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Exposure Methods and Measurements Division
Air Quality Branch

STANDARD OPERATING PROCEDURE

SOP Title: Determining Aerosol Size Distribution Using the Multisizer 4 Coulter Counter

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SOP Contact Signature

Name: Robert Vanderpool
Signature/Date:

ROBERT VANDERPOOL

Digitally signed by ROBERT VANDERPOOL
DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=ROBERT VANDERPOOL, dnQualifier=0000023954
Date: 2017.12.13 08:28:02 -05'00'

Management Signature

Name: Surender Kaushik
Title: AQB Branch Chief
Signature/Date:

SURENDER KAUSHIK

Digitally signed by SURENDER KAUSHIK
DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=SURENDER KAUSHIK, dnQualifier=0000033892
Date: 2017.12.19 10:36:52 -05'00'

QA Signature

Name: Sania W. Tong Argao
Title: EMMD QA Manager
Signature/Date:

Sania W. Tong Argao

Digitally signed by Sania W. Tong Argao
DN: cn=Sania W. Tong Argao, o=US EPA, ou=ORD/NERL/EMMD, email=Tong-Argao.Sania@epa.gov, c=US
Date: 2017.12.19 10:39:21 -05'00'

*See discipline descriptions on the NERL Scientific and Technical SOP intranet site

Determining Aerosol Size Distribution Using the Multisizer 4 Coulter Counter

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Process Modeling Research Branch
National Exposure Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Prepared by: Carlton L. Witherspoon
Carlton Witherspoon, Jacobs Senior Scientist
Date: 11/13/15

Approved by: Laurie L. Brixey
Laurie Brixey, Jacobs Department Manager
Date: 11/13/15

Zora E Drake-Richman
Zora Drake-Richman, Jacobs QA Officer
Date: 11/13/15

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1.0 Scope and Application

This standard operating procedure (SOP) provides a detailed description of the procedures and data analysis necessary to determine aerosol size distribution using the Beckman Coulter, Inc. Multisizer 4 Coulter counter. Specific details are provided for creating a standard operating method (SOM) within the Multisizer 4 software specifically for analyzing samples containing commercially available polydisperse size standards collected in the EPA's Aerosol Test facility (ATF) aerosol wind tunnel.

2.0 Summary of Method

Filter samples are collected in the EPA aerosol wind tunnel to determine the concentration of airborne particles. The particles are extracted from filter media using electrolyte solution and analyzed using a Beckman Coulter Multisizer 4. The analytical data are then reduced, and size-differentiated particle concentration is determined for each sample. The resulting data output is then used to determine the size-dependent effectiveness of various candidate samplers.

3.0 Definitions

aLpm	actual liters per minute	µm	micrometer
ATF	Aerosol Test Facility	MeOH	methanol
CV	coefficient of variation	mL	milliliter
DI H_2O	deionized water	mm	millimeter
GISO	mixture of glycerol and Isoton	m^3	cubic meters
Lpm	liters per minute	SOM	standard operating method
µL	microliter	SS	stainless steel

4.0 Health and Safety Warnings

Standard laboratory personal protective equipment, including safety glasses and lab coats, should be worn at all times during the operation in accordance with the U.S. Environmental Protection Agency (EPA) Chemical Hygiene Plan.

5.0 Cautions

The concentration of the sample must be ≤ 10% solids to reduce coincidence errors during analysis. If average sample concentration is greater than 10% solids, the sample should be diluted. Refer to Appendix C in SOP-J15-002.0, "Collection and Extraction of Challenge Aerosol for Subsequent Coulter Analysis," for specific instructions detailing the dilution of sample extractions.

6.0 Interferences

To mitigate the risk of contamination, laboratory instrumentation and glassware should be washed with a dilute solution of laboratory detergent (e.g., Sparkleen) in warm water to remove particles prior to use.

7.0 Personnel Qualifications

7.1 Personnel must have knowledge of laboratory safety practices.

7.2 Personnel should have sufficient background in aerosol science to perform the procedure without direct supervision.

8.0 Equipment and Supplies

8.1 Equipment

Desktop computer with Microsoft Windows XP, 2002, Service Pack 3 or newer version and Beckman Coulter Multisizer 4 software

Multisizer 4, Beckman Coulter, Inc. (Figure 1)

Electrolyte filtration assembly (Figure 2)

Figure 1. Multisizer 4 (Beckman Coulter, Inc.)

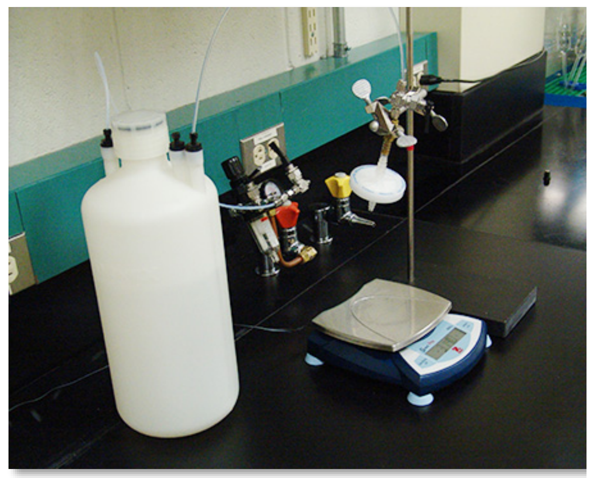


Figure 2. Electrolyte filtration assembly.

8.2 Materials

Methanol (MeOH)

Deionized water (DI H_2O)

Isoton II (P/N: 8546719, Beckman Coulter, Inc.)

Non-ionic dispersant Type IC (Beckman Coulter, Inc., item no. 6600705)

Glycerol, laboratory grade, 99.7% (VWR stock no. BDH1172-1LP)

Mild laboratory detergent (Sparkleen) for washing sampler components (Fisher catalog no. 04-320-4)

8.3 Supplies

Nitrile gloves, powder free (VWR stock no. 82026-428)

Capsule filters for liquid, 0.45-µm pore diameter (Aqua Prep, Pall Life Sciences, P/N: 4270)

50-µm aperture tube for Multisizer 4 (Beckman Coulter, Inc. P/N: A36392)

70-µm aperture tube for Multisizer 4 (Beckman Coulter, Inc. P/N: A36393)

100-µm aperture tube for Multisizer 4 (Beckman Coulter, Inc. P/N: A36394)

140-µm aperture tube for Multisizer 4 (Beckman Coulter, Inc. P/N: A36395)

200-µm aperture tube for Multisizer 4 (Beckman Coulter, Inc. P/N: A36396)

400-mL Coulter beaker(s) (Beckman Coulter, Inc. P/N: A35597)

1-L stainless steel (SS) containers with lids (Polar Ware 300 series, VWR stock no. 36312-004)

Accuvette cups (Beckman Coulter, Inc. P/N: A35473)

Coulter CC size standard L10 (Beckman Coulter, Inc. P/N: 6602796)

9.0 Instrument Calibration and Standardization

The Multisizer 4 particle counter should be calibrated on an annual basis to conform to the manufacturer’s recommendation. Beckman Coulter Inc. will dispatch a service technician who will conduct the annual calibration on site.

10.0 Sample Collection, Handling, and Preservation

Samples containing particles should be discarded after results of analysis have been reviewed. Refer to section 16 for sample validation criteria.

11.0 Procedures

11.1 Selecting and Changing the Aperture Tube

1. Turn on the Multisizer 4 and the desktop computer.
2. Open the Multisizer 4 software and choose “connect to Multisizer 4”.
3. Determine the physical size distribution of the challenge aerosol that will be contained in sample extractions based on the commercially available particle size analysis data sheet provided by the manufacturer.

4. Select the appropriate aperture tube to analyze the size range of the commercially available size standard used. Refer to the table in Chapter 3 of the Multisizer 4 user's manual for details regarding selecting an aperture size. Section 8.3 of this SOP lists the aperture tubes on hand in the ATF; any others must be ordered from Beckman Coulter, Inc.

5. Open the Change Aperture Tube Wizard in the Multisizer 4 software. The wizard walks the user through several steps required when changing an aperture tube. Keep the following items in mind as you follow the necessary steps to change the aperture:

   a. Place a clean Coulter beaker filled with filtered electrolyte solution on the sample platform within the Multisizer 4. Be sure to use a beaker and electrolyte solution that match what will be used for upcoming analyses.

   b. Measure the noise level to obtain the sizing threshold. This will establish a threshold of electrical resistance to distinguish a particle from background signals or "noise". Upon measurement, the instrument will return the measured value in micrometers (µm). The lower sizing threshold must be ≥ 0.2 µm above the noise level. Otherwise, the bin range will be adjusted automatically to meet this criterion.

      This step should be conducted in a manner consistent with upcoming analyses. Operating parameters such as stirrer speed, stirrer angle, beaker volume, and electrolyte solution have a significant impact on the measured noise level. Keeping these parameters consistent will ensure accuracy of measurement. If the noise level is too high, confirm that all wetted components and lab ware are clean and electrolyte solution is properly filtered, and then rerun the measurement. Adjusting some of the parameters detailed above might also improve the measurement.

   c. Exit the Change Aperture Tube Wizard.

   d. Open the Edit the SOM menu and select "Parked out of beaker" on the Stirrer tab.

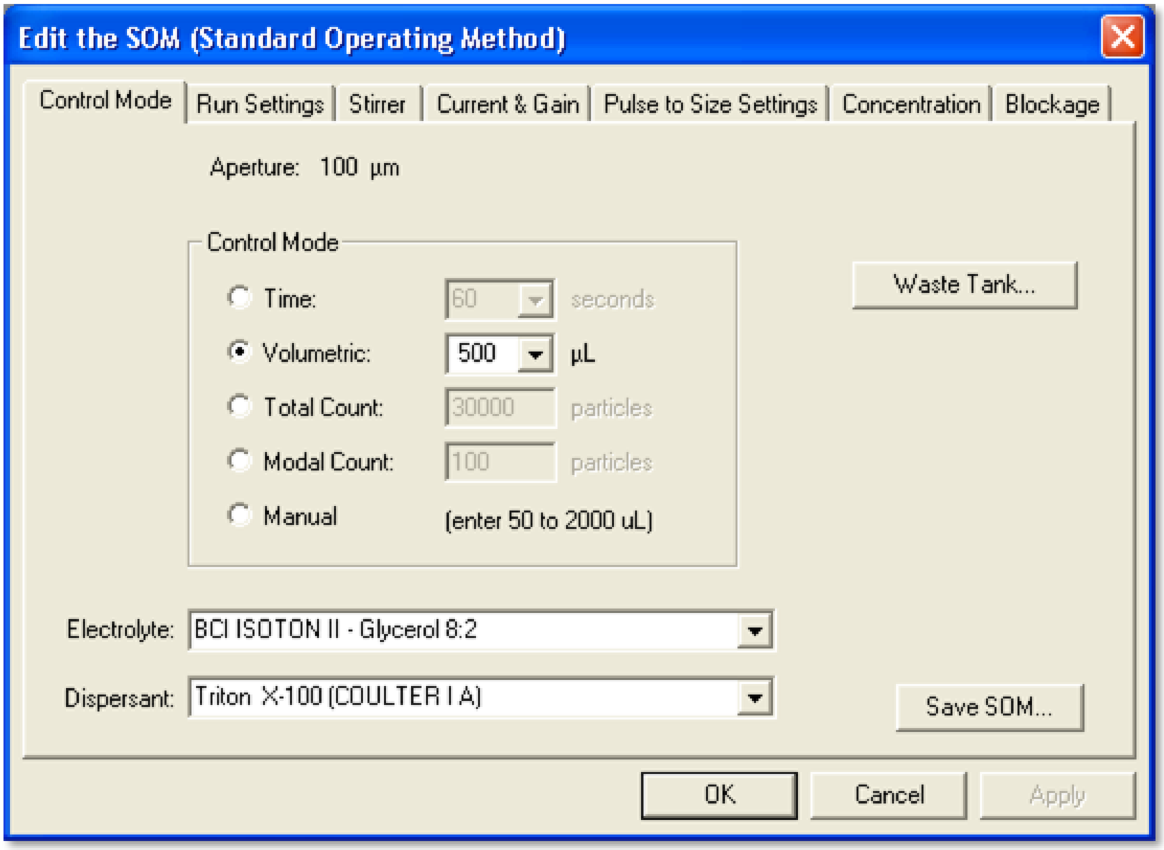
   e. Reopen the Change Aperture Tube Wizard and proceed with the calibration verification.

   f. Verify the calibration by transferring 20 mL of filtered electrolyte solution to a 25-mL Accuvette and add 10–20 drops of the L10 size standard to the Accuvette.

11.2 Selecting a Standard Operating Method (SOM)

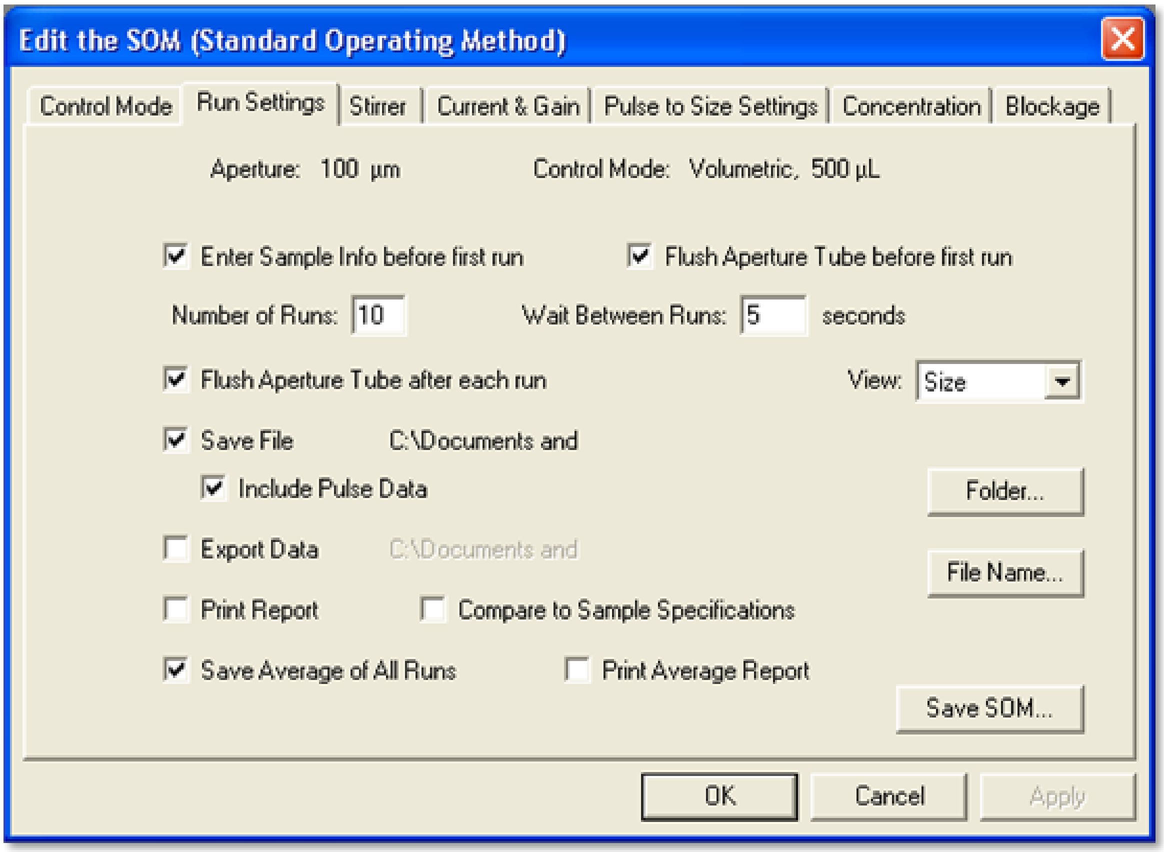
11.2.1 Refer to the Multisizer 4 user's manual for detailed instructions on navigating the Multisizer 4 software.

11.2.2 Open the appropriate SOM. The SOM can be edited or a new one created. The following figures should be helpful in either case.



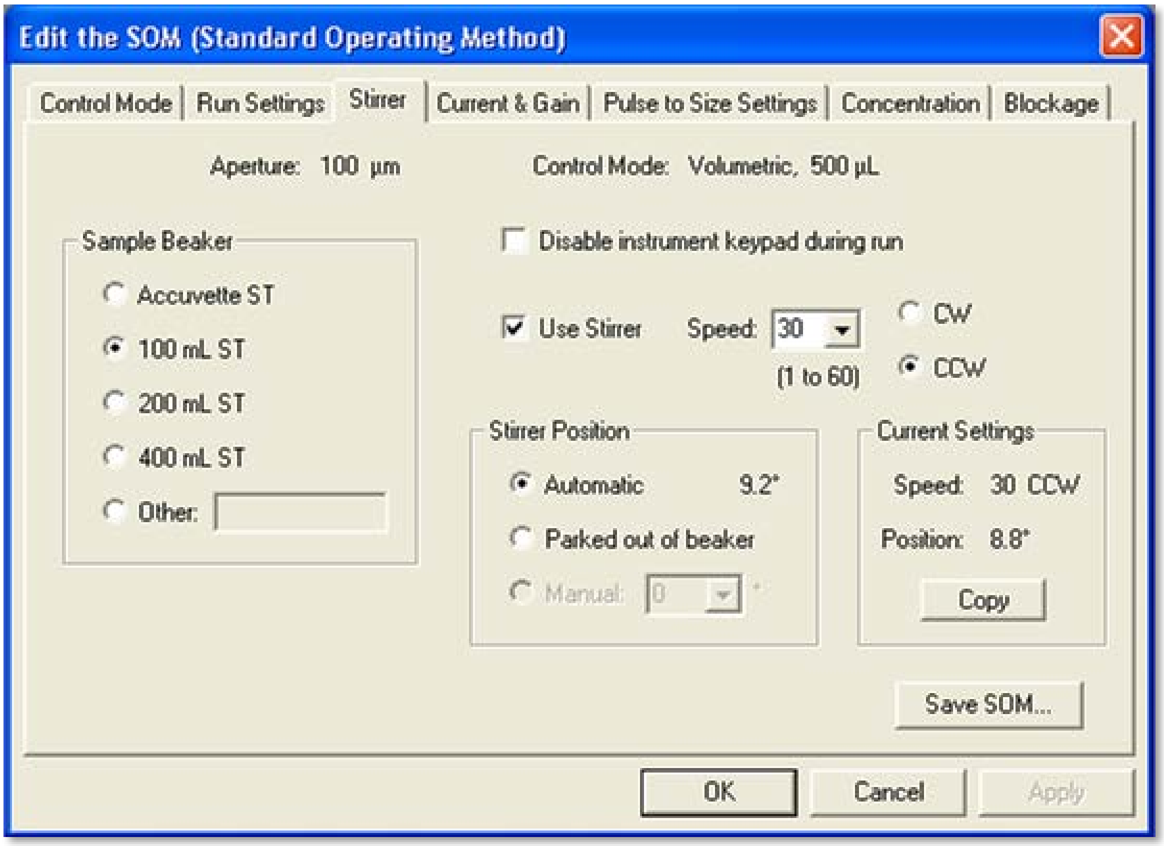
In the field…	Select or enter…
Control Mode	Volumetric: 500 µL
Electrolyte	BCI ISOTON II–glycerol 8:2
Dispersant	Triton X-100 (Coulter I A)
Waste Tank…	Empty Waste Tank when 80% Full

Figure 3. Edit the SOM – Control Mode.



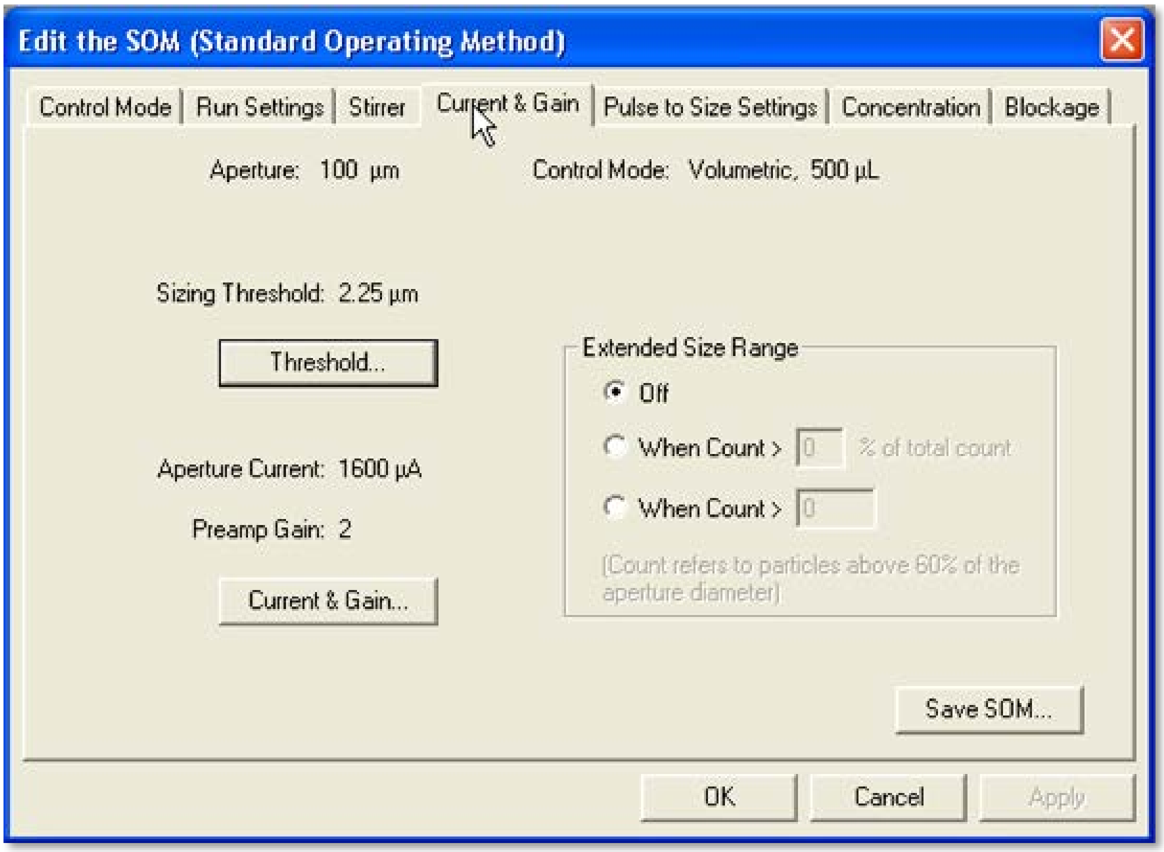
In the field…	Select or enter…
Enter Sample Info before first run	Yes
Flush Aperture Tube before first run	Yes
Number of Runs	10
Wait Between Runs	5 seconds
Flush Aperture Tube after each run	Yes
Save File	Yes
Include Pulse Data	Yes
Save Average of All Runs	Yes
View	Size
Folder…	Create or select a specific folder
File Name…	Create a convenient file name template

Figure 4. Edit the SOM – Run Settings.



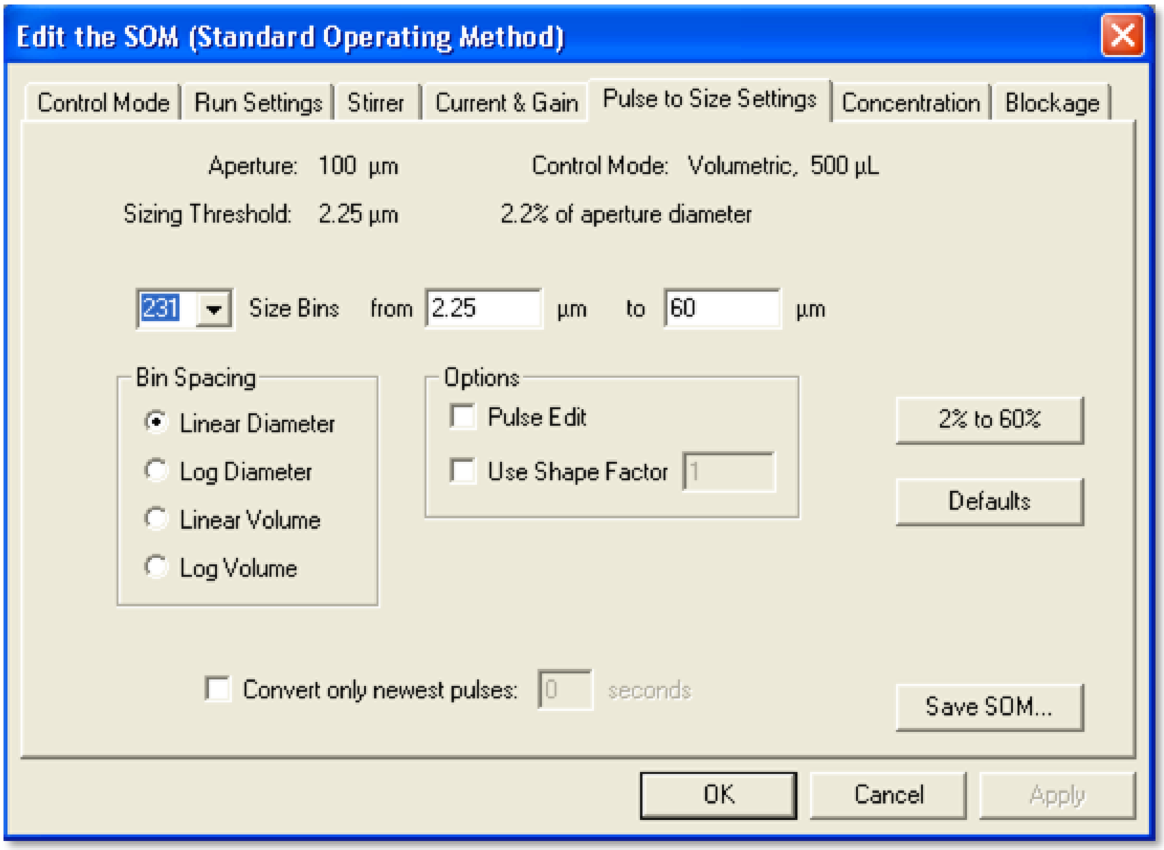
In the field…	Select or enter…
Sample Beaker	Intended Coulter beaker (100 mL or 400 mL)
Stirrer	Use Stirrer
Speed	CCW, 30 for 100-mL Coulter beaker and 40 for 400-mL Coulter beaker
Stirrer Position	Automatic

Figure 5. Edit the SOM – Stirrer.



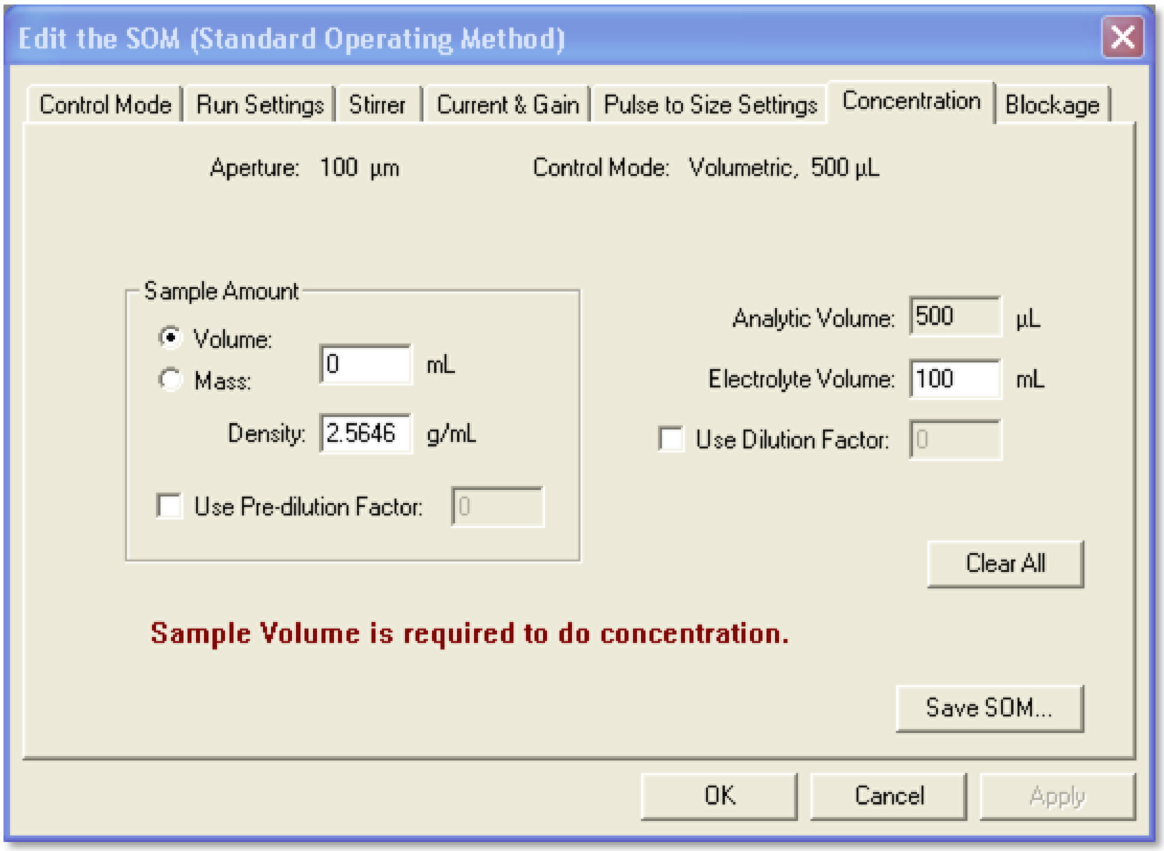
In the field…	Select or enter…
Threshold…	2.25 µm
Current & Gain…	Auto-Set
Extended Size Range	Off

Figure 6. Edit the SOM – Current & Gain.



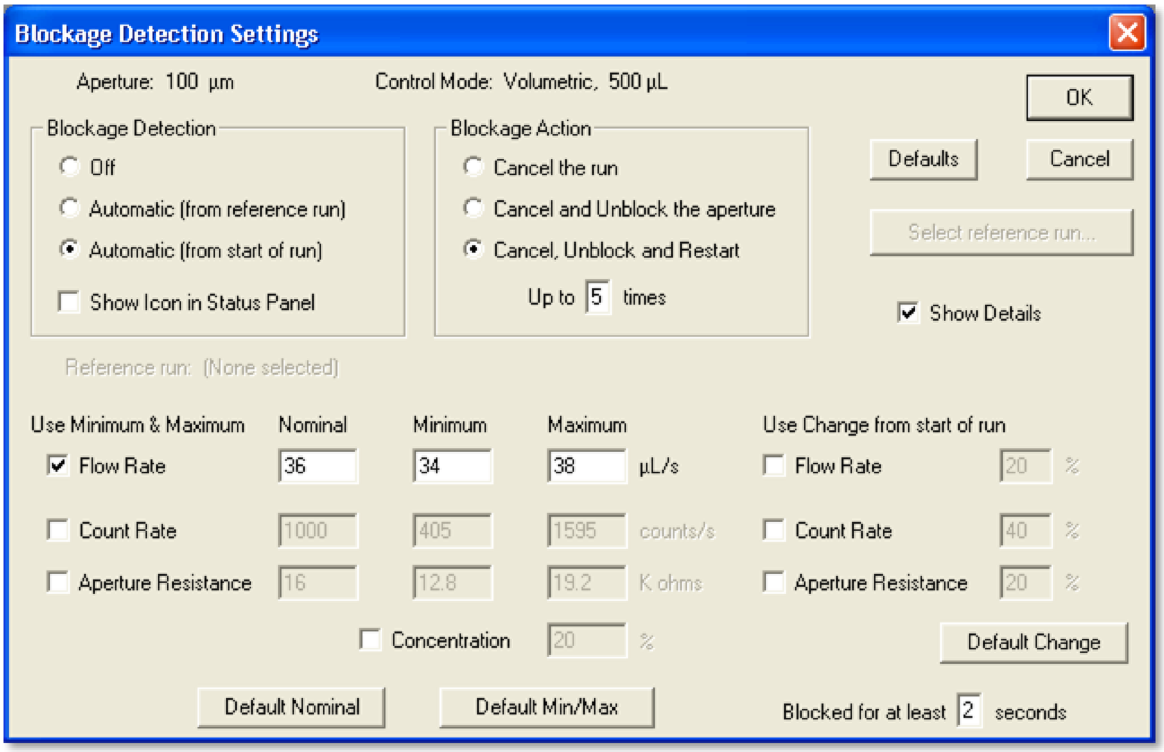
In the field…	Select or enter…
Size Range	231 Size Bins; from 2.25 µm to 60 µm
Bin Spacing	Linear Diameter

Figure 7. Edit the SOM – Pulse to Size Settings.



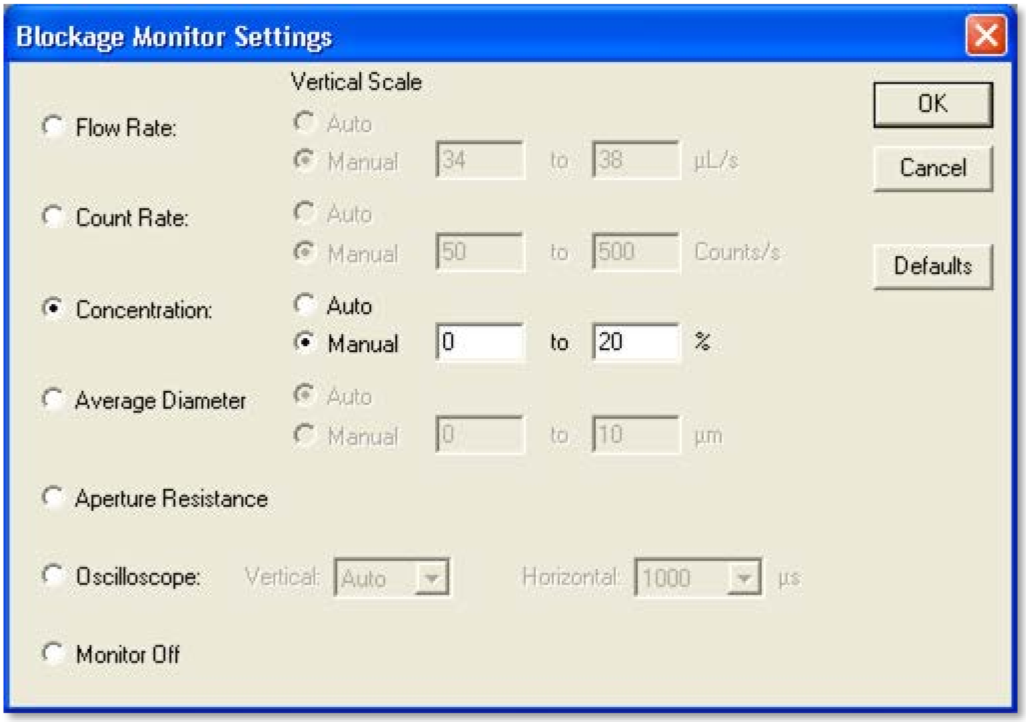
In the field…	Select or enter…
Sample Amount	Measured by volume; Density of particle in upcoming sample analyses (useful in plotting particle mass distributions)
Electrolyte Volume	Specific based on Coulter Beaker volume

Figure 8. Edit the SOM – Concentration.



In the field…	Select or enter…
Blockage Detection	Automatic (from start of run)
Use Minimum & Maximum	Flow Rate: Nominal - 36, Minimum - 34, Maximum - 38
Blockage Action	Cancel, Unblock and Restart Up to 5 times
Blocked for at least	2 seconds

Figure 9. Edit the SOM – Blockage Detection Settings.



In the field…	Select or enter…
Concentration	Manual, 0 to 20%

Figure 10. Edit the SOM – Blockage Monitor Settings.

11.3 Sample Analysis

Samples extracted according to SOP-J15-002.0 are analyzed using the Multisizer 4 as follows:

1. Open the sample compartment door and lower the sample platform by depressing the small stainless steel lever just inside the compartment door (Figure 11).

Figure 11. Multisizer 4 sample platform lowered to install sample.

2. Place a Coulter beaker containing the sample on the sample platform.
3. Load the appropriate SOM for the size of the Coulter beaker and the aperture installed. Click SOP on the Main menu bar of the Multisizer 4 software window and select Load an SOM (Figure 12).
4. After loading the appropriate SOM, raise the sample platform by hand and close the sample compartment door.
5. Click the Start button on the instrument toolbar at the bottom of the Multisizer 4 software window (Figure 13).
6. In the "Enter Sample Info for Next Run" dialog box, enter an appropriate sample identifier and then change the run number to 1 (Figure 14). This creates the file name for the upcoming sample analysis (see section 15).
7. Click OK and wait for the analysis to begin. The software will automatically analyze the number of runs specified in the SOM. During analysis, the concentration bar on the status panel (Figure 15) indicates sample concentration in percent solids in the sample. Sample concentrations should be ≤ 10% solids in order to avoid coincidence errors that could bias the analysis.

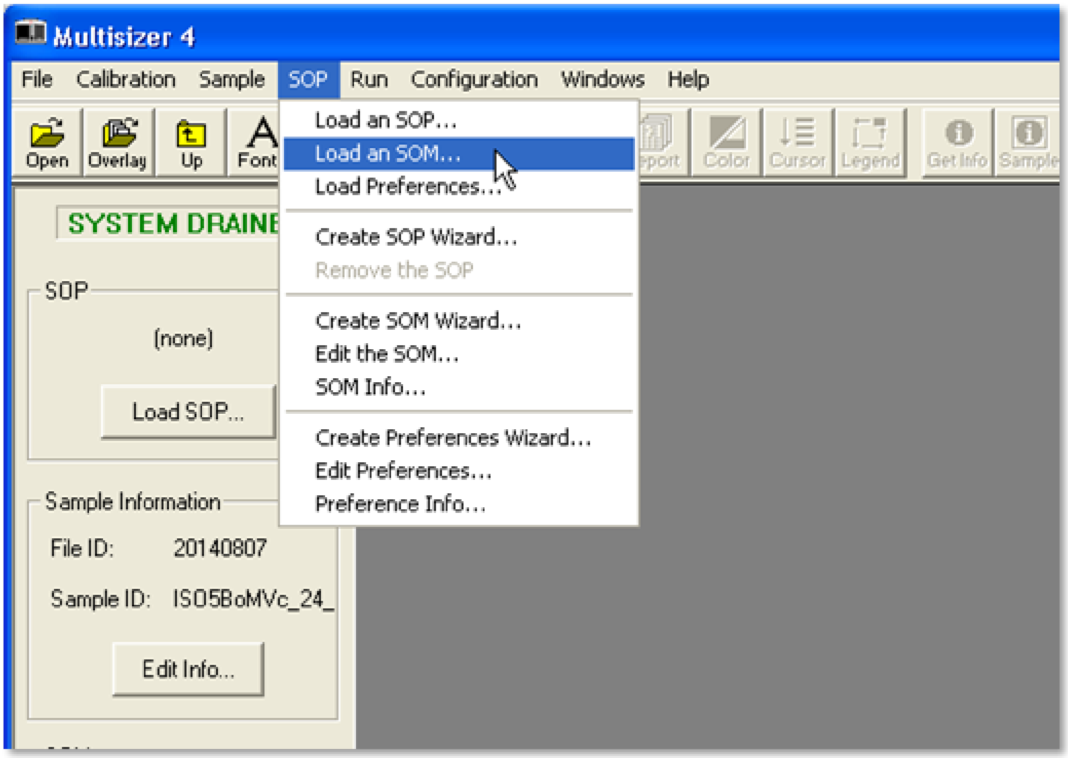


Figure 12. Load appropriate SOM.

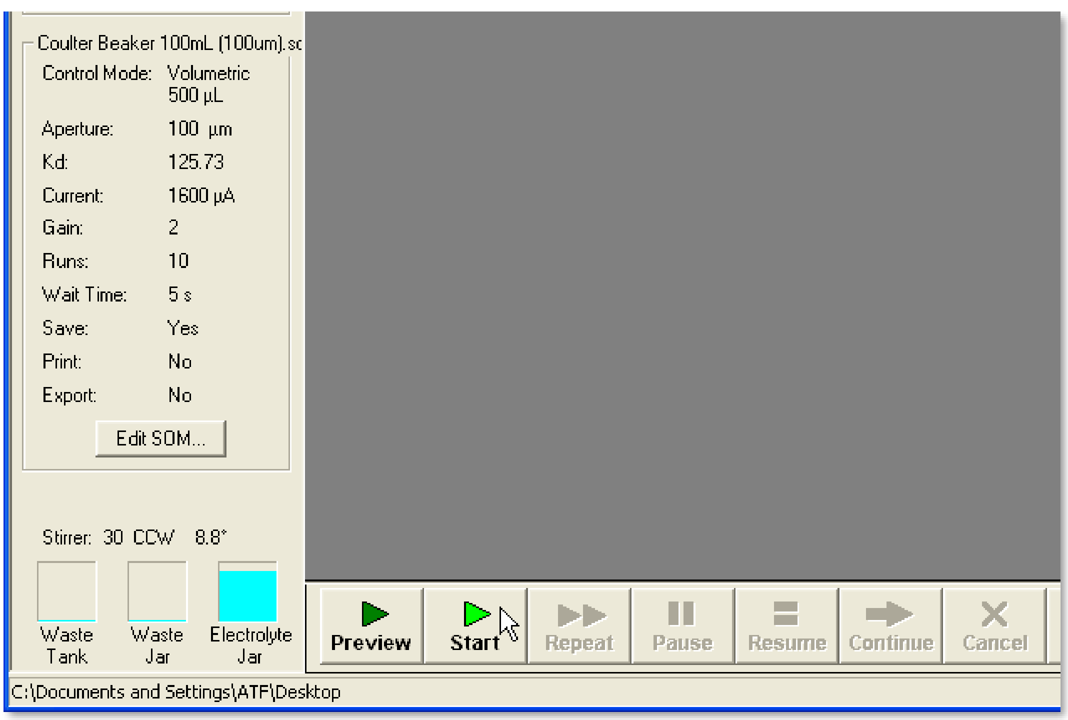


Figure 13. Start sample analysis.

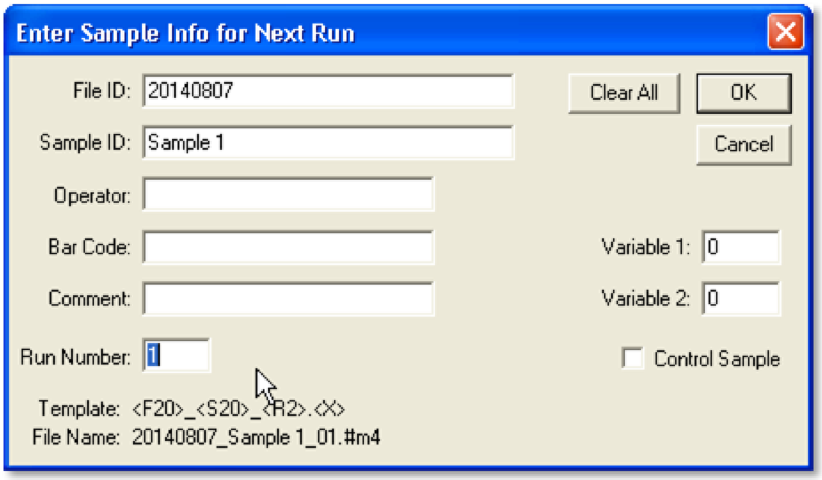


Figure 14. Enter sample information.

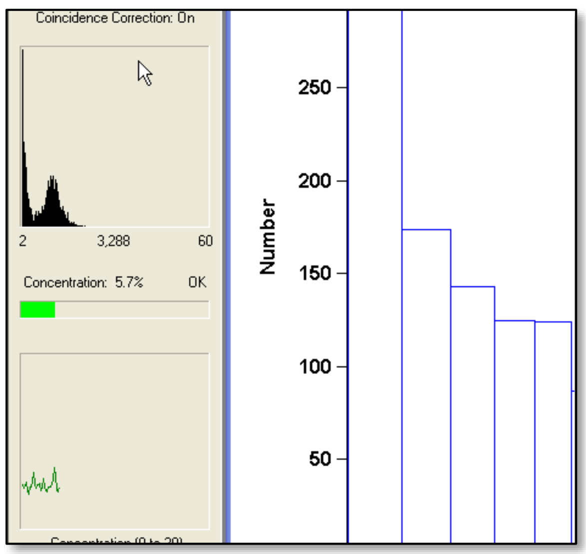


Figure 15. Multisizer 4 concentration bar in % solids (left side of screen).

8. When analysis is complete, open the sample compartment door and lower the platform.
9. Remove the Coulter beaker from the sample compartment and load the next sample for analysis.
10. Rinse the aperture tube and stirrer with filtered DI H_2O using a squirt bottle or beaker.
11. Repeat steps 1 through 10 for all remaining samples.
12. When all samples have been analyzed, overlay the files by choosing Overlay on the instrument toolbar and selecting the 10 run files just captured. Then select RunFile and

"Export Data…." from the menu (Figure 16). Exported data will be saved as an Excel file (e.g., 20140807_sample1.xls).

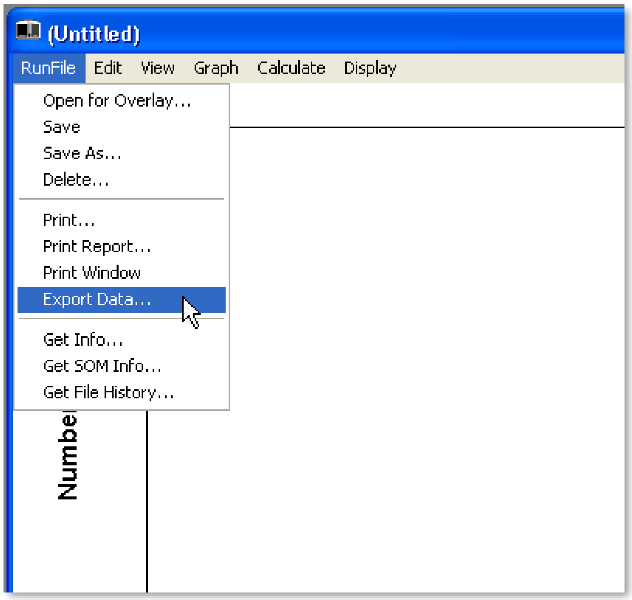


Figure 16. Export data.

12.0 Maintenance

Monitor the following items to maintain an efficient run schedule:

12.1 The electrolyte solution contained within the electrolyte jar is used to fill the system, flush particles out of the system, rinse the aperture tube, and unblock the aperture. The electrolyte dip tube must remain submerged in order to keep air from being pulled into the system during flush, fill, or unblocking procedures. Refer to SOP-ZED-12-01 (Standard Operating Procedure for the Operation and Maintenance of the Beckman Coulter Multisizer 4 Particle Analyzer) for specifics involved in filling the electrolyte jar.

12.2 During analysis, the electrolyte solution that is pumped through the aperture, is then guided to the waste tank inside the Multisizer 4. By default, the contents of the waste tank are automatically sent to the waste jar when 80% capacity is reached. The waste jar is a removable container located next to the electrolyte jar in the Multisizer 4. This process overrides any other process. In order to prevent any interruption during sample analysis, it is good practice to initiate the "Empty" process prior to analyzing a sample by clicking the Empty button on the instrument toolbar at the bottom of the Multisizer 4 software window.

12.3 Keep a beaker or squirt bottle of filtered DI H_2O nearby to rinse the aperture tube and stirrer between samples.

12.4 Maintain an annual calibration and maintenance schedule with a Beckman Coulter, Inc. service representative to ensure all system components are operating correctly and to eliminate any instrument performance issues.

13.0 Troubleshooting

The troubleshooting guide in the Multisizer 4 user's manual provides some guidance to help the user work through issues associated with sample analysis. The table below contains some additional items that have been determined empirically to reduce the need to call the manufacturer when common problems occur.

Issue	Recommended Solutions
(1) Sample concentration too high, indicated by concentration bar on status panel in Multisizer 4 software window.	Could be due to one of two issues: (1) The average concentration of solids in solution is higher than 10%. (2) There is extraneous material blocking the flow of electrolyte through the aperture. For solutions to the latter, see issue 3. Sample requires dilution to lower particle concentration to ≤ 10% solids. Click the Cancel button on the instrument toolbar, and then select RunFile > GetInfo… and scroll through the sample information dialog window to find the sample concentration. Determine the appropriate dilution to bring the particle concentration into the correct range. Remove the Coulter beaker and transfer its contents to a clean 800-mL SS container. Then dispense an appropriate volume of filtered electrolyte to a clean SS container for dilution. Use the new electrolyte to rinse the particles remaining in the Coulter beaker to the SS container with the original extract. Mix the newly diluted sample by transferring the solution back and forth between the two SS containers at least 10 times. Then transfer enough diluted sample into the Coulter beaker to reach the fill line and rerun the sample. If the dilution was successful, record the information on the data sheet and dilute identical samples accordingly.
(2) Sample concentration seems correct but concentration bar spikes above the 10% line.	Sample contains material that is causing momentary aperture blockage. This is a common occurrence that requires immediate attention to ensure particle counts and size information are accurately recorded by the Multisizer 4. Several approaches are recommended in a stepwise process to improve the sample analysis. Click the Unblock button on the instrument toolbar, and then click Repeat on the instrument toolbar. If the issue continues try any one of the following approaches along with the first: - Remove the Coulter beaker from the sample compartment and place a container on the sample platform with enough filtered DI H_2O to submerge the aperture. A Coulter beaker or general-use laboratory beaker will suffice. After the aperture unblock procedure is complete, replace the sample container and click Flush and then click Repeat on the instrument toolbar. - Place the sample container in the ultrasonic bath for 10 seconds. - Add 5–10 drops of non-ionic dispersant to the sample. - Pour the sample through a clean SS sieve to remove extraneous material.
(3) Sample concentration depicted in status panel is significantly higher than 10%, but sample has been diluted to correct concentration.	Aperture is blocked. Remove sample container and aperture tube. Empty the aperture tube into the sink, and then rinse and fill with filtered DI H_2O. Place a thumb on top of the aperture tube and apply downward pressure to force liquid through the aperture. Repeat the procedure until a steady, uninterrupted stream is seen squirting from the aperture. Then empty the aperture tube. Replace the aperture tube and click Fill on the instrument toolbar. Then rerun the sample. If this does not fix the issue, consider reviewing your dilution information and/or refer to the solutions to issue 2 in the troubleshooting guide.

14.0 Data Acquisition, Calculations, and Reduction

14.1 Overlay and Review Data Files Collected in Multisizer 4 Software Window

1. Overlay the average files from each analyzed sample (e.g., R1, candidate, and R2) and export the size listing by using the Overlay and Export Data options (see section 11.3, step 12).
2. Review the overlay statistics from the Excel data file containing the exported data. A coefficient of variation (CV) ≥ 5% indicates that a significant issue occurred during analysis that biased the readings of one or more of the runs. In this case, more analyses of the sample should be captured until a CV of ≤ 5% is achieved.

14.2 Reduce Data and Determine Sample Particle Concentration Using Microsoft Excel

1. Open the Excel data file containing the exported data.
2. Calculate particle concentration in counts/m^3 for each sampler across the entire size range of interest using the following equation:

$$\text{Particle concentration, } \frac{\#}{\mathrm{m}^3} = \frac{\#}{\mathrm{mL}} \times \frac{V_{extract}}{Q \times t} \times 1000,$$

   where

   \# = particle counts

   $V_{extract}$ = extract volume in mL

   Q = sample volumetric flow rate in Lpm

   t = test duration in minutes

3. Calculate the particle concentration ratio between R1 and R2 across the entire size range of interest.
4. Calculate the candidate sampler effectiveness across the entire size range of interest. That is, the particle concentration ratio as measured by the candidate sampler to the average wind tunnel concentration as measured by the reference samplers:

$$\text{Candidate sampler effectiveness} = \frac{2 \times C_{candidate}}{(C_{R1} + C_{R2})},$$

   where

   C = particle concentration in #/m^3

15.0 Data and Records Management

All electronic data files should be saved to the desktop hard drive and backed up on an external hard drive for protection. This includes raw Multisizer 4 data files as well as Microsoft Excel files containing wind tunnel aerosol concentration data and size-selective performance data of candidate sampler inlet(s). Using a consistent naming structure will ensure the integrity of data records is maintained throughout the project.

Example: YYYYMMDD_SSS_WS_RN

Where:

YYYY – 4-digit year

MM – 2-digit month

DD – 2-digit day

SSS – sample source identifier

WS – 2-digit wind speed designation (e.g., 02 km/h, 24 km/h)

RS – 2-digit replicate number

16.0 Quality Control and Quality Assurance

16.1 The CV between successive runs of a sample should be less than or equal to 5%.

16.2 Sample concentration should be near but not overshoot 10% solids in order to increase particle count precision across successive analytical runs.

16.3 Verification of Multisizer 4 calibration should be conducted monthly or when an aperture tube is changed. This includes verifying counting accuracy using the concentration control standard and particle sizing accuracy using the Coulter size standard. For specific details regarding calibration and verification, refer to the Multisizer 4 user's manual or SOP-ZED-12-01, "Standard Operating Procedure for the Operation and Maintenance of the Beckman Coulter Multisizer 4 Particle Analyzer."

17.0 References and Supporting Documentation

SOP-J15-002.0 (formerly draft SOP-ZD-14-02(1) prepared by Alion Science and Technology and RTI International), Collection and Extraction of Challenge Aerosol for Subsequent Coulter Analysis, August 2014. Research Triangle Park, NC: Jacobs Technology Inc.

Multisizer™ 4 Particle Analyzer User's Manual. Beckman Coulter, Inc., 250 Kraemer Blvd., Brea, CA 92821.

SOP-ZED-12-01, Standard Operating Procedure for the Operation and Maintenance of the Beckman Coulter Multisizer 4 Particle Analyzer, January 2012. Research Triangle Park, NC: Alion Science and Technology.