Optimizing the Beckman Coulter Multisizer 4e for use with small apertures

Introduction

The purpose of this application note is to describe a method which achieved successful testing on a Multisizer 4e Coulter Counter when employing apertures that are 20 µm and smaller. Additional information for changing and working with small size apertures is given in Chapter 4 of the User's Manual. Here, we provide process steps for instrument setup and preparation, as well as sample measurement, based on a protocol that was successfully applied when working with small aperture tubes.

Using aperture tubes below 30 µm on a Multisizer instrument can be challenging due to the small size of the aperture which becomes challenging because they can become easily clogged by larger particles. Many of these particles may come from random dust and other contaminating materials in the instrument environment.

It is necessary to take some proactive steps to ensure a smooth and accurate testing process when working with smaller apertures. Placing the instrument in a controlled environment such as a laminar flow hood (low airborne particulate levels and reduced electrical noise) is highly recommended. In addition the instrument should be thoroughly cleaned as recommended in Chapter 4 of the User's Manual.

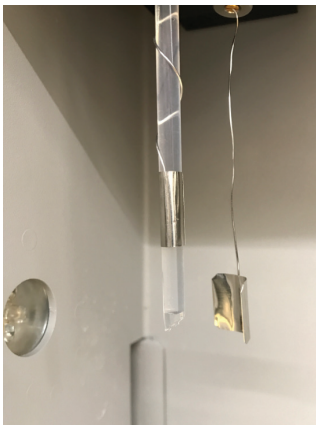

Figure 1. Electrode positioning

Filtering Isoton and electrolyte solutions

For easy and accurate dispensing, a graduated dispenser was used, which fits different bottles to dispense the Isoton II electrolyte solution. For convenience a 2 L bottle of IsoFlow Sheath Fluid, PN 8547008, was used.

The dispenser has a nozzle to which a syringe filter or series of syringe filters can be fitted to remove any stray particles and ensure the cleanest possible electrolyte solution for use with the sample.

Beckman Part Number	Description
8320309	Fixed volume repipet dispenser
8547008	IsoFlow Sheath Fluid
C96980*	Isoton II diluent 10L (Americas)
8448011*	Isoton II diluent 20L (Europe/METAI)
8546719*	Isoton II diluent 10L (East Asia/Australia)
	PALL Acrodisc® Syringe Filters
4611	0.1 µm, 25 mm
4612	0.2 µm, 25 mm

*Part number varies depending on the region.

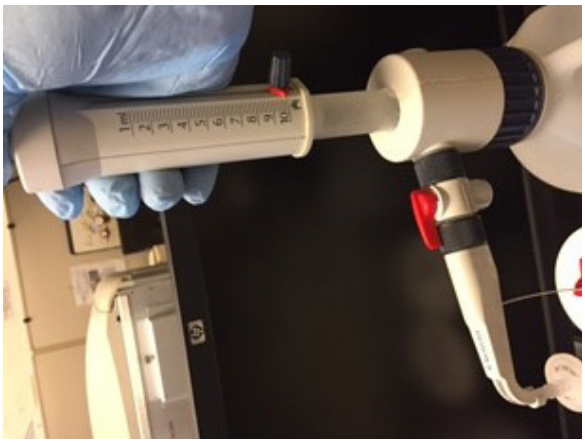

Figure 2. 8320309 Graduated 10 mL dispenser

Figure 3. Syringe filters

Process steps for instrument setup and preparation

1. Remove the Electrolyte Jar from the Multisizer 4e Coulter Counter and perform a thorough cleaning of the jar and ensure the final rinse is done with a laboratory grade DI water.
2. Fill the Electrolyte Jar with fresh Isoton II that has been filtered through at least a 0.2 µm filter.
3. Ensure that the internal electrode is ~1/2" above the tip of the filling Teflon tubing. If not, carefully wind its wire around the filling tubing until the electrode is at ~1/2" above the tip of the tubing as shown in Figure 1.
4. Follow the "Change Aperture Tube Wizard" function located in the Run menu to install the new aperture. Before installation of the new aperture ensure it is clean and the orifice inlet is clear of debris. Do not touch the orifice, and it is essential to use particle-free gloves.
5. Using a 200 mL beaker of clean, 0.2 µm filtered DI water, rinse the outside of the aperture tube and the electrode by completely immersing them in the water, gently swirling the fluid as you rinse down the aperture tube.
6. Use the Beckman Coulter Life Sciences Accuvette ST when performing small particle sampling. Ensure the new accuvettes and caps are rinsed thoroughly with lab grade filtered DI water, preferably filtered down to a minimum of 0.1 µm.
7. If using a separate precision fluid dispenser ensure that a minimum of a 0.1 µm disc filter is used when dispensing new Isoton II into the rinsed accuvette. Fill accuvette to either the 10 mL or 20 mL level depending on the application or material requirements.
8. After the "Change Aperture Tube Wizard" steps have been completed, add the suitable particle size control standard to the accuvette. Attach the cap and roll the accuvette gently between your hands to adequately suspend the particles and prevent bubbles in the sample.
9. Perform a Calibration if the aperture was not previously calibrated, or perform a Verify if the aperture was previously calibrated.
10. After calibration/verification is complete, repeat step 5 only to thoroughly rinse the aperture tube and electrode prior to running samples.

Process steps for running samples

1. Install cleaned accuvette with filtered electrolyte into the Multisizer 4e Coulter Counter and perform preview, flush and preview again to ensure zero counts in the progress bar. When reviewing the real time result the graph will indicate if the blank fluid and containers are clean and your process is sound.
2. When satisfied with Preview, cancel it and run three blanks using the same Control Mode that will be used in your SOM when formally running the samples, e.g., Volumetric or Time mode; flush before run, etc. See Figure 4 below.
3. Ensure that the SOM is set up for only 1 run at a time, not multiple runs, to reduce blockages. Set the pre–flow stabilizing time to 10 seconds and total stabilizing time to 15 seconds respectively for best accuracy as shown in Figure 5 below.
4. Perform “Unblock” upon completion of each run to ensure there is no buildup of material at the aperture inlet. “Unblock” will disperse the particles that gathered at the aperture by inertia after a run.
5. Some samples, such as proteins may require you to clean between runs with a 5% solution of Micro 90 Cleaner because of the tendency of protein to stick to the aperture surface and create noise. Have a squirt bottle of DI water to spray the aperture after the Micro 90 Cleaner.
6. In general, it is good practice to clean with Micro 90 solution between samples and at the end of the last sample to maintain the aperture in optimal condition.

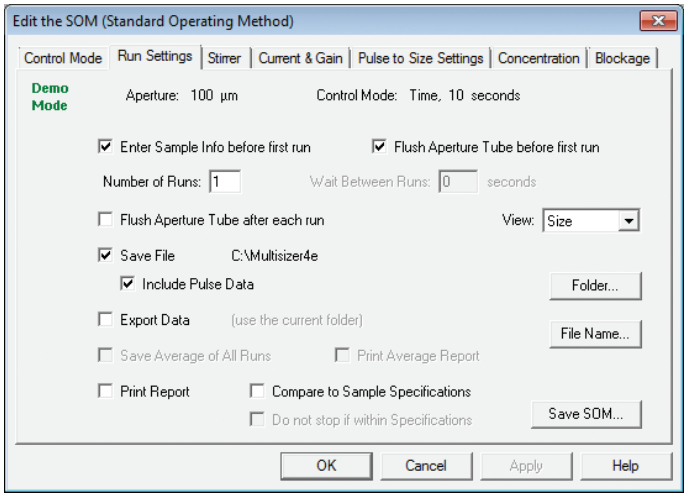

Figure 4

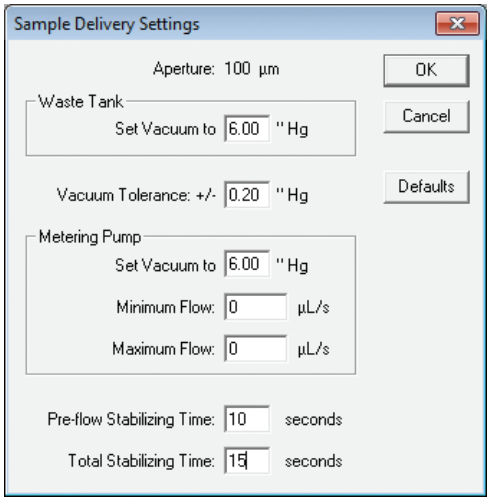

Figure 5

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

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