (file name format) under file name are entered.

Figure 6.20 Step 2 of SOM Development



5 In Step 3 (figure 7) offsets, alignment and background are selected. The **Run Cycle Options** button will take you to the MLM Run Cycle Options dialog box (figure 8) in which the measurement times for offsets, background, and alignment are set.

Figure 6.21 Figure 7 Step 3 of SOM Development

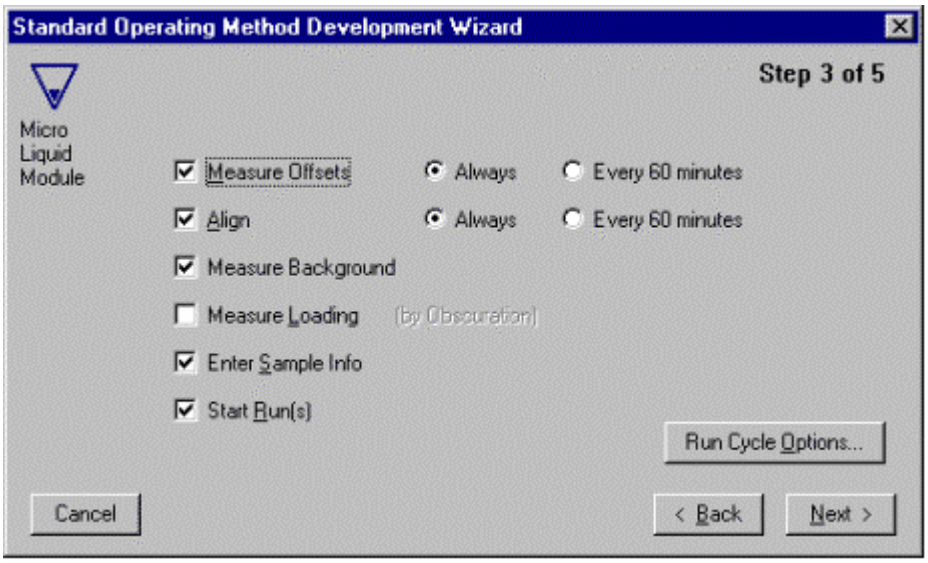
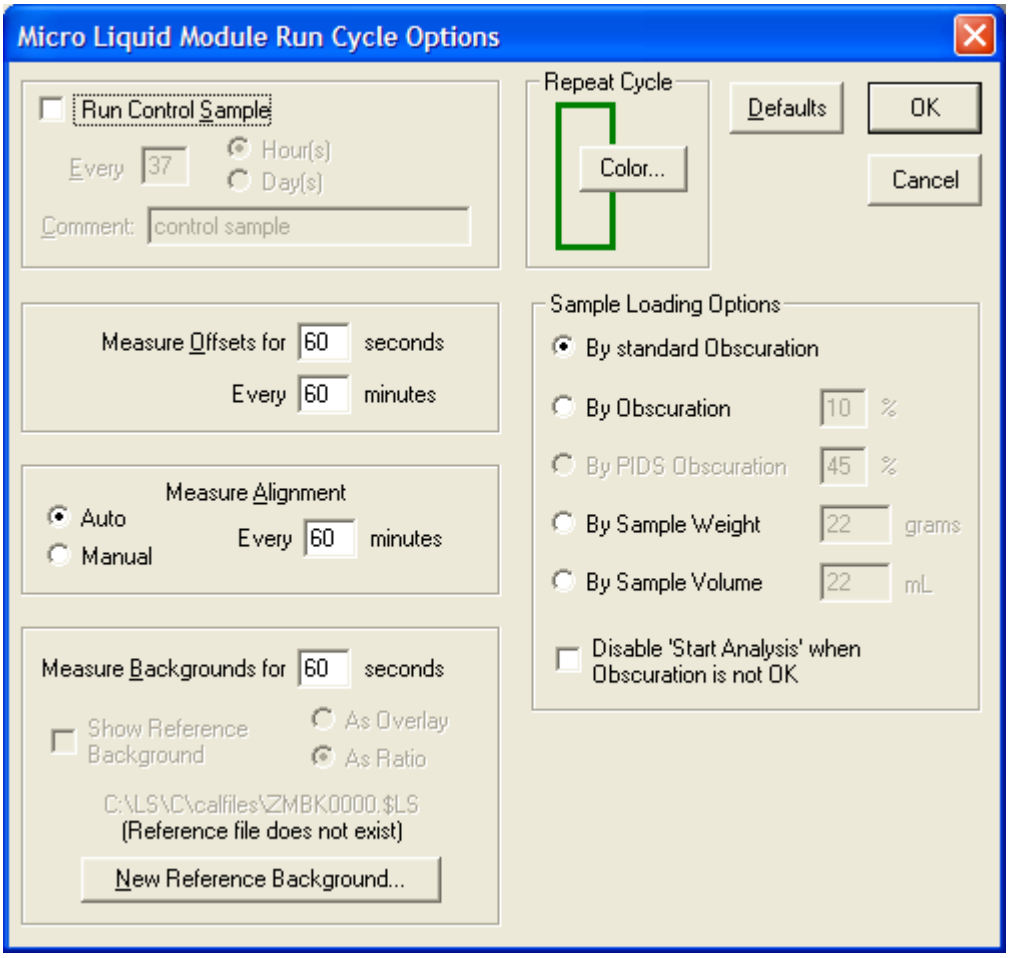
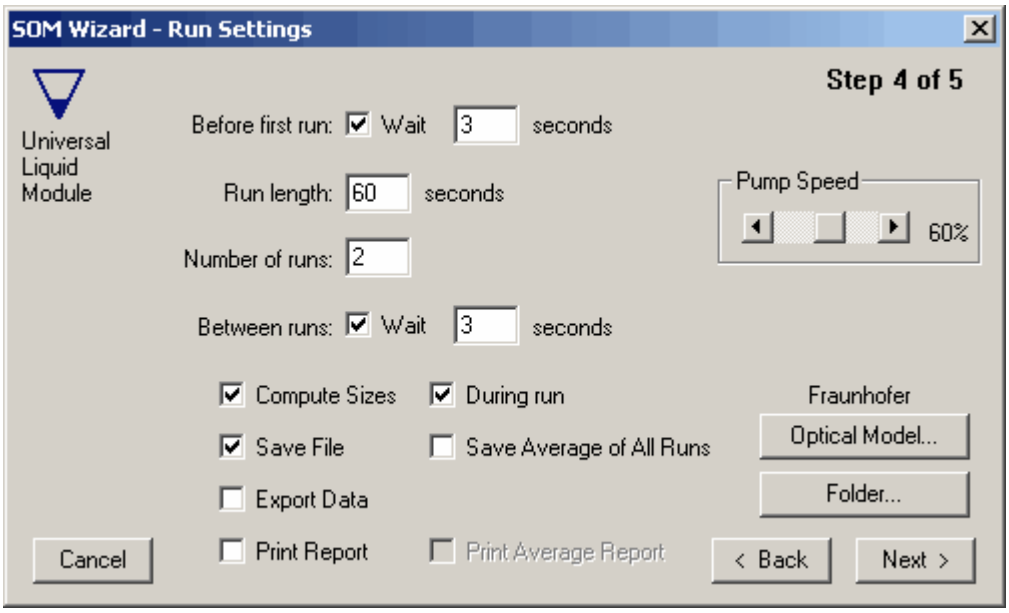


Figure 6.22 Figure 8 Run Cycle Options Dialog



6 In Step 4 (figure 9) the run length is set. Sixty seconds is the recommended time for an analysis, though analyses may be as short as 10 seconds. The Stirrer Speed is also adjusted in this dialog screen. It is recommended for samples that require some type of surfactant that the stirrer speed be kept at low speeds, i.e. 30%, to prevent the formation of air bubbles.

Figure 6.23 Figure 9 Step 4 of SOM Development

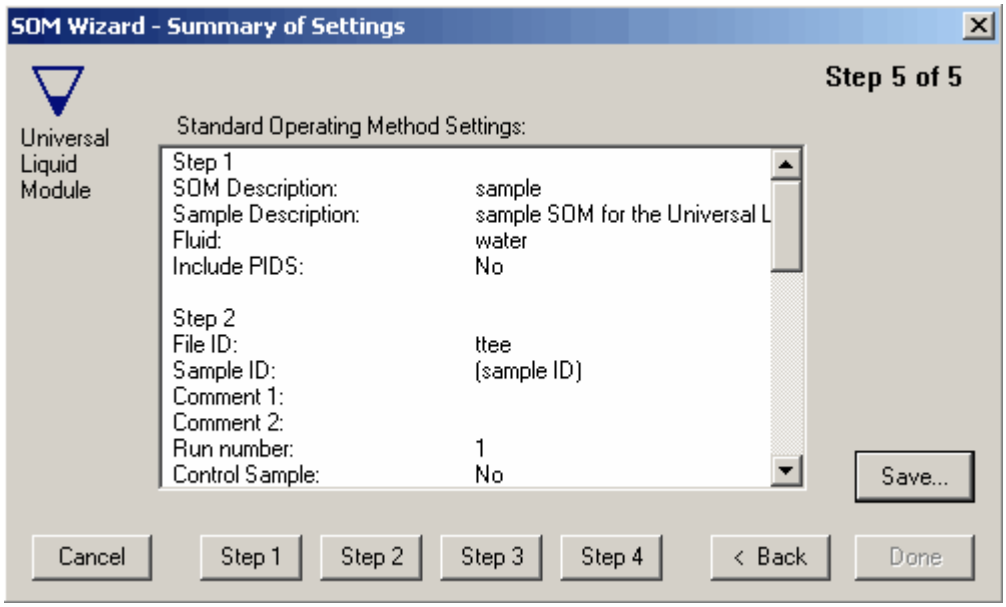


Optical models are also selected from this dialog box. For more on optical models refer to APPENDIX C, *Optical Models*.

7 Step 5 (Figure 6.24) provides the user with a summary of the options selected in the previous steps. The SOM is saved during this step.

IMPORTANT SOM files, by default, are saved under the SOP folder, but they can be saved to the folder of your choice as well.

Figure 6.24 SOM Summary



Under high security (21 CFR Part 11, for compliant version of software only), SOMs can only be saved by the Administrator. Under Operator mode, the **Save** button in step 5 of the SOM creation is not displayed.

Loading an SOM

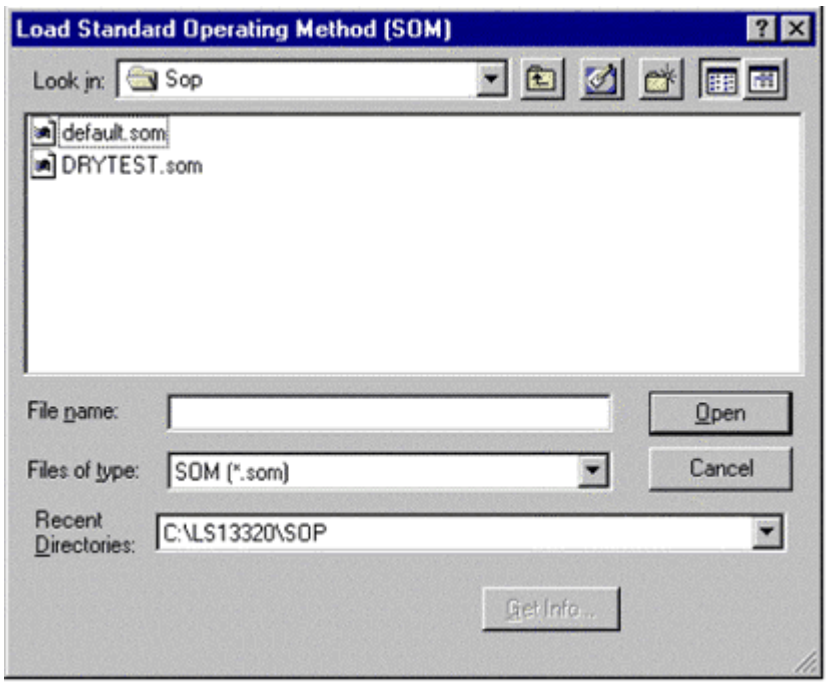
To load an SOM:

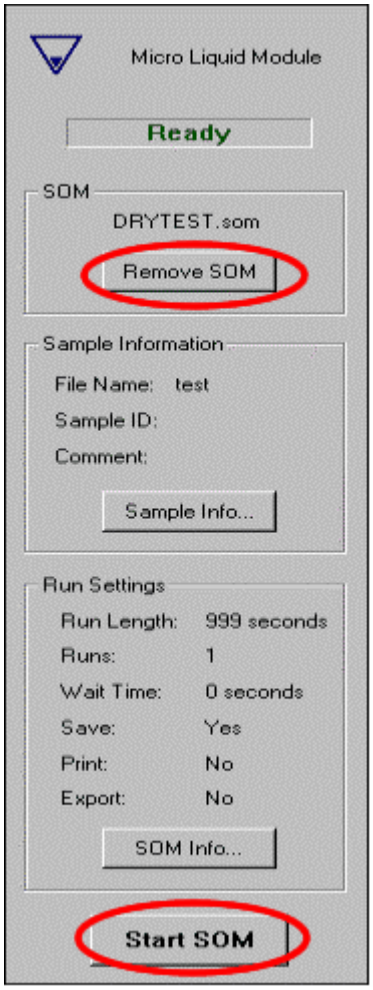
1 **Run.**

2 Select **Load an SOM**.

3 Select the SOM file from the list (Figure 6.25).

Figure 6.25 SOM List Dialog





When an SOM is loaded, the status panel will show information about the loaded SOM and the **Start Cycle** button will change to **Start SOM**. Clicking on this button will start an analysis using all the settings unique to that SOM. Clicking on the **Remove SOM** button will re-activate the **Start Cycle** option.

Standard Operating Procedures (SOP)

Standard Operating Procedures (SOPs) are the last step in setting all the parameters necessary to fully define restricted operation of the instrument.

Creating an SOP

To create an SOP:

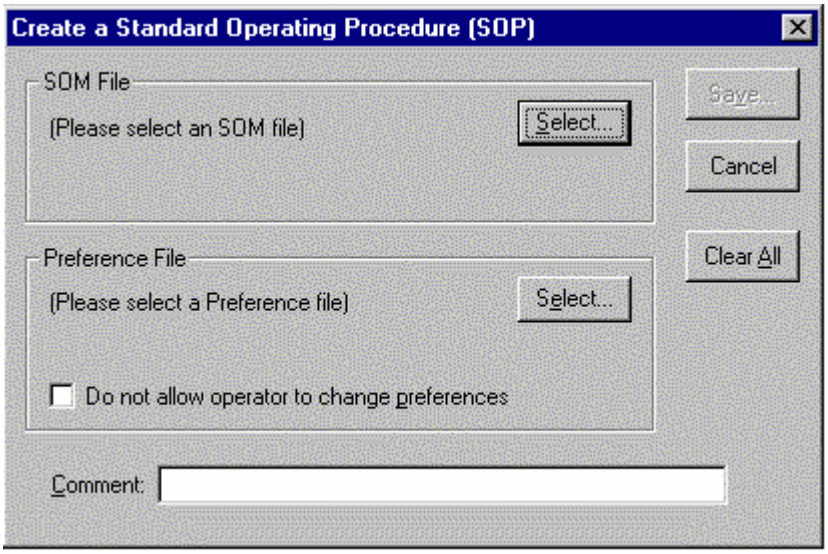
1 **Run.**

2 Select **Create an SOP**.

3 From the Create an SOP dialog box (Figure 6.26), select an SOM and a preference file.

4 Select **Save**. By default the SOP file will be saved in the SOP folder.

Figure 6.26 Create an SOP Dialog



Loading an SOP

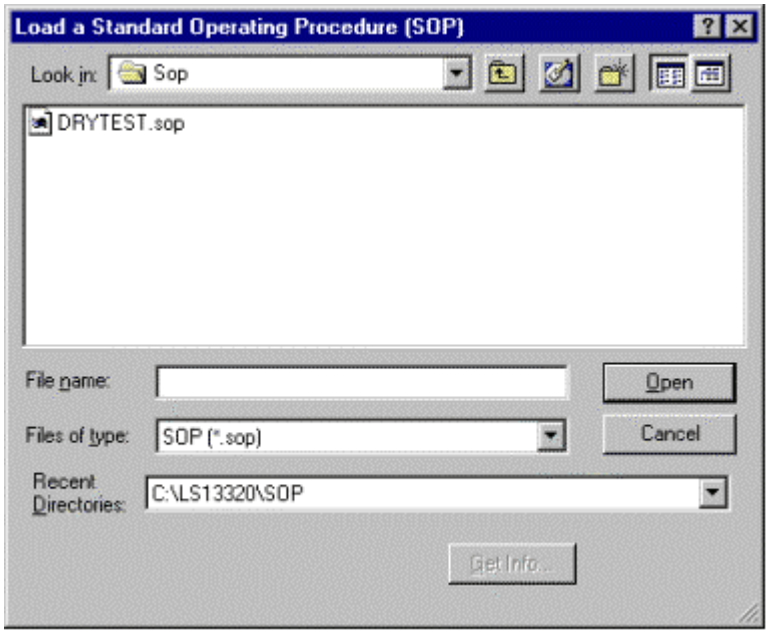
To load an SOP:

1 **Run**.

2 Select **Load an SOP**. SOPs can also be loaded from the status panel by clicking on the **Load SOP** button.

3 Select the desired SOP file from the SOP subfolder or the subfolder where the file was last saved.

Figure 6.27 Figure 13 SOP List Dialog



Suspension Fluids

The materials used in the construction of the MLM that come in contact with the suspension fluid are Teflon®, 316 stainless steel, glass, and Kal-rez®.

Only use suspension fluids that have flash points greater than 10.0°C (50°F) and meet the requirements listed below. Examples of fluids that can be used in the MLM are water, acetone, weak aqueous-based acids or bases, mineral oils, ethylene glycol, hexane, etc. The suspension fluid used depends on the type of sample to be analyzed.

Suspension fluid requirements:

- **Chemical Compatibility** - does not adversely react with the MLM components mentioned above.
- **Transparency** - transparent in the 450nm to 900 nm range
- **Clarity** - no contamination or extraneous particles
- **Consistency** - no bubbles
- **Density** - lighter than the sample particles
- **Wettability** - able to deflocculate the sample particles
- **Fluidity** - low viscosity for rapid stirring
- **Insolubility** - no dissolution of particles
- **Chemical neutrality** - neutral to the sample and the MLM

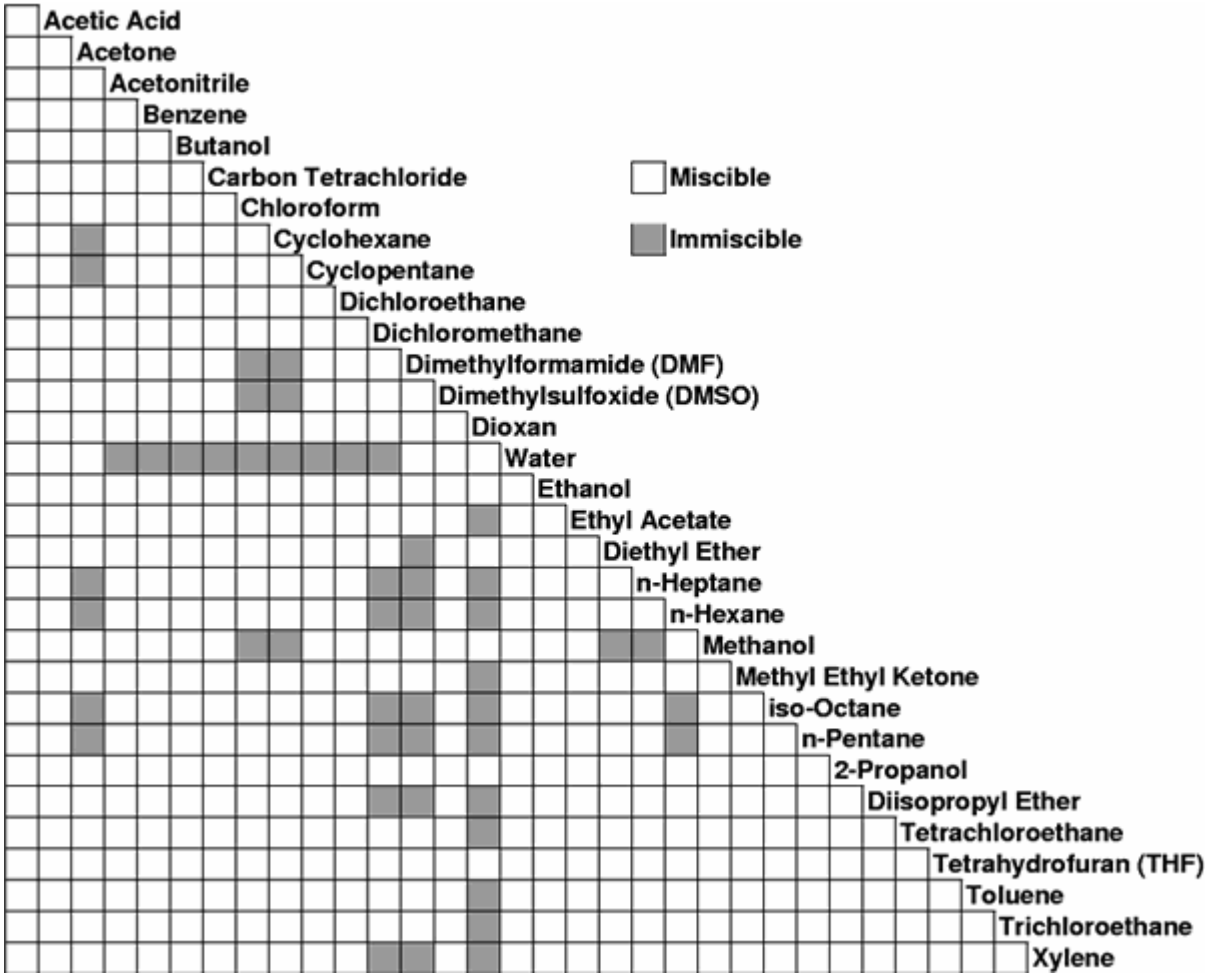
Listed below are acceptable suspension fluids for the MLM. If your application requires a fluid not listed here, check with your local Beckman Coulter representative for chemical compatibility.

Butanol	Butanone
Carbon Tetrachloride	Chloroform
Ethanol	Ethylene Glycol[a]
Glycerol[a]	Heptanes
Hexanes	Jet Fuels
Kerosene	Ketones
Methanol	Methylene Chloride
Mineral Oil[a]	Pentanes
Petroleum Ether	Polyethylene Glycol[a]
Propanol	Silicone Oil[a]
Toluene	Trichloroethane
Trichloroethylene	Water
Weak acid and base solutions (pH 4-10)	

a. These fluids are highly viscous and result in a slower flow rate. Use only when diluted so that viscosity does not exceed 5 centipoise.

NOTE Before changing from one suspension fluid to another, make sure the two fluids are miscible with one another. If the two fluids are not miscible, replace the current fluid with a suspension fluid with which they are both miscible and then change to the new suspension fluid. Mixing of immiscible fluids will form an emulsion that may be very difficult to remove from the system. Figure 6.28 provides a miscibility guide for some common solvents.

Figure 6.28 Miscibility Guide

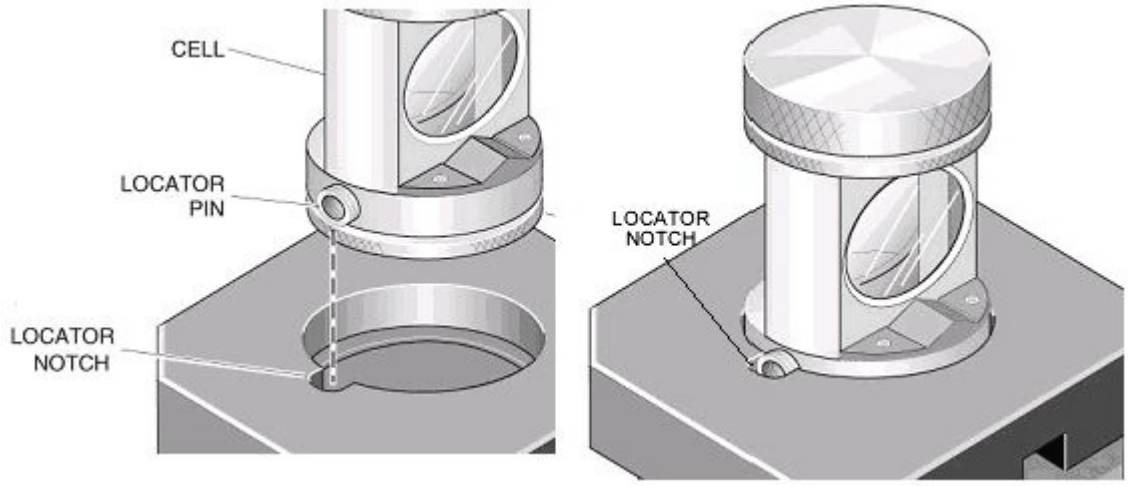


MLM System Preparation

Placing the Cell on the Sample Stand

Open the door to the optical bench. If the module has been docked, undock it. Place the sample cell on the sample stand with the locator pin fitting into the locator notch on the sample stand as shown in figure 14.

Figure 6.29 Locator Notch



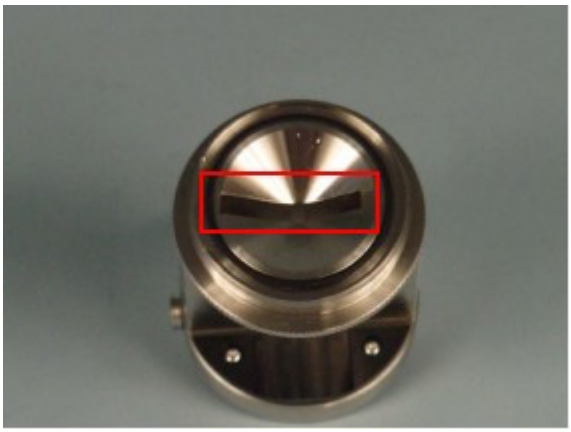
Filling Micro Liquid Module with Diluent

1 Open the door to the optical bench. If the sample cell is placed on the stand, remove it.

2 Remove the cap.

3 Tilt the cell toward the back (away from the locator pin) in order to trap less air inside the cup. Pour fluid slowly into sample entry (Figure 6.30).

Figure 6.30 Cell Diluent / Sample Entry



4 To remove air bubbles after the sample cell has been filled, tilt the cell to the side to allow trapped air to escape. Tilt it the opposite direction and repeat this procedure. Alternatively, place the cell on the sample stand and turn the stirring motor on at a low speed. With high viscosity fluids, you may have to turn off the motor periodically to let the bubbles rise.

5 Screw the cap securely onto the cell. Ensure that the O-ring is in place before screwing on the cap.

6 Place the cell back on the MLM stand.

Adding Sample

Sample is added to the sample cell until the correct obscuration is obtained. To add sample:

1 Open the Optical Bench door.

2 Remove the cell cap and add a small amount of sample to the sample cell.

3 Close the door and note the obscuration.

4 Repeat the previous two steps until the correct obscuration is obtained.

IMPORTANT Do not allow liquid to spill inside the optical bench while loading sample into the cell.

Setting the Stirring Motor

The speed of the stirring motor may need to be adjusted, depending on the sample being analyzed. For most applications, maximum speed is appropriate. Lower speeds may allow larger or denser particles to settle, skewing the size distribution. Lower speeds have been provided so that delicate samples, or those likely to flocculate when agitated, can be run.

Cleaning the MLM

IMPORTANT Always remove the Micro Liquid Cell from the sample stand to add fluid or sample in order to prevent spills on the Micro Liquid Sample Stand or inside the optical module. If you follow this advice, only the Micro Liquid Cell will require occasional cleaning.

Disassemble the cell

Refer to Figure 6.31.

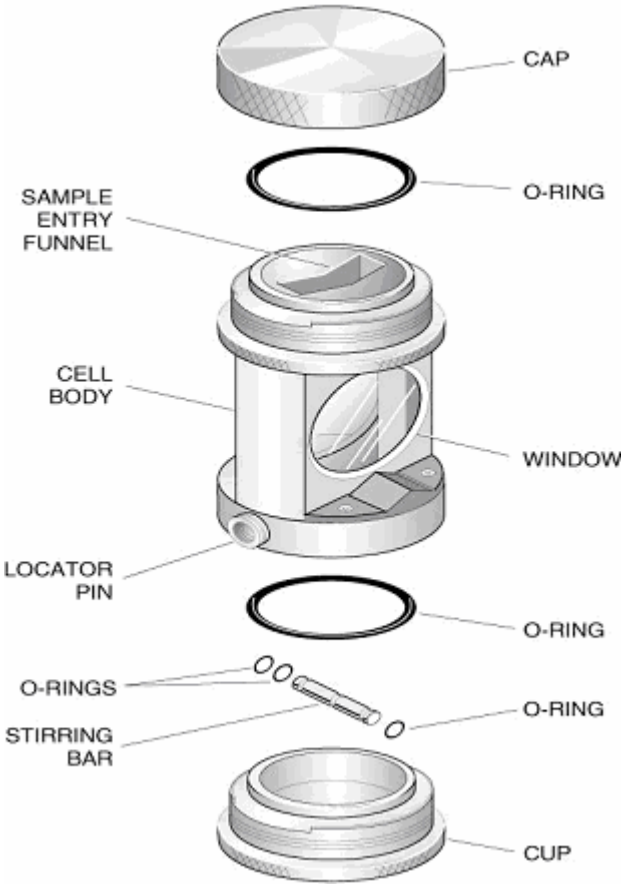
1. Unscrew the cell cap.
2. Pour the fluid out of the cell.
3. Remove the O-ring from the top of the cell.
4. Unscrew the cell body from the cup and remove the stirrer bar.
5. Remove the O-rings from the stirring bar.

Clean the cell components

1. Wash everything except the cell body in a 2% solution of MICRO Critical lab cleaner. Rinse and dry.
2. Clean the inside of the cell body by rinsing with acetone or isopropyl alcohol.

   IMPORTANT Do not use any abrasive cleaners in the cell.
3. If any particles or dirt remain on the inside of the cell windows after rinsing, use lens cleaning fluid and the swab to clean the windows. Clean the windows with a circular motion.
4. Clean the outside of the cell windows with lens cleaning fluid and lens tissue using a circular motion.

Figure 6.31 Sample Cell Components



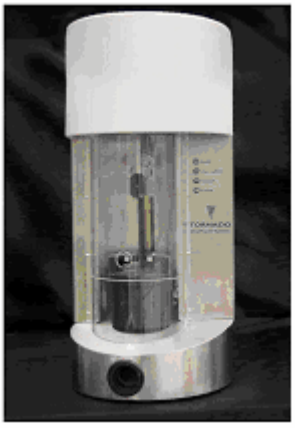
It is not necessary to take the cell apart every time there is a need for cleaning. Adding a small amount of cleaning solution and scrubbing with the swab supplied can also clean the inside of the cell.

Figure 6.32 Figure 17 Cleaning the Cell With a Swab

MLM Troubleshooting

Problem	Possible Cause/Solution
No communication with optical bench	• Module docked incorrectly. Eject module and re-dock.
Stirrer will not turn	• Liquid too viscous • Cell not mounted properly • O-rings missing from stirrer bar
Sensor light not on	• Module not docked properly. Eject module and re-dock. • Cell not mounted properly
Obscuration too high	• Dirty windows. Clean windows.
Module leaking	• Check fittings

Tornado Dry Powder System



The Tornado Dry Powder System is intended for use with the LS 13 320 Optical Bench. It is capable of feeding and measuring dry powder samples in the size range 0.4 µm to 2000 µm. The Tornado DPS measures the entire sample presented to the instrument, with a sample volume that allows an analysis that will provide statistically accurate results. The range of sample volumes the instrument can accept should provide for a minimum 10 second run at controlled obscuration. There is no need for sample preparation and operator intervention is minimal. The system is controlled via software commands that make it completely hands-free. The sample is placed in a sample holder and delivered to the sensing zone in the optical bench by a vacuum.

Tornado Description

The Tornado DPS system consists of:

- The Tornado DPS module
- A vacuum source
- A vacuum hose that connects the Tornado DPS to the vacuum
- Sample tubes

The Tornado DPS provides automatic feed rate (obscuration) control. The set point for the obscuration is user selectable between 4% and 8%. The accuracy of the average obscuration control is better than ±2% from the set point. It is measured using the average obscuration set in the Run Settings dialog box (see the Software section of this manual).

The system disperses cohesive powders without milling fragile materials. The dispersion of the dry powders is comparable to the dispersion achieved when the samples are run wet with proper manual pre-dispersion.

The sample to be measured is contained in the system to prevent airborne contamination of the work area throughout. This is accomplished by maintaining a negative pressure (vacuum) system for the sample path and trapping the sample via a filtration system in the vacuum.

Indicators

A set of LEDs mounted in the front panel of the module provide visual information about the status of the system when it is operational. The indicators are active in manual mode as well as indicating functions accessed through software.

Figure 6.33 Tornado DPS LED Indicators

Error

A red LED which will flash on any error condition.

Load Sample

Green when sample loading is possible.

Red at all other times while running.

Vacuum

Green when vacuum is within range.

Solid red when vacuum is low.

Flashing red if vacuum is high.

Power

Green when power is applied to the module's Control PCB.

Docking the Tornado DPS

To dock the Tornado DPS:

1 Open the LS 13 320 door to load the sample cell by undocking the auto-docking tray. Press the **OPEN** button on the instrument and the sliding door moves to the left. When it is open, press the **EJECT MODULE** button to extend the tray toward the user.

2 In the auto-dock tray, you will notice stainless steel pins extending into the center tray area both at the back of the tray and in the front of the tray. These pins fit into slots on the Tornado. The back pins prevent the module from tipping out and the forward pins locate the module in the tray.

3 Hold the Tornado DPS by placing one hand on the top and the other hand on the bottom, tip the DPS to the rear at an approximately 30 degree angle into the auto-dock tray. This is done to clear the forward pins and engage the back pins first.

4 When these pins have engaged into the slots on the back of the DPS, tip the Sample Cell up to its vertical position. The Tornado module should now be resting on the auto-dock tray.

5 Press the **EJECT MODULE** button and the Tornado will be drawn into the LS 13 320.

⚠ CAUTION

Do not place fingers inside bench as automatic sliding door closes. Do not place fingers inside bench as module docks into bench.

It is sometimes necessary to manually guide the module into the LS. When the module is successfully docked, the Power LED of the Tornado will turn ON. The Tornado will go through a diagnostic self-test routine, turning LEDs ON and OFF, spinning and raising the sample lift until the self-routine is over. If the Power LED does not turn ON, it means the Tornado did not properly dock into the LS 13 320. Gently push the Tornado both at the top and bottom into the LS 13 320 and it should then properly dock.

⚠ CAUTION

Do not place fingers/hand between sample lift and suction nozzle during operation.

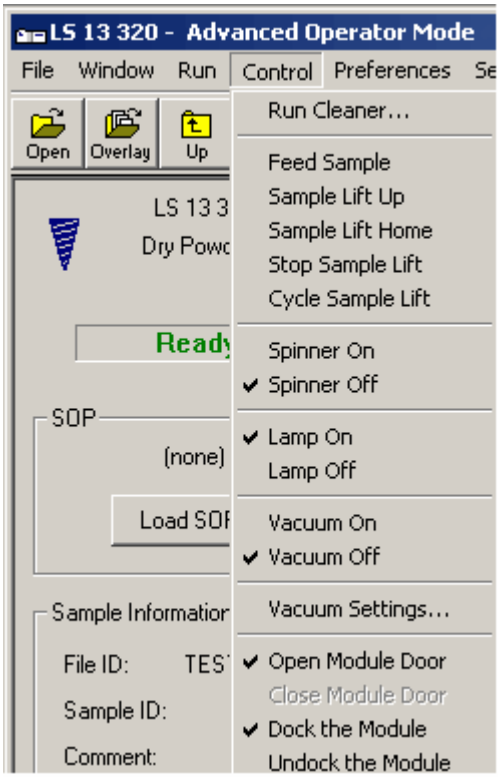
Software

This section covers the Wizards (Standard Operating Methods, SOMs) and controls found in the software program to operate the Tornado DPS.

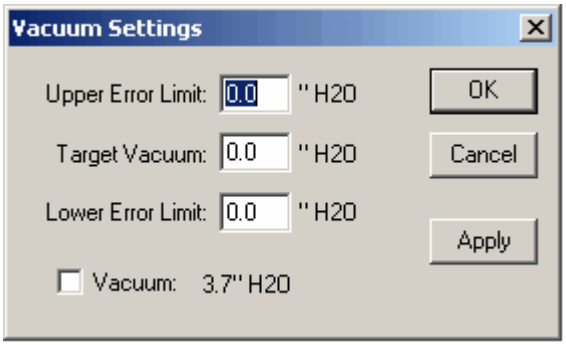
8.4.3.1 Control Menu

The Tornado DPS system can be operated through a series of manual controls accessed through the software.

Control and select the function you want to activate from the menu shown in the figure below.



- **Run Cleaner** - Runs a cycle that runs the cleaning material (provided with your module) through the Tornado DPS.
- **Feed Sample** - Raises the sample lift. This cycles with either the vacuum on/vacuum off options selected, allowing for feeding the sample to the system and maintaining the obscuration that has been pre-set in the preference file. Selecting Sample Lift Home stops this command.
- **Sample Lift Up** - Sample Lift Home moves the lift to its maximum height and back to its home stage. Useful when cleaning the module.
- **Stop Sample Lift** stops the sample lift during its movement at its current position.
- **Cycle Sample Lift** cycles the sample lift up and down. Use the Sample Lift Home option to stop this action.
- **Spinner On** - Spinner Off turns the spinner on and off.
- **Lamp On** – Lamp Off turns the sample lamp on and off.
- **Vacuum On** - Vacuum Off Use this option to turn the vacuum on and off when cleaning the sample lift.
- **Vacuum Settings** - This option is available only under Administrator or Supervisor mode.



These limits must be set so that the vacuum value falls between the two limits. The vacuum LED on the front of the module will provide a guide as to which value needs to be adjusted. If the LED light is solid red, indicating the vacuum is low, adjust the Lower Error Limit to be less than the vacuum pressure reading. If the LED light is flashing red, indicating the vacuum is high, adjust the Upper Error Limit to be greater than the vacuum pressure reading.

8.4.3.2 Standard Operating Methods (SOM)

The Standard Operating Methods (SOMs) are used in part to assure consistency in the way analyses are performed. The options available when creating an SOM are similar to those in the Run Cycle dialog.

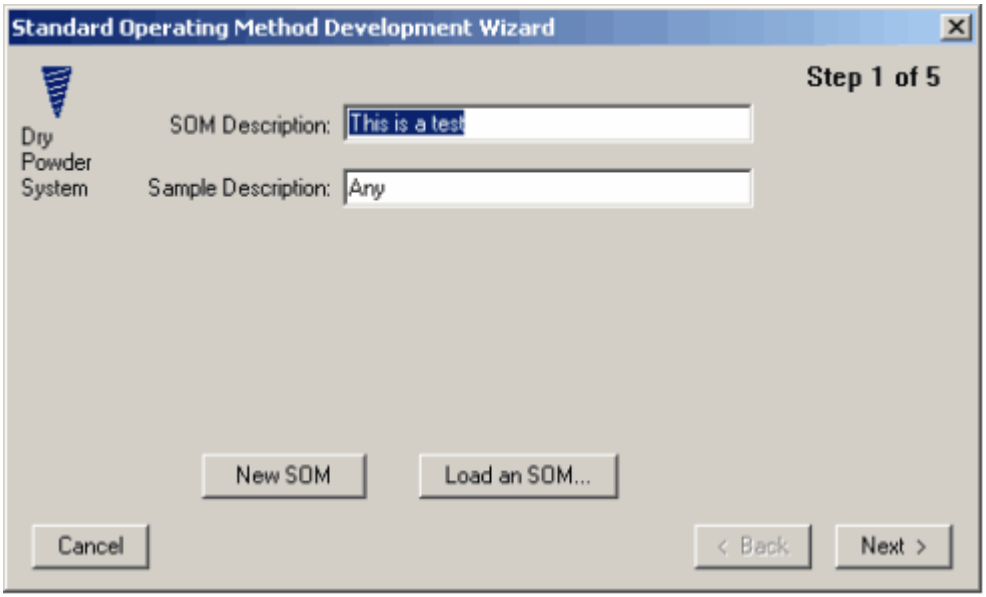
Creating SOMs

To create an SOM:

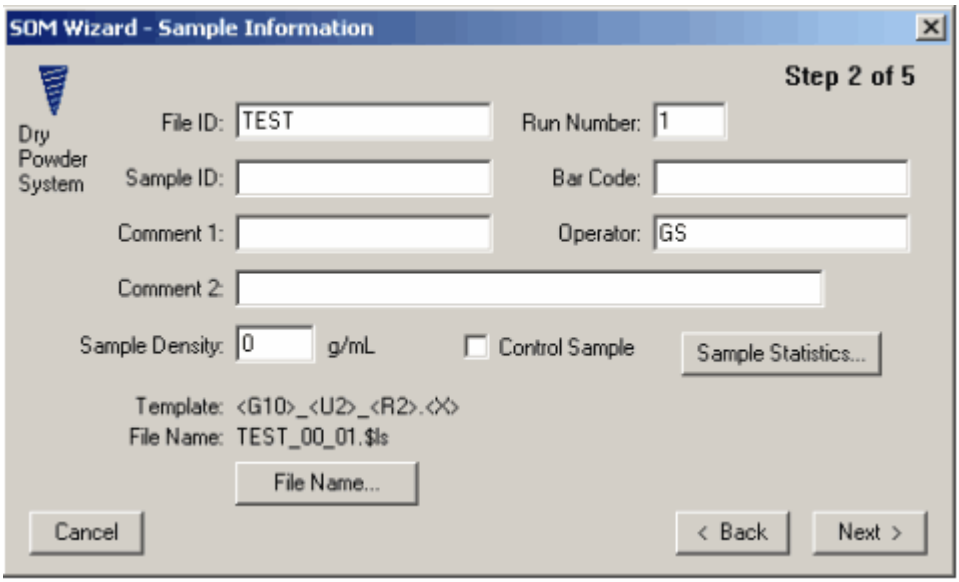
1 **Run**.

2 Select **Create an SOM**. A sequence of screens will guide you through the creation of an SOM.

3 Step 1 prompts you to input descriptions of the sample and the SOM for this particular sample.

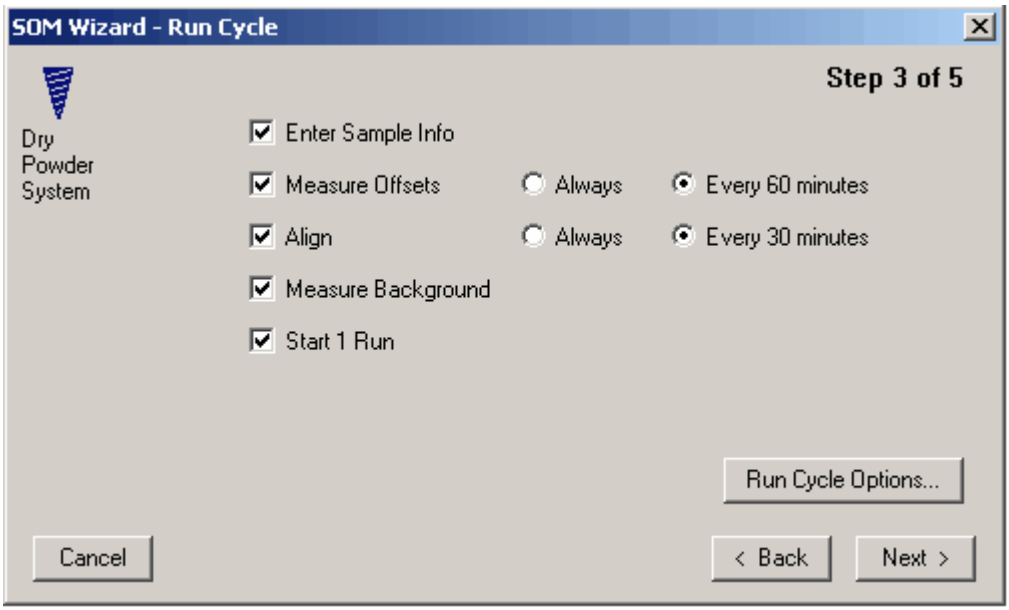


4 Step 2 information about the sample as well as the (file name format) under the file name is entered.

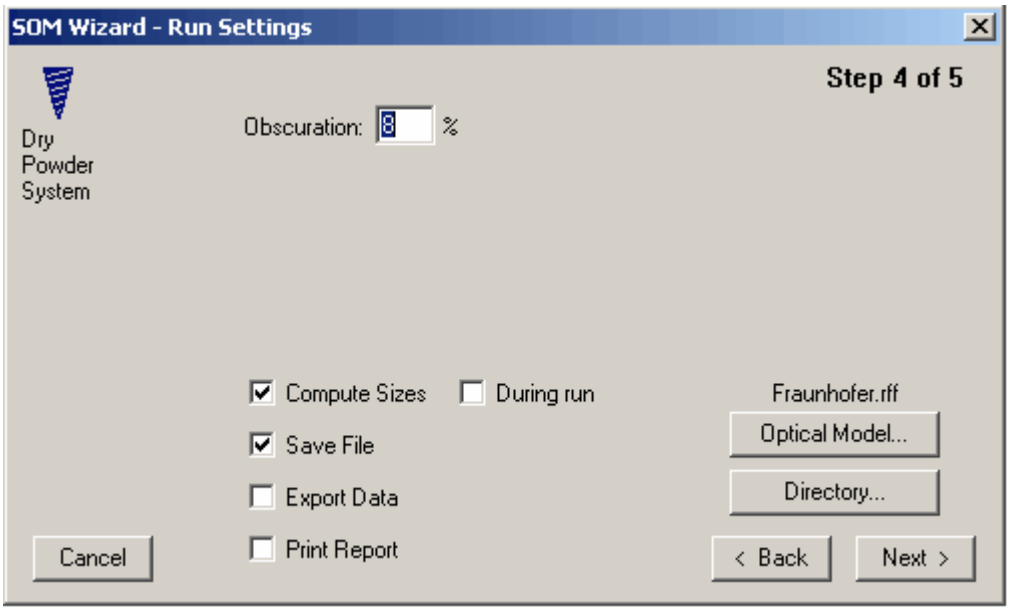


5 Step 3 **Run Cycle** options such as offsets, alignment and background are set during this step.

The **Run Cycle Options** button will take you to the **Run Cycle Options** dialog box in which the measurement times for offsets, background and alignment are set.



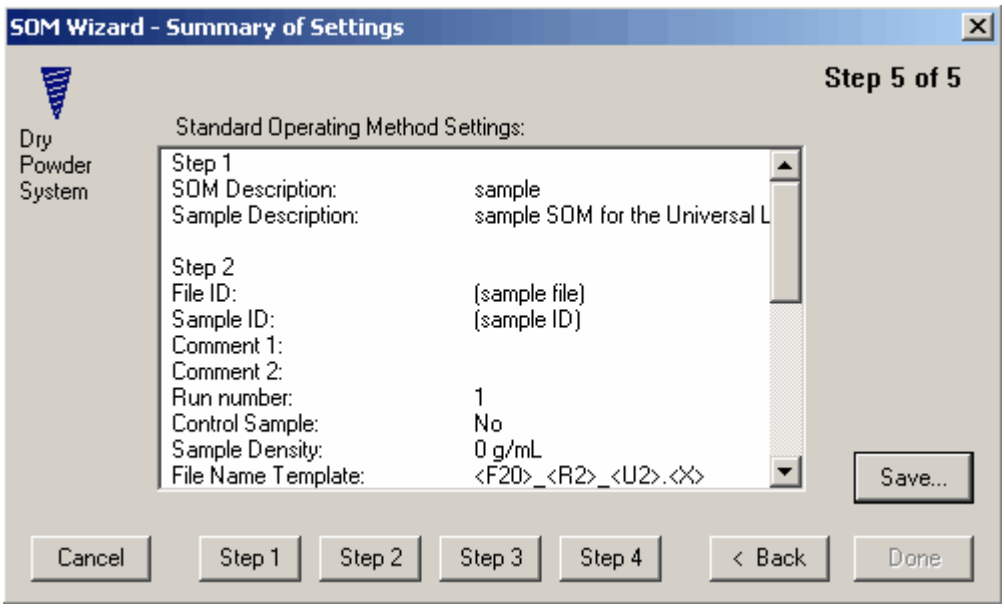
6 In Step 4 the obscuration value (%) at which the analysis will be performed is selected. For the Tornado DPS this value should be between 4% and 8%. The ISO 13320 standard suggests an obscuration value of 5% (ISO 13320-1 Particle size analysis – Laser diffraction methods. Part 1: General Principles. Section 6.2.4, page 8).



The **Directory** option allows the user to select a specific folder to save the data in or to simply utilize the current folder for saving data.

7 Step 5 summarizes the options selected in the previous steps. The SOM is saved during this step.

IMPORTANT SOM files, by default, are saved under the SOP folder, but they can be saved to any other folder.



Under high security (21 CFR Part 11, for compliant version of software only), SOMs can only be saved by the Administrator. Under Operator mode, the **Save** button in step 5 of the SOM creation is not displayed.

8.4.3.3 Loading an SOM

To load an SOM:

1 **Run**.

2 Select **Load an SOM**.

3 Select the desired SOM file from the SOP subfolder or the subfolder where the file was last saved.

When an SOM is loaded, the status panel will show information about the loaded SOM and the **Start Cycle** button will change to **Start SOM**. Clicking on this button will start an analysis using all the settings selected when the SOM was created. Clicking on the **Remove SOM** button will re-activate the **Start Cycle** option.

8.4.3.4 Standard Operating Procedures (SOP)

The Standard Operating Procedures (SOPs) bring together the SOMs and the preference files, thus setting all the parameters necessary to fully control the operation of the instrument, as well as data presentation.

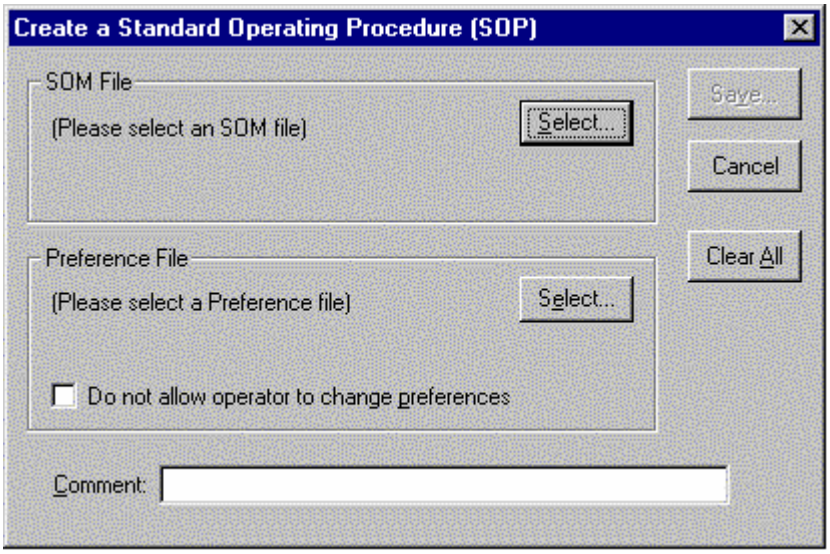
Creating SOPs

To create an SOP:

1 **Run**.

2 Select **Create an SOP**.

3 From the Create an SOP dialog box, select an SOM and preference file.



4 Select **Save**. By default the SOP file will be saved under the SOP folder.

Loading an SOP

To load an SOP:

1  **Run**.

2 Select Load an SOP. SOPs can also be loaded from the status panel by clicking on the **Load SOP** button.

3 Select the desired SOP file from the SOP subfolder or the subfolder where the file was last saved.

Cleaning the Tornado DPS

It is important for all glass surfaces in your sample system to be clean, as dusty or coated optical surfaces can cause erroneous results. Some samples may coat the inside of the sample cell windows. If the instrument is in a dusty or smoky environment or if vapors are present, the lenses and outer surfaces of the windows may become coated. Check the optical surfaces for cleanliness at least every three months, and clean if needed. Depending on the environment, cleaning may be required more frequently.

Using the Cleaning Sample

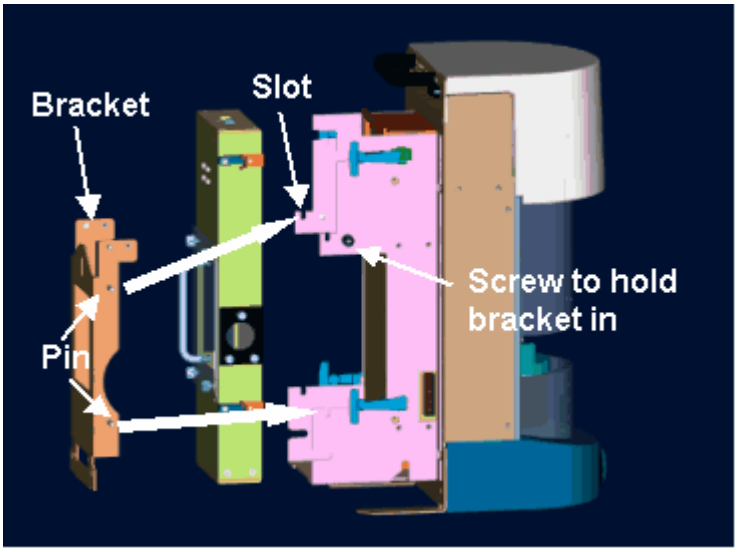
The sample cell and tubing may be cleaned by using the provided cleaning sample. Load approximately 15 cc to 25 cc of the cleaning sample into the sample holder and select the **Run Cleaner** option from the Control menu.

Cleaning the Cell Windows

To clean the windows the sample cell must be removed from the module housing.

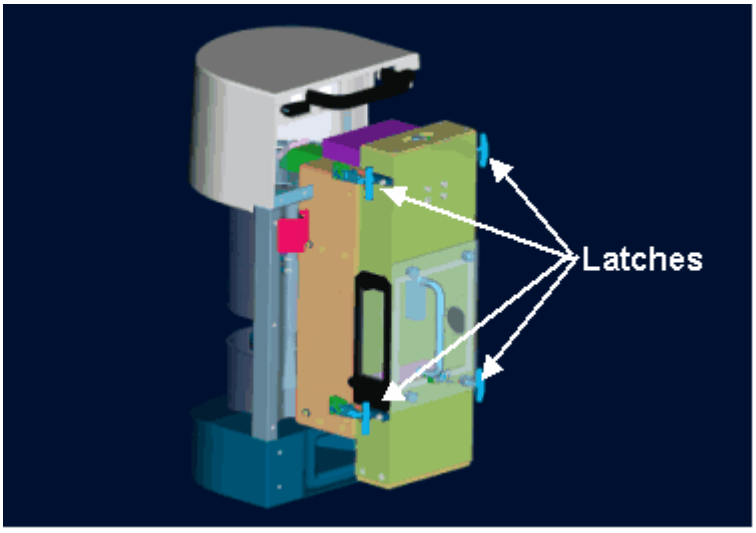
1 Loosen the screws that hold the bracket in place. Lift the bracket to disengage the pins from the pin slots. See Figure 6.34.

Figure 6.34 Figure 2 Bracket Screws



2 Undo the four (top and bottom) latches found in the back of the module that attach the sample cell to the module housing (Figure 6.35).

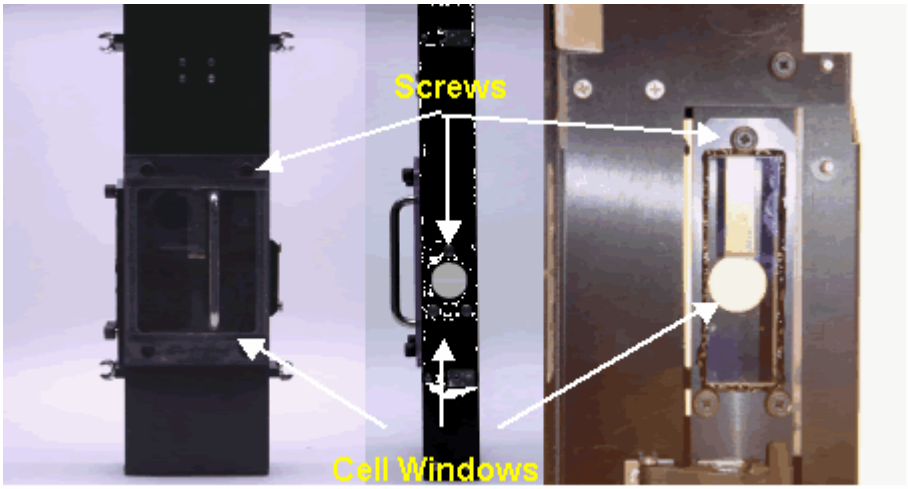
Figure 6.35 Sample Cell Latches



3 Remove the sample cell.

Clean the inner and outer surfaces of the sample cell windows (figure 4) whenever they become coated. To clean these windows:

Figure 6.36 Figure 4 Sample Cell Windows



1 Remove the set of screws holding the window you wish to clean. Remove the window.

2 Fold three to four lens tissues in half.

3 Apply lens cleaner onto the creased area of the folded lens tissue.

4 Place the lens tissues on the window surface, holding the tissue in place with two fingers.

5 Slowly, wipe the window.

6 Discard the used lens tissue.

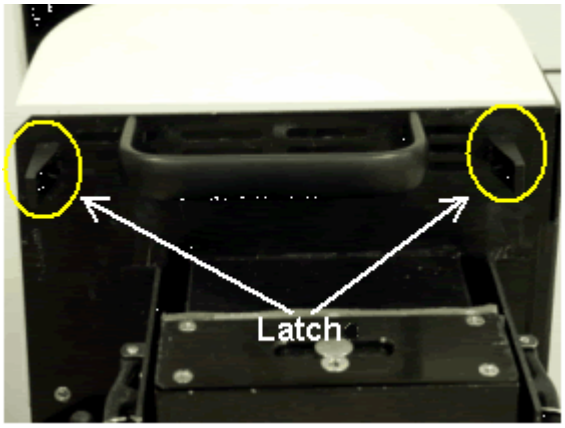
7 Re-assemble the windows and sample cell.

Cleaning the Sample Tubing

The lines that carry the sample into the sample cell may need to be cleaned separately when the sample cleaner cannot clean them completely.

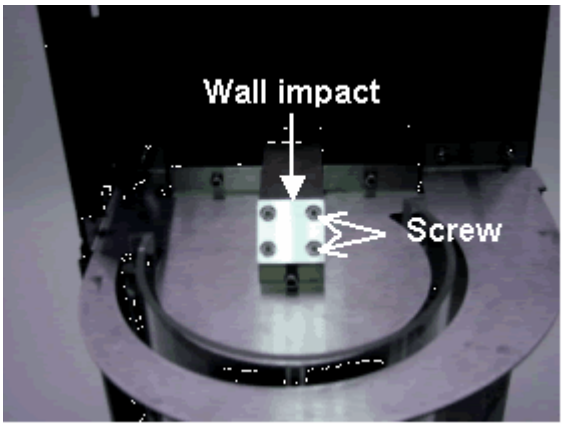
1 Remove the module cover by undoing the two latches found in the back of the module (Figure 6.37).

Figure 6.37 Cover Latches



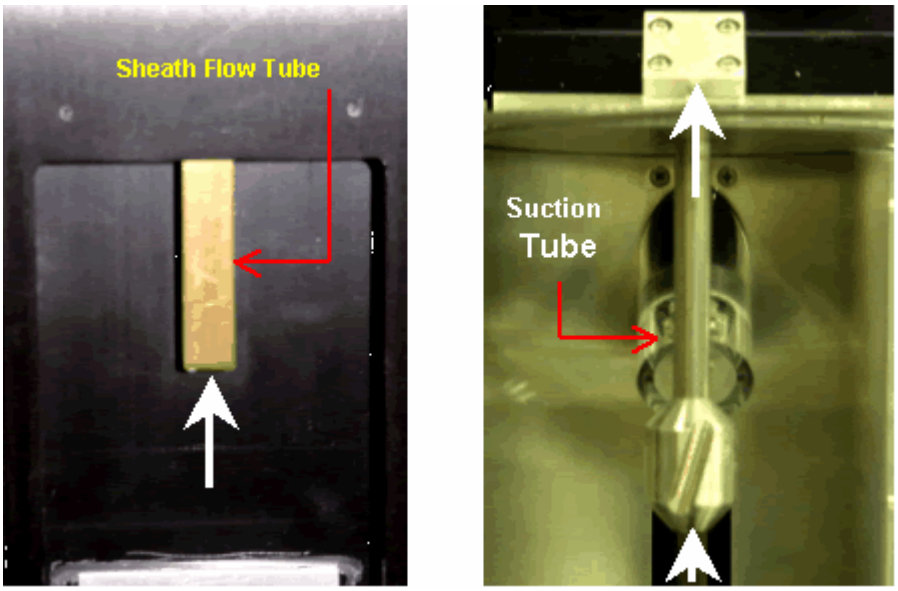
2 Remove the wall impact plate by removing the four screws. (Figure 6.38)

Figure 6.38 Wall Impact Plate



3 Use the brush provided in the Tornado DPS accessory kit to clean the suction and sheath flow tubes in the direction shown by the arrows in Figure 6.39. Use the same brush to clean the tube that runs perpendicular to the suction tube and runs towards the sheath flow tube.

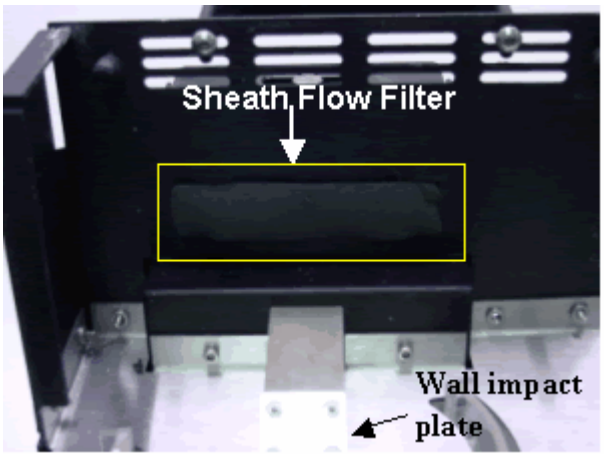
Figure 6.39 Suction and Sheath Flow Tubes



Cleaning the Sheath Flow Filter

1 Remove the module cover by undoing the two latches found in the back of the module (Figure 6.37).

Figure 6.40 Sheath Flow Filter



2 Remove the filter (figure 8) and clean with soap and water. Allow it to dry before re-installing. The filter can also be cleaned by applying vacuum to it.

Tornado DPS Troubleshooting

The LEDs on the Tornado DPS will aid you in troubleshooting the module. Refer to figure 1 for a description of the function of each LED.

Problem	Possible Cause/Solution
Module not responding	• Module docked incorrectly (LEDs will not light). — Eject module and re-dock. — Apply slight pressure on the front of module while docking. If LEDs turn on, check cable from optical bench to the computer.
Vacuum will not start	• Check that vacuum is plugged-in. • Check that vacuum remote power switch is in the ON position. • Check that remote power switch cable is connected to the optical bench.
Sample not being suctioned	• Check that the vacuum limits are set properly (Administrator only). • Check that sample tubing is not plugged.
Error LED is RED	• Power instrument down and back up. If error light is still red, call service.
High background	• Dirty windows. Clean all the windows.
Low vacuum pressure	• Sheath flow filter missing. Install filter. • Leaks. Tighten windows screws. • Sheath flow tube plugged. Clean tube. • Vacuum bag full or filter clogged. Replace vacuum bag and/or filter.
High vacuum pressure	• Suction nozzle plugged. Clean nozzle. • Sheath flow filter dirty. Clean or replace filter.

Tornado DPS Specifications

Item	Specification
Power	The Tornado DPS derives its power directly from the optical bench < 50 W peak-power
Temperature	10° to 40° C (50° to 104° F)
Humidity	0 to 90% without condensation
Dimensions	Height: 46.4 cm (18.25 in) Width: 20.3 cm (8.0 in) Depth: 21.6 cm (8.5 in)
Weight	8.2 kg (18 lb)
Vacuum System	Flow rate: 420 L/min Pressure: 740 to 750 torr (15 cfm at 5 to 10 inches of H2O below atmospheric pressure)

Universal Liquid Module

The Universal Liquid Module (ULM) is intended for use with the LS 13 320 Optical Bench. It is capable of suspending samples in the size range of 0.017 µm to 2000 µm.

The ULM measures the entire sample introduced to the instrument by re-circulating the sample. The amount of sample needed depends on its size and concentration. Prior sample preparation is often needed to achieve proper dispersion (see APPENDIX B, *Sample Handling* for more information on how to disperse samples) in order to obtain accurate and valid results.

ULM Description

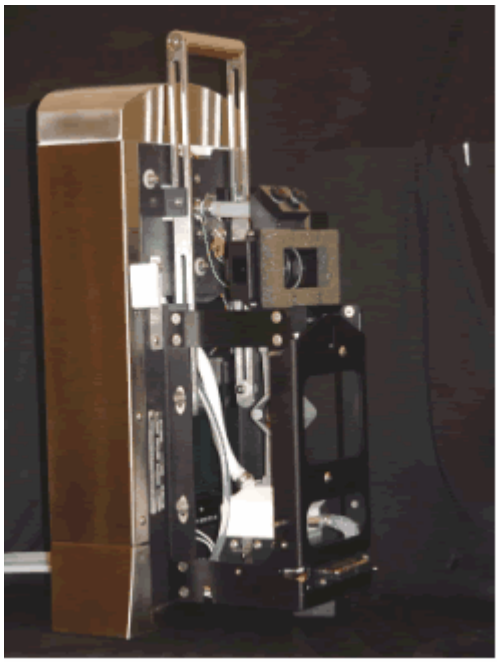
The ULM system consists of:

- The ULM module
- Two hoses: one for fluid delivery and another for waste.

The ULM requires relatively small volumes of sample and dispersing liquid (total volume 125 mL). It is compatible with both organic and aqueous media. The set point for the obscuration is user selectable or may be attained automatically as the sample is added to the vessel.

This sample module has the capability of auto-filling and auto-rinsing using a self-contained internal pump. The pump is controlled by an automated level sensor that signals when the container is full or empty. The pump fills and rinses the unit automatically, expelling all waste into a waste container (provided with the module).

Loading the ULM



1 Open the LS 13 320 door to load the ULM by undocking the auto-docking tray. Press the **OPEN** button on the instrument and the sliding door moves to the left. When it is open, press the **EJECT MODULE** button to extend the tray toward the user.

2 In the auto-dock tray, you will notice stainless steel pins extending into the center tray area both at the back of the tray and in the front of the tray. These pins fit into slots on the ULM. The back pins prevent the module from tipping out and the forward pins secure the module in the tray.

3 Hold the ULM by the top with one hand and on the bottom with the other hand. Place it in the auto-dock tray by tipping the ULM to the rear at an approximately 30 degree angle into the auto-dock tray. This is done to clear the forward pins and engage the back pins first.

4 When these pins have engaged into the slots on the back of the ULM, tip the module up to its vertical position. The ULM should now be resting on the auto-dock tray.

5 Press the **EJECT MODULE** button and the MLM will be drawn into the LS 13 320.

⚠ CAUTION

Do not place fingers inside bench as automatic sliding door closes.

⚠ CAUTION

Do not place fingers inside bench as module docks into bench.

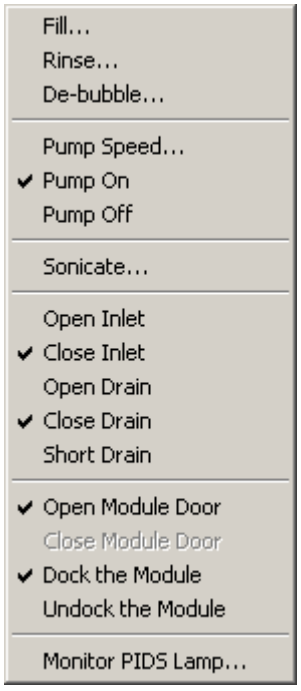
ULM Software

This section covers the controls found in the software program to operate the ULM. It also covers the parts of the Wizards (Standard Operating Methods (SOMs)) that deal with the ULM.

8.5.3.1 Control Menu

The ULM system can be operated through a series of manual controls accessed through the software.

Control and select the function you want to activate.

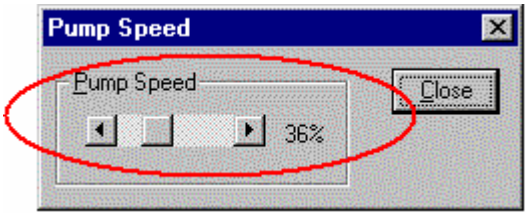


Fill
This option opens the inlet valve to fill the vessel with the diluent. The drain valve, if open, will be closed. Make sure the fill hose is completely submerged in the diluent.

Rinse
Selecting this option will start an automatic rinse cycle. The order of operations is Open Drain, Close Drain, Fill, and Pump On. This cycle is repeated for a set number of times (set in the Run Cycle Options dialog box).

De-bubble
This routine may be used to de-bubble the diluent when the presence of too many bubbles produces high backgrounds.

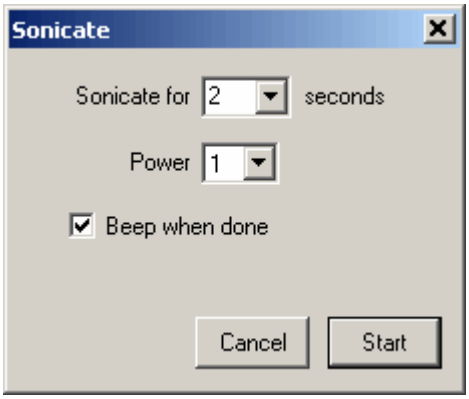
Pump Speed
This option opens a dialog box that allows you to set the speed of the pump, by either clicking on the arrows or by dragging the indicator.



Pump On – Pump Off
Selecting one of these options will turn the pump On or OFF.

Sonicate

You may sonicate your sample before an analysis by selecting this option. The following dialog box is displayed with options to select the time for sonication and the power at which the sonication is to be done.



Open Inlet - Close Inlet

By default, the inlet valve is closed. When using the Rinse or Fill options the valve is automatically opened during the filling process and returned to its default setting after the vessel is full. You can also fill the vessel by selecting Open Inlet.

Open Drain - Close Drain

Opening the drain will stop the pump automatically when the fluid level falls below the bottom sensor. If Fill or Rinse is selected the drain is automatically closed. You can have the drain open while adding fluid to the vessel by selecting both the Open Drain and Open Inlet options at the same time. Only in this set up will the pump stay on.

8.5.3.2 Standard Operating Methods (SOM)

The standard operating methods are used in part to assure consistency in the way analyses are performed. The options available when creating SOMs are similar to those available when using the Run Cycle dialog box.

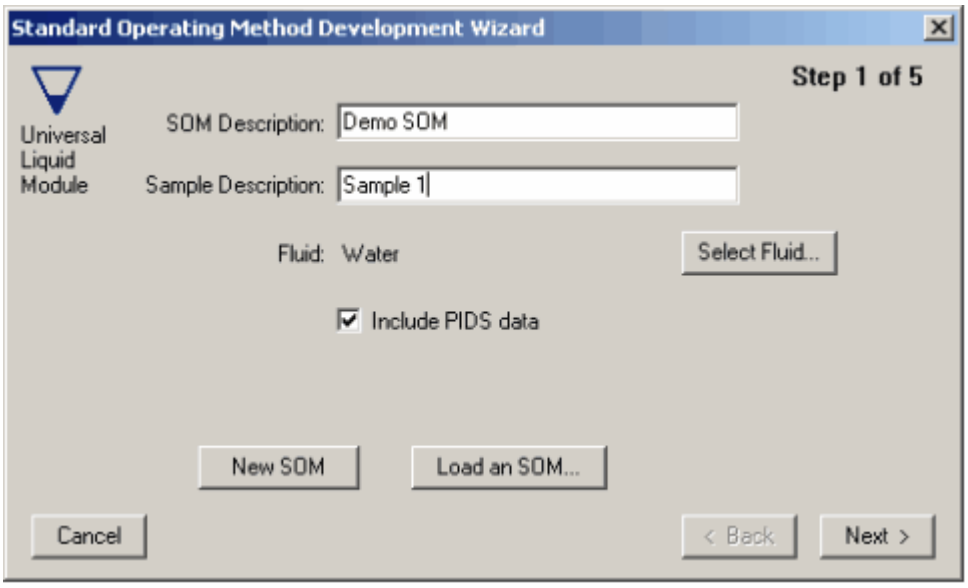
Creating SOMs

To create an SOM:

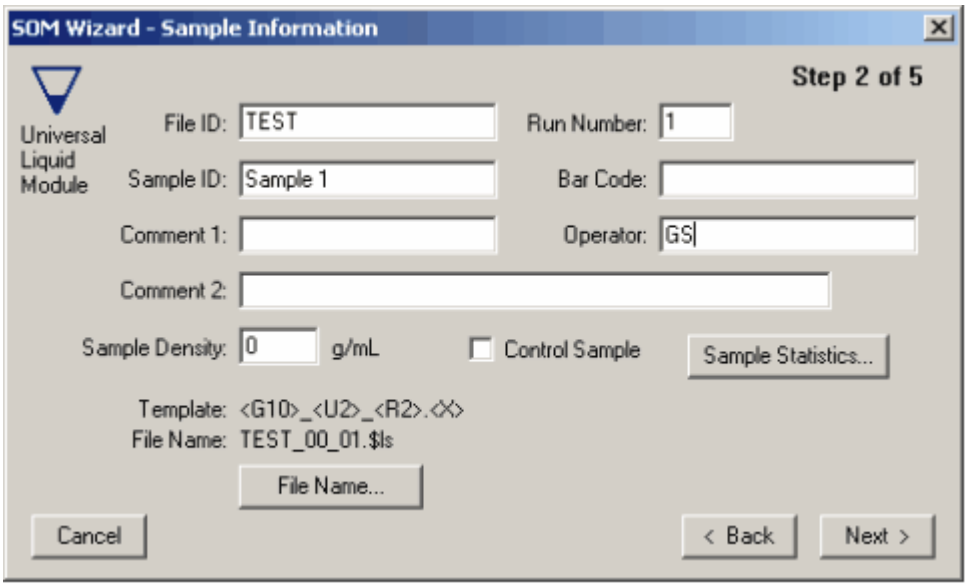
1 **Run**.

2 Select **Create an SOM**. A sequence of screens will guide you through the SOM creation.

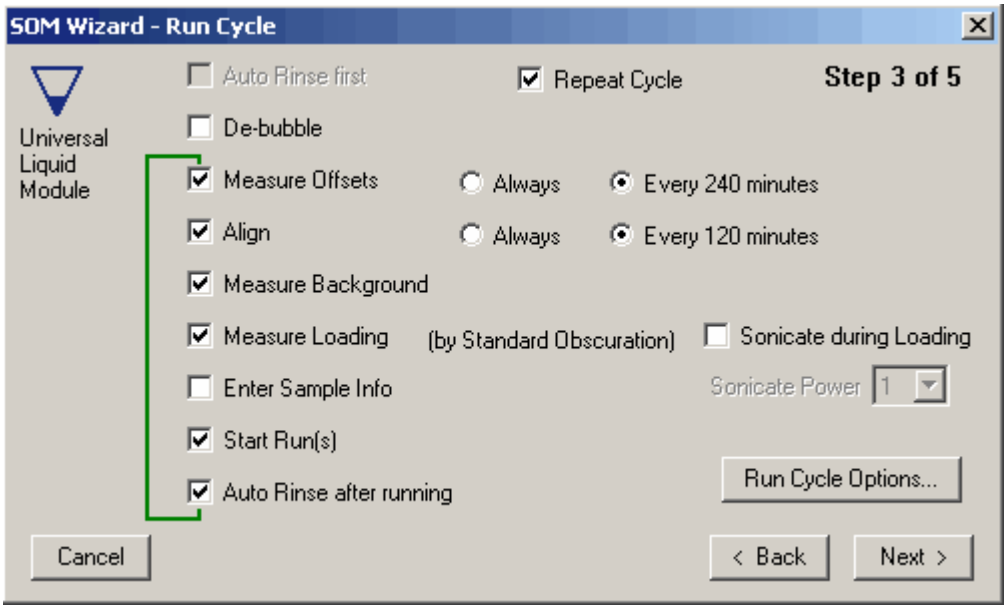
3 Step 1 prompts you to input a description of both your sample and the SOM you are creating for this particular sample.



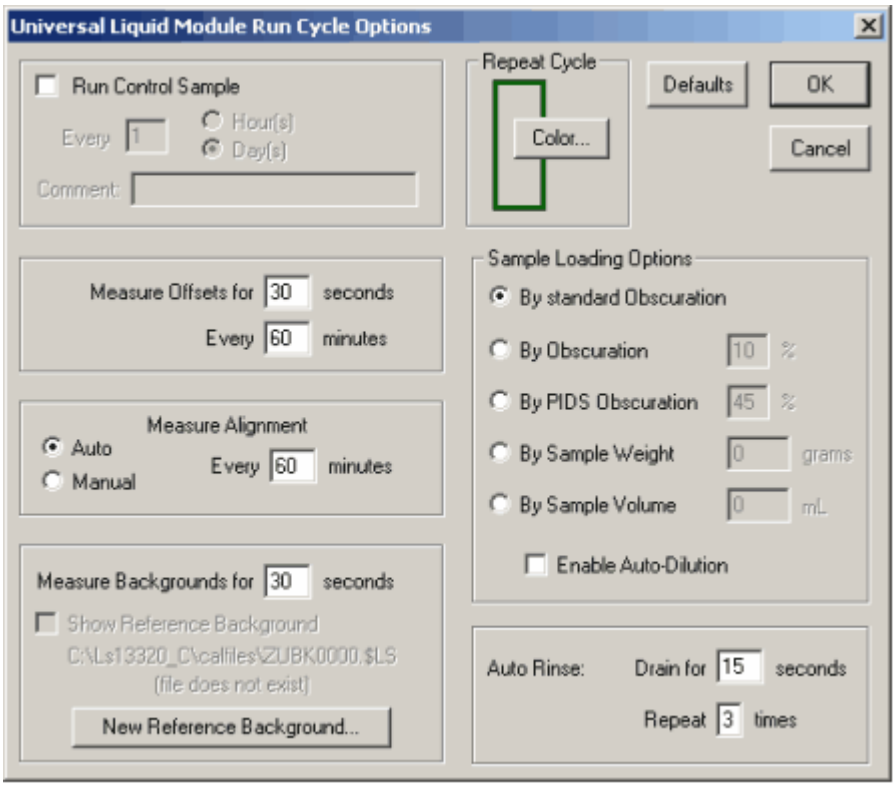
4 Step 2 information about the sample as well as the (file name format) under the file name is entered.



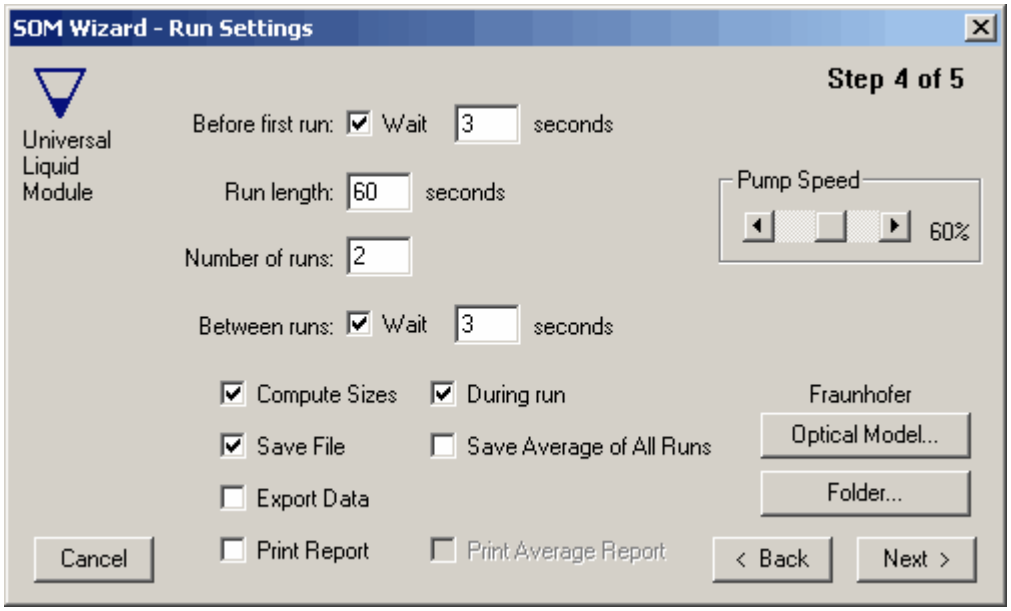
5 Step 3 **Run Cycle** options such as offsets, alignment and background are set during this step. The **Run Cycle Options** button will take you to the Run Cycle Options dialog box in which the measurement times for offsets, background and alignment are set.



Run Cycle Options are selected from this screen as well.

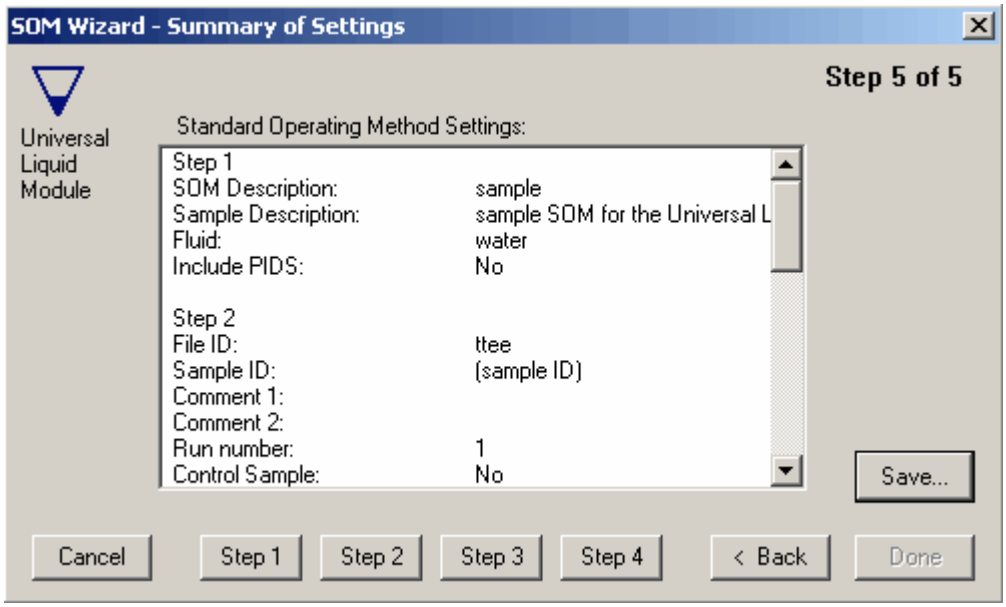


6 In Step 4 the run length is set. For analyses that require collection of PIDS data, a run length of at least 90 seconds is recommended. For analyses without PIDS, 60 seconds is the recommended time for the analysis, though analyses may be as short as 10 seconds. The optical model can be selected during this step. For more on optical models see APPENDIX C. The Pump Speed can be adjusted in this dialog. It is recommended for samples that require surfactant that the pump speed be kept low, i.e. 30%, in order to prevent the formation of air bubbles. Should bubbles be present, alternating high pump speed with low pump speed is useful when trying to de-bubble the suspending fluid. If the pump speed is increased to its maximum (100%) and then reduced to about 50%, air bubbles can be eliminated. This procedure may need to be repeated two to three times to eliminate the bubbles. High pump speeds are also useful when measuring large, dense particles. You can access the manual speed control from the Control menu by selecting Pump Speed.



7 Step 5 provides the user with a summary of the options selected in the previous steps. The SOM is saved during this step.

IMPORTANT SOM files, by default, are saved in the SOP folder, but they can be saved to a folder of your choice as well.



Under high security (21 CFR Part 11, for compliant version of software only), SOMs can only be saved by the Administrator. Under Operator mode, the **Save** button in step 5 of the SOM creation is not displayed.

8.5.3.3 Loading an SOM

To load an SOM:

1 **Run**.

2 Select **Load an SOM**.

3 Select the desired SOM file.

When an SOM is loaded, the status panel will show information about the loaded SOM and the **Start Cycle** button will change to Start SOM. Clicking on this button will start an analysis using all the settings selected during the SOM creation. The **Remove SOM** button re-activates the Start Cycle option.

8.5.3.4 Standard Operating Procedures (SOP)

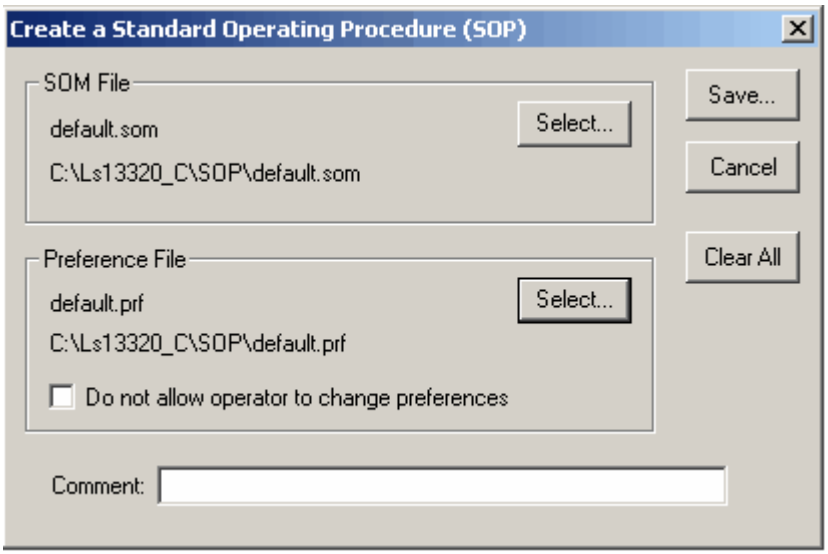
Creating SOPs
Standard Operating Procedures (SOPs) is the last step in setting all the parameters necessary to fully control the operation of the instrument.

To create an SOP:

1 **Run**.

2 Select **Create an SOP**.

3 From the Create an SOP dialog box, select an SOM and preference file.



4 Select **Save**. By default the SOP file will be saved under the SOP folder.

Loading an SOP
To load an SOP:

1  **Run**.

2 Select **Load an SOP**. SOPs can also be loaded from the status bar by clicking on the **Load SOP** button.

3 Select the desired SOP file from the SOP subfolder or the subfolder where the file was last saved.

Suspension Fluids

The materials used in the construction of the ULM that come in contact with the suspension fluid are Teflon®, 316 stainless steel, glass, and Kal-rez®.

Only use suspension fluids that have flash points greater than 10.0°C (50°F), with specific gravities greater than or equal to 0.6, and that meet the requirements listed below. Examples of fluids that can be used in the ULM are water, acetone, weak aqueous-based acids or bases, mineral oils, ethylene glycol, hexane, etc. The suspension fluid used depends on the type of sample to be analyzed.

Suspension fluid requirements:

- **Chemical Compatibility** - does not adversely react with the ULM components mentioned above.
- **Transparency** - transparent in the 450 nm to 900 nm range
- **Clarity** - no contamination or extraneous particles
- **Consistency** - no bubbles
- **Density** - lighter than the sample particles
- **Wettability** - able to deflocculate the sample particles
- **Fluidity** - low viscosity for rapid stirring
- **Insolubility** - no dissolution of particles
- **Chemical neutrality** - neutral to the sample and the ULM

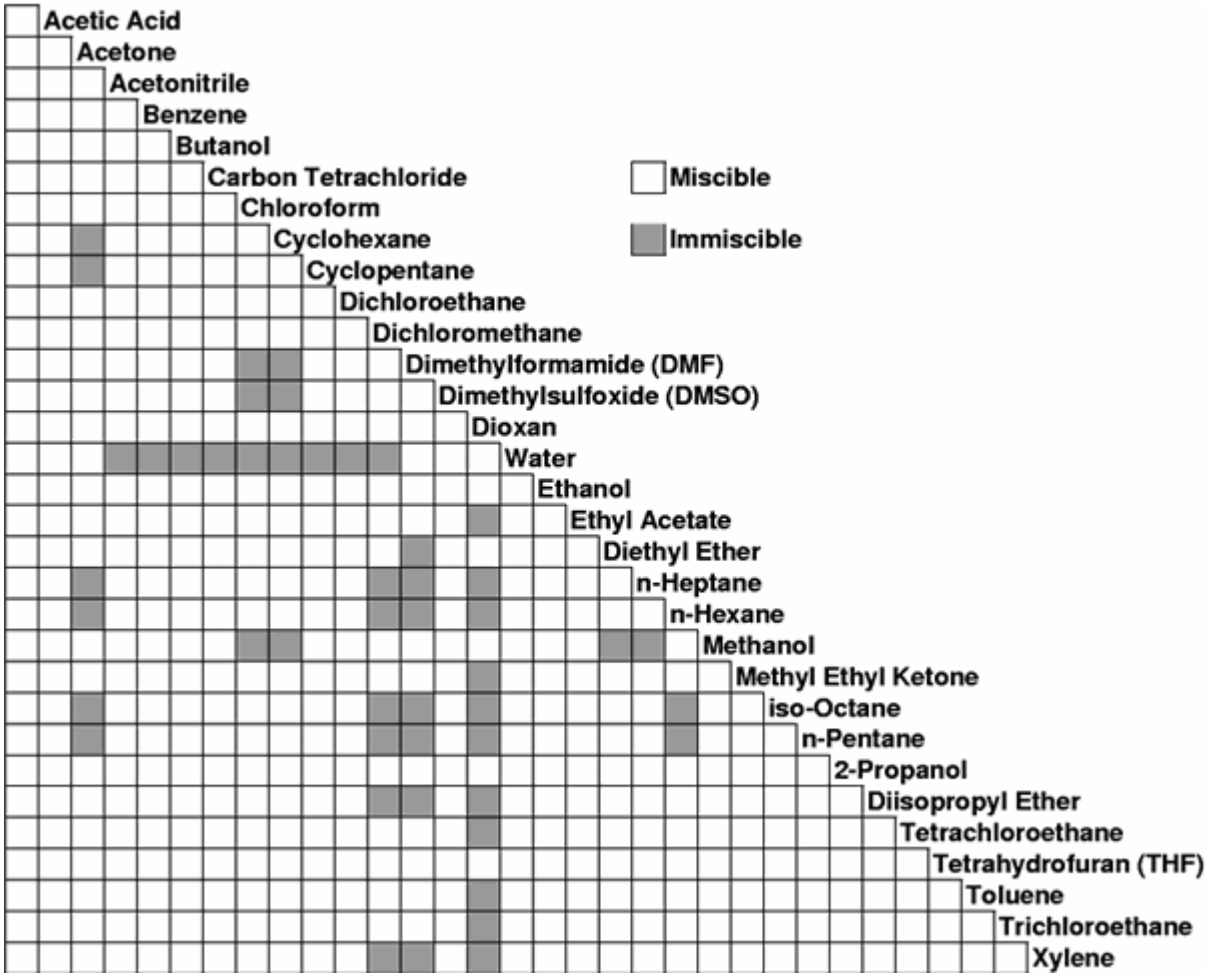
Listed below are acceptable suspension fluids for the ULM. If your application requires a fluid not listed here, check with your local Beckman Coulter representative for chemical compatibility.

Butanol	Butanone
Carbon Tetrachloride	Chloroform
Ethanol	Ethylene Glycol[a]
Glycerol[a]	Heptanes
Hexanes	Jet Fuels
Kerosene	Ketones
Methanol	Methylene Chloride
Mineral Oil[a]	Pentanes
Petroleum Ether	Polyethylene Glycol[a]
Propanol	Silicone Oil[a]
Toluene	Trichloroethane
Trichloroethylene	Water
Weak acid and base solutions (pH 4-10)	

a. These fluids are highly viscous and result in a slower flow rate. Use only when diluted so that viscosity does not exceed 5 centipoise.

NOTE Before changing from one suspension fluid to another, make sure the two fluids are miscible with one another. If the two fluids are not miscible, replace the current fluid with a suspension fluid with which they are both miscible and then change to the new suspension fluid. Mixing of immiscible fluids will form an emulsion that may be very difficult to remove from the system. Figure 6.41 provides a miscibility guide for some common solvents.

Figure 6.41 Miscibility Guide



Cleaning the ULM

It is important for all glass surfaces in your sample system to be clean, as dusty or coated optical surfaces can cause erroneous results. Some samples or suspension fluids may coat the inside of the sample cell windows. If the instrument is in a dusty or smoky environment or if vapors are present, the lenses and outer surfaces of the windows may become coated. Check the optical surfaces for cleanliness at least every three months, and clean if needed. Depending on the environment, cleaning may be required more frequently.

Cleaning Fluids

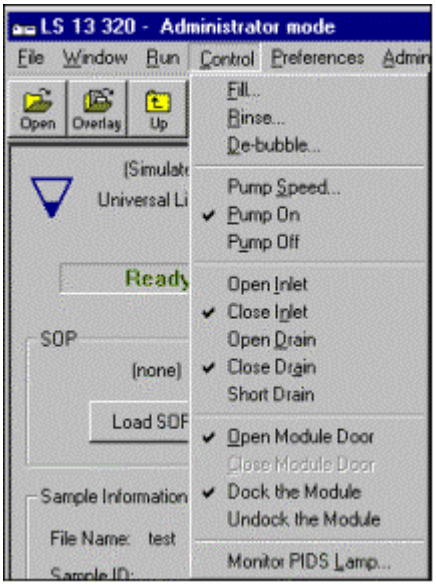
Beckman Coulter recommends using the cleaning solution supplied with the module for cleaning the inner surfaces of the ULM sample system. Beckman Coulter recommends lens cleaning paper for cleaning the exterior surfaces of the lenses and sample cell windows.

Cleaning the Cell

Clean the inner surfaces of the sample system (pump, hoses and within the sample cells) whenever they become coated.

To clean the sample system:

1 Select **Control**.



2 Select **Rinse**

3 Select **Open Drain** after the Rinse routine has finished.

4 Prepare the cleaning solution.

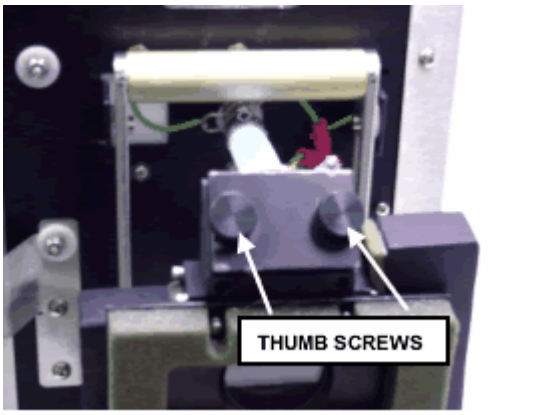
5 Place the inlet hose into the container of cleaning solution.

6 Select **Fill**.

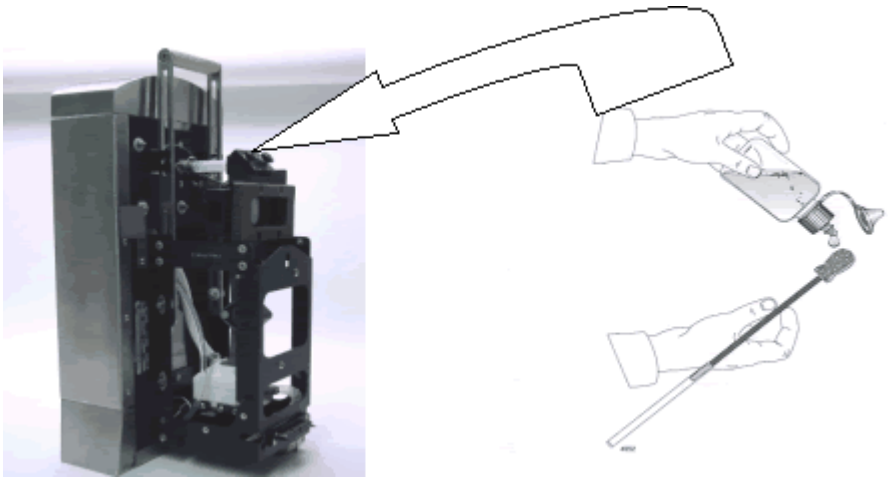
7 Let the cleaning solution circulate for 15 minutes to 1 hour, depending on how dirty the cell is.

8 Select **Control > Rinse**.

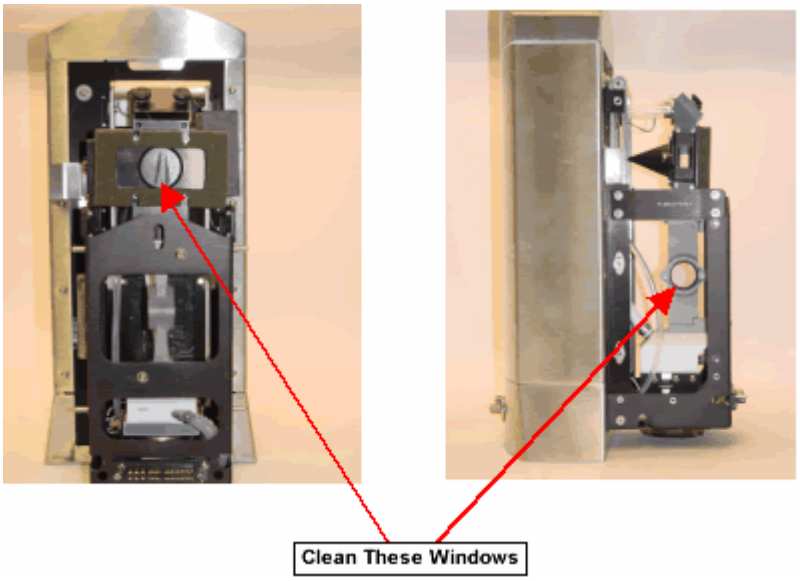
9 You can also clean the windows by removing the thumbscrews that hold the cover, as shown in this figure.



10 After removing the cover you will have access to the inside of the cell. This will allow you to insert a swab with an extension (provided with your accessory kit) to clean the inside of the cell windows.



11 Use the same solution prepared in step 1 to clean the windows with the swab.



Replacing the Diffraction Cell Window

Equipment Needed

- Lens tissues
- Lens cleaning solution
- Window seating tool

Procedure

Use this procedure to replace a diffraction sample cell window that is scratched or damaged.

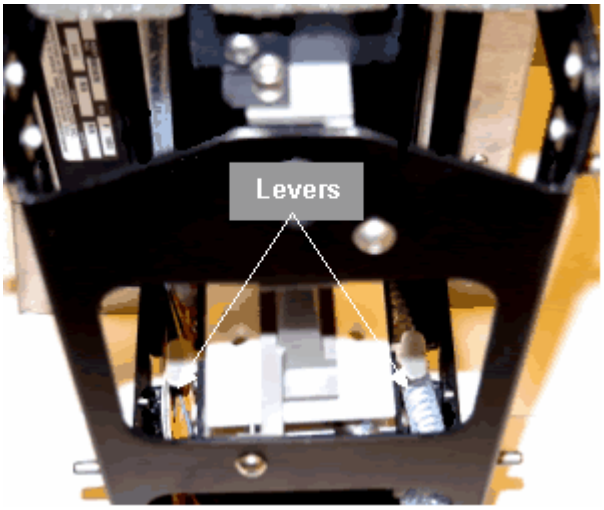
To replace a diffraction sample cell window:

1 Drain liquid from module.

⚠ CAUTION

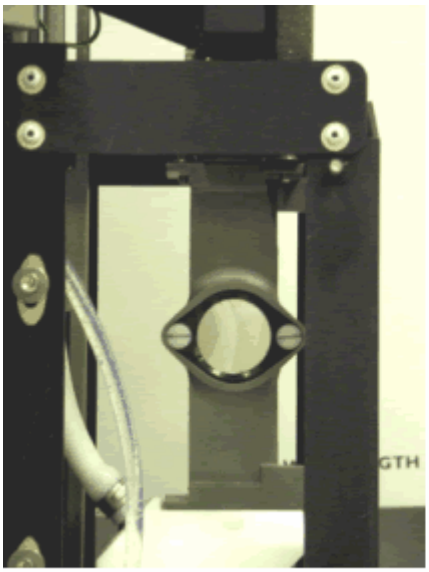
Wear safety equipment appropriate for the suspension fluids being handled.

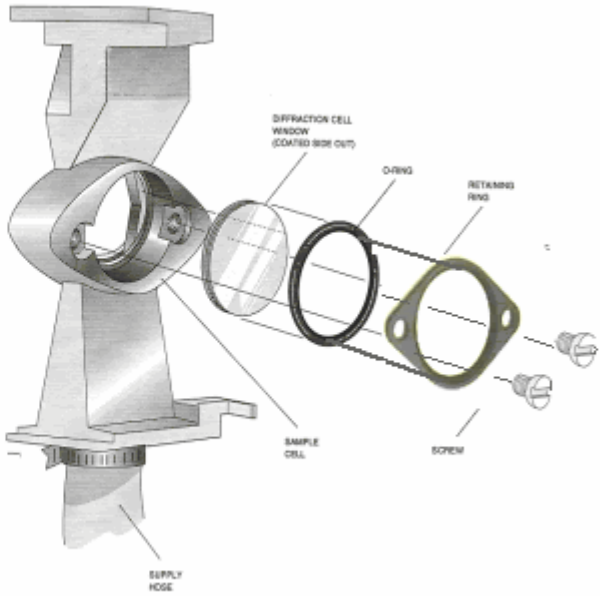
2 Eject module.



3 If there is liquid remaining in the cell, drain it by applying pressure on the two levers at the same time (see figure above).

4 Loosen the two nylon screws to remove each window.





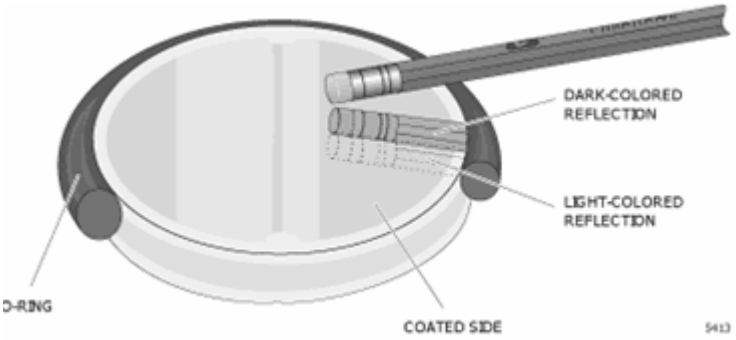
5 Cup your hand over the loose retaining ring and tilt the sample cell stand until the retaining ring falls into your hand.

NOTE You can now lay down the sample stand so the window to be replaced is facing up.

6 Stick a piece of masking tape on the window and lift the tape up to remove the O-ring and window. This may take several tries.

7 Discard the old O-ring, window and nylon screws.

8 Hold the new window by its edge to determine the coated side. Refer to the figure below to determine which side is the correct one.



a. Take the eraser end of a pencil and hold it close to the window, near its edge. Note the two reflections of the pencil, dark- and light-colored.

b. Turn the window over and repeat step a.

c. The side that reflected a dark reflection is the coated side.

⚠ CAUTION

Use lens tissues only once, then discard. Never use silicone-coated, eyeglass lens tissues to clean lenses or sample cell windows, as this type of tissue may leave a film. Wash your hands thoroughly before cleaning any lens or sample cell window. Do not touch optical surfaces with your fingers or skin. Body oils are difficult to remove without harming the anti-reflective coating.

9 Lay the window, coated side down, on a piece of lens tissue.

10 Clean the window with lens tissue wetted with lens cleaner.

11 Gently place the window, coated side to the outside, into the sample cell window opening. It must be flat to avoid scratching the other window.

12 Lubricate the O-ring with lens cleaning solution.

13 Carefully place the new O-ring on top of the window.

14 Insert the grooved end of the window-sealing tool into the sample cell. Push in gently and twist from side to side to seat the O-ring.

15 Remove the tool and check that the O-ring is completely seated down and around the window.

16 Clean the retaining ring, then gently place it on top of the window.

17 Replace the two new nylon screws that hold the retaining ring.

18 Clean the outer surface of the diffraction window as described in step 10.

19 Replace the window on the other side if needed using steps 4 through 18.

20 Insert both hoses into the bottom of the sample cell stand. Make sure they are tight.

21 Load the ULM into the optical bench and dock it.

22 Perform a leak test.

- **a.** Select **Control > Fill**.
- **b.** After the sample vessel is filled, select Control, Pump On.
- **c.** If any leaks are noticed after 2 to 3 minutes, remove the retaining ring and check that the window and O-ring are seated correctly, then repeat the leak test.

ULM Troubleshooting

Problem	Possible Cause/Solution
No communication with optical bench	• Module docked incorrectly. Eject module and re-dock. Apply slight pressure on the front of module while docking.
Module will not fill	• Check to see if it is already full • Check that connections are air-tight • Check that the fill hose is submerged in the diluent. • The valve should make an audible sound. If it does not, contact Beckman Coulter service.
Module will not drain	• Check that the waste container is not full. • Check that the drain hose is clear of obstructions.
Pump will not work	• Module is not completely full of fluid. • Check power to the system. • Check belt drive.
Module leaking	• Check fittings. • If leakage is inside, contact Beckman Coulter • service.

ULM Specifications

Item	Specification			
Power	The ULM derives its power directly from the optical bench < 50 W peak power			
Temperature	10° to 40° C (50° to 104° F)			
Humidity	0 to 90% without condensation			
Dimensions	Height 49.5 cm (19.5 in)	Width 21.0 cm (8.25 in)	Depth[a] 11.4 cm (4.5 in)	Depth[b]
Weight	8.85 kg (19.5 lb)			

a. Visible portion of module once docked into the optical bench

b. Includes optical cell

APPENDIX A

Specifications

Item	Specification			
Power	100 ±10 V, 120 ±10 V, 220 ±20 V, or 240 ±20 V 50/60 Hz ≤6 A			
Temperature	10° to 40° C (50° to 104° F) **IMPORTANT** If room temperature varies 2° C/hour, perform alignment and offsets tests more often, at least once an hour.			
Humidity	0 to 90% without condensation			
Dimensions	**Unit**	**Height**	**Width**	**Depth**
	Optical Module	44.5 cm (17.5 in)	101 cm (40 in)	25.4 cm (10 in)
	Computer Processor with Disk Drive	13 cm (5 in)	36 cm (14 in)	41 cm (16 in)
	Monitor	32 cm (12.5 in)	32 cm (12.5 in)	41 cm (16 in)
	Printer	23 cm (9 in)	39 cm (15.5 in)	41 cm (16 in)
Weight	71.5 lbs (32.5 kg)			
Altitude	Sea level to 3,000 m (10,000 ft)			
Laser	**Classification** I for operator (no access to radiation) IIIb for service and maintenance (trained Beckman Coulter personnel only). **Power** 5 mW 4 mW operating power **Wavelength** 750 nm or 780 nm **Note**: 750 nm laser has been discontinued **Life Expectancy** 70,000 hours			

Item	Specification
PIDS Lamp	**Type** Tungsten-halogen **Ratings** 6.0 V, 1.7 A, 10 W **Output** 150 lumens at 2,900° K **Life Expectancy** 2,000 hours
Particle Sizing Range	**Aqueous Liquid Module and Universal Liquid Module:** 0.017 µm to 2000 µm **Micro Liquid Module and Tornado Dry Powder System:** 0.4 µm to 2000 µm
Size Channels	Micro Liquid Module and Tornado Dry Powder System: 92 Aqueous Liquid Module and Universal Liquid Module: 116
Repeatability	1% about mean size (repeat runs of the same sample)
Run Time	1 to 999 seconds (30 to 90 seconds typical)
Sample Requirement	All quantities will vary considerably depending on material density and particle size distribution Aqueous Liquid Module: approx. 10 mg to 100 g Universal Liquid Module: approx. 1 mg to 10 g Micro Liquid Module: approx. 1 mg to 50 mg Tornado Dry Powder System: approx. 5 cc to 30 cc
Data Presentation	Video display and hard-copy printouts
Data Formats	**Listing, Intensity** • Detector group intensity flux, picoamps and millivolts • Background, flux and picoamps • Offset, millivolts • Gains for the diffraction and PIDS detectors
Listings, Size	• Channel group size and particle diameter for differential and cumulative greater than or less than distributions in volume %, surface area % and number %
Graphs, Intensity	• Detector flux, picoamps or millivolts • Background, flux or picoamps • Offset, millivolts • Gains for the diffraction and PIDS detectors
Graphs, Size	• Particle size by volume %, surface area %, or number % in differential or cumulative (greater than or less than) distribution
Calibration	None

APPENDIX B

Sample Handling

The probability of obtaining a sample, which perfectly represents the parent distribution, is remote. For example, when powder is poured into a heap, size separation or partitioning occurs with the fine particles located at the center of the heap. Also, when a container of powder is subjected to vibration the fine particles percolate through the coarse particles. Therefore, it is not a good practice to just scoop off a small sample and attempt to predict the properties of the bulk from an examination of that sample. In addition, fine powders suspended in a liquid medium often tend to partition, meaning that larger particles will migrate downward while smaller particles remain in suspension. The viscosity of the liquid plays a key role in the rate that particles migrate through a medium. Although sampling is only one step in the overall process of characterizing the size distribution of the bulk, care in performing this step will produce a representative size distribution.

Material Handling

Liquid Sampling

The sampling of powders suspended in a liquid medium requires the use of devices such as rollers, magnetic stirrers and tube rotators as well as manual inversion and aspiration with a pipette, etc. These are generally used to keep particles in suspension while attempting to draw a measurement sample. Even though these techniques are effective in obtaining a representative sample from liquid, the overall sampling for powder samples will be representative only when the initial solid sampling is performed correctly.

Solid Sampling

The sampling of powders involves a reduction process from bulk stage down to a measurement stage, which must be representative of the bulk sample. Clearly before any sampling is performed the bulk material must be well mixed.

Stationary non-flowing materials, such as fine cohesive powders, sticky materials, moist materials, or fibrous solids, do not have a tendency to segregate but may not be uniform. Hence it is necessary to pass these materials through a mixer before storage. Surface sampling with a scoop usually yields a good representation of the bulk. If more than one sample is taken and analyzed separately, the variations in the results are usually minimal.

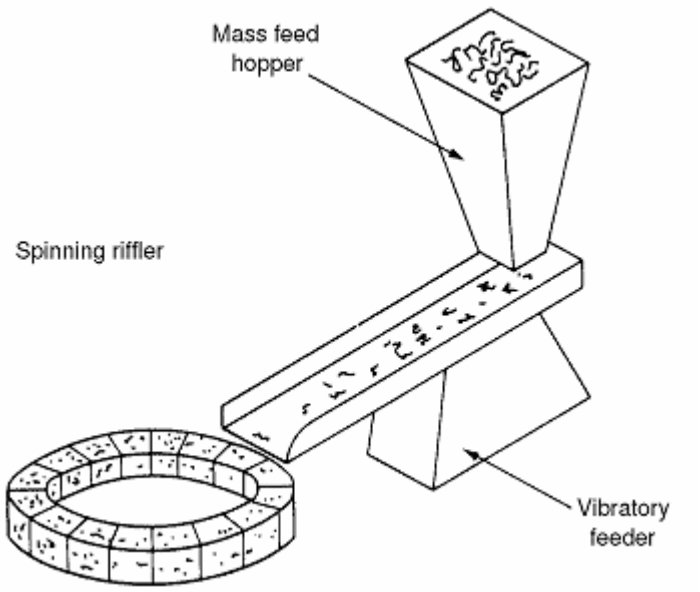
Stored free-flowing materials will segregate by particle size. When poured into a heap, fines tend to percolate to the center while coarse particles roll down to the outside. In vibrating containers, coarse material will migrate to the surface even if larger particles are denser than the smaller particles, for example, a steel ball placed in a beaker of sand. It is not recommended to remove a sample from the surface due to the tendency to segregate.

A representative sample can be obtained by implementing one of the five common sampling procedures. The five procedures are listed in descending order with respect to their efficiency at getting good sample representation.

Spinning Riffler

The spinning riffler consists of a rotary sample tray, hopper, and a vibratory feeder. The sample fills the mass flow hopper without heaping in piles and falls from the hopper to the chute of the vibratory feeder. The feeder supplies a constant flow rate to the collection boxes, which move in a circular motion.This method, according to statistics, yields the most accurate results. It is recommended that samples be subdivided to the amount of a gram and the sample must be free flowing.

Figure B.1 Figure 1. Spinning Riffler



Chute splitter

The sample is placed in a V-shaped hopper and migrates down a series of chutes that alternately feed two trays placed on either side of the trough. The sample is poured into the hopper and repeatedly halved until a desired sample amount is obtained. Satisfactory sample division can be obtained only if the operator loads the sample into the trough carefully without segregation occurring.

Figure B.2 Figure 3. Table Sampling

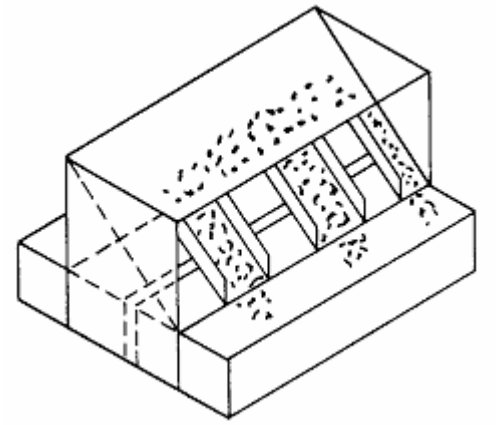
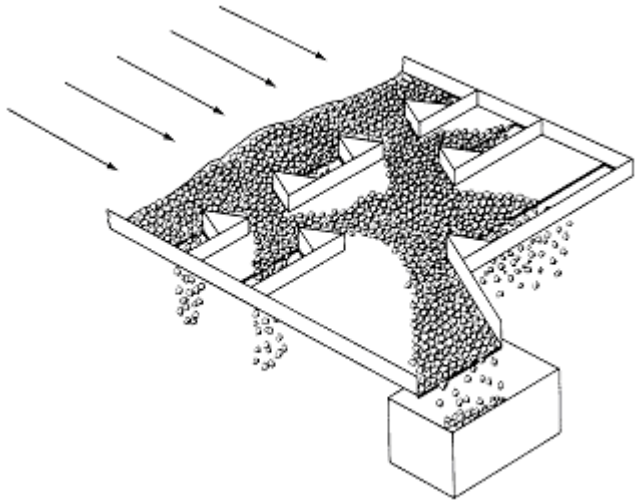


Table sampling

The sample is fed to the top of an inclined plane. The sample stream flows downward and is broken up into fractions as it encounters prisms and holes. The draw back of this device is that it depends on the initial feed being uniformly distributed and complete mixing after each separation. Thus, accuracy is low since errors are compounded at each separation.

Figure B.3 Figure 3. Table Sampling



Scoop sampling

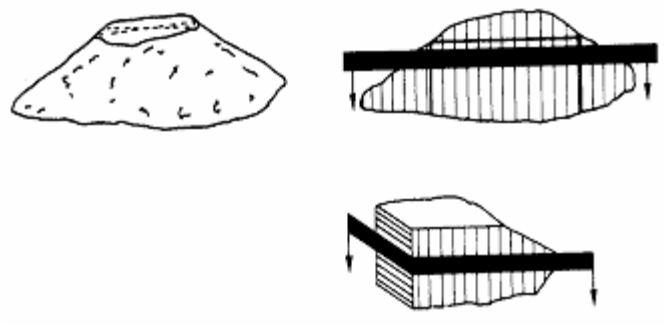
This technique is prone to error since the whole sample does not pass through the sampling device. Sample is taken from the surface, where it may not be typical of the bulk. To eliminate segregation, it is usual to shake the sample in a container using different modes of shaking.

Figure B.4 Figure 4. Scoop Sampling

Cone and quartering

Cone and quartering consists of pouring the material into a conical heap and hoping that all four parts are symmetrical. If symmetry does not exist in the cutting and flattening of the heap, size error will occur. This method is greatly dependent on the skill of the operator and consequently should not be used.

Figure B.5 Figure 5. Cone and Quartering



Liquid Sample Dispersion

Dispersion can make or break the analysis regardless of the particle analyzer being used in the experiment. It allows us to see the correct particle distribution for a given sample. Factors that govern the dispersion method that is to be utilized are:

- Solubility (e.g., fertilizer)
- Reactivity (e.g., cement, lithium hydride with water)
- Suspendibility (e.g., use of glycerol)
- End use (e.g., pigments, flour)

Liquid Sample Dispersion

Liquid sample dispersion is when we attempt to suspend a dry powder in a liquid medium before analysis. This medium can be aqueous or non-aqueous. Characteristics of the dry powder dictate what medium is to be utilized as a diluent. Factors such as solubility, reactivity, suspendibility and end use govern the choice of the media. For example, a non-aqueous medium such as isopropanol is chosen over water as a diluent when determining particle size distribution for a sample of fertilizer, which is soluble in water.

Many pharmaceutical powders, such as niacin, experience this solubility problem and require the use of non-aqueous media. Powders can also be highly reactive. For instance, lithium hydride explodes when in contact with water and cement cures in water. Next, suspendibility of large or heavy powders in a medium may prompt you to use a highly viscous solvent such as glycerol. Finally, pigments are often dispersed in toluene because toluene is the major component of the end product. In the preceding examples, we illustrate why sometimes it is beneficial to select a non-aqueous medium over an aqueous one. Nevertheless, aqueous systems have fewer precautions in the handling and disposal of waste, and water is far less costly than any organic solvent.

⚠ CAUTION

The safe handling and disposal of hazardous organic materials have to be practiced according to government regulations such as Occupational Safety and Health Administration (OSHA) and Environment Protection Agency (EPA) regulations.

The techniques that are used in the dispersion of powders in both aqueous and non-aqueous media are quite similar. Dispersants aid wetting and stabilization of a suspension and can be ionic (anionic or cationic) or non-ionic. However, concentrations of dispersants must be low. Otherwise, their properties are reduced or even inverted (i.e., cause aggregation or flocculation). In dispersing a powder in a nonaqueous medium, it is critical to select an organic solvent that is a good diluent as well as dispersant, although there are many organic dispersant aids. Aqueous surfactants are primarily responsible for reducing surface tension, hence aiding in the dispersion of powders in aqueous systems. The first task in dispersing a dry powder is the wetting of the sample with diluent or dispersant, if needed. Gentle spatulation of the resulting paste prior to addition of diluent further aids the dispersion process. Once the diluent is added, a sample of the slurry can be placed on a slide for a microscopic evaluation of the effectiveness of dispersion. Providing that the sample is well dispersed, the use of a magnetic stir bar is suggested to keep the sample fully dispersed, so a representative sample can be drawn for analysis while stirring is in progress. For example, in some cases it is necessary to use sonication. This is the case with strongly aggregated pigments of earth elements. Ultrasonics must be used with caution since it can cause aggregation, shattering and heating if the duration is too long or the ultrasonic power is too strong.

Physical Methods of Liquid Dispersion

- **Spatulation:** use spatula to break up large clumps but not individual particles.
- **Sonication:** use sound waves to break up aggregates; use either ultrasonic bath or probe.
- **Dilution:** high dilution can aid in dispersion by allowing more space between particles.
- **Degaussing coil or heat:** can aid in the dispersion of magnetic particles.

Chemical Methods of Liquid Dispersion

- **Wetting agents:** used to lower surface tension (e.g., hydrophobicity) between diluent (typically water) and particle (usually nonionic agent).
- **Surfactants:** "Surface active agents" increase the surface charge of the particles in order to cause them to repel one another so that they remain properly dispersed while in the suspension. Types of surfactants include:

  Anionic: imparts negative charge

  Cationic: imparts positive charge

  Amphoteric: imparts both negative and positive charges

  Nonionic: wets particle without imparting charge

Stabilization by surface charge

- Adjustment of pH for surface ionization. Adjusting the pH so that the surface is charged.
  - Amine, hydroxyl, and carboxyl groups all adsorb a hydrogen ion below their isoelectric pH value (obtained via zeta potential analysis) and result in a positive (+) charge on the group.
  - Amine, hydroxyl, and carboxyl groups all lose a hydrogen ion above their isoelectric pH value and result in a negative (-) charge on the group.
  - Typically, 2 pH units above or below the isoelectric pH value will result in stabilization. Stabilization occurs when the zeta potential is at least +30 mV.
- Common Ions
  - A solution containing a dissolved ion which is the same as one found in the sample's molecular lattice may help disperse that sample by adsorbing and charging the surface of the particles. However, be careful for solvation effects.
- Multiple-charged Ions
  - For ionic particles or particles with polar bonds in water, multiply-charged ions that are not part of the crystal lattice, may adsorb to give a surface charged with soluble salts. (Examples: polyphosphates; hexametaphosphate, pyrophosphate, polysilicate ions)
  - For nonpolar organic particles in polar organic media, the surface can be charged by adsorbing a neutral ion-pair. Dissociation then occurs with one part of the ion-pair, desorbing and leaving a charged particle. (Example: trimethyldodecylamine hydroxybenzoate dissociates into a quaternary amine(+) and a polar organic acid(-).)
- Surfactant Ions which charge the surface
  - Organic powders: these can adsorb the organic ion of a surfactant with the inorganic counter-ion dissolved in solution, allowing the particle to be wetted and charged, thus repelling each other.
  - Organic amines adsorb a hydrogen ion to become positively charged when pH is below the pKb.
  - Organic acids lose a hydrogen ion to become negatively charged when pH is above the pKa.
  - Again, zeta potential of + 30 mV is achieved when pH is 2 units above pKa or 2 units below pKb.

Steric Stabilization

- Best in organic solutions. Can be anionic, cationic, or nonionic dispersants or block copolymers. Optimal structure has anchor region that adsorbs strongly on the organic solid (example: alkane chains, aryl groups), and another region that is highly soluble in the liquid (example: polyethylene oxide chains are soluble in water).

Physical and Chemical Methods of Liquid Dispersion

Usually, the best dispersions are achieved when both methods are employed on a sample. A combination of wetting the sample with a dispersant followed by spatulation and sonication has proven to give the best dispersions.

Special cases

- Emulsions: special care must be taken when dispersing emulsions. Dispersants and sonication can cause de-emulsification.
- Liposomes: special care must be taken when dispersing liposomes. Dispersants can disrupt the liposome wall and sonication can rupture the liposome wall.

A Practical Recipe For Liquid Sample Dispersion

- **Dispersant check**: use watch glass/weigh boats to determine which dispersant has the best interaction with particles.
- **Wetting:** add just enough dispersant or diluent to the sample to form a thick paste.
- **Spatulate:** use spatula, rubber policeman, etc. to mull the sample and the dispersant into a paste.
- **Add diluent:** add diluent to form a slurry.
- **Optical check:** use a microscope to visually check the status of the dispersion. If the dispersion appears incomplete, then either try another dispersant or add more energy to the system taking care not to disrupt the integrity of the particles.
- **Add energy to system:** use sonication (usually best), stirring, heat, etc., to de-agglomerate particles.
- **Check for Stability:** Sometimes the sample may re-agglomerate or flocculate over time due to dilution, change in pH, ionic concentration, or improper dispersant. If this happens, we say the dispersion is not stable. Puffy, loosely held "flocs" may be analyzed with good results if there is enough shear from the pump or circulating system to separate the particles. Tightly held "flocs" or "re-agglomerates" may need more energy added to the system; to be dispersed with a different dispersant; or put under different conditions such as change in pH, ionic concentration, etc. Adsorbing polymers onto the surface can help stabilize the dispersion.
- **Sampling:** While adding sample to the sample vessel, always try to obtain a representative sample with each addition. A stir bar or pipette aspiration can be utilized while sampling in order to keep particles suspended.

Solid Sample Dispersion

Solid sample dispersion is an attempt to disperse a dry powder with the intent of suspending it in air. As previously mentioned, dry powders encounter problems with solubility, reactivity and suspendibility, when suspended in liquid media. Due to these problems, analysis of dry systems is quite often preferred. In many instances, selection of solid sample dispersion is dependent on the end use of the product. For example, particle size measurement of flour must be carried out using a dry system, hence requiring solid sample dispersion. Dry powders that are free flowing, anti-caking and anti-clogging require no external dispersion aids. However, if these conditions do not hold true, external dispersion aids (additives that have a much lower or distinguishable size from sample) such as fumed silica (size ~1 nm), tricalcium phosphate, and carbon black are available. Fumed silica, when added to particulate systems (0.5-1 wt%), can help the flow of the dry powder because of its "ball-bearing" effect. The same mixing ratio (0.5-1%) can be used for tricalcium phosphate. Also, its overall effect on particle size measurement can be negligible. Sometimes, anti-static aids such as sprays and a static bar are needed to eliminate static charges.

Diluent Selection

Consider Solvation Effects

- If the sample dissolves, it may be necessary to use a saturated solution to perform the measurement.
- If the sample does not dissolve, then this may be a good diluent.

Consider Other Effects

- Does the diluent react chemically with the sample to alter the sample?
- Does the diluent react physically with the sample to cause agglomeration, flocculation, aggregation, clumping, etc.?
- Does the sample shrink or swell in the proposed diluent?

Consider the Effects the Diluent Has on the Sample's Suspension Fluid

- Does the diluent react chemically with the sample's suspension fluid?
- Does the diluent and the sample's suspension fluid act together to cause agglomeration, flocculation, aggregation, clumping, etc.?

Consider Characteristics of the Diluent

Viscosity:

1. Too high:
   - Entraining of bubbles
   - Inadequate sampling of particles due to slow movement of diluent
   - Pump may work too hard
   - Difficult to disperse sample in diluent properly
2. Too low may cause formation of explosive mixture of air and vapor

Active life:

1. Will the diluent absorb unwanted water?
2. Will the diluent allow growth of bacteria, yeast, etc. (may use preservatives)?

Reactivity:

1. Will the diluent degrade the instrument's polyethylene fill and waste tanks over time?
2. Will the diluent degrade the instrument's stainless steel fill and waste tanks over time?
3. Will the diluent degrade any wetted materials in the instrument?

Purity:

1. Is the diluent chemically pure?
2. Is the diluent free of interfering particles? (If not, filtering may be appropriate.)

Refractive Index:

Is the R.I. of the diluent the same or very close to that of the sample? If so, the instrument may not be able to see the sample.

Color:

1. If the color of the diluent is similar to the color of the laser, the instrument may not be able to see the sample.
2. If the color of the diluent causes the sample to be too opaque, the instrument may not be able to see the sample.

APPENDIX C

Optical Models

Introduction

The Fraunhofer optical model preprogrammed in your system is a general optical model that can be used for any sample (and suspension fluid) on the LS 13 320.

The LS 13 320 also lets you make optical models using the complete Mie theory of the scattering of light by spherical particles. The Mie theory takes into account the complex refractive indices of both the particles and the suspension fluid. For particles larger than 100 microns in diameter, the Fraunhofer model will produce results almost identical to those obtained with an optical model generated for the material and fluid. Between 10 and 100 microns, the difference will be observable but slight, and is generally considered negligible. If a sample contains a significant fraction of material below 10 microns, however, the correct optical model will produce significantly more accurate size distributions than the Fraunhofer model.

Please note that for an optical model to be “correct”, the model must match the sample. Since the Mie theory assumes spherical particles with a homogeneous refractive index, if a material deviates significantly from these conditions (for example needles or rods, or birefringent materials), the resulting size distribution may not be accurate.

Refractive Index

The complex refractive index consists of a real part and an imaginary part. The real part is what is generally thought of as the refractive index; the imaginary part represents the absorption coefficient of the material. The real part of the refractive index of most samples and suspension fluids are listed in reference books such as the Merck Index or the CRC Handbook of Chemistry and Physics. This number must be known to within 0.01 to 0.03 units. The real part of the refractive index for most samples ranges from 1 to 3; the real part for most suspension fluids ranges from 1 to 2.

The imaginary or absorptive part of the refractive index for a sample is hard to find in any reference. Use the guidelines below to enter the imaginary part of the refractive index. This does not affect the results substantially unless the value of this parameter changes by a factor of greater than approximately 3.

Guidelines to use as an estimation of the imaginary part of the refractive index:

- White or transparent powders - 0 to 0.1

- Clear materials, glass, clear polymers - <0.001
- Latex, translucent materials, quartz, polymer resins, crystallization processes - <0.01
- Lightly colored translucent material - 0.01 to 0.1
- Gray or lightly pigmented materials, metal oxides, highly-colored materials - 0.1 to 1
- Black or highly pigmented materials, metallic particles, carbon black (imaginary values for metals are similar in magnitude as the refractive index) - 1 to 10

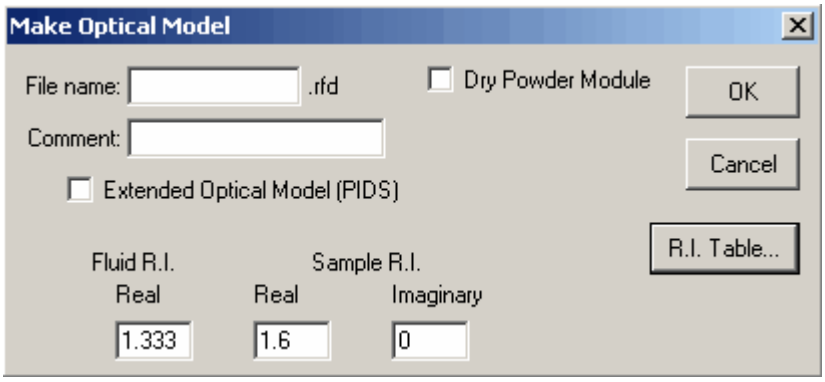
Extended Optical Model

Although refractive indices are generally wavelength dependent, only use this option in the rare cases when the wavelength dependence of the refractive index is large, such as with colored pigments and toners.

Creating an Optical Model

To make an optical model:

1 **Run** and select **Make Optical Model**.



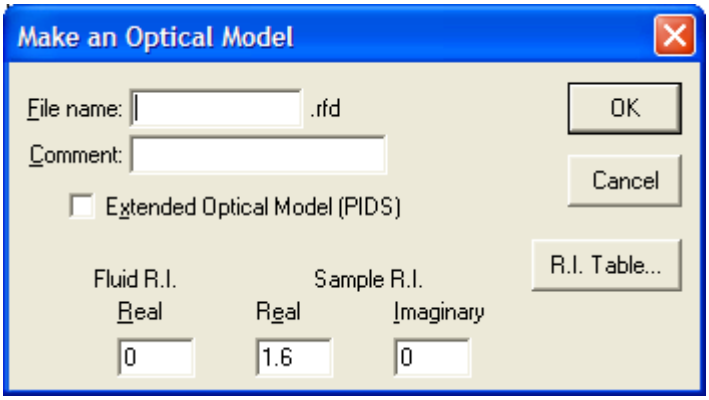
IMPORTANT Clicking the **R.I. Table** button will open the Refractive Index table.

Clicking on the **Dry Powder Module** check box will disable the **Extended Optical Model (PIDS)** and will switch to the .rff file name extension.

2 Enter a name for the optical model in the **File name** field.

3 Enter any description about the Optical Model in the **Comment** field.

4 If using PIDS, check the **Extended Optical Model (PIDS)** box.



5 Enter the real part of the refractive index for the fluid (suspension fluid). See Table 1. If the Tornado DPS is being used, enter **1** in this field.

6 Enter the real and imaginary parts of the refractive index for the sample to be analyzed.

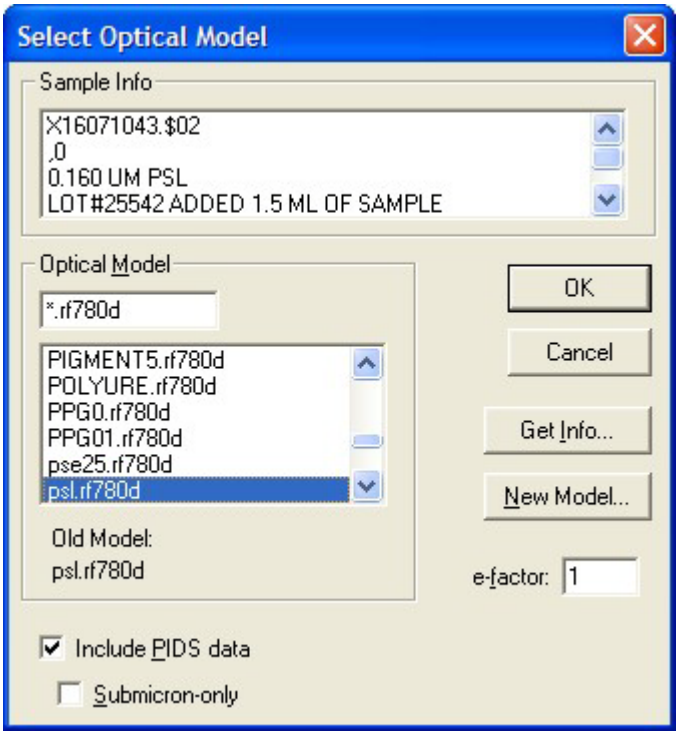
An optical model can also be created from the RunFile menus or during the creation of a Standard Operating Method (SOM).

Creating an Optical Model from the RunFile Menu

From the RunFile menus:

1 **Analyze**.

2 **Compute Sizes.** The Select Optical Model dialog is displayed.



3 the **New Model** button to display the Make Optical Model dialog. Follow steps 2 through 6 above under Creating an Optical Model.

Creating an Optical Model through SOMs

In step 4 of the SOM creation you're given the option to select an optical model. This is similar to creating an optical model from the RunFile menus. Once you select Optical Model the dialog box Make Optical Model is displayed. Follow steps 2 through 6 above under Creating an Optical Model.

Optical models are files that are saved in the “opmodels” folder under the LS 13 320 main folder. These files use extensions that indicate the type of analysis that the model is to be used for. All optical model files used with LS 13 320 benches that are equipped with the 750 nm laser use a file extension that begins with the characters “rf” while benches equipped with the 780 nm laser will use the file extension “rf780”. The last character of the optical model file extension uses the following identification:

z =analyses that do not use PIDS

d = analyses using PIDS

f = analyses using the Tornado Dry Powder System

Statistics

The LS13 320 program calculates a number of statistical quantities to characterize the particle size distribution. The statistics are calculated as they would be for a frequency distribution, with the volume percent (or surface area percent or number percent) in a certain size channel being analogous to the frequency of occurrence of a certain value.

Size channels in the LS 13 320 are spaced logarithmically, and are therefore progressively wider in span toward larger sizes. Statistical calculations are made based on the logarithmic center of each channel.

Statistics can be calculated either arithmetically or geometrically (logarithmically); that is, the statistics are based on either the value of a channel center or the logarithm of that value. Arithmetic statistics are more appropriate for particle size distributions that are similar to a Gaussian, or normal distribution. Geometric statistics are more appropriate for particle size distributions that are closer to log-normal. A log-normal distribution is symmetrical when plotted on a logarithmic size scale.

The statistical formulas used by the LS 13 320 software, and a brief description of each, are presented in the next sections.

User-Defined Mean

There are two general formulas for the user-defined mean diameter D(p,q), one for arithmetic means and one for geometric means. These functions allow you to view one type of weighted distribution while obtaining a mean normally displayed only with another weighting. For example, the surface-area weighted arithmetic mean diameter can be available when you are viewing a volume weighted distribution. You can also define weightings other than the three standard ones. The definitions are:

for p = q (arithmetic mean diameter), and

$$D(p,q) = \left[\frac{\sum n_i x_i^p}{\sum n_i x_i^q} \right]^{\frac{1}{(p-q)}}$$

for p = q (geometric mean diameter),

Where:

xi = the channel center

ni = the percentage of particles in the i'th channel

If D(p,q) is chosen in the Size Statistics Preferences dialog box, and integer values for p and q are entered in the provided fields, D(p,q) will appear with other statistics when **Analyze**, **Statistics** is chosen.

The mean diameters (m.d.) represented by some choices of p and q are shown in Table C.1, along with the LS 13 320 software equivalent.

Table C.1

D(p,q)	Standard Name (LS 13 320 Program Equivalent)
D(0,0)	Geometric mean diameter (Geometric mean size in Number % mode)
D(1,0)	Arithmetic mean diameter (Arithmetic mean size in Number % mode)
D(2,2)	Surface weighted geometric m.d. (Geometric mean size in Surface Area % mode)
D(3,2)	Surface weighted m.d. (Arithmetic mean size in Surface Area % mode)
D(3,3)	Volume-weighted geometric m.d. (Geometric mean size in Volume % mode)
D(4,3)	Volume weighted m.d. (Arithmetic mean size in Volume % mode)

The default mean value in the LS 13 320 output is D(4,3).

Median and Mode

Median

The median is the particle diameter at which half of the distribution (half of the volume percent, surface area percent, or number percent) is larger and half is smaller.

Mode

The mode is the value that occurs most frequently in a set of data. The value of the mode corresponds to the channel center with the maximum volume percent, surface area percent, or number percent, depending on the weighting chosen.

Characterizing the Distribution

The variance, standard deviation, coefficient of variation, confidence limits, skewness and kurtosis characterize the shape of the particle size distribution. A normal distribution is symmetric in shape in a linear horizontal axis and a log-normal distribution is symmetric in shape in a logarithmic horizontal axis. Many statistical measurements use the normal curve or log-normal curve as a reference.

Variance

The variance is defined as:

Arithmetic

$$V_a = \frac{\sum [f_i (x_i - x)^2]}{\sum f_i}$$

Geometric

$$V_g = \exp\left[2\sqrt{\frac{\sum\left[f_i\left(\ln x_i - \ln \bar{x}\right)^2\right]}{\sum f_i}}\right]$$

Where fi = bin height.

Standard Deviation

The standard deviation, SD, is the square root of the variance:

Arithmetic

$$SD_a = \sqrt{\frac{\sum\left[f_i\left(x_i - \bar{x}\right)^2\right]}{\sum f_i}}$$

Geometric

$$SD_g = \exp\left[\sqrt{\frac{\sum\left[f_i\left(\ln x_i - \ln x\right)^2\right]}{\sum f_i}}\right]$$

Coefficient of Variation

The coefficient of variation, CV, is the standard deviation divided by the mean. It relates the breadth of the particle size distribution (in percentage) to the mean about which it is measured. The CV's utility is in measuring relative variation as opposed to absolute variation.

$$CV = \frac{SD * 100\%}{\bar{x}}$$

Skewness

Skewness, g1, is the degree of distortion from symmetry of a distribution. Both the degree and direction of skewness may be determined from the following equations:

Arithmetic

$$g_1 = \frac{\sum\left[f_i\left(x_i - \bar{x}\right)^3\right]}{SD^3 \sum f_i}$$

Geometric

$$g_1 = \frac{\sum\left[f_i\left(\ln x_i - \ln \bar{x}\right)^3\right]}{\left(\ln SD\right)^3 \sum f_i}$$

When a distribution is perfectly symmetrical, the values of mean, median, and mode coincide. Under these circumstances, the coefficient of skewness equals zero (g1 = 0).

For right-skewed distributions, the mainly larger values increase the value of the mean, while the value of the mode is unaffected and the skewness coefficient is positive (g1 > 0).

For left-skewed distributions, the mainly smaller particles reduce the value of the mean, while the value of the mode is unaffected and the skewness coefficient is negative (g1 < 0).

Kurtosis

Kurtosis, g2, is a measure of the peakedness of a distribution. It is calculated by determining moments about the mean from the following equation:

Arithmetic

$$g_2 = \frac{\sum\left[f_i\left(x_i - \bar{x}\right)^4\right]}{SD^4 \sum f_i} - 3$$

Geometric

$$g_2 = \frac{\sum\left[f_i\left(\ln x_i - \ln \bar{x}\right)^4\right]}{\left(\ln SD\right)^4 \sum f_i} - 3$$

The normal, or Gaussian, distribution is the standard against which the peakedness of other curves is measured. A normal distribution is referred to as mesokurtic (g2 = 0).

If the distribution has a higher, sharper peak than the normal curve, then it has a larger degree of kurtosis and is termed leptokurtic (g2 > 0). In a leptokurtic distribution most of the particle sizes are close to the mean size.

If the distribution has a lower, broader peak than a normal curve, then it has a smaller degree of kurtosis and is termed platykurtic (g2 < 0). In this type of distribution, the particle sizes are more widespread.

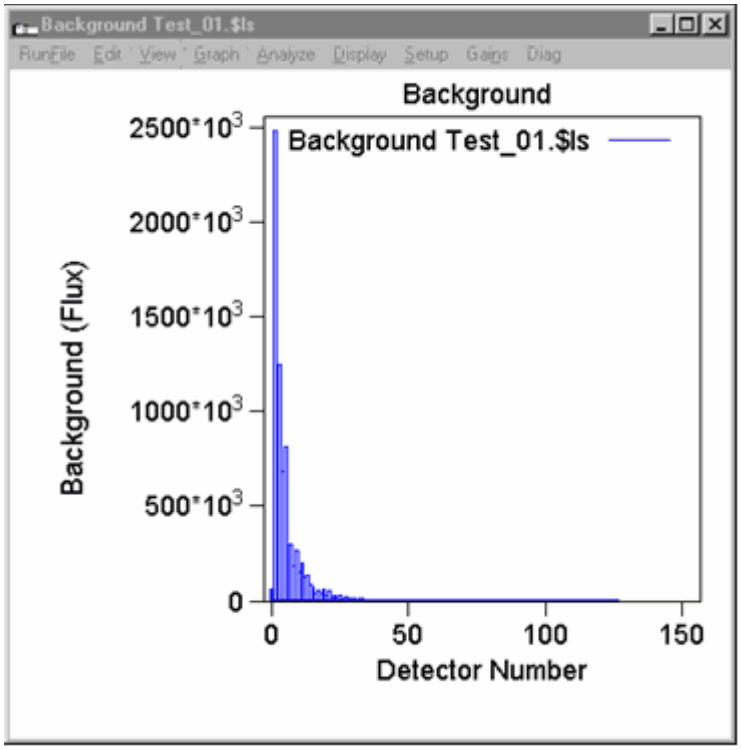
APPENDIX D

Troubleshooting

Background

The background flux pattern can offer information about the cleanliness and integrity of the diluent. It is good practice to become familiar with this flux pattern. Figure D.1 is an example of a good background pattern.

Figure D.1 Normal Background



If the current background flux pattern is higher in any channel by a factor of two, the cause should be determined and remedied before running further samples. Figure D.2 and Figure D.3 are examples of bad backgrounds and their causes.

To check the background flux pattern on a sample run:

1 **View.**

2 **Intensity.**

3 **Graph > Background (Flux).**

Figure D.2 Background Showing the Presence of Bubbles

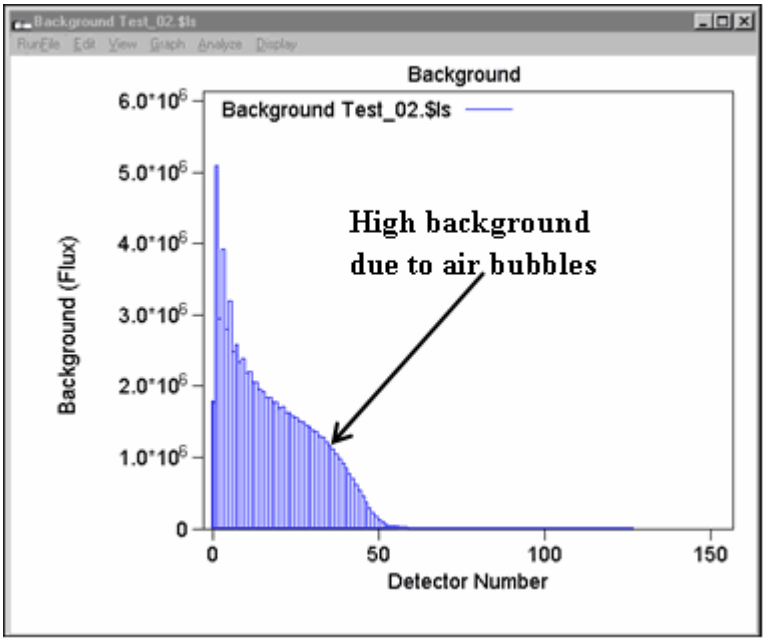
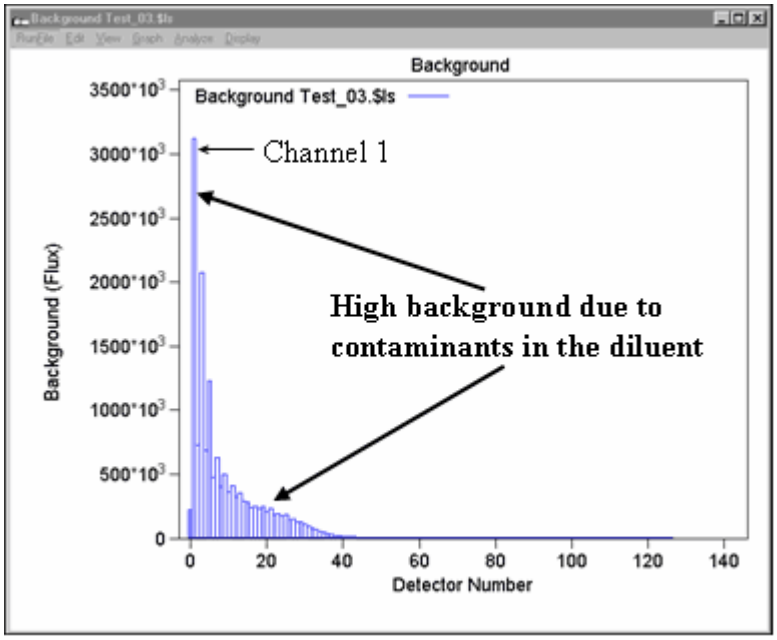
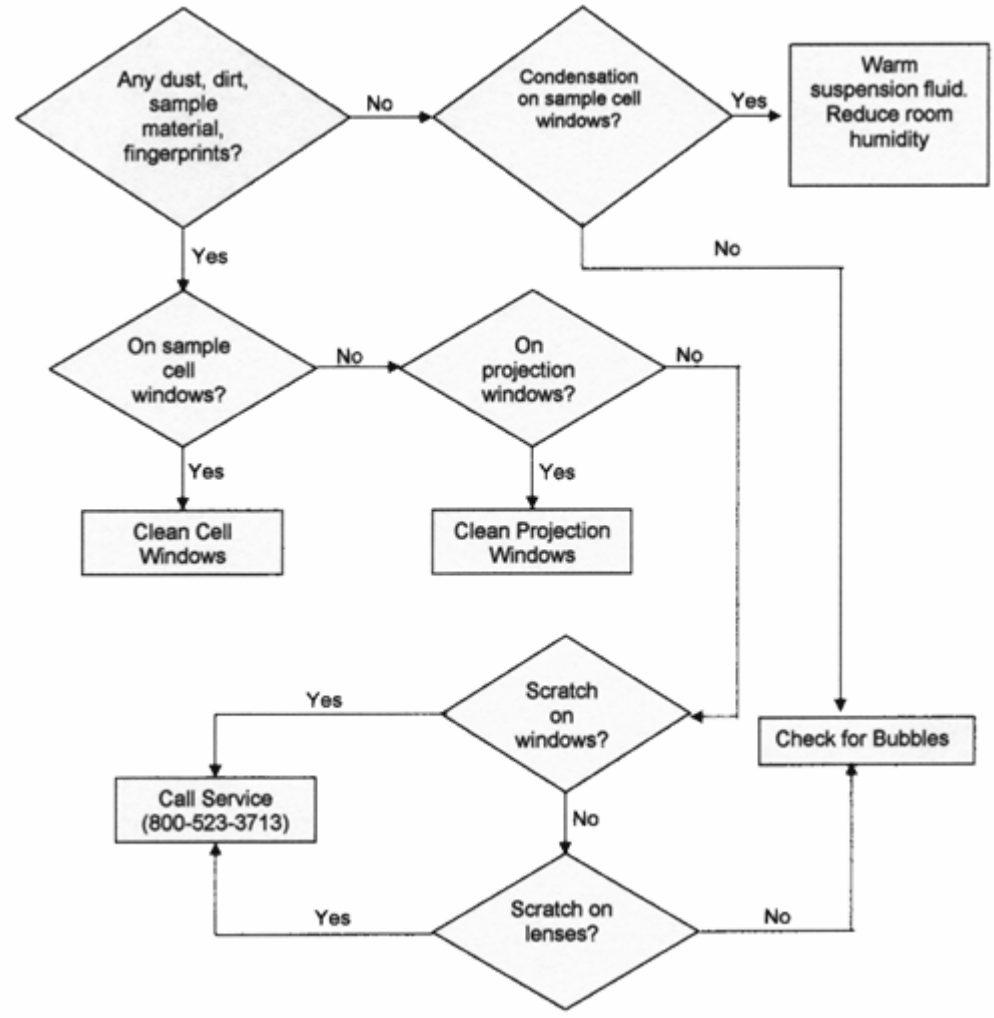


Figure D.3 Background Showing the Presence of Contaminants



Troubleshooting High Backgrounds

If the background intensity is too high, you need to determine the reason for the high background. After making a diagnosis and performing the appropriate corrective action, you will need to check the background again to verify if the problem is solved. Use the following diagram as a guide in checking the background.



Reference Background Set-Up

Backgrounds are an integral part of a particle size analysis to obtain correct and accurate results. This document will guide you through the necessary steps to set up a reference background that can be used to compare the background on a new analysis as it is taking place. This side-by-side comparison will allow you to determine if the background on an analysis being done is within an acceptable range.

NOTE A reference background file is linked to a specific optical bench (serial number) and sample module being used. This means that a reference background obtained from one optical bench cannot be used on a different optical bench. Likewise, a reference background obtained on an MLM cannot be used on an ULM, etc.

Part 1 - Setting Up a Reference Background

An acceptable reference background will be one that is within the recommended flux intensity. These values are:

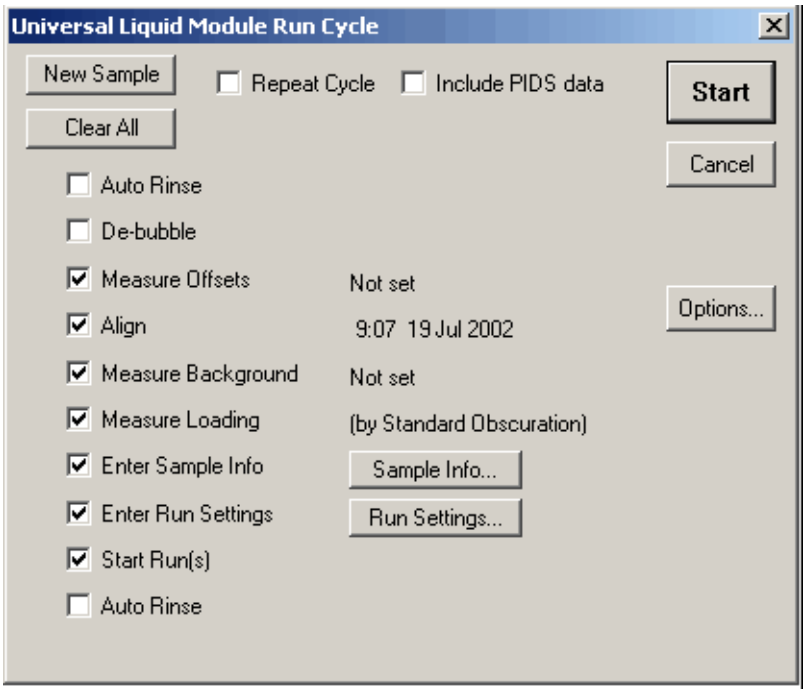
- 2.5x106 for either channel 1 or 2
- 2.0x106 for the sum of channels 10 to 40
- up to 20 for the highest channel between 95 and 126

---

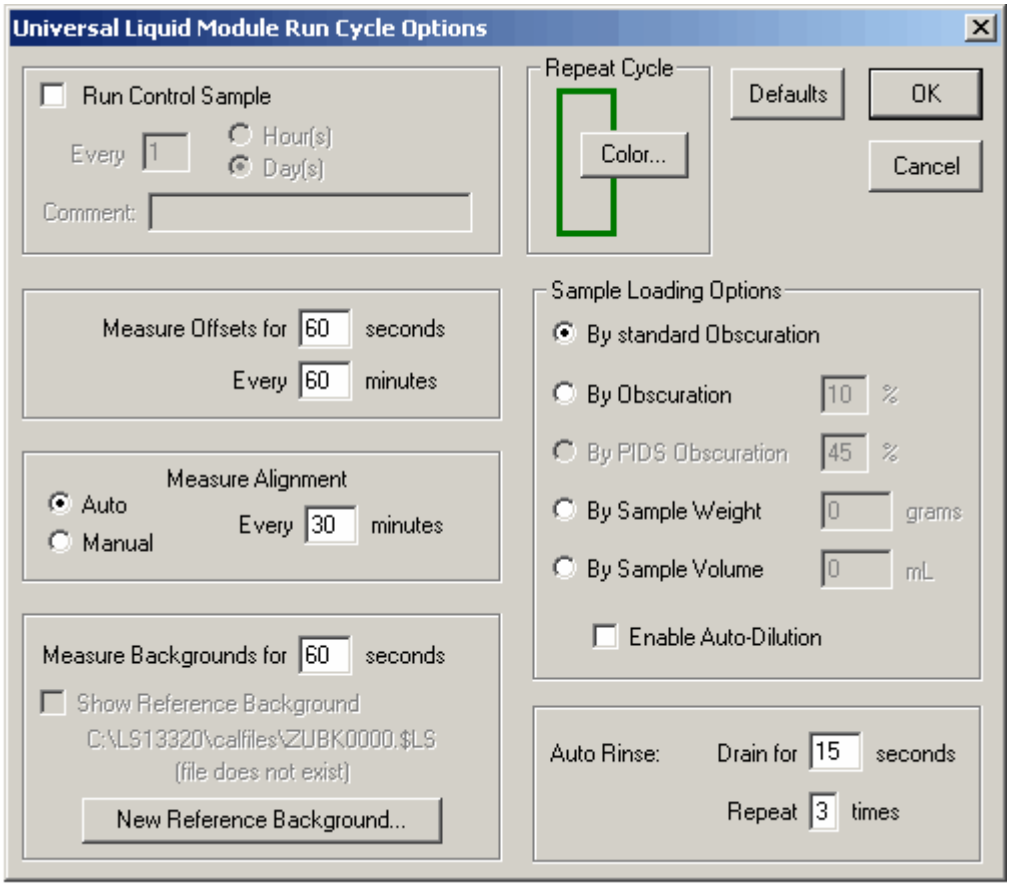
1 Thoroughly clean the optical system and fill with clean diluent.

---

2 Select **Run > Run Cycle**.

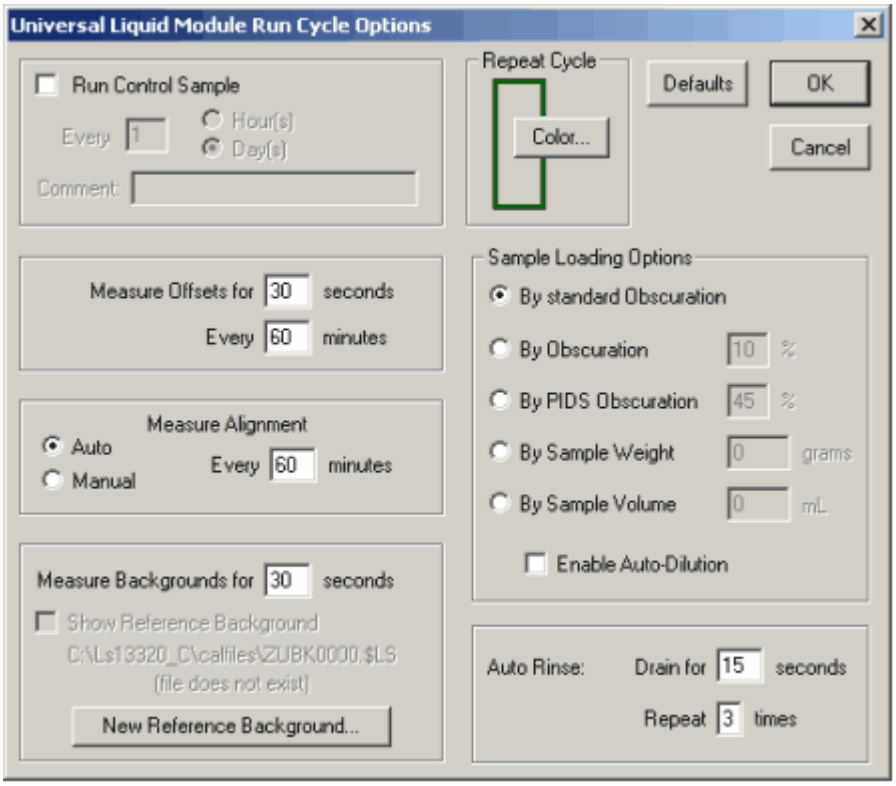


3 Select all options as shown in the figure above.



4 From the Run dialog select **Options** to display the Run Cycle Options dialog screen.

5 In the **Run Cycle Options** page ensure that the box for **Show Reference Background** is not checked if there is a loaded background file. (If no background file is loaded the screen will appear as shown in the figure below.)

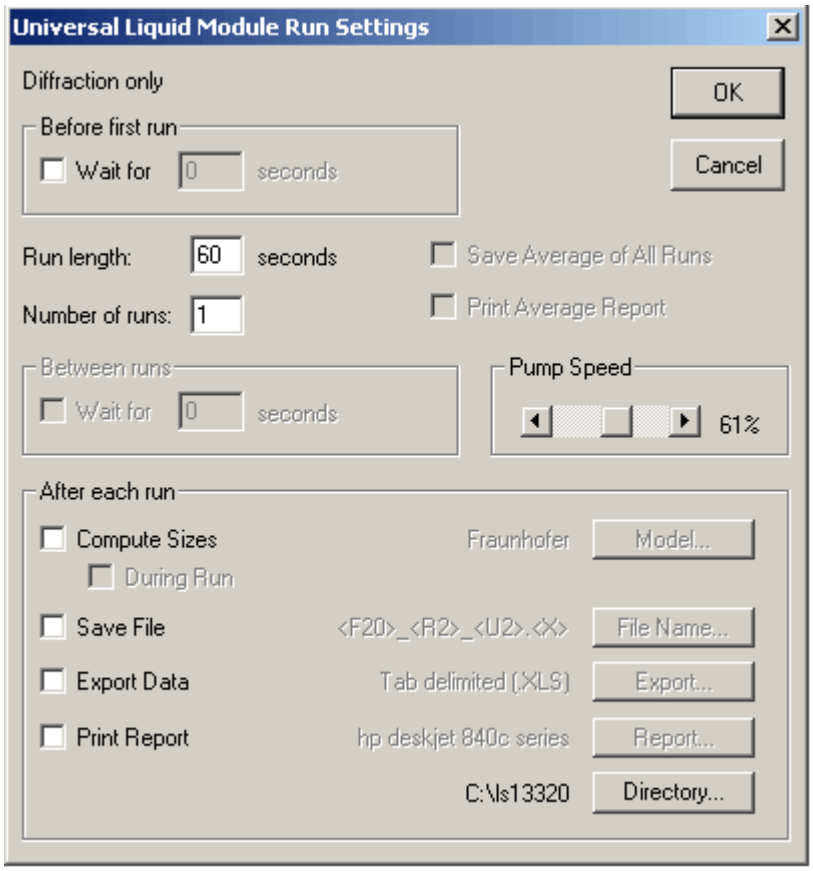


a. Select **60** sec for **Measure Offsets**.

b. Select **60** sec for **Measure Backgrounds**.

c. Then click **OK**.

Under **Run Settings** ensure that "Compute Sizes", "Save File" and "Print Report" are NOT selected, as shown in the figure below.



d. Then click **OK**.

6 When prompted, select a File ID to save the run.

7 After the run is complete select **Run Cycle Options** from the **Run** menu.

8 Select **New Reference Background**. Select the file you saved in step 6. This file will be assigned a new name under the following format:

Filename changes as follows: Z*BKxxxx.$ls. Where * is substituted by the module code (U for the ULM, A for the ALM, P for the Tornado DPS, and M for the MLM). The last 4 digits of the optical bench serial number substitute for xxxx.

Part 2 - Using the Reference Background

1  **Run**.

2  **Run Cycle Options**.

3 In the **Run Cycle Options** page, select: **Show Reference Background**. Select the file created under step 8 above. Click **OK**.

4 When the software gets to the Background graphics page during measurement there should now be 2 curves showing. One is the reference curve and the other is the current measurement labeled “untitled”.

APPENDIX E

Maintenance

Cleaning Procedures

It is important that all glass surfaces in your instrument and sample system are clean, as dusty or coated optical surfaces can cause erroneous results. Some samples or suspension fluids may coat the inside of the windows of the sample cell(s). If the instrument is in a dusty or smoky environment or if vapors are present, the lenses and outer surfaces of the windows may become coated. Check the optical surfaces for cleanliness at least every three months, and clean if needed. Depending on the environment, cleaning may be required more frequently.

Cleaning Fluids

Beckman Coulter recommends the cleaning solution provided with your instrument to clean the inner surfaces of the sample modules. Beckman Coulter also recommends LENS CLENS NO. 1 lens cleaner for cleaning the lenses and sample cell windows.

Cleaning the Lens

High backgrounds or odd backgrounds can indicate dirty lenses. Inspect each lens' surface carefully with a flashlight for dust, particles or smudges. Only clean a lens when needed. These lenses have a special anti-reflective coating on them that is easily scratched.

Fourier Lenses

Use this procedure to clean the upper and lower Fourier lenses, Figure E.1. To clean the Fourier lenses:

1 If a sample module is docked, undock it, if not, open the Optical Module's door.

⚠ CAUTION

Use lens tissues only once, then discard. Never use silicone-coated, eyeglass lens tissues to clean lenses. They can leave a film. Wash your hands thoroughly before cleaning any lens or sample cell window. Do not touch optical surfaces with your fingers or skin. Body oils are difficult to remove from lenses without harming the anti-reflective coating.

2 Fold three to four tissues in half.

3 Squirt lens cleaner onto the creased area of the folded lens tissue.

4 Place the lens tissues across the upper Fourier lens, holding the tissue in place with two fingers. See figure 1.

5 Wipe down slowly, keeping the tissues about 3 mm (1/8 in.) below the evaporation line formed.

6 Discard the used lens tissue.

7 Repeat steps 2 to 6 to clean the lower Fourier lens.

⚠ CAUTION

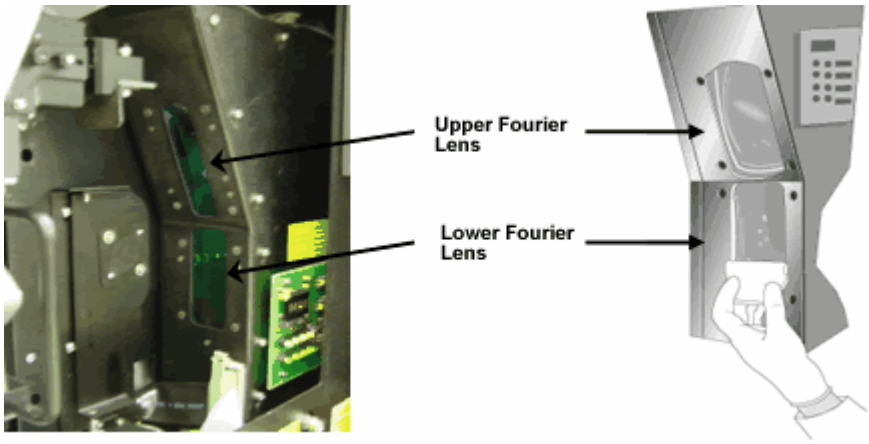
If the lens or soft anti-reflective coating is scratched, the lens must be replaced. Call your local Beckman Coulter Representative.

8 Carefully inspect the lens' surface for cleanliness by shining a bright light on it and looking at the surface from all angles.

9 Dock the sample module back into the instrument.

10 Select the **Measure Offsets**, **Auto-align** and **Measure Background** functions in the Run Cycle dialog box before running your next sample.

Figure E.1 Fourier Lens Location and Cleaning Technique



Projection Lens

Use this procedure to clean the projection lens, Figure E.2.

1 If a sample module is docked, undock it, if not, open the Optical Module's door.

⚠ CAUTION

Use lens tissues only once, then discard. Never use siliconecoated, eyeglass lens tissues to clean lenses. They can leave a film. Wash your hands thoroughly before cleaning any lens or sample cell window. Do not touch optical surfaces with your fingers or skin. Body oils are difficult to remove from lenses without harming the anti-reflective coating.

2 Fold two or three lens tissues in half.

3 Squirt lens cleaner onto the folded lens tissues.

4 Place your finger in back of the lens tissue(s).

5 Starting at the outermost edge of the component, wipe in a spiral pattern to the middle. See Figure E.2.

6 Discard the used lens tissue(s).

⚠ CAUTION

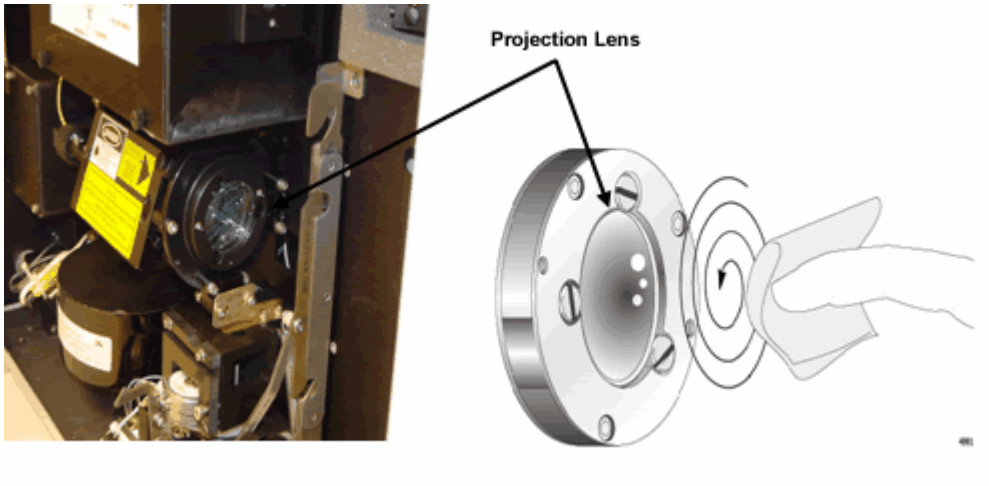
If the lens or soft anti-reflective coating is scratched, the lens must be replaced. Call your local Beckman Coulter Representative.

7 Carefully inspect the lens surface for cleanliness by shining a bright light on it and looking at the surface from all angles.

8 Dock the sample module back into the instrument.

9 Select the **Measure Offsets**, **Auto-align** and **Measure Background** functions in the Run Cycle dialog box before running your next sample.

Figure E.2 Projection Lens Location and Cleaning Technique



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