TABLE OF CONTENTS

SECTION 9   CREATING A STANDARD OPERATING PROCEDURE (SOP)

SECTION 1 INTRODUCTION

1.1   The Coulter Principle

In the late 1940's a technique that allowed particles homogeneously suspended in a conducting liquid to be simultaneously counted and sized was developed and patented by Wallace Coulter. Instruments employing this technique were originally designed to facilitate blood cell analyses but over the years have become invaluable tools in industrial applications as well. The Coulter Principle, also known as the Electrical Sensing Zone (or ESZ) method, is referenced in many national and international standards, and analyses as well as analytical instruments based on the Coulter Principle often serve as references in the evaluation of both other particle size analyzers and sizing techniques.

In the ESZ method a suspension is made to flow through a small cylindrical opening (the aperture) separating two electrodes between which an electric current flows. Although the magnitude of this current may be small, (typically about 1 mA), the resistance, or "pinch", created by the restriction separating the electrodes produces a considerable current density within the aperture. As each particle passes through the aperture (or "sensing zone") it displaces its own volume of conducting liquid, momentarily increasing the impedance of the aperture.
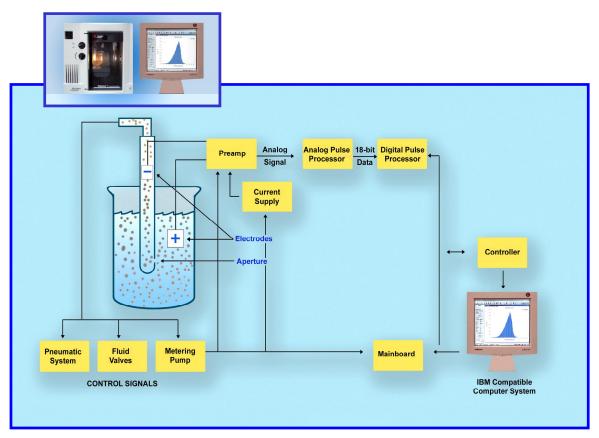
Figure **1.1**-1 Diagrammatic illustration of the Coulter Principle applied in the Multisizer 3

This change in impedance produces a tiny but proportional current flow into an amplifier that converts the current fluctuation into a voltage pulse large enough to measure accurately. The Coulter Principle states that the amplitude of this pulse is directly proportional to the volume of the particle that produced it. Scaling these pulse heights in

volume units enables a size spectrum to be acquired and displayed. In addition, if a metering apparatus is used to draw a known volume of the suspension through the aperture, a count of the number of pulses will yield the concentration of particles per unit volume in the suspension.

In industrial applications historically it has been common practice to report particle 'size' in linear terms, and so the volume of a particle is converted into an equivalent spherical diameter (ESD). In this case, the "particle size" is the diameter of a sphere whose volume is equal to that of the particle. The biological and biomedical sciences traditionally (when reporting the results of cellular analyses) are more accustomed to using actual volumetric metric units, and in these applications "particle size" is usually presented in terms such as femtoliters or cubic microns (fL, or μL^3).

Particle surface areas can be calculated, with the assumption that the surface area is that derived from (and equates to) the surface area of a perfect sphere whose ESD is the same as that of the volume of the particle.

The International Standard ISO 13319 Determination of Particle Size Distributions-Electrical Sensing Zone Methods describes and gives guidance on the measurement of the size distribution of particles using the Coulter Principle.

1.2   About the Multisizer™ 3 COULTER COUNTER®

The BECKMAN COULTER Multisizer 3 is a flexible, multi-channel analyzer employing the Coulter Principle (or Coulter electrical impedance method) together with state-of-the-art Digital Pulse Processing (DPP) technology to provide both particle sizing and counting within an overall size range of 0.4 µm to 1200 µm. Data is acquired and processed using proprietary DSP circuitry and may be archived, displayed and re-analyzed on any PC equipped with a Pentium® II or similar microprocessor running Beckman Coulter's Multisizer™3 software on Microsoft's Windows® operating systems versions 95, 98, 2000 or NT 4.0.

The Multisizer 3 uses a unique, mercury-free system to draw suspensions through the aperture at a steady rate and to meter these liquid volumes precisely enough to yield accurate particle concentration measurements. The instrument is also equipped with a continuous flow alternative for applications where accuracy of liquid metering is unimportant or less critical, as well as options that allow the user to define analysis parameters based on elapsed time or particle count.

By using the very latest in digital pulse measurement techniques, an entire analysis (which may consist of many thousands of particle pulses) can be stored either in memory, or on disk, for recall and reprocessing. This technology allows, for the first time, the resolution of an analysis to be increased up to a hundred-fold by narrowing the size range of interest in real time, eliminating the need to re-run the sample. Using the "Convert Pulses to Size" feature in the software, measurements from the lowest to the highest possible resolution can be made on just one pass of sample through the aperture.

Storing the individual values of pulse heights means that it is not necessary to predetermine the number of size classes (also called "channels" or "size bins") into which any size distribution can be divided. This choice is at the discretion of the operator and can be chosen either before or after the analysis or even changed during analysis. Any number of channels from 4 up to 300 may represent a partial or full range of an aperture. A smaller number may improve the appearance of a size distribution by reducing statistical scatter in the results, while details in the results may be enhanced by

increasing the number of size classes in regions of particular interest. A higher number of channels provides more detail about the size of the particles The advanced digital electronics used in the Multisizer 3 allow the number of channels to be changed in real time without losing any of the information acquired with other channel spacing.

Results can also be stored in a fully processed (abridged) form that conserves disk space. Although in this form the raw pulse data is lost and so cannot be re-analyzed at a different resolution, the processed results still can be enlarged, or a region of interest selected, using the "Zoom" facility in the software.

Both number data and size distributions are corrected by proprietary algorithms for the occurrence of multiple particle passage (coincidence) in the aperture. An indicator provides real time particle concentration in the analysis suspension. The Multisizer 3 software includes a pulse monitor window that offers a guide to the electronic behavior of the aperture. The time base of this window can be adjusted to show either pulse concentration or the shapes of individual pulses. Electronic pulse editing facilities ensure that very narrow size distributions can be highly resolved by minimizing the effect of line width broadening in the sensing aperture.

Data can be displayed graphically as number, volume, mass and surface area size distribution plots, or as tables of values versus size. Both display types can show data as differential or cumulative frequencies. Full data reduction facilities are included for statistical and trend analysis, with export and import capabilities to text and spreadsheet programs via disk files or the Windows Clipboard. All results can be printed/plotted on any printer that has an installed driver compatible with the PC's Windows platform.

A report facility allows results to be combined with sample information in a user selectable way to produce a systematic reporting configuration. Report formats can be individually named for ease of archiving and retrieval. Number and date formats are supported in both conventional as well as in ISO 9276-1 forms.

The Multisizer 3 system, illustrated in figure 1.2-1, comprises an integrated fluid handling and pulse processing unit (the Analyzer Module), and a personal computer with an XVGA monitor. (A keyboard, mouse and an ink-jet printer are also part of the system but are not shown in the figure.)
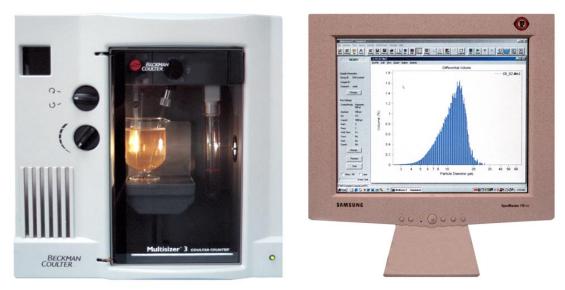

Figure 1.2-1 The Multisizer 3 system

With the pre-amplifier design used by Beckman Coulter in the Multisizer 3, there is no voltage rise. A sensitive oscilloscope attached to the aperture electrodes will detect no pulses at all. Instead, the pre-amplifier offers a momentary parallel path for the current that cannot pass through the aperture. The sum of these two currents, one through the aperture, the other through the amplifier, exactly equals the constant current that flows when no particle is present. The electrical current flowing in the aperture electrode circuit therefore always remains fixed.

A current pulse is converted into a series of voltage pulses of different heights by main amplifiers of different but exactly known gain. By scanning and digitizing all of these pulses at extremely high speed and storing the parameters that are required, it is possible to describe the original pulse. It creates for each pulse a unique set of numerical integers that represent the pulse characteristics to the required precision.

In the Multisizer 3, these numbers are large enough to describe any particle pulse within the aperture measurement range to better than 1% accuracy. All pulse parameters are recorded as a file of data in the order in which they arrive (list mode data). If for any reason, pulse data arrive quicker than they can be stored, the values are held temporarily in a buffer that continues to be emptied to the main file after an analysis has been completed. This updating process ensures that no data is lost except for the statistical losses due to coincident particle arrival within the aperture's sensing zone.

Because pulses are digitized to maximum resolution, the resolution of the Multisizer 3 can be increased or decreased before, during or after an analysis. The number of "channels" or "size classes" into which the data is divided (as well as how these intervals are spaced over the aperture range) represents the extent of the instrument's resolution. The same size distribution can be shown as 100 channels of results, 256 channels, or any other number from as few as 3 to as many as 300 size classes over any aperture's working range as required by the operator. All measurements, however presented, actually contain the maximum resolution and can be saved with this high degree of precision, though this will increase the size of the data file significantly. Using a setting of 256 channels (as opposed to the maximum the software will allow) reduces the statistical scatter (white noise) and improves the appearance of the results. If the number of particles measured is not sufficient to provide a size distribution without significant scatter the Multisizer 3 allows the analysis to be continued without losing previous data, even when the continuation is at a different level of resolution than the initial result.

1.3   About this Manual

This manual is intended to provide the user with all the information needed to operate and maintain the Multisizer 3 system safely and effectively within the stated working range and conditions given in **Section 11**.

1.3.1 Conventions

- The fluid handling and pulse processing unit of the Multisizer 3 system is referred to as the "Analyzer Module". The PC, keyboard, monitor and printer are all referred to collectively as "the computer".

- ⌃ means "click the mouse button". Unless the mouse has been inverted for left-handed users, this will be the left mouse button.

- ⌃⌃ means "double click the (left) mouse button".

- ☒, ☑ and ☉ mean "check the box or radio button", and   and ○ mean "uncheck the box or button" or leave unchecked.

1.3.2   Warnings and Cautions

Indicates a situation or condition that might result in personal injury if not corrected.

Indicates a situation or condition that, if ignored or proceeded with, may result in damage to the instrument or serious error in the results produced by it.

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

1.4   Warnings

1.4.1   Electrical

(1)   High voltages are present inside the instrument. Always disconnect the instrument from the power supply before removing the cover.

(2)   The instrument must be grounded correctly.

1.4.2   Chemical

(1)   Do not use any electrolyte solutions or diluents that are not compatible with the materials of construction. Consult Beckman Coulter Inc. or its local representative before using any concentrated acids or alkalis in the instrument.

(2)   Toxicity, safety, and proper handling procedures for diluents and reagents used in particle and cell analysis should be adhered to at all times. Consult appropriate safety manuals and Material Safety Data Sheets for the items.

(3)   Care should be taken when mixing or exchanging diluents. Reactions can occur between incompatible solvents and electrolyte salts, and may be violent.

(4)   Do not use azide and cyanide salts in acid solutions.

(5)   Flammable electrolyte solutions should be prepared for use in an appropriate environment and brought to the instrument only when required for an analysis.

1.4.3   Mechanical

(1)   Do not interfere with or attempt to disable the interlocks incorporated in the door and beaker platform assembly.

(2)   Do not use force to remove or replace any glass item. In the event of difficulty, consult Beckman Coulter Inc. or its local representative.

(3)   Replace an aperture tube empty of diluent. Do not attempt to pre-fill a tube before locating it in the aperture tube connector.

1.4.4   Fire

(1)   Many non-aqueous electrolyte solutions are flammable and contain organic salts that can support or enhance fire. Where possible choose less flammable alternatives.

(2)   Prepare and filter flammable solvents and diluents away from the Multisizer 3 itself, preferably under a suitable fume hood. Extinguish all open flames in the vicinity and observe normal precautions against ignition by hot surfaces or electrical sparks.

1.5   Cautions

1.5.1   Electrical

(1)   Use the Windows® Shut Down command to turn the computer off before switching off power. Switching off power first will generate a Windows error message at next computer start up.

(2)   Power must be largely free from interference. The Multisizer 3 is CE, UL and CSA compliant with regards to electrical interference but some circumstances may benefit from a constant voltage transformer or regulator.

1.5.2   Chemical

(1)   If Beckman Coulter ISOTON® II diluent is used, the Multisizer 3 must be drained and flushed through with distilled water before carrying out a bleach disinfection procedure.

(2)   If the Multisizer 3 has been subjected to a bleach (sodium hypochlorite solution) disinfection procedure, the instrument must be drained and flushed through with distilled water before refilling with ISOTON® II.

(3)   Take care disconnecting diluent lines -- open-ended tubing may allow liquid to siphon out of containers.

(4)   Never stand containers of liquids on top of the Multisizer 3. Repair of instruments damaged or affected by spilt liquids will not be covered by any warranty or guarantee.

1.6   Warming up

As with all sensitive electronic instruments, the Multisizer 3 components offer best performance once they have reached a steady working temperature. This may typically take 15 minutes from power on.

1.7   Sources of Error

1.7.1   Calibration constant

Size distributions are only correct if the calibration constant (Kd) used to construct the size scale is correct. Instructions on how to calibrate an aperture are contained in **Section 5**. If a different constant is entered manually, the software will use the manually entered value despite the fact that this may result in an incorrect calculation of the size range appropriate for that particular aperture.

1.7.2   Damaged apertures

Aperture damage, such as a cracked wafer, can exhibit a variety of symptoms. Usually, it results in sporadic bursts of high counts at the lower aperture size limit. It is often the cause of deterioration in blank count in a tube previously giving quiet and reliable blank counts.

1.7.3   Deposits on and around aperture wafers

Protein and others biological materials may create a deposit on apertures that could alter their calibration and background count characteristics. Apertures used for these applications should be subjected to appropriate cleaning procedures at frequent intervals. It is advisable to perform a cleaning procedure to avoid any material deposit or microbial growth inside the system (see **Section 6.10**).

1.7.4   External sound and mechanical vibration

Loud sounds and vibrations may register in the aperture as measurable pulses.

1.7.5   Electrical interference

Electrical interference both from the power line and radiated may, if outside the scope of EMC compliance requirements, produce erroneous measurements.

1.7.6   Particles behind the aperture

In the case of either very large or unusually dense materials, particles may settle inside the aperture tube when the instrument is standing idle. Sediments of such particles have been known to swirl up behind the aperture when the instrument is then used and cause interference with subsequent counts and size distributions.

1.7.7   Sample dispersion

Measurements of powder dispersions are only as good as the dispersed state of the sample and the sampling procedure allows. Incorrectly dispersed or unstable dispersions will give rise to incorrect results.

1.7.8   Excessive aperture current

Excessive current causes the electrolyte solution in the aperture to heat, or even to boil, causing noise (see **Section 4.4.9**).

SECTION 2 INSTALLATION

2.1   Location

Ideally the instrument should be located in a dust free room. Dusty environments should be avoided to minimize sample contamination. The instrument should be placed on a bench that is not subject to:

(1) Excessive airborne dust

(2) Strong vibrations

(3) Spillage or splashing

(4) Sounds of high intensity (e.g., ball mills, ultrasonic baths or probes); electrical noise (e.g., electrical switching gear, water baths or gas chromatographs)

2.2   Unpacking

The Multisizer 3 will normally be installed only by Beckman Coulter approved and trained installation engineers. Unless otherwise agreed to by Beckman Coulter, **DO NOT UNPACK** the Multisizer 3.

🛑 *The Multisizer 3 is a heavy instrument. Follow the instructions below. Failure to do so may result in personal injury and/or damage to the instrument. Such damage may not be covered by warranty*.
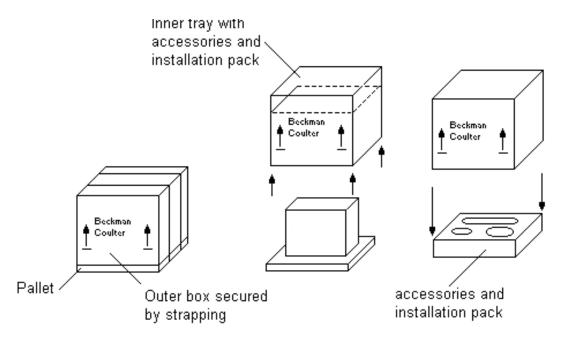
Figure 2.2-1   Unpacking the Multisizer 3 Analyzer Module

(1) Lift the Analyzer Module (requires two people) off the pallet and onto the bench taking care not to exert any force on or lift by the plastic front compartment molding (see Figure 2.2-1).

(2) Turn the outer box over to gain access to the Accessories and Installation Pack fastened to the inside top of the outer box. Slide out the Pack and inspect all installation items for damage. Compare the contents with the Packing List.

(3) Report any missing items or damage to any part of the instrument to Beckman Coulter or their local distributor, and to the carrier responsible for delivery. Do not send back any items before receiving instructions to do so from the manufacturer.
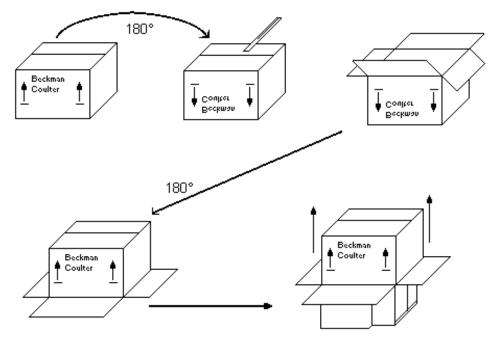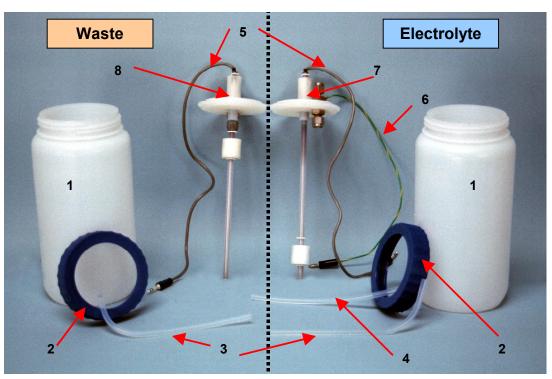
Figure 2.2-2   Unpacking the computer monitor

(4) Open the carton containing the computer monitor and remove the monitor as shown in figure 2.2-2 by inverting the carton and slitting the tape along the box bottom flaps. Turn the carton back upright and slide it upward from the monitor.

(5) Unpack the computer and printer by placing upright on the bench and slitting the tape around all box flaps. Lift the items out from the boxes. Compare the computer and printer manufacturers' packing lists and report any deficiencies or damage as in (3) above.

2.3   Connections

1.   Remove the tubing and containers kit from its package.

2.   Prepare the lids for each container. The waste lid uses one curve tube to connect the container to the waste outlet of the instrument and has only one connection cable. The electrolyte lid uses two tubes and it has a connector cable and ground cable. The straight tube, the intake tube, goes from the inner side of the lid to the bottom of the electrolyte container. The curve tube connects the container to the electrolyte intake on the side of the instrument (See Figure **2.3.1** and **2.3.2**).

3.   Leave the waste container empty and screw the lid with the sensor and tube. Fill the electrolyte container with the required electrolyte and screw the lid down.

4.   Attach the appropriate tube from each container to the fittings marked "Diluentl" (Electrolyte) and "Waste" at the top of the left side panel (See Figure **2.3.2**).

1. Containers
2. Lids
3. Connecting Tubes
4. Electrolyte Intake Tubing
5. Connecting Cables
6. Ground Cable
7. Electrolyte Sensor
8. Waste Sensor
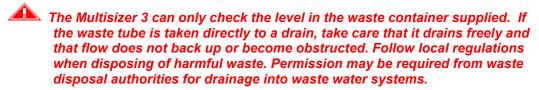
Figure 2.3.1 Assembly of the Electrolyte/Waste System

Figure 2.3-2   Left side panel showing waste and diluent connections

5. Plug the connector cables from the Waste and Diluent sensors into the dual sockets immediately behind the pipe fittings on the left-hand side of the instrument. Either cable may go into either socket. Plug the ground cable from the electrolyte sensor into the ground socket (See Figure 2.3.2).

⚠️ *The Multisizer 3 can only check the level in the waste container supplied. If the waste tube is taken directly to a drain, take care that it drains freely and that flow does not back up or become obstructed. Follow local regulations when disposing of harmful waste. Permission may be required from waste disposal authorities for drainage into waste water systems.*

6. Use the EtherNet cable supplied with the instrument to connect the Multisizer 3 to the PC's network card. (See Figure 2.3.3). The parallel and serial connectors are intended to be used by Beckman Coulter authorized service personnel.

7. Check that the voltage selection indicator located on the rear side of the instrument is set for the voltage (line voltage) to which the instrument is to be connected. (see **Figure 2.3-4**).
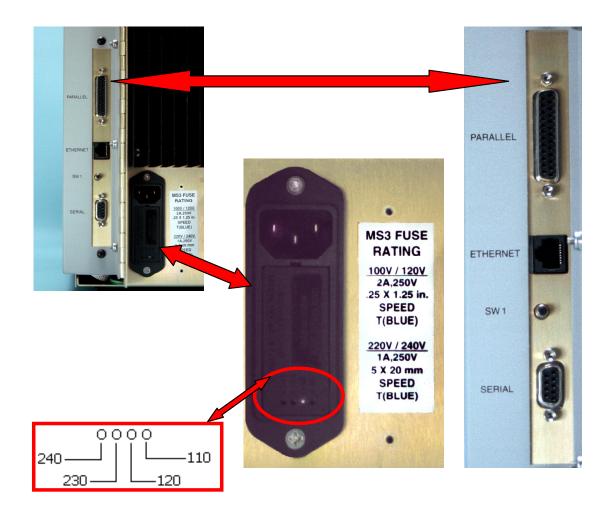
⚠️ *All items comprising the Multisizer 3 system must be set for the correct voltage. Connecting the instrument to an incorrect voltage may cause damage to the instrument and invalidate any warranty. Different voltages require appropriate fuses.*

8. If the selector does not indicate the correct voltage setting, change it as follows: Remove the fuse holder and cover plate by inserting a small bladed screwdriver (~3 mm blade width) into the recess at the top (figure 2.3-4). Lever upwards to "pop out" the holder. Using needle nosed pliers, pull out the voltage selector board. Do not pull on the plastic voltage indicator pin. Voltage values are marked around the four corners of the plate (**Figure 2.3-4**). Holding the board as shown in Figure 2.3-4B, pull out the plastic pin as far as it will go (Figure 2.3-4C) and rotate it so that it points outwards towards the cover plate. Figure 2.3-4D shows the orientation of the pin for 120 V.

   (a)   Push the pin back in towards the board (the pin will locate in a recess unique to the chosen voltage). Turn the board until the voltage required faces inwards (towards the instrument). Replace the selector board and push firmly into place.

   (b)   If fuses need replacing use only 250 V, 3.15 A, "T" (time delay), IEC standard 20 mm fuses.

(c)   Fit the cover plate so that the appropriate small hole at its base fits over the white plastic indicator pin and push firmly at the top until it clicks into its seating. The white pin should now indicate the correct voltage for the supply. The positions left to right are: (**See Figure 2.3-3**).

9.   Make sure all the power switches of the Multisizer 3, the computer, and the printer are set to O (=OFF) then connect these items to power with the cables supplied.

10. First switch the Multisizer 3 analyzer unit power switch to | (=ON), followed by the computer and printer. To find out how to use the instrument's control program turn to **Section 3.3.1**

Figure 2.3-3   Detail from the bottom left side of the rear panel showing the connections to the PC and the power socket with the fuse holder
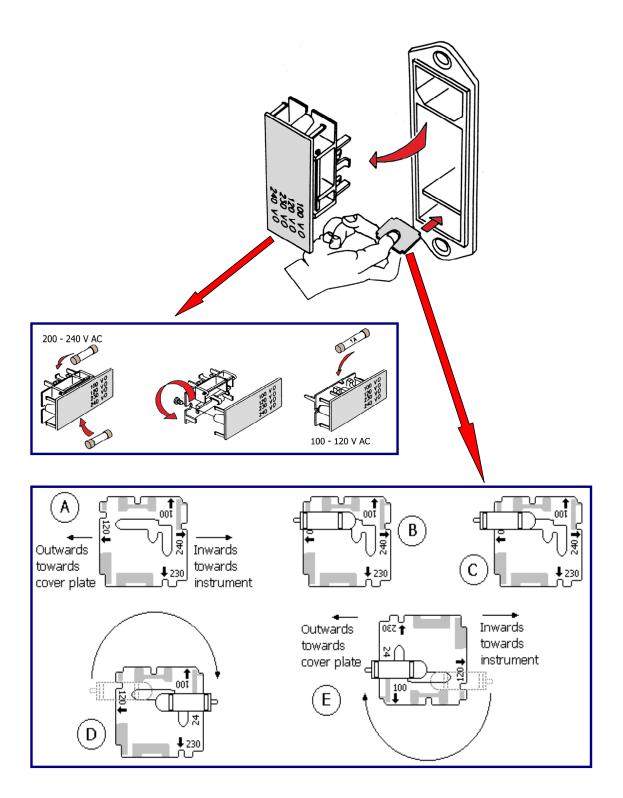
Figure 2.3-4 The voltage selector board: B shows the orientation for 240 V; C & D show the conversion from 240 V to 120 V, and E the final orientation for 120 V before re-insertion.   **Back to TOC**

SECTION 3 BASIC OPERATIONS

The software controls all the operations on the Multisizer 3. Only the stirrer speed and the sample platform need to be adjusted manually on the analyzer unit.

3.1 Starting the Instrument and its Control Program

1. If the analytical system has been started before the program has been launched, the first view presented by the program is the copyright acknowledgement box (Figure 3.1-1). If the checkbox is unchecked, the program will behave purely as a data processing program and the Multisizer 3 control menu options will be either "greyed out" or not displayed.

Figure 3.1-1 Copyright Acknowledgement Box

2. Check the box **Connect to Multisizer 3**, ⊕➔**OK** to launch the control program.

   ✓☺ *If the analyzer unit has not already been switched on, do not check the "Connect to Multisizer 3" box. Connection can be made later under "Run" in the Main Menu Bar.*

3. Once connection has been made, a series of status messages illustrate the initializing process of the Analyzer Module. These normally start with "Waiting" and/or "Initializing valves" but the precise sequence will depend on the actual start condition of the Analytical Module. After initializing the complete system, the status message will change to "Ready" (**see Figure 3.1-2**).

4. The Security Set Up dialog box is shown the first time that the program is started. At this moment the security level can be selected. These security options can also be selected at a later time (see **Section 10.7.1**). It is advisable to read **Section 10** before selecting the security level.

5. To set up the security options at a later time, ⊕➔**Cancel** in the Security Set Up dialog box (see **Figure 3.1-3**). Refer to Section 10 to set up security at a later time.

6. To select the security level at this time choose the required level option (see **Figure 3.1-3**). ⊕➔**OK** and go to **Section 10**.

7. Disconnection from the Multisizer 3 can be made at any time by ☝➔**Run** then ☝➔**Disconnect from Multisizer 3**. (When disconnection has been made this way the fact is "remembered" when the program is closed down. The checkbox in figure above will be then be unchecked next time the program is launched.)

Figure 3.1-2   Screen view when is "Ready".

Figure 3.1-3   Security Set Up dialog box

3.2   Instrument Controls

1. Control of the Multisizer 3 is essentially accomplished via the drop-down menus enabled when a connection to the Analyzer Module has been established. Without a connection, the Main Menu bar defaults to the standard menu set for data review,

editing, and printing. In this case the control menus are no longer visible. (The Multisizer 3 software, because of the digitization of pulse data, has the ability to re-process previously acquired results, as long as the data was stored with the option to include the pulse data selected.)

Figure 3.2-1 The Status Panel

2. Provided connection to the Analyzer Module has already been established, stopping and starting an analysis is controlled by "Buttons" on the Status Panel shown on the left side of the Control Page. These buttons change their function and annotation according to the status of the analysis.

3. The instrument can also be directly controlled via the drop-down menus, for example:

⟡➔**Run** ⟡➔**Start** has exactly the same function as the Start button on the control panel. Likewise, other analysis controls are available via **Run Menu.**

Figure F3.2-2 The Run Menu

3.3   Shutting down the Multisizer 3

At any time, the software program can be closed down by any of the standard Windows applications closing actions such as:

⟡➔**File** on the Main Menu Bar then ⟡➔**Close**,

or ⟡➔⊠ on the Window top right-hand corner.

(1)   When the program has been closed, the Analyzer Module power switch can be switched off.
(2)   Closing down the program leaves the Multisizer 3 connection in its last state. If connection has been enabled, the copyright acknowledgement box will show "**Connect to Multisizer 3**" checked next time the program is started. If the connection has already been disabled through "**Run**", the copyright acknowledgement box will show the "**Connect to Multisizer 3**" box unchecked.
(3)   If the Analyzer Module is switched off before the program has been closed, connection to the PC is automatically broken, and may be recorded as a disconnection. It will then be necessary to re-enable the connection by ⟡➔**Run**⟡➔ **Connect to Multisizer 3.**
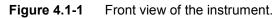
SECTION 4   GETTING STARTED

4.1   Locating and Identifying Controls and Parts You Need to Know

4.1.1   The Frontal Panel of the Multisizer 3

Figure 4.1-1   Front view of the instrument.

On the front panel of the instrument you will find:
- **Aperture Viewer:** This screen allows visual monitoring of the aperture.
- **Stirrer Rotation Control:** This knob changes the direction the stirrer rotates.
- **Stirrer Speed Control:** This knob is used to set the rotation speed of the stirrer and to turn it on and off. The stirrer can also be turned on or off from the Multisizer 3 software.

4.1.2   The Sample Compartment

The sample compartment is enclosed in a Faraday Cage to avoid electric interference. Opening the glass door of the sample compartment you can find the Sample Platform and the places where the electrode and stirrer have to be attached (See Figures **4.1.2** and **4.1-3**).
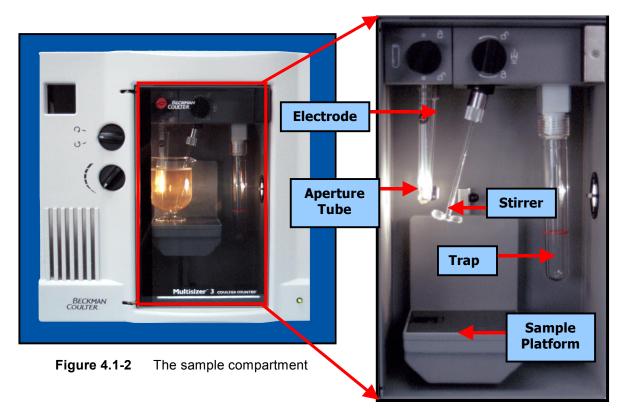
Inside the Sample Compartment you will find:

- **Electrode**
- **Stirrer**
- **Aperture Tube**
- **Sample Platform**

- **Trap:** Its function is to prevent large and dense particles to go into the system. When the sample sediment reaches the red mark it is necessary to clean the trap.

- **Aperture Tube Knob:** This knob is used to hold or release the Aperture Tube.

- **Stirrer Knob:** This knob is used to fix the position of the stirrer.

Figure 4.1-2   The sample compartment
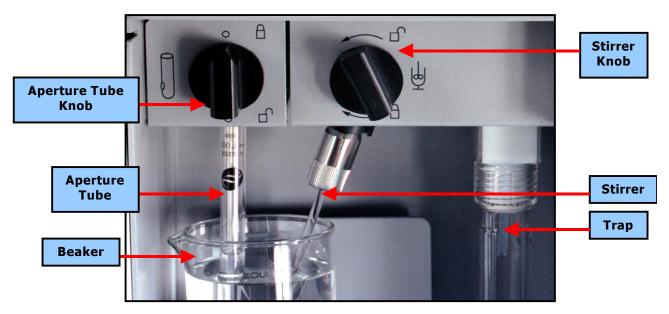
Figure 4.1-3   Inside the sample compartment

4.2   Fitting the Electrode and Stirrer

1.   Carefully remove the electrode and stirrer from their packages.

2.   Hold the electrode only by its metal connector and insert it into the electrode socket located on the upper side of the sample compartment. Rotate it until the platinum spade is positioned roughly parallel with the lamp lens axis behind the aperture tube then push up to complete the connection. (See Figure 4.2-1).
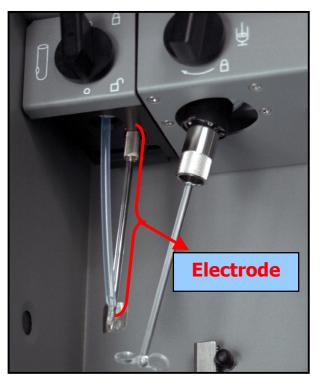
Figure 4.2-1   Electrode.

🛑   *The electrode stem is made of glass and is therefore fragile. If the connector does not enter its socket easily, do no use undue force. Do not twist, push or pull the glass stem in any way. Hold the electrode only by the connector.*

⚠️ *The wire in a broken electrode which may appear to have electrical continuity but the break may still cause erroneous results.*

3.   Hold the stirrer, paddle downwards, about 2 cm from the tapered top, and push firmly into the collet of the stirrer motor as far as it will go. It helps to support the collet with the finger and thumb of the other hand. The black elastomer band around the collet will provide enough securing tension to hold the stirrer in place (see **Figure 4.2-2**).

🛑   *If the stem does not enter the collet easily, do not use undue force. Do not twist or flex the glass stem in any way. Hold only as described.*

4.   Adjust the vertical position control of the stirrer motor until the stirrer paddles sweep at or just below the bottom of the aperture tube. Test the clearance

available to the paddles by rotating the collet between finger and thumb. The stirrer must not encounter the electrode or the aperture tube when it rotates.

Figure 4.2-2 Stirrer.

🛑 *Do not hold the stirrer by its paddles or push or pull on them to provide additional leverage.*

✔☺ *Glass stirrers are fragile and knocking them against beaker walls or subjecting them to ultrasound from probes and baths easily damages the paddles. Multisizer 3 glass stirrers cannot be used with very small beakers (e.g., less than 150 mL). A small helical agitator can often be used with beakers down to 50 mL squat form. Neither can be used with ACCUVETTE® II vials.*

4.3   Fitting the Aperture Tube

The first time you use the instrument familiarize yourself with the operations for fitting and changing an aperture tube. You should practice several times until you get used to performing this routine.

1.   Wash all items in filtered liquid.

✔☺ *For all aqueous diluents, well-filtered distilled quality (DQ) water will be found suitable for washing and rinsing items. For most non-aqueous electrolyte solutions, filtered propan-2-ol (iso-propanol, I.P.A.) is a useful rinsing liquid. When using electrolyte solutions that are incompatible with solvents containing -OH groups, filtered butan-2-one (also known as methyl ethyl ketone} or a low boiling point petroleum can be used instead of I.P.A.*

2. **Figure 4.1-1** shows a frontal view of the instrument. Open the sample compartment door. The trigger release for the beaker platform is located under the front lip of the platform itself. This trigger is designed to be squeezed while supporting the platform with fingers and thumb. Move the platform down until it is at its lowest position.

3. Rotate the aperture tube clamp counter-clockwise to the "open seal" position. Insert the aperture tube in the connection head as far as it will go with the logo facing the front towards you. **DO NOT GREASE** the aperture tube. The Multisizer 3 aperture tube connection is sealed by O-rings and forms a greaseless seal.

4. Tighten the **Aperture Tube Knob** clockwise to the closed (tighten seal) position (see **Figure 4.3-1**).

✓☺ *There is no need to over tighten the knob to obtain a leak proof tight seal. A correct seal is obtained when the aperture tube cannot easily be rotated or pulled down when gripped by fingertips alone. Using just enough lock on the aperture tube will prevent excessive wear and tear on the O-ring seal and extend its life.*
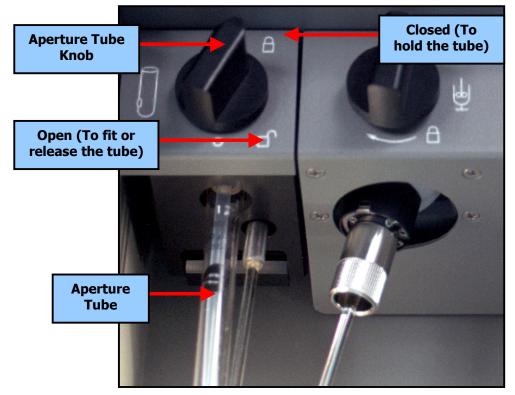
Figure 4.3-1   Aperture Tube

4.4   Setting Up the Aperture Tube

Before setting up the new aperture tube, the instrument must be in the following condition:

(1)   The electrolyte container is sufficiently filled (enough to raise the level sensor).

(2)   The Waste container is empty (enough to lower the level sensor).

(3)   The Analyzer Module and the computer have been powered on with the control program running.
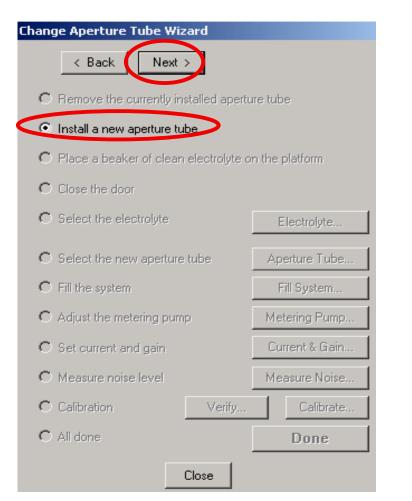
On the Main Menu Bar, ☝➜**Run,** then, ☝➜**Change Aperture Wizard.**

The wizard will guide you through the process.

1. Removing the currently installed aperture tube.

Rotate the aperture tube clamp counter-clockwise to the "open seal" position and remove the aperture tube (see **Figure 4.2-2**) ☝➜ **Next**

2. Installing a New Aperture Tube.

From the table below, select an aperture tube appropriate to the sample to be analyzed.

Aperture Size (μm)	Analysis Range (μm)
20	0.4 - 12.0
30	0.6 - 18.0
50	1.0 - 30.0
70	1.4 - 42.0
100	2.0 - 60.0
140	2.8 – 84.0
200	4.0 – 120.0
280	5.6 – 168.0
400	8.0 – 240.0
560	11.2 – 336.0
1000	20.0 – 600.0♦
2000	40.0 – 1200.0♦

♦ Range depends on sample density

Install the selected tube following the procedure described in **Section 4.2.** ⊕➔ **Next.**

3. Placing a beaker of clean electrolyte on the platform.

After placing the beaker with clean electrolyte on the platform, fill the electrolyte container with the same kind of electrolyte being used in the beaker. ⌂➔ **Next.**

Change Aperture Tube Wizard

< Back   Next >

○ Remove the currently installed aperture tube

○ Install a new aperture tube

⦿ Place a beaker of clean electrolyte on the platform

○ Close the door

○ Select the electrolyte   [Electrolyte...]
   ISOTON II

○ Select the new aperture tube   [Aperture Tube...]

○ Fill the system   [Fill System...]

○ Adjust the metering pump   [Metering Pump...]

○ Set current and gain   [Current & Gain...]

○ Measure noise level   [Measure Noise...]

○ Calibration   [Verify...]   [Calibrate...]

○ All done   [Done]

[Close]

Change Aperture Tube Wizard

< Back   Next >

○ Remove the currently installed aperture tube

○ Install a new aperture tube

○ Place a beaker of clean electrolyte on the platform

⦿ Close the door

○ Select the electrolyte   [Electrolyte...]
   ISOTON II

○ Select the new aperture tube   [Aperture Tube...]

○ Fill the system   [Fill System...]

○ Adjust the metering pump   [Metering Pump...]

○ Set current and gain   [Current & Gain...]

○ Measure noise level   [Measure Noise...]

○ Calibration   [Verify...]   [Calibrate...]

○ All done   [Done]

[Close]

4. Closing the Sample Compartment door.

Raise the platform with the beaker to the appropriate height and close the door. ⌂➔ **Next.**

5. Selecting the electrolyte.

✆➜ **Electrolyte**. From the drop down list select the electrolyte to be used. If the electrolyte is not on the list, type the name in the box. You can also select the dispersant to be used, or type its name if it is not on the list. After selecting the electrolyte and dispersant,✆➜ **OK**. Be Sure to have the beaker and electrolyte container filled with the same electrolyte selected

6. Selecting the aperture tube.

✆➜ **Aperture Tube** From the drop down list Select the aperture tube installed in **4.4 step 2**. The first time a new aperture is installed, the default aperture is selected from the list.✆➜ **OK.**

7. Filling the System.
   ⍟➔ **Fill System**

8. Adjusting the Metering Pump.
   ⍟➔ **Metering Pump**

9.   Setting Current and Gain.

✋➔ **Current & Gain**, ✋➔ **Auto Set**. When the instrument is done setting the current and gain, ✋➔**OK**. If you are analyzing cells, ✋➔**Cells**, and ✋➔**OK**.

⚠️ *If cells are to be analyzed, it is very important to select cells in this step. Not selecting cells could cause their damage due to a higher current setting.*

10. Measuring Noise Level.

🖱️➔ **Measure Noise.**

11. Calibration and Verification of the Calibration

If the installed aperture is going to be used for first time, it needs to be calibrated. Now is the time to do this. For a previously calibrated aperture, the verification of the calibration can be done at this time. For details on the calibration and verification procedures, refer to **Section 5**. If calibration is not necessary, 🖱️➔ **Next,** 🖱️➔ **Done**

4.5   Aligning the Optics

The three knobs on the side of the instrument are used to align and focus the aperture image in the display window. The two bottom knobs are labeled with a horizontal and a vertical arrow. The horizontal adjusting knob causes the aperture image to move left and right on the display window. The vertical adjusting knob causes the aperture image to move up and down on the display window. The top knob labeled with an "eye", focuses the image on the display window. Adjust the three knobs until the aperture image is centered on the Aperture Viewer (see Figure 4.5.1).

Re-adjust the focus to obtain a well contrasted aperture image. If good contrast is impossible to obtain, it may be necessary to reposition the beaker slightly, particularly if the beaker has a flat bottom and tall form.
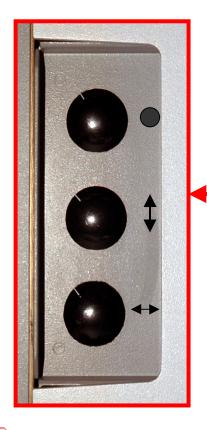
Figure 4.5.1   Optics Alignment Knobs

Remember that the image in a microscope is always upside down and horizontally reversed - it always moves the opposite way to the adjustment.

SECTION 5 CALIBRATION AND VERIFICATION OF CALIBRATION

5.1 Choosing the Calibrator

After installing the aperture as described in **Section 4,** choose the appropriate calibrator size according to the installed aperture. **The calibrator must to be standardize for the modal value and provide its value**. Beckman Coulter offers a variety of calibrator suitable for the Multisizer 3.

Aperture Size (µm)	Calibrator Nominal Modal Size (µm)	Recommended Beckman Coulter CC Calibrator Nominal Modal Size (µm)
20	2 – 4	2
30	3 – 6	3
50	5 – 10	5
70	7 – 14	10
100	10 – 20	10
140	14 – 28	20
200	20 – 40	30
280	28 – 56	30
400	40 – 80	43
560	56 –128	65
1000	60 – 150	90
2000	90 – 260	90

Table 5.1-1 Calibrators and size range for the calibration run
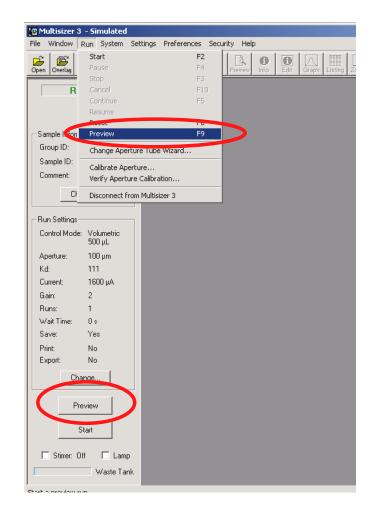
⚠️ *Beckman Coulter is not responsible for erroneous results if the proper calibrator and calibration procedure is not used.*

5.2 Preparing the Calibrator
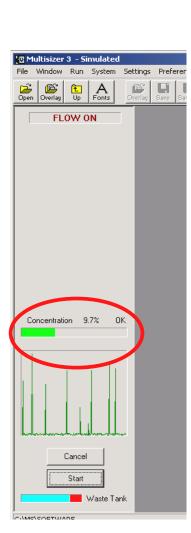
1   Place an ACCUVETTE® or a beaker* with the electrolyte and a few drops of the calibrator into the analyzer unit.

*An ACCUVETTE® (20 mL) is appropriate for apertures up to 100µm, for larger apertures a larger beaker should be used.

2   On the Status Panel
    ☝➔**Preview,** or in the Main
    Menu, ☝➔**Run,**☝➔**Preview.**

3. On the concentration index bar, verify that the concentration indicates OK; try to be as close as possible to 10%. If the concentration is high it can lead to an incorrect calibration. If the concentration is too low, the calibration will take a longer time to be completed. Adjust the concentration if it is necessary. When done, ☝➔**Cancel.**

5.3   Running the Calibrator

1. On the tool bar click on the calibration icon, or on the **Main Menu Bar**, ⊕→**Run**, ⊕→**Do a Calibration Run.**

2. On the Calibrator Size box enter the modal value of the calibrator. This value appears on the assay sheet of the calibrator.

3. 🖰➜**Start.**

4. When the calibration run is completed, 🖰➜**Accept New Kd.** The instrument is calibrated

It is convenient as a future reference to clearly identify the calibration files. These files can be saved in the folder Calibration located in C:\MS.

5.4   Obtaining the mean Kd

For more consistent results we recommend the first time an aperture is calibrated to perform the following procedure:

(1) Complete steps **5.1** through **5.3** ten times.

(2) Record the Kd value for each calibration. Calculate the mean for the 10 values.

(3) The mean for the Kd is only valid for the set aperture tube/electrolyte/instrument used for the calibration. In future calibrations the Kd should always be within ±4% of the mean value from Step 5.4.2. The next table shows an example using a 10 µm calibrator and a 100µm aperture tube.

Run	Kd
1	124.15
2	125.82
3	125.13
4	125.23
5	125.43
6	125.13
7	124.25
8	125.62
9	124.25
10	125.03
MEAN	125.00
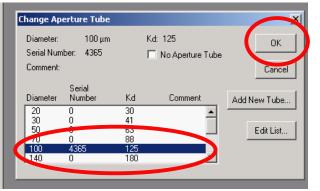
5.5   Entering the mean value for the Kd in the aperture list

1.   On the **Main Menu Bar**, ↰→**Run**, then ↰→**Change Aperture Wizard**.
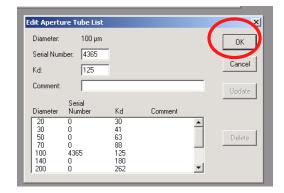
2.   On the Change Aperture Wizard, ↰→**Next** to skip the steps up to **Aperture Tube.** ↰→**Aperture Tube,** in the new box, ↰→**Edit List.** On the **Edit List Box**, ↰→on the list the aperture size you just calibrated. Enter the serial number for the aperture tube, mean Kd value and ↰→**Update.**

3.   🖰➔**OK**

4.   🖰➔the edited aperture and 🖰➔**OK**

5.   🖱➔**Close**

5.6   Verifying the calibration
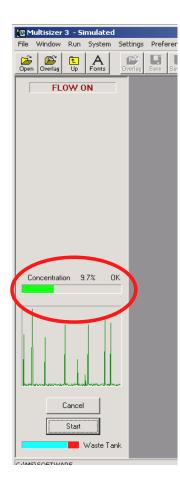
As the Multisizer 3 uses constant current, the instrument holds the calibration practically for indefinite time provided that the same aperture tube/electrolyte combination is used and the working conditions are stable. The calibration can be verified using the following procedure:

1.   Place an ACCUVETTE® or a beaker* with the electrolyte and a few drops of the calibrator into the analyzer unit.

      *An ACCUVETTE$^®$ (20 mL) is appropriate for apertures up to 100µm, for larger apertures a larger beaker should be used.

2.   On the Status Panel 🖱➔**Preview,** or in the Main Menu, 🖱➔**Run**, 🖱➔**Preview.**
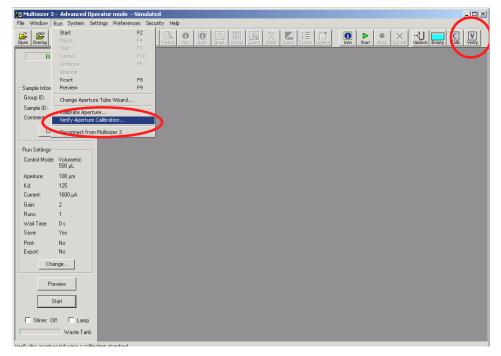
3. On the concentration index bar, verify that the concentration indicates OK. Try to be as close as possible to 10%. If the concentration is high it can lead to an incorrect result. If the concentration is too low, the verification will take a longer time to be completed. Adjust the concentration if it is necessary. When done, ☝➔**Cancel.**

4. On the tool bar click on the verification icon, or on the **Main Menu Bar** ☝➔**Run** and ☝➔**Verify Aperture Calibration.**

5.   On the Calibrator Size box enter the modal value of the calibrator. This value appears on the assay sheet of the calibrator. To choose the appropriate calibrator, refer to **Table 5.1.1**

6.   ☝➔**Start.**

7.   When the run is completed, the dialog box will indicate the status of the calibration. If it indicates verification is OK, ☝➔**Close.** If the instrument is out of calibration, go to Step **5.2** and **5.3.**

It is convenient as a future reference to clearly identify the verification files. These files can be saved in the folder Verification located in C:\MS.   **Back to TOC**

SECTION 6 SAMPLE ANALYSIS
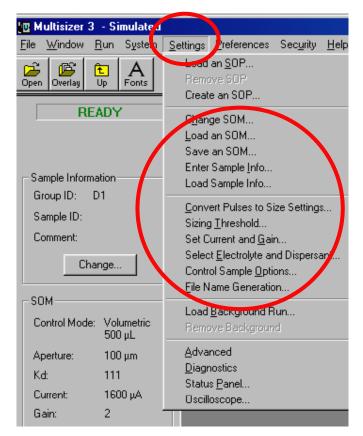
6.1   Introduction

At the start of each analysis, it is good practice to check each item in the following list even though no change may be required from previous instrument settings.

Before running an analysis, the instrument must be in the following condition:

(4)   The system has been filled with electrolyte.

(5)   The correct aperture has been fitted.

(6)   A beaker with clean electrolyte has been placed on the platform.

(7)   The aperture is immersed into the electrolyte contained in the beaker

(8)   The electrolyte container is sufficiently filled (enough to raise the level sensor).

(9)   The Waste container is empty (enough to lower the level sensor).

(10)   The Multisizer 3 and the computer have been powered on with the control program running.

(11)   The appropriate analysis settings have been selected.

These settings are the essential parameters that must be established before the analysis to ensure that the particle pulses measured by the Multisizer 3 are optimally positioned within the digital converter range and have the best signal to noise ratio. Other parameters that in previous instrument designs have had to be preset, such as the number of channels of data, can be changed after, or even during an analysis. The **Analysis Settings** menu can be found on the main menu bar.

6.2   Changing Standard Operating Method (SOM).

🖱➜**Change** on the **SOM** sub-panel in the **Status Panel**, or on the Main Menu bar, 🖱➜**Settings**, and 🖱➜**Change SOM.**

6.2.1   Selecting the Control Mode

Select the appropriate control radio button on the Control sub-panel (refer to **Table 6.2-1**). Enter the variable associated with the method in the field.

- **Time.** Any time up to 99999 seconds can be entered. The analysis will stop when the selected time has elapsed.

- **Volumetric.** Any volume up to 2000µL can be entered. The analysis will stop when the selected volume has been analyzed. Use this mode only with apertures not larger than 280µm. (See **Section 6.8**).

- **Total Count.** The number for total count can be selected up to 525,000. The analysis will stop when the number corrected for coincidence corresponding to the selected raw count number has been accumulated.

- **Modal Count.** This is the number of particles counted in the modal channel. The modal count number can be selected up to 525,000 counts.

- **Manual.** The analysis has to be manually stopped.

Analysis for:	Control Mode				
	Manual	**Modal Count**	**Total Count**	**Time**	**Volumetric**
Size Distribution	√	√	√	√	√
Particle Concentration	No	No	No	♦	√

♦ Refer to **Section 6.8**

Table 6.2.1 Selecting the control mode for the analysis

6.2.2 Selecting the number of runs

You can select to run single or multiple runs. Selecting multiple runs allows you to insert a delay time between runs. When multiple runs are selected, the average of the runs can be automatically saved and printed by checking the appropriate boxes.

6.2.3 Selecting the threshold

1.   In the **SOM** dialog box
🕭➔**Threshold**

⚠ *The threshold is the size above which particles are counted. It is also the lower limit of the size distribution. This setting CAN NOT be changed during or after the analysis.*

2. ⊕→Measuring Noise Level.
The instrument determines the lowest optimum threshold based on the measured noise level. Any number, however, can be manually entered for the threshold. If a number smaller than the size selected by the instrument is chosen, probability of noise interference will increase.

6.2.4 Adjusting current and gain.
   1. ⊕→Current & Gain

2. �👆➔Auto Set

3. Select Particles or Cells. �👆➔**OK**

⚠️*If cells are to be analyzed, it is very important to select cells in this step. Not selecting cells could cause them to be damaged due to a higher current setting.*

⚠️*Every time the current and gain are adjusted, it is necessary to measure the noise level.*

6.2.5   Changing the Electrolyte

If you need to use a different electrolyte with the same aperture tube, perform the following procedure:

1. 🖱➔**Electrolyte**

2. Select the electrolyte by double clicking in the drop down list or typing its name in the box.

3. If a dispersant agent is used, select it by double clicking in the drop down list or typing its name in the box.

⚠️ *Every time the electrolyte is changed, it is necessary to adjust current and gain and measure the noise level. If this is the first time using the new electrolyte, it is necessary to calibrate the aperture. If the electrolyte has been used before, it is necessary to verify the calibration (See Section 5)..*

6.2.6   Flushing the Aperture Tube

By selecting this option, the instrument will automatically flush the aperture every time a run is completed. To activate auto flush, check the appropriate box.

6.2.7   Saving the Data.

To automatically save the results after each analysis, check the **Save File** box. By checking this box, the run files are saved without the pulse data. To save all the original information from the pulses, check the **Save Pulse** Data box. Saving the pulse data allows you to change resolution, range and other parameters of the analysis without needing to re-run the sample.

✓☺ **The size of the files containing the pulse data is significantly large. Depending on the use of the instrument, it is advisable to transfer the files regularly to an appropriate storage media. A CD writer or similar storage device can be used.**

6.2.8   Generating the File Name

To identify the files from different analyses, a file name should be generated. The operator can customize it by checking the boxes of the information to be part of the file name. On the **Standard Operating Method** dialog box, ⊹➔**File Name**. In the File Generation Name Dialog box, customize name, ⊹➔**Use Example** and ⊹➔**OK.**

6.2.9   Exporting Data

To automatically export the analysis data after each run is completed, check the Export Data box. To select the export data format and destination, ⊹➔**Export,** select the options and ⊹➔**OK.**

6.2.10 Printing the Analysis Report

To automatically obtain a printed report of the analysis after completing each run, �🖰➔**Report.**

Select the content of the printed report and �🖰➔**OK.**

6.2.11 Changing the Destination Directory.

�🖰➔**Directory,** by checking the appropriate boxes, the run may be saved in the current directory or in a different directory. To select the new directory 🖰➔**Browse** or　🖰➔ the drop down arrow. When done, �🖰➔**OK.**

At this moment the Standard Operating Method has been selected. In the Standard Operating Method Panel, ⏚→**OK.**

6.3 Convert Pulses to Size Settings

Although these settings can be changed during or after the analysis, it is convenient to select these settings before the analysis. In this way, they can be saved with the Standard Operating Method.

1. ⏚**L→Settings,** ⏚**L→Convert Pulses to Size Settings.**

2. Select the settings

- **Size Bins.** The number of size bins can be selected from 4 up to 300. Higher resolution is achieved when a larger number of channels is selected.

- **Bin Spacing.** Use Logarithmic when the X axis units are logarithmic, use Linear when the X axis units are linear. Use Diameter to have the X-axis is in diameter units, use volume to have the X axis in volume units.

- **From – To**. Enter the desired range for the analysis. The range can be selected from the threshold size up to the size of the aperture.
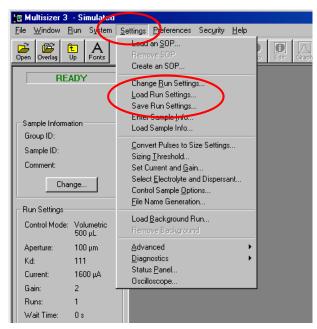
⚠️ *The size distribution is guaranteed to be linear from 2 to 60% of the aperture diameter.*

- **Multisizer II Edit.** This function is similar as in the Multisizer IIe. It is used to correct for non-axial pass of particles through the aperture. It can be used only with narrow size distributions.
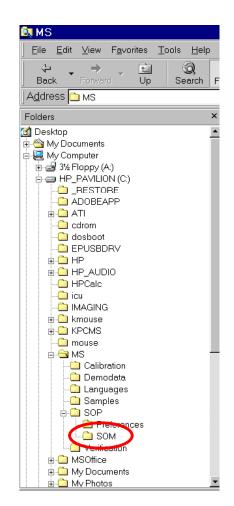
6.4   Saving Standard Operating Method

All the settings for the analysis can be saved for future use. The saved file containing the Standard Operating Method can also be used to create a **Standard Operation Procedure (SOP)** (see **Section 9**).

To save the Standard Operating Method 🖑➔**Settings** on the Main Menu bar and then 🖑➔**Save SOM**.

The **SOM** file can be saved in the **SOM** folder located in C:\MS\SOP. Any number of **SOM** files can be saved; they can be used at a later time for creating a **Standard Operating Procedure** (see **Section 9**).

To use a **Standard Operating Method** previously saved, ⌁**Settings** on the Main Menu bar, then ⌁**Load SOM**. To change the **Standard Operating Method**, ⌁**Change SOM.**

6.5   Entering Sample Information

By entering the sample information, it is possible to save all the information regarding the sample in the same file for the analysis. When the determination of concentration is needed, (counts/ml or counts/g), it is necessary to enter additional information in order for the software to do the calculation.

1.   ⌁**Change** on the Sample Information sub-panel in the Control Panel,

     or ⌁**System** on the Main Menu bar ⌁**Enter Sample Info...**

2. In the dialog box, enter all the required information.

Group ID, Sample ID, Operator, Bar Code and Comment are optional fields and their only purpose is to identify and classify the analyses. The run number will track the analyses sequence of each Group ID.

⚠️ *If it is necessary to determine the concentration of particles in the sample (counts), the followings fields must be filled in.*

Sample volume or mass: This is the weight or volume of sample used.for the analysis.
Electrolyte volume: This is the volume of electrolyte used for the analysis.
Analytical volume: This is the volume of sample analyzed.

Other fields in the Enter Sample Info dialogue box.

Variable 1 and Variable 2: These fields won't affect the results and are just to enter any variable you would like to save in the sample information.

Shape Factor: If a constant correction is needed, a factor can be entered and it will affect the results. For example, if a fibrous material is analyzed and instead of the equivalent spherical diameter, the length is required, a conversion factor can be entered to compensate for the difference in shape.

File Name: This button has the same function as described in **Section 6.2.8**

Control Sample: By checking this box, you indicate that the sample to be analyzed is a control sample.

Density: This information needs to be entered if the mass size distribution is required.

Use Dilution Factor: By checking this box, it is posible to use a factor if dilution has been used when preparing the sample. The dilution factor will affect the results.

Sample Statistics: When a sample is selected as "Control Sample", its statistical parameters and acceptable limits can be entered here.

✓☺ **To use the Sample Statistics as a reference, save this control sample under an easily identifiable name.**
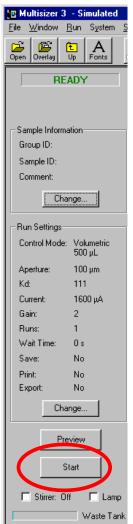
This information can be use as reference or specification for other samples. When done, ⌐**OK.**

6.6   Running a Background.

The background is an analysis of the electrolyte without sample material; it is also called a "Blank Analysis". It is advisable to run a background for every batch of analyses in a single day using the same electrolyte.

⚠️   *When analyzing low concentration samples, it is absolutely necessary to run a background.*
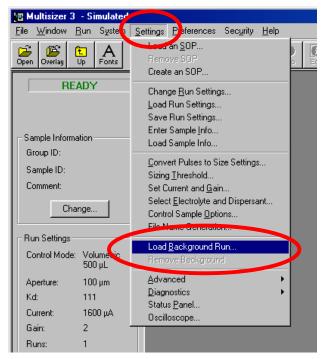
1. Select the same settings and parameters that will be used for the sample analyses.

2. Place the beaker with clean electrolyte in the analyzer unit.

3. ⌐→**Start** in the Status Panel.

After the run is completed and saved, it can be automatically subtracted from each subsequent analysis.

6.7   Subtracting the Background
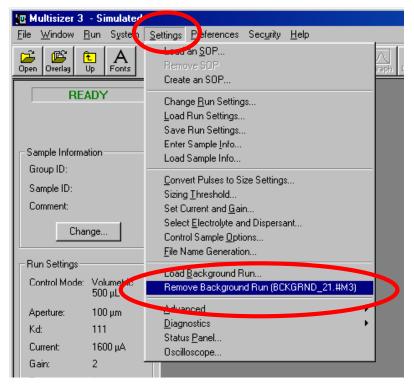
1.   On the main menu, 🖰➔**Settings,** 🖰➔**Load Background Run.**

2.   Select the background run.
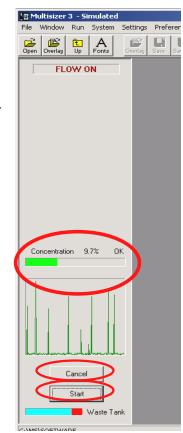
3.   Double click on the background file or select the file and 🖰➔**Open**. From this point on, the background run will be automatically subtracted from each

subsequent analysis. To disable background subtaction, on the main menu, ⁣🖰➔**Settings,** then ⁣🖰➔**Remove Background Run.**

6.8   Running the Sample

1. Place the beaker with the sample in the analysis unit.⁣🖰➔**Preview** in the Status Panel, or ⁣🖰➔**Run** on the Main Menu bar ⁣🖰➔**Preview**.
2. Check the sample concentration. The index meter must be below 10%. Adjust the concentration as necessary. When done ⁣🖰➔**Start** in the Status Panel.

6.9   Determination of Particle Concentration Using a 400µm Aperture or larger

When using a large aperture (400µm and larger) to determine particle concentration, it is not practical to use the volumetric control mode. If the volumetric control mode is used, the maximum volume allowed by the metering pump is 2 mL. Using a large aperture takes a very short time to pass 2 mL through the aperture. Since there is a limit for the appropriate concentration of sample, the numbers of particles counted are not enough to obtain good statistical results and therefore a poor reproducibility will achieved. The electrolyte used with large apertures should be thickened to avoid noise due to a high flow rate. It will also help in suspending heavy particles. As thickening agents, glycerol, sucrose and others can be used.

⚠️   *Measure exactly the volume of electrolyte needed to fill the beaker to about 9/10 of its capacity.*

1. Set the instrument in Time Mode to 60 seconds.

2. Run the electrolyte.

3. When the run is completed, measure accurately the volume of electrolyte left in the beaker.

4. Subtract the volume left from the original electrolyte volume.

5. Divide the resulting volume by 60. The result is the flow rate in ml/sec for this specific combination of aperture and electrolyte.

6. Knowing the flow rate, the Time Mode can be used to determine particle concentration. The volume of sample for the analysis is calculated using the flow rate. This volume is the "Analytical Volume".
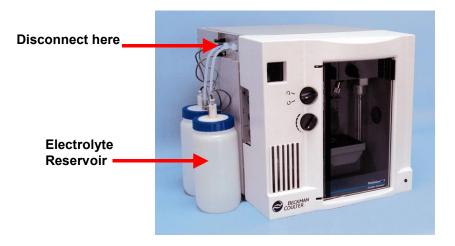
6.10   Cleaning the System

It is advisable to clean the system at the end of the day if the Multisizer 3 has been under any of the following working conditions.

• Analysis of biological samples

• Analysis of foods

• Use of corrosive fluids

6.10.1 Cleaning Procedure

1. Disconnect the electrolyte reservoir from the instrument.

Disconnect here

Electrolyte Reservoir

2. On the main menu, ☝➔**System,** ☝➔**Drain System.**

3. After the drain of the system is completed, in place of the electrolyte reservoir, connect a reservoir dedicated to holding the cleaning agent. The recommended cleaning agents for biological or food applications are Coulter Clenz or 2% bleach solution. For all other applications, DI water is suitable.

4. On the main menu, ☝➔**System,** ☝➔**Fill System.**

5. Repeat step 3.

6. Leave the system filled with the cleaning agent until the next working day.

7. Before start using the instrument on the next working day, follow steps 1 trough 4, but in this case, switching back to the electrolyte.
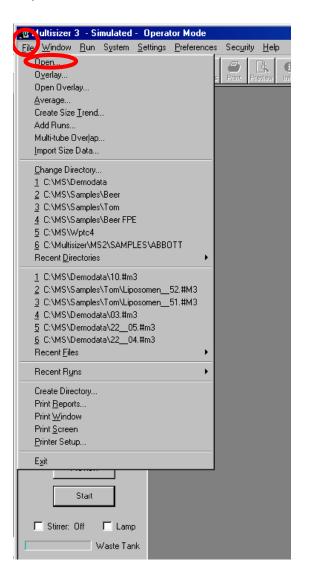
SECTION 7 REVIEWING AND SAVING THE RESULTS

7.1   Introduction

While an analysis file is open it can be viewed, changed and saved in many different formats by using the commands and options described in this section.

7.2   Opening a File

To open a previously saved file, on the main menu, ⏳➔**File,** ⏳➔**Open**.

Or on the tool bar ⏳➔the **Open** icon.

The Open command automatically displays the Open dialog box. To open the **Demodata** file, ⤷→ the file and**, ⤷→Open.**

By checking the **Quick View** box, it is possible to preview the files. The files can be previewed for size, trend or pulses by selecting the option from the drop down menu at the right of the **Quick View** box. To preview a file, ⤷→ the file name.

To open any of the files, ✋➔ the file name and ✋➔**Open.**

7.3   <u>Overlaying Files</u>

The Multisizer 3 software allows you to view more than one data file on the same graph. This is useful when you want to view point-by-point data comparisons using the same graph.

To open the data files for overlay, ⍾➔**File** then ⍾➔**Overlay** on the main menu, or on the tool bar, ⍾➔the **Overlay** icon.

The **Overlay** dialog box will be displayed.
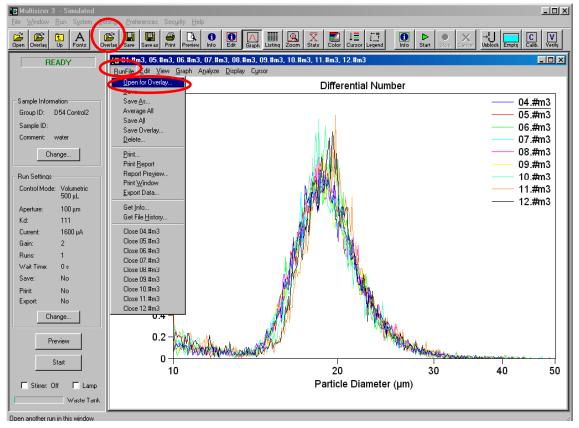
To **Overlay** files, ⏷🖱➔to highlight the file name. ⏷🖱➔**Add** in the bottom of the dialog box. Repeat this step to select all the files to overlay. When you have finished choosing the files, ⏷🖱➔**OK.** To overlay consecutive files, ⏷🖱➔to highlight the first file name, hold down the left mouse button and drag to highlight all the required files then ⏷🖱➔**Add**, and ⏷🖱➔**OK**.

A graph containing data from all selected files is displayed. The legend that appears in the upper right-hand corner of the graph displays the names of the data files used in the overlay.

To add an additional file to an already existing overlay, ✌➜**Run File,** then ✌➜**Open for Overlay** on the second level menu, or ✌➜**Overlay** (second icon) on the tool bar.
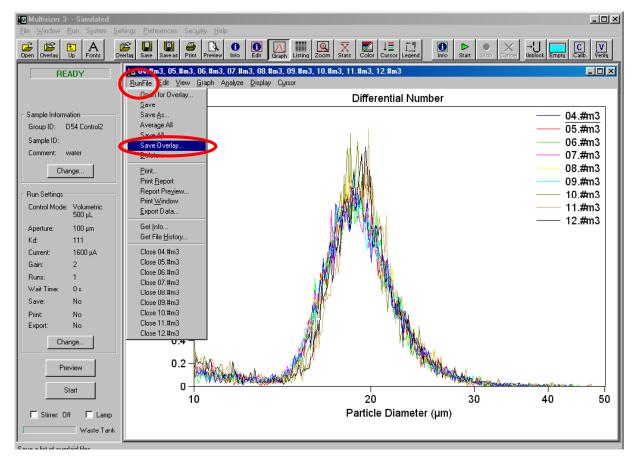

The **Open for Overlay** dialog box will appear automatically. Select the file to be added to the existing overlay and ✌➜**Open**.

The selected file will be added to the existing overlay.

The **Overlay** can be saved as an **Overlay File**. To save an **Overlay**, on the second level menu,🖑➔**Run File,** 🖑➔**Save Overlay**.

The **Save Overlay** dialog box will appear automatically. Assign a name for the **Overlay File** and 🖑➔**Save**.

To open an **Overlay File**, on the main menu, ↱**File,** ↱**Open Overlay**.

The **Open Overlay** dialog box will appear automatically. Select the file and ↱**Open**.
The file will be added to the **Overlay**.

⚠️ *This file is linked to the source files. It means that the file may only be opened when the original files are in the location from where the overlay file was saved.*

7.4   Averaging Files

The averaging function allows data from multiple data files to be averaged together. Data from individual files are combined, channel contents are averaged, sample statistics for each channel are calculated and the distribution is plotted on a single graph. The resultant file provides a graphical representation of the averaged contents of each channel.
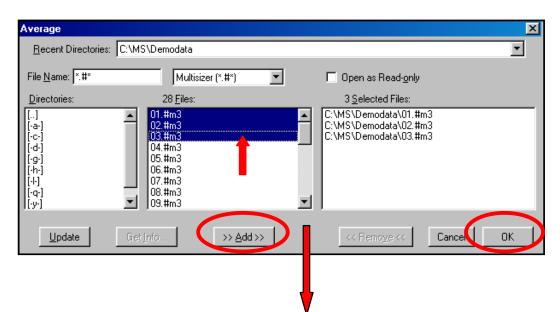
To average files, on the main menu, ✋➔**File,** ✋➔**Average**.

The **Average** dialog box will appear automatically.
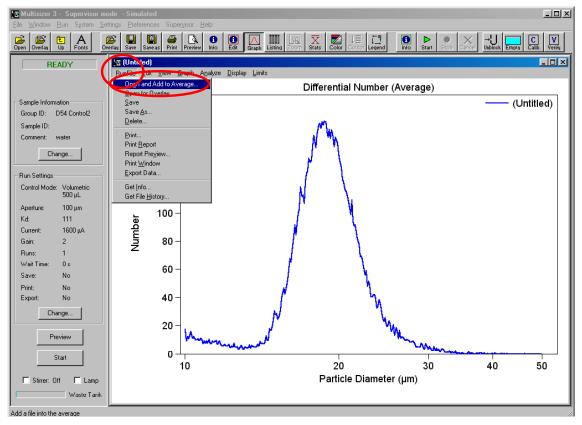
For averaging files, ✋➔ file name. ✋➔**Add** in the bottom of the dialog box. Repeat this step to select all the files to overlay. When you have finished choosing the files, ✋➔**OK.** To **Average** consecutive files, ✋➔to highlight the first file name, hold down the mouse left mouse button and drag to highlight all the required files, ✋➔**Add** and then ✋➔**OK**.
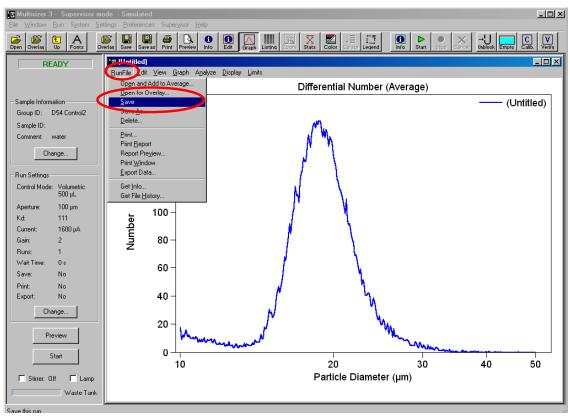
To add an additional file to an already existing **Average**, on the second level menu, ☝➔**Run File,** ☝➔**Open and Add to Average**.
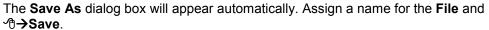
The **Open** dialog box will appear automatically. Select the file and ᗡ→**Open**. The file will be added to the **Average**.

The **Average** file can be saved. On the second level menu, ᗡ→**Run File,** ᗡ→**Save**.

The **Save As** dialog box will appear automatically. Assign a name for the **File** and ☝️➔**Save**.

7.5   Creating a Size Trend

Size Trend is most often used to show statistics from a number of sample runs on one graph or report. This would be useful, for example, in a manufacturing process where a sample is measured with the Multisizer at specific time intervals, (minutes, hours, days etc.). These data files can be used to create a trend graph or listing at the end of a chosen period of time to track changes of selected statistical values. User-selectable pass/fail limits can also be shown on trend graphs and listings. The Size Trend can be updated at any time by adding new points.
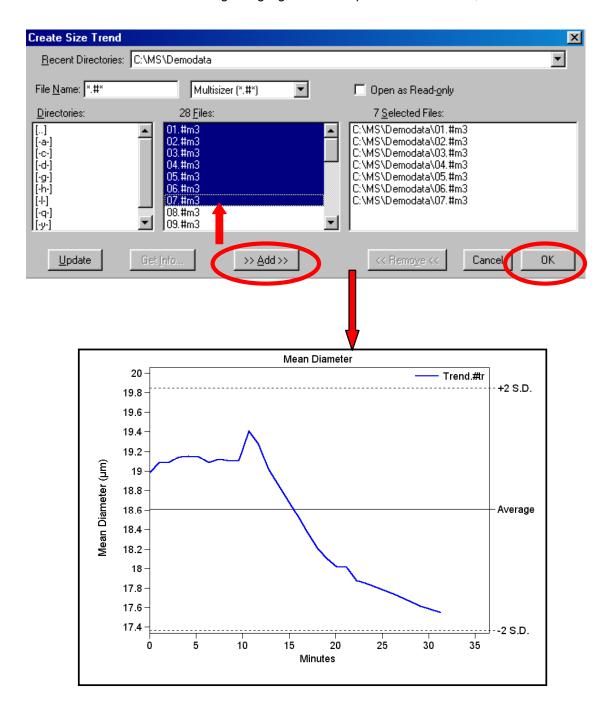
To create a **Size Trend**, on the main menu, ⊕➔**File,** ⊕➔**Create Size Trend**

The **Create Size Trend** dialog box will appear automatically.
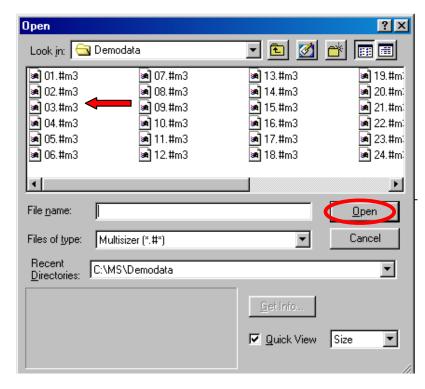
To select the files to be included in the **Size Trend**, ☝➔ the file name. Once you have highlighted the file name, ☝➔**Add** in the bottom of the dialog box. Repeat this step to select all the files for the **Size Trend**. When you have finished selecting the files, ☝➔**OK.** To select consecutive files, ☝➔to highlight the first file name, hold down the left mouse button and drag to highlight all the required files. ☝➔**Add**, and ☝➔**OK**.
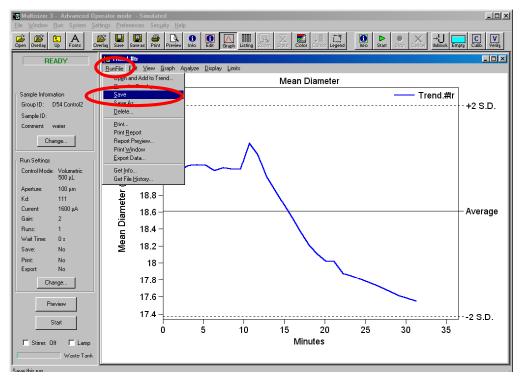
Additional files may be added to an already existing **Size Trend**. On the second level menu,✋➜**Run File,** ✋➜**Open and Add to Trend**.

The **Open** dialog box will appear automatically. Select the file and ✋➜**Open**. The file will be added to the **Size Trend**.
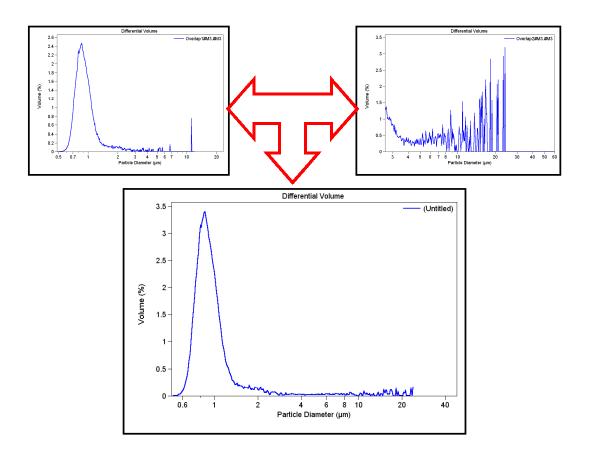
The **Trend** file can be saved. On the second level menu, ⊖➔**Run File,** ⊖➔**Save**.

The **Save As** dialog box will appear automatically. Assign a name for the **File** and ⊖➔**Save**.

7.6   Overlapping Files from Different Aperture Sizes.

The **Multi-Tube Overlap** procedure is used to combine up to 5 distributions into a single distribution with a new file name. This merging routine is able to create particle size distributions outside the range of any single aperture by combining 2 to 5 aperture size analyses from the same sample.

Obtain a size distribution on the Multisizer analyzer with an aperture tube suitable for the coarsest material in the sample. After analysis, sieve, filter or use any other suitable method to remove particles from the remaining suspension which are likely to block the smaller aperture. Continue the analysis with the next smaller aperture.
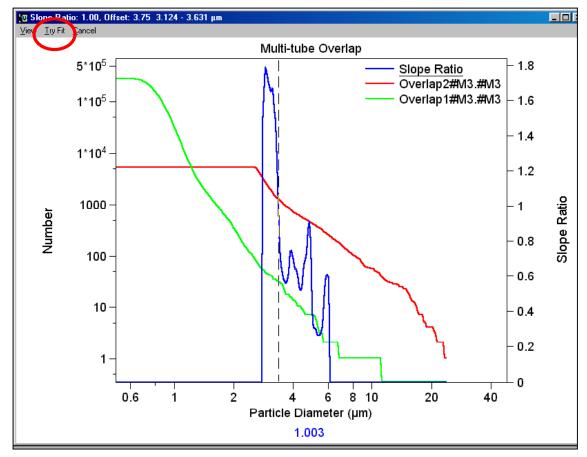
To find the combined distribution, find the point or range where the plots are most "parallel". This point, or range, is called the "offset point". At this point the vertical displacement (or offset), between the two graphs is measured. For example, if at the chosen point the cumulative number oversize is 1,000 from the small aperture and 10,000 from the large aperture, then the dilution is exactly 10:1.To combine the two plots the Multisizer 3 program multiplies the sample data from the small aperture by 10 and overplots it on the large aperture plot. The program will then exclude the small aperture data with a size larger than the offset point. The result is the combined distribution.
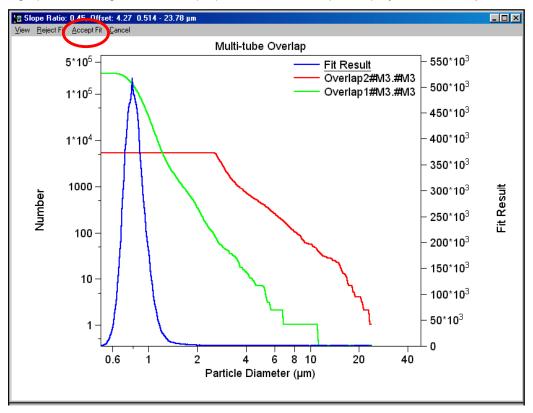
To create a **Multi-Tube Overlap** file, on the main menu,🖰➔**File,** 🖰➔ **Multi-Tube Overlap.**

The **Multi-Tube Overlap** dialog box will appear automatically. Up to five files may be selected to be included in the **Multi-Tube Overlap**. To select files ⌖➔ the file name. Once you have highlighted the file name, ⌖➔**Add** in the bottom of the dialog box. Repeat this step to select all the files for the **Multi-Tube Overlap**. When you have finished selecting the files, ⌖➔**OK.** To select consecutive files, ⌖➔to highlight the first file name, hold down the left mouse button and drag to highlight all the required files, ⌖➔**Add,**.and ⌖➔**OK**.

A graph showing the slope ratio and offset appears automatically ⌖➔**Try Fit.**

A graph containing the software proposal for the overlap is displayed. 🖰➔**Accept Fit.**

The size distribution graph for the resultant file will appear.

To save the file, on the second level menu,✋➔**Run File,** ✋➔**Save**.

The **Save As** dialog box will appear automatically. Assign a name for the **File** and ✋➔**Save**.

SECTION 8 PRINTING A REPORT

8.1   Introduction

This section describes how to set up the **Preferences Menu** in order to customize the printed report from the analysis. **The Preferences Menu** is found on the main menu, and after selecting the desired options, they can be saved as a preference file or as the **Default Preferences** file. Saving these files allows the user to recall them in the future and also to be used in the creation of a **Standard Operation Procedure (SOP)** (see **Section 9**).
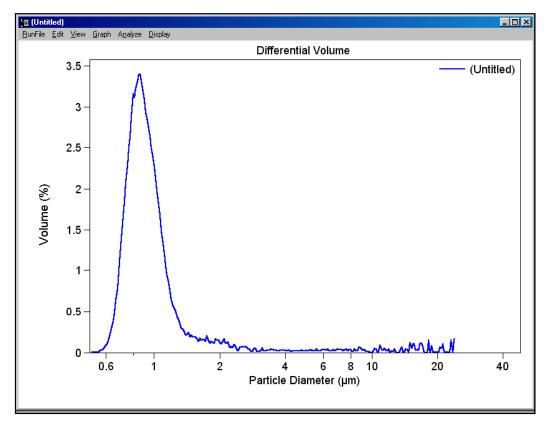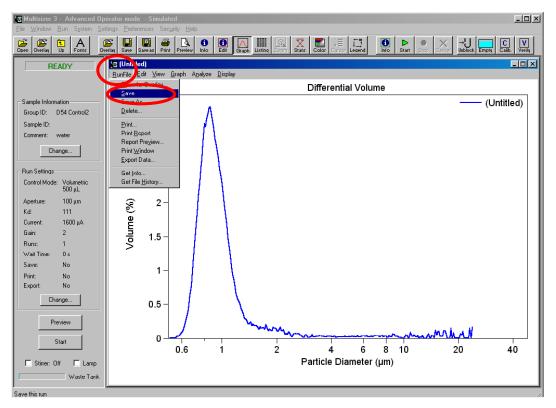
The **Default Preferences** file will determine how the format of the on-screen graph and data is displayed when the program is started at the beginning of a working session.
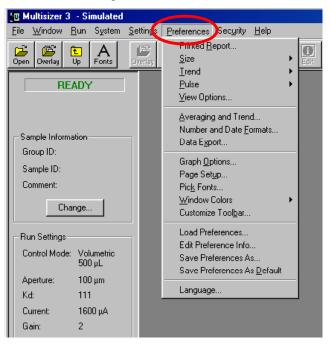
The **Preferences File** in use will determine the format of the on-screen graph and data displayed in the following situations:

- During the length of the analysis.

- After the analysis is completed.

- When a file is opened

Samples of data files, chosen to illustrate how to work with them, are included in the software's **Demodata** directory and are installed in **C:/MS/Demodata**.

The way the on-screen results are displayed can be changed at any time while the file for the run is open, but the printed report will be changed only if the Preferences File is altered.

To access the **Preferences Menu**, go to the main menu and ☝➜**Preferences.**

8.2   Selecting the Content of the Printed Report

The **Printed Report** submenu is where the choices for the content of the report are found. Once the selection of the content is done, they can be customized on the subsequent submenus.

In the **Preferences Menu**, ☝➜**Printed Report.**

8.2.1   Sample Info.

Checking the **Sample Info** box allows you to place basic information about the analysis on the report. The sample information enterd by the operator in **Section 6.5** can be reported in two formats, standard and short.

Example for standard **Sample Info**:

```
Multisizer 3                    3.51
20 May 2001
File name: 14931s1.#01
Group ID: 14931s1
Sample ID: A11
Operator: AL
Run number: 1
Electrolyte: 4% NaCl IN H2O
Dispersant: None
Aperture diameter: 30 µm Kd: 42.393
Aperture current: -800 µA Gain: 4
Size bins: 256 from 0.6 µm to 18 µm
Sigma: 7087 (Coincidence corrected)
Count > 0 µm: 0 Coincidence corrected: 0
Coincidence correction: 0.0%
Control method: Volumetric, 500 µL
Acquired: 9:19 30 Mar 2000
Electrolyte volume: 20 mL
Analytic volume: 500 µL
Sample: 2.5 mL
Background run: 14931.#01
```

Example for short **Sample Info**:

Multisizer 3	**3.51**

20 May 2001
File name: 14931s1.#01
Group ID: 14931s1
Sample ID: A11
Size bins: 256 from 0.6 µm to 18 µm
Sigma: 7087 (Coincidence corrected)
Acquired: 9:19 30 Mar 2000
Background run: 14931.#01

8.2.2 Size Section of the Printed Report

1. Size Graph

By checking the Graph box, the size distribution histogram for the samples will appear on the Printed Report. Different options are available for the size distribution **Graph**. To select these options, ⏁➔**Select** and a dialog box containing all the options for the Graph will pop up. After selecting the format of the **Graph**, ⏁➔**OK.**

2. Size Statistics

The **Statistics** for the samples will be contained in the Printed Report by checking the corresponding box. In Section 8.3 is described how to select the statistics parameters to be shown on the report.

3. Averaged Statistics

When several runs are averaged, the statistics of the resultant run can be obtained on the printed report by selecting the **Averaged Statistics** option.
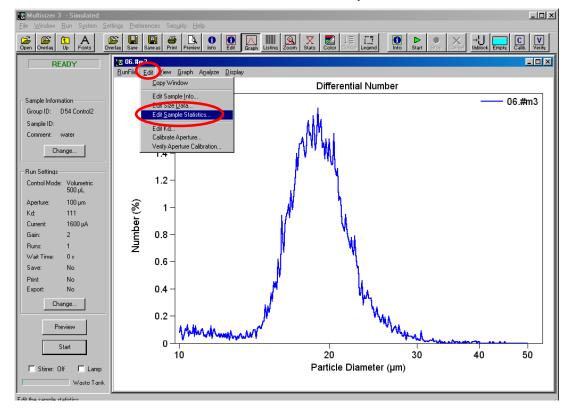
4. Compare Statistics

The **Multisizer 3** software allows one data file to be used as a statistical standard to which other data files may be compared. Limits from this standard file can also be applied to other data files.

To use the **Compare Statistics** function set up, as described below, a data file to serve as a standard file against which other files will be compared. When a sample is selected as control and its statistics are entered in the **Sample Info** box, this run can be used as a reference to compare to other runs (see **Section 6.5**). If a file from a sample was not selected as **Control Sample** at the time of the analysis, and it is required to **Compare Statistics,** the following procedure can be used**.**

1. Use the **Open** command as described in **Section 7.2** to open the required file.

2. On the second level menu, ᶿ➔ **Edit**, ᶿ➔**Edit Sample Statistics.**

3. In the **Edit Sample Statistics** dialog box, enter the statistics information: ᶿ➔**OK.** Save the file.

✓☺ **To use the Sample Statistics as a reference, save this file under an easily identifiable name.**

To display on the **Printed Report** the **Compare Statistics**, check the appropriate box in the **Printed Report** dialog box.

5. **Size Listing**

By selecting the **Listing** option, a section containing a list of the data for each channel of the size distribution will be placed on the **Printed Report**.

The format and content of the Listing can be selected by ↗⊕→**Select** next to the **Listing** box in the **Printed Report** dialog box.

The **Size Listing** dialog box appears automatically. Select the desired options, then ↗⊕→**OK.**

6.   **Size Interpolation**

The **Multisizer 3** software can be used to create sample data reports that will arrange data to interpolate data to conform to a set of user defined boundaries, also called "channels", "bins", or "size classes". (This is similar to how particle data points are reported when different size sieves are used.) The Multisizer 3 software refers to this process as **Size Interpolation**.

To include on the **Printed Report** the **Interpolation Points**, check the appropriate box in the **Printed Report** dialog box.

The format and arrangement of the data for the **Interpolations Points** can be selected by ↗⊕→**Select** next to the **Interpolation** box in the **Printed Report** dialog box.

The **Size Interpolation** dialog box appears automatically. When finished selecting the options, ↗⊕→**OK.**

8.2.3 Size Statistic Section of the Printed Report

This section of the Printed Report dialog box allows a choice of the statistic parameters to be printed on the report. Here the formats of the size distributions from which the statistic is calculated can be selected as well.

The kind of size distribution can be selected by checking the appropriate box.

❑ Mass	❑ Volume %
❑ Number	❑ Volume/mL
❑ Number %	❑ Surface Area
❑ Number/mL	❑ Surface Area %
❑ Volume	❑ Surface Area/mL

The **Size Statistics** dialog box appears when ⁀🖱➔**Select** inside the **Size Section**. Choose the parameters needed on the Printed Report and ⁀🖱➔**OK.**

8.2.4 Pulses Section of the Printed Report

1. Pulse Graph

If Pulse Data is saved as described in **Section 6.2.7**, the options to display and print this information can be selected now. Check the appropriate box for including the **Pulse Graph** in the **Printed Report** and select options by ⌂➔**Select.**

The **Pulse Graph** dialog box will appear. After selecting the options,⌂➔**OK.**

2. **Pulse Listing**

Check the appropriate box for including the **Pulse Listing** in the **Printed Report** and select options by ⁣🖰➔**Select.**

The **Pulse Listing** dialog box will appear. After selecting the options,🖰➔**OK.**

8.2.5 Trend Section of the Printed Report

1. **Trend Graph**

    Check the appropriate box for including the **Trend Graph** in the **Printed Report** and select options by ☝➜**Select.**

The **Trend Graph** dialog box will appear. After selecting the options,☝➜**OK.**

2. Trend Listing

Check the appropriate box for including the **Trend Listing** in the **Printed Report** and select options by ⊕➔**Select.**

The **Trend Listing** dialog box will appear. After selecting the options, ⊕➔**OK.**

8.2.6 Printer Section

The default printer can be used to print the report or another printer attached to the computer can be selected.

8.2.7 Closing the Printing Report Dialog Box

At this moment all the selections for the Printed Report have been made. ☝➔**OK.**

8.3   Customizing the Preferences

This section describes how to set up the preference file. **Section 8.2** described how to select the options for some of the preferences, in this section they are explained in more detail.

Size Graph	**Trend Graph**
Size Listing	**Trend Listing**
Size Statistics	**Pulse Graph**
Size Interpolation	**Pulse Listing**

If you already made these selections, it is not necessary to modify them again.

8.3.1   Size

To set up the **Size Preferences**, on the main menu ✛**Preferences** and ✛**Size**. A submenu showing the options for **Size** will appear.

1. Size Graph

In the **Size** submenu ↱→**Size Graph**. The dialog box containing the options to display and print the **Size Graph** will appear. A separate graph will be printed for every box checked in the **Distribution Types** section.These graphs will be printed in the order they are selected, and the printing order will be displayed in the lower left-hand corner of the dialog box.

Types of Distribution

For each kind of **Size Distribution**; (mass, number, number %, number/mL, volume, volume %, volume/mL, surface area, surface are % and surface area/mL), there are five different types of graph to choose from.

Differential Distribution.

Cumulative <

Cumulative >

Differential + Cumulative <
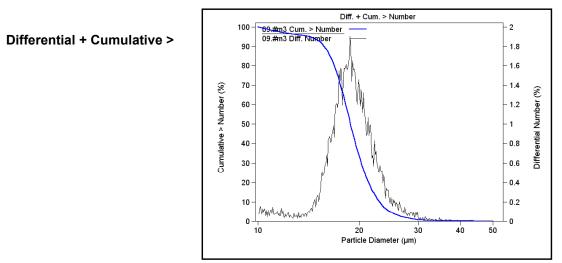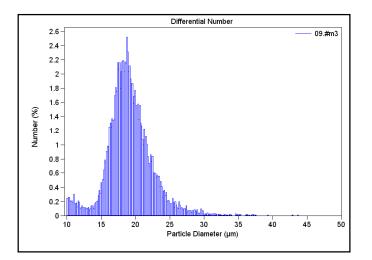
Differential + Cumulative >
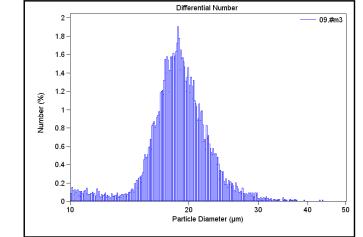
X Axis Scale

The X axis scale may be displayed in either linear or logarithmic. The logarithmic scale offers more detail for the lower side of the distribution. When a file acquired on a Multisizer IIe is open using the Multisizer 3 software, it is advisable to choose the option "as accumulated".

Linear X axis

Logarithmic X axis

Other Options for the Size Graph

❑ **Logarithmic Y axis**

❑ **Multisizer Cursors.** This option shows where the cursors are placed when a file acquired on the Multisizer IIe is open using the Multisizer 3 Software.

❑ **Y/dX Graph.**

❑ **Use Sample ID as Title.** It places the sample ID entered in Step 6.5 as the graph title.

When done selecting the **Size Graph** options, ☝➜**OK** in the **Size Graph** dialog box.

2. **Size Listing**

In the **Size** submenu, ☝➜**Size Listing,** the dialog box containing the options to display and print the **Size Listing** will appear.

✓☺ **Selecting "Between Cursors" will display the Listing for only the channels within the range being used. When "All Bins" is selected, all the channels for the entire range of the aperture (2% to 60%), will be listed. "From" allows selection of the channel range for the Listing.**

When done selecting the **Size Listing** options, ☝➜**OK** in the **Size Listing** dialog box.

3.   Size Statistics

In the **Size** submenu, ↻➔**Size Statistics.** The dialog box containing the options to display and print the **Size Statistics** will appear.

Selecting **"Between Cursors" will calculate the Statistics for only the size data within the range selected with the cursors on the graph. When "All Bins" is selected, all the channels for the entire range of the aperture (2% to 60%), will be used in the calculations. "From" allows selection of the size range for the calculations.**

When done selecting the **Size Statistics** options, ↻➔**OK** in the **Size Statistics** dialog box.

4. Size Interpolation

The Multisizer 3 software may be used to create sample data reports that will arrange data to interpolate in a set of user defined boundaries. This is similar to how particle data points are reported when different size sieves are used.

Two types of interpolation format may be used. In the first format, the boundaries represent size classes. In the second format, the particle data are contained in percentage classes. There are 3 choices: "Over", "Under", and "Differential". You can redefine both the number of boundaries and the value assigned to each boundary.

In the **Size** submenu,🖰➔**Size Interpolation.** The dialog box containing the options to display and print the **Size Interpolation** will appear.

✓☺ **Selecting "Between Cursors" will calculate the Interpolation points for only the size data within the range selected with the cursors on the graph. When "All Bins" is selected, all the data for the entire range of the aperture (2% to 60%) will be used in the calculations. "From" allows selection of the size data range for the calculation of the Interpolation points.**

When done selecting the **Size Interpolation** options, 🖰➔**OK** in the **Size Interpolation** dialog box.

8.3.2 Trend

Trend data is used to show statistics from a number of sample runs on one graph or report. This would be useful, for example, in a process where a sample is measured at specific time intervals. Trend files are widely used in quality control to track the compliance of the product to established specifications. These data files can be used to create a trend graph or listing at the end of any desired period of time, and can be updated as often as is necessary. On the main menu ⏚➔**Preferences** and ⏚➔**Trend.** A submenu showing the options for **Trend** will appear.

1. **Trend Graph**

   In the **Trend** submenu, ⏚➔**Trend Graph.**
   The parameters in the larger section at the left side of the dialog box can be selected for the Y-axis. The parameters in the smaller section can be used on the X-axis. When done selecting the **Trend Graph** options, ⏚➔**OK** in the **Trend Graph** dialog box.

2. **Custom Points**

   Up to three points can be customized**.**

Trend Listing
In the **Trend** submenu, ☞➔**Trend Listing.** When finished selecting the **Trend Listing** options, ☞➔**OK** in the **Trend Listing** dialog box.

8.3.3 Pulses

If Pulse Data is saved as described in **Section 6.2.7**, the options to display and print this information may be selected at this time. On the main menu ☞➔**Preferences** and ☞➔**Pulse.** A submenu showing the options for **Pulse** will appear.

1.   Pulse Graph

In the **Pulse** submenu, ⁸➜**Pulse Graph.**

Pulse Maximum High can be used for the Y-axis and Pulse Sequence or Pulse Width on the X-axis. When finished selecting the **Pulse Graph** options, ⁸➜**OK** in the **Pulse Graph** dialog box.

2. **Pulse Listing**
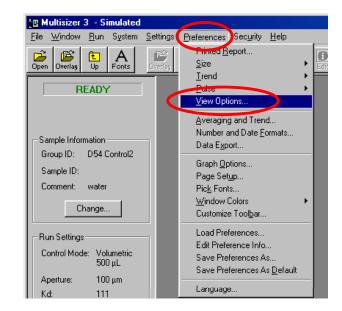
    In the **Pulse** submenu, ⁸➜**Pulse Listing.**

When finished selecting the **Pulse Listing** options, ⁸➜**OK** in the **Pulse Listing** dialog box.

8.3.4   Other Options for the Preference File

On the main menu, ⁸➜**Preferences** and ⁸➜**View Options.** A dialog box will appear.

1. **X-Axis Section**

    The units for the X-axis can be expressed as diameter, volume or surface area.
    Diameter: this is the most common use for the X-axis for industrial applications.
    Volume: this is widely used in biological applications
    Area: this is used for specialized studies.
    As Accumulated: this option is used when a file acquired on the Multisizer IIe is opened using the Multisizer 3 software.

2. **Measuring Section**

    Particles or Cells can be selected. This selection is used only for changing the X-axis units for displaying the data and /or printing the report. Where it is critical to select Cells is in **Step 6.2.4**.

3. **Volume Units Section**

    When Diameter is used for the X-axis, units can be μ^3 or fL. In cell biology, fL is more commonly used

4. **Specific Surface Area Units**

    When Surface Area is used for the X-axis, units can be selected as cm^2/mL or m^2/mL.

5. **Multisizer 3 Pulse Data**

    In this box the number of pulses to be saved can be selected. Up to 525,000 pulses may be saved.

6. **Z2 Data**
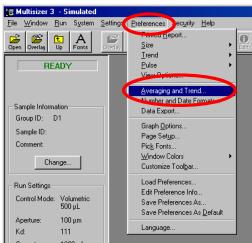
    If a file acquired on the Z2 is opened using the Multisizer 3 software, the last channel can be suppressed by checking this box.
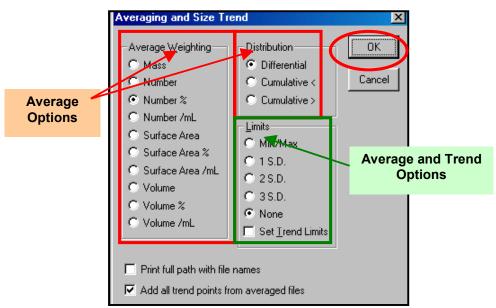
7. **ISO 9276 (Preliminary)**

    The choice of having all units represented in accordance with ISO 9276-1 (preliminary) is also included as an option for both graphs and listings.

8.3.5   Averaging and Trending

The options for the weighting and type of distribution for the average and also the setting for the limits averages and trends are located under **Averaging and Trend**.

On the main menu ☝➜**Preferences** and ☝➜**Averaging and Size Trend.** The following dialog box will appear.

The two boxes at the bottom of the dialog allow file names to be printed with their full path, and another option to add to the report all data points from the averaged or trend files.

Once the options have been selected, ☝➜**OK.**

8.3.6   Number and Date Formats

The **Number and Date Format** dialog specifies what number format will be used for data files that are being exported to other applications. This function also determines how numbers are displayed in tabular data listings, and determines whether the Multisizer software represents the decimal point as a space, a comma or a period. The date can also be formatted under this **Preferenc**e option.

On the main menu, ☝➜**Preferences** and ☝➜**Number and Date Format**

Once the options have been selected, ☝➜**OK.**

8.3.7   Data Export
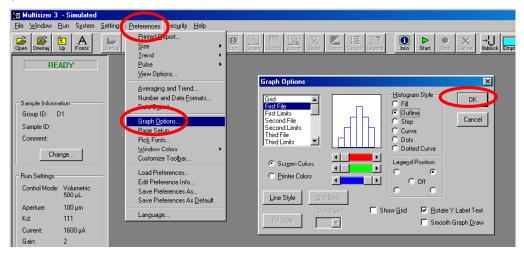
Options in the **Data Export** dialog allow the user to specify which subsections of the runfile (e.g., the file header, size data, pulse data, etc.) will be exported. An extension, such as .xls, may be entered so that another application will recognize the exported data. Formatting options are also included in the **Data Export** dialog. On the main menu, ⊕➔**Preferences** and ⊕➔**Data Export.**

Once the options have been selected, ⊕➔**OK.**

8.3.8   Graph Options

The **Graph Options** dialog box allows customizing the appearance of the graphs. In this dialog, separate color, line or dot styles may be specified for each data file, and the dialog also includes options for grid lines and legend position. The colors and styles for each file may be defined differently depending on whether the choices refer to a printed document or an on-screen display. On the main menu, ⊕➔**Preferences** and ⊕➔**Graph Options.**

Once the options have been selected, ⊕➔**OK.**

8.3.9   Page Setup

Select this menu item to control printer functions, including printer set-up, margins, titles and page orientation of printed output. **Page Setup** also allows inserting a company logo as a page header by entering the file name of a **custom logo** in bitmap format using the **Custom Bitmap** option. This can be created using Microsoft Paintbrush to make a black and white or 16-color bitmap up to 600 pixels wide by 200 pixels high.

The full path to the bitmap must be entered, e.g. ("c:\windows\ls\mylogo.bmp",). This bitmap will show up at the top left of every page printed, replacing the Beckman Coulter logo .   On the main menu, ✋➔**Preferences** and ✋➔**Page Setup.**

When the page setup has been selected, ✋➔**OK.**

8.3.10 Pick Fonts

Use **Pick Fonts** to make changes in font type and font size. Separate settings are available for graphs, listings, statistics, and headers. The program allows previewing font size and font samples before making the choices. On the main menu ✋➔**Preferences,** then ✋➔**Pick Fonts.**

After making changes**,** ✋➔**Apply** to implement changes, then ✋➔**OK.**
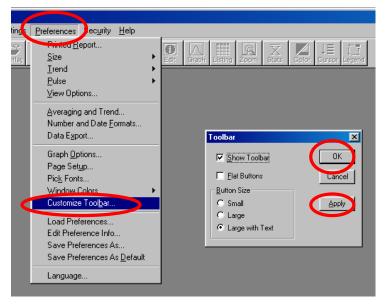
8.3.11 Windows Colors

Choices here will control the default colors for the text, warning text and the window background. ↷➔**Preferences,** and ↷➔**Windows Colors.** After selecting colors for each option, ↷➔**OK.**

✓☺ **These choices will not affect any other Windows applications.**

8.3.12 Customize Toolbar

This option allows you to select wether to show or to hide the toolbar and the way the toolbar buttons will be displayed. On the main menu ↷➔**Preferences,** and, ↷➔**Customize Toolbar.**

After selecting options, ↷➔**Apply** to implement changes, then ↷➔**OK.**

8.3.13 Selecting the Language

To select the language, 🖱️➔**Preferences** then 🖱️➔**Language.**
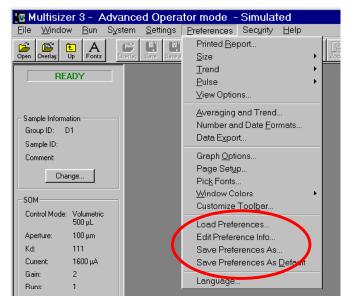
The available languages will appear in the drop down menu of the language dialog box. After selecting the language, 🖱️➔**OK.**
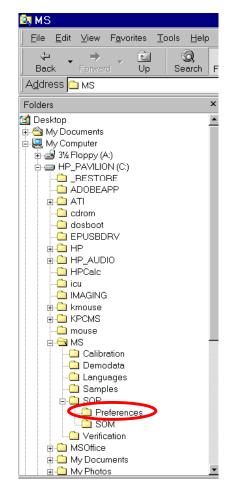
8.3.14 Saving Preferences

At this moment all the options for the **Preferences** have been selected.

All the options for the **Preferences** can be saved for future use. To save the **Preferences,** 🖱️➔**Settings** on the Main Menu bar and then 🖱️➔**Save Preferences As**.

After assigning a name to identify it, the **Preferences** file can be saved in the sub-folder **Preferences** located in C:\MS\SOP. Any number of **Preferences** files can be saved; they can be used at a later time as the options to view the results and print the analysis report or for creating a **Standard Operating Procedure** (see **Section 9**).

To use a Preferences file previously saved, ⟡➔**Preferences** on the Main Menu bar, then ⟡➔**Load Preferences**. To change a **Preferences** file, ⟡➔**Edit Preferences Info.**

The **Preferences** options can also be saved as the **Default Preferences.** These default options will appear every time the Multisizer 3 program is started. To save **Preferences** as default, ⟡➔**Preferences** on the Main Menu bar, then ⟡➔**Save Preferences As Default.**

SECTION 9   CREATING A STANDARD OPERATING PROCEDURE (SOP)

9.1   Introduction

This section describes how to create and use **Standard Operating Procedures (SOP)**. This feature of the software allows you to combine **Preferences** files and **Standard Operating Methods (SOM)** files into one single file. This new file will include all the necessary settings and options described in any user defined **SOP**. Creating and saving SOP's is specially useful when more than one operator is using the Multisizer 3 or when the instrument is used for more than one type of application. The use of SOP's also ensures the consistency of the analyses by preventing an unauthorized operator to change any of the settings or options included in the **SOP**, (see **Section 10**).

9.2   Creating and Saving an SOP

Before creating an SOP, it is necessary to create and save the **Standard Operating Method (SOM)** file and the **Preferences** file (see **Section 6.2 to 6.4** and **Section 8**).

To create the **SOP** file, on the main menu ⌐**Settings** and in the drop down list select **Create an SOP**.

The dialog box to select the necessary files for creating the **SOP** will appear.

9.2.1 Selecting the SOM file.

In the **SOM** file section of the **Create a Standard Operating Procedure** dialog box ↷➔**Select.**

A dialog box will display the folders under C:\MS. Select the folder **SOP** and ↷➔**Open.**

A dialog box will display the folders under C:\MS\SOP. Select the folder **SOM** and ↷➔**Open.**
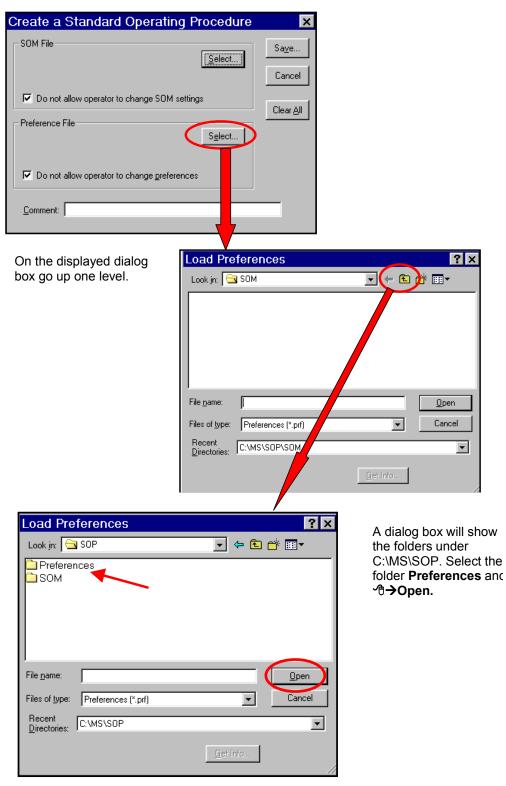
After selecting the folder **SOM**, ☝➔**Open.** All the previously saved **SOM** files will be shown (see **Section 6.4**). Select the necessary file to be included in the **SOP** and ☝➔**Open.**

9.2.2   Selecting the Preferences File

In the **Preference** file section of **Create a Standard Operating Procedure** dialog box 🖱→**Select.**

On the displayed dialog box go up one level.

A dialog box will show the folders under C:\MS\SOP. Select the folder **Preferences** and 🖱→**Open.**

After selecting the folder **Preferences**, 🖰➔**Open.** All the previously saved **Preferences** files will be shown (see **Section 8.3.14).** Select the necessary file to be included in the **SOP** and 🖰➔**Open.**

9.2.3 Saving the SOP

At this moment the two files needed to create an **SOP** have been selected. A comment can be added and it will be shown in the Info box for the **SOP** (see **Section 9.2)**. To save the **SOP** 🖰➔**Save.**
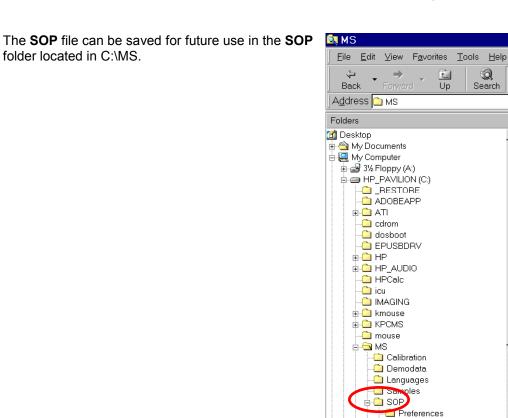
The **SOP** file can be saved for future use in the **SOP** folder located in C:\MS.
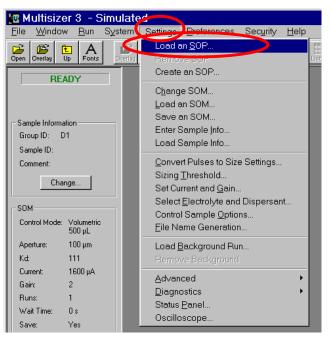
9.3   Using an SOP

Any number of SOP files can be created and saved for future use (see **Section 9.1**). To use a previously saved SOP file, ⊕➔**Settings** on the main menu and select. **Load an SOP.**

A dialog box will show all the **SOP** files previously saved. Select the required **SOP**. The **Get Info** button shows a display of all the information about the selected SOP file. To use the SOP file for analyzing samples in the Multisizer 3, ⏁➔**OK.**

9.4   Removing the Active SOP

If during a working session an **SOP** file is in use and a different file is needed, just follow instructions in **Section 9.2** and load a new **SOP** file. This action will remove the previous file from use. When there is no need for using an SOP file, the current file in use can be removed.. ⏁➔**Settings** on the main menu and select **Remove SOP.**

SECTION 10 REGULATORY COMPLIANCE

10.1 21 CFR Part 11

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 Multisizer™ 3 generate electronic records, these systems must comply with the Electronic Records Rule.

Here are described the relevant portions of the 21 CFR Part 11 regulations and their implementation using the Multisizer™ 3 control software explained. It is important to realize that Implementation and compliance of the rule remains the responsibility of the organization or entity creating and signing the electronic records in question. Proper procedures and practices, such as GLP and GMP, are as much part of overall compliance with these regulations as are the features of the Multisizer™ 3 control software.

10.2 Electronic records

Section 11.3 subpart A of 21 CFR Part 11 deals with the definitions assigned to electronic records, 'electronic record means any combination of text, graphics, data, audio, pictorial, or other information representation in digital form that is created, modified, maintained, archived, retrieved or distributed by a computer system'. In reality this refers to any digital computer file submitted to the agency, or any information not submitted but which is necessary to be maintained. Public docket No 92S-0251 of the Federal Register (Vol.62, No 54) identifies the types of documents acceptable for submission in electronic form and where such submissions may be made.

10.3 FDA Requirements

In the general comments section of the ruling the following is stated: ' The agency emphasizes that these regulations do not require, but rather permit, the use of electronic records and signatures'. In the introduction to the final ruling the following statement is made The use of electronic records as well as their submissions to FDA is voluntary'.

If electronic submissions are made, Section 11.2 subpart A comes into play: ' persons may us electronic records in lieu of paper records or electronic signatures in lieu of traditional signatures….provided that: (1) The requirements of this part are met; and (2) The document or parts of a document to be submitted have been identified in public docket No. 92S-0251'.

The Multisizer™ 3 control software have been designed to allow users to comply to the electronic records and signatures rule. Any organization deciding to employ electronic signatures must declare to the FDA their intention to do so.

10.4 Implementation of electronic records and electronic signatures

Section 11.3 Subpart A describes two classes of systems; "closed systems" and "open systems". A closed system is one "in which system access is controlled by persons who are responsible for the content of electronic records". In other words, the people and organization responsible for creating and maintaining the information on the system are also responsible for operating and administering the system. In contrast, an open system

is one "in which system access is not controlled by persons who are responsible for the content of electronic records".

A typical Multisizer™ 3 installation will need to have a procedure designed to ensure proper operation, maintenance and administration for system security and data integrity. Anyone who interacts with the system, from administrators to users, must abide by these procedures. Therefore the ultimate responsibility is with the organization generating electronic records and signatures. The Multisizer™ 3 software is a component, albeit a vital one, of the overall process.

10.5   Controls for electronic records

Subpart B, Section 11.10 describes the controls to be applied to a *"closed system"*. Section 11.30 describes the controls for an *"open system"*, which include *"those identified in § 11.10, as appropriate, and additional measures such as document encryption and use of appropriate digital signature standards".* Since a typical Mulitsizer™ 3 system can be regarded as a closed system, additional controls for open systems will not be discussed in this document. The primary thrust of these controls is *"to ensure the authenticity, integrity, and, when appropriate, the confidentiality of electronic records, and to ensure that the signer cannot readily repudiate the signed record as not genuine"*. In other words, to protect the data and to make it difficult for someone to say that this is not their "signature". Many of the controls described in Section 11.10 refer to written procedures (SOP's) required of an organization by the agency, for the purpose of data storage and retrieval, access control, training, accountability, documentation, record keeping, and change control. The other controls are addressed either by the Multisizer™ 3 software itself, or in combination with end-user procedures.

Of the other controls, perhaps the foremost is described in Section 11.10 Paragraph (a): *"Validation of systems to ensure accuracy, reliability, consistent intended performance, and the ability to discern invalid or altered records."* It is the complete and overall validation of the system, as developed by the organization, which ensures the integrity of the system and the data within. It is to this end that the features of the Multisizer™ 3 software comply with the specifications of these regulations.

10.6   Establishing an electronic record control with the Multisizer™ 3

The Multisizer™ 3 software employs a system of usernames and passwords, consistent with the specifications of Subpart C, Section 11.300, "to ensure that only authorized individuals can use the system, electronically sign a record, access the operation or computer system input or output device, alter a record, or perform the operation at hand".

10.6.1 Setting up security

If you selected the security option as described in **Section 3.1**, a dialog box appear requiring you to enter a user name and password. If the security option was not selected in **Section 3.1**, it can be selected at this time. On the main menu ⚲➔ **Security Set Up**.

The security level option dialog box is displayed.
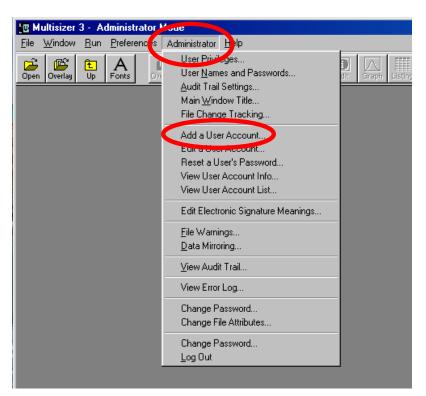
After selecting the required option ⚙➔**OK.** The **Add User Account** dialog box will appear.

10.6.2 Adding a user account

Add an Administrator account when prompted. The administrator can then add and configure the level of access for all other users, when finished, ⚙➔**OK.** If more user accounts are needed, they can be added at this moment or at a later time by ⚙➔ **Administrator** on the main menu and then ⚙➔ **Add a User Account** .
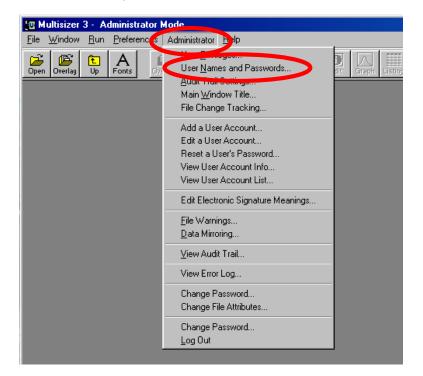
In the **Administrator** drop down menu select **Add a User Account**. The **Add a User Account** dialog box is displayed. Follow the instructions at the beginning of **Section 10.7.2**

10.6.3 Selecting user names and passwords

In the **Administrator** drop down menu select **User Names and Passwords**.

The entire Login process is controlled via the user names and passwords dialog. This ensures that the system login is controlled via time dependant metrics, such as password expiry and dialog inactivity, and has the required password lengths. Select the options in the dialog box and ⚲➔**OK.**

✓☺〉 **Note that the dialog has a button marked 21 CFR 11. When activated this ensures all options are set so that the settings meet with the rules as applicable to 21 CFR part 11.**

10.6.4 File History and Audit Trail

The Multisizer™ 3 software also performs data input and "operational checks", as specified in Subpart B, Section 11.10, "to determine, as appropriate, the validity of the source of data input or operational instruction", and "to enforce permitted sequencing of steps and events". These two features ensure that, as much as possible, valid data are being 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 is described in Section 11.10, Paragraph (b): "The ability to generate accurate and complete copies of records in both human readable and electronic form suitable for inspection, review, and copying by the agency". Consequently, strict procedures can be enforced within the Multisizer™ 3 software system to record all changes that are made to data generated from within Multisizer™ 3 software, as defined in Section 11.10, Paragraph (e).

Any file created by the Multisizer™ 3 software can have '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 Multisizer™ 3 software automatically records the identity of the user making the change, the date and timestamp of the change, the parameter that has 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 **File History** inside 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 a complete and continuous record of all changes to the file is maintained. Through the file protection and archiving capabilities of the Multisizer™ 3 software, it can be ensured, in compliance with Section 11.10, Paragraph (e), that "Such audit trail documentation shall be retained for a period at least as long as that required for the subject electronic records".

To access the **File History** information, open a file as described in **Section 7.2**, ⊕➔**Run File** and select **Get File History**
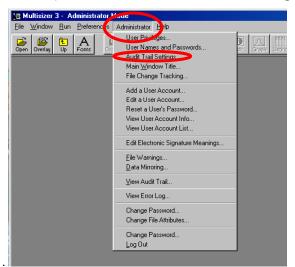
10.6.5 Setting Audit Trail
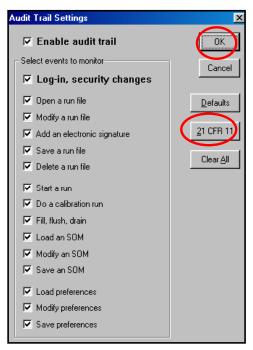
In addition to the auditing associated with the electronic file itself the Multisizer™ 3 software offers two additional levels of auditing. First, a general system audits trail that records and stores information at a system level, for example who logged in when, when users were added to the system etc. This is analogous to the security features of a Windows NT© operating system; this can be useful for systems using other operating systems. Second, the Multisizer™ 3 software generates an error log that stores and records errors associated with the system e.g. missing files, bad communications etc.

In the **Administrator** drop down menu select **Audit Trail Settings.**

Select the options in the dialog box and ⌕➔**OK.**

✓☺ **Note that the dialog has a button marked 21 CFR 11. When activated this ensures all options are set so that the settings meet with the rules as applicable to 21 CFR part 11.**

10.6.6 Assigning user privileges

The level of access to the Multisizer™ 3 software system is controlled by the rights granted through the **User Privileges** dialog box. The operations granted through these options are linked to the level of security chosen and to the **"User Category"** to which a user is assigned. Users can be classified into five different categories.

- Reviewer
- Operator
- Advanced Operator
- Supervisor
- Administrator

To access the **User Privileges** dialog box, in the **Administrator** drop-down menu select **User Privileges**. At this moment the access level to the software can be assigned to the different categories of users.

If all the user accounts have not been created yet, at this moment they can be created, as well as the **User Names and Passwords** and the **Audit Trial Settings**. These settings can be accessed using the three buttons located in the right lower part of the **User Privileges** dialog box.

Select the options in the dialog box ⍦➔**Set** and then ⍦➔**OK.**

✓☺〉 **Note that the dialog has a button marked High Security 21 CFR 11. When activated this ensures all options are set so that the settings meet with the rules as applicable to 21 CFR part 11.**

10.6.7 Electronic Signature

In Subpart A, Section 11.3, an electronic signature is 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". Subpart C, Section 11.100 of the regulation defines the general requirements of such a manifestation. Paragraph (a) states that "each electronic signature shall be unique to one individual and shall not be reused by, or reassigned to, anyone else". These two paragraphs, taken together, mean 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 procedural aspect of Section 11.100 requires that before any such electronic representation is applied, the organization first must "verify" the identity of that individual. The subsequent use of electronic signatures as the "legally binding equivalent of traditional handwritten signatures" then must be "certified" to the agency in writing

Subpart C, Section 11.200, refers to biometric and non-biometric forms of electronic signature. Biometric signatures are defined in Subpart A, Section 11.3 as a "a method of verifying an individual's identity based on measurement of the individual's physical feature(s) or repeatable action(s) where those features and/or actions are both unique to that individual and measurable". 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, as per Section 11.200, "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 Multisizer™ 3 software.
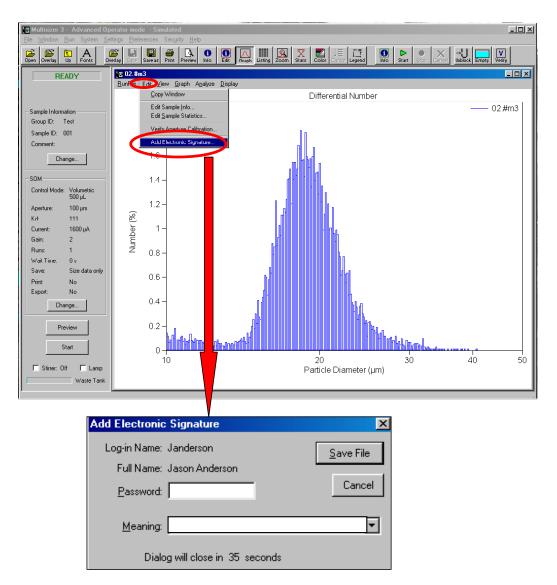
10.6.8 Generating electronic signatures

The Multisizer™ 3 software employs User ID's and passwords to verify the identification of each user logging into the system. When using this technique, Subpart C, Section 11.300 of the regulation requires "maintaining the uniqueness of each combined identification code and password, such that no two individuals have the same combination of identification code and password". This section also requires that the "identification code and password issuance's are periodically checked, recalled, or revised". Multisizer™ 3 software supports both of these provisions.

The administration of the system requires that individuals are added to the list of valid Multisizer™ 3 users via the **Add a User Account** dialog screen. The "identification code" or username of each Multisizer™ 3 user must be unique. No two users on the same Multisizer™ 3 system can have the same user name. It is also required that these users supply a password to access the Multisizer™ 3 software, thus satisfying the requirement to "employ at least two distinct identification components such as an 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 Multisizer™ 3 software can satisfy the requirement that "identification code and password issuance's 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 can electronically sign a file. To generate the electronic signature, open a file as described in **Section 7.2**, ⇧➔**Edit**, and select **Add Electronic Signature.**

10.6.9 Applying electronic signatures

Subpart C, Section 11.200 stipulates several requirements for the control of electronic signatures. Procedurally, the regulations require that electronic signatures "be used only by their genuine owners" and that they "be administered and executed to ensure that attempted use of an individual's electronic signature by anyone other than its genuine owner requires collaboration of two or more individuals". Through the application of Multisizer™ 3 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.

Section 11.200 further specifies the use of electronic signature components during a period "when an individual executes a series of signings during a single, continuous period of controlled system access", and "when an individual executes one or more signings not performed during a single, continuous period of controlled system access". This section of the document represents the "heart" of electronic signature application. To comply with these provisions, the Multisizer™ 3 software uses the application of the username 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".

10.6.10   Additional security features

The Multisizer™ 3 software offers two important additional levels of security, that whilst 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 that can only be accessed under administrator mode.

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

10.7   Starting the Multisizer 3 software with enabled security.

After all settings for security have been configured, every time the Multisizer 3 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.

⚠ **After using the Administrator, Supervisor and/or Advance Operator modes, in order to log off you must close down the Multisizer 3 program.**

SECTION 11   SPECIFICATIONS

11.1   Dimensions and weight

Weight, Dimensions and Power (excluding computer)

Unpacked weight:	45 kg (99 lb)
Width:	45 cm (18 in)
Depth:	60 cm (24 in)
Height:	60 cm (24 in)

Input Voltage within Set Ranges:

100 - 120 VAC; 230 - 240 VAC $\pm$ 10%; single phase

Supply Frequency:	47 to 63 Hz inclusive
Power:	Less than 55 volt-amps (watts)
Fuse types:	240 V, IEC (5x20 mm), Time delay (T), 3.15 A

⚠️ **The use of other fuse types may result in damage to the instrument.**

11.2   Environmental Conditions

a)   This instrument is safe for indoor use only.
b)   Installation category: 11.
c)   Pollution degree: 1.

Operating Temperature:	5°C to 40°C
Relative Humidity:	10% to 80% without condensation
Altitude:	Up to 2000 m (5500 ft). Above 7300 m (20,000 ft) unpressurized transport may damage the monitor.
Electrolyte Solutions:	All aqueous and non-aqueous electrolyte solutions devised for use with aperture technology will be suitable for use with the Multisizer 3. Electrolytes should be compatible with glass, fluoropolymers, fluoroelastomers, and polypropylene.

⚠️ **Solvents unsuitable for use with silicone rubbers and silicone adhesives are unlikely to be suitable for the Multisizer 3.**

11.3   Operational Parameters

Particle Size Range:	0.4μm to 1200μm in diameter. 0.0336fL to 904.8 x 10^6 fL or μm^3 in volume. The upper size limit is dictated by solution viscosity and particle density, and the lower limit by the aperture size, electrolyte conductivity and environmental conditions.
Orifice Tube Sizes:	20 μm to 2000 μm apertures (nominal sizes). Each size allows the measurement of particles in a range of 2% to 60% of aperture diameter.

Aperture (µm)	Range (µm)	Range (fL or µm^3)
20	0.4 – 12	0.0336 - 905
30	0.6 – 18	0.113 – 3054
50	1.0 – 30	0.524 – 14140
70	1.4 – 42	1.440 – 38800
100	2.0 – 60	4.189 – 113130
140	2.8 – 84	11.49 – 310330
200	4.0 – 120	33.5 – 904830
280	5.6 – 168	92.05 – 2.483x10^6
400	8.0 – 240	268 – 7.238 x10^6
560	11.2 – 336	735.6 – 19.86 x10^6
1000	20 – 600	4189 – 113.1 x10^6
2000	40 - 1200	33540 – 904.8 x10^6

Number of Channels:	Pulse data is digitized and can be processed to achieve up to 300 size channels for any sleeted range. The number of channels and range can be reprocessed as necessary.
Digital Pulse Processor:	High-speed digitalization of the signal allows the use of pulse area analysis and other techniques for additional particle characterization. This information may be used at a later time to recalculate the size distribution using different parameters.
Pulse Data:	Up to 525,000 pulses per analysis.
Linearity:	± 1 % for diameter
	± 3 % for volume.
Aperture Current Range:	30 µA – 6000 µA in 0.2 µA steps
Aperture Current Accuracy:	± 0.4% of setting
Polarity error:	Less than 0.5%
Time mode range:	0.01 to 1000 seconds, selectable in 10 ms increments
Metering System:	Mercury-free wide range metering pump
Metered Sample Volumes: **(Volumetric mode only)**	Continuously selectable from 50µl to 2000µl
Volumetric pump accuracy:	Better than 99.5%

11.4   Regulatory Compliance

The software is 21 CFR part 11 compliant