



**Brookhaven
Instruments**

BI-DCP User's Manual

Disc Centrifuge Particle Size Analyzer

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About this manual

This manual covers the operation, care, and analytical methods of the Brookhaven Instruments BI-DCP disc centrifuge. It is intended to be used alongside the instrument documentation shipped with your unit. Where the two disagree, the methods given here take precedence: they reflect later development work and supersede the procedures described in earlier manuals.

How the manual is organized

Chapters 2 and 3 cover care, maintenance, and detector alignment. Chapter 4 covers the line start (LIST) analysis. Chapter 5 covers the homogeneous (HOST) analysis, including negative delta rho work. Chapters 6 and 7 cover light scattering correction files and baseline adjustment. Reference tables are collected in the appendices.

The injection techniques described here are straightforward. Once you have mastered the internal gradient technique for the LIST analysis in section 4.5, you will also have mastered the injection technique used for the HOST analysis.

Reporting errors and suggestions

Please report any section you find confusing, ambiguous, or incorrect. Send comments to service@brookhaveninstruments.com or call +1 (631) 758-3200. The fax-back form in Appendix C may also be used.

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CHAPTER 1

Introduction

What the BI-DCP measures, and what this manual assumes you already have.

The BI-DCP determines particle size by centrifugal sedimentation. Particles suspended in a spin fluid are driven outward in a rotating optical disc and pass a fixed or scanning photodetector. The instrument records the change in transmitted light intensity against time and converts that record into a particle size distribution using Stokes' law.

Four quantities must be known before a size can be calculated: the density of the particle, the density of the spin fluid, the viscosity of the spin fluid, and the distance the particles travel through the spin fluid to reach the detector. The first three are entered by the operator. The fourth is fixed by the alignment of the detector head, which is why Chapter 3 matters as much as the analytical chapters that follow it.

The two measurement modes

The instrument supports two distinct analyses. In the line start (LIST) analysis, a small volume of concentrated suspension is injected onto the surface of a spin fluid column carrying a density gradient. All particles begin at the same radial position and separate as they sediment, so the raw data is differential.

In the homogeneous (HOST) analysis, the particles are dispersed uniformly through the whole spin fluid volume before the run begins. The suspension clears from the inside outward and the raw data is cumulative. LIST gives higher resolution; HOST handles broad distributions, low density particles, and solvent systems that would flocculate under LIST conditions.

Software versions covered by this manual

Two separate Win32 applications drive the instrument, one per measurement mode. Both are at their final stable release; no further versions are planned, and the version numbers below are the terminal releases for each application.

Installer	Mode	Version	Release date
dcplwSetup_v5.92.exe	LIST, line start	5.92	20 December 2019
dcphwSetup_v5.84.exe	HOST, homogeneous	5.84	31 May 2016

USE THE PACKAGE THAT MATCHES THE MODE

The LIST and HOST packages calculate the signal differently and are not interchangeable.

Chapter 6 describes the earlier DOS scattering correction utility, DCP-SCATT.EXE. Its PRM and SCT file formats and the SCATT.NDX index are carried forward by both Windows packages, so correction files built under DOS remain usable.

Before you begin

- Confirm the measurement disc material is compatible with your spin fluid. See Appendix A.
- Confirm the instrument is aligned and reads a NIST traceable standard correctly. See Chapter 3.
- Confirm the instrument baseline sits within the accepted range. See Chapter 7.

CHAPTER 2

Maintenance and handling

Disc handling, cleaning, and the routine maintenance schedule.

The BI-DCP is a durable instrument that is equally at home on a production floor or in a laboratory. Following the handling procedures in this chapter will give many years of service.

2.1 Special handling concerns of the measurement disc

The measurement disc is made of either PMMA or polycarbonate. PMMA is suitable for non-aggressive solvents, weak acid and base solutions, and other non-aggressive aqueous systems. The polycarbonate disc (trade name Homalite) is suitable for aggressive solvents, ketones, aldehydes, and strong acid and base solutions. Check the compatibility table in Appendix A before using any unusual solvent or solution.

CAUTION

Never use chlorinated solvents with a PMMA disc. They will degrade it immediately.

Handle the disc carefully to avoid scratching its surface. When a disc is not on the instrument, either wrap it in protective material and store it safely or, for short periods, set it down hub-down with the measurement face pointing upward. Each disc is individually balanced and tested. Discs are replaceable but expensive to manufacture.

Optical integrity matters most for HOST scanning head measurements, where a scratch travelling under the detector can be read as a change in turbidity and recorded as false data. The LIST analysis is less sensitive because the head is held fixed relative to the disc, so surface defects contribute only to a constant background. A disc that becomes badly scratched or soiled will still raise the instrument baseline and reduce the concentration range available to you. See section 4.6.

2.2 Attaching and removing the measurement disc

The BI-DCP ships with the disc removed to protect it in transit. Inspect a new disc for surface defects before fitting it and report any defect to Brookhaven Instruments immediately.

DO NOT REMOVE THE BALANCE MATERIAL

Sections of the disc circumference may have been drilled out, and small amounts of gray epoxy may be present on the outer rim. Both are balancing corrections. Leave them in place.

Attaching the disc

1. Turn the BI-DCP on.

2. Inspect the disc and confirm both hub screws are present and backed off far enough that they do not protrude into the keyway slot inside the hub.
3. Press CUT on the front panel to bring up the selection screen on the LCD.
4. Press 1 to retract the measurement head.
5. Inspect the end of the motor spindle. The notch running through it is the keyway; it holds a rectangular metal key. The key bridges the spindle keyway and an identical keyway cut into the disc hub, and is what keeps the disc from slipping during a run. Two lock-down screws over the disc keyway secure the key and lock the disc to the spindle.
6. Rotate the spindle so the keyway points straight up and seat the key in it. The key must be fully inserted, with its tip flush with or slightly proud of the end of the spindle.
7. Open the small square door above the disc chamber. Look down through the opening and locate the spindle, the key, and the keyway. Reach in with the supplied Allen wrench and hold the key in place.
8. Pick up the disc by its face so the hub projects toward the spindle. Rotate the disc so its internal keyway lines up with the key already seated in the spindle.
9. Holding the key with the wrench so it cannot back out, push the disc onto the spindle. Watch through the top opening and confirm the key stays seated. A key that slides back into the spindle keyway does not do its job, and the disc can spin free.
10. Press the disc firmly against the spindle and tighten both lock-down screws on the hub with the Allen wrench until they will not turn further. Loose screws can back out during a measurement.

Confirming the disc is properly attached

- The key is securely captured in both the spindle and the disc keyways.
- The disc hub is pressed firmly against the end of the spindle. Press on the center of the disc to confirm.
- The measurement head is free to travel across the disc face.
- Both lock-down screws on the hub are firmly tightened onto the key.

As a final check, slide a small piece of paper flat against the forward face of the disc. It should pass easily between the detector head and the disc. If the paper will not pass behind the head, the disc is not fully seated.

STOP

Do not restore the head or start the motor until the disc is fully seated on the spindle. Running a partially seated disc can permanently damage the disc and the instrument.

11. Press CUT to bring the selection screen back up.
12. Press 2 to restore the head to its previously aligned position if you simply removed and cleaned the same disc. Press 3 to realign the system if you have fitted a different disc with different volumetric dimensions.

Removing the disc

- 13.** Turn the BI-DCP on.
- 14.** Press CUT to bring up the selection screen.
- 15.** Press 1 to retract the measurement head.
- 16.** Open the square door above the disc chamber and locate the spindle, key, and keyway.
- 17.** Reach in and rotate the disc until the two hub lock-down screws point straight up.
- 18.** With the Allen wrench, loosen both screws until they turn freely. Do not back them all the way out of the hub or they may be lost.
- 19.** Take hold of the disc and pull it off the spindle gently but firmly, then turn it slightly to clear the door. Pulling too hard makes the disc come free suddenly and strike the door enclosure, which can damage both.
- 20.** If the key stayed in the disc keyway, remove it and store it somewhere secure.
- 21.** Wrap and store the disc, or set it down hub-down with the face upward.

2.3 Washing the measurement disc

The easiest way to clean the disc is on the instrument. Inject 16 ml of 2 vol% Micro cleaning solution and press MIX. Allow the solution to mix for three to five minutes, then remove it. Rinse with water in the same way. After the rinse water is removed, spin the disc at 5000 RPM for 15 seconds and stop it, then draw out the remaining water and dry the disc. A folded paper towel turned slowly against the disc picks up the bulk of the water; a syringe fitted with a short length of rubber hose removes what is left.

The disc may also be removed if cleaning it in place proves awkward. Brookhaven Instruments uses specialized cleaning and buffing materials to lift light scratches and remove adhered chemicals. If soap and water will not clean your disc, call us before attempting anything more aggressive.

Severely damaged discs can be returned for resurfacing. The process is costly and leaves you without a disc while it is here, so many users keep a second disc on hand.

2.4 Cleaning the disc chamber

Clean the chamber at the end of each day. The chamber includes the clear Homalite door, the measurement head, the chamber walls, and the overflow catch basin below the disc chamber. The basin catches run-off when more than 17 ml is used in a measurement; the maximum usable volume is 25 ml. Remove the basin by pulling gently on the small circular handle below the front door panel, empty and clean it, and slide it back into place.

Wipe the chamber down with a damp paper towel. Keep soap and water away from the optical components of the measurement head, and take care not to scratch the disc. Clean the disc surface whenever oils, soaps, or sample material have contacted it.

2.5 Cleaning the dust filter

Turn the screw at the rear of the top panel; it loosens without coming out. The top panel then opens to expose the gray dust filter. Lift the filter out carefully so trapped dust does not fall onto the instrument. Shake it clean outdoors or in a fume hood, or blow it out with compressed air, then refit it and re-secure the top panel.

2.6 Routine maintenance

Treat the following as a starting point. Actual intervals depend on how heavily the instrument is used and on the environment it operates in.

Interval	Task
After every analysis	Clean the measurement disc, unless serial injections are being made over the same gradient. See section 4.7.
Daily	Clean the disc and store it dry on the instrument. Empty and clean the catch basin as required. Clean the measurement chamber.
Weekly	Remove the fill tube and clean it in a sonic bath. Check instrument alignment per Chapter 3. Check that the disc hold-down screws in the hub are tight.

CHAPTER 3

Aligning the detector head

Setting and verifying the detector position that all size calculations depend on.

The software calculates particle size from the particle density, the density and viscosity of the spin fluid, the centrifugal field, and the distance the particles travel to reach the detector. This chapter deals with the last of these. The software must know where the detector head is relative to the fluid column, and the head must actually be there. Checking alignment periodically is the only way to be sure both conditions hold.

Every measurement disc ships with a page giving its volumetric dimensions, principally the disc cavity radius and the disc cavity width. From these two values the height of any column formed in the spinning disc can be calculated for a given injected volume. The same page gives the detector head position relative to the center of the disc. That position corresponds to the radial position of the meniscus when 5 ml of water is spun in the disc at 1000 RPM.

3.1 Warm-up period

Unless otherwise stated, allow the BI-DCP five minutes of warm-up before aligning the head. The detector contains an internal heater that holds the electronics at a constant operating temperature, and five minutes is what it needs to stabilize.

3.2 Aligning the BI-DCP manually

This section describes manual alignment. The instrument can also align itself; see section 3.5, and note the limitation described there.

- 22.** If the instrument has not been in use, turn it on and let it warm up for at least five minutes.
- 23.** Open the door and confirm no fluid remains in the disc. Inspect the disc surface for deposits.
- 24.** Inject exactly 5.0 ml of water using a Class A precision pipette. Do not use a syringe.
- 25.** Press CUT to bring up the selection screen, press 3 to enter alignment mode, then press 1 for manual alignment.
- 26.** The disc now spins at 1000 RPM. Record the signal voltage shown on the LCD; it should normally read between 0.2 and 0.7 volts. Confirm the step position reads zero, which is the home position of the detector.
- 27.** With the door open, press and hold CUT. The head travels to the left, toward the center of the disc. Release CUT when the dark shadow cast by the meniscus is close to but still right of the detector slit. The voltage should be about what it was at home and the step number may be near 200. Now approach the slit in single steps, noting voltage and step number as you go. The signal voltage rises as the dark band

nears the slit. Continue until the voltage reaches a maximum, and record that voltage and its step position. A typical maximum is around 2.2 volts near step 230, though systems vary.

FINDING THE MAXIMUM WITHOUT BACKLASH ERROR

You have to pass the maximum to know where it is, but do not hunt back and forth across it. Repeated reversals introduce error from play in the drive screws.

Once you have found the maximum approximately, use BOOST to move the head at least 10 steps to the right, then step left with CUT only and take the step position at which the signal peaks.

- 28.** Subtract 5 from the step position of the maximum. Use BOOST to move to that step position. This is the correct alignment position.
- 29.** Press MOTOR. The instrument leaves alignment mode and stores the alignment step position. The instrument is now aligned.

3.3 Confirming the alignment

Confirm alignment by analyzing a NIST traceable standard. The 0.993 μm polystyrene sphere ($\rho = 1.05 \text{ g/cc}$) is the standard most commonly used. Any sub-micron sphere may be substituted, but the disc speed must then be adjusted to suit it.

3.3.1 Run parameters, sample preparation, and injection order

Set the parameter page up as follows, using the 0.993 μm standard:

Parameter	Setting
Disc speed	5000 RPM
Spin fluid volume	15 ml deionized water
Gradient	0.2 ml 100% methanol
Evaporative shield	0.1 ml 100% dodecane
Temperature	Measure the water temperature before the run and enter that value

Suspension preparation

Add 3 ml of deionized water to a 20 ml vial. Add three drops of the 0.993 μm standard and mix gently by hand until homogeneously dispersed. Add 1 ml of methanol and mix gently again.

Syringe setup

- 2 ml glass syringe with 0.1 ml dodecane, 16 gauge needle.
- 2 ml plastic syringe with 0.2 ml 100% methanol, 16 gauge needle.

- 20 ml glass syringe with 15 ml deionized water.

Injection order

- 30.** Inject 0.2 ml of 100% methanol into the dry, non-spinning disc.
- 31.** Press MOTOR and inject the 15 ml of deionized water.
- 32.** Inject the 0.1 ml of dodecane. Wait five minutes.
- 33.** Inject 0.2 ml of the sample suspension and press START at the same moment.

Interpreting the result

Accepted values for NIST standards are printed on the container the sample came from. For the 0.993 μm standard the accepted value is 993 nm plus or minus 21 nm. If your result falls outside the stated tolerance, work through the checks below before re-aligning.

- Confirm the correct disc parameters are entered in the software.
- Confirm the run conditions on the setup page: fluid volume, temperature, and spin fluid identity. The software calculates density and viscosity for water only. For any other spin fluid you must enter density and viscosity manually.
- Confirm the correct particle density is entered.

If none of these accounts for the error, proceed in order:

- 34.** Repeat the injection directly over the same spin fluid column. Serial injection is covered in section 4.7. If the result repeats, go to step 2.
- 35.** Empty the disc, rebuild the gradient, and inject a fresh sample. If the result repeats, go to step 3.
- 36.** Read section 3.4, re-align the system, and repeat the test. If the result still repeats, contact the manufacturer of the standard to confirm it is correctly labeled and within its shelf life.

3.4 Common alignment errors

The most common error is misidentifying the meniscus shadow produced by 5 ml in the spinning disc. Concentric scratches in the disc can be mistaken for the meniscus. The simplest defense is to turn the room lights off while aligning. Automatic alignment is particularly prone to this failure.

The second most common error is not using exactly 5 ml. Use a Class A precision pipette. If none is available, weigh the aliquot instead: water is close enough to 1 g/cc at room temperature that 5 ml should weigh 5 grams.

3.5 Automatic alignment

To align automatically, empty the disc of all fluid, inject exactly 5.00 ml of deionized water, and press CUT to bring up the selection screen. Press 3, then press START. The instrument aligns the detector head on its own.

Manual alignment is still preferred. Automatic alignment is prone to error whenever the disc is anything less than new, for the reason given in section 3.4.

CHAPTER 4

The LIST analysis

Line start measurements: fluid selection, run conditions, gradient formation, and injection concentration.

4.1 Selecting the spin fluid and buffer fluid pair

A LIST analysis begins with the choice of the fluid the particles will sediment through. This is not a trivial choice; the spin fluid has to satisfy four separate requirements.

Requirement 1: the spin fluid must not dissolve, attack, or alter the particles

If the particle size is to be measured, the particles cannot be allowed to dissolve. The point sounds obvious, but users routinely attempt to suspend materials in solutions that attack them.

Osmotic potential has to be considered as well. When sizing liposomes, match the osmotic potential of the spin fluid to that of the fluid inside the liposomes. Otherwise the liposomes will burst or collapse, depending on which way the imbalance runs, as soon as they enter the suspending medium.

Requirement 2: the spin fluid must have a miscible, less dense partner to act as a buffer

Every LIST analysis uses a spin fluid and a matching buffer fluid. The buffer slows the injected particles so they can settle under Stokes forces alone. On injection the particles pick up a great deal of momentum from striking the back wall of the rapidly spinning disc, and the low viscosity of air lets them reach high speed before they meet the fluid.

Stokes' law, which relates sedimentation rate to particle size and density in a medium of known density and viscosity, is the basis of every size calculation the instrument makes. It holds only if the particles begin their fall from rest. The buffer fluid is what brings them to rest.

The buffer must be miscible with the spin fluid. It is not enough for a less dense fluid to lie on top of the column; it has to blend into the spin fluid and establish a concentration gradient through it. Oil over water will not do, because the two would simply form two distinct layers with no gradient between them.

Some proven pairs:

Spin fluid	Buffer / gradient fluid
Water	Methanol
Water	Ethanol
Sucrose solution, 1 to 50 wt%	Water, or a more dilute sucrose solution

Spin fluid	Buffer / gradient fluid
Glycerol	Water
MEK	Dodecane

This list is a starting point rather than a catalog. Applying the rules above will turn up many other workable pairs.

Requirement 3: the viscosity must bring particles to the detector on a usable timescale

Viscosity is one of the factors setting how quickly particles reach the detector. A viscous spin fluid slows them down.

Timing matters in both directions. Enough time has to pass before the first particles arrive, and the last particles must not take so long that the run becomes impractical.

Arriving too early causes a specific problem. Because sedimentation rate goes as the square of the diameter, the size being measured changes very rapidly at the start of a run. Press START and watch the measured size for the first 20 seconds and the progression looks roughly like this:

Elapsed time (s)	Diameter being measured
1	10 μm
2	8 μm
3	6 μm
4	5 μm
5	4.5 μm
10	4 μm
20	3.5 μm

A well designed analysis therefore needs 15 to 30 seconds of baseline before the measured turbidity begins to change appreciably. There is always a small error between the moment you press START and the moment the computer begins collecting data. If particles arrive too soon, that error lands in the steepest part of the size-versus-time curve and small differences in the determination of time zero produce large errors in the first sizes reported.

This is why fluid selection matters. Large, dense particles need slowing down with a viscous solution such as sucrose so the first and largest of them take long enough to arrive. Run the same sample in water and the largest particles may reach the detector in under 15 seconds, and their reported size will be unreliable.

Requirement 4: the spin fluid must not attack the measurement disc

Compatibility tables for the PMMA and polycarbonate discs are given in Appendix A.

4.2 Determining the optimum run conditions

The time particles take to reach the detector can be varied through several instrument parameters. The most direct route to good conditions is the modeling utility, reached with F7 from the main menu. Changing values in the function key field at the bottom of the screen shows immediately how each change affects arrival time.

MODELING TIP

When modeling an unknown size group, force the modeled polydispersity to 1.0001. The distribution then plots as a single line, effectively a pointer in time space, which makes it easy to read arrival time directly.

Four variables control arrival time, and all four are under your control:

- **Disc speed.** Arrival rate goes as the square of disc speed, so a particle taking 16 minutes at 5000 RPM arrives in 4 minutes at 10,000 RPM. This is the usual adjustment. The operating range is 600 to 15,000 RPM.
- **Delta rho,** the density difference between the particle and the spin fluid. A larger delta rho drives particles faster. Particle density is fixed, so delta rho is manipulated by changing the spin fluid.
- **Spin fluid viscosity.** Arrival time scales with viscosity. If particles arrive too quickly even at low disc speed, move to a more viscous solution.
- **Column height.** Arrival time scales with the height of the spin fluid column, so injecting a larger spin fluid volume makes particles travel farther and takes longer.

4.2.1 Narrow samples

Set conditions so the first particles reach the detector between one and two minutes after START. These conditions give the highest available resolution without spending an unreasonable amount of laboratory time. As a rule, a longer LIST analysis resolves better. If the signal has not returned to its initial baseline within 30 minutes, consider increasing disc speed.

4.2.2 Broad samples

Set conditions so the first particles arrive between 15 and 30 seconds. Resolution on the largest particles suffers, but running fast substantially shortens the analysis; trimming 15 seconds off the initial baseline can save 30 minutes or more at the tail, depending on polydispersity. If the sample is very broad, consider moving to the HOST method in Chapter 5 with the scanning detector head instead.

4.3 Determining the optimum injection concentration

The BI-DCP will size micron and sub-micron ceramics as well as other dense materials such as diamond and crystalline powders, but these measurements require attention to injection concentration.

Stokes' law holds only where the injected particles do not interact with one another. An overly concentrated suspension lets particles fall together through the spin fluid, and the resulting distribution reports sizes larger than the true ones. This hydrodynamic instability is called streaming.

Recognizing streaming

Streaming is most often identified with the strobe. Turn the strobe on before injecting, then watch the turbidity and concentricity of the injected band as it develops. Because the sample is being separated by size, you should not expect uniform turbidity from the meniscus out to the detector. You should expect the same turbidity everywhere on a given concentric ring. Departures from that condition indicate streaming.

There are two distinct causes, and they look different. An unstabilized gradient produces one appearance; an overly concentrated suspension produces another, usually recognizable by arms that form in the injected band, similar to the arms of a spiral galaxy. Mistaking the first for the second is a common error.

The Applications Laboratory routinely injects a NIST traceable standard through a new gradient system first. If the standard sizes correctly and its calculated polydispersity is within limits, the gradient is sound and a series of sample injections can be made over it. See section 4.7. Once the gradient is known to be stable, any streaming that appears has only two possible causes:

- The concentration of injected solids is too high.
- The disc speed is too high, and the particles are travelling through the spin fluid under non-laminar conditions.

Establishing the optimum concentration

The simplest approach is to prepare a highly concentrated stock suspension, around 0.1 wt%, and work with dilutions of it. Suspend the particles in a fluid that disperses them well. For ceramics, a 0.1 wt% solution of tetrasodium pyrophosphate (TSPP) is a frequent choice; in TSPP the particles carry a strong negative surface charge and disperse well from one another.

Method 1: working up from a low initial concentration

Dilute the stock roughly 1:8 by adding 0.5 ml of the concentrated suspension to 2.5 ml of the pure suspension fluid, then adding 1 ml of the gradient fluid. Where the spin fluid is water the gradient is usually established with 100% methanol, so the 1 ml added here would be methanol. Inject this diluted suspension and collect the distribution.

The first differential distribution may be very noisy because so little material was injected and the signal to noise ratio is poor. Make a serial injection over the same gradient at roughly twice the concentration and compare.

Overlaid, the two distributions should agree closely. If the second injection was too concentrated, the distribution shifts generally toward larger sizes. If it was much too concentrated, the streaming arms will be visible in the disc.

WHY THE NOISE MATTERS MORE THAN IT LOOKS

The light scattering correction amplifies the smallest-size region of the distribution. That region often carries the weakest signal, so a correction applied to an under-concentrated run amplifies the worst resolved part of the analysis and returns a poorly resolved curve.

Method 2: working down from a high initial concentration

Start with a concentrated injection suspension and repeat the measurement at progressively lower concentrations. This is usually faster, because once two successive dilutions overlay to within 1% you can stop. Working upward from a low concentration instead produces distributions that are true but jagged, often unrepresentable, and prone to losing information in the tails.

A nominal 1.0 μm silica powder illustrates the method. Transmission electron microscopy gave a number average of roughly 1 μm and showed a population of doublets and triplets, though the photographs could not settle whether those particles were genuinely fused or merely overlaid in preparation. The BI-DCP resolved the question.

The first injection, at full concentration, did not resolve the doublets and triplets at all. Those particles made the suspension hydrodynamically unstable and appeared to tumble through the gradient. Only after a series of dilutions did the true distribution emerge, with the fine detail appearing as the streaming was eliminated.

The primary peak near 1 μm also shifts toward larger sizes at higher concentration. That shift comes from larger particles streaming through the injected band and dragging smaller particles along in their turbulent wake. The peak position is only established once two successive dilutions overlay to within 2%.

Over-diluting the injection suspension

Dilution reduces streaming and brings out fine detail, but below a critical concentration resolution is lost. The instrument calculates the distribution from the intensity of transmitted light against time, so reducing concentration reduces the information available.

In the silica example, the doublet, triplet, and quadruplet peaks resolved at the quarter dilution disappear again at the eighth dilution: the suspension is too dilute for the instrument to detect the turbidity changes those particles produce. The quarter dilution is therefore the optimum. A small residual shift remains in the primary peak at that concentration, but it can be explained by the relative reduction of the other peaks, and losing the multiple-peak information would be a far worse trade.

THE PROCEDURE ONLY HAS TO BE RUN ONCE PER MATERIAL

Once the optimum concentration is established for a material, unknown samples of similar size distribution are simply run at that concentration. Combined with serial injections, the protocol is much less laborious in practice than it first appears.

4.4 Stability of the gradient and serial injections

The buffered gradient formed in the BI-DCP is stable for a minimum of two hours and forty minutes. Over one such period, a mixture of four NIST traceable standards was injected over a single gradient seventeen times, with results as follows.

Conditions

- Initial spin fluid volume: 15.0 ml deionized water.
- Disc speed: 8000 RPM.
- Injection: 0.2 ml of a mixture of 993, 720, 502, and 398 nm Duke Scientific NIST traceable microspheres.
- Serial runs were calculated using the previous run's spin fluid volume plus the 0.2 ml of suspension injected in that run.
- Laboratory temperature 21.1 C; initial spin fluid temperature 19.1 C. A slight temperature rise was therefore included for the first five runs, after which the fluid held at ambient.

Results

No significant drift in the size of any of the four modal peaks was detected over the two hour forty minute period, and every modal value deviated from the established value by 1% or less. Modal values are weight (mass) averages.

Run	Temp (C)	Volume (ml)	993 nm	720 nm	496 nm	398 nm
1	19.1	15.0	993	724	499	398
2	20.0	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	21.0	15.8	993	720	497	398
6	21.1	16.0	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	17.0	993	721	497	*

Run	Temp (C)	Volume (ml)	993 nm	720 nm	496 nm	398 nm
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	18.0	996	720	500	400
17	21.1	18.2	994	721	498	398
Average			993.8	720.8	497.9	398.8
NIST value			993	720	496	398
Deviation			0.08%	0.11%	0.38%	0.19%

* Run 11 was stopped prematurely by accident. The 398 nm peak had not reached the detector and was not recorded. Deviation is calculated as (averaged modal value – established modal value) divided by the established modal value.

4.5 Forming the LIST internal gradient

- 37.** Determine the optimum run conditions with the modeling utility (F7).
- 38.** Enter the disc speed from the model into the Run Parameters menu.
- 39.** Press F1 to continue, and confirm the correct speed reached the instrument by reading the RPM on the LCD.
- 40.** Prepare all syringes before starting the motor. Fill a 2 cc glass syringe with 0.1 ml dodecane, which goes in last as the evaporative shield. Fill a 1 cc syringe with 0.2 ml of 100% methanol. Fill a 20 to 30 cc syringe with the spin fluid volume the model called for.
- 41.** Inject the 0.2 ml of methanol into the dry, non-spinning disc. Place the spin fluid needle in the injection port but do not inject yet. Press MOTOR. As soon as the disc begins to spin, inject the 10 to 25 ml of spin fluid over the methanol at a steady, brisk rate, taking no more than three seconds. The smoother the injection, the better the gradient. As soon as the spin fluid is in, inject the 0.1 ml of dodecane.
- 42.** Allow the gradient five minutes to stabilize before injecting the sample. The program timer can be used for this: press F1 or START after the dodecane goes in, and when five minutes have elapsed press ESC to abort the run, re-enter the setup page, and press F1. Alternatively just note the time and wait five minutes.
- 43.** While waiting, prepare the sample. Add 3 ml of deionized water to a 20 ml vial, add enough drops of the concentrated stock to give a good signal, swirl by hand until homogeneous, then add 1 ml of 100% methanol and mix by hand again.

- 44.** At the end of the five minutes, draw 0.2 ml of the injection suspension into a clean syringe and place the needle in the injection port. Confirm the computer is ready to accept data. With one finger on START, inject the 0.2 ml firmly and press START at the same instant. A short beep confirms the computer has begun collecting data.

DO NOT INJECT THE SAMPLE EARLY

The gradient stabilization period is not optional. Sample injected into an unstabilized gradient produces exactly the appearance that would otherwise be diagnosed as concentration streaming.

4.6 Troubleshooting the LIST analysis

Symptom	Checks
Standard sizes incorrectly	Confirm the disc parameters in software. Confirm fluid volume, temperature, and spin fluid identity on the setup page. For any fluid other than water, enter density and viscosity manually. Confirm the particle density entered.
Distribution shifted to larger sizes	Injection concentration too high; dilute and repeat until two successive dilutions overlay to within 2%. Disc speed too high, producing non-laminar flow.
Streaming arms visible under the strobe	Injection concentration much too high. Confirm the gradient stabilized for five minutes before injection.
Jagged, noisy differential curve	Injection concentration too low. Increase, typically by a factor of two, and re-inject over the same gradient.
First particles arrive in under 15 seconds	Reduce disc speed, increase spin fluid viscosity, or increase column height.
Signal has not returned to baseline in 30 minutes	Increase disc speed, or reduce viscosity or column height.
Baseline signal high, limited concentration range	Clean the disc; see section 2.3 and Chapter 7.

CHAPTER 5

The HOST analysis

Homogeneous measurements, including negative delta rho work on low density materials.

5.1 Introduction

In the LIST analysis described in the preceding chapter, particles are injected onto a spinning disc holding a pure spin fluid with a density gradient. Larger particles move outward faster than smaller ones, and as each group passes the detector it briefly blocks light travelling from emitter to detector. All particles start at the same radial position and separate, so the raw data is differential and the cumulative curve is obtained by integration.

In the HOST or homogeneous analysis, the sample is suspended in a much larger volume of fluid and that whole suspension is injected. The particles do not start at a common radial position; they are dispersed uniformly throughout the disc. The suspension becomes gradually less turbid as they sediment, and that change is again recorded against time. The particles are suspended in spin fluid alone, with no gradient fluid added to the sample, which helps considerably when the gradient fluid would agglomerate the particles.

The two modes also differ in the form of the raw data. A HOST analysis begins with the suspension at its most turbid, so the signal starts low, perhaps 0.2 volts, and rises to a maximum near 2.2 volts once all particles have been centrifuged clear of the detector. Where the LIST signal starts at baseline, rises as the band passes, and falls back to baseline, the HOST signal climbs from a lower baseline to an upper baseline and stays there.

Because HOST raw data is already cumulative, it must be differentiated to produce a differential curve. Differentiating a cumulative curve is unforgiving: subtle changes in inflection can generate false peaks. The HOST technique is therefore intrinsically lower in resolution than LIST.

When to prefer HOST

- Very broad samples, or samples with low density and small diameter. A narrow distribution is better served by LIST, where the band passes the detector quickly; a broad one keeps passing the detector for a very long time.
- Particles less dense than the suspending fluid. A LIST analysis is impossible here, because the particles are driven toward the center of the disc rather than through the column. Anything below 1 g/cc dispersed in an aqueous solution must be run as HOST.
- Particles suspended in a solvent that does not suit LIST, such as carbon black or organic dyes in MEK, or ceramics in phenol. In LIST work the gradient fluid makes up around 25% of the injection volume and can flocculate the particles. In HOST the particles meet only the spin fluid; the gradient fluid is pushed through the column but stays isolated from them.

5.1.1 Positive delta rho conditions

The positive delta rho (PDR) analysis suits broad samples and samples denser than their spin fluid, for example polystyrene at 1.05 g/cc in water at 0.998 g/cc. Under these conditions particles move radially outward under the centrifugal field.

In a typical PDR raw data curve for a mixture of five NIST traceable standards, turbidity is greatest at the start of the analysis, with the signal near 0.9 volts at time zero. Once a given group, say the 720 nm standard, has passed the detector head it no longer contributes to the turbidity of the system. That is how the software extracts size: a rapid change in turbidity marks the passage of the last of a group of particles.

Note that the signal rises slightly at the very start of the analysis, roughly 0.1 volts over the first minute, even though no group has completely left the suspension. This is radial dilution, discussed in section 5.6.1. Under negative delta rho conditions the signal would instead fall over the same period.

5.1.2 Negative delta rho conditions

Users are often required to size materials less dense than the fluid they are suspended in. Because sedimentation rate depends on the difference between particle and fluid density, it can pay to replace water with a denser fluid such as 40 wt% sucrose at 1.18 g/cc, which increases delta rho.

There is always a trade. Adding sucrose raises viscosity along with density, so the particles are driven harder by the larger delta rho while being held back by the thicker fluid. Whether the change helps depends on the numbers, which is what the modeling utility is for.

5.1.2.1 Forced negative delta rho analyses

You may find it more efficient to run a sample under negative rather than positive delta rho conditions when the particle density is only slightly above that of the spin fluid, typically 1.01 to 1.07 g/cc. Because rate depends on the density difference, the modeling utility will often show that a low density particle of a given size takes longer to analyze under PDR conditions than under NDR conditions. The example in section 5.2 works through exactly this case.

5.2 Using the modeling utility

Press Modeling Utility on the main screen and enter the sample and spin fluid information on the parameter page: densities, viscosity, temperature, volume. Make a first guess at run time and disc speed, enter those, and press Calculate Size Range. The software returns the particle size window those conditions will cover.

In general, set up first for the largest particles in the suspension, then extend the run time to accommodate the smallest particles, which reach the detector last.

Worked example: a 1.02 g/cc material

Modeled under positive delta rho conditions at 22 C in water, 11 ml of spin fluid, 5000 RPM, with a run time of 60 minutes, the utility returns a size range of 5.515 um down to 0.243 um, a diameter ratio of 22.7. In other words, a suspension of 330 nm particles plus or minus 50 nm would need a full hour.

Now model the same sample under negative delta rho conditions, driving the spin fluid density to 1.100 g/cc by dissolving 24 grams of sucrose in 76 grams of water to give 24 wt% sucrose. The particles then travel faster, in the opposite direction.

Modeled condition	Positive delta rho	Negative delta rho
Spin fluid	Water	24 wt% sucrose
Spin fluid density (g/cc)	0.998	1.100
Spin fluid viscosity (cP)	0.955	2.280
Particle density (g/cc)	1.020	1.020
Disc speed (RPM)	5000	5000
Spin fluid volume (ml)	11.0	11.0
Temperature (C)	22.0	22.0
Run time (min)	60	30
High diameter (um)	5.515	4.218
Low diameter (um)	0.243	0.263
Diameter ratio	22.7	16.0

Changing only the spin fluid cuts the analysis from one hour to 30 minutes. Even though 2.28 cP is well above the 0.955 cP of water, the larger delta rho still drives the particles faster overall. This is a useful technique for any material with a density close to that of water.

5.3 Preparing a sample for the HOST analysis

The preparation steps are broadly the same as for LIST. Chapter 4, section 4.1 covers spin and buffer fluid selection in full and should be read first.

5.3.1 Selecting the spin fluid

Use the modeling utility to find optimum conditions for either polarity of delta rho. The aim is always to minimize run time while maximizing resolution, and the utility supports that directly by reporting the size range a given set of conditions will cover.

A density gradient is required in the HOST analysis just as it is in LIST, because the HOST system is equally sensitive to streaming. The guidance on run speed applies as well, although the scanning head allows the disc to be slowed somewhat relative to a comparable LIST run.

5.3.2 Determining the optimum injection concentration

Optimum concentration depends on the breadth of the distribution, the size of the particles, and the opacity of the sample in the spin fluid. Concentration is expressed here in terms of the voltage differential the suspension produces on the BI-DCP.

Term	Voltage differential
Dilute suspension	0.2 to 0.5 volts
Moderate suspension	0.5 to 1.25 volts
Concentrated suspension	1.25 to 2.2 volts

NEVER RUN AT ZERO VOLTS

If the front panel reads 0.0 volts at the start of an analysis, the suspension is too concentrated to pass any light at all, and no turbidity change can be measured. Dilute and start again.

Experiment with your own samples to learn what voltage differential a given concentration produces. As general rules:

- Very broad, polydisperse samples: maximize turbidity, and allow the head to scan.
- Narrow distributions between 1000 and 500 nm: use a moderate concentration.
- Bimodal samples whose peaks are separated by at least five times the size of the smaller peak, for example 100 nm and 500 nm: use a moderate concentration.
- Very small diameter and low density: minimize concentration, or consider a negative delta rho measurement.

5.4 The HOST internal gradient method

A HOST analysis needs a stable density gradient through the spin fluid, made the same way as for the line start analysis. Compatible spin fluid and buffer fluid pairs are tabulated in section 4.1. Negative delta rho work requires a different gradient arrangement; see section 5.7.

45. Select the sample to be analyzed.

46. Select a spin fluid to disperse it in.
47. Select the matching buffer fluid.
48. Dilute the sample into the spin fluid. Aim for a signal differential between 0.5 volts for narrow samples and 1.5 volts for broad ones.
49. Determine the disc speed and run time needed to cover the expected size range, adding 20% at each end if you are not certain of the range. Press Modeling Utility on the setup page to send those parameters to the Sample Run menu.
50. From the main screen, press Sample Run to enter the parameters.
51. Press OK on the Sample Run menu to prepare the instrument to accept data.
52. Press Start on the main screen. The run parameters go to the instrument and the software prepares to collect data.
53. Prepare the syringes. Draw 0.1 ml of 100% dodecane into a glass syringe with a small gauge needle and set it aside. Draw 0.2 ml of the selected buffer into a 1 ml syringe and place it in the injection port, without starting the disc. Draw the modeled volume of suspension into a 20 to 30 ml glass syringe.
54. Inject the 0.2 ml of buffer fluid into the dry, non-spinning disc. Immediately place the needle of the suspension syringe into the injection port, holding the plunger against the side of your finger so nothing leaks out.
55. Press MOTOR and begin injecting immediately into the now-rotating disc, smoothly, over about two seconds. As soon as the fluid is fully in, press START on the front panel. Any delay in pressing START will render the analysis incorrect.
56. Immediately after pressing START, inject the 0.1 ml of dodecane. This oil is an evaporative shield; it prevents cooling and loss of spin fluid volume to evaporation.
57. Press STROBE and inspect the turbidity inside the disc.

WHAT A VALID HOST INJECTION LOOKS LIKE

Every point at a given radius should show the same turbidity as every other point at that radius. Turbidity may vary from the meniscus outward, but it must be uniform around each concentric ring.

If it is not uniform, the particles are streaming and the analysis is not valid.

5.5 Setting the calculation baselines

Two horizontal baselines define the limits of integration over which the raw data is calculated. In a typical moderately concentrated run these might sit at 0.900 and 1.970 volts, a differential of 1.1 volts. Placing them is a critical step and is done at the end of every run.

The lower baseline is the voltage corresponding to the light reaching the detector when the sample is at its most concentrated, or homogeneous. The instrument sets it automatically, but a spike in the signal can place it artificially, in which case set it manually.

Placing the upper baseline

- 58.** Expand the region where the raw data curve reaches its maximum, using the up button beside Upper in the Move Baselines panel. Voltages can also be typed in directly.
- 59.** Type lower and upper Y axis limits that put the baseline in the center of the graph window. For a maximum near 1.97 volts, set Ymin to 0.8 and Ymax to 2.1.
- 60.** With the region expanded, use the up and down arrows to place the baseline exactly at the maximum, or the minimum for the lower baseline, of the raw data curve.
- 61.** Press Close to continue and view the completed analysis.

Previously analyzed data files can be reopened later and the baselines placed again.

5.5.1 Radial dilution and radial concentration

A physical effect that concerns users who have not met it before takes place whenever a homogeneous suspension begins to centrifuge. It is called radial dilution, and it occurs because particles moving outward in equal numbers occupy progressively larger volumes.

Consider monodisperse 1 μm particles of density 1.05 g/cc suspended in deionized water, uniformly dispersed at the start of the analysis. Two minutes in, at 5000 RPM, the main body of particles has not yet reached the detector; that happens near three minutes under these conditions. But every particle in the disc is nevertheless moving outward, from a smaller volume into a larger one. Turbidity is measured per unit volume, so the measured turbidity begins to fall before any of the 1 μm group has passed the detector.

This is radial dilution, and it always occurs in HOST mode. When the particles are less dense than the fluid they are suspended in, the opposite happens and the suspension concentrates radially, so the signal falls for the first part of the run, in one three-standard example until about 2.5 minutes in.

BASELINE PLACEMENT RULE

Unless otherwise stated, always place baselines at the minima of the raw data curve, after any radial dilution or radial concentration has run its course.

5.6 Conducting a negative delta rho analysis

A negative delta rho (NDR) analysis is intended for materials that are less dense than the spin fluid, and for particles whose density is close to that of the spin fluid. The gradient has to be prepared differently than for a positive delta rho run: because the particles travel inward rather than outward, the density gradient must be inverted.

A second density gradient may still be needed at the meniscus, as in a positive delta rho analysis. Its function is to trap the centrifuged particles near the meniscus and keep them away from the dynamic air/water interface there. Without it, particles that have reached the end of the column can remix into the suspension.

The protocol below was developed for fat and liposome emulsions in aqueous media. Non-aqueous suspensions can also be run on the HOST platform, but their protocols are specific enough that a general manual is not the right place for them. Contact the Applications Laboratory.

Protocol for aqueous liposome and fat suspensions

- 62.** Prepare 20 ml of a 1:1 mixture of ethylene glycol and water. Inject 1 ml of this solution into the dry, non-spinning disc.
- 63.** Disperse the low density sample into 100 ml of deionized water.
- 64.** Draw 0.2 ml of 100% dodecane into a 2 ml glass syringe.
- 65.** Draw 15 ml of the suspension into a 20 to 25 ml glass syringe and remove all air bubbles from the syringe and needle.
- 66.** Carefully draw 1.0 ml of 100% methanol into the same syringe and keep the syringe upright. It is essential that the methanol and the suspension do not mix in the syringe.
- 67.** Press MOTOR to start the disc. Place the needle in the injection port and inject the fluid smoothly and firmly.
- 68.** Press START, then inject the 0.2 ml of dodecane.

Why the gradient is inverted

Particles being centrifuged need a density gradient through the spin fluid. Here they begin at the outer edge of the disc and travel inward, so the gradient must be denser than the particles, the reverse of the positive $\Delta\rho$ case where the particles are denser than the spin fluid and the gradient fluid is the lighter of the two, as with PVC and methanol.

Methanol is still required on the inside of the disc, nearest the center. As the low density particles travel toward the meniscus, the methanol keeps them from reaching it. Having a density between that of the spin fluid and the methanol, they come to rest in the region of the gradient where their density and the fluid density match. Conditions there are more hydrodynamically stable than at the meniscus itself, so holding them away from the meniscus prevents them from remixing back into the column.

Use the fixed head for these measurements. With two gradients in the disc there are optical regions produced by gradient mixing that must be avoided, and scanning the head increases the chance of entering one. The distance at which the detector fixes itself from the meniscus can be set by the operator.

Detector position and the reduced volume technique

The software knows the density of both the spin fluid and the particles, so it identifies an NDR condition on its own. Set up for an NDR analysis, it moves the detector head toward the inside of the disc, near the meniscus, because the particles travel from the outside inward and the inside position is where the largest particles are detected. The head moves inward so that the greatest column height is available for those particles to travel through.

The last particles measured in an NDR analysis start at the outside of the disc and travel inward. The distance that matters is therefore from the outer radius of the disc cavity to the detector, not the position of the meniscus. This holds only as long as the column has enough volume to extend to or beyond the detector head; a column that stops short of the head means the particles are never measured at all.

Once particles have passed the detector, how much farther they travel is irrelevant; they are detected once. So if gradient effects are disturbing the signal, you can tell the software there is 10 ml in the disc when there is really 15 ml. The instrument moves the head to a position a fixed number of steps in from the calculated meniscus, so a declared 10 ml puts the head where 10 ml minus those steps would be, and gradient effects occurring at, say, 13 ml are pushed outside the measurement window.

COST OF THE REDUCED VOLUME TECHNIQUE

The measurement range shortens along with the column, restricting the analysis to smaller sizes. A short column suits particles below 500 nm and monodisperse distributions well.

If a broad sample must be run this way, extend the run time so the smallest particles are still captured.

5.7 Troubleshooting the HOST analysis

The HOST and LIST analyses share most of their failure modes, so read section 4.6 first. Problems specific to HOST are listed below.

Symptom	Checks
Raw data signal drops off suddenly, then rises again	Check alignment and confirm the detector is not scanning over the meniscus. The sample may be too concentrated, with refractive index differences between sample and spin fluid bending light away from the detector. Reduce the concentration.
Particles re-enter the measurement region after being detected	Use 0.1 to 0.2 ml of 100% dodecane over the spin fluid surface to stabilize conditions. Increase disc speed to raise the centrifugal force on the particles.
Bumps in the raw data	Confirm the disc is clean inside and out. Use a fixed detector head. Use the fast scan analysis mode.
Signal falls at the start of a run	Expected behavior under negative delta rho conditions: radial concentration. Place the lower baseline at the minimum of the curve.
Front panel reads 0.0 volts at the start	Suspension too concentrated to transmit light. Dilute and repeat.

Further questions may be sent to service@brookhaveninstruments.com or raised by phone at +1 (631) 758-3200.

CHAPTER 6

Light scattering correction files

Building a scattering correction for a new particle and spin fluid combination.

These instructions assume DOS 5.0 or later, the BI-DCP software installed on the C: drive in C:\DCP, and a floppy drive at B:.. For earlier versions of DOS or another operating system, refer to that system's documentation for editing files.

6.1 Installing the scattering software

- Insert the disk labeled DCP-SCATT.EXE into the floppy drive.
- Copy the contents to the SCATT subdirectory: `copy b:\*.* c:\dcp\scatt`
- Change to the C: drive by typing `c:`
- Change to the directory by typing `cd dcp\scatt`
- Confirm the file arrived by typing `dir dcp-scatt.exe`. The file should be listed; if it was not copied, the computer reports file not found.

6.2 Creating a scattering correction file

Building the PRM file

- 69.** Edit the existing PRM template. For a tungsten-halogen source, edit `tungsten.prm`; for an LED source, edit `led.prm`. The DOS editor opens with the file displayed. Replace the line marked for sample information with a brief description of the sample, for example PMMA in 5% Sucrose.
- 70.** Enter the refractive index of the spin fluid in the `n(med)` column, replacing the zeros. Copy the format exactly, using typeover mode with insert off, and fill in the same number of digits shown on the template. For LED sources you need only the value at 700 nm. For tungsten-halogen sources you must supply values from 400 to 1100 nm; if you have data at only one wavelength, use it in every row. A close estimate is better than nothing. For 5% sucrose you would type 1.3703 in the 700 nm row.
- 71.** Enter the refractive index of the sample in the `n(re)` column the same way. For PMMA you would type 1.4847 in the 700 nm row.
- 72.** Save the file under the sample name with a PRM suffix, using Save As from the File menu, for example PMMA5SUC.PRM. Do not save under the original template name or the template will be overwritten.

Converting the PRM file to an SCT file

- 73.** Type `dcp-scatt` at the DOS prompt.
- 74.** Enter the PRM file name without the extension, for example PMMA5SUC.

- 75.** The PRM file appears on screen. Check it for correct digits and no extra spaces, then press Enter.
- 76.** The program processes the file and creates an SCT file with the same prefix, confirming on screen when it completes.

6.3 Adding the SCT file to the index

- 77.** Type edit SCATT.NDX
- 78.** Insert an entry for your SCT file at the appropriate position, in the form: number on list, name of SCT file, name as it should appear in the index. The display name may be up to 28 characters including spaces. Exclude the SCT extension from the file name field.
- 79.** For example, to add PMMA5SUC.SCT between entries 25 and 27, place the cursor on 27, press Return to open a line, and type: 26,"PMMA5SUC","PMMA / 5% Sucrose". The correction then appears as number 26 in the list.
- 80.** Save with Alt, F, X.
- 81.** Type DCP or DCPH and go to the Standard Sample Run (F1). Your new file should be listed in the scattering correction index; use PgUp and PgDn to find it depending on its position.

6.4 Additional notes

- Follow the existing code number scheme. If your new file belongs to a family that already exists, use a nearby number; several carbon black corrections occupy numbers 40 to 50.
- The PRM name given to DCP-SCATT.EXE must match the PRM file exactly, and must not carry the SCT extension.
- Follow the established on-screen label scheme: particle name, slash, liquid name, as in PVAC / 5% Sucrose.
- If DCP-SCATT.EXE will not run properly, the PRM file is almost certainly at fault. Compare it against a template in the DOS editor; typically a line is missing or a value is short a digit.
- Calculation time depends on machine speed and source type, from a few minutes on a 486 or 386/387 with an LED source to about 30 minutes on a 286/287 with a tungsten-halogen source. Wavelength and particle size display during calculation to show progress.
- Refractive index at the exact wavelengths of interest is usually unknown, and so is the wavelength dependence. Use whatever value you have in every row; a correction built on incomplete data is better than no correction. If the material is colored it probably absorbs in the visible, and if you have no value for the imaginary part of the refractive index, 0.1 is a reasonable first guess.
- Refractive indices for aqueous sucrose, ethylene glycol, and glycerol solutions differ from that of pure water. These values are readily found in reference handbooks.

CHAPTER 7

Baseline adjustment

Accepted baseline values and what to do when the baseline drifts high.

7.1 Accepted baseline values

The instrument sizes particles by correlating the change in optical turbidity of the suspension against time, differentially in the LIST analysis and cumulatively in the HOST analysis. Both need a baseline value to measure the changing signal against.

Brookhaven Instruments sets this baseline to 30 mV plus or minus 10 mV with pure deionized water spun in a new PMMA disc, under the BI-DCP software package. The BI-DCPH package calculates its signal differently, so be careful to use the right package when checking. Baselines are higher for Homalite discs, around 0.6 volts, because polycarbonate is more optically opaque.

In general, accepted baseline values fall between 0.1 and 0.4 volts, measured with 15 ml of filtered deionized water in the disc at 1000 RPM.

7.2 Adjusting the baseline

Baseline values drift upward over time. A high baseline does not make the data incorrect, but it does narrow the concentration range the instrument can work over.

The common causes are:

- The disc has clouded, usually from oils or sample adhered to the surface, or from contact with an aggressive solvent that damaged it.
- Something is blocking the emitter/detector optical path in the measurement head. This tends to make the baseline fluctuate, because material in the optical slit can move during a run.
- The electrical resistance of the emitter/detector circuit inside the instrument has changed. In this case the baseline is adjusted manually at an internal variable resistor, by the user or at the factory.

If the baseline is too high, work through the following:

- 82.** Clean the measurement disc. If it is contaminated beyond cleaning, the remaining options are resurfacing at Brookhaven Instruments or replacement.
- 83.** Inspect the emitter/detector system. Retract the head, remove the disc, and look at the optics. The path should be clear and the beam reaching the detector slit should be uniform. Clean any obstruction gently with a new cotton swab or a piece of lens paper.

- 84.** If the disc is clean and the optics are clear, the cause is likely a change in the internal impedance of the instrument circuitry. Contact Service and Support at service@brookhaveninstruments.com before adjusting the internal variable resistor.

CHAPTER A

Chemical compatibility

PMMA and Homalite (polycarbonate) measurement discs at 20 C.

THIS CHART IS A GENERAL GUIDE ONLY

Test the chemical in question against a sample of the disc material before running an analysis if you are unsure of its effect.

Chemical class at 20 C	PMMA disc	Homalite (polycarbonate) disc
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
Ketones	N	N
Oxidizing agents, strong	N	N
Chlorinated solvents	N	F

Key	Meaning
E	No damage after 30 days of 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.

More complete charts appear in laboratory supply catalogs such as the Cole-Parmer catalog.

CHAPTER B

Viscosity reference tables

Where to find the fluid property data the software does not calculate.

The software calculates density and viscosity for water only. For every other spin fluid, these values must be entered manually at the temperature of the fluid. Tables are required for:

- Sucrose solutions at various concentrations.
- Ethylene glycol and water solutions at various concentrations.
- Glycerol solutions at various concentrations.

For other solutions and chemicals, consult the CRC Handbook of Chemistry and Physics. Current tables for the fluids above are available from the Applications Laboratory on request; write to service@brookhaveninstruments.com.

CHAPTER C

Reader response form

Your feedback helps us improve this manual.

Copy this page, complete it, and return it by fax to +1 (631) 758-3255, or send the same information by e-mail to service@brookhaveninstruments.com.

Field	Response
Date	
Company name	
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