Brookhaven Instruments Corporation

BI-DCP Users Manual

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by Jean-Luc Brousseau, PhD

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Please Note:

This manual is being presented in it unfinished form. Errors and omissions in the manual may be expected. Please make notes of any errors and or questions you may have concerning this manual and report them directly to BIC. Any suggestions made will be greatly appreciated by the author.

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Additions and corrections made in version 2.0

- Detector alignment section (Chapter 3) was modified and expanded.
- Reference to the *buffered* line start in Chapter 4, section 6 has been removed to avoid confusion on how the density gradient is to be made.

Additions and corrections made in version 3.0

- A discussion concerning the DCP LIST run conditions was added (chapter 4.2)
- A discussion concerning radial dilution and concentration has been added (chapter 5.5)
- The BI-DCP HOST analysis chapter has been expanded to include methodology for negative delta rho (NDR) analysis (chapter 5.6)
- Additional editorial corrections were made throughout the manual

DCP Users Manual Table of Contents

Chapter 1 Introduction

This manual is intended to assist users of the Brookhaven Instruments Corporation BI-DCP disc centrifuge. Advancements in the BI-DCP techniques has warranted the creation of this manual and users are strongly encouraged to read its entire contents. It is the intention of Brookhaven Instruments that this manual be used in conjunction with the supplied users manual that supplied with each instrument. It should be noted, however, that the techniques presented in this manual are to be considered superior to those presented in the earlier manuals and should be used in place of those found in above-mentioned manuals.

The manual is broken up into five chapters. Chapters two and three deal with the care, maintenance, and alignment of the BI-DCP. Chapter four deals specifically with the line start, or LIST, analysis technique. Chapter five focuses on the use of the BI-DCP in making a homogeneous, or HOST, analysis. Readers of this manual will find valuable information concerning sample preparation, gradient preparation, proven injection techniques, and trouble shooting techniques.

This manual represents the final product of many years of research and development. It is the hope of this author that the manual be used to solve many frequently asked questions and that it serves a reference source to refer back to. New users of the BI-DCP who did not have the benefit of on-site training will also benefit greatly by the reading of this manual.

In general the injection techniques presented here are simple and straightforward to follow. Users will discover that by mastering the internal gradient (section 4.3) injection technique for the LIST analysis, they will have also have simultaneously mastered the injection technique for the HOST analysis. Additionally, advancements in making the HOST Negative Delta Rho (NDR) analysis have made this type of measurement uncomplicated to perform and they provide users of the BI-DCP with a useful alternative method of making a particle size analysis.

Readers are encouraged to identify and report back to BIC any sections of this manual that they find confusing or ambiguous. Brookhaven Instruments is constantly seeking ways to improve our customer service and support, so any suggestions are greatly appreciated.

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Chapter 2 Maintenance and Handling Of The BI-DCP

The Brookhaven Instruments BI-DCP is a durable instrument which resides equally well on the production floor as in the laboratory. By following simple handling procedures the users of the BI-DCP can guarantee many long years of service.

The following sections outline the recommended cleaning and maintenance schedule of the instrument and discuss the removal and attachment of the measurement disc. Special disc handling precautions are discussed in the final section of this chapter.

2.1 Special handling concerns of the measurement disc

The BI-DCP measurement disc is made of either PMMA or Polycarbonate. The PMMA is suitable for non-aggressive solvents, weak acid and base solutions, and other non-aggressive aqueous systems. The Polycarbonate (trade name Homalite) disc is suitable for aggressive solvents, ketons, aldihydes, and strong acid and base solutions. Please check the chemical compatibility table found in the appendix of this manual before using any exotic solvents or solutions in the measurement disc(s). Avoid using any chlorinated solvents with the PMMA discs as they will degrade it.

The measurement disc should be handled with extreme care to avoid scratching its surface. Discs, when not attached to the instrument, should be either wrapped in protective material and stored in a safe location or, for short periods, placed with its hub down and face pointing upward. Each disc has been carefully balanced and tested and should be treated with great care. Although the discs are replaceable, they are expensive to manufacture and test.

The optical integrity of the measurement disc becomes very important if the user is intent upon making BI-DCPH (or HOST analysis, chapter 5) scanning head measurements. A scratch on the surface of the disc may be interpreted incorrectly as change in the turbitity in the test suspension which will cause false information to be recorded.

The line start analysis (LIST analysis, chapter 4) is less susceptible to scratches on surface of the disc as the measurement head is held fixed, relative to the disc, throughout the duration of the analysis. Thus any scratches on the surface of the disc become part of a constant background, or baseline, signal. Trouble could arise, however, if the disc should become too scratched or dirty. Should this happen the instruments baseline signal will rise and will reduce the signal range over which suspension may be concentrated (chapter 4.4).

2.2 Attachment and removal of the measurement disc

The BI-DCP is shipped with its measurement disc removed. This is done to protect it during transportation. Once the instrument has been unpacked the measurement disc must be attached before any analyses may take place. The user is encouraged to carefully inspect the new disc to ensure that its surface is free of any defects and/or scratches. Any defects must be reported back to BIC immediately so that we may correct the problem. Please note that sections of the circumference of the disc may have been drilled out. There may also be small amounts of a gray epoxy on the outside rim of the disk**.** ***Do not remove this material*** as it is used to balance the disc*.*

Once the disc has been removed from its shipping box and inspected for defects it is ready to be attached to the BI-DCP. Follow the directions below. Please remember that attaching the disc for the first time is difficult and new users can be expected to make several attempts at it before succeeding. Once the user gets some experience, however, the procedure becomes much easier.

Attaching the disc:

1) Turn the BI-DCP on.
2) Inspect the measurement disc to be attached to ensure that both of its hub screws are present and loosened enough so as not to protrude into the keyway slot inside the discs hub.
3) Press the **CUT** button on the front panel of the BI-DCP. This will bring up a user selection screen on the front LCD panel.
4) Press the number **1** on the front panel to retract the measurement head.
5) Inspect the axial of the motor. It will have a notch running through its end. This is called the **keyway** and it holds a rectangular metal pin called a **key**. The key slips into the keyway in the axial of the motor and into an identical keyway that is cut into the hub of the measurement disc. It is responsible for keeping the disc from slipping off during the measurement. Two lock down screws located directly over the disc keyway secure the key in place and lock the disc to the motor axial.
6) Position the motor axial so that the keyway slot points directly upwards. Place the key into the motor axial keyway. Make sure that the key in fully inserted into the keyway and that its tip is flush with or sticks out slightly from the end of the motor axial.
7) Open the small square door located directly over the disc chamber on the BI-DCP. Look down into the chamber through the opening and locate the motor axial. You should be able to identify the key and the keyway in the axial. Reach down into the disc chamber with the supplied disc (Allen) wrench. Use the wrench to hold the key in place while you push the disc onto the axial.

8) Reach over with your free hand and pick up the measurement disc which is to be attached. Hold the disc by its face so that the discs hub sticks out. Rotate the disc, if necessary, so that when you place it on the motor axial the keyway located inside of the disc is lined up with the key which is already inserted into the motors keyway.
9) Hold the key in place with the Allen wrench so that while you push the disc onto the motor axial it is not allowed to slip out. Many users accidentally allow the key to slide out of the keyway while attempting to attaching the disc. Make sure, by looking though the open section on the top of the BI-DCP, that the key is staying securely in place while the disc is inserted. Take great care not to allow the key to slide back in the axial keyway or the key will not perform its function and the disc may spin free of the disc.
10) Press the disc firmly against the motor axial. Tighten the two lock down screws located on the top of the disc hub. This is accomplished by reaching down through the disc chamber door with the supplied Allen wrench and tightening the screws until they can not be turned any further. Make sure that the screws are firmly in place or they may come loose during a measurement.

11) The disc is considered well attached when the following criteria are met;

- The key is securely fixed inside of both the motor and the disc keyways.
- The disc hub is pressed firmly against the end of the motor axial. This is assured pressing firmly on the disc's center after it has been attached.
- The measurement head is free to travel over the disc surface. This is tested by placing a small section of paper flush on the surface of the measurement disc and ensuring that the plane of the paper does not extend into the measurement head.
- The two lock down screws located on the disc hub are firmly screwed down on the key.
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12) To make sure that the disc is inserted all the way into the motor axial the user is encouraged to gently place a section of paper over the forward facing plane of the disc. The plane of the paper should easily extend between the detector head and the measurement disc. If the disc is not attached fully the paper will not slide behind the detector head. **DO NOT go to step 13 before ensuring that the disc is fully fastened to the motor axial. Failure to do so can cause permanent damage to the disc and to the BI-DCP unit.**

13) Press **CUT** on the front panel of the BI-DCP to bring the user selection screen back up on the front LCD panel.

14) Press **2** to restore the head to its previously aligned position (if the user simply removed and cleaned the disc) or press **3** to realign the system (if the user has placed a new disc on the instrument which has different volumetric dimensions.

The measurement disc has now been properly attached. Should the user wish to remove the disc simply follow the directions printed below;

Removal of the measurement disc

1) Turn the BI-DCP on.
2) Press the **CUT** button on the front panel of the BI-DCP. This will bring up a user selection screen on the front LCD panel.
3) Press the number **1** on the front panel to retract the measurement head.
4) Open the small square door located directly over the disc chamber on the BI-DCP. Look down into the chamber through the opening and locate the motor axial. You should be able to identify the key and the keyway in the axial.
5) Reach into the disc chamber through the front door and rotate the measurement disc until the two lock down screws located on the discs hub are pointing directly upwards.
6) Reach down into the disc chamber with the supplied disc removal tool Allen wrench. Find the lock down screws with the wrench and loosen them to the point that they turn with little effort. Do not take the screws all the way out of the disc hub or they may be lost.
7) Reach into the disc chamber through the front door and take hold of the measurement disc. Gently, but firmly, pull the disc off of the motor axial. Take great care not to pull too hard on the disc. After the disc becomes free turn it slightly to allow it through the door. If the user pulls too hard it will cause the disc to rapidly come free and to possibly hit the front door enclosure which would damage the disc and possibly the instrument itself.
8) Remove the key from the disc keyway if it is found to remain inside upon taking the disc off of the instrument. Place the key in a secure place so that it is not lost.
9) The removed measurement disc should be handled with extreme care to avoid scratching its surface. Discs, when not attached to the instrument, should be either wrapped in protective material and stored in a safe location or, for short periods, placed with its hub down and face pointing upward in a safe location.

2.3 Washing the measurement disc

The measurement disc is most easily cleaned injecting into it 16 ml of 2vol% Micro™ cleaning solution and pressing the **MIX** button. This soap solution may mix in the disc for 3 to 5 minutes before being removed. Water is then placed inside of the disc and allowed to rinse once more. Once the water is removed the disc may be spun at 5000 RPM for 15 seconds and stopped. All of the remaining water in the disc is drawn out and the disc is then dried. The water may be removed by simply placing a folded paper towel into the measurement disc and turning it (the disc) slowly to pick up the excess water. A syringe with a rubber hose attached to the end of it is normally used in conjunction with a paper towel to remove what water may remain.

The disc may also be removed if cleaning it on the instrument is found to be too difficult (see **removing the disc** above). Brookhaven Instruments uses specialized cleaning and buffing materials to bring out small scratches and to remove adhered chemicals from the disc. If your disc can not be cleaned by simple soap and water the user is encouraged to phone BIC to receive instruction from us as to how to clean the disc.

Discs which are severely damaged may be sent back to BIC for re-surfacing. This process is costly, however, and the user is inconvenienced while the disc is at BIC. Many users choose to buy two measurement disc so that should one be made unusable for any reason the second is always near by.

2.4 Cleaning the disc measurement chamber

The disc measurement chamber should be cleaned at the end of the day. The chamber includes the clear Homalite door, the measurement head, the walls of the disc chamber, and lastly the overflow catch basin located directly below the disc chamber. This is used to capture excessive run-off from the disc if over 17 ml is used in making a measurement (a maximum volume of 25 ml can be used).

The overflow basin is removed by pulling gently on the small circular handle located directly below the front door panel. Empty the container, clean it, and replace it back into the slot below the BI-DCP door panel.

The chamber should be cleaned by a simple wiping down with a wet paper towel. Make sure not to get the optical components of the measurement head wet with the soap or water. When cleaning the chamber take care not to scratch the measurement disc. The disc itself should have its surface cleaned whenever any oils, soaps, or sample materials come in contact with it.

2.5 Cleaning the dust filter

The dust filter may be found by turning the rear screw located in the rear of the BI-DCP on its top. Once the screw is turned (it does not come all the way out) the top panel will open up exposing the gray dust filter. Take care when removing the dust filter not to allow any of the trapped dust to fall out over the instrument. Carefully bring the filter outside or into a protected fume hood and shake the filter free of dust. Compressed air, when available, also aids in blowing out trapped particles of dust from the filter. Once the filter has been cleaned it should be placed back into the instrument and the top panel is re-secured.

2.6 Routine maintenance

The following time table is intended as a reference guide. The actual amount of maintenance required is dependent upon the uses of the BI-DCP and the environment in which it is operated.

After every analysis

- The measurement disc is to cleaned (unless serial injections are being made, more on this in chapter 4 section 5).

After every day

- The measurement disc is to cleaned and stored (on the unit) dry.
- The catch basin is emptied (as necessary) and cleaned.
- The measurement chamber is cleaned.

Once every week

- The fill tube is removed and cleaned in a sonic bath.
- The instrument is checked for alignment (see chapter 3).
- The disc hold down screws (located in the disc hub) are checked to ensure that they are securely tightened down.

Chapter 3 Aligning the BI-DCP Detector Head

Introduction:

The BI-DCP disc centrifuge is able to calculate the size of the particles passing its detector based on a set of known parameters. Those known parameters are the particle density, the density and viscosity of the fluid which the particles are moving through (the spin fluid), the centrifugal forces exerted on the particles, and the distance that the particles must travel through the spin fluid to reach the detector.

It is the later parameter that is to be discussed in this chapter. In order for the instrument to make proper calculations of particle size it is necessary for the software to know the correct position of the detector head relative to the column that the particles are moving through. Furthermore the actual position of the detector head must be correct. Because of this it is always a good idea to check the alignment of the BI-DCP to ensure that the head is in the proper position and thus the calculation of particle size is correct.

Included with every measurement disc, either when sold separately or when sold with a BI-DCP system, is a page containing the volumetric dimensions of the disc, mainly the disc cavity radius and the disc cavity width. From these two values one may calculate the height of any column formed in the spinning disc based on the volume of the injected fluid. The final parameter listed on the disc parameter page is the position of the detector head relative to the center of the disc. This position corresponds to the radial position of the meniscus when 5 ml of water is injected into the disc and spun at 1000 RPM.

In order to check ensure the proper alignment of the BI-DCP the user is encouraged to analyze NIST standard micro-spheres (the Duke 0.993 um NIST traceable micro-sphere is most commonly used). Clearly if the standards are found by the instrument to that of the accepted value of the standard than the instrument is properly aligned.

Standard alignment testing conditions may be found in this chapter, section **3.3**.

3.1 Warm-Up Period

Unless otherwise stated the standard warm up time required by the BI-DCP prior to aligning the detector head is 5 minutes. The BI-DCP's detector has an internal heating device to ensure that electronic components are kept at a constant operating temperature. The required five minutes allows that heating system to bring the detection system to a constant temperature.

3.2 Aligning the BI-DCP[1]

Procedure:

1) If prior to the alignment, the instrument has not been in use it must be allowed to warm up. Turn on the BI-DCP and simply allow the unit to warm up for at least 5 minutes.

2) Open the door and ensure that there is no fluid remaining in the disc. Inspect the disc to ensure that it is free of any deposits on its surface.

3) Inject into the disc exactly 5.0 ml using a grade A precision pipette. Do not use a syringe.

4) Press the CUT button to bring up the selection screen on the instruments LCD panel. Press the number 3 on the panel to enter into the alignment mode and then press 1 to manually align the system.

5) The disc will now be spinning at 1000 RPM**. Record the signal voltage** (shown in the front panel of the BI-DCP's LCD screen). Normally it should be in the range of 0.2 to 0.7 volts. Verify that the "step" position is zero. This is the "home" position of the detector.

6) With the instrument's door open, press and hold down the CUT button. This will cause the head to travel to the left (relative to the center of the disc). Stop pressing the CUT button when the dark shadow cast from the meniscus appears close to, but to the right of, the detector slit. The voltage should still be about the same as it was in the home position and the step number may be around 200. Now slowly approach the detector slit by pressing the CUT button in single steps. As the dark band approaches the slit, you will be able to observe an increase in the signal voltage. Keep pressing the CUT button all the while making note of the voltage and step number. Keep moving the head towards the meniscus until the signal voltage has reached a maximum. Record this signal voltage and its corresponding step number.

   A typical maximum might be 2.2 volts with a corresponding step position of 230. Systems vary. These are approximate values.

[1] This section discusses the MANUAL alignment of the detector head. The BI-DCP is capable of aligning the detector head automatically. This is discussed in further detail at the end of this chapter.

To determine a maximum, you must go beyond it. But do not determine the maximum by going back and forth many times. To do this will cause an error due to the play in the drive screws. So after determining the maximum approximately, use the BOOST button to move the head to the right at least 10 steps and then stepping only to the left with the CUT button, determine the step position corresponding to the maximum.

7) Subtract 5 from the step position corresponding to the maximum. Use the BOOST button to move to that step position. This is the correct alignment position.

8) Once you have determined the alignment position press the MOTOR button. This will cause the instrument to exit out of the alignment mode and save the alignment step position. The instrument is now aligned.

3.3 Confirming the Proper Alignment of the BI-DCP

It is necessary to analyze a NIST traceable standard in order to confirm the alignment of the BI-DCP. The most common standard used is the Duke Scientific[2] 0.993 um polystyrene (Rho=1.05 g/cc). If this standard is not available the user may select any sub-micron micro-sphere in its place, although the speed at which the analysis is conducted must be modified to optimize the analysis.

3.3.1 Instrumental Run Parameters, Sample Preparation, and Order of Injection

Using the Duke NIST traceable 0.993 um standard,

Instrumental Setup Parameters (at 5000 RPM):
Set the BI-DCP parameter page up with the following fields;

Disc Speed:	5,000 RPM
Spin Fluid Volume:	15 ml
Gradient:	0.2 ml 100% MeOH
Evaporative Shield:	0.1 ml 100% Dodecane
Temperature:	Measure temperature of water prior to analysis and use this value.

Suspension Preparation Protocol:

Add 3 ml of DI water into a 20 ml vial. Add 3 drops of the of the Duke 0.993 um NIST traceable standard into the water and mix gently by hand until it is homogeneously dispersed. Add 1 ml of MeOH to the suspension. Mix gently by hand.

Syringe Setup Conditions;

[2] Duke Scientific Company, 1135D San Antonio Road, Palo Alto, California 94303. 1-800-334-3883

1. Fill the 2 ml glass syringe with 0.1 ml of Dodecane. Use a 16 gauge needle on the syringe.
2. Fill the 2 ml plastic syringe with 0.2 ml 100% MeOH (Methyl Alcohol). Use a 16 gauge needle on the syringe.
3. Fill the 20 ml glass syringe with 15 ml of DI water.

Order Of Operation For Injection:

1. Inject into the dry, non-spinning, disc the 0.2 ml of 100% MeOH.
2. Press the ***MOTOR*** Button and inject the 15 ml of DI water into the disc.
3. Inject the 0.1 ml of Dodecane into the disc. Wait 5 minutes.
4. Inject in 0.2 ml of the sample suspension and immediately press the ***START*** button.

Results:

The accepted values for the various NIST standards are normally written on the container from which the sample was taken. The accepted values for the Duke 0.993 um standard is, accordingly, 993 nm +/- 21 nm. If your values differ by more than the accepted value on the bottle the user is encouraged to refer to the trouble shooting table below. If the solution can not be found below than the user should refer to section 4 in this chapter.

If the accepted NIST value is incorrectly sized on the BI-DCP;

- Check to make sure that the ***proper disc parameters*** entered into the BI-DCP's software.
- Ensure that the proper run conditions are entered into the Setup Parameters page. This includes checking that the ***correct volume*** of fluid is reported in the Fluid Volume field, the ***correct temperature*** has been entered and that the correct spin fluid has been used. This will ensure that ***the proper values of density and viscosity*** have been calculated. Remember, the BI-DCP's software makes calculations of density and viscosity for WATER ONLY. If the user is using anything other than water he must MANUALLY enter in the values of viscosity and density of the spin fluid.

- Ensure that the proper ***particle density*** has been entered into the software.

If none of the solutions listed above can be attributed to the error in sizing the NIST standard the user is encouraged to follow the steps outlined below:

1. Repeat the sample injection directly over the same spin fluid column. This is type of injection procedure is covered in more depth in chapter 4 section 6 of this manual. If the user gets the same results go to step 2.

2. Clean out the disc of the spin fluid, re-prepare the gradient and re-inject a fresh sample. If the user gets the same results go to step 3.

3. Re-align the system (see 3.4 below first), repeat the alignment test. If the user gets the same incorrect results contact the maker of the NIST standard to ensure that it was properly labeled and that it is not beyond its shelf life.

3.4 Common Errors in Alignment

The most common errors in aligning the BI-DCP is the incorrect identification of the position of meniscus produced when 5 ml is spun in the disc. This can be caused by concentric scratches in measurement disc which can in turn lead to the user incorrectly believing that the scratch is actually the shadow cast by the meniscus. The best way to avoid this error is to turn out the lights in the room while making the alignment. The automatic alignment mode is especially sensitive to this sort of problem (see below).

Another problem that can lead to an improper alignment is not using ***exactly 5 ml*** to align with. The user is encouraged to use a Class A precision pipette to make the alignment with. If none is available it is suggested that the user weigh out the volume of the alignment fluid to ensure that the proper amount of fluid is actually being dispensed (water has a density of approximately 1 g/cc at room temperature so 5 ml should weigh 5 grams).

3.5 Automatic Alignment of the BI-DCP

The BI-DCP has the ability to automatically align itself. This is done by simply cleaning out the disc of any fluid, injecting into the measurement disc 5.00 ml of DI water and pressing the CUT button on the front panel. This will bring up a selection screen (see above). At this point press the number key ‘3’ and then press the START button. This will cause the BI-DCP to perform an automatic alignment of the detector head. The user is encouraged to align the instrument manually, however, as the automatic alignment is prone to error when measurement discs of less than perfect (new) quality are used (see above, section 3.4).

Chapter 4 The BI-DCP LIST Analysis

4.1 Selection of the spin fluid buffer fluid pair

The BI-DCP list analysis begins with the determination of the spin fluid in which to suspend the particles intended for the analysis. This is no trivial matter as the spin fluid must meet a demanding set of criteria. The list below summerizes the requirements for choosing a spin fluid:

1) The spin fluid must not dissolve, attack, or change in any way the particles to be analyized.

Clearly if the particle size is to be measured the particles themselves can not be allowed to dissolve. This statement may appear trivial but in many cases users have attempted to suspend materials in solutions that attack the particles.

Considerations such as the the osmotic potential of the spin fluid must be taken into account. In the case of liposome particle sizing the users must take into account the osmotic potential of the fluid inside of the liposomes and bring the osmotic potential of the spin fluid to match it. Otherwise the liposomes will explode (or implode, depending upon the solution conditions) upon introduction into the suspending medium.

2) The spin fluid must have a misable, less dense, fluid which can be used as a buffer.

All line start (LIST) analyes are made using a spin fluid and a corresponding buffer fluid. The purpose of the buffer fluid is to slow the injected particles down so that they can settle under Stokes forces alone. When the particles are injected into the disc they obtain a large amount of momentum from hitting the back wall of the rapidly spinning disc. This aquired energy, coupled with the low viscosity of air, brings the particles up to a great speed.

In order for Stokes Law to be held valid (Stokes law is the mathmatical formula that describes the rate of sedimentation of a particle of known size and density through a medium of known density and viscosity. It is the formula that is used for the calculation of particle size from the raw data on the BI-DCP) the particles must begin their "fall" from a state of rest. The buffer fluid is responsible for bringing about this state of rest by slowing the injected particles down.

The buffer fluid must be micable with the spin fluid because it is not enough to simply have the lower density fluid laying over the spin fluid colloum, it must actually blend into the spin fluid creating a concentration gradient throughout the colum made from the

spin fluid. Clearly a mixture of, say, oil and water won't mix. If the user tried to use these two fluids for the buffer fluid and spin fluid, respectively, a concentration gradient could not exsist. The oil would simply layer itself over the spin fluid creating two distinct solution regions.

A partial list of micable buffer/spin fluid pairs are listed below:

Spin Fluid	Gradient
Water	Methonal
Water	Ethonal
Sucrose Solution (1-50wt%)	Water (or less concentrated sucrose solutions)
Glycerol	Water
MEK	Dodecane

This list only covers a few of the applicable buffer/spin fluid pairs. By following the rules for spin fluid/ buffer fluid selection the user will discover many such pairs exist.

3) The spin fluid must have a viscosity large or small enough to allow the particles to reach the detector in a timly manner.

One of the parameters that determines the rate at which the particles being analized reach the detector is the viscosity of the spin fluid solution. Clearly if the spin fluid is very viscus the particles will travel more slowly through the column.

It is of great importance that the particles reaching the detector do so in a timly manner. This means that enough time is allowed to pass before any particles reach the detector. It also means that particles are not slowed down to such a great extent so that they reach the detector too slowly, thus make the run time unaccetablely long.

If the particles reach the detector too quickly two problems can arise. The first has to do with the rate at which particles centrifugate through the column. Because the rate of centrifugation is inversely proportional to the **square** of their diameter the diameter being measured at the onset of an analysis is changing very rapidly.

Because of the inverse square relationship at the onset of an analysis the measured particle size is changing very rapidly with respect to time. You can demonstrate this by simply pressing the START button on the BI-DCP and watching how quickly the measured particle size changes for the first 20 seconds or so.

The progression goes something like this (as an example);

Sec	Diameter Being Measured
1	10 um
2	8 um
3	6 um
4	5 um
5	4.5 um
10	4 um
20	3.5 um
ect...	

In order to make a well designed analysis you need to have a minimum of 15 to 30 seconds of baseline before the measured turbitity begins to change appreciably. That is to say, you need fifteen seconds of run time prior to any particles reaching the detector system.

The reason for this is that there is a slight error associated with the time between when the user presses the START button on the instrument and when the computer begins to collect data. If the particles reach the detector too quickly there will be an error in the size distribution because the measured size is changing so rapidly at the onset of the analysis that slight changes in conditions (such as the determination of 'time zero' at the onset of the analysis) can make for large shifts in the first particles to be measured.

It is for this reason that the selection of the spin fluid is so important. If the user is working with large particles of a high density than it will be necessary to so the particles down using a high viscosity solution, such as sucrose, so that the first (largest) particles reach the detector in a long enough time period. If water is used for the above mentioned sample the user will find that the larger particles may reach the detector in a time period less then 15 seconds and thus the determination of the particle size of this group of large particles may be incorrect.

4) The spin fluid can not dissolve or damage the measurement disc.

This statement requires no explaination. A listing of fluids compatable with the BI-DCP PMMA and Polycarbonate (solvent resistant) discs may be found in the appendex of this manual.

4.2 Determination of the Optimum Run Conditions

The user of the BI-DCP may vary the length of time that particles reach the detector by changing a number of variables. The most direct way to determine the best conditions over which to run the analysis is to use the BI-DCP modeling utility. This can be done by selecting the function key F7 from the Main Menu. By altering the various values found in the function key field located at the bottom of the sceen the user can see how the changes in operational conditoins affect the rate at which the particles reach the detector.

Hint: In modeling an unknown particle size group force the modeled polydispersity to a value of 1.0001. This will make the particle size distribution on the modeling utility page appear to be a straight line, or pointer in time space. Then, after entering in to the modeling utility all of the information on the spin fluid density,viscosity and the particle density, the user enters into the instrument

The time dependence of the particles reaching the detector is determined by the following factors, all of which can be adjusted by BI-DCP user:

♦ The most direct way to adjust the rate at which the particles reach the detector is by modifying the centrifuge disc's **rotational speed**. The rate of which particles reach the detector goes to the square of the disc speed. Thus a particle taking 16 minutes to reach the detector at 5000 RPM will reach the detector in only 4 minutes at 10,000 RPM. Modification of the disc speed is the most common way to adjust the conditions of the analysis. The BI-DCP has an operational range from 600 RPM up to a maximum of 15,000 RPM.

{ The **difference in densities** between the particle and the spin fluid through which it centrifugates: This value is known as the delta rho quoent. The larger the delta rho, the faster the particles will travel through the disc. As the particles density is fixed the only way to manipulate delta rho is by changing the spin fluid.

♦ Users may adjust the rate at which particles reach the detector by adjusting the **viscosity** of the spin fluid. The rate at which particles reach the detector is directly proportional to the viscosity of the fluid in which they are traveling through. If the particles in question are reaching the detector in too small of a time frame, even at low disc speeds, the user may opt for the use of higher viscosity solutions that will impede the motion of the particles.

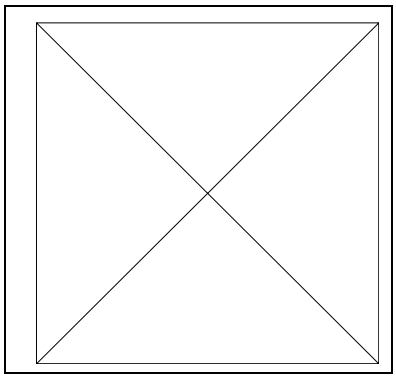
♦ The height of the **spin fluid** column: The length of time required to reach the detector is proportional to the height of the spin fluid column. Thus, by changing the volume of the injected spin fluid, the user may adjust the rate at which the particles reach the detector. A larger column (more spin fluid) forces the particles to travel farther through the spin fluid, thus the length of time necessary to reach the detector is increased.

Just how these variables are adjusted depends upon the type of sample to be run. The sections below describe the different conditions that the BI-DCP user faces and the optimum solutions for these conditions.

4.2.1 For narrow samples:

Particle groups that contain only narrow distributions of particles should be analized under conditions such that the first particles in the population reach the detector from between **1 and 2 minutes**. These conditions are optimized for the highest resolution analysis possible, without sacraficing too much laboratory time. As a general rule the longer the BI-DCP LIST analysis, the greater the resolution. Please remember, however, that if the instrument signal has not returned to its initial baseline value in under 30 minutes the user may consider speeding the disc up.

4.2.2 For broad samples:



Particle groups that contain broad distributions of particles should be analized under conditions such that the first particles in the population reach the detector from between **15 and 30 seconds.** Although the resolution of the analysis will suffer in characterizing the larger particles, operating the instrument at high speeds when working with broad samples will significantly reduce the time over which the analysis will take. By cutting 15 seconds off the initial baseline time the user can save, depending upon the polydispersity of the sample, upwards of 30 minutes or more in analysis time. If the sample is too broad, however, the user should consider going to the BI-DCPH (chapter 5) method with the scanning detector head. The user is encouraged to use the BI-DCP modeling utility to determine the optimum conditions over which to run the analysis.

4.4 Determination of the Optimum Injection Concentration

Introduction:

The Brookhaven Instruments BI-DCP is capable of making particle size analyses on micron and sub-micron sized ceramics, as well as other high density materials such as diamond and other crystalline powders. This type of measurement, however, must be made with certain considerations. The first of these considerations is the concentration of the injected suspension.

One of the primary conditions under which Stokes' Law is held valid is when the injected particles do not interact with one another. If the suspension is overly concentrated, the particles may "fall" with one another while moving through the spin fluid. If this occurs, the particle size distribution usually reflects larger than real sizes. This type of hydrodynamic instability is called streaming.

Determination of Streaming: Gradient vs. High Injection Concentrations

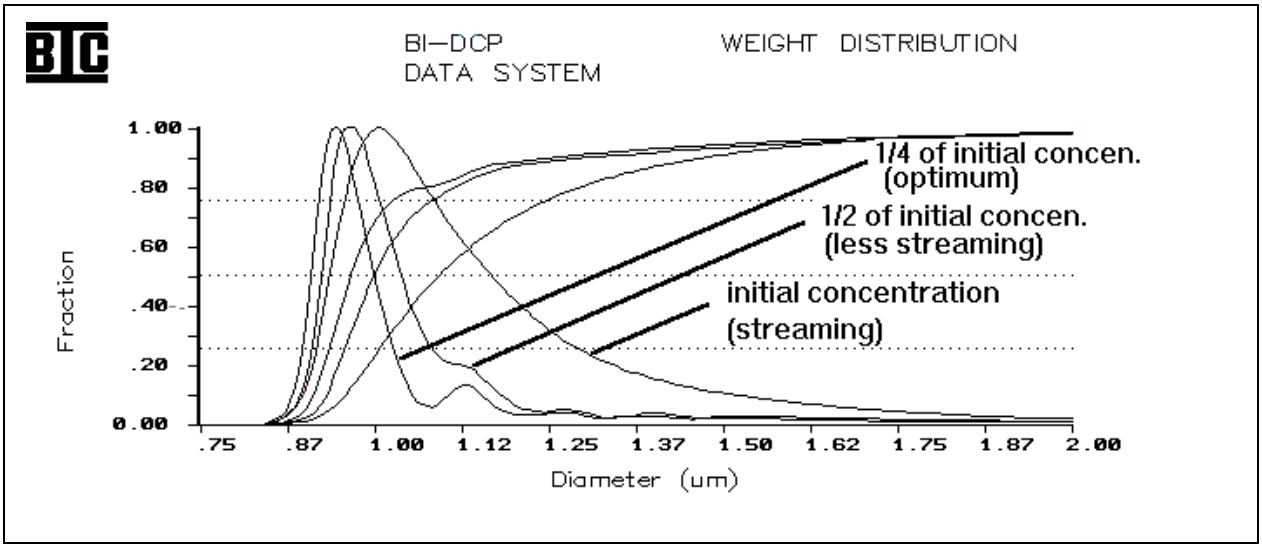
Most often, streaming is identified by turning on the BI-DCP's strobe prior to injecting the sample and, upon injecting the sample, observing the changes in turbidity and concentricity of the injected sample using the strobe. Because samples in the BI-DCP are separated by particle size, the user should not expect to see uniform turbidity from

the meniscus to the detector. However, the same particles (and thus the same level of turbidity), should be visible in the same concentric region of the disc. The diagrams below demonstrate two types of streaming.

Figure 1
Gradient Streaming vs. Overly Concentrated Suspension Streaming

Figure 1 shows two distinct types of streaming. If the gradient has not yet stabilized, the user can incorrectly think the suspension is streaming due to it being overly concentrated. The type of streaming found in overly concentrated injection suspensions is usually apparent by "arms" (similar to those found in a spiral galaxy) which form in the injected band.

Often, the BIC Applications Laboratory will inject a known, NIST traceable, particle size standard through new gradient systems. If the size and calculated polydispersity of the standard is within accepted limits, the user may make a series of injections using sample suspension over the same gradient system (see Stability of the Internal Gradient Over a Series of Injections on the BI-DCP). Furthermore once the user has established that the gradient system is stable any observed streaming can not be blamed on the gradient. The only remaining causes of any observed streaming can be;



1) the concentration of the injected solids is too high, or 2) the disc's RPM is too high and the particles are streaming because they are traveling through the spin fluid under non-laminar conditions.

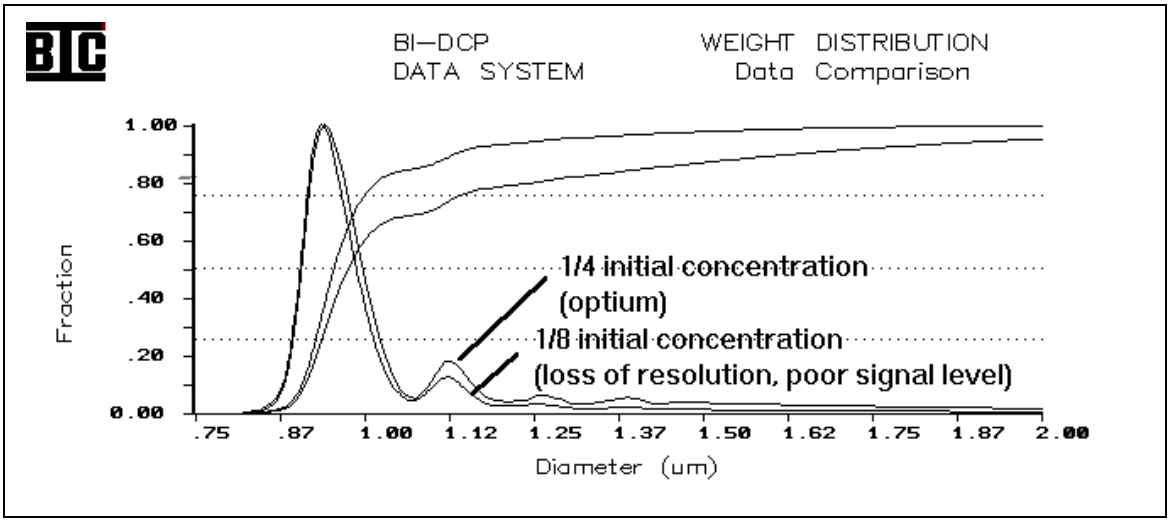
Determination of Optimum Injection Concentrations:

The simplest way to determine the optimum injection concentration is to make a highly concentrated suspension of the particles and then work with dilutions of this suspension. The concentration of this primary suspension should be around 0.1wt%. The particles should be suspended into a fluid that is conducive to dispersing the particles. Frequently, the suspension fluid of choice for ceramics is a 0.1wt% solution of Tetra-Sodium Pyrophosphate (TSPP). In the TSPP solution, the particles are given a strong negative surface charge and disperse well from one another.

Method #1; Working with low initial injection concentrations.

From this primary concentration (above), the user dilutes the sample by roughly 1:8. This is done by adding 0.5 ml of the concentrated suspension to 2.5 ml of the pure suspension fluid, then adding 1 ml of the same fluid used to make the gradient. In most cases, the gradient is established with 100% MeOH when the selected spin fluid is water, so the 1 ml of gradient fluid to be added to the diluted suspension would be, in this case, 100% MeOH. This diluted suspension is then injected into the BI-DCP and the distribution data is collected. This initial distribution curve may show a very noisy differential distribution because of the low concentration of the injected sample. The signal to noise ratio is very low. The user then should make a serial injection over the same gradient using a more concentrated suspension, typically twice that of the injection suspension concentration that was just analyzed.

The two particle size distribution curves (obtained from the two injections described above), when overlaid, should overlap very closely. If streaming has occurred because the second injection's concentration was too high, there will be a general shift to larger sizes in the particle size distribution. If the concentration is much to high the user will be able to see the streaming "arms" depicted in Figure 1B .
The figure below shows the results of following the above protocol. The graph depicts the same sample run under the same conditions, but at different concentrations. The first curve, although having correct distribution information, was made at such a low concentration that the differential curve is subject to instrumental noise. Furthermore, af-



ter applying the light scattering correction, the region of the distribution curve having the smallest sizes will be amplified. Because the signal may, in some cases, be lowest in this smallest sized region the user will find that the light scattering correction amplifies the poorest resolved section of the analysis and provides a poorly resolved distribution curve.

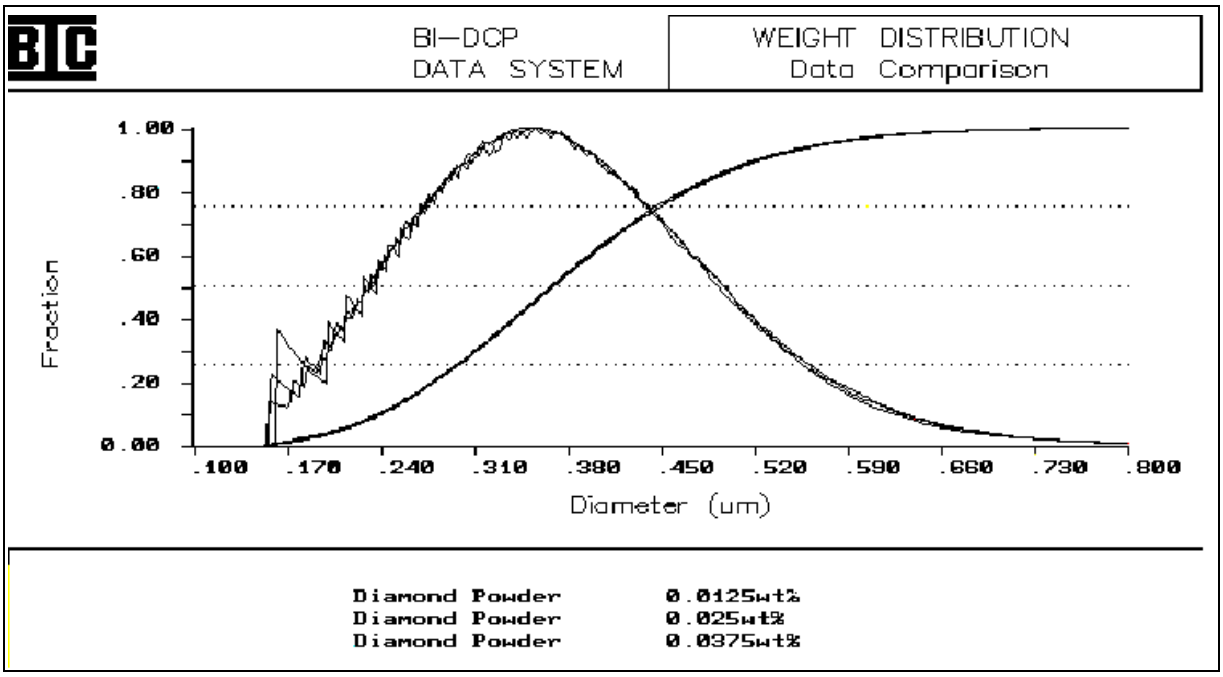


Figure 2
Increase in Resolution by Increasing Concentration of Injection Suspension

Method #2; Working with a high initial injection concentration:

A second approach to the same type of analysis is to start with a highly concentrated injection suspension, and then to make repeated measurements at progressively lower concentrations. This can speed up the process because once two progressive dilutions overlay to within 1%, the user may then stop. The alternative (starting with a low

Figure 3
Reduction in Streaming and Increase in Resolution by Dilution of Injection Suspension

concentration suspension and working up), will produce distributions, although true to the real distribution, that are jagged and in many cases are un-presentable. The low concentrations also suffer from a general loss of information at the tail ends of the distributions, as depicted above.

Figure 3 is a graphical representation of the process outlined above. The sample analyzed was a nominal 1.0 um Silica powder. Transmission Electron Microscope photographs identified the material as having, by number, an average particle size of roughly 1 um. The TEM also identified a number of fused together doublets and triplets. It was difficult to tell from a TEM photograph if the particles were actually fused together, or simply overlaid or prepared in a way that causes them to appear fused. The BI-DCP was able to resolve the question.

As depicted above in Figure 3, the first injection (initial concentration) did not initially identify the doublets and triplets. Indeed, these particles proved to make the injection suspension very hydrodynamically unstable. They appeared prone to "tumbling" through the spin fluid/gradient system. Only after a series of dilutions was the true distribution of the powder identified. It is apparent that the fine details of the distribution became resolved only after the streaming was eliminated. This was done by reducing the concentration of the initial injection fluid.

It is also evident from the above graph that the primary peak, at around 1 um, shifts to larger sizes at higher concentrations. The shift is caused by the larger particles streaming through the injected suspension and pulling the smaller particles along with them in their turbulent wake. The final position of the peak is only truly established after two successive dilutions distribution curves overlay to within 2% of each other.

Over Diluting Injection Suspensions:

Although diluting the injection suspension reduces streaming, and the ability to identify small details is enhanced, resolution will be lost below a critical concentration. Because

Figure 4
Loss in Resolution by Over-Dilution of Injection Suspension

the BI-DCP calculates the distribution of particle size by measuring the intensity of transmitted light through the spin fluid vs. time, a reduction in concentration will also result in a general reduction of information.

This loss of information is clearly demonstrated above in Figure 4. Shown here is the same Silica material as depicted in Figure 3. The graph above, however, depicts the loss of resolution of the smaller doublet and triplet peaks because the concentration of the suspension was made too dilute, thus not allowing the instrument to detect changes in turbidity that would have been attributed to the doublets, triplets, and other larger particles. The doublets, triplets, and quadruplets seen in the 1/4 dilution are no longer resolved in the final, 1/8 diluted, injection suspension.

This confirms the optimum concentration to be the 1/4 concentration injection suspension. Although there is still a very small shift seen in the primary peak, this can be caused simply by the relative reduction in the other peaks (thus bringing up the primary peak's overall relative cumulative percentage). In any case, the loss of information concerning the multiple peaks is too important to be sacrificed for the small shift seen in the primary peak.

Conclusion:

Although it may appear somewhat tedious to carry out the series of dilutions outlined in this protocol, the user may take relief in the following way. Once the optimum concentration for the material in question is established, the user does not need to repeat the entire procedure for a new sample (if its size distribution is known to be roughly in the same region as the first material). Once the ideal concentration conditions have been found, the user simply runs unknown samples at that concentration.

Although this protocol is lengthy, the quality of the user's data will benefit by its use. Furthermore, with the use of serial injections, the user will find that it is not as labor or time intensive as it initially seems.

4.6 Stability of the Gradient System and Serial Injections

Overview:

It is shown that the buffered gradient prepared by the Brookhaven Instruments Photo-Disc Centrifuge is stable for a minimum of 2 hours 40 minutes. Furthermore over this time period a mixture of four NIST traceable standards was injected over the gradient *seventeen times* all being analyzed with unprecedented accuracy and reproducibility.

Operational Parameters and Environmental Conditions:

- Initial spin fluid injection volume was 15.0 ml of DI H_2O.
- The disc speed was set to 8000 rpm.
- The suspension fluid injection volume was 0.2 ml of a mixture of 993, 720, 502, and 398 nanometer Duke Scientific NIST traceable micro-spheres. Serial runs were calculated using the spin fluid volume from the pervious run plus the additional 0.2 ml of the injected suspension fluid volume from the pervious run.
- The laboratory temperature was measured at 21.1 degrees C. The initial temperature of the injected spin fluid was measured at 19.1 degrees C. A subtle temperature rise was thus included for the first 5 runs, after which the temperature was held constant at that of the ambient laboratory temperature.

Analysis Results:

Over the two hour forty minute run period no significant drift in particle size of any of the four modal peaks was detected. Furthermore the modal values identified for each of the four standards deviated from the established values by 1% or less for every analysis.

The table below indicates the modal values of the weight (mass) averages all of the reported peaks. Operational and environmental parameters are also included in this table for reference.

Serial Injection Analysis Results

Run #	Temperature (degrees C)	Spin Fluid Volume (ml)	**993** nm Duke Std.	**720** nm Duke Std.	**496** nm Duke Std.	**398** nm Duke Std.
1	19.1	15	993	724	499	398
2	20	15.2	992	721	498	397
3	20.3	15.4	994	722	498	397
4	20.7	15.6	993	721	499	398
5	21	15.8	993	720	497	398
6	21.1	16	991	721	497	398
7	21.1	16.2	994	721	497	399
8	21.1	16.4	996	720	498	399
9	21.1	16.6	992	719	497	401
10	21.1	16.8	992	719	498	398
11	21.1	17	993	721	497	*
12	21.1	17.2	994	721	497	398
13	21.1	17.4	995	719	497	401
14	21.1	17.6	997	721	498	400
15	21.1	17.8	996	722	499	400
16	21.1	18	996	720	500	400
17	21.1	18.2	994	721	498	398

Averaged Particle Size From Table	993.8 nm	720.8 nm	497.9 nm	398.8 nm
Established Diameter (NIST)	993 nm	720 nm	496 nm	398 nm
% Deviation**	0.08%	0.11%	0.38%	0.19%

* Run #11 was accidentally stopped prematurely. The 398 nm peak did not reach the detector and was thus not recorded.

** calculation method: $\frac{\text{((averaged modal value)-(established modal value))}}{\text{(established modal value)}}$

4.6 Protocol For The Formation Of The BI-DCP LIST Internal Gradient

1. Determine the optimum run conditions using the BI-DCP modeling utility (F7).
2. Enter into the Run Parameters menu the disc speed as determined from above.
3. Press F1 to continue, confirm that the proper disc speed has been sent to the instrument by observing the RPM speed displayed in the LCD panel.
4. Prepare your syringes for injection prior to starting the motor. First fill a 2 cc glass syringe with 0.1 ml of dodecane. This will be injected over the spin fluid last to act as an evaporative shield. Next fill a 1 cc syringe with 0.2 ml of 100% methanol. Set this syringe aside as well. Lastly fill a 20 to 30 cc syringe with the amount of spin fluid (water in this example) that was determined to be the optimum amount from the modeling utility.
5. Follow the next steps as precisely as possible: Inject into the disc the 0.2 ml of MeOH. Place the needle of the spin fluid syringe into the BI-DCP injection port, do not inject the fluid yet. Press the MOTOR button on the front panel of the BI-DCP. As soon as the disc begins to spin begin injecting in the 10 to 25 ml of spin fluid (water) over the already injected 0.2 ml MeOH. The rate of injection should be steady and brisk taking no longer then 3 seconds to completely inject in the fluid. The smoother the injection, the better the gradient. As soon as the fluid is completely injected into the disc, inject in the 0.1 ml of dodecane. DO NOT inject your sample yet. The sample should only be injected after a 5 minute gradient stabilization period has expired. You may wish to use the timer in the BI-DCP program to time out this period for you. Simply press the F1 key or the START button after you have injected the dodecane. After 5 minutes is up (as displayed on the screen) simply press ESC button to abort the run. Re-enter the Setup Page, press F1 and your ready to inject your sample. The alternative to this is to simply, after injecting in the dodecane, make note of the time and wait 5 minutes before injecting your sample.
6. While you waiting for the gradient to stabilize you may prepare the sample for injection. This is done by adding 3 ml of DI water to a 20 ml vial, then add as many drops of your concentrated suspension to the water as necessary to get a good signal. Swirl the suspension by hand until homogeneously dispersed, and finally inject into the suspension 1 ml of 100% MeOH (mix again by hand).
7. At the end of the 5 minute period pull 0.2 ml of the injection suspension into a clean syringe. Place the needle of the syringe containing the injection suspension into the injection port of the BI-DCP. Make sure that the instrument is ready to accept data by confirming the proper screen displayed on the computer monitor. With one finger on the START button firmly inject into the disc the 0.2 ml of the injection suspension. At the same time that you inject in the suspension press the START button. You should hear the computer make a slight "BEEP" at the point that it begins to take data.

Chapter 5 The BI-DCP HOST Analysis

5.1 Introduction

The BI-DCP is capable of measuring particle size distributions using two different techniques. The most commonly used technique is the Line Start Analysis, or the LIST technique as it is commonly called. Under this set of conditions the particles are injected onto a spinning disc containing a pure spin fluid with a density gradient. The larger particles centrifugate in an outward direction at a faster rate then the smaller particles. As the particles pass the detection head they temporally block the transmitted light from the emitter to the detector. This produces a corresponding signal change which is measured as a function of time. Using this technique all of the injected particles begin at the same radial location in the fluid and separate, or differentiate, apart from one another. Thus the raw data is collected in the form of a differential curve. By integrating under the differential curve a cumulative curve is calculated. This technique has been described in detail in the preceding chapters.

The second technique that may be used is the **BI-DCPH**, **HOST**, or **homogeneous mode**, analysis. Using this technique the sample to be analyzed is suspended in a much larger volume of fluid then as compared to the LIST analysis. This suspension is then injected into the BI-DCP. Unlike the LIST analysis the particle do not all start at the same radial position in the disc. In the homogeneous analysis mode the particles to be analyzed are uniformly, or homogeneously dispersed, throughout the disc. This suspension gradually becomes less turbid, again recorded as a function of time. This change in turbitity is correlated to particle size, just as in the LIST analysis. Also, the particles are suspended in the spin fluid alone. There is no necessity to add a gradient fluid to the suspension, as with the LIST analysis. This can be especially helpful if the gradient fluid agglomerates the particles.

The major differences between the LIST and the HOST analysis are the method under which the changes in turbitity is calculated to particle size. Unlike the LIST analysis which records information as a differential curve, the HOST analysis begins with a suspension at it most turbid. Thus, the signal that is recorded at the onset of the analysis begins at a low value, say 0.2 volts. After enough time has passed so that all of the particles have been centrifuged from the suspension the signal will reach a maximum value, in general about 2.2 volts. Where the LIST analysis produces data that starts at a baseline value, climbs up when particles pass the detector, then drops back down to a baseline value once again after the particles have passed by,

the HOST analysis data begins at a low signal value referred to as the lower baseline and only climbs up over time to what is referred to as the upper baseline value.
Because the HOST analysis produces raw data in the cumulative form (starts from 0% and goes to 100%) it must be differentiated to produce a differential curve. Determining a differential curve from a cumulative can misleading as subtle changes in the inflection of the cumulative curve may cause false peaks to be calculated in the differential curve. Thus the HOST technique is intrinsically lower in resolution then the LIST technique.

When the HOST technique is most appropriate:

Generally there are three cases under which the HOST analysis is to be the preferred technique of particle sizing.

The first case in which the DCPH analysis is to be used is for very broad samples, or samples with low densities and small diameters. If a distribution is narrow it is best to analyze the particles using the LIST technique as the particle band will be narrow and will pass the detector over a short period of time. If, however, the sample is very broad the particles will continue to pass by the detector over a much longer period of time.

The second case is one in which the HOST analysis is not only warranted but necessary. This is when the particles to be analyzed have a density less than that of the suspension fluid in which they are dispersed. Under these conditions it is impossible to make a LIST analysis as the injected particles would not travel through the spin fluid (because they are less dense then the spin fluid they feel forces pushing them to the center of the disc). Users with particles of a density of less than 1 g/cc that are to be dispersed in an aqueous solutions must use this technique.

The final case in which a user would be inclined to use the HOST technique is when the particles in question are suspended in a solvent that does not lend itself to the LIST analysis. An example of this is carbon black or organic dyes suspended in MEK, ceramics suspended in phenol, and other exotic systems. In these cases it is best to use the HOST analysis because of its ease of application. In the case of the LIST analysis many times the addition of the gradient fluid (at 25% by volume) into the injection fluid containing the particles and the spin fluid (75% by volume) can cause the particles to flocculate. For this reason the HOST analysis is best suited for the particle size analysis as the particles need only be suspended in the spin fluid. The gradient fluid is pushed through the spin fluid but remains isolated from interacting with the particles. This will be discussed in greater detail throughout this chapter.

5.1.1 Positive delta Rho conditions

As mentioned above the positive delta Rho (PDR) analysis is best for broad samples and samples that are denser than the spin fluid that the particles are suspended in. An example of this would be polystyrene (density=1.05 g/cc) in water (density 0.998 g/cc)[1]. Under these conditions the particles spinning in the BI-DCP measurement disc will move radially outward under the influence of centrifugal force.

Below is a typical raw data curve which was produced analyzing a mixture of 5 NIST traceable standards. As is clear from the graph, the turbitity of the suspension is at its greatest level at the onset of the analysis (time=0 seconds, signal =0.9 volts). Once all of one particular group of particles, the 720 nm standard for example, has passed by the detector head it can no longer contribute to the turbitity of the system. This is how the software makes the calculation of particle size from the raw data. When the turbitity of the suspension changes rapidly over time it indicates the passage of the last group of any set of particles.

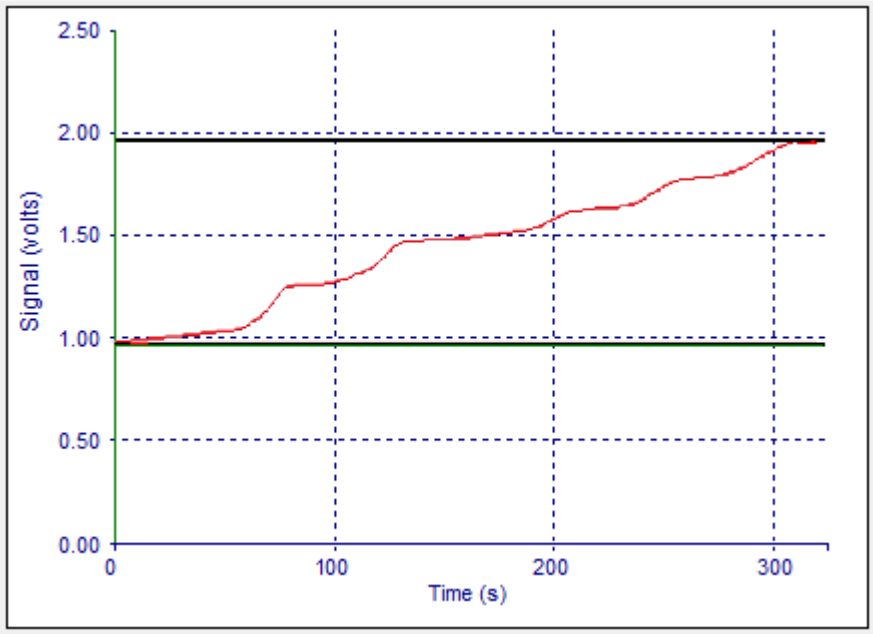


Figure 5.1
Changes in turbitity Vs.time
HOST analysis, positive delta Rho conditions

Note that at the onset of the analysis there is a rise in the signal voltage. Even though no group of particles has completely left the suspension from zero through one minutes the signal rose 0.1 volts. The reason for this is

[1] The density of water is 0.998 g/cc at 20 degrees C.

because the suspension concentrates radially at the onset of the analysis. If the particles were centrifuging in the opposite direction (as they would under negative delta Rho conditions) the signal would have dropped to a lower value over the same period. Radial dilution and concentration are discussed in greater detail in section 5.4.3.

5.1.2 Negative delta Rho conditions

Many times users have been required to measure the particle size of materials having a density less than that of the spin fluid in which they are suspended in.

Because the rate of centrifugation is dependent upon the differences (delta) between the density of the particle and the fluid in which they are traveling through, the user may wish to use in place of water a more dense fluid, such as 40wt% sucrose, which has a density of 1.18 g/cc and thus makes for a larger delta Rho. There is always a tradeoff, however, because another factor that determines how fast the particles move through the centrifugal field is the viscosity of the spin fluid. As you increase the density of the fluid by adding, say, sucrose, the viscosity is also increased. Thus the particles are inclined to move faster because of the larger delta Rho but are slowed down by the increased viscosity of the solution.

5.1.2.1 Forced negative delta Rho analysis

The user may determine that it is more efficient to run their samples under the negative, rather than positive, delta Rho conditions as the density of the particles may in fact be only slightly greater than that of the spin fluid. In these cases the user generally has particles having a density of 1.01 to 1.07 grams per cubic centimeter.

Because the rate of centrifugation is dependent upon the difference in density between that of the particles and the spin fluid the user may find, using the modeling utility, that a particle of a given low density and size will take longer to analyze under positive delta Rho conditions than under negative delta Rho conditions. This issue is used below for an example of using the BI-DCPH modeling utility.

5.2 Using the BI-DCPH modeling utility

Below is an example taken directly from the modeling utility in the BI-DCPH program. For this example we will investigate the differences in run time when analyzing a particle that has a density close to that of water (ρ=1.02

g/cc). The modeling is carried out under both positive and negative delta rho conditions.

To use the modeling utility the user simply presses the "Modeling Utility" from the main screen. Then the user enters into the parameter page all of the pertinent information concerning the sample and the selected spin fluid (density, viscosity, etc..). First the user makes a guess of the run time and disc speed, enters those values into the proper fields, and presses "Calculate Size Range" button. The software will automatically calculate the particle size window over which the analysis conditions will be able to cover.

In general the user will first set up the conditions necessary to measure the largest particles in the suspension. Then the user will advance the time of the run to accommodate the smaller particles which will reach the detector last.

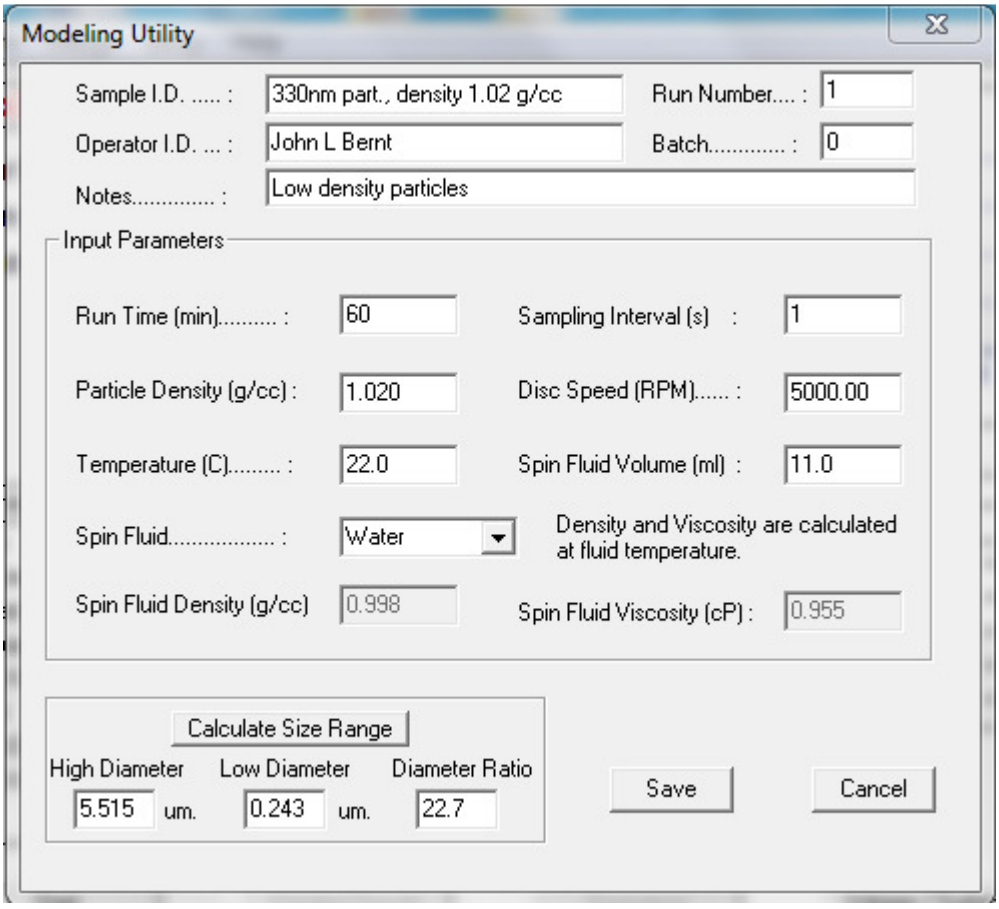


Figure 5.2
Modeling A Particle Size Analysis Under Positive Delta Rho Conditions

What the modeling utility tells us is that given a material with a density of 1.02 g/cc the instrument can analyze over a size range of 5.52 um down to 243 nm in 60 minutes, or one hour (at 22 degrees C, in water, with 11 ml

spin fluid, and spinning the disc at 5000 RPM). In other words, if the user had a suspension of particles having a diameter of 330 nm +/- 50 nm, under these conditions the user would require one hour to make the analysis.

Now let's take a look at the same sample modeled under negative delta Rho conditions. In this case we will drive the density of the spin fluid to 1.1 g/cc by adding 24 grams of sucrose into 76 grams (ml) of water, or 24wt% sucrose. Under these conditions the particles will travel faster, but in the opposite direction.

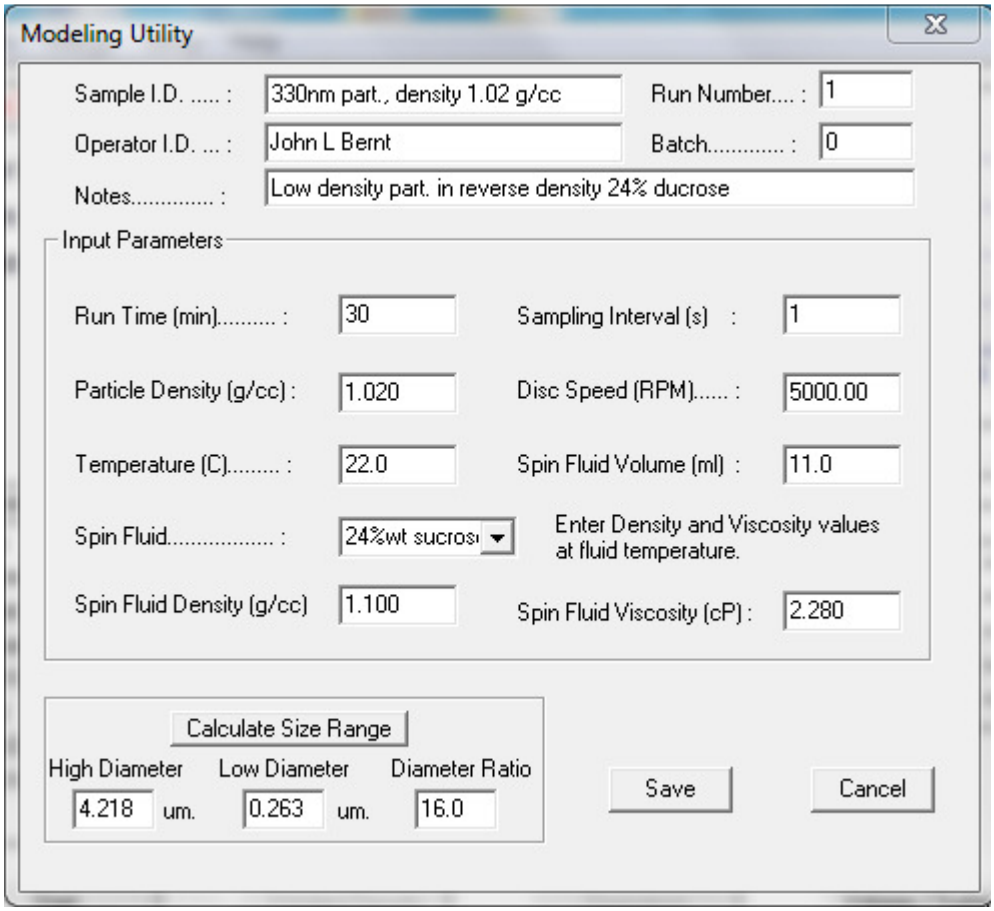


Figure 5.3
Modeling a 1.02 g/cc material under negative delta Rho conditions

As is evident from the above modeling to analyze the same 330 nm particle, changing only the spin fluid from water to 24wt% sucrose, the analysis has been decreased in time from one hour to 30 minutes. Even though the viscosity of the spin fluid, 2.28 cP, is considerably larger than the water, 0.9548 cP, the larger delta Rho still has the end result of driving the particles faster through the spin fluid. This is a very useful technique for materials having a density very close to that of water.

5.3 Sample preparation

Several steps are required in making a particle size analysis on the BI-DCP. The procedures are, in general, the same for both HOST and LIST techniques. Below the steps for selecting the spin fluid, the buffer fluid, and determining the optimum sample concentrations. Please refer to chapter 4, section one in this manual for additional details on the selection of the spin and buffer fluids.

5.3.1 Selection of the spin fluid

It is always recommended to use the modeling utility in the BI-DCPH software to help determine the optimal conditions under which to run the analysis are, for either positive or negative delta Rho analyses. In general users tries to minimize the run time while maximizing the resolution of the analysis. The modeling utility is capable of determining the most optimum conditions under which to suspend the particles because it supplies the user with the size range over which he may make the analysis (see above).

As with the LIST technique it is also necessary to use a density gradient in the HOST analysis. The host system is as sensitive to streaming as the LIST analysis. The user is strongly advised to review chapter 4 of this manual which contains information on selecting spin and buffer fluid pairs. In addition to the selection of the suspending fluids the user is further advised to review the section on selection of a ideal run speed, as the same principles apply to the HOST analysis as they do the LIST analysis, although the use of a scanning head may allow the user to slow the disc down somewhat.

5.3.2 Determination of the optimum injection concentration

In determining the optimum injection concentration several different variables must be taken into consideration. The optimum injection concentration is determined by the broadness of the sample, the size of the sample being measured, and the opacity of the sample when suspended in the spin fluid.

Terms:

Dilute Suspension	A suspension registering a voltage differential of 0.2 - 0.5 volts on the BI-DCP.
Moderate Suspension	A suspension registering a voltage differential of 0.5 - 1.25 volts on the BI-DCP.
Concentrated Suspension	A suspension registering a voltage differential of 1.25 - 2.2 volts on the BI-DCP.

Users of the BI-DCP are encouraged to experiment with their specific samples to help them in determining the voltage differential associated with different concentrations of the samples. Never concentrate the samples to the point that a voltage of 0.0 is registered on the front panel of the BI-DCP at the onset of the analysis. This means that your suspension is too concentrated to allow any light to pass through the disc, thus changes in voltage due to the changing of the turbitity of the sample cannot be measured.

In general the following rules are to be applied in preparing a suspension for a BI-DCPH analysis:

Optimum concentrations and conditions for various types of samples run in the BI-DCPH analysis mode

- If a sample is very broad (polydisperse) the turbitity of the suspension should be maximized. Furthermore the head should be allowed to scan.
- If the sample has a narrow distribution and ranges from 1000 to 500 nm the concentration of the suspension should be moderate.
- If the sample is bi-modal (having two modes/populations) and the peaks are separated by at least 5X the size of the smaller peak (example: peak 1 @ 100 nm and peak 2 @ 500 nm) the user should make the moderately concentrate the sample.
- If the sample has a very small diameter and a low density the user should minimize the concentration or think about a negative Rho measurement.

5.4 The HOST internal gradient method

In conducting a DCP HOST analysis the user must, as with the line start analysis, create a stable density gradient throughout the spin fluid in the measurement disc. The gradient is made in the same way that it is for the line start analysis.

The general order of operation for the creation of a stable density gradient is listed on the following page. Please note that a table of compatible spin fluid/buffer fluid pairs can be found in chapter 4, section 1 of this manual.

Special considerations must be taken into account when the user is making a negative delta Rho analysis (an analysis in which the density of the particles being measured is less than the density of the fluid in which the particles are moving through). Details concerning this type of analysis are discussed further in section 5.6.

General order of operation for the injection and analysis of HOST samples:

1) Select a sample to be analyzed.
2) Select a spin fluid to disperse the sample to be analyzed in.
3) Select a buffer fluid pair to be used with the spin fluid.
4) Dilute the sample into the spin fluid. In general the turbitity of the suspension should be made so that a signal differential measured on the BI-DCP is between 0.5 (for narrow samples) and 1.5 volts (for broad samples).
5) Determine the optimum speed and run time necessary to cover the particle size range over which you believe your suspension to cover, be sure to add +/- 20% if you're not sure. Press Modeling Utility setup page to send the parameters to the Sample Run menu.
6) From the main screen press Sample Run button to enter the parameters.
7) Prepare the BI-DCP to accept data by pressing OK on the Sample Run menu.
8) Press the start button on the main screen. This will cause the instrument to send the run parameters you have selected to the BI-DCP and will set the software package up to collect data.
9) Prepare the syringes for injection. First draw 0.1 ml of 100% dodecane into a glass syringe using a small gauge needle. Set this syringe aside. Next, draw into a 1 ml syringe 0.2 ml of the buffer that was selected. Place this syringe into the injection port of the BI-DCP *but do not start the disc spinning.* Lastly draw up into a 20-30 ml glass syringe the volume of the suspension that was determined from the modeling utility to be the optimum amount for the analysis.
10) Inject into the dry, non-spinning disc, the 0.2 ml of buffer fluid. Immediately place the needle of the glass syringe containing the sample suspension into the injection port on the BI-DCP. Make sure to hold the

syringe plunger with the side of your finger to ensure that no fluid leaks out of the syringe.

11) Press MOTOR on the front panel of the BI-DCP and immediately start injecting into the (now rotating) measurement disc. Make sure to make the rate of injection smooth. The total time to inject your sample into the spinning disc should be around 2 seconds. **Once the fluid has been injected fully into the measurement disc press the START button located on the front panel of the BI-DCP. Do not delay in pressing the START button or the analysis will render incorrect results.**

12) Immediately after pressing the START button on the BI-DCP inject into the measurement disc the 0.1 ml of dodecane. This oil acts as an evaporative shield which prevents cooling and loss of volume of the spin fluid due to the effects of evaporation.

13) Turn on the STROBE by pressing the STROBE button on the front panel of the BI-DCP. Inspect carefully the changes in turbitity observed concentricity inside of the measurement disc. **A proper BI-DCPH analysis will create conditions inside of the measurement disc such that any radial point on the disc will have the same turbitity as any other point which lies at the same radius.** In other words, points that lie on the same concentric ring inside of the measurement disc will have the same turbitity. If this is not the case than the particles are streaming and thus the analysis is not valid.

5.5 Setting the calculation baselines of the analysis

Referring to the raw data graph taken from a typical BI-DCPH analysis below please note the two horizontal lines at 1.97 and 0.9 (1.1 volt signal differential, moderately concentrated). These two "baselines" represent the lower and upper limits of integration over which the raw data "function" will be calculated.

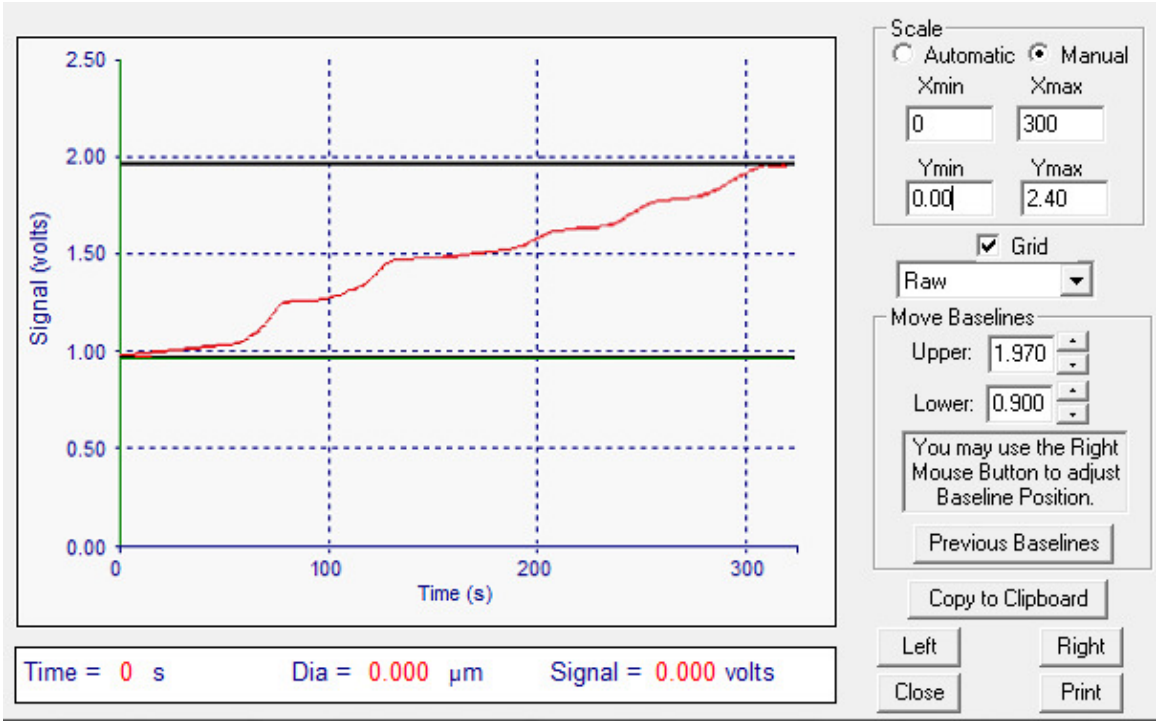


Figure 5.4 The upper and lower baselines of integration

Placement of the upper and lower baselines is a critical step which is taken at the end of every run. The lower baseline, is the voltage level associated with the intensity of light reaching the detector when the sample is most concentrated (or homogenous) is set automatically by the BI-DCP. Sometimes, however, the user may have to set the lower baseline manually as a spike in the signal can cause the artificial placement of it.

The upper baseline is set by first expanding the region at which the raw data curve reaches its maxima (1.97 volts in the above example). This is done by pressing the up button next to the Upper word in the Move Baselines. Alternatively, the voltages can be typed in. Now expand the region of interest by typing into the keyboard the lower and upper limits of the Y axis that places the upper (or lower) baseline directly in the center of the new graph window. In the above example the Y axis should be expanded by setting the Ymin at 0.8 and the Ymax at 2.1. This will expand the graph in this region allowing the user to determine the correct position of the true baseline. Now use the up and down arrows next to the upper (lower) to place the baseline directly at the maxima or minima of the raw data curve. After you have placed the baseline integration points press the close button to continue and view the completed analysis.

Previously analyzed data files can be re-analyzed at a later time at which the user may again place the upper and lower baselines of integration.

5.5.1 Radial dilution and concentration

An expected physical event takes place when a homogeneous suspension begins to centrifugate in a centrifuge disc. This event, known as radial dilution, can cause some concern amongst BI-DCP users if they do not understand its mechanism. Simply put, radial dilution occurs when particles in a given volume begin to concentrate by moving in equal numbers to larger volumes.

Consider a group of mono-dispersed particles having a diameter of, for example, one micron with a density of 1.05 g/cc being suspended in DI water. At the onset of the analysis all of the particles are uniformly dispersed. Keep in mind that the BI-DCP HOST mode analysis measures particle size by correlating the changes in turbidity with the passage of time. Now after 2 minutes, at a disc speed of 5000 RPM, the major body of particles has still not passed by the detector (that will occur at around 3 minutes under these conditions). But during this time all of the particles in the disc are centrifugating nevertheless. While all of the particles are moving to the outside of the disc they are traveling from a smaller volume into a larger volume. Because turbidity is measured as a function of particles per unit volume the turbidity measured by the BI-DCP detector starts to decrease, even though no particles from the 1 micron group have passed the detector.

This event is radial dilution and it always occurs in the BI-DCP HOST analysis mode. If the particles being measured are less dense than the fluid in which they are suspended the opposite will occur, radial concentration. Below is an example of radial concentration. A mixture of 3 NIST traceable standards was used. Notice how the signal decreases until about 2.5 minutes into the analysis.

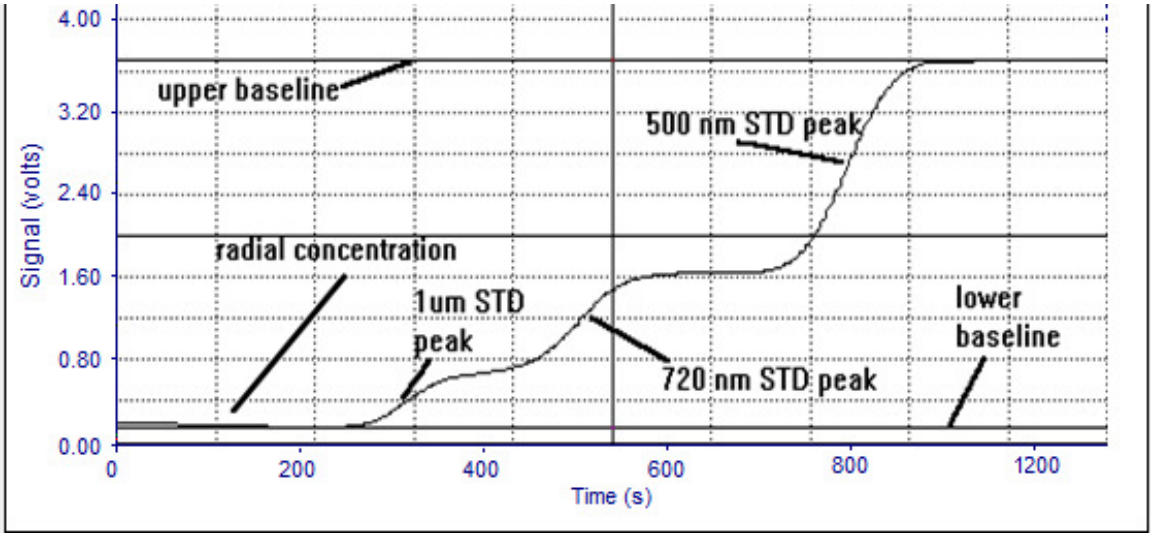


Figure 5.5
Radial concentration, negative delta Rho conditions

Please note the position of the lower baseline placement. It was placed at the minima of the raw data curve. Unless otherwise stated user of the BI-DCP HOST software should always place their baselines as shown above, at the minima of the raw data curve.

5.6 Conducting a BI-DCPH Negative Delta Rho Analysis

As mentioned previously in this chapter the analysis of particles under negative delta rho (NDR) conditions is designed for materials that are 1) less dense than the spin fluid, and 2) particles that have densities close to that of the spin fluid. When making an NDR analysis the user must prepare the gradient in a different fashion when compared to the positive delta rho analysis. Indeed, the density gradient must be inverted as the motion of the particles through the spin fluid will be reversed (as opposed to the positive delta rho analysis).

Additionally a second density gradient may be still be necessary at the meniscus, as with the positive delta rho analysis. This second gradient's usefulness is in 'trapping' the centrifugated particles near the meniscus and keeping them from interacting with the dynamic air/water interface that is present in this region. In other words the purpose of the second gradient is to keep the particles that have reached the end of the fluid column from re-mixing back into the suspension.

The protocol listed below was worked out for fat and liposome emulsions suspended in an aqueous medium. Non-aqueous suspensions can also be analyzed on the BI-DCP Host platform but the protocols can be rather specific and describing them here in a general manual is not entirely appropriate.

For aqueous liposome and fat suspensions:

Prepare a 20 ml solution of a 1:1 mixture of Ethylene Glycol and water. Inject 1 ml of this solution into the dry, non-spinning disc. Disperse your low density sample into 100 ml of DI water. Draw 0.2 ml of 100% dodecane into a glass 2 ml syringe. Draw 15 ml of the suspension fluid up into a 20 to 25 ml glass syringe. Remove all air bubbles from the syringe and the needle. Now carefully draw up into the syringe 1.0 ml of 100% MeOH. Keep the syringe upright, it is of the utmost importance that you do not allow the MeOH and the suspension fluid to mix in the syringe. Refer to 4.6 and 5.5 of this manual for details on the proper procedure for injecting spin fluids into the BI-DCP.

Now press the MOTOR button to start the disc spinning. Quickly place the needle containing the suspension fluid/MeOH into the injection port in the BI-DCP and smoothly but firmly inject the fluid into the disc. Press the start button and then inject into the disc the 0.2 ml of Dodecane.
This technique makes an ***inverted gradient***. It is necessary for the particles that are being centrifugated to have present a density gradient through the spin fluid. Because the particles begin at the outside edge of the disc and travel inward (this situation is referred to as a negative delta-rho condition, or NDR) it is necessary to have that density gradient be more dense than the particle, as opposed to when the particles are more dense than the spin fluid and gradient fluid is less dense (PVC and MeOH, for example).

It is still necessary to have the MeOH present on the inside (nearest the center) of the disc. This is so that when the less dense particles travel towards the meniscus they will be kept from reaching it. The particles, having a density between that of the spin fluid and the MeOH, will come to rest in a region of the gradient where the particle and spin fluid densities are the same. At the meniscus itself less hydrodynamically stable conditions are present than the region that the particles will now, with the MeOH, come to rest in into. Holding the particles away from the meniscus keeps them from entering this dynamic region of the spin fluid and re-mixing back into the column.

The fixed head analysis is used for making measurements such as these because with the dual gradients present in the disc optical regions due to the mixing of the gradients must be avoided. If you scan the head you will increase your chances of entering into one of those regions. Incidentally you may actually set the distance that the detector fixes its position to from the meniscus.

Because the BI-DCP H software knows the density of both the spin fluid and the particle it can easily identify a NDR condition. When the instrument is set to do an NDR analysis it will move the detector head to the *inside* of the disc, towards the meniscus. This is because the particles are going to travel from the outside to the center. The detector being in the inside position is situated to detect the largest particles present in the suspension. Therefore it must move the inside of the disc so that the greatest column height is available for the larger particles to centrifugate through.

The last particles to be measured in any NDR analysis start at the outside of the disc and travel inwards through the spin fluid column towards the center of the disc. For an NDR measurement the most important distance the particles must travel is from the outside radius of the disc cavity to the detector, not the position of the meniscus[2]. Thus if the user of the BI-DCP (in homogeneous mode)

Once the particles have passed the detector is doesn't matter *how* far they travel, they are only detected once. Thus if the user has difficulties with the gradient affecting the signal he or she may simply instruct the BI-DCP software that there is in fact 10 ml in the disc, and not 15---EVEN IF THERE REALLY IS 15 ML IN THE DISC. The DCP will move the detector head to a location X steps from the position of the *calculated* meniscus. If the user says there is only 10 ml in the disc it will move the detector to 10 ml minus X steps, so now the gradient effects which were occurring at- for example 13 ml, are now pushed outside of the measurement window.

The only drawback using the reduced volume technique is that the measurement range over which the particles will fall through will be shorter, thus you will be restricting the analysis to smaller size ranges. A shorter column height is ideal for the analysis of smaller particles (< 500 nm) or mono-disperse distributions. If the reduced volume method must be used for broad samples the user may have to extend the run time to accommodate all of the samples particles.

5.7 Troubleshooting the BI-DCP HOST analysis

[2] This statement is only valid if the column of spin fluid has enough volume to create a column that extends to or beyond the detector head. Clearly if the column lies below the detector head the particles will never be measured.

As the BI-DCP HOST analysis is very similar to the LIST analysis the reader is encouraged to read chapter 4 section 6 of this manual for immediate troubleshooting questions.

Problems specific to the BI-DCP HOST analysis are listed below, along with common solutions:

Problem	Solution
The raw data signal suddenly drops off and then rises again.	1) Check the alignment of the BI-DCP to ensure that the detector is not scanning over the meniscus. 2) The sample is too concentrated and the refractive index differences between the sample and the spin fluid are bending the detectors light away. Reduce the concentration of the particles.
Particles stream or go back into the measurement region after already having been detected	1) Make sure that you use 0.1-0.2 ml of 100% dodecane over the surface of the spin fluid to stabilize the conditions. 2) Increase the run speed, thus supplying greater centrifugal forces to the particles.
'Bumps' occur in the raw data	1) Make sure the disc is clean, both inside and out. 2) Used a fixed detector head 3) Use the Fast Scan Analysis Mode

Additional questions may be addressed to info@brookhaveninstruments.com or by phone at +1-631-758-3200.

Chapter 6 Light Scattering Correction Files

6.1 Making A Light Scattering Correction File

Installing the Software

All instructions are given for a computer with **DOS 5.0** or higher, BI-DCP software installed on the **C:** drive located in the subdirectory **C:\DCP**, and using the **B:** drive for the floppy. If you are using a earlier version of DOS, or another operating system, refer to the system manual on editing existing files.

- Insert the disk labeled **DCP-SCATT.EXE** into your floppy drive.
- Copy the complete contents of the disk to your DCP **SCATT** sub-directory by typing ***copy b:*.* c:\dcp\scatt***.
- Change from drive **a:** to drive **c:** by typing ***c:***.
- Change to the DCP SCATT directory by typing ***cd dcp\scatt***.
- Make sure the **DCP-SCATT.EXE** file is in your SCATT subdirectory by typing ***dir dcp-scatt.exe***. The computer should respond by listing your file. If the file was not copied the computer will respond ***file not found***.

6.1.1 Creating a Scattering Correction File

Make A PRM File

1. Edit the existing **PRM** template. If your BI-DCP uses a Tungsten-Halogen light source type ***edit tungsten.prm***. If the light source is an LED type ***edit led.prm***. The DOS editor should appear with the **PRM** file on the screen. Replace the line ***Put your sample information here*** with a brief description of the sample you are creating the scattering file for.

   Example: If your sample is PMMA and it is being analyzed through using a 5% sucrose solution as the spin fluid, you would type ***PMMA in 5% Sucrose***.

2. Enter the refractive index of the spin fluid into the template. In the column labeled **n(med)** enter the refractive index (R.I.) of the spin fluid in place of the set of zeros. Copy the format *exactly* as you see it on the screen. Use typeover mode (insert off) and fill in the R.I. to the same number of digits that appear on the template. For LED's you need only enter the R.I. at 700 nm. For Tungsten-Halogen sources you must determine the R.I. for each of the wavelengths from 400 to 1100. If R.I. information is available for only a specific wavelength use it in all of the rows. It is better to use a close estimation than none at all.

   Example (from previous page): For 5% Sucrose you would type (in the 700 nm row) ***1.3703*** in place of **0.0000**.

3. Enter the R.I. for the sample in the **n(re)** column as you did for the spin fluid.

Example: For PMMA you would type (in the 700 nm row) ***1.4847*** in place of **0.0000**.

4. Save the file using the name of the sample with the **PRM** suffix. Use the mouse to select **file** at the top of the DOS editor screen. If you don't have a mouse type ***Alt***, ***F*** at the same time. Select **save file as** by typing the letter **a** and ***Enter*** or select it using the mouse. When asked what to name the file, use the name of the sample.

Example: If you are making a **PRM** file for PMMA in 5% sucrose type ***PMMA5SUC.PRM*** in the *name* and press ***Enter***.

Note: ***DO NOT*** save the file using the original name or your template will be altered.

Using the DCP-SCATT program to make a .SCT file from a PRM file

5 From the DOS prompt type ***dcp-scatt***.

6 When asked enter the name of the **PRM** file (without the **PRM** extension).

Example: If the name of the **PRM** file is **PMMA5SUC.PRM**, you would type ***PMMA5SUC,*** and press ***Enter***.

7. The **PRM** file should now appear on the screen. Inspect it to ensure that the information is correct (same number of digits, no extra spaces, etc.). Press ***Enter***.

8. The DCP-SCATT.EXE program will now process the **PRM** file and create a **SCT** file with the same prefix. The program will display confirmation of the newly created **SCT** file upon completion.

6.1.2 Insert the New SCT File Information into the SCATT.NDX Index File

1. Type ***edit SCATT.NDX***.

2. Insert information concerning your **SCT** file in the appropriate position in the index. Make sure to use the notation **"number on list","name of SCT file","name of file as you wish it to appear on the index list"** Note: The name as it appears in the index list can be a maximum of 28 characters, including spaces. Remember to exclude the **SCT** file extension when entering in the **"name of SCT file"**.

   Example: In the DOS editor you see the following list;

   20,.....
   25,"PMMA","PMMA / Water"
   27,"PMMA-SUC","PMMA / 10% Sucrose"
   28,.....

   And you wish to insert information about the PMMA in 5% Sucrose file (**PMMA5SUC.SCT**). Place your cursor on the number 27 in the list and press return. This will open a line up on the list. Now type in the open space;

   26,"PMMA5SUC","PMMA / 5% Sucrose".

   The scattering correction will appear as **# 26** on the list of scattering files an will have the name **PMMA / 5% Sucrose**.

3. Save the **SCATT.NDX** file with the new additions. Type **Alt, F**, **X** in that order to save the **SCATT.NDX** file with the new modifications.

4. Type ***DCP*** or ***DCPH***. Go directly to the **Standard Sample Run *(F1)***. When asked to enter a scattering correction you should see your new scattering correction file listed in the pop-up scattering correction index. In either case it may be necessary to use ***PgUp*** or ***PgDn*** to see a listing of your SCATT file, depending upon its position in the index.

6.1.3 Additional Information on the Creation of the Light Scattering Correction File

We suggest you follow the code number scheme that already exists. That is, if you add a new SCT file to a family of SCT files that already exists, then use a similar code number. For example, several carbon black corrections exist between code numbers 40 and 50.

Remember, the **PRM** file name you enter into the DCP-SCATT.EXE program must agree exactly with the **PRM** file name you created previously. Do not use the extension SCT when naming the file. We also suggest you follow the scheme already established for the on-screen labels, namely: the name of the particle, slash (/), the name of the liquid. For example, "PVAC / 5% Sucrose" follows this scheme.

If the **PRM** file was not created properly, the program, DCP-SCATT.EXE, will not execute properly. In this case return to the **PRM** file using the DOS editor. Look for the difference between the new .PRM file and one of the template **PRM** files. Typically, a line is missing or not all the digits are expressed.

The time to calculate the resulting SCT file (for example, PMMA.SCT) depends on the computer speed and whether you have the LED or Tungsten-Halogen source. The fastest time (486 or 386/387 with LED source) is just a few minutes. The slowest time (286/287 with Tungsten-Halogen source) is 30 minutes. During calculation wavelength and particle size appears on the screen as an indication of how much longer you have to wait.

The refractive index for most particles is not known at exactly the wavelengths of interest. Do not worry. Take whatever information you have. The wavelength dependence is also not known in most cases. Then use the value that is known. It is better to make a correction than none at all, even without complete wavelength information. If the material is colored, it probably absorbs in the visible. If you have no value for the imaginary part of the particles refractive index (absorption), try 0.1 as a reasonable guess. Remember: the refractive index for water solutions of sucrose, ethylene glycol and glycerol are not the same as for pure water. These values are easily found in reference books.

Chapter 7 Adjustment of the BI-DCP baseline

7.1 Acceptable BI-DCP baseline values

The BI-DCP measures particle size by correlating the change in optical turbitity of the suspensions within the disc verses time. The turbitity changes are registered differentially when making the LIST analysis, and in a cumulative fashion when making the HOST analysis. Both analyses require for the calculation of particle distribution a baseline value from which to compare changing turbitity (signal voltage) against. This baseline value is set at Brookhaven Instruments to be 30 mV +/- 10 mV when pure DI water is spun in a new PMMA disc in the BI-DCP software package. The BI-DCPH software package calculates its signal differently from the BI-DCP software so please be careful to use the correct software package. The baseline values are higher for Homalite discs, around 0.6 volts, because they are more optically opaque.

7.2 Adjustment Of The Baseline

The value of the baseline can drift to higher values over time. A high baseline value does not make the data collected on the BI-DCP incorrect. If the baseline values is too high, however, the concentration range over which the instrument can operate becomes limited.

Below is a list of the most common causes responsible for a rising baseline.

- The measurement disc has become clouded. This can be caused by oils and or samples adhered to the surface of the disc. Sometimes users accidentally allow the disc to come into contact with aggressive solvents which damage the disc.
- There is blockage in the emitter/detector optical system in the measurement head. This can cause the baseline value to fluctuate as the material in the optical slit may move during the analysis.
- The electrical resistance in the emitter/detector circuit, internal to the BI-DCP's circuitry, has changed. In this case the user or factory will adjust the baseline manually by adjusting an internal variable resister.

In general the accepted baseline values for the BI-DCP fall between 0.1 volt and 0.4 volts. This value is obtained, as mentioned above, by placing 15 ml of DI filtered water into the measurement disc and allowing the disc to spin at 1000 RPM.

If the baseline value is found to be too high the user is encouraged to attempt the following;

- Clean the measurement disc. The measurement disc may have been contaminated beyond the point that the user can clean it out. If this is so, the only recourse left to the user is to either send the disc back to BIC for re-surfacing or simply buy a replacement disc.
- Check the emitter/detector system in the measurement head. Retract the head, remove the measurement disc, and visually inspect the optical system. It should be free of any obstructions. The beam of light which travels to the detector slit should be uniform. If any obstruction was found carefully brush its surface with a new Q-Tip cotton swab or a section of lens paper.
- If the disc is clean and the detector head is found to be free of obstructions the cause of the increased baseline voltage may be in increase in the internal impedance of the BI-DCP circuitry. If this is the case the user is encouraged to follow the instructions for manually adjusting the baseline found in Appendix C of this manual.

Appendix

Contents

A Chemical compatibility charts for the BI-DCP PMMA and Homalite measurement discs

Please note: This chart is intended as a general guide only. Test the chemicals in questions with a sample of the disc material prior to conducting the analysis should the user be unsure as to its effects.

Chemical @20 degrees C	PMMA Disc	Homalite Disc (Polycarbonate)
Acids, weak or dilute	G	E
Acids, strong or concentrated	N	N
Alcohols, aliphatic	N	G
Aldehydes	G	F
Bases	F	N
Esters	N	N
Hydrocarbons, aliphatic	G	F
Hydrocarbons, aromatic	N	N
Hydrocarbons, Ketones	N	N
Ketones	N	N
Oxidizing agents, strong	N	N

Key

E- No damage after 30 days constant exposure.
G-Little or no damage after 30 days of constant exposure.
F-Some effect after 7 days of constant exposure
N-Not recommended, immediate damage may occur.

Note: A more complete chart may be found in the Cole-Parmer Instrument Company catalog as well as many other chemical and laboratory supply catalogs.

B Commonly used viscosity tables

The tables that follow pertain to:
1) various concentrations of sucrose solutions;
2) various concentrations of Ethylene Glycol and water solutions;
3) various concentrations of Glycerol solutions.

For further solutions and chemical the user is encouraged to reference the CRC Chemistry and Physics Handbook.

C Reader response fax-back page

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Please make a copy of the following page and complete it accordingly.

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List of figures found in this manual

Figure Description

Chapter Four, The LIST Analysis

Chapter Five, The HOST Analysis