Instruction Manual

for

90Plus/BI-MAS

Multi Angle Particle Sizing Option
Operation Manual

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Manual contains FAQ
and What's New?

PLEASE READ

This is your instruction manual for the Brookhaven BI_MAS option. Please read it carefully before making measurements. The INSTALLATION section describes procedures for checking that the instrument and software are working properly. Familiarize yourself with the software by running the program with some of the data sets that are provided for this purpose. If you have any questions, comments or suggestions, please contact Brookhaven.

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First Printing : January, 1995
Catalog Number : MASMAN, Ver. 1.0

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I Introduction

The MAS OPTION is an automatic particle sizer designed for use with either concentrated suspensions of small particles or solutions of macromolecules. Generally speaking, sizes from 2nm to 3μm can be measured. The software for instrument control and data analysis is written for use in the Microsoft Windows environment though a DOS version is also available.

The technique employed - photon correlation spectroscopy (PCS) of quasi-elastically scattered light (QELS) - is based on correlating the fluctuations about the average, scattered, laser light intensity. It has several advantages including:

• Speed	(Typically 1 to 2 minutes)
• Accuracy	(± 1% on monodisperse samples)
• Sample Volume	(0.5 to 3 ml)
• Calibration	(None required)
• Versatility	(Measures particles, polymers, emulsions, colloids, etc. in any suitable liquid)

Sample preparation is relatively quick and easy with dust being the major problem. Using a few simple "tricks of the trade" and the unique built in "dust filter" will allow a high percentage of acceptable measurements to be made by the novice user.

Computer control of the MAS OPTION makes the instrument easy to operate. The software is menu driven with friendly screens designed to guide and inform at each stage of the operation. Common problems are identified and accentuated and simple remedies are suggested by the software. After pressing the **Start** button, everything else is automatic, including the calculations.

Please read the chapters on **Installation**, **Self Test**, the **MAS OPTION Software** and **Diagnostics** before attempting to use the instrument. The chapters entitled Quasi Elastic Light Scattering (QELS) Theory and Data Interpretation are not essential for correct instrument setup and operation. However, an understanding of these principles will help the user to apply the results in a better informed fashion.

The technical and sales personnel at Brookhaven would be glad to render any assistance regarding questions that you might have about the MAS OPTION that have not been clarified despite reading this manual.

I.I Specifications

Size Range : 2nm to 3μm

Diffusion Coefficient Range : 10^{-6} to 10^{-9} cm^2/sec

Accuracy : ± 1% to 2% with monodisperse samples

Repeatability : ± 1% to 2% with dust free samples

Laser : 15mW solid state laser. Complies with BRH 21CFR 1040:10 as applicable.

Temperature Control (OPTIONAL) : 5 °C to 75 °C on steps of 0.1 °C

Sample Volume : 0.5 to 3ml

Measurement Time : Typically 1 to 2 minutes

Results : Mean and Standard Deviation calculated for size distribution by weight assuming a Lognormal distribution. All results are displayed on the monitor and can be printed. Text file reports are provided and the raw data can be exported into an ASCII text file as well. **Multimodal Size Distribution** analysis (MSD) is optional.

II Software Installation

If Brookhaven supplied the computer, the software is already installed on your hard drive.

If you supplied the computer, insert the CD with the **9KPSDW** software into the CD drive. In the MAS subdirectory, find and double click on Setup.Exe. Follow the suggestions on screen. When you are finished, the software can be found under START/ALL PROGRAMS/Brookhaven Instruments Corp (Win 32). You may find it convenient to create a shortcut to your desktop.

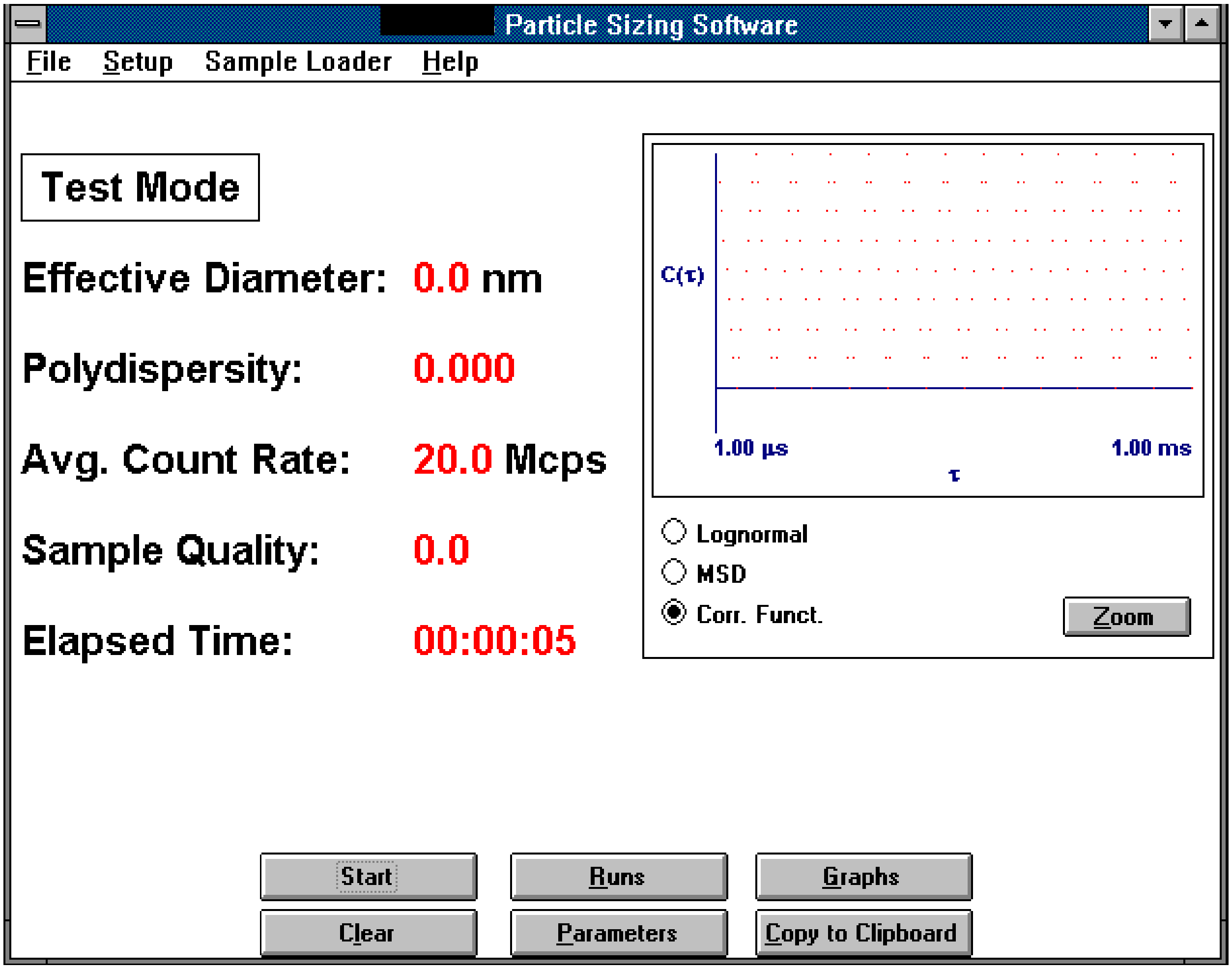
The software will be installed in the sub-directory **9KPSDW** in the directory **BICW32.** It is strongly recommended that the installation directories be allowed to remain as named by the setup software and not be altered in any way.

A data directory for the storage of measurement data will also be created. This will be a sub-directory of the **9KPSDW** directory called **DATA**.

To install the Multimodal Size Distribution (MSD) software (or to update the version of the MAS OPTION software at a later time) insert the MSD (or the updated MAS OPTION software) into the drive and follow steps shown above for initial installation.

II.3 Self test

As we have discussed at various points in this manual, the heart of the MAS OPTION is a digital autocorrelator that processes information for the instrument and generates an autocorrelation function. It is from this function that vital information such as particle size and polydispersity are obtained. The correlator is equipped with self diagnostic capabilities. Before attempting to make a measurement, it is advisable to use these self-test functions to ascertain that the correlator is functioning properly. To execute the diagnostics, press **Ctrl+T** in the main menu of the program. This puts the correlator into its test mode wherein it correlates 200 channels linearly spaced from 5 micro seconds to 1 second and accumulates for 5 seconds. At the end of this test procedure, the screen should look like the one shown in figure (2b).



Test Mode

Effective Diameter: 0.0 nm

Polydispersity: 0.000

Avg. Count Rate: 20.0 Mcps

Sample Quality: 0.0

Elapsed Time: 00:00:05

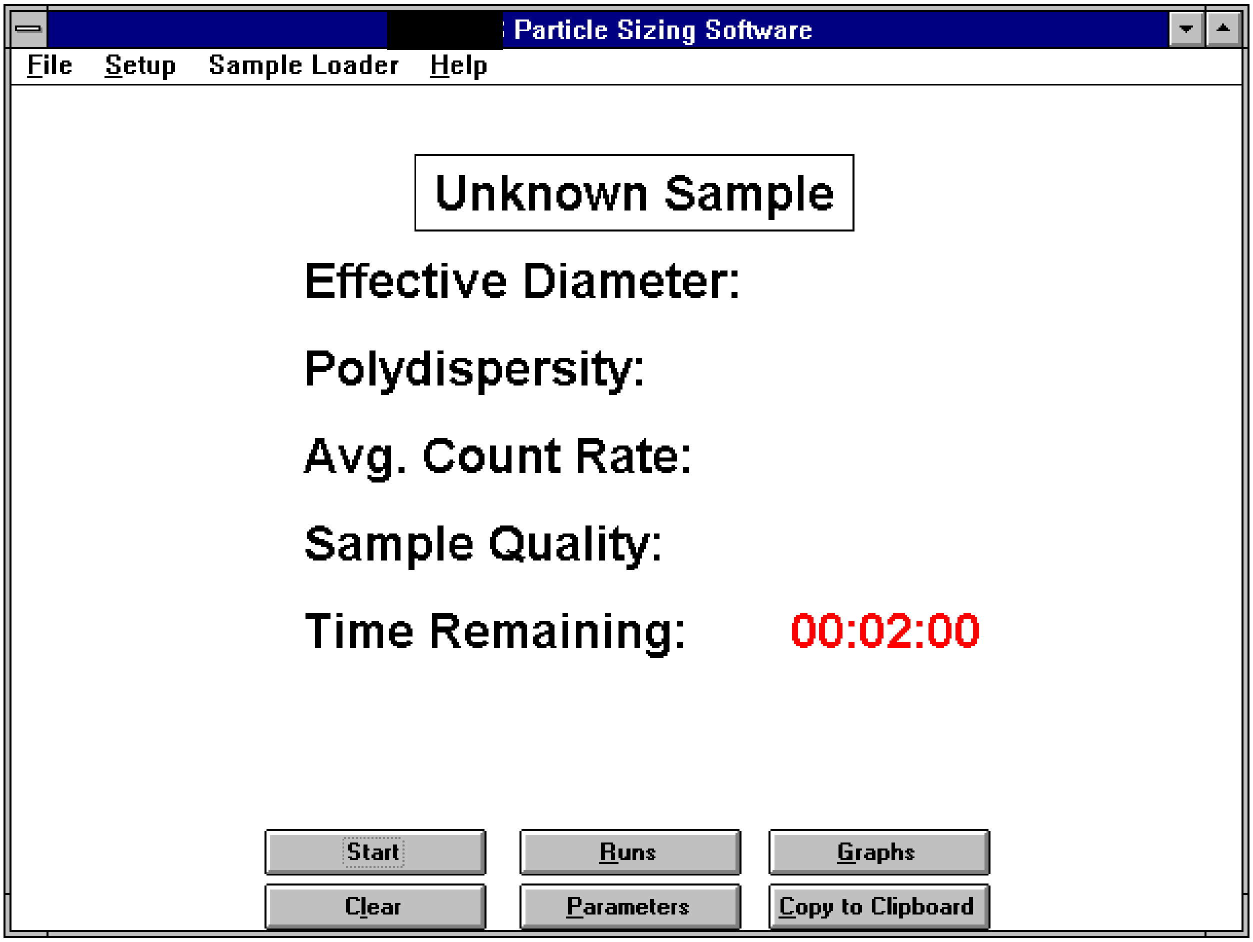
○ Lognormal
○ MSD
● Corr. Funct.

Figure 2b
Testing the correlator

III The MAS OPTION Software

The software for your Brookhaven MAS OPTION has been designed to run in the Microsoft Windows environment. Users who wish to familiarize themselves with Microsoft Windows should read their Windows documentation before attempting to use the MAS OPTION software.

The MAIN MENU for the MAS OPTION software is the menu page through which all the features of the software can be accessed. Figure (3a) shows the MAIN MENU as it appears when the program is first executed.



Unknown Sample

Effective Diameter:

Polydispersity:

Avg. Count Rate:

Sample Quality:

Time Remaining: 00:02:00

Figure 3a
The Main Menu

Before we begin taking any new data, let us familiarize ourselves with the operation of the software by learning how to load a data set and how to analyze it. As mentioned before, the software is designed to be compatible with the philosophy behind most Windows based programs. The menu bar is located at the top of the MAIN MENU and remains accessible so long as the window bearing the MAIN MENU is active. To access a data file one needs to go through the following simple steps:

III.1 LOADING DATA

Move the mouse cursor over to **File** on the Menu bar of the MAIN MENU and click on it. The **File** menu will now become accessible:

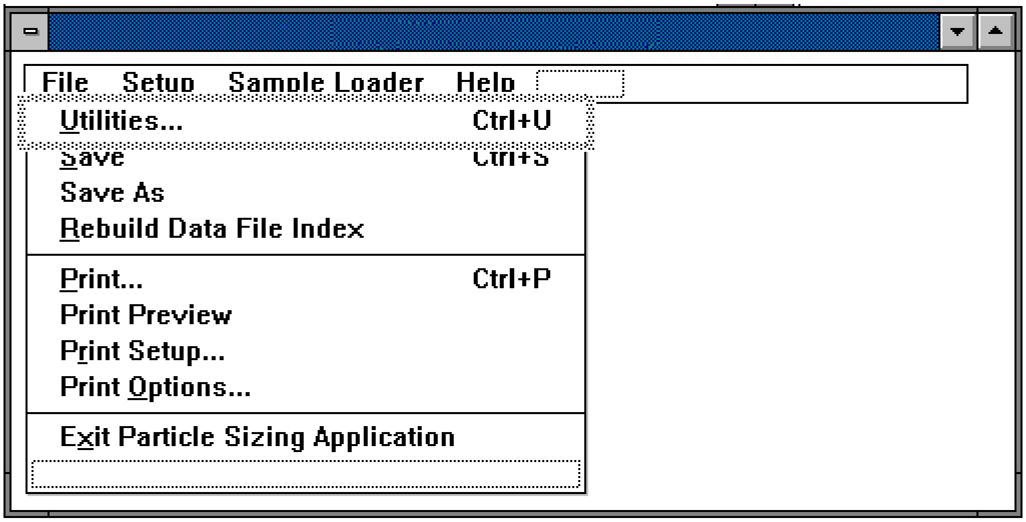


Figure 3b
The File Menu

Selecting the **Utilities** option in this menu will open the File Utilities page (Figure 3c) and afford access to the data files stored in the various folders. The buttons on the bottom of the page give you access to the various features of this menu.

1. Select a Folder by clicking on it.

A list of files stored in that folder appear.

2. Select a file by clicking on it.

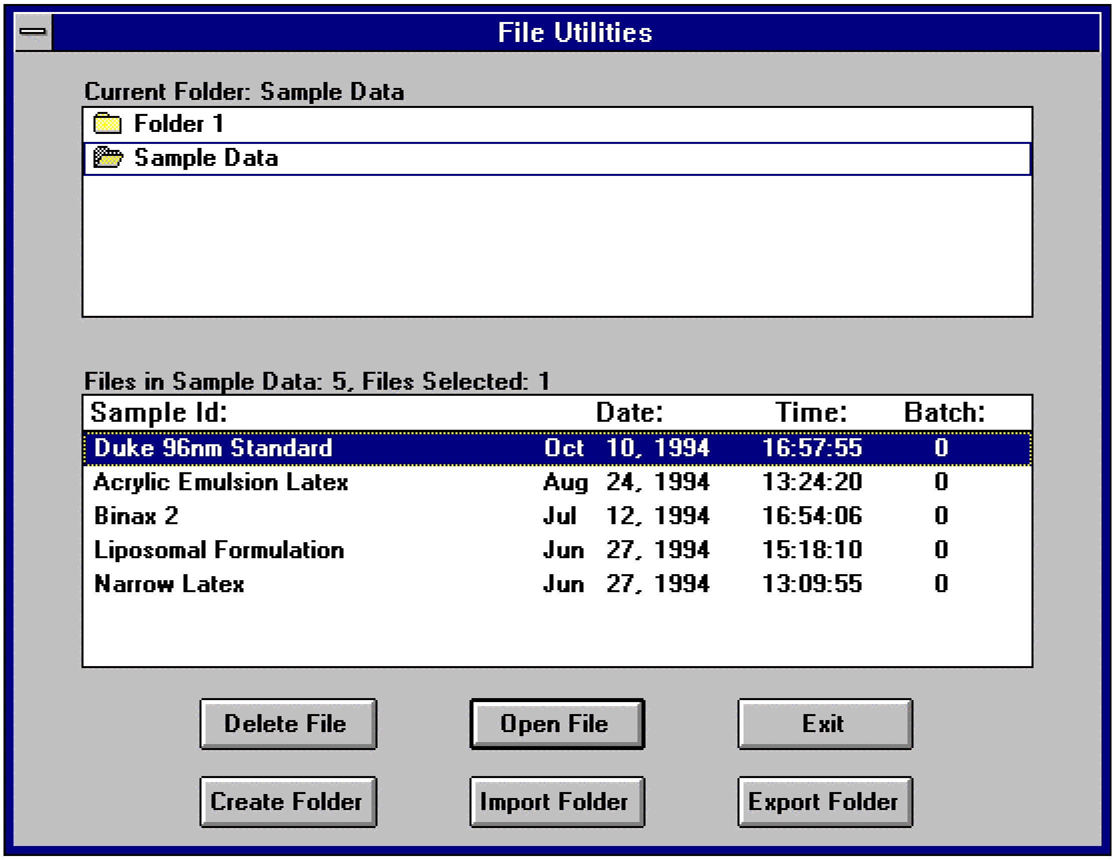
The buttons on the screen are now active pertaining to the file and/or the folder that you have selected. Files can be opened (**Open File** - you can also do this by double-clicking on any file) or deleted (**Delete File** - this becomes active when a file has been selected You will be asked for confirmation before the file is actually deleted). You can also create a folder (**Create Folder** - you will be prompted for a folder name limited to 30 characters), delete a folder (**Delete Folder** - this becomes active when a folder has been selected, again you will be asked for confirmation), import a folder (**Import Folder**) from another directory or from a disk and export a folder (**Export Folder**) to another directory or to a floppy disk.

In this case, we see that two folders exist. **Folder 1** has nothing in it, it is meant to store any new data that you may generate. At the present time, the folder called

Sample Data is of more interest to us. We can select this folder by double clicking on it. We can now see a list of the data files that are stored in the folder **Sample Data** (Figure 3c). The data is identified primarily by the **Sample Id** though the automatically attached **Date** and **Time** stamp can also be used for this purpose. A **Batch** number may also be provided by the user for additional identification. Let us select the first sample.

Double click on **Duke 96nm Standard**.

We have now loaded the data associated with the **Duke 96nm Standard** sample.



Current Folder: Sample Data

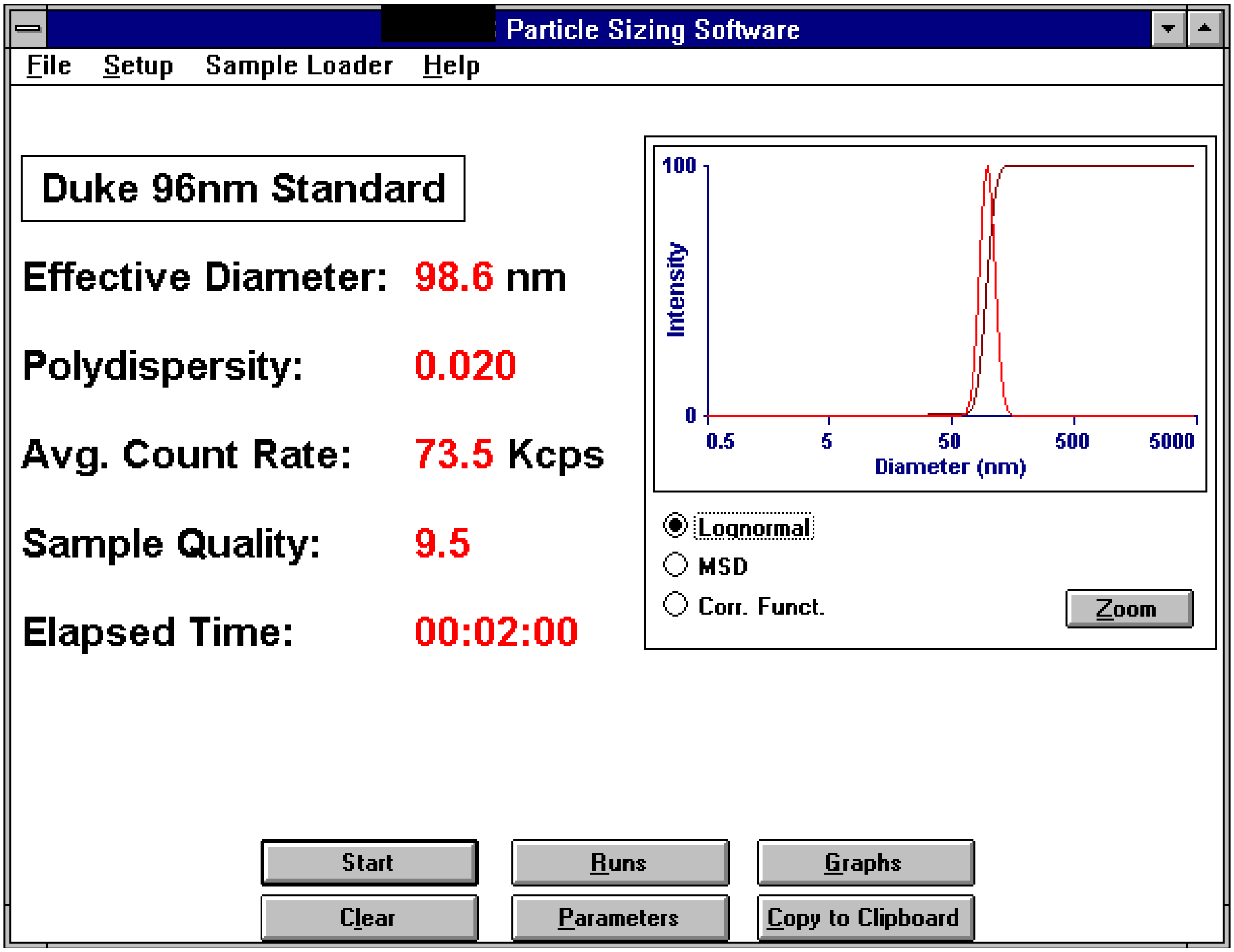
Folder 1
Sample Data

Files in Sample Data: 5, Files Selected: 1

Sample Id:	Date:	Time:	Batch:
Duke 96nm Standard	Oct 10, 1994	16:57:55	0
Acrylic Emulsion Latex	Aug 24, 1994	13:24:20	0
Binax 2	Jul 12, 1994	16:54:06	0
Liposomal Formulation	Jun 27, 1994	15:18:10	0
Narrow Latex	Jun 27, 1994	13:09:55	0

Figure 3c
The File Utilities Page

Figure 3d shows the screen that results from loading a data file. The results appear conveniently tabulated just below the Sample Id. Each of these quantities are discussed in greater detail in the chapter on Data Interpretation. For now we will accept them at face value keeping in mind that the **Effective Diameter** represents an average size of the particles in the sample and that the **Polydispersity** is a measure of the non-uniformity's that exist in the particle size distribution, Avg. Count Rate is a measure of signal intensity (Kilo Counts **Per Second - KCPS**) and **Sample Quality** is an indicator of the difference between the measured and calculated baselines of the correlation function. The highest number (best quality) is 10.



Duke 96nm Standard

Effective Diameter: 98.6 nm

Polydispersity: 0.020

Avg. Count Rate: 73.5 Kcps

Sample Quality: 9.5

Elapsed Time: 00:02:00

● Lognormal
○ MSD
○ Corr. Funct.

Figure 3d
A reloaded data set is displayed on this screen.

At the bottom of the screen are six buttons, each affording access to additional features of the software.

The **Start, Clear** and **Runs** buttons pertain to collecting data from your sample. For the time being we will leave it at that and return to them later.

III.2 Parameters

The **Parameters** button provides us access to the **Parameter** page as shown in Figure 3e. This page serves the twofold purpose of sample archiving as well as entering the parameters needed for running the sample and for data analysis. Let us examine these a little more closely:

Sample ID : Enter the name by which you wish to identify your sample. (30 characters maximum).

Operator ID : Enter your name (30 characters maximum).

Notes : Enter any notes that you might have on this sample (42 characters maximum).

Batch # : Enter a Batch Number for your samples (5 Digits maximum)

Runs : Enter the number of successive runs you wish to make on this sample.

Run Duration: Enter the desired duration for each run.

The preceding parameters are mainly archival in nature and are optional. The following however, are used for calculations and should be entered with due care.

Temp.: Enter the temperature at which the sample will be controlled.
Suspension : Enter the medium in which the sample is suspended. A list of common media is made available by clicking on the ↓ symbol.

Viscosity : The viscosity of the medium (Automatically calculated from the temperature).

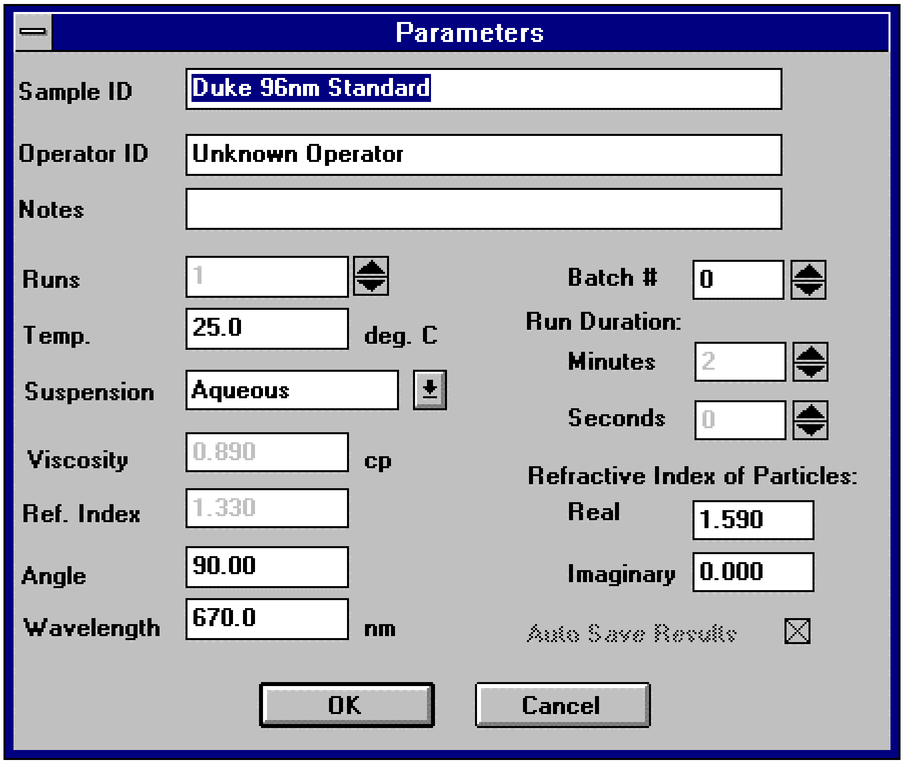
Ref. Index : The Refractive Index of the medium (Automatically calculated and inserted).

Refractive Index of Particles

Real : Real component of the particle refractive index.

Imaginary : Imaginary component of the refractive index.

Enabling the **Auto Save Results** feature (by clicking in the box) will result in the results being saved at the end of every run. If the **Auto Save Results** feature is not turned on, you will have to manually save the data after each run.



Sample ID: Duke 96nm Standard
Operator ID: Unknown Operator
Notes:
Runs: 1
Temp.: 25.0 deg. C
Suspension: Aqueous
Viscosity: 0.890 cp
Ref. Index: 1.330
Angle: 90.00
Wavelength: 670.0 nm
Batch #: 0
Run Duration:
Minutes: 2
Seconds: 0
Refractive Index of Particles:
Real: 1.590
Imaginary: 0.000
Auto Save Results ☒

Figure 3e
The Parameters Page

To save any changes that you make to the Parameters Menu, click on the **OK** button, to exit without saving any of the changes, click on the **Cancel** button.

III.3 Copy to Clipboard

This is a very useful feature for transporting results from one Windows application to another. By clicking on this button the data represented on the screen (both tabular and graphical) is copied to the Windows clipboard. It can now be exported to any application by using standard Windows clip and paste techniques. For example you could incorporate a graph or a table into a document created by using Microsoft Word for Windows.

III.4 Graphs

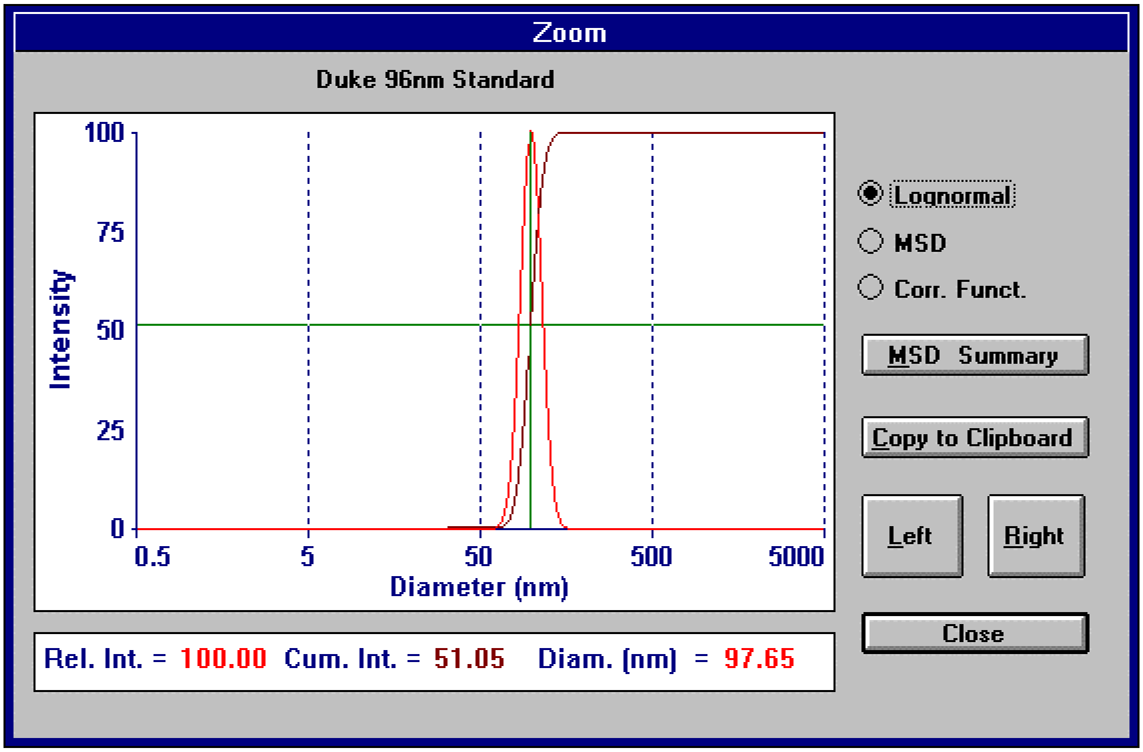
This button allows you to change the screen to either add a graphical representation of the data, or to erase the graph if one is already present. Once the **Graphs** feature has been activated, you can select the type of graph to view.

The simplest graphical representation available is the **Log Normal** where the **Effective Diameter** and the **Polydispersity** are used to generate a log normal distribution of particle sizes.

You can click on **MSD** to see a more sophisticated representation of the size distribution. The **MSD** uses the Non-Negatively constrained Least Squares (NNLS) algorithm to fit the data. This is a model independent technique and it is possible to achieve multi-modal distributions using it.

Clicking on the **Corr. Funct.** will display the autocorrelation function obtained from the sample. This is the primary result measured by the instrument and all the other information is computed from this curve. A more detailed description of the correlation function is given in the chapter dealing with the theory of the MAS OPTION.

Clicking on the **Zoom** button will allow you to examine whichever graph that you might be viewing in greater detail (Figure 3f). In this expanded screen, it is possible to move the cursor along the curve to examine, at each point, the values of the x and y axes. A feature that some users might find useful is the ability to hide (or display) the cursors and the numerical values of the axes. Clicking on any portion of the Graphs window outside the rectangle bounding the graph itself will hide the cursors and the data associated with the graph. Clicking inside the graph itself will restore the graph highlight and the data values. Again the **Copy to Clipboard** button will enable you to transport the graph to other Windows applications.

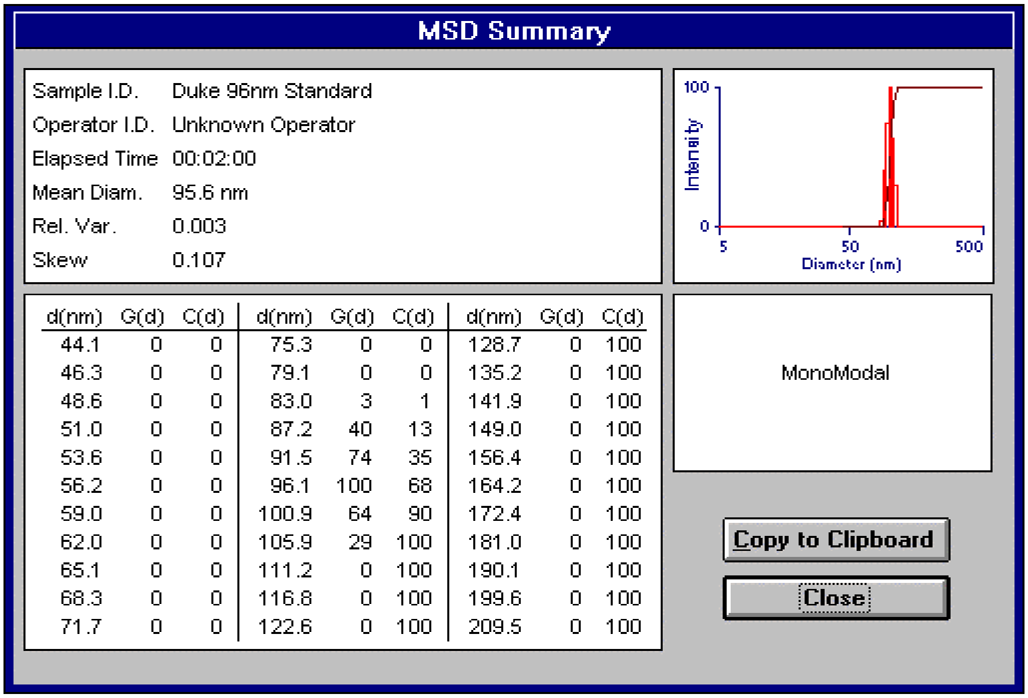


Duke 96nm Standard

Rel. Int. = 100.00 Cum. Int. = 51.05 Diam. (nm) = 97.65

Figure 3f
Zooming into a graph

Clicking on the **MSD Summary** button will show a table of particle sizes and the corresponding values of intensity weighted sizes obtained from the NNLS algorithm (Figure 3g).



Sample I.D. Duke 96nm Standard
Operator I.D. Unknown Operator
Elapsed Time 00:02:00
Mean Diam. 95.6 nm
Rel. Var. 0.003
Skew 0.107

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
44.1	0	0	75.3	0	0	128.7	0	100
46.3	0	0	79.1	0	0	135.2	0	100
48.6	0	0	83.0	3	1	141.9	0	100
51.0	0	0	87.2	40	13	149.0	0	100
53.6	0	0	91.5	74	35	156.4	0	100
56.2	0	0	96.1	100	68	164.2	0	100
59.0	0	0	100.9	64	90	172.4	0	100
62.0	0	0	105.9	29	100	181.0	0	100
65.1	0	0	111.2	0	100	190.1	0	100
68.3	0	0	116.8	0	100	199.6	0	100
71.7	0	0	122.6	0	100	209.5	0	100

MonoModal

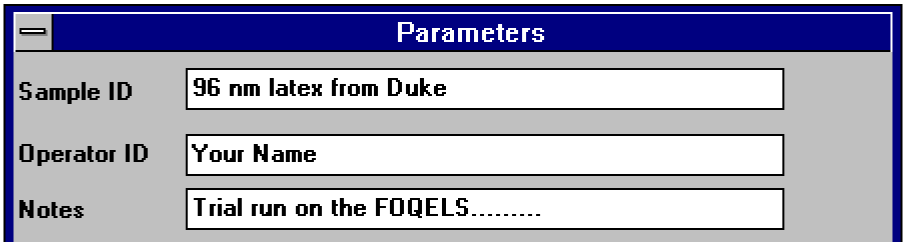
Figure 3g
MSD table

III.5 Collecting New Data

Before beginning to seriously collect data with the instrument, it is usually a good idea to make a few trial measurements on a well known sample. A latex particle size reference material is well suited for this purpose and we recommend the 96nm latex manufactured by Duke Scientific Corporation.

We are now ready to attempt a trial measurement.

Clear any Data that might already be loaded into the MAS OPTION software. You can do this by clicking on the **Clear** button in the Main Menu screen. Click on **Parameters** page and enter the Parameters as discussed earlier. To start with it might be a good idea to leave most of the Parameters in their default settings and entering only the **Sample ID**, **User ID** and **Notes**.



Sample ID: 96 nm latex from Duke
Operator ID: Your Name
Notes: Trial run on the FOQELS.........

Figure 3i
Parameters for a trial run.

Click on **Start** to commence the measurement. Indication that the measurement has started successfully will be provided in the following ways:

1. The button labeled **Start** will be re-labeled as **Stop**.
2. Numbers will appear on the screen for **Effective Diameter**, **Polydispersity** and **Avg. Count Rate**, **Sample Quality** and the **Time Remaining** clock will count down to zero. If Graphs has been activated, a graph will also appear.

If a single measurement has been completed and a second measurement has not yet been started then the operator may either extend the duration of the of the current measurement or request additional runs by calling up the **Parameters** page. The **Runs** button can be used to change the number of runs that you desire for this sample - overriding any request that may have been made in the **Parameters** page. The **Stop** button (that replaced the **Start** button) can be pressed at any time to pause the measurement. Once it has been pressed, it will be replaced by a **Continue** button. Your data is not lost by pressing the Stop button and the Continue button will allow the run to continue for its original duration.

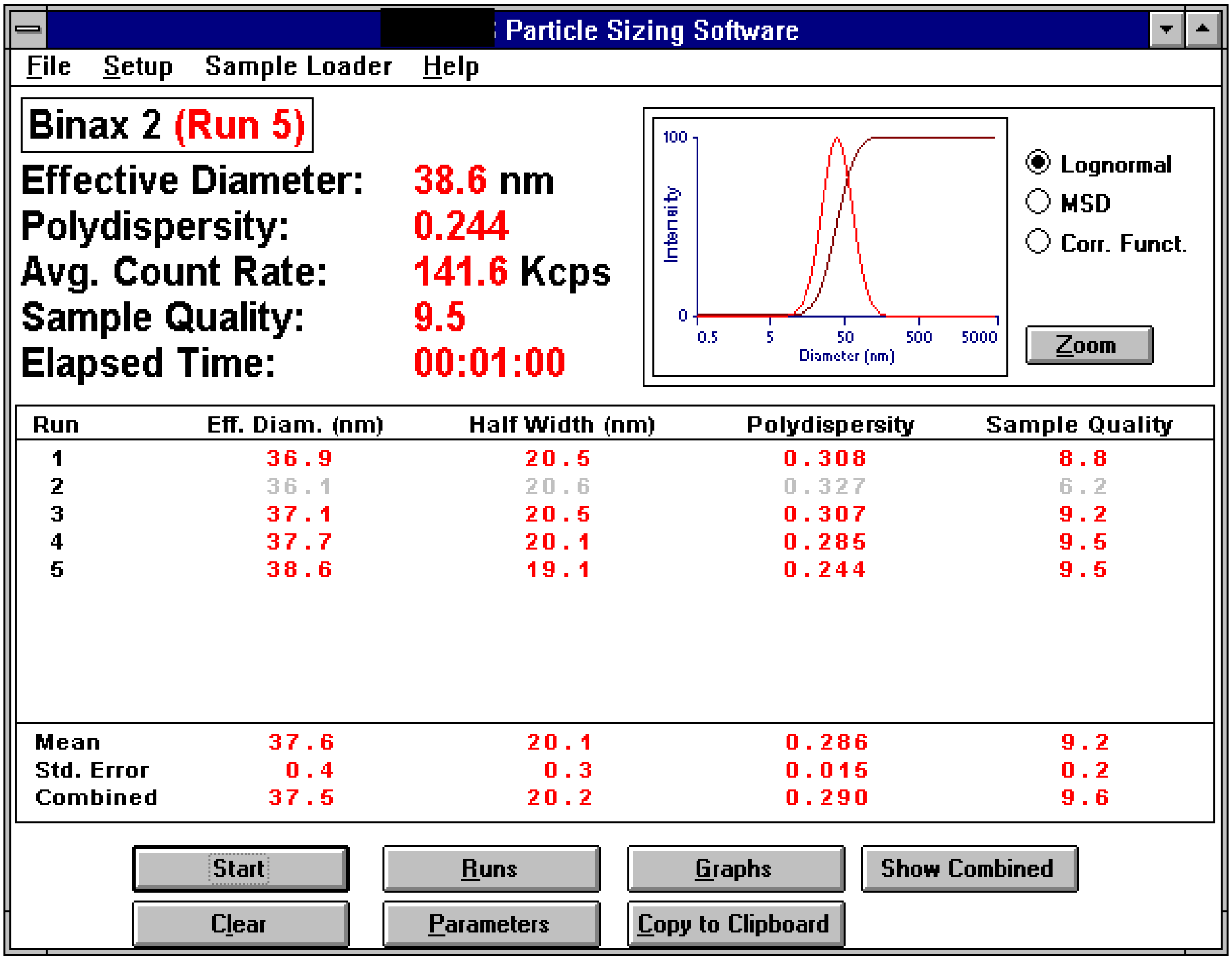
The **Parameters** button can be pressed at any time. If it is pressed while a measurement is in progress, it will display the current settings without allowing you to change them. You can however modify certain **Parameters** while the measurement is in the **Stop** mode.

Allow the measurement to run for its complete duration. You should now be able to examine the results and satisfy yourself that they are acceptable.

III.6 Multiple Measurements

Figure 3j shows the screen when multiple measurements of a sample have been requested. You will notice that the lower part of the screen has been re-structured to incorporate a data table. This displays a brief “history” of the runs completed thus far along with some simple statistics showing the **Mean** and the **Standard Error** of the results.

Individual measurements may be deactivated from this table. You can deactivate a particular measurement either by double clicking on it or by pressing the number associated with the run. This will cause it to be grayed out in the table and its values will not contribute to the computation of the statistics for the table. Double clicking (or pressing the number) on a deactivated measurement will reactivate it.



Binax 2 (Run 5)

Effective Diameter: 38.6 nm
Polydispersity: 0.244
Avg. Count Rate: 141.6 Kcps
Sample Quality: 9.5
Elapsed Time: 00:01:00

● Lognormal
○ MSD
○ Corr. Funct.

Run	Eff. Diam. (nm)	Half Width (nm)	Polydispersity	Sample Quality
1	36.9	20.5	0.308	8.8
2	36.1	20.6	0.327	6.2
3	37.1	20.5	0.307	9.2
4	37.7	20.1	0.285	9.5
5	38.6	19.1	0.244	9.5
Mean	37.6	20.1	0.286	9.2
Std. Error	0.4	0.3	0.015	0.2
Combined	37.5	20.2	0.290	9.6

Figure 3j
Multiple Measurements. Run 2 has been deactivated.

Another feature that becomes available during multiple measurements is the **Show Combined** button. If the **Show Combined** button is clicked the program combines all the measurements into a single data set. It is as if the data from all the runs had been accumulated during the process of a single measurement. Obviously the **Show Combined** feature is possible only when more than one measurement is available. Once the **Show Combined** button is selected, it will be replaced by the **Show Current** button which enables the user to return to viewing the data from the most recent measurement.

The combined data should not be confused with the Mean displayed in the statistics. The Mean is an average of the completed measurements that are still activated. The combined data is generated by using the sum of the raw data from all of the completed measurements (that are activated) and recalculating the cumulant values. Also, to avoid confusion, note that while a measurement is in progress, the information in the upper portion of the screen is updated with data from both the current and the completed measurements that are activated. The information shown in the table in the lower portion of the screen is made up of completed measurements only.

III.7 Data Files

The MAS OPTION particle sizing software has a complete file system which maintains as many as 250 "folders" that may be used to store up to 500 data files each. A data file may contain as many as ten measurements of a single sample. Data files are saved automatically if the "autosave" option is selected in the parameters dialog. Files are always saved in the current active folder. When the program is installed the active folder is called Folder 1.

Data files are stored in a binary format and cannot be modified by the user. When a data file is saved, a copy of the Sample ID, date and time of the first measurement are saved in a folder file index. If a file is saved again after additional measurements of the same sample are made (without clearing previously measured data), then the same file will be updated to contain all the completed measurements.

The user may choose **Save As** from the file menu to save an additional copy of the current data. If the file is re-saved under a new name, then the new name is assumed to be a new sample identification. If previously saved, the original file will still exist under the previous sample identification. The operator may choose to save the file in a different folder or may even create a new folder from the **Save As** dialog. The folder chosen will become the current active folder and any further saved data will be placed in the current active folder.

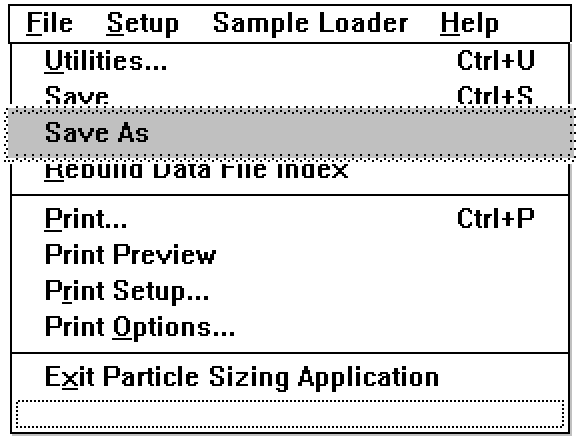
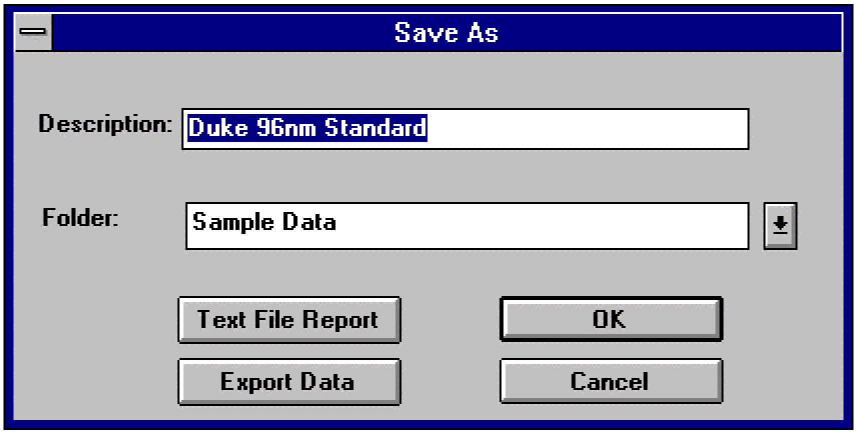


Figure 3k

Saving Files

You can also save the data in text format from the **Save As** dialog by selecting the **Export** button. The software will prompt the operator for the name and location of the text file to be generated. If more than one measurement has been completed, then all active measurements will be combined into a single measurement including all the data from all the completed measurements. If multiple measurements have been completed, and an operator wants to save data from a single measurement then all other measurements should be deactivated before **Exporting** the data file. A deactivated measurement will appear gray on the main screen. Active measurements appear in red. The format of the data file generated is described in appendix I.

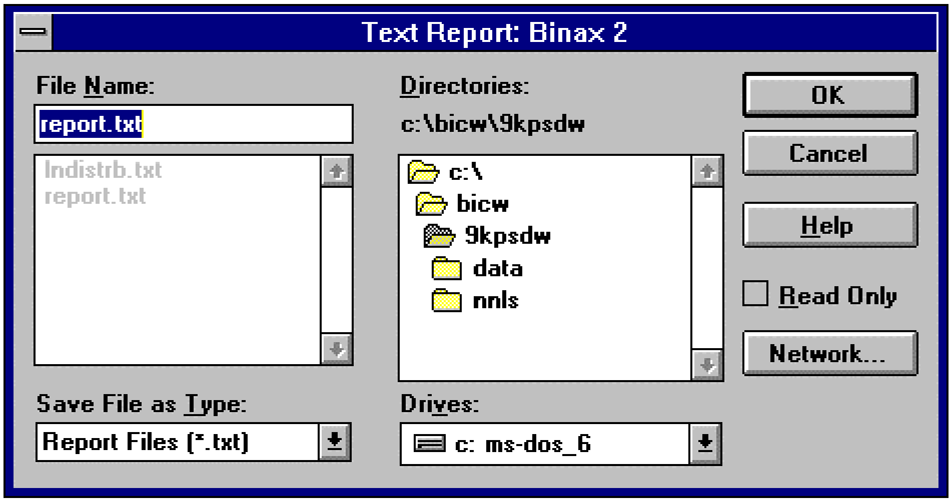


Description: Duke 96nm Standard

Folder: Sample Data

Figure 3l

Saving Files in various formats



File Name: report.txt

Directories: c:\bicw\9kpsdw

☐ Read Only

Save File as Type: Report Files (*.txt)

Drives: c: ms-dos_6

Figure 3m

Saving data as a Text File Report

Clicking on the **Text File Report** button will provide you with a report of the results for the sample in standard ASCII text format. You will be prompted for a file name (for the text report). A example of a Text File Report is shown in appendix II.

An operator may save the current data at any time by choosing "Save" from the file menu. If no measurements have been completed then a partial measurement will be saved reflecting the current elapsed time. If one or more measurements have been completed then only completed measurements will be saved. Any partial measurements started after the first run is completed must also be completed in order to be included in the data file.

Data file folders may be maintained by choosing **Utilities** from the **File** menu on the main screen (Figure 3c). A dialog box will appear showing a list of folders and files. The current active folder is indicated by the open folder icon displayed to the left of the folder name. Please note that data folders do not correspond to individual DOS directories and all folders are maintained through software indexing exclusively. The files list box show files that have been stored under the current active folder only. Files stored in other folders will only be listed if the alternative folder is selected by "double clicking" the desired folder name in the folders list box.

Pressing the tab and shift-tab keys will move the focus of the dialog box from item to item. When the focus is placed in the folders list box, the currently selected item will be highlighted. This should not be confused with the current active folder which is marked with the "open folder" icon.

If the folder list box is selected, pressing the **Delete Folder** button will allow the user to delete the entire highlighted folder and all of the data file contained in it. Similarly, if the files list box show one or more selected files they may be deleted by pressing the "Delete File" command button. The operator will be given one chance only to verify the intention to delete files or folders. **After that there is no way to recover deleted information.**

Multiple files may be selected by pressing the Ctrl key while "single clicking" an individual file. A contiguous section of the file list may be selected by single clicking the first file and then the last file desired while pressing the shift key.

Files may be **moved** from one folder to a new folder by "dragging" the currently selected files to the new folder and "dropping" then into the new folder.

Files may be **copied** to a new folder by "dragging" the currently selected files to a new folder, while pressing the Ctrl key, and "dropping" them into the new folder.

Users should be aware of the current active folder because any further data saved will be placed in this folder until the active folder is changed.

Occasionally, one or more data file will become damaged and will not be able to be reloaded by the software. This could happen for a variety for reasons including unexpected power interruption during a save operation. If this occurs, then the file list may inaccurately represent the available data file by displaying the names of un-readable sample data files. If the operator suspects that any data corruption has occurred, then the **Rebuild Data File** Index option should be invoked from the **File** menu. This will initiate a routine where the software will attempt to validate all data files and rebuild the appropriate folder index which will include only valid readable data files.

The File Utilities dialog also supports the archiving of complete file folders through use of the "Export Folder" command button. When selected, the user will be asked where to save a copy of the data included in the current highlighted folder.

The output of the export folder option is a binary file that contains all the sample data from all measurements included in the folder. The file is generated with a file extension of ".FDR". The file is saved in a binary format that is not compatible with any other software or word processors. Do not confuse this binary format with the text format used by the Export option available in the Save As dialog.

An operator may import the exported data folder back into the current highlighted folder by choosing the **Import Folder** button.

The import/export features can be used as a safety measure in order to "back up" your data files. It is also a convenient method of transporting data files from one computer to another.

III.8 Printer Support

All printer output must use standard window printer drivers and it is assumed that a printer has been previously installed through the Windows control panel. The standard Windows print setup and print preview dialogs are supported. Printer output can only be generated correctly on a printer that supports bitmap graphics and True Type fonts.

The software has the ability to generate a variety of printed data. The operator may select the type of data to output by choosing **Printer Options** from the File menu, and selecting the items of interest .

The most common printer output format used is the "Results Summary" which includes the information displayed on the main screen as well as the parameters in effect when the measurement was made. When printing the results summary, the current graph displayed on the main screen will be included in the results summary page. If no graph is displayed on the main screen then no graph will be included on the results summary page.

If more than one additional graphs are to be printed, they may be printed on a single page or on separate pages.

Please note that the date/time printed on each page header is the date/time of when the measurement was made and not the data/time of when the data is being printed.

IV Quasi Elastic Light Scattering (QELS) - Theory of Operation

IV.1 Light Scattering

The information in this chapter is not necessary for operating the MAS OPTION particle sizer. If the operating instructions are followed correctly, good results will be obtained. However, if you wish to understand the basis for the measurement, and if you wish to interpret the results in a better informed fashion, then this chapter may be of considerable value.

Of the many techniques available for particle sizing light scattering offers many advantages: speed, versatility, small sample size, non-destructive, and measurement time independent of particle density. For sub-micron sizes it is sometimes the only viable technique.

IV.2 What Causes Light Scattering?

A little over a hundred years ago J.W. Strutt answered that question, and, in doing so, also answered the age-old question of why the sky is blue. Strutt became Lord Rayleigh, and the basics of light scattering were laid.

Light may be treated as an electro-magnetic wave. The oscillating electro-magnetic field induces oscillations of the electrons in a particle. These oscillating changes form the source of the scattered light.

Over the years many features of the scattered light have been used to determine particle size. These include:

- Changes in the average intensity as a function of angle.
- Changes in the polarization.
- Changes in the wavelength.
- Fluctuations about the average intensity.

This later phenomenon is the basis for QELS, the technique employed in the MAS OPTION, and it arises in the following manner: imagine a detector of light fixed at some angle with respect to the direction of the incident light beam and at some fixed distance from the scattering volume which contains a large number of particles. Scattered light from each particle reaches the detector. Since the small particles are moving around randomly in the liquid, undergoing diffusive Brownian motion, the distance that the scattered waves travel to the detector varies as a function of time. Electromagnetic waves, like water and sound waves, exhibit interference effects. Scattered waves can interfere constructively or destructively depending on the distances traveled to the detector. The result is an average intensity with superimposed fluctuations. Figure 5a illustrates this.

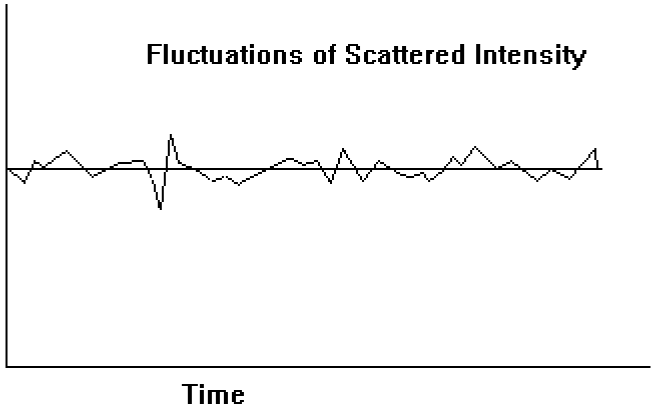


Figure 5a
Fluctuations about the average scattered intensity.

The decay times of the fluctuations are related to the diffusion constants and, therefore, the sizes of the particles. Small particles moving rapidly cause faster decaying fluctuations than large particles moving slowly. The decay times of these fluctuations may be determined either in the frequency domain (using a spectrum analyzer) or in the time domain (using a correlator). The correlator generally offers the most efficient means for this type of measurement.

IV.3 Correlation

The concept of correlation is familiar to most people. If two variables or two signals are highly correlated then a change in one can be used to predict, with confidence, the change in the other. Mathematically, correlation is defined as the average of the products of the two quantities. Autocorrelation is just the average of the product of variable with a delayed version of itself.

In QELS the total time over which a measurement is made is divided into small time intervals called delay times. These intervals are selected to be small compared with the time it takes for a typical fluctuation to relax back to the average. The scattered light intensity in each of these intervals, as represented by the number of electrical pulses registered during each delay time, fluctuates about a mean value. The intensity autocorrelation function is formed by averaging the products of the intensities in these small time intervals as a function of the time between the intervals (delay times).

A computer automatically controls the buildup of the function including the choice of delay times, the length of the experiment, display of pertinent information, data analysis, and the printing of results.

The Brookhaven MAS OPTION has several advantages over conventional QELS systems:

- The delay time intervals are not linearly spaced (in the default mode).
  This allows broad distributions to be sampled properly.

- An algorithm is provided which removes signals affected by dust.

- Unimodal fits to an assumed lognormal size distribution is standard. This distribution is the single most common one used in particle sizing. Multimodal and model independent size distribution software is also available as an option.

- Automatic repetition of measurements with statistical averaging is provided.

- The software is Windows based with easy to use graphical user interfaces.

IV.4 Basic Equations

As described earlier, the random motion of small particles in a liquid gives rise to fluctuations in the time intensity of the scattered light. The fluctuating signal is processed by forming the autocorrelation function, C(t), t being the time delay. As t increases correlation is lost, and the function approaches the constant background term B. For short times the correlation is high. In between these two limits the function decays exponentially for a monodisperse suspension of rigid, globular particles and is given by

$$C(t) = A\, e^{-2\,\Gamma\, t} + B \qquad 5\text{-}1$$

where A is an optical constant determined by the instrument design, and Γ is related to the relaxation of the fluctuations by,

$$\Gamma = D\, q^2 \qquad [\text{rad/sec}] \qquad 5\text{-}2$$

The value of q is calculated from the scattering angle θ (eg. 90°), the wavelength of the laser light λ_o (eg. 0.635 μm), and the index of refraction n (eg. 1.33) of the suspending liquid. The equation relating these quantities is

$$q = \frac{2\pi\, n}{\lambda_0}\, 2Sin(\frac{\theta}{2}) \qquad 5\text{-}3$$

The translational diffusion coefficient, D, is the principle quantity measured by QELS. It is an inherently interesting property of particles and macromolecules. QELS has become the preferred technique for measuring diffusion coefficients of sub-micron particles.

Notice that no assumption about particle shape has to be made in order to obtain D other than assuming a general globular shape. Not until needle shape particles have aspect ratios of greater than about 5, will a rotational diffusion term appear in the equation (1).

Particle size is related to D for simple common shapes like a sphere, ellipsoid, cylinder and random coil. Of these, the spherical assumption is most useful in the greatest number of cases. For a sphere,

$$D = \frac{k_B\, T}{3\pi\ \eta(t)\, d} \qquad [\mathrm{cm}^2/\mathrm{sec}] \qquad \text{5-4}$$

Where k_B is Boltzmann's constant (1.38054×10^{-16} ergs/deg), T is the temperature in °K, η(t) (in centi poise) is the viscosity of the liquid in which the particle is moving, and d is the particle diameter. This equation assumes that the particles are moving independently of one another.

In summary, the determination of particle size consists of:

- Measurement of the autocorrelation function.
- Fitting the measured function to equation (5-1) to determine Γ.
- Calculating D from equation (5-2) given n, θ, and Γ.
- Calculating particle diameter d from equation (5-4) given T and η.

The MAS OPTION makes the measurements, fits the function, calculates D and d automatically. The measured autocorrelation function is more complex than equation (1). A preliminary discussion of the techniques used for handling polydisperse systems is included in the chapter on Data Interpretation.

IV.5 References

For more information on laser light scattering consult the following references:

1) B. Chu, "Laser light scattering", Academic Press, New York, 1974

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V DATA INTERPRETATION

V.1 Preliminaries

The type of diameter obtained with photon correlation spectroscopy (PCS) is the hydrodynamic diameter (the particle diameter plus the double layer thickness) which is the diameter that a sphere would have in order to diffuse at the same rate as the particle being measured. This diameter may also be called the equivalent sphere diameter (ESD).

When a distribution of sizes is present, the effective diameter measured is an average diameter which is weighted by the intensity of light scattered by each particle. This intensity weighting, which is discussed below, is not the same as the population or number weighting used in a single particle counter such as in electron microscopy. However, even for narrowly dispersed samples, the average diameters obtained are usually in good agreement with those obtained by single particle techniques.

V.2 Narrow Distribution of Spheres

For monodisperse, rigid spheres the measured autocorrelation function (ACF) determined using the MAS OPTION is analyzed using eqs. 5-1, 5-2, 5-3, and 5-4.

How accurate are these equations and the MAS OPTION? Standard Reference Material (SRM) 1691 was obtained from the U.S. National Institute of Standards and Technology (NIST). The number average diameter, as determined by transmission electron microscopy (TEM) calibrated using NBS SRM 1690, is stated as 269 +/- 7nm, where the uncertainty includes both systematic and random errors as well as sample-to-sample variations. The MAS OPTION results are 273 +/- 5nm, where the uncertainty represents the average of 5 repeated measurements with 2 more measurements rejected as obvious outliers.

These measurements show that the MAS OPTION is capable of very accurate measurements on monodisperse spheres. (Please note that so-called standard latex spheres from other sources are not usually as well-known as the NBS SRM's, regardless of what is printed on the bottle.) It is also obvious from the uncertainty of the NBS standard that attempts at or claims of accuracy better than +/- 2.6% (7/269) are suspect.

V.3 Broad Distribution of Spheres

When a broad distribution of spheres is present in the sample eq. 5-1 must be modified before data analysis of the measured ACF can proceed. Each size contributes its own exponential, and the mathematical problem becomes

$$g(t) = \int G(\Gamma)\, e^{-\Gamma t}\, d\Gamma \qquad 6\text{-}1$$

Here the baseline B of eq. 5-1 has already been subtracted and the square root of the remaining part has been taken eliminating the factor of 2 in the exponent.

The left hand side of eq. 6-1 is the measured data, and the desired distribution information, G( ), is contained under the integral sign on the right hand side. The solution to this Laplace Transform equation is nontrivial. The ill-conditioning of this transform, combined with measurement noise, baseline drifts and dust make the function particularly difficult to solve. It is therefore imperative to acquire the best possible statistics for the purposes of the measurement.

Several schemes have been proposed for obtaining information from eq. 6-1. These schemes may be grouped as follows:

- Fit to a known distribution.
- Cumulant analysis.
- Inverse Laplace Transform.

V.3.1 Fit to a known distribution.

This method requires the assumption of a particular distribution. For example, if it is assumed that the distribution consists of only 2 peaks, each of which is very narrow, then eq. 6-1 reduces to the sum of 2 exponentials. The measured ACF is then fit to this sum of exponentials. The ratio of the fitted, pre-exponential factors contains information on the ratio of scattered intensities for each of the 2 peaks. And, in turn, this ratio of intensities can be related to the mass ratio for the two peaks. Likewise, the fitted Γ's contain information on the diameters of the 2 peaks.

The principal problem with this approach is the assumption of a given form (here, bimodal) for the distribution. One might think that different models (unimodal, bimodal, trimodal, etc.) could be compared using a statistical criteria such as a goodness-of-fit parameter like chi-squared. Unfortunately it is well-known that this approach often leads to ambiguous results with ill-conditioned equations like eq. 6-1.

An alternative approach is to assume an analytic equation for the distribution, integrate eq. 6-1 (numerical integration is usually required), and fit the results to the measurements. Typical analytic equations may have 2 or 3 parameters which are to be determined by the fitting. This approach again suffers from the assumption of a particular form of the distribution and has generally not proven successful.

V.3.2 Cumulant analysis.

The method of cumulants is more general (1). An understanding of this method provides the user with an insight into the "intensity" weighted character of the results from PCS. Cumulant results are very often reported in the scientific literature. They are often the starting point for further analysis. During a measurement with the MAS OPTION the cumulant results (effective diameter and polydispersity index) are calculated and displayed on an ongoing basis. Finally, the 2 cumulants are used to calculate the 2 lognormal parameters.

In the method of cumulants no assumption is made about the form of the distribution function. The exponential in eq. 6-1 is expanded in a Taylor series about the mean value. The resulting series is integrated to give a very general result. This result shows that the logarithm of the ACF can be expressed as a polynomial in the delay time, t. The coefficients of the powers of t are called the cumulants of the distribution. In practice, only the first couple of cumulants are obtained reliably, and these are identical to the moments of the distribution. (In general, the first moment of any distribution is the average and the second is the variance.)

The first two moments of the distribution $G(\Gamma)$ are as follows:

$$\Gamma \quad = Dq^2 \qquad 6\text{-}2$$

$$\mu_2=(D^{2*}-D^{*2})q^4 \qquad 6\text{-}3$$

where q is the scattering vector given in eq. 5-3 and D* is the average diffusion coefficient.

The effective diameter (hereinafter designated d_{pcs}) is calculated from D using equation 5-5.

Equation 6-3 shows that μ_2 is proportional to the variance of the "intensity" weighted diffusion coefficient distribution. As such it carries information on the width of the size distribution. The magnitude and units of μ_2 are not immediately useful for characterizing a size distribution. Furthermore, distributions with the same relative width (same shape) may have very different means and variances. For these reasons a relative width (reduced second moment) is conveniently defined as follows:

$$\text{Polydispersity} = \mu_2 / \Gamma^2 \qquad 6\text{-}4$$

Polydispersity (Poly) has no units. It is close to zero (0.000 to 0.020) for monodisperse or nearly monodisperse samples, small (0.020 to 0.080) for narrow distributions, and larger for broader distributions.

Now lets examine the "intensity" weighting of the averaging process.

The intensity of light scattered by a suspension of particles with diameter d is proportional to the number of particles N, the square of the particle mass M, and the particle form factor P(q,d) which depends on size, scattering angle, index of refraction, and wavelength. Using "intensity" weighting, the following equations may be derived for the moments of the diffusion coefficient distribution:

$$D = \Sigma\, NM^2P(q,d)D / \Sigma NM^2P(q,d) \qquad 6\text{-}5$$

$$D^2 = \Sigma NM^2P(q,d)D^2 / \Sigma NM^2P(q,d) \quad . \qquad 6\text{-}6$$

Here the sums are carried out over all the particles.

For particles much smaller than the wavelength of light (say, 60nm and smaller), P(q,d) equals 1. Likewise, it equals 1 if measurements are extrapolated to zero angle. For narrow distributions an average value of P(q,d) can be used. In this case it cancels out of both equations. In other cases suitable approximations exist for P(q,d) (2).

To further understand the "intensity" weighting process and how it relates to other techniques, consider the simple case where P(q,d) = 1. Then,

$$D = D_z = \Sigma\, NM^2 D / \Sigma NM^2 \quad . \qquad 6\text{-}7$$

This is called the z-average. More familiar are the number, area, and weight or volume averages. Furthermore, since the diffusion coefficient is inversely proportional to the diameter for a sphere, the "average" obtained in this type of light scattering experiment is the inverse z-average given by

$$(1/d_z) = \Sigma NM^2 (1/d) / \Sigma NM^2 \quad . \qquad 6\text{-}8$$

Since M is proportional to d^3, and since we desire an average rather than an inverse average, the effective diameter, in this case, is defined by the following equation:

$$d_{pcs} = (1/d_z)^{-1} = \Sigma Nd^6 / \Sigma Nd^5 \qquad 6\text{-}9$$

Compare this to the number average,

$$d_n = \Sigma Nd / \Sigma N \qquad 6\text{-}10$$

the area average

$$d_a = \Sigma Nd^3 / \Sigma Nd^2 \qquad 6\text{-}11$$

the weight average diameter

$$d_w = \Sigma Nd^4 / \Sigma Nd^3 \qquad 6\text{-}12$$

All four averages are equally descriptive of the <u>same</u> sample. One may be preferred for a particular application: d_n when numbers of particles are important; d_a when surface area is important; d_W when mass or volume is important; and d_{pcs} when light scattering dependent properties are important.

Examination of eqs. 6-9 to 6-12 shows that, quite generally,

$$d_n \leq d_a \leq d_W \leq d_{pcs} \qquad \text{6-13} \qquad .$$

The equal sign occurs only for monodisperse samples. **For this reason <u>the weight average diameter is always less than or equal to the effective diameter</u>**. For narrow distributions they are nearly equal, and for broader distributions they will differ considerably.

In order to transform the cumulant results to weight average results, it is necessary to assume a form for the size distribution. (Please note that this is quite different from assuming a form prior to determining the cumulants as discussed in Section VI-2.A.). The lognormal distribution by weight has been chosen for use in the MAS OPTION because of its wide application in particle sizing. The lognormal distribution by weight is given by

$$dW = \{\frac{1}{\ln \sigma_g \sqrt{2\pi}}\, e^{-(\frac{\ln d\,(-\ln MMD)^2}{\ln \sigma_g \sqrt{2}})^2}\}\, d(\ln d) \qquad \text{6-14}$$

where MMD is the mass median diameter (50% by mass, weight, or volume is above and 50% is below this value) and σ_g is the geometric standard deviation (GSD). With these 2 parameters all other distributions, averages, tables, and graphs can be produced.

For narrow, unimodal size distributions any other 2-parameter equation is equally adequate. For broad, unimodal distributions the lognormal is a better assumption than a symmetric form like a gaussian (neither grinding, accretion, nor aggregation lead to symmetrical size distributions). Furthermore, manipulation of the lognormal equation is particularly convenient. The lognormal parameters are described in any number of standard texts on size distributions (3). To monitor relative changes in a size distribution either the cumulant results or the lognormal fit will usually suffice. However, when one requires more detailed information (for example, is it bimodal or just broad and skewed?) then the cumulant/lognormal analysis is no longer adequate.

V.4 Nonspherical particles

Describing the "size" distribution of nonspherical particles presents difficulties for any technique including PCS. If shape information is needed, then an image analyzer is necessary. Even then problems arise with sample preparation, statistical relevance of the small number of particles sized, and, finally, choosing the right statistical descriptors of "size".

Other techniques have their pitfalls. The settling velocity of nonspherical particles depends, in a nontrivial fashion, on shape. Thus, results from sedimentation techniques are shape dependent. Highly porous, rough, or multifaceted particles present much more surface area than smooth spheres of equivalent volume, and, therefore, size distributions based on surface area measurements may be highly biased toward larger sizes. Techniques based on elution through a column often depend on hydrodynamic interactions. Nonspherical particles may interact quite differently with the flow than spherical ones.

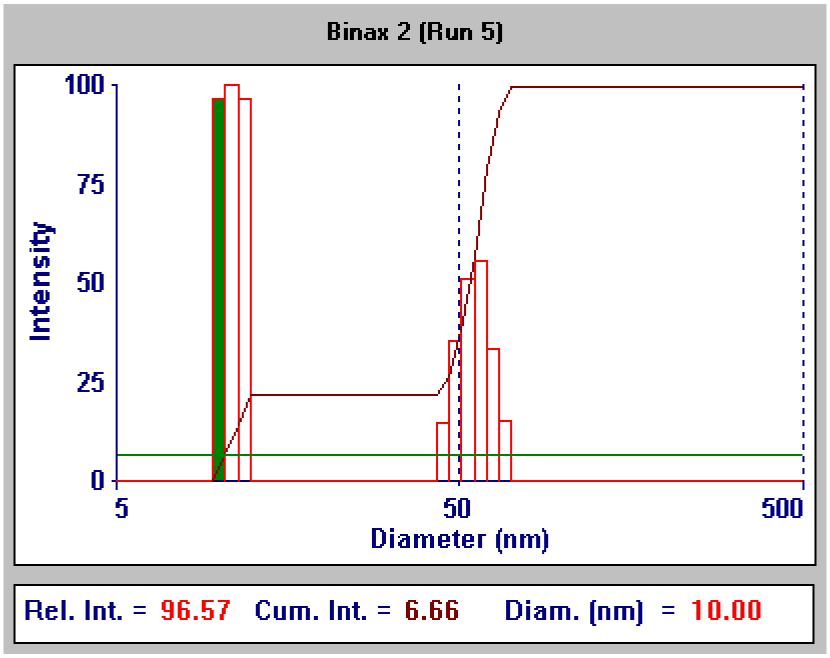
Angular light scattering measurements have the capability, in some circumstances, of distinguishing between simple shapes like spheres, ellipsoids, rods, and random coils (polymers). However, this is not possible with only one scattering angle unless extra information is supplied. For example, if it were known that the particles were all prolate ellipsoids (cigar shaped), with the same aspect ratio (major/minor axes ratio), then the diffusion coefficient results given by the MAS OPTION could be used in conjunction with an equation relating the translational diffusion coefficient of an ellipsoid to its aspect ratio to obtain the other dimension (4).

V.5 Advanced data interpretation

As explained in Section VI-3, the general solution of eq. 6-1 is surprisingly difficult. Several approximate solutions have been tried over the more than 20 years since the initial quasi-elastic light scattering measurements were performed (5). Most are of very limited utility. The reason for the many failures has been addressed in a paper by Pike and coworkers (6). Grabowski and Morrison (7) formulated an approach for the solution of this problem. This approach called the Non-Negatively constrained Least Squares (NNLS) algorithm is used at the heart of the MSD option in the Brookhaven MAS OPTION.

The prime assumptions with NNLS are these.

- Only positive contributions to the intensity-weighted distribution are allowed.
- The ratio between any two successive diameters is constant.
- A least squares criterion for judging each iteration is used.



Rel. Int. = 96.57 Cum. Int. = 6.66 Diam. (nm) = 10.00

Figure 6a

Output from the MSD software

In order to calculate the Weight (or Mass or Volume, since we are assuming the same density for all the particles) and Number Fractions from the Intensity Fractions, scattering factors have to be calculated. These are call spherical Mie factors, after Gustave Mie, who in 1908 was the first to solve the equations exactly. In order to calculate these corrections the complex refractive index of the particle (in addition to the refractive index of the liquid) must be known. In this example the REAL part was 1.59 and the IMAGINARY part was 0.0. These values are valid for polystyrene latex. The 0.0 indicates this particle does not absorb any light at this wavelength. Appendix V discusses RI REAL and RI IMAG.

It is worth repeating that the most important pieces of information obtained in PCS using any multimodal size distribution analysis are the positions of the peaks and the ratio of the peak areas. The widths of the peaks, however, are not particularly reliable. They will narrow, typically, with increasing experiment duration, until a limit is reached.

The above discussion is predicated on the idea that no assumptions are made about the distribution. If prior information is available, then the fitting of ACF's can produce correspondingly more resolution.

V.6 REFERENCES

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APPENDIX I

A list of common liquids and their compatibility with acrylic cells.

COMPATIBLE	NOT COMPATIBLE
Water	Benzene
Methanol	Toluene
Ethyl Alcohol	Acetone
Hexane	Butatone
Cyclohexane	Carbon Tetrachloride

APPENDIX II

Raw Data File Format

1. Run Number
2. Total Counts - A Input
3. Total Counts - B Input
4. Number of Samples
5. Not Used
6. First Delay (μsec)
7. Number of Data Channels
8. Determines whether the ISDA programs will prompt the user for which baseline to use.
   0 = will prompt
   1 = use calculated baseline
   2 = use measured baseline
9. Angle
10. Lambda (nm)
11. Temperature (°K)
12. Viscosity (centipoise)
13. Not Used
14. Refractive Index of liquid
15. Refractive Index of particle - REAL
16. Refractive Index of particle - IMAGINARY
17. One less than the number of parameters preceding the x and y data in this file. As of the date of this document the number of parameters is 37
18. First channel used for calculations
19. Time Delay Mode
    -2 = Constant ratio spacing
    -1 = Spacing from delay file
    1 = Linear Spacing
20. Analysis Mode
    2 = Auto Correlation
    3 = Cross Correlation
    4 = Test
21. Number of extended baseline channels
22. Calculated Baseline
23. Measured Baseline.
24. Last Delay (μsec)
25. Sampling time used to generate number of samples (μsec)
26. First delay used for high speed section
27. Number of high speed channels used
28. Middle speed sampling time (μsec)
29. Number of middle speed channels
30. Low speed sampling time (μsec)
31. Number of low speed channels used

32. Not used
33. Not used
34. Not used
35. First measured channel number
36. Last measured baseline channel number
37. Not used
38. Delay time #1 Channel Contents #1
39. Delay time #2 Channel Contents #2
40. Delay time #3 Channel Contents #3
41. Delay time #4 Channel Contents #4
42. etc.

SAMPLE ID
OPERATOR ID
DATE
TIME

APPENDIX III

A text file report from the MAS OPTION software.

```
**** Brookhaven Instruments Corp.****
MAS Particle Sizing Software Beta Version 1.13
Sample Identification: Binax 2 (Run 5)
Operator Identification: Brookhaven Instruments
Measurement Date: Jul, 12, 1994
Measurement Time: 16:54:06
Batch: 0

    **** Measurement Parameters ****
Temperature = 25.0
Suspension = Aqueous
Viscosity = 0.890 cp
Ref.Index Fluid =1.33
Angle = 90.000
Wavelength = 635 nm
Runs Completed = 5
Run Duration = 00:01:00
Total Elapsed Time = 00:04:00
Average Count Rate = 141.6 Kcps
Ref.Index Real = 1.590
Ref.Index Imag = 0.000

    **** Measurement Results ****
Binax 2 (Run 5)
Effective Diameter: 38.6
Polydispersity: 0.244
Sample Quality: 9.5
Elapsed Time = 00:01:00

 Run           Eff. Diam. (nm)   Half Width (nm)   Polydispersity   Sample Quality
----------------------------------------------------------------------------------
  1              36.9              20.5                      0.308          8.8
  2 (inactive)    36.1             20.6                      0.327          6.2
  3              37.1            20.5                        0.307          9.2
  4              37.7            20.1                        0.285          9.5
  5              38.6            19.1                        0.244          9.5
----------------------------------------------------------------------------------
Mean             37.6            20.1                        0.286          9.2
Std.Error         0.4             0.3                        0.015          0.2
Combined         37.5            20.2                        0.290          9.6
```

```
    **** Lognormal Size Distribution Results ****
GSD: 1.595
   d(nm)  G(d) C(d) |   d(nm)  G(d) C(d) |   d(nm)  G(d) C(d)
--------------------------------------------------------------
    17.9   26    5  |    34.3   97   40  |    52.9   80   75
    21.2   44   10  |    36.4   99   45  |    57.2   70   80
    23.8   58   15  |    38.6  100   50  |    62.6   58   85
    26.0   70   20  |    40.9   99   55  |    70.2   44   90
    28.2   80   25  |    43.4   97   60  |    83.2   26   95
    30.2   87   30  |    46.8   92   66  |
    32.2   93   35  |    49.3   87   70  |

    **** Multimodal Size Distribution Results ****
Mean Diameter: 29.134
Relative Variance: 0.580
Skew: 0.455

Percent         | Low Peak    High Peak
---------------|----------------------------
By Intensity:  |  29              71
By Weight:     |  98               2
By Number:     | 100               0

   d(nm)  G(d) C(d) |   d(nm)  G(d) C(d) |   d(nm)  G(d) C(d)
--------------------------------------------------------------
    10.0   97    7  |    24.9    0   22  |    62.2   33   93
    10.9  100   14  |    27.1    0   22  |    67.6   15  100
    11.8   97   22  |    29.4    0   22  |    73.4    0  100
    12.8    0   22  |    32.0    0   22  |    79.8    0  100
    13.9    0   22  |    34.8    0   22  |    86.7    0  100
    15.1    0   22  |    37.8    0   22  |    94.2    0  100
    16.5    0   22  |    41.1    0   22  |   102.4    0  100
    17.9    0   22  |    44.6   14   26  |   111.3    0  100
    19.4    0   22  |    48.5   35   38  |   120.9    0  100
    21.1    0   22  |    52.7   51   57  |   131.4    0  100
    23.0    0   22  |    57.2   56   79  |   142.7    0  100
```

APPENDIX IV

Operating Environment Definitions.

In order to support particle sizing with a variety of instruments, the windows particle sizing software supports multiple operating environments where parameters such as wavelength and scattering angle may be defined.

The parameters for one or more operating environments are stored in two .INI files that are installed by the particle sizing setup program. These files use the standard windows .INI file protocol and are located in the windows system directory after successful installation.

The first of these files is named **9KBOARDS.INI,** and is used to name the available operating environments and provide information about the correlator card that has been allocated for processing the signal for each environment. Each "Board" has an associated an I/O port address associated with it. The I/O port address refers to a hexadecimal port address of the correlator card that is assumed to have been already installed in the computer.

```
[Boards]

Board1BasePort=300

Board2BasePort=(None)

Board3BasePort=(None)

Board4BasePort=(None)

Board5BasePort=(None)

Board6BasePort=(None)

Board7BasePort=(None)

Board8BasePort=(None)
```

In the example above, the 9KBOARDS.INI file is set up for one correlator board with a baseport address of 300 hex.

More than one operating environment may be defined for each correlator. This allows operating parameters to be defined that represent different signal sources such as a BI-90plus, MAS OPTION, a ZetaPlus or a FOQELS. If more than one operating environment is defined then the software must be told which environment will be used before any particle sizing measurements are to be made.

The operating environment has been identified the particle sizing software will access an additional file (**9KPSDW.INI),** to determine the operating parameters for the selected environment. The file is broken down into sections to define general parameters and instrument specific parameters. Many of these parameters are modifiable from the software's parameters page dialog. These parameter values remain in effect for all future measurements until they are modified again. Parameters indicated as optional in the comments section of this document are generally not necessary, but are used to override default values that have been "hard coded" in the software.

Please note that when a data file is loaded from disk, the parameters page will reflect the parameters that were in effect during the time the measurement was made. Parameters from data files may be modified where applicable in order to recalculate particle size. When a data file is cleared the program will re-read values from the 9KPSDW.INI file in order to restore the current parameter values.

9KPSDW.INI

[General]	*General Information Section*
Aloader=FALSE	*TRUE if automated sample loader hardware is available*
ShowParamOnStartUp=FALSE	*Parameters dialog will be shown on startup if set to TRUE*
AutoSave=TRUE	*If set to TRUE then data is automatically saved at completion of each measurement*
CorrFunc=TRUE	*If set to TRUE then correlation function graph will be available for viewing*

CombineFunc=TRUE	*If set to TRUE then combined function data will be calculated and displayed when making multiple measurements*
UpdateInterval=2500	*Interval in milliseconds for timer to trigger requests for data update*
Nnls=TRUE	*Value of TRUE indicates Multi Modal Size Distribution software option has been installed*
[90Plus]	*Instrument definition section used for external signal source.*
Runs=1	*Number of measurements to make*
Duration=120000	*Duration in milliseconds of a single measurement Displayed as minutes/seconds in parameters dialog*
RefractiveIndexFluid=1.331	*Refractive index of suspension fluid (range 1 - 9)*
RefractiveIndexReal=1.590	*Refractive index of particle (real) (range 1 - 9)*
RefractiveIndexImaginary=0.000	*Refractive index of particle (imaginary)*
Theta=90.0	*Angle of scattered laser light*
ThetaLimit=90.0,15.0	*Range of angles allowed*
WaveLength=635.0	*Wavelength of laser in nm*
WaveLengthLimit=635.0	*Range of wavelengths allowed.*
Viscosity=0.89	*Viscosity of suspension fluid in cp (see “suspension” below)*
Temp=25.0	*Temperature in degrees C*
TempMin=6.0	*Minimum Allowed temperature setting*
TempMax=74.0	*Maximum Allowed temperature setting*
TempControlGain=33000	*Value of hardware gain used for the temperature controller. (only acceptable values are 45450 (old) or 33000(new))*
Suspension=Aqueous	*Description of suspension*

	note: *if any of the suspensions listed below are used, then the refractive index and viscosity are calculated by software and may not be modified by the user.* *Aqueous, Ethanol, Methanol, Toluene, Hexane, Cyclohexane, Heptane, Benzene*
BypassTempInit=TRUE	*If TRUE will not wait for temperature stabilization on start.*
BypassNDFInit=TRUE	*If true will not maximize scattered intensity on start.*
Instrument=ZPLUS	*From the hardware poin of view the base instrument is a ZetaPlus.*
[Environment]	*This section defines all the environments that this software is set up for supporting.*
Environment1=90Plus	*The first environment is defined as a 90Plus*
BoardNum1=1	*The hardware base address will be gotten from Board1BasePort (defined in the 9KBoards.INI file).*
Environment2=(None)	*Additional environments and base addresses can be defined here.*
BoardNum2=1	
Environment3=(None)	
BoardNum3=1	
Environment4=(None)	
BoardNum4=1	
Environment5=(None)	
BoardNum5=1	
Environment6=(None)	
BoardNum6=	
Environment7=(None)	
BoardNum7=	
Environment8=(None)	

BoardNum8=

Appendix V

Index of Refraction of Particles

For monodisperse and narrow distributions the particle refractive index is not needed to calculate results. Even in broad and bimodal distributions the particle index of refraction is not needed if one is satisfied with the intensity weighted size distribution. Only when transforming from intensity-weighted to mass and number-weighted distributions is this information required.

If the particle index of refraction is not known, enter 1 for RI Real in the Parameters page. The angular light scattering factors, the Mie factors will be set to 1. The transformations will still include d^3 and d^6 calculations. For particle sizes below about 60 nm, the Mie factors are, in fact, 1, independent of the particle refractive index.

For polystyrene latex spheres or any other non-absorbing, white, opaque particle in the visible, use RI Real = 1.55 to 1.65 and RI Imaginary = 0.000.

For carbon black, perhaps the most highly absorbing particle in the visible, use RI Real = 1.84 and RI Imaginary = 0.85.

For other particles please consult various texts and references or contact BIC.

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Features Added to the Particle Size Distribution Software

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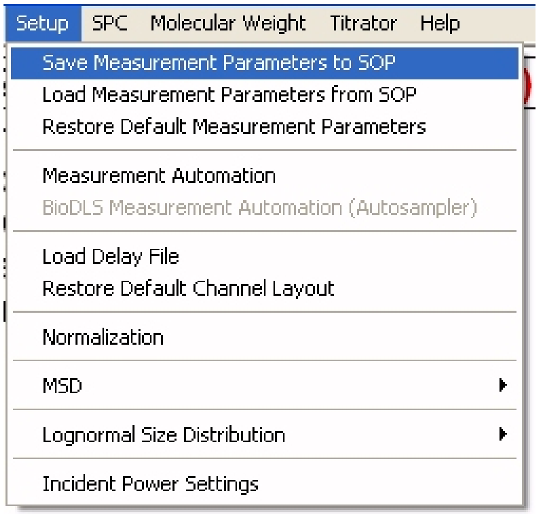
Features Added to the Particle Size Distribution Software

Saving/Loading SOPs, Standard Operating Procedures

Standard operating procedures are useful for enforcing uniformity in measurement conditions and data analysis. Pharmaceutical companies are particularly found of SOPs. In the current context, procedures are the parameters (number of runs, duration, refractive index, etc.) that are used to make the measurement and to analyze the data. The software can create an SOP from whatever parameters are currently set.

Click *Setup/Save Measurement Parameters to SOP*. Name the file that will be created. It will be saved with the file extension *.sop*. It is saved in a subdirectory called SOP. You can create an SOP before you start a measurement, after it is finished, or by recalling a previous measurement. In all three cases, whatever parameters are currently set becomes the basis of the SOP.

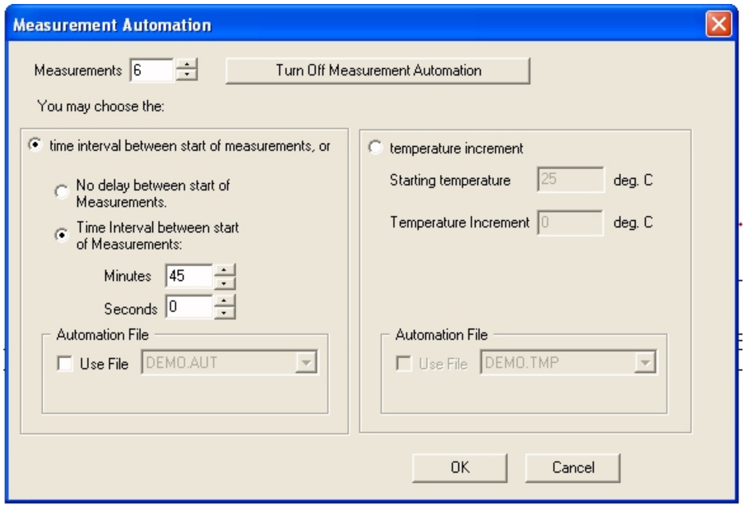
To use a particular SOP for a new measurement, click *Setup/Load Measurement Parameters from SOP*. Click on the filename from the list of SOPs displayed. Click *Open*. Change the Operator ID or any of the other values shown in the Parameters window. However, changing too many values defeats the purpose of the SOP. Click *Okay*. Click *START*.



Measurement Automation: Time and Temperature

Measurements consist of one to 10 runs. The number of runs and the duration per run are selected in the *Parameters* window. You can make automatic, unattended measurements to see either the effect of time or temperature.

Click *Setup/Measurement Automation*. Select either time interval or temperature measurement.



Measurement Automation

Measurements 6

You may choose the:

● time interval between start of measurements, or

○ No delay between start of Measurements.

● Time Interval between start of Measurements:

Minutes 45

Seconds 0

Automation File

☐ Use File DEMO.AUT

○ temperature increment

Starting temperature 25 deg. C

Temperature Increment 0 deg. C

Automation File

☐ Use File DEMO.TMP

The time interval between the start of each new measurement must be greater than the number of runs per measurement times the duration per run. The time intervals selected this way are constant. If you require a selection of times that are not constant, you can create an *.aut* file, a simple ASCII file, and recall it when required. Such files are located in the 9kpsdw/data subdirectory. The default.aut file has instructions and an example of how to create such a file.

Suppose you are interested in the overnight stability of a suspension: Does it increase size over time? A measurement might consist of 3 runs, 3 minutes each, for a total of 9 minutes. Perhaps you will leave for the evening starting at 17:00 hrs and return the next morning at 09:00 hrs. If you set the time interval between start of measurements at 30 minutes, then including the measurement at 5 pm, you will have 33 measurements covering 16 hours.

You can use either the SPC feature or the *Log File Settings* (under *File*) feature to look at the data. With the SPC chart, it is assumed the data are linearly spaced. In this example they were; in other cases they may not be. With the *Log File Settings* you create a new ASCII file with a limited set of information from each measurement, the set being determined by highlighting only the data you want saved from a long list of choices. The information in this *logfile.txt* is located in the 9kpsdw/data subdirectory and can be imported into Excel or any other plotting or spreadsheet program. Note: Log file data use the mean values and not the combined values from each file.

Automatic temperature measurements require a start temperature and an increment. If the number of measurements times the increment plus the start temperature exceed the capacity of the temperature controller, the run sequence will be automatically terminated after the last attainable temperature. If you require a selection of temperatures that are not linearly related, you can create a *.tmp* file, a simple ASCII file, and recall it when required. Such files are located in the 9kpsdw/data subdirectory. The default.tmp file has instructions and an example of how to create such a file. Using the *Log File Settings* in combination with the automatic temperature measurements is a powerful way to collect temperature dependent size information without having to attend the instrument.

APD Detector

The avalanche photodiode detector has a much greater sensitivity (5-10 times) to photons in the red (620-680 nm, approximately) than a PMT. Thus, with weakly scattering systems, such as globular proteins, micelles, quantum dots, dendrimers, and other small nanoparticles and polymers, an APD has great advantages over an expensive, short-lived, higher power laser such as the frequency-doubled, 532 nm, and solid-state laser. Most 90Plus instruments are supplied with the BI-APD option.

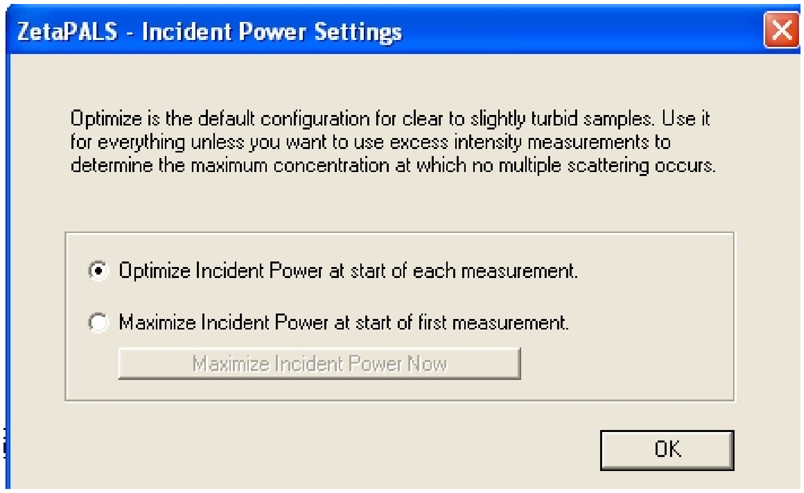
The APD, however, has a drawback. Due to its higher sensitivity, strong scatters can easily produce too many photons, resulting in distortion of the correlation function from which all size distribution information is ultimately calculated. To prevent this, a variable neutral density filter wheel is introduced and automatically varied to obtain a count rate less than 500 kcps. Note: With a PMT, the maximum, automated count rate is 750 kcps.

Optimize/Maximize Count Rate

For a long time this software had a feature that was applicable if the instrument possessed a variable neutral density filter. The feature was the ability to maximize the intensity by automatically rotating the position of a variable neutral density filter located between the laser and the sample. All instruments for zeta potential determination have such a filter. All particle sizing instruments with the BI-APD option have such a filter. All instruments with the 50 mW, 532 nm laser have such a filter.

An additional choice is now included: Optimize incident power at the start of each measurement.

To access either feature, click *Setup/Incident Power Settings*. Then choose either *Optimize Incident Power at the start of each measurement* or *Maximize Incident Power at the start of the first measurement*. Optimize is the default and should be used for particle size measurements except those where you are determining intensity vs. concentration in order to determine the range over which multiple scattering is a problem. In this case use the maximize feature.



ZetaPALS - Incident Power Settings

Optimize is the default configuration for clear to slightly turbid samples. Use it for everything unless you want to use excess intensity measurements to determine the maximum concentration at which no multiple scattering occurs.

● Optimize Incident Power at start of each measurement.

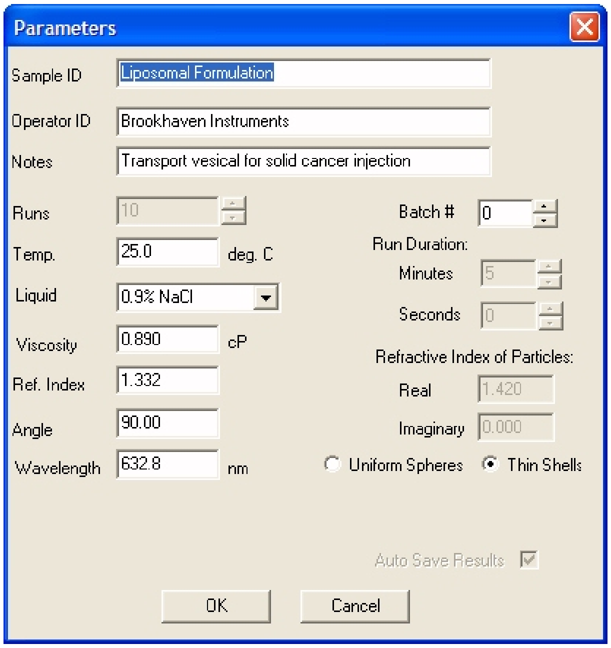
○ Maximize Incident Power at start of first measurement.

Thin Shell Choice for Calculation of Mie Scattering Correction: Liposomes Primarily

In the Parameters window, on the right side, under "Refractive Index of Particles", there is now a new line. Choose "Uniform Spheres" and fill in the particle's real and imaginary refractive index (if it absorbs light at the laser wavelength), if the particles are uniform (homogeneous, i.e. constant composition throughout particle), and if and only if you want to calculate the volume-, surface-area-, or number-weighted MSD results. This is the standard choice as it has always been.

The new choice is "Thin Shells", also known as core/thin-shell. This model is appropriate for a liposome where the core of the particle has a refractive index equal or close to that of the liquid surrounding it and the shell's thickness is small compared to the diameter of the particle. In addition, this model is appropriate for diameters generally less than 300-400 nm. These assumptions are valid for most liposomes. When these assumptions are fulfilled, then the Mie scattering coefficient (same as the light scattering correction) is independent of the shell's refractive index and the calculation is made without reference to the particle's refractive index.

An interesting example of the change you will see can be seen in the Liposomal Formulation file in the Sample Data folder. Use the MSD, Uniform Sphere choice with the real part of the particle refractive index at 1.35, an average value between 1.33 for water that comprises most of the liposome and a higher value for the lipid layer that comprises a small fraction of the particle. Then recalculate selecting Thin Shell. The peaks stay in the same position but the fraction in each peak changes.



Sample ID: Liposomal Formulation
Operator ID: Brookhaven Instruments
Notes: Transport vesical for solid cancer injection
Runs: 10
Temp.: 25.0 deg. C
Liquid: 0.9% NaCl
Viscosity: 0.890 cP
Ref. Index: 1.332
Angle: 90.00
Wavelength: 632.8 nm
Batch #: 0
Run Duration:
Minutes: 5
Seconds: 0
Refractive Index of Particles:
Real: 1.420
Imaginary: 0.000
○ Uniform Spheres ● Thin Shells
Auto Save Results ☑

Marquardt Baseline Algorithm

For noisy correlation functions, such as one might find with weakly scattering globular proteins, the measured baseline is sometimes hard to establish using our standard fitting routines. The Levenburg-Marquardt, often abbreviated Marquardt, algorithm makes it easier to establish the baseline. This improvement is now standard. It is transparent to the user. It works on all correlation functions, with or without noisy baselines.

Molecular Weight by MHS Equation: Main Menu Selection

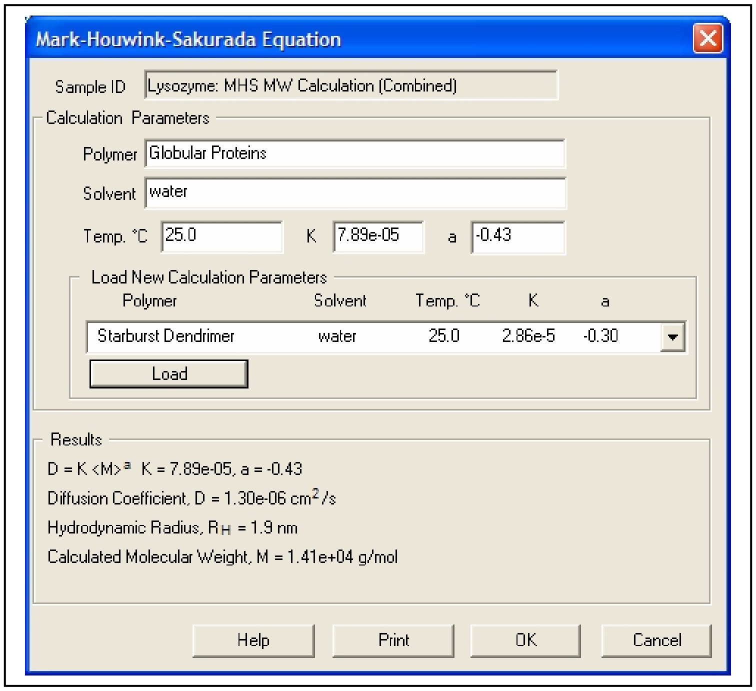
Over limited ranges of molecular weight, there is an empirical relationship between the translational diffusion coefficient and the molecular weight. It is known variously as a Mark-Houwink (MH), a Mark-Houwink-Sakurada (MHS) or a Mark-Houwink-Kuhn-Sakurada (MHKS) relationship:

$$\boxed{D_T = K \bullet M^a}$$

where {K, a} are a pair of constants and depend on the polymer, the solvent, and the temperature as well as the range of molecular weights examined. This equation has a more famous analogue where the intrinsic viscosity is related to molecular weight using a different pair of constants.

Such an empirical equation allows the calculation of a diffusion-coefficient averaged molecular weight if the pair of constants is known. The calculated molecular weight is empirical and not easily related to the weight- or number-averaged value obtained with static light scattering and osmometry, respectively, except for narrow distribution such as unaggregated globular proteins. This average value is convenient because the DLS instrument, unlike a static light scattering instrument, does not have to be calibrated nor does the differential refractive index increment, dn/dc, need to be known. In addition, a set of accurate polymer solution concentrations do not have to be prepared. A single, dilute solution will suffice. However, for large random coils, one has to watch out for modes of motion other than translational diffusion contributing to the correlation function.

To access this standard feature, click *Molecular Weight* in the main menu bar. For more information, read the *Help* command button in the MHS window.



Mark-Houwink-Sakurada Equation

Sample ID: Lysozyme: MHS MW Calculation (Combined)

Calculation Parameters

Polymer: Globular Proteins

Solvent: water

Temp. °C: 25.0   K: 7.89e-05   a: -0.43

Load New Calculation Parameters

Polymer	Solvent	Temp. °C	K	a
Starburst Dendrimer	water	25.0	2.86e-5	-0.30

Results

$D = K \langle M \rangle^a$ K = 7.89e-05, a = -0.43

Diffusion Coefficient, D = 1.30e-06 cm^2/s

Hydrodynamic Radius, R_H = 1.9 nm

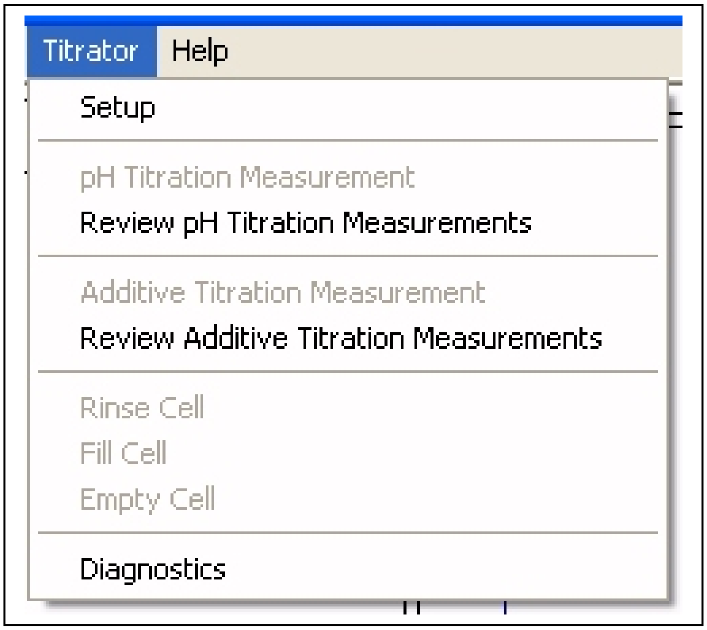
Calculated Molecular Weight, M = 1.41e+04 g/mol

90PDP, Absolute Molecular Weights Using A Debye Plot: Refer to Separate Manual

For a more accurate determination of the well-defined, weight-average molecular weight of a polymer or protein in dilute solution, M_w, consider the 90PDP option that consists of a 40 µL flow cell and software. With this option, a so-called absolute Mw is determined if and only if the molecular size is small enough. Generally, this means radii of gyration less than about 12 nm, a criterion satisfied by globular proteins, highly branched polymers, dendrimers, and random coils up to approximately 100 kDa. A complete manual is available that describes the measurement and calculation that requires the value of dn/dc as well as a calibration, typically with toluene.

BI-ZTU Titrator Software Now Works with PSDW & Additives Other than Acids/Bases

The BI-ZTU, zeta titrator unit, is used primarily with the zeta machines (ZetaPlus or ZetaPALS or the BI-Zeta or BI-PALS options) and acids/bases to determine the isoelectric point. However, there are cases where one would want to automatically determine zeta potential or particle size as a function of pH, concentration of added surfactant, concentration of added salt, and in general the concentration of an added reagent that is expected to affect the surface potential. The latest PSDW software incorporates this software, though it is not enabled unless the BI-ZTU hardware is attached.



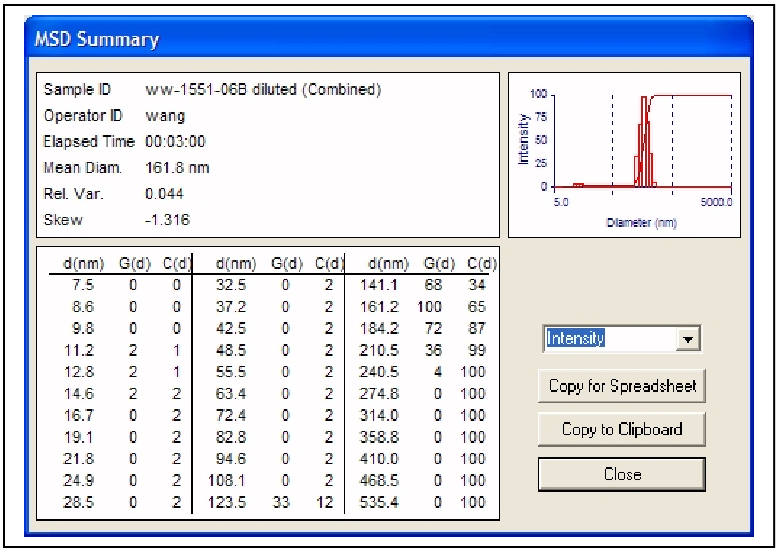
The *Setup* window is used to enter up to four different reagents, their names, concentrations and units. Pumps are assigned. In the “pH Titration Measurement” or the “Additive Titration Measurement” windows the number of points and desired concentrations are specified. After the measurements are made, the full curves of particle size vs. pH or additive concentration can be reviewed. For more detailed information, see the BI-ZTU manual.

Copy for Spreadsheet, Copy to Clipboard

There are times when the standard printouts are not enough. In these cases, you can take advantage of the clipboard to produce either a printable and editable picture of the displayed graph or a coma-delimited set of values suitable for re-plotting using a spreadsheet or plotting program.

In the example shown below, click *Copy to Clipboard*. Open the *Paint* application or another graphics program. Then *paste* the information in the clipboard. The result is the same as the image shown below without the copy and close commands. Such an image can be resized, printed, and even edited if you have the right tools.

Alternatively, you may want to plot the differential distribution, d vs. G(d), or the cumulative undersize distribution, d vs. C(d) on different scales or to label the axes differently. In this case, click Copy for Spreadsheet. Then *paste* the information into a text editor such as the Notepad program found in Windows. Save it as a .txt file. Use the *Import External Data* or equivalent tool in your spreadsheet or plotting program to separate the columns



Sample ID ww-1551-06B diluted (Combined)
Operator ID wang
Elapsed Time 00:03:00
Mean Diam. 161.8 nm
Rel. Var. 0.044
Skew -1.316

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
7.5	0	0	32.5	0	2	141.1	68	34
8.6	0	0	37.2	0	2	161.2	100	65
9.8	0	0	42.5	0	2	184.2	72	87
11.2	2	1	48.5	0	2	210.5	36	99
12.8	2	1	55.5	0	2	240.5	4	100
14.6	2	2	63.4	0	2	274.8	0	100
16.7	0	2	72.4	0	2	314.0	0	100
19.1	0	2	82.8	0	2	358.8	0	100
21.8	0	2	94.6	0	2	410.0	0	100
24.9	0	2	108.1	0	2	468.5	0	100
28.5	0	2	123.5	33	12	535.4	0	100

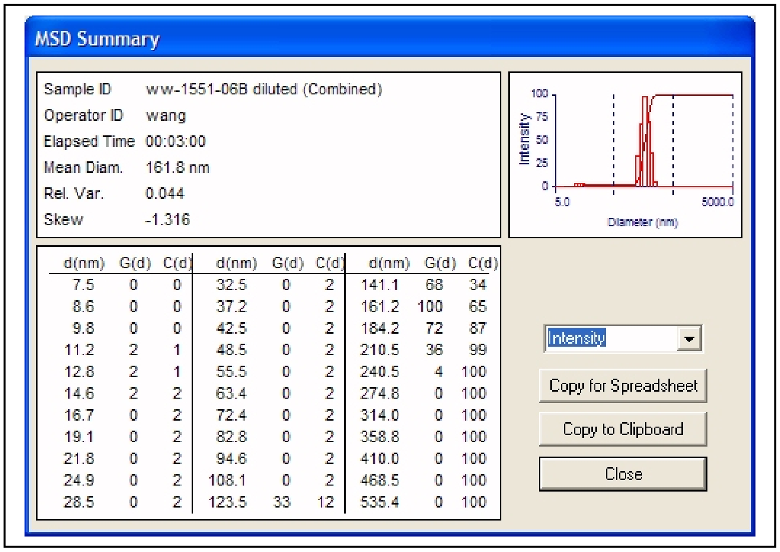
Using the Dust Cutoff to Improve Results

Dust is a generic term referring to a few, large particles that prevent a flat baseline. Without a flat baseline, it is difficult to properly normalize the data and therefore difficult to calculate good results. Dust can be a few, very large agglomerates coming from the sample itself, dust from the air or from cell and transfer vessel walls, or tiny bubbles. It is best to remove dust mechanically by filtration or centrifugation or by waiting for settling. A few seconds in a sonic bath is normally enough to drive air bubbles up and out.

However, there is usually residual dust and further procedures are necessary. One is an electronic filter known as the *Dust Cutoff* found in the *Parameters* window. This is a number used to filter out short-term spikes in the intensity data. Such spikes presumably come from rare, dust events. If the number is set high (100 or more), all data is accepted and none is filtered. If the number is set low (less than 5), real fluctuations might be clipped and the results distorted towards smaller sizes.

When the dust filter is on, the percent data retained is displayed to the right of the baseline index. The dust filter is toggled on and off using the command button in the main window.

Start with a dust cutoff value of 30. The optimal value of the dust cutoff is actually related to the expectation of outliers in intensity, which is related to the size of particles being measured. For particle sizes on the order of 10 nm, use a value of about 15. For particle sizes on the order of several 100 nm, use a value of 30. For particle sizes on the order of 1 micron, use a cutoff value of 80. To truly optimize the dust cutoff value, lower the dust cut-off filter until about 98% of the data is retained.



Sample ID ww-1551-06B diluted (Combined)
Operator ID wang
Elapsed Time 00:03:00
Mean Diam. 161.8 nm
Rel. Var. 0.044
Skew -1.316

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
7.5	0	0	32.5	0	2	141.1	68	34
8.6	0	0	37.2	0	2	161.2	100	65
9.8	0	0	42.5	0	2	184.2	72	87
11.2	2	1	48.5	0	2	210.5	36	99
12.8	2	1	55.5	0	2	240.5	4	100
14.6	2	2	63.4	0	2	274.8	0	100
16.7	0	2	72.4	0	2	314.0	0	100
19.1	0	2	82.8	0	2	358.8	0	100
21.8	0	2	94.6	0	2	410.0	0	100
24.9	0	2	108.1	0	2	468.5	0	100
28.5	0	2	123.5	33	12	535.4	0	100

Definitions and Calculations in the MSD Summary Window

The mean diameter is defined as the weighted sum over the diameters, d, with the weighting factor G(d). Thus,

$$\text{Mean Diameter in MSD Window} = \bar{d} = \frac{\sum d \cdot G(d)}{\sum G(d)}$$

It is a measure of central tendency for the distribution.

A relative measure of the width is the square of the standard deviation of the distribution, the variance, divided by the mean diameter squared.

Relative Variance = Variance/(Mean Diameter)^2

$$\text{Variance} = \frac{\sum (d-\bar{d})^2 \cdot G(d)}{\sum G(d)}$$

Narrow distributions have smaller relative variances than broader distribution. A monodisperse distribution would have a zero relative variance.

Peaks can be skewed to the left (negative skew) or to the right (positive skew) of the modal value. A relative measure of skew is obtained by dividing the third weighted moment about the mean diameter [the weighting is once again G(d)] by the square root of the cube of the variance. This relative measure, like the relative variance, has no units.

$$\text{Skew} = \frac{m_3}{\sqrt{(Variance)^3}}$$

$$m_3 = \frac{\sum \left(d - \bar{d}\right)^3 \cdot G(d)}{\sum G(d)}$$

The cumulative undersize distribution, C(d), is the percent of the size distribution at or below the diameter d. It is calculated by dividing the sum of the G(d) values at and below d by the total sum of G(d). The differential size distribution is a continuous curve connecting the values of G(d). Thus, C(d) is the integral of G(d) and G(d) is the differential of C(d).

Weighting: Intensity, Volume, Surface Area, and Number

The MSD feature takes the accumulated, combined, autocorrelation function and uses the NNLS, non-negatively constrained least-squares, algorithm to produce an intensity-weighted differential size distribution: d, $G_I(d)$. When these values are used to produce a mean, relative variance, skew, and cumulative undersize distribution, it should be understood that these results are all intensity-weighted. Intensity weighting favors larger particles except in those rare cases where the light scattering coefficient of the larger particles is close to zero.

The volume-weighted differential size distribution $G_V(d) = G_I(d)/d^3M$, where M is the light scattering coefficient, also called the Mie scattering coefficient. M is a function of d, wavelength, scattering angle, and the refractive index of the particle (real and imaginary if it absorbs at the wavelength used) and the suspension liquid. For $d < 25$ nm, $M = 1$. This defines a Rayleigh particle, one with a diameter much smaller than the wavelength of light. It scatters equally in all directions. M decreases smoothly with size until the size approaches the wavelength of light. Then oscillations can occur.

With d and $G_V(d)$, the mean, relative variance, skew, and volume-weighted cumulative undersize distribution are easily calculated. Similarly, surface-area weighted size distribution information is obtained by noting that $G_{SA}(d) = G_V(d)/d$. Finally, to obtain number-weighted distribution information, $G_N(d)$ is calculated from $G_V(d)/d^3$.

While these definitions are straight forward and the calculations of $G_V(d)$, $G_S(d)$, and $G_N(d)$ are trivial, one should not assume the final results are accurate or meaningful unless they are repeatable and even then are in agreement with other information about the size distribution. Since all the calculations depend on the accuracy of G_I, look for repeatability in the intensity-weighted distribution before proceeding. Artifacts at low values of d in G(d) due, typically, to noise in the accumulated function, dominate the other differential distributions because of the division of d raised to higher and higher powers.

FAQ: **90Plus**

Questions and Answers for Driving the Brookhaven **90Plus**

By Jeffrey Bodycomb, Ph.D.

Table of Questions

1. What is the baseline index?

The baseline index (BI) is a measure of how much the autocorrelation function was distorted by a few large particles. Often referred to as "dust", they could be a few aggregates, too few for this technique to characterize properly. A high value (10) corresponds to no dust and a low value (0) corresponds to a noticeable amount of dust. During data analysis, the experimental autocorrelation function must be normalized. In theory, one should use the infinite time baseline B_∞ obtained from the square of the average counts per sampling time. However, in practice, in order to eliminate the effect of a few large particles (dust), a baseline obtained from the measured correlation function at some long delay time is a better choice for normalization. During measurement, this constant background term is measured using a variety of possible methods (see below) to obtain a measured baseline value B_m. For $(B_m/B_\infty-1) < 0.01$, that is a less than 1% difference in baselines, the index is defined by

$$\mathbf{BI = 10\,[\,1 - 100\,|(B_m/B_\infty - 1)|\,],} \qquad \mathbf{if\ \ |(B_m/B_\infty-1)| < 0.01,}$$
$$\mathbf{otherwise,\quad BI = 0.0}$$

If the measured baseline is 1% or more above the infinite time baseline, or the measured baseline is below the infinite time baseline, the baseline index is 0. If the baselines agree exactly (a good thing), the baseline index equals 10. At a 0.1% baseline difference, the baseline index is 9 (which is quite good). If the BI value is 8 or higher, it means that the two measures of the correlation function baseline are in good agreement. Even if the value is lower than 8, if you have reasonable results and repeatable results, then the software has taken care of the less than perfect sample. The low value for baseline index is to alert you about what is going on. If you make a new sample, consider removing the few large dust particles by, for example, filtration or centrifugation. In previous versions of the **90Plus** software the BI was referred to as Sample Quality. The two names refer to exactly the same value, only the name was changed.

2. Why are baselines important?

The first step of data analysis is to normalize the experimental autocorrelation function by dividing each data point by the baseline. These normalized data are then analyzed to obtain particle size. In the most common case, cumulant analysis, the logarithm of the normalized data minus one is fit to a second order power series in delay time. A good baseline index indicates that the data is of high quality, e.g., free of dust. Don't worry if the index is poor; the **90Plus** uses advanced algorithms to recover as much information as possible. If you have reasonable results and repeatable results, then the software has taken care of the less than perfect sample.

3. How are baselines changed in the software?

Click on the menu item: `Setup` → `Normalization`. Select the method desired. Normally the "Autoslope (Slope Analysis)" method is selected. This is described below.

4. Why are different baseline options available?

This is one way that you can recover from an imperfect sample. Varying the baselines changes the way large dust particles are treated in the analysis.

5. What is *Autoslope* (Slope Analysis) normalization?

In this case, the data are examined starting halfway down the autocorrelation function and a series of straight line fits are performed until the slope is less than a small, positive value. Effectively, this is where the decaying autocorrelation function is flat or nearly so. For most samples this works nicely and we recommend using the Autoslope baseline. Sometimes, there are correlation functions that are very noisy or have two distinct decays. In these cases, the Autoslope (Slope Analysis) routine may set the baseline prematurely. In such cases we recommend either “Last Channels” or “Calculated” as baseline choices.

6. What is *Last Channels* normalization?

This places the last eight baseline channels at the last eight delay times. If the baseline index (BI) improves and repeatability is acceptable then the results may be better. However, if there are large variations in Effective Diameter and Polydispersity then there may be some dust and you are better off with Autoslope.

7. Must a measurement be made again in order to use a different baseline?

No. Just recall the data (file/database) and change the normalization method to see the results.

8. What is the *Dust Filter*?

The dust filter is an algorithm that rejects data that are corrupted by scattering from dust. You can turn the “dust filter” on or off during a measurement and you can recall data and turn the dust filter on and off retroactively. However, during and after a measurement, a new filter value cannot be entered. If the dust cutoff value is too high, 100% of the light is accepted for correlation and you have not “clipped” the high or “dust” contributions. Note that for very clean samples, one expects that 100% of the data is retained, provided the dust cutoff is not set too low. If the dust filter value is too low, then the reported effective diameter will be too low.

9. What is the exact algorithm for the *Dust Filter*?

In the data collection the scattered intensity is measured over many short, consecutive time intervals. The data are divided into two sets. The first data set, "unfiltered" includes all of the collected data. The second data set, "filtered" is filled by using the following procedure. The moving mean, I_{mean}, and standard deviation, σ_m, of five sequential intensity values is calculated. On the sixth measurement, the intensity, I_{new}, is compared with the average of the previous five using the dust filter cutoff value "C." If the following test is true, the data is accepted into the second "filtered" data set.

$$| I_{new} - I_{mean} | < C\sigma_m$$

If the test is false, then the data from this measurement is not added to the "filtered" data set. Thus, the "filtered" set is only the result of measurements that meet the inclusion criteria for the dust filter. The autocorrelation function is accumulated and kept for both sets. In this way, the user can turn the dust filter on or off during or after a measurement to observe the effects of data filtering on the results. However, the dust filter cutoff value cannot be changed once a measurement has begun.

10. What value is appropriate for the *Dust Filter*?

Start with a value of 30. The optimal value of the dust filter cutoff is actually related to the expectation of outliers in intensity, which is related to the size of particles being measured. For particle sizes on the order of 10 nm, use a value of about 15. For particle sizes on the order of several 100 nm, use a value of 30. For particle sizes on the order of 1 micron, use a cutoff value of 80. To truly optimize the dust cutoff value, lower the dust cutoff filter until about 98% of the data is retained.

11. How do I know how much data is retained for a given *Dust Filter* setting?

When the dust filter is on, the percent data retained is displayed to the right of the baseline index.

12. What is the *Effective Diameter*?

The effective diameter is calculated from the Stokes-Einstein equation (for spheres, see question 27) from the experimentally determined, translational diffusion coefficient. The translational diffusion coefficient is calculated from the accumulated autocorrelation function of the scattered light using the method of cumulants. Thus, the translational diffusion coefficient is intensity-weighted. The Stokes-Einstein equation shows that the hydrodynamic diameter is inversely proportional to the diffusion coefficient. Therefore, it is wrong to say the diameter determined this way is intensity-weighted. The weighting is actually the inverse of the intensity weighting of the reciprocal of the diameter. An average calculated in this way is sometimes called the harmonic mean.

13. What is the *Mean Diameter*?

Consider N runs, each yielding an Effective Diameter. The average of these Effective Diameters (sum divided by N) is called the Mean Diameter.

14. What is the *Combined Diameter*?

The combined diameter is the diameter calculated by treating the data as if it were taken in one long run rather than a series of individual runs. Deselected runs are ignored.

In a size measurement, there are typically multiple runs. Each run results in an autocorrelation function made up of M correlation coefficients (one for each delay time, τ). Summing the correlation coefficient for a particular delay time over all runs will give the correlation coefficient that would result from a single, long run. Repeating the process for all delay times will then give the correlation function that would result from a single, long run. Finally, this averaged function is analyzed using cumulants to obtain an intensity-weighted average translational diffusion coefficient. The Stokes-Einstein relation (see question 27) is then applied to the averaged translational diffusion coefficient to calculate an average diameter. This is the Combined Diameter.

15. Which one do I use, the *Mean Diameter* or the *Combined Diameter*?

If the Mean Diameter and the Combined Diameter are equal to within one or two standard errors, then there are no statistical outliers and the data are acceptable. In this case, use the Mean Diameter to characterize the central tendency of the size distribution. This is one way to characterize the "size" of a distribution. It is often the safest way to do so.

16. What if there are outliers?

If the Effective Diameter of a particular run differs from the Mean Diameter by more than twice the standard error, consider it an outlier. Delete the outlier by double clicking on the line of data corresponding to the run. The text on that line will turn gray. Deletion can be reversed by double clicking on the same line.

17. What is the upper limit for count rate?

The upper limit for count rate is 500 kcps (kilocounts per second) for the avalanche photodiode (APD) detector and 1 Mcps (1 million counts per second) for the photomultiplier tube (PMT) detector.

18. What is the lower limit for count rate?

The lower limit for count rate is 2 kcps, about five times the count rate for a typical liquid. A lower count rate means a measurement will take longer to complete. At low count rates, over a given time period, fewer photons are reaching the detector and there is less data with which to build the correlation function. Thus, at low count rates increase the duration to get more repeatable results.

19. How do I enable or initiate the automatic adjustment of the incident intensity and thereby the count rate?

Instruments with a BI-APD (avalanche photodiode) high sensitivity detector and instruments with zeta potential measurement options have two features that allow for adjustment: adjustable laser power or adjustable neutral density filter. Automatically adjust the count rate by choosing `Setup → Incident Power Settings`. Choose the radio button "Optimize Incident Power at start of each measurement." Close the dialog box. When the start button is pressed, the count rate will be optimized so that it is below the upper limit for the count rate. In this process, if the instrument has an adjustable laser, it will be used as it is the more sensitive. If it does not have the adjustable laser, then the neutral density filter wheel will be adjusted such that the count rate is lower than the maximum count rate for the instrument.

20. How do I optimize the count rate?

To optimize the count rate, choose `Setup → Incident Power Settings`. Choose the radio button "Optimize Incident Power at start of each measurement." Close the dialog box. When the start button is pressed, the count rate will be optimized so that it is below the upper limit for the count rate. If your instrument has an adjustable laser, the laser power will be automatically adjusted until the count rate is at or below the maximum for the type of detector you have. If your instrument does not have an adjustable laser, but it has a variable neutral density filter, then the filter position will be automatically adjusted until the count rate is at or below the maximum for the type of detector you have. In either case, you will see the count rate change automatically. If you do not, then you have an older system with neither an adjustable laser nor a neutral density filter wheel. In this case, your only choice is to dilute the sample until the count rate is below the maximum for the type of detector you have. Finally, if your software does not allow for optimization, then contact the factory for the latest version.

21. How do I manually adjust the incident intensity, and thereby the count rate?

Manually adjust the count rate by choosing `Setup → Incident Power Settings`. Choose the radio button "Maximize Incident Power at start of first measurement." Close the dialog box. When the start button is pressed, the count rate will be maximized by adjusting the laser to maximum power, if you have an adjustable laser, and by position of a neutral density filter wheel. During this process, click "Bypass" when the desired count rate appears. Or, the software will automatically maximize the count rate. Of course, count rate can also be decreased by lowering the concentration of particles in your sample.

22. How do I export results as plain text (ASCII) to a spreadsheet such as Excel?

Open up a measurement and choose the data you wish to export, such as MSD. Press the "Zoom" button in the upper right of the screen. If you choose Lognormal or MSD data, then press "Lognormal Summary" or "MSD Summary". In the resulting window, press "Copy for Spreadsheet." The data will then be placed on the clipboard as comma delimited data. You can save the data as a text file and import it into Excel, choosing a comma as a delimiter.

23. How do I send data to Brookhaven Instruments when I have a problem?

See question 24, "How do I save the data so I can analyze it at my desk?" Then, e-mail the resulting file to Brookhaven Instruments.

24. How do I save the data so I can analyze it at my desk?

Within the software, click on `File` → `Database`. Then, highlight the measurement(s) you wish to send and press the "Archive Selected File(s)" button. A standard save file dialog will appear. Choose a descriptive file name then save the file. The extension ".bak" will automatically be appended to the file name. This file contains many details about the measurement that are not available in a screen shot or printed report.

25. Why is 10 mM KNO_3 used as a diluent for the latex reference material?

See question 26, "Why not just use distilled water as a diluent?"

26. Why not just use distilled water as a diluent?

In order to calculate particle size from dynamic light scattering results, the Stokes-Einstein equation is used (see question 27). An underlying assumption in the calculation is that the particles are freely diffusing. For dilute electrostatically stabilized dispersions, long-range interactions distort free, translational diffusion. Adding salt screens the particle-particle interactions, making the assumption of free diffusion valid and therefore the use of the Stokes Einstein equation correct. 10 mM aqueous KNO_3 is often a good choice for a diluent. Most aqueous dispersions, such as the anionic latex used to validate the performance of the 90Plus, are electrostatically stabilized.

27. What is the Stokes-Einstein Equation?

The Stokes-Einstein equation relates the diameter of a spherical particle to the translational diffusion coefficient. It is

$$D = \frac{k_B T}{3\pi\eta\, d}$$

Where:

D is the translational diffusion coefficient,

k_B is Boltzman's constant,

T is the absolute temperature in Kelvin,

η is the liquid viscosity, and

d is the hydrodynamic diameter.

In biochemistry this same equation is used but often with six substituted for three in the denominator and R_H, the hydrodynamic diameter substituted for d.

This equation is used to transform diffusion coefficients determined by dynamic light scattering into diameters presented as measurement results.