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CPS Disc Centrifuge Operating Manual

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This manual supports Version 9.x Operating Software and Models DC12000/DC18000/DC20000/DC24000 centrifuges. All information contained in this document is believed to be accurate and is presented in good faith; however, no warranty or guarantee as to the accuracy of the information is given. Copyrights to this document and the Version 9.x Operating Software are the property of CPS Instruments, Inc.

Updated June 2004

System Description

General

The CPS Disc Centrifuge is a particle size analyzer for measuring particles in the range of 0.01 micron to 40 microns. The system is most effective with particles between 0.02 and 30 microns. The analyzer measures particle size distributions using centrifugal sedimentation within an optically clear spinning disc that is filled with fluid. Sedimentation is stabilized by a density gradient within the fluid, and accuracy of measured sizes is insured through the use of a known size calibration standard before each test.

The concentration of particles at each size is determined by continuously measuring the turbidity of the fluid near the outside edge of the rotating disc. The turbidity measurements are converted to a weight distribution using Mie Theory light scattering calculations. The weight distribution is converted to a surface area or number distribution if required.

The Disc Centrifuge Control System (DCCS) software, which runs on a personal computer, handles all data collection and analysis, and also sets the speed of rotation for the disc based upon the type material being analyzed and the range of sizes being measured. All distributions are recorded on a hard disc in the personal computer for later retrieval. Distributions can be viewed on the computer monitor, and can be printed on the Windows default printer, whether this printer is local or on a network. The presentation format for the printed distributions has a wide range of options. The personal computer has an internal tape drive to allow rapid data back-up.

System Components

1. CPS Disc Centrifuge - Model DC12000, DC18000, DC20000, or DC24000

2. Computer* - 2.3 GHZ Pentium microprocessor, 60 gigabyte hard disc, super VGA color monitor, and mouse.

3. Hewlett - Packard DeskJet printer*.

* If purchased.

Safety Warnings

Shock Danger

The CPS Disc Centrifuge is internally powered by 100 to 220 Volt AC (single phase) and up to 440 Volt AC (3 phase). If you disassemble the centrifuge housing while the power is connected, you risk ELECTROCUTION! The housing should only be opened by a qualified electrician or electrical/instrumentation technician, who is aware of the potential hazard. The Disc Centrifuge housing is grounded through the incoming power cord ground wire; this power cord has a three-prong grounded plug. You should be **certain that the power outlet you use has a proper ground connection**. In most cases, the ground wire inside an electrical outlet box is either bare copper or has green colored insulation. **Operation of the CPS Disc Centrifuge without a proper ground connection is NOT safe and not recommended.** Operation without a ground will also lead to noisy particle size distributions.

The personal computer, monitor, and printer included in the CPS Disc Centrifuge system also present shock hazards if their cabinets are opened with the power connected. You should read the owners manuals for each of these to review the requirements for safe operation.

The Rotating Disc

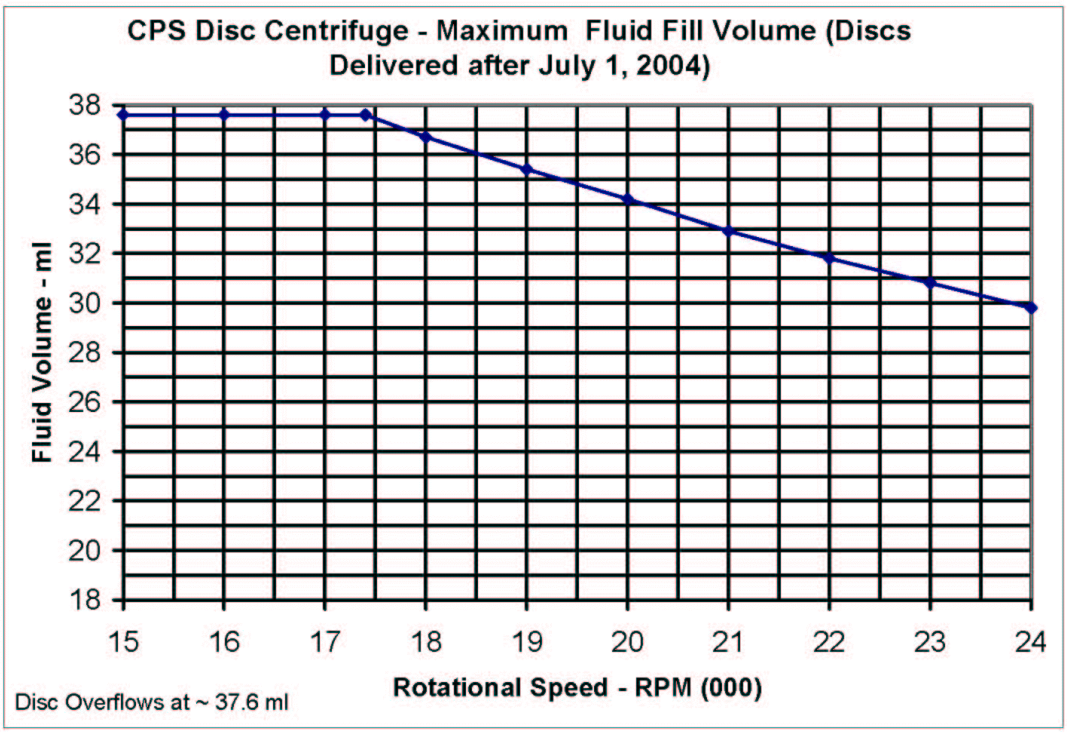
The disc spins at very high speed during normal operation, up to 24,000 RPM, and stores a tremendous amount of kinetic energy. The centrifuge will not start with the door open, and the door locks shut when the centrifuge is started. The door remains locked until the centrifuge comes to a stop.

NEVER, under any circumstances, defeat the safety interlocks and operate the centrifuge with the door open. NEVER attempt to touch the disc or do any maintenance work on the system while the disc is spinning. REMEMBER: With the door open, the rotating disc can easily entangle LONG HAIR, LOOSE CLOTHING, or a NECKTIE, with very harmful or fatal results!

Volume Fill Limit

Stress on the faces of the disc depend on both disc speed and fill volume. The disc chamber is normally filled with between 15 and 22 ml of fluid; more fluid is not needed. All users of the instrument should be aware that if the chamber is **overfilled** and the

centrifuge then run at **high speed**, then the disc may not be able to withstand the stess and may fail. Failure of the disc at high speed will caus significant internal damage to



the instrument**. The Maximum Fill Limit shown on the graph below MUST be adhered to.**

Getting Started

If the CPS Disc Centrifuge is delivered by a CPS representative, then the representative will unpack the system and set it up for operation. If the system is shipped directly to you, or if the components must be disconnected from each other and moved to a different location, then use the following instructions.

Unpacking

The CPS Disc Centrifuge in its shipping box weights about 45 kilograms, so it is better if two people are available for unpacking.

1. Open the top of the centrifuge shipping box (you may have to cut reinforcing bands off the box before you can open it), and then open the inner shipping box. Remove all the items packed with the centrifuge. Put all of these items aside for later use.

2. Lift the centrifuge out of the shipping box. The centrifuge is tightly packed in polystyrene foam, so removing it may require two people: one to hold the box and the other to lift the centrifuge.

3. Place the centrifuge upright on a laboratory bench or table, and open the centrifuge door. You will see that the disc has been secured to the light source/detector assembly with a combination of spacers and nylon bands. Cut the nylon bands and remove the spacers.

4. Loosen the bolt that holds the light source detector assembly in place so that the assembly can slide left and right. Cut the nylon band that secures the disc to the light assembly, then slide the light source/detector assembly to the right and remove the protective spacer that is between the disc and the light source. Finally slide the assembly left as far as it will go, and then tighten the bolt that secures the assembly in place. Spin the disc by hand to be sure that it is free spinning and not in contact with the light source/detector assembly.

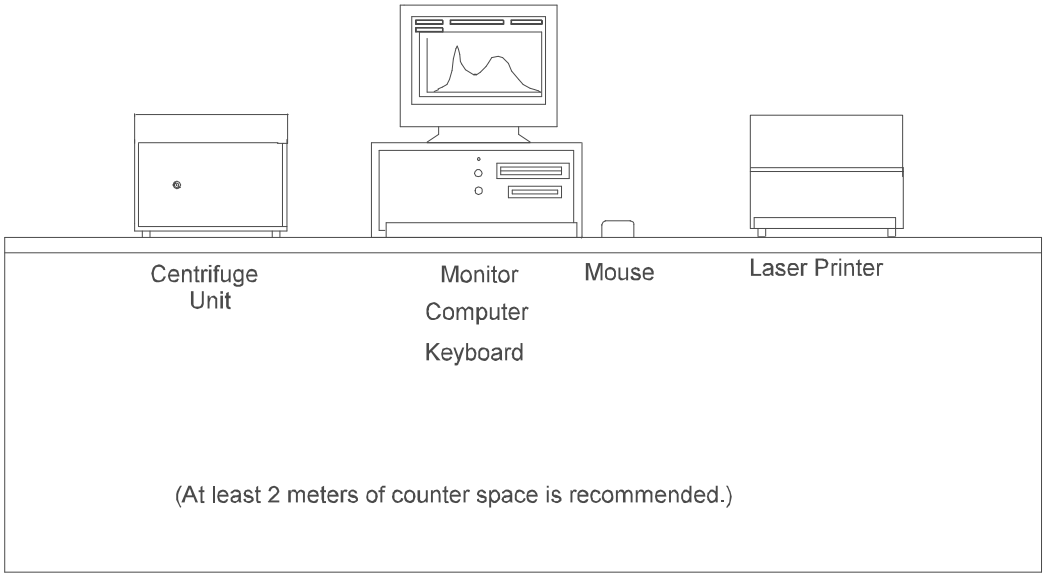
5. If you have purchased a computer system, unpack the personal computer, color monitor, and printer from their shipping containers and place them on the lab bench or table with the centrifuge unit. The recommended layout is shown in Fig 1.

6. Follow the instructions that come with the printer to remove any plastic shipping spacers from inside the printer housing, install the ink cartridges that come with the printer, and load the printer with paper.

It is recommended that you keep all shipping containers in case you need to ship the system in the future.

Figure 1

Recommended Layout



Power Requirements

The power requirements for each of the system components is shown below:

Centrifuge Unit - 100 to 220 VAC (50 to 60 Hz, single phase) 7.5 amperes maximum (the instrument is configured at the factory to match your local AC voltage).

Personal Computer - 100 - 220 VAC (single phase) 2.0 amperes maximum

Monitor - 100 - 220 VAC (single phase) 1.2 amperes

Printer - 100 - 220 VAC (single phase) 2.0 amperes maximum

Normal operating loads for the centrifuge unit, personal computer, and printer will be significantly less than the above maximum values.

Cable Connections

1. Connect the three prong power cord to the back of the centrifuge and to a grounded power outlet. Connect the computer, monitor, and printer power cords to appropriate power outlets. Be sure all components are OFF before making any other connections.

2. Connect a printer cable from the computer parallel port to the local printer (if one is present).

3. Connect the video signal cable (permanently attached to the monitor) to the computer.

4. Connect the mouse cable to the mouse port on the computer.

5. Connect the keyboard cable to the computer keyboard port.

8. Connect the 9-pin serial cable from the instrument to a **Serial Port** on the computer. Any available serial port (no. 1 to no. 4) is acceptable.

You can now safely turn on all of the components in the system. If you have purchased a computer with the CPS Disc Centrifuge, then there is a icon on the Windows "desk top" that you use to start the software.

If you did not purchase a computer, then you must install the operating software. The software and all supporting files are on an installation set of 5 floppy diskettes. Place diskette #1 in A: drive and run "setup.exe". Setup will guide you through the installation process. Setup will ask for confirmation of an installation directory. CPS recommends that you do not accept the default directory, but rather specify the installation go to "C:\analyzer" directory. Setup will create this directory for you once you have typed in this directory name. If you are **upgrading** from an earlier software version, then you **MUST** specify the C:\ANALYZER directory if this is where the old software is installed.

The software completes the installation process the first time it is run. A directory will be created for the "default" operating procedure, and several particle size distributions will be copied to the default operating procedure. You can then view these distributions and print them if you want.

Solutions For Operating the CPS Disc Centrifuge

The CPS Disc Centrifuge requires several solutions to operate. These must be

available in order to use the system to run analyses. A starter set of 9 aqueous solutions for forming a density gradient (125 ml each) is normally provided with the system. Aqueous sample preparation fluid and dodecane are also provided. You can prepare additional solutions, or CPS can provide these solutions in larger quantities for you if you would like. Complete sets of preserved solutions (eight 1 liter containers of density gradients, one 250 ml container of dodecane, and one 3.8 liter container of sample preparation solution) are available. The price for the complete set as of February 2003 was US$600, plus shipping. If you want to purchase the prepared solutions, contact CPS for current pricing. Special density gradient solutions (such as based biological buffers) are available by special order.

If you have purchased the CPS Density Gradient Builder, then you receive only two density gradient solutions: a high density stock solution and a low density stock solution. The Density Gradient Builder mixes these stock solutions in continuously changing proportions as the density gradient is being formed.

1. Aqueous Density Gradient Solutions

For most aqueous analyses, the disc is filled with a series of sucrose (table sugar) solutions to establish a density gradient within the disc. For low density materials (specific gravity of the particles less than 2), the start-up set of gradient fluids will normally be:

24.0% (by weight) sucrose in distilled water
22.0% sucrose in distilled water
20.0% sucrose in distilled water
18.0% sucrose in distilled water
16.0% sucrose in distilled water
14.0% sucrose in distilled water
12.0% sucrose in distilled water
10.0% sucrose in distilled water
8.0% sucrose in distilled water

The startup set of solutions normally have about 0.05% of an anionic surfactant to insure that sample particle do not coagulate during sedimentation. If the samples are cationically stabilized, then sucrose solutions should be prepared using a cationic stabilizer should be used. If the samples are not compatible with any type of ionic emulsifier, then solutions can be prepared using a non-ionic emulsifier. If your samples are either very low or very high in density, or very large in particle size, then you may receive different density gradient fluids that are more suitable for your samples. The gradient is formed while the disc is spinning at constant speed, so be sure the centrifuge is running at constant speed before you start to build the density gradient. If the disc is accelerating while the gradient is being formed, then the

gradient will be completely disrupted and the instrument will not operate properly.

2. Sample Preparation/Injection Solution

Concentrated samples must be prepared for analysis by dilution to a low concentration. For aqueous based samples, the dilution is normally done in a stock solution of the following weight composition:

99.9% Distilled Water
0.1% Emulsifier (same type as in the density gradient solutions)

The relatively high concentration of emulsifier insures that samples do not coagulate when they are diluted.

3. Calibration Standard

Unless you have your own calibration standard available, the CPS Disc Centrifuge is shipped with two reference calibration standards. These calibration standards are usually well defined, narrow polyvinyl chloride latexes. You must have an accurate calibration standard in order to use the CPS Disc Centrifuge. If you wish to develop your own calibration standards, you should read the section of this manual called "Principles of Operation" for recommended techniques to develop a calibration standard.

Several additional calibration standards are available from CPS at reasonable cost. These samples are narrow, well characterized polyvinyl chloride, polymethyl methacrylate, or polyvinylidene chloride latexes.

4. Anti-evaporation Cap

All instruments shipped after May 2002 include a small plastic “cap” that fits into the central opening of the disc. The cap has a small central hole that is treaded to accept a handle which allows easy insertion and removal, and is also equipped with an “o-ring” on the outside edge that seals against the machined surface of the central opening. This cap essentially eliminates evaporation from the fluid surface, and also eliminates drag on the fluid surface from motion of the air relative to the fluid. Use of this disc is required for any volatile gradient fluid, and is recommended for use with all gradients, even those that are not volatile, because it will increase the useful lifetime of all gradients.

5. Dodecane Cover Layer

With water based spin fluids, a thin cover of dodecane or tetradecane is injected into the instrument to inhibit evaporation of fluid from the rotating disc. The dodecane allows operation of the disc for at least several hours without significant degradation of the density gradient. Do not substitute other materials for dodecane or tetradecane. Dodecane and tetradecane normally attack and swell rubber seals on disposable syringes, so all-plastic or all-glass syringes should be used to handle these liquids. A small container of dodecane is normally shipped with the CPS Disc Centrifuge.

6. Running Low Density Samples

If you have purchased the optional disc for **low density samples**, then you have the option to run samples that are either higher in density than the fluid in disc (as described above), or samples that are **lower** in density than the fluid in the disc. Low density samples start analysis at the bottom of the centrifuge chamber and float toward the surface. Low density samples are injected into a "V" shaped groove in the front of the low density disc;at the base of this “V” shaped groove there are four capillary tubes which lead to the outside edge of the disc chamber. The density gradient may be identical to that used for high density samples, but the prepared sample (fluid plus particles) **must** be more dense than the most dense fluid that is used to form the gradient. If you are using an aqueous gradient based on 24% to 8% sucrose, then the sample preparation fluid could be 30% sucrose in water, along with ~0.1% of a suitable surfactant. When the prepared sample reaches the bottom of the chamber, it spreads over the outside edge of the chamber, and forms a narrow initial band of particles.

The centrifuge is started with the standard injection port, and the gradient fluids are added to the spinning disc in the normal way. Once the disc gradient has been formed, the normal injection port is removed, and replaced with a special angled injection port, which guides the After the centrifuge is spinning at constant speed, inject the density gradient solutions in the same way as normal. After the gradient is formed, you must **remove** the normal injection port by turning counterclockwise, then install the special angled injection port for low density samples. The special port directs the injection syringe needle toward the “V” shaped injection groove.

Trying out the System

The steps listed below take you on a brief tour of the operating software (the DCCS) and explain how to run samples on the CPS Disc Centrifuge. It is best if you read the Operating Manual cover to cover before you explore the system. However, the following steps will give you the minimum information you need to find your way through the system. The steps below are for samples higher in density than water.

1. Confirm that the disc centrifuge is empty and clean, then close the front access

door.

2. Turn on the Power ON-OFF switch located on the back of the analyzer. This will turn on the idle cooling fan located inside the centrifuge housing, and produce a steady discharge of air from the back of the centrifuge housing.

3. Turn on the computer and monitor, and start the CPS V9.x software.

4. Use the mouse to point at the Procedure Definition button, and press the left mouse button one time. The DCCS will present all of the operating parameters for the default procedure, usually called "DEMO". When the system is shipped, DEMO contains the parameters that CPS believes are suitable for your application. You have the option to change any of the operating parameters as needed, and also to define an unlimited number of different operating procedures. At a minimum, you should look at all of the “What is this?” help screens on the Procedure Definition window before you change or create a new operating procedure. It is better if you read the section of this manual called "Procedure Definition Window" before you create any new operating procedures. Point at and "click" the Exit Without Saving button to return to the Main Menu window.

5. Use the mouse to point at and click the Retrieve Distribution button. The DCCS will present a window titled “Retrieve Distributions - Select Files”. This window has a list of all of the available data files for the default operating procedure, as well as spaces for “Averages” of files and “Saved” files. You select files for viewing by pointing at the desired file and clicking the left mouse button once to highlight the file. You can view the selected files by clicking the View button. The software will present a new window titled “Retrieve Distributions - View Files”, on which the size distribution graphs for the selected files are displayed. You can change the presentation format by clicking the presentation options near the top of the window. After making changes in the presentation format, you must click the Apply Changes button to force the software to recalculate the size distribution(s) using the new format. You should feel free to explore all of the options within the "View Distribution" window. You can't change the original data in any way with the options on the screen, so there is no danger that you will do any damage to the data files or the operating software. You can print distributions by clicking the Print button on this window. Printed distributions are sent to the Windows default printer, so you may want to be sure what printer is set as the default before trying to print any graphs. The Windows default printer may be set for any printer that is available, including network printers. You may want to try adding notations to the graph using the Add Text option at the top of the screen. The text you add can be moved around on the graph, via “click-and-drag”, or deleted via a double click. All of the other items you see on the distribution graph can also be moved around via “click and drag”, or can be minimized to a command button by double

clicking on the item. Minimized objects will not be printed. You return to the "Select Files" window with the Exit button, and to the Main Menu window by clicking the Exit button from the "Select Files" window. When you have finished exploring the retrieve distribution section of the program, you should return to the Main Menu.

6. Use the mouse to point at the Start button on the left side of the screen, then press the left mouse button one time. The disc will accelerate to the set point speed over about 10 to 140 seconds, depending on what that speeds is. The set point depends upon the conditions defined by the default operating procedure. The set point is “Automatic”, because the software is selecting the speed. Click the Stop button to stop the disc, then click the “Manual” speed control option button. The speed reported under the Start and Stop buttons will be "Manual", and a slider bar will appear near the bottom of the window that allows you to select the centrifuge speed manually. Note that you can only change the speed set point when the centrifuge is stopped.

7. Inject the series of density gradient solutions, starting with the highest density (8% sucrose in water), and ending with the lowest density solution (normally pure water). The quantity injected should be about 1.6 ml of each solution. Inject 1 ml of dodecane, after the density gradient is complete, to inhibit evaporation of the water.

8. Use the mouse to select the Operate Analyzer button. The DCCS will present a Window that controls data collection. You can use a calibration standard (either your own, or a standard provided with the system) both to calibrate the analyzer and as a dummy "unknown". (Please note that the calibration standard you are using must be accurately described in the procedure definition for "DEMO".) Running a dummy unknown will help you become familiar with operation of the instrument. To get started, you key in a sample identification and then click the Start button. At each step in the operation, the DCCS will give you instructions near the top of the screen in bright red letters. You will be asked to inject the sample or calibration standard **and** hit the space bar at the same time. Please note that the accuracy of your results (especially with larger, fast moving particles) depends on the injection and strike of the space bar taking place at the same time. You should practice this step several times with dummy samples before beginning to run real samples. When you have finished running a sample, the DCCS will show the completed distribution on the screen, and also show a Next Sample button that you click to start the process over again. You can click the Exit button at any time you want to return to the main menu. If you are collecting data when you exit, then data collection continues in the background. The words “Collecting Data” flash on the upper right of the main menu window so long as data is being collected.

You can open other Windows based applications (word processors, spreadsheets, etc.)

while the CPS Disc Centrifuge is operating, so long as those applications do not attempt to use the same serial port as the CPS Disc Centrifuge, and so long as your computer is fast enough to run all of the open applications while still keeping up with the data stream coming from the CPS Disc Centrifuge. When you return to the Main Menu window, click the 

button so that you can view and print the distributions you have just run, as well as the distributions that were installed with the software.

Operating Software - The DCCS

The software that comes with the CPS Disc Centrifuge is called the Disc Centrifuge Control System (DCCS). This software is custom designed for the CPS Disc Centrifuge; it handles all the collection and analysis of particle size distribution data, storage and retrieval of distributions, and control of the disc speed.

The DCCS runs on any personal computer with an Intel compatible microprocessor. The software operates under Windows 95, and Windows 98, Windows NT, Windows 2000, and Windows XP.

The DCCS stores a permanent record on the hard disc of every distribution that is run. The capacity of a 20 gigabyte disc is enough to hold at least 300,000 distributions, so at a use rate of 30 analyses per day, it would take >25 years to use up all available disc space; the hard drive will certainly fail before this. Data files can be archived to floppy diskettes or to network drives. It is strongly recommended that this be done regularly to insure that a hard disc failure does not lead to lost data. With regular use of the system, a large volume of data will be accumulated by the DCCS; good organization of the data will make it much easier to access the data when needed. Please see the section of this manual called "Organizing and Protecting the Data" for some suggestions about organizing data storage.

Interacting with the DCCS

The DCCS interface is a standard Windows type interface, and you use mostly command buttons and option buttons to tell the software what you want to do. In the few places where you must key in data, the DCCS limits your keystrokes to numerical values in number fields, and also checks for keying errors (non-sense values are detected).

Getting Help

The software has two kinds of on-screen help. The simplest form of help is the **"Quick Tip".** You simply point at any active object on the screen with the mouse (for at least 2 seconds), and a single line "tip" that describes the object appears just under the mouse pointer. Second, you can access the full **"Help"** system by clicking the "Help" menu option on the upper left of each window. You can then search for information by keyword or select any of the help files listed in the help index. (Note: The full help system my not be available until mid 2003.)

Looking Over your Shoulder

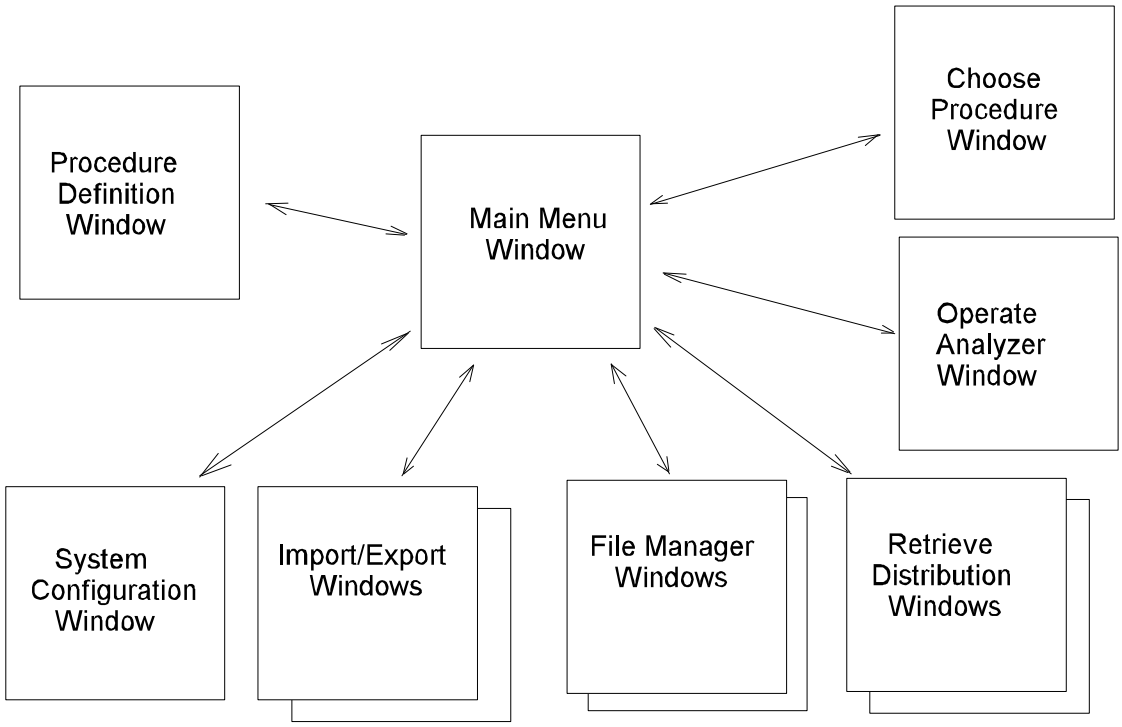
The DCCS warns you when there is a situation which may cause problems with the

operation of the analyzer, which may lead to inaccurate analyses, or which could cause a loss of data. The DCCS will not allow you to enter data which is impossible to use, or which is internally inconsistent. For example, if you attempt to define an operating procedure where the minimum particle size is larger than the maximum particle size, then the DCCS will not accept the new minimum value, and will issue a warning that explains the problem.

Organization and Logical Structure of the DCCS

Figure 2 shows the overall organization of the DCCS. The arrows show the paths that can be followed within the software.

Figure 2



The Active Operating Procedure

Whenever the software is running there is always an active operating procedure, which tells the DCCS what conditions to use for operation of the disc centrifuge, and also which data files can be accessed by the file manager. The active operating procedure is always shown on the Main Menu window.

There may be many different operating procedures that have been defined and can be run, but only one may be active at a time. You may change the active operating procedure by selecting the Choose Procedure command from the Main Menu screen. All of the available procedures are then presented on the screen, and any procedure can be made the active procedure by pointing at the name on the screen with the mouse and pressing the left mouse button. One (and only one) operating procedure is designated as the default operating procedure. The default procedure is automatically loaded whenever the DCCS software is started. You have the option to designate any of the available procedures as the default procedure. This is done in two steps:

1. Select Choose Procedure from the Main Menu, then select the name of the procedure that you want to be the default procedure. Return to the Main Menu by clicking the Change to Selected Procedure button.

2. Select Procedure Definition from the Main Menu. All of the parameters for the active procedure (chosen in step 1) will be presented. Check the box that says "Make this the default procedure", and then click Save and Exit to return to the Main Menu.

The "Retrieve Distribution" part of the software allows access to data files from any operating procedure **without** changing the active operating procedure. This allows you retrieve and print distributions that were created by one procedure while running the instrument (collecting data) under another procedure. If you change the active procedure **during an analysis** (that is, while data is being collected), then the instrument will operate under the original procedure until the run is finished **and** you click the Operate Analyzer button on the main menu window.

Control of the Disc

The parameters that make up the active operating procedure determine the speed at which the disc must spin during an analysis. A larger maximum particle size requires the disc to spin at lower speeds, while a smaller maximum particle size allows the disc to spin at higher speed. Particles that are higher in density (relative to the gradient fluid in the disc) also require a slower disc speed, while lower density particles allow increased disc speed. You must start the disc spinning by clicking the Start command **before** the DCCS will allow you to click the Operate Analyzer command.

Once the disc is spinning, it will continue to spin until either you:

1. Select the Stop command from the Main Menu window, or
2. Turn off the power switch on the back of the centrifuge unit.

Once the disc is spinning, the DCCS will not normally allow you to change the disc operating speed unless you first stop the disc to empty all fluid from the disc. Stopping the disc is required because a change in disc speed would disrupt the density gradient in the disc and make proper operation impossible. A special optional disc allows for "speed ramping" during an analysis. Speed ramping broadens the dynamic range of the instrument. The special speed ramping disc also allows you to change speed between analyses without having to stop and re-fill the disc.

> ***FAQ: What Speed to Run?***
> *The software calculates a conservative speed for each operating procedure. This is the speed the centrifuge will run if you operate with "Automatic" speed control. The this speed is calculated so that the largest particle in the analysis range arrives at the detector ~1.0 second after the sample is injected (based on an assumed fluid volume of ~15 ml) .*

You can manually force the centrifuge to run at any desired speed, regardless of the speed the software recommends. This might be required if you have added much more fluid to the disc than normal (for example, 24 ml instead of the more typical 15 - 18 ml). To manually force a particular speed, stop the disc and then click the "Manual" option button. A slider bar will appear near the bottom of the screen, which allows you to set the speed manually. Please note that the accuracy of results may be reduced for large particles if you force the disc to run at a higher speed than is normal and have not increased total fluid volume. CPS recommends that the largest particle in the distribution *arrive at the detector no less than 1 second after the sample is injected, unless you can tolerate some loss of accuracy in the coarse end of the distribution.* If the centrifuge is run at a speed that allows the coarsest particles to reach the detector in less than 0.5 second, then the reported distribution may be far from accurate, certainly for the coarsest particles.

Operator Interface Windows

The following section describes several of the operator interface windows, including all of the available options on each window.

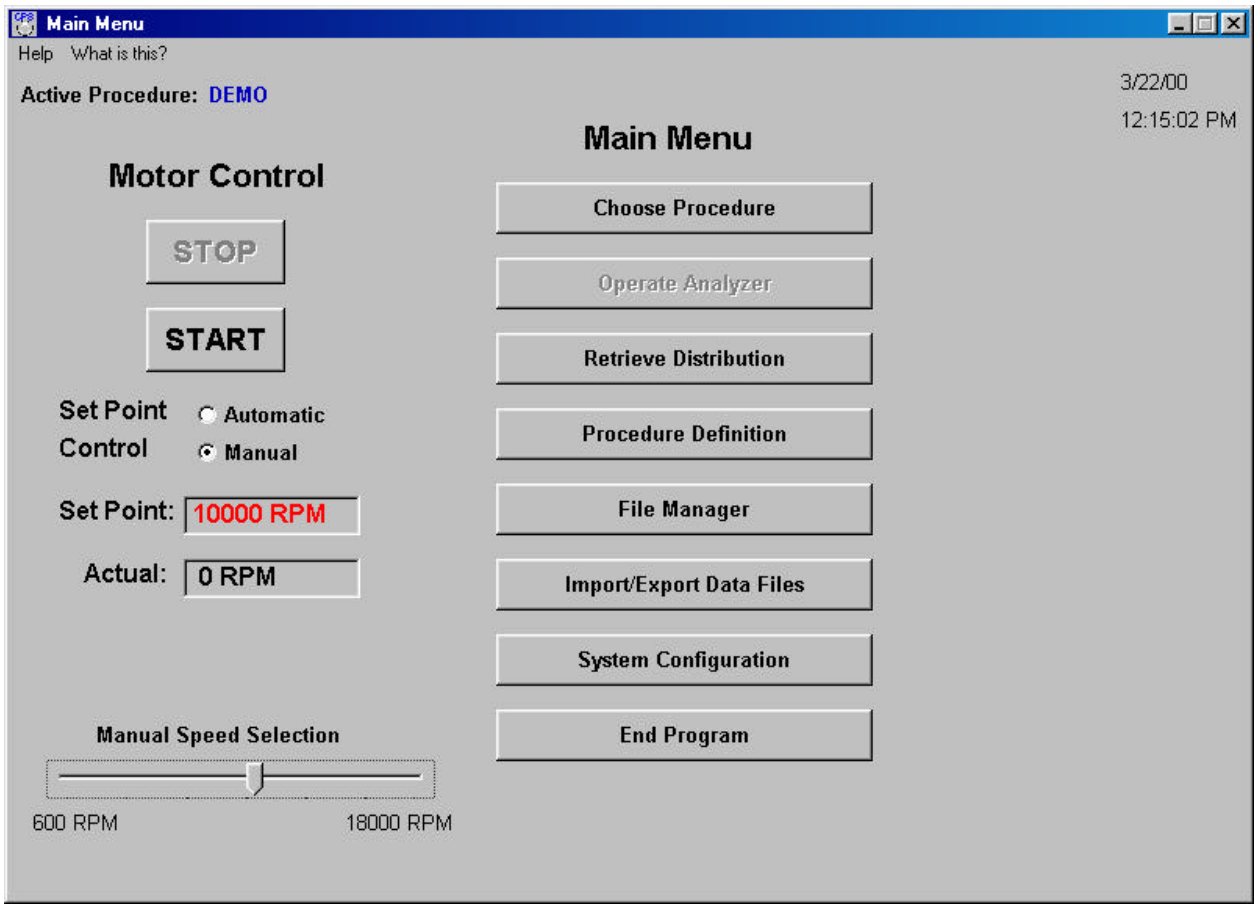
Main Menu Window

Figure 3 shows an image of the Main Menu screen. This window is where each of the main functions of the DCCS can be accessed. Descriptions of the command options follow.

Stop and Start

These commands stop and start the motor that drives the centrifuge. If there is no instrument attached, then all motor controls are hidden; motor controls are only

Figure 3



only available when the software has established serial communication with the instrument. When you start or stop the motor, it accelerates or decelerates over 10 to 140 seconds, depending on the speed set point.

The stop command is disabled if the motor is not spinning. The start command is disabled if the motor is already spinning. The stop command is also disabled when data is being collected from the instrument (even when the motor is spinning!). This avoids the possibility of stopping the motor by mistake while a run is in progress.



This command allows the operator to make any of the available operating procedures the currently active procedure. Activating a procedure allows the following:

1. Analyses may be run using conditions specified in that procedure, and

2. Distributions which were run using the procedure may be accessed by the File Manager utilities (Copy, Delete, Move, or Archive).

Operate Analyzer

This command initiates sample analysis. This command is not available unless the disc is already spinning.

Retrieve Distribution

This command allows you to retrieve, view, and print any distribution that is accessible to the software. "Accessible" means a data file that is stored under one of the system operating procedures, was exported to a disc file, or was archived to a disc file. Exported and archived files can be on either local disk drives (hard or floppy), or on a network drive. Retrieved distributions may be viewed and/or printed singly, in groups of two or more, or combined into average distributions.

Procedure Definitions

This command presents all of the data which defines the current procedure. Each piece of data is presented in a separate field. You can revise any of the fields to modify the current procedure, or can change the current name to an new name and create a new operating procedure. Changing the procedure definition does not change any distributions that have already been run. For example, suppose a procedure, which requires particle size analysis over a range of 3.5 microns to 0.15 microns, is modified to require analysis over 2.5 to 0.15 microns. All of the particle size distributions run up until the change will continue to cover the original range (3.5 to 0.15), while all later distributions will cover only the new range (2.5 to 0.15). The DCCS does not allow you to change the data stored for a distribution once the analysis is completed, except by "recalculating" the distribution using new procedure parameters. The recalculation function is found on the first of the Retrieve Distribution" windows.

File Manager

This command gives access to File Manager utilities. To access the utilities, you must first enter a password. Password protection keeps unauthorized people from deleting

data files. The File Manager allows data files (distributions) to be deleted, moved from one procedure to another, copied from one procedure to another, or archived (which means exported to another location and then erased from DCCS data system). File Manager also allows operating procedures to be removed from the system. The initial password to enter the File Manager is "cps". This can be changed by entering "cps" when the password is requested, and then entering a new password is the provided space. Any combination of characters may be used in the password, including spaces. The password is case sensitive; you must duplicate the password exactly, including upper/lower case.

Import/Export

The Import/Export functions are useful for sending files between two CPS Disc Centrifuge Systems, or to retrieve distributions that have been archived. If you export to a floppy diskette, then keep in mind that an empty diskette will hold only ~ 25 data files. In order to export files, the files must first be selected and "saved" from within the Retrieve Distributions screens. See the section below called **Retrieve Distributions Screens** for information about "saving" distributions for export.

System Configuration

This command gives you access to the system configuration settings. The configuration provides the DCCS information about the environment in which the software in running, including the instrument model number, the serial port being used, the size paper used for printing, and whether the printer is color or black and white. Once the configuration is initially set to the correct values, you should not have to modify it.

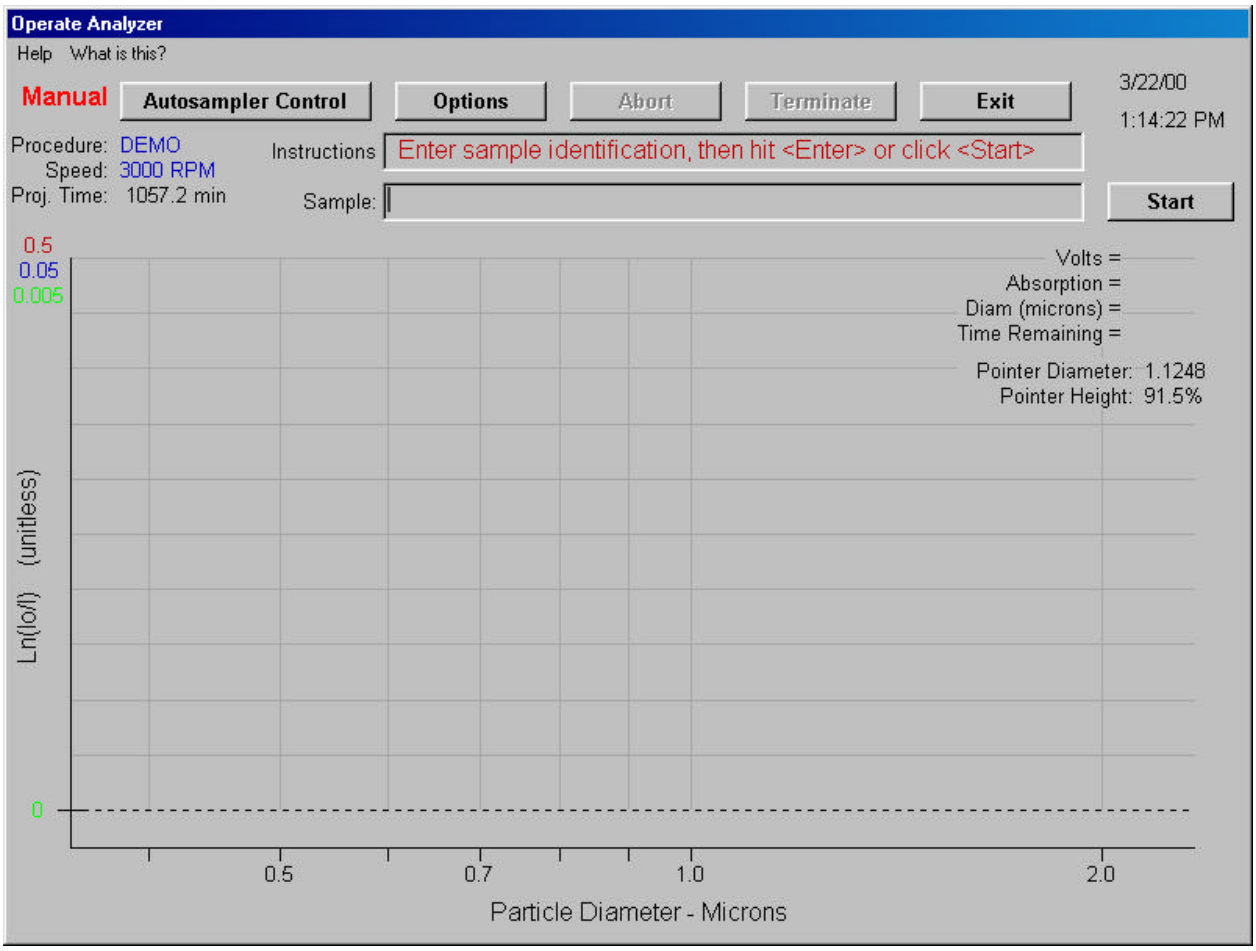
Exit Program

This command terminates the DCCS program and returns to the Windows operating system. This command is not available if the centrifuge motor is in operation. When you exit the DCCS, the procedure you are operating under is **not** retained; the next time the DCCS is started, the default procedure will be activated, even if a different procedure was active when you last exited.

The Operate Analyzer Windows

Figure 4 shows the principle window in the "Operate Analyzer" part of the DCCS. All data collection is under the control of this window. During operation of the disc centrifuge, the DCCS guides you by prompting for actions as needed, and issuing warnings if there is a problem.

Figure 4



Running each sample is normally a three step operation:

1. Enter the sample identification. (This identification becomes part of the data file.)
2. Inject the calibration standard and hit the space bar on the computer at the same time.
3. After the calibration is complete, inject the sample and hit the space bar on the computer at the same time.

The DCCS software collects data over the particle size range described by the procedure definition, and presents the raw data (absorption data) on the screen as the run is in progress. The signal voltage, absorption value, particle diameter, and remaining time for the run are continuously reported on the upper right side of the distribution graphic during both calibration and sample runs.

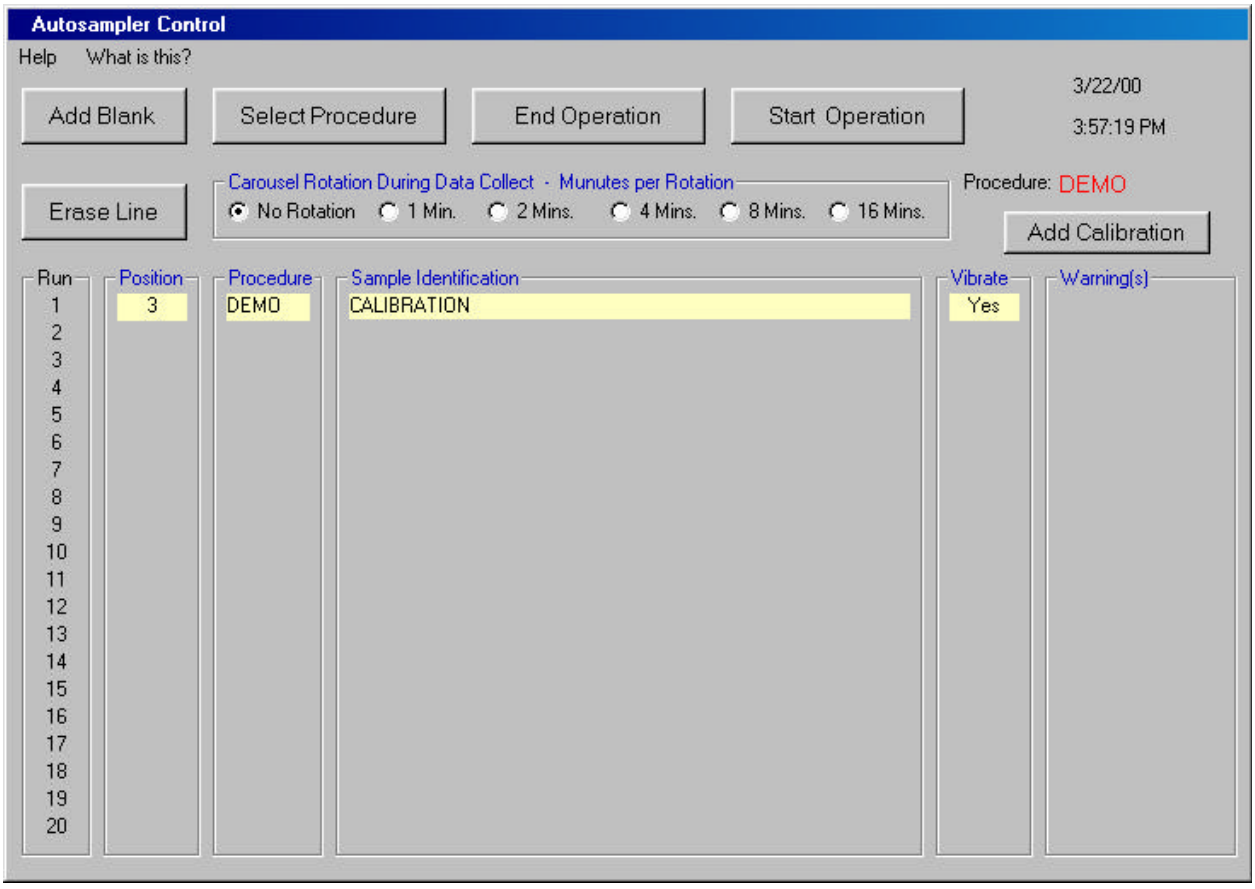
When the analysis is completed, the DCCS converts the raw data into a particle size

distribution in the mode specified by the procedure definition (weight, surface, number, or absorption), and presents the completed distribution. You then click a Next Sample button to start the process over again.

The Terminate command allows you to end data collection (for either a calibration standard or a sample) when you believe that all of the sample has already passed the detector. Terminating data collection early does not cause loss of data.

The Exit command takes you back to the Main Menu window. If you are collecting data, then data collection will continue in the background. Once back at the Main Menu, you can minimize the software to a button on the bottom (or side) of the Windows "Desktop", and work with other Windows applications if you desire.
The Autosampler Control command is only visible if you have configured the system as having an Autosampler. The Autosampler control window is shown in Figure 5.
The lower part of the Autosampler Control window is where you specify the sample identifications, operating procedures, order of sample injections, and whether or not to

Figure 5



vibrate a sample just prior to injection. The upper part the window includes command buttons and options for automatic (slow) rotation of the sample carousel. Rotation insures that samples do not settle while awaiting injection.

You receive detailed information about operation of the Autosampler and entry of data in the Autosampler Control window when you purchase the CPS Instruments Autosampler. The Autosampler allows a total of 20 calibration standards and samples to be injected without operator attention. Please contact CPS Instruments or your local representative for more information about the Autosampler.

The Exit command takes you back to the Main Menu window. If you are collecting data, then data collection will continue in the background. Once back at the Main Menu, you can minimize the software to a button on the bottom (or side) of the Windows "Desktop", and work with other Windows applications if you desire.

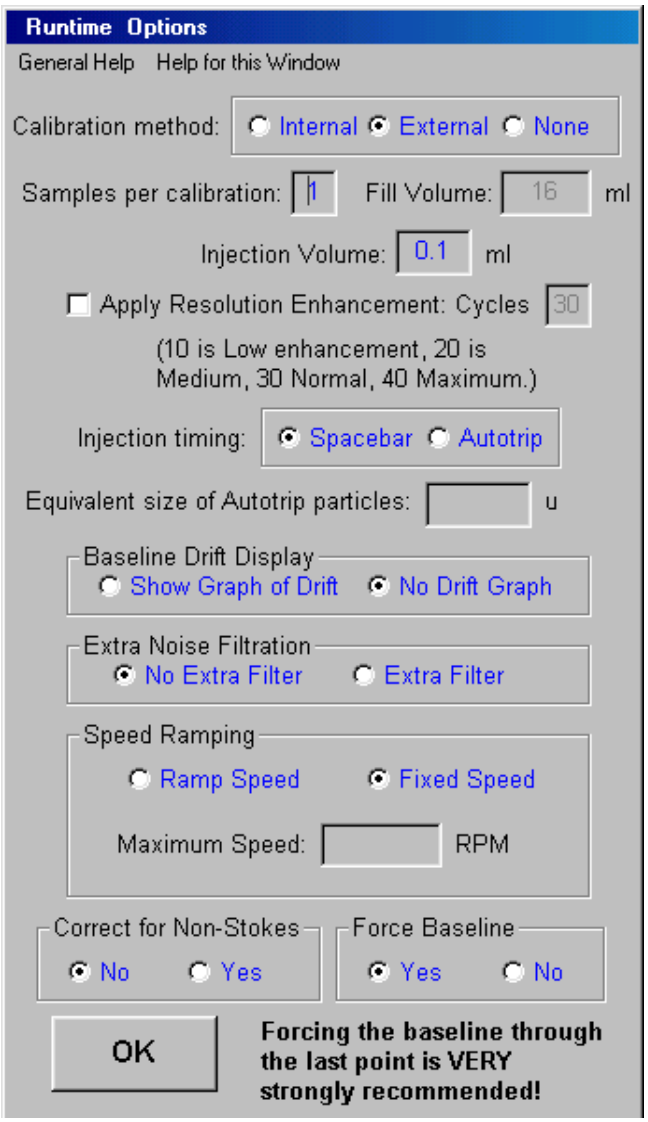
The Abort command ends data collection **and discards any collected data**. You will always be asked to confirm that you want to abort the run when you click this command. Please remember that any particles still sedimenting inside the centrifuge when you abort a run may interfere with the next sample if you do not give them sufficient time to sediment to the bottom of the disc chamber.

There is an Options button on the operate analyzer screen. Clicking this button brings up a window with options for how samples will be run. The window is shown on the next page. The runtime options are:

- *Internal or External Calibration*
- *Number of sample runs per calibration run*
- *Injection Volume*
- *Resolution Enhancement*
- *Injection Timing ("Space bar", or large "Autotrip" particles)*
- *Baseline Drift Display*
- *Speed Ramping*
- *Correct for Non-Stokes Sedimentation*
- ***Force or don't force the baseline through the final data point***

"*External*" calibration means that you will run the calibration standard separately (before) the unknown sample(s), while **"*Internal*"** calibration means that you will mix a small quantity of a narrow standard into each sample. When you select internal calibration, the DCCS software looks for the calibration standard peak inside the distribution of the unknown sample, and then adjusts calibration based on the reported size for the standard peak. (The software takes into account any differences in particle density between the calibration standard and sample.) Please note that you must run the calibration standard in "*External*" mode one time before changing to "*Internal*" calibration. This lets the software know approximately where to look for the internal standard peak. Without an external run, the software might pick the wrong peak as the internal standard.

When you are using "*External*" calibration, you may calibrate at any frequency between once per sample run (that is, just prior to each sample) up to once per 9 sample runs. Please keep in mind that the absolute accuracy of the reported size distribution declines with each injection following a calibration run. Typical loss of accuracy is ~0.1% to ~0.6% per run, depending on operating conditions and type of sample. For maximum accuracy, you should calibrate prior to each run, or calibrate each run internally.



Injection Volume tells the software how many milliliters each injection will be. The default volume is 0.1 ml. The software uses this volume to account for the effect of increasing total fluid volume in the disc with each injection. This parameter is especially important if you will be injecting more than one sample for each calibration run, since the accuracy of second and later measurements will depend on accounting for the increase in volume. Each time the calibration standard is run, the accumulated volume is accurately accounted for, even if the *Injection Volume* value is not exactly correct.

FAQ: How many samples can you run?

You can continue to run samples until the density gradient begins to degrade. The useful lifetime with water based density gradients is normally at least several hours (in most cases 6-8 hours), so long as a cover layer of dodecane or tetradecane has been used to reduce evaporation from the surface of the fluid. The DCCS evaluates the condition of the density gradient by comparing the known broadness of the calibration standard with the measured broadness from the calibration run. The DCCS will not allow a sample to be run if the density gradient has significantly degraded, so you will have to stop the disc and build a new density gradient before resuming operation. If the analysis time for your samples is not too long, then you will be able to run many samples without stoppling the centrifuge.

The ***Resolution Enhancement*** check box tells the software whether or not to apply a deconvolution algorithm at the end of the analysis. The software is able to remove most of the effect of the detector beam width from the final distribution, and so produces a distribution that is close to "ideal", as if the detector beam were near zero width. With a zero width beam, the instrument contributes very little to the total width of distribution peaks. The value for "Cycles" tells the software how hard to work toward removing the effect of the detector beam. More cycles means more beam effect is removed, but at the expense of some increase in noise and a possibly long calculation cycle. The deconvolution process uses a lot of computer power, so if you have a slow computer (less than 300 MHZ), the calculations may take up to 5-10 minutes. Resolution enhancement is really only needed when you have samples with very narrow peaks; most samples have distributions that are so broad that the instrument contributes almost nothing to the measured width of the distribution. You should consider using resolution enhancement if your samples have peaks with a half height width of less than 20% of their peak diameter. (See the Figure 14 in the "Procedure Definition" section for an explanation of half-height width.)

"Space bar" timing means that you must manually strike the space bar on the computer keyboard at exactly the same time as you inject the sample to tell the software to start collecting data. ***"Autotrip"*** timing means that you will add some large particles (of known size) to both calibration standard and samples, and the software will begin collecting data when these particles are detected. The DCCS software looks for a rapid deflection in the instrument voltage, which indicates the passage of the large "trip" particles, and then initiates data collection. If you select "*Autotrip"* timing, then you must also key in the "*Equivalent size*" of the trip particles; which just means the size the particles would be if they were of the same material as the sample. (For example, if you are measuring particles of 1.4 g/ml density in water, but your large "trip" particles are only 1.2 g/ml, then the equivalent size would be (0.2/0.4)^0.5 = 0.707 times the actual size of the large particles, because it will take the less dense "trip" particles longer to reach the detector than if they were the same density as the sample.) "Trip" particles should always have an equivalent diameter that is at least 100% larger than the largest particles you want to measure. This insures that there is no "overlap" of the "trip" and sample particles.

The ***Baseline Drift Display*** option allows you to graphically view the stability of the instrument baseline before injecting a calibration standard or sample. When this option is selected, the software displays a graph of the baseline signal while it is waiting for you to inject a calibration standard or sample. The graph is displayed on the screen as if a calibration standard or sample was being run, even though no injection has been made. When the graph reaches the smallest size on the display, the screen is erased and the graphing process begins again. When you inject a sample and strike the space-bar, the screen is erased and the distribution for the standard or sample is displayed normally. You may want to use this feature if you are running samples that give a relatively weak signal and have a relatively long analysis time, where baseline stability is critical for getting accurate results. The graph of the baseline allows you to

confirm that the baseline signal is stable enough to give a good distribution.

The ***Speed Ramping*** option is only available if you have purchased the optional speed-ramping disc and specified this in the instrument configuration. When you select this option, you specify a second speed (higher than the starting speed), and the software ramps the disc speed very gradually during both calibration runs and sample analyses up to the higher speed. At the end of each run, the software returns the disc to its original speed. Speed ramping is useful in cases where you have samples with very wide distributions. Speed ramping is advantageous when the dynamic range of the sample (ratio of largest diameter to smallest diameter) is greater than about 40. For example, an acrylic latex with particles between 2 microns and 0.075 micron would not require a speed ramping disc, while a polyvinyl acetate latex with particles between 30 microns and 0.1 micron could not be measured except with a speed ramping disc. You receive detailed instructions and recommendations for using speed ramping when you purchase a speed ramping disc.

The ***Correct for Non-Stokes Sedimentation*** option tells the software that you want to automatically adjust for sedimentation that may be outside the "Stokes sedimentation region". The Stokes region is where the Reynolds number for flow of fluid around the particles is completely laminar. The Reynolds number for fluid flow around a sedimenting sphere is given by:

$$Re = (\rho_f V D) / \eta$$

Where ρ_f is the fluid density in **g/cc**
V is the sedimentation velocity in **cm/second**
D is the particle diameter in **centimeters**
η is the fluid viscosity in **poises** (NOT centipoises)

So long as the Reynolds number is less than about 0.01, measurement errors from non-Stokes sedimentation are quite small. For example, assume that you are measuring 2 micron particles in water, and that the detector beam is 1 cm below the fluid surface. If the particles require 2 seconds to reach the detector, then the Reynolds number is 0.01, and the measurement error from ignoring non-Stokes effects is about 0.63%. You should use the automatic non-Stokes correction whenever the maximum Reynolds number is more than about 0.01. Large particles (>10 microns) will normally have Reynolds numbers much larger than 0.01, and so always require correction to get maximum accuracy.

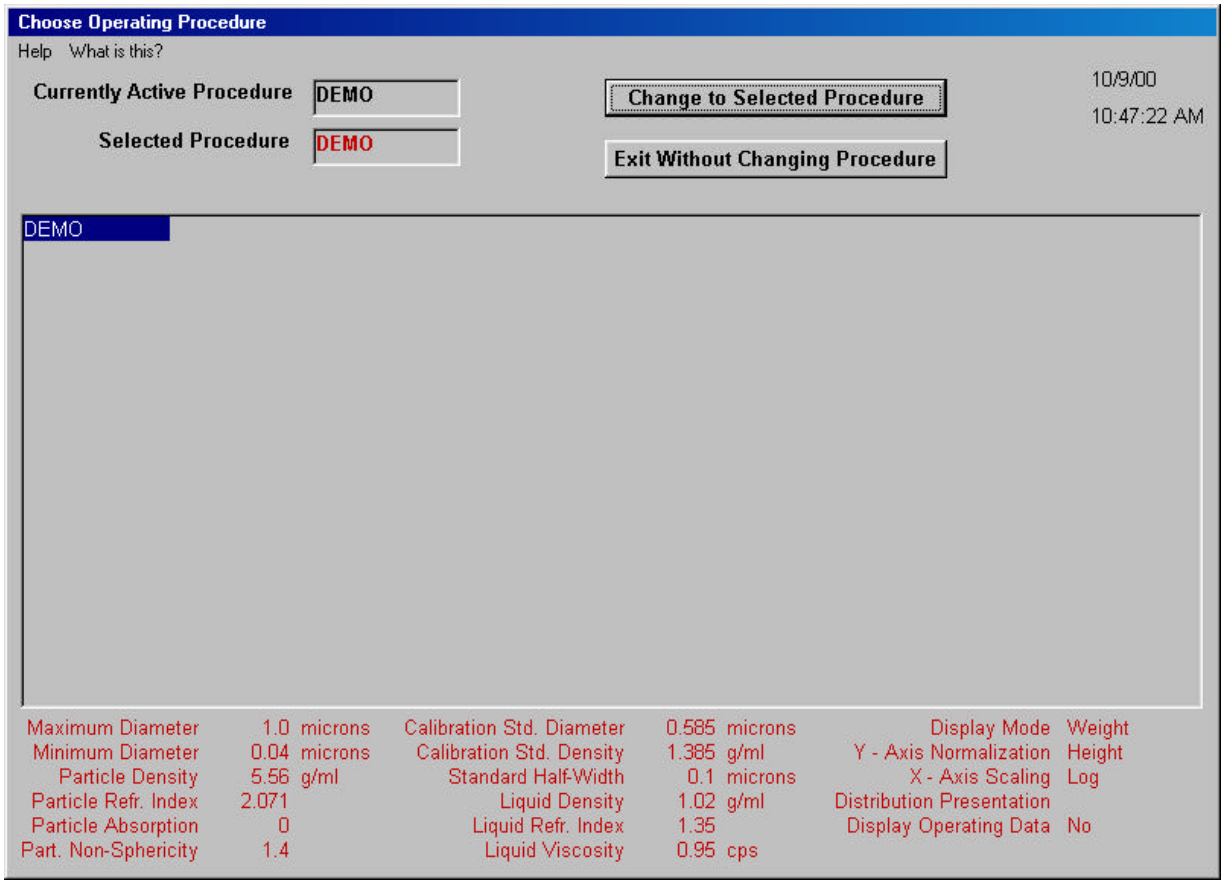
Choose Operating Procedure Window

Figure 6 shows an image of the window where the active operating procedure is selected. The DCCS displays this window when the Choose Procedure command is selected from the Main Menu. This same window is also used in several other places within the software, for example, when you specify a destination for data files that are

being imported.

Each available operating procedure name is listed on the screen. You select the procedure by pointing at the name with the mouse and pressing the left mouse button once. The procedure name is then shown in the "Selected Procedure" box near the top of the screen. At the bottom of the screen, all of the information about the procedure is displayed in red letters. This information can be used to confirm that the correct procedure has been selected.

Figure 6



An unlimited number of procedures can be displayed. If there are too many procedures to fit on the window, then a scroll bar is automatically added so that you can scroll down though all of the procedures. Once the desired procedure has been selected, click the Change to Selected Procedure command to return to the Main Menu. Upon return to the Main Menu, the DCCS will be operating under the newly selected procedure.

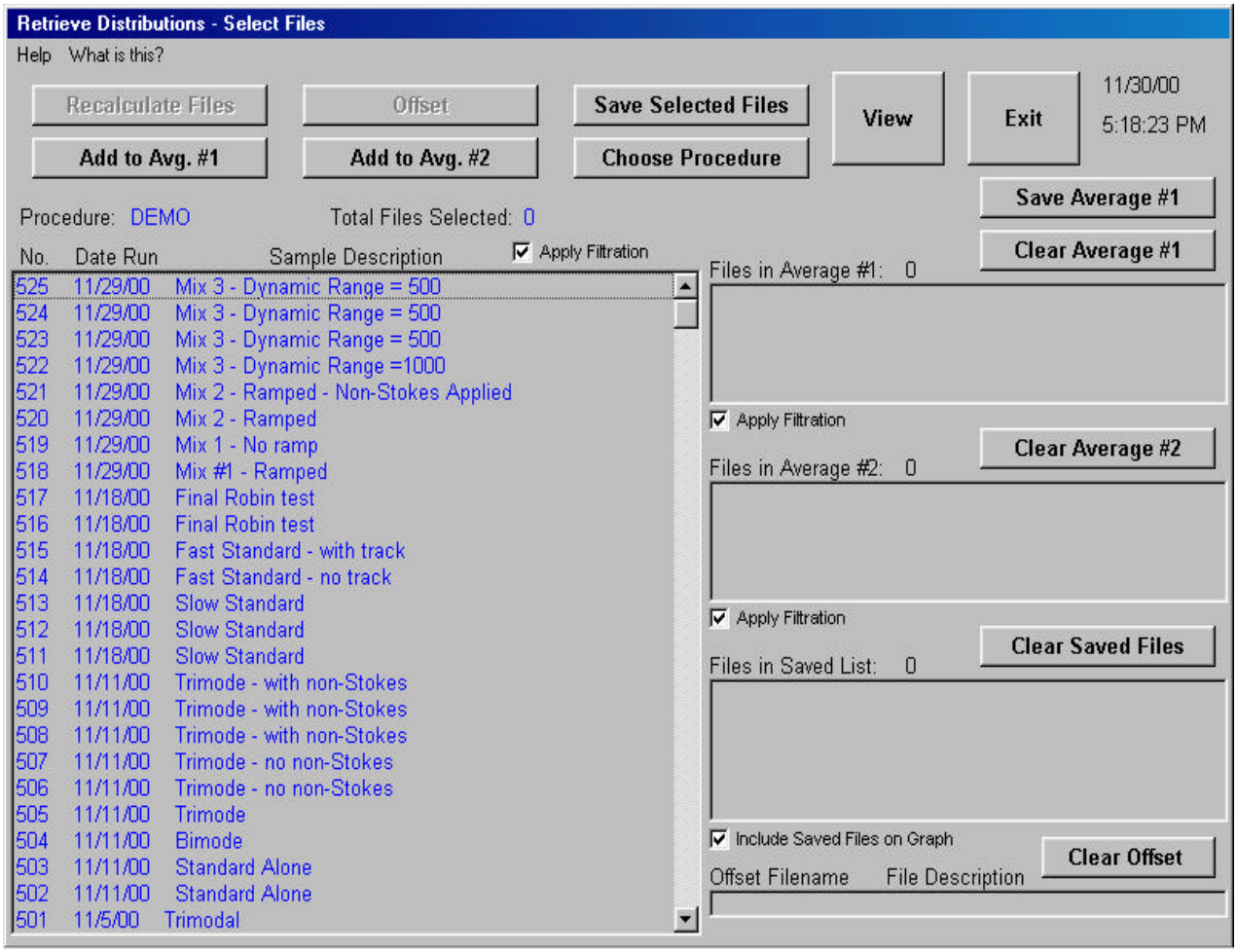
Retrieve Distribution Windows

The retrieval of stored distributions is done with two different windows that work together: one to select the desired distribution(s), and a second to view the distribution(s), modify presentation, and print if desired.

1. Select Files Window

Figure 7 shows an image of the Select Distribution window. This window serves several different functions:

Figure 7



a. Provides access to data files that have been stored under any operating procedure
b. Allows you to view distributions in “exported” or “archived” files (outside of the DCCS data structure)
c. Allows you to generate averages of multiple files
d. Allows you to generate a permanent “average file” that is the average of files that are placed in Average #1.
e. Allows you to “save” data files for later export
f. Allows “recalculation” of an existing data file using the current procedure

definition.

g. Allows one data file to be subtracted from one or more others (“Offset”)

Selecting Files

The Select Files window offers powerful options for comparing and evaluating size distributions. All data files in the current operating procedure are listed in the box on the lower left of the window. The files are listed in reverse order, with the most recently run file at the top of the list, and the oldest file at the bottom. The file number, the date the file was created, the operating procedure under which the file was created, and the sample identification information are all shown. The sample identification is the information entered by the operator when the sample was run on the analyzer. When there are many files, a scroll bar is shown on the right side of the list to make it easier to look at all of the files in the list.

To "select" a file for viewing, you point at the line which has the desired file, and then press the left mouse button one time. The file information is highlighted in beep blue when a file has been selected. To "unselect" the file, just point at the file a second time and press the left mouse button again; the blue highlight disappears, indicating that the file is no longer selected for viewing.

When there are many files in the list, you may be selecting files from widely separated parts of the list, so the DCCS helps you keep track by printing the number of "selected" files near the top center of the screen. Each time a file is "selected" or "unselected", the number increases or decreases by one. When the View command is clicked, all of the "selected" files are shown together on a single graph. Viewing two or more files together produces a direct (overlay type) comparison of the distributions. You may select a total of up to 20 distributions for overlay comparison. (The total of twenty overlay distributions includes Average #1, Average #2, and “Saved” files. See the below sections for explanations of what these other files are.)

Averages #1 and #2

If you click the Add to Average #1 command with one or more files already selected, then the DCCS copies the file information into the “average #1" file list. You can add as many files to this list as desired. When you later click the View command, the DCCS will calculate the mathematical average of all the distributions in the Average #1 list, and present the average on the View window. The Add to Average #2 command works in exactly the same way, but creates a separate average. If you have files in both Average #1 and Average #2, then you will see a comparison of averages when you click the View command. If you have files in one or both average lists and also have selected individual files, then you will see a comparison of “individuals” with “averages”. This especially useful in quality control applications when you need to see how much an individual sample deviates from the “average” sample. The averages have many other uses, including:

a. Comparing the average distribution from two different processes or procedures.

b. Creating distributions for hypothetical mixtures of individual samples; for example, add sample A to the average 7 times, sample B 2 times, and sample C

1 time, then the average is a distribution for a 70:20:10 mixture.

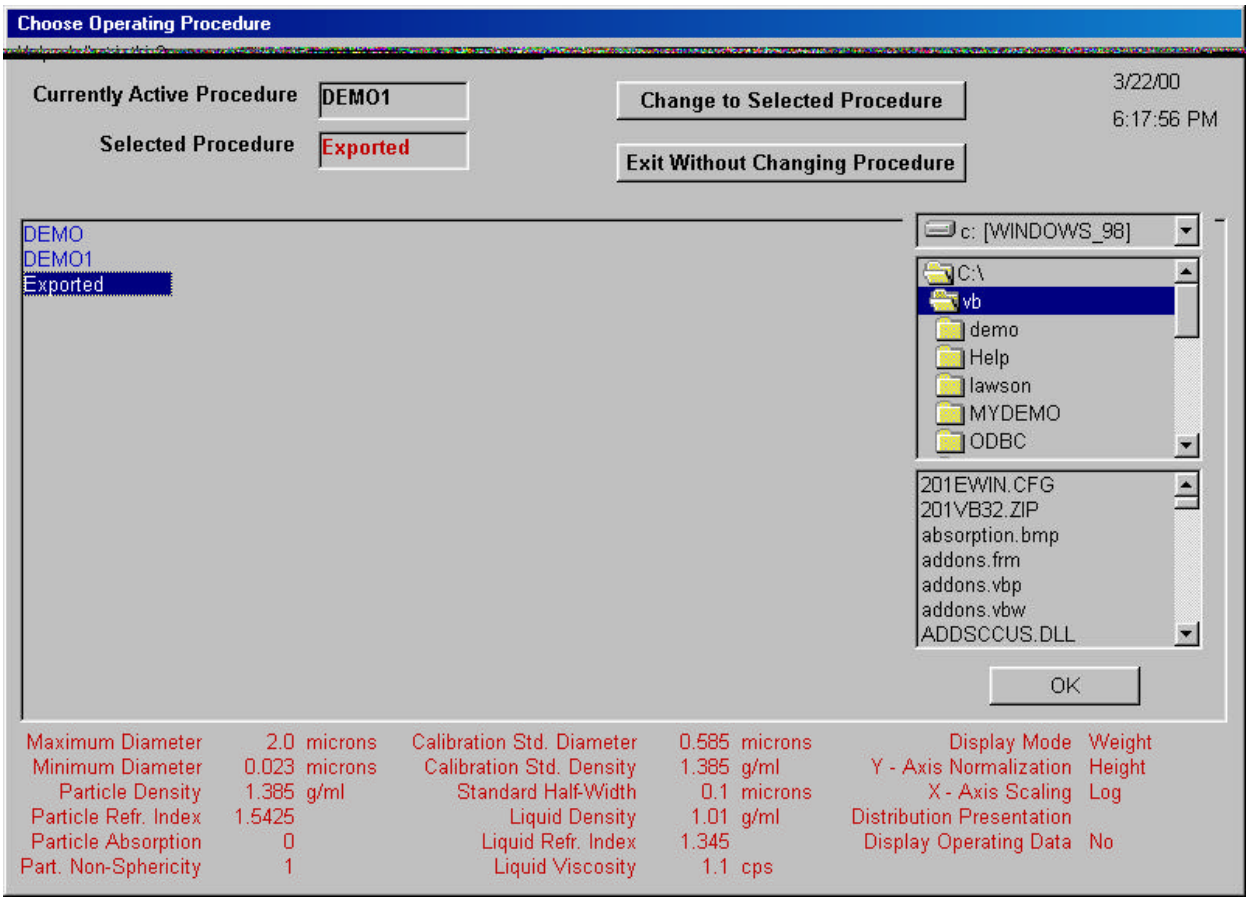
c. Evaluating long term trends in a process (average May 2000 versus 1999).

d. Creating permanent data files that are the mathematical average of the files placed in Average #1. You create a permanent data file by clicking the Save Average #1 command button.

Choosing Procedures

Within the Retrieve Distributions windows you are not limited to working on the currently active operating procedure (the operating procedure used in running the Disc Centrifuge); you can retrieve **any** data file that the DCCS has access to. When you click the Choose Procedure command, you will see normal the Choose Procedure

Figure 8



window (the same used to select the active procedure), showing all the operating procedures that are available. When you select one of the other operating procedures, the list of files shown on the “Select Files” window will not be the files for the active procedure, but rather all the files in the newly selected procedure. When activated from within the Retrieve Distributions windows, the Choose Procedure window has an additional “procedure” called “**Exported**”. When you select “exported” as the procedure, you are given access to any exported data files that are available to the computer, including files on network drives. The Choose Procedure window with

"Exported" selected is shown in Figure 8.

You select the disc drive and directory where the exported files (or archived files) are located, using the selection boxes on the right side of the window, followed by the OK command. You must confirm that the exported files are present in the directory. These files always have files names that begin with "999" followed by a file number identification (for example 99900001.dat, 99900002.dat, etc.). You will also see the file "Export.ctr", which is a counter file the software reads to know how many exported files are present in the directory you have selected. **Exported and archived files are identical for the DCCS**, and are imported and viewed in the same ways.

"Saved" Files

The saved file list is on the lower right of the Select Files window. Data files are added to this list by first selecting the files (just as if you were going to view them) and then clicking the Save Selected Files command. This copies the selected files onto the Saved Files list and "unselects" the files. The saved file list has two important functions:

a. Allows files from different operating procedures to be directly compared, and
b. Allows files to be selected for later "export" using the Import/Export window of the DCCS.

You have the option to include saved files in the files that are viewed, or to exclude these files from the files that are viewed. When you want to compare files from different operating procedures, you simply copy the files you want from each procedure onto the "saved" list, and view the files together. All the files that are on the Saved list are available for export using the Import/Export command from the Main Menu.

> ***Hint: Save Time "Unselecting" Files***
> *If several files are already selected for viewing, but you no longer want to view those selected files, then you need to "unselect" the files before selecting other files for viewing. You can save yourself time by just clicking the* Save Selected Files *followed by the* Clear Saved Files *command. This process quickly "unselects" all selected files.*

Offset Distribution

When a single file has been selected, the Offset command is enabled. When you click this command, the single selected file is copied to the "Offset Filename" box on the lower right side of the screen. When you select other files for viewing, the "Offset" file is subtracted mathematically from the other distributions. You can adjust the amount of offset on the "View Distributions" window. An offset file is subtracted from **all other distributions,** even average distributions. Offset is useful for removing internal calibration peaks, and for "separating" a known distribution from a mixture with an unknown distribution.

Recalculating Distributions

You may sometimes need to change the physical parameters that the DCCS uses

during calculation of a particle size distribution. For example, you may discover that a sample has a different particle density than at first believed, or perhaps an incorrect value was entered for particle refractive index, and the error was discovered only after a series of samples have already been run. Rather that repeating all the analyses that were run with incorrect parameters, you can just put the correct parameters in the procedure definition (and remember to save the corrected procedure definition!). Once the parameters in the procedure definition are correct, select the distributions that you want to recalculate by clicking those files (just like you would to view the files), and then click the Recalculate Files command. You will be asked to confirm that you want the files recalculated before the recalculation is started. Once the files have been recalculated, the distributions will look the same as if they had been run using the correct parameters. You can recover from errors in any of the following parameters:

Particle density
Particle refractive index
Particle light absorption
Particle non-sphericity
Fluid density
Fluid refractive index
Calibration standard density
Calibration standard size

Great care should be used when you are going to recalculate a distribution, since **ALL** of the parameters in the current procedure definition will be applied to the original data during the recalculation. The software will carry out the recalculation exactly in accordance with the current procedure definition, just as if you were running the sample again on the disc centrifuge, so you must be certain that all of the parameters are correct before you recalculate. If there are any errors in the parameters used to generate a **recalculated** distribution, then it becomes more difficult to backtrack and return to the original parameters, since the revised parameters (not the original ones) are stored in the data file each time the distribution is recalculated. The recalculated distribution appears just as if the revised parameters had been in the procedure definition when the analysis took place. If you look at the operational data for the file following recalculation, all of the revised parameters are present, not the original ones.

The DCCS generates (or adds to) a special log file called " mmm0nnnn.ORG" whenever you recalculate a distribution. The main part of the file name (" mmm0nnnn") is the same as the calculated data file (.DAT) and raw data file (.RAW) from the same analysis. This file is an ASCII text file, and consists of 21 data lines (the original file header lines, prior to recalculation) followed by a line of repeated asterisks "**********". Each time the same data is recalculated, 22 new lines are added to the end of the .ORG file. You can recover the original parameters, as well as a history of recalculations, by examining the .ORG file with a word processor or with Windows Notepad. The most important parameters are in the file according to this scheme:

Line 1: Original procedure name
Line 4: P(particle density)C(standard density)L(liquid density)
Line 6: Diameter of calibration standard
Line 9: Refractive index of particles

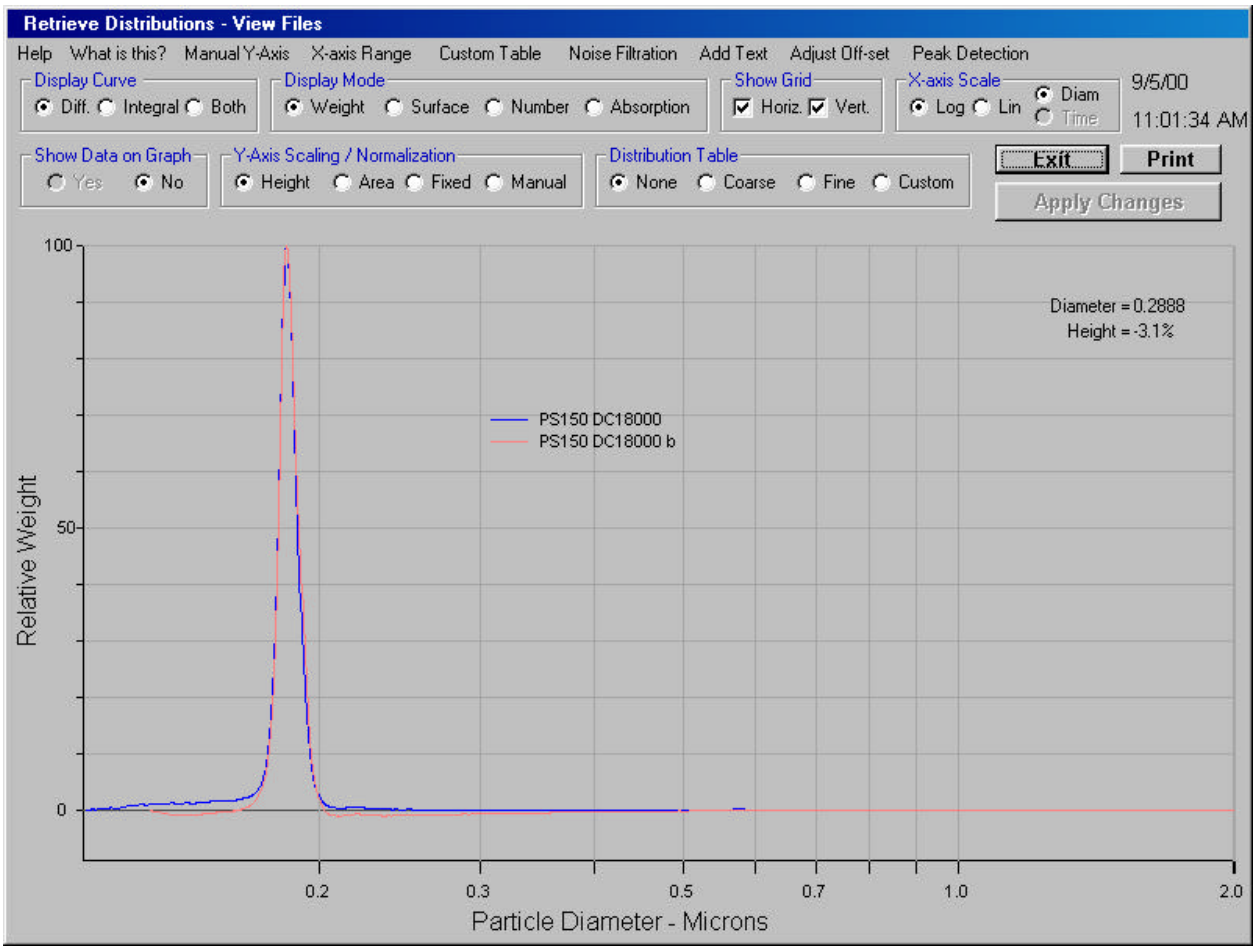
Line 10: Refractive index of liquid

By reviewing the contents of the .ORG file, it is be possible (with some effort) to return to the original operating conditions in case a recalculation is done with incorrect parameters. **It is always best to recalculate only when you are certain that all the parameters in the current procedure definition are correct.**

2. The View Distribution Window

Figure 9 shows an image of the View Distribution window. The View Distribution window presents a graph of one or more distributions, and allows the presentation of the distribution(s) to be revised and printed in the desired format. All presentation options that are part of the procedure definition can be modified.

Figure 9



The Exit command returns you to the Select Distributions window, and the Print command displays the "Print Options" window (described below). All presentation option buttons are near the top of the window. The presentation options are initially set for the values that are in the procedure definition being used on the "Select Distributions" window. The presentation options follow.

<Display Operating Data> (Yes, No)

When you are viewing a single distribution and select “Yes”, basic information about the analysis is displayed on the screen inside a box. If you print the distribution with the Display Operating Data option set to “Yes”, then the DCCS will print both a graph and a detailed data page that describes the conditions under which the analysis was run, the graphical presentation format, and distribution statistics (mean, median, coefficient of variation, etc.) If you are viewing more than one distribution (overlay comparison) and select “Yes”, then no box is display on the screen, but a separate data page will printed for each distribution when you print the graph. The data page for **average** distributions has a slightly different format than for individual distributions. A data page for a single distribution is shown in Figure 10.

Figure 10

CPS Disc Centrifuge Summary of Operating & Distribution Data

********** Summary of Distribution **********
Distribution Identification: DEMO1\00200002 - SiO2 30N50PHN 008/VH00340 #1
Date of Analysis: 2/17/00
Time Completed: 5:26:24 PM
Date Printed: 3/23/00
Time Printed: 6:03:16 PM
Maximum Diameter in this Distribution: 0.4984
Minimum Diameter in this Distribution: 0.0254
Disc Speed: 18000 RPM
Cal. Standard Diameter: 0.585 Microns
Cal. Standard Density: 1.385 g/ml
Particle Density: 2.1 g/ml
Fluid Density: 1.01 g/ml
Number of Data Points: 1142
Analysis Time: 13.76 Minutes

********** Graphical Presentation Data **********
Graph Mode: Weight
Differential Curve: Yes
Integral Curve: No
Off-Set: --- None ---
Noise Filtration: --- None ---
Maximum Graphed Diameter: 0.4984 Microns
Minimum Graphed Diameter: 0.0254 Microns
X - Axis Scaling: Log
Y - Axis Scaling: Height Normalized
Peak Detection Factors:
Mininum Height: 5.0% of full scale
Detection Window: 5.0% of peak diameter
Detected Peak Diameters (Microns)
0.0736

Total Weight: 2578.16 Micrograms

Distributions Statistics:

Weight Mean: 0.0747	Weight Median: 0.075	Weight CV: 15.43%
Surface Mean: 0.0721	Surface Median: 0.0739	Surface CV: 18.35%
Number Mean: 0.0624	Number Median: 0.0703	Number CV: 27.08%
Absorption Mean: 0.081	Absorption Median: 0.0784	Absorption CV: 27.07%

Polydispersity Index: 1.197 (Weight Mean / Number Mean)

<Display Curve> (Diff., Integral, Both)
Any particle size distribution can be displayed in to different ways: the differential distribution, which shows the quantity of material at each size, and the integral (or

cumulative) distribution, which shows the total quantity larger than the minimum size at each size in the distribution range. These two distributions contain the same information, and each can be derived from the other by mathematical manipulation. The differential distribution is converted to the integral distribution by integration with respect to diameter, and the integral distribution is converted to the differential distribution by applying differentiation with respect to diameter. You have the option to display either or both of these curves.

<Display Mode> (Weight, Surface, Number, and Absorption)
The distribution can be displayed as a weight distribution (weight of particles plotted against diameter), surface area distribution (surface area of the particles plotted against diameter), number distribution (number of particles plotted against diameter), and absorption (light absorbed/scattered plotted against diameter). The absorption distribution is the raw data that comes from the instrument. The absorption distribution is converted to the other distributions by applying "correction" factors that are based on the efficiency of light scattering/absorption by the particles at different sizes. The weight, surface, and number distributions are different representations of the same data; each of these distributions can be calculated from either of the others. The DCCS stores only the absorption and weight distributions when a sample is run; the surface and number distributions are calculated from the weight distribution as needed.

<Y-Axis Scaling/Normalization> (Height, Area, Fixed, Manual)
When one or more distributions are plotted, there are several ways that the same y-axis data can be shown. Which of these is best depends on what you are trying to learn about the distribution(s).

When you select "Height" scaling, each distribution will be plotted so that the highest point in that distribution is scaled to 100% of the y-axis.

When you select "Area" y-axis scaling, one of the distributions is scaled to 100% at its highest point, and all other distributions are scaled so that the total **area** under each distribution is equal. In this case, different distributions will have different maximum heights.

When you choose "Fixed" scaling, the y-axis scale maximum value is set equal to the highest **absolute** point found among all of the selected distributions. This point will be at 100%, and all other points in all other distributions will be scaled relative to this maximum point. "Fixed" scaling allows you to see the absolute distributions, so if you inject the same sample at several different concentrations, then you will see distributions with the same shape, but with very different heights; in other words, you see the amount of injected sample. "Height" and "Area" scaling hide the amount of sample injected, and make replicate runs appear identical, even if different amounts of sample were injected.

When you choose "Manual" scaling, you can manually select the y-axis maximum value. Manual scaling allows you to expand the y-axis so that you can look at the details of the particle size distribution that are too small to be seen at normal y-axis size. To set the

manual y-scale value, you first select “Manual” scaling, then click on the menu item “Manual Y-Axis” that is at the top of the screen. You see a text box where you can key in a manual value, or you can use the mouse to point at the maximum y-value (on the graph) that you want.

> ***FAQ: What Y-Axis Scale method is best?***
> *The best scaling method depends on what you want to learn about your distributions. If you are comparing distributions that are very similar in size and width, then “Height” scaling is adequate to show differences. If you are comparing distributions that are significantly different in size and width, then “Area” scaling is probably best, since the differences become more obvious, especially differences in width. If you are concerned about the absolute quantity of material (such as “How many micrograms of the active component is in this sample?”), then you should use “Fixed” scaling or “Manual” scaling.*

<Show Grid> (Check boxes: Horizontal and Vertical)
When these boxes are checked, the graph is drawn with faint “grid” lines which line up with x-axis and y-axis markers. When these boxes are unchecked, the graph is drawn without a “grid”.

<X-Axis Scale> (Log or Linear)
You can choose whether the x-axis scale will drawn with a log or a linear format. If you select the linear format, then the distribution will initially be drawn between the maximum diameter in the distribution and zero. If you select the log format, then the distribution will be initially drawn with a log scale where the minimum size is the smallest size in the distribution and the maximum size is the largest size in the distribution. The log scale can cover up to 3 orders of magnitude between the smallest and largest sizes.

> ***FAQ: Should I use a log or linear x-axis scale?***
> *If your distributions cover a relatively narrow size range (for example, 0.3 microns to 1.5 microns), then a linear scale is adequate and is more intuitive to interpret. If your distributions cover a relatively broad size range (for example 0.04 micron to 2 microns), then a log scale is better, because it avoids having the small end of the distribution “compressed” into a small portion of the graph. Log scaling also shows “relative” width more clearly: a 1.5 micron peak with a total width of 0.3 microns appears to be “narrow”, while a 0.4 micron peak that is 0.3 microns wide appears quite “broad”. The terms “narrow” or “broad” are normally used to describe the distribution width* ***relative to the mean particle diameter****.*

Please note that the distribution curve changes in shape when you change between log and linear x-axis scaling. The shape changes because the height of the curve (y-axis) must be adjusted to account for the change in the x-axis scale. With a linear scale, the

physical distance on the graph between pairs of points that are the same distance apart (in microns) is independent of size, while this physical distance depends on size when you use log scaling. For example, consider the distance between 1 micron and 1.1 micron and compare it to the distance between 0.2 micron and 0.3 micron. On a linear scale these pairs of points are the same physical distance apart along the x-axis, but on a log scale, the 0.2, 0.3 micron points are more than 4 times further apart than the 1.0, 1.1 micron points. A log scale always plots small diameter peaks with less height, and large diameter peaks with more height, compared to a linear scale. The adjustment preserves the "area" under the curve as you change x-axis scale.

> ***FAQ: What do the units on the Y-axis scale mean?***
> *Most particle sizing instruments report a size distribution in only "relative" terms; which means that the y-axis scale can simply be labeled "Relative Weight" or "Relative Number". You have the option to view your distributions this way, but you can also view them using an "absolute" scale, where you see the actual weight, actual surface, or actual number of particles over the entire range of sizes. When you use linear scaling, the y-axis units are "Micrograms per Micron" (in weight mode), "Square microns per Micron" (in surface mode), or "Particles per Micron" (in number mode). If you integrate the distribution over the entire range of sizes you generate the total weight, total surface, or total number of particles in the sample. (Note that the units are consistent: (weight/micron) x (microns) = weight!) When you view the distribution using log x-axis scaling, then the y-axis units change to simply "Weight", "Surface", or "Number", in order to preserve area under the curve (see above paragraph).*

<Distribution Table> (None, Coarse, Fine, Custom)
You may want to know the fractions of the distribution that are between different particle sizes. For example, your application may require that less than 5% of the particles be larger than 1 micron or smaller than 0.1 micron. The distribution table shows the fractions in different size ranges or "channels". "None" means no table, "Coarse" means five evenly spaced "channels" across the distribution, "Fine" means 10 evenly spaced "channels" across the distribution, and "Custom" means that the channel breaks are set according to sizes that are either: a) entered as part of the operating procedure definition, or b) manually selected on the View Files window. To manually set "custom" channels, select the Custom option, and then click the "Custom Table" menu at the top of the screen. You can then either key in the channel limits you want, with limit values separated by commas, or use the mouse to point at the limits you want on the graph. When you move the mouse pointer over the graph, a red vertical line appears. Each time you click the left mouse button, a new channel limit is recorded. You can edit the limits manually to get "round numbers" for the channel limits; just be sure that each limit is separated by a comma.

When a single distribution is being viewed, the distribution table is displayed on top of the distribution graph, and you can move the table around on the screen using the "click-and-drag" technique, or minimize the table to "command button" at the bottom of the window by double-clicking the table. When two or more distributions are displayed,

the tables for these distributions are not shown, but are printed as part of the "data page" when you have selected yes for the "Show Operating Data" option.

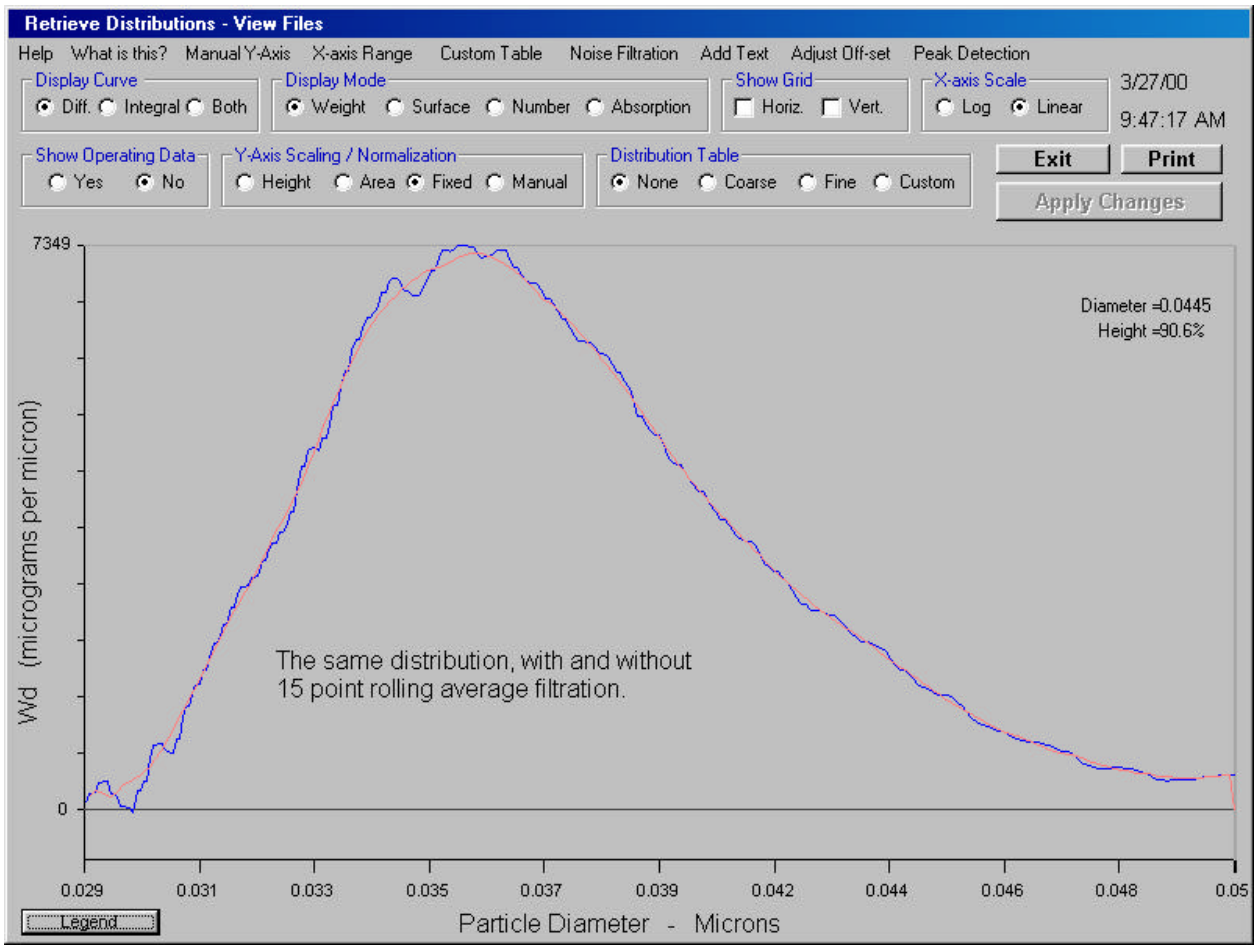
Hint: Using Distribution Tables in QC Applications
Quality control specifications often have "not more than x larger than" or "not less than y larger than" values for particle sizes. You can use a custom distribution table, along with the appropriate channel limits, to give an automatic "yes or no" result when a sample is run; "yes", the product is in specification, or "no" the product is out of specification.

The Menu Bar
The top of the View Files window has a "Menu Bar" with several different options. Some of these menu items work with the option buttons described above (Manual Y-axis, and Custom Table). The others are:

X-axis Range
By default, you view the entire distribution, but you can click "X-axis Range" to choose only a portion of the distribution for viewing. You select the desired range by moving the mouse pointer over the distribution graph; the DCCS draws a red vertical line, and you move the line to the lower and upper limits you want to view, each time clicking the left mouse button. You can look at any portion of the distribution that you desire. If you view less than 100% of the distribution, you should keep in mind that all distributions statistics are calculate based **only on the portion of the distribution you are viewing.** If you print the distribution, the graph that is printed will only cover the size range that you have selected. You can return to the full distribution width by clicking the "X-axis Range" menu option, followed by clicking the **right** mouse button when the mouse pointer is over the distribution graph.

Noise Filtration
Sometimes, you may have a distribution with a very weak signal that appears "noisy" when plotted on the View Files window. You can improve the appearance of a noisy distribution by selecting the "Noise Filtration" menu option. You can adjust the amount of noise filtration, from no filtration (a "1-point" rolling average) to a 51-point rolling average. High rolling average values strongly reduce noise, but also reduce the resolution of the distribution. In most cases, between 5 to 15 points is sufficient to substantially improve a noisy distribution, without too much loss of resolution. An example of the effect of added noise filtration is shown in Figure 11.

Figure 11



Add Text

Clicking the Add Text menu option displays a text entry box near the top of the distribution graphic. You can key in any text that you desire, and choose the font size for that text. If you want to enter multi-line text, just hit the <Enter> key to start a new line. When you are finished entering text, hold down the <Shift> key and hit the <Enter> key. You can move the text to any location on the graphic by pointing at the text with the mouse, holding down the left mouse button, and dragging the text to a new location. You can erase the text by just "double-clicking". You can add up to 20 different text entries to your graph. The text on Figure 11 was added to the graph using the Add Text menu option.

Adjust Offset

If you are using an offset distribution (that is, subtracting one distribution from one or more others), then you can adjust the "amount" that is subtracted by clicking the Adjust Offset menu option. You can adjust the offset from 0.0% to

200.0% of the original distribution, in steps of 0.1%.

Peak Detection

The DCCS software determines what is a “peak” in the distribution based on two peak discrimination factors: Peak Height and Window Width. For a peak to be reported, it must be higher than the Peak Height value, and must also be the highest point in the distribution within a “window” that is described by the Window Width. The Peak Height is in units of % of full scale, and the Window Width is in units of +/- % of peak diameter. Peak Detection parameters allow the software to discriminate between real peaks and false peaks that are just the result of signal noise.

<Print>

Once the distribution is displayed in the format you want, you can print the distribution using the Print command. You can print a full page graph, a half page graph, or an image of the View Files window. (A printed image of the window is only available if you have specified a color printer in the system configuration.) You can print up to 99 copies, and you can add number identifiers to the individual curves if desired. The number identifiers are small black numbers that are printed on top of each distribution line. Number identifiers help you identify each curve in a multiple overlay comparison, especially if you have black and white printer.

Hint: Printing a “clean” distribution graphic.

You may not want to have all of the information that is displayed on the View Files window printed along with your distribution. Any object that the DCCS places on the graphic can be “minimized” to a button at the bottom of the screen by double clicking the object. Any minimized object is ***not printed*** *with the distribution(s). You can later restore minimized objects to their original locations by clicking each minimized button with the mouse. You can also* ***move*** *any object that is added to the graphic using “click-and-drag”, so that no object obscures the distribution.*

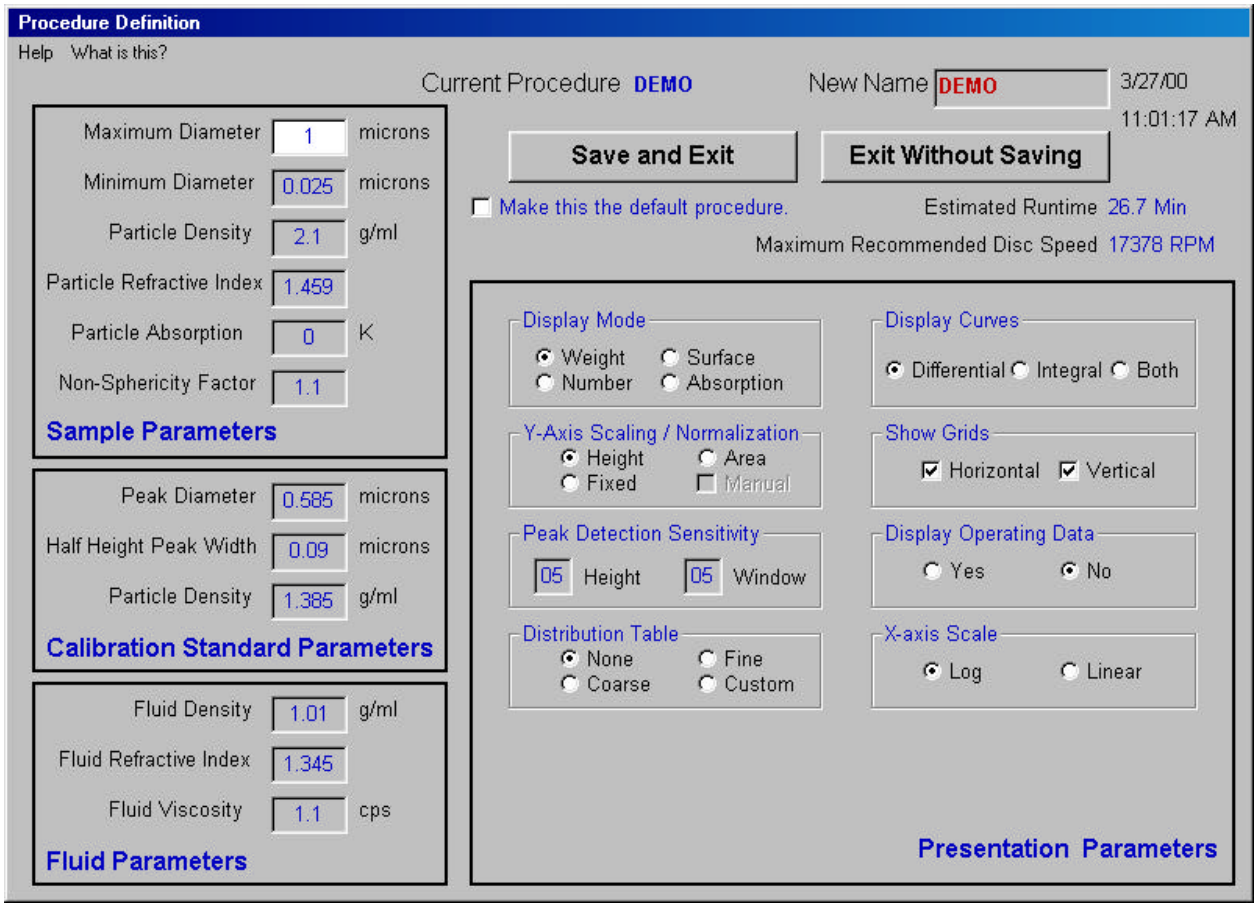
FAQ: How can I put the printed output in a Word or WordPerfect document?

The software prints to whatever you have set as the “default” Windows printer. If you want to place the printed output in a word processing document, then you need to have the output in a form that can be imported, not sent to a printer that creates an image on paper. The simplest way to do this is to use what is called a “print driver” to create a graphic format file that can be added later to a document. Several of these are available for a modest price; we recommend installing the “Miraplacid Publisher" (www.miraplacid.com), which generates either JPEG or BMP files.

The Procedure Definition Window

Operation of the CPS Disc Centrifuge is always based on an “Operating Procedure”, which includes all relevant physical data about the unknown sample, calibration standard, and density gradient fluid, as well as the desired analysis range (maximum and minimum sizes) and format in which the data will be presented. The accuracy of your particle size results depends on having a Operating Procedure with accurate values for several key parameters. The Procedure Definition window is where Operating procedures are created or modified. Figure 12 shows the Procedure Definition window. All physical parameters are on the left size of the screen, while all presentation parameters are on the right side of the screen.

Figure 12

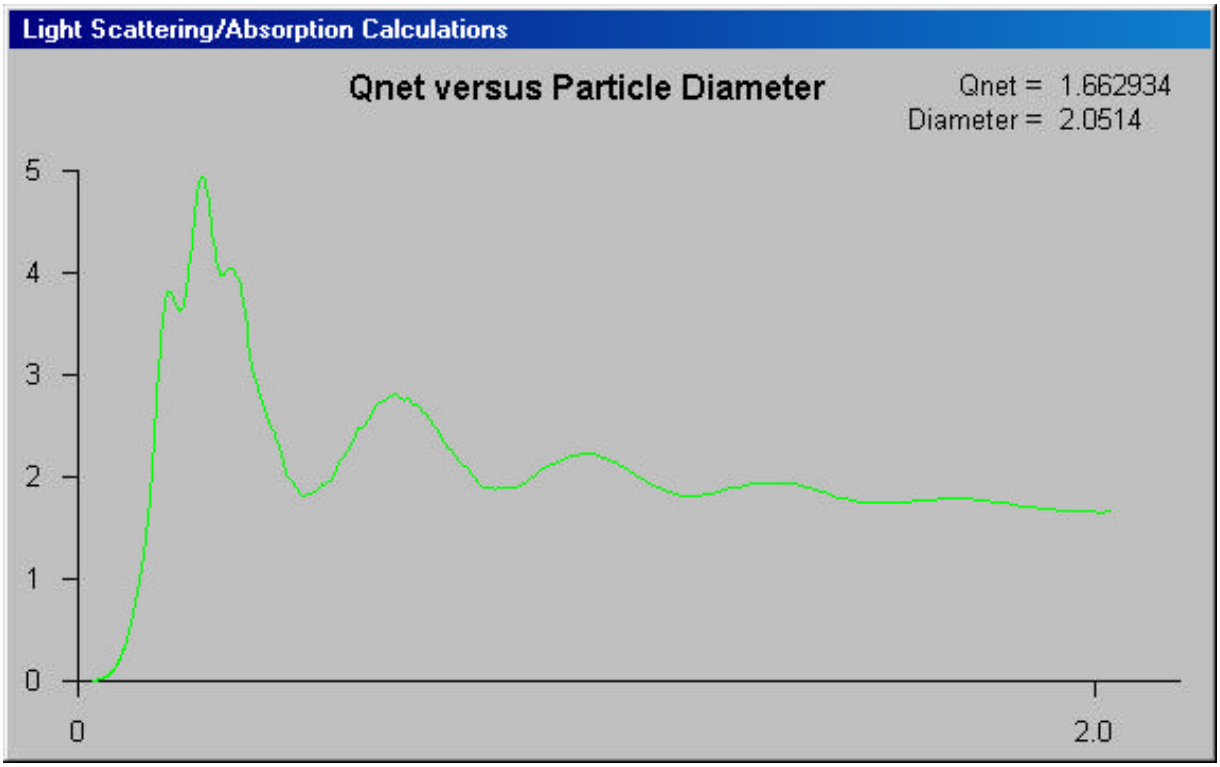


This window contains all of the operating parameters for the currently active operating procedure. You have the option to change any of the parameters listed on the screen, so long as the values used are within the operating limits for the system, and so long as the values are internally consistent. (For example, the maximum size for the analysis range must be larger than the minimum size.) When the DCCS software is installed, the operating procedure named DEMO normally has parameters that CPS believes are suitable for the specified application.

To modify an existing procedure or create a new procedure, you first enter the new physical parameters and presentation parameters. You can move between the different numerical entry boxes using the up and down arrow keys or by clicking a box with the left mouse button. You change the presentation options by clicking the desired option buttons. When you want to create a **new operating procedure** you enter a new procedure name in box labeled "New Name". When you want to modify the existing procedure, **do not change the name in the "New Name" box**.

If you change your mind about modifying or creating a new procedure, then just click the Exit Without Saving command button to return to the Main Menu Window. When you are sure all the parameters are correct, click the Save and Exit command button. The new or modified procedure definition will be saved. Each time that a new procedure is created, the DCCS shows you a graphic of the light scattering efficiency of the sample versus particle diameter. This calculation is based on Mie Theory light scattering. You will also see this graphic any time you modify an existing procedure in a way that changes the light scattering function. The DCCS uses the light scattering efficiency curve to convert the raw data from the Disc Centrifuge (absorption versus diameter) into the particle size distribution (weight versus diameter). The light scattering curve for commercial titanium dioxide pigment in water (with slightly non-spherical particles) is shown in Figure 13.

Figure 13



FAQ: What is Qnet?

Q_{net} is the effective light scattering cross section of a particle compared to its physical cross section. Note that very small particles give very small Q_{net} values, which just means that very small particles (those much smaller than the wavelength of light) scatter very little light; in other words, a suspension of extremely small particles becomes nearly transparent, even a white pigment like titanium dioxide!

Physical Parameters in the Procedure Definition

Each of the physical parameters, and how the DCCS uses it, are described below.

Maximum Diameter is largest size that the DCCS will collect data for, even if there are larger particles in the distribution. When the maximum diameter is increased, the Disc Centrifuge may operate at a slower speed (when on automatic speed control), because the DCCS adjusts the disc speed so that particles of the maximum diameter reach the detector ~1 second after the injection. Increasing the maximum diameter value with the same minimum diameter value will lead to longer total analysis times if the DCCS operates the disc at a slower speed to accommodate the larger maximum size. You can see the effect of changing the maximum size by looking at the "Recommended Maximum Speed" that is shown on the upper right of the window. It is best to operate with a maximum diameter as large as the largest size expected to be actually contained in the sample, but no larger. If you learn that a certain type of sample does not contain particles as large as initially believed, then you should reduce the maximum diameter value to reduce analysis time.

Minimum Diameter is the smallest size that the DCCS will collect data for, even if there are smaller particles in the distribution. When the minimum diameter is decreased, the DCCS will collect data for a longer time, because it takes longer for smaller particles to reach the detector light beam located near the outside edge of the disc. In general, the total analysis time increases as the inverse square of the minimum particle size. For example, analysis to 0.08 micron minimum diameter will take 4 times longer than analysis to 0.16 micron. It is therefore important to choose the minimum diameter carefully. Too small a minimum size will make the total analysis time unnecessarily long. Too large a minimum size may stop the analysis while some portion of the distribution remains suspended in the density gradient fluid inside the disc. This lowers the accuracy of the analysis, and also may cause inaccuracy in the next sample, since some of the first sample may remain in the disc when the next sample is injected. The minimum size used should be no smaller than the smallest particle size expected to be in the samples. When you learn that a certain type of sample does not contain particles as small as initially believed, then you should increase the minimum diameter value to reduce analysis time. When the instrument is collecting data, you have the option to override the minimum diameter value during an analysis by clicking the 

command on the Operate Analyzer window.

Particle Density is the density (or specific gravity) for the particles being measured, in units of grams per ml. Remember that the Particle Density is the density of the pure

material that the particles are made of, not the density of the particles as an emulsion or suspension in a liquid. Particle Density is used by the DCCS in conjunction with the "Calibration Standard Density" to adjust calibration when these two densities are not the same. The particle density can't be too close to the "Fluid Density", or the particles will not sediment during the analysis. (Neutral buoyancy is not allowed!)

Particle Refractive Index is the refractive index of the pure material that the particles are made of, not the refractive index of a dispersion of the particles in a liquid medium. The refractive index value should be reasonably accurate to get accurate size distributions, especially for small particles (<0.5 microns) or when the refractive index of the particles is close to the refractive index of liquid inside the disc centrifuge. Refractive indexes are know for a wide variety of materials (see for example the *"CRC Handbook of Chemistry and Physics"*, CRC Press, any edition).

Particle Absorption is a constant that describes the rate at which light intensity falls with distance as it passes through the particle. This is also sometimes called the *imaginary part of the refractive index*. Particles that absorb light (non-dielectric particles) attenuate the detector light beam in two ways: first by scattering, and second by absorption. Particle Absorption is used in the calculation of Q_{net}. Non-absorbing materials (like most polymers, SiO_2 particles, oil droplets, white pigments, etc.) have absorption values near zero. Strongly colored materials (like many colored pigments and amorphous carbon) usually have absorption values between 0.1 and 1.0. Metallic materials (those that are highly reflective when polished, such as silver, aluminum, mercury, etc.) have very high absorption values (>>1). You might not expect metals to have strong absorption constants, but they are "reflective" because their absorption constant is so high that light can't really penetrate their surface at all, and so is reflected away. Good values for absorption constants are often not available, but any material that appears white or near white when finely divided has an absorption constant near zero. (Compare ice and snow.) Please contact CPS Instruments, Inc. for recommended Particle Absorption values for strongly colored or black materials.

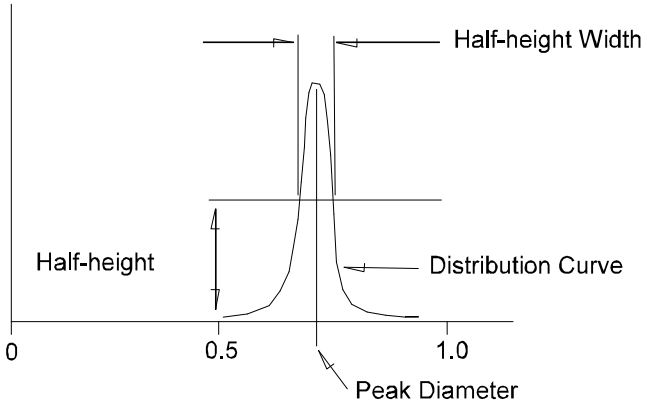
Non-Sphericity Factor is used by the DCCS to account for non-spherical particles. When the particles are not spherical, they scatter light differently than do spherical particles, so Mie theory light scattering calculations must be modified to account for non-spherical particles. The value you enter in this field is the average aspect ratio for the particles for all possible orientations. Spherical particles have a factor of 1. Non-spherical particles always have a factor greater than 1. For example, rods with an aspect ratio of 2 would have a non-sphericity factor of about 1.5. The maximum practical non-sphericity is 3, which corresponds to extremely non-spherical particles (rods with an aspect ratio of ~6). Cubes have a factor of ~ 1.2, while regular polygons approach 1 as the number of sides increases; eicosahedrons have an aspect ration of <1.03.

(Standard) Peak Diameter is the diameter of the calibration standard at maximum light scattering. This is used by the DCCS to calibrate the instrument. This value is very critical and must be correct to get accurate particle size distributions from the CPS Disc

Centrifuge. If you are planning to use your own calibration standard, keep in mind that the accuracy of the results will only be as good as the accuracy of the calibration standard diameter.

Half Height Peak Width describes the broadness of the calibration standard distribution. The value entered is the "half-height" width of the distribution. The meaning of "half-height" width is shown in Figure 14. This value is used by the DCCS to evaluate the condition of the density gradient. If the gradient has degraded significantly, then when the calibration standard is run the measured half-height width will be larger than the known half-height width. The Principles of Operation section of the manual has a more complete discussion of calibration standard width.

Figure 14



Standard Particle Density is the density (or specific gravity) for the calibration standard. Remember that the value used is the density of the **pure material** that the particles are made of, not the density of the particles as an emulsion or suspension in a liquid. The value used is very critical for getting accurate particle size distributions if the calibration standard and the material being analyzed are different materials, but not critical if the two are the same material.

Liquid Viscosity is the approximate viscosity, in centipoise, of the liquid that makes up the density gradient at the temperature where the analysis is run. For common aqueous fluids (like dilute sucrose in water) the value is 0.95 - 1.30. The Liquid Viscosity value is not critical, and can be off by up to 25% with little effect on the reported distribution. The DCCS uses the Liquid Viscosity only to calculate an optimum speed for the analysis; the viscosity does not enter into calculation of particle size. An accurate value for liquid viscosity **is required** to get accurate **absolute** weight distributions (that is, if you wish to see the absolute weight of the injected sample), but not to get accurate relative distributions.

Liquid Refractive Index is the refractive index of the liquid in the disc at the point where the detector beam passes through the disc. This value is used in the calculation of light scattering efficiency. The fluid inside the disc centrifuge is **not** homogeneous in

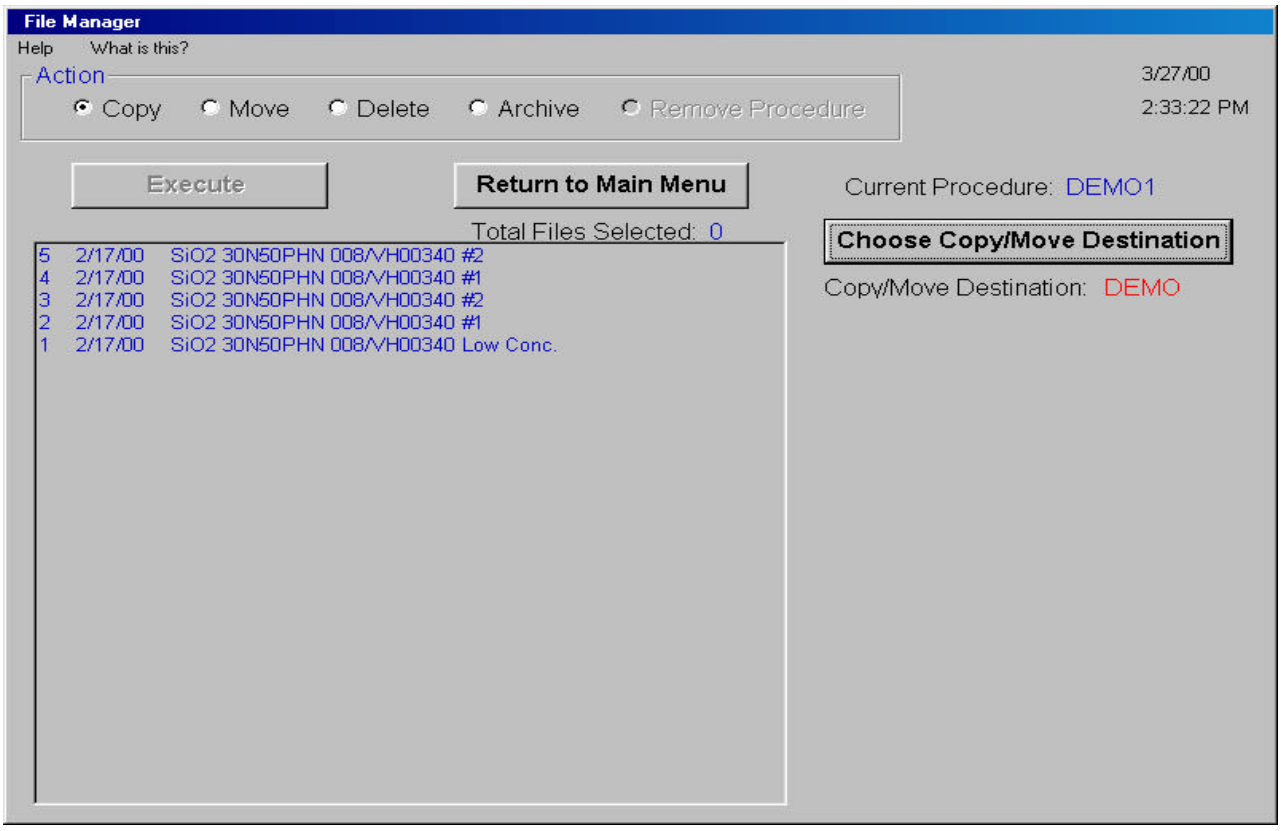
composition, because of the density gradient, so the refractive index normally changes from top to bottom. For example, 6% sucrose in water has a refractive index of 1.3418, while pure water is 1.3330. The detector beam normally passes through the fluid about 20% to 30% from the outside edge of the disc, so it is not difficult to estimate a good value for Liquid Refractive Index. The value you use is not critical (so long as it is reasonable close to correct!) except in cases where the particles being measured have a refractive index close to the refractive index of the fluid. For example, the Liquid Refractive Index is more critical for SiO_2 particles (1.450) than for polystyrene (1.591), because 1.450 is much closer to 1.3418 than is 1.591.

Liquid Density is the average density between the surface of the liquid and the detector light beam. For 0% to 8% sucrose in water, the average sucrose concentration between the surface and the detector beam is ~1.01. The liquid density value is not critical unless 1) the density of the material to be analyzed is close to the density of the liquid **and** 2) the calibration standard and sample are of different densities. When the calibration standard and sample are the **same** material (same density!) it is not necessary to know the particle density exactly, because the calibration standard and sample pass through the same liquid.

The File Manager Window

Figure 15 shows the File Manager window, where you can copy files between

Figure 15



procedures, move files from one procedure to another, delete files, and archive files.

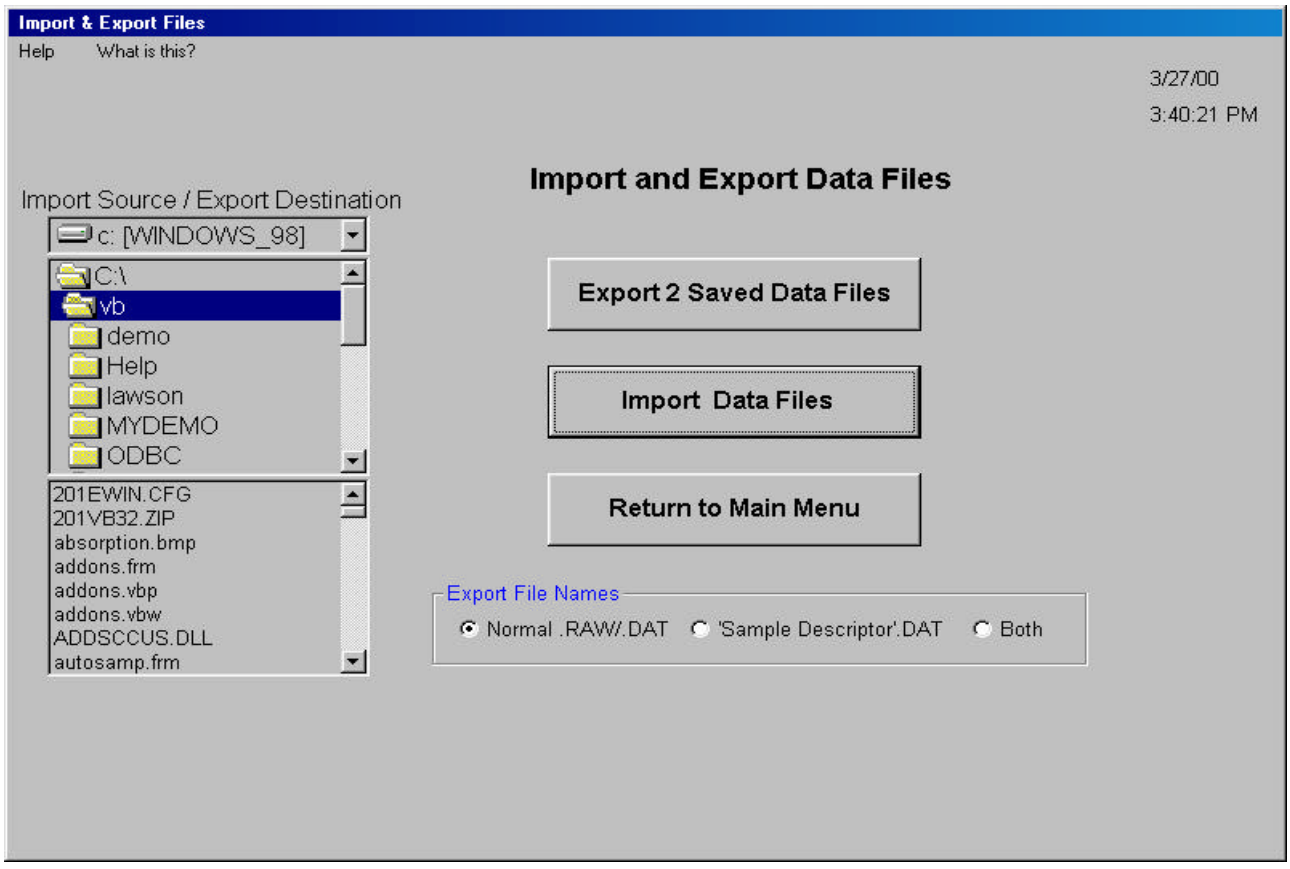
Operating Procedures that contain no data can be removed from the DCCS system. The File Manager always works with files in the currently active procedure. Files can be copied or moved from the currently active procedure to any other procedure, can be deleted, or can be archived. The "Copy" and "Move" functions require a destination procedure, which is chosen by clicking on the Choose Copy/Move Destination command. "Archived" files are exported to a target destination outside the DCCS and then deleted from the DCCS data structure.

Files that are to be acted on are first selected from a list of all files, by clicking on the desired files with the left mouse button. The selection process is exactly like that in the Retrieve Distributions part of the DCCS; selected files are highlighted in deep blue color, and clicking an already selected file "unselects" the file. "Copy" creates an identical file in the destination operating procedure for each selected file. "Move" transfers each selected file to the destination procedure. Delete permanently removes all selected files from the system. (Use caution with Delete!) All actions by the File Manager are initiated by clicking the Execute command button.

The Import/Export Files Windows

You may sometimes need to export distribution files from the DCCS data structure (where all files are stored in Operating Procedure directories) to outside the DCCS, or

Figure 16



need to import data files from outside the DCCS into the DCCS data structure. The Import/Export window allows you to accomplish these actions easily and quickly. Figure 16 show the Import/Export Files window.

To Export Files
Exporting files is a four step process:

1. Select the files you want to export using the "Retrieve Distributions - Select Files" window. All files that you want to export **must** be placed on the "Saved" files list.
2. Select the destination for export using the drive and directory boxes on the left side of the Import/Export Files window.
3. Select the format you wan the files exported in. Your choices are **"Normal"** name format, where file names like 9990xxxx.DAT and 9990xxxx.RAW are used and an "EXPORT.CTR" file is created to account for all exported files, **"'Sample Descriptor'.DAT",** where the sample identification is used to create a recognizable filename, or **"Both",** where both types of files are created in the destination directory.
4. Click the Export n Data Files command button (where 'n' is the number of files awaiting export.

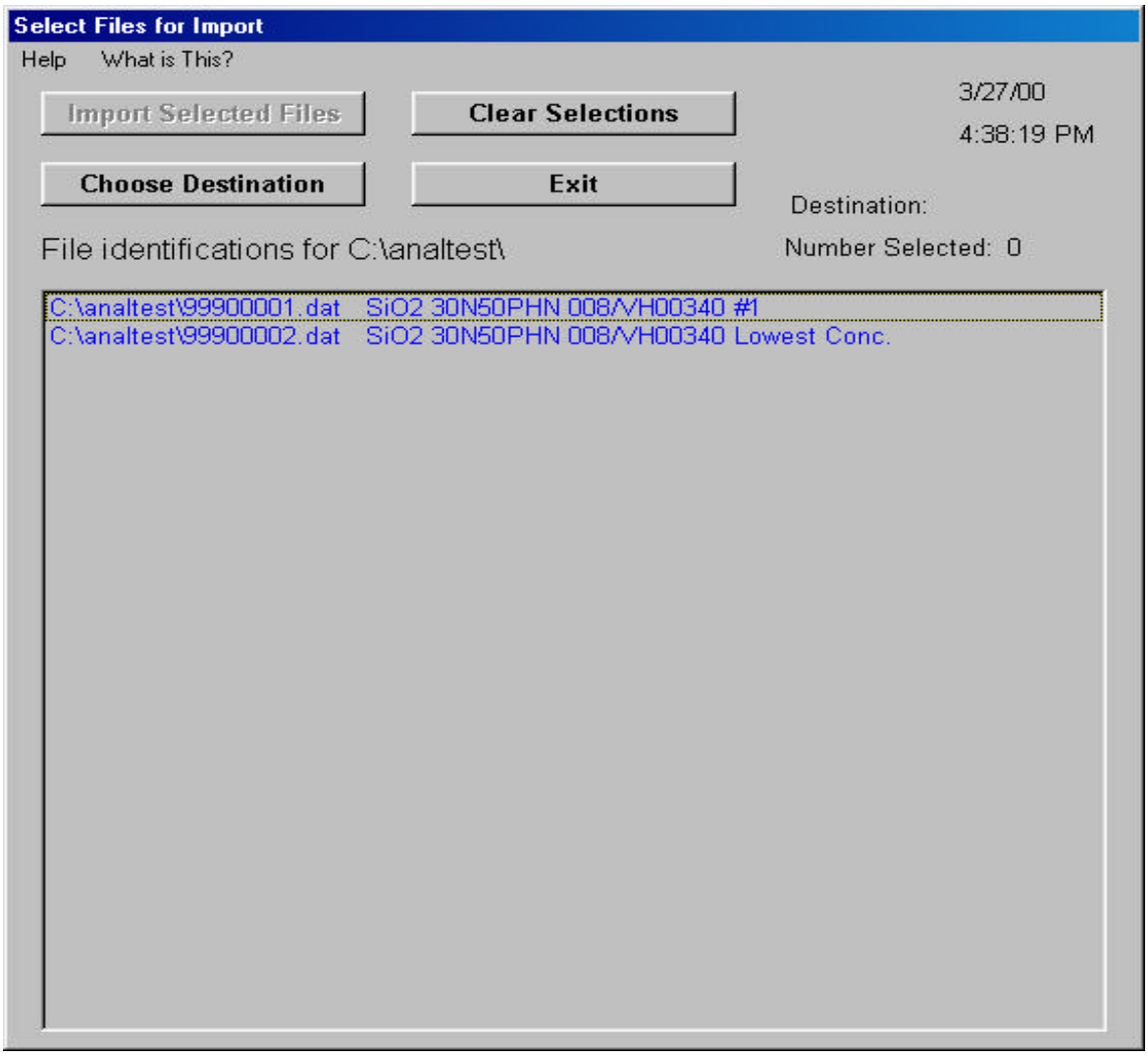
Exporting files does not remove them from the DCCS system. You should use the "Normal" name format if the exported files will be used from within the DCCS. Use the "Sample Descriptor" name format if you plan to work with the files using a different program (for example, Microsoft Excel). The file name that is created from the file descriptor will be easier to identify with this type of name. The file descriptor is converted to a file name by stripping leading and trailing blanks, stripping all illegal characters, and substituting an underscore for all spaces. The descriptor "Type b3, bad sample - 10/22 AM" would become: "Type_b3_bad_sample__1022_AM.DAT". All data files are stored as ASCII text files with commas separating numbers. The data file has 22 header lines with information about analysis conditions and operating procedure, followed by pairs of numbers with a comma between (diameter, weight).

To Import Files
Previously exported data files, that were exported using the "Normal" name format can be imported into the current operating procedure. This is a five step process:

1. Select the disc drive and directory where the files you want to import reside.
2. Click the Import Data Files command button. This will bring up the "Select Files for Import" window, which is shown in Figure 17.
3. Select the files you wish to import from the list of all of the exported files.
4. Click the Choose Destination command button to tell the DCCS where you want the imported files to go.
5. Click the Import Selected Files command button to import the files.

Imported files become part of the DCCS data structure and can be treated just like any other data files. You can copy/move/delete/archive imported files, and also export them.

Figure 17



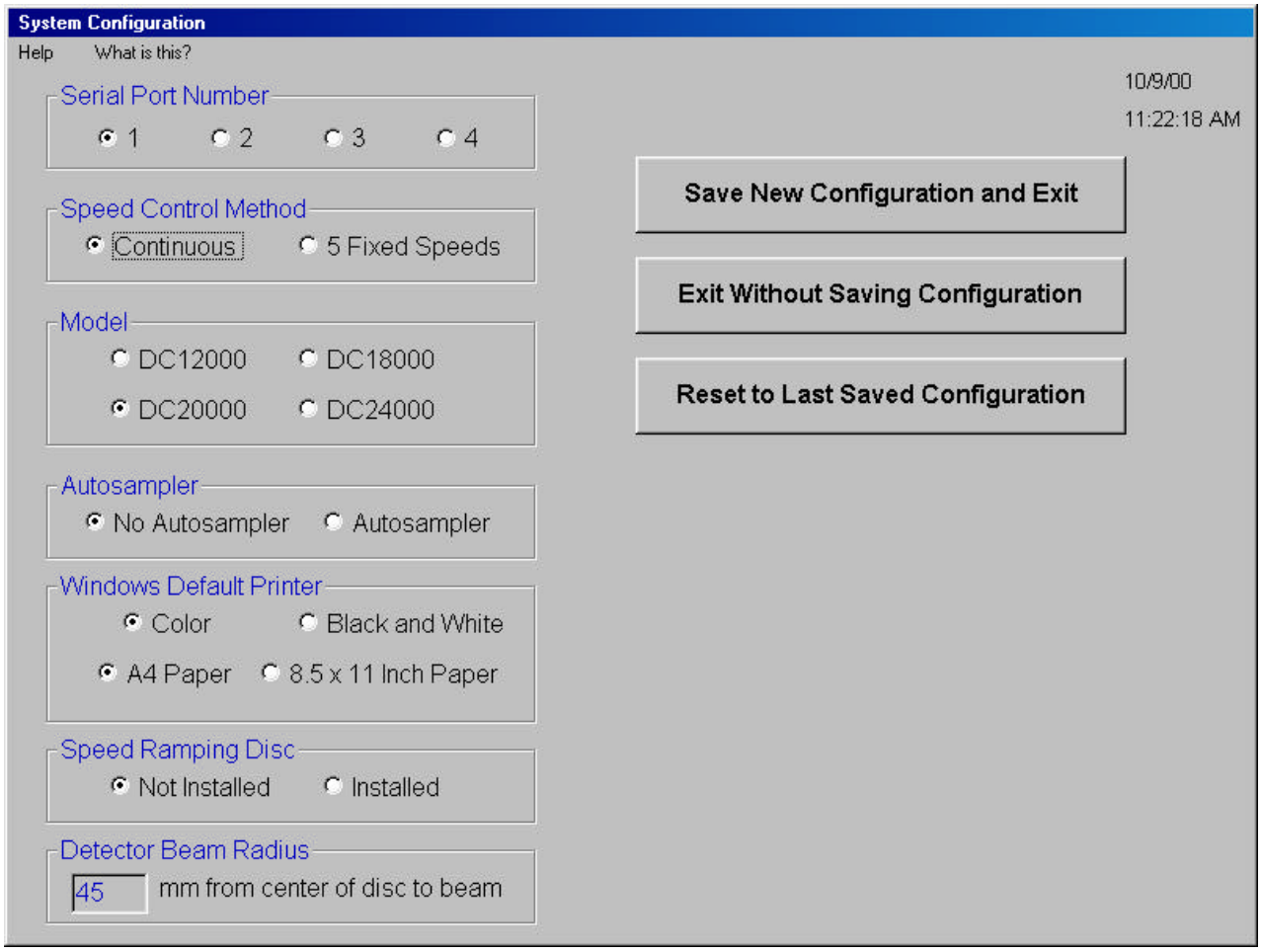
The System Configuration Window

The DCCS must know the environment in which it is operating in order to function correctly. The System Configuration window allows you to set the configuration correctly. Figure 18 shows the System Configuration window. The configuration parameters are:

Serial Port Number (1, 2, 3, or 4)
The selected port must be the port which the CPS Disc Centrifuge is connect to.

Speed Control Method (Continuous, 5 Fixed Speeds)
You should choose "Continuous" control if you have a new CPS Disc Centrifuge. You should choose "5 Fixed Speeds" if you have an older CPS Disc Centrifuge and you have received this manual along with a software upgrade. **You must not select "5 Fixed Speeds" if you have continuous speed control.**

Figure 18



Model (DC12000, DC18000, DC20000, DC24000)

Select the correct model number so that the DCCS knows the maximum speed for your instrument.

Autosampler (No Autosampler, Autosampler)
Select Autosampler only if you have purchased a CPS Disc Centrifuge Autosampler.

Windows Default Printer (Color, Black and White; A4 Paper, 8.5 x 11 Inch Paper)

Speed Ramping Disc (Not Installed, Installed)
This must be set to Not Installed, unless you have purchased the optional disc for speed ramping.

Detector Beam Radius (mm from center of rotation)
The detector beam is normally set at ~45 mm from the center of rotation at the factory. If you change the position of the detector, then you should measure the new radius and enter the new value. The DCCS must have a accurate detector radius to measure

accurate absolute weight distributions.

Organizing and Protecting Your Data

The data produced by the CPS Disc Centrifuge will become, over a period of months or years, a valuable asset: a wealth of historical data that you can use to better understand your process, to improve product quality, and to help solve process problems. The value of the data often become far greater than the value of the system itself. This part of the operating manual covers how to best organize your data so that it is easy to access, and how to protect your data so that it is never lost because of an operator error, equipment failure, or accident.

Organization of the Hard Disc Files

The entire CPS Disc Centrifuge Control System normally resides in a directory called "ANALYZER" (although you can specify a different directory when you install the software the first time). All of the data files reside in sub directories within the ANALYZER directory. Each time you create a new operating procedure using the "Procedure Definitions" window, a new sub directory with the name of that new procedure is created. The files in the ANALYZER directory are:

CPSV9x.EXE
This is the Disc Centrifuge Control System (DCCS) main program.

HELP.xxx.pdf
These are the help files that are called when you ask for on-line help.

CPS.CFG
This is the file that holds the system configuration information. Each time the DCCS starts, this file is read. When you modify the configuration, this file is changed.

DEFAULT.PRO
This file contains the name of the current default operating procedure. Each time the default procedure is changed, this file receives the new name.

DRIVR201.CFG
This is a file of configuration constants used for the serial interface on the CPS Disc Centrifuge. These configuration values are automatically loaded each time the DCCS is started; you should never need to change this file.

MAXNUM.MAX
This file keeps track of the total number of operating procedures on the system.

PROCLIST.LST
This file contains a complete list of the names of operating procedures in the order that they were created.

SPEEDS.LST
On older systems, this file holds a list of the five distinct speed settings for the variable frequency drive. On newer systems, this file is a dummy file and exists only to maintain

software compatibility.

BEAM.TXT
This file describes the intensity profile for the detector beam across its width. The DCCS uses this file when you select *Enhance Resolution* on the Runtime Options menu.

The Analyzer directory contains a sub directory for each procedure that is created. When the software is first installed, there is only a single procedure (named DEMO) and a single sub directory (named DEMO) for that procedure. Each new operating procedure creates a new sub directory. Within each procedure sub directory (like the DEMO sub directory), there are always four files that contain information about the procedure, and as many pairs of data files as there have been analyses run under the procedure. Each pair of data files has the same file name followed by ".RAW" and ".DAT". Each pair of data file represents a particle size distribution that was run on the CPS Disc Centrifuge. A procedure sub directory is structured as follows:

'procname'.IDN contains the identification number for the procedure.

'procname'.DEF contains all of the procedure definition data. When you change a procedure definition, this is the file that gets changed.

'procname'.CTR keeps track of the number of data files that are in the sub directory.

'procname'.SCA is a file with the light scattering correction data for the procedure. This file is generated when the procedure definition is saved; Mie theory scattering calculations are done to generate the file. Each time a sample is run, the DCCS retrieves the scattering correction file and uses it to convert the ".RAW" (absorption) distribution into a ".DAT" weight distribution.

nnn0mmmm.DAT are the weight data files. nnn is the procedure number (contained in the "procname".IDN file), and mmmm is the run number. The first data file created under an operating procedure is nnn00001.DAT, the second is nnn00002.DAT and so forth; each new file is given a name consisting of the procedure number joined with the next higher file number within the procedure. Each .DAT file has a 22 line header section, followed by pairs of numbers that represent diameter and weight. The may be up to several thousand pairs of numbers.

nnn0mmmm.RAW are raw data files. nnn is the procedure number (contained in the "procname.IDN" file), and mmmm is the run number. The raw data files contain the original analysis data, without any corrections applied for baseline drift or light scattering efficiency. The line by line format of the files is as follows:

Sample identification text
absorption value, time, particle diameter
absorption value, time, particle diameter
.

The absorption value is the natural log of the ratio of the starting light intensity at the detector to the light intensity reaching the detector during the run. An absorption value of

zero indicates no change in light intensity (no particles in the light beam). The time is in seconds, and the particle diameter is in microns. The file format is standard ASCII text (comma delimited), so you can easily import the file into a word processor, spreadsheet, or other software. Raw data files are used when you view a distribution using "Absorption" mode. ".DAT" files are used when you view files using weight, surface, or number modes. The raw data files are also used by the DCCS when you "recalculate" a distribution using different operating procedure parameters. In this case, a new ".DAT" file is generated for each recalculated file, and the old ".DAT" files erased.

Naming and Organizing Operating Procedures

Quality Control Applications

The number of operating procedures and the names chosen for the procedures will depend upon how the CPS Disc Centrifuge is being used. If the primary function of the analyzer is quality control of an ongoing process, then it is best to set up operating procedures that fit a quality control environment. For example, in many production facilities, there are several distinct products or several distinct product grades of the same basic product: a batch polymerization plant that produces acrylic latexes for paint and coating applications might produce five or six different grades of latex, each with its own characteristics (monomer combination, particle size, etc.). In other production facilities, there may be several different production trains, each of which operates independently of the others, but all of which make the same basic product(s) at the same time.

The procedures that are defined need to correspond to the actual process that is being monitored. The key consideration is to define procedures in a way which will cause related distributions all to reside in a single procedure. Suppose an emulsion plant has four continuous emulsion polymerization trains, and that each of four different product grades are produced on each polymerization train at various times during a month. In this case, it would probably be best to have a different operating procedure defined for each combination of product grade and production train: 4 trains X 4 grades = 16 operating procedures. The conditions used by the DCCS in the 16 procedures might even be the same, but separating the data into related groups will be a big help in identifying trends and solving process problems.

The other major consideration for quality control applications is planning for the volume of data that will be generated. A single operating procedure can accommodate an unlimited number of data files, but too many data files will make file retrieval cumbersome. If a process is checked on a frequent basis (say 6 times per day), then it won't be too long before many hundreds of files are stored in the procedure directory. It this case, it is better to plan on creating a group of identical procedures, each of which represents a distinct time period for the process. In the case of a process that is checked six times a day, perhaps procedures with names like TP1A194 (Type 1A, first quarter 2000), TP1A294 (Type 1A, second quarter 2000), etc, would be a good way to organize the large volume of data files.

Research Applications

The procedures defined in a research (or pilot plant) application are generally quite

different than in a quality control application. The research lab tends to explore many more (new) things than a production plant, so the tendency is to create many different operating procedures. The problem is that each procedure may contain only a few (in some cases only one!) data files.

When there are several different researchers who rely upon or operate the CPS Disc Centrifuge, it is a good idea to have all of the people agree to a consistent procedure naming scheme. Typically, all the analyses run for a project can be run under a single procedure name (even if the actual conditions used to run the analyses are very different; it is easy to change the conditions of the analysis in the Define Procedure part of the DCCS). Alternatively, all the analyses run by a single person can be held in a single (or several) operating procedure(s) used only by that individual.

In any case, all the people who use the instrument should understand how files are stored by the DCCS and agree upon a procedure naming scheme, so that there is no chance of data files getting put in the wrong procedure directory and "lost".

Backing Up Your Data

Hard disc drives do not last forever. The expected lifetime depends upon frequency of use and also upon how "disc-intensive" the applications used with the hard disc are. The expected lifetime is normally in the range of 2 to 10 years. Unfortunately, the hard disc could fail at any time, and depending on the mode of failure, it may be difficult or impossible to recover the information stored on the hard disc after failure.

CPS recommends that you make provision for a reliable method of backing up the data on the C: drive. The most secure backup for stand-alone (non-networked) systems is probably a tape drive, though other backups are faster and easier. If you install a second hard disc, it is very easy and fast to copy an image of one drive onto the other. Of course, physical damage to the computer (fire, smoke, water, etc.) could easily destroy both hard discs at the same time.

Networked computers should have all critical files backed up on a separate network drive. This can be done automatically on many networks.

Please read the section below on erasing data files!!!

********** Do Not Erase Files **********

Warning!

Do not erase ANY files from the DCCS directory or the operating procedure sub directories! DO NOT use Windows Explorer, or a command line to delete or rename ANY DCCS files. This will cause many problems, including making the system inoperable. The DCCS expects to find all of the files that it uses to set up and run the system, and also expects to find all of the data files it has created. The only way a data file may be safely removed from the hard disc is with the File Manager Window. DO NOT EVER erase data files to make room on the hard disc!

****

Density Gradient Fluids and Sample Dilutions

The CPS Disc Centrifuge operates with fluids that form a density gradient inside the disc. The density, refractive index, and viscosity of the fluids must be known in order to get accurate analyses.

The Standard Density Gradient Fluids

The standard fluids used to form a density gradient inside the rotating disc are dilute solutions of sucrose (table sugar) in distilled water, along with a very low concentration of an emulsifier. The density gradient is created by injecting 1.4 to 2.0 milliliters of each solution into the disc, in order of decreasing concentration. The highest concentration solution (4% to 8% by weight) has the highest density, while the lowest concentration (pure water) has the lowest density. The density gradient created in this fashion allows the sedimentation of particles to remain "stable", and produces accurate and reproducible results. The mechanism by which the density gradient stabilizes the sedimentation is explained in detail in the Principles of Operation section of this manual. The refractive index, viscosity, and density of the standard density gradient fluids are different than pure water, due to the dissolved sucrose. The refractive index, viscosity, and density values must be included in each operating procedure definition.

Figure 19

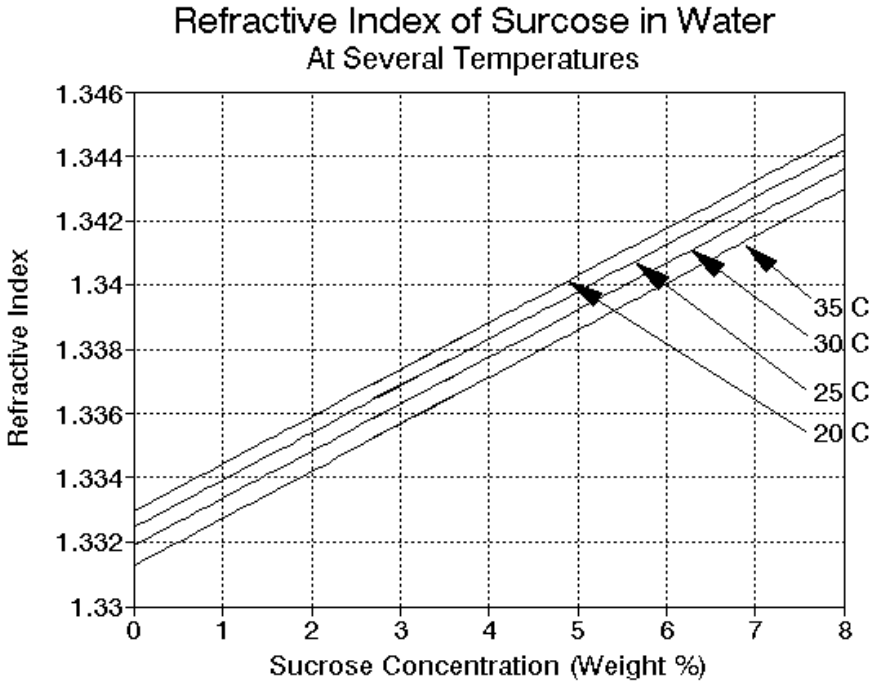


Figure 19 shows the refractive indexes of dilute sucrose solutions in water at several different temperatures. The value of refractive index used depends (slightly) on the ambient temperature in the laboratory where the CPS Disc Centrifuge is used. The disc

housing normally increases about 2° to 5° C above the ambient temperature in the laboratory. The concentration of sucrose inside the disc where the light beam passes through is about 6.0% by weight if you are using a 0% to 8% sucrose gradient. If the ambient laboratory temperature is 25°C, then the best value to use for refractive index is 1.3418. The data in Figure 19 can be used to determine the correct refractive index with other sucrose concentrations if you use a density gradient with higher concentration. Changes in ambient temperature of only a few degrees do not change the refractive index very much, so such changes can usually be ignored.

Figure 20 shows how the density of dilute sucrose solutions changes with temperature and sucrose concentration average concentration. When a 0% to 8% sucrose based density gradients is used, the average concentration of sucrose in the fluid the particles pass through on their way to the detector is about 3%, so if the ambient temperature in the laboratory is 25° (up to 30° inside the disc), then the best value to use for average fluid density is 1.007 g/ml. If sucrose solutions with higher concentrations are used instead of the normal solutions, then Figure 20 can be used to determine the correct average density.

Figure 20

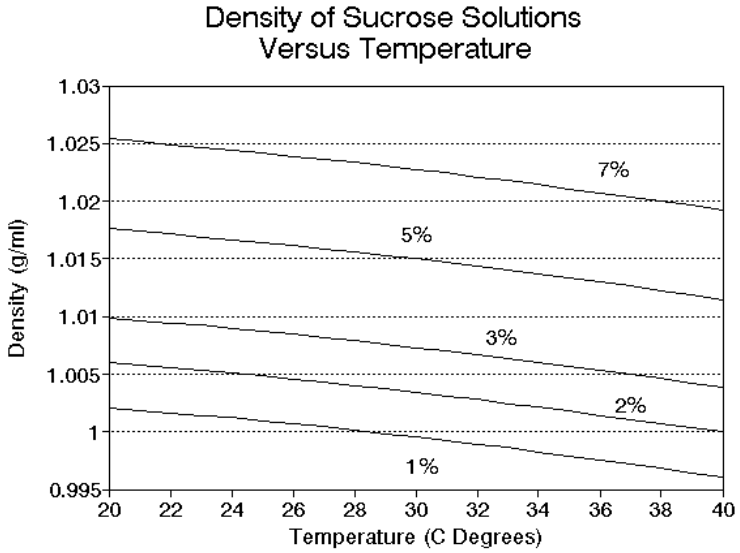
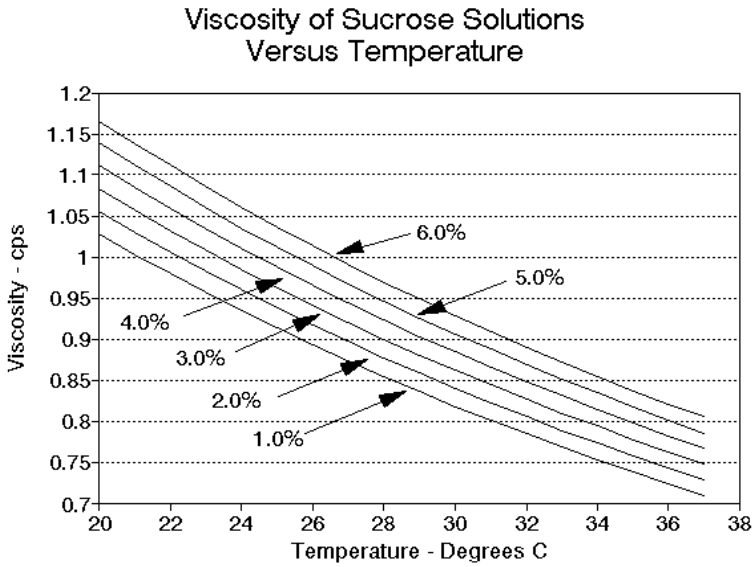


Figure 21 shows how the viscosity of dilute sucrose solutions changes with temperature. As you can see in Figure 21, the viscosity changes very little as the concentration of sucrose changes, but that the viscosity changes markedly as the temperature changes. Fortunately, the value for fluid viscosity is normally not very critical to get accurate particle size distributions. Any value within ~ 20% of the true value is good enough to produce accurate analyses. When 0% to 8% sucrose density gradient is used, the average concentration of sucrose that the particles pass through is 3%. If the ambient temperature in the laboratory is 24°, then the average viscosity will be between 1.0 and 0.9 centipoise, depending of centrifuge speed. Figure 21 can be used to determine the viscosity of

sucrose in water density gradient solutions at different sucrose concentrations and at different temperatures.

Figure 21



Density Gradient Fluids for Unusual Circumstances

The 0% to 8% sucrose/water gradient is suitable for very wide range of materials and particle sizes. However, there are some situations where changing the density gradient fluid will improve the accuracy of the distributions, save analysis time, or make an analysis possible when it might not be possible. The circumstances that may require a change in the fluids fall into four categories:

1. When the particles are extremely small.

2. When the density of particles is close to or lower than the density of the fluid.

3. When the particles are very dense and/or the distribution is very narrow.

4. The sample is not compatible with an aqueous analysis.

Each of these circumstances are discussed below.

Very Small Particles
When the particles to be measured are extremely small (less than 0.05 micron minimum diameter), the analysis time may be unacceptably long. As a general rule, the total analysis time increases as the inverse square of the smallest size measured, and

increases as the inverse of the density difference between the particles and the fluid. If the density of the particles is close to the density of the liquid, see the discussion below on low density particles.

The first thing to consider when analyzing small particles is the maximum analysis size. If the maximum size is set to too high a value, then the DCCS may set the disc speed to less than the maximum value (12,000, 18,000, 20,000, or 24,000 RPM). The total analysis time will be reduced if the maximum size is set so that the disc runs at maximum speed.

If the difference in density between the particles and the fluid is sufficient for good sedimentation (>0.05 g/ml difference), and the operating procedure definition makes the disc run at maximum speed, then the simplest approach is to **reduce the volume of the fluid in the disc.** This will reduce the distance that the particles must settle to reach the detector, and reduce analysis time considerably. For example, if 1.2 ml of each solution is used when the density gradient is built (instead of 1.6 - 1.8 ml), then the analysis time will be reduced by about 50%. However, you should keep the following things in mind if you reduce the fluid volume:

1. The resolution of the analysis will be lower because the particles travel a shorter distance before reaching the detector. This means that the distribution will appear slightly wider than normal, and that peaks that are close to each other in size will not be as completely separated.

2. The density gradient may degrade more quickly because the high concentration sucrose solution is physically closer to the low concentration solution. This means that the density gradient may have to be replaced more often.

3. The values of viscosity, density, and refractive index used in the procedure definition may have to be revised to reflect the lower volume of fluid. The location of the light beam is not changed when the volume of fluid is increased, so the average concentration of sucrose that the particles pass through is reduced with lower fluid volume, and the concentration where the light beam passes through the disc also is reduced slightly.

Low Density Particles

When the material to be measured has a density close to or lower than the density of the normal sucrose/water density gradient fluids, it may not be possible to run an analysis using the standard method. The minimum practical difference in density is about 0.03 g/ml, and 0.05 g/ml or more is better. If the particles are less than 0.03 g/ml more dense than the fluid in the disc, then there will not be enough density difference to make the particles sediment in a reasonable time, and the accuracy of the analysis will be lower than normal. If the particles are lower than the density of the liquid, then they will actually float on top of the fluid, and never sediment. Low density samples like these can be run using the optional CPS centrifuge disc that is designed for **low density particles**. Please

contact CPS or your local representative if your system does not have a low density disc and you need to purchase one.

The optional CPS Low Density disc delivers particles to the **bottom** of the centrifuge chamber when they are injected into the centrifuge, rather than the surface. Low density particles float toward the surface during the analysis, following the same equations of sedimentation, but with a negative value for motion. **Any** sample that is compatible with an aqueous environment can be measured using the optional disc:

1. If the particles are significantly more dense than water, they can be measured in the normal way, using the normal sucrose/water gradient.

2. If the particles are significantly less dense than water (<0.95 g/ml), they can be measured using the low density disc and a normal sucrose/water gradient.

3. If the particles are near the density of water (0.95 to 1.05 g/ml), they can be measured using the low density disc and a sucrose density gradient based on **deuterium oxide** (heavy water) in place of water. Deuterium oxide has a density of 1.107 g/ml at 20°C, so it can be used to measure particles that are exactly the same as the density of water (1.00 g/ml).

The central opening to the disc is filled with a removable plastic cap whenever low density samples are to be run. Injections of density gradient fluids are made using the normal (straight through) injection port. Once the gradient is in place, the normal injection port is removed and a angled low density port is installed. The angled injection port directs calibration standards and samples into a “V” shaped grove that is machined into the front of the disc. The "V" shaped groove catches injected liquid, and carries it to four capillary channels. The capillary channels transport the fluid to the bottom of the centrifuge chamber.

Calibration standards and samples **must** be prepared for injection so that they have a net fluid density (liquid plus particles) that is **higher** in density than the liquid at the bottom of the centrifuge chamber. The higher density insures that the samples spread quickly on the bottom of the chamber before sedimenting toward the surface. The following solutions are recommended.

Example D_2O Density Gradient

Sucrose Concentration	Density (20°C)
0%	1.1070
0.5%	1.1089
1.0%	1.1109
1.5%	1.1128
2.0%	1.1148
2.5%	1.1167
3.0%	1.1187
3.5%	1.1207

4.0%	1.1226

Example D_2O Sample Dilution (including 0.1% anionic surfactant)

Sucrose Concentration	Density (20°C)
6.0%	1.1306

Analyzing Dense Particles with Narrow Distributions

In a few cases, samples will have a combination of high density (>2.0 density) particles and narrow particle size distributions, where most of the weight of the sample is in one or more narrow peaks. This combination has the potential to cause some instability in sedimentation and loss of resolution. You can determine if there is a problem by injecting the same sample two times, but with only half the normal sample volume the second time. The completed distributions should be very nearly identical; if they differ significantly, then there is probably some instability in the sedimentation.

There are two ways to handle this situation:

1. Reduce the sample concentration to the lowest practical level. The absorption signal should probably not pass 0.05.

2. Increase the steepness of the density gradient by using higher sucrose concentrations. Instead of 6% to 16%, use 8% to 24%, or 8% to 32%. This increases the "steepness" of the gradient. A steeper gradient will improve stability for high density materials with narrow distributions, approximately in proportion to it's steepness.

Non-aqueous Systems

It is possible to prepare density gradient solutions from other liquids, such as hydrocarbon oils using the standard polymethyl methacrylate disc. You can contact CPS or your representative for help with setting up a non-aqueous gradient. In general, aliphatic oils (like tetradecane) are acceptable. n-propanol or n-butanol can be added in different proportions to give a range of densities for creating a density gradient in the disc. If you do attempt to use a non-aqueous with the standard disc, then, you must be **certain** that the liquid does not dissolve or swell polymethyl-methacrylate, or else the disc could be destroyed. Be sure to expose a sample of poly- methyl methacrylate to the fluid you plan to use for at least several days to be **certain** that there is no effect on this type of polymer before you use it in the Disc Centrifuge.

If you need to analyze samples in a solvent that would attack poly-methyl methacrylate, then you will need a solvent resistant disc. Please contact CPS or your representative to discuss your application and the solvent resistant disc.

Sample Dilutions

In nearly every case, the sample you want to measure will be much too concentrated to use directly in the CPS Disc Centrifuge. You will need to dilute the sample to a low enough level that the sample can be accurately measured. Too high a concentration will cause instability in the sedimentation, and loss of resolution.

Samples are normally diluted to well under 0.5%solid content, and in many cases to under 0.1% solid content. The required dilution rate depends upon the average size of the distribution, the refractive index of the particles, and the width (or dispersity) of the distribution. Very wide distributions, which contain a wide range of particle sizes, require less dilution than narrow distributions, because the particles will spread out during the analysis so that only a small fraction of the total weight of the sample is in the detector light beam at any moment. Samples with one or two very narrow peaks may need to be diluted to well under 0.1% solid content, since the particles in each peak do not spread out during the analysis; this means a large fraction of the total sample can be in the path of the light beam at one time.

Materials with higher refractive index usually require more dilution than materials with lower refractive index, because they have higher turbidity at equal concentration. There must be some difference in refractive index between the particles and the density gradient fluid. If the refractive indexes are exactly the same, then the particles become "invisible", since with equal refractive index the particles will not scatter light. Fortunately, virtually all solid materials have a refractive index higher than most fluids.

As a general guideline, samples with relatively dense particles (>2 g/ml) and a broad distribution, should be diluted so that the absorption does not pass about 0.2. Samples that consist of one or more narrow peaks will often go "off-scale", passing an absorption value of 0.5. If this happens, the sample should be diluted several fold, since the sedimentation may become unstable with such a high sample concentration.

Coagulation During Sample Dilution

When the sample is diluted, it is subjected to some "shock" from the dilution process. Some very sensitive materials (especially elastomer latexes and adhesives) may coagulate due to this shock. Samples that consist of hard particles are normally more resistant to this type of coagulation, unless their particle size is very small (<0.1 micron).

A sample can also coagulate if it is cationically stabilized (positively charged particles) and the dilution fluid contains an anionic emulsifier. It this case, the emulsifier used in the dilution fluid and in the density gradient fluids must be changed to a cationic type of emulsifier to avoid coagulation.

It is normally possible to see when a sample has coagulated during dilution based upon its appearance. If there are large particles or floc particles, or if the sample appears to be at all inhomogeneous, then the sample may be partially coagulated. If you see any evidence of coagulation, there are three ways to eliminate it:

1. Add an excess of emulsifier to the concentrated sample before attempting to dilute it.

2. Add more emulsifier to the dilution fluid before diluting the sample.

3. Dilute the sample in two steps: first about 1:5 in distilled water that contains about 0.5% emulsifier, then in the normal dilution fluid. Lowering the concentration of the sample before mixing with the dilution fluid reduces the chance of coagulation.

If you are ever unsure of whether or not a sample has been partly coagulated, you can try one of the above techniques, and run particle size distributions using both the normal preparation and the special preparation. If the particle size distribution is the same for both preparations, then coagulation probably did not occur. If the particle size distribution is significantly different using different sample preparation techniques, then the sample has at least partially coagulated during sample preparation.

Principles of Operation

This section explains in some detail how the CPS Disc Centrifuge operates, including the function of the density gradient. The information presented in this mostly theoretical rather than operational, and is not required in order to successfully operate the CPS Disc Centrifuge. The information is presented for people who want to have a more complete understanding of how the Disc Centrifuge operates.

Sedimentation under Stokes Conditions

The rate of sedimentation inside the rotating disc is controlled by four factors: the size of the particles, the difference in density between the particles and the fluid through which they pass, the viscosity of the fluid, and the strength of the centrifugal field (rotational speed). The sedimentation of particles in a gravitational field was first systematically investigated by Sir G. G. Stokes (Mathematical and Physical Papers, 11). Stokes built upon earlier work by Newton (Principia, Lib 11, Loc. Cit. 21) and Rayleigh (Scientific Papers, 6, Art. 392) which described how drag force on a spherical particle moving through a fluid depends upon the diameter of the particle, the viscosity of the fluid, and the velocity of movement.

Stokes showed that when particles settle in a gravitational field under a certain set of conditions, the forces acting on the particle are in perfect balance, and the particle moves at a constant velocity (which can be predicted) after a very brief period of initial acceleration. The required conditions are:

1. The particle must be smooth, spherical, and rigid enough to not deform due to the forces acting on it.

2. The particle must be small compared to the container of fluid: the fluid must be essentially infinite in size compared to the size of the particle.

3. The particles which make up the fluid (that is, the molecules) must be much smaller than the settling particle, so that the fluid is essentially homogeneous in how it acts on the particle. (Brownian motion of small particles is the first evidence of non-homogeneous interaction between the fluid and the particle.)

4. The settling speed must be slow enough that all viscous forces come from smooth (non-turbulent) flow.

These conditions are normally satisfied for most samples run on the CPS Disc Centrifuge using normal operating parameters. There are two fairly common situations where these conditions are not completely satisfied.

Non-Spherical Particles

The requirement for smooth, spherical particles must be satisfied to get accurate absolute measurements of particle size. Non-spherical particles always settle at a rate which is lower than the rate for a sphere of the same weight, so non-spherical particles are reported as smaller than their correct size. All non-spherical particles can be measured to determine the "equivalent spherical diameter" distribution. While this distribution is not correct in an absolute sense, it does allow accurate comparisons of size for similar samples. For example, an inorganic pigment could consist of roughly cubic crystals. So long as the shape of the particles does not change significantly from one sample to the next, the equivalent spherical diameter measurement will be a good representation of the relative particle size, and will allow accurate comparison of different samples.

If you are looking for reasonably accurate weight distributions for non-spherical particles, then there are two steps that you can use to produce distributions that are very close to the correct “weight” distribution:

1. You should enter the appropriate “non-sphericity” factor in the procedure definition. This makes the software calculate a light scattering function that is close to the correct scattering function for the particles. The non-sphericity index is equal to the average aspect ratio for the particle when viewed in all possible orientations. Spheres have a value of 1. Roughly cubic crystals have a value of about 1.55.

2. You should reduce the particle density in the procedure definition to compensate for slower than expected sedimentation. The adjustment that is required depends on how non-spherical the particles are. A reasonable adjustment is to reduce the density value for the particles according to the following empirical equation:

   Adjusted Den. = Fluid Den. + (True Den. - Fluid Den.) * (1 - 0.16 * (NSI - 1))

   Where NSI is the non-sphericity index for the particles. In the case of cubic crystals with a density of 2, the adjusted density would be 1.912. If this density value is entered into the procedure definition, then the reported weight distribution will be very close to the correct weight distribution. The above adjustment works quite well for particles a with non-sphericity index up to 3. Rigid rods that are 5 - 6 times as long as they are wide have a non-sphericity index of about 3.

Brownian Motion Broadening

The requirement for a fluid which appears completely "homogeneous" compared to the size of the particle is not completely satisfied when the particle is small enough to exhibit "Brownian motion". Brownian motion is random, irregular motion of very small particles suspended in a liquid. It is caused by momentary unevenness in the impacts of fluid molecules on the surface of the particle. The unevenness is due to the completely random nature of the impacts. When particles are very small, there is a finite probability that, during a very brief time period, the net force on the particle from molecular impacts on one half of the surface will be greater than on the opposite half of the surface. This causes the

particle to briefly move (appears to "jump") in the direction of the net force. Significant Brownian motion only occurs for particles that are smaller than about 1-2 microns in diameter. Larger particles have enough surface area and enough total mass so that Brownian motion becomes negligible.

Brownian motion is, in reality, a diffusion process. The individual particles "diffuse" with time according to their size: large particles diffuse very little, small particles diffuse more. Inside the disc centrifuge, a narrow band of particles broadens during the sedimentation at a rate that depends on the particle size. Normally, the rate of diffusion (called the diffusion constant) is proportional to the inverse square root of the particle diameter. The table below shows the approximate diffusion distance in one second for particles suspended in a liquid with viscosity of 1 centipoise.

Diameter (microns)	**Average Displacement (microns in 1 second)**
1.0	0.754
0.5	1.052
0.25	1.49
0.1	2.36
0.05	3.331
0.01	7.54

Note that these displacement values are for **linear displacement**, no matter what direction. Since Brownian motion is completely random (the second-to-second displacement can be in any direction), most of the motion of a particle cancels over time (sometimes up, sometimes down, sometimes left, sometimes right). The most probable location for a particle after a long diffusion time is **its original location**. In the disc centrifuge, a very narrow band of particles gradually broadens due to diffusion as the particles sediment, and the band assumes a Gaussian shape, but the center of the band always arrives at the detector beam at exactly the same time as it would had there been no Brownian motion. The net effect of Brownian motion is loss of resolution, not an error in reported peak size.

FAQ: How much is Brownian motion broadening my peaks?
This depends on both the particle size and the time for the particles to reach the detector. Particles larger than about 0.2 micron will have negligible broadening under nearly any circumstances. The diagrams below (Figures 22 and 23) show the expected broadening for bands of 0.04 micron and 0.1 micron particles over 15 minutes. The net change in resolution is very small for the 0.1 microns particles. With a total sedimentation depth of ~7 mm, perfectly uniform 0.04 micron particles will be measured as a band starting at ~0.041 and going to ~0.039 micron. If you are measuring 0.02 micron particles with 60 minute analysis time, then the broadening effect will be significant: the measured band will be from ~0.022 to ~0.018 micron.

Figure 22

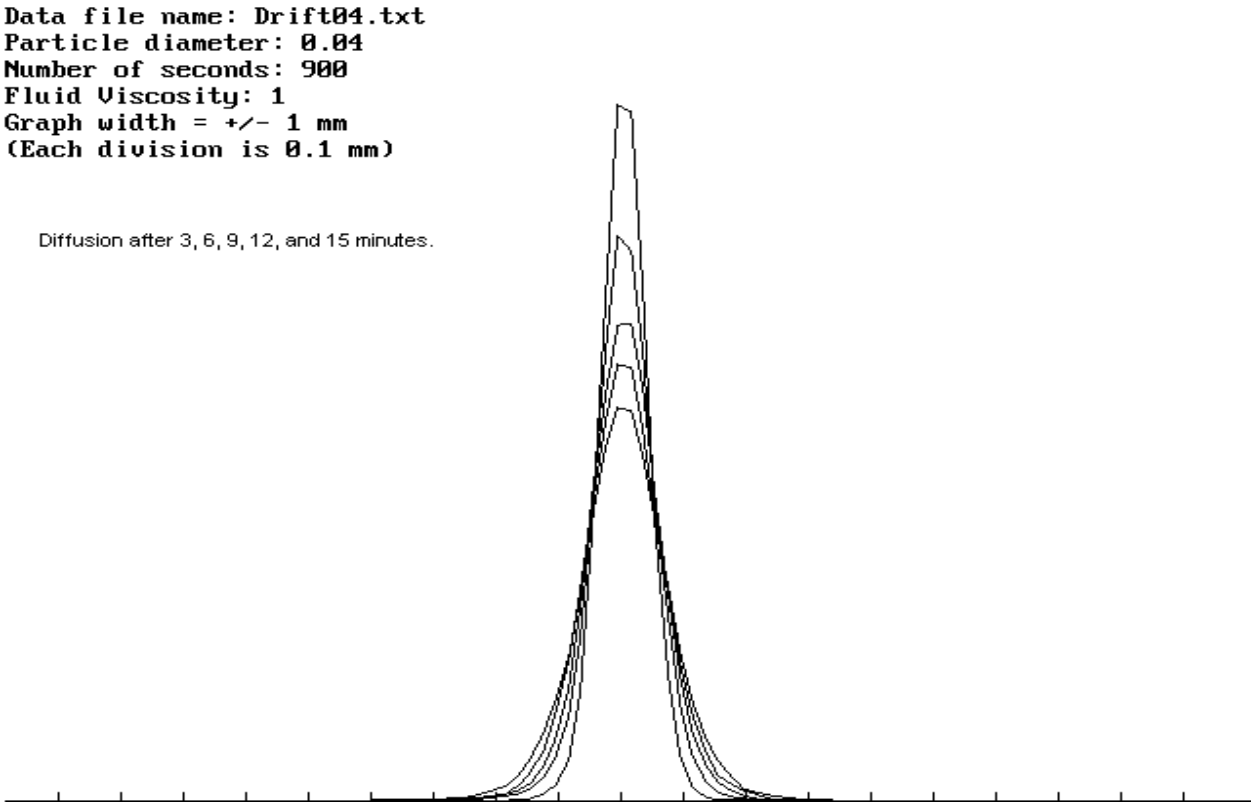
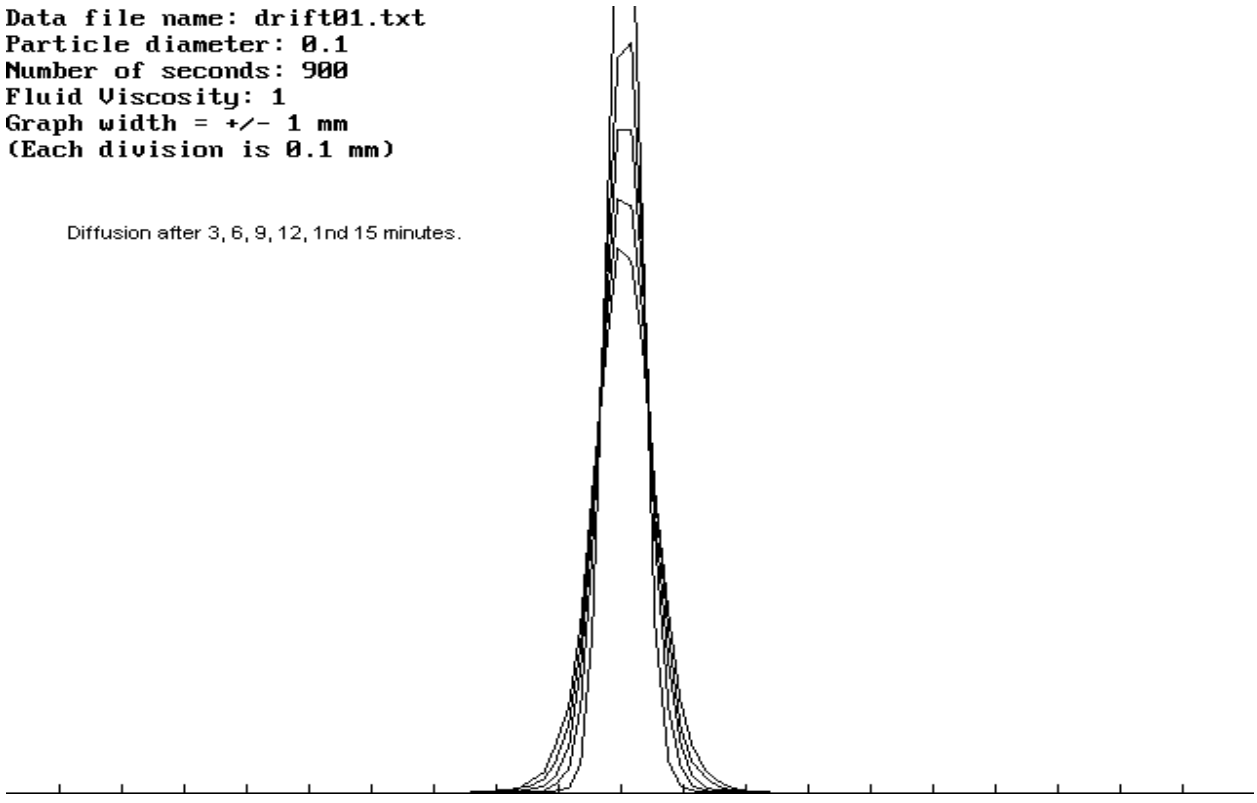


Figure 23



Stokes Equation

If all the conditions specified by Stokes are satisfied, then the viscous drag force acting on the particle is given by:

$$Drag\ Force = 3\pi D\eta V \qquad \text{(Equation 1)}$$

where D is the particle diameter
η is the fluid viscosity
V is the settling velocity of the particle

In a constant gravitational field, the gravitational force acting on the particle is given by:

$$Gravitational\ Force = (\pi/6)D^3(\rho_p - \rho_f)g \qquad \text{(Equation 2)}$$

where ρ_p is the particle density
ρ_f is the fluid density
g is the gravitational acceleration

When the two forces are equal:

$$(\pi/6)D^3(\rho_p - \rho_f)g = 3\pi D\eta V \qquad \text{(Equation 3)}$$

or

$$V = (D^2(\rho_p - \rho_f)g)/(18\eta) \qquad \text{(Equation 4)}$$

Equation 4 (Stokes Equation) shows that the velocity of the particle is proportional to the square of the particle diameter, proportional to the difference in density between the particles and the fluid, proportional to the gravitational acceleration, and inversely proportional to the fluid viscosity. The time (t) required for the particle to move from the surface of the fluid to a distance X below the surface is given by:

$$t = X/V = (X18\eta)/(D^2(\rho_p - \rho_f)g)$$
(Equation 5)

$$D = (X18\eta)/(t(\rho_p - \rho_f)g)^{1/2} \qquad \text{(Equation 6)}$$

Equation 6 shows that the diameter of a particle can be determined by measuring the time required to sediment a known distance. Since all of the parameters in Equation 6 are constant during a sedimentation (except for D and t), Equation 6 can be restated

as:

$$D = K/t^{1/2} \qquad \text{(Equation 7)}$$

where K is a combination of constants from Equation 6

Of course, inside the Disc Centrifuge the centrifugal acceleration replaces the gravitational field. The centrifugal acceleration increases as the particles move from the surface of the liquid toward the outside edge of the disc chamber, because the radius of rotation increases as the particle moves outward.

$$Centrifugal\ Acceleration = \omega^2 R \qquad \text{(Equation 8)}$$

where ω is the rotational speed in radians
R is radius of rotation

This value for acceleration is substituted for 'g' in Equation 4:

$$V = (D^2(\rho_p - \rho_f)\omega^2 R)/(18\eta) \qquad \text{(Equation 9)}$$

The settling velocity 'V' can be restated as the first derivative of the distance from the center of rotation with time:

$$\frac{\delta R}{\delta t} = (D^2(\rho_p - \rho_f)\omega^2 R)/(18\eta) \qquad \text{(Equation 10)}$$

This differential equation can be rearranged to separate variables, and then integrated to get a solution for settling time as a function of the starting radius (R_o), the ending radius (R_f), and several constants:

$$\frac{\delta R}{R} = (D^2(\rho_p - \rho_f)\omega^2)/(18\eta)\delta t \qquad \text{(Equation 11)}$$

$$\int \frac{\delta R}{R} = (D^2(\rho_p - \rho_f)\omega^2)/(18\eta)\int \delta t \qquad \text{(Equation 12)}$$

$$\ln \frac{R_f}{R_o} = ((D^2(\rho_p - \rho_f)\omega^2)/(18\eta))t \qquad \text{(Equation 13)}$$

Equation 13 can be rearranged to the same form as Equation 7:

$$D = ((18\eta \ln(R_f/R_o))/((\rho_p - \rho_f)\omega^2))^{1/2} / t^{1/2} \qquad \text{(Equation 14)}$$

or:

$$D = K/t^{1/2} \qquad \text{(Equation 15)}$$

where K is a combination of constants

Even though the conditions within the disc centrifuge are quite different from sedimentation with gravity, the same type of simple equation describes both processes.

The DCCS software uses the sedimentation time for a calibration standard of known diameter to precisely determine the value of K in Equation 15. Each time a sample is run, a calibration standard run is used to determine a new value for K. In this way, the accuracy of each particle size distribution is assured. The exact values for the individual constants in Equation 14 do not need to be determined, since it is only the combined constant K that must be known. This means that it is not necessary to use exactly the same operating conditions each time the Disc Centrifuge is run. Variation in the conditions are automatically accounted for by the use of an accurate calibration standard.

As the particles near the outer edge of the disc, they pass through the detector light beam, and scatter a portion of the beam. The intensity of the beam that reaches the detector is reduced by the particles, because the scattered portion of the light does not reach the detector. The reduced light intensity reduces the voltage produced by the detector. The detector voltage is amplified and sent to the computer via an analog to digital converter. The DCCS software converts the raw voltage signal into an absorption value, and then generates a particle size distribution by calculating the weight of material at each size based upon the elapsed time of sedimentation, the light absorption, and the efficiency of light scattering by the particles as a function of particle diameter.

The calculated distribution will be accurate so long as the particles are spherical (or nearly spherical) and so long as the refractive index of the particles and fluid and the absorption constant for the particles are accurate. If these values are not known accurately the absolute accuracy of the reported distribution will be reduced. However, even under these circumstances, the CPS Disc Centrifuge will continue to give accurate relative distributions. While it may not be possible to determine the exact

fraction in a particular size range, it is always possible to compare distributions and to determine which has a greater fraction in a particular size range.

The Function of the Density Gradient

The Stokes equation applies to individual particles that are settling in a fluid. A single particle settles just as the equation predicts. However, a sample injected into the disc is not a single particle, but rather millions of individual particles suspended in a fluid. If you attempt to operated the Disc Centrifuge using a homogeneous fluid (for example, water) inside the disc, and inject a sample of particles suspended in water, then the analysis always fails, and no information about the particle size distribution is collected.

The analysis fails because a suspension of more dense particles in a less dense fluid makes the suspension behave like a fluid of higher density, rather than like individual particles suspended in a fluid of lower density. For example, if you measure the density of a 50% (by weight) emulsion of poly methyl methacrylate in water, the measured density is not the density of water, but rather the average density of water and polymethyl methacrylate. A hydrometer will show the density of the latex is much higher than water. If you add a drop of this latex to a container of water, the drop will rapidly fall through the water toward the bottom of the container, leaving a stream of particles behind as it falls (due to some mixing of particles into the bulk water). The drop settles very rapidly, not at all according to the size of the particles that make up the emulsion, but rather only according to the apparent density of the emulsion relative to water.

The bulk settling of a suspension as if it were a homogeneous fluid is called "streaming"; streaming makes measurement of the particle size distribution impossible.

The density gradient that is used in the CPS Disc Centrifuge counteracts the destabilizing effect of higher apparent density in the sample. The sedimentation of particles inside the disc will be stable if (and only if) the apparent density of the fluid inside the disc increases continuously from the surface of the fluid to the outside edge of the disc at all times during the analysis. This requirement is stated by the following equation:

$$\frac{\delta \rho}{\delta R} \geq 0 \qquad \text{(Equation 16)}$$

where ρ is the apparent density of the fluid
R is distance from the center of the disc

The density gradient that is established inside the disc allows Equation 16 to be satisfied during the analysis. When a very dilute sample is injected onto the surface of

the fluid inside the disc, the apparent density of the sample could be very slightly higher than the fluid at the surface of the fluid in the disc, but the fluid just under the surface is also higher in density (due to the density gradient), so there is no instability. The particles sediment according to the Stokes Equation, and the there is no significant "streaming" of the sample during the analysis.

The required "steepness" of the density gradient depends upon the density of the sample to be injected. A sample with higher apparent density requires a steeper density gradient than a sample with lower apparent density. Samples with narrow distributions also require steeper density gradients for optimum analysis, because a narrow distribution tends to form a narrow ring of particles inside the fluid, with higher apparent density and greater potential for streaming. It is therefore better if the samples injected into the Disc Centrifuge are very low in concentration, especially when the particle size distribution is narrow, since this reduces the steepness of the density gradient that is required for stability.

The use of a density gradient to stabilize centrifugal sedimentation was first reported by M. K. Brakke (Brakke, M.K., *Arch. Biochem. Biophys.*, 45 275-290 (1953)). Brakke used a density gradient to stabilize sedimentation of tobacco mosaic virus particles separated from a puree of tobacco leaf cells. Brakke used a sucrose in water gradient, and showed that the virus particles formed a very narrow band during sedimentation (because all the virus particles were the same size). Later, several other researchers independently discovered the same stabilizing effect of a density gradient on sedimentation.

Degradation of the Density Gradient

The density gradient that is established inside the disc centrifuge gradually degrades until it is no longer sufficient to stabilize the sedimentation process. The degradation is by two separate routes: surface evaporation and molecular diffusion. If a thin layer of a low density oil (like dodecane or tetradecane) is placed on top of a water based density gradient, evaporation is greatly reduced.

Diffusive and Evaporative Degradation

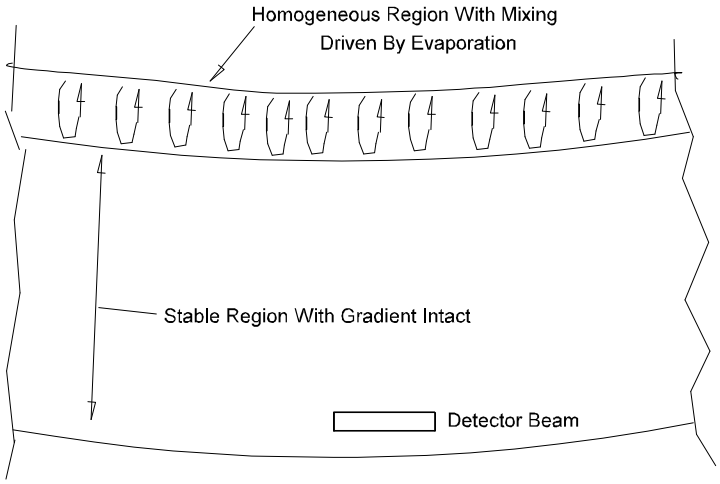
The sucrose molecules in the density gradient diffuse with time, which causes the steepness of the density gradient to decline. The diffusion of sucrose is a fairly slow process, although it is accelerated at higher temperature. The thermodynamic driving force for the diffusion of sugar molecules is an increase in the total entropy of the system as the concentration of sucrose becomes more uniform. The degradation of the density gradient due to diffusion can be thought of as a relaxation of the original concentration gradient.

Sucrose is not volatile, so when water evaporates from the surface of the density gradient fluid, all sucrose molecules are left behind. With time, the concentration of sucrose tends to increase at the surface, until the density of the fluid at the surface becomes slightly higher than the density of the fluid just below the surface. Under these conditions, Equation 16 is no longer satisfied, and the surface fluid falls through and mixes with the fluid just below the surface. The process produces a layer of

homogeneous sucrose concentration, and no density gradient. Figure 24 shows a closeup view of a section of the disc after a homogeneous layer has started to form due to evaporation. With time, the depth of the homogeneous region increases. (Dodecane is added after the gradient is formed to inhibit evaporation of water and extend the lifetime of the density gradient.)

Figure 24

Closeup of Disc SectionWith Partial Degradation of Density Gradient



Any sample will mix into the homogeneous region and form a broad initial band.

Any sample injected onto a homogeneous layer will immediately stream into that layer, and form a broad initial band of particles, instead of the normal narrow initial band. This process causes a loss of resolution, since the apparent width of the entire distribution will be increased. When the calibration standard is injected under these conditions, the DCCS software will "reject" the calibration as "no-good" because the DCCS compares the measured broadness for the standard with the expected broadness, as defined in the procedure definition. A thin layer of a non-volatile oil on the surface of the water, such as dodecane or tetradecane, reduces evaporation of water because water has very low solubility in the oil. This nearly eliminates degradation of the density gradient due to evaporation, and extends the useful life of the gradient to at least several hours.

The injection of calibration standards and samples actually helps to extend the effective

life of the density gradient. The calibration standard and sample dilutions do not normally contain any sucrose, so each injection reduces the concentration of sucrose at the surface of the fluid, and compensates for evaporation from the surface. In addition, the calibration standard and sample dilutions may contain a fraction of ethanol. Ethanol is much lighter than water, and each injection actually **increases the steepness** of the density gradient near the surface as the ethanol diffuses into the bulk of the solution. The effect of these injections is so favorable that the useful lifetime of a density gradient can be greatly extended with frequent injections. For example, if the analysis time for a particular procedure is 10 or 15 minutes, the lifetime of the gradient will probably be at least 8 hours (so long as a new sample is run each time an analysis is completed), while it may be only 3 or 4 hours if the analysis time is 45 minutes.

The volume of fluid in the disc gradually increases as samples and calibration standards are injected. At some point, the time required to run an analysis may become unacceptably long, even though the density gradient is still in acceptable condition. In this case, it is better to stop and empty the disc and then build a new density gradient, since the time needed to replace the gradient is only about 5 minutes. Each injection of a calibration standard plus sample (about 0.1 ml to 0.2 ml total) increases the analysis time about 1% to 2%, so after 20 samples have been run, the analysis time will increase about 40% compared to the first analysis.

New Calibration Standards

Each Operating Procedure Definition requires that a calibration standard of some kind be specified. You can choose any dispersion of particles as a calibration standard that satisfy the following requirements:

1. **The distribution must be fairly narrow.**

   The more narrow the distribution, the better it will perform as a calibration standard. At a minimum, the smallest particles in the distribution must be no less than 0.50 times the peak (highest concentration) diameter. For example, if a potential calibration standard has a particle size of 0.9 micron, then the smallest particles in the distribution should be no less than 0.45 micron.

2. **The particle size must be known accurately.**

   There are a couple of different ways to determine the peak particle size of a potential calibration standard. If the standard is a commercially produced calibration standard (for example, a 2 micron polystyrene latex), then the producer will provide certification of size. If the standard has been carefully measured (average results of several analyses) using the CPS Disc Centrifuge, and the calibration standard supplied with the system, then this should be a reliable size. Analysis with the CPS Disc Centrifuge also supplies the information needed for the Half-height Width measurement (see below).

3. **The density of the particles must be known accurately.**

   This is especially important if the calibration standard is going to be used in the measurement of materials that have density different than the calibration standard. If the calibration standard is only going to be used in the measurement of samples made of the same material (same density) then the need to know the density exactly is less critical.

4. **The half-height width of the standard must be known accurately.**

   The DCCS uses a calibration standard for two purposes: to determine the value of K in Equation 15, and also to determine if the density gradient has degraded significantly. The value of K is determined by measuring the time required for the peak of the calibration standard distribution to reach the detector. The condition of the density gradient is determined by comparing the measured (half-height) width of the calibration standard with the width value that is part of the procedure definition. Commercially prepared calibration standards (such as the one supplied with the CPS Disc Centrifuge) normally provide some measure of the distribution width. If a new calibration standard **does not** include a value for distribution width, then the half-height width can be measured directly using the CPS Disc Centrifuge.

   To do this, the operator initially uses an arbitrary (wide) value for half-height width in the procedure definition, but the correct peak size. The operator then runs the analyzer using a **new density gradient**. The new calibration standard is used **both as the calibration standard and as the "unknown" sample.** The complete distribution for the new calibration standard can then be used to determine the correct half-height width to be used in the procedure definition. The new density gradient insures that the sedimentation in the disc will be stable, and that the measured distribution will be accurate, even with an arbitrary (wide) value used for half-height width in the procedure definition.

Refining a Calibration Standard

The most common problem with developing a new calibration standard is that the initial distribution is too wide to make an acceptable calibration standard. Any dispersion that will settle on standing to the bottom of its container in a reasonable time (less than a week or two) can be "refined" by eliminating the fine and coarse portions of the distribution. Refining a distribution has the following steps:

1. Measure the initial peak size and distribution width.

   This can easily be done on the CPS Disc Centrifuge using an existing calibration standard. Once the initial distribution is known, those portions of the distribution which must be eliminated can be determined. Keep in mind that the smallest size in the final (refined) distribution must contain no material less than 0.65 times the peak size.

2. Calculate the settling rate for the smallest acceptable size in the distribution.

   The settling rate can be calculated using Equation 4:

$$V = (D^2(\rho_p - \rho_f)g)/(18\eta)$$

   where: V = settling velocity in cm/sec
   g = 980 cm/sec^2
   η = viscosity of the dispersing liquid in poises
   ρ_p = density of the particles in g/cm^3
   ρ_f = density of the liquid in g/cm^3
   D = the smallest acceptable diameter **in centimeters** (1 cm = 10,000 microns)

3. Allow a sample to settle for about 40% of the time required for the smallest acceptable diameter to settle from the surface to the bottom of the container.

4. Carefully decant the liquid from the container, leaving the "sediment" on the bottom.

5. Disperse the sediment in clean liquid (perhaps containing some emulsifier).

6. Repeat steps 3, 4, and 5 at least three times.

7. Calculate the settling time (for the **largest** acceptable particle) from the surface of the liquid to the bottom of the container.

8. Disperse the product from step 6 in clean liquid, and allow the dispersion to settle for about 60% of the time calculated in step 7, then decant the liquid and discard the sediment from the bottom of the container.

9. Repeat Step 8.

Steps 1 to 6 remove fine particles from the initial distribution. Steps 7 to 9 remove coarse particles from the initial distribution. The refined distribution should easily satisfy the requirements of a calibration standard. Please note that all settling steps in the refining process must be carried out under fairly constant temperature, or convective currents in the liquid will interfere with the settling of the particles.

Maintenance of the CPS Disc Centrifuge

The CPS Disc Centrifuge is designed and constructed to require very little maintenance. The most important maintenance is the regular tape backup of the system files and data files. The only other normal maintenance procedures are cleaning of the disc and disc housing, and replacing the intake air filter.

Cleaning of the Disc

The disc should be emptied of all used density gradient fluids after each use. It is also recommended that the disc be cleaned after each use, or daily (if used several times per day) according to the following procedure:

1. Add approximately 10 ml of clean distilled water (containing a trace of surfactant) to the disc, then turn the disc by hand for several revolutions.

2. Remove the rinse water with a 10 or 20 ml syringe body or rubber bulb attached to a thin flexible plastic or rubber tube. **Do not use** a syringe needle or any other metal object, since this could scratch the disc and reduce its optical clarity.

3. Push a clean cotton rag, cotton towel, or paper towel into the disc chamber until it reaches the outside edge, then slowly turn the disc by hand to soak up any residual liquid from inside the disc, and to insure that all solids and liquids are wiped from the internal surfaces of the disc.

4. Repeat steps 1 to 3 a second time

5. Use a moist, clean rag or moist, clean cotton towel to wipe both the front and back faces of the disc, especially where the detector light beam passes through the disc.

Never use an abrasive cleaning compound or an abrasive pad to clean the disc (either inside or out) since these will severely scratch the disc surfaces and may make the disc unusable.

Cleaning the Disc Housing

When samples are injected into the disc, sometimes a few drops of the liquid splash off the rotating disc. CPS recommends that the interior of the Disc Housing be inspected for fouling each 1 to 2 weeks, and cleaned using a damp cotton rag or towel if there is any material on the surfaces.

Air Filter

The air filter located in the top of the instrument should be removed and cleaned each several months. If the instrument runs frequently, or if the operating environment is dusty, cleaning should be more frequent. The filter can be blown off with compressed air or gently washed with water and detergent followed by rinsing and drying.

Technical Specifications and Information

System Name: CPS Disc Centrifuge, Model DC12000
CPS Disc Centrifuge, Model DC18000
CPS Disc Centrifuge, Model DC20000
CPS Disc Centrifuge, Model DC24000

General Specifications

Maximum Disc Speed	12,000, 18,000. 20,000 or 24,000 RPM
Minimum Disc Speed	600 RPM
Maximum Measurable Size	> 40 Microns
Minimum Measurable Size	<0.01 Micron
Minimum Resolution (Size difference for narrow peaks)	10 %
Typical Resolution (Size difference for narrow peaks)	<5 %
Minimum Accuracy (Compared to calibration standard)	+/- 2 %
Typical Accuracy	+/- 0.5 %
Minimum Repeatability	+/- 2 %
Typical Repeatability	+/- 0.5 %
Calibration Method	Internal or External Standard
Reporting Modes	Weight, Surface Area, Number

Computer System Requirements (minimum)

CPU	Pentium Class 500+ MHZ
Hard Disc Capacity	3+ Gigabyte available
Random Access Memory	min needed for Windows
Display Type	Color Super VGA At least 800 x 600 Pixels With 16,000 colors or more
Installed Software	Windows 95, 98, NT, ME, XP CPS DCCS Software
Printer	Any Windows compatible, Color is preferred‡

‡ Print drivers may be used instead of a physical printer to create graphic output files in several different formats, such as .PDF, .BMP, or .JPG.

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