

# BD FACScan System

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After completing this module, you will be able to:

- Describe the BD FACScan™ system and how it works.
- Perform instrument startup and shutdown procedures.
- Describe instrument maintenance procedures.

# BD FACScan™ System

The FACScan™ system is an automated flow cytometer. It analyzes cells as they pass through a focused laser beam one at a time in a moving fluid stream. A BD FACScan flow cytometer can be used for routine research applications, immunophenotyping, and DNA cell-cycle analysis.

The FACScan system consists of a sensor module (Figure 2-1), computer module, and several software packages. Many of the instrument functions are controlled by the software.

## Cytometer

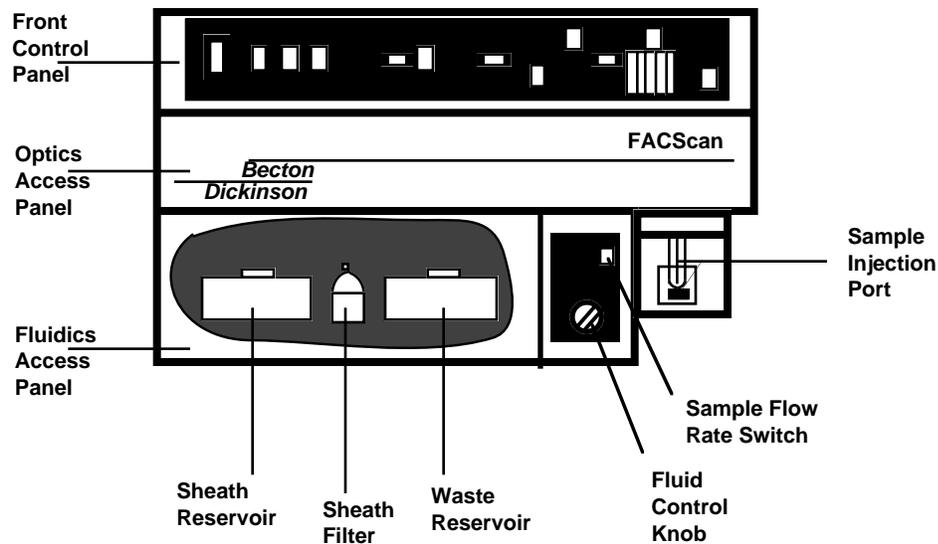


Figure 2-1 BD FACScan Sensor Module

## Power Switch

This switch, located on the left side of the Front Control Panel, turns the cytometer on and off.

## Fluid Control Panel

This panel consists of the fluid control dial and the sample flow rate switch (Figure 2-2).

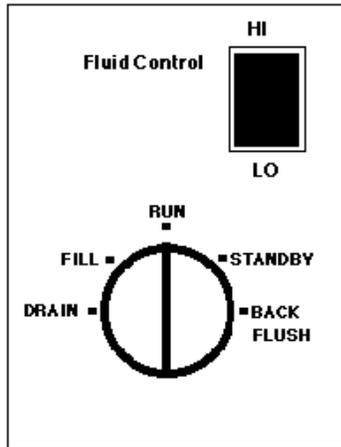


Figure 2-2 Fluid Control Panel

### Fluid Control Dial

This rotary dial allows you to select from five fluidic modes.

**DRAIN**—forces liquids out of the flow cell and fluid lines leading to the waste.

**FILL**—fills the flow cell and fluid lines with sheath fluid at a controlled rate, which prevents bubble formation.

**RUN**—pressurizes the sample tube to transport the cell suspension through the sample injection tube and into the flow cell. When a tube is not on the Sample Injection Port (SIP), the cytometer goes into auto standby. Sheath flow is restricted and laser power is lowered.

**STANDBY**—stops sheath flow and lowers laser power to extend the laser life. Hard Standby and Auto Standby are defined under Status Controls of the Control Panel.

**BACKFLUSH**—reverses the flow of sheath fluid and flushes fluid out of the sample injection tube to remove clogs.

### Sample Flow Rate Switch

The HI/LO flow rate switch selects the sample flow rate.

- **LO**—12  $\mu\text{L}/\text{min}$  of sample through the flow cell.
- **HI**—60  $\mu\text{L}/\text{min}$  of sample through the flow cell.

## Fluidics Access Panel

The fluidics access panel (the lower left panel of the instrument) opens down for easy access to the fluidics reservoirs and sheath filter. See Figure 2-1.

## Sheath Reservoir

This 4-liter container, located on the left, holds enough sheath fluid for approximately 3 hours of run time. It is equipped with a low-level fluid detector which indicates via software a near-empty condition.

## Waste Reservoir

This 4-liter container, located on the right, collects the fluid waste. A fluid-level detector indicates via software a near-full condition.

**⚠ CAUTION:** If potentially biohazardous materials are run through the instrument, treat the materials as biohazardous waste.

## Sheath Filter

This 0.22- $\mu\text{m}$  filter cleans the sheath fluid before it enters the flow cell. This reduces the amount of debris in the sheath fluid.

## Vent Valve Toggle Switch

A switch that when set in the direction of the arrow, relieves the sheath reservoir of air pressure. This allows for the removal of the reservoir when refilling.

## Air Filter

This component filters the air that cools the laser.

## Optics Access Panel

This door shields the compartment that houses the flow cell assembly. Open this door to view the flow cell while draining and filling.

## Flow Cell

This quartz cuvette is where the laser intercepts the sample stream. The dimensions are 430  $\mu\text{m}$  x 180  $\mu\text{m}$ .

## Sample Injection Port

The SIP is the area where the sample tube is installed. It consists of the sample injection tube through which the sample travels to the flow cell, the tube support arm, and the droplet containment system (Figure 2-3).

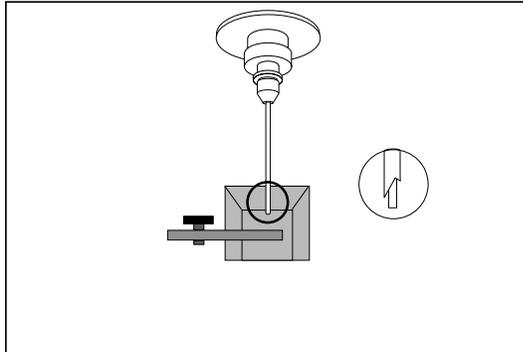


Figure 2-3 Sample Injection Port

## Sample Injection Tube

This is the stainless steel tube that carries the cells from the sample tube to the flow cell. The tube is covered with an outer sleeve that serves as part of a droplet containment system. See Figure 2-4. The droplet containment system employs a vacuum pump to aspirate fluid and prevent drips from splashing onto the countertop.

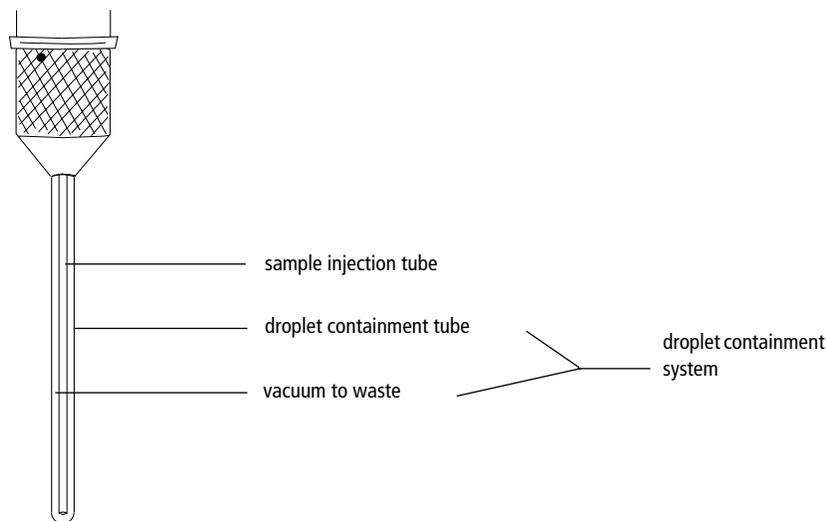


Figure 2-4 Sample Injection Tube and Droplet Containment System

## Tube Support Arm

This arm supports the sample tube. The arm has three positions: centered below the sample tube, to the right, or to the left of the sample tube. Its main function is to activate the droplet containment vacuum. The vacuum is activated when the arm is in the right or left positions and off when the arm is centered.

## Droplet Containment System

This system consists of a vacuum pump and an outer tube that surrounds the sample injection tube. When the tube support arm is on either side of the sample injection tube, the vacuum pump is activated, removing sheath as it backflushes from the tube. See Figure 2-3. When the tube support arm is centered, the pump is deactivated.

**⚠ CAUTION:** When placing a tube on the SIP, be sure to center the tube support arm as soon as possible to avoid having the sample aspirated and removed to the waste reservoir.

## BD FACScan Control Panel

The BD FACScan instrument controls are located on the top panel of the instrument. See Figure 2-1.

### Front Control Panel

The panel contains these controls and indicators:

**GAINS**—for adjusting the PMTs and FSC photodiode.

**THRESHOLD**—for setting the threshold level on the triggering channel.

**COMPENSATION**—for setting the compensation networks.

**PULSE AMPLITUDE**—for displaying the peak amplitudes of the five signal pulses.

**STATUS**—for verifying the cytometer is ready for acquisition.

**TEST**—for verifying all parameter channels are operating correctly.

## GAINS Selections

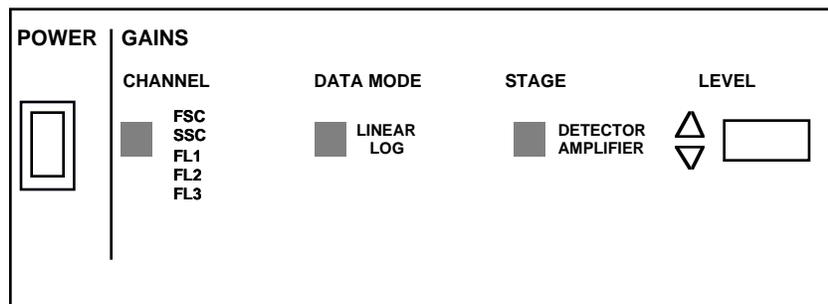


Figure 2-5 GAINS Selections

Five parameters are available by cycling through the CHANNEL touchpad.

The DATA MODE selects LINEAR or LOG amplification for each of the five parameters.

The STAGE selects whether the detector (DET) or amplifier (AMP) levels will be displayed.

The LEVEL of the detector or amplifier is adjustable by means of the up and down switches located immediately to the left of the level display.

## THRESHOLD

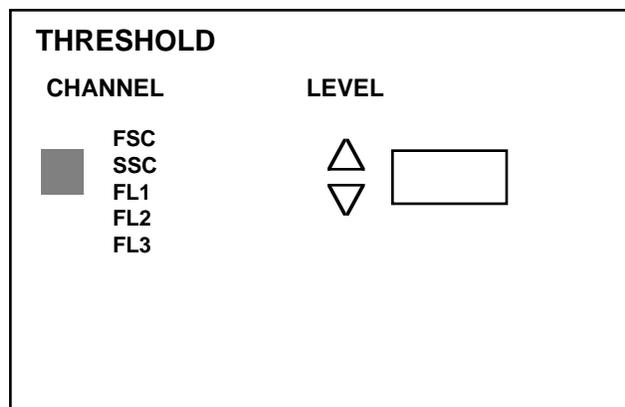


Figure 2-6 THRESHOLD Control

THRESHOLD control sets a lower-level discrimination on the current threshold (trigger) parameter selected on the left. The threshold level is adjusted with the up and down level switches next to the level display. See Figure 2-6. The instrument ignores signals that do not exceed this threshold setting which is actually a channel number. This provides a method for eliminating background or small particles that are not to be acquired. The range is 0 to 996 in steps of four.

## COMPENSATION

COMPENSATION controls adjust the electronic circuitry that reduces the unwanted signal of a fluorochrome that overlaps the signal of another fluorochrome.

The four compensation networks are selected by cycling the COMPENSATION touch pad. The compensation value is adjusted with the level switches to the left of the level display. The values are displayed on the level indicator and range from 0.0 to 99.9 in steps of 0.1 (Figure 2-7).

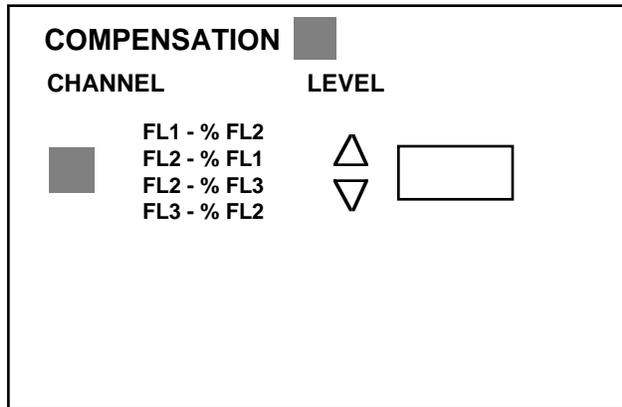


Figure 2-7 COMPENSATION Controls

## PULSE AMPLITUDE

The PULSE AMPLITUDE displays the peak amplitudes generated by the five measurement parameters (FSC, SSC, FL1, FL2, and FL3). Pressing the touch pad toggles the display on and off. See Figure 2-8.

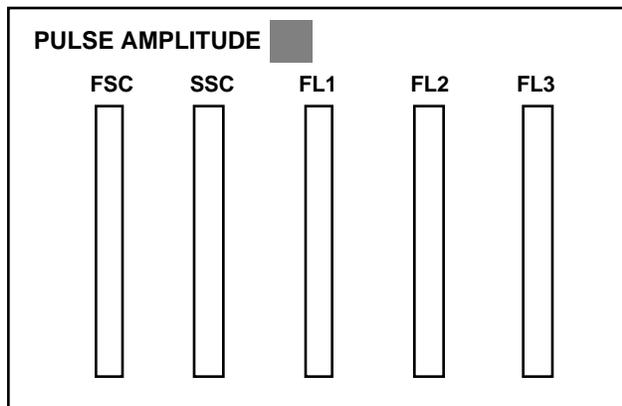
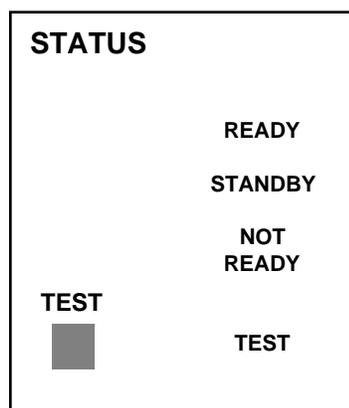


Figure 2-8 PULSE AMPLITUDE Display

## STATUS



**Figure 2-9** STATUS Control and TEST Mode Switch

**READY**—pressurizes the sample tube to transport the cell suspension through the sample injection tube and into the flow cell.

An illuminated **READY** indicates that a sample has been installed on the SIP, the fluid control dial is in the **RUN** position, and data is being collected and processed under the conditions set by the instrument controls (Figure 2-9). The computer can then be used to view, store, and analyze the data.

**STANDBY**—stops sheath flow and lowers laser power to conserve sheath fluid and prolong laser life.

- **Hard STANDBY**—When the fluid control dial is manually turned to **STANDBY** all fluid valves are closed except for one valve that vents the sample test tube air pressure to the atmosphere. Additionally, the laser input current is reduced to a standby level and **STANDBY** is illuminated.

After turning the fluid control dial back to **RUN**, **STANDBY** will continue to be illuminated until you introduce a sample tube. After a sample has been introduced, **READY** will then be illuminated.

- **Auto STANDBY**—If the instrument is in **RUN** and the sample test tube is removed, **STANDBY** is illuminated. This is called auto standby. Twelve seconds later, the system shuts off the sheath flow by means of a pinch valve between the flow cell and the waste reservoir. Additionally, the laser input current is reduced to a standby level. In this mode, back pressure is maintained to flush the sample injection tube. The system returns to **RUN** mode when you reinstall the sample tube. The pinch valve is opened, the laser returns to full power, and **READY** is illuminated.

## NOT READY

There are three conditions that cause NOT READY to be illuminated:

- The sheath fluid reservoir is empty.
- The waste fluid reservoir is full.
- The five-minute laser warm-up period is in progress.

**⚠ CAUTION** There is nothing to prevent you from running samples when NOT READY is illuminated, assuming the sheath and waste levels are in running condition. However, running the instrument when the laser is not completely warmed up may result in low signal strengths and decreased precision for all optical signals.

## TEST PULSES

The TEST mode switch activates system-generated signals that verify that all parameter channels are operating correctly. See Figure 2-9. It is used by field service engineers for troubleshooting. Depress the TEST touchpad once and test signals are generated for all parameters. Depress it again, and only FSC signals are generated. In this mode, TEST flashes; it is used while executing BD FACSComp software. Depress the touchpad a third time to turn the test signals off.

# Startup and Shutdown Procedures

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## Startup Procedure

Always turn on the cytometer before turning on the computer when acquiring data. This enables the computer to recognize that the cytometer is connected. When analyzing data, it is not necessary to turn on the cytometer.

- 1 Turn on the cytometer.

The power switch is located in the upper left corner of the instrument.

- 2 Turn on the computer.

## Filling the Sheath Tank

- 1 Open the fluidics access panel, and flip the vent valve toggle in the direction of the arrow.

The switch, located between the sheath and the waste reservoirs, relieves the sheath reservoir of air pressure.

- 2 Disconnect the sheath tubing (white) and the air tubing (blue) from the cytometer by squeezing the metal clip on the quick-disconnect while simultaneously pulling the plastic connector.

- 3 Disconnect the fluid detection probe connector by squeezing the sides while pulling the plastic connector.

- 4 Remove the sheath reservoir, unscrew the fluid detection probe, and fill the reservoir to 3/4 its capacity with the recommended sheath fluid.

**⚠ CAUTION** Avoid filling the sheath reservoir to its maximum capacity. When a full reservoir is pressurized, fluid may be forced into the air supply tubing, preventing proper pressurization.

## Recommended Sheath Fluids

- BD FACSTFlow™ sheath fluid (BD Biosciences)
- Phosphate-buffered saline (PBS) (Dulbecco's Ca<sup>2+</sup> and Mg<sup>2+</sup> -free) for sorting applications.

## Non-recommended Sheath Fluids

- Fisher Hematology Diluent
- Isoton III
- Isolac D
- Deionized (DI) water

**NOTICE** If you make your own sheath fluid in the lab, be sure to pass it through a 0.22- $\mu$ m filter before running it on the instrument.

- 5** Connect the sheath fluid detection probe connector.
- 6** Replace the sheath reservoir (make sure to position the tubing so there are no kinks) and snap the fluid and air supply tubing into place by pushing firmly until you hear a click.
- 7** Flip the vent valve toggle switch to pressurize the reservoir.
- 8** Check the sheath reservoir to make sure it is properly pressurized. A properly pressurized sheath reservoir will not be able to move.

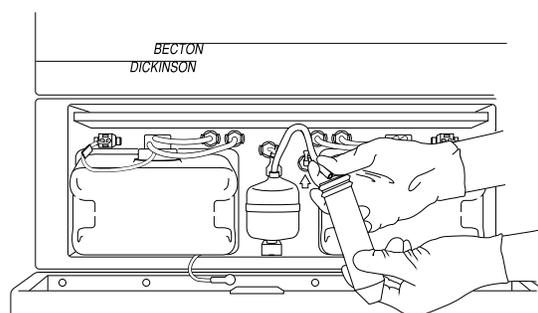
## Emptying the Waste Tank

- 1** Disconnect the waste tubing (orange) and the air tubing (white) from the cytometer by squeezing the metal clip on the quick disconnect and pulling.
- ⚠ CAUTION** It is good practice to empty the waste reservoir when you fill the sheath reservoir. This prevents the waste reservoir from overflowing. Follow good laboratory practice: wear gloves when handling waste materials.
- 2** Disconnect the fluid detection probe connector by squeezing the sides and pulling.
- 3** Remove the waste reservoir, remove the fluid detection probe, and empty the reservoir according to local, state and federal hazardous waste handling regulations.
- 4** Fill the waste container with 400 mL of undiluted household bleach.  
  
This will make a 10% solution of bleach in the waste container once it is full.
- 5** Connect the fluid detection probe connector.
- 6** Replace the waste reservoir (make sure to position the tubing so there are no kinks) and snap the waste and air vent tubing into place by pushing firmly until you hear a click.

## Purging Air Bubbles

- 1 Check the sheath filter to be sure that no air bubbles are trapped inside.

If bubbles are visible, gently tap the filter body to dislodge them and force them to the top. Squeeze the vent line and carefully remove the vent cap to allow the pressurized sheath fluid to force the air bubbles into a beaker, then replace the vent cap (Figure 2-10).



**Figure 2-10** Venting Air from Sheath Filter

- 2 Check the sheath line and saline filter line for bubbles.

If bubbles are present in either line, disconnect the tubing at the quick-disconnect port and press the tip of the valve against the side of a waste beaker. The pressurized sheath will force bubbles and sheath fluid out of the tubing.

- 3 Remove the tube of distilled water from the SIP.
- 4 Open the optics compartment door.
- 5 To clear the flow cell of trapped bubbles, view the flow cell while turning the fluid control dial to DRAIN until you see that all of the fluid has drained from the flow cell.
- 6 Turn the fluid control dial to FILL and watch as the flow cell steadily fills with sheath fluid.
- 7 Repeat the DRAIN and FILL as necessary until no bubbles are visible upon filling.
- 8 When filling the flow cell for the last time, replace the tube of distilled water back on the SIP and place the support arm directly underneath.
- 9 Leave the fluid control dial on FILL until the line carrying sheath into the waste reservoir is completely filled with fluid.
- 10 Turn the fluid control dial to STANDBY until you are ready to run your samples.

**NOTICE** Allow the laser to warm up for 5 minutes after turning on the instrument, before running samples.

## Shutdown Procedure

Always clean the cytometer before you power it off at the end of the day. Proper cleaning will ensure that your instrument will function consistently.

To prevent the sample tube from becoming clogged and to remove adhesive dyes that could remain in the tubing causing carryover, run a bleach solution through the SIP at the end of each day followed by a distilled water rinse. Follow this procedure immediately after running viscous samples or dyes such as propidium iodide (PI), acridine orange (AO), or thiazole orange (TO).

### Bleaching the Droplet Containment Tubing and the Sample Injection Tube

- 1 Turn the fluid control dial to RUN, install a tube containing 3 mL of a bleach solution on the SIP with the support arm to the side (vacuum is on), and let it run for 1 minute.

Use BD FACSTM clean solution or a 1:10 dilution of bleach in DI water as the bleach solution.

BD FACS clean solution is a bleach-based cleaning agent for daily use in cytometer maintenance.

- 2 Move the support arm under the tube (vacuum is off). Allow the a bleach solution to run for 5 minutes on HI.

### Rinsing the Droplet Containment Tubing and the Sample Injection Tube

- 1 Install 3 mL of distilled water on the SIP with the support arm to the side (vacuum is on). Let it run for 1 minute.
- 2 Move the support arm under the tube (vacuum is off). Allow the water to run for 5 minutes on HI.
- 3 Set the fluid control dial to STANDBY.

- 4 Place a tube containing no more than 1 mL of distilled water on the SIP.

**⚠ CAUTION** Sheath fluid will backflush into the tube and may cause the tube to overflow if more than 1 mL of distilled water is on the sample tube. This could affect instrument performance.

- 5 If you are finished running samples, choose Apple menu > shut down to turn off the computer and then shut off the power to the cytometer.

The tube of distilled water should remain on the SIP to prevent salt deposits from forming in the injection tube.

## Shutdown Procedure (BD FACS Loader Option)

See the Loader module for the daily cleaning procedure.

## Maintenance and Care Procedures

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### Monthly Maintenance

Perform overall system fluidics cleaning at least once a month or more frequently if you are running high volumes of samples or dyes such as propidium iodide (PI), acridine orange (AO), or thiazole orange (TO).

- 1 Turn on the cytometer.
- 2 Remove the sheath reservoir.
- 3 Disconnect the upper tubing of the sheath filter from the SALINE FILTER port by squeezing the metal clip on the quick-disconnect plug and pulling the connector from the fitting.
- 4 Connect the sheath tubing (white) from the reservoir to the upper connector for the sheath filter.

This bypasses the sheath filter and allows fluid to travel from the sheath reservoir through the tubing directly to the flow cell and to the waste reservoir (Figure 2-11).

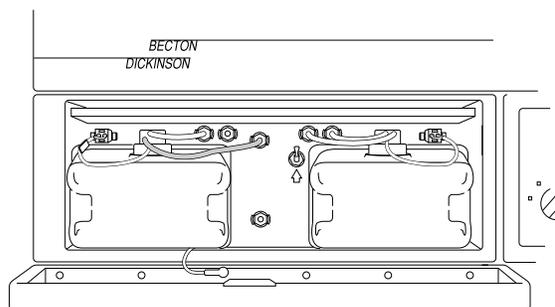


Figure 2-11 Bypassing the Sheath Filter

- ⚠ CAUTION** Bleach or detergent run through the sheath filter will break down the filter paper within the filter body, thus sending small fragments of the filter through the fluidic system including the flow cell. This will require a Field Service Engineer to come into your lab and clean the fluidic system of your cytometer.

- 5 Install a spare reservoir with 1-2 L of a bleach solution.

Use BD FACSTM clean solution or a 1:10 dilution of bleach in DI water as the bleach solution.

- 6 Set the sample flow rate switch to HI and install a tube containing 3 mL of the bleach solution on the SIP.

- 7 Set the Fluid Control Knob to RUN and allow the sample to run for 20-30 min.

- 8 Remove the tube of 10% bleach from the SIP.

Optional Rinse: Repeat steps 5 through 8 using BD FACS rinse solution.

BD FACS rinse solution is a detergent-based cleaning agent.

- 9 Repeat steps 5 through 8 using DI water.

Replace the tube on the SIP with a tube containing 3 mL of DI water. Replace the 10% bleach reservoir with a spare reservoir containing 1 to 2 L of DI water.

- 10 Remove the tube of DI water from the SIP.

- 11 Replace the original sheath reservoir and reconnect the sheath filter. Place a tube containing 1 mL of distilled water on the SIP.

- 12 Set the fluid control dial to STANDBY.

At this point, you may turn off the power to the cytometer if you are finished running samples. The tube of distilled water should remain on the SIP to prevent salt deposits from forming in the injection tube.

**NOTICE** If you will be using the cytometer to acquire samples, you will need to drain and fill the fluidics. If you will not be using your instrument for a week or longer, perform the monthly maintenance procedure and keep the distilled water in the fluidic system until you use the instrument again.

Conrad™ 70 is an excellent solution for removing clogs and wetting the fluidics. A 2% solution of Conrad 70 may be substituted for 10% bleach if necessary.

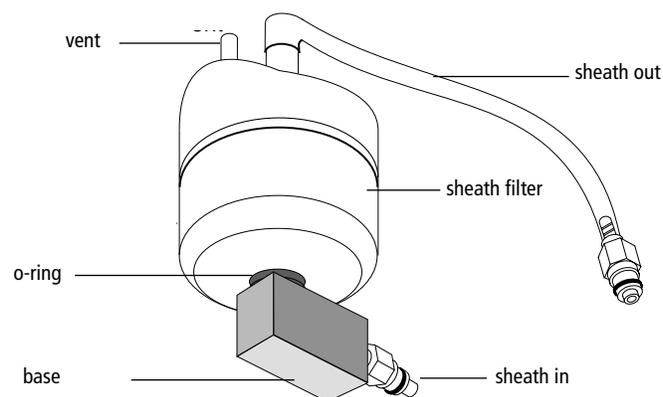
## Periodic Maintenance

Several components of your instrument may not require regular maintenance, but should be checked occasionally and cleaned as necessary. This will depend on how frequently you run your instrument.

## Sheath Filter

The sheath filter, located between the sheath and waste reservoirs, filters the sheath fluid as it comes from the sheath reservoir. When you start to notice an increase in the amount of debris seen in the FSC vs SSC plot, this may be an indication that your sheath filter should be replaced. Although the recommended time to change your filter is every 3 to 6 months, this will depend on how frequently you run the instrument and the quality of your sheath fluid. An increase in FSC noise in your BD FACSComp results is another indication that your sheath filter should be replaced. See Figure 2-12 for components of the filter.

### To replace the sheath filter



**Figure 2-12** Sheath Filter

- 1** Push the vent valve toggle switch in the direction of the arrow to release the pressure from the sheath reservoir.
- 2** Squeeze the metal clips of the output and input quick-disconnects. Disconnect the air vent tubing from the filter by unscrewing the fitting from the filter vent port.

The filter should be free of the instrument and the air vent tubing should remain attached to the instrument.

- 3** Unscrew the base of the filter to remove it from the filter body. Save this piece to attach to the new filter.

Discard the old O-ring.

- 4** Disconnect the output tubing from the output port by pulling. Leave the tie wrap in position. Save the tubing to attach to the new filter.
- 5** Install a new O-ring on to the threaded end of the filter. Wrap the filter threads with teflon tape and then attach the base (from step 3) to the new filter by screwing until snug.

- 6** Attach the output tubing (from step 4) to the new filter by pushing the tubing into the output port, and check that the tie wrap is in place.
- 7** Snap the new filter into place by pushing each quick-disconnect firmly until you hear a click.
- 8** Reattach the air vent tubing to the new filter by screwing the fitting onto the vent port.
- 9** Pressurize the instrument by pulling the vent valve toggle switch forward.
- 10** Fill the newly installed filter with fluid by pushing the RUN fluid control button and pushing the roller in the pinchcock forward to allow air to escape as the filter fills with fluid.  
  
**NOTICE** If bubbles are visible in the filter, gently tap the filter body to dislodge them and force them to the top. Push the roller in the pinchcock forward to allow the pressurized sheath fluid to force air bubbles into the waste reservoir. When the air bubbles are expelled, return the roller to its original position to stop the flow of sheath to the waste reservoir.
- 11** Discard the old filter.
- 12** Record the replacement date on the outside of the filter.

## **Air Filter**

The air filter located above the fluid reservoirs cleans the air that cools the laser. The filter may be vacuumed or washed with water, then air dried as necessary. When the filter is put back be sure it is dry and the arrows along the edge of the filter are pointing up.

# Troubleshooting

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Refer to the *BD FACScan System User's Guide* for Troubleshooting the FACScan instrument.

**NOTICE** In BD CellQuest Pro software, choose cytometer > status to help diagnose STATUS problems.

## System Stays in NOT READY

### Check the following:

- Sheath reservoir empty
- Waste reservoir full
- Initial 5-minute warmup is not complete
- Top cover of instrument is loose causing laser not to light (tighten thumb screws on top cover)
- Electrical connector on sheath probe is loose or disconnected

## System Stays in STANDBY (System Not Pressurized)

When the system is not pressurized, installation of a sample tube with the fluidics control knob set to RUN will not change the status to READY. It will remain in STANDBY. Pressure may be leaking from the reservoir cap, the vent valve, or the sample tube. The sample either does not flow to the flow cell or flows poorly, and the instrument produces poor data.

### Check the following:

- Air escaping from the sheath reservoir (tighten cap)
- Vent valve in down position
- Cracked sample tube
- Bal seal worn out
- Blue connector for sheath reservoir not correctly seated

## Excess Noise (Bubbles in the Sheath Fluid)

Bubbles are registered as events, producing spurious data. Bubbles can also cause alteration in the sample flow path resulting in less than optimal data. The fluidic system should be re-primed.

If the sheath reservoir was run dry, refill sheath and RUN system for 5–10 minutes with a tube of distilled water before attempting sample acquisition. This will remove bubbles/air from the sheath lines.

## No Events on the Computer Screen

### Check the following:

- If the system remains in STANDBY, check items under *System Stays in STANDBY*.
- If READY light is illuminated, make sure sample concentration is adequate and the sample is properly mixed.
- Ensure instrument settings are correct for applications being run.
- Ensure threshold is not eliminating the populations by being set too high.
- In BD CellQuest Pro software, choose cytometer > status to see if readings are being updated. If readings are not being updated, communication between the instrument and the computer is not present. Turn off the FACScan and the computer, turn them back on, and resume where you left off.
- Drain and fill the fluidics to remove any air bubbles that may be trapped in the flow cell. These trapped air bubbles may deflect the sample stream away from the laser beam causing no events to be detected.

## Sheath Fluid Dripping from the Sample Injection Tube

### Check the following:

- Ensure that the droplet containment tube is seated as far up into flow cell assembly as possible. Loosen the retainer and push droplet containment tube up, then retighten the retainer.
- Replace the O-ring inside the droplet containment tube retainer.
- Listen to hear if the droplet containment system pump is rotating. If you cannot hear the pump with the tube support arm to the side, the pump may have stalled. Turn off the cytometer and turn it on. If the pump is still not rotating call BD Biosciences customer support.

## Replacing Consumable Parts

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Certain items such as connectors, filters, O-rings, and tubing are considered consumable items which you may need to replace routinely or if there is a failure of the consumable item.

**NOTICE** Consumable part numbers are available in the BD Biosciences Product Catalog.

### Bal Seal

The Bal seal is a Teflon™ ring on the sample injection port which fits snugly inside an installed sample tube. See Figure 2-13. When a Bal seal is damaged, the sample test tube will not become pressurized.

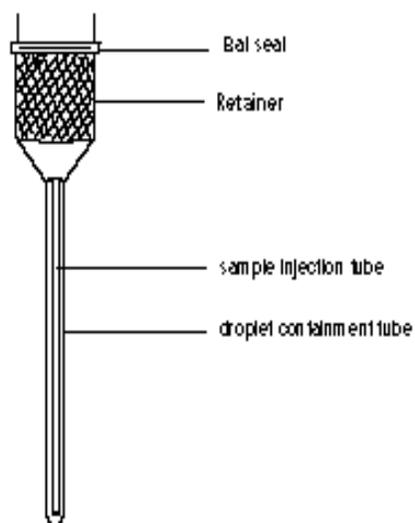


Figure 2-13 Bal Seal

#### To replace the Bal seal:

**⚠ CAUTION** Wear protective gloves when performing this procedure.

- 1 Remove the droplet containment tube retainer by turning it counterclockwise.  
The droplet containment tube will usually come off with the retainer.
- 2 Remove the old Bal seal by gripping it between your index finger and thumb and pulling down.

- 3** With one hand, install the new Bal seal spring side up around the sample injection tube. Screw on the retainer and the droplet container tube to seat the Bal seal.
- 4** Once the Bal seal has been seated, loosen the retainer enough to adjust the droplet containment tube. Push the tube up until it contacts the bottom of the sample injection tube and retighten the retainer.
- 5** Install a sample tube on the SIP. Go to BD CellQuest Pro software and choose cytometer > status to verify that the sheath pressure voltage is stable.

**NOTICE** A new Bal seal may cause some initial resistance to the installation of a sample tube. Be sure to push the sample tube up as far as possible.

## Sheath and Waste Connectors

The sheath and waste sensor connectors are color coded. Simply pull the old connector from the tubing and replace with a new properly color-coded connector.

## Sheath Filter Connector

Unscrew the connector in the base of the sheath filter. Screw the new connector into the black base. It may be necessary to use Teflon tape on the threads of the connector to prevent leakage.

## Hydrophobic Filter

The hydrophobic filter is located in the fluid compartment assembly behind the fluidic control dial. The hydrophobic filter needs to be replaced if it gets wet from fluid overflowing from the sample tube on the SIP. This may happen if the sample tube is too full.

To replace the hydrophobic filter, first open the door to the sheath and waste compartment. Loosen the thumb screw on the upper left of the fluid compartment assembly. The thumb screw is on a spring, pulling it out will allow the fluid compartment assembly to move forward. Locate the hydrophobic filter attached to a black pressure transducer and a green air line. Unscrew the green air line by turning the green air cap and remove the filter.

Install the new filter by screwing the green cap onto the threaded end of the filter and slipping the other end into the pressure transducer tubing.

## Fluidic Tubing

Fluidic tubing can be replaced as a troubleshooting measure. You may want to call BD Biosciences Customer Support for assistance with the tubing replacement.