Job Aid

BD FACSymphony™ A1 System: Performing the initial small particle detector setup procedures

This job aid contains instructions for the initial setup of the BD® Small Particle Detector (SPD) when using the BD FACSymphony™ A1 Flow Cytometer. See the Small particle detector chapter in the *BD FACSymphony™ A1 Flow Cytometer user guide* for more information.

Before you begin

- Create the SPD QC template described in the BD FACSymphony™ A1 System: Creating the small particle detector QC experiment template job aid.
- Start the cytometer and run a performance check.

Materials needed

- BioCytex™ Megamix-Plus SSC Beads (BioCytex reference: 7803)
- Filtered diluent (0.2 µm-filtered 1X PBS)
- 1.5% dilution of BD® Detergent Solution Concentrate
  NOTE: Dilute before use. Mix one full 15 mL bottle of BD® Detergent Solution Concentrate into 985 mL of DI water to make 1 L total.

Preparing the Samples

Preparing the Megamix bead tube:

- Label a clean, capped 12x75 tube *Megamix beads*.
- Mix the Megamix-Plus SSC beads by gently pipetting the mixture.
- Pipet 500 µL of the Megamix-Plus SSC beads into the Megamix beads tube.

Preparing the diluent tube:

- Label a clean, capped 12x75 tube *Diluent*.
- Pipet 3 mL of the 0.2 µm-filtered 1X PBS into the Diluent tube.

Preparing the cleaning tube:

- Label a clean, capped 12x75 tube *Detergent solution*.
- Prepare a tube with 3 mL of a 1.5% dilution of BD® Detergent Solution Concentrate.

NOTE: Never mix BD® Detergent Solution Concentrate and bleach because they create chlorine gas.

SPD tube removal process

Additional precautions are required when using the BD® Small Particle Detector to prevent sample carryover and fluidic fluctuations. Before you begin this procedure, practice loading tubes to ensure that you can perform all the steps in around 10 seconds.

To avoid adverse fluctuations in background event rate, ensure that the tube support arm is to the side for no longer than 15 seconds.

① Remove the existing tube from the SIP.

② Wipe the SIP with a delicate task wipe to remove any residual liquid from outside the SIP.

③ Install a new tube on the SIP.

④ Place the tube support arm under the tube.

If needed, repeat this procedure until you can complete the steps in around 10 seconds.

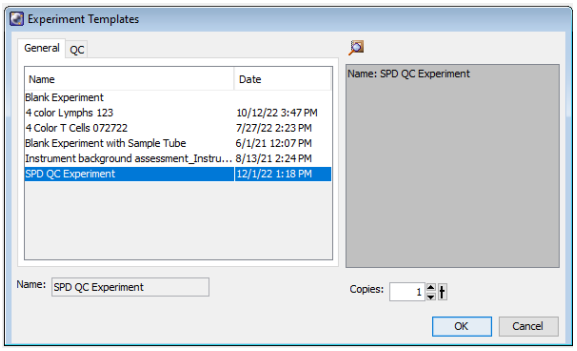
Performing the initial SPD setup procedures

Creating the initial settings

Create initial settings by placing the beads onscale using the SPD QC Experiment template.

① On the cytometer control panel, press the RUN and LOW buttons.

NOTE For best results when working with small particle detection, the cytometer should stay in run mode with a low flow rate.



② Import the SPD QC Experiment.

a. Select a folder in the Browser, then select **Experiment > New Experiment**.

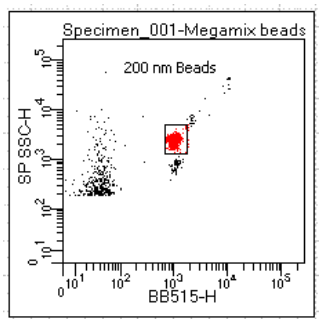
b. Select the SPD QC Experiment template in the Experiment Templates wizard and click **OK**.

③ Set the current tube pointer to the Megamix beads tube.

④ Install the Megamix beads tube onto the cytometer and click **Acquire Data** in the Acquisition Dashboard.

NOTE Leave the bead tube on the cytometer until the directions tell you to remove the tube. If you must remove the tube, follow the SPD tube removal procedure.

⑤ In the Cytometer window, roughly adjust the BB515 voltage until the bead population is placed in the middle of the SP SSC-H vs BB515-H dot plot.

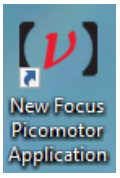


Aligning the small particle detector

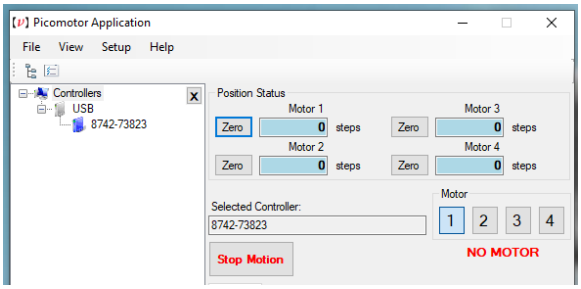
Next, you will optimize the signal by aligning the SPD using the New Focus Picomotor Application.

Setting up the application and determining the motors

① Double-click the New Focus Picomotor Application icon on the desktop to open the application.



② In the Picomotor Application dialog, ensure that the **Controller** is selected.



③ Determine if your system is configured with a Motor 1/2 set or a Motor 3/4 set.

a. In the Motor area, select Motor 1.

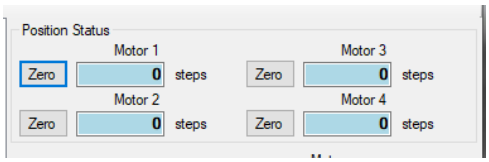
A NO MOTOR message will be displayed if the selected motor is absent from your system.

b. Select Motor 3 to confirm the appropriate motor set.

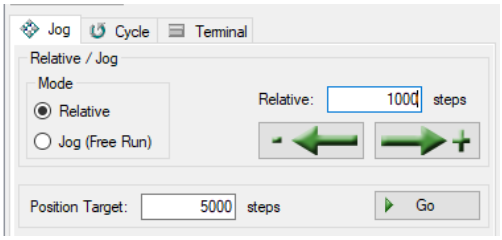
Your system will be configured either with Motors 1 and 2 or Motors 3 and 4.

Setting up the application and determining the motors, continued

④ Under Position Status, click the **Zero** button for Motor 1 or Motor 3 and for Motor 2 or Motor 4.



⑤ Under Mode, select **Relative** and enter *1000* steps in the Relative field.



Adjusting the motors

① Arrange your workspace to display both New Focus Picomotor Application and the SPD QC worksheet plots.

② Select Motor 1 or Motor 3, depending on your setup.

③ In the Relative steps area, click the – (minus) or + (plus) arrows to move the motor stage in small increments.

You should see the bead populations move as you adjust the steps.

④ Repeat for Motor 2 or Motor 4.

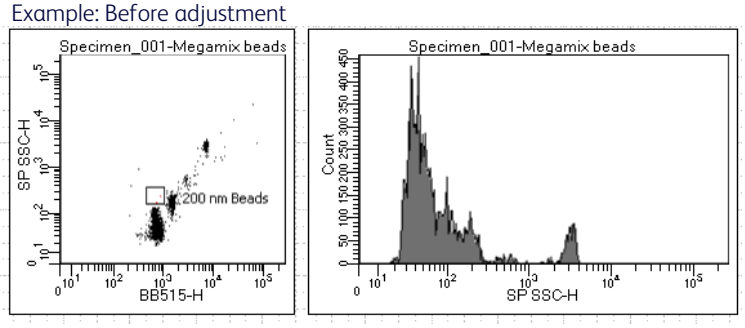
a. Select the appropriate motor.

b. Click the arrows to adjust the motor.

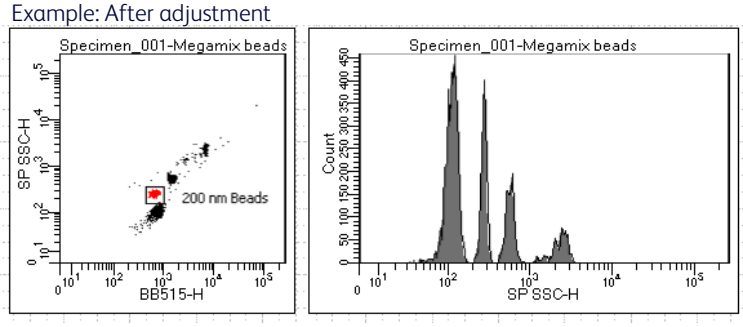
⑤ Adjust each motor one step at a time until:

- For the SP SSC-H v BB515-H plot, the bead populations are as compact as possible.
- For the SP-SSC-H histogram, the bead peaks are well separated.

Example: Before adjustment



Example: After adjustment

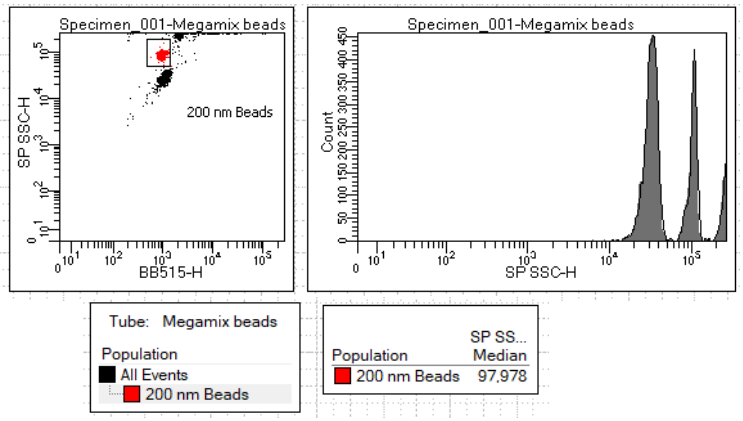


⑥ Close the application.

Recording data

Record data for the Megamix beads and Diluent tubes.

Recording data for the Megamix bead tube



① In the Cytometer window, adjust the SP SSC voltage to place the 200 nm SP SSC-H median at around 100,000.

The bead peak at 500 nm will be offscale, and the bead peak at 240 nm will be either offscale or close to it.

② Move the 200 nm bead gate to fully encompass the 200 nm population.

③ Click **Record Data**.

Removing bead traces from the system

Next, run the Detergent solution tube to remove traces of beads from the system.

① Remove the Megamix beads tube from the SIP.

② Wipe the SIP with a delicate task wipe.

③ Install the Detergent solution tube on the SIP.

④ Place the tube support arm under the tube.

⑤ Run the detergent solution for 15 minutes.

Recording the Diluent tube

Next, run the Diluent tube to check for background.

① Remove the Detergent solution tube from the SIP.

② Wipe the SIP with a delicate task wipe.

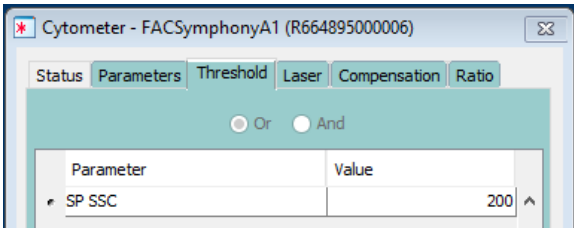
③ Install the Diluent tube on the SIP.

④ Place the tube support arm under the tube.

⑤ Set the current tube pointer to the Diluent tube.

Recording the Diluent tube, continued

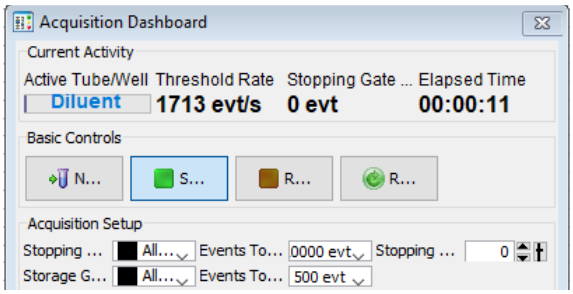
⑥ In the Threshold tab, select **SP SSC** as the parameter and verify that the threshold value is set to 200.



⑦ Click **Acquire Data.**

⑧ In the Acquisition Dashboard, observe the threshold rate for approximately 5 minutes.

- The threshold rate should slowly decrease and then plateau.
- If the threshold rate has not plateaued after 5 minutes, see the Small particle detector troubleshooting section in the BD FACSymphony™ A1 Flow Cytometer user's guide.



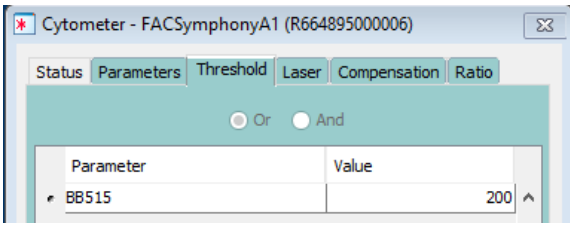
⑨ Click **Record Data.**

⑩ Record for 1 minute, then click **Stop Recording.**

Saving application settings

Save the settings as application settings to use as a starting point for subsequent experiments.

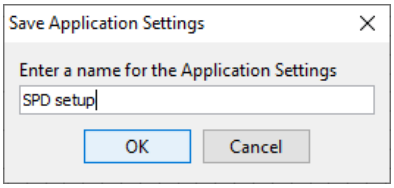
① In the Threshold tab in the Cytometer window, reselect the BB515 parameter.



② Right-click the Cytometer Settings icon and select **Application Settings > Save.**

③ Rename the settings, *SPD setup*.

④ Click **OK**.



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