MicroCuvette Measurement Accessory Option Guide for the DAWN® HELEOS™ and miniDAWN TREOS™ Light Scattering Instruments

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MWMC Revision A

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MicroCuvette Measurement

When the amount of available sample is limited, sample recovery is necessary, or time-dependent studies are required, the MicroCuvette measurement option can be used. The MicroCuvette makes batch measurements possible for sample volumes as small as 12 microliters. This manual describes the installation and use of the MicroCuvette in the miniDAWN TREOS and DAWN HELEOS.

CONTENTS **PAGE**

1 Overview

The MicroCuvette measurement accessory for the miniDAWN TREOS and DAWN HELEOS is designed for making batch light scattering measurements with sample volumes as small as 12 µL. In particular, it is an ideal substitute for more accurate flow cell measurements when sample volume is limited, sample recovery is necessary, or time-dependent studies are required.

The MicroCuvette accessory for making simultaneous dynamic and static light scattering measurements is easily installed in the miniDAWN TREOS or DAWN instrument. In the miniDAWN TREOS, dynamic and 90° static light scattering can be measured. In the DAWN HELEOS, dynamic and static light scattering at seven different angles can be measured. With the MicroCuvette measurement accessory, accurate molar mass and size can be determined for sample sizes that have traditionally been too small for the standard scintillation vial.

Productive use of the MicroCuvette requires careful observation of the instructions. In particular, it is absolutely necessary to follow the guidelines for cleanliness in order to obtain accurate results. Please read the instructions carefully before using the MicroCuvette.

2 Installation

The installation instructions are slightly different for each instrument type. Please follow the instructions for the appropriate instrument type.

2.1 miniDAWN TREOS

Items needed for installation:

- 2.5 mm ball driver
- Two 1/4" crescent wrenches for disconnecting the in-line unions
- MicroCuvette measurement accessory kit

Installation steps (see Figure 1 for an exploded view):

1. Make sure the instrument power is off, and that the autocorrelator power is off if the instrument has the QELS option.
2. Put on the anti-static wrist strap.

   This is an important step. The strap keeps the flow cell glass and windows from building up a static charge and attracting particles while being handled.
3. Slide open the batch door to expose the flow cell.
4. Using the 2.5 mm ball driver and 1/4" crescent wrenches, remove the read head cover plates and flow cell assembly from the instrument (see Step 1 of “Cleaning the Flow Cell and Windows” in the miniDAWN TREOS *Hardware Manual*). In order to keep the flow cell assembly clean and dust free, place it in an antistatic bag for storage.
5. Insert the batch manifold and secure it with the two M3 screws. Note the orientation of the manifold. The manifold is keyed such that it is not possible to put it in backwards.
6. Install the current ASTRA software on your data collection computer.
7. The miniDAWN TREOS is now ready for use with the MicroCuvette. Please read the rest of the instructions before introducing sample into the MicroCuvette or inserting the MicroCuvette into the instrument.

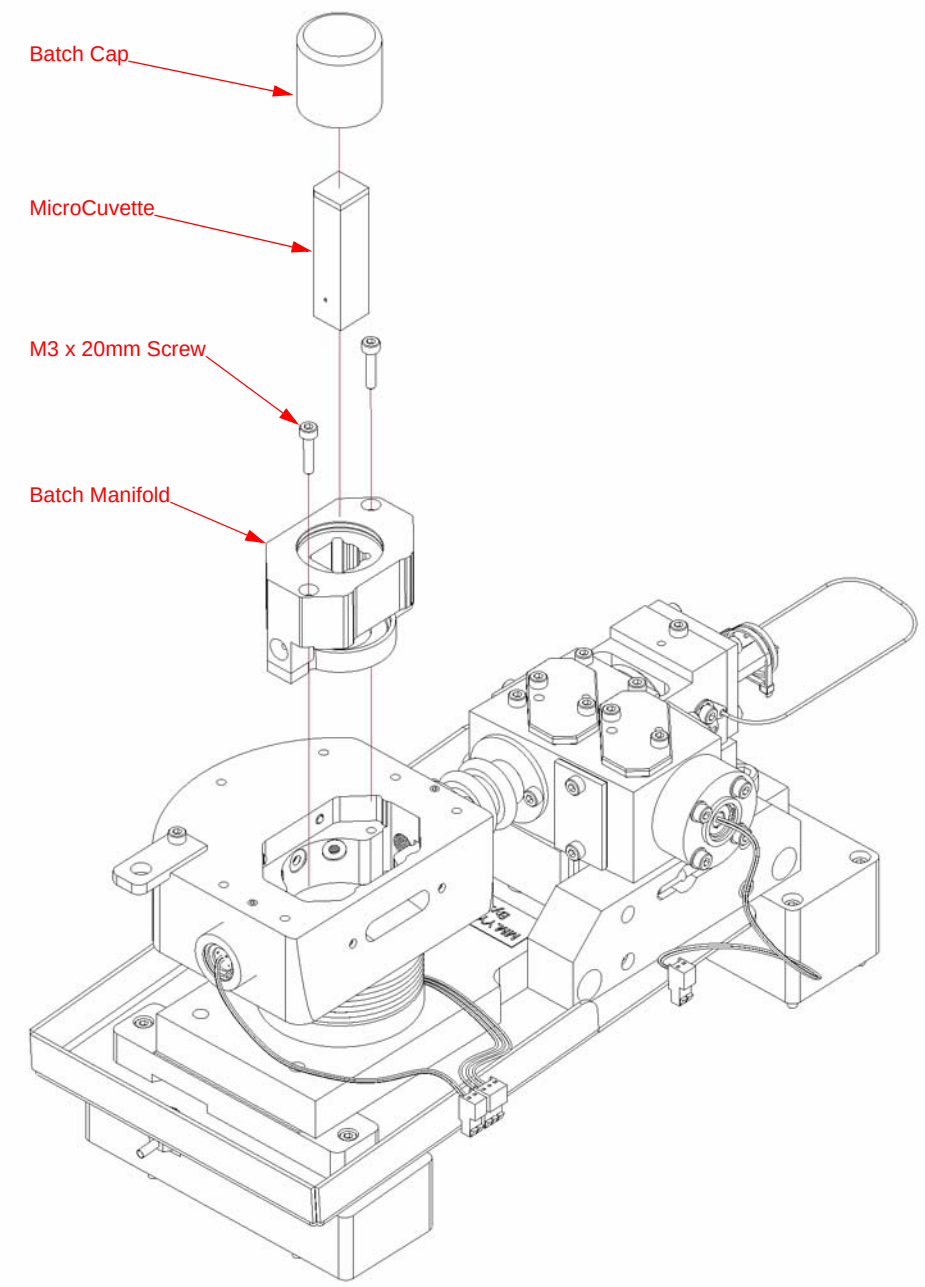

Figure 1: miniDAWN TREOS flow to MicroCuvette conversion kit, exploded.

2.2 Ambient DAWN HELEOS

Items needed for installation:

- 2.5 mm ball driver
- Two 1/4" crescent wrenches for disconnecting the in-line unions
- MicroCuvette measurement accessory kit

Installation steps (see Figure 2 for an exploded view):

1. Make sure the instrument power is off, and that the autocorrelator power is off if the instrument has the QELS option.
2. Put on the anti-static wrist strap.

   This is an important step. The strap keeps the flow cell glass and windows from building up a static charge and attracting particles while being handled.
3. Remove the bib from the top cover of the instrument.
4. Using the 2.5 mm ball driver and 1/4" crescent wrenches, remove the read head cover plates and flow cell assembly from the instrument (see Step 1 of “Cleaning the Flow Cell and Windows” in the DAWN HELEOS *Hardware Manual*). In order to keep the flow cell assembly clean and dust free, place it in an antistatic bag for storage.
5. Insert the batch manifold and secure it with the two M3 screws. The manifold is keyed such that it is not possible to put it in backwards.
6. Install the current ASTRA software on your data collection computer.
7. The DAWN HELEOS is now ready for use with the MicroCuvette. Please read the rest of the instructions before introducing sample into the MicroCuvette or inserting the MicroCuvette into the instrument.

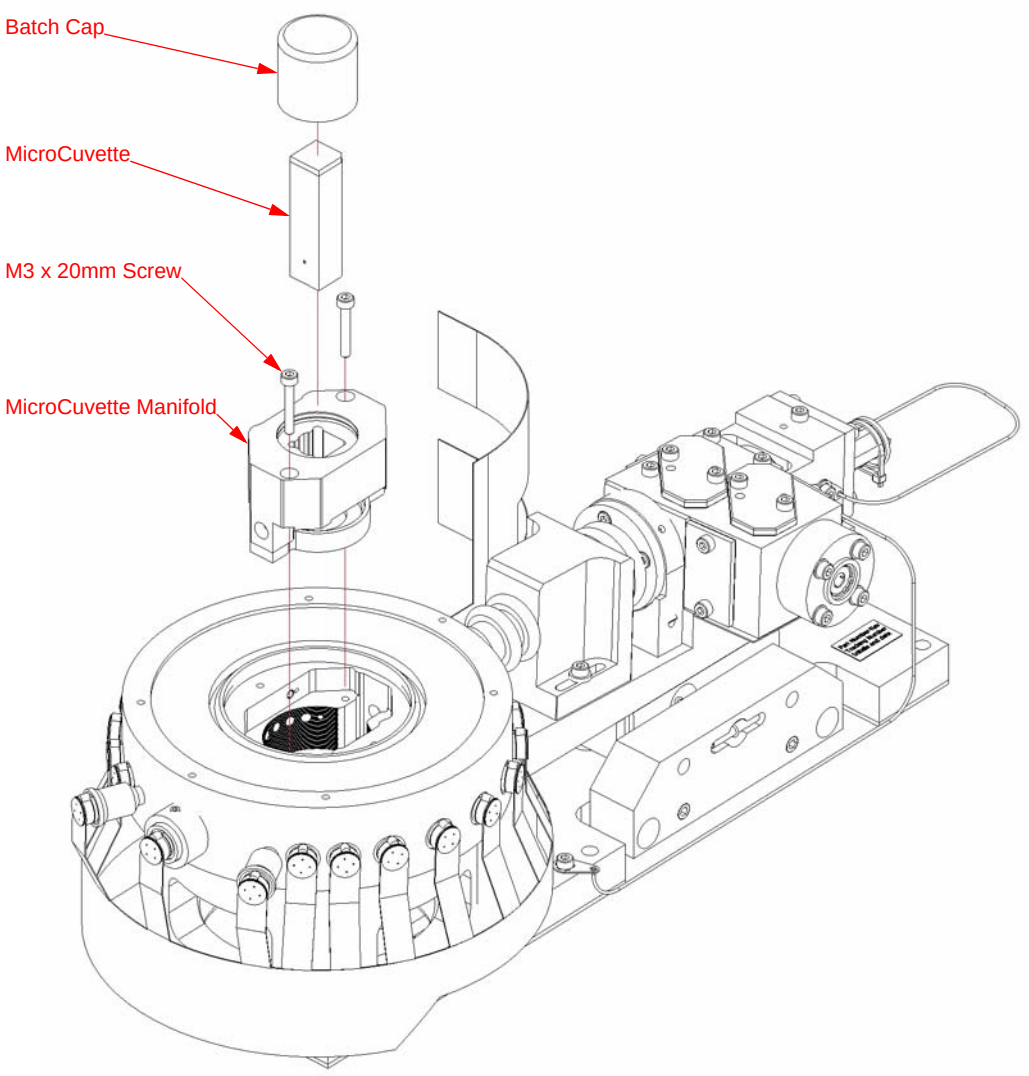

Figure 2: Ambient flow to MicroCuvette conversion kit, exploded.

2.3 Heated/Cooled DAWN HELEOS

Items needed for installation:

- 2.0 mm and 2.5 mm ball driver
- Two 1/4" crescent wrenches for disconnecting the in-line unions
- Phillips screw driver
- MicroCuvette measurement accessory kit

Installation steps (see Figure 3 for an exploded view):

1. Make sure the instrument power is off, and that the autocorrelator power is off if the instrument has the QELS option.
2. Make sure the read head cavity is cooled down to room temperature before performing the installation.
3. Put on the anti-static wrist strap.

   This is an important step. The strap keeps the flow cell glass and windows from building up a static charge and attracting particles while being handled.
4. Remove the bib from the top cover of the instrument.
5. Using the 2.5 mm ball driver and 1/4" crescent wrenches, remove the read head cover, the flow cell cover plates, and the flow cell assembly from the instrument (see the section “Removing the Cell Assembly” in the DAWN HELEOS *Hardware Manual*). In order to keep the flow cell assembly clean and dust free, place it in an antistatic bag for storage.
6. Using the 2.0 mm ball driver install four shoulder screws as shown in Figure 3.
7. Insert the batch manifold and secure it with the two M3 screws. The manifold is keyed such that it is not possible to put it in backwards.
8. Ensure the foam insulation rings are securely in place.
9. Install adapter plate, MicroCuvette, and batch cap
10. Install batch cover, as shown, by twisting it into place on the shoulder screws.

Note: The knob on top of the batch cover is not functional with the MicroCuvette option.

11. Install the current ASTRA software on your data collection computer.
12. The DAWN HELEOS is now ready for use with the MicroCuvette. Please read the rest of the instructions before introducing sample into the MicroCuvette or inserting the MicroCuvette into the instrument. In particular, be certain to read the special instructions for using the MicroCuvette at elevated temperatures.

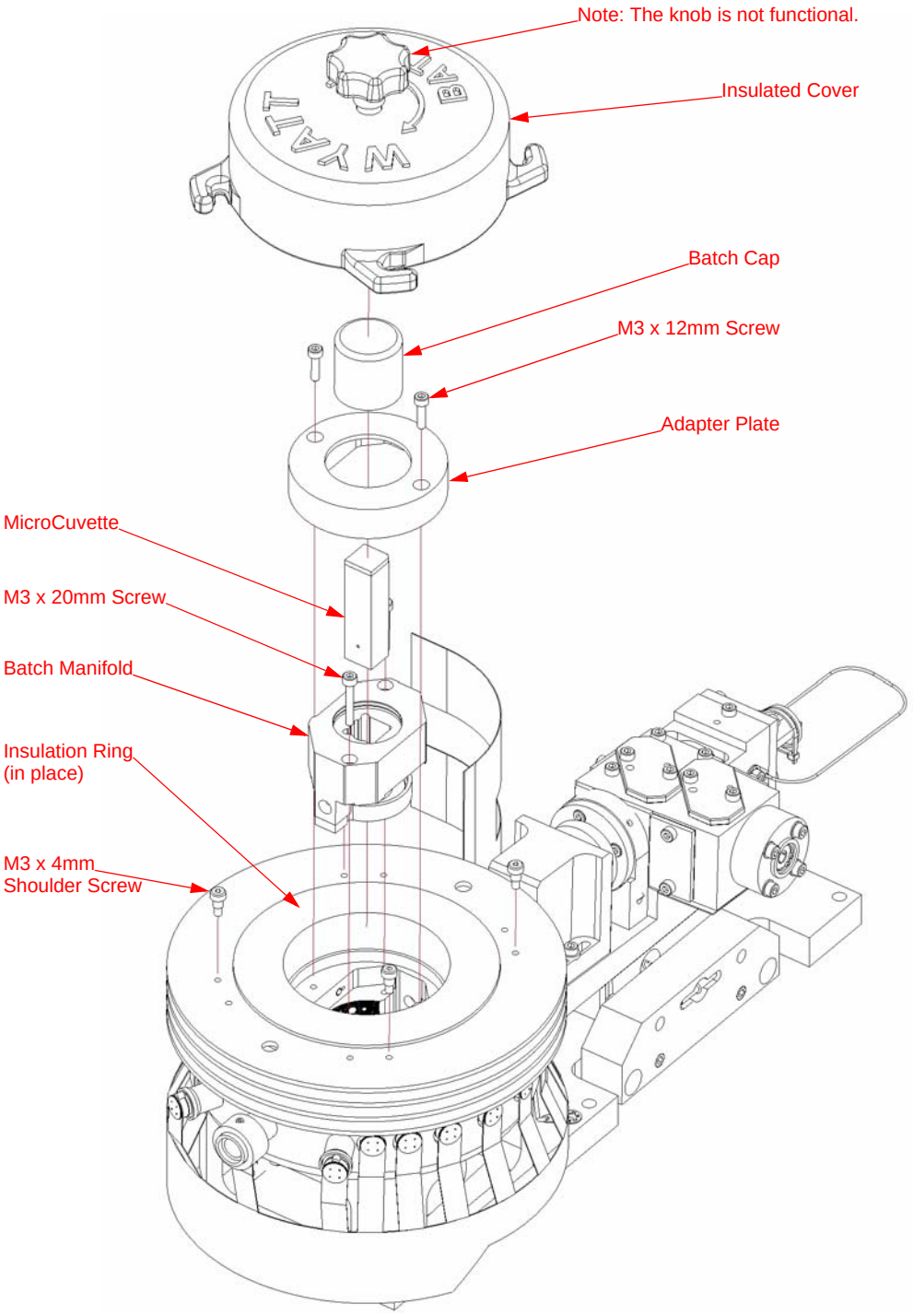

Figure 3: Temperature controlled flow to MicroCuvette conversion kit, exploded.

3 MicroCuvette Essentials

3.1 Handling

The MicroCuvette is made of fused suprasil quartz, and has an anti-reflection coating on each of the four windows. It is a precision optical component, and should be treated as such. When handling the MicroCuvette, avoid touching the lower windows so as not to scratch or dirty them. In addition, the MicroCuvette should not be subjected to extreme shocks, either mechanical or thermal.

3.2 Cleaning

Outside surfaces

To avoid scratching the anti reflection coating on the windows, the outside window surfaces should only be wiped with lens cleaning paper wetted with a few drops of solvent. For organic and oily deposits, 99.9% hexanes is recommended. For other dirt, 99.9% methanol is recommended.

Inside surfaces

The simplest solution to keeping the inside of the MicroCuvette clean is to never let it get dirty. When using the MicroCuvette with actual sample, never let it dry out without first cleaning it. This prevents sample from precipitating onto surfaces. If the MicroCuvette is not cleaned between uses, store it in a solvent bath to prevent sample from drying on the surfaces.

When the MicroCuvette needs to be cleaned, it is important to determine the residual material that must be removed. The suggested methods for cleaning depending on solute and solvent are listed in Table 1. Definitions for the different methods and materials in Table 1 follow the table.

Ultrasonic cleaners

WARNING! Ultrasonic cleaners can operate at frequencies that are resonant with the MicroCuvette, which can cause it to break. Wyatt Technology Corporation does not recommend the use of ultrasonic cleaners, and will not warranty MicroCuvettes that have been cleaned in ultrasonic cleaners.

MicroCuvette cap

If the sample cap is dirty, clean with detergent, followed by a copious water rinse, alcohol rinse, and nitrogen blow dry.

Table 1: Suggested cleaning methods

Solvent	Material	Suggested cleaning method
aqueous	protein, DNA, biologics	1. warm water with detergent 2. mild acid rinse 3. copious water rinse 4. alcohol rinse and nitrogen blow dry
aqueous	salt solutions	1. strong acid rinse 2. copious water rinse 3. alcohol rinse and nitrogen blow dry
aqueous	basic solutions	1. warm water with detergent 2. mild acid rinse 3. copious water rinse 4. alcohol rinse and nitrogen blow dry
organic	oil based	1. rinse with solvent 2. warm water with detergent 3. mild acid rinse 4. copious water rinse 5. alcohol rinse and nitrogen blow dry
organic	alcohol solutions	1. rinse with solvent 2. copious water rinse 3. alcohol rinse and nitrogen blow dry
organic	acidic solutions	1. rinse with solvent 2. copious water rinse 3. alcohol rinse and nitrogen blow dry

Definitions for Table M-1

- **mild acid**—dilute hydrochloric acid (approximately 1 M).
- **strong acid**—less dilute hydrochloric acid (approximately 5 M).
- **solvent rinse**—rinse with the solvent that solvated the sample.
- **copious water rinse**—rinse at least 10 times with pure water (deionized, distilled, or reverse osmosis filtered).
- **detergent**—a neutral pH detergent. In general, a mild acid rinse should follow the detergent to remove residues.
- **alcohol rinse**—rinse with HPLC grade ethanol until remaining water is displaced.
- **nitrogen blow dry**—compressed chromatographic grade nitrogen gas through a 0.2 µm filter. The use of canned dusting gas to dry the cell is not recommended, since it contains contaminants.

3.3 Storage

Make sure MicroCuvette is clean and dry before storing. Use the sealing cap to insure that dust and dirt do not settle inside. Wrap in lens cleaning paper and return to case.

4 Sample Handling

Cleanliness is essential in introducing sample into the MicroCuvette. Dirt and dust can destroy a light scattering measurement, and great pains should be taken to prepare the sample correctly in a clean, dust-free environment. After the MicroCuvette and sealing cap have been cleaned, only remove the cap to insert the sample. Keeping the sealing cap on the MicroCuvette will prevent dust from contaminating the sample.

4.1 MicroCuvette Preparation

If the MicroCuvette is not clean and dry, first clean and dry according to the above instructions. If repeated measurements are to be made using the same solvent, it is usually not necessary to completely clean the MicroCuvette between samples. Instead, wash the old sample out with a copious (at least 10 times) rinse of the solvent being used, then blow dry with filtered nitrogen. If the solvent is aqueous, rinse with HPLC grade ethanol following the solvent rinse, then blow dry with filtered nitrogen.

4.2 Sample Preparation

Clean, dust free sample is necessary for accurate light scattering measurements. A syringe/filter combination is provided for filtering the sample into the MicroCuvette. For pure solvent, use the syringe, 0.02 µm filter, and needle to introduce the solvent into the MicroCuvette. Push at least one or two drops of solvent through the filter/needle to clean it out before introducing the sample into the MicroCuvette.

The filtration method for the actual sample depends on the amount of sample available. For sample sizes greater than 300 µL, use the supplied syringe, 0.10 or 0.20 µm filter, and needle to introduce the sample into the MicroCuvette. Push at least one or two drops of sample through the filter/needle to clean it out before introducing the sample into the MicroCuvette.

For sample sizes less than 300 µL, there is not sufficient sample volume to use traditional syringes and syringe filters. To effectively use sample volumes in the 30 to 300 µL range, the Wyatt Technology NanoFilter is recommended. Please contact Wyatt Technology for more information on obtaining the NanoFilter. Finally, it is also possible to use centrifugal filtering devices for small sample volumes, but these do not have filtration membranes comparable to the 20 and 100 nm anodisc membranes in the NanoFilter. Therefore, the NanoFilter is recommended for small sample sizes.

4.3 Sample Injection

Remove the sealing cap on the MicroCuvette, place the needle all the way into the sample trough, and inject the sample. The sample volume is 10 µL, but at least 12 µL of sample are needed to raise the meniscus above the window level. After the sample is injected, replace the sealing cap.

Check for bubbles in the sample. Hold the MicroCuvette such that it is possible to look through the 1 mm square windows. A diffuse, bright background will help. Small bubbles in the sample should be obvious. If bubbles are present, gently tap the MicroCuvette on a hard surface to dislodge the bubbles. It may take several taps to dislodge the bubbles.

WARNING! Don't break the MicroCuvette when tapping it on a hard surface to dislodge bubbles. Wyatt Technology Corporation will not be responsible for bubble induced collateral damage.

If dripping the sample into the MicroCuvette, it is possible for the sample to get stuck completely above the sample viewing area - in essence, there is one big bubble in the sample volume. However, the viewing path can appear perfectly clear. As described in the section on taking data, a quick check of the forward monitor reading will reveal this situation. If this is the case, remove the MicroCuvette from the instrument and gently tap it on a hard surface until the sample falls into place.

4.4 Sample Recovery

Remove the sealing cap on the MicroCuvette, tip it slightly, and insert the recovery needle fully into the trough to remove the sample. Be careful not to scratch the inner surfaces of the optical windows. Make sure to clean the MicroCuvette or store it in clean solvent after sample recovery, since it is easier to keep it clean if sample does not dry onto the inner optical surfaces.

5 Data Collection and Analysis

5.1 Inserting the MicroCuvette in the Instrument

WARNING! There is only one correct orientation for inserting the MicroCuvette in the holder: the 45 degree beveled corner should be in contact with the ball plungers. Trying to force the MicroCuvette into the holder in any other orientation can damage the MicroCuvette.

As shown in Figure 4, the batch manifold has ball plungers that push the MicroCuvette against two reference surfaces for accurate positioning. The bottom of the manifold is the third reference surface, setting the height of the MicroCuvette. When inserting the MicroCuvette, position the MicroCuvette so that the 45° beveled corner is in contact with the ball plungers, then gently push the MicroCuvette into the manifold until it rests firmly on the bottom. Be careful not to insert the MicroCuvette so forcefully that it "smacks" the bottom of the manifold; this can break chips off of the corners of the MicroCuvette.

The forward monitor of the miniDAWN TREOS or DAWN HELEOS is used to determine the positioning of the MicroCuvette. With the laser on, note the forward monitor reading without the MicroCuvette in position. Then insert the MicroCuvette. If bubble-free sample is in the viewing volume of the MicroCuvette, then the forward monitor should decrease by no more than 4% when the MicroCuvette is correctly inserted. A decrease of around 15% is an indication that sample is not in the viewing volume (i.e., the sample might be "stuck" above the viewing volume). Even smaller percentages are an indication that there is a bubble in the sample, or that the MicroCuvette is not properly positioned. Check for bubbles and reinsert the MicroCuvette, making sure that the MicroCuvette is resting firmly against the reference surfaces. If alignment problems persist, verify that the batch manifold is installed correctly, and that the batch spacer plate is clean and properly positioned.

Once the MicroCuvette is properly inserted, use the batch cap or close the batch access on the cover to eliminate stray light. The MicroCuvette is now ready for data collection.

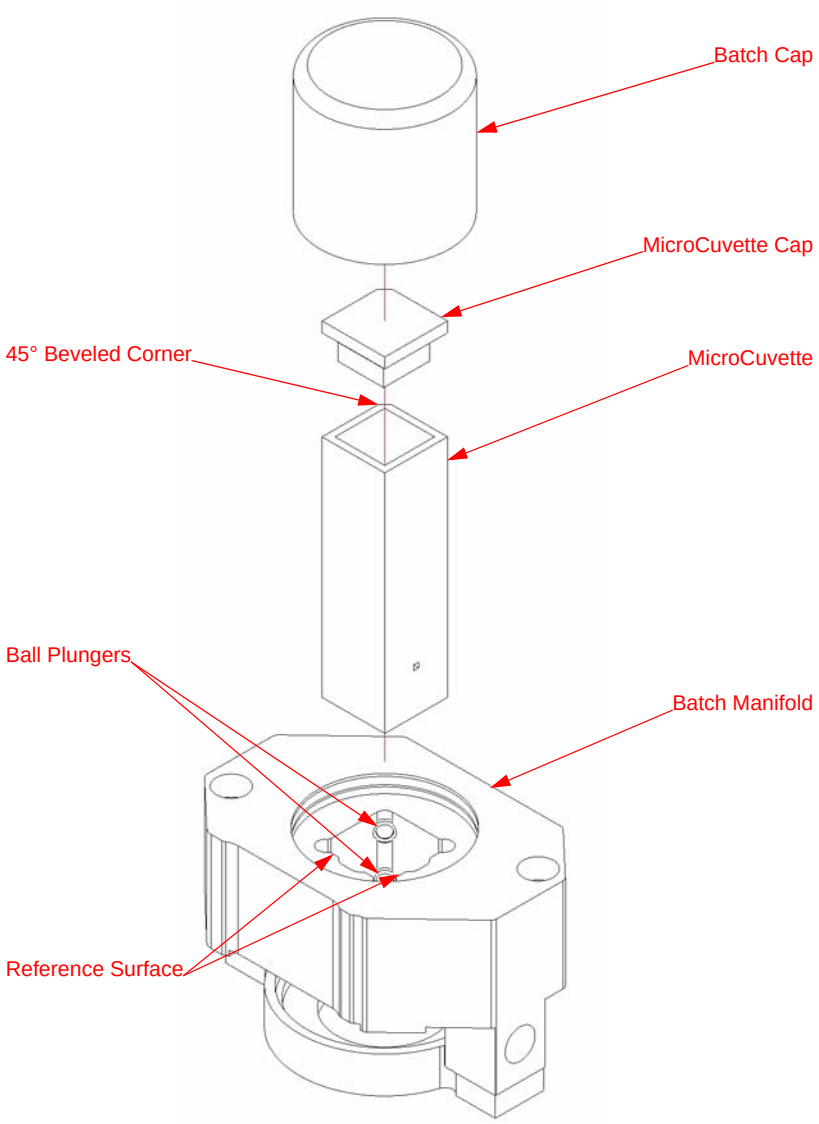

Figure 4: MicroCuvette insertion in batch manifold.

5.2 ASTRA Settings and Angles

Data collection in ASTRA with the MicroCuvette is very similar to batch mode data collection with scintillation vials. See "Appendix B: Batch Mode Data Collection and Analysis" in *ASTRA for Windows User's Guide*. It is only necessary to choose the MicroCuvette cell type. On the Collect menu, choose System Setup. Under Cell Type, choose "MC" for MicroCuvette.

5.3 Calibrating

Follow the usual calibration procedures in the *ASTRA for Windows User's Guide*. MicroCuvette calibration differs from scintillation vial calibration in that it is not necessary (or even possible!) to rotate the MicroCuvette and perform multiple calibrations in order to obtain an average calibration constant. Please note, however, that if multiple MicroCuvettes are to be used, each MicroCuvette can have a different calibration constant. Calibrate each MicroCuvette, and record the calibration constant for each one. Each MicroCuvette has a unique serial number to help in identification.

5.4 Normalizing

For the miniDAWN TREOS, only the 90° detector is used for static light scattering, so it is not necessary to normalize. For the DAWN HELEOS, follow the usual normalization procedure, choosing an isotropic scatterer in the solvent to be used. Do not forget to choose the "MC" cell type in Systems Setup. See the *ASTRA for Windows User's Guide* for more information on normalization.

5.5 QELS Data Collection

QELS data collection is essentially the same as for the standard flow cell. When using ASTRA, the correct angle for the QELS detector is calculated automatically, as long as the correct solvent and cell type have been chosen in System Setup. For WyattQELS, it is necessary to provide the QELS detector angle. For the miniDAWN TREOS, it is 90°. For the DAWN HELEOS, the correct angle can be retrieved from the list of actual angles in the Processing Parameters dialog from the Options menu. For example, detector 13 in the DAWN HELEOS for aqueous solvent is 103.4°.

In the DAWN HELEOS, it is possible to move the QELS detector to different angular positions. To avoid clipping the QELS viewing area on the corners of the MicroCuvette, it is recommended that only detector positions 8 through 13 be used for the QELS detector.

Finally, if a significant drop in count rate is observed when going from the flow cell to the MicroCuvette, it might be necessary to re-optimize the fiber position. Please see the QELS instruction manual.

5.6 Static Light Scattering and Analysis

Determining molar mass and size using static light scattering is identical to the methods for batch and micro-batch analysis described in "Appendix B: Batch Mode Data Collection and Analysis" in *ASTRA for Windows User's Guide*. Please see that appendix for details.

5.7 Making Measurements with Multiple Angles

For the DAWN HELEOS, it is possible to use detectors 7 through 14 to make angular measurements of the light scattering in order to determine the rms radius. To do this, take great care when removing and reinserting the MicroCuvette to insure that it returns to its former position. This can be done by observing the forward monitor when inserting the MicroCuvette, and making sure that the forward monitor signal returns to its previous value.

Also, normalization can be aided by first recording a baseline with solvent alone, and then adding the normalization standard into the MicroCuvette without removing the MicroCuvette. The resulting normalization constants can then be used with the Live data display in the Collect menu under Inject to verify that the signal levels on the various detectors return to their previous values when reinserting the MicroCuvette, and that the correct ratios of signals are observed when measuring the actual sample.

In general, for samples with an rms radius less than 10 nm, it is recommended that only the 90 degree detector be used. For larger radii, additional detectors about 90 degrees can be added to resolve the angular variation. It is a good idea to practice angle measurements with a known standard before attempting them on an unknown sample.

5.8 Notes on Dust and Signal Stability

The presence of dirt and dust in a sample can lead to oscillating signals. The static light scattering detectors are far more susceptible to this than the QELS detector, since the static detectors view a greater illuminated sample volume. If oscillating signals are observed, the simplest solution is to wait a few minutes before taking data. It is often the case that waiting will give time for the dust and dirt to settle out of the illuminated scattering volume. Another solution is to recover and refilter the sample. However, the best solution is to avoid dirt and dust from the start. Be meticulous in keeping the MicroCuvette clean and dust free, and use proven methods to obtain clean, filtered sample.

6 Using the MicroCuvette above Room Temperature

When using the MicroCuvette at elevated temperatures in a temperature controlled DAWN HELEOS, there are several additional points to consider:

The MicroCuvette can be damaged if subjected to thermal shock. Keep heating and cooling rates below 1° C/minute.

Two caps are provided with the MicroCuvette. The sealing cap is made of polyethylene, and has a melting temperature of approximately 100° C. It is recommended that the polyethylene sealing cap not be used at temperatures greater than 80° C. The second cap is made of Teflon, and may be safely used to 210° C. The Teflon cap is non-sealing, so greater care must be taken to keep dust out of the MicroCuvette.

12 µL of sample can evaporate quickly at elevated temperatures. Be sure that adequate solvent is used, and be aware that concentrations can change due to evaporation. A good trick for heating aqueous samples is to place a layer of low vapor pressure oil on top of the sample. This provides a "cap" that prevents evaporation. Take great care to completely clean the oil from the MicroCuvette after the measurement is complete.