PROCEDURE

1.0 Start Up

1.1. Check that the sheath tank is full and the waste tank is empty. Add 250 ml of bleach to the empty waste tank if necessary. Check that the air line and the blue fluidics line is connected to the sheath tank. Check that the auxiliary compressors are on and that the air and vacuum valves on the wall are open.

1.2. Turn on the computer. Log in to Windows as the Operator. The password is “BDIS”.

1.3. Double click the DIVA icon to open the instrument acquisition software. Log in to the software as the Administrator. The password is “loki”. The software will indicate that the cytometer is not connected.

1.4. Turn on the instrument system power by pressing the green button on the right side of the instrument. Wait until the software connects to the cytometer.

1.5. On the main toolbar select Cytometer > Fluidics Startup and follow the software prompts.

Note: The closed loop nozzle is not inserted during fluidics startup or shutdown and all steps referencing it can be ignored.

1.6. Ensure that the sort block and plates are clean and free of residue. To clean them wet a Kim wipe with diH2O or ethanol, wipe the plates and block thoroughly, then dry them with a new Kim wipe.

2.0 Quality Control

2.1. Turn on the cabinet fan and set the AMS controls to “Low”.

2.2. Turn on the stream by clicking the red “X” on the breakoff window. Ensure that the stream is centered in the waste catcher, close the sort block, and allow the stream to stabilize.

2.3. Create a new folder and name it with the date using the form YYYYMMDD. Open the CST experiment template from the Flow Core folder.

2.4. Place the CST beads into the cytometer loading port. Set the flow rate to 2.0 on the Acquisition Dashboard, click “Load” and verify that two scatter populations and three fluorescent populations are discernable. Click “Unload”.

2.5. Ensure that Sweet Spot is turned off on the breakoff window. On the main toolbar select Cytometer > CST and wait until the cytometer connects to the CST software.

2.6. Ensure that the correct CST bead lot number is selected then click “Run”. Review the Performance Tracking Report, and make a note of any problems or errors on the whiteboard.
2.7. Remove the CST beads from the loading port, exit the CST software, and allow the instrument to reconnect to DIVA.

2.8. Turn on Sweet Spot in the breakoff window. Ensure that the center stream is bright, tight, and centered in the side stream window.

2.9. Open the Accudrop experiment template from the Flow Core folder. Place the Accudrop beads into the cytometer loading port, and click “Load”. Adjust the flow rate so that the event rate is between 1,000 and 3,000 events per second.

2.10. Turn on the voltage in the side stream window. Click “Sort” on the Sort Layout window, then click “Cancel” on the dialog box that comes up. Turn on the Optical Filter on the side stream window and ensure that the sorting stream is inside the filter box. Adjust the voltage slider if necessary.

2.11. Optimize the drop delay until 98% of beads or more are being sorted. Unload the beads and record the date, nozzle size, drop delay, sort efficiency, 1st drop, and amplitude in the “Aria Sort Settings” log on the clipboard next to the sorter.

3.0 Shut Down

3.1. Place a tube with Contrad in the sample loading port, set the flow rate to 11.0 on the Acquisition Dashboard, and click “Load”. Allow Contrad to run for at least one minute, then click “Unload”.

3.2. Replace the Contrad tube with a tube of diH₂O, and click “Load”. Allow diH₂O to run for at least one minute. Click “Unload” and leave the diH₂O in the sample loading port.

3.3. Turn off the stream by clicking the green check mark on the breakoff window. Ensure that the stainless steel ethanol tank is full. Connect the air line and the blue fluidics line to the ethanol tank.

3.4. On the main toolbar select Cytometer > Fluidics Shutdown and follow the software prompts.

   Note: The closed loop nozzle is not inserted during fluidics startup or shutdown and all steps referencing it can be ignored.

3.5. Ensure that the sort block and plates are clean and free of residue. To clean them wet a Kim wipe with diH₂O or ethanol, wipe the plates and block thoroughly, then dry them with a new Kim wipe.

3.6. Empty the waste tank and add 250 ml of bleach to the empty waste tank. Reconnect the air line and the blue fluidics line to the sheath tank.

3.7. Exit DIVA and shut down the computer. Turn off the instrument system power by pressing the green button on the right side of the instrument.

RELATED PROCEDURES

This handout is related to SCFC SOP IN005. Please see the full SOP for further information.