Instruction Manual

for

BI-200SM Goniometer
Ver. 2.0

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PLEASE READ

This is your instruction manual for your Brookhaven goniometer. Please read it carefully before attempting to use the equipment. The INSTALLATION section describes initial checks which must be made to ensure proper operation.

DO NOT shine light directly into the photomultiplier when the high voltage is attached and power is on. This will ruin the photomultiplier tube and void the warranty. Align the system as described in the manual. Brookhaven Instruments is not responsible for photomultiplier tubes ruined by ignoring this warning.

DO NOT scratch the glassware. Alignment is degraded, especially at the lowest and highest angles, by scratches and pits in the index matching vat and sample cells. Follow carefully the instructions for cleaning vats and cells. Brookhaven Instruments is not responsible for glassware scratched by ignoring this warning.

COPYRIGHT NOTICE

Third Printing : August 1993 with changes to Alignment sections

Catalog Number : BI-200SMMAN, Ver. 2.5

WARRANTY

Brookhaven Instruments Corporation (hereinafter known as BIC) warrants that the product is free from defective material and workmanship. Under the terms of this warranty BIC agrees to correct by repair, or at BIC's election by replacement, any parts which prove to be defective through no fault of the user.

This warranty is limited to the original purchaser of the product.

The product shall be shipped, freight prepaid and insured in full, or delivered to a facility authorized by BIC to render the service provided hereunder, in either the original package or a similar package affording an equal degree of protection. The purchaser must contact BIC for instructions prior to returning the product.

The product shall not have been previously altered, repaired or serviced by anyone other than a service facility authorized by BIC. The product shall not have been subjected to accident, misuse, or abuse, or operated contrary to the instructions contained in the operating manual or manuals.

BIC shall not be liable for direct, indirect, incidental, consequential, or other types of damages resulting from the use of this product other than the liability stated above. These warranties are in lieu of all other warranties, expressed or implied, including, but not limited to, the implied warranties of merchantability or fitness for a particular purpose.

The BIC warranty extends for a period of 90 days. This period begins from the date of receipt of the equipment, and it applies only to the original purchaser. The warranty period is automatically extended to 1 year (except as noted below) from the date of receipt of the equipment provided all invoices for said equipment, including transportation if applicable, are paid within 30 days after receipt of invoice.

The BIC warranty extends for a period not exceeding the warranty period of the original equipment manufacturer where applicable. The typical warranty period on printers and computer peripherals is 90 days, and on photomultiplier tubes it is 180 days. Please contact BIC for copies of applicable OEM warranties.

The risk of damage passes to the buyer upon receipt of all glassware associated with the goniometer and its options and accessories including vats, cells, filters, and lenses.

TABLE OF CONTENTS

Section I: INTRODUCTION

General Description

This manual describes the BI-200SM goniometer, version 2.0. Version 2.0 differs in three ways from the original goniometer. First, an additional 2mm aperture and mount are provided to make the initial alignment of the laser beam much easier. Second, a completely new, compact and much improved detector optic is provided. It is now much easier to view the alignment needle and to make the center of rotation adjustments. All lenses are coated achromats. This allows wavelength changes with minimal or no changes in alignment. Third, a more universal filtration/circulation system is provided. This system is an option for use with the index matching fluid. We now use Teflon tubing and a gear pump.

The BI-200SM is designed for making precision measurements of scattered laser light. The average, time-integrated intensity (Classical), and the intensity fluctuations (QELS) can both be measured. From the average intensity measurements on a polymer solution, one may calculate the molecular weight, radius of gyration and second virial coefficient. From the QELS measurements, one calculates diffusion coefficients, particle size, and particle size distribution information.

The design of the BI-200SM incorporates an open, modular concept. This allows you, the user, to modify the existing design and to add your own components should the need arise. The laser and detector rails are industry standards. If you wish to add optical components please contact Brookhaven for suggestions with mounts for these rails.

The specimen cell assembly has separate flow paths for the index matching liquid and the liquid used for temperature control. Although the plumbing for these separate systems is standard, the options themselves--an external temperature controller, the BI-TCA or TCD, and the filtration/circulation system, the BI-FC--are not. If you have chosen to supply your own temperature controller or filtration/circulation system, please contact the factory for advice.

Organization of this Manual

The sections in this manual are organized into three groups: the introduction, sections I-III; installation, section IV; and measurement, sections V-VIII. Look at section III for the configuration of your instrument. The options that you purchased are listed there as well as the high voltage setting for the

photomultiplier tube. Parts of the goniometer are described in Section IV-1. Laser safety features and precautions are described in Section IV-2. Section IV-3 is the heart of this manual. The alignment of the goniometer is described in this section. If the goniometer is not properly aligned, then measurements will not turn out as well as expected. Pay particular attention to this alignment section.

The last four sections are devoted to measurements including a measurement to verify alignment. Sample preparation is discussed in Section V. Poor sample preparation, especially for low angle aqueous measurements, is the leading cause of bad light scattering results. Photon correlation spectroscopy (PCS) measurements are discussed in Section VI. A more complete discussion of PCS measurements is found in Brookhaven correlator manuals. Intensity measurements are discussed in Sections VII and VIII.

It is assumed that you are using the goniometer with a Brookhaven signal processor, either a photon counter or correlator. If you are using your own signal processor, please make the appropriate revisions to the suggestions in this manual. Brookhaven software has been explicitly written for use with Brookhaven goniometers and signal processors.

Please read the manual carefully. Every attempt has been made to be thorough. Designs do change, however, and documentation is not always perfect or current. Occasionally an error or omission is discovered. If you find a problem with the instrument or the manual, we would appreciate hearing from you.

Section II: SPECIFICATIONS

Goniometer

200mm turntable mounted on circular base with holes for mounting to a supporting table. Manual or motor driven selection of angles with 0.01° steps. Rigid arm for mounting beam alignment apertures and neutral density filter in common holder and for mounting detector rail. Fine-thread screws for center of rotation adjustments.

Detector Optics

Adjustable eyepiece for viewing scattering volume. Two hundred micron wide slit with adjustments to determine center of rotation. All lenses are coated achromats. Filter wheel with narrow-band optical filters for laser wavelengths of 633, 514.5, 488nm, one open and two blank positions. Pinhole turret with 100, 200 and 400μ pinholes for selecting coherence areas (QELS measurements) and 1, 2 and 3mm apertures for adjusting intensity (classical measurements).

Detector

Selected photomultiplier tube (PMT), shielded housing, socket, dynode chain, integral amplifier/discriminator. The low voltage power requirements (± 5vdc) are supplied from a Brookhaven correlator or photon counter. A separate high voltage supply (-1,500 to -3,000vdc, 3mA, ±15mvdc for ±10% line voltage fluctuations) is required.

ND Filter

A neutral density filter with an optical density of 3.0 is mounted on the rigid arm after alignment is complete. This reduces the main beam intensity by approximately a factor of 1000 for angles below approximately 8°.

Beam Focus

A 10cm focal length coated achromat with adjustments is used for focusing and steering the beam onto the center of rotation. Entrance and exit apertures block stray light.

Sample Cell Assembly

Insulated, anodized metal pot with special glass vat for containing an index matching liquid. Inlet/outlet ports for circulating index matching liquid. Inlet/outlet ports for circulating a temperature controlled fluid around the sample cell holders. Temperature range approximately +5°C to +80°C. Cylindrical and square cell holders. Alignment cell for use in determining the center of rotation.

Cells

Five, nominal 12mm outside diameter, round cells with Teflon caps are provided as standard. Cells are nominally 55mm tall and require a minimum of 1.5mL of sample.

Options

BI-LRM

Laser rail and mounts for small, cylindrical HeNe lasers, 35mW and lower power HeNe lasers with rectangular cross sections, and most Argon-ion lasers. Includes laser rail of appropriate length, foot with mounting holes and rail slider. Two adjustable laser mounts included.

BI-FC

Gear pump, 47mm filter holder, set of filters, smooth-tip forceps for handling filters, Teflon tubing and connectors. For use with most index matching fluids like toluene or decahydronapthalene (trade name, Decalin).

BI-TCA or TCD

Temperature controller with external circulation. Stability of ±0.1°C over the range of temperatures suitable for use with the BI-200SM. Proportional control with temperature selection either analog (TCA) or digital (TCD).

BI-HV

High voltage power supply suitable for use with BI-200SM.

BI-RC26

Nominal 26mm outside diameter cylindrical cells with Teflon stoppers. Best for wide angle measurements and aqueous suspensions.

Section III: CONFIGURATION

Model Number: BI-200SM
Detector Type: BI-DS1
Dark Count Rate: 275 ±25 cps @ 24°C

Goniometer S/N: 80451
PMT S/N: HC1303

OPTIONS

Correlator Type: PCI BI-9001AT Di

Correlator S/N: 4001136

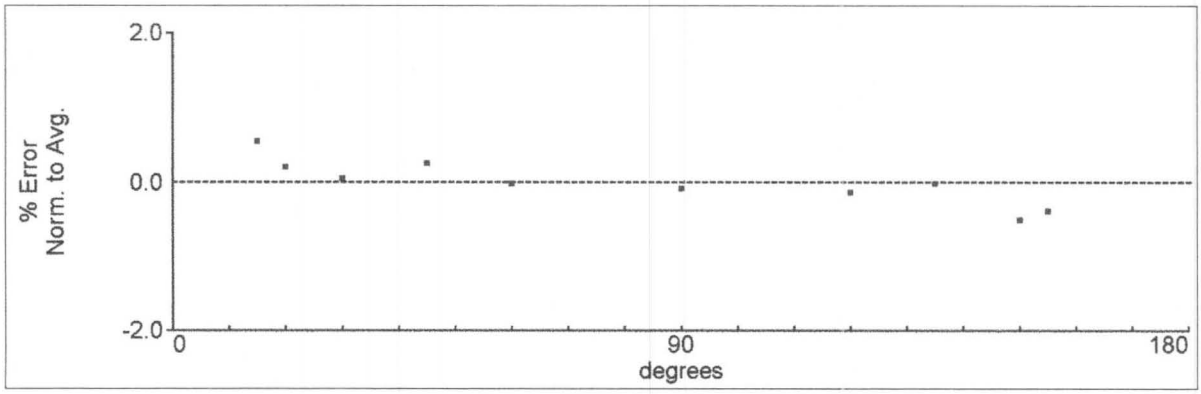
NOTES:

ORIGINAL PURCHASER: Prof. Sinha
ORGANIZATION: University of CA, San Diego
DELIVERY DATE: December 3, 2004

Brookhaven Instruments Corp.
Goniometer Alignment Software Ver. 3.15

Date: Dec 1, 2004
Time: 12:24:20

Sample ID **U of CA San Diego setup**
Operator ID **G.Comstock**
Notes **633nm**



"A" Dark Count Rate:	243 cps	**Refractive Index of Sample Liquid:**	1.474
"B" Dark Count Rate:	0 cps	**Refractive Index of Sample Cell:**	1.500
Repeats:	5	**Refractive Index of Vat Liquid:**	1.474
Duration /Rep:	1.0 sec	**Pinhole size:**	1 mm
Dust rejection ratio:	1.33	**Polarization Analyzer:**	None
		Interference Filter:	In

Angle	I'	I' · sin θ = I	% Error Norm. to 90	% Error Norm. to Avg.
15.00	66135	17117	0.64	0.56
20.00	49875	17058	0.30	0.22
30.00	34062	17031	0.14	0.06
45.00	24136	17067	0.35	0.27
60.00	19652	17019	0.07	-0.01
90.00	17008	17008	0.00	-0.08
120.00	19629	16999	-0.05	-0.13
135.00	24069	17019	0.07	-0.01
150.00	33873	16936	-0.42	-0.50
155.00	40124	16957	-0.30	-0.38

Section IV: ALIGNMENT

Unpacking

Carefully unpack the goniometer. Be sure to remove and inspect all items. Some pieces are small, packed separately, and are easy to overlook if you are not careful. Please inspect carefully any packing material before discarding.

If there are any signs of external damage to the crates or cartons, you must make note of them on the delivery receipt and/or freight bill. Notify the carrier immediately. Save all packing material for their inspection. Contact Brookhaven Instruments for further advice.

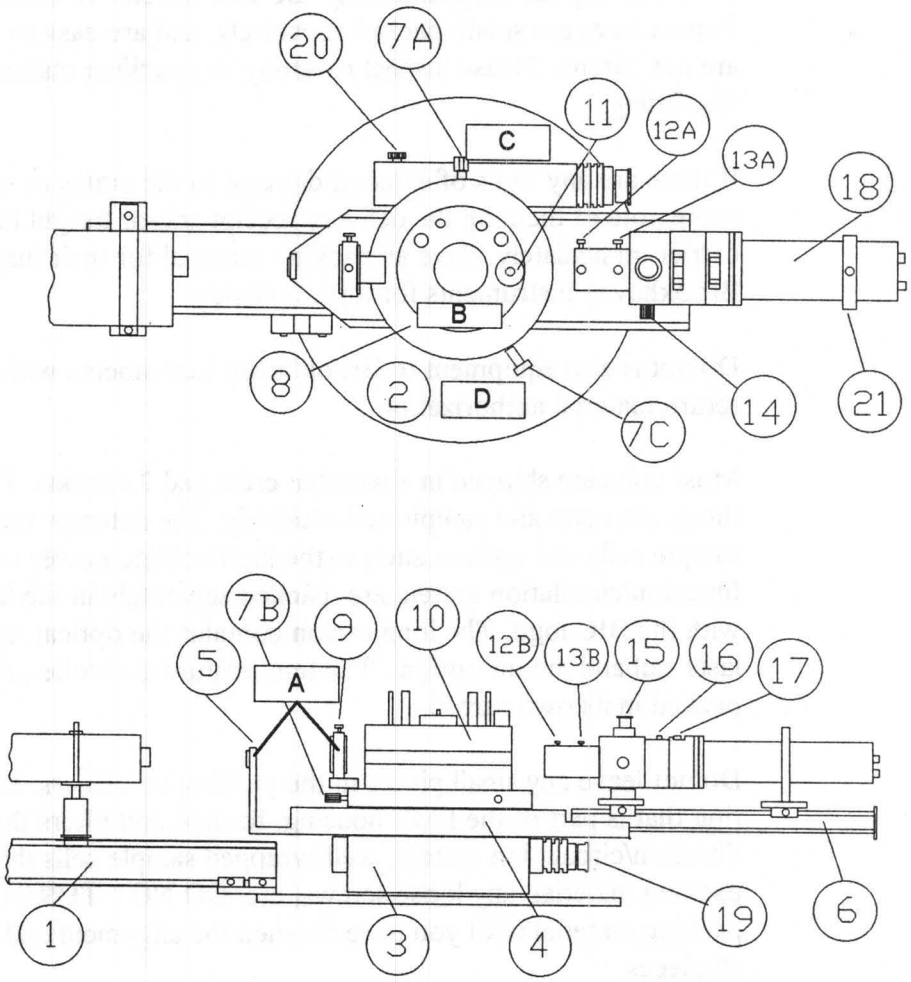
Do not return equipment to Brookhaven Instruments without requesting a return material authorization.

Most units are shipped in a wooden crate and 2 cartons. The crate contains the goniometer and sample cell assembly. The detector optics, PMT, sample cells and options such as the high voltage power supply and the filtration/circulation system are packed separately in the large white box with the BIC logo. The long carton contains the optical rail, part of the laser rail and mount option. The temperature controller, also an option, is packed in its own carton.

Do not leave any small pieces in the packing containers. Look for the o-ring that is part of the PMT housing, forceps and filters that are part of the filtration/circulation system, well-wrapped sample cells that look like packing material, any loose screws, etc. DO NOT THROW AWAY any packing material until you have finished the alignment and have identified all pieces.

Some of the parts of the goniometer are illustrated in Figure IV-1. These parts are listed in the legend that follows. Additional pieces not shown include the universal cell holder and the alignment cell. The following optional items are also not shown: the temperature controller, the filtration/circulation system, and the high voltage power supply.

Figure IV-1: Top & Side View of Assembled Goniometer



Legend

1. Laser rail and mounts, optional
2. Precision-machined base
3. Turntable
4. Rigid rotating arm
5. Upright for 2 mm alignment aperture
6. Detector rail
7A,B. Center of rotation adjustment screws
7C. Center of rotation locking screw
8. Center of rotation adjustment table
9. Beam focusing and steering lens assembly
10. Sample cell assembly
11. Beam Stop
12A. Lens adjustment, horizontal
12B. Lens adjustment, vertical
13A. Slit adjustment, horizontal
13B. Slit adjustment, vertical
14. Mirror adjustment
15. Eyepiece
16. Pinhole wheel
17. Filter wheel
18. Photomultiplier housing
19. Angle adjustment
20. Clutch release
21. Support ring

Laser Safety

BIC manufactures your goniometer in compliance with United States Laser Safety Regulations 21 CFR Subchapter J. This Federal regulation is administered by the Bureau of Radiological Health (BRH) under the auspices of the Food and Drug Administration.

BIC does not manufacture lasers, only the means for mounting the laser and adjusting the beam position. It is the user's responsibility for maintaining the laser as prescribed in the laser operator's manual.

Figure IV-1 depicts the positions of the warning and informational labels with capital letters. Figure IV-2 shows facsimiles of these labels in more detail.

Figure IV-3 shows the position of the rigid rotating arm for adjustments at or near zero scattering angle. After initial alignment, a black Delrin disk is mounted in the alignment upright (5). Previously, a neutral density filter was supplied. This item is no longer necessary. You should not look directly at the laser beam in the following alignment procedures.

This manual refers to laser power of 15 mW or less. CAUTION: HIGHER POWER LASERS MAY CAUSE BRH SAFETY LIMITS FOR VIEWING OPTICS TO BE EXCEEDED. The BRH limit is often too high for comfortable viewing. If you feel you need to reduce the intensity even further, please contact BIC for suggestions.

As a general rule: DO NOT LOOK DIRECTLY INTO THE LASER OR AT ITS DIRECT REFLECTION. Laser light is hazardous to the eyes. When the laser is in operation but the beam is not in use, block the radiation by using the beam attenuator. Most often this is a shutter located on the laser head. This is an excellent habit to form. It will also keep the output mirror of the laser clean, ensuring a longer time between cleaning.

Never turn on the laser while looking through the eyepiece (15). Likewise, never rotate the mirror into the down position (14) while looking through the eyepiece. Place a piece of white paper a few inches above the eyepiece. If you can see any light from the eyepiece falling on it, then reduce the beam intensity before looking into the eyepiece.

Figure IV-2: Laser Safety Labels

A

AVOID EXPOSURE
LASER RADIATION IS EMITTED
FROM THIS APERTURE

B

DANGER

LASER RADIATION WHEN OPEN.
AVOID DIRECT EXPOSURE TO BEAM

C

BROOKHAVEN INSTRUMENTS CORPORATION
750 BLUE POINT ROAD
HOLTSVILLE, NEW YORK 11742

Manufactured: April 1996
Model: BI-200SM
Serial No.:400
Power:15mW MAX Made in U.S.A.

THIS PRODUCT COMPLIES WITH
21 CFR 1040 AS APPLICABLE

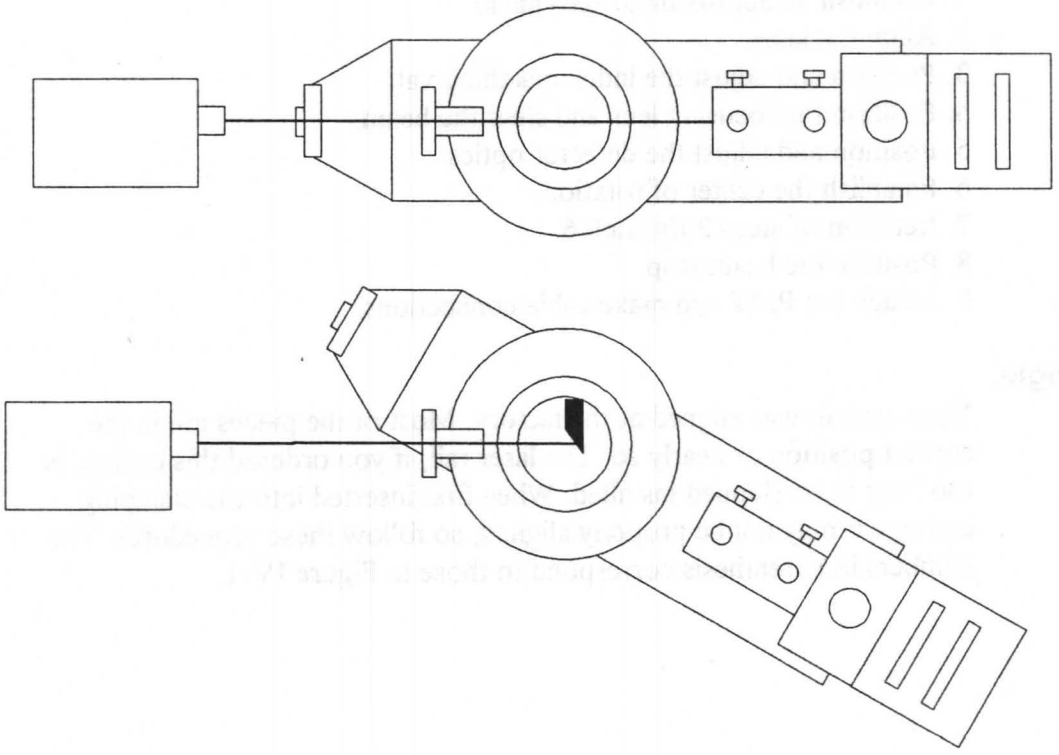
D

DANGER

LASER RADIATION
AVOID DIRECT EXPOSURE
TO BEAM

CLASS IIIb LASER PRODUCT

FIGURE IV-3



Alignment Introduction

When the system is properly aligned, the laser beam will be focused horizontally onto the center of rotation of the sample cell. The cell will be concentric with the vat. Scattered light from the center of rotation will be focused onto the slit in front of the photomultiplier. To achieve this, it is necessary to mount various optical and mechanical pieces in sequence.

It is assumed that all the pieces have been purchased from Brookhaven Instruments. If the user has supplied components, appropriate modifications to these procedures are the user's responsibility.

A set of metric Allen wrenches, a flat-head screw driver, and a small pen light are the only tools needed for alignment.

Please read the previous section on laser safety. During the alignment procedure, the laser shutter is opened and shut in a precise sequence to avoid directly viewing the laser beam.

It is difficult to judge the size and position of very bright beams. If your laser power is adjustable, reduce it below 15 mW.

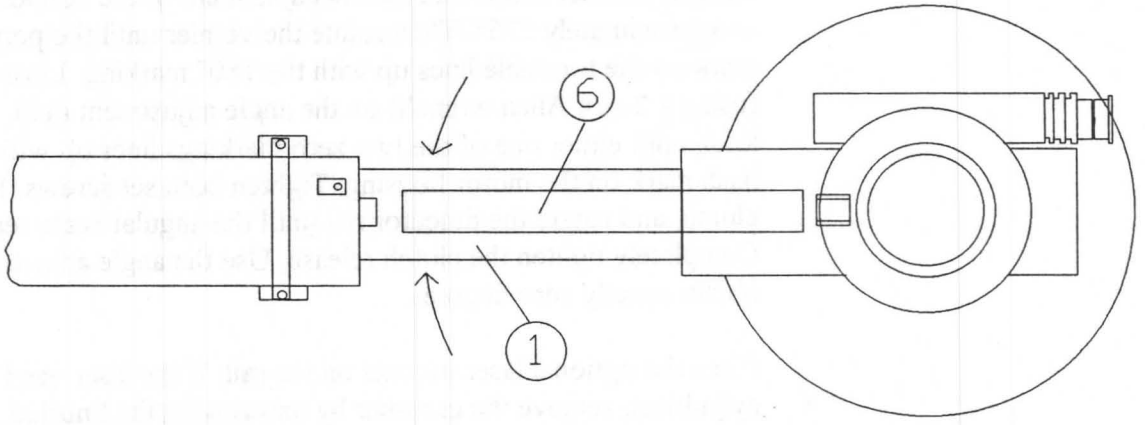
These are the steps you will follow to align your system:

1. Establish an approximate zero angle.
2. Align the laser.
3. Position and adjust the index matching vat.
4. Position the focusing lens and steer the beam.
5. Position and adjust the detector optics.
6. Establish the center of rotation.
7. Iteration of steps 3 through 6.
8. Position the beam stop.
9. Attach the PMT and make cable connections.

Zero Angle

Your system was aligned at the factory. Most of the pieces are in the correct position or nearly so. The laser rail, if you ordered this option, is too long to be shipped installed. When first inserted into the clamping carriage it may not be properly aligned, so follow these procedures. The numbers in parenthesis correspond to those in Figure IV-1.

Figure IV-4: Establishing An Approximate Zero Angle



Place the machined base (2) on a sturdy table. Securely fasten the base to the table using screws or clamps. Slide the laser rail (1) into the clamping carriage on the base. A separate foot/carriage is provided with the laser rail option. Slide it onto the free end of the laser rail. Position the foot approximately one-third the distance from the free end. *Lightly* tighten the screws on the base clamping carriage. Securely tighten the screws on the separate clamping carriage, but do not secure the foot to the table.

Remove the black top and outer jacket from the sample cell assembly (10). Remove the three screws from the top of the brass manifold, and lift the manifold out of the assembly. Set it aside. Loosen the four set screws on the focusing lens (9) mount and slide the lens off. Remove the alignment upright (5) with the 2 mm aperture, located on the opposite side of the goniometer from the laser rail, and replace it with the detector rail (6). Fasten the rail to the rotating detector arm (4) using the two screws that are shipped installed in the rail.

Completely loosen the clutch release (20), allowing the rotating arm (4) to swing freely. Rotate the detector rail (6) until the angular scale reads 180°. The angular scale is located on the turntable (3). Tighten the clutch release. Sight along the detector rail toward the laser rail (1). They should be parallel with each other and with the focusing lens mount on the adjustment table (8). See Figure IV-4. If necessary, adjust the angle with the fine adjustment knob (19) until they are. You may need to gently tap the laser rail with a your hand. If necessary, loosen locking screw (7C) and rotate the adjustment table (8) until the center line of the lens mount is parallel to the detector and laser rails as shown in Figure IV-4. **Do not turn the adjustment screws (7A & 7B) to adjust table.** Secure the laser rail foot to the table, and gently tighten locking screw 7C.

Look at the coarse vernier scale on the turntable (3). If the angle no longer reads 180°, loosen the set screw (using a 2 mm Allen wrench) in the vernier. The set screw that allows adjustment of the vernier scale is located at approximately 175°. Then rotate the vernier until the permanent hash mark on the turntable lines up with the 180° marking. Loosen the set screw (using a 2 mm Allen wrench) on the angle adjustment (19), and rotate the knob until either one of the two zero markings lines up with the permanent hash mark on the motor housing. Tighten both set screws. Release the clutch, and rotate the detector rail until the angular scale reads zero. Completely tighten the clutch release. Use the angle adjustment (19) to obtain exactly zero degrees.

Place the optional laser mounts on the rail. If the laser used is not cylindrical, remove the crossbar by unscrewing the knurled, plastic-topped screws located on the mount posts. See Figure IV-5.

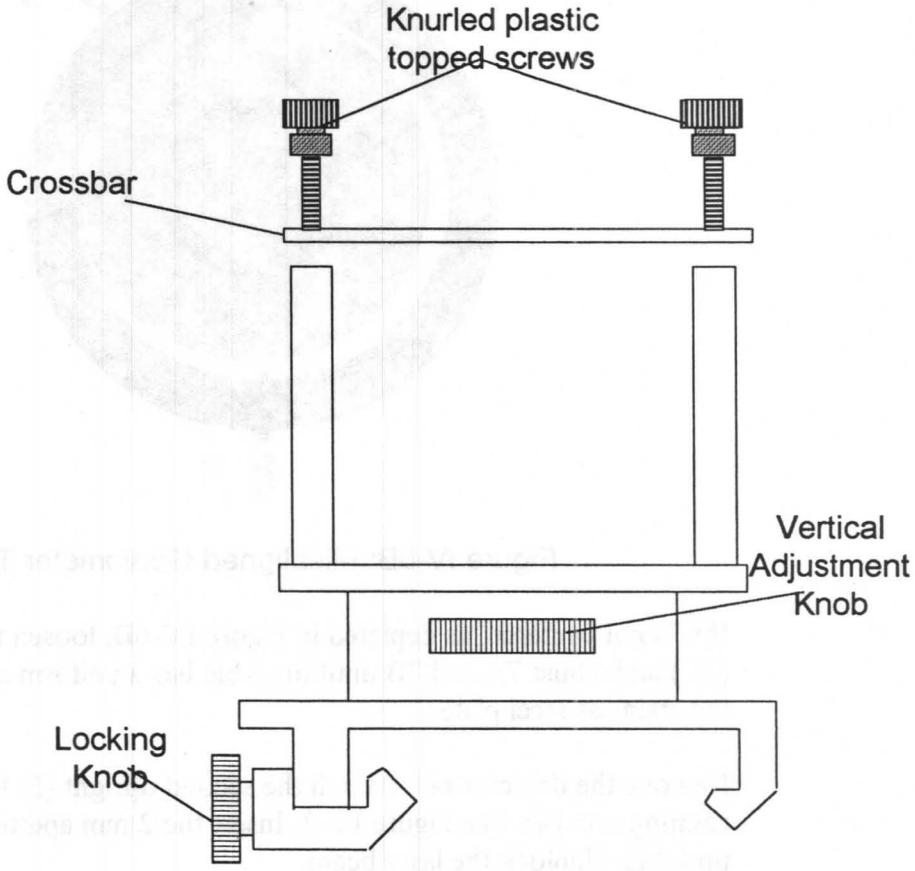
Position the mounts and laser as far as possible from the goniometer. Divergent stray light is then more easily blocked by the first aperture in the beam steering and focusing assembly.

Place the laser in the mounts. Cylindrical laser users should ensure that the linear polarization of the laser is vertical. Non-cylindrical lasers must possess linear polarization in the vertical direction. Contact Brookhaven Instruments if you are unsure of your laser's polarization.

Make the power and any cooling-water or duct connections to the laser. Turn it on. Warm it up for at least 15 minutes or until the laser has reached maximum beam pointing stability as described in the laser manual.

<u>BLOCK THE BEAM WITH THE MECHANICAL SHUTTER LOCATED ON THE HEAD OF THE LASER. IF YOUR LASER IS NOT EQUIPPED WITH A SHUTTER, PLACE A ND3 FILTER IN THE PATH OF THE LASER, OR BLOCK WITH A NON-REFLECTIVE OBJECT.</u>

Figure IV-5: Optional Laser Mount (BI-LRM)



Look underneath the center of rotation adjustment table (8). The table should be centered, with an equal gap as depicted in Figure IV-6A.

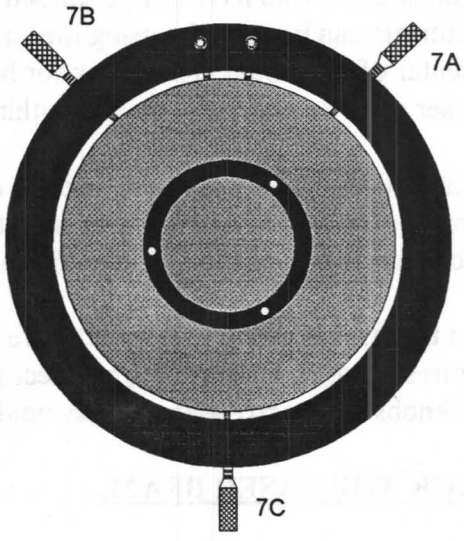


Figure IV-6A: View from Bottom of BI-200SM

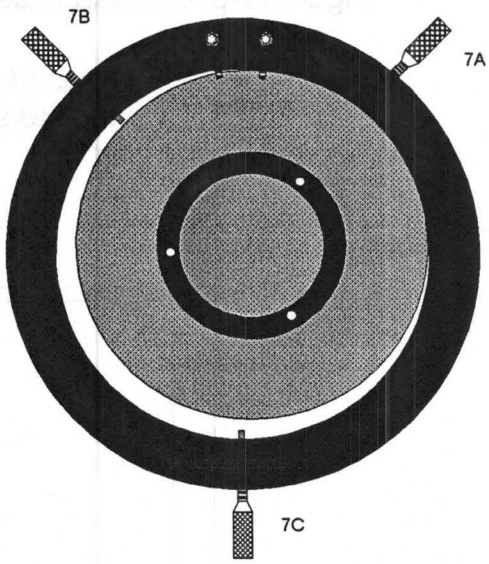


Figure IV-6B: Misaligned Goniometer Table

If it is not centered, as depicted in Figure IV-6B, loosen the locking screw (7C) and adjust 7A and 7B until the table has a uniform gap between it and the stainless steel plate.

Remove the detector rail. Attach the second upright (5) to the rigid rotating arm (4). See Figure IV-7. Insert the 2 mm apertures into the uprights. Unblock the laser beam.

With the laser warmed up, use the laser-mount adjustments to steer the beam cleanly through *both* apertures.

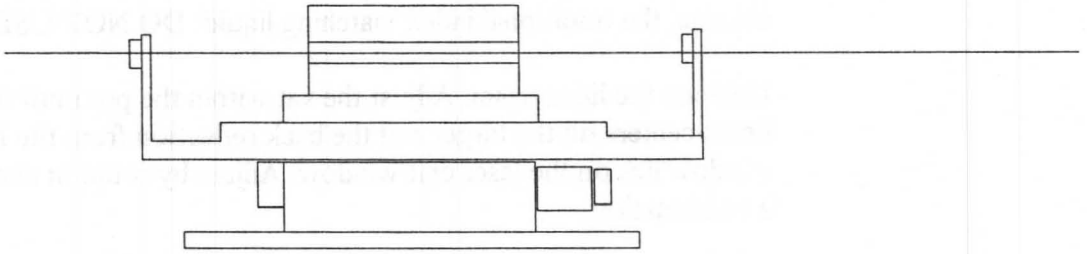
For most users who have not purchased cylindrical lasers, vertical adjustments can be made by using the large, knurled brass knob located in the center of the mount. To correct for horizontal beam placement errors, the laser must be physically moved within its mount.

For users with cylindrical lasers, use the bottom two positioning screws for height adjustment. Rotate the laser until the polarization mark ensures vertical, linear polarization. Secure the laser with the top screw.

When the laser is properly aligned, there are no bright spots visible on the apertures; instead, a slight corona effect is observed. Tighten the knurled brass knobs so the crossbar securely holds the laser in the mount.

BLOCK THE LASER BEAM.

Figure IV-7: Laser Alignment



Place the target on the last page of this section (page 4-31) on the wall, about 1 - 3 meters from the base of the goniometer. Center the target on the laser beam and tape the target to the wall, being careful to keep the spot centered.

Vat Placement

Unpack and inspect the index matching vat very carefully. DO NOT HOLD THE VAT BY ITS CYLINDRICAL SURFACES. It should be clean with only dust clinging to its surfaces. Use dry air or compressed gas to remove the dust. Hold the vat securely while doing this.

Eventually the vat will need additional cleaning. Use a warm, mild soap solution. DO NOT USE ABRASIVES. Drip or blow-dry the vat. DO NOT DRY THE VAT WITH TOWELS. The beam enters the vat on the polished flat that is parallel to the axis of the cylinder. The part of the vat that must be free of water spots is a one centimeter-wide band running counterclockwise around from the flat toward zero degrees. The band starts about 1 cm from the base of the vat.

When dry, gently wipe this region by dragging a lens tissue across the surface of the vat onto which you have dripped reagent grade methanol. Use only the highest quality lens tissue on the vat. DO NOT USE ORDINARY LAB TOWELS OR TISSUES. Hold the tissue with forceps being extremely careful not to allow contact between the vat and forceps. DO NOT RUB THE VAT USING FINGER PRESSURE. A piece of dirt or dust trapped between the tissue and your fingers could scratch the surface. Brookhaven Instruments is not responsible for scratched glassware after delivery.

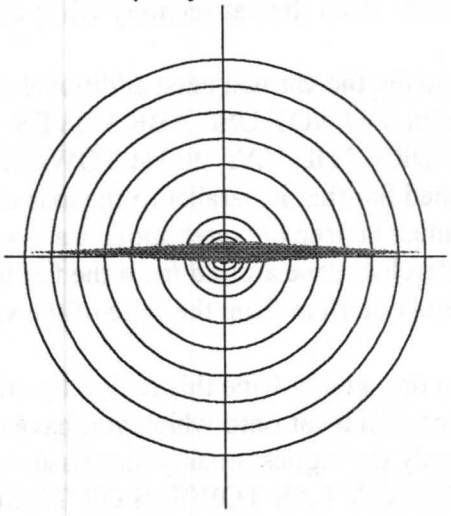
Clean any grit or dust from the inside of the black metal pot. Holding the the back of the vat with your fingertips (the "Brookhaven" label is at 90°, the back of the vat is opposite the "Brookhaven" label), carefully lower the vat into the metal pot, centering the flat entrance window perpendicular to where the laser beam will enter. Fill the vat with approximately 100ml of Decalin (cis+trans decahydronaphthalene), being very careful not to spill any Decalin on the outside surfaces of the vat. If you drip any Decalin,

repeat the cleaning steps described above. Decalin has a refractive index close to that of glass, and its vapor pressure is much less than that of toluene, the traditional index matching liquid. DO NOT USE WATER.

Unblock the laser beam. Adjust the vat within the pot until the exit beam centers on the target and the back reflection from the flat entrance window lies on the laser exit window. Adjust by rotation and translation or a combination.

Due to the cylindrical shape of the vat, the beam exiting the vat and striking the target will assume an elongated, horizontal elliptical shape. Center this shape on the target. See Figure IV-8. The back reflection from the flat entrance window is a spot. Center it on the laser exit window. This spot is superimposed on a weaker, elliptical reflection from the rear of the vat. It is the spot that you want to superimpose on the input beam, not the elliptical reflection. If the spot is not obvious to you, then temporarily block the beam inside the vat.

Figure IV-8: Vat Placement -Target Image of Laser when Vat is Properly Positioned



Lightly secure the vat using the three nylon screws around the sides of the pot while pressing firmly down on the top edges of the vat. Use a thin bladed, small handled screwdriver to tighten. Utilize only enough pressure such that the vat cannot be twisted by hand. Check that the image on the target and back reflection is correct.

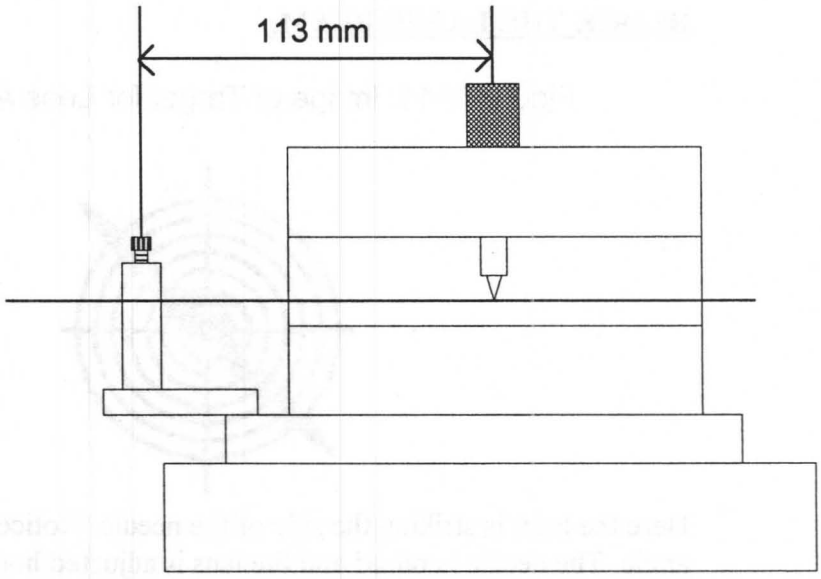
CAUTION: VATS ARE VERY DELICATE AND EXPENSIVE. USE ONLY LIGHT, EVEN PRESSURE ON THE NYLON SCREWS. A HEAVY HAND ON THE SCREWDRIVER WILL CRACK THE VAT.

Carefully replace the brass manifold, securing it finger-tight with the three screws. Refit the black outer jacket and the top to the sample cell assembly. Check that the image on the target and back reflection is correct.

BLOCK THE LASER BEAM.

CAUTION: VATS ARE VERY DELICATE AND EXPENSIVE. THEY CAN BE CRACKED WHEN REPLACING THE MANIFOLD IF YOU ARE NOT CAREFUL.

Figure IV-9: Position of Steering Lens Assembly



Unblock the laser beam. Move the vertical lens adjustment, which is the brass screw on the top of the lens assembly, until the beam is vertically centered on the target. The image on the target is now a vertical oval. See Figure IV-10.

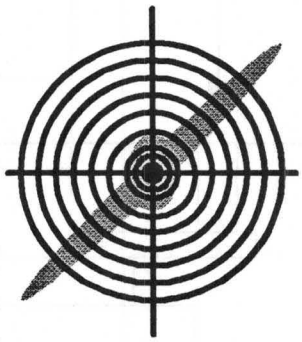
Figure IV-10: Image of Laser on Target

With the needle tip well below the beam, move the horizontal adjustment (located on the side of the lens assembly) until the beam strikes the needle. Notice the image on the target - it should show flare light as depicted in Figure IV-11. (Of course, the flare light could be off in the other direction, which would cause a mirror image of Figure IV-11.) Raise the needle by

turning it counterclockwise about one quarter turn. Turn the horizontal adjustment of the lens assembly until the laser beam strikes the needle again. Repeat this step until the beam is centered on the tip of the needle. (Many needle tips become hooked or bent, in which case it is sufficient to center the beam horizontally on the thicker needle shaft while maintaining the beam vertically centered on the target.) The flare light should now be centered horizontally as depicted in Figure IV-12.

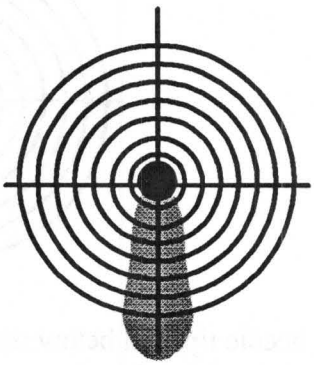
BLOCK THE LASER BEAM.

Figure IV-11: Image of Target for Lens Adjustments



Here the laser is striking the side of the needle. Notice the flare is on an angle. The needle is raised and the lens is adjusted horizontally until the laser strikes the tip of the needle, causing the flare to appear on the target as depicted in Figure IV-12. Some systems will cause a 4-pointed star pattern to appear instead of the more predominate downward flare seen below. When the laser strikes the tip of the needle and either pattern appears, the lens is properly focused.

Figure IV-12: Target Image with Properly Aligned Focusing Lens



BLOCK THE LASER BEAM.

Detector Optics Adjustments

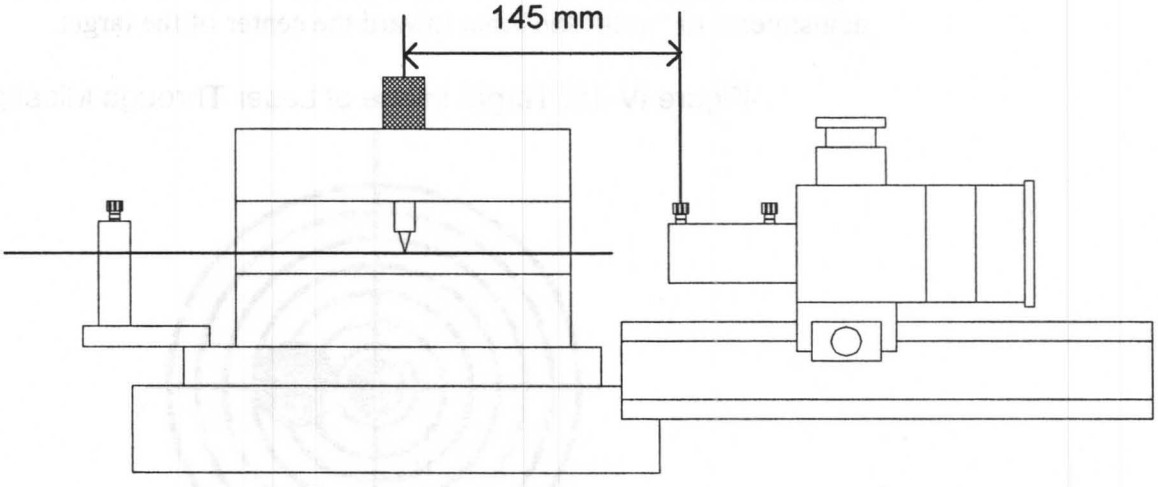
The front part of the detector optic consists of an adjustable lens that focuses light onto a slit. Behind the slit is a two-position mirror. When the mirror adjustment (14) is rotated counterclockwise, light focused onto the slit can be seen in the eyepiece (15). When the mirror adjustment is rotated clockwise, the light passes through the slit onto a variable pinhole and then through a filter into the photomultiplier tube.

Change the angle of the rotating arm to 20°. Mount the detector optic on the detector rail. Rotate the mirror adjustment (14) counterclockwise. Shine a penlight into the small entrance hole of the detector optic. Move the eyepiece up and down until the slit is sharply in focus. Lock down the eyepiece. Rotate the mirror adjustment clockwise.

Slide the detector optic along the rail until it is 145 mm from the center of rotation. Measure from the center of the vertical adjustment knob 12B. See Figure IV-13. Lock the detector optic slider so it cannot move along the detector rail.

Rotate the pinhole wheel (16) to the 3 mm position and the filter wheel (17) to the position marked "O" for open. Change the angle of the rotating arm to zero degrees. Unblock the laser beam.

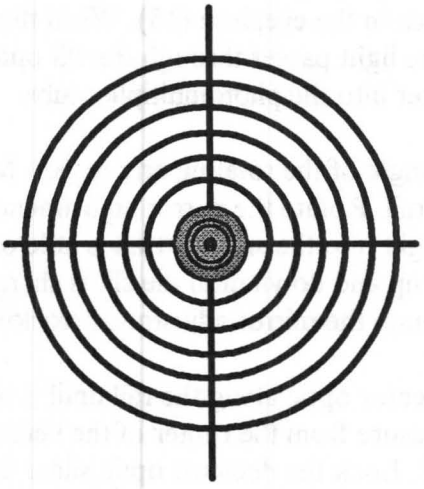
Figure IV-13: Position of Detector Optics.



Use the horizontal and vertical adjustments on the lens (12A & B) to maximize the intensity of the beam falling on the target. You will also need to move the slit in the horizontal direction (13A) and adjust the angle (19). Rotate the pinhole wheel to the 1mm position. Adjust the lens, slit, and

angle until the intensity of the image on the target is maximized. The image should be a bright circular spot with visible diffraction rings. If possible, reduce the intensity of the laser and turn off the room lights to better see the diffraction rings.

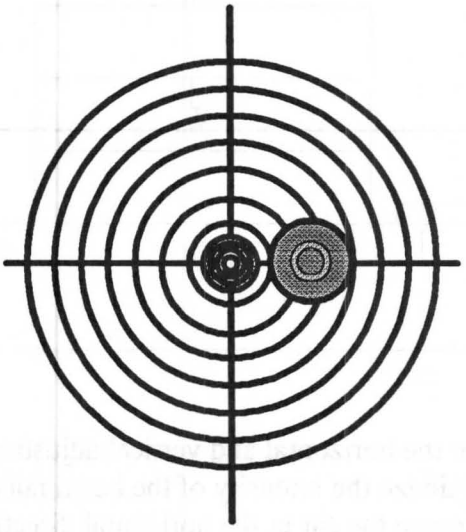
Figure IV-14: Target Image of Laser Through Detector Optics



Most likely you will not be able to superimpose the light onto the center of the target like it is in Figure IV-14. At this point, it is more important to get the image centered vertically. Use adjustment 12B to accomplish this.

Rotate the pinhole wheel. Notice the position and uniformity of the image at each position. If the image on the target is not centered, as depicted in Figure IV-15, use the lens (12A & B) and the horizontal slit (13A) adjustments to "walk" the beam toward the center of the target.

Figure IV-15: Target Image of Laser Through Misaligned Detector



To "walk" the beam, do the following:

Move the slit horizontally (13A), turning clockwise if the image is to the right of being centered on the target, counterclockwise if to the left, until the image begins to disappear. Then adjust 12A to maximize the laser intensity image. Change the angle (19) slightly until the maximum intensity is observed. Repeat this process until the uniformly bright image is centered on the target.

Check the images using the other pinhole positions. Find the lens and slit position where all the images are uniformly bright, maximized, and without halos.

BLOCK THE LASER BEAM.

Reset the zero angle using the vernier scale on the turntable and the fine adjustment knob (19). This is your new zero angle.

Center of Rotation

Rotate the mirror adjustment (14) counterclockwise. Aim a penlight into the initial aperture of the detector optics. Observe the light as it passes through the slit. Adjust the eyepiece until the slit appears focused.

Insert the alignment cell into the sample cell assembly. Release the clutch (20) and change the angle to 20°. Aim a penlight at the needle. Hold the penlight at the smallest angle possible between the light and the front of the detector optic. The needle of the alignment cell should be visible, though probably not centered, in the eyepiece. Rotate the needle adjustment up and down until you can easily see the tip. The reflection off the side of the needle will be a bright band of light, on an angle in relation to the slit. The tip of the needle will appear to point toward the center of the goniometer. If necessary, adjust the slit horizontally (13A) until the needle is exactly centered in the slit.

Unlock the center of rotation adjustment table (7C).

A. Change the angle to 155° while looking through the eyepiece. Unless the sample cell assembly is perfectly centered on the turntable, the needle will appear to move across the slit.

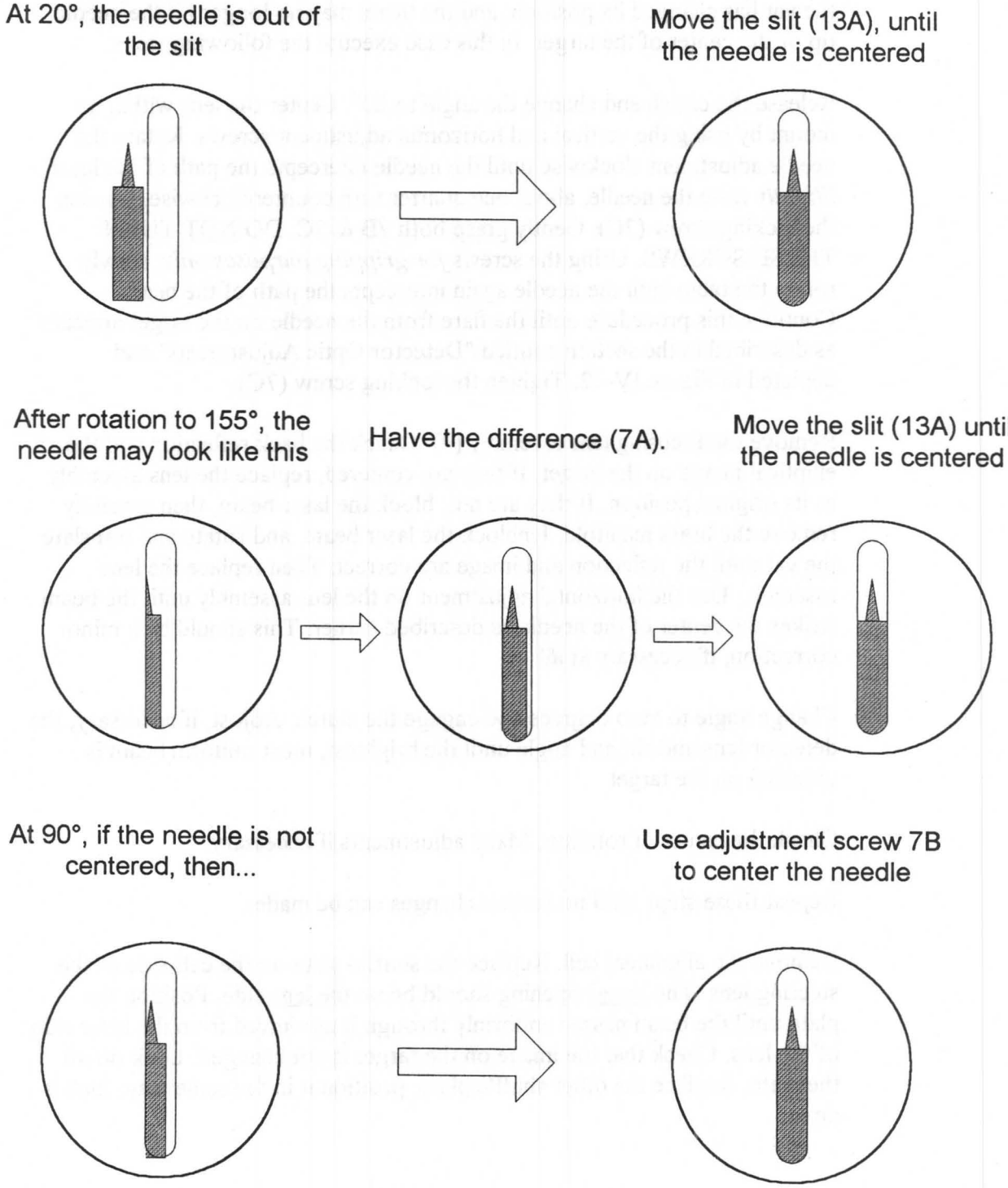
B. Use adjustment screw 7A to halve the distance the needle tip is displaced from the center of the slit. This should be a minor correction. Do not rotate the screw more than one half turn in either direction. If greater adjustment appears to be needed to center the needle image, refer to Figure IV-6A and Figure IV-6B. If the rotation table appears centered, contact Brookhaven Instruments for assistance.

C. Change the angle to 20° while looking through the eyepiece. The needle should appear to move much less. Center the slit on the needle using the horizontal adjustment (13A). Repeat steps A, B and C until the needle is centered within the slit.

D. Change the angle to 90°. Use adjustment 7B to center the needle in the slit.

Repeat steps A, B, C, and D until the needle does not appear to move in the slit. When you are satisfied, use adjustment 7C to lock the center of rotation table. Return the detector optic to 0° and engage the clutch (20). Rotate the mirror adjustment (14) clockwise. Unblock the laser. *If the image is still uniformly bright, and the intensity is maximized and centered on the target,* skip to the section entitled "Beam Stop." If not, continue with the next section entitled "Iterations."

Figure IV-16 Center of Rotation Adjustments



Iterations

If the center of rotation adjustment table has been moved significantly, then the vat has changed its position, and the beam may no longer hit the needle tip or the center of the target. In this case execute the following steps.

Release the clutch and change the angle to 20°. Center the lens within its mount by using the vertical and horizontal adjustment screws. Rotate the needle adjustment clockwise until the needle intercepts the path of the laser. *Slightly* raise the needle, about one quarter turn counterclockwise. Loosen the locking screw (7C). Gently grasp both 7B & 7C. DO NOT TURN THESE SCREWS. Using the screws *for gripping purposes only,* slowly rotate the table until the needle again intercepts the path of the beam. Continue this procedure until the flare from the needle on the target appears as described in the section entitled "Detector Optic Adjustments" and depicted in Figure IV-12. Tighten the locking screw (7C).

Remove the focusing lens assembly (9). Check the back reflection and the elliptical image on the target. If they are centered, replace the lens assembly to its original position. If they are not, block the laser beam, then carefully remove the brass manifold. Unblock the laser beam, and rotate and translate the vat until the reflection and image are correct. Then replace the lens assembly. Use the horizontal adjustment on the lens assembly until the beam strikes the center of the needle as described earlier. This should be a minor correction, if necessary at all.

Change angle to zero degrees and engage the clutch. Adjust, if necessary, the detector lens and slit and angle until the brightest, most uniform beam is obtained on the target.

Check the center of rotation. Make adjustments if necessary.

Repeat these steps until no further changes can be made.

Remove the alignment cell. Replace the shuffle plate on the cell-side of the steering lens. The larger opening should be on the lens side. Position the plate until the beam passes uniformly through it as viewed from the laser side of the lens. Check that the image on the target is not changed. Lock down the plate. Replace the other shuffle plate; position it in the same way; lock it down.

Beam Stop

Mount the periscopic beam stop (11) on the top of the sample cell assembly. Twist it until the back reflection, as seen on the shuffle plate of the steering lens assembly, is centered on the optical axis. Secure the beam stop mount by tightening the two screws on the top of the mount.

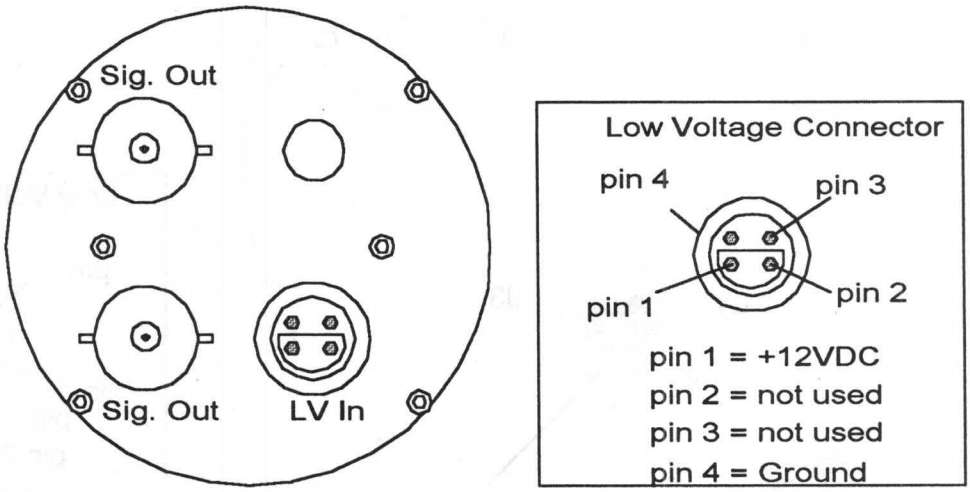
Mounting The PMT

Make sure that the o-ring in the PMT housing (18) flange is seated correctly. Hold the housing up against the end of the detector optic. Use the three, M5 screws in the rear of the detector optic to secure the PMT housing. The screws have brass washers. First, use your fingers to tighten the screws evenly all around. Then use an Allen wrench to tighten the screws. Do not over tighten.

Slide the PMT support ring (21) onto the detector rail. This piece is necessary in systems where the PMT housing is longer than our standard. It is not supplied unless the BI-DS2 option is purchased. Brookhaven's standard PMT and housing do not require additional support. Position the ring about 1/4 the distance from the end of the PMT housing. Lock the slider on the rail. Support the PMT in the ring by lightly tightening the three support screws with your fingers. These screws are only for support, not for positioning. DO NOT TIGHTEN with a wrench.

Power & Signal Connections

Figure IV-17: Standard Configuration for BIC PMT

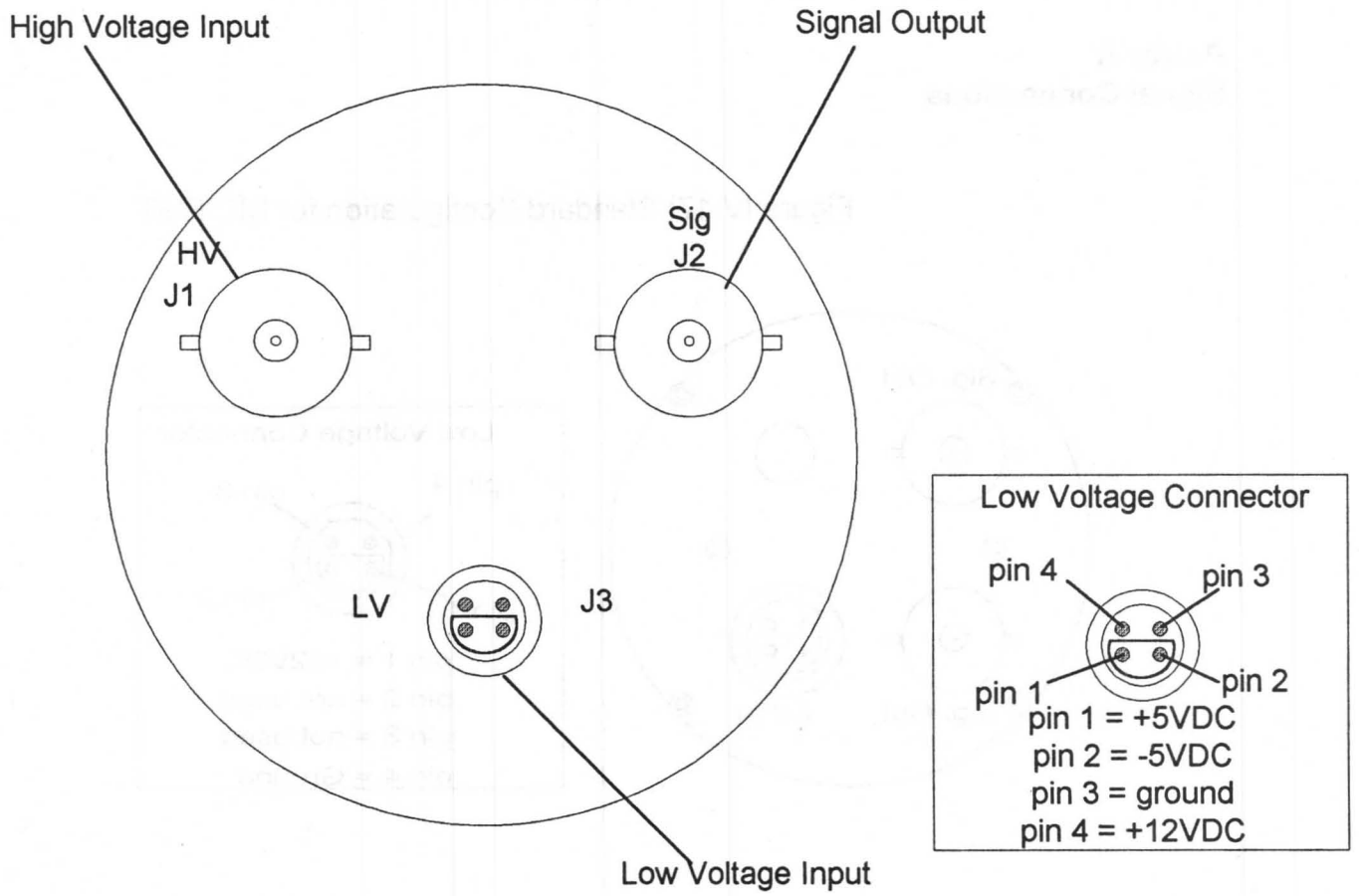


The standard Brookhaven PMT has the High Voltage power supply internally, so only signal and low voltage cables are required.

For signal hook up, attach the long, thin coaxial cable (RG 174) with one BNC and one miniature Lemo to either connector marked "Sig. out" on the PMT and the connector mark "A" on the BI-9000AT correlator board. The signal output employs TTL levels with a 50 Ohm output impedance.

For low voltage, attach the long, gray coaxial cable supplied with LEMO connectors at both ends to either low voltage output of the correlator to the low voltage input on the tube. This supplies the power to the amplifier/discriminator *and internal high voltage. Be sure to make this connection after the PMT housing is attached to the detector optics.* **Never supply low voltage to the PMT when it is not attached to the detector optics.** Doing so can easily destroy the PMT.

Figure IV-18: Power and Signal Connections for Optional BI-DS2



Signal

There are three connectors on the rear of the BI-DS2 PMT housing marked J1 H.V., J2 SIG, and J3 L.V. For signal hook up, use the short, thin coaxial cable (RG 174) with a BNC connector on each end to connect the PMT and the BI-HV connector marked "Sig. A in." Then attach the long, thin coaxial cable with one BNC and one miniature Lemo to the connector marked "Sig. A out" on the BI-HV and the connector marked "A" on the BI-9000AT correlator board. The signal output employs TTL levels with a 50 Ohm output impedance.

Use a coaxial cable (RG 58U) with BNC connectors at either end to connect the signal output (J2 SIG) to Input A on your BI-2030AT correlator.

High Voltage

For the high voltage connections, use the short, thicker, coaxial cable (RG 59) supplied with MHV (mini high voltage) connectors at both ends to connect the high voltage input (J1 H.V.) to the negative output on your HV supply. **Never connect or disconnect cables with the high voltage on.** Your BI-HV power supply has been preset at the factory to operate your PMT at peak performance. Contact the factory if you need to change the HV setting. **Do not turn on the high voltage power supply at this time.**

Do not try to force a BNC connector onto the MHV connector. The connectors should lock together easily by pushing and twisting. If excessive force is required to lock them together, then you have probably tried to mate the wrong pair. The female BNC and MHV connectors on the rear of the PMT housing look similar. The males, on the cables, however, are different. The male MHV pin is further recessed, and it is surrounded by thicker insulation. **If high voltage is applied, mistakenly, to the signal output on the PMT housing, damage to the internal amplifier/discriminator circuit will occur.**

Low Voltage

For the optional EMI tubes, use the short, gray coaxial cable supplied with LEMO connectors at either end to connect the low voltage input (J3 L.V.) to the low voltage output on the BI-HV, marked "LV out." Connect the long, gray coaxial cable supplied with LEMO connectors at both ends to either low voltage output of the correlator to the low voltage input on the BI-HV, marked "LV in."

Motor Connections

If you ordered the BI-CON stepping motor controller option with a Brookhaven correlator, then use the thick, round, gray cable to make the connection from the 15-pin delta connector on the motor housing of the goniometer to the port marked MOTOR on the rear of the BI-2030AT correlator or on the rear panel of the computer for the BI-8000AT or BI-9000AT.

Experience has shown that many problems can be solved by making sure that all connectors are securely fastened before making measurements.

Temperature Control

For some light scattering measurements it is important to maintain a constant and known temperature. This is particularly true for diffusion coefficient measurements. The diffusion coefficient is inversely proportional to the viscosity of the solvent or suspending liquid. Viscosity is strongly temperature dependent, and the percentage change per degree can be large.

For those cases where temperature control is necessary the plumbing has been provided in the goniometer. Many standard temperature baths with external circulation capability are suitable. The ones provided by BIC are typically capable of regulating temperatures over a larger range than the sample cell assembly can maintain (approx. +5° to +80 °C). It is up to the user to recognize this fact and maintain the temperature within these limits.

At the low end of the range, condensation may become a problem. Consider insulating all hoses, inlet and outlet ports, and any exposed metal surfaces on the sample cell assembly. It may be necessary to blow a dry gas onto the exposed portions of the index matching vat to prevent condensation there. At the higher end of the temperature range a heat loss may require additional insulation.

Above approximately 80°C temperature stability decreases. Gradients in the index matching vat and sample cell contribute to systematic and random errors.

The system has been designed to maintain ± 0.2 °C stability over the range +5° to +80 °C, and over a length of about 3 cm from the bottom of the cell. Thus, the temperature control is suitable for use with most light scattering experiments within the range specified, except critical point studies. In this case the user is advised to provide his or her own

temperature control. For the most exacting work the user is advised to measure the temperature of the sample directly.

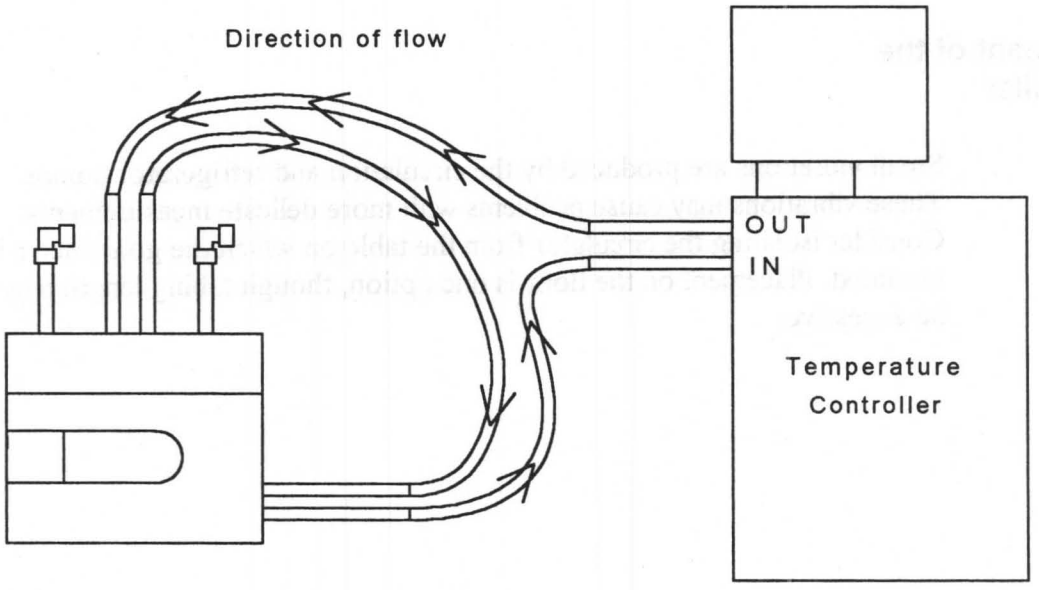
Setting Up the Controller

Be sure to read carefully the instructions supplied with the temperature controller before turning on the power. The reservoir in the controller needs to be covered with the bath liquid to within 2 or 3 cm from the top, and care should be taken when externally circulating liquid since the bath level will drop. Never expose the heater coils to air while operating because severe overheating and damage may occur. **Do not use pure water as the temperature control fluid in the bath**. Instead of water, use ethylene glycol (antifreeze). It is less corrosive and does not evaporate as easily. A 50% v/v aqueous mixture is also suitable.

Use hose clamps on all I/O ports. The inside diameter of the tubing should be nominally 3/8 inch (9.5 mm) as this matches the outside diameter of the 4 ports of interest.

Liquid circulates through a copper coil surrounding the sample cell holder sleeve. Then it flows through a hollow chamber under the sample cell assembly. This combination should prevent severe temperature gradients over the range specified. Severe gradients in the cell can cause convection. This may result in sinusoidal features in the correlation function, which complicate, unnecessarily, the data analysis.

Figure IV-19: Connecting the Temperature Controller



Attach the circulator output to either large-diameter port on the top of the sample cell assembly. See Figure IV-19. Connect a short hose from the other port to one on the sample cell assembly's bottom edge. Finally, connect the remaining port to the return port on the circulator. Tygon tubing is sufficient for use with ethylene glycol. Test the compatibility of the tubing with other liquids before using.

Arrange the hoses such that they do not interfere with any of the mechanical parts of the goniometer, especially when changing angles. It is convenient to use a ring stand for this purpose.

Equilibration Time

When changing temperature, you should allow enough time for the sample to come to thermal equilibrium. The actual time depends on the circulator bath size, flow rate, tubing length, and the temperature change. There is also a lag in time between thermal equilibrium of the circulator bath and the sample. With circulators supplied by BIC it takes about 15 minutes for the sample to reach 40 °C starting from room temperature. An additional 15 minutes is required from 40 °C to 70 °C, and the sample temperature will be about 0.5 °C less than the circulator bath temperature unless the hoses, brass I/0 ports, and black, metal dust cap are insulated.

When cooling it takes longer to reach equilibrium. From room temperature to 5 °C it takes about 1 hour for the temperature of the circulator bath to stabilize. The sample temperature is about 1.5 °C higher unless extra, external insulation, is provided.

Placement of the Controller

Small vibrations are produced by the circulation and refrigerator pumps. These vibrations may cause problems with more delicate measurements. Consider isolating the circulator from the table on which the goniometer is mounted. Placement on the floor is one option, though tubing length may be excessive.

Circulation/Filtration System

To reduce stray light, index matching liquid surrounds the sample cell. This translates the air-glass interface to the outer surface of the vat which has a much larger diameter than the cell. Thus, a much wider range of scattering angles is usable without interference from stray light. In addition, stray light from scratches on the outer cell walls is minimized.

The ideal index matching liquid has a refractive index around 1.5, a low vapor pressure, and is easy to pump around a filtration system to remove dust. We recommend using cis-trans decahydronaphthalene, trade name Decalin, as the index liquid. Toluene, a known carcinogen, is no longer recommended for index matching. **Do not use water.**

The optional BI-FC filtration/circulation system consists of a pump, tubing, filter holder, clamp, tweezers, filters and connectors. The Teflon, graphite gear pump turns at 1550 rpm, delivers about 0.130 liters per minute of a 1 centipoise liquid and has a normal operating pressure of 15 to 20 psi. Operate the pump only when it is necessary to remove dust from the index liquid. Initially, for a dusty liquid in a dusty vat, this may take several minutes. Thereafter, the pump need only be used for a minute or two. A filter change will also be required after the first few minutes.

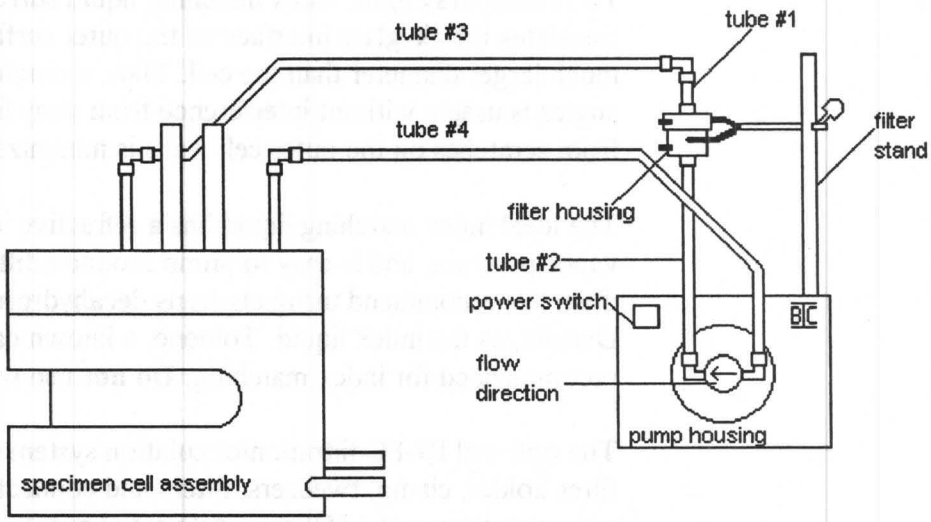
BI-FC Connections

See Figure IV-20. Attach tube #3 to one of the Teflon elbow connectors. Insert the tube until it bottoms in the connector housing, and then tighten the nut by hand. Attach the other end of the connector to one of the small diameter ports located on the specimen cell assembly. Use the same procedure to attach tube #4 to the other port.

The NPT elbow connectors should already be attached to the pump head. If they are not, remove the pump head to attach them. Otherwise, they can be cross threaded and damaged. Attach tube #4 to the inlet side of the pump head. (Look at the arrow on the pump head.) Attach tube #2 to the outlet side. Attach the filter housing, with the filter installed, to the other end of tube #2. Clamp the filter housing to the filter stand. Attach tube #1 to the output side of the filter using one of the Teflon elbow connectors. Fasten this connector to the free end of tube #3. Check all connections.

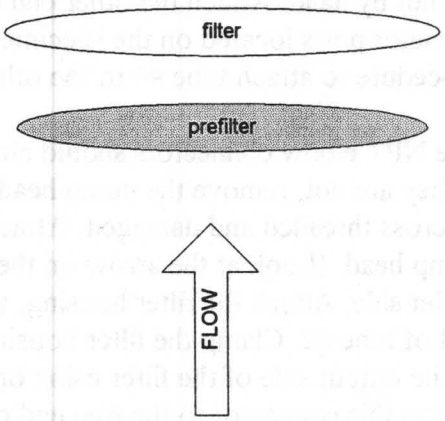
The filter housing, a 47 mm in-line type that is self-sealing (no o-ring), is also made of Teflon. The flow direction is marked on the side of the clear section. One hundred nylon filters are provided with the BI-FC. The pore size is 0.22 micron. This filter is compatible with most liquids. Always handle the membrane with the smooth-tip forceps that are included with the BI-FC system. Also supplied are 100 prefilters.

Figure IV-20: Filtration/Circulation System



Open the filter housing using the two, green plastic wrenches supplied, one on each end, fitted over the housing. Wet a filter with the index matching liquid and place the filter in the housing. Wet the prefilter, and place it with its smooth side against the filter. Center it as best you can; this will help prevent leakage. To seal the filters inside, tighten the housing with the wrenches. Later, if leaks develop around the housing, tighten it further. See Figure IV-20.

Figure 21: Direction of flow for the BI-FC



If you need more tubing, you may order it directly from the Cole Parmer Instrument Company, Chicago, Illinois, telephone 1-800-323-4340 (from the U.S.). Ask for the ¼ inch O.D. Teflon PFA tube, part number E06375-02.

Running the BI-FC

Before starting the pump you must add more liquid. Fill the vat until the level of the liquid reaches the middle of the cell holder sleeve. Turn the pump on.

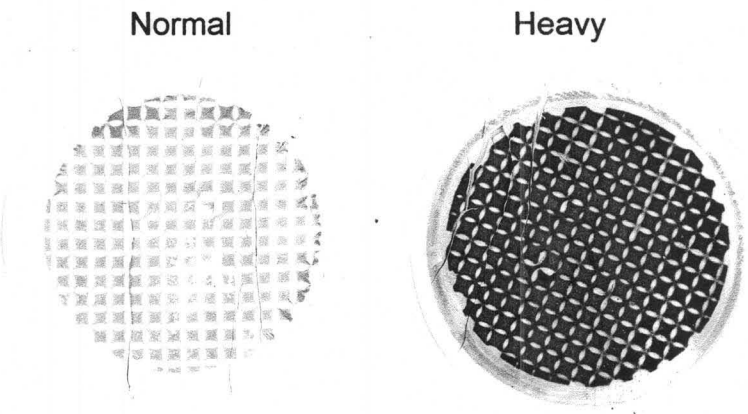
While it is running, check the level in the sample cell area. If necessary, add liquid until all the tubes and the filter housing is full. When the system is full, the level of the index liquid should be a few millimeters above the bottom of the cell holder sleeve. Run the pump until, at low angles, little or no dust is visible. Check for leaks. Tighten connections until the leaks stop.

With the pump stopped, the filter holder may be opened for filter replacement. If the amount of dust is not noticeably reduced after several minutes, change the filter. To change the filter, use the plastic wrenches to open the filter. Place a clean beaker under the filter to catch the liquid that spills. This liquid can be reused.

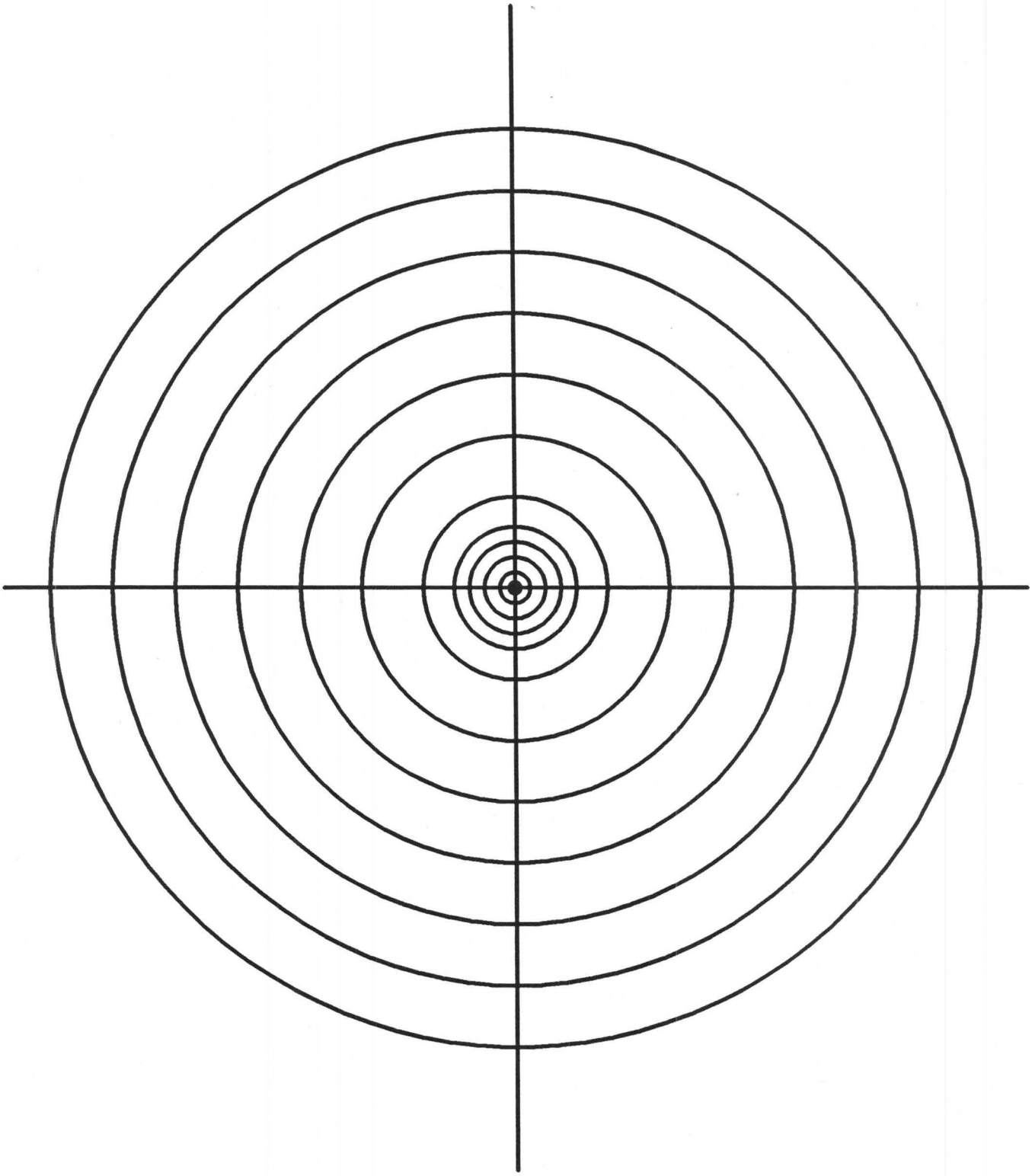
Do not pump the liquid during measurements of the scattered light. Pump vibrations may interfere with measurements.

Consider purchasing the optional BI-SFS sample filtration system for cleaning pure liquids and sometimes the samples themselves.

Figure IV-22: Examples of filter discoloration



TARGET



Section V: SAMPLE PREPARATION

Introduction

Sample preparation consists of three parts: solvent purification, cell cleaning, and solution/suspension preparation. The first two parts are described in varying levels of detail in this section. The last part depends on the particular type of sample. Some general guidelines are given at the end of this section, and the preparation of a polystyrene latex suspension in water is described in detail.

Good sample preparation is still an art that requires practice and patience. Once you have established a procedure that works you will find that it is worth the effort.

Dust

The two major problems in all types of light scattering measurements are stray light and dust. Brookhaven has designed the goniometer to minimize stray light. If you follow the alignment procedures, maintain properly your laser, and do not scratch your vat or sample cells, then stray light will not be your most significant problem. Dusty samples, however, will be a continuing problem. Dust is a general name given to any undesirable, large scatterer that contributes to the signal.

Your success in making good light scattering measurements will depend on your ability to prepare clean samples. Most often dusty samples limit the reproducibility of the results. Unfortunately, dust can also yield systematic errors. Molecular weights calculated from a Zimm plot may be much too high; the average size and breadth of the distribution calculated from a PCS measurement may shift towards higher values; and the size distribution algorithms used in PCS are particularly prone to adding artificial components to the distribution when measurements are made in the presence of dust particles.

Dust is much more of a problem in highly polar liquids, especially in water. It is often visible through the detector eyepiece as a short burst of light. Since dust is usually a large particle, the light scattered from it is more visible at lower angles. Sometimes its presence is easily detected during a PCS measurement by watching the buildup of the autocorrelation function on the video monitor. Sudden jumps in the entire function are due to dust. An obviously high value in a string of repeated intensity measurements indicates the presence of dust. Good sample preparation begins with removal of dust from the liquid used to dissolve or suspend the sample.

Dust can be removed by filtration or distillation, or it can be suppressed by centrifugation. Filtration is the easier method thanks to the availability of inexpensive, disposable filters. Multiple filtration is the key to effectively removing dust. Multiple passes are necessary because filters do not trap all the dust on the first pass. Distillation, however, may be necessary in order to deionize the liquid and to remove trace impurities. Centrifugation requires that a suitable method for cushioning the cell is employed. This technique is used if the sample cannot be filtered. The dust will eventually rise up into the path of the laser beam.

The next few sections describe several techniques for cleaning solvents. Choose the one most suitable for your particular application. Try the simplest one first.

Filtration

Water

To filter small quantities use a 20ml syringe with a 25mm, 0.2μ pore size, disposable filter. Choose one with a Luer-lock fitting. If a needle is required in your application, use a large diameter (18 gauge). Airborne dust is attracted to the increased surface area obtained by atomizing the water through the tip of a narrow-gauge needle.

Clean and flush a syringe and a needle several times to remove coarse particles which will clog a filter prematurely. If possible use filters manufactured without adhesive residues, often polymeric coatings, which add particles to the solvent being filtered. Flush the filter several times before using to remove these residues. Consider pre-filtering if liquids are heavily laden with particles. This will extend the life of the fine-pore filters.

To filter large quantities use a 47mm or larger filter housing with a 0.2μ filter. Attach the inlet of the filter housing to a water faucet. Run tap water through the filter for an hour prior to use with samples. This flushes the residue on the surfaces of the filter, though it also reduces the filter's life. In use, let the water drip into the cell or dilution bottle to avoid creating a large surface area.

Figures V-1 and V-2 demonstrate these two techniques.

Organic Solvents

If the solvent is compatible with the plastic filter housing and the filter material, then use the same procedures described above for water. If not, replace the housing with the stainless steel variety and the filter material with one suitable for use with the particular solvent. Compatibility charts are available from the manufacturers of filters.

Figure V-1: Solvent filtration, small quantities

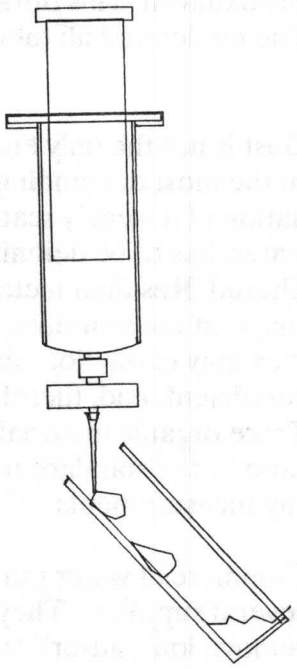
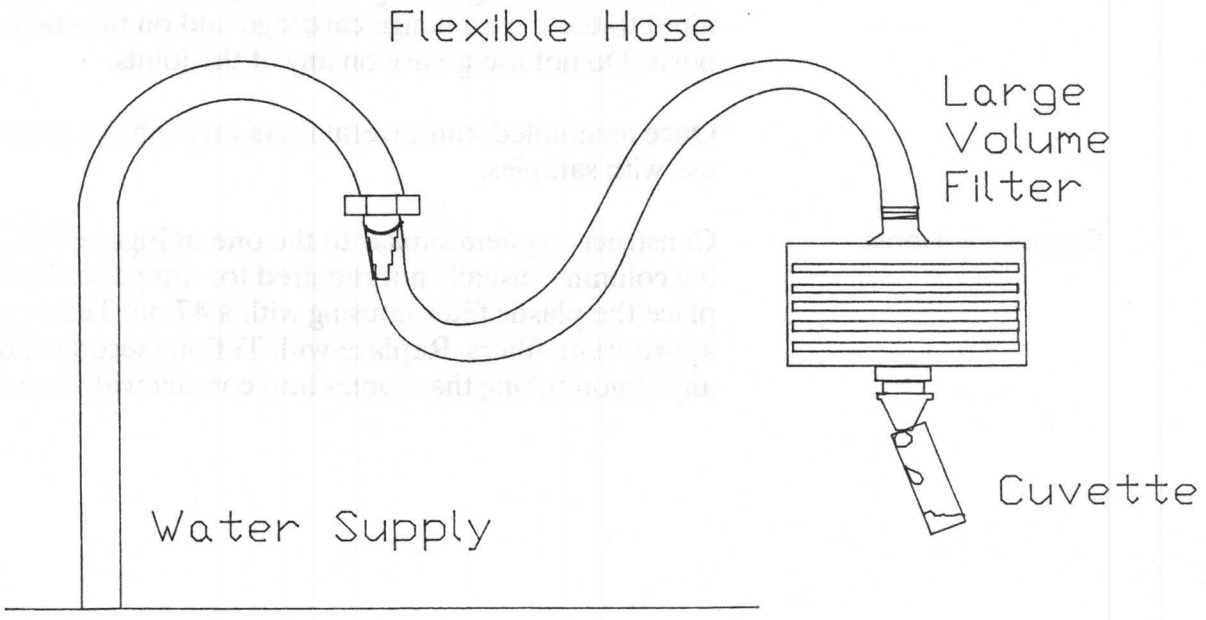


Figure V-2: Water filtration, large quantities



To filter small quantities use a 13mm or 25mm diameter, stainless steel filter holder and filters with a glass syringe. Be sure to choose o'rings that will not dissolve. Handle filters with smooth forceps to avoid puncturing them.

For larger quantities consider purchasing a second BI-FC from Brookhaven. This filtration system uses a 47mm filter holder. The holder and all tubing are made of Teflon.

Purification

Water

Dust is not the only enemy of light scattering found in liquids. In the most demanding situations--molecular weight determination of a weakly scattering biomacromolecule--the solvent, water, has to be deionized, free of trace organic impurities, and filtered. Residual metal ions may affect the chemistry and shape of the scatterer. Ions may help to stabilize a sample, or they may cause flocculation. Shape may affect the diffusion coefficient, and, therefore, the results of PCS measurements. Trace organic material may significantly alter the Rayleigh ratio of the standard used to calibrate the instrument for intensity measurements.

Commercial water purification systems are available from several suppliers. They consist of replaceable cartridges that remove ions, adsorb trace organic material, and filter to 0.2μ. They are convenient, compact, safe, and easy to maintain. They are, however, relatively expensive.

An alternate approach is shown in Figure V-3. An ion exchange cartridge is connected to a boiling flask/condenser followed by a collection bottle and a 0.2μ filter.

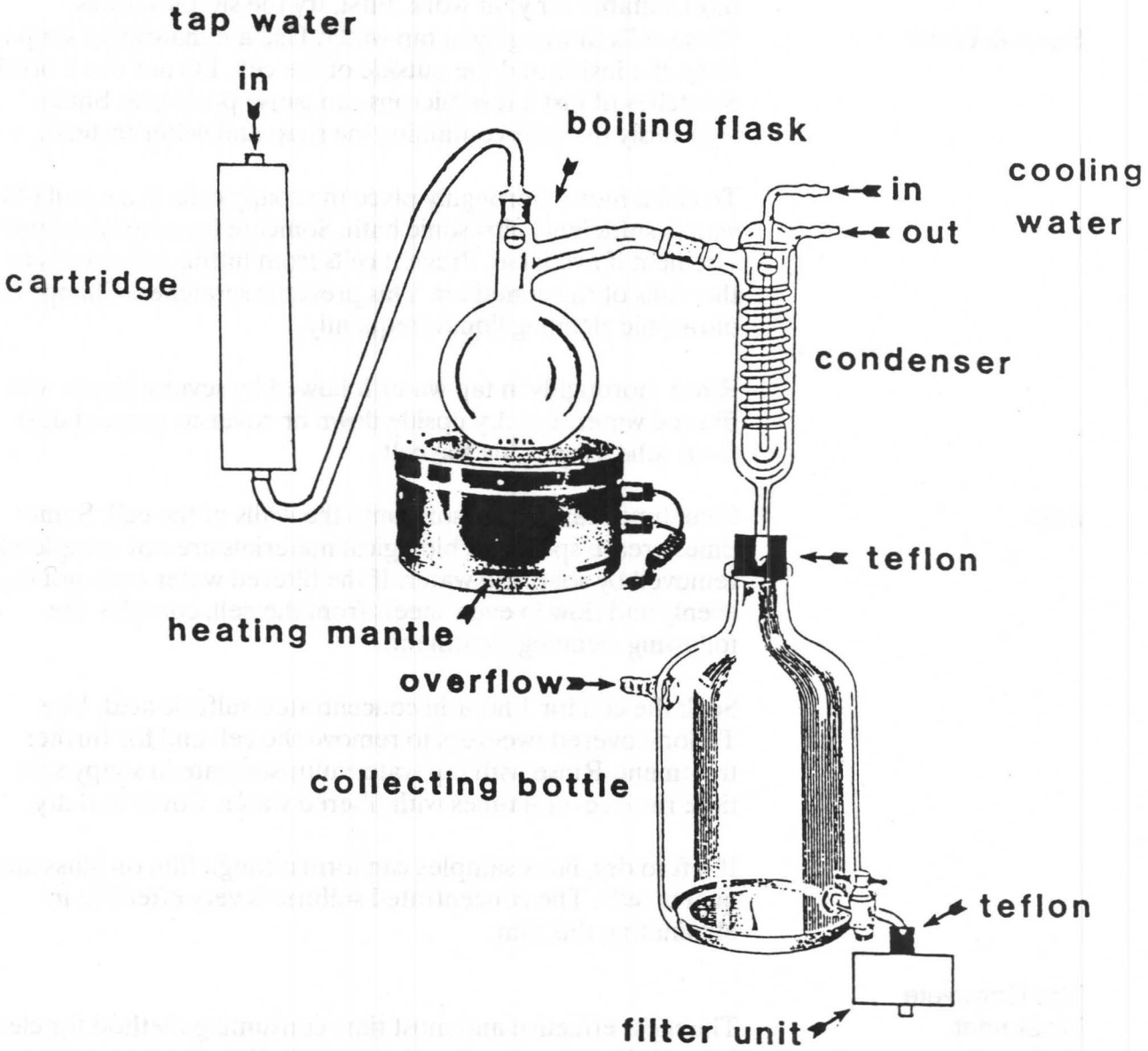
Use Teflon tubing throughout except use Tygon tubing on the input to the ion exchange cartridge and on the cooling water ports. Do not use grease on any of the joints.

Once assembled, run several liters through the system prior to use with samples.

Organic Solvents

Construct a system similar to the one in Figure V-3. A deionizing column is usually not required for nonpolar liquids. Replace the plastic filter housing with a 47mm Teflon one and appropriate filters. Replace with Teflon (second choice, Viton) any Tygon tubing that comes into contact with the solvent.

Figure V-3: Water purification. Deionize, distill, filter.



Cell Cleaning

Like solvent purification, the degree of cell cleaning depends on the application. The simplest case--for use with disposable, individually packed, clean cells--involves blowing off dust using compressed air. Avoid finger prints in the area where light enters or exits by holding the cell near its top.

More rigorous cleaning procedures follow. Choose the one most suitable for your work. First, try the simplest ones.

Soap & Water

Rinse cells thoroughly in tap water. Use a nonabrasive soap to clean the inside and the outside of the cell. Do not use a brush. Scratches of just a few microns can cause problems. Shake vigorously the cells containing the soap and water instead.

To clean more thoroughly place the soapy cells in a small (40 watt is sufficient) ultrasonic bath. Sonicate for several minutes. Use heat if available. Prevent cells from hitting other cells or the walls of the container. This prevents scratches. Change the ultrasonic cleaning liquid frequently.

Rinse thoroughly in tap water followed by several rinses with filtered water. Let dry upside down or cover to prevent dust from collecting inside the cell.

Acid

Sometimes samples adsorb onto the walls of the cell. Sometimes grease spots and biological materials are not completely removed by soap and water. If the filtered water does not dry evenly and flow in even sheets from the cell, consider the following cleaning treatment.

Soak the cell for 1 hour in concentrated sulfuric acid. Use Teflon-covered tweezers to remove the cell and for further treatment. Rinse with tap water; ultrasonicate in soapy solution; rinse several times with filtered water. Cover and dry.

If left to dry, latex samples can form a tough film on glass and quartz cells. The concentrated sulfuric is very effective in eliminating this film.

The Complete Treatment

The most effective and most time consuming method for cleaning cells involves a series of steps including: a strong acid, a strong oxidizing agent, a strong reducing agent, steam test and oven dry.

Prepare the following solutions using distilled and deionized water.

Solution A: 16g NaOH in 200mL water. Add 0.7g $KMnO_4$. Swirl until uniformly purple. This is a strong oxidizing agent.

Solution B: Dilute 32mL of conc. HCl to 200mL. Add 70mg hydroxyl amine hydrochloride. This is a strong reducing agent.

Store these solutions in reduced light at room temperature. They have a useful shelf life of 2 to 4 weeks. Excessive contamination will reduce their effectiveness. Pour about 100mL of each of these solutions and conc. sulfuric acid into 3 separate 200mL beakers or dishes. Cover the these containers when not in use to prevent contamination. Clean these containers and covers prior to use with soap and water. Rinse with filtered water.

During the cleaning procedures outlined below use Teflon-covered tweezers to handle the cell. Use the tweezers to grab the cell around its edges at the bottom or top, but never touch the cell surfaces directly, especially where the light beam will enter or exit.

Follow these procedures to clean each cell:

1. Soak cells for 30min in conc. H_2SO_4. Rinse thoroughly in tap water.

2. Soak for 10min in Solution A. Rinse in tap water.

3. Dip cells in Solution B two or three times. Empty cell after each dipping. Rinse in tap water.

4. Rinse cells, inside and out, several times with deionized, distilled, filtered water.

5. Steam the cell as shown in Figure V-4.

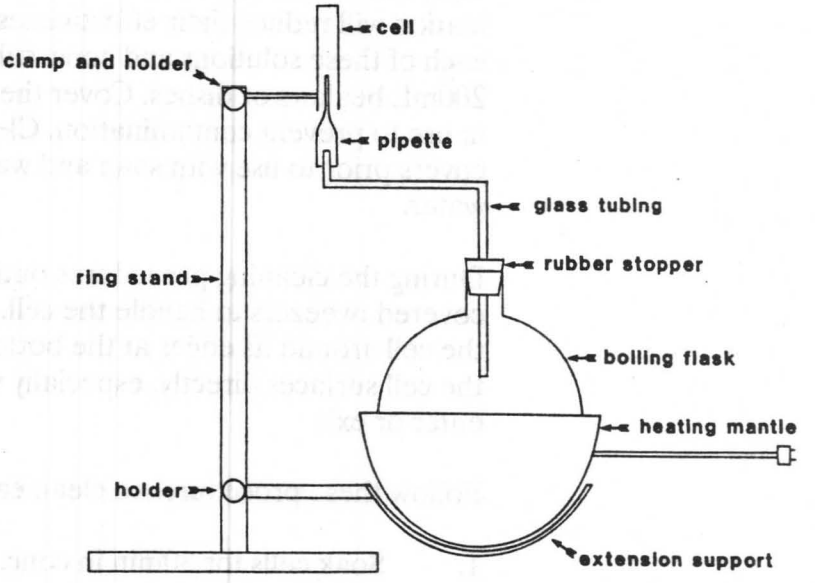
If the cell has been cleaned well then the outside will dry without spotting, and water will evaporate in a continuous sheet. Water will not condense on the inside of the cell, and the steam will roll off in a continuous sheet.

If the steam test fails, do not proceed. Start over. Try longer soakings and cleaner solutions. Particles which remain on the cells act as nucleation sites for the steam to condense in a discontinuous manner.

6. Place the cell in a smooth piece of loosely fitting aluminum foil. Dry in an oven at 80°C for 1 hour.

7. Keep cells covered until ready for use.

Figure V-4: Apparatus for steam-testing the cells.



The steamer is constructed from standard parts. The glass tubing between the boiling flask and pipette must be made by hand. The pipette is a standard Pasteur type that has been cut to a suitable length. It rests freely on top of the glass tubing. Use glass wool between the heating mantle and boiling flask. Use a gram of boiling chips in the flask. Clean the pipette, tubing, flask and boiling chips with deionized, distilled, filtered water prior to use. Let the steam flow for about 5 minutes before clamping the cell into position. Cover the pipette tip when not in use.

Solution/Suspension Preparation

Generalizations

The first and last rule is this: clean everything with purified and filtered solvent. The list is endless: cells and caps, dilution bottles and caps, transfer pipettes or syringes, glassware for buffer solutions and surfactants, etc. Prepare buffer and surfactant solutions with purified and filtered solvent.

Compressed air is usually sufficient for removing dust from plastic, disposable pipette tips, but not from glass ones, especially if they have been left uncovered collecting grease and dust.

Minimize the contact between air and liquids used in the final phase of sample preparation. This reduces the chance of reintroducing dust. Wherever possible, use direct connections, avoiding contact with air. Do not store filtered water for long periods. Bacteria, which scatter light nicely, grow in stored water. Prepare small amounts of fresh solutions daily if possible. If not, filter prior to use.

Where possible, never spray liquids into flasks, bottles or sample cells. Allow liquid to run down the side of a clean, smooth surface. Less dust is reintroduced this way.

Never shake samples violently once prepared. This may entrap dust laden air and dissolve air in the solvent. Bubbles ,too small to see, scatter more light than most particles of interest. Gentle swirling is best. Adding diluent to a concentrated drop of sample promotes uniform distribution faster than adding the drop to the diluent.

When making true molecular solutions, follow the advice given in specialized literature sources. Beware of gels and other supramolecular species which can dominate the light scattering and lead to false conclusions, especially at low angles.

When preparing stable suspensions for particle sizing , t is not always easy to decide about wetting agents, surfactants, and sonication.

If particles clump together , and obviously do not go into sus pension, try wetting the particles with a few drops of ethanol first, followed by water.

Do not overuse surfactants. Beyond the critical micelle concentration (typically around 10^{-4} to 10^{-5} vol. %) a surfactant can actually promote flocculation.

A useful list of wetting and dispersing agents is found in Table 8.1 in Particle Size Measurements, 3rd edition, T. Allen author, Chapman and Hall publishers, New York. A new edition is expected soon.

The world is divided about sonication. In some cases it breaks apart unwanted agglomerates. In other cases it promotes agglomeration because of increased collision frequency. In still other cases it is the presence of agglomerates that you want to measure. Try measurements with and without sonication. Can you explain the differences?

Sample preparation is, ultimately, a topic under the more general category of colloid stability. Colloid stability is an old, rich, and interesting field of study. Without knowing the surface chemistry of the particles you are trying to suspend, you may, by addition of surfactants and ions, either stabilize or destabilize the suspension.

Some systems require special knowledge. Silver halides used in the photographic industry are often prepared with a gelatinous binder. Light scattering measurements are dominated by large gel and gel/particle agglomerates. unless this binder is digested with a special enzyme.

The best chance you have of making a good measurement is to consult the literature. Contact Brookhaven for sources and suggestions.

Preparing a Latex Suspension

For your first PCS measurement ,choose a narrowly distributed, polystyrene, standard latex of about 90nm. A dilute aqueous suspension of this standard will scatter very well and serve as one test of the alignment. You will also gain experience using the goniometer and correlator by measuring the particle size of this standard.

While there are several sources of polystyrene standards, not all sources are equally reliable. The Duke Scientific Corporation in Palo Alto, California and Bangs Laboratories, Inc. in Carmel, Indiana supply consistent standards.

Make 200ml of a 10mM NaCl solution. Use a disposable 0.2μ filter to clean the liquid. Rinse thoroughly a 25mL dilution vial and its screw-on cap with the filtered solution. Rinse a suitable sample cell and its cap. Cover both until ready for use.

The latex samples come in small, plastic, squeeze bottles. They are usually full of surfactant. No more need be added. The concentration varies from 2 to 10% solids by weight. Squeeze about 4 drops into the dilution vial. Add about 20ml of filtered water. The result is a slightly turbid dispersion suitable for measurement with a 5mW, HeNe laser. If your laser is considerably more powerful, use only one drop, or dilute in two stages.

Place the vial in the path of the laser beam. A distinct scattering line should be visible. If the line is thick and fuzzy, the sample is too concentrated. Dilute it. If the line is extremely weak, not much more visible than filtered water, the sample is too dilute. Add more latex. If random bursts of light are readily apparent, prepare a new sample paying more attention to cleaning the dilution vial and water.

Ultrasonicate the sample in the dilution vial for a total of about 2 minutes using short burst, perhaps 10 seconds, of sonic energy followed by a few seconds of no energy.

Clean a BI-RC12 or equivalent round glass cell. Use the soap and water technique described in this section. Do not use anything abrasive. Rinse thoroughly with tap water followed by filtered water. Rinse the Teflon cap. Gently pour the sample from the dilution vial into the cell. Cap it.

If wet, dry the outside of the cell prior to placing it in a sample holder. Do not wipe the cell with an abrasive towel. Pat it dry, or roll it gently on an adsorbent paper, preferably one designed for use with optical quality glass. Keep this sample for use with PCS measurements as described in the next section in this manual.

Section VI: PCS MEASUREMENTS

Introduction

This entire section is dedicated to the novice. Its purpose is to teach the first-time user of laser light scattering equipment how to make a PCS measurement from start to finish. A 90nm standard polystyrene latex suspension has been chosen as an example.

Latex spheres have an honored place in these types of measurements. They were first used to demonstrate the technique of light beating spectroscopy (1), the precursor of PCS, and they are often used as a check that the entire system is operating correctly. In addition, PCS measurements on latexes are quite commonly made for quality control and research purposes.

Several useful generalizations will be given for making and interpreting measurements. Some of these rules are documented in the literature. Others are approximations. The user is urged to read the extensive literature on PCS. Some of the early reviews on the subject are listed as references (2-7) at the end of this section.

It is assumed that the user has a Brookhaven goniometer and correlator. Reference will be made to features on these instruments. It is further assumed that the goniometer is aligned; the laser is warmed up and the beam is blocked; the high voltage is on; the filtration/circulation system has been used to remove dust from the index matching liquid; and all the cables have been connected.

Measurement Conditions

Rotate the detector arm to 90° At this angle the effects of flare, dust, and misalignment should be insignificant.

Use the sample prepared in the last section. Place the 12mm cell in one of the brass, round-cell holders. Choose the one with the closest fit. Do not force it into the cell holder. Clean out any dust in the holders prior to inserting the cell to prevent scratching.

Make sure the laser beam is blocked. It is a good practice to block the beam whenever it is not in use. Do this for safety reasons and because it prevents a flash of very intense light from damaging the photocathode whenever the cell is changed. Place the cell and holder in the goniometer. Unblock the beam. Rotate the mirror in the detector optic to the down position

(counterclockwise). View the scattering volume through the eyepiece. Look for a line of light across the slit in the detector optic. The line should be uniformly bright with no random bursts from dust.

Interference Filter

Use the filter turret on the detector optic to select the interference filter corresponding to your laser wavelength. These positions are marked 488, 514, 633, O for open, and C for closed. The filters prevent room light from reaching the detector. They allow you to run experiments in room light without covering the entire goniometer. However, the filters do reduce the amount of scattered light reaching the detector. Use the O position, which has no filter at all, to increase the signal. In this case, turn off the room lights. Light scatterers are known for working in the dark. Use the closed position as a shutter to protect the photomultiplier tube when not making measurements.

Pinhole Selection

Select the 200μ pinhole using the pinhole turret on the detector optic. This corresponds to about one coherence area, which is the optimum choice for PCS measurements. For weak signals you may have to compromise by using the 400μ pinhole, and for strong signals choose the 100μ pinhole. For PCS measurements never use the 1, 2 or 3mm pinholes.

Temperature/Viscosity

For this preliminary measurement it is not necessary to control the temperature. It is, however, necessary to know what the temperature is in order to obtain the viscosity of water. The viscosity is needed to calculate the particle size from the measured diffusion coefficient. A brief table follows:

Viscosity of Water in Centipoise

t(C°)	n(cP*)	t(C°)	n(cP)
15	1.14	26	0.871
16	1.11	27	0.851
17	1.08	28	0.833
18	1.05	29	0.815
19	1.03	30	0.798
20	1.00	31	0.781
21	0.978	32	0.765
22	0.955	33	0.749
23	0.933	34	0.734
24	0.911	35	0.719
25	0.890	36	0.705

1 cP = 1 mPa•s

If the temperature of the sample changes by as much as a degree during long measurements, then an error of about 2% occurs in the calculation of the particle size.

If you choose to use the temperature controller, wait for the sample to reach equilibrium before making measurements.

Making the Measurement

Turn on the correlator as described in the BI-2030AT or BI-8000AT manual. Wait until the default values have all been set. Both correlators use control files that automatically set defaults such as angle, temperature, wavelength, and other parameters. These defaults are contained in the auto-zero control file as explained in the correlator documentation. If these values do not correspond to the configuration you want, change them by ollow ing the instructions in the correlator manual .Alternatively, press the PAGE SELECT key and PAGE 1 to select appropriate values.

Rotate the mirror in the detector optic to the up position (clockwise). Scattered light is now able to reach the detector.

Count Rate

Check the count rate as follows. Set a duration of 1 second and a sample time of 10μsec. Press the START key. The count rate is displayed in the middle of the screen in Kcps, kilocounts per second. It should be between, say, 10^4 and 2×10^5 counts per second, depending on your laser power and latex concentration.

The measurements will fluctuate about a mean. The standard deviation of the distribution of count rates is the square root of the number of counts. Therefore, low count rates will fluctuate more than high count rates.

If the count rate is zero, check that the HV is on, set properly, and the cables are all connected. If your count rate is below 1000, check that the beam is not blocked, the interference filter is correct, the aperture is 200μ, and the mirror is up.

You can increase the count rate by increasing the laser power, removing the interference filter (the open position), increasing the aperture to 400μ, or increasing the concentration.

If the count rate is greater than 2×10^5, lower it. Decrease the laser power; decrease the aperture to 100μ; or decrease the concentration.

CTRL File F1

NUe a control file to automatically make the measurement. Hold the CTRL key down and press F1. A 100 second measurement is initiated with the following steps. First, a short measurement is made to estimate the 1/e point of the correlation function. Second, the correlator settings are adjusted such that the last channels nearly touch the baseline. Third, after the measurement is complete, the results are automatically calculated using the measured baseline. Fourth, if you purchased the optional BI-ISDA set of programs, then the NNLS algorithm is employed to determine the size distribution.

Auto Sample Time

A special command is available for setting the sample time spacing. This command is initiated by pressing SHIFT 2 (@). Press it to initiate a measurement. This is the same command used in the control files.

You may wish to set the correlator controls manually. A lot can be learned by the novice doing it manually the first few times. First you need to estimate an appropriate sample time.

Sample Time Calculation

To calculate a sample time you will need an estimate of the particle size, in this case 90nm. Calculate the diffusion coefficient from the Stokes-Einstein equation

$$D = k_B T/(3\pi n d) \qquad \text{where,}$$

k_B = 1.38054×10^{-16} erg/°K, Boltzmann's Constant
T = Absolute temperature in °K = t(°C)+273.15
n = Viscosity of liquid in which particle is suspended
d = Particle diameter

At 22.5°C, $D = 5.12 \times 10^{-8}$ cm^2/s for a 90nm latex particle in water.

The linewidth, Γ, is calculated from D and the magnitude of the scattering wave q as follows:

$$\Gamma = Dq^2 \qquad \text{where}$$

$$q = 4\pi n \sin(\Theta/2)/\lambda_o \quad \text{where,}$$

n = index of refraction of suspending liquid
Θ = scattering angle
λ_o = laser wavelength in air

For the assumed case of water, n = 1.33, and for a HeNe laser, the wavelength is 0.6328 micron. With Θ = 90°, Γ = 1787 rads/s.

For a monodisperse sample set the sample time such that the measured autocorrelation function spans 4 exponential decays. This criterion results in a total span T of,

$T = 2/\Gamma$, which is 1120μsec for this example.

For a linearly spaced correlator with 72 channels, such as the BI-2030AT without the BI-4T multiple sample time option in use, set the sample time at $1120/72 \approx 15\mu$sec. For 136 linearly spaced channels set the sample time at approximately 8μsec.

If you are using the BI-4T option, then adjust the base sample time and the exponents in the multiple sample time option untilthe last correlation channel stretches out to approximately 1120μsec. For example, set the base sample time at 2μsec and the multiple sample time exponents at 1, 2, and 3 for a 136 channel BI-2030AT with the BI-4T. See the correlator manual for details.

If you are using the BI-8000AT correlator adjust the number of channels, the ratio spacing between successive channels, and the first sample time such that the last correlation channel stretches out to approximately 1120μsec. For example, set the number of channels at 10, the ratio spacing at 2, and the first sample time at 2μsec. See the correlator manual for details.

If you have no idea what the sample time should be use the @ command to make an estimate. Often this will find the best choice. If not it will be very close, and you can manually increase or decrease the span of the correlation function to make a better choice.

Overflows

Once the sample time is set properly check for overflows on the BI-2030AT. (The BI-8000AT does not overflow.) Use either the 4-BIT OVERFLOW LIGHT on the correlator or the OVERflow counter displayed on the video monitor to estimate the overflows. When the light flashes occasionally or the OVERflow counter is less than about 0.1% of the A TOTAL counter, then do not worry about overflows. If the light flashes semi-continuously or more, increase the integer exponent by one, using PRESCALE A, until the flashing decreases to occasional flashes or stops all together.

Make a Run

Set the experiment duration for 100 seconds. Press START. If you have followed all the suggestions above you should see a smooth correlation function buildu-p which will decay smoothly to the baseline.

Press the CALC key. You are asked to choose a baseline, either the measured or calculated. If they agree to within 0.1%, choose the calculated; otherwise, choose the measured.

The calculations are described more fully in the correlator manuals. HCncentrate on two values: the EFF. DIAM. and the POLY. The EFF. DIAM. should be within a few percent of the reference value printed on the bottle, and the POLY should be 0.02 or less, indicating a relatively narrow distribution.

Variations on a Theme

Once you have a sample and preparation techniques which result in repeatable results try the following variations to familiarize yourself with the system and technique.

Angle

Repeat the measurements at 30°, 45°, 60°, 120°, 135°, and 150°. At each angle determine the count rate, set the appropriate sample time, check for overflows (if any), and set the duration. Determine the angular range over which your system gives the same results. Eventually, as the angle decreases, the correlation function will decay more slowly resulting in a larger EFF. DIAM. and POLY. This occurs because of either alignment, stray light, true aggregation, or dust.

Alignment and stray light can be checked by measuring the scattering from a pure liquid as described in the section on intensity measurements. Change your sample or sample preparation techniques to check for aggregates. Compare with other techniques such as a disc centrifuge. Dust is the most likely cause of failure with aqueous suspensions at low angles. Try again.

Concentration

At one angle vary the sample concentration. At low concentrations longer durations are necessary to get smooth, statistically meaningful results. At high concentrations there will be interparticle effects. Plot the size versus the concentration. Extrapolate into the dilute regime. It is in this regime where the Stokes-Einstein equation is valid. Count rates vary with concentration. The PRESCALE on the 2030AT may need changing.

Duration

The random error in the results is a function of the smoothness of the correlation function. The longer the duration the smoother the function. becomes Like all counting experiments the decrease in the random error goes, roughly, as the square root of the duration. This has a diminishing returns effect. Try a few different durations to see if the results are worth the extra time.

Sample Time

Vary the sample time. Notice its effect on the results. Short sample times emphasize the faster moving, smaller particles in any distribution. Longer sample times emphasize the slower moving, larger particles. Even with a monodisperse sample, some changes in calculated results will occur. Be sure to keep the duration the same for this type of comparison.

Baseline

Repeat the calculations using both baselines. Note the differences. At lower angles, where dust is the most likely problem, the measured baseline is higher and gives better results than the calculated baseline. Also, the POLY should decrease, under these conditions, when using the measured baseline. Do not be fooled by statistical errors. If the baselines agree to within 0.1% or so, then no statistically significant difference between the sets of results will occur. As the baseline differences increase, the results will differ. Negative baseline differences indicate the run was too short. Except for statistical error, the calculated baseline should always be lower than the measured baseline.

Prescaling Effects

Prescaling is only of importance with the 2030AT correlator. To test prescaling, choose a high sample concentration or low angle or high laser power where prescaling exponents of 2, 3 or 4 are necessary to keep the overflow light from blinking more than occasionally. Repeat experiments as a function of the prescale exponent. In order to keep the statistics constant, you will have to double the duration each time the exponent increases by 1.

When the prescaling is too high some distortion in the function will occur; however, the largest effect is to increase unnecessarily the experiment duration. If the prescaling is too low, the correlator channels fill up rapidly with a more or less constant value. Indeed, with significant overflowing of the 4-bit input counter, a nearly flat correlation function obtains. Little or no useful information can then be calculated.

Coherence Area

The pinhole size affects the ratio of coherently scattered to detected light. The coherence area surrounding a point in space is the area over which the phase of the scattered light does not differ enough to cause significant destructive interference. TI is the area over which there is a bright speckle of light. This area is surrounded by other such speckles separated by dark areas representing destructive interference. These light and dark areas are shifting around randomly in time because the source of the scattered light, the particles, are moving. If a light sensitive detector is placed behind an aperture, the intensity registered by the detector will fluctuate in

time as the speckle pattern moves randomly across the detector aperture.

If the detector aperture is large compared to a single speckle, a coherence area, then la highintensity is registered with very little fluctuation since, for a large number sof peckles, as many speckles enter as leave the detector area. Use the 800μ or 1mm pinhole setting to test this. The count rate is high, but the exponential decay of the correlation function is hardly noticeable above the baseline. (Use the RAW DATA mode to see this effect. Be sure to decrease the scattered light such that the count rate is less than a few hundred thousand counts/second. Use a low power setting on the laser, low concentration, and 90°.)

If the detector aperture is comparable in size to a coherence area, then less intensity is registered with, however, large fluctuations. Every speckle entering and leaving the detector area causes vast swings in the instantaneous intensity. Use the 200 or 400μ pinholes to test this. Since the count rate is lowered by approximately the ratio of the square of the detector diameters, you must collect data for a longer time to get smooth curves. However, the rise above the baseline is 50% or more. Since this rise contains the significant information, this is the reason the final pinhole should be comparable in area to the coherence area.

References

For more information on making measurements, the theory of PCS, and data interpretation, consult some of the following references:

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Section VII: INTRODUCTION TO INTENSITY MEASUREMENTS

Introduction

In this section intensity measurements are described for testing the goniometer alignment. In addition the basic concepts for any intensity measurement are presented.

The formalism of light scattered is expressed in terms of the radiant intensity (watts/steradian) of the scattered beam and the irradiance (watts/cm^2) of the incident beam. Both these quantities are proportional to the radiant flux (watts), the total radiation per unit time received from a source. The source can be either the mean beam or the scattering particle. The radiant flux is often called the intensity, even though this can be confused with the radiant intensity. In photon counting measurements the radiant flux is proportional to the number of photons per second.

Counting

Count Rates

The number of photon counts per second is called the count rate. In photon counting measurements the terms count rate and intensity are used interchangeably, even though they are only proportional to each other.

In the latest versions of Brookhaven correlator control programs the count rate in kilocounts/second (Kcps) is displayed in the middle of the video monitor during a measurement. Alternatively, divide the total counts measured by the total duration. Even easier, set the duration to 1 second and the sample time to 1μsec. Press START. The total counts are then equal to the count rate. Use the REPEAT MEASUREMENT key to initiate multiple measurements of the count rate.

The count rate is displayed in the upper right hand corner of the video monitor when running the alignment software, BI-IST, or the Zimm plot software, BI-ZP. During the measurement the total counts for the duration set (it may be more than 1 second) are displayed in the middle of the screen.

Counting Statistics

Repeated measurements of the total counts from a source with constant intensity are not, themselves, constant. The measurement process is statistical in nature as is the emission of light from the source. The distribution follows Poisson statistics; therefore, the relative standard deviation of the total number of counts I_{tot} is given by,

$$\sigma/I_{tot} = (I_{tot})^{-1/2}$$

The relative standard deviation as a percentage is shown below for various total counts.

I_{tot}	Rel. Std. Dev.
10^2	10.0%
10^3	3.2%
10^4	1.0%
10^5	0.3%
10^6	0.1%

With the above as a guide it is easy to estimate the total counts necessary to achieve a specified level of statistical error. Suppose you are aligning the goniometer, and you want the systematic error (center of rotation, flare, etc.) to be less than 1%. Random errors at this level could be confused with systematic errors. You decide, therefore, to count up to at least 10^5 counts at each angle. If your count rate is 20,000 cps, you need to measure for 5 seconds to achieve your goal.

Dark Counts

Even in the absence of any light the detection system will register some counts. These are called the dark counts, and the number of dark counts per second is called the dark count rate.

Dark counts are due to a number of effects within the photomultiplier tube, chief among them is thermionic emission. Cooling the tube below ambient lowers the dark count; however, it produces afterpulsing, which is very bad for PCS measurements. Lowering the high voltage lowers the dark counts, but it also lowers the gain and stability. Leave the high voltage at the setting specified in the configuration section.

Dark count rates vary considerably between tubes, even tubes of the same type. Photon counting tubes with dark count rates from under 10 to over a thousand per second are available. As long as the dark counts are stable and much smaller than the signal of interest, any rate can be tolerated.

Measure the dark count rate as follows. Block the light using one of the closed (C) positions on the detector optic. Make several one second measurements. Notice that the values seem to fluctuate. This is a direct consequence of the counting statistics discussed in the previous section. To reduce the error in measuring the dark count rate make measurements for longer periods, subsequently dividing the total counts by the period to obtain the rate.

Repeat the measurements with the room lights off or by covering the detector optic. Is there a systematic difference between the rates? Be careful! Statistical counting errors can easily be mistaken for systematic errors. If there is a real difference, locate its origin.

Dead Time

Every photon counting system has a dead time, T_d, during which incoming photons are not recorded. The measured count rate I_m, therefore, is always lower than the true count rate I_t. The dead time is 100nsec when using the 2030AT correlator; it is 10nsec when using the 8000AT correlator.

A first-order correction, useful when the correction is 10% or less, is given by,

$$I_t = I_m/(1-I_m T_d)$$

The percent error in neglecting the dead-time correction is given by,

$$\% \text{ Error} = 10^2 \cdot I_m \cdot T_d$$

The magnitude of this error depends on the dead time and the measured count rate. For dark count rates it is negligible. For the 2030AT with a dead time of 100nsec the correction is 1% at 10^5 counts/sec. Above 10^6 cps, the correction is no longer very accurate. Count rates above a million, however, are not likely to occur in practice.

Laser Stability

The long-term stability of a laser may be the limiting factor in intensity measurements. Allow your laser to warm up for the time specified in its operator manual.

The small, cylindrical HeNe lasers warm up in about 20 minutes. Their stability is better than ±0.5%, provided room temperature fluctuations are less than ±2 or 3°C. The newer Argon-ion lasers, especially the ones with light control mode, are also stable to better than 1% after warming up. The 35mW, HeNe lasers are also stable to 1% or better. It is the older 15mW, HeNe lasers that can drift by as much as 5% over an hour.

Test the stability of the laser by making intensity measurements as a function of time at 90° on a dust-free liquid like toluene. Make the measurements every minute or so for an hour.

Angular Intensity Measurements

The alignment of the goniometer can be checked by measuring the intensity scattered from a pure fluid such as the index matching liquid. Do not attempt these measurements until the filtration/circulation system has thoroughly removed most of the dust; otherwise, your measurements at low angles will continually fail. Make sure to turn off the pump when making these measurements.

At first make measurements without any sample cell. Look at the scattering from the index matching liquid. In this way you can separate sample cell problems from the rest of the alignment.

If you are using cis-trans decahydronapthalene (decalin) as the index matching liquid and a 5 or 10mW, HeNe laser, then you may have problems getting enough scattering. In this case fill the largest round cell you have with filtered toluene. Make the measurements described below on the toluene.

Rayleigh Scatterer

A Rayleigh scatterer is defined as one that scatters equally in all directions. The scatterers are much smaller than the wavelength of light. Molecules of toluene and decalin satisfy this criterion If the intensity from these scatterers is measured as a function of angle, then the results should be independent of angle if the system is working properly.

The detector does not register the intensity from a single scatterer. It registers the signal from a cylindrical scattering volume. At 90° the minimum scattering volume is in view, and as the angle varies on either side of 90° the length of the cylinder contributing to the scattering increases. The length increases as $1/\sin(\Theta)$. This is called the volume correction. By multiplying the intensity by the sine of the scattering angle, a value proportional to the intensity per scatterer is obtained. It is this value that should be angle independent for a Rayleigh scatterer.

The optional, BI-IST software is designed to help the user check and maintain alignment. Measurements of $I \cdot \sin(\Theta)$ are measured and plotted as a function of Θ. The results are normalized first to the intensity at 90° and then to the average intensity. Various errors in alignment can be seen from these plots.

$I \cdot \sin(\Theta)$ measurements can be made by hand. The principles are described below.

The Count Rate and Duration

Rotate the detector arm to 90°. Measure the count rate. Adjust the laser power and/or the final aperture until the count rate is approximately 100,000cps. Then the dead-time corrections can still be made, even at 15° where the count rate will be approximately 386,400cps [100,000/sin(15)].

With an Argon-ion laser use the 1 or 2mm aperture to achieve the 100,000cps target. With a low power HeNe or with decalin as the index matching fluid, you may measure considerably less than 100,000cps. In this event use the 3mm aperture. Also, increase the duration from 1sec to 2, 3, 4, or 5 seconds until approximately 100,000 total counts are registered. Remember, the statistical error is related to the total counts, not the count rate. Increasing the duration has a disadvantage: the number of measurements ruined by dust.

Measurements & Calculations

Make 5 measurements at each of the following angles: 20°, 30°, 45°, 60°, 90°, 120°, 135°, 150°, and 160°.

At each angle calculate the average after deleting any values that are too high. These were probably affected by dust.

Convert the average to a count rate by dividing by the measurement period.

Correct each average count rate for dead time.

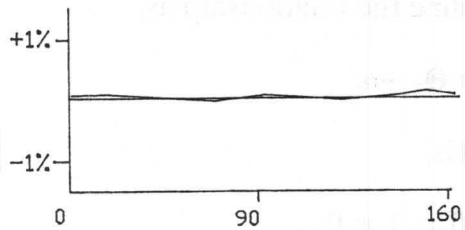
Subtract the dark count rate from each corrected average.

Multiply the result by the sine of the scattering angle.

Divide each value by the result at 90°.

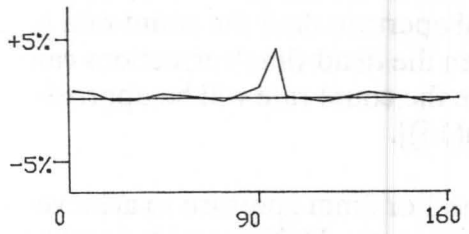
Subtract 1 from this result and plot as a function of angle.

Data Interpretation

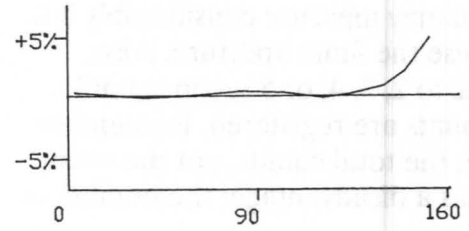


If the alignment was perfect, then the resulting I·sin(Θ) plot would be a horizontal with an intercept of zero. If the deviations are less than ±1% over the angular range, then the alignment is excellent. Repeat the measurement to be sure that any trends are indeed systematic and not the result of either large statistical errors or dust. Several common patterns, including their causes are described below.

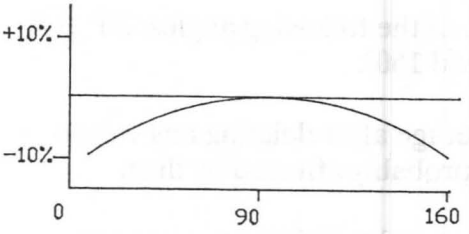
Sine wave centered at 90°. Recheck the center of rotation. The alignment needle should not move more than ½ the width of the slit over the angular range of interest.



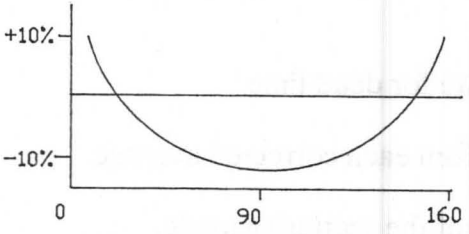
Large deviation at an interior angle. Remove vat. Clean exterior. Look for smudges, scratches, occlusions, schliers and other imperfections. When you reposition the vat the offending angle may have moved, because the angular region of the vat through which the scattered light is observed is very small. Over time imperfections will appear, especially scratches. Do not make measurements at those angles which, after an alignment, are still bad.



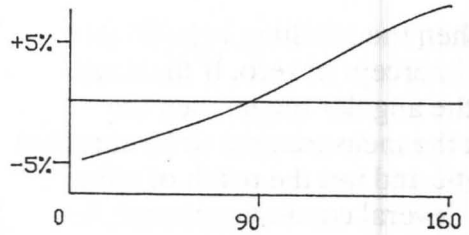
Large, positive deviations at the extremes. Check for smudges on the entrance window of the vat. Use a x10 magnifier to look for scratches where the beam enters the vat. Twist the vat slightly to avoid scratches.



Symmetrical negative curvature centered on 90°. Remeasure the dark count rate. It is probably much higher. Also, this cannot be the problem if the count rate is 100,000cps. If you are using a 5mW HeNe laser and decalin, then the count rate may be less than 20,000cps. In this weak scattering situation a dark count of 1,000cps represents 5%. If you mistakenly subtracted only 500cps as the dark count rate, then a noticeable, symmetric, negative curvature is observed.



Symmetrical positive curvature centered on 90°. Is your laser vertically polarized and positioned correctly? If you are using a cylindrical laser make sure it is positioned correctly. You may have overestimated the dark count rate. Again, this is only likely in a weak scattering situation. Finally, the amplifier/discriminator may have developed a problem. Check by decreasing the count rate and repeating the measurement. If the alignment is good at low count rates, but it seems to be bad at count rates above 50,000cps, contact the factory for advice.



Cotangent corrections. If the $I \cdot \sin(\Theta)$ plot looks like the letter S lying on its side, then the true zero angle is not correct. The true scattering angle Θ_T differs from the measured angle Θ_M by a small amount ε, where we define the relationship as,

$$\Theta_M = \Theta_T + \varepsilon$$

A perfectly aligned system yields,

$$I \cdot \sin(\Theta_T)/I_{90} - 1 = 0 \quad ,$$

where I is the intensity corrected for dead time and dark counts and averaged to remove high values due to dust.

When ε does not equal 0, then the following function is the one plotted

$$f(\varepsilon,\Theta_M) = I\cdot\sin(\Theta_M)/I_{90+\varepsilon} - 1$$

Substituting for Θ_M, expanding using a Taylor series, and assuming small errors results in the following approximation,

$$f(\varepsilon,\Theta_M) = \varepsilon\cdot\cot\Theta_M \quad ,$$

where ε is now in radians.

This approximation works best when the data are normalized at 90° since the sine function is quite flat around 90°.

A cotangent curve looks like the letter S lying on its side. Depending on the sign of the error ε, the curve goes from positive to negative or negative to positive. Quite small ε's can give rise to appreciable errors at the high and low angles. For example, a +0.3° error yields a +2% error at 15° and a -2% error at 165°.

The optional BI-IST software allows the user of a 2030AT or 8000AT correlator to make $I\cdot\sin(\Theta)$ measurements automatically. A least squares routine is included to calculate ε.

If the $I\cdot\sin(\Theta)$ plot shows any indication of positive or negative slope, even roughly centered on 90°, then it is worthwhile applying the cotangent correction. As an example, it can be estimated as follows. If the error is positive at low angles then ε is positive and Θ_T is less than Θ_M. In this case use the PAGE SELECT and Page 1 (parameters) to set the current angle at, say, 0.2° less than the current setting. Repeat the $I\cdot\sin(\Theta)$ measurement. Iterate until the best compromise is reached. Manually reset the verniers on the goniometer until they correspond to the proper angle.

Periodic Alignment Verification

Once aligned the goniometer should stay aligned indefinitely provided no changes are made. Lasers do drift, adjustments are inadvertently moved, and glassware becomes scratched, however. Therefore, periodically check the alignment. If it has not systematically changed beyond acceptable limits, then do not change it. If the alignment has changed, then only small adjustments are usually necessary.

1. Most often the laser beam position has drifted a little bit. The zero angle needs adjusting. Use the cotangent correction to guide you.

2. If the beam has drifted it may strike an imperfection on the entrance window which causes large, positive deviations at the extremes. Twist the vat slightly. Then adjust the lens to redirect the beam to strike the needle tip. Repeat the $I \cdot \sin(\Theta)$ measurement.

Cell Alignment

Check the alignment with a sample cell filled with toluene. Twist the cell until no bright spots is observed coming from the region where the beam enters the cell. For the best measurements you need to reposition cells in the same position that gave the best $I \cdot \sin(\Theta)$ results. Mark the outside of the cell, near the top, with a grease pen. In this way you can position the cell in the same position each time.

Handle the best cells very carefully. Clean cell holders often. Remove dust which may scratch the cell.

Alignment is the second most difficult part of intensity measurements; cleaning samples is the most difficult. Verify the alignment once a month and before any crucial set of measurements is attempted. Keep a cell filled with toluene ready for an alignment check, or use the vat without a cell.

Section VIII: RAYLEIGH RATIOS

Introduction

To obtain a value from the measured intensity that depends only on the property of the scatterer and not on the source or detector optics one uses the Rayleigh factor which is defined as follows,

$$R_\Theta = I_\Theta r^2/(I_{inc} V_{obs})$$

where I_Θ is the radiance scattered from the observed and illuminated volume V_{obs} at a distance r from the detector, and I_{inc} is the incident irradiance. Rayleigh factors, in the cgs system, have units of cm^{-1}·$steradian^{-1}$, and they represent the fraction of light scattered per unit length per unit solid angle. They are not really ratios at all. Ratios have no units; the Rayleigh factor does have units. Historically they have been called ratios, and we will continue this traditional misnomer in this document.

For a solution of macromolecules the difference between the measured Rayleigh ratio of solution and the solvent is called the excess Rayleigh ratio. It is given by,

$$\Delta R_\Theta = R_\Theta(\text{solution}) - R_\Theta(\text{solvent})$$

Excess Rayleigh ratios are used in the determination of molecular weight from a Zimm plot. The Rayleigh ratio is not measured directly; an intensity is. Corrections must be applied to the measured intensities. Also, the BI-200SM is not an absolute photometer; it must be calibrated. The appropriate corrections and calibration are described in the following sections.

Corrections

Polarization

In principle there are 4 combinations of polarization that may be of interest in a light scattering experiment: the incident light can be vertically or horizontally polarized; and the detected light can be analyzed for vertical or horizontal polarization. Classical measurements were typically made with the unpolarized incident intensity derived from a Hg-arc lamp. The measured intensities were then multiplied by $C_p = 1/(1+\cos^2\Theta)$. The BI-200SM goniometer will most probably be used with a vertically polarized laser. In this case $C_p = 1$.

Adsorption

If the scatterer of interest absorbs at the laser wavelength then the calculated Rayleigh ratio must be increased before comparison with theoretical expressions. This is done by multiplying the measured intensity by $C_a = 1/T_i$, where T_i is the internal transmittance of the scattering system.

In most cases of practical interest $C_a = 1$. Furthermore, if the transmitted intensity at $\Theta = 0°$ is used as the reference intensity, then any absorbance is automatically corrected when using a cylindrical sample cell. Alternatively, use a spectrophotometer to measure the transmittance.

Volume

As the detector arm moves the observed scattering volume changes. At 90° the observed volume is least. The correction depends on the geometry of the incident beam as seen by the detector. In the BI-200SM this geometry is cylindrical. The correction factor, therefore, is $C_v = \sin\Theta$. The validity of this correction for the BI-200SM has been verified many times, and it is the basis of the alignment procedure known as the $I \cdot \sin\Theta$ plot.

Refraction

To first order the acceptance solid angle of the scattered light is inversely proportional to the refractive index of the scattering solution, n_s. Also, to first order the length of the observed scattering volume is inversely proportional to n_s. Then, for goniometer systems where the ratio of the sample cell radius to the detector distance, r, is small, as is the case for the BI-200SM, the raw intensity measurements must by multiplied by the factor $C_n = (n_s/n_v)^2$, where n_v is the refractive index of the liquid in the vat.

A similar correction applies when calibration is performed. In that case n_s is replaced by n_c, the refractive index of the calibration liquid. In the final calculation n_v cancels, and the correction becomes $C_n = (n_s/n_c)^2$. In the case that n_s is very close to n_c, for example toluene as the solvent and benzene as the calibration liquid, then C_n is sufficiently close to 1 that it can be ignored. If water, however, were the solvent, then $C_n = .79$, and this correction is important.

Reflection

Reflection occurs at any interface between 2 dielectrics with different indices of refraction. The worst case occurs when a collimated beam strikes an air/glass interface. The back reflectance, measured at Θ, gives rise to a scattered component at $180-\Theta$, the supplementary angle. By immersing the cell in an index matching liquid and by using a beam stop in that liquid, the major reflections are minimized. There remains, however, the reflection from the cell/solution interface which is most serious in the case of scattering from aqueous solutions.

The reflection coefficient, f, is calculated from the Fresnel equation for perpendicular incidence as

$$f_{12} = [(n_1 - n_2)/(n_1 + n_2)]^2$$

where the n's are the refractive indices on either side of the interface. Even for angles of incidence up to 20° this equation is satisfactory for our purposes.

The worst case is water/glass. Then f = 0.0045. For most organic solvents and glass f≤ 0.0001 which is negligible.

The measured intensity is then the sum of the intensity scattered at Θ and 180-Θ. Letting a prime denote a measured quantity and an unprimed value denote the true value we have,

$$I'_{\theta} = I_{\theta} + f \cdot I_{180-\theta}$$

A similar relationship holds for the measured intensity at 180-Θ. Combining these two results one finds,

$$I_{\theta} = (I'_{\theta} - f \cdot I'_{180-\theta})/(1 - f^2)$$

It is this value of the intensity which is then used for further calculation.

For Rayleigh scatterers the intensity at any angle and its supplement are equal; therefore, the reflection-corrected intensity for an aqueous solution is about 0.5% smaller than the uncorrected value.

Only when there is a significant angular dependence to the scattered light from aqueous solutions is this correction worth considering. For a nonaqueous system the correction is insignificant.

Reference Intensity

The Rayleigh ratio is defined by reference to the incident intensity I_{inc}, where this is the value of the intensity incident on the scatterers in the sample cell. This value is proportional to the laser intensity I_0. This is one of two intensities that are commonly used as a reference. The second one is the transmitted intensity I_t.

Either reference intensity may be used to normalize the data. The transmitted intensity is useful if absorption is a problem. Sometimes both references are used. In this way data can be rejected that do not show a constant ratio between the two references. Neither reference is necessary if the laser is stable during the measurements. With modern lasers this is most often the case. For small, long-terms drifts one can also recalibrate between measurements. The optional BI-ZP software offers this feature.

Calibration

Combining all the corrections the Rayleigh ratio is given by,

$$R_\Theta = K \cdot \sin\Theta \cdot (n_s/n_v)^2 \cdot (1/I_{ref}) \cdot (I_\Theta - I_{180-\Theta})/(1 - f_s^2)$$

where K is a calibration constant, I_{ref} is proportional to either the laser or transmitted intensity, and f_s is the reflection coefficient for the solution/cell interface. For simplicity the primes have been dropped. Also, it has been assumed that the reference intensity has not changed during the time it took to measure the intensity at angle Θ and its supplement.

The calibration constant K is determined by measuring the scattered intensity at 90° from a sample of known Rayleigh ratio. Rayleigh ratios for pure liquids like toluene and benzene are the most well known at the laser wavelengths of interest. At 90° the result is,

$$R_c(90°) = K \cdot (n_c/n_v)^2 \cdot (1/I_{ref}) \cdot I_c(90°)/(1 + f_c)$$

where f_c is the reflection correction for the calibration liquid/glass interface. Again, the primes were deleted for simplicity. Notice at 90° that the reflection correction is simplified.

Since the calibration liquid has a refractive index extremely close to that of the glass cells, f_c is negligible compared to 1. It will be neglected from now on. Also, notice that it has again been assumed that the reference intensity has not changed.

Eliminating K between the last two equations one finds for the Rayleigh ratio the following expression,

$$R_\Theta = (1/I_c) \cdot R_c(90°) \cdot (\sin\Theta) \cdot (n_s/n_c)^2 \cdot (I_\Theta - f \cdot I_{180-\Theta})/(1 - f_s^2)$$

For the case of polymer solutions in organic solvents a further simplification is possible. In that case $n_c \approx n_s$ and $f_s \approx 0$. The result, assuming the reference intensities cancel, is

$$R_\Theta = R_c(90°) \cdot \sin(\Theta) \cdot (I_\Theta/I_c)$$

Calibration consists of measuring I_c at 90° for a pure liquid several times. Outliers are discarded. The remaining values are averaged and corrected for dark count rate and dead-time. The liquid should be spectrograde and redistilled in a nonbubbling Pyrex still to leave behind any traces of high molecular weight impurities.

Rayleigh Ratios

In the preceding sections it was shown how to calculate the Rayleigh ratio from measured intensities. The following gives literature values of the Rayleigh ratio for 2 pure liquids. In addition various theoretical relationships are given which are useful for manipulating literature values measured under a variety of conditions.

Typically the measurement involves a vertically polarized laser and no polarization analyzer. In this case the Rayleigh ratio is given by a sum of the vertically and horizontally scattered light as

$$R_v = V_v + H_v$$

where the subscript describes the state of the laser polarization.

The V and H components are often expressed in terms of the isotropic (F) and anisotropic (Γ) components as follows,

$$V_v = F + 4\Gamma \quad , \text{ and } \quad H_v = 3\Gamma$$

Historically the anisotropic part of the scattering was expressed using depolarization ratios which are defined as follows:

$$p_v = H_v(90°)/V_v(90°) \text{ and } p_u = H_u(90°)/V_u(90°) = 2p_v/(1 + p_v)$$

where the subscript u indicates measurements were made with an unpolarized light source.

It follows that $\Gamma/F = p_v/(3 - 4p_v) = p_u/(6 - 7p_u)$. Combining these equations gives,

$$R_v = F\cdot\{6/(6 - 7p_u)\} = F\cdot\{(3 + 3p_v)/(3 - 4p_v)\}$$

Similar expressions can also be written for R_h and R_u. These Rayleigh ratios are for horizontal and unpolarized incident light, respectively. The expressions in the brackets are commonly known as Cabannes-Rocard factors, and similar factors exist for R_h and R_u. The expressions above are correct for vertical polarization of incident light with unpolarized detection, the most common situation.

If a polarization analyzer is employed to make the measurements, then the Rayleigh ratio R_v is replaced by either,

$$V_v = F + 4\Gamma = F\cdot\{3/(3 - 4p_v)\} = F\cdot\{(6 - 3p_u)/(6 - 7p_u)\}$$

$$\text{or} \qquad H_v = 3\Gamma = F\cdot\{3p_v/(3 - 4p_v)\} = F\cdot\{3p_u/- 7p_u)\}$$

Toluene & Benzene

The Rayleigh ratio of a pure liquid is a strong function of the wavelength, and it is best to use values measured at the wavelength of interest. In laser light scattering this means 632.8, 514.5 and 488.0nm. Unfortunately, until recently, good values of R at these wavelengths were not plentiful. Classical measurements were made at 546, 436, and, occasionally, 366nm. Also, $R_u(90°)$ and p_u were measured, not $R_v(90°)$ and p_v. A little manipulation of the above equations yields

$$R_v = 2 \cdot R_u(90°)/(1 + p_u) \quad .$$

An instrument designed by Kaye[1] for absolute measurements of Rayleigh ratios using a vertically polarized HeNe laser gave the following results:

Absolute Rayleigh Ratios at 632.8nm

	$R_v = R_c(90°)$	p_v	p_u
Toluene	$14.0 \times 10^{-6}\ cm^{-1}$	0.325	0.491
Benzene	12.6×10^{-6}	0.265	0.419

The values in the first two columns were measured by Kaye, and the value in the last column was calculated from the equation in the previous section which relates p_v and p_u.

The absolute errors in R_v are estimated at ±2%. A very sensitive, but not absolute, light scattering instrument was constructed by Cannell and coworkers[2]. They also used a vertically polarized HeNe laser. As a test of the instrument Cannell measured several Rayleigh ratios, three of which were liquids measured by Kaye. After calibrating using Kaye's value for toluene, the values of the 2 other liquids were found to agree to within 0.5% with Kaye's results.

Such excellent agreement between two completely different types of light scattering devices over a 10 year period strongly suggests that the values given above are quite good. Brookhaven recommends the values in the above table for $R_c(90°)$ when calibrating the goniometer.

Values at 488 and 514.5nm are not as well known. We estimated them by interpolation of Coumou's data[3]. Coumou's data on several liquids are considered to be quite self-consistent, and they are often referenced for use in calibration. The interpolation was done by noting that the expression

$$R_u(90°)\cdot(\lambda^4/n^2)\cdot\{(6 - 7p_u)/(6 + 6p_u)\}$$

is a very mild function of wavelength. For benzene we used only Coumou's data to plot this function at the classical wavelengths; for toluene we used Coumou's single value at 546nm and Kaye's value at 632.8nm. Then the values at 488 and 514.5nm were interpolated. The results are as follows:

Absolute Rayleigh Ratios at 514.5nm

	$R_v = R_c(90°)$	p_v	p_u
Toluene	$32 \times 10^{-6}\ cm^{-1}$	0.31	0.47
Benzene	30×10^{-6}	0.26	0.41

Absolute Rayleigh Ratios at 488nm

	$R_v = R_c(90°)$	p_v	p_u
Toluene	$40 \times 10^{-6}\ cm^{-1}$	0.31	0.47
Benzene	38.6×10^{-6}	0.27	0.42

where p_v was calculated from the measured p_u. The absolute errors in R_v for benzene are estimated at ±5% and ±10% for toluene. Therefore, we recommend using benzene as the calibration liquid at these wavelengths.

It is important to report the value of $R_c(90°)$ used for calibration. If two molecular weight measurements disagree, it may be due to different choices of the Rayleigh ratio of the calibration liquid. Also, look in the literature for more recent values, especially values measured at 488 and 514.5nm. These values should be close to the ones given above. If the author also measured Rayleigh ratios of toluene or benzene using a HeNe laser, then compare the results with those given above. If they are not within ±2%, then the results at the Argon-ion wavelengths may also be suspect. This statement reflects our confidence in the values given for 632.8nm.

(1) W. Kaye and J.B. McDaniel, Applied Optics, 13, 1934, (1974).

(2) Cannell, et. al., Review of Scientific Instruments, 54, 973 (1983).

(3) Coumou, et. al., Journal of Colloid Science, 15, 408 (1960).
Coumou, et. al., Transactions of the Faraday Society, 60, 1726 (1960).
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Absolute Rayleigh Ratios at 532 nm

	$R_v = R_c(90°)$	P_v	P_u
Toluene	$28.0 \times 10^{-6} cm^{-1}$	0.31	0.47
Benzene	26.0×10^{-6}	0.26	0.41

The values in the table above were estimated using the expression at the top of page 8-7. The expression was evaluated and plotted as a function of wavelength. A value at 532 nm was graphically interpolated. R_u was then calculated and, finally, R_v.